

Biodegradation of Petroleum Degrading Microorganism Present in Soil

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ABSTRACT

In this present study, the soil sample was collected from various petrol bunks around Trichy and Tanjore. Bacterial and fungal species were isolated from the soil sample and they were identified. Among this bacterial isolates, two dominant species, *Bacillus subtilis* and *Pseudomonas aeruginosa* were selected for petroleum biodegradation and one dominant fungal species *Aspergillus niger* was also selected for biodegradation. The degradation ability was screened based on the soil characteristics. After treatment all the parameters were analysed which was compared with initial values. All the values were reduced after bacterial and fungal treatment. Among this highest degradation was noted in bacteria than in fungi. The plasmid was isolated from the isolated bacteria, *Pseudomonas aeruginosa*. Also to study the protein nature SDS PAGE was also performed. In 1979, for the first time Anand mohan Chakrabarty, an India borne American scientist (at the time with general electric Co USA) obtained a strain of *Pseudomonas putida* that contained the XYL and NAH plasmid as well as a hybrid plasmid derived by recombining parts of CAM and OCT (these are . The bacteria laden straw can be stored until required. When the straw was spread over oil slicks, the straw soaked up the oil and bacteria broke up the oil into non polluting and harmless products. Finally conclude that the isolated bacterial strains efficiently degrade the petroleum contaminated soil, so that bacterial strains such as *Bacillus subtilis* and *Pseudomonas aeruginosa* were recommended for petroleum biodegradation.

Keywords: Biodegradation, Petroleum, Microorganism, Soil

INTRODUCTION

Petroleum (L. *petroleum*, from Greek: *petra* (rock) + Latin: *oleum* (oil)) is a naturally occurring, flammable liquid consisting of a complex mixture of hydrocarbons of various molecular weights and other liquid organic compounds, that are found in geologic formations beneath the Earth's surface. A gas well produces predominantly natural gas. However, because the underground temperature and pressure are higher than at the surface, the gas may contain heavier hydrocarbons such as pentane, hexane, and heptane in the gaseous state. The proportion of light hydrocarbons in the petroleum mixture varies greatly among different oil fields, ranging from as much as 97% by weight in the lighter oils to as little as 50% in the heavier oils and bitumens.

The hydrocarbons in crude oil are mostly alkanes, cycloalkanes and various aromatic hydrocarbons while the other organic compounds contain nitrogen, oxygen and sulfur, and trace amounts of metals such as iron, nickel, copper and vanadium. Major methodological breakthroughs in microbial biodegradation have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of microorganisms to adapt to changing environmental conditions. Products that contain biodegradable matter and non-biodegradable matter are often marketed as biodegradable.



Methods of measuring biodegradation

In nature, different materials biodegrade at different rates. To be able to work effectively, most microorganisms that assist the biodegradation need light, water and oxygen. Temperature is also an important factor in determining the rate of biodegradation. This is because microorganisms tend to reproduce faster in warmer conditions. Biodegradation can also be measured by anaerobic microbes and the amount of methane or alloy that they are able to produce. In formal scientific literature, the process is termed bio-remediation.

Bacillus subtilis, known also as the **hay bacillus** or **grass bacillus**, is a Gram-positive, catalase-positive bacterium commonly found in soil.

Pseudomonas aeruginosa is a common bacterium that can cause disease in animals, including humans. It is a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility.

Aspergillus niger is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. Some strains of *A. niger* have been reported to produce potent mycotoxins called ochratoxins but other sources disagree, claiming this report is based upon misidentification of the fungal species. Recent evidence suggests some true *A. niger* strains do produce ochratoxin A

Biosurfactants

Biosurfactants are surface-active substances synthesised by living cells; they are generally non-toxic and biodegradable. Interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under extreme conditions, and potential applications in environmental protection.

Biosurfactants enhance the emulsification of hydrocarbons, have the potential to solubilise hydrocarbon contaminants and increase their availability for microbial degradation. The use of chemicals for the treatment of a hydrocarbon polluted site may contaminate the environment with their by-products, whereas biological treatment may efficiently destroy pollutants, while being biodegradable themselves. Hence, biosurfactant-producing microorganisms may play an important role in the accelerated bioremediation of hydrocarbon-contaminated sites. These compounds can also be used in enhanced oil recovery and may be considered for other potential applications in environmental protection. Other applications include herbicides and pesticides formulations, detergents, healthcare and cosmetics, pulp and paper, coal, textiles, ceramic processing and food industries, uranium ore-processing, and mechanical dewatering of peat.

Several microorganisms are known to synthesise surface-active agents; most of them are bacteria and yeasts. When grown on hydrocarbon substrate as the carbon source, these microorganisms synthesise a wide range of chemicals with surface activity, such as glycolipid, phospholipid, and others. These chemicals are synthesised to emulsify the hydrocarbon substrate and facilitate its transport into the cells. In some bacterial species such as *Pseudomonas aeruginosa*, biosurfactants are also involved in a group motility behavior called swarming motility.

Growth curve

A **growth curve** is an empirical model of the evolution of a quantity over time. Growth curves are widely used in biology for quantities such as population size, body height or biomass. Values for the measured property can be plotted on a graph as a function of time; see Figure 1 for an example.

Growth curves are employed in many disciplines besides biology, particularly in statistics, which has an extensive literature on growth curves. In mathematical statistics, growth curves are often modeled as being continuous stochastic processes, e.g. as being sample paths that almost surely solve stochastic differential equations



SDS-PAGE

SDS-PAGE, **sodium dodecyl sulfate polyacrylamide gel electrophoresis**, describes a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility (a function of the length of a polypeptide chain and its charge). In most proteins, the binding of SDS to the polypeptide chain imparts an even distribution of charge per unit mass, thereby resulting in a fractionation by approximate size during electrophoresis. The sample to be analyzed is mixed with SDS, an anionic detergent which denatures secondary and non-disulfide-linked tertiary structures, and applies a negative charge to each protein in proportion to its mass. Heating the samples to at least 60°C further promotes protein denaturation, helping SDS to bind. A tracking dye may be added to the protein solution.

Electrophoresis

Various buffer systems are used in SDS-PAGE depending on the nature of the sample and the experimental objective. The buffers used at the anode and cathode may be the same or different. An electric field is applied across the gel, causing the negatively-charged proteins to migrate across the gel towards the positive (+) electrode (anode). Depending on their size, each protein will move differently through the gel matrix: short proteins will more easily fit through the pores in the gel, while larger ones will have more difficulty (they encounter more resistance). The proteins will have differentially migrated based on their size; smaller proteins will have traveled farther down the gel, while larger ones will have remained closer to the point of origin. Proteins may therefore be separated roughly according to size (and thus molecular weight); however certain glycoproteins behave anomalously on SDS gels.

Plasmid

Plasmid is a DNA molecule that is separate from, and can replicate independently of, the chromosomal DNA. They are double-stranded and, in many cases, circular. Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms. Plasmid sizes vary from 1 to over 1,000 kbp. The number of identical plasmids in a single cell can range anywhere from one to thousands under some circumstances. Plasmids can be considered part of the mobilome because they are often associated with conjugation, a mechanism of horizontal gene transfer. Plasmids are considered *replicons*, capable of replicating autonomously within a suitable host. Plasmids can be found in all three major domains: Archaea, Bacteria, and Eukarya. Similar to viruses, plasmids are not considered by some to be a form of *life*. Unlike viruses, plasmids are *naked* DNA and do not encode genes necessary to encase the genetic material for transfer to a new host, though some classes of plasmids encode the sex pilus necessary for their own transfer. Plasmid host-to-host transfer requires direct, mechanical transfer by conjugation or changes in host gene expression allowing the intentional uptake of the genetic element by transformation.

Plasmid DNA extraction

Plasmids are often used to purify a specific sequence, since they can easily be purified away from the rest of the genome. For their use as vectors, and for molecular cloning, plasmids often need to be isolated. The yield is a small amount of impure plasmid DNA, which is sufficient for analysis by restriction digest and for some cloning techniques.

In recent times, many commercial kits have been created to perform plasmid extraction at various scales, purity, and levels of automation. Commercial services can prepare plasmid DNA at quoted prices below \$300/mg in milligram quantities and \$15/mg in gram quantities (early 2007).

COD (Chemical Oxygen Demand)

Chemical Oxygen Demand is a vital test for assessing the quality of effluents and waste waters prior to discharge. The Chemical Oxygen Demand (COD) test predicts the oxygen requirement of the effluent and is used for monitoring and control of discharges, and for assessing treatment plant performance. The impact of an effluent on the receiving water is predicted by its oxygen demand. This is because the removal of oxygen from



the natural water reduces its ability to sustain aquatic life. The COD test is therefore performed as routine in laboratories of water utilities and industrial companies. COD - Chemical Oxygen Demand is a laboratory test that takes two hours to complete and calculates the oxygen demand of a discrete sample under specific conditions, used to indicate water quality.

MATERIALS AND METHODS

Collection of samples

Petroleum contaminated soil samples were collected from petrol bunks around Trichy and Tanjore. Clean and sterile sampling bottles were used for the collection of samples and were transported to the laboratory for further analysis.

Processing of the sample

The collected samples were serially diluted 0.1ml of samples were aseptically spread on to petriplates containing about 15 ml of pre sterilized molten medium of Nutrient agar and potato dextrose agar. The plates were then incubated for 24 to 48 hours at 37 C. Nutrient agar and potato dextrose agar were used for the isolation of bacterial and fungal colonies respectively.

Selection of bacterial strains

Bacterial colonies with different morphologies were selected and pure cultures of the same were maintained. From the isolated strains, based on earlier literatures and preliminary screening a few (four) strains of bacteria were selected for further analysis. Selected single colony to identify the colony morphology Gram staining and motility test was performed.

Selection of fungal strains;

The fungal colonies was identified by Rose bengal aAgar medium. The fungal colonies identified by lactocotton blue dye method.

ISOLATION AND IDENTIFICATION OF BACTERIA

The media is of complex type that is rich in vitamins and nutrients. The following components were used to prepare Nutrient agar medium.

Isolation of Bacteria

Gram Staining

This method was developed by Hans Christian's gram a Danish bacteriologist. It is used to differentiate the gram positive and gram negative bacteria.

BIOCHEMICAL TESTS

The following biochemical tests were performed to characterize the isolates:

Indole,MR_VP,oxidase TSI,citrate utilization test

Soil Analysis; Detection of nitrogen,organic carbon,

ESTIMATION OF PHOSPHORES

Estimation of lead, Mercury, were performed by standard methods.

Determination by pH Paper

Method

pH of water samples are determined using pH Solution . The pH solution is mixed with water samples. The sample attains a colour depending upon the hydrogen ion concentration of the sample and the colour can be compared with the colour given the pH charts.

Procedure

A small amount of sample is taken in clean test tube add pH solution into sample with the help of a pipettes the colour attained on the tube is compared with that of the colour on the pH chart. The approximate pH value is thus determined.

The same procedure is repeated and the procedure for the degradative analysis and pH values is recorded.

Measurement of growth by optical density (OD)value

After 48 hrs aliquots were withdrawn from the respective flasks inoculated with the same quantity of isolate under sterile condition was centrifuged at 8000 rpm for 10 minutes. The pellets were resuspended in know volume of distilled water (5ml) and the absorbance was measured at 420 nm. The plain media without inoculum served as the control.

Extraction of crude oil after degradation for quantification

The culture broth incubated for different time intervals with different concentrations of crude oil was transferred to separating funnel at the end of the incubation period. It was extracted with 10 ml toluene. The funnel was shaken vigorously for 5 minutes and allowed to settle. The toluene oil mix was collected in to sample bottle and analyzed for the estimation of crude oil content by diluting with known of fresh toluene (5ml) (odu,1985). The values were compared with known concentration of crude oil.

Estimation of degradation

The amount of crude oil degraded is the difference between initial and final concentration of oil content in medium and percentage of degradation was calculated.

Estimation of biomass

10 ml of sample (inoculated broth) was taken at every 48 hours and centrifuged at 4000x g for 20 minutes. The pellets were dried in an oven at 110 C. The dry weight was calculated. The supernatant was used for biosurfactant extraction.

Extraction of biosurfactants

Surfactants were extracted following Itoh and Suzuki (1972). The cells were first separated from supernatant by centrifugation at 6800 x g. The supernatant was then acidified using 12 M hydrochloric acid to Ph 2.0 and the precipitate was collected by centrifugation (12100 x g) and then extracted three times with a chloroform-ethanol (2:1) mixture, which was then evaporated away leaving behind relatively pure rhamno lipids having an oil-like appearance (Zhang and Miller,1992). The oily residues were dissolved in an appropriate volume of methanol and transferred to a previously weighed container. The methanol was evaporated and the weight of the recovered product was determined to calculate the total biosurfactant yield.

Plasmid DNA Extraction Using Alkaline Lysis Method

Plasmids can be isolated by a variety of methods many of which rely on the differential denaturation and reannealing of plasmid DNA compared to chromosomal DNA. One commonly used technique developed by



Birnboim and Doly involves alkaline lysis. This method essentially relies on bacterial lysis by sodium hydroxide and sodium dodecyl sulfate (SDS), followed by neutralization with a high concentration of low-pH potassium acetate. This gives selective precipitation of the bacterial chromosomal DNA and other high molecular-weight cellular components. The plasmid DNA remains in suspension and is precipitated with isopropanol.

SDS-PAGE

Sample preparations

- Take a defined amount of Sample in an eppendorf tube.
- Add 4X sample buffer to make it 1X.
- Boil the samples for 5 min.
- Cool down the samples to RT before loading.

Running gel

- Set up the gel apparatus.
- Transfer the gel plates from the gel casting apparatus to the running unit.
- Add the running buffer in the reservoirs.
- Load carefully the samples.
- Connect the power cords and run the gel at 200 mA.

Processing

- After the gel run is over with the dye front reaching the end of the gel, stop the power.
- Lift the plates and transfer the gel for further analysis like staining or for Western blot.

Chemical Oxygen Demand

Reagents

Potassium dichromate solution 0.1 N

3.67 g of potassium dichromate solution was dissolved in 1 litre of distilled water.

Sulphuric acid solution 2M

1.84 ml of sulphuric acid was dissolved in 25 ml of distilled water

Sodium thiosulphate solution 0.1 M

7.9055g of sodium thiosulphate were dissolved in 100 ml of distilled water.

Starch solution 1%

1 g of starch was added to 10 ml of hot distilled water and few drops of formaldehyde were added.



Procedure

50 ml of serially diluted soil sample was taken in a conical flask and similarly 50 ml of distilled water was taken in another flask. 5 ml of potassium dichromate was separately added to each flask. The flasks were kept in boiling water bath 100 °C for 1 hour. After one hour the flask were cooled for 10 minutes. 5 ml of KI and 10 ml of sulphuric acid solution was added into the flasks. Then it was filtrated against 0.1 M sodium thiosulphate kept in burette. It was titrated until pale colour disappears. 1 ml of starch solution was added to both the flasks. The colour turned blue. Again it was titrated with sodium thiosulphate until the blue colour disappears.

Calculation

$$\text{COD}(\text{mg/l}) = 8 \times C \times V_b \times V_a / V_s$$

C = concentration of titrant

V_a = volume of titrant used for control

V_b = volume of titrant used for soil sample

V_s = volume of sample used

Oil and Grease Content

One hundred ml of sample was taken and pH was adjusted to 4.0. Six ml of carbon tetrachloride was added and shaken vigorously. A layer of organic matter was collected and transferred to the clean dried pre weighed container. Then it was placed in a hot air oven at 50 °C for 30 minutes and weighed. Amount of oil and grease content was calculated using the formula.

$$\text{Oil and grease content} = \frac{\text{weight of beaker with sample} - \text{weight of beaker without sample}}{\text{Volume of sample}}$$

RESULTS AND DISCUSSION

The present study is carried out for the isolation and identification of bacteria and fungi from petroleum contaminated soil sample. The physicochemical characteristics of the soil were analysed before and after treatment with isolated bacteria and fungi.

Isolation of bacteria and fungi

From the nutrient agar plates the number of bacterial colonies were observed after incubation. Among these colonies two dominant colonies were noted. That colony was used for degradation process.

Similarly number of fungal colonies was also recorded in PDA plates. From these fungal colonies single predominant colony was selected for degradation study.

Identification of bacteria and fungi

The isolated bacterial and fungal strains were identified. The bacterial strain cultural, morphological and biochemical characteristics results were compared with Bergey's manual systematic bacteriology and identified as *Bacillus subtilis* and *Pseudomonas aeruginosa*.

The isolated fungal colony identified based on the lactophenol cotton blue staining results and it was confirmed as *Aspergillus niger*.



Soil analysis

Before and after the treatment the soil characteristics were estimated and analysed. The rate of heavy metals decreased after the biodegradation process. The amount of organic carbon was also reduced. The pH of the soil sample was high (pH 8) After treatment the pH was also reduced.

Chemical oxygen demand (1960 mg/ml) is reduced . It indicates the measure of oxygen consumed during the oxidation of the utilizable organic matter.

Plasmid DNA Isolation

The electrophoresis was run for 5 hours at a constant voltage of 150 V. To confirm the purity and identification of the plasmid DNA, a restriction endonuclease Sma I was used. The Fig shows an illustration of fragments produced from each plasmid of PI, and P2. By comparing the density and the composition of fragments separated by electrophoresis, the purity of the plasmids was determined.

SDS PAGE

The page was successfully run using the vertical electrophoresis. The fragmentation was occurred between 14.4 to 97 Kda. The sample showed two protein bands and the molecular size is 14.4 KDa and 45 KDa respectively. No significant polymorphism was evident through PAGE as Seed and fruit isolates revealed more or less uniform profile. The variation of fragmentation was occurring due to the nature of the sample and amount of protein. The result showed in fig.

Cod and Oil Content

Many microorganisms have the ability to utilize hydrocarbons as sole source of energy. The hydrocarbon utilizing microbes including fungi and bacteria degrade very high concentrations of oil that pollutes the soil environment. Diverse groups of microbial populations were frequently isolated from soils polluted with hydrocarbons in the soil environment has been well established (Atlas, 1981)

In their present study totally fifteen species that belonged to bacteria or fungi were isolated from oil sludge contaminated soils. Contaminated tests proved that among the bacteria, *Pseudomonas aeruginosa* and *Bacillus cereus* and among fungi *Aspergillus niger* and *Penicillium granulatum* were potential organisms that could effectively degrade hydrocarbon contents of the soil. COD values during biodegradation was measured and expressed as mg/l. Walker et al (1975) compared the abilities of bacteria and fungi in the degradation of hydrocarbons. They studied *Candida sp* and found many species of *Pseudomonas* are capable of utilizing petroleum hydrocarbons. Amount of COD varied among the organisms. *Pseudomonas aeruginosa* treated MSM produced 0.136 mg /l, of COD where as *Bacillus subtilis* produced 0.176 mg/l of COD on the 10th day of incubation. Likewise *Aspergillus niger* produced 0.144 mg/l of COD on the 13th of incubation. Among the test organisms, *Pseudomonas aeruginosa* showed maximum reduction (198-177 mg/l) in the total oil and grease contents on the 10th day followed by *Aspergillus niger* (259-229 mg/l). Walker et al (1975) compared the abilities of bacteria and fungi in the degradation of hydrocarbons. They studied *Candida sp*, *Hansenula sp*, *Rhodotorula sp* and found large amount of *Pseudomonas* species are capable of utilizing petroleum hydrocarbons. Swaranjit singh and his group (IMTECH, Chandigarh) have isolated both bacterial and fungal culture from the petroleum sludge. The fungal culture could degrade 0.4% sludge in 3 weeks. Degradation of petroleum sludge occurred within two weeks when the bacterial culture (*Bacillus circulans* C1) was used. More over, significant degradation of petroleum sludge was noticed in 10 days when the fungus + *B. circulans* and a prepared surfactant were exogenously added to petroleum sludge. Hayumanavan et al (2001) tested the effect of microbial consortium on the degradation of petroleum refinery effluent plant sludge applied to sterile and nonsterile red soil and found fungal population, *Aspergillus*, *Cladosporium*, *Rhizopus* etc to be effective.

Soil Analysis

FIGURE : 1 Estimation of Nitrogen

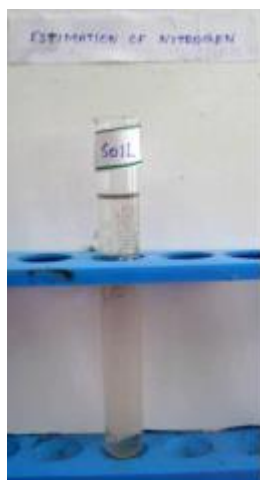


FIGURE: 2 Estimation of Lead



FIGURE: 3 Estimation of Mercury

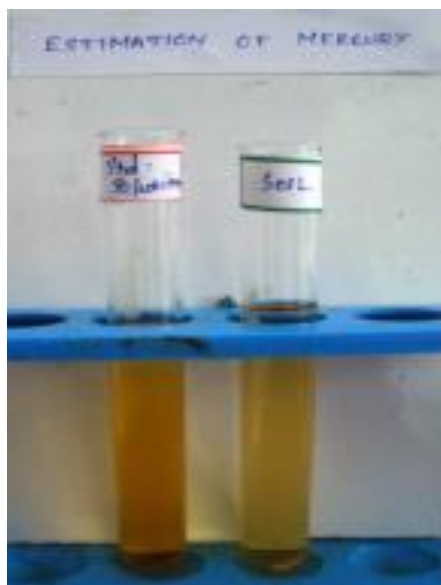


FIGURE: 4 Estimation of Phosphorus



FIGURE: 5 Estimation of Organic Carbon

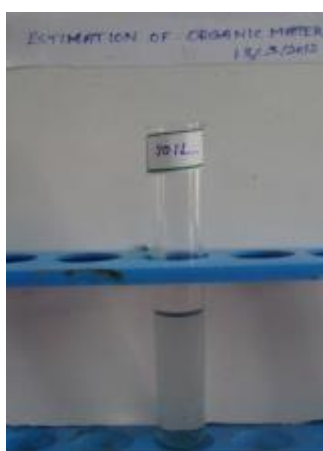


FIGURE: 6 Grams Staining



FIGURE; 7 Lactophenol Cotton Blue Staining

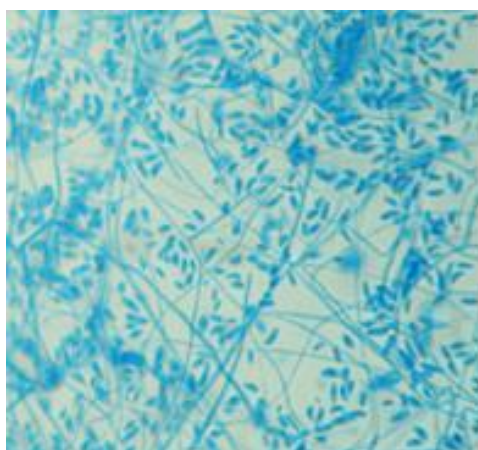


FIGURE: 8 *Pseudomonas aeruginosa*

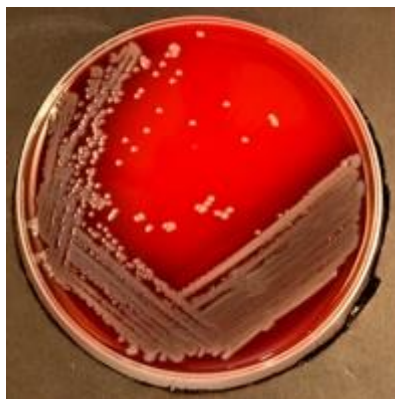


FIGURE: 9 *Bacillus subtilis*



FIG :10 Identification Of Bacteria By Biochemical Tests



Bacillus subtilis

INDOLE: NEGATIVE

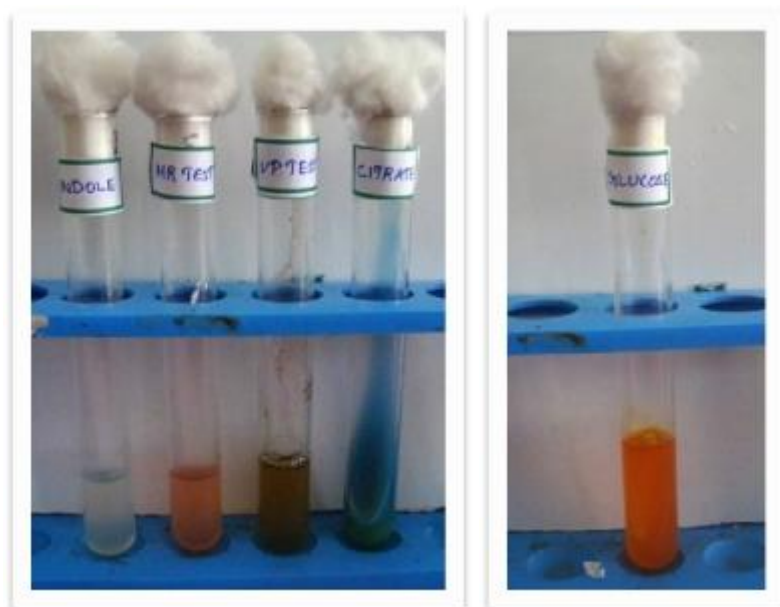
METHYL RED: POSITIVE

VOGES PROSKAUER: POSITIVE

CITRATE : POSITIVE

GLUCOSE UTILISATION: POSITIVE

Identification of Bacteria by Biochemical Tests



Pseudomonas aeruginosa

INDOLE : NEGATIVE

METHYL RED : NEGATIVE

VOGES PROSKAUER : NEGATIVE

CITRATE : POSITIVE

GLUCOSE UTILISATION: POSITIVE

Table 1 SOIL ANALYSIS

S No	Element	Before degradation	After degradation
1	Nitrogen	0.351	0.517
2	Phosphorous	1.04	1.734
3	Mercury	1.317	0.832
4	Iron	0.525	0.325
5	Lead	1.297	0.912
6	Carbon	1.007	0.764

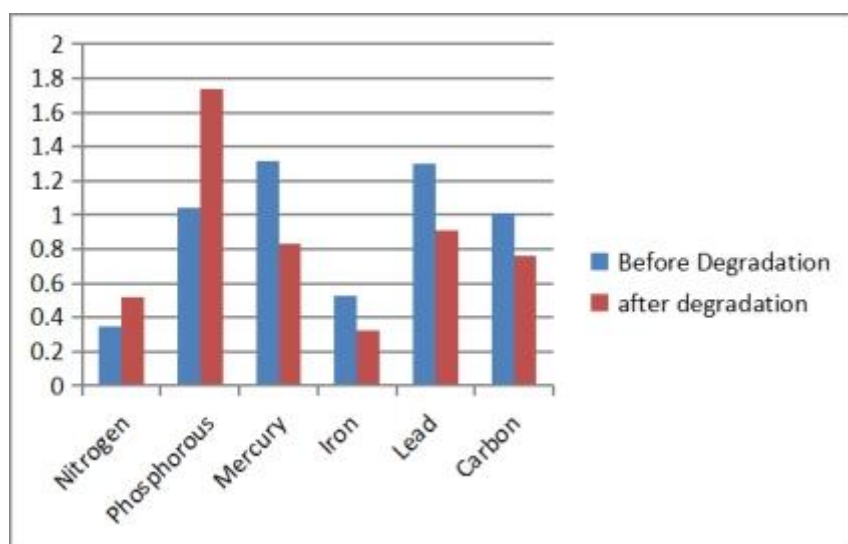
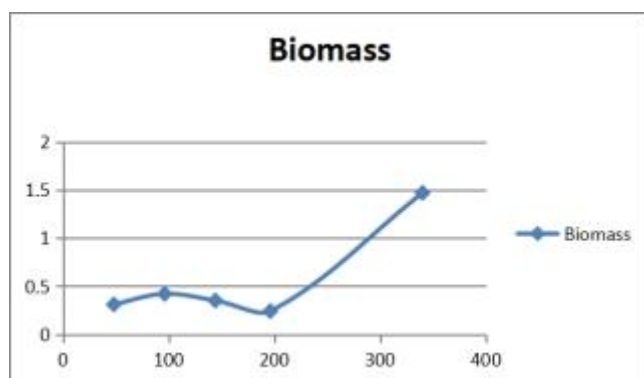


Figure- 12 **Soil Analysis**

TABLE 2 **Estimation of Biomass**

Time interval	Estimation of Biomass
48	0.31
96	0.42
144	0.35
196	0.24
340	0.21

Figure – 13 **CELL DRY WEIGHT**

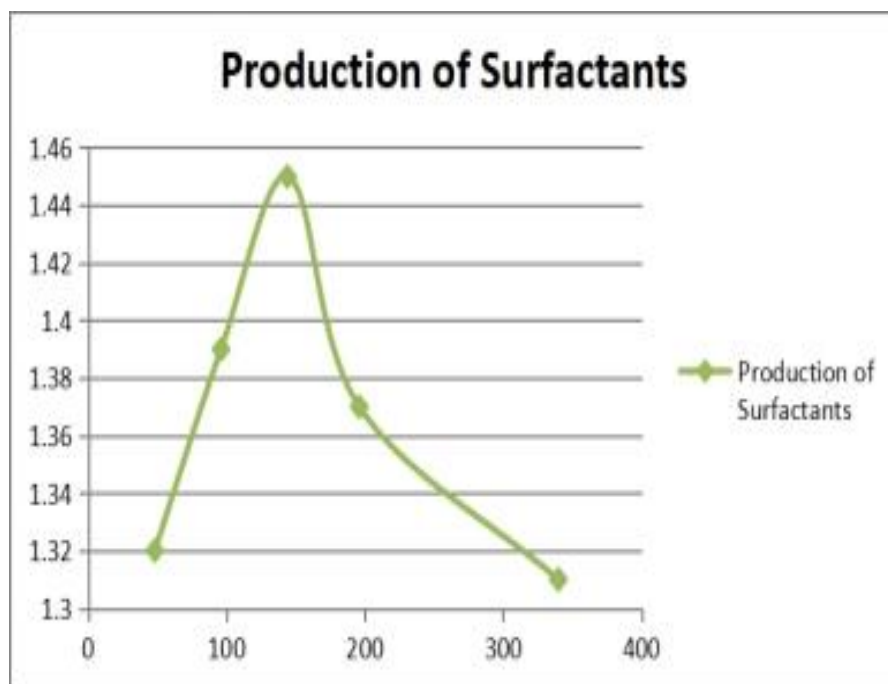


X axis – time interval, Y axis - biomass

TABLE 3 **Production of Biosurfactant**

Time interval	Production of Surfactants
48	1.32
96	1.39
144	1.45
196	1.37
340	1.31

FIGURE :14 Production of Surfactants at different time intervals

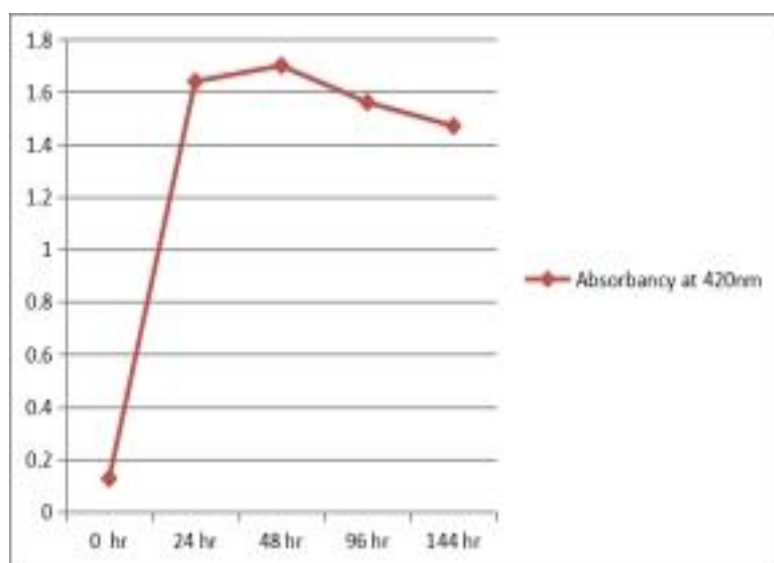


X axis – time interval, Y axis – biosurfactant production

TABLE 4 Growth at different time interval

Time interval	Absorbancy at 420nm
0 hr	0.126
24 hrs	1.64
48 hrs	1.7
96 hrs	1.56
144 hrs	1.47

FIGURE ;15 Bacterial Growth



X axis : Time interval Y axis : Absorbancy

FIGURE: 16 SDS FOR *Pseudomonas aeruginosa*

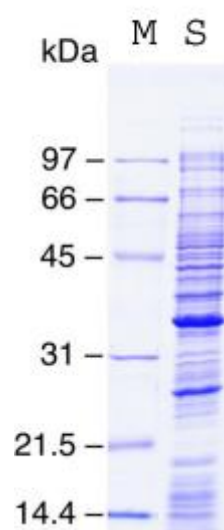


FIGURE :17 Fig: Isolation of Plasmid DNA from *Pseudomonas*



Table - 5 Amount of cod and grease content in microbial isolates isolates

	COD		Oil and grease content	
	5 th day	10 th day	5 th day	10 th day
Test isolates	C T1 T2	C T1 T2	C T1 T2	C T1 T2
<i>Pseudomonas aeruginosa</i>	0.184	0.178	259	258
<i>Bacillus subtilis</i>	0.160	0.136	232	177
	0.152	0.144	219	169
	0.200	0.196	290	286
	0.192	0.176	285	269
	0.172	0.168	273	251
<i>Aspergillus niger</i>	15 TH day	30 th day	15 th day	30 th day
	0.200	0.196	286	279
	0.168	0.144	268	235
	0.160	0.124	251	229

SUMMARY AND CONCLUSION

In this present study, the soil sample was collected from various petrol bunks around Trichy and Tanjore.



Bacterial and fungal species were isolated from the soil sample and they were identified. Among this bacterial isolates, two dominant species, *Bacillus subtilis* and *Pseudomonas aeruginosa* were selected for petroleum biodegradation and one dominant fungal species, *Aspergillus niger* was also selected for biodegradation.

The degradation ability was screened based on the soil characteristics. After treatment all the parameters were analysed which was compared with initial values. All the values were reduced after bacterial and fungal treatment. Among this highest degradation was noted in bacteria than in fungi. The plasmid was isolated from the isolated bacteria, *Pseudomonas aeruginosa*. Also to study the protein nature SDS PAGE was also performed. In 1979, for the first time Anand mohan Chakrabarthy, an India borne American scientist (at the time with general electric Co USA) obtained a strain of *Pseudomonas putida* that contained the XYL and NAH plasmid as well as a hybrid plasmid derived by recombining parts of CAM and OCT (these are incompatible and cannot co-exist as separate plasmids in the same bacterium. This strain could grow rapidly on crude oil because it was capable of metabolizing hydrocarbons more efficiently than any other single plasmid. In 1990, the USA government allowed him to use this superbug for cleaning up of an oil spill in water of state of Texas. Superbug was produced on a large in laboratory, mixed with straw and dried. The bacteria laden straw can be stored until required. When the straw was spread over oil slicks, the straw soaked up the oil and bacteria broke up the oil into non polluting and harmless products. Finally conclude that the isolated bacterial strains efficiently degrade the petroleum contaminated soil, so that bacterial strains such as *Bacillus subtilis* and *Pseudomonas aeruginosa* were recommended for petroleum biodegradation.

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