Bioremediation of Automobile oil effluent by *Pseudomonas* sp.

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Abstract

Automobile workshops are an important component of the service sector industry. The most significant environmental impact associated with the existing workshops is the seepage of used engine oil and washed water into the soil. Contamination of the soil by oil causes it to lose its useful properties such as fertility, water-holding capacity, permeability and binding capacity. In this study was to degrade the oils from the automobile effluent by *Pseudomonas* sp. with the best carrier based formulation. The oil degrading *Pseudomonas* sp. was isolated by enrichment technique from the automobile oil effluent polluted soil, which had been contaminated over a period of time. In this study, two liter capacity of batch type mini fermentor was used for the production of *Pseudomonas* sp. Two different carrier formulations like Diatomaceous and Diatomaceous earth with lignite (8:2 ratios) were used as a carrier for the preparation of carrier based biodegrader. The automobile effluent was analyzed by various methods like pH, acidity, Total Dissolved and suspended solids, hardness, BOD, COD. Addition of Inorganic salts found to be maximum degradation of 93.18 % at the 6th day. In the combination of Glycerol and Inorganic salts was showed 100 % oil degradation and which was found to be a most effective treatment. 96.97 % of oil degradation was recorded in the treatment of glycerol with inorganic salts.

Key words: auto mobile effluent, carrier, Diatomaceous earth, oil, *Pseudomonas* sp.

Introduction

Oil released into the environment is a well-recognized problem in today's world. Oil spills affect many species of plants and animals in the environment, as well as humans. Biodegradation is a natural process by which microbes alter and break down oil into other substances. The resulting products can be carbon dioxide, water, and simpler compounds that do not affect the environment.

Pseudomonas sp. ubiquitous in soil and water, are of considerable scientific and technological importance and comprise a taxon of metabolically versatile organisms capable of utilizing a wide range of simple and complex organic compounds. They are known to be involved in biodegradation of natural or man-made toxic chemical compounds (Holloway (1992) *Pseudomonas* in the late 20th century. Further more, the bacterial genus *Pseudomonas* is a prolific producer of a number of extra cellular enzymes, including lipase.

Bioremediation is the optimization of biodegradation. Two forms of technology can accomplish this acceleration: (1) fertilizing (adding nutrients) and/or (2) seeding (adding microbes). These additions are necessary to overcome certain environmental factors that may limit or prevent biodegradation.

Petroleum is a complex mixture of many thousands of compounds. These can be divided into four major groups: the alkanes, the aromatics, the resins, and the asphaltenes. In general, the alkane fraction is the most biodegradable, whereas the polar fraction (i.e., the resins and asphaltenes) is resistant to biological degradation. The aromatic compounds, especially the polycyclic aromatic hydrocarbons (PAHs) are of intermediate biodegradability, but these are of most concern owing to their toxicity and tendency to bioaccumulate (Atlas, 1981).

The bioavailability of weekly soluble hydrophobic compounds for microbial conversion is usually low and thus limits their degradation rate in aqueous medium. The use of surfactants has been found to enhance degradation of crude oil (Urum *et al.,* 2003; Balba *et al.,* 2002) or other hydrocarbons (Nakahara *et al.,*1981). Among various surfactants, rhamnolipids are considered to be the most in degrading hydrocarbons (Itoh and Suzuki, 1972). For example, the biodegradation of long-chain alkanes was stimulated by addition of rhamnolipid (Woutter and Dick, 2002). This facilitated biodegradation is probably due to increased cell surface hydro-phobility after extraction of lipopolysaccharides from the cellular envelope by rhamnolipids, which subsequently stimulates uptake via direct contact between cells and hydrocarbon droplets. Thus, the integration between addition of rhamnolipid and biodegradation of hydrocarbons seems to be highly specific.

Automobile workshops are an important component of the service sector industry. With rapid socioeconomic and infrastructure development, the number of vehicles in India, especially in the urban areas, has increased significantly. The most significant environmental impact associated with the existing workshops is the seepage of used engine oil into the soil. Contamination of the soil by oil causes it to lose its useful properties

such as fertility, water-holding capacity, permeability and binding capacity. Contamination of groundwater is also a potential problem. The other significant impact observed was on surface water, mostly the nearby streams, which receive a lot of untreated effluent from service stations containing oil and grease as well as non-biodegradable detergents.

Many species of microorganisms: bacteria, yeasts, and fungi obtain both energy and tissue-building material from petroleum. The fuel-eating bacteria, known as *Pseudomonas*, have evolved a taste for hydrocarbons, the major component of fossil fuels. In this study was to degrade the oils from the automobile effluent by *Pseudomonas* sp. with the best carrier based inoculum.

Materials and methods

Isolation of oil degrading bacteria

The oil degrading *Pseudomonas* sp. was isolated by enrichment technique from the automobile oil effluent polluted soil, which had been contaminated over a period of time. The cells were grown on a rotary shaker in one-liter flasks each containing 500 ml of basal salt medium with 0.5 ml of crude oil. The cells were recovered by centrifugation after 72 h of growth, washed and resuspented in normal saline. The isolated bacterial cultures were characterized by their morphological characteristics and maintained in the nurtrient agar medium and stored at 4° C.

Mass production of *Pseudomonas* sp. by batch fermentor

In this study, two liter capacity of batch type mini fermentor was used for the production of *Pseudomonas* sp. Starter culture at the rate of 3% was added aseptically to the nutrient in the fermentor. Sterile air from air compressor was bubbled to the medium. Stirred with built in stirrers and incubated for 48 hrs. After fermentation, the quality culture broth was checked by the bacterial population by dilution method. Population should be above 10^9 viable cells per ml. After fermentation the culture broth was collected in sterile container and stored at 4° C.

Preparation of carrier based biodegrader

Two different carrier formulations like Diatomaceous and Diatomaceous earth with lignite (8:2 ratios) were used as a carrier for the preparation of carrier based biodegrader. (i) Diatomaceous earth (grainy, porous, and sand-like material) was obtained as a gift from M/s Seema Minerals and Metals; Rajasthan, India. (ii) Diatomaceous earth and lignite (8:2): The diatomaceous earth 800 g was mixed thoroughly with 200g of Lignite powder (Received from Neyveli Lignite Corporation, India). The carrier material was first dried in the hot air oven at 80° C for 24 hr and then cooled to room temperature for further treatments. The pH solution of carrier materials were carried out in 60cm^3 glass bottles where 2.5 g of carrier material and 25cm^3 of de-ionized water were added. The mixture was then agitated for 24 hr. The pH of the mixture was recorded using a pH meter. The carriers were neutralized with organic acetic acid (pyroligneous acid). The neutralized carries were sterilized and after cooling it was mixed with the cultured broth *Pseudomonas sp.* in the ratio of 3:1 (w/v), for carried based inoculam composition. After proper mixing, the biodegrader was transferred to sterile polypropylene cover and sealed tightly. This Carrier Based Microbial Inoculam (CBMI) was used for biodegradation of auto mobile oil effluent.

Characterization of automobile effluent

The automobile effluent was collected form automobile work shops (Chennai, India) and its characteristic features were analyzed such as pH, Temperature, total solids, total dissolved solids, acidity, hardness, chlorides, biological oxygen demand and chemical oxygen demand.

Experimental setup for Auto mobile oil effluent treatment

Automobile effluents (100 ml) were taken in 42 Erlenmeyer cleaned flask and then following experimental setups were followed, (i) The first six flasks were served as a control. (ii) 7-12 flasks were treated with 0.5 g of CBMI. (iii) 13-18 flasks were added with the following mixture of inorganic nutrients like 0.25 g ammonium phosphate; 0.05 g magnesium sulfate; 0.25 g potassium phosphate; 1.25 g non-iodinated sodium chloride with 0.5 g CBMI. The inorganic nutrients provide nitrogen and minerals to the organisms. (iv) 19-24 flasks were treated with CBMI along with 0.5 ml of Glycerol. Glycerol served as a carbon source for the microbial inoculums. (v) 25-30 flasks were treated amended with 0.1 ml surfactant (Fatty alcohol alkoxylate) and CBMI. (vi) 31-36 flasks were treated with the combination of 0.5 ml Glycerol and 0.5 g of CBMI. (vii) 37-42 flasks were amended with both organic nutrients and Glycerol along with CBMI. The above flasks were tightly cotton plugged in order to avoid evaporation. The flasks were incubated for a week under room temperature. Results were recorded from each flask every day up to 6 days.

Estimation of oil degradation

Oil degradation is defined as the process by which oil is converted to a form that is no longer extractable by benzene. Oil conversion was determined by extracting 100 ml of incubation mixture with 10 ml of benzene; the aqueous phase was extracted a second time with 10 ml of benzene and the combined benzene fractions were filtered through Whatman no. 1 filter paper in order to clarify the extract. The benzene was evaporated at 37^{0}C to constant weight in a tared large petridish. The rate of oil degradation was expressed in milligrams as well as in percentages.

Conversion of milligrams to %,

Rate of oil degradation (z) = x/y *100

Rate of oil degradation (%) = z 100.

x = periodical Benzene extractable oil (mg);
y = Control Benzene extractable oil (mg)

Determination of shelf life of carrier based inoculums

Serial dilution of CBMI

Thirty gram of CBMI was taken in sterilized Erlenmeyer flask containing 270 ml of sterile water and shacked for 10min on a reciprocal shaker. Serial dilution was made up to 10^{-8} dilutions. 1 ml from each of 10^{-6} and 10^{-8} dilutions of each carrier inoculums were taken and pour plated in nutrient agar medium following strict sterile handling techniques.

The inoculated plates were then incubated for 24 hours at 37°C. A replica of this work was done again for reference and the average colonies would be verified and consolidated as the final colony count. Isolated colonies formed in the next day of pour plating, indicates the number of colony forming units of *Pseudomonas sp.* to be sustained in the carrier inoculums. The results were expressed in Colony Forming Units (CFU).

Results

Characterization of automobile effluent

The automobile effluent was analyzed by various methods like pH, acidity, Total Dissolved and suspended solids, hardness, BOD, COD, etc., The analyzed results were tabulated (Table-1).

Testing parameters	Results
pH	6.5
Total solids (incl.oil)	7g/l
Total suspended solids	6.7 g/l
Total dissolved solids	250 mg/l
Total acidity	6.3
Chloride	23 mg/l
Calcium	29 mg/l
Magnesium	78 mg/l
Total hardness	110 mg/l
BOD	230
COD	650

Carrier Based Microbial Inoculums

Microbial consortium developed with suitable carrier material, with the combination of diatomaceous earth and lignite, which is an environment friendly product. The survivability of the consortium in the carrier-based condition was determined periodically at ambient temperature. The carrier based microbial inoculums was put into sterile polyethylene bags and sealed aseptically (8:2).

Bioremediation of automobile oil effluent

The rate of biodegradation of oil was performed every day up to 6 days. The results were expressed in percentage of oil degradation. 70.61% of oil degradation was showed in the treatment of CBMI. Amendment of glycerol with CBMI to the effluent and which was found to be higher degradation rate than the CBMI treatment 85.61%. Addition of Inorganic salts found to be maximum degradation of 93.18% at the 6th day. Incase of surfactant added effluent was recorded 81.82% of oil degradation at the final day of incubation. In the combination of Glycerol and Inorganic salts was showed 100% oil degradation and which was found to be a most effective treatment. 96.97% of oil degradation was recorded in the treatment of glycerol with inorganic salts (Fig-1).

Shelf life of CBMI

The shelf life of CBMI was determined by viable plate count method. The initial days of colony count was showed 294CFU and it was steadily maintained up to final days of analysis with decrease of colony count. In this results shows the carrier was effectively preserving the microbial population.

Discussion

The carrier-based inoculums of *Pseudomonas sp.* was degraded the automobile oil effluent. However, oil is composed only of hydrogen and carbon, and the bacteria need additional nutrients to grow for fast degradation rate. The inorganic nutrient mixture provides nitrogen and several essential minerals. Other source like glycerol, surfactant was also contributing the oil degradation.

Oxygen was not a rate limiting factor in biodegradation in Alaska "because of the generally large size of the sediment, it's high permeability into sea water and the ample content of dissolved oxygen in seawater flushed through the sediments during each of the two daily tide cycles" (Bragg *et al.,* 1992).

Bioremediation is not only used to clean up after oil spills; the same basic science discussed in this report can be used to remediate many environments. The EPA claims that bioremediation is a technology with enormous promise for the future. The process of bioremediation is similar to wastewater treatment where we rely on microbes to clean our water for us. As long as we give them a favorable environment they do a pretty good job.

Conclusion

Oil spills are a major menace to the environment as they severely damage the surrounding ecosystems. Bioremediation is best way to treat the oil contaminant that employs the oil degrading microorganisms. These organisms have the capability to degrade toxic contaminants for the reclamation of polluted sites.

Pollution of water bodies is mainly caused by spillage of used engine oil. Therefore, spillage of oil at the source should be controlled.

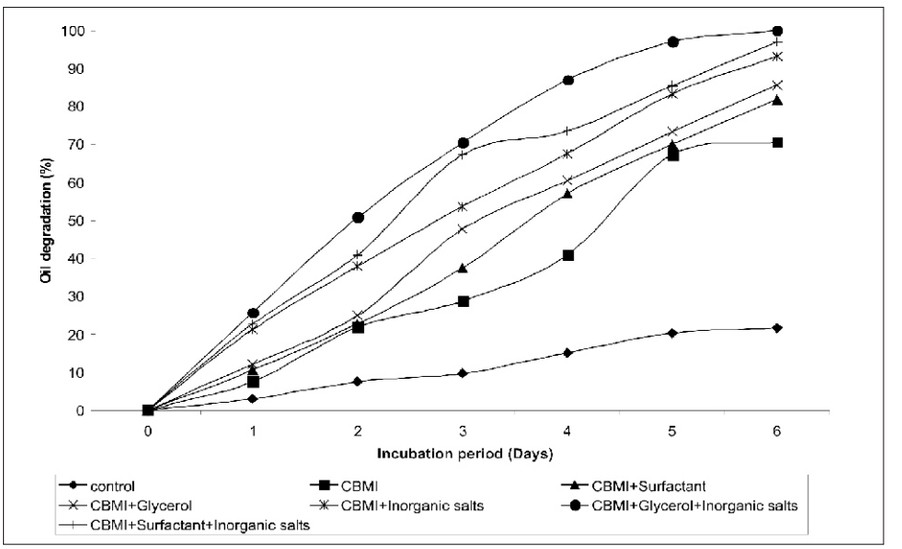

Figure-1 Degradation of automobile oil effluent by carrier bases microbial inoculam with the effect of various nutrients

Waste oil should be transferred to metallic drums (200-liter capacity) with proper labels and stored in closed areas. The workshop owners need to cooperate and maintain strict operating procedures with the contractor collecting the used oil.

The effluents from washing activities should not be released into open drains and streams but treated properly before being discharged. Because most automobile workshops in India are clustered together, it is recommended that they have a common effluent treatment system. The cost of operating this system could be shared among workshop owners.

Some of the areas where bioremediation is still relatively new but it has the potential of saving money, being ecologically sound, destroying contaminates and allowing for the treatment waste on site (EPA 1991, p.7). The application of bioremediation will be an important aspect of waste management now and in the future.

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