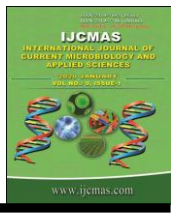
Int.J.Curr.Microbiol.App.Sci (2020) *9*(1): 1134-1139

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 1 (2020)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2020.901.127

Hydrocarbon Degradation by Bacteria Isolated from Cow Dung

S. K. Mondal*, D. P. Samantaray and B. B. Mishra

Department of Microbiology, Odisha University of Agriculture and Technology Bhubaneswar- 751 003, India

**Corresponding author*

Keywords

CD, Cow dung, Hydrocarbon degradation, *Pseudomonas* sp.

Article Info

Accepted:
15 December 2019
Available Online:
20 January 2020

A B S T R A C T

Petrochemical is the fastest growing industry, also manufacturing variety of chemicals. However the waste generated during the manufacturing is a major environmental concern due to its persistence in soil and water bodies. An alternative to chemical treatment is bioremediation which aims to enhance the normal processes of microbial degradation and clear the pollutant from the environment. Cow dung is a nitrogen-rich material and is of economic importance as organic fertilizers and also contributes diverse species of microorganisms which further has capability to degrade pollutants like hydrocarbon. Therefore an attempt was made to evaluate potential of the bacteria isolated from cow dung for hydrocarbon degradation in MSM media. Two bacterial isolates CD-5 *Bacillus* sp. BBMRH (KF175230) and CD-7 *Pseudomonas* sp. BBMB (KF965279) were found effective and further utilised for degradation of diesel, petrol, kerosene, and petrol-ether.

Introduction

Life's quality on earth is connected to the environment. Releases of recalcitrant and toxic petrochemicals into the environment have a negative impact on biosphere and the environment. Crude oil when released to the environment causes a variety of pollutions. It physically, chemically and biologically degrades the soil and water because of the presence of toxic compounds, such as polycyclic aromatic hydrocarbons, benzene and its substituted cyclo-alkane rings, in relatively high concentrations. These hazardous contaminants find their way into the plants, animals and human beings by direct or indirect pathways in food chain (Adams *et al*., 2014). Crude petroleum is a complex mixture of aliphatic and aromatic hydrocarbons which includes volatile constituents of gasoline, petrol, kerosene, lubricating oil and solid asphaltene. Serious calamities have been seen in developed and developing countries, due to contamination of

soil and marine environment by crude oil and petroleum products .The main sources of this pollution can originate through oil leaks and human actions and during extraction, refinement, transportation, utilization and storage of petroleum products (Agarry and Ogunleye, 2012). For reduction of hydrocarbon pollution chemical and mechanical methods are used which are very expensive and time consuming.

Hydrocarbons including PAHs have been foretold as substrates which supports microbial growth (Bushnell and Haas, 1941; Speight, 1991; Ehrlich, 1995). Bioremediation is a process in which indigenous oil–consuming microflora, called petrophiles, can naturally degrade large hydrocarbons and utilize them as an energy source by using enzymes and can be useful in cleaning up of the contaminated sites (Harder, 2004 and Alexander, 1999). Microbes isolated from cow dung have considerable potential for biodegradation and biotransformation of petroleum product, further contributing to plant production and biogeochemical processes (Adegunloye *et al*., 2007). There are reports that bioremediation of benzene using cow dung microflora in a bioreactor (Akinde and Obire, 2008) and it was found that *Pseudomonas putida* isolated from cow dung micro flora was potential benzene degrader at various concentrations. Keeping in view of this, the objective of this study was to investigate the efficiency of cow dung microflora in the bioremediation of diesel, petrol, kerosene and petrol-ether under controlled conditions.

Materials and Methods

Screening for hydrocarbon utilization

The bacteria isolated from fresh cow dung (Mondal *et al*., 2015, 2016) were inoculated on enrichment Mineral Salt Medium (MSM) which is composed of K_2HPO_4 (1.8 g/L); NH_4Cl (4 g/L); $MgSO_4.7H_2O$ (0.2 g/L); NaCl (0.1 g/L); $Na_2SO_4.7H_2O$ (0.01 g/L) and distilled water (1L) Jyothi *et al*., (2012).The medium was supplemented with 1% (v/v) filter sterilized hydrocarbons (petrol, diesel) to serve as the only source of the carbon and energy. The organisms were inoculated and screened on the Mineral Salt Agar medium (MSM+ Agar-20 g/L) with diesel as a sole of carbon source. The plates were incubated at 37°C for 5-7 days.

Hydrocarbon degradation assay

For the study of bio-degradation of hydrocarbon by bacterial isolates four sources (diesel, petrol, kerosene and petrol-ether) was taken in to consideration. Sterile autoclaved Mineral Salt medium was prepared, dispensed into 100 ml conical flask and inoculated with freshly prepared pre-selected bacterial isolates (1%) with varying percentages of filtered-sterilized hydrocarbon source concentration (1%, 2%, 3%, 4%, 5% and etc.) and were incubated in rotary shaker incubator for 24 hours at optimum temperature and pH at 100-120 rpm. After 24 hours of incubation these flasks containing varying concentrations of hydrocarbon sources were analysed spectro-photometrically at 595nm against control as standard which contain 24 hours incubated Mineral Salt broth medium and bacterial isolates.

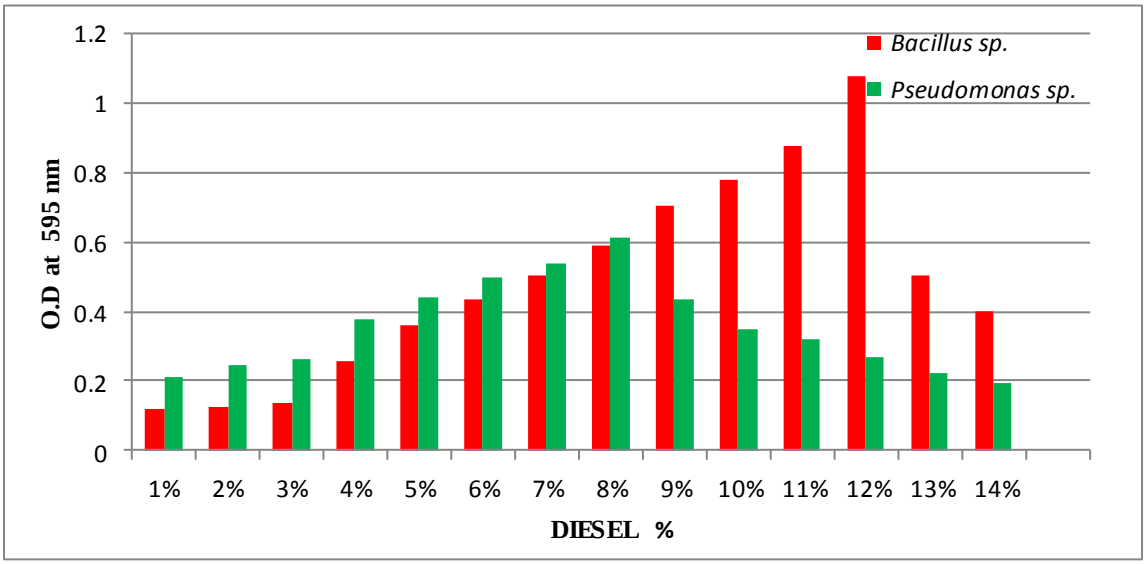
Results and Discussion

Screening for hydrocarbon utilization

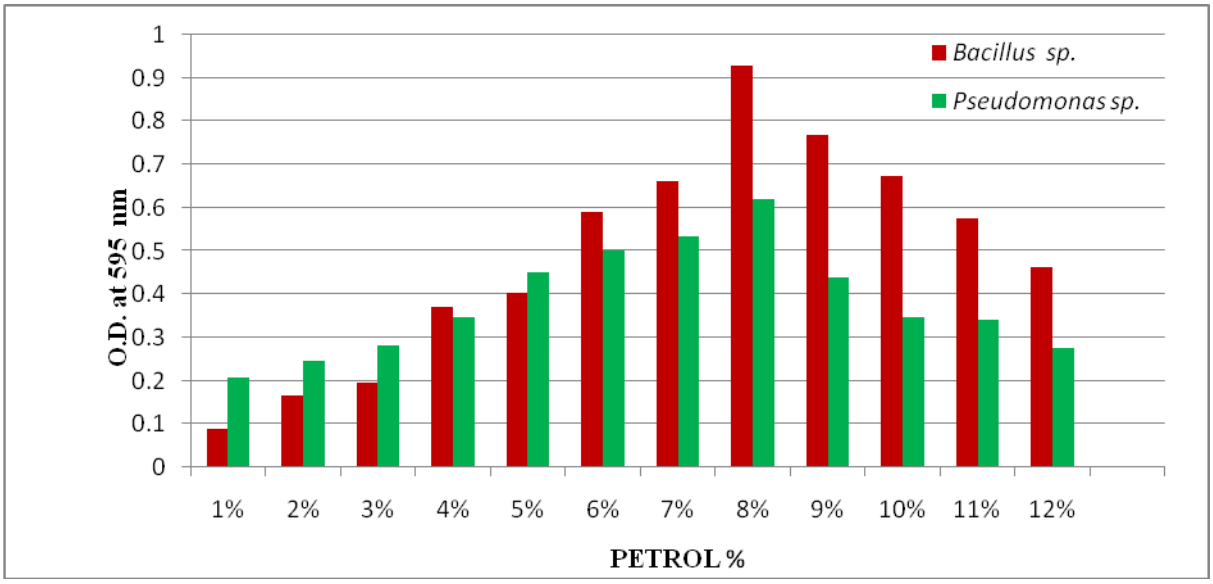
During screening of bacterial isolates for the utilisation of hydrocarbon it was found the bacteria coded as CD-5(*Bacillus* sp. BBMRH-accession number KF175230) Mondal *et al*., (2015) and CD-7 (*Pseudomonas* sp. BBMB - accession number KF965279)Mondal *et al*., (2016),utilized Diesel up to 1% in Mineral

salt media. Sukumar and Nirmala, (2016) isolated four bacterial strains from diesel contaminated soil which were *Pseudomonas aeruginosa*, *P. putida*, *Arthobacter* sp. and *Bacillus* sp., the diesel degrading efficiency of isolated organisms was tested in BH medium supplemented with diesel and DCPIP for 14 days.

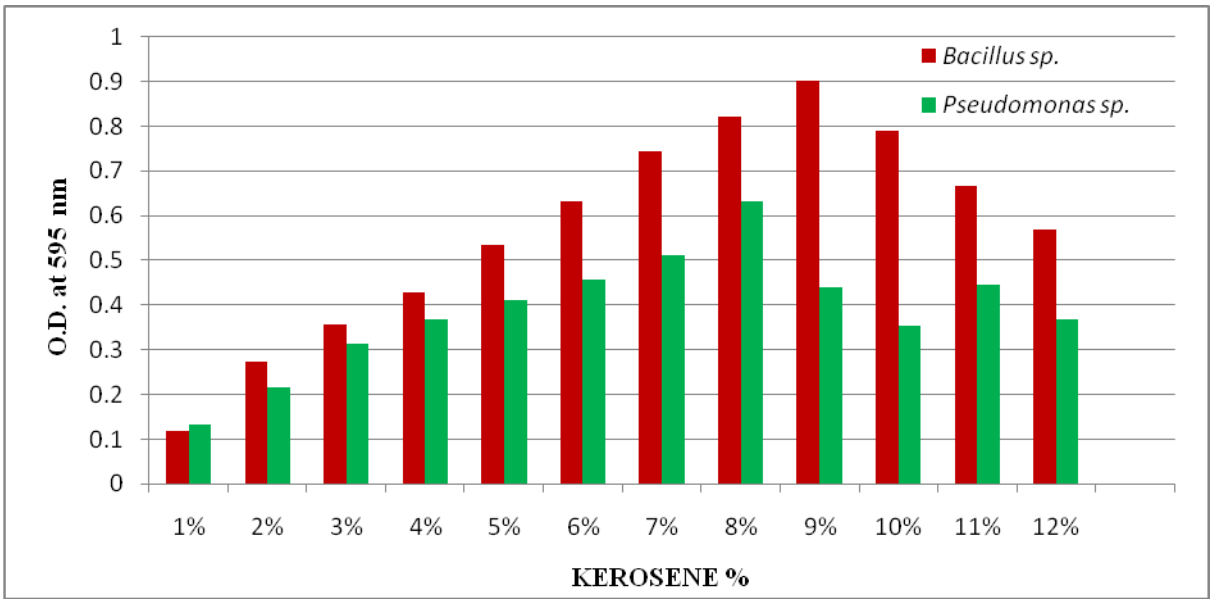
Graph.1 Growth of *Bacillus* sp. &*Pseudomonas* sp. in MSM medium containing diesel as the carbon source



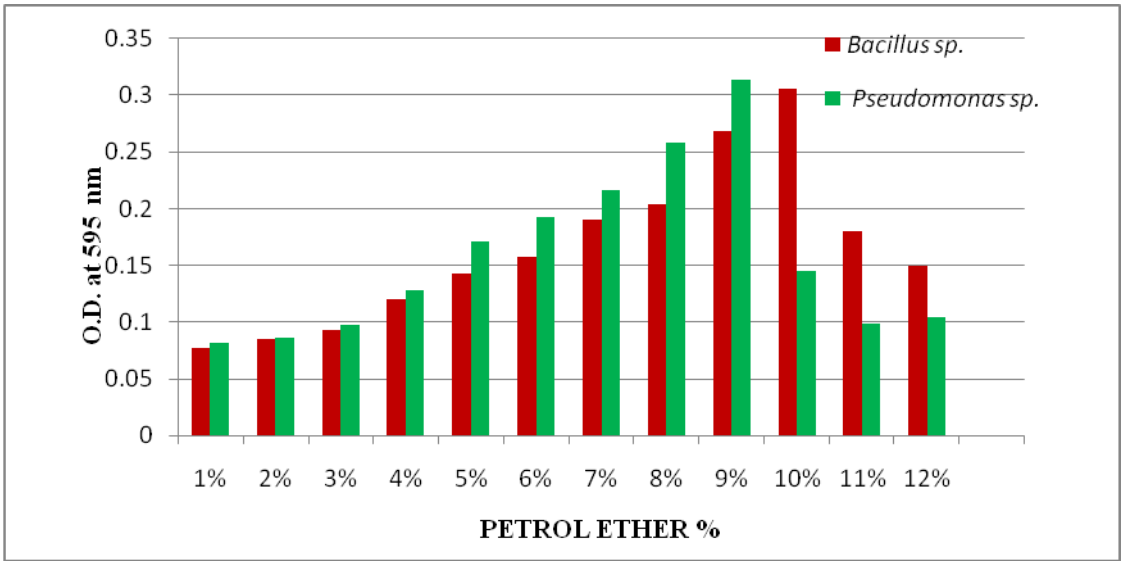
Graph.2 Growth of *Bacillus* sp. & *Pseudomonas* sp.in MSM medium containing petrol as the carbon source



Graph.3 Growth of *Bacillus* sp. &*Pseudomonas* sp.in MSM medium containing kerosene as the carbon source



Graph.4 Growth of *Bacillus* sp. &*Pseudomonas* sp.in MSM medium containing petrol ether as the carbon source



Hydrocarbon degradation assay

The bacteria inoculated in Mineral Salt medium broth were supplemented with varying percentage (v/v) of filter sterilized hydrocarbons (diesel, petrol, kerosene and petrol-ether) as single source of the carbon. The inoculated organisms after incubating at 34°C, pH 7 for *Bacillus* sp.and 36°C, pH 7 for *Pseudomonas* sp. in optimised conditions (temperature and pH) for 24hours. Optical density (OD) were measured at 595 nm and found that *Bacillus* sp. degraded maximum diesel at 12%,petrol at 8%, kerosene at 9%

and had maximum growth at10% on petrol-ether, whereas *Pseudomonas* sp. degraded maximum diesel at 8 %, petrol at 8%, kerosene at 8% and had optimal degradation rate at 9% in petrol-ether (graph-1, 2,3and 4). Jyothi *et al*., (2012) isolated and identified the hydrocarbon degrading bacteria associated with environmental samples collected from soil near petrol, diesel pumps and water samples and chlorine water from swimming pool. These organisms were studied for their biodegrading activities on hydrocarbons (diesel and petrol) using enrichment medium. The microbial growths were determined using calorimeter blanked at 595 nm, which revealed that bacteria *Bacillus megaterium*, *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Lactobacillus acidophillus*, *Neisseria flavescence* and *Corynebacterium xerosis* were the potent degraders of hydrocarbons (petrol and diesel).

It is evident from the findings that the two isolates viz. *Bacillus* sp. And *Pseudomonas* sp. isolated from cow dung has the potential to degrade hydrocarbon and can be employed in hydrocarbon contaminated sites for biodegradation.

References

Adams, G.O., Tawari-Fufeyin, P. and Ehinomen, I. 2014. Laboratory Scale Bioremediation of Soils from Automobile Mechanic Workshops Using Cow Dung. *Journal of Applied & Environmental Microbiology*, 2(4):128-134.

Adegunloye, D. V., Adetuyi, F.C., Akinyosoye, F.A. and Doyeni, M.O. 2007. Microbial analysis of compost using cow dung as booster. *Pakistan Journal of Nutrition*, 6(5): 506-510.

Agarry, S.E and Oladipupo, O.O. 2012. Factorial Designs Application to Study Enhanced Bioremediation of Soil Artificially Contaminated with Weathered Bonny Light Crude Oil through Biostimulation and Bioaugmentation Strategy. *Journal of Environmental Protection*, 3:748-759.

Akinde, S.B. and Obire, O.2008. Aerobic heterotrophic bacteria and petroleum-utilizing bacteria from cow dung and poultry manure. *World J. Microbiol Biotechnol*, 24: 1999–2002.

Alexander, M. 1999. Biodegradation and Bioremediation (2nd edition)Academic Press, San Diego

Bushnell, L.D. and Haas, F.H. 1941. The utilization of hydrocarbons by microorganisms. *J. Bacteriol*.,41: 653–673.

Ehrlich, H.L .1995. Geomicrobiology. Marcel Dekker, Inc., New York, N.Y..

Harder, E.2004. Bioremediation of engine oil. Little Flower Academy. Dallas, Texas.

Jyothi, K., SurendraBabu, K., Nancy Clara, K., and Kashyap, A.2012. Identification and Isolation of Hydrocarbon Degrading Bacteria by Molecular Characterization. *Helix* , 2:105-111.

Mondal, S. K., Samantaray, D. P. and Mishra, B. B. 2015**.** Optimization of Pigment Production by a Novel *Bacillus* sp. BBMRH isolated from Cow Dung. *J. Pure and Applied Microbiology*. 9(3):2321-2326.

Mondal, S. K., Samantaray, D. P. and Mishra, B. B. 2016. Standardization of bio-parameters for pigment production by *Pseudomonas* SP. BBMB isolated from cow dung. *The Bioscan*. 11(3): 1541-1545.

Speight, J.G. 1991. The chemistry and technology of petroleum. Marcel Dekker, New York, N.Y.

Sukumar, S. and Nirmala, P. 2016. Screening of diesel oil degrading bacteria from petroleum hydrocarbon contaminated soil. *International Journal of Advanced Research in Biological Sciences*, 3(8): 18-22.

How to cite this article:

Mondal, S. K., D. P. Samantaray and Mishra, B. B. 2020. Hydrocarbon Degradation by Bacteria Isolated from Cow Dung. *Int.J.Curr.Microbiol.App.Sci.* 9(01): 1134-1139.
doi: https://doi.org/10.20546/ijcmas.2020.901.127