Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Pelagia Research Library

Advances in Applied Science Research, 2014, 5(6):91-96

Pelagia Research Library

ISSN: 0976-8610
CODEN (USA): AASRFC

Optimization of growth condition for diesel oil degrading bacterial strains

Mahalingam P. U. and Nithya Sampath*

Department of Biology, Gandhigram Rural Institute–Deemed University, Gandhigram, Dindigul, Tamil Nadu, India

---

ABSTRACT

Three efficient diesel oil degrading bacterial strains were isolated from soil sample collected at diesel contaminated sites in Dindigul,Tamilnadu and were identified as Pseudomonas sp, Bacillus sp and Micrococcus sp. The results of the study reveals that the better degradation of diesel oil was recorded in Pseudomonas sp ans Bacillus sp with 1% diesel oil at 37 ^{0}C on 7th day. The higher percentage of microbial biomass was recorded in Bushnell-Haas medium supplemented with sunflower oil as carbon source and ammonium sulphate as nitrogen source. Among the three bacterial isolates Pseudomonas sp was identified as efficient diesel oil degrader.

Keywords: Hydrocarbon, bacterial strains, diesel oil, Bushnell-Haas medium, microbial biomass.

INTRODUCTION

Petroleum is a complex mixture of hydrocarbon and other organic compounds including some organometallo constituents, most noticeably vanadium and nickel (1). Petroleum hydrocarbon can be divided into four classes: saturates, aromatics, asphaltenes (phenols, fatty acid, ketones, esters and porphyrins) and resins (pyridines, quinolines, carbazoles, sulfoxides and amides) (2). Petroleum continues to serve as a priniciple source of energy. Crude oil and its products are vital to modern society and thus vast quantities are consumed each year. As the necessity increases, the rate of hydrocarbon contamination also increases day by day. These oil contaminations have made severe impacts on the plant and animal ecosystem including human health (3). Biodegradation by natural population of microbes is the most reliable mechanism by which thousands of pollutants including crude oil are eliminated from the environment. Soil is a rich source of microbes which promotes the biodegradation of hydrocarbon and residual oil (4). There are so many bacteria possesing the ability to utilize hydrocarbon as their sole source of carbon, thus transforming hazardous component into non hazardous, biodegrable and ecofriendly compounds (5). Several reports on isolation of diesel degrading bacteria were well documented and still intensive research is need to isolate versatile bacterial strain for effective degradation of hydrocarbon. Hence the present study is focussed on isolation and characterization of efficient hydrocarbon degrading bacteria with better diesel oil degrading ability.

MATERIALS AND METHODS

Screening for diesel oil degrading bacterial isolates

Potential hydrocarbon degrading bacterial strains were isolated from diesel oil contaminated soil collected near automobile stations in Dindigul(10.3540^{0}N,77.9850^{0}E),Tamil Nadu, India using the standard methods (5).

One gram of soil sample was transferred to the 100ml of Bushnell Hass (BH) medium in 250 ml of conical flask with 2% (V/V) of diesel oil as a carbon source. The flask was incubated at 30^0 C on a rotatory shaker at 200 rpm for 7days. Then 1ml of culture was transferred to fresh media containing diesel oil and incubated for another 7 days. After 3 successive cycles of such enrichment, 1ml of the culture was plated on BH agar medium containing 2% (V/V) diesel oil as a carbon source and incubated at 30^0 C for 7 days. The prominent bacterial colonies were selected and pure cultured for further study.

Optimization of growth condition for diesel oil degrading bacterial strains

Optimization of growth condition for three selected diesel oil degrading bacterial isolates were determined by standard methods (7) as follows:

Effect of pH on bacterial growth during biodegradation of diesel oil

The effect of hydrogen ion concentration on the growth of three bacterial isolates and their ability to degrade diesel oil was determined using Bushnell-Haas medium with the different pH ie., 5, 7 and 9 supplemented with 1% diesel oil as carbon source at 37°C. The bacterial growth of different isolates were determined spectrophotometrically at 600nm and expressed in terms of microbial biomass.

Effect of temperature on bacterial growth during biodegradation of diesel oil

The influence of temperature (15°C, 30°C and 45°C) on the growth of three bacterial isolates and their ability to degrade diesel oil at different time intervals was studied using Bushnell-Haas medium with pH 7.5 supplemented with 1% diesel oil. The bacterial growth in terms of microbial biomass were determined spectrophotometrically at 600nm.

Effect of various concentrations of diesel oil on bacterial growth during biodegradation

The effect of substrate concentration on the growth of three bacterial isolates and their ability to degrade diesel oil was determined using BH medium with pH 7.5 and supplemented with various concentration of diesel oil ie.,1,3 and 5% at 37°C. The growth of three bacterial isolates were determined spectrophotometrically at 600nm and expressed in terms of microbial biomass.

Effect of carbon source on bacterial growth

The utilization of various oil substrates as carbon sources by the bacterial isolates was determined using BH medium with pH 7.0 supplemented with 1% of different carbon sources such as diesel oil, corn oil, sunflower oil and incubated at 30°C for 7 days. The growth of three bacterial isolates were determined spectrophotometrically at 600nm and expressed in terms of microbial biomass.

Effect of nitrogen source on bacterial growth during biodegradation of diesel oil

The utilization of diesel oil by the three bacterial strains in the presence of different nitrogen source were determined using BH medium in the pH 7.0 incorporated with 1% diesel oil and with different Nitrogen sources viz.,Sodium Nitrate,Ammonium Sulphate and Urea at 37°C for 7 days. The growth of each bacterial isolates were determined using Spectrophotometer at 600nm and expressed in terms of microbial biomass.

Antagonistic assay

Antagonistic effect among the three bacterial isolates was determined by streaking them as thick bands on edges of the nutrient agar and incubated at 37°C for 2-3 days. Bacterial growth pattern was recorded and expressed the antagonistic effect as strong, moderate, and weak and no effect (8).

Statistical analysis

All the experiments were carried out with three independent replicates.Inorder to verify significant difference, results were evaluated statistically,at 95% confidence level ($p<0.05$) using Graphpad Prism 5. All the graphical representations are carried with Graphpad Prism 5 and Origin 6.0 software.

RESULTS AND DISCUSSION

The enrichment technique resulted in 10 bacterial isolates having the ablility to grow in Bushnell-Haas medium with diesel oil as sole source of carbon. Out of the 10 bacterial isolates three predominant bacterial isolates such as *Bacillus* sp, *Pseudomonas* sp and *Micrococcus* sp were identified as hydrocarbon degraders possessing higher

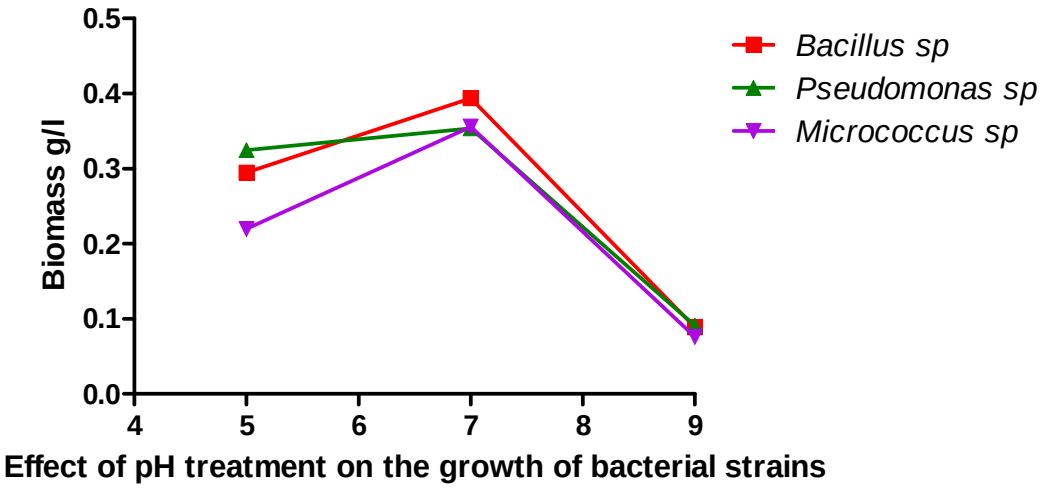
efficiency to grow on diesel and able to produce biosurfactant. In the earlier ecological studies the above mentioned genera were identified among hydrocarbon degrading microorganisms (9). The addition of hydrocarbon to an ecosystem, due to accident oil spills may selectively increase or decrease size of microbial population depending on various factors such as temperature, pH , chemical composition of the contaminant and the species of microorganism present within the microbial community of the particular ecosystem (10 and 5).

In order to enhance the bacterial growth, optimization of environmental conditions is very significant . The pH variation in culture medium is due to the accumulation of metabolic waste products by the bacterial cells which strongly affects its growth. Hence it is very important to maintain optimum pH condition for bacterial growth medium. Different buffers with pH such as 5,7 and 9 were used to study the optimal growth of the three bacterial isolates. At pH 7, *Pseudomonas* sp showed a higher biomass production comparing to the others ($p<0.005$) (Fig1). The optimal growth of many other diesel degrading bacteria were reported at neutral or near neutral pH (11,12 and 13).

Bacterial strains from hydrocarbon contaminated strain able to grow in a wide range of temperature (14). The selected three bacterial isolates were able to grew at different temperature condition viz.,4^0C, 28^0C and 37^0C . The higher microbial biomass production were observed at 37^0C for all the three bacterial isolates and showed a positive correlation between the temperature and the biomass production ($p<0.005$) (Fig2). Similarly, the optimal temperature for bacterial growth on diesel degradation was reported at 30^oC (15 1nd 14).

Diesel oil is needed as a carbon source but with certain level of contamination it can be toxic to microorganism due to the solvent effect which destroy bacterial cell membrane. Hence many biodegradation studies on diesel were carried out using lesser diesel contamination ranging from 0.5 to 1.5 %. It has been found that degradation is generally retarded with contamination increase of 1% or 1.5% (15). In this study, on optimization of different substrate concentration revealed that, 1% diesel oil supports excellent growth for all the three isolates with no significant difference ($p<0.005$) (Fig3). However, the bacterial strains P*seudomonas sp.* and *Bacillus sp* were able to tolerate increased diesel oil concentration.

Fig:1 Effect of different pH on the growth of selected three bacterial strains



Different carbon sources such as diesel oil, corn oil and sunflower oil were used to study the bacterial growth. Excellent growth of all the three selected bacterial isolates were observed in sunflower oil and there was a positive correlation with the carbon sources and the bacterial growth ($p<0.005$) (Fig4). Similiar results were also observed in many hydrocarbon degradation studies (16).

Different nitrogen sources such as sodium nitrate, ammonium sulphate, urea were used at an concentration of 1% (w/v) in BH medium supplemented with 1% of diesel to monitor their effects on bacterial growth. Ammonium sulphate optimally supports the growth of all the three bacterial strains (Fig5). Analysing satistically, it shows a

positive correlation between the nitrogen source and the biomass which results in no significant difference ($p<0.001$). Ammonium sulphate was chosen as the principle nitrogen source due to its widespread usage as a cheap source of nitrogen for bioremediation. Optimization with 2% concentration of ammonium sulphate will support maximal growth of bacteria (14).

The study on the antagonistic assay infers a mutualistic association among three bacterial isolates and hence these strains could used as an bacterial consortium for diesel oil biodegradation for future studies.

Fig:2 Effect of Temperature on the growth of selected three bacterial strains

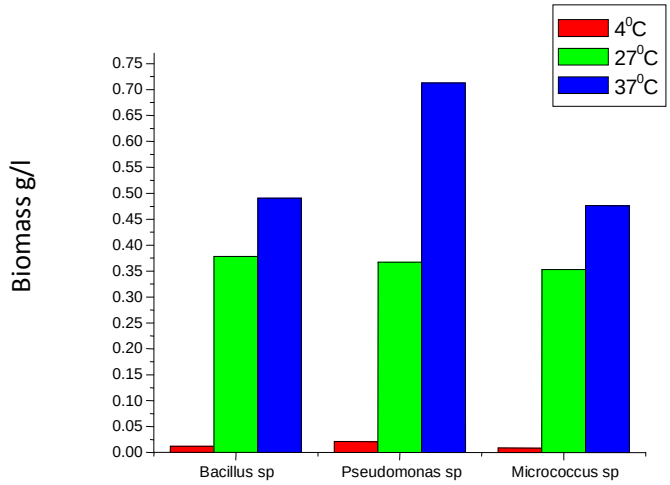
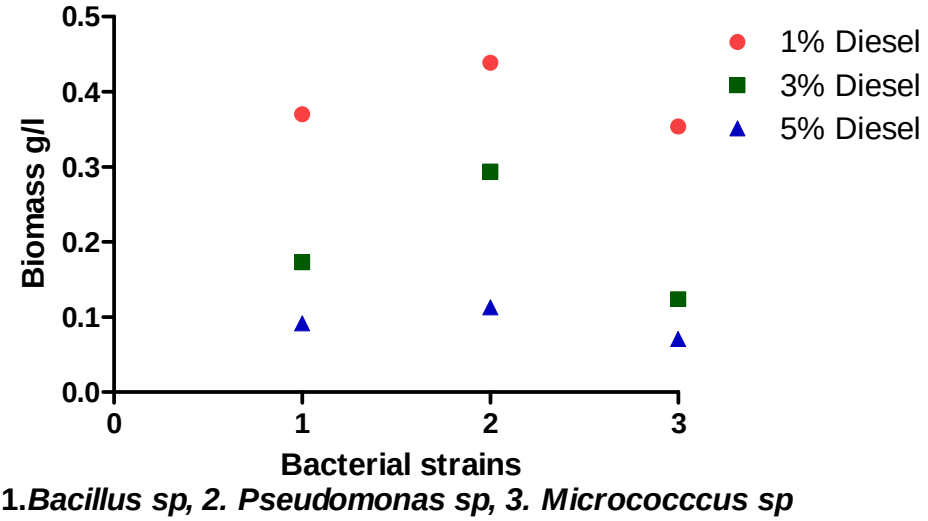


Fig:3 Effect of substrate concentration on the growth of selected three bacterial strains



1.*Bacillus sp, 2. Pseudomonas sp, 3. Micrococccus sp*

Fig:4 Effect of carbon source on the growth of selected three bacterial strains

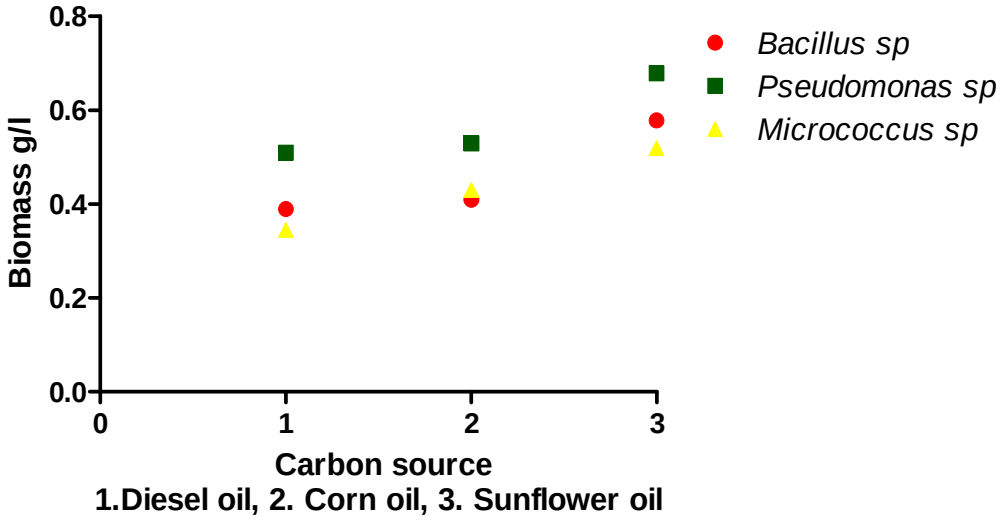
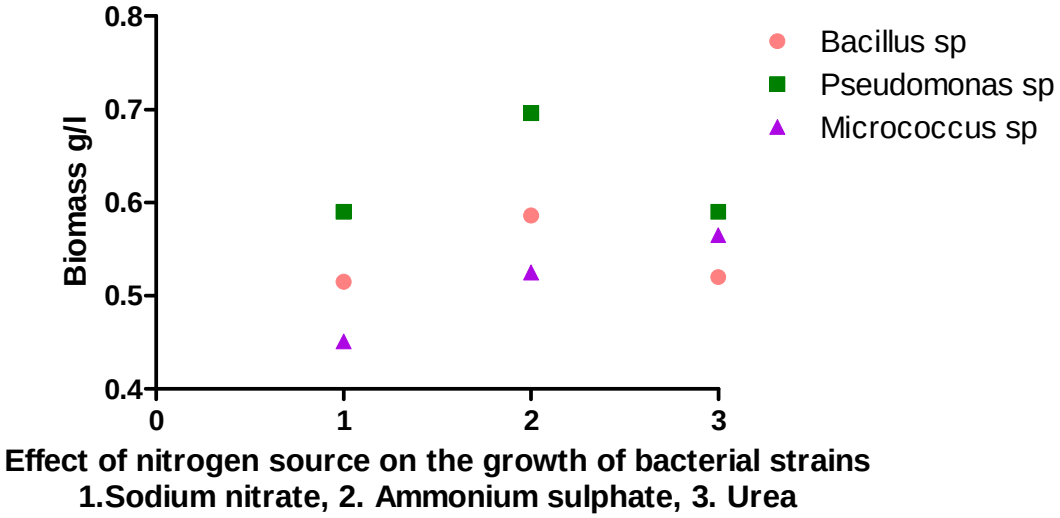


Fig:5 Effect of Nitrogen source on the growth of selected three bacterial strains



REFERENCES

[1] Van Hamme, J.D., Singh A., Ward O., **2003**. *Microbial Mol Biol. Rev.*, 67,503-549.
[2] Leahy J. G. and R. R. Colwell. **1990**. *Microbiol*. Rev.,54,305-317.
[3] Mandal Ajoy Kumar, Priyangshyu Manab Sarma, Manish Dwivedi, Abu Swaleh, Banwari Lal, A. Agnihotri, U. S. Hazra, A. Doraiah and S. S. Patidar,**2007**. Bioremediation Of Oil Contaminated Soil At South Santhal CTF,ONGC, Mehsana Asset, India. Proceedings of **2007** Asia Pacific Oil and Gas Conference and Exhibition, Jakarta, Indonesia, *Society of Petroleum Engineers (SPE)*, Paper no. 109571.
[4] Atlas RM and Bartha R. **1998**. *Microbial Ecology*: Fundamentals and applications 4th Edition. Benjamin cummings publishing company Inc.Addison Wesley Longman Inc. pp.,300-350.
[5] Bharathi S. and Vasudevan N. **2001**. *Environ. Int.*, 26, 413 – 416.
[6] Rahman, K. S. M., Rahman T. J., Kourkoutas Y., Petsas I., Marchant R. and I. M. Banat. **2003**. *Bioresour. Technol.*, 90,159–168.

[7] Hamzah, A., Rabu, A., Azmy, R.F.H.R. and Yussoff, N.A. **2010**. *Sains Malaysiana*, 39(2), 161-168.
[8]. Boer ,W.D., Gunnewiek P.J.A.K., Lafeber.P., Janse J.D., Spit B.E and woldendrop J.W.**1998** *Soil biology and Biochemistry*, 30, 193-203.
[9]Marquez-Rocha, Olmos-Soto F.J.J., Concepcion Rosano-Hernandez M. and Muriel-Garcia M.**2005**. *Int. biodeterior. Biodegrad*., 55, 17-23.
[10] Lal, B. and S. Khanna.**1996**.: *J. Appl. Bacteriol*., 81, 355-362.
[11] Rajasekar, A., Babu T.G., Pandian S.T., Maruthamuthu S., Palaniswamy N. and Rajendran A.**2007**. *J. Ind. Microbiol. Biotechnol*.,34, 589-598.
[12] Ueno, A., Ito Y., Yumoto I. and Okuyama H. **2007**. *World J. Microbiol. Biotechnol*.,23, 1739-1745.
[13]Kwapisz, E., J. Wszelaka, O. Marchut and S. Bielecki **2008**.: *Int. Biodeterior. Biodegrad*., 61, 214-222.
[14]M.Y. Shukor, F.A. Dahalan, A.Z. Jusoh, R. Muse, N.A. Shamaan and M.A.Syed **2009**, *J. Environ. Biol*., 30(1), 145-150.
[15] Lee, M., M.K. Kim, I. Singleton, M. Goodfellow and S.T. Lee **2006**: *J. Appl. Microbiol*.,100, 325-333.
[16] Angaleswari, C., Suji, L., Mahalingam, P.U. **2012** *Advances in Applied Science Research*, 3(6),4030-4032.