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RESEARCH ARTICLE

BIODEGRADATION OF AUTOMOBILE OIL EFFLUENT BY *Pseudomonas aeruginosa*

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Abstract

The used engine oil and washed water of automobile workshops causes soil to lose its useful properties such as fertility, water-holding capacity, permeability and binding capacity. Biosurfactant, synthesized by microorganisms, is a structurally diverse group of surface-active molecule can be employed to degrade the automobile waste engine oil. In the present study, it was observed that *Pseudomonas aeruginosa* MCCB 0037 showed high potential to produce biosurfactant and showed best degradation of the automobile effluent oils in Diatomaceous earth carrier based formulation. It was observed that oil degradation was found to be 75% and 47.50% in the third week, when CBMI was supplemented with inorganic nutrient and glycerol, respectively. Also, shelf life of CBMI was determined by serial dilution method that indicated CFU count of *Pseudomonas aeruginosa* MCCB 0037 was 1760 cfu/mL.

INTRODUCTION

Bioremediation means to use living things to solve an environmental problem such as contaminated soil or groundwater. The microorganisms are capable to change these chemicals into water and harmless gases, such as carbon dioxide (Whang et al., 2008). Petroleum oil pollution is an environmental problem of the present scenario. Petroleum is a complex mixture of many thousands of compounds. These can be divided into four major groups: the alkanes, the aromatics and the resins (Colwell et al., 1977). In general, the alkane fraction is the most biodegradable, whereas the polar fraction 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 bioaccumulation (Banat et al., 2000). The hydrocarbon-degrading microorganisms produce biosurfactants of diverse chemical nature and molecular size. These surface-active materials increase the surface area of hydrophobic water-insoluble substrates and increase their bioavailability (Desai and Banat, 1997), thereby enhancing the growth of bacteria and the rate of bioremediation. The diversity of biosurfactants makes them an attractive group of compounds for potential use in a wide variety of industrial and biotechnological applications (Banat, 1995). Surfactants are amphiphilic molecules consists of a hydrophilic and a hydrophobic domain (Georgiou et al., 1992). Biosurfactants grouped as low molecular weight biosurfactants as well as high molecular weight biosurfactants (bio-emulsifiers) composed of glycolipids, flavolipids, phospholipids and polysaccharides, proteins, lipopolysaccharides, lipoproteins, respectively (Calvo et al., 2009). Biodegradation by microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment (Okoh, 2006). Some Fungi, such as *Fusarium oxysporium*, and Bacteria *P. cepacia, P. putida, P. aeruginosa* are used to degrade these pollutants (Kumar and Sharma, 1992), but genus *Pseudomonas* is capable of using different substrates, such as glycerol, mannitol, fructose, glucose, n-paraffins and vegetable oils, to produce Rhamnolipid-type biosurfactants (Kretschmer et al., 1982). It was also reported the isolation of *Pseudomonas* sp. from hydrocarbon contaminated region and its growth on crude oil associated with the production of surfactants (Desai and Banat, 1997).

Many types of bio-surfactant are being utilized for the bioremediation of petroleum oil but unable to resist in the market, due to their high production costs involved (Brady and Trams, 1964). Hence, the present study emphasis on the screening of maximum biosurfactants producing *Pseudomonas aeruginosa* from the selected strains and degradation of used engine oil by them.

MATERIALS AND METHODS

Procurement of microbial strains

Three bacterial strains procured from the Microbial Culture Collection Bank, Department of Microbiology and Fermentation Technology, SHIATS, Allahabad were *Pseudomonas aeruginosa* MCCB 0035, *Pseudomonas aeruginosa* MCCB 0036 and *Pseudomonas aeruginosa* MCCB 0037 and cultured in the nutrient media for the experimental study.

Screening of biosurfactants producing micro-organisms

The procured bacterial strains were tested for their biosurfactant production by the Oil Spreading Technique (Rodrigues et al., 2006), Blood Haemolysis Test (Anandaraj and Thivakaran, 2010) and Emulsification Stability (E24) Test (Aparna et al., 2011). After screening the potential strain of *Pseudomonas aeruginosa* was taken for the further experimental study.

Mass production of *Pseudomonas aeruginosa* MCCB 0037

5% of the selected *Pseudomonas aeruginosa* culture was inoculated in the production media and incubated for 48h. Bacterial population should be above 10^9 viable cells per mL, was checked by dilution method. The culture broth was stored at 4ºC.

Production of Carrier Based Microbial Inoculums

In the carrier formulation, Diatomaceous earth (grainy, porous, and sand-like material) was used as a carrier for the preparation of carrier based bio-degrader. The carrier material was first dried in the hot air oven at 80ºC for 24h and then cooled to room temperature for further treatments. The neutral pH solution of the carrier material was made using 2.5g of carrier material and 25mL of de-ionized water and agitated for 24h. The pH of the solution was recorded using a pH meter (Moorthi et al., 2008). The carrier was then sterilized and it was mixed with the potential strain cultured broth in the ratio of 3:1 for carried based inoculums composition. After proper mixing, Carrier Based Microbial Inoculums (CBMI) was then used for biodegradation of auto mobile oil effluent (Moorthi et al., 2008).

Auto mobile oil effluent treatment

Automobile effluents (100mL) were collected in 10 cleaned test tubes and one test tube was served as a control while three tubes were treated with 0.5 g of CBMI and next three test tubes were added with the mixture of inorganic nutrients (g/L: 0.25, ammonium dihydrogen orthophosphate; 0.05, magnesium sulfate; 0.25,dipotassium hydrogen orthophosphate; 0.5, CBMI). The last three test tubes were treated with 0.5g CBMI along with 0.5mL of Glycerol. Glycerol served as a carbon source for the microbial inoculums (Moorthi et al., 2008). The flasks were then cotton plugged in order to avoid evaporation. The flasks were incubated for a 3 week under room temperature. Results were recorded from each flask every week up to 21 days (Ganesh and Lin, 2009).

Determination of used engine oil degradation

For gravimetric analysis degraded engine oil, the aqueous culture phase was dispensed into separating funnel and the organic hydrocarbon phase (engine oil) was extracted with 50 mL of dichloromethane, an organic solvent (Marquez et al., 2001). The 2.5 pH was adjusted with 1M HCl (Boonchan et al., 2000). The organic phase was then filtered through 10g of sodium sulfate, collected in the pre–weighed flasks and left overnight in the fume cupboard to enable evaporation of the organic solvent. Combined weight of the pre–weighed flask and oil was recorded and the pre–weight subtracted to ascertain the weight of the oil remaining. This remaining oil was then subtracted from the control and multiplied by 100 to attain the percentage of oil degradation (Marquez et al., 2001).

Determination of shelf life of carrier based inoculums (Serial dilution of CBMI)

The shelf life of CBMI was determined by viable plate count method (Moorthi et al., 2008). The results were expressed in Colony Forming Units (CFU).

Statistical analysis

The data recorded during the course of investigation was statistically analyzed using χ^2 -Test. Chi square statistic compares the observed count in each table cell to the count which would be expected under the assumption

of homogeneity. Formula for χ^2–Test= $\Sigma(O_i–E_i)^2/E_i$ (Formula for χ^2–Test, if d.f.=1), $\chi^2=\Sigma\{(O_i–E_i)–0.5\}^2/E_i$; Where, O_i= Observed frequency in each category, E_i=Expected frequency in each category, d.f.= Degree of freedom (n-1), χ^2= Chi square.

RESULTS AND DISCUSSION

Screening of biosurfactant producing micro-organisms

Oil spreading test

In the oil spreading technique, all the three strains of *P. aeruginosa* showed biosurfactants surface activity for the auto mobile oil effluent. *P. aeruginosa* MCCB 0035 and *P. aeruginosa* MCCB 0036 produced 3.7mm and 3.5mm clear zone but *P. aeruginosa* MCCB 0037 showed higher surface activity with 4.0mm zone formation. On analyzing the data statically, it was found non-significant (Table 1). Similar results were reported for the *P. aeruginosa* with zone displacement of 7mm and it was suggested to the use of oil spreading method to detect biosurfactant producing micro-organism in natural environment (Anandaraj and Thivakaran, 2010).

Blood Haemolysis Test

Blood Haemolysis test was carried out on all the three procured strains and α-haemolytic activity was recorded (Table 2). It was observed that the strain MCCB 0037 showed higher degradation zone at 37ºC after 48h incubation periods s shown in the Fig.1. Samanta et al. (2012) and Sneha et al. (2012) reported the similar results for the culture supernatant of *P. aeruginosa* during screening of biosurfactant producing organisms using blood haemolysis test.

Emulsification Stability (E24) Test

Results for the emulsification test showed that the bacterial strain MCCB 0035, MCCB 0036 and MCCB 0037 produced 22.5%, 27.5% and 32.5% stability, respectively as depicted from the Fig.2. It was also recorded that *P. aeruginosa* MCCB 0037 produced higher emulsification stability in automobile oil effluent. After analyzing the data statistically, it was observed that the difference was non-significant for the procured strains as concluded in the Table 3. The *Pseudomonas aeruginosa* 181 was reported with 85% of emulsification stability (Laith et al., 2007).

Determine the Shelf Life of CBMI Produced from *P. aeruginosa* MCCB 0037

The shelf life of CBMI was determined by viable plate count method which expressed in cfu/mL. First day colony count of CBMI was 176×10^{-1}cfu which decrease to 160×10^{-1}cfu at the final day of analysis shown in Fig. 3 and Fig. 4, respectively. On analyzing the data statistically the shelf life of CBMI was found to be significant (Table 4). Ghazali et al. (2004) and Moorthi et al. (2008) reported the CFU count of 7.76×10^8cfu/g to 6.35×10^8cfu/g with slight decrease in colony count calculated by viable plate count method. It was concluded that the presence of carrier in the mixture didn't affect the growth of microbial population but it maintains its growth and activity. Higher survivability exhibited by *Pseudomonas aeruginosa* when Diatomaceous earth was used as carrier material, can be attributed to fact that micro-environment of this carrier is better suited to the growth requirement of the bacteria.

Determine extent of degradation of used engine oil

A good surfactant can increase degradation rate of crude oil by using micro-organism. Degradation results were expressed in percentage of oil degradation for the procured strains represented in Fig. 5. It was observed that 57.5% of oil degradation resulted in treatment with CBMI. Addition of CBMI with glycerol showed 47.5% degradation and with inorganic nutrients showed 75%, found to be maximum degradation. After analyzing the data statistically the difference in degradation of used auto mobile engine oil was found to be significant (Table 5). Similar incidences were observed for the *P. aeruginosa* with 60% and 81% degradation of engine oil reported by Moorthi et al. (2008) and Thenmozhi et al. (2011), respectively.

Table 1. Observations for the Oil Spreading Technique

Culture Sample	Zone Formation (mm)	Oil displacement area(mm^2)
P. aeruginosa MCCB 0035	3.7	10.75
P. aeruginosa MCCB 0036	3.5	09.62
P. aeruginosa MCCB 0037	4.0	12.57

For oil spreading: χ^2_{cal} (0.0339) < χ^2_{tab} (5.9915); NS= Non significant.

Table 2. Blood Haemolysis Test

Culture Sample	α-Haemolysis
P. aeruginosa MCCB 0035	++
P. aeruginosa MCCB 0036	++
P. aeruginosa MCCB 0037	+++

++ = moderate activity, +++ = maximum activity

Table 3. Emulsification Stability (E24) Test

Culture Sample	Emulsified layer (mm)	E24 (%) Emulsification activity
P. aeruginosa MCCB 0035	4.5	22.5
P. aeruginosa MCCB 0036	5.5	27.5
P. aeruginosa MCCB 0037	6.5	32.5

For E24 test: χ^2_{cal} (1.818) < χ^2_{tab} (5.9915); NS= Non significant

Table 4. Determine the Shelf Life of CBMI

Number of Days	Population of CBMI (cfu/mL)
1	1760
21	1600

***** For shelf life: χ^2_{cal} (7.5242) > χ^2_{tab} (3.8415); S= Significant.

Table 5. Determine Extent of Degradation of Used Engine Oil.

Sample	1st Week Degradation (%)	2nd Week Degradation (%)	3rd Week Degradation (%)
CBMI	7.5	35	57.5
CBMI+ Inorganic Nutrients	12.5	55	75
CBMI + Glycerol	5	25	47.5

For degradation of oil: χ^2_{cal} (1.9498) < χ^2_{tab} (12.5916); NS=Non Significant.

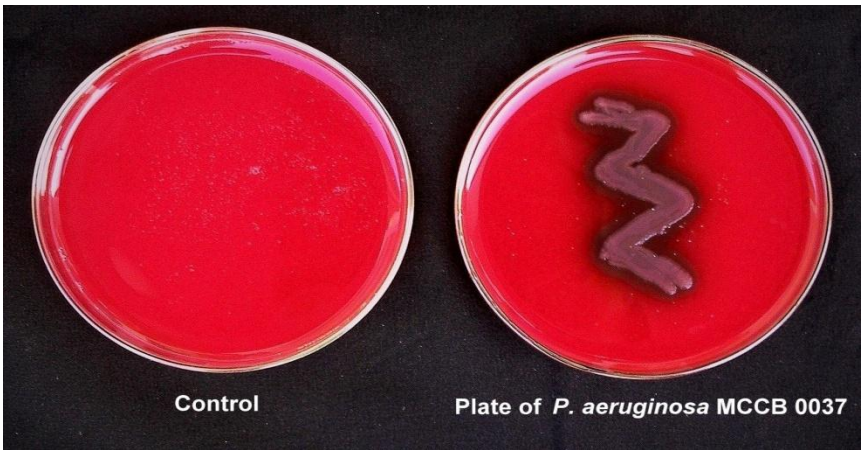

Fig.1. Blood Haemolysis Test for the Strain MCCB 0037

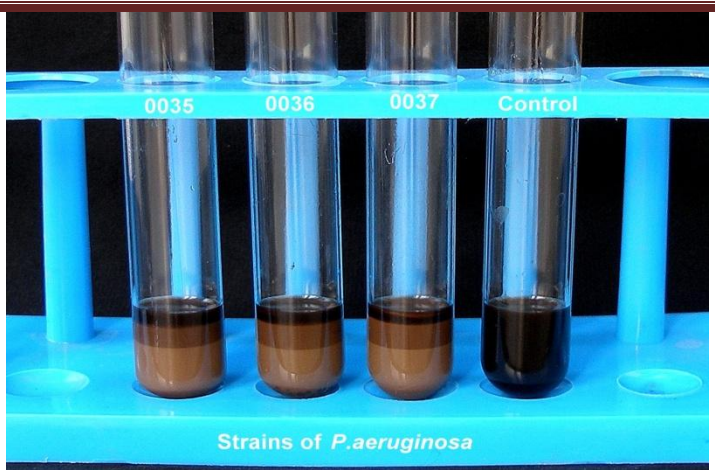

Fig.2. Emulsification Stability Test for the three bacterial strains of *Pseudomonas* sp.

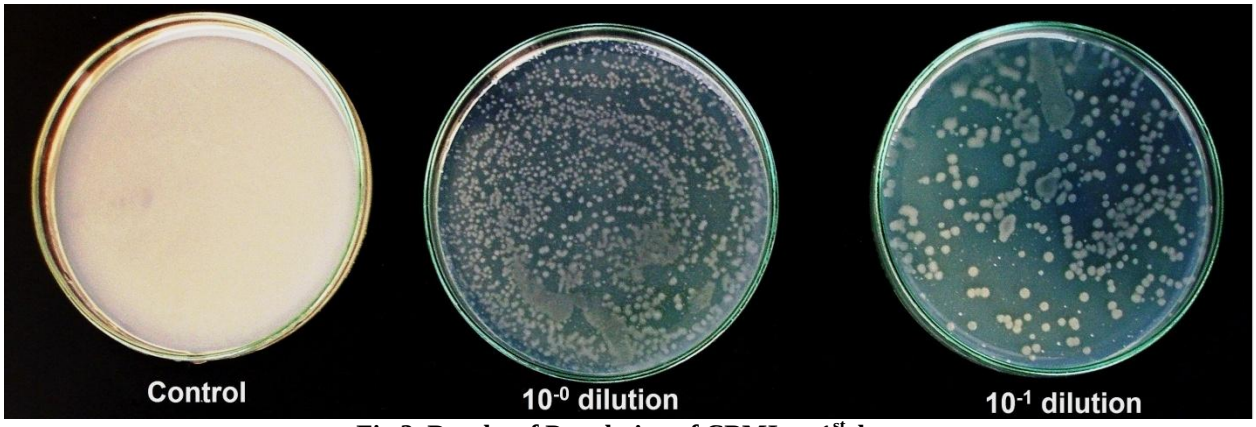

Fig.3. Results of Population of CBMI on 1st day

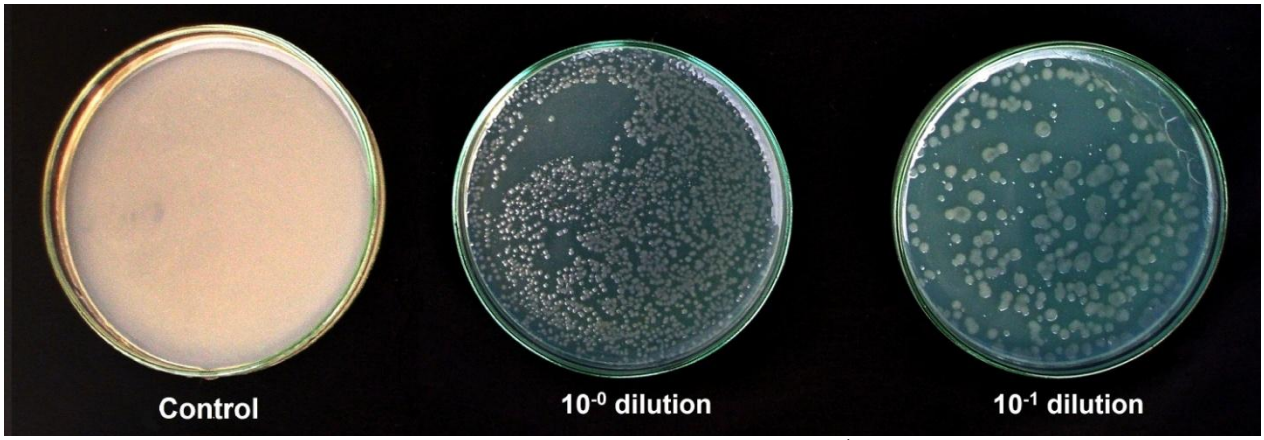

Fig.4. Results of Population of CBMI on 21st day

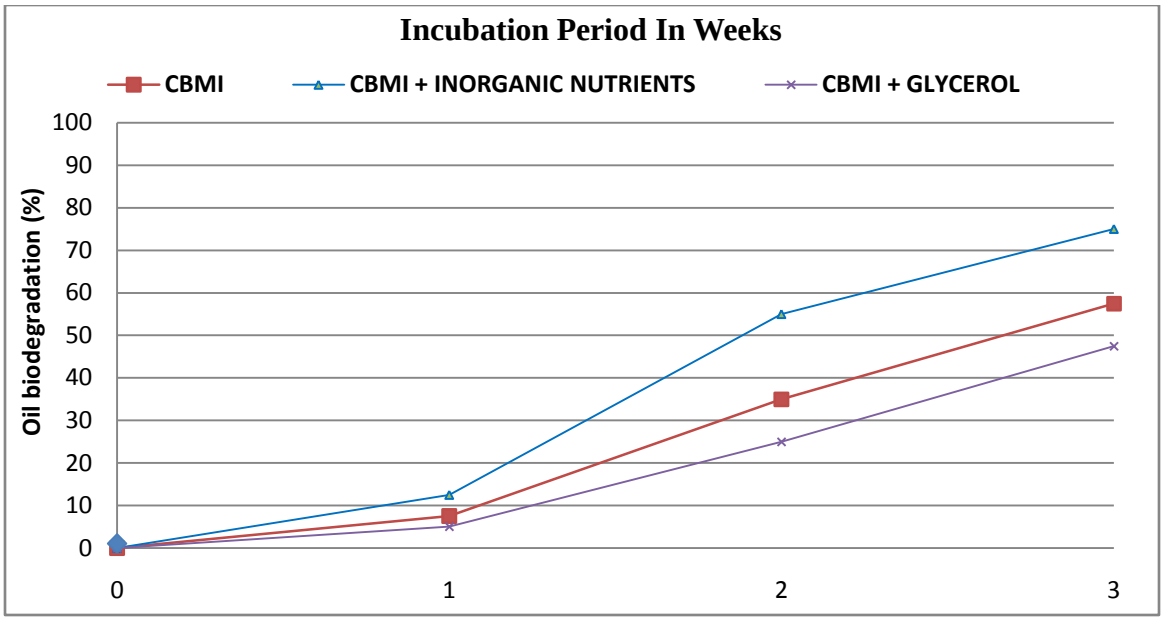

Fig.5. Degradation of automobile oil effluent by Carrier Bases Microbial Inoculums.

CONCLUSION

The present study inferred that bacterial strain of *Pseudomonas aeruginosa* MCCB 0037 showed satisfactory results in all three screening tests in comparison to other two procured strains of *Pseudomonas aeruginosa* MCCB 0035 and MCCB 0036. Also the CBMI Diatomaceous earth showed best self-life for the *Pseudomonas aeruginosa* MCCB 0037. Since, advantage of using biosurfactant in biodegradation of hydrocarbons is that they are extracellular, less toxic and easily degradable in the environment. Hence, the present study recommend the use of *Pseudomonas aeruginosa* MCCB 0037 for producing biosurfactant that further could be used to enhance the degradation of crude oil instead of chemical surfactant. The present study also recommended the comparison study between the CBMI Diatomaceous earth and other carrier using *Pseudomonas aeruginosa* MCCB 0037.

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REFERENCES

Anandaraj, B. and Thivakaran, P. (2010) Isolation And Production of Biosurfactant Producing Organism from Oil Spilled Soil. Journal of Bioscience and Technology.1 (3):120-126

Aparna, A., Srinikethan, G. and Hegde, S. (2011) Effect of Addition of Biosurfactant Produced by *Pseudomonas* sp. on Biodegradation of Crude Oil. Environmental Science and Technology.6:71–75

Banat, I.M. (1995) Characterization of Biosurfactants and their use in Pollution Removal-State of Art (reviews). Acta Biotechnology.15:251-267

Boonchan, S., Britz, M.L. and Stanley, G.A. (2000) Degradation and Mineralization of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons by Defined Fungal-Bacterial Cocultures. Applied Environmental Microbiology.66:1007–1019

Brady, R.O. and Trams, E.G. (1964) The Chemistry of Lipid. Annual Review Biochemistry.33: 75–78

Calvo, C., Manzanera, M., Silva-Castro, G.A., Uad, I. and González-López, J. (2009) Application of Bioemulsifiers in Soil Oil Bioremediation Processes. Future prospects of Science Total Environment.407:3634–3640

Colwell, R.R., Walker, J.D. and Cooney, J.J. (1977) Ecological Aspects of Microbial Degradation of Petroleum in the Marine Environment. Critical Reviews in Microbiology.5:423–445

Desai, J.D. and Banat, I.M. (1997) Microbial Production of Surfactant and their Commercial. Microbiology and Molecular Biology Reviews.61:47-64.

Ganesh, A. and Lin, J. (2009) Diesel Degradation and Biosurfactant Production by Gram-Positive Isolates. African Journal of Biotechnology.8 (21):5847-5854

Georgiou, G., Lin, S.C. and Sharma, M.M. (1992) Production of Biosufactant using Substrate from Renewable Resources. Biotechnology.10:60–65

Ghazali, F.M., Rahman, R.N.Z.A., Salleh, A.B. and Basri, M. (2004) Biodegradation of Hydrocarbons in Soil by Microbial Consortium. International Biodeterioration and Biodegradation.54:61-67

Kumar, R. and Sharma, C.B. (1992) Biodegradation of Carbamate Pesticide Propoxur in Soil. Environment and Biodegradation.137-148.

Kretschmer, A., Bock, H. and Wagner, F. (1982) Chemical and Physical Characterization of Interfacial-Active Lipids from *Rhodococcus erythropolis* Grown on n-Alkanes. Applied Environmental Microbiology.44: 864–870.

Laith, A., Rahman, R.N.Z.A., Basri, M. and Salleh, A.B. (2007) The Effects of culture Conditions on Biosurfactant Activity of *Pseudomonas aeruginosa* 181 using response Surface Methodology. Medical of Biological Sciences.1(1).

Marquez-Rocha, F.J., Hernandez V., Rodriguez, M.T. and Lamela (2001) Biodegradation of Engine and Diesel Oil in Soil by a Microbial Consortium. Water, Air and Soil Pollution.128: 313-320.

Moorthi, P.S., Deecaraman, M. and Kalaichelvan, P.T. (2008) Bioremediation of Automobile oil effluent by *Pseudomonas* sp. Advanced Biotechnology.34–37.

Okoh, A.I. (2006) Biodegradation Alternative in the Cleanup of Petroleum Hydrocarbon Pollutants. Biotechnology and Molecular Biology.1: 38–50.

Rodrigues, L.R., Teixeira, J.A. and Mei, H.C. (2006) Oliveira Physicochemical and Functional Characterization of a Biosurfactant Produced by *Lactococcus lactis* 53. Colloids and Surfaces B: Biointerfaces.49: 79-86.

Samanta, A., Pal, P., Mandal, A., Sinha, C., Lalee, A., Das, M., Kaity, S. and Mitra, D. (2012) Estimation of Biosurfactant Activity of an Alkaline Protease Producing Bacteria Isolated from Municipal Solid Waste. Central European Journal of Experimental Biology.1(1): 26-35.

Sneha, K.S., Padmapriya, B. and Rajeswari, T. (2012) Isolation and Screening of Biosurfactants Produced by *Pseudomonas aeruginosa* from Oil Spilled Soils. International Journal of Pharmaceutical and Biological Archives.3(2): 321-325.

Thenmozhi, R., Nagasathya, A. and Thajuddin, N. (2011) Studies on Biodegradation of used Engine Oil by Consortium Cultures. Advances in Environmental Biology.5(6): 1051-1057.

Whang, L.M., Liu, P.W.G., Ma, C.C. and Cheng, S.S. (2008) Application of Biosurfactant, Rhamnolipid, and Surfactin, for Enhanced Biodegradation of Diesel-Contaminated Water and Soil. Journal of Hazards Materials.151: 155–163.