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Comparative Analysis of Oil Degrading Bacteria and Fungal Species to Manufacture Biosurfactant Using Neem Oil

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Abstract

Biosurfactant are attractive attention in recent year because they offer several advantages over chemical surfactants. Such as low toxicity, good biodegradability and ecological friendliness. In this respect, selected two oil degrading bacteria and two oil degrading fungal species. The selected microbes play major role in oil recovery, environmental bioremediation, food processing and pharmaceuticals owing to their unique properties such as higher biodegradability. The ability of oil degrading bacteria's such as *Pseudomonas putida, Pseudomonas aeruginosa* and fungal *species Aspergillus oryzae, Penicillium chrysogenum* to utilize Neem oil to produce biomass. The energy sources of neem contain different biologically active compounds undergoes into simpler compounds by the microbial activity. This will be helpful for environmental safe agricultural development. Bacterial species such as *Pseudomonas putida and Pseudomonas aeruginosa* could grow well in stone medium using water and hexane soluble fractions of neem oil and the presence of $(NH_4)_2$ HPO_4 seemed to be important for better production of biomass and Biosurfactant under the laboratory and neutral PH conditions than the fungal species. The composition of water and hexane soluble fractions of neem oil, non-degraded and degraded neem oil by microbes were analysed by using gas chromatography. The result suggested most of the components in hexane soluble extract were degraded by *Pseudomonas putida*. Hence, the present study aims to find out, oil degrading microbes for maximizing Biosurfactant productivity using Neem oil as an energy source.

Keywords: Biomass; Biosurfactant; Oil degrading; Neem oil; Hydrocarbon-degrading.

Introduction

Biosurfactant have advantage over synthetic surfactants, it can only replace the synthetic if the cost of the raw material and the process is minimal. So far, several renewable substrates form various sources; especially from industrial wastes have been intensively studied for microorganism's cultivation and surfactant production at an experimental scale. A variety of cheap raw materials, including plant-derived oils, oil wastes, starchy substances have been reported to support biosurfactant production. Researchers have used variety of vegetable oils from canola, corn, sunflower, safflower, olive, rapeseed, grape seed, palm, coconut, fish and soybean oil.

Azadirachta indica AJuss.; The neem tree is consider a good purifier of air due to its large leaf area. Parts of the plants are used medicinally. Neem oil contain several terpenoids, steroids, alkaloids, flavonoids, glycosides, etc., The isolated constituents are margosic acid, Nimbin, nimbidin, kaemperol,

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azadirone, quercursertan, b-sitosterol, praisine, vanilic acid, nimbicetin, meliacins, etc., Garg and Bhakuni, 1984 reported salanolide (a meliacin) as one of the bitter principles in neem seed oil. Furthermore, (Raman and santhanagopalan, 1979) reported tignic acid (5-methyl-2-butanoic acid as parted of the seed constituents. This compound is believed to be responsible for the distinctive odour of the neem oil. The limonoids are freely soluble in organic solvents such as hydrocarbon, alcohol and ketones, but are sparingly soluble in water. The tetranotriterpenoid compound azadirachtin exposed to neem compounds (Isman, 1990, Mordue and Black well, 1993, Verkerk and Wright, 1993). Different biologically active compounds have been isolated from neem seed (Lee et al., 1991). All biologically active neem components are suspected to be derived from one parent component which is tetra cyclic triterpenoid tirucallol (Ascher, 1993).

The complex biological structure of neem undergoes a break down into simpler compounds resulting in microbial succession favouring heterotrophic nitrogen fixers. These slow and gradual changes result in the formation of nitrogen pool which is available for plant growth and development (Barbara W. Ellis and Fern Marshall Bradley, 1996). Neem tree has identified as one of the most suitable candidate for environmentally safe agricultural development (Dhillon and Khajuria, 1996).

The oil degrading bacteria of *P.putida*, called "multi-plasmid hydrocarbon-degrading *Pseudomonas*," is the first patented organism in the world. It demonstrates a very diverse metabolism, including the ability to degrade organic solvents such as toluene. This ability has been put to use in bioremediation, or the use of microorganisms to biodegrade oil. Use of *P.putida* is preferable to some other *Pseudomonas* species capable of such degradation, as it is a safe species of bacteria, unlike *P. aeruginosa*, for example, which is an opportunistic human pathogen. This research uses the neem tree components like neem oil as a natural resource and the act of *P.putida* and *P.aeruginosa* on it in different environmental conditions.

Materials and Methods

Inoculum and Media

The bacterial species *Pseudomonas putida, Pseudomonas aeruginosa* and the fungal species *Aspergillus oryzae, Penicillium chrysogenum* were obtained from laboratory. The strain was cultured in 50 ml of stone medium, 10 ml of distilled water, and acetone and hexane extracts of Neem oil was amended in the medium as energy source. For further study analysis, Neem oil is used as substrate.

Control and Growth Conditions

Un Inoculated medium with the carbon sources (extract of neem oil) maintained at room temperature with neutral PH served as control. Another control was maintained with inoculated stone medium with carbon sources. The culture conditions are as follows PH4 - PH9, temperature 25c, 35c, 40c in BOD incubator, and 20mg of Nitrogen sources viz., $(NH_4)_2HPO_4$ (di-ammonium hydrogen orthophosphate), (NH4) 2SO4 (Ammonium sulphate), NH_4Cl (Ammonium chloride) and KNO_3 (Potassium nitrate) per 50 ml of stone medium with neutral PH was amended medium.

Biomass and Biosurfactant Production by Bacteria and fungi Using Neem Oil

Distilled water, acetone and hexane extracts of 5 ml of oil was obtained using 10 ml of extractives. The extract was centrifuged and the supernant was taken in conical flask. This extract served as carbon source. To each of these extracts 50 ml of stone medium was added and inoculated with bacterial species, Pseudomonas putida and Pseudomonas aeruginosa and fungal species *Aspergillus oryzae, Penicillium chrysogenum* are incubated for 6 days.

Fig. 1:

Fig. 2:

Isolation of Biosurfactant (Swaranjit Camorra, 1995)

After separating the biomass, the culture filtrate was centrifuged at 10,000 rpm for 30 minutes to remove any debris. The clear supernant was then treated with 3 volumes of ice cold acetone. The precipitate formed is collected by centrifugation at 5,000 rpm for 30 minutes.

Gas Chromatography Analysis

Working standard of 2 µl were prepared from the stock solution by dilution and used in finding of the retention times and qualification of the compounds in GC-ECD.

End Analysis

Oil was estimated by gas chromatography model varian Cp 3800 equipped with electron capture detector (ECD) fitted with capillary column. The following were the operating parameters.

Detector	ECD
Temperature C	Column – 25^{0}C Injector – 25^{0}C Detected – 25^{0}C
Column	1/8 inch 55 Packing – OV17
Nitrogen flow rate	30ml/min
Threshold	10 µv
Volume injected	2 µl

Analytical Methodology

Sample (2 µl) oil was injected into the injection port by using the injection needle. In GC unit carrier gas used will ensure the migration of the components of the sample. The column used was 1/8 inch 55 packing OV-17. The ECD detector was used to observe free electrons entered by radioactive sources. The current produced by free electrons were detected by detector, when current is decreased. The response of the detector is plotted by the recorder which furnishes the chromatogram. Then the physical measurement like retention time, peak height and areas were measured.

Results and Discussion

Biomass and Bisurfactant Production by Bacteria

Water and hexane extracts enhanced the biomass and biosurfactant production and the results were significant when $(NH_4)_2HPO_4$ was amended in the medium. When water and hexane extract was used as energy source. In the absence of this nitrogenous source also using water and hexane extracts the bacteria's produced Biosurfactant significantly (Table 1).

Biomass and Biosurfactant production by Fungi

Both the fungi produced biosurfactant during the degradation process at normal laboratory temperature of 30c and the presence of nitrogenous sources expecting KNO_3 favoured Biosurfactant production. The best result were obtained in the presence of $(NH_4)_2HPO_4$ for both *Aspergillus oryzae* and *Penicillium chrysogenum.* But the comparison with the fungal species, bacteria showed better production. Hence, it has been taken for further analysis.

GC Analysis of Neem Oil

To find out the components present in the neem oil was analysed to compare the components of hexane extract of Neem oil. The results showed 14 peaks representing the presence of number of 14 components of which 4 peaks appeared to be significant (Fig. 3).

GC Analysis of Hexane Extact of Neem Oil

Since hexane extract of Neem oil supported the biomass and biosurfactant production to identify the components in the oil. About 11 peaks were identified and one significant peak with 21 percent

Table 1: Biomass and Biosurfactant production by Bacteria

Factors	Water Extract				Acetone Extract				Hexane Extract			
	P.putida		*P.aeruginosa*		*P.putida*		*P.aeruginosa*		*P.putida*		*P.aeruginosa*	
	BM	BS	BM	BS	BM	BS	BM	BS	BM	BS	BM	BS
C1	-	-	-	-	-	-	-	-	-	-	-	-
C2	-	-	-	-	-	-	-	-	-	-	-	-
C3	0.72	0.48	0.58	0.32	0.56	0.25	0.27	0.12	0.80	0.53	0.50	0.27
$(NH_4)_2HPO_4$	1.27	0.59	0.80	0.45	0.59	0.28	0.52	0.31	1.38	0.55	0.92	0.43
$(NH_4)_2SO_4$	0.94	0.30	0.27	0.13	0.30	0.16	0.28	0.09	1.02	0.48	0.63	0.38
$(NH_4)Cl$	1.24	0.52	0.68	0.34	0.25	0.10	0.43	0.20	0.47	0.25	0.28	0.12
KNO_3	-	-	-	-	-	-	-	-	-	-	-	-
pH_4	-	-	-	-	-	-	-	-	-	-	-	-
pH_9	0.26	0.13	0.32	0.16	0.15	0.07	0.33	0.17	0.24	0.12	0.32	0.18
25°C	0.31	0.10	0.23	0.11	-	-	-	-	0.32	0.15	-	-
30°C	0.76	0.46	0.58	0.32	0.56	0.25	0.29	0.13	0.80	0.53	0.51	0.24
35°C	-	-	-	-	-	-	-	-	-	-	-	-
40°C	-	-	-	-	-	-	-	-	-	-	-	-

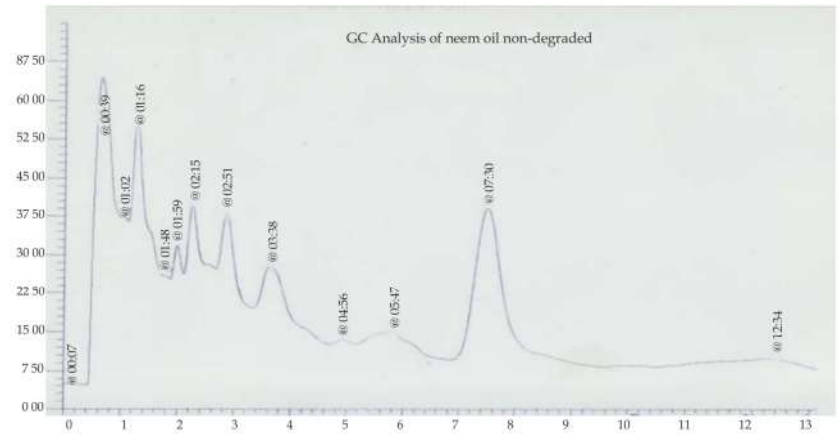

Fig. 3:

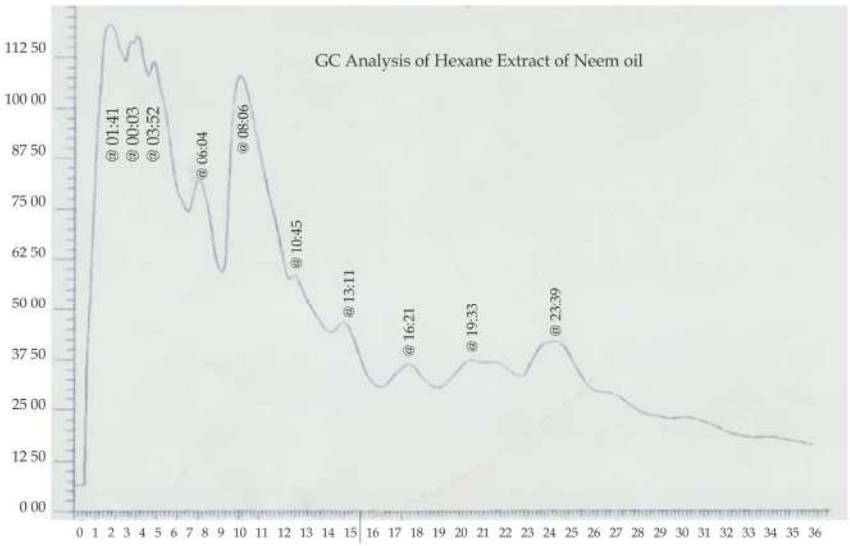

Fig. 4:

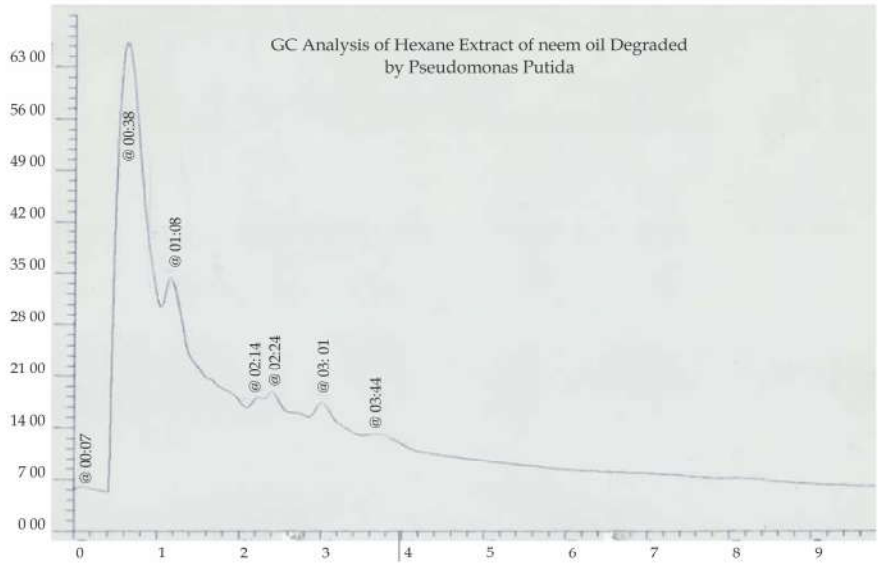

Fig. 5:

area of 21 appeared after 8 min (Fig. 4).

GC Analysis of Neem Oil Degraded by Pseudomonas Putida

This experiment was done to find out whether all the components in the results were significant when hexane soluble fractions of Neem oil was degraded and utilized by the microbes, as the results were significant when hexane soluble fractions were used as energy source. Only 7 peaks were recorded, which one peak appeared to be significant (Fig. 5).

Discussion

In the present investigation, efficiency of microbes is enhancing the biosurfactant production of neem oil has been tested. The experiment performed to find out the suitable medium suggested that in stone medium with water and hexane extracts of Neem oil as energy source. The oil degrading Bactria *Pseudomonas putida* and *Pseudomonas aeruginosa* could produce appreciable amount of biomass and biosurfactant than the fungal species. Presence of a nitrogenous source in the medium such as $(NH_4)_2HPO_4$ seems to play significant role under laboratory conditions and neutral PH. GC analysis of hexane extract of Neem oil degraded by *Pseudomonas putida* showed that almost all the components were progressively utilized by the microbes and therefore only one peak appeared in the GC analysis. Comparison of Neem oil and hexane extract of Neem oil showed no significant similarity. All the peaks in the Neem oil could be recorded by 15 minutes whereas the hexane extracts of Neem oil the peaks appeared for about 23 minutes.

Conculsion

As a result suggests *Pseudomonas putida* degraded most of the components, so *pseudomonas putida* is the best and safest oil degrading organism. Hence, Surfactants have several applications in agriculture and agrochemical industries. This study will help in replacing the harsh chemical surfactants with green ones. Several researchers indicate that variety of environmental niches such as soil, water, and leaf surface are explored for Biosurfactant producing bacteria. Plant associated microbes are known to produce biosurfactant indicating the potential role of biosurfactant in plant-microbe interaction and further application of biosurfactant in agriculture.

References

1. Adkins JP., RS Tanner, EO Udegbunam, M J JMcinerney and RM Knapp. Microbially enhanced oil recovery from unconsolidated lime-stone cores. Geomicrobiology journal. 1992;10:77-86.
2. Ascher KRS. Non-conventional insecticidal effect of pesticides available from the neem tree, Azadirachtin indica. Arch.insect Biochem. Physiol. 1993;22:433-49.
3. Atlas RM. Effect of temperature and crude oil composition on petroleum biodegradation Appl. microbil. 1975;30:396-403.
4. Banat IM. Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation. A Review Bio resource Technology. 1995;51:1-12.
5. Buchner B.R. Global phenotypic characterization of bacteria. FEMS Microbial. Rev. 2009;33:191–205.
6. Broderick LS and JT cooney. Emulsification of hydrocarbon by bacteria from fresh water ecosystem. Dev.ind.microbial. 1982;23:423-34.
7. Cooney J, SA Selver and EA Beck. Factors influencing hydrocarbon degrading in fresh water lakes. Microbial. Ecol. 1985;11:127-137.
8. Desai JO, Banat IM.Microbial production of Biosurfactants and their commercial potential. Microbial.Mo.Rev. 1997;61:47-64.
9. Dhillion, M.S and Khajuria, N.S. Neem for environmental safe agricultural development. Neem newsletter, 1996 April-June 13:17.
10. Fiechter A. Biosufactant moving towards industrial application, Trends in Biotech. 1992;10:208-17.
11. Garg HS, Bhakuni DS. Hand book of African medicinal plants. CRC Press, London. 1984.pp.124–128.
12. Georgiou VG, S. Lin and MM Sharma. Surface-active compounds from microorganism. Biotechnology. 1992;10:60-65.
13. Haba E, Abalos A, Jauregui O, Espuny MJ, Manresa A. Use of liquid chromatography-mass spectroscopy for studying the composition and properties of rhamnolipids produced by different strains of Pseudomonas aeruginosa. J Surfactants Deterg. 2003;6:155–61.
14. Isman M.B., O. Koul A. Luczynski and J. Kaminski. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J. Argic. Food Chem. 1990;38:1406-11.
15. Jain DK, S. Collins, D.L Thomson, H. Lee and

JT Trevors. Effect of addition of Pseudomonas aeruginosa UG-2 inoculant or biosurfactant on biodegradation of selected hydrocarbons. 1992.

16. Lee SM, Kloco JA, Barnby MA, Yamasaki RB and MF Balandrin. Insecticidal constituent of Azadirachtin indica and Melia Azadirachta. Acs symp.series. 1991;449:293-304.
17. Mercade ME, Manrewa MA, Robert, Espuny, MJ Andres, Guniea J. Olive oil mill effluent (OOME): Neem substrate for biosurfactant production. Bioresour. Technol. 1993;43:1-6.
18. OudotJ, J. Dupont, S. Halouri and M.F. Roquebert Biodegradation potential of hydrocarbon assimilating tropical fungi. Soil.Bio.Biochemistry. 1993;25(9):1167-73.
19. Partricia cunnif. Official methods of analysis of A.O.A.C. International. Chap. 1995;36:9-10.
20. Raman H, Santhanagopalan S. Hand book of African medicinal plants. CRC Press, London. 1979. pp.124–28.
21. Robert M, Mercade ME, Bosch MP, Parra JL, EspunyMJ, Manresa MA and Guinea J. Effect of the caron source on Bio.prod by Pseudomonas aeruginosa. Singer ME. 1985. Microbial biosurfactant. Microbes and oil recovery. 1989;1:19-38.
22. Swaranjit C, Cameotra. biosurfactant production by an oil field bacterial stain. J. Microbial. Biotech. 1995;10(1):8-16.