ISSN: 2394-1391

Original Article

Indian Journal of Biology
Volume 6 Number 2 / July – December 2019
DOI: http://dx.doi.org/10.21088/ijb.2394.1391.6219.2

Biomass and Biosurfactant Manufacturing of Neem Cake by Hydrocarbon Degrading Microbes

Lasitha K[1], M Arul Sheeba Rani[2]

How to cite this article:
Lasitha K, M Arul Sheeba Rani. Biomass and Biosurfactant Manufacturing of Neem Cake by Hydrocarbon Degrading Microbes. Indian J Biol. 2019;6(2):75–81.

Abstract

Naturally occurring surface active compounds drived from microorganism also called Biosurfactant. They offer several advantages over the chemical surfactants. Such as low toxicity, inherent good biodegradability and ecological acceptability. Biosurfactant which are revealed to be produced by bacteria, yeast and fungi can serve as green surfactants. The biosurfactant synthesizes by environmental isolates also has promising role in the agricultural industry. Based on the above assets, selected two oil degrading fungal species from the laboratory for the further studies. The selected microbes *Aspergillus oryzae* and *Penicillium chrysogenum* play major role in oil recovery, environmental bioremediation and pharmaceuticals owing to their unique properties such as biodegradability. The ability of oil degrading fungal species utilize neem cake to produce biomass and biosurfactant. Neem cake as it is the cheapest carbon source as compared to glucose, fructose, and other petroleum based substrate for the production of biosurfactant. The energy sources of neem cake contain different biological active compounds which undergoes by the microbial activity can provide added benefits. This will be helpful for environmental safe agricultural development. Fungal species such as *Aspergillus oryzae* and *Penicillium chrysogenum* could grow well in stone medium using water and hexane soluble fraction of neem cake in the presence of $(NH_4)_2HPO_4$ seemed to be important for better production of biomass and biosurfactant under laboratory and neutral pH conditions. Hence, the present study aims to find out, oil degrading microbes for maximizing biosurfactant productivity using neem cake as an energy source.

Keywords: Biomass; Biosurfactant; Oil degradation; Neem press cake; Hydrocarbon-degradation.

Introduction

A number of microorganisms are known to synthesise surface-active agents, most of them are bacteria and yeast (Banat, 1995). But, in recent years, it has been produced by filamentous fungi as well (Castiglioni, 2009), (Velioglu and Urek 2015). Filamentous fungi are capable of degrading the whole array of hydrocarbon containing compounds (Adekunle and Oluyode, 2005); (Saratale et al., 2007; George-Okafor et al., 2009, Reuben et al., 2011) by producing capable enzymes. Hence, they are called nature's original recyclers. Furthermore fungi yield a good amount of biosurfactant when compared to bacteria. The reason might be presence of rigid cell wall in them (Kim et al., 1999).During biological process, the microbes are utilizing natural and synthetic chemicals for their metabolism, which are less expensive and more environment friendly

Author's Affiliation: [1]PhD Research Scholar, [2]Assistant Professor, Department of Botany, Nirmala College for Women, Coimbatore, Tamil Nadu 641018, India.

Corresponding Author: M Arul Sheeba Rani, Assistant Professor, Department of Botany, Nirmala College for Women, Coimbatore, Tamil Nadu 641018, India.

E-mail: sheebam582@yahoo.com

Received on 19.08.2019, **Accepted on** 05.12.2019

(Sasikumar and Papinazath, 2003). Sanyaolu et al., (2018) reported that *Aspergillus oryzae* has the ability to utilize different types of petroleum and vegetable hydrocarbon compounds.

When the microbes grown on hydrocarbon substrate as carbon source, these microorganisms synthesise a wide range of chemicals with surface activity such as glycolipid, phospholipid and others (Desai and Banat, 1997). These chemicals are apparently synthesised to emulsify the hydrocarbon substrate and facilitate its transport into the cells.

More over Factors playing important role for the production of biosurfactants, there are nature of the carbon source and the concentrations of nutrients such as nitrogen, phosphorus, magnesium, iron, and manganese, as well as the pH, temperature (Banat et al., 2010; Fontes et al., 2008; Pirôllo et al., 2006). These factors can make the production of biosurfactants more valuable than that of synthetic surfactants (Thavasi, et al., and Banat, 2007).

The end-product of Biosurfactants are structurally and functionally diverse amphiphilic, surface active compounds which lower the surface and interfacial tension between individual molecules at respective surfaces and interfaces. Hence, these are very important in the living systems to transport and exchange of the various important Materials (Lang, 2002; Banat, 1995). Biosurfactants are ecologically safe and can be applied in various industries. Although using biosurfactant are relatively high in production cost. The utilization of alternative substrate (Agro industrial waste) is the possible way to reduce the cost. The main problem related to the usage of alternative substrate is the final product depends on the composition of culture media. Hence, the present study is focused on Neem cake as it is the cheapest carbon source as compared to glucose, fructose, and other petroleum based substrate for the production of biosurfactant.

Fig. 1:

Fig. 2:

Azadirachta indica A. Juss. (Meliaceae) has been identified as one of the most suitable candidate for environmentally safe agricultural development. Neem cake is the residue obtained from neem seed (Fig. 1 & 2). It is an excellent organic fertilizer. The composition of Neem Cake after the extraction of oil varies widely depending on the raw material. It contain crude protein, carbohydrate, crude fibre, fat, ash, acid insoluble ash. The component of azadiractin in neem cake protects crops against parasite and enriches the soil (Mishra and Prasad, 1974). The neem cake is rich in most of the amino acids. It is a potential source of organic manure and contains many plant nutrients. The neem cake contains a large number of triterpenoids, more of which are being discovered. The important characteristic of the Neem cake is to reduce the alkalinity in the soil by producing organic acids when mixed with the soil. The composition of calcium and magnesium present in neem cake also aid in removing alkalinity (Gupta et al., 1993). The biological structure of neem cake undergoes a break down into simpler compounds resulting in microbial succession favouring heterotrophic nitrogen fixers. These slow and gradual changes results in the formation of nitrogen pool which is available for plant growth and development (Barbara W. Ellis and Fern Marshall Bradley, 1996). Neem cake has identified as one of the most suitable candidate for environmentally safe agricultural development. (Dhillon and Khajuria, 1996)

Materials and Methods

Micro organisms

- *Aspergillus oryzae*
- *Penicillium chrysogenum*

Materials used

- Neem press cake

Nitrogen source

- Di-ammonium hydrogen orthophosphate [$(NH_4)_2HPO_4$]
- Ammonium sulphate [$(NH_4)_2SO_4$]
- Ammonium Chloride [NH_4Cl]
- Potassium Nitrate [KNO_3]

Medium used (Outdo et al.*, 1993)*

- Bushnell and hash medium
- Mineral medium
- Nutrient broth medium
- Stone medium

Inoculum and Media

For further study, Neem press cake used as substrate. The fungal species *Aspergillus oryzae*, *Penicillium chrysogenum* were obtained from the laboratory, and the fungal species were maintained in potato dextrose agar medium. The strain was cultured in 50 ml of stone medium, 10 ml of distilled water, acetone and hexane with 5 gm of neem cake extract of the medium served as energy source.

Control and Growth Conditions

Un Inoculated medium with the carbon sources (extract of neem press cake) 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 25°C, 35°C, 40°C in BOD incubator, and 20 mg of Nitrogen sources viz., $(NH_4)_2HPO_4$ (di-ammonium hydrogen orthophosphate), $(NH_4)_2SO_4$ (Ammonium sulphate), NH_4Cl (Ammonium chloride) and KNO_3 (Potassium nitrate) per 50 ml of stone medium with neutral pH.

Biomass and Biosurfactant Production by Fungi Using Neem Cake

To 50 ml of stone medium, 10 ml of distilled water, acetone and hexane extracts of 5 gm of neem press cake powder was centrifuged and the supernant was taken in conical flask. This extract served as carbon source. Fungal species are incubated for 7–8 days.

Extraction of Fungal Biomass

The mycelium was separated from the medium by filtering through pre-weighed Whatman's No. 41 filter paper. The filter paper along with mycelial mat was dried at 80°C in the hot air oven until constant weight and this weight was recorded. Difference between the weight of the filter paper bearing mycelial mat and the weight of only the filter paper represented biomass of fungal mat. Biomass was quoted in terms of mg ml^{-1} (dry weight).

Isolation of Biosurfactant

After separating the Biomass, Biosurfactant in the culture filtrate was estimated according to the procedure described by Swaranjit Cameotra, (1995) and the weight was expressed as mg ml^{-1}. The culture filtrate was centrifuged at 10,000 rpm for 30 minutes at 4°C to remove any debris. The clear supernant was then treated with 3 volumes of ice cold acetone. The precipitate is collected by centrifugation at 5,000 rpm for 30 minutes.

Results

Suitable Medium for the Growth of Neem Cake Degrading Microbes using as Energy Source

In this experiment, using four different liquid medium such as Bushnell and Hass, Nutrient broth, Mineral medium and Stone medium were tested for their efficiency in supporting the growth of the neem cake degrading microbes with water, acetone, and hexane extract, under normal laboratory conditions. Two controls were maintained simultaneously to verify whether the growth of the microbes enhanced by the extract. Control I was only medium with uninoculated microbes with no energy source and Control II was medium with extract without microbial inoculants. The experimental flask contained inoculated medium with extract. Optical density of the cultures were measured at 420 nm at the end of the log phase and the results were recorded. The results suggested that stone medium is suitable for the present study.

Table 1:

Medium (O.D at 420 nm)	Organism	Control 1 (M + I)	Control 2 (M + E)			Control 3 (M + E + I)		
			Water	Acetone	Hexane	Water	Acetone	Hexane
Bushnall & Hass medium	A.O	0.13	–	–	–	0.30	0.22	0.35
	P.C	0.12				0.25	0.19	0.21
Mineral medium	A.O	0.11	–	–	–	1.0	0.47	0.85
	P.C	0.09				0.90	0.40	0.81

Medium (O.D at 420 nm)	Organism	Control 1 (M + I)	Control 2 (M + E)			Control 3 (M + E + I)		
			Water	Acetone	Hexane	Water	Acetone	Hexane
Nutriant broth medium	A.O	0.55	–	–	–	1.4	0.56	1.3
	P.C	0.40				1.3	0.85	1.2
Stone medium	A.O	0.80	–	–	–	1.7	0.90	0.5
	P.C	0.72				1.5	0.85	1.3

(A.O ≥ *Aspergillus oryzae*; P.C ≥ *Penicillium chrysogenum*

Biomass Production by the fungi Aspergillus oryzae and Penicillium chrysogenum using the Neem Cake

To find out whether the degrading microbes have the ability to produce biomass and biosurfactant utilizing neem cake. The fungi *Aspergillus oryzae* and *Penicillium chrysogenum* were inoculated in stone medium with extracted neem cake as an energy source. At the end of log phase the biomass was separated and culture filtrate tested for the presence of biosurfactant. Effect of various environmental parameters such as nitrogenous source, pH, temperature and nutritional conditions of the medium were also studied.

Biomass Production

Aspergillus oryzae produced significant amount of biomass in the presence of $(NH_4)_2HPO_4$ and $(NH_4)_2SO_4$. Alkaline conditions and room temperature (30°C) also favoured growth of *Aspergillus oryzae*. Acidic condition did not show much influence. All the other factors had no favourable effect. The result showed in Table 2. Figs. 1 & 2.

Table 2: Biomass Production

Factors	Fresh Weight Mg/50 ml of medium		Dry Weight Mg/50 ml of medium	
	I	II	I	II
Control I	1.98	0.89	0.88	0.76
Control II	–	–	–	–
$(NH_4)HPO_4$	5.16	4.39	2.09	1.38
$(NH_4)_2SO_4$	4.92	3.85	1.96	0.97
$(NH_4)CL$	4.11	4.11	1.76	0.82
KNO_3	2.34	2.19	1.76	0.82
pH-4	2.02	2.89	0.78	0.63
pH-9	3.29	3.96	1.15	1.01
25°C	3.23	3.01	1.18	1.02
30°C	4.96	4.26	2.23	1.08
35°C	2.04	2.08	1.07	0.56
40°C	1.92	1.08	0.99	0.48

I-*Aspergillus oryzae*; II-*Penicillium chrysogenum*

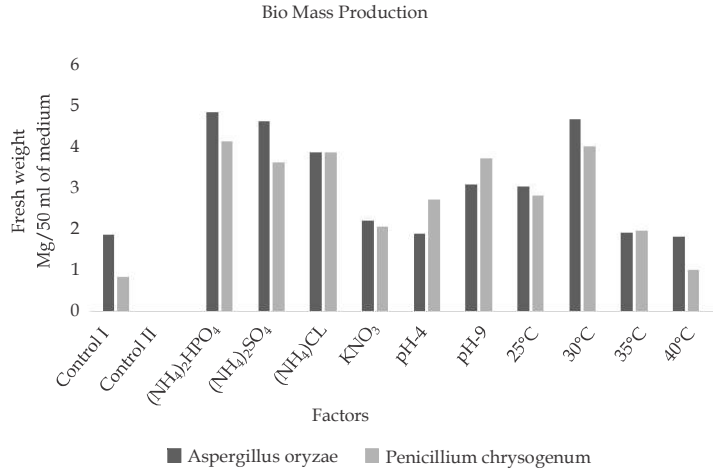

Fig. 1: Biomass Production (Fresh Weight)

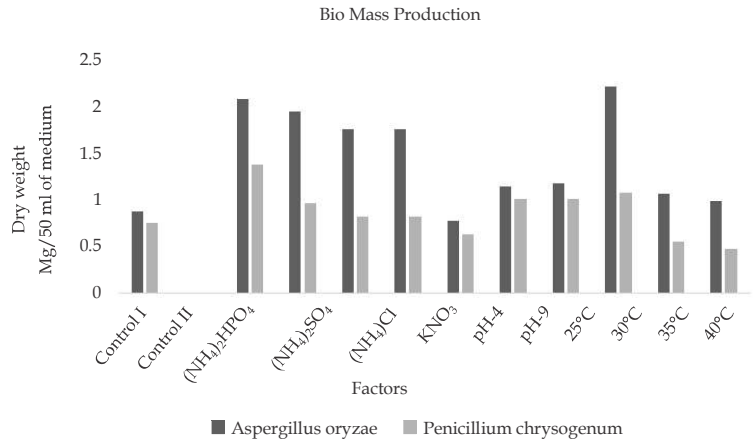

Fig. 2: Biomass Production (Dry weight)

The nitrogenous source $(NH_4)_2HPO_4$ enhanced the biomass production by *Penicillium chrysogenum*. $(NH_4)_2SO_4$ also enhanced biomass production. Other factors had influencing effect on this fungi excepting KNO_3 and higher temperature.

Biosurfactant Production

Both the fungi produced biosurfactant during the degradation process. But *Aspergillus oryzae* shows better production then the *Penicillium chrysogenum*. Normal laboratory temperature 30°C and the presence of nitrogenous sources excepting KNO_3 favoured biosurfactant production. The result showed in Table 3 & Fig. 3.

Table 3: Biosurfactant Production

Factors	Biosurfactant Mg/50 ml of medium	
	I	II
Control I	0.03	0.025
Control II	–	–
$(NH_4)_2HPO_4$	0.40	0.32
$(NH_4)_2SO_4$	0.28	0.21
$(NH_4)CI$	0.37	0.28
KNO_3	0.08	0.04
pH-4	0.06	0.03
pH-9	0.13	0.11
25°C	0.16	0.10
30°C	0.8	0.4
35°C	0.5	0.35
40°C	0.4	0.09

I-*Aspergillus oryzae*; II-*Penicillium chrysogenum*

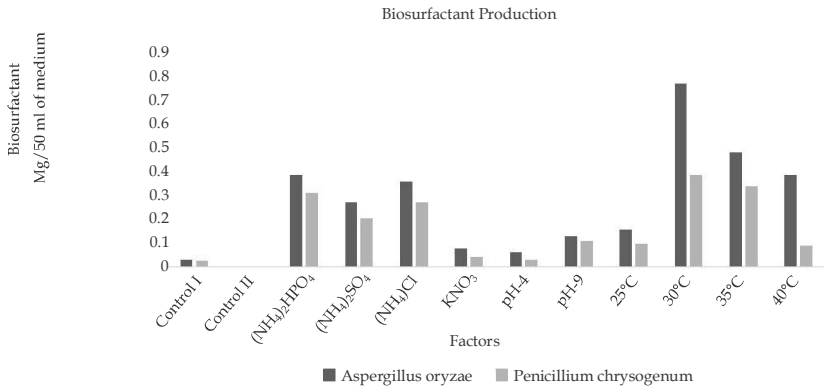

Fig. 3: Biosurfactant Production

Discussion

Biosurfactants are widely used in many industries such as agriculture, food production, chemistry, cosmetics and pharmaceutics. Biosurfactants increase the bioavailability of hydrocarbon resulting in enhanced growth and degradation of contaminants by hydrocarbon-degrading microbes present in polluted soil. Therefore, to meet these requirements, the present investigation focused on Biosurfactant production used by cheaper carbon source like Neem press cake. The possibility of biosurfactant production using cheaper carbon sources was already reported by Thavasi et al. (2008) in peanut cake. The efficiency of microbes is enhancing the biosurfactant production of neem cake has been tested. The experiment performed to find out the suitable medium suggested that in stone medium with water and hexane extracts of Neem cake as energy source. The oil degrading Fungi *Aspergillus oryzae* could produce appreciable amount of biomass and biosurfactant compare to *Penicillium chrysogenum* 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.

Conculsion

As a result suggests *Aspergillus oryzae* could grow well on the medium and produces biosurfactant than *penicillium chrysogenum*, so *Aspergillus oryzae* is the best and safest oil degrading organism. Hence, Surfactants have several applications in agriculture and agrochemical industries. This studies 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. Adekunle AA and Oluyode TF. Biodegradation of crude petroleum and petroleum products by fungi isolated from two oil seeds (melon and soybean). J Environ Biol. 2005;26(1):37–42.
2. Adrio JL and Demain AL. Fungal biotechnology. International Microbiology. 2003;6(3):191–99. View at Publisher; View at Google Scholar; View at Scopus.
3. Banat IM. Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: A review. Bioresource Technology. 1995;51:1–12.
4. Castiglioni GL, Bertolin TE, and Costa JA. Produção de biossurfactante por Aspergillus fumigatus utilizando resíduos agroindustriais como substrato. Química Nova. 2009;32(2)292–95. View at Publisher; View at Google Scholar.
5. Desai JD, Banat LM. Microbial production of surfactants and their commercial potential. Microbiology and Molecular Biology Reviews. 1997.pp.47–64.
6. Fontes GC, Amaral PF, and Coelho MA. Produção de biossurfactante por levedura. Química Nova, 2008;31(8):2091–99. View at Publisher; View at Google Scholar.
7. George-Okafor U, Tasie F and Muotoe-Okafor F. Hydrocarbon degradation potentials of indigenous fungi isolated from petroleum contaminated soils. Journal of Physical and Natural Sciences. 2009;3(1):1–6.
8. Gupta P, Sebastil S, Sobita SR and Mishra SM. Neem Newletter. 1993;10(1&2):37.
9. Haba E, Abalos A, Jauregui O, et al. 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.
10. Isman MB, Koul O, Luczynski A and Kaminski J. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J. Argic. Food Chem. 1990;38:1406–11.
11. Kim HS, Yoon BD, Choung DH, et al. Characterization of a biosurfactant mannosylerythritol lipid produced from Candida sp. SY 16. Appl Microbiol Biotechnol. 1999;52:713–21.
12. Lang S. Biological amphiphiles (microbial biosurfactants). Curr Opin Colloid Interphase Sci. 2002;7:12–20.
13. Mishra SD and Prasad SK. Effect of Soil Amendments on Nematodes and Crop Yields. Indian J. Nematol. 1974;4:1–19.
14. M. Pirôllo, Estudo da Produção de Biosurfactantes Utilizando Hidrocarbonetos, Universidade Estadual Paulista, Sao Paulo, Brazil, 2006.
15. Reuben NO, Josiah MA, Davidson DD. and M. Allagoa. Mycoremediation of polycyclic aromatic hydrocarbons (PAH) contaminated oil-based drill-cuttings. African Journal of Biotechnology. 2011;10(26):5149–56.
16. Sanyaolu AAA, Adekunle AA and AA. Osuntoki. Bioremediation Potentials of a Petroleum Hydrocarbon Polluted Soil by

Aspergillus oryzae (JQ675305.1) Isolated from Diseased Irvingia gabonensis Seeds. The Pacific Journal of Science and Technology. 2018;19(1):321–33.

17. Saratale G, Kalme S, Bhosale S and S. Govindwar. Biodegradation of kerosene by Aspergillus ochraceus NCIM – 1146. Journal of Basic Microbiology. 2007;47:400–06.
18. Sasikumar CS and Papinazath T. Environmental Management and Bioremediation of Polluted Environment. In: Burch MJ., Suresh VM. and Kumaran TV. (Eds). Proceedings of the Third International Conference on Environment and Health, Chennai-India. 2003:469.
19. Satpute SK, Banpurkar AG, Dhakephalkar PK, et al. Methods for investigating biosurfactants and bioemulsofers: a review. Crit Rev Biotechnol 2010;30:127–44.
20. Shin-foon C. The active principles and insecticidal properties of some Chinese plants, with special reference to Meliaceae. 1984:255–62.Ibid.
21. Swaranjit C. Cameotra. Biosurfactant production by an oil field bacterial strain. J. Microbial. Biotech. 1995;10(1):8–16.
22. Thavasi R, Jayalakshmi S, Balasubramanian T, Banat IM. Biosurfactant production by Corynebacterium kutscheri from waste motor lubricant oil and peanut oil cake. Letters in Applied Microbiology, 2007;45(6):686–91. View at Publisher; View at Google Scholar; View at Scopus.
23. Velioglu Z and Urek RO. Biosurfactant production by Pleurotus ostreatus in submerged and solid-state fermentation systems. Turkish Journal of Biology, 2015;39(1)160–66. View at Publisher; View at Google Scholar; View at Scopus.
24. Vererk RHJ and DJ. Wright. Biological activity of neem seed kernel extracts and synthetic azadiractin against larva of Plutella xylostella. Pesticide sci. 1993;37:83–91.
25. Vipulanandham C. Pollution prevention by bioconversion of industrial waste material of biosurfactant. Geo. Environment. 2002.
26. Warthen JD Jr, Uebel EC, Dutky SR, et al. An adult housefly feeding deterrent from neem seeds. UD Agric. Res. Results RR-NE2. 1978.