OPTIMIZATION OF PROCESS PARAMETERS FOR PRODUCTION OF BIOSURFACTANTS BY RHIZOPUS SP

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Abstract: Biosurfactants are a group of microbial molecules identified by their unique capabilities to interact with hydrocarbons. Biosurfactant production is a secondary metabolism. It is directly related with growth phase. Deproteinised leaf extracts (DPE) of different plants like Eucalyptus (*Eucalyptus sp.* L.), Castor (*Ricinus communis* L.), Soyabean (*Glycine max* L.), and cauliflower were used. Soil fungi were screened for biosurfactant production and efficient fungi were identified i.e. *Aspergillus niger, Aspergillus flavus, Penicillium sp* and *Rhizopus* sp. Effect of various culture conditions like pH, Temperature, Substrate concentration, incubation period and aeration for optimum growth and biosurfactant production were determined. Amongst selected isolates *Rhizopus* sp. showed maximum biosurfactant production at pH- 6.5, Temperature - 28°C, 2.5% of substrate concentration & 0.1% KNo_3 as nitrogen source, 7-days of incubation period. GN- medium was used as control.

Key words: Biosurfactant, Deproteinised leaf extracts, GN- medium, Isolated fungi.

Introduction: Biosurfactants are amphiphilic compounds made by a wide variety of microbes, effectively lower surface and interfacial tensions and are valued as emulsifying, foaming, and wetting agents. Exhibiting low toxicity and a high degree of biodegradability, biosurfactants appear to be safer than synthetic surfactants Kaelynn Williams (2009).Biosurfactants could replace synthetic (chemically-produced) surfactants that are currently used due to reports of adverse reactions with long term use [Lourith N, Kanlayavattanakul M. N. 2009]. Like synthetic surfactants, biosurfactants are excellent emulsifiers and maintain wetting and foaming properties, characteristics that are valued in several applications including the cosmetics industry. Biosurfactants are readily biodegradable contributing to environmental compatibility. Biosurfactants are likely to gain wide acceptance since they are readily biodegradable and have lower toxicity as compared to their chemically synthesized counterpart. Effect of environmental factors on growth and biosurfactant production by microorganisms were carried out (Kim, 1993, L. Rodrigues *et al.*, 2006, Abu-Ruwaida *et al.*, 1991, Banat I. M., 1993, and Aulwar U. L. and Awasthi R. S., 2009). The objective of the study was to determine the culture conditions for maximum biosurfactant production by the *Rhizopus* sp. effecient isolate amongst the screened fungi i.e. *Aspergillus niger, Aspergillus flavus,* and *Penicillium* sp. The Deproteinised leaf extract (DPE) is rich in water soluble plant nutrients. It contains water soluble carbohydrates, minerals, free amino acids etc. (Barnes 1976, Shahane and Mungikar 1985). Deproteinised leaf extract supports to the growth of *Rhizoctonia* and also used for alcohol production by yeast (Doiphode 2003, Mungikar and Gogle 2003).

Materials And Methods: Isolation of fungi: The hydrocarbon-utilizing fungi were isolated from different soil samples. The isolated fungi were screened for biosurfactant production on the basis of lipolytic activity. These fungi were identified as *Aspergillus niger, Aspergillus flavus, Rhizopus* sp. and *Penicillium* sp. Fungal cultures were maintained on potato dextrose agar slants at 4°C.

Medium: Deproteinised leaf extract (DPE) was used. GN medium in modified form was used as control (Glucose-10gm, KNo_3-2.5gm, KH_2PO_4-1gm, $MgSO_4$-0.5gm in 1000ml distilled water).

Method of inoculation: A loopful of spore suspension was standardize to contain 20-30 spores per field (10X10) was inoculated in 50ml Deproteinised leaf extract &modified GN medium.

Incubation: In 250ml Erlenmeyer flask 50ml sterilized Deproteinised leaf extract (DP) & modified GN medium was inoculated with fungal culture and incubated in static condition for 6 days (unless stated otherwise).

Assessment of Growth: Growth was assessed interms of dry weight at the end of incubation period by gravimetric method.

Biosurfactant production:Biosurfactant production was determined by measuring emulsion index. The emulsification index was measured by kerosene emulsification method (Singer N.E. 1985).

After separation of biomass the cell free broth (supernatant) was used. 4 ml of cell free broth was taken and to it 2 ml of kerosene was added, mixed well on cyclomixer for 2 minutes. The tubes were kept undisturbed for 12 hours. After 12 hours height of emulsion and height of kerosene was measured.

Effect of culture conditions: pH in the range of 6 to 7.5, temperature like 28°C, 37°C, 40°C, substrate concentration from 0.5% to 2.5% (w/v) and incubation period from 4 to 12 days, aeration at 80rpm to 160rpm were checked with respect to growth and biosurfactant production.

Table no. – 1 Growth and emulsification activity of *Rhizopus* sp. in DPE - medium

DPE of plants	Dry weight (gm/lit)	Emulsion Activity (%)
Eucalyptus	347	25.02
Castor	271	15.17
Soybean	282	16.68
Cauliflower	233	11.48
Modified GN medium (control)	330	21.23

Table no. 2- Growth and emulsification activity of *Rhizopus* sp. in suspension culture

Medium	Dry weight (gm/lit)	Emulsion activity (%)
GN medium	330	21.33
DPE- medium	347	25.02

Table –3 Effect of DPE – medium concentration on growth and biosurfactant production of *Rhizopus* sp.

DPE- medium (%)	Dry weight (Mg/100ml)	Emulsion Index
0.5	173	16.78
1.0	257	19.99
1.5	280	22.01
2.0	331	23.63
2.5	381	24.86
30	383	24.52
3.5	387	21.31
4.0	390	18.01
4.5	392	13.33
5.0	395	13.29
5.5	398	10.7
6.0	401	10.1
6.5	404	9.97
7.0	405	9.82
7.5	405	8.01
8.0	399	7.59
8.5	352	7.1
9.0	303	5.87
9.5	271	5.23
10.0	202	5.11
Total	6909	277.23
Mean	345.45	13.8615
S.D.	72.64294	6.903982

Table –4 Effect of pHon growth and biosurfactant production of *Rhizopus* sp. in DPE- medium:

pH	Dry weight (Mg/100ml)	Emulsion Index
4.0	243	15.05
4.5	282	19.75
5.0	302	20.92
5.5	322	22.05
6.0	341	23.92
6.5	383	24.87
7.0	347	16.02
7.5	206	10.05
Total	2426	152.63
Mean	303.25	19.07875
S.D.	58.05355	5.024945

Table –5 -Effect of temperature on growth and biosurfactant production of *Rhizopus* sp. in DPE- medium:

Temperature (^{0}C)	Dry weight (Mg/100ml)	Emulsion Index
25^{0}C	285	22.01
28^{0}C	381	24.85
37^{0}C	302	11.61
40^{0}C	217	8.87
45^{0}C	110	6.61
Total	1295	73.95
Mean	259	14.79
S.D.	101.703	8.1456

Table –6-Effect of aeration on growth and biosurfactant production of *Rhizopus* sp. in DPE- medium:

Shaking (rpm)	Dry weight (Mg/100ml)	Emulsion Index
80rpm	258	20.12
100rpm	337	23.90
120rpm	381	24.98
140rpm	342	21.15
160rpm	298	10.21
Total	1616	100.36
Mean	323.2	20.072
S.D.	46.82627	5.855883

Table –7-Effect of incubation period on growth and biosurfactant production of *Rhizopus* sp. in DPE- medium:

Incubation period (days)	Dry weight (Mg/100ml)	Emulsion Index
2	113	5.21
3	131	7.34
4	145	10.03
5	180	19.87
6	280	24.82
7	382	25.02
8	384	24.91
9	390	9.75
10	393	9.40
11	394	9.11
12	396	8.02
13	396	7.71
14	397	6.01
15	288	5.02
Total	4269	172.22
Mean	304.9286	12.30143
S.D.	114.1406	7.705772

Results And Discussion: Fungi isolated from soil samples were screened for biosurfactant production. They were identified as *Aspergillus niger, Aspergillus flavus*, *Rhizopus* sp. and *Penicillium* sp. All the isolates grew well at 28°C, pH-6.5 and 2.5% concentration of glucose substrate. Maximum growth was observed on 12 days of incubation. Amongst the isolates *Rhizopus* sp. showed optimum growth and emulsification activity at pH-6.5 (Table no.-4), temperature 28° (Table no.-5), 2.5% (w/v) glucose (Table no.3) as substrate concentration, 7- days of incubation period (Table no. -7) and 0.1% KNo_3 as nitrogen source. Growth and optimization studies were carried out. On the basis of the same growth and emulsification activity of all these isolates were measured in static condition and in suspension culture by using modified GN medium. Amongst these three isolates *Rhizopus* sp. was found suitable for biosurfactant production. In suspension culture growth measured was 330 mg/100ml and emulsion activity measured was 21.33%. *Rhizopus* sp. was selected for further studies. DPE- medium of different plants i.e. Eucalyptus, Castor, Soyabean, Cauliflower was used for biosurfactant production by *Rhizopus* sp. Optimum growth and maximum activity was measured in DP- medium of *Eucalyptus*. In suspension culture *Rhizopus* sp. showed maximum growth and optimum activity in DPE- medium.

Emulsion activity was found more in DPE- medium as compared with activity in GN medium.Microorganisms used various renewable sources, especially agro-industrial wastes a potential carbon sources. Olive oil, mill effluent, animal fat, frying oil, babassu oil, molasses, whey, starch rich wastes were used for biosurfactant production (Deshpande and Daniel 1995; Haba *et al.* 1999; Christen *et al.* 2000; Morques 2001; M.H. Vance Harrop *et al.* 2003). DPE- medium was used for growth, biomass and alcohol production (A.M. Mungikar and D.P. Geogle 2000; D.A. Doiphode 2005). The nitrogen source can be important key to the regulation of biosurfactant synthesis. *Arthrobactor paraffineus* ATCC 19558 preferred ammonium nitrate as inorganic nitrogen source for biosurfactant production (Ruwalda et al. 1991). Results obtained in present study are in agreement with the results reported by Ruwalda et al. 1991.Temperature had direct influence on biosurfactant production by *Pseudomonas sp.* strain DSNC 874 was reported by Drawin and Cooper 1992, Syldatk C.S. et al. 1985. In the present study 28°C temperature was found best for growth and biosurfactant production by fungal isolates. At higher temperature growth and biosurfactant production

was found less. *Lactobacillus sp.* produced biosurfactant optimally at 72 hours of incubation when strains were in stationary growth phase at initial stage of four hours of incubation biosurfactant production was less. Activity was found decreased upon prolonged incubation (L. Rodridrigues et al. 2006). Growth and biosurfactant production was found increased up to 7 days of incubation. However on 12 days of incubation biosurfactant production was decreased. Results obtained are in agreement with the report of L. Rodridrigues et al. 2006. 2% glucose as carbon source at pH-7.2 was found best for biosurfactant production by *Pseudomonas sp* as reported by Yamaguchi et al. 1976. However for fungal isolate *Rhizopus* sp. 2.5% glucose as carbon source at pH-6.5 was found best for biosurfactant production.

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