

Screening and optimization of biosurfactant producing bacteria from oil mill area MIDC, Parbhani (M.S.)

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Abstract

Biosurfactant are amphiphilic compounds produced on living surfaces, mostly on microbial cell surfaces. They are biodegradable non-toxic and ecofriendly materials. *Pseudomonas aeruginosa* isolated from oil mill area produced biosurfactant at 37°C at pH 7 on YPG medium and mineral salt medium. The biosurfactant production depends on the fermentation conditions, environmental factors and nutrient availability. The extraction of the biosurfactant from the cell free supernatant using the solvent extraction procedure & the qualitative and quantitative analysis has been discussed with appropriate equipment details.

Keywords: Biosurfactant, Rhamnolipid, Bioremediation, Emulsification, Qualitative analysis, Quantitative analysis.

INTRODUCTION

Petroleum hydrocarbon continues to be used as the principle source of energy and hence an important global environmental pollutant. Apart from accidental contamination of the ecosystem, the vast amounts of oil sludge generated in refineries from water oil separation systems and accumulation of waste oily materials in crude oil storage tank bottoms pose great problems because of the expensive disposal methods. Biosurfactants are amphiphilic compounds that reduce surface and interfacial tensions by accumulating at the interface of immiscible fluids or of a fluid and a solid increase the surface areas of insoluble compounds leading to increased mobility. They are produced by many bacterial strains that can degrade or transform the components of petroleum products. They are non-toxic, non-hazardous biodegradable and environmentally friendly compounds [1]. Different variety of microorganisms are able to produce the surface active agents, including bacteria, *actinobacter sp.*, *Pseudomonas sp.*, *Bacillus Sp.* Yeast, *Rhodotorula sp.*, *candida sp.* and some algae. Crude oil can be accidentally, deliberately released in to the environment leading to serious pollution problems. Even small releases of petroleum hydrocarbons in to aquifers can lead to concentration of dissolved hydrocarbons in excess of regulatory limits. These pollution problems often result in huge disturbance of both the biotic and abiotic components of the ecosystems [2]. There are many advantages of biosurfactants if compared to their chemically synthesized counterparts. Some of these are,

Biodegradability.

Generally low toxicity

Biocompatibility and digestibility

Which allows their application in cosmetics, pharmaceuticals and as functional food additives.

MATERIALS AND METHODS

A) Screening programme

Isolation of biosurfactant producing microorganisms from oil mill area MIDC parbhani. Soil Sample and waste oil sample were collected and inoculated in mineral salt medium containing 2% of olive oil. Simultaneously the method of serial dilutions of the sample and plate count in selective medium cetrimide agar [3] The selected colonies are used for biochemical test

1. Methyl Red
2. Voges Proskaur test
3. Indol Test
4. Citric acid test
5. Hydrogen sulphite test
6. Manitol salt agar test
7. Catalase test

After biochemical test the culture was checked by gram staining.

B) Media and cultivation condition

Nutrient broth was used for preparation of the inoculums. The cultures were grown in this broth for 16-18hrs. at room temperature. This was used as inoculums at the 2%(v/v) level for biosurfactant synthesis a mineral salt medium was used. [4].

Medium optimization

The medium optimization was conducted in a series of experiments changing one variable at a time, keeping the other factors fixed at a specific sets of conditions. Different carbon and nitrogen source are used for higher productivity of the biosurfactant. Carbon source – Glycerol (2% w/v), glucose (20g/l) Nitrogen source – NH₄Cl, NaNO₃, NH₄NO₃ were employed at a concentration of mg/l with the optimum carbon source. [3]

Received: Oct 02, 2011; Revised: Nov 03, 2011; Accepted: Nov 17, 2011.

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C) Biomass and pH measurements

The dry weight technique was used to quantify microbial growth as bacterial density through the cultures absorbance at 600nm. using vv – v/s – spectrophotometer Biomass obtained after filtration on 0.2 u milipore was dried over night at 105°C & weighted. The pH of the supernatant was measured with a digital pH meter. [5]

Surface tension measurement

The surface tension measurement (s) of cell free supernatant was determined by using stalagmometer.

Emulsification index (E 24)

E 24 of culture samples was determined by adding 2ml of a hydrocarbon (Olive oil/ glycerol) to the same amount of culture, mixing with a vortex for 2 min. and leaving to stand for 24 h. E 24 index is given as percentage of height of emulsified layer (mm) divided by total height of the liquid column (mm) [6]

Biosurfactant Vortex.

The culture broth was centrifuged (10000g, 15 min) to remove the cells and thereafter sterilized with milipore membrane filter. The biosurfactant was recovered from the cell free culture supernatant by cold acetone precipitation method [7].

RESULTS AND DISCUSSION

Microbial isolation and identification

The biochemical characters were confirmed by using Bergeys manual.

Sr. No.	Test	Result
1	Methyl Red	Positive
2	Voges-Proskaur	Positive
3	Indol Test	Positive
4	Hydrogen Sulphite Test	Positive
5	Mannitol salt agar Test	Positive
6	Catalase Test	Positive

From above table it was confirm that isolated strain was *Pseudomonas aeruginosa*.

The present investigation was depends on production and characterization of biosurfactant producing *Pseudomonas spp.* collected from oil mill area MIDC, Parbhani.

Optimization of cultivation medium

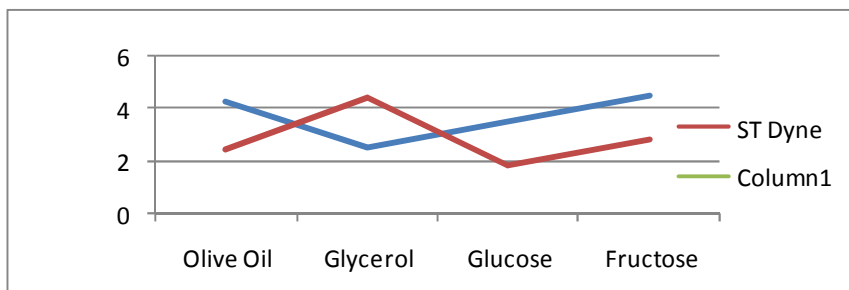
The biosurfactant production was carried out by using different substrate such as carbon and nitrogen sources the isolated strain has efficiently grow on all the carbon and nitrogen source but among these the olive oil and peptone are efficient for maximum production of biosurfactant.

Effect of carbon source

The production of biosurfactant by the *Pseudomonas aeruginosa* strain using substrate such as olive oil, glycerol, glucose and fructose is displayed in table and graph.

Table1. Effect of carbon source on biomass, E24 index, surface tension and final pH during biosurfactant production by *Pseudomonas aeruginosa sp1*

Sr. No.	Carbon Source	Biomass (g/l)	E24Index (%)	ST (dyne/cm ²)	pH
1	Olive Oil 2% v/v	0.21	70	37	6.21
2	Glycerol 2% v/v	0.12	60	38	6.12
3	Glucose 15 g/l	0.04	43	41	3.10
4	Fructose 15 g/l	0.03	33	42	4.06



Graph 1. Effect of carbon source on biosurfactant activity.

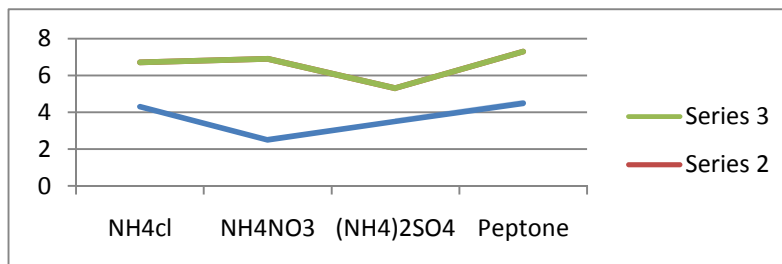
The strain was able to use olive oil as carbon source to produce biosurfactant seems to be interesting and low cost alternative [8]. Similar results were found with *Pseudomonas aeruginosa* 44Tv [9] a probable reason for this tendency that *Pseudomonas spp.* are lipase positive which facilitate assimilation of fatty acids contained in olive oil fraction.

Effect of Nitrogen source

Peptone and ammonium nitrate were the two best sources of nitrogen for growth and biosurfactant synthesis the biomass formed is higher in case of peptone as nitrogen source about 2.8g/l biomass is formed and they shows higher emulsifying activity (E 24 is 537) and surface tension was lower down by 31 dyne/cm³

Table 2. Effect of Nitrogen source on biomass, E24 index, surface tension and final pH during biosurfactant production by *Pseudomonas aeruginosa* sp1.

Sr. No.	Nitrogen Source	Biomass (g/l)	E24Index (%)	ST (dyne/cm ²)
1	NH ₄ cl 1 g/l	0.8	37	36
2	NH ₄ NO ₃ 1g/l	1.6	47	32
3	(NH ₄) ₂ SO ₄ 1g/l	1.2	44	37
4	Peptone 1g/l	2.8	53	29



Graph 2. Effect of nitrogen source on biosurfactant activity.

Similar results were observed in [10] Evaluation of different carbon and nitrogen sources in production of biosurfactants by *Pseudomonas fluorescens*.

CONCLUSION

The strain isolated from oil mill area was identified as *P.aeruginosa*, it has the capacity to use different and nitrogen sources, for production of biosurfactant. Fructose, glucose, olive oil, glycerol etc. are carbon sources among these olive oil is the best for maximum emulsifying activity and for surface tension reduction at 2% v/v. While peptone is the best nitrogen source, it produces high biomass concentration (5g/l) and biosurfactant production was also high.

ACKNOWLEDGMENT

The authors wish to thank Dr. Khandare sir, Principal of Shri Swami Vivekananda College, Mantha. For giving a moral support.

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