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FULL LENGTH ARTICLE

Studies on Biosurfactant Production from Soluble and Non-Soluble Source of Carbon by *Nocardia hydrocarbonoxidans*

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ABSTRACT

Surfactants of biological origin are gaining importance in recent years due to their biodegradability and nontoxic nature. In present work the synthesis of trehalose-lipid biosurfactant by Nocardia hydrocarbonoxidans NCIM-2386 was studied in batch experiments. Since the concentration of carbon source plays a major role in the synthesis of biosurfactants, the experiment was conducted with different kinds of carbon sources such as soluble and non soluble source of carbon. In this work an attempt has been made to understand the effect of soluble and non soluble source of carbon on biosurfactant production.

INTRODUCTION:

Surfactants are compounds that reduce the surface tension of a liquid, the interfacial tension between two liquids, or that between a liquid and a solid. Surfactants are characteristically organic compounds containing both hydrophobic groups and hydrophilic groups Therefore, a surfactant molecule contains both a water insoluble (and oil soluble component) and a water soluble component [1]. Biosurfactants are valuable amphiphilic molecules with effective surface-active and biological properties, which are produced on the living surface, mostly microbial cell surfaces, or excreted extracellularly, by a variety of yeast, bacteria and filamentous fungi from various substances including sugars, oils and wastes. Biosurfactants are mostly combinations of lipids, sugars and proteins. Microbial surfactants are known for their usefulness in enhanced oil recovery [2, 3]. Further they are almost as effective in application as many conventional synthetic surfactants. Bacterial surfactants are known to reduce surface tension of aqueous solutions to about 27 mN/m and interfacial tension against octane or decane, to 10-12 mN/m, thereby competing favourably with synthetic surfactants [4]. The usage of biosurfactant through bulk production however depends chiefly on the economical feasibility of such operations [5]. Insoluble source of carbon for production of biosurfactants with the help of bacteria might help in identification of bacteria which has the ability to degrade oil at the site of oil contaminated soil.

MATERIALS AND METHODS:

Microorganism

Nocardia hydrocarbonoxydans (NCIM-2386) is an actinomycetes, chosen for the present study by virtue of its effectiveness to degrade hydrocarbons. It is procured from National Collection of Industrial Microorganisms (NCIM), a division of National Chemical Laboratories, Pune. The strains are periodically sub cultured once in 15 days on agar slants and are stored at 40^{o}C.Nocardia is a genus of Gram-positive, catalase-positive, rod shaped bacteria; some species are pathogenic (nocardiosis). Nocardia are found worldwide in soil that is rich with organic matter. Soluble source of carbon medium: Inoculum preparation: About 5 % inoculum from cultures grown on medium as mentioned in table 1.1. This culture at about 48 hours was found to be in the exponential growth phase and hence was used as inoculum to batch experiments with different sucrose concentrations.

Table 1.1 Medium components and their concentrations:

Medium components	Concentration
Sucrose	10 to 60 g/l
$NaNO_3$	1 g/l
$MgSO_4 . 7H_2O$	0.1 g/l
Yeast extract	0.2 g/l
K_2HPO_4	0.1 g/l
$CaCl_2$	0.1 g/l

Non soluble source of carbon medium: Inoculum preparation: About 5% inoculum from cultures grown on medium mention below-

Table 1.2 Medium components and their concentration

Medium components	Concentration
$NaNO_3$	15g/l
KCl	1.1g/l
$FeSO_4.7H_2O$	0.00028g/l
KH_2PO_4	4.4g/l
$MgSO_4.7H_2O$	0.5g/l
Yeast Extract	0.5g/l
Crude Oil	2 g/l

Sampling:

10ml of the culture solution was taken as sample after every 24 hrs. Samples were centrifuged at 10000 rpm for 15 min. The supernatant was analyzed for trehalose lipid and sucrose. The residue was analyzed for cell biomass after washing and drying to constant weight.

Determination of biomass:

Dry Centrifuge tubes were taken and their empty weight was determined.12 ml of culture broth was centrifuged at 10000 rpm for 10 min. The supernatant was removed completely and collected in separate test tube. The Centrifuge tube containing wet biomass (cell pellets) is kept for drying in a hot air oven at an interval of one hour. After every hour the Centrifuge tube is weighed and the value is reduced by its empty weight. The drying is continued till we get a constant value of dry biomass.

Estimation of Biosurfactant:

The trehalose-lipid was estimated by Phenol-Sulphuric acid method [6]. This method is sensitive to micro quantities of sugar present in the sample. Biosurfactant concentrations were measured as trehalose-lipid. To measure the concentration of trehalose-lipid in the fermentation broth, the supernatant obtained after centrifugation was adjusted to pH 2. It was subjected to liquid-liquid extraction using chloroform and methanol in the ratio of 2:1. The extract was kept at room temperature till the solvent was evaporated completely.

The residue was dissolved in 0.1N NaHCO3 and the trehalose lipid concentration was measured as below:

1 ml of suitably diluted sample prepared as mentioned above was treated with 1 ml of 5% phenol and shaken well.5 ml of concentrated sulphuric acid was added rapidly. The stream of acid being directed against the liquid surface rather than against the sides of test tube in order to obtain good mixing and the tubes were kept at the room temperature was 10 min. Then the tubes were kept in the water at room temperature for color to develop, after which absorbance was read at 480nm in a spectrophotometer. A graph of absorbance Vs standard concentration of trehalose was plotted in the concentration range of 0 to 100 mg/ml. Trehalose-lipid were estimated by Phenol Sulphuric acid method and expressed in terms of trehalose (Yaraguppi *et al* 2013)

RESULTS AND DISCUSSION

Estimation of biomass is performed using centrifuge in order to identify the effect of carbon source with that of biomass. Following table shows the biomass produced with soluble source of carbon after 72 hours.

Table 1.3: Amount of Biomass with soluble source of carbon

Initial sucrose concentration (g/l)	Y x/s (g dry wt. of cells/g of substrate)
10	0.31
20	0.04
30	0.20
40	0.07
50	0.09
60	0.07

Amount of biomass produced with non soluble source of carbon is as shown in table below after 72 hours

Table 1.4: Amount of biomass with non-soulble source of carbon

Initial crude oil in %	Biomass in gms
0.5	0.07
1.0	0.10
1.5	0.08

As in case of several bioproducts the synthesis of biosurfactant is known to be influenced by the concentration of carbon source. The types of sugar influence the hydrophilic moiety of glycolipid. Alkanes are also known to decide the type of fatty acid moieties present in the gycolipid [5]. The effect of sucrose on product formation was studied for 10-50g/l and the amount of trehalose lipd formed was more in 50g/l after 72 hrs of incubation and it was found to be 29 mg/l

The effect of crude oil has shown the production of 14mg/l of trehalose lipid with 1% of crude oil as carbon source after 72 hours.

Table 1.3: Trehalose lipid production with soluble source of carbon

Sl.No	Sucrose in g/l	Trehalose lipid in mg/l after 72 hrs
1	10	15
2	20	19
3	30	24
4	40	27
5	50	29
6	60	28

Table 1.3: Trehalose lipid production with non-soluble source of carbon

Sl.No	Crude oil in %	Trehalose lipid in mg/l after 72 hrs
1	0.5	8.74
2	1	14.08
3	1.5	12.74

CONCLUSION

From the results we can observe that the biomass production was high 10g/l of sucrose and the lowest is with 20% of sucrose. The results were inaccurate may be because of human error. But in case of non soluble source of carbon 1.0% of oil showed higher amount of biomass, this might be because oil is non soluble and it does not get easily available for microorganisms as carbon source and in the initial stage microbe synthesizes very little amount of biosurfacant and further this helps in oil mobilization which in turn help microbe to degrade it. In the present study we performed the biosurfactant production with two different source of carbon one is soluble and the other one is insoluble source of carbon. In soluble source we used sucrose as sole carbon source. In the soluble source of carbon study initial concentration of 50g/l of sucrose, was found to be optimum concentration, at which 29mg/l of trehalo-lipid was synthesized at 72 hrs. The minimum trehalo-lipid production was observed to be at initial sucrose concentration of 10g/l.

In non soluble source of carbon, crude oil was used for the study and in this study 1% of oil showed higher amount of biosurfactant by Nocardia hydrocarbonoxydans (14.08 mg/l) at 72 hrs.

From the above study we can confirm that soluble source of carbon has shown higher amount of biosurfactant production compared to that of soluble non source of carbon.

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