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Production of Biosurfactant (Rhamnolipid) and its efficacy to remove Oil and Ink stains

Meshram Swetal and Chandorkar Vaidehi*

Department of Microbiology, Shivaji Science College, Nagpur, INDIA
E.mail: vaidehic21@rediffmail.com

ABSTRACT

Biosurfactants are produced mostly by microorganisms on their cell surface. Rhamnolipid is a glycolipid type of biosurfactant. *Pseudomonas aeruginosa* have been known to produce biosurfactant, rhamnolipid by fermentation process. The present study was carried out under the utilization of milk whey as a source of carbon for the production of rhamnolipid. Screening of rhamnolipid was carried by oil spreading technique, drop collapse method and characterized by thin layer chromatography. The production was carried out in two different fermentation medium, one medium consistedof milk whey as a source of carbon and other consisted of steamed milk whey from which casein was discarded, where rhamnolipid concentration obtained was 2.4mg/ml and 3.5mg/ml respectively. The produced rhamnolipid was examined as a cleaning agent in competition with synthetic detergent which removed approximately upto 60-80% of the oil and 40-50% of ink stain.

KEYWORDS:*Biosurfactant, Whey, Pseudomonas aeruginosa, Rhamnolipid, Detergent*

***Corresponding author**

Vaidehi Chandorkar

Department of Microbiology

Shivaji Science College,Congress Nagar, Nagpur, INDIA

E.mail: vaidehic21@rediffmail.com Ph: 9822828142

INTRODUCTION

Surfactant or surface-active agents are amphiphilic compounds which lower the surface tension and orient the amphiphilic compound in an immiscible solvent, in such a way that the compound becomes miscible i.e. polar (hydrophilic head) group lies in water and non-polar (hydrophobic tail) group is placed out of it. They are widely used in industrial processes like bioremediation of oil polluted soil and water, enhanced oil recovery, used in the detergent industry, formation of stable oil in water emulsions for the food and cosmetics industries the total surfactant production was more than 2.5 million tons in 2002 for many uses like polymers, lubricants and solvents[1]expected to increase to over 24 million tons annually by 2020 [2].

Synthetic surfactant causes environmental pollution that represents great risk to natural ecosystem [3]. Almost all the surfactants are chemically synthesized which have adverse effect on the environment and also, they are partly toxic and readily biodegradable. In the past few decades, surface active molecules having microbial origin has gained interest[4].

Biosurfactants can be replaced over chemically synthesized surfactants because of their low toxicity, excellent foaming properties, biodegradability, environmentally friendly nature and also due to the fact that they can be produced from industrial wastes and by products [4,5,6,7,8,9,10].Due to these properties biosurfactants have gained potential use in agriculture, bioremediation, petrochemical, cosmetics and detergents and food industries.

Different types of biosurfactants are synthesized by variety of microorganisms during their growth on water-immiscible substrates. Bacteria produce majority of biosurfactants. The major classes of biosurfactant includes glycolipids, lipopeptides and lipoproteins, fatty acids, phospholipids, neutral lipids and polymeric microbial surfactants [11,12].

Pseudomonas aeruginosa is a gram negative, rod shaped bacterium. It is found in soil, water, skin flora, most manmade environments throughout the world. P. aeruginosa has been studied for use in bioremediation and in processing polyethylene in municipal solid waste[13].*P.aeruginosa*a strain capable of rhamnolipid biosurfactant production from whey and studied the specific growth rates of *P.aeruginosa*strain [14]. Rhamnolipid is one type of glycolipid.It is the biosurfactant produced by *pseudomonas* species with surface-active properties when grown on different carbon substrates [15].

Presently the production of biosurfactants is highly expensive due to the use of synthetic culture media. Therefore, greater emphasis is being laid on procurement of various cheap agro-industrial substrates including vegetable oils, distillery and dairy wastes, soya molasses, animal fat, waste and starchy waste as raw materials. These wastes can be used as substrates for large-scale production of biosurfactants with advanced technology which is the matter of future research[16].

The industrial effluents have ability to be used as substrates for biosurfactant production.water-miscible waste like molasses, milk whey and distillery waste have been suggested for rhamnolipid production. Dairy wastewater, have been used as a cheap raw material for growth of microorganism and rhamnolipid production [14].Study have showed that, compared to synthetic media whey waste may be better substrate for rhamnolipid production at the commercial scale[17,18].

The objective of this study was the production of rhamnolipid from the waste milk whey and to examine the produced rhamnolipid as a cleaning agent i.e. for removing the oil and ink stain in competition with the synthetic detergent or surfactant.

MATERIALS AND METHOD

1. Preparation of culture medium (seed culture):

The organism was inoculated into nutrient broth and was incubated at 37˚c for 24 hours.

2. Preparation of Fermentation medium:

The fermentation medium was prepared in two different flask:

Flask 1: Mineral salt (MS) medium consisting of (g/L): NH4NO3, 4; KH2PO4, 4.08; Na2HPO4, 5.68;CaCl2, 7.77 × 10^{-4}; MgSO4.7H2O, 0.2; sodium EDTA, 1.49 × 10^{-3}; FeSO4·7H2O, 5.56 × 10^{-4} was prepared in 250 ml flask containing 50 ml of MS medium and 100 ml of milk whey. Medium was autoclaved and inoculated with seed culture, was incubated at 20˚C for 48hrs at 120 rpm

Flask 2: 100ml of milk whey was neutralized by 5N NaOH (ph-7). Milk whey was steamed for 10 min, Casein gets settled at the bottom. Supernatant was separated, MS medium was added to supernatant to make up the volume upto 150ml before sterilization. Medium was autoclaved at 15 lbs pressure for 10 min. Medium was inoculated with 5ml of seed culture and incubated at 20˚c for 48 hours and 120 rpm in an incubator shaker.

(ph was adjusted to 2 with 1N HCl and the sample from each flask was centrifuged for 20 min and 9000 rpm. Further supernatant was used).

Screening of Rhamnolipid:

1. ***Oil spreading technique*****:** 30 ml distilled water was taken in petriplate. 1ml of oil was added to surface of water. 500 µl of supernatant was added at the centre. The organism producing biosurfactant can displace the oil.

2. *Drop collapse method*: 2µl of oil was placed on cavity slide. 5µl of supernatant was added to oil surface. After 1 min shape of drop was observed. Shape of drop becomes flat after adding supernatant indicating ability of biosurfactant production.

3. ***Thin layer chromatography;***Slide with silica gel was prepared. Sample was spotted and was placed in the solvent system $CHCl_3$: CH_3OH: CH_3COOH (15: 3.46: 0.46). It was allowed to dry then it was stained with orcinol sulfuric acid reagent. RF value was calculated and compared with the standard.

Rhamnolipid Estimation:

For estimation of Rhamnolipid cell free culture 1ml, sulfuric acid 4.5 ml (mixture heated at 100^0 C for 10 min then 3% Thioglycollic acid 0.1 ml was added and after 3 hours absorbance was recorded at 450 nm. Standard curve of Rhamnose was prepared. O.D was plotted and concentration of Rhamnolipid was estimated.

Biosurfactant as cleaning agent:

Preparation: The 10 ml cell free culture was mixed with Sodium sulphate and Sodium dihydrogen phosphate, 2 spatula each and diluted with water and applied to washing pieces of cloth which were stained with edible oil and ink which was washed after 24 hours and 5 days to check its efficiency the cloth was also washed with available detergent by diluting it with water.

RESULT AND DISCUSSION

The rhamnolipid producing organism showed the growth in the medium containing milk whey.

Screening of Rhamnolipid:

1. *Oil spreading technique*: The cell free culture obtained by centrifugation was added to the oil containing plate which showed zone of displacement (Figure 1).The diameter of the zone correlates with the surfactant activity.

1: Demonstration of Oil Spreading by Biosurfactant

2. *Drop Collapse method*: The cell free culture obtained after centrifugation was added over the surface of oil placed on slide. The shape of the drop becomes flat which indicates the presence of biosurfactant in the sample, Drop may spread or collapse because force between the liquid and the hydrophobic surface is reduced.

3. ***Thin layer chromatography*:** Spot appeared after staining slide with orcinol sulfuric acid reagent. Spot indicated the presence of biosurfactant. The result obtained in both sample contained rhamnolipid with RF value of 0.73 comparable with the standard values of rhamnolipid.

Rhamnolipid Estimation:

Concentration of rhamnolipidfor sample 1was found to be 2.4 mg/ml and for sample 2 was 3.5 mg/ml.

Use of rhamnolipid as cleaning agent:

Approximately 70- 80% of oil stain was removed with both the samples and 50% was removed by Detergent (Figure 2).Approximately 60-70% of ink stain was removed with both samples but Best results were obtained for the oil stains.

Oil stain With Detergent With produced Biosurfactant

2: Effect of washing with Biosurfactant and Detergent for Oil Stains

CONCLUSION

The above study concluded that *Pseudomonas aeruginosa* produced the biosurfactant rhamnolipid by utilizing the raw milk whey as a carbon source, demonstrating best utilisation of the waste.Produced Rhamnolipid efficiently cleaned the oil stains than the ink stains at par with the synthetic detergent. biosurfactant be used over synthetic surfactant in upcoming years as it is cheaper, have low toxicity which would be totally harmlessto the environment.

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