Global Journal of Biotechnology & Biochemistry 5 (4): 216-219, 2010
ISSN 2078-466X

Production of Biosurfactant Using Cashew Nut Shell Liquid as the Carbon Source from *Pseudomonas aeruginosa* MTCC 424

[1]*B.V. Praveesh,* [2] *A.R. Soniyamby,* [3]*C. Mariappan,* [4]*P. Kavithakumari and* [2]*M. Palaniswamy*

[1]Karpagam Arts and Science College, Coimbatore, Tamil Nadu, India
[2]Karpagam University, Coimbatore, Tamil Nadu, India
[3]Sivanthi Aditanar College, Nagercoil, Tamil Nadu, India
[4]Cashew Export Promotion Council Laboratory and Technical Division, Kollam, Kerala, India

Abstract: Biosurfactants or microbial surfactants are surface-active biomolecules that are produced by a variety of microorganisms. The features that make them commercially promising alternatives to chemically synthesized surfactants are their lower toxicity, higher biodegradability and, hence, greater environmental compatibility, better foaming properties (useful in mineral processing) and stable activity at extremes of pH, salinity and temperature. Nevertheless, biosurfactants can be produced with high yield by some microorganisms, especially *Pseudomonas* sp. These microorganisms can use the various renewal resources. This work deals with the production of a biosurfactant by *Pseudomonas aeruginosa* MTCC 424. Biosurfactant synthesis was followed by measuring surface tension and emulsifying index E24. In our study, the biosurfactant productivity was enhanced in cultures grown in a medium supplemented with 2.0% cashew nut shell liquid (CNSL) adjusted to pH 7.0 and incubated at 35°C. The productivity at the optimum condition (5.7 g/L) was approximately 4.3 folds higher compared to the initial productivity (1.3 g/L). This is the first report using CNSL as the carbon source for biosurfactant production.

Key words: Rhamnolipid · *Pseudomonas aeruginosa* · Cashew nut shell liquid · Biosurfactant

INTRODUCTION

Due to the features of high surface activity and biodegradability, biosurfactants produced by a variety of microorganisms have been studied extensively in the recent years. Biosurfactants are amphiphilic compounds produced on living surfaces, mostly microbial cell surfaces or excreted extracellularly and contain hydrophobic and hydrophilic moieties that reduce surface tension and interfacial tension between individual molecules at the surface and interface respectively [1]. Biosurfactants are produced by different microorganisms such as bacteria, fungi and yeast. Biosurfactants have many advantages over their chemical counterparts because they are biodegradable [2], have low toxicity [3], are effective at extreme temperatures or pH values [4] and show better environmental compatibility [5]. Biosurfactants have gained importance in the fields of enhanced oil recovery, environment bioremediation, food processing and pharmaceuticals [6].

Although biosurfactants have many interesting properties, their industrial importance is dependent upon ease of production. Low yields of biosurfactant are a major factor jeopardizing its popularity. Recently, efforts have been made to increase yields by focusing on nutritional and environmental factors [7].

Various types of biosurfactants are synthesized by a number of microbes particularly during their growth on water-immiscible substrates. A majority of biosurfactants are produced by bacteria. Among the bacteria, the *Pseudomonas* species is well known for its capability to produce rhamnolipid biosurfactants with potential surface-active properties when grow on different carbon substrates. Rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa*, in particular offer special advantages due to their potent emulsifying activity and low critical micelle concentration [8]. The genus *Pseudomonas* is capable of using different substrates, such as glycerol, mannitol, fructose, glucose, n-paraffins and vegetable oils, to produce rhamnolipid-type

Corresponding Author: B.V Praveesh, Department of Microbiology, Karpagam Arts and Science College, Pollachi Main Road, Eachanari Post, Coimbatore-641 021, Tamil Nadu, India.
Mob: +91 9600637071, E-mail: praveesh_bv@yahoo.co.in.

biosurfactants [9, 10]. Several studies have been carried out to define the best ratio between carbon, nitrogen, phosphorus and iron needed to obtain high production yields. Optimizing factors that affect growth in biosurfactant producing organisms with potential for commercial exploitation is of paramount importance [11].

In this study Cashew Nut Shell Liquid (CNSL) is used as energy source for the production of biosurfactant by *Pseudomonas aeruginosa*. Cashew Nut Shell Liquid (CNSL) is a versatile by-product of the cashew industry. CNSL has innumerable applications in polymer based industries such as friction linings, paints and varnishes, laminating resins, rubber compounding resins, cashew cements, polyurethane based polymers, surfactants, epoxy resins, foundry chemicals and intermediates for chemical industry. This work also reports the optimal condition on the production of biosurfactant by *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

Microorganisms: *Pseudomonas aeruginosa MTCC 424* strain was used in this work.

Growth Conditions: The composition of the basal mineral salt medium (MS) used in this study was the following (gl^{-1}): K_2HPO_4. $3H_2O$, 4.8; KH_2PO4, 1.5; $(NH_4)_2SO_4$, 1.0; Na_3 ($C_6H_5O_7$). $2H_2O$, 0.5; $MgSO_4.7H_2O$, 0.2; yeast extract, 0.1. For biosurfactant production the medium (BMS) was supplemented with trace elements solutions with the following composition (mg l^{-1}): $CaCl_2$. $2H_2O$, 2.0: $MnCl_2$. $4H_2O$, 0.4; $NiCl_2$. 6H2O, 0.4; ZnSO4. 7H2O, 0.4; FeCl3. 6H2O, 0.2; Na2MoO4. 2H2O, 0.2; and 2% CNSL as sole carbon source, pH 7.2. CNSL was sterilized through 0.2 μmm membrane filters (Milipore Corp., Bedford, Mass.).

Analytical Techniques

Biosurfactant Activity: The oil-displacement method was used to detect the activity of biosurfactant. Forty milliliters of distilled water was added to the Petri dish followed by the addition of 10μl of crude oil to the surface of the water, 10 μl of sample was added onto the centre of the oil film. The diameters of the clear zone on the surface were measured and compared with the control using uninoculated medium [12].

Emulsification Activity: The emulsification capacity was determined by adding 2ml of oil to the same amount of cell-free culture broth, mixed for 2min on a vortex mixer and allowed to stand for 24 h. E24 index is defined as percentage of the height of emulsified layer divided by the height of the liquid column [13].

Quantification of Rhamnose: The quantification of rhamnolipid expressed in rhamnose (g/l) was measured in the cell-free culture medium, using the phenol sulfuric acid method [14, 15].

Optimization of Growth Condition: Biosurfactant production was optimized using different parameters like pH (5-10), temperature (20-40°C) and incubation period (1-10 days).

RESULT AND DISCUSSION

Biosurfactants can be produced with high yield by some microorganisms, especially *Pseudomonas* sp [16]. *Pseudomonas aeruginosa* used in this study, produced rhamnolipid biosurfactants when grown with CNSL as the carbon and energy source. Few reports have been published on the use of waste as substrates for rhamnolipid production. In this study we have investigated the production of rhamnolipid at a concentration of 5.7g/l using CNSL as the carbon source. This is the first report using CNSL as the carbon source for biosurfactant production.

Oil Displacement Test: The area of clearly formed oil displacement area (circle) was measured as the activity of surfactant. It was a circle of 5mm diameter.

Emulsification Stability (E24) Test: The emulsification index of the produced biosurfactant, determined at 24h (E24), was found to be the highest when kerosene was considered (65%). As shown in Figure (1), all the hydrocarbons tested served as substrates for emulsification by the biosurfactant. Vegetable oil was the lowest substrates for emulsification (45%).

Effect of Incubation Period on Biosurfactant Production: The production of rhamnolipid was studied during the tenth day of incubation period. The strain was able to use CNSL and producing 4.3g/L of rhamnose at the end of seven days of fermentation (Figure 2). The same incubation period was observed in the study of *Rashedi et al.* [17], they are also worked with *Pseudomonas aeruginosa* which produced 4.2g/l when paraffin and glycerol were used as carbon source.

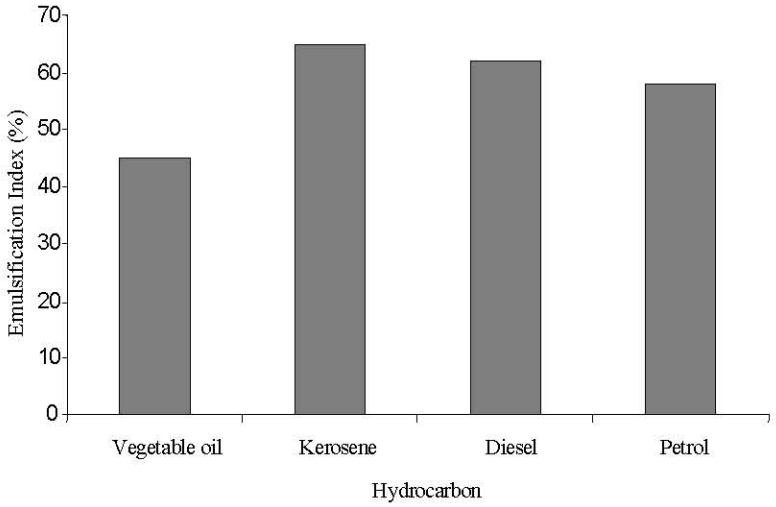

Fig. 1: Emulsification activity (E24) of biosurfactant against different hydrocarbons

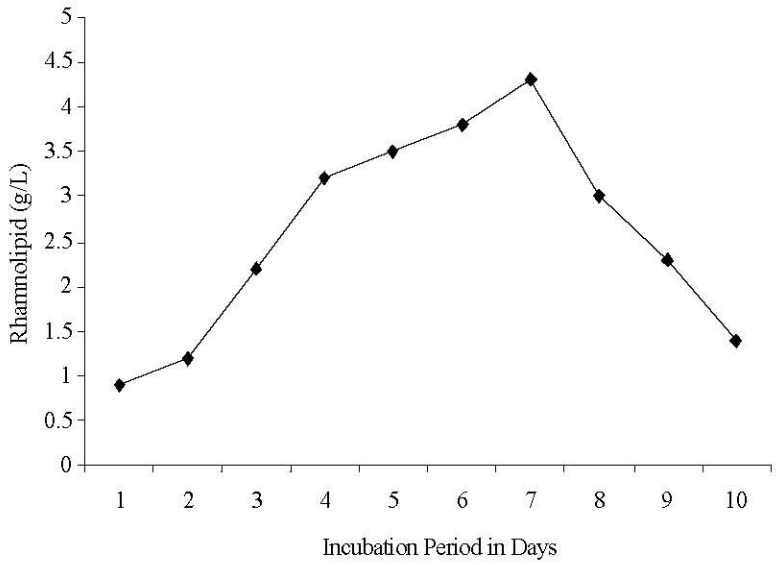

Fig. 2: Effect of Incubation Period in Rhamnolipid Production

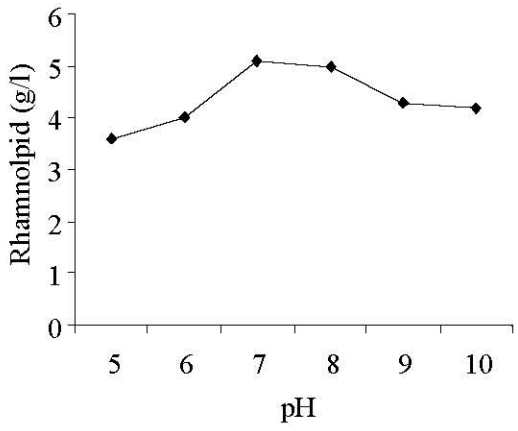

Fig. 3: Effect pH on rhamnolipid production

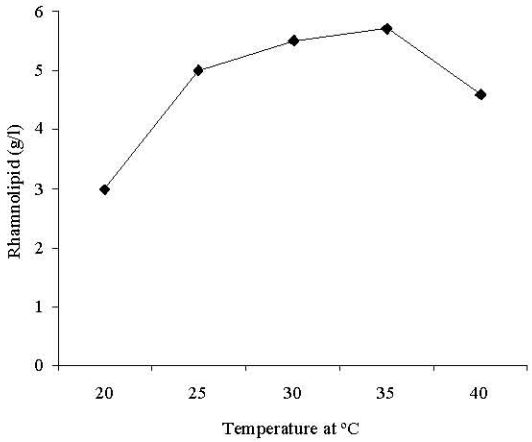

Fig. 4: Effect of Temperature on Rhamnolipid production

Effect of pH on Biosurfactant Production: The experiments were conducted in the fermentation medium with different pH (5-10). The effect of pH in production of rhamnolipid was shown in (Figure 3). The maximum rhamnolipid concentration (5.1g/l) was found in medium with pH 7. These values agree with those previously reported for rhamnolipid by the authors *Priya* and *Usharani* [18]. They showed maximum production of rhamnolipid at pH 7 using *Pseudomonas aeruginosa.*

Effect of Temperature on Biosurfactant Production: Temperature ranges from 20-40°C were studied for biosurfactant production. At 35°C *Pseudomonas aeruginosa* showed highest rhamnolipid concentration of 5.7g/l (Figure 4). This result is comparable with the results obtained by the authors *Priya* and *Usharani* [18]. They showed maximum biosurfactant production at 37°C. Temperature and pH were two environmental factors that majorly affect biological activities of prokaryotes [19].

CONCLUSION

Based on the experimental result it can be concluded that *Pseudomonas aeruginosa* can utilize CNSL as carbon source for rhamnolipid production. Therefore, it is feasible to use cheaper substrates for rhamnolipid production. And also the production was found to be affected by the incubation period, pH and temperature.

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