# Enhanced Production of Biosurfactant from Isolated Pseudomonas Sp Growing On Used Edible Oil

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**Abstract:** The production of surface active compounds or biosurfactants by microorganisms has been a subject of increasing interest in recent years especially due to the potential applications in enhanced oil recovery. A number of studies have indicated that the type of medium and growth conditions can influence the type and yield of biosurfactants. The present work demonstrated that the isolated bacteria, *Pseudomonas sp* from used edible oil was able to utilize the used edible oil as carbon and energy source to produce rhamnolipid at a concentration of 7.6 g/L. The temperature, incubation period, and nitrogen source optima of biosurfactant production was found at 36 °C, 72 hr and sodium nitrate respectively.
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**Key words:** Rhamnolipid. *Pseudomonas* sp. Used edible oil. Biosurfactant

## 1. Introduction

Surfactants are amphiphilic molecules that tend to lower the interfacial tension. Biosurfactants are microbially produced surface-active compounds. They are amphiphilic molecules with both hydrophilic and hydrophobic regions causing them to aggregate at interfaces between fluids with different polarities such as water and hydrocarbons (Banat, 1995; Fiechter, 1992; Georgiou *et al*., 1990 and Karanth *et al*., 1999). Biosurfactants are widely used in the petroleum, pharmaceutical, cosmetic and food industries. Most of these compounds are chemically synthesized and it is only in the past few decades that surface-active molecules of biological origin have been described. At present biosurfactants are readily biodegradable and can be produced from renewable and cheaper substrates, they might be able to replace their chemically synthesized counter parts (Arino *et al*., 1996).

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 them, the *Pseudomonas* species is well known for its capability to produce rhamnolipid biosurfactants with potential surface-active properties when grown on different carbon substrates. Rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa*, in particular offer special advantages because of their potent emulsifying activity and low critical micelle concentration (Cooper *et al*., 1981).

There are several renewable substrates from various sources, especially from industrial waste have been intensively studied for microorganism's cultivation and production at an experimental scale. The genus *Pseudomonas* is capable of using different substrate such as glycerol, vegetable oil, mannitol, fructose, glucose, n-paraffin, soap stock, molasses, to produce rhamnolipid type biosurfactants.

Used edible oils and fats are considered as a problematic waste, contributing to the environmental pollution. It is well known that microorganisms are able to grow on vegetable oils or fats and produce new products with potential industrial application such as lipase (Haba *et al*., 2000) and biodiesel (Alcantara *et al*., 2000 and Haba *et al*., 2000) and used olive or sunflower cooking oil as carbon source for biosurfactant production.

The purpose of this work was to study the production of rhamnolipid-type biosurfactants by a strain isolated from used edible oil and produce biosurfactant using used edible oil as energy source, as well as to evaluate different production parameters on biosurfactant production.

## 2. Materials and Methods

***Bacterial isolates:***

*Pseudomonas* sp was isolated from used edible oil. The culture was identified as per IS 13428:1998 Annex D.

***Screening for Biosurfactant Activity:***

Biosurfactant activity of isolated *Pseudomonas* sp was detected by using oil-displacement method (Youssef *et al*., 2004 and Praveesh *et al*., 2010]. Forty milliliters of distilled

water was added to a petri dish followed by the addition of 10 µl of crude oil to the surface of the water, ten 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 control using uninoculated medium.

***Biosurfactant Production using different carbon source*:**

Production of the emulsifier was carried out in 250 mL Erlenmeyer flasks containing 50 mL of the medium composed of (g/l): KH2PO4: 0.5, K2HPO4: 1, KCl: 0.1, MgSO4.H2O: 0.5, FeSO4.7H2O: 0.008, CaCl2: 0.05, Urea: 6 and 0.05 mL of trace elements solution (Br: 0.026, Cu: 0.05, Mn: 0.05 and Zn: 0.07) (Sifour *et al*., 2004), carbon source (used edible oil, rice water, diesel. petrol and whey) was added at 4% (wt or vol/vol), pH was adjusted to 7.0. The medium was inoculated with 5% of the 18 hours bacterial culture grown on nutrient broth. Incubation was carried out at 37 ºC in an incubator shaker at 150 rpm for 48 hours.

***Quantification of Biosurfactant:***

A modified orcinol method was used to assess the amount of rhamnolipids in the sample (Chandrasekaran *et al*., 1980). A volume of 200 mL of the acidified culture supernatant was extracted three times with 1 mL of diethyl ether, and then the fractions were pooled, dried and resuspended in 1 mL of 0.05 M sodium bicarbonate. A 200 mL sample was treated with 1.8 mL of a solution of 100 mg of orcinol in 53% $H_2SO_4$ and boiled for 20 min. After cooling at room temperature for 15 min, the A421 was measured. Rhamnolipid concentrations were calculated from standard curves prepared with L-rhamnose and expressed as rhamnose equivalents (in milligrams per milliliter).

***Optimization of culture conditions:***

The factors such as temperature, incubation period and nitrogen sources affecting production of biosurfactant were optimized by varying parameters one at a time. The experiments were conducted in 200 mL Erlenmeyer flask containing production medium. After sterilization by autoclaving, the flasks were cooled and inoculated with culture and maintained under various operational conditions separately such as temperature (20, 25, 30, 35 and 40 ºC) , incubation period (24, 48, 72, 96 and 120 h), and nitrogen source (ammonium sulfate, sodium nitrate and urea each at 0.5%). After incubation the culture filtrate was assayed for biosurfactant productivity.

***Determination of the emulsifying activity:***

To estimate the emulsifying activity, two equal volumes of supernatant and different hydrocarbon such as kerosene, sunflower oil and petrol (2mL each) were added in a test tube and mixed at high speed for 2 min. The emulsion stability was determined after 24 hr. The emulsification index, E24 (%) was the ratio of the height of the emulsion layer by the total height of the mixture (Iqbal *et al*., 1995).

**Result and Discussion**

***Isolation and Screening of Biosurfactant Producing Pseudomonas sp*:**

A total of 13 strains of *Pseudomonas* sp were isolated from used edible oil. All isolates were screened for extracellular biosurfactant by oil displacement method. Of these, one strains showed very strong positive reactions indicated by clearing of oil more than 6mm in diameter. The bacterium was identified as *Pseudomonas* sp (Table 1) and used for further study.

Table 1. Identification of *Pseudomonas* sp

| Test Performed | Results |
|---|---|
| Gram staining | Gram Positive |
| Morphology | Rod |
| Skim milk agar | Greenish yellow colony with clearing of medium |
| Oxidase test | Positive |
| Catalase test | Positive |
| Hugh-Liefson test | Non fermentative |
| Gelatin Liquefaction | Positive |
| Asparagine proline broth | Turbidity, Fluorescence under UV |

***Biosurfactant Production:***

Biosurfactants can be produced with high yield by some microorganisms, especially *Pseudomonas* sp. (Maneerat, 2005). The *Pseudomonas* sp used in this study, produced rhamnolipid biosurfactants of 2.24 g/L when grown with used edible oil. The production of rhamnolipid using used edible oil as carbon source was higher than the other sources such as rice water, diesel. petrol and whey (Fig.1). Few reports have been published on the use of vegetable oil as substrates for rhamnolipid production. From olive oil, a production of 2.7 g/L was produced by Haba *et al*, (2000).

***Effect of Temperature on Biosurfactant Production:***

Biosurfactant production was studied for different incubation temperature and the result is presented Fig.

2. In our study the maximum production of 2.78 g/L was found at 36 ºC. With a rise in temperature the biosurfactant production was decreased. This was in good agreement with the results obtained earlier for biosurfactant from *Pseudomonas aeruginosa* (Praveesh *et al*., 2010).

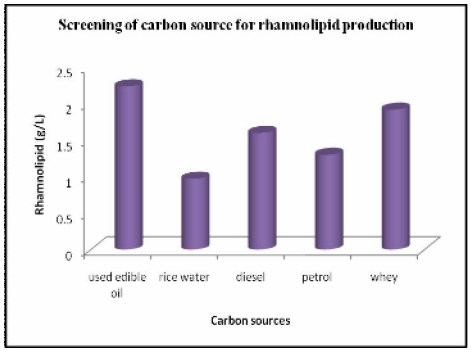


Figure 1

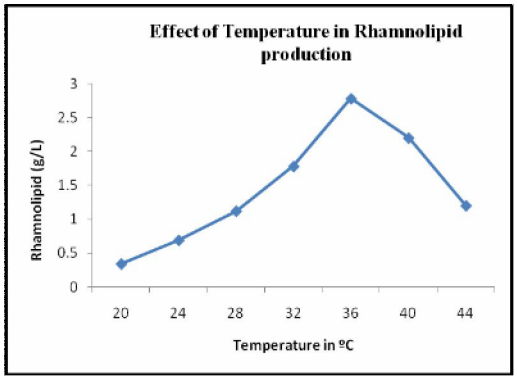


Figure 2

***Effect of Incubation period:***

The effect of incubation time on biosurfactant production was determined by incubating the culture medium at different time intervals (24 – 168 h) with an interval of 24 h. Rhamnolipid production was found to be maximum (5.86 g/L) at 72 h (Figure 3). The production gradually decreases from 72 to 168 h.

***Effect of Nitrogen source:***

The nitrogen source can be an important key to aim at increasing the production of rhamnolipids by *Pseudomonas* sp. Different nitrogen sources were tested to determine the best source for biosurfactant production. Figure 4 showed that sodium nitrate (7.6 g/L) is more effective than ammonium sulfate and urea. *Pseudomonas sp* is able to use nitrogen sources such as ammonia or nitrate. However, in order to obtain high concentrations of rhamnolipids it is necessary to have restrained conditions of this macro-nutrient. Our studies showed that nitrate is more effective in the production of rhamnolipids than ammonia and urea, which is in agreement with other studies reported in the literature (Cooper and Zajic, 1980; Edwards and Hayashi, 1965 and Guerra-Santos *et al*., 1983).

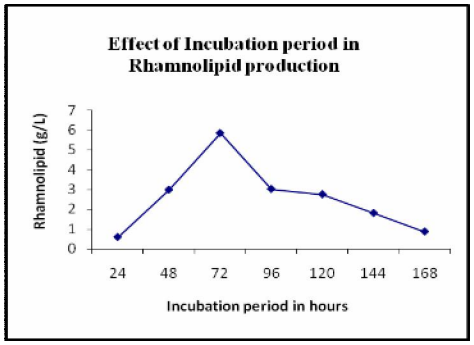


**Figure 3**

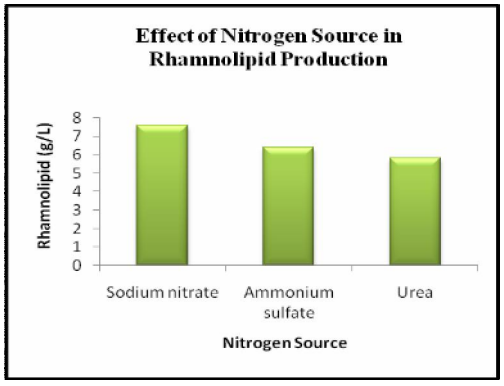


Figure 4

***Emulsifying activity:***

The highest value of emulsification index ($E_{24}$) was observed with kerosene (68%) during our study (Fig 5). Sunflower showed lower emulsification index when compared to kerosene, but diesel did not showed much difference.

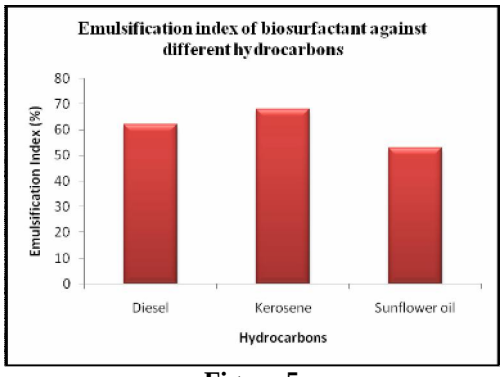


**Figure 5**

**Conclusion**

The strain isolated from used edible oil was identified as *Pseudomonas* sp. It has the capacity to use used edible oil as energy sources to produce rhamnolipids, seems to be an interesting and low cost alternative.

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