

## USE OF POTATO PEEL AS CHEAP CARBON SOURCE FOR THE BACTERIAL PRODUCTION OF BIOSURFACTANTS

Faiza A. Ansari<sup>1</sup>, Shahid Hussain<sup>1</sup>, Basheer Ahmed<sup>1</sup>, Jameela Akhter<sup>2</sup> and Erum Shoeb<sup>1\*</sup>

<sup>1</sup>Department of Genetics, University of Karachi, Karachi-75270, Pakistan

<sup>2</sup>Centre for Molecular Genetics, University of Karachi, Karachi-75270, Pakistan

\*Corresponding author e-mail: erumsh@uok.edu.pk

### ABSTRACT

Production of environmental friendly biopolymers from microbes such as bacteria by using low cost substrate as a nutrient source is the matter of great interest to the biological researchers. In the present study effect of potato peels as a cheap nutrient source was observed on biosurfactant production of bacterial isolates. It was also compared with the additional Sodium Nitrite and Urea as nitrogen sources and discussed the influence of various parameters on the growth of bacterial isolates and yield of biosurfactants. This study revealed that the simple potato peel enhances the growth rate of culture and positively affect the biosurfactant production. Addition of Urea and Sodium nitrite as Nitrogen source showed positive impact on growth of bacterial isolates but no effect was observed on biosurfactant production. Such studies suggest cost effective solution to the production of commercially important biosurfactant.

**KEYWORDS:** Cheap-carbon source, Biosurfactant, Bacteria, BATH assay

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### INTRODUCTION

Surfactants and emulsifiers are indispensable components of daily life. Many different types of surfactants are already being used in industry, but it is important to develop even more new compounds to broaden the spectrum of specific properties and applications (Cameotra and Makkar, 1998). Most of these compounds are synthesized chemically and are of petroleum origin. Most of them are toxic to the environment, not easily biodegradable, and their manufacturing processes and byproducts can be environmentally hazardous. Naturally occurring surface active compounds derived from microorganisms are called biosurfactants. Biosurfactants are amphiphilic biological compounds produced extracellularly or as part of the cell membrane by a variety of yeast, bacteria and filamentous fungi (Chen, 2007). These surface-active agents occur in nature as chemical entities such as glycolipids, phospholipids and lipopeptides. These molecules have attracted considerable scientific attention due to lower toxicity, higher biodegradability, activity at extremes of temperature, pH and salinity and possibility of their production through fermentation using cheap agro-based substrates (Desai and Banat, 1997). Different strategies including the use of inexpensive substrates have been suggested towards making their production economically viable (Mukherjee *et al.*, 2006). Carbon substrate is an important limiting factor affecting the production of microbial surfactants (Sen, 1997). Biosurfactants can be produced by microbial fermentation processes using cheaper agro-based substrates and waste materials (Mukherjee *et al.*, 2006). This aspect is very important because the production of microbial surfactants on a commercial scale has not been realized because of their low yields and high production costs. As biosurfactants are readily biodegradable and can be produced from renewable and cheaper substrates, they might be able to replace their chemically synthesized counter parts (Patel and Desai, 1997). Several carbon sources such as ethanol, glucose, vegetable oil, and hydrocarbon have been used to produce biosurfactant. A variety of these cheap raw materials supporting biosurfactant production include plant derived oils like sunflower oil, oil wastes like soapstock, cassava wastewater and oil refinery wastes (Nitschke and Pastore, 2006). The low cost agro-industrial wastes include lignocellulosic residues (barley bran husks, trimming vine shoots, corn cobs and *Eucalyptus globules* Hips), jute, plant polymer, oil extracts, distillery and whey wastes, potato process effluent and pea nut cake, dates syrup, sugar beet, sugar cane or sugar sorghum (Wang *et al.*, 2008; Chowdhury *et al.*, 2011). Starch and cellulose are the components of *Madhuca indica*, corn, tapioca, wheat bagasse, sugarcane bagasse, soyabean and potato peel powder. Evaluation of different carbon substrates may help to overcome the problem of high production cost.

In the present study we have used potato peel waste to evaluate its effect on biosurfactant producing capability of bacterial isolates and have also checked the effect of additional nitrogen sources on the emulsification and adherence activities of the isolates.

### MATERIALS AND METHOD

**Microorganisms and their culture:** Previously isolated and preserved bacterial isolates were utilized in the present study (Shoeb *et al.*, 2012). For enrichment Luria Bertani broth (Peptone 10g, Sodium chloride 5g and yeast extract 5g, in one liter of distilled water) was used (Bertani, *et al.*, 1951). Cultures were grown at 37°C and stored at 4°C for further use. Strains utilized were DGEF01-06.

**Conditions for biosurfactant production:** For biosurfactant synthesis a Tris-minimal medium (Mergeay *et al.*, 1985) was used for cultivation of bacterial strains. Cultivations were performed in 250 mL flasks containing 100 mL medium supplemented with potato peel (2% w/v) as substrate were added to Erlenmeyer flask. In combination of Potato peel sodium nitrite and urea (1% w/v) were used as nitrogen source. Erlenmeyer flasks were left in incubation of 150 (rpm) rotation in a temperature of 37°C for various time periods (24, 36, 48, 72, and 96 h). Uninoculated flasks and flasks without substrate(s) served as controls. Samples were collected at pre-defined intervals of time, growth was recorded and measured spectrophotometrically as values of OD<sub>600nm</sub>, and cells were separated by centrifugation at 10,000g for 2 min and the supernatant was promptly submitted to analysis of Bath Assay and emulsification index (E24).

**Preparation of substrate:** Potato peels were collected from University canteens and washed first with tap water followed by distilled water to remove the adhered surface dust particles. Then blanching was carried out by immersing the peels. Peels were then oven dried for 48 h. The dried material was sterilized at 121°C, 15 lbs pressure for 15 min and stored at 4°C before further use.

**Bacterial growth:** The growth curves of biosurfactant producing isolates were constructed for the determination of increase in cell number, for five consecutive days, in presence of potato peel only and with two additional nitrogen sources sodium nitrite and Urea. Cell growth was monitored with DU 730 spectrophotometer of Beckman Coulter which was used to measure the optical density at 600 nm (OD<sub>600</sub>).

**Emulsification test (E24):** The emulsification index (E24) was measured using the method described by (Ilori *et al.*, 2005) to check the stability of the biosurfactant extracted. Biosurfactant activity was measured by adding 2 ml of crude oil to 2 ml of cell-free extract and vortexing at high speed for 2 minutes. Measurements were taken 24 hours later. Emulsions formed by the isolates were compared to those formed by a 1% (w/v) solution of the synthetic surfactant, Sodium Dodecyl Sulphate (SDS) in deionized water, as proposed by (Das *et al.*, 1998). The emulsification activity was determined using the formula:

$$E24 = (\text{Height of emulsion layer} / \text{Height of liquid column}) \times 100$$

**BATH assay:** Bacterial adherence to hydrocarbon (BATH) was performed according to Rosenberg *et al.*, method (1980). Cell pallet was harvested from overnight culture, washed with 1ml buffer (K<sub>2</sub>HPO<sub>4</sub>+ KH<sub>2</sub>PO<sub>4</sub>), resuspended in 5ml buffer in test tube. Initial O.D at 600nm was taken. 1ml of hydrocarbon (Xylene) was added; shaken for two minutes and left for 1hr at undisturbed area. Final O.D<sub>600</sub> was taken and compared with initial O.D<sub>600</sub>, decrease O.D in %age was calculated by using formula:

$$(\text{Final O.D} / \text{Initial O.D}) \times 100$$

## RESULTS

All the isolates (DGEF01-06) were successfully grown with potato peel as carbon source without any nitrogen source and with both the nitrogen sources: sodium nitrite and urea.

**Bacterial growth curves:** Growth increased continuously in all six strains (Fig. 1). The maximum growth was recorded on 5<sup>th</sup> day in strain DGEF04 (0.483). Two nitrogen sources were tested: sodium nitrite (NaNO<sub>2</sub>) and Urea. Results showed that in presence of sodium nitrite growth was better than without any nitrogen source but not as good as Urea. Potato peel with urea was the best combination for growth curve of all the isolates. Maximum growth was observed in DGEF06 on 5<sup>th</sup> day in medium containing urea.

**Emulsification test (E24):** For emulsification activity (E24) best results were obtained when grown with potato peel without any nitrogen source. Highest Emulsification activity of 70% was obtained on 5<sup>th</sup> day of incubation (Fig. 2), and similar results were obtained with isolate DGEF01 and DGEF03 (47% and 50%, respectively). For DGEF06, lowest emulsification activity was obtained on 5<sup>th</sup> day (11%).

**Bath assay:** Results of BATH assay indicated maximum adherence in presence of potato peel only. With the addition of any nitrogen source adherence of cells found to be decreased. Results are shown in Fig. 2.

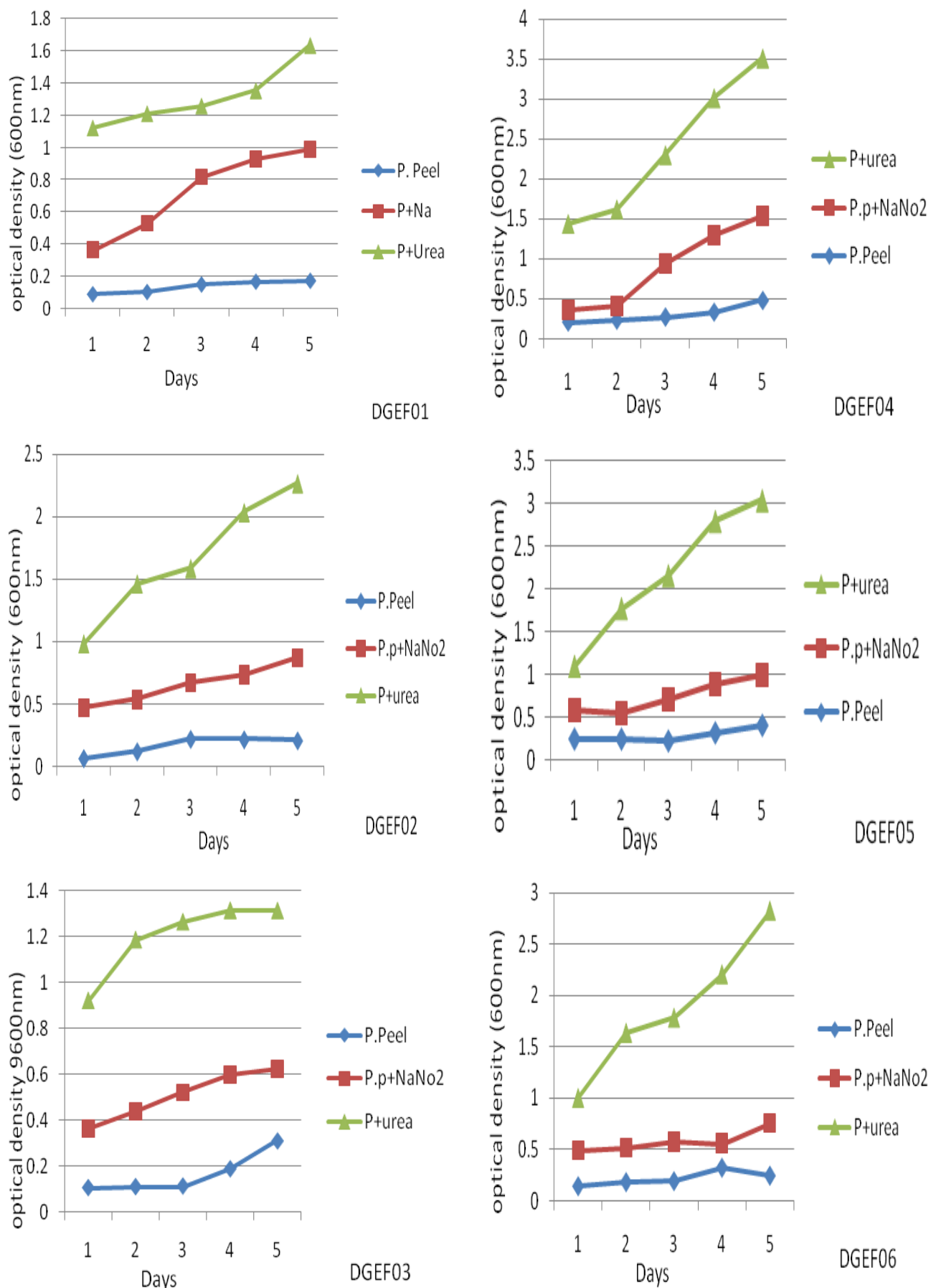


Fig. 1. Showing growth curves at OD<sub>600</sub> indicating growth with potato peel only, potato peel + sodium nitrite, and potato peel + Urea.

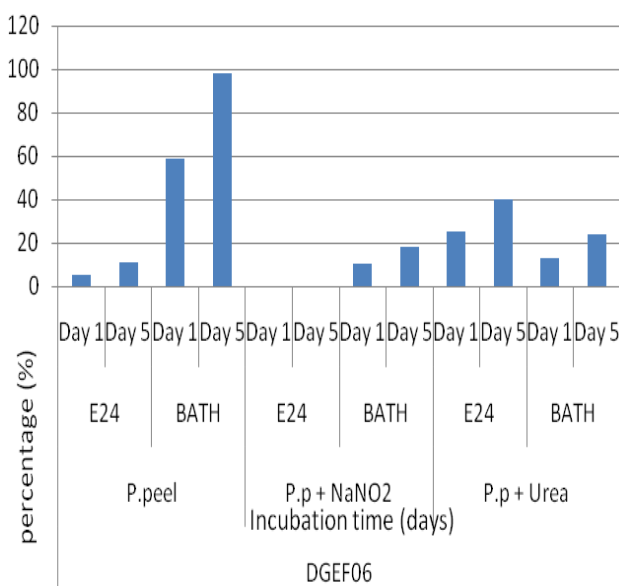
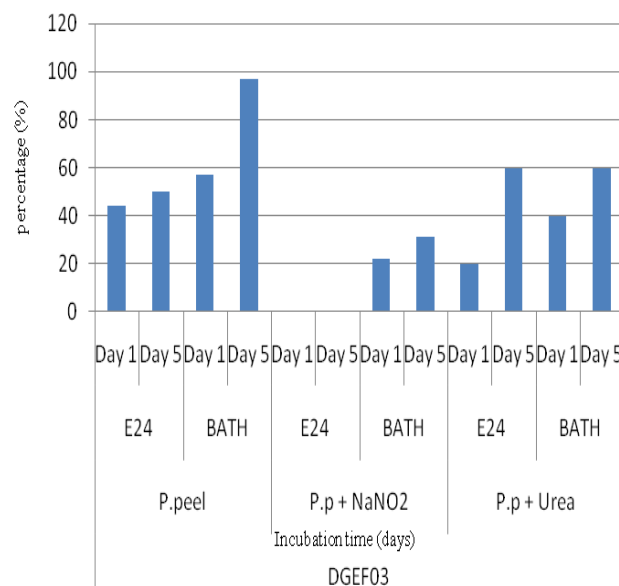
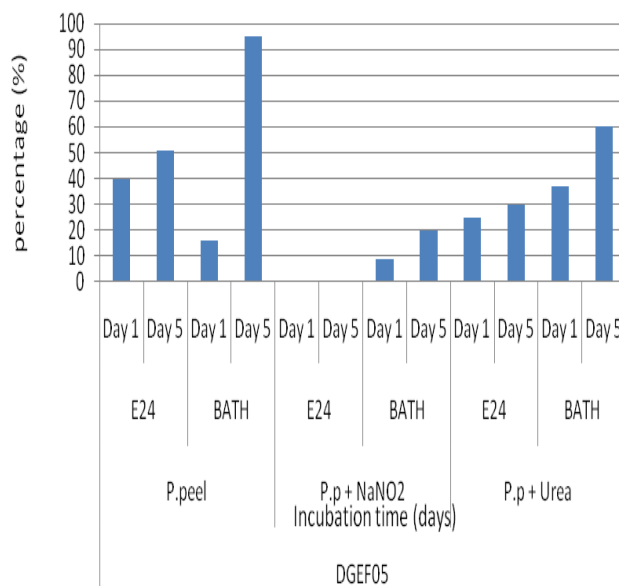
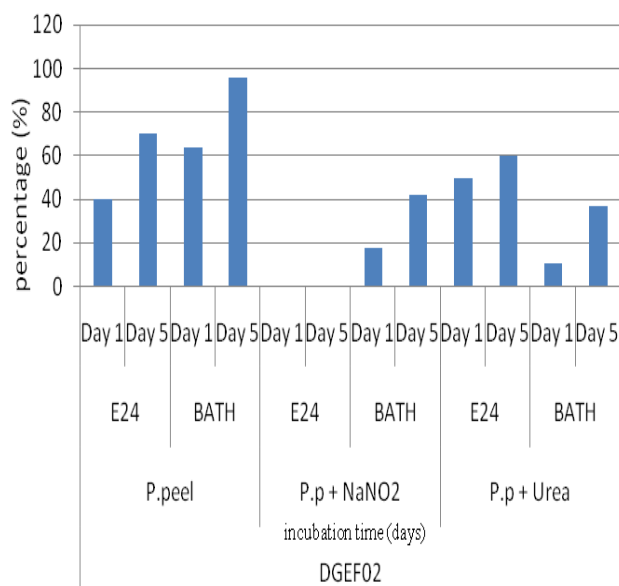
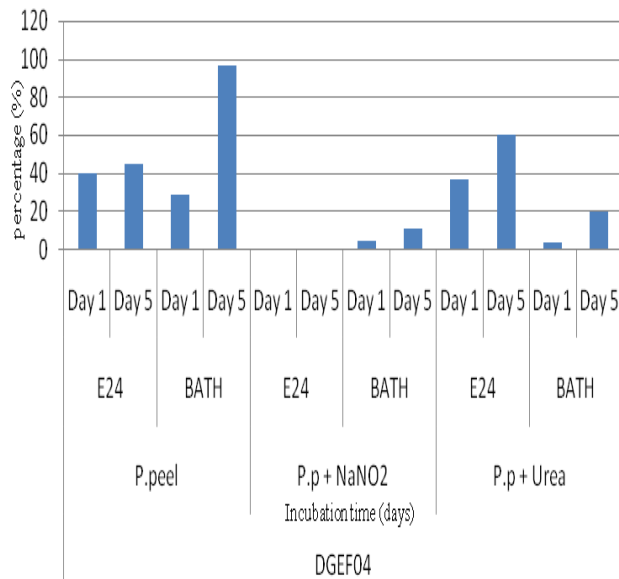
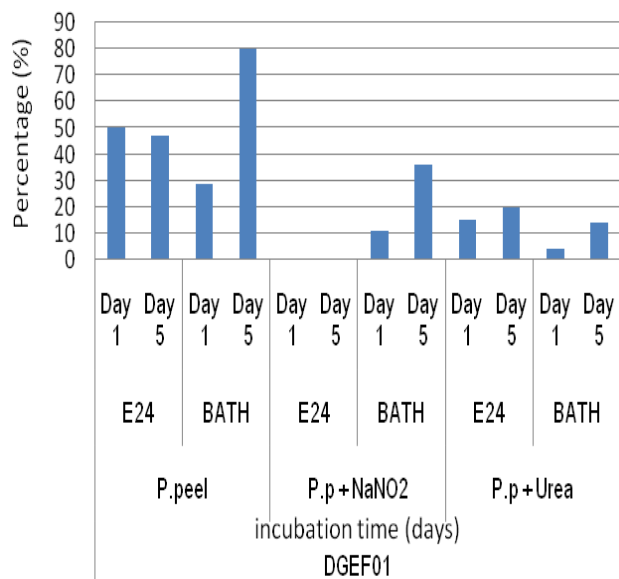


Fig. 2. Showing Emulsification Index (E24) and BATH assay results for first and fifth day of incubation of all the six isolates.

## DISCUSSION

To determine the optimal conditions that yield the highest biosurfactant production by strain DGEF01-06, the effect of potato peel as carbon source and sodium nitrite and urea as nitrogen sources were evaluated. We selected three parameters to check the performance of our biosurfactant producing isolates. First one was growth curve to check effect of cheap carbon source with and without any nitrogen source on growth of bacterial cells. Then we selected two screening tests for biosurfactant production in order to evaluate performance of our isolates when cheap carbon source is used. One screening test was Emulsification Index (E24) and other one was Bacterial Adhesion to hydrocarbon (BATH) assay.

According to Chen *et al.* (2007) biosurfactants act by emulsifying hydrocarbons, increasing the solubilization of crude oil and subsequent availability for microbial degradation. The biosurfactant production and bath assay dependent on growth of culture in the fermentation medium. In the presence of potato peel the bacterial growth was not as good as when grown in presence of nitrogen source. Isolates showed enhanced growth with Urea as compare to sodium nitrite. Considering good effect of nitrogen sources on growth it could be speculated that these sources might enhance the biosurfactant production of these isolates. But the results indicated best Emulsification Index (E24) and BATH assay results in presence of potato peel only but no positive effect was observed with additional nitrogen sources.

These results do not support the findings that the surfactant biosynthesis occurred predominantly during the exponential growth phase (Cunha *et al.*, 2004), since with the addition of nitrogen sources growth of bacterial cells was enhanced which must have increased the biosurfactant production. Contrary to that when we tested our isolates through the emulsification index and bacterial adhesion to hydrocarbon assay, which are considered as an efficient method to screen bacteria for biosurfactant production (Bonilla *et al.*, 2005; Rosenberg *et al.*, 1980), we found isolates giving best results when grown only with potato peel without any nitrogen source. It has indicated that non-dividing cells must be able to produce biopolymer more efficiently as compared to dividing cells.

Our studies are significant from two aspects. We have tested the role of cheap carbon source, potato peel, for the production of commercially important biosurfactants in order to reduce the cost of its production and results came out to be positive. Secondly, we have been able to propose a theory regarding biosurfactant production. When additional nitrogen sources (sodium nitrite and urea), were added growth rate was higher but biosurfactant production was lower- indicating efficiency of stationary phase culture for surfactant biosynthesis.

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