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AN EFFECTIVE MOVE TOWARDS USE OF AGRO-INDUSTRIAL & DAIRY WASTES FOR PRODUCTION OF BIOSURFACTANTS USING *BACILLUS SUBTILIS* AND *PSEUDOMONAS AERUGINOSA*

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Abstract

From an environmental viewpoint, biosurfactants are more acceptable for the remediation process both at sea and land. Recently, the biosurfactant production has been growing up, because this substance has amphiphilic nature with high emulsifying and surface interaction. They are produced through metabolism of microorganisms and many applications are mentioned in industry (chemical, food, pharmaceutical etc.). The current study describes the biosurfactant production by Bacillus subtilis and Pseudomonas aeruginosa using agroindustrial and dairy wastes containing high levels of glucose. The biosurfactant production was evaluated according to the emulsification index (using oils), surface tension analysis. The biosurfactant produced using 10% of inoculums of Bacillus subtilis showed highest surface tension reduction and emulsification index than 5% and 7% inoculum. Pseudomonas aeruginosa showed biosurfactant production but the yield is very low. Hence, current research invented an alternative way to process industrial wastes and the use of natural surfactants, or biosurfactants, is among one of the most promising methods due the fact that such compounds are metabolic products of fungi, bacteria and certain strains of yeast.

Keywords: Biosurfactants, Bacillus subtilis, Pseudomonas aeruginosa, Industrial wastes

I. INTRODUCTION

Microbial surfactants are structurally different group of surface-active biomolecules produced by a variety of microorganisms and are receiving considerable attention due to their unique properties such as higher biodegradability, lower toxicity, and greater stability [1,2]. Research in the area of biosurfactants has expanded quite a lot in recent years due to its potential use in different areas, such as the food industry, agriculture, pharmaceutics, the oil industry, petrochemistry and the paper and pulp industry amongst others. The development of this line of research is of paramount importance, mainly in view of the present concern with protection of the environment. Therefore, the most significant advantage of a microbial surfactant over chemical surfactants is its ecological acceptance because it is biodegradable and nontoxic to natural environments [3]. Plant biomass is a valuable resource to man and the value of the biomass contents is related to the chemical and physical properties of its molecules [4]. It is the main foreseeable sustainable source of organic fuels, chemicals and bio-materials, and significant efforts are made to make the 21st century one that is based on renewable substrates.

In addition the bioconversion of waste materials is considered to be of prime importance for the near future because of its favorable economics, low capital and energy cost, reduction in environmental pollution, and relative ease of operation [5, 6, 7, 8]. Producing usable products from agro industrial waste is therefore a feasible and favorable option [9,10,11].

The biotechnological processes underlying microbial surfactants production should be based on the utilization of cheap substrates, in order to make commercialization possible. In addition to the cost of the substrates, the operating cost for the production of microbial surfactants is also a major concern in the commercial viability of biosurfactants. Many researches choose using waste like medium of fermentation to biosurfactant production. Bibliography [12] used wastewater from a local dairy industry that was simultaneously utilized and treated for the production of sophorolipids (SLs), a glycolipids type of biosurfactant, by Candida bombicola. Bibliography [13] used glycerine and clarified cashew apple juice as feed stocks for the microbial surfactant synthesis by Yarrowia lipolytica. Bibliography [14] studied two wastewater systems: corn steep liquor and ground–nut oil refinery as low cost nutrients for the production of a biosurfactant by Candida sphaerica. Cassava flour wastewater was used as culture media for biosurfactant production by Bacillus sp. and results showed a surface tension of 59 to 26 mN/m [15].

This study has aimed to investigate biosurfactant production by *Bacillus subtilis* and *Pseudomonas aeruginosa*, using an industrial wastewater, cheese whey and molasses as agro-industrial wastes or by-products which contain high glucose as substrate, and are easily available in the Vidarbha region, so that the process is economically viable and the evaluation of the use of these unconventional substrates as alternatives for biosurfactant production.

II. MATERIAL AND METHOD

A. **Innoculum Preparation and Standardization:** The microorganisms used in fermentation for biosurfactant production were the *Bacillus subtilis,* and *Pseudomonas aeruginosa*. The medium used for inoculum preparation was Nutrient Broth (1 % glucose, 0.46 % peptone, 0.3 % meat extract and 0.06 % sodium chloride). Pre-inoculum was prepared 15 mL (same composition the medium) in a 50-mL Erlemeyer flask, which received the microorganism (for adaptation) and incubated in an orbital shaker for 24 h at 37 °C. And inoculum (150 mL of sterile nutrient broth into a 250-mL Erlemeyer flask) received the pre-inoculum and incubated in shaker for another 24 h at 37 °C. The standardization of the inoculum was performed using a nutrient broth (same composition used above), adjusted in a spectrophotometer (625-nm-wavelength), to a range from 0.08 to 0.1 in absorbance, according to the method of McFarland (cell concentration around) [16].

B. **Substrate:** The industrial wastewater, Cheese whey and Molasses were used as substrate in the current investigation. The waste was collected immediately after the process output without undergoing any treatment. This residue was kept frozen until its use.

C. **Fermentation:** The fermentations were conducted in Erlenmeyer Flasks of 250 ml capacity containing 80 mL of industrial waste containing high rate of glucose that were inoculated with 5, 7 and 10% inoculum. The flasks were autoclaved, inoculated and brought to an orbital shaker (150rpm) under stirring and at 37 °C for 18 h. The measured parameters were glucose, surface tension and emulsification index.

- **Surface tension:** The surface tension measurements were determined according to Behring (2004) [17] methodology. The samples were centrifuged at 3,500 rpm for 10 minutes. The burette was calibrated with sodium dodecyl sulphate solutions at various concentrations and tested with pure water before each fermentation. All tests were performed in triplicate.
- **Emulsification Index (E24)**: The emulsification index was determined according to the methodology proposed by Cooper and Goldenberg (1987) [18]. To analyze the emulsification index (E24), the fermented medium was centrifuged (8000 rpm, 15 min, 2 ºC) to obtain cell-free supernatant. 2 mL were collected from the supernatant to mix with 2 mL of Toluene in test tubes. It was stirred by vortexing for 2 min and the mixture was allowed to stand for 24 h. The EI24 was calculated by following equation:

$$\% = \frac{\text{Height}_{\text{emulsion}}}{\text{Total Height}_{\text{mixture}}}$$

- **Recover the crude biosurfactant:** To recover the biosurfactants for measure yield, cell-free supernatants from fermentation were subjected to an acid precipitation. Briefly, the supernatants were acidified (pH~2.0) with HCl 1 M and left overnight at 7 ºC. Afterwards, it was centrifuged

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(8,000 rpm, 15 min, 2 ºC), the supernatant was discarded and the precipitate was washed with acidified water and saved. All assays were performed in duplicate.

III. RESULTS AND DISCUSSION

In the current study the results obtained for the decrease in surface tension during the fermentation as function of the percentage of inoculums is shown in figure 1-3. Out of the two microorganisms used in the study, *Bacillus subtilis* showed promising reduction in the surface tension with respect to time where as *Pseudomona aeruginosa* does not showed any significant decrease in surface tension. The decrease in of superficial tension is the primary means one can elect microorganisms capable to produce biosurfactants, although emulsifying and dispersing agents will not necessarily decrease the superficial tension [19, 20]. A decrease in surface tension is a strong indication of biosurfactant production, being confirmed by its emulsion index.

The major decrease in surface tension was obtained by using 10% inoculum level. The initial value was 69 mN/m, which drop down to 35mN/m and 50mN/m by *Bacillus subtilis* and *Pseudomonas aeruginosa* respectively. These values indicate that the prominent decrease in surface tension value was by *Bacillus subtilis*. Later on it has been found that for the 7% and 5% inoculum of both the microbes, fraction showed less decline in surface tension. It may be noted thus, the higher the percentage of the inoculum, the greater the reduction in surface tension. Studies conducted by bibliography [21] using cassava as substrate and *Bacillus subtilis* in the synthesis of biosurfactant (surfactin) showed a reduction of surface tension by 37% relative to the starting point, reaching levels below 30 mN/m.

Our results are also in accordance with bibliography [22] where the initial value was 70 mN/m, reaching 44 mN/m after 22 hours of fermentation (a 36% drop). For the 7.5% and 5% inoculum fraction showed a decline in surface tension of 14% and 20%, respectively.

Figure 1: Analysis of surface tension with 5% inoculum of *Bacillus subtilis* and *Pseudomonas aeruginosa*

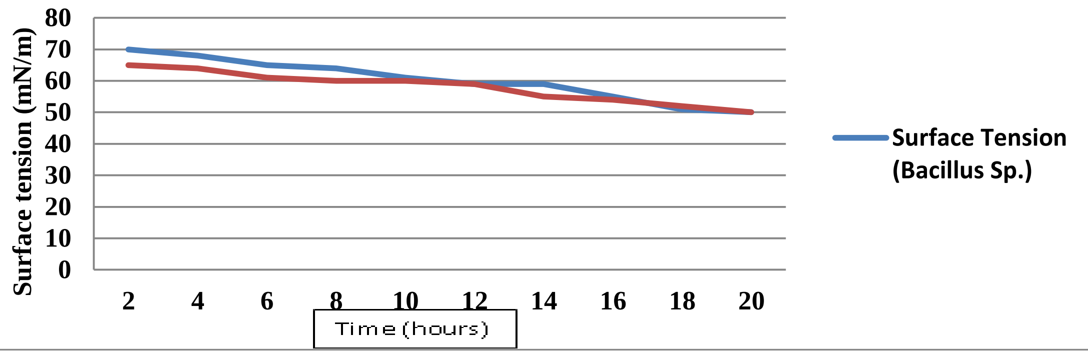


Figure 2: Analysis of surface tension with 5% inoculum of *Bacillus subtilis* and *Pseudomonas aeruginosa*

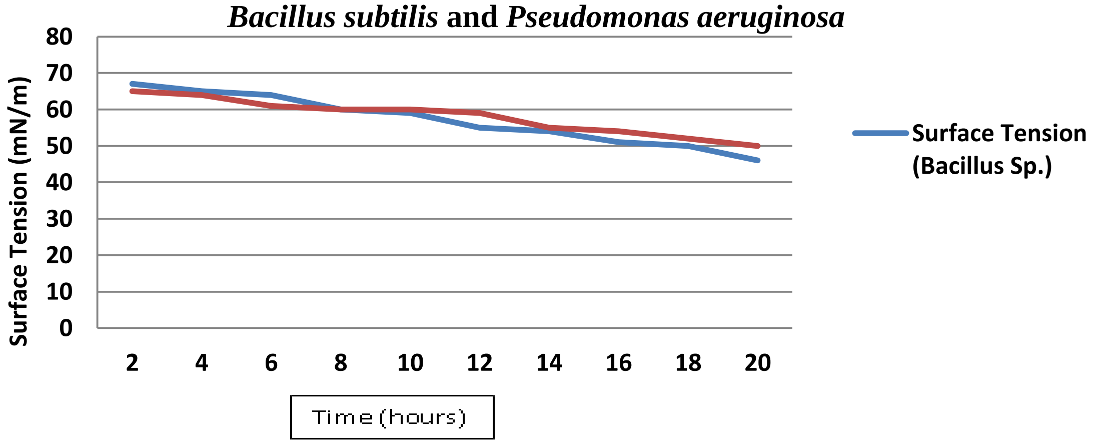
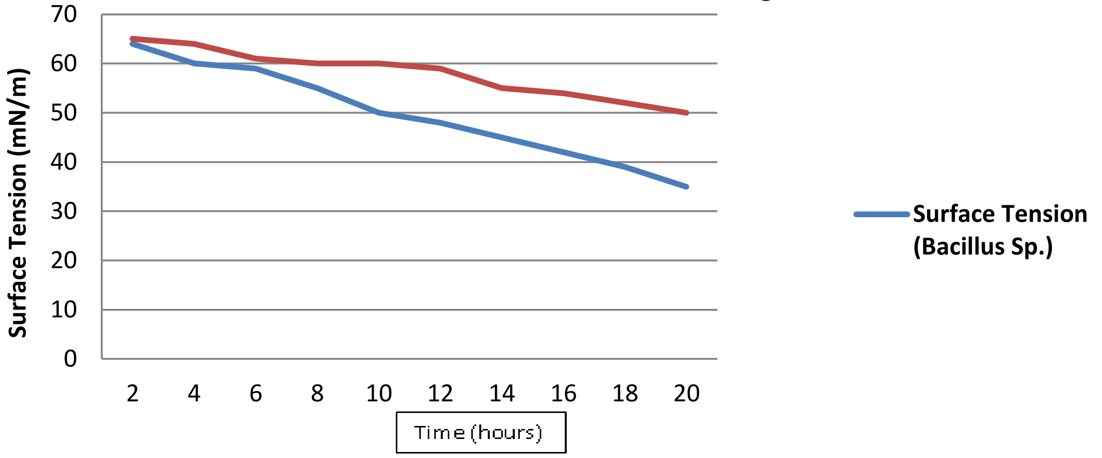


Figure 3: Analysis of surface tension with 5% inoculum of *Bacillus subtilis* and *Pseudomonas aeruginosa*



In the current study the emulsification index was also calculated during the fermentation. The emulsification index also evaluates biosurfactant production. Tests were made with oil. The emulsification index expresses the ability of produced biosurfactants to emulsify hydrocarbons. According to bibliography [23], one criterion considered to select a good emulsifier is the capability of forming the emulsion with a hydrocarbon and that it remains emulsified above 50% for 24 hours or more. Therefore, according to the results, the tests showed a good emulsifying power, achieving up to 85% of emulsification. Bibliography [22] showed up to 75% of emulsification for soy oil and 93% for motor oil.

Bibliography [24] showed that the emulsification activities of the biosurfactant produced by Pseudomonas aeruginosa 2297 from different fermentation substrates and synthetic surfactants were tested with diesel, petrol, olive oil, and groundnut oil. When petrol and olive oil tested, maximum emulsification activity of 72.25± 2.47 and 59.23± 0.19 % was shown by biosurfactant produced from groundnut husk and coconut oil, respectively, used as a substrate and was comparable to all synthetic surfactants. In diesel and groundnut oil, the synthetic surfactants (SDS) had the highest activity of 70.41± 0.56 and 62.31± 0.28 %, respectively, compared to all biosurfactants. From the quantification experiment, the highest biosurfactant production was observed in sawdust with 4.53± 0.03 mg/ml followed by groundnut husk and glycerol as substrates.

Bibliography [25] evaluated the emulsion obtained using the biosurfactant produced by *Bacillus subtilis*, cassava wastewater as substrate and several hydrocarbons and vegetable oil. Their results showed high emulsification index values for most of carbon sources used, including cyclic and aliphatic hydrocarbons, and vegetable oils with different fatty acids.

IV. CONCLUSION

The use of waste in biosurfactant production come increase due strong appeal from their characteristic and applications, beside low cost. In current work, strategies were investigated to use only waste (no synthetic medium) to propose a culture medium for bio-surfactant production. The results showed that the biosurfactant produced using glucose-rich industrial wastewater successfully emulsified oil samples and provided a significant decrease in surface tension when a culture medium containing 10% inoculum is used. Thus, the use of this low-cost substrate, whose treatment is expensive and costly, for biosurfactant production is feasible, since such compounds have a wide variety of applications in many industries.

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