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# Effect of Substrate Concentration and pH on Biosurfactant Production from Pineapple Peel

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**Abstract—***Increase in processing of agricultural commodities has also resulted in generation of a huge quantity of wastes. The non-edible portion (waste) of fruits and vegetables after processing like peels, pods, seeds, skins, etc., contribute to about 10-60% of the total weight of the fresh produce. In some fruits the discarded portion can be very high (mango 30-50%, banana 20%, orange 30-50%, pineapple 40-50%). Such waste results in increasing disposal and severe pollution problems and represents a loss of valuable biomass and nutrients as it has significant presence of cellulose, hemicelluloses, pectin, minerals, vitamins, fats, phytochemicals etc. This waste offers a huge potential for its microbial conversion into useful products. In this research study, biosurfactant was produced from culture media, prepared by utilizing the pineapple peel waste as the carbon source substrate and fermenting microbe Bacillus subtilis. Pineapple peel has been taken in three different concentrations (10%, 30% and 50%) and pH (6, 7 and 8). The selected high yield strain Bacillus subtilis showed the maximum biosurfactant yield of 24.3±0.1 g/L with sample having 10% Pineapple peel fermented at pH 7. The biosurfactant obtained showed emulsification capacity from 62 to 79% and critical micelle concentration range from 11±0.8 to 7±0.2 mg/L. Moreover, the highest reduction in the surface tension of water from 72.1±0.02 mN/m to 21.7±0.01 mN/m was also observed. All these characteristics indicate that bioconversion and biodegradation of waste pineapple peel by Bacillus subtilis is a promising and commercial way of waste utilization for biosurfactant production.*

## 1. INTRODUCTION

Surfactants are surface active compounds with both hydrophilic and hydrophobic domain. They are capable of reducing surface and interfacial tension at the surface and interface between liquids, solids and gases. These chemically derived surfactants are toxic and non-biodegradable to the environment. The disadvantage of using petroleum-based surfactants is that they contribute to the depletion of a non-renewable resource and are highly polluting. They can release toxic chemicals when they decompose. Tightening environmental regulation and increasing awareness for the need to protect bionetwork have effectively resulted in an increasing interest in biosurfactants as promising alternatives over synthetic surfactants.

Biosurfactants are amphiphlic molecule produced by a wide variety of plants, animals and microorganisms (bacteria, yeast and fungi) and the microbial derived surfactants are either adhere to cell surface or excreted extra-cellularly in the growth medium [1]. Biosurfactants could easily be produced from renewable resources via microbial fermentation, having an additional advantage over synthetic surfactants.

The production of biosurfactant is inexpensive while using alternative substrates and their industrial potential [2]. Biological surfactants are easily degraded by microorganism. They can be produced from very cheap raw materials which are available in large quantities. Many biosurfactants are not affected by environmental factors such as temperature, pH and ionic strength tolerances. Biosurfactants are biocompatible and digestible which allows their application in cosmetic, pharmaceuticals and as functional food additives [3].

Research on the selection of suitable substrates has mainly centered on tropical agro industrial crops and residues. These include crops such as cassava [4], soybean oil [5], sugar beet [6] , sweet potato, potato, and sweet sorghum, crop residues such as bran and straw of wheat and rice; hull of soy, corn and rice; bagasse of sugarcane and cassava; residues from the coffee processing industry such as coffee pulp, coffee husks, spent coffee grounds; residues of the fruit processing industries such as pomace of apple and grape, waste from pineapple and carrot processing, banana waste; waste from oil processing mills such as coconut cake, soybean cake, peanut cake, canola meal and palm oil mill waste; and others such as sawdust, corn cobs, carob pods, tea waste, chicory roots etc. [7].

Additional substrates have been suggested for biosurfactant production, especially water-miscible wastes, molasses, whey milk or distillery wastes [8]. Diesel, crude oil, glucose,

sucrose, glycerol have been reported to be a good source of carbon substrate for biosurfactant production [9].

*Pseudomonas aeruginosa* 44T1 used hydrocarbons with carbon chains C12 and olive oil as carbon sources to produce rhanmolipids [10]. *Arthrobacter protophormiae* MTCC 688 is used to produce biosurfactant at different salt concentrations [11]. *Bacillus subtilis* ATCC 21332 was used as a carbon source potato (60 g/L) for the production of Surfactin (decreased surface tension of the medium was 41.8 mN/m) [12].

*Pseudomonas aeruginosa* strain U129791, used corn oil as a carbon source and produced rhamnolipids, the highest production of rhamnose (5.4 g/L) was obtained when the concentration in culture medium was 40 g/L of corn oil [13]. *Cellulomonas cellulans* produced glycolipids (8.9 g/L, expressed as glucose) when it grew in liquid medium with 30 g glycerol/L [14].

Various strains have been used for production of biosurfactant like *Bacillus subtilis* [15], *Arthrobacter protophormiae* [11], *Corynebacterium lepusc* [9], *Pseudomonas aeruginosa* [13], *Acinetobacter calcoaceticus* [16], *Cellulomonas cellulans* [14], in which it was suggested by the researchers that some strains like *Bacillus subtilis, Pseudomonas aeruginosa, Corynebacterium lepusc* and *Acinetobacter calcoaceticus* give better yield of biosurfactants from agro waste [17]. So, three common microbial strain has been chosen for this study as *Bacillus subtilis.*

The composition and emulsifying activity of the biosurfactant not only depends on the producer strain but also on the culture conditions. Thus, the nature of the carbon source, the nitrogen source as well as the C:N ratio, nutritional limitations, chemical and physical parameters such as temperature, aeration, divalent cations and pH should be taken in consideration as these factors not only influence the amount of biosurfactant produced but also the type of polymer produced.

Thus, this project study has been carried out to utilize the pre-treated selected pineapple peel waste for the preparation of culture medium and also study the effect of concentration of carbon source and pH on the various properties of biosurfactant as yield, emulsifying activity and surface activity which is considered as very important factor from research point of view.

## 2. MATERIALS AND METHODS

### 2.1. Microbial Strain

The biosurfactant producing microbial strain *Bacillus subtilis* (*B. subtilis*) was procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The culture was maintained on nutrient agar slants and stored at 4°C.

### 2.2. Pretreatment of Waste

Pineapple peels were collected from local Nawabganj market juice shop near HBTU, Kanpur. Foreign materials and dirt were removed from Pineapple peel and dried at 55°C for 4 days in convective hot air drying oven (REMI, RDHO 80). The dried peels was crushed using a high speed grinding machine (LG, Seoul) and ground dried peel powders was passed through a 70-mesh sieve. The powdered sample was stored in desiccators packed in air tight pouches at room temperature until needed [19].

### 2.3. Media Preparation

Liquid media was prepared from a stock solution with compositions $KH_2PO_4$ (0.68g), $Na_2HPO_4$ (4.5g), $MgSO_4.7H_2O$ (0.1g), $NaNO_3$ (6.5g) and yeast extract (0.5g) per liter. The natural waste materials pineapple peels were evaluated as carbon sources in the above medium at the concentration of 10% , 30% and 50% (weight/volume) for the production of biosurfactant [19]. The pH of medium was adjusted to three different levels as at 6, 7 and 8 with a solution of 1M NaOH.

### 2.4. Production of Biosurfactant

Fermentation was carried out in 250 ml Erlenmeyer flasks containing 100 ml of the medium inoculated with 5% (v/v) inoculum and incubated at 37°C for 96 hours [19]. Nine samples were obtained with three different substrate concentrations at three different fermentation pH conditions.

### 2.5. Recovery of Biosurfactant

At the end of the fermentation, the culture was centrifuged (4000 rpm, 20min) to remove the cells. The cell-free supernatant was adjusted to pH 2 with 6M HCl and was subsequently incubated overnight at 4°C to promote the biosurfactant precipitation.

Further, the precipitate (crude biosurfactant) was collected by centrifugation (9000rpm, 20min, and 4°C). The crude biosurfactant was dissolve in a minimal amount of distilled water and the pH was adjusted to 7 using 1M NaOH. The biosurfactant solution was dried and the products obtained was weighed and stored at -20°C [20].

### 2.6. Physico-chemical Analysis of Biosurfactant

The analyses of each eighteen samples will be done individually for all the parameters.

#### 2.6.1. Yield

The biosurfactant yield was determined for each sample after the recovery process is done. The final weight of the recovered biosurfactant amount produced from the fermentation process is represented as the yield of process.

$$Yield = \frac{Final\ recovered\ weight}{Amount\ of\ waste\ taken\ for\ fermentation} \times 100$$

### 2.6.2. Emulsification Capacity

To analyze the emulsification index (EI24), the fermented medium was centrifuged (8000 rpm, 15 min, 2ºC) to obtain cell-free supernatant. 2 mL sample was 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:

$$EI_{24} = \frac{HE_{24h}}{HE_t} \times 100$$

Where, $HE_{24h}$ is the height of the emulsion formed in 24 h and $HE_t$ is the height of the solution (Ehrhardt *et al.,* 2015).

### 2.6.3. Surface tension measurement

Surface tension measurement was done by Du-Nouy-Ring method as described by Abouseoud et al. [21] and Devesa-Rey et al. [22]. The surface tension (ST) was measured by means of a tensiometer (3B Scientific@ product U20030) by the ring method with the slight modification suggested by Maufo et al., 2018. The formula used to calculate surface tension was:

$$ST = \frac{F - P_0}{4\pi r} \times 1000$$

Where, $F$ represents the force measured, $P_0$ the force read before removing the ring, and r the radius of the ring.

The existence of biosurfactants in the solution was confirmed on the basis of decrease in the value of surface tension of the supernatants against the distilled water taken as control.

### 2.6.4. Critical Micelle Concentration measurement

Critical micelle concentration (CMC) is known as the concentration of an amphiphilic component in solution at which the formation of micelles is spontaneously initiated. It is important for several biosurfactants applications to establish their CMC, as above this concentration no further effect is expected in the surface activity. The CMC was determined by plotting the

All these measurements were done in triplicate.

## 3. RESULTS AND DISCUSSION

All the nine samples were subjected to determination of biomass yield, emulsification index measurements, surface tension and CMC.

### 3.1. Biomass Yield

In the Table 1 the yields of biosurfactants extracted from the different supernatants vary significantly ($p< 0.05$) from one biosurfactants produced at different pH levels and from one concentration of substrate to another. The yields of biosurfactants with 10% substrate was higher than 30% as suggesting that higher initial substrate concentration (30%) led to lower consumption at the end of fermentation resulting less biosurfactant productivity. Also, it is concluded that the peel have good ability to be used as substrate for a low cost production of biosurfactants by *B. subtilis*. The highest yield of biosurfactants was recorded with 10% substrate at pH level 7. It could be explained by the presence of compounds other than Carbohydrates in peel which may have contributed to the increased production of biosurfactants and also the pH level 7 is suggested best suitable condition for growth of *B. subtilis* [23]. Sharma et al. [24] found a yield of 0.80 g/L with *Lactobacillus helveticus* MRTL91 while using cheese whey as an alternative nutrient source. Distilled grape marc residues were used by *Lactobacillus pentosus* to produce 4.8mg/L of biosurfactants [25].

The cell mass decreased by about twice when the substrate concentration was increased from 10 to 20 %. Cell mass yield continued to decline with increasing initial substrate concentration because of substrate inhibition. This variability in yield with the pH and substrates could be explained by the fact that the metabolism of substrates to produced biosurfactants depends on the variation in enzymatic action of *B. subtilis*.

### 3.2. Emulsification Capacity

Emulsifying capacity permits the homogenous distribution of biopreservatives in all food matrixes and optimizes their efficacy. Emulsifying activity of the crude biosurfactants produced by three concentration of pineapple peel as substrate was determined and shown in Table 1. The emulsifying activity which has been observed could be due to the fact that adsorption of biosurfactants at the interface between water and oil allows a decrease in energy required to generate interfacial area, thus facilitating in obtaining the drops with small diameter during emulsification and thus allowing the formation of emulsions.

Therefore, it can be concluded that the crude biosurfactants produced by 10% substrate concentration has are able to stabilize emulsions for 72 h. The ability of biosurfactants to stabilize emulsions could be explained by the fact that adsorption of biosurfactants to oil/water interface by with agitation resulted in repulsion of drops.

However, it was observed that the the emulsifying capacity of crude biosurfactants obtained was quite stable after 72 h in comparison to synthetic surfactants who lost their activity after 48 h. This concludes that the crude biosurfactants produced by Bacillus strains with 10% pineapple peel as substrate and at pH level 7 can effectively be used to substitute chemical emulsifiers in food industry.

### 3.3. Surface tension measurement

The surface tension of the different supernatants was measured and the results are presented in Table 1. The results

showed that biosurfactants present in the different supernatants caused a significant ($p$<0.05) reduction of surface tensions.

A decrease in surface tension from 72.1±0.02 to values ranging from 49.5±0.02 mN/m to 23.4±0.03 mN/m was observed in case of 10% substrate concentration at different pH ranges. Concerning broth made with 20% pineapple peel as substrate, a decrease to values ranging from 37.9±0.01 mN/m to 21.7±0.01 mN/m was observed. A lower reduction of surface tension was observed with 30% pineapple peel as substrate.

The high surface tension reduction properties of the different supernatants observed in the present study could be explained by the fact that supernatants may probably contain biosurfactants composed of a mixture of several compounds with significant surface activity [26].

The results obtained in this study are in accordance with the data previously reported for lactobacilli species. Rodrıguez et al. [27] obtained a reduction of surface tension to 41.1mN/m with biosurfactants of *Lactococcus lactis*.

20% substrate concentration resulted in the smallest value of surface tension at the end of fermentation. The low values of surface tension obtained here were in accordance with the values of surface tension in the literature for Bacillus species strains [28-30].

The graphical representation of surface tension along with emulsification index has been shown in Figure 1 for the 10% substrate at different levels of pH.

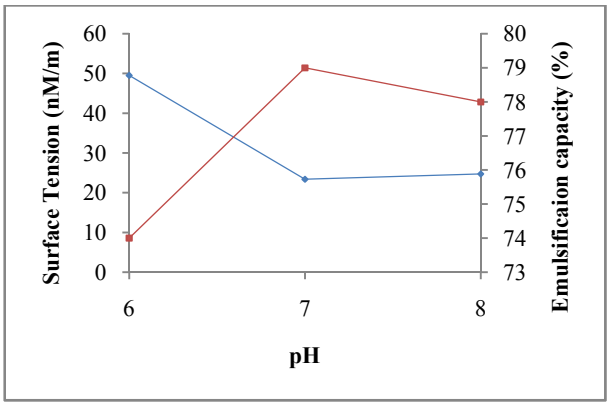


**Figure 1. Effect of pH on the surface tension and emulsification capacity ($EI_{24}$) of biosurfactant produced from 10% peel substrate medium.**

### 3.4. Critical Micelle Concentration measurement

Table 1 presents the various values of CMC for biosurfactant produced by *B. subtilis* at different process parameters.

**Table 1. Effect of substrate concentration and pH on the produced biosurfactant properties.**

| Substrate conc. (%) | pH | Yield (g/l) | Emulsification capacity (%) | CMC (mg/l) | Surface tension (mN/m) |
|---|---|---|---|---|---|
| 10 | 6 | 21.2±0.1 | 74 | 9.1±0.3 | 49.5±0.02 |
| | 7 | 24.3±0.1 | 79 | 8.3±0.2 | 23.4±0.03 |
| | 8 | 19.4±0.2 | 78 | 10.0±0.2 | 24.7±0.01 |
| 20 | 6 | 18.3±0.1 | 68 | 7.1±0.1 | 37.9±0.01 |
| | 7 | 18.5±0.4 | 73 | 7.0±0.2 | 21.7±0.01 |
| | 8 | 17.6±0.3 | 71 | 7.3±0.3 | 23.2±0.02 |
| 30 | 6 | 12.0±0.1 | 62 | 10.8±0.5 | 53.2±0.04 |
| | 7 | 12.4±0.2 | 67 | 10.2±0.4 | 31.1±0.03 |
| | 8 | 11.9±0.2 | 65 | 11.0±0.8 | 37.7±0.02 |

This variation in CMC values is due to the properties of the solvent used for dissolving the biosurfactant. The biosurfactant showed the most efficient performance, with a CMC value of 7.0±0.2 mg/L. The CMC values obtained here declined from 8.3±0.2 to 7.0±0.2 mg/L with substrate concentration 10% and 20%, respectively, and are in agreement to those obtained from the literature [31]. This finding is reasonable because the biosurfactant concentration reached its highest value at the pH 7 and higher surface tension for 10% (23.4±0.03 mN/m) was also obtained compared to the value for 20% (21.7±0.01 mN/m).

## 4. CONCLUSION

The results of this study showed the good ability of pineapple peel to be used as low-cost substrates in the production of biosurfactants by *B. subtilis*. Statistical analysis showed that the properties of the produced biosurfactants are correlated with the concentration of substrate used and pH. Higher initial substrate concentration (30%) led to lower consumption at the end of fermentation resulting less biosurfactant productivity.

The pH level 7 is best suitable for the biosurfactant production with 10% substrate concentration. Emulsions obtained with crude biosurfactants are stables on storage at room temperature, suggesting that they are effective in forming and stabilizing emulsions. This study suggested the use of pineapple peel as substrate by *B. subtilis* strains to produce biosurfactants which can be used in food industry as emulsifier or biopreservatives.

## 5. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## 6. ACKNOWLEDGEMENTS

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