Journal of Agricultural Engineering and Food Technology
p-ISSN: 2350-0085; e-ISSN: 2350-0263; Volume 6, Issue 4; July-September, 2019, pp. 268-270

http://www.krishisanskriti.org/Publication.html

Effect of Process Parameters on Yield of Biosurfactant by *Bacillus Subtilis*

Smriti Pandey[1], Dr. Vivek Kumar[2] and Anjali Srivastava[3]

[1]PG Student, Department of Food Technology,
School of Chemical Technology Harcourt Butler Technical University,
Kanpur, Uttar Pradesh
[2]Faculity, Department of Food Technology, School of Chemical Technology,
Harcourt Butler Technical University, Kanpur, Uttar Pradesh
[3]Faculity,Department of Food Technology, Institute of Engineering and Technology,
Bundelkhand University Jhansi
E-mail[1]: smriti.shilpipandey@gmail.com,
[2]viveksachan99@rediffmail.com,
[3]anjali011bt@gmail.com

Abstract–*Biosurfactants are amphipathic compounds produced extracellularly by microorganisms on cell surfaces, or excreted extracellularly. They contain hydrophilic and hydrophobic moieties that reduce surface and interfacial tension between molecules at the surface and interface respectively. The present study was focused on development of economical methods for biosurfactant production by the use of unconventional substrates. The research investigated the potential of utilizing agro industrial wastes (orange peel, pineapple peel & to tomato pomace) to replace synthetic media for cultivation of Bacillus subtilis MTCC 2388, and biosurfactant production.One-variable-at-a-time (OVAT) research wascarried out to enhance the production of biosurfactant by B. subtilis MTCC 2388 the maximum yield of biosurfactant 2.0±0.3g/L in medium: tomato pomace powder 3%, ammonium ferric citrate, pH 7, 2% inoculum, 30°C temperature.*

1. Introduction

Biosurfactants are surface active fragments produced by extensive variety of microorganisms. Owing its amphipathic nature, these biomolecules are accomplished of dropping the surface tension, interfacial tension and establishing micro-emulsion to allow involvement of two immiscible solutions. Such belongings show admirable detergency, emulsifying, foaming, and dispersing traits. Some of the features, which mark them favourable substitutes to chemically derived surfactants, are their poorer toxicity, higher biodegradability, superior stability at wide range of pH and temperature, and better environmental compatibility [1]. Thus, attention towards these biomolecules has improved consideration in recent years, as they are potential compound for several commercial applications in the petroleum, pharmaceuticals, biomedical and food processing industries[6]. In spite of such benefits, biosurfactant have not been completely commercialized due to exclusive raw material and lower yields. One of the strategies to expand production is to enhance the growth media in order to get maximum production. Formulation of an optimized production medium involves selection of the precise nutrients at their exact levels to deliver an ideal microenvironment for auxiliary growth and metabolite fabrication[5]. Microorganisms usevariability of organic mixtures as the cause of carbon molecules and energy for their evolution. Nitrogen compoundplays an important role in the synthesis of biosurfactants. There are limited researches on the temperature dependence for biosurfactant production. The strain of *Bacillus subtilis*was grown in salt medium at 25-47˚C to explore the influence of culture temperature on biosurfactant production. Metabolism is pH sensitive because pH is the important factor that affects the chemical reactions of the living cells[3].

2. Materials and methods

2.1 Raw materials

Orange peel and pineapple peel was collected from local Nawabganj Market juice shop near HBTU, Kanpur. Tomato pomace was collected from MORTON Allahabad canning company. Biosurfactant producing freeze-dried microbial strains *Bacillus subtilis* MTCC 2388 (*B. subtilis*), was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India in freeze-dried form. Foreign materials and dirt was removed from orange peel, pineapple peel and tomato pomace, washed them with running tap water and each of these separately dried at 70°C for 4 days in convective tray drying oven. The dried peel was crushed using a high speed grinding machine and each grinded dried peel powders were passed through a 70-mesh sieve separately. The three powdered samples was collected and stored in desiccators at room temperature until needed[4]. Three samples were prepared Orange peel with *B. subtilis* (OPB), Pineapple peel with *B. subtilis* (PPB), and Tomato pomace with *B. subtilis* (TPB)

2.2 Media preparation

Aliquots of 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 orange peel, pineapple peel and tomato pomace was evaluated as carbon sources in the above medium at the concentration of 3% (weight/volume) for the production of biosurfactant [2]. Nine samples were prepared with 3 different substrates and then media was sterilized in autoclave at 121°C, 15 psi for 20 minutes. After that all the samples were cool down and the liquid media is cultured by microbial strains. Incubation was carried out for 96 hours at 37°C for production of biosurfactants.

2.3 One-Variable at a-Time (OVAT) for Growth and Biosurfactant Production

The optimization method was charted by changing the concentration of one-variable-at-a-time (OVAT) while keeping all others at their selected levels. Once a factor was optimized, this optimal level was used for the next step of OVAT research conducted with the other variable and so on. Firstly, the effect of different nitrogen sources (ammonium nitrate, ammonium ferric citrate &ammonium sulfate,) was determined on the production of biosurfactant. An uninoculated control medium was also maintained. The medium were maintained at 30°C in an incubator shaker at 160 rpm. Then, the effects of numerous environmental factors, such as, pH (6, 7 &8), inoculum size (1, 2 & 3%) and incubation temperature (25, 30 &37◦C) on the growth and biosurfactant production by *B. subtilis MTCC 2388* were determined[7].

2.4 Time Duration of Biosurfactant Production in Optimum Condition

The outcome of incubation duration on the biosurfactant production in OVAT optimized medium was observed over the period of 72 h. Biosurfactant was extracted, purified, and expressed in terms of its dry weight [7].

2.5 Determination of Media Components for the production of Biosurfactant

Factors taken into consideration included media components, viz., agro waste (orange peel, pineapple peel and tomato pomace), ammonium ferric citrate, ammonium sulfate and ammonium nitrate and environmental factors, such as pH, inoculum size, and incubation time. Media were set using the 27 combinations of factors and inoculated with the stated amount of over-night grown inoculum. Flasks were incubated on the shaker at 160 rpm, at 30◦C for different time periods. All the experiments were performed in triplicate and average of the three responses are reported[7].

2.6 Recovery of biosurfactant by Solvent extraction method

The supernatant containing Biosurfactant was treated with the combination of extraction solvent (methanol/ chloroform/ acetone, 1:1:1 by volume). The mixture was continuously shaken at 200 rpm, 30°C for 5 hrs inside incubator shaker. Two layers of precipitate were obtained. The upper layer was discarded. Dry powder obtained at the end [8].

2.7 Yieldof biosurfactant

The final weight of the recovered biosurfactant amount produced from the fermentation process is represented as the yield of process.

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

3. Results & Discussions

It is shown in Table 1 that the yields of biosurfactants extracted from the different supernatants vary significantly ($p < 0.05$) from one sample to other.

Table 1: Effect of carbon source on yield of biosurfactant

Carbon Source	Biosurfactant Yield (g/L)
Orange peel	2.4±0.6
Pineapple peel	2.4±0.7
Tomato pomace	3.6±0.6

Table 1: shows the effect of carbon source (orange peel, pineapple peel and tomato pomace) concentration, on the yield of biosurfactants. It was observed that biosurfactant increased till 3% agro waste concentration. The highest yield obtained by the tomato pomace 3.6±0.6 and the lowest yield is 2.4±0.6 in orange peel. It could be explained by the presence of compounds other than carbohydrates particularly lipids in seeds of tomato which may have contributed to the increased production of biosurfactants.

Table 2: Effect of nitrogen source on yield of biosurfactant

Nitrogen Source	OPB	PPB	TPB
NH4NO3	1.3±0.2	1.1±0.3	1.7±0.7
$(NH_4)_5[Fe(C_6H_4O_7)_2]$	1.8±0.1	1.5±0.4	2.0±0.3
$(NH_4)_2SO_4$	0.9±0.5	1.1±0.8	1.2±0.6

The effect of different nitrogen sources(ammonium ferric citrate, ammonium sulfate and ammonium nitrate) was investigated using 3% agro waste as the carbon source; it was observed in table 2 that biosurfactant production was in the range of 3.6 – 2.4 g/L and it fluctuate little with the nature of the nitrogen source. However, the growth of the isolate was better with nitrogen sources, urea. It is clearthat there was a steady increase in biosurfactant yield with addition of ammonium ferric citrate as nitrogen source. The highest biosurfactant yield obtained by ammonium ferric citrate with tomato pomace is 2.0±0.3 g/L and the lowest biosurfactant yield obtained by ammonium nitrate with pineapple peel 1.1±0.3 g/L.

Table 3: Effect of temperature on yield of biosurfactant

Temperature (°C)	OPB	PPB	TPB
25	1.5±0.01	1.3±0.4	2.4±0.3
30	2.2±0.3	2.0±0.8	3.1±0.1
37	1.9±0.4	1.7±0.3	2.9±0.7

Data showing in table 3 the effect of incubation temperature indicated that 30°C is the optimum temperature for maximum biosurfactant production (3.1±0.1 g/L with tomato pomace)

Table 4: Effect of pH on yield of biosurfactant

pH	OPB	PPB	TPB
6	1.5±0.8	1.3±0.8	2.1±0.3
7	2.4±0.3	2.4±0.2	3.6±0.5
8	2.0±0.06	1.9±0.5	3.2±0.1

In table 4: Acidic pH showed inhibitory effect on the biosurfactant production, by *B. subtilis* MTCC 2388. The isolate produced 1.5±0.8, 1.3±0.8 & 2.1±0.3 of biosurfactant at pH 6 with, 2.4±0.3, 2.4±0.2 & 3.6±0.5 g/L at pH 7 and 2.0±0.06, 1.9±0.5 & 3.2±0.1g/L at pH 8 with orange peel, pineapple peel and tomato pomace respectively.

Table 5: Effect of inoculum of microbial strain on yield of biosurfactant

Inoculums (%)	OPB	PPB	TPB
1	1.0±0.3	0.8±0.9	2.2±0.4
2	2.3±0.5	2.2±0.7	3.1±0.2
3	0.9±0.2	0.5±0.1	1.5±0.8

The maximum biosurfactant yield of 3.1±0.2 g/L was obtained on employing 2% concentration of the*B. subtilis* MTCC 2388 inoculum with tomato pomace.

4. Conclusion

Use of cheap raw materials for the growth and production is a common strategy, for reducing the production cost of biosurfactants. Orange peel, Pineapple peels and Tomato pomace were used in the production media, for the production of biosurfactant. The yields of biosurfactants with varies in each sample with initial substrate concentration (3%) 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* MTCC 2388. The highest yield of biosurfactants was recorded with tomato pomace substrate it could be explained by the presence of compounds other than carbohydrates particularly lipids in tomato pomace which may have contributed to the increased production of biosurfactants with ammonium ferric citrate as a nitrate compound at pH level 7, 30°C temperature with 2% inoculum is concluded as the best suitable condition for the production of biosurfactants. 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 microbial strain.

5. Acknowledgement

All the research of this paper is done in food technology lab of HBTU, Kanpur, by Author.

References

[1] Desai J.D. and Banat I.M. Microbial production of surfactants and their commercial potential. Microbiology and Molecular Biology Reviews. 1997;61(1):47-64.

[2] George &JayachandranK.., Analysis of Rhamnolipid Biosurfactants Produced Through Submerged Fermentation Using Orange Fruit Peelings as Sole Carbon Source. Applied Journal of Biochemistry and Biotechnology 2009:158:694-705

[3] Guerra-Santos,L.H.,.KappeliO.and FiechterA., Dependence of *Pseudomonas aeruginosa* continuous cultures biosurfactants production on nutritional and environmental factor. Applied Microbiology Biotechnology 1986:24:443-448.

[4] Kulkarni P.P., Kanekar J.P., Jog, S.S., Sarnaik S.S., Production of copolymer, poly (hydroxybutyrate-co-hydroxyvalerate) by Halomonascampisalis MCM B-1027 using agro-wastes. International Journal of Biological Macromolecules2014,.

[5] Mukherjee S, Das P, Sivapathasekaran C and Sen R. Enhanced production of biosurfactant by a marine bacterium on statistical screening of nutritional parameters. Biochemical Engineering Journal. 2008;42:254-260.

[6] Parveen J, Wan M and Md ZA. Optimum Medium Components for Biosurfactant Production by *Klebsiellapneumoniae*WMF02 Utilizing Sludge Palm Oil as a Substrate. Australian Journal of Basic and Applied Sciences. 2012;6(1):100-108.

[7] Rane A. N., Vishakha V. Baikar, V. Kumar R and Deopurkar R L., Agro-Industrial Wastes for Production of Biosurfactant by *Bacillus subtilis* ANR 88 and Its Application in Synthesis of Silver and Gold Nanoparticles. Frontiers in Microbiology 2017:8:492.

[8] Shah M.U.H, Sivapragasam M., Moniruzzaman M., Yusup S.B., A comparison of recovery methods of rhamnolipids produced by *Pseudomonas aeruginosa*. Procedia Engineering2016:148:494-500