Statistical Optimization of Sophorolipids Production using Sunflower Oil By *Starmerella bombicola*

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Abstract-Sophorolipids (SL) is widely used in various industries like environmental, pharmaceutical, cosmetic and food. In this work, sophorolipids production is performed using *Starmerella bombicola*. The effect of media components and its composition is investigated and optimized using Box-Behnken design (BBD). Composition of media components viz. glucose, potato extract, urea and carbon source are optimized. In this work, sun flower oil (SF) is employed as carbon source for SL production. Optimum condition for maximum yield of SL is found. The SL production using sun flower combined media is 35.70 g/l. The statistical parameter values suggest that BBD can be effectively utilized for SL production. The results suggest that sunflower oil can be utilized for SL production using the *Starmerella bombicola*.

Key words: *Sophorolipids, Carbon source, sun flower oil, BBD, Optimization.*

Introduction

Research on bio surfactants has drawn curiosity from all around the globe owing to the prevalent use of these substances in a variety of industries. Paraszkiewicz et al., (2018) found that microbial bio surfactants are better for the environment than chemical surfactants. According to Varjani and Upasani (2017), bio surfactants are unique products that are both biodegradable and relatively harmless. They have excellent foaming ability, high selectivity, and may be used in environments with harsh pH, heat, and salt. Elshikh et al., (2016) classified bio surfactants as either high molecular weight (lipoprotein and polymeric molecules) or low molecular weight (rhamnolipids, trehalolipids, sophorolipids, and lipopeptides). Researchers are required to describe the glycolipids nature of bio surfactant (Fred et al., 2010; Astuti et al., 2019; Dwivedia et al., 2019), and industry have gained preference for lipoproteins and glycolipids (Geys et al., 2014). Amphipathic micro-bio surfactants have hydrophobic and hydrophilic sections that allow them to partition at the interface of oil and water, two immiscible liquid phases with different polarity (Ohadi et al., 2017). Bio surfactants are mainly categorized as fatty acids, polymeric compounds, glycolipids, phospholipids, lipopeptides, and lipoproteins according to their origin and chemical composition (Wang et al., 2018).

Bacillus aryabhattai is used for bio surfactant production by Deepak et al. (2020) using response surface approach. An optimal growth medium was prepared with 4.0% crude oil, 0.7% yeast extract, and 3.0% $NaNO_3$, and the yield was 8.86 g/l. They tested the bio surfactant's stability at temperatures up to 100 °C, pH levels between 5 and 10, and concentrations of NaCl up to 8%. Electron microscopy paired with energy-dispersive X-ray analysis was used to characterize the morphology. Bio surfactant ZDY2 generated by B. aryabhattai strain was identified as a lipopeptide by Fourier-transform infrared spectroscopy. In the medical and pharmaceutical fields, the bio surfactant is useful.

Using response surface methodology (RSM), Yuning Feng et al., (2019) enhanced the fermentation process of sophorolipids. The three main criteria that were determined based on the findings of the preliminary experiment were the temperature before fermentation, the period between temperature shifts, and the temperature after fermentation. Then, in order to maximize sophorolipid yields, response surface approach was used. A very high coefficient of determination (0.9914) and a probability (P) value of less than 0.001 were found in the second-degree equation for the yield of SLs in the regression model. The results demonstrated that a pre-fermentation temperature of 26 °C, a temperature shift duration of 60 h, and a late fermentation temperature of 30 °C were the ideal conditions for the real fermentation production of SLs (Daniel et al., 2007). The sophorolipid yield was 99.08 g/L under these circumstances, an increase of 11.97% over the yield when maintained at a steady temperature alone. In this study, SL production was optimized using RSM and the variables like glucose, potato extract, urea and carbon source were optimized.

Materials and Methods

Materials

The strain *Starmerella bombicola*, was isolated from oil polluted region of CPCL, Chennai, were used in this work. The potato extract and sunflower oil was purchased from Namakkal district Greens supermarket. The glucose, peptone, yeast extract was used in the media formulation was purchased from Himedia chemicals.

Investigation of the Box-Bhenken Model

The Box-Bhenken Design (BBD) test (Box and Behnken, 1960) was used to produce SL for hydrophobic carbon sources such as glucose, urea extracted from potatoes, sunflower oil. The important factors of these experiments were determined using the ANOVA. The data is tabulated according to the quadratic model, and the validated model is examined through the use of R^2, adjusted R^2 and residual plots. Using this data, the best circumstances for producing SL were discovered. The ideal circumstances were validated by conducting more laboratory experiments and comparing the expected and real values. Design expert was used to construct the 3D surface plots for sophorolipid yield.

Optimization and Formulation of Media

Potatoes were obtained from the local market of Tiruchengode district and served as the carbon source. Grocery stores were the source of sunflower oil. Table 1 illustrates the implementation of the Box-Behnken design (BBD) to enhance the production of sophorolipids from these compounds. In addition to the aforementioned sources of nitrogen and carbon that were considered to be enhanced minerals in the media, the media was elevated in nutrients. The following trace elements were incorporated into the media formulation: 5-g/l of yeast extract, 5-g/l of peptone, 0.5 g/l of $MnSO_4.7H_2O$, 0.1 g/l of $CaCl_2$, 0.4 g/l of KH_2PO_4, and 0.1 g/l of $COCl_2$. The Box=Behnken design (BBD) is implemented in sets, employing a 500 ml conical flask, as detailed in rhe Table 1.

Production Fermenter

The media was transferred to a 2.5-liter fermenter that was maintained at a temperature of 25°C, a pH of 7, and agitation at 200 rpm after being decontaminated. The sophorolipids were extracted after a 7-day bulk fermentation, and hexane was employed to filter out any remaining media, trace elements, and impurities. The crude SL was evaporated and processed with a 4:1 volume ratio of methanol to water after ethyl acetate concentration.

Table 1 BBD and the results for SL production

A	B	C	D	SL using SF (expt)	SL using SF (pred)
3	2	0.2	10	29.15	27.45
3	3	0.5	10	25.36	25.52
2	3	0.8	10	30.42	29.89
2	1	0.5	15	30.42	27.20
2	3	0.2	10	25.41	24.80
2	2	0.5	10	33.2	33.20
2	2	0.8	15	30.55	31.66
2	2	0.5	10	33.2	33.20
2	1	0.5	5	16.78	16.34
2	2	0.2	15	26.44	28.17
3	1	0.5	10	19.75	20.12
2	2	0.2	5	25.45	25.30
2	3	0.5	15	22.16	21.95
1	1	0.5	10	18.35	19.15
3	2	0.8	10	30.04	28.88
1	3	0.5	10	20.64	21.23
2	3	0.5	5	28.46	29.06
1	2	0.2	10	21.26	21.77
2	1	0.8	10	25.24	25.54
1	2	0.5	5	22.55	21.60
3	2	0.5	5	24.85	26.56
2	2	0.5	10	33.2	33.20
1	2	0.5	15	27.82	25.80
1	2	0.8	10	28.25	29.31
3	2	0.5	15	25.46	26.10
2	1	0.2	10	21.44	21.66
2	2	0.8	5	31.56	30.79

RESULTS AND DISCUSSION

Optimization of media composition for SL production

Box Behnken design (BBD) is employed for optimization of media components, including glucose, potato extract urea, and sun flower oil, which serve as hydrophobic carbon sources. Table 1 illustrates the findings. An equation for the model of SL production based on the media components employing sun flower oil is provided beneath:

SL Production, g/L = 33.20 + 1.31 A + 1.87 B + 2.24 C +0.94 D + 0.83 AB – 1.53 AC – 1.17 AD + 0.30 BC – 4.49 BD - 0.50 CD - 5.16 A^2 – 6.54 B^2 - 1.19 C^2 - 3.03 D^2

Where A - glucose, B- potato extract, C- urea and D – SF Oil (Carbon source)

The experimental results of SL production are analyzed using ANOVA and are summarized in Table 2. Results show that the model's F value is statistically significant (27.41) (Table 2). Sources of SL production that are presumed to be significant are those with p values less than 0.05 (95% confidence level). Considerable sources for SL production using sun flower oil are shown in table 2 as the square effect of all nutrients, the interactive effects of glucose and urea, the concentration of sun flower oil and potato extract, and the linear effect of all nutrients. The statistical characteristics indicate that the model created by BBD for SL synthesis is good for hydrophobic carbon sources, including a high R^2 (>0.92), low standard deviation, CV value, and others.

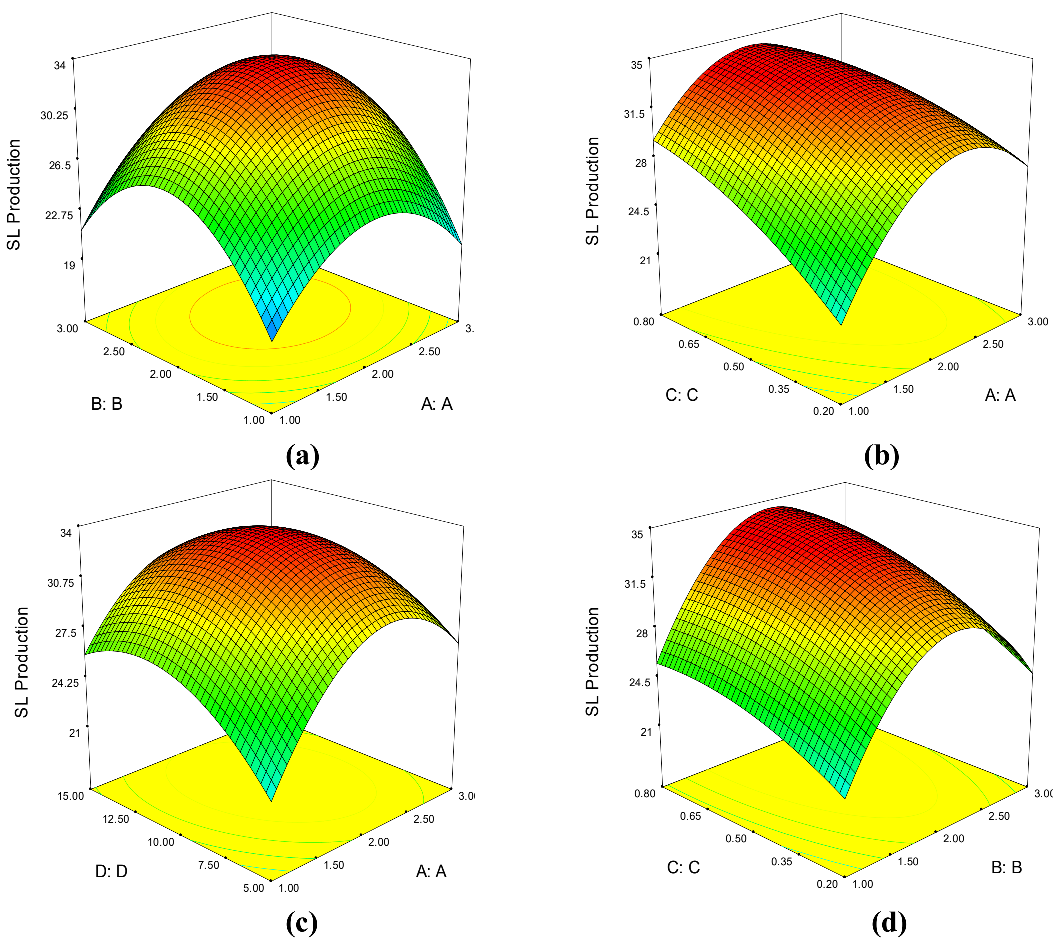
Table 2. ANOVA for SL production

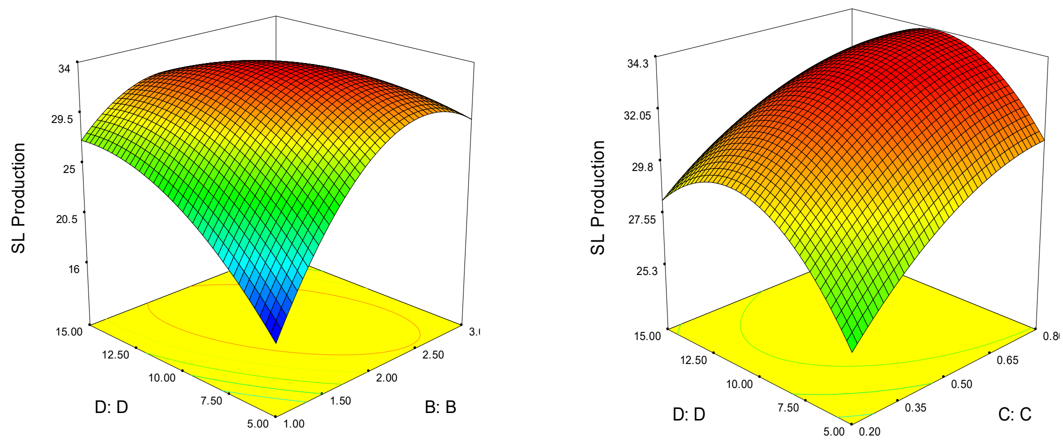
Source	**SF Oil**	
	F Value	**P value**
Model	**27.40941**	**< 0.0001**
A-A	12.61881	0.0032
B-B	25.64807	0.0002
C-C	36.88387	< 0.0001
D-D	6.423453	0.0238
AB	1.68425	0.2153
AC	5.685779	0.0318
AD	3.318197	0.0899
BC	0.223718	0.6435
BD	49.34311	< 0.0001
CD	0.61121	0.4474
A^2	105.4918	< 0.0001
B^2	169.3565	< 0.0001
C^2	5.633951	0.0325
D^2	36.32859	< 0.0001
SD	1.28	
Mean	26.62	
C.V. %	4.81	
PRESS	131.94	
R-Squared	0.9648	
Adj R-Squared	0.9296	
Pred R-Squared	0.7973	
Adeq Precision	18.329	

The optimization results for SL production using *Starmerella bombicola* is depicted in Fig.1. BBD was employed to optimize media preparations for the synthesis of SL, and the obtained results are illustrated in Fig. 1. The optimal composition of the carbon source (glucose and potato extract), secondary nitrogen source (urea), and hydrophobic carbon source (sunflower oil) is achieved to maximize SL production. Fig. 1 (a) demonstrates that the production of SL increased from 20% to 35.85% as the glucose concentration increased from 1 to 2.01%. This suggests that the organism is able to effectively utilize its carbon source and thrive at this glucose level. The microorganism's extraordinary utilization of carbon sources for the production of sophorolipids is revealed in this experimental study.

Figure 1 (b) also indicates that an increase in urea concentration from 1 to 2.19% has resulted in an increase in SL production. Secondary nitrogen sources are indispensable for the organism's proliferation and for the synthesis of sophorolipids. The optimal concentration for the microbial growth and synthesis of SL is determined by experimenting with various urea concentrations. In Fig. 1, the interactive effect of C and A demonstrates that the maximal SL production is achieved when the urea concentration is increased from 0.2 to 0.7939 g/l. In terms of the maximal production of sophorolipids, this is substantiated by Vedaraman and Venkatesh (2010). Additionally, the production is increased to 35.85 g/l when the SF oil concentration is 9.6465%. The SL producing organism requires an oil source to enhance the growth and conversion of lipids to fatty acids (Yoshihiko Hirata et al., 2021). In their published article, Kim, et al. (2021) reported comparable outcomes when applied to residual frying oil. Vinit Bajaj et al. (2012) and Vedaraman and Venkatesh (2010) provide substantial support for the findings of this investigation. The results obtained in this work are comparable with the literature.

In a research article, Yoshihiko Hirata et al. (2021) elucidated these comparable results. The identical experiment was conducted by Davila, et al. (1997) to achieve the highest possible yield using a dual carbon source. The production yield of sophorolipids was approximately 180 g/l, as confirmed by Renata Raianny da Silva et al. (2023).





(e) (f)

Fig. 1. 3D graph showing the interactive effect of variables on SL production using SF oil (a) glucose – PE (b) glucose – Urea (c) Glucose – SF oil (d) PE – Urea (e) PE-SF oil (f) Urea-SF oil

The optimizer tools in Minitab is employed to determine the optimal values for the composition of the media components. Fig. 2 (a) illustrates the optimal condition for the highest possible production of SL. The optimal values are as follows: 0.7939 g/l urea, 2.01 % glucose, 2.19% potato extract, and 9.6465% sun flower oil. Experiments are conducted at the optimal values of media components, and the maximal SL production is determined to be 35.70 g/l.

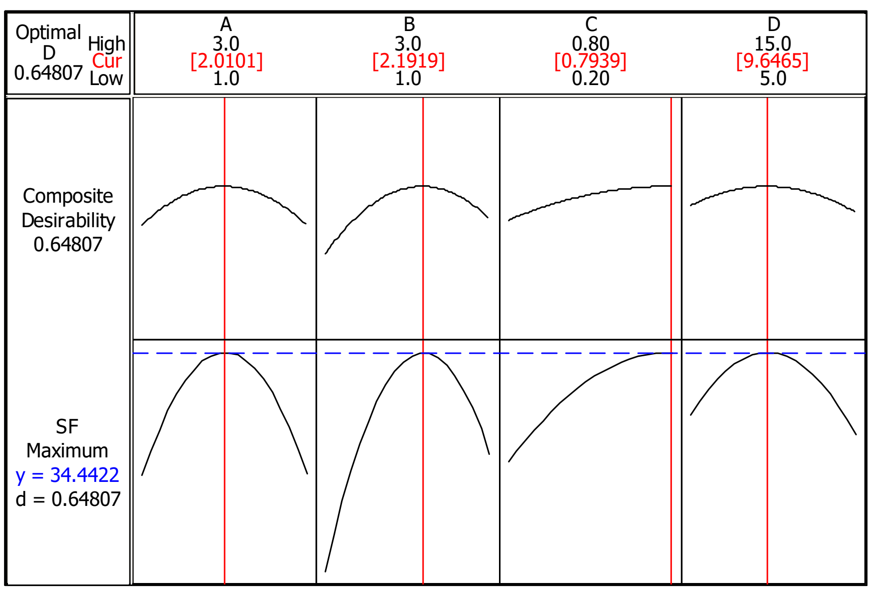


Fig.2. Optimizer tool show the optimum values for SL production

Conclusion

In this investigational study, Box-Behnker Design (BBD) optimizes substrates, sun flower oil (SF), in conjunction with carbon sources such as glucose, potato extract, urea, and hydrophobic carbon source for the formation of SL. After conducting an analysis, BBD determined the ideal parameters for achieving the highest yield SL for hydrocarbon sources.

The greatest yield of SL was identified by comparing the expected and actual values. Under ideal circumstances, sun flower oil and glucose combination medium, the yield SL production is 35.70 g/l. According to the outcomes, sunflower oil can be used to produce SL by *Starmerella bombicola*. The values of the statistical variables demonstrate that BBD can be implemented in SL production in an efficient manner.

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