ORIGINAL ARTICLE

Statistical optimization of medium components for biosurfactant production by *Achromobacter xylos GSR21*

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Abstract

Introduction: Biosurfactants have picked up an impressive consideration as of late due to their potential uses in an expansive scope of use territories, including environmental remediation, agriculture, biofilm formation, quorum sensing, textile, pharmaceuticals, cosmetics, and the food, oil, and petrochemical industries. **Aim:** In the present study, optimization of the critical medium components for biosurfactant production by *Achromobacter xylos* strain GSR21 using statistical experimental design was studied. **Materials and Methods:** Response surface methodology (RSM) was employed to determine the optimal level of the four medium variables (agar powder, yeast extract, $FeSO_4 7H_2O$, and KH_2PO_4). Central composite design of RSM was applied to study the four variables at five levels, and biosurfactant concentration was measured as a response. **Results:** Regression coefficients were calculated by regression analysis and the model equation was determined. R^2 value for biosurfactant (g/L) was calculated as 72%, and it indicates that the model was well fitted with the experimental results. Surface plots were made, and the maximum biosurfactant production (*A. xylos* strain GSR21) (10.20 g/L) was predicted at the optimized values of agar powder 90 g/L, yeast extract 5 g/L, $FeSO_4 7H_2O$ 0.05 g/L, and KH_2PO_4 0.15 g/L. The obtained mathematical model was verified by performing the experiment with the predicted optimized values, and the yield of bio-surfactant was found to be 9.69 g/L. Validation of the predicted model was fitted 96.9% with the experimental results conducted at the optimum conditions. **Conclusion:** Results of this statistical analysis showed that agar powder and yeast extract had found significant medium components for biosurfactant (*A. xylos* GSR21) production.

Key words: *Achromobacter xylos,* biosurfactant, central composite design, response surface methodology

INTRODUCTION

Surfactants are generally natural intensifies that are amphiphilic in nature containing both hydrophobic and hydrophilic gatherings, and it is utilized to bring down the interfacial tension between two fluids.[1-3] Surfactants may go about as detergents, wetting, emulsifiers, foaming agents, and dispersants.[4] Biosurfactants got from microorganisms are observed to be better interchange for the synthetic surfactants. They are complex particles that can be grouped in light of various structures that incorporate lipopeptides, glycolipids, polysaccharide-protein buildings, unsaturated fats, and phospholipids.[5] The real points of interest of utilizing biosurfactants are biodegradability, low toxicity and can be created from inexhaustible and less expensive substrates.[6] Biosurfactants are fundamentally utilized for bioremediation to treat hydrocarbon contaminated destinations and furthermore for oil recuperation. They are likewise utilized as one of the fixings in the definition of pesticides, medicinal services, and beautifiers, mash and paper, and nourishment ventures.[7,8] Microorganisms, for example, *Bacillus subtilis, Bacillus licheniformis, Bacillus pumilis*, and *Pseudomonas putida* are equipped for delivering biosurfactant.[9-12] Lipopeptides got

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Table 1: Range of variable levels for RSM experiment

Factors (g/L)	Symbol	2	1	0	−1	−2
Agar powder	A	70	60	50	40	30
Yeast extract	B	7	6	5	4	3
$FeSO_4.7H_2O$	C	0.06	0.055	0.05	0.045	0.04
KH_2PO_4	D	0.25	0.2	0.15	0.1	0.05

RSM: Response surface methodology

Table 2: Central composite design matrix with the experimental and predicted values of biosurfactant produced by *Achromobacter xylos* strain GSR21

Run order	Medium components				Biosurfactant (g/L)		
	A	B	C	D	Experimental	Predicted	Residual
1	30	3	0.04	0.05	7.33	7.98	−0.65
2	70	3	0.04	0.05	8.53	9.79	−1.26
3	30	7	0.04	0.05	8.67	9.34	−0.67
4	70	7	0.04	0.05	9.33	8.26	1.07
5	30	3	0.06	0.05	5.33	5.33	0.00
6	70	3	0.06	0.05	9.33	7.98	1.35
7	30	7	0.06	0.05	7.33	6.63	0.70
8	70	7	0.06	0.05	5.33	6.38	−1.05
9	30	3	0.04	0.25	5.33	5.08	0.25
10	70	3	0.04	0.25	7.33	7.73	−0.40
11	30	7	0.04	0.25	6.58	7.62	−1.04
12	70	7	0.04	0.25	6.57	7.37	−0.80
13	30	3	0.06	0.25	4.55	5.32	−0.77
14	70	3	0.06	0.25	8.67	8.80	−0.13
15	30	7	0.06	0.25	8.25	7.79	0.46
16	70	7	0.06	0.25	9.33	8.38	0.95
17	10	5	0.05	0.15	8.53	7.92	0.61
18	90	5	0.05	0.15	10.2	10.32	−0.12
19	50	1	0.05	0.15	6.35	5.79	0.56
20	50	9	0.05	0.15	6.67	6.73	−0.06
21	50	5	0.03	0.15	8.67	7.17	1.50
22	50	5	0.07	0.15	4.53	5.53	−1.00
23	50	5	0.05	−0.05	8.67	8.66	0.01
24	50	5	0.05	0.35	8.25	7.76	0.49
25	50	5	0.05	0.15	7.33	8.04	−0.71
26	50	5	0.05	0.15	9.33	8.04	1.29
27	50	5	0.05	0.15	7.33	8.04	−0.71
28	50	5	0.05	0.15	8.25	8.04	0.21
29	50	5	0.05	0.15	7.33	8.04	−0.71
30	50	5	0.05	0.15	8.67	8.04	0.63

from *B. subtilis* are especially intriguing a result of their high surface action and restorative potential.[13,14]

Optimization of medium and fermentation conditions is a basic advance in bioprocess improvement, and it includes a few variables.[15] One factor at a time optimization is accepted method; however, it has numerous weaknesses such as more trial runs and time.[16] Response surface method (RSM) is an accumulation of measurable devices to outline and examinations the analyzes concentrated

Table 3: ANOVA statistics for biosurfactant production by *Achromobacter xylos* GSR21

Factors	Sum of squares	df	Mean squares	F value	p-value	Significance
Model	48.10	14	3.44	2.79	0.0292	Significant
A-Agar powder	8.63	1	8.63	6.99	0.0184	Significant
B-Yeast extract	1.32	1	1.32	1.07	0.3172	
C- $FeSO_4.7H_2O$	4.03	1	4.03	3.26	0.0909	Significant
D- KH_2PO_4	1.22	1	1.22	0.99	0.3359	
AB	8.40	1	8.40	6.81	0.0198	Significant
AC	0.70	1	0.70	0.57	0.4625	
AD	0.69	1	0.69	0.56	0.4651	
BC	0.00	1	0.00	0.00	0.9523	
BD	1.39	1	1.39	1.12	0.3058	
CD	8.31	1	8.31	6.74	0.0203	Significant
A^2	1.99	1	1.99	1.61	0.2235	
B^2	5.42	1	5.42	4.39	0.0535	Significant
C^2	4.88	1	4.88	3.96	0.0652	Significant
D^2	0.05	1	0.05	0.04	0.8420	
Residual	18.50	15	1.23			
Lack of fit	14.89	10	1.49	2.06	0.2203	Not significant
Pure error	3.62	5	0.72			
Cor total	66.61	29				

ANOVA: Analysis of variance

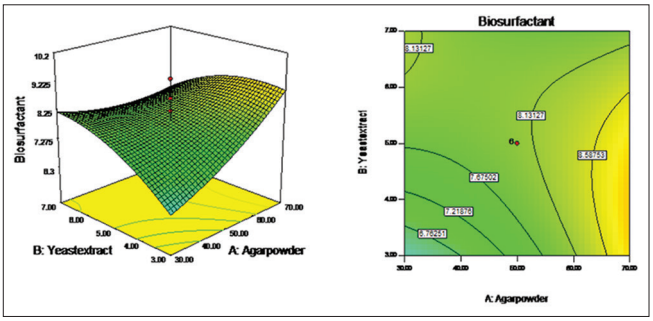

Figure 1: Three-dimensional and contour surface plots showing the mutual effect between pair of variables agar powder (A) and yeast extract (B) on biosurfactant production

on improvement.[17] RSM is effectively utilized to decide the ideal states of the chose factors associated with the procedure.[9,18,19] The fundamental preferred standpoint of utilizing RSM is to assess the cooperation impact of the factors under investigation with the assistance of reaction surface plots produced by the product.

The objective of this study is to determine the optimal levels of the medium components for biosurfactant production from *Achromobacter xylos* strain GSR21 by RSM.

MATERIALS AND METHODS

Microorganism

The microorganism *A. xylos* GSR21 used in this study was obtained from Environmental Microbiology Laboratory culture collection of the Department of Biotechnology at K L University Andhra Pradesh, India. The culture was maintained in Luria Bertani (LB) Agar plates incubated at 30°C and subcultured at regular intervals. Inoculums were prepared by transferring a loopful of culture to 100 mL of sterilized LB broth and kept in a rotary shaker incubator at 200 rpm at 30°C for 48 h. All the chemicals used in the study are of analytical grade and procured from quality-control, Hyderabad, India.

Fermentation Conditions

Nearly 2% (W/V) of the seed culture was inoculated in the production media containing (g/L): Glycerol, 5 g; asparagine 1 g; KH_2PO_4, 1 g; $MgSO_4.7H_2O$, 5 g; KCl, 1.0 g; agar powder, 15 g; and 1 mL of trace solution containing (in 1 L of distilled water) $MgSO_4.7H_2O$, 0.5 g, $CuSO_4.5H_2O$, 0.16 g, and $FeSO_4.7H_2O$, 0.015 g. The initial pH of the medium was

adjusted to 8.0.[20] All fermentations were carried out at 30°C in shaker flask held on a rotary platform shaker at 200 rpm. For statistical optimization experiments, 100 mL of medium was prepared in 250 mL conical flask according to the central composite design (CCD) given in Table 1.

Biosurfactant Precipitation

About 1.5 mL of fermented broth was collected in 2 mL Eppendorf tube and centrifuged at 10,000 rpm for 10 min. After centrifugation, the supernatant was used for the extraction of biosurfactant. 6N HCl was added in the Eppendorf containing supernatant and kept it for overnight incubation. Then, the sample was centrifuged at 6000 rpm for 10 min, and the precipitated biosurfactant was collected in the form of pellet. The precipitated biosurfactant was dried in hot air oven at 80°C for overnight and weight of the crude biosurfactant was determined.

Experimental Design

Four medium variables (agar powder, yeast extract, $FeSO_4.7H_2O$, and KH_2PO_4) were selected for RSM optimization studies based on preliminary screening studies. The range of the level of four variables was given in Table 1. 30 experiments were carried out according to CCD shown in Table 2. The relationship between the variables and the response is generally represent by the second order polynomial equation (Eqn. 1).

$$Y = \begin{aligned} &\acute{a}_0 + \acute{a}_1 X_1 + \acute{a}_2 X_2 + \acute{a}_3 X_3 + \acute{a}_4 X_4 + \\ &\acute{a}_{11} X_1^2 + \acute{a}_{22} X_2^2 + \acute{a}_{33} X_3^2 + \acute{a}_{44} X_4^2 + \\ &\acute{a}_{12} X_1 X_2 + \acute{a}_{13} X_1 X_3 + \acute{a}_{14} X_1 X_4 + \\ &\acute{a}_{23} X_2 X_3 + \acute{a}_{24} X_2 X_4 + \acute{a}_{34} X_3 X_4 \end{aligned} \quad (1)$$

RESULTS AND DISCUSSION

Response Surface Optimization

Statistical optimization for biosurfactant production was carried out according to the CCD of RSM using Design expert software. The response, biosurfactant concentration was estimated for 30 experiments and represented in Table 2. The response data were subjected to regression analysis to estimate the regression coefficient. The estimated coefficients were presented in Table 3 and a second-order polynomial equation (final equation in terms of coded factors) (Eqn. 2) and final equation in terms of actual factors (Eqn.3) for biosurfactant production was constructed using the coefficients.

$$Y_{Biosurfactant(\frac{g}{L})} = \begin{aligned} &+8.04 + 0.60A + 0.23B - 0.41C - 0.23D - 0.72AB + \\ &0.21AC + 0.21AD - 0.017BC + 0.29BD + 0.72CD + \\ &0.27A^2 - 0.44B^2 - 0.42C^2 + 0.043D^2 \end{aligned} \quad (2)$$

Final Equation in Terms of Actual Factors

$$\begin{aligned} Y\_(\text{Biosurfactant}\ (\tfrac{g}{L})) = {} & 1.96086 - 0.014745 \times \text{Agarpowder} \\ & +1.95536 \times \text{Yeast extract} + 224.80208 \\ & \times FeSO_4 7H_2O - 52.13854 \times KH_2PO_4 \\ & -0.018109 \times \text{Agarpowder} \times \text{Yeast extract} \\ & +1.04687 \times \text{Agarpowder} \times FeSO.7H_2O \\ & +0.10406 \times \text{Agarpowder} \times KH_2PO_4 \\ & -0.84375 \times \text{Yeast extract} \times FeSO_4.7H_2O \\ & +1.47188 \times \text{YeastYeast extract} \\ & \times KH_2PO_4 + 720.62500 \times FeSO_4.7H_2O \\ & \times KH_2PO_4 + 0.000673177 \times (\text{Agarpowder})^2 \\ & -0.11112 \times (\text{Yeast extract})^2 - 4219.79167 \\ & \times (FeSO_4.7H_2O)^2 + 4.302081 (KH_2PO_4)^2 \end{aligned} \quad (3)$$

The adequacy of the model was checked using analysis of variance, and the results were shown in Table 3. The model F=2.79 implies the model is significant. There is only a 2.92% chance that a "model F-value" this large could occur due to noise. The high value of F-test for regression indicating that the model is fit well and can adequately explain the variation observed in biosurfactant concentration with the designed levels of variables. Probability value ($p<0.0500$) is usually used to check the statistical significance of the parameters. Results represented in Table 3 explained that the individual effect of agar powder (A), agar powder*yeast extract (AB), $FeSO_4.7H_2O$* KH_2PO_4 (CD), and square effect of yeast extract (B^2), and $FeSO_4.7H_2O$ (C^2) was found significant in the production of biosurfactant. R^2 value was observed as 0.7222, and this value shows that the model was fitted for 72.2% of biosurfactant production. These results showed that the model chosen can satisfactorily explain the linear effects and square effects of the variables selected for the biosurfactant production.

Figure 1 represents the combined effect of agar powder and yeast extract, and maximum biosurfactant production (10.2 g/L) was observed at the low level of yeast extract (4.53 g/L). There was a significant increase in the product concentration when agar powder concentration increased from 30 g/L to 70 g/L Makkar and Cameotra; Kumar *et al.*[21,22] reported that agar powder was most suitable carbon source for biosurfactant production by *glycolipid* among the other carbohydrates studied. Several researchers concluded that the presence of yeast extract in low concentration increases the biosurfactant synthesis.[22,23] Supplementation of yeast extract (4 g/L) in the production medium was sufficient for enhancing biosurfactant production as the amino acids are required for the formation of the glycolipid biosurfactant by *A. xylos* GSR21 Casas and García-Ochoa; Kumar *et al.*[22,24] also reported that a low level of yeast extract enhances the biosurfactant production.

Figure 2 demonstrated that increase in both agar powder and $FeSO_4.7H_2O$ improves the biosurfactant production. It was observed that the $FeSO_4.7H_2O$ in the medium plays a significant role in productivity. When agar powder concentration increases from low to high level, the productivity was also increased whereas an increase in concentration of KH_2PO_4 does not show any impact in the biosurfactant production [Figure 3].

From Figure 4, it was observed that the production of biosurfactant decreased when the yeast extract increased from low to high level stating that 4.53 g/L is sufficient for optimum productivity, whereas the productivity increased when the concentration of $FeSO_4.7H_2O$ increased from low to high level.

In Figure 5, biosurfactant production was decreased when yeast extract concentration increased from low to high whereas static condition is prevailed in KH_2PO_4 indicating the contribution for biosurfactant production by KH_2PO_4 is minimum. It is observed that the productivity of biosurfactant increased when the concentration of ferrous sulfate increased from low to high [Figure 6].

Point prediction tool of design expert software was used to determine the optimal level of each variable in the process. The maximum biosurfactant concentration (10.20 g/L) was predicted by the software at an optimal level of agar powder - 90 g/L, yeast extract - 5 g/L, $FeSO_4.7H_2O$ - 0.055 g/L, and KH_2PO_4-0.15 g/L.

Model Validation

To check the accuracy of the predicted model, experiments were carried out at the predicted optimal concentration of agar powder - 90 g/L, yeast extract - 5 g/L, $FeSO_4.7H_2O$ - 0.055 g/L, and KH_2PO_4-0.15 g/L. In the validation experiment, maximum biosurfactant concentration of 9.69 g/L was obtained. The time course profile of biosurfactant and biomass production by *A. xylos* GSR21 at predicted optimal level of the medium components is shown in Figure 7. The validation result indicates that predicted model was fitted 96.9% with the experimental results.

CONCLUSION

RSM was successfully applied to optimize the four media components to enhance the biosurfactant production. Four variables (agar powder, yeast extract, $FeSO_4.7H_2O$, and KH_2PO_4) were optimized according to the CCD of RSM. Surface plots were made and the optimized values obtained for the maximum production of biosurfactant were agar powder - 90 g/L, yeast extract - 5 g/L,

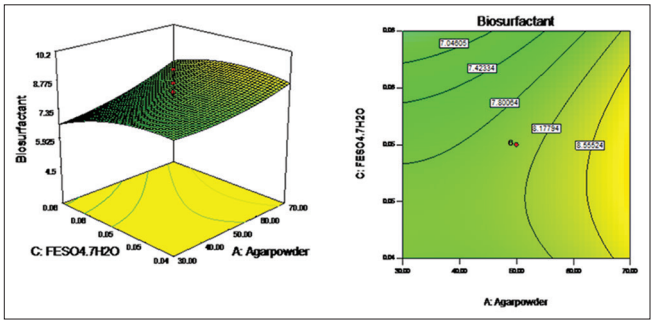

Figure 2: Three-dimensional and contour surface plots showing the mutual effect between pair of variables agar powder (A) and $FeSO_4.7H_2O$ (C) on biosurfactant production

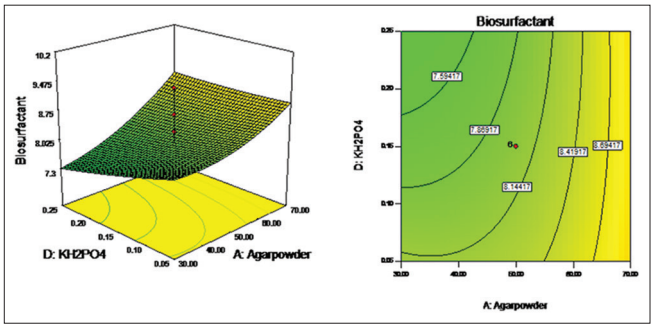

Figure 3: Three-dimensional and contour surface plots showing the mutual effect between pair of variables agar powder (A) and KH_2PO_4 (D) on biosurfactant production

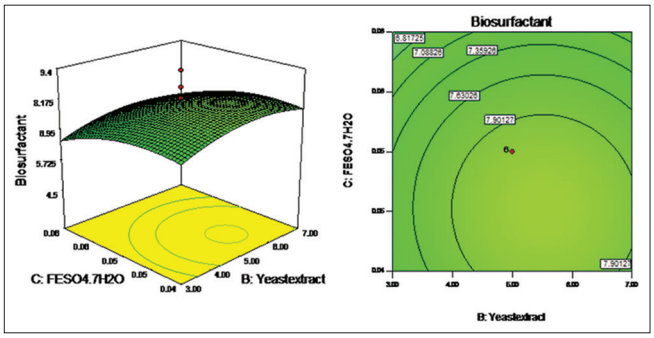

Figure 4: Three-dimensional and contour surface plots showing the mutual effect between pair of variables yeast extract (B) and $FeSO_4.7H_2O$ (C) on biosurfactant production

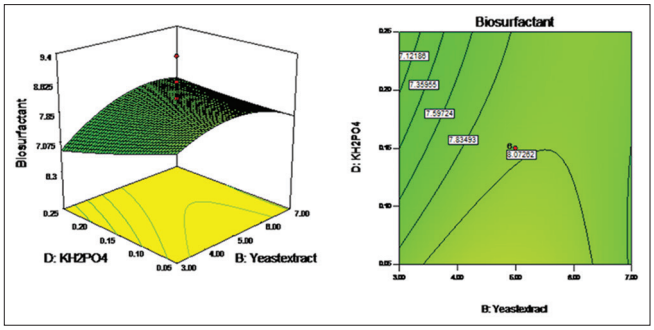

Figure 5: Three-dimensional and contour surface plots showing the mutual effect between pair of variables yeast extract (B) and KH_2PO_4 (D) on biosurfactant production

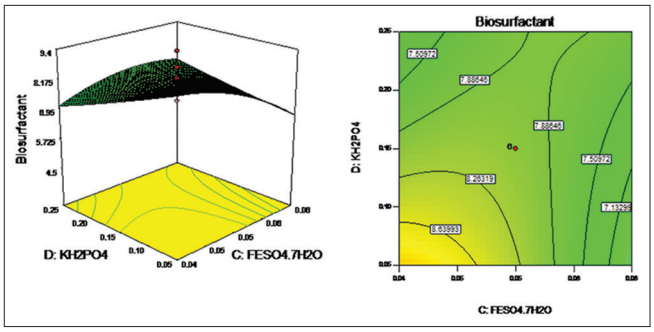

Figure 6: Three-dimensional and contour surface plots showing the mutual effect between pair of variables $FeSO_4.7H_2O$ (C) and KH_2PO_4 (D) on biosurfactant production

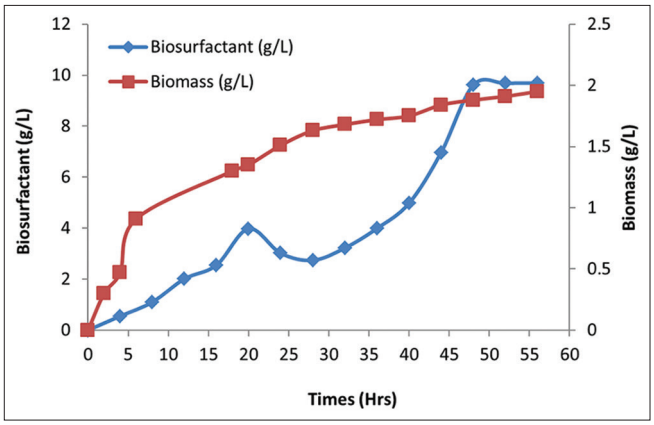

Figure 7: Time course profile of biosurfactant and biomass production by *Achromobacter xylos* GSR 21 predicted the optimal level of the selected medium components in the validation experiment

$FeSO_4.7H_2O$ - 0.055 g/L, and KH_2PO_4-0.15 g/L. Validation of the experiment was performed, and it indicates that the model was well fitted with the experimental results. Application of RSM illuminates the optimal levels for enhanced production of biosurfactant with less experimental runs and interaction effects of the variables.

AUTHORS' CONTRIBUTIONS

GSR and BM conceived the study. GSR carried out the laboratory analysis. GSR, BM, and RSR participated in the study design and coordination and drafting of the manuscript. All authors read and approved the final manuscript.

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COMPETING INTEREST

The authors declare that they have no competing interests.

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