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Statistical Approach to Optimize Production of Biosurfactant by *Achromobacter Xylos*

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Abstract: This study aimed to optimize medium composition biosurfactant production of achromobacter xylos using response surface quadratic model . Lipoprotein and lipopeptides are used in many industries such as petroleum refining, pharmaceutical, mining, agriculture and bioprocess industries. The point of this assessment was to pull out and portray the biosurfactant passing on restriction of microorganisms from oil corrupted soil and considering their advancement energy at various temperatures and pH. The separation and growth study was directed in MSM medium using lamp fuel oil as sole carbon hotspot for bacterial turn of events. Confined strains were found to be Gram positive bacillus and in general Gram's positive minuscule life forms can convey lipopeptides type biosurfactants. The ideal conditions for achromobacteria xylos growth were discovered to be at pH seven (7) and temperature 30°C. Central composite design (CCD) was utilized to pick the following medium components ($MgSO_4$, $NaNO_3$, $CaCl_2$, $(NH_4)_2SO_4$, $FeSO_4$, and KH_2PO_4). Central composite arrangement (CCD) of RSM was utilized to analyze the four parts at five stages, and biosurfactant fixation was evaluated as reaction. Backslide coefficients were directed by backslide examination, and the quadratic model condition was settled. R^2 an impetus for bio-surfactant was endeavored to be 0.7527, showing that the quadratic model was basic with the exploratory outcomes. Confirmation of the numerical model was driven by playing out the assessment with the normal overhauled values, and bio-surfactant production was found to be 10.53 g/L. Underwriting of the normal quadratic model was 97.3% exact with the test results facilitated under the ideal conditions. $CaCl_2$, $(NH_4)_2SO_4$, $FeSO_4$, and KH_2PO_4 were perceived as successful portions for bio-surfactant delivering 98% of achromobacter xylos microorganism.

Keywords: *Lipoprotein and Lipopeptide*, Biosurfactant Production, Response Surface Model, Central Composite Design, Surface Tension.

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1. INTRODUCTION

Lipoprotein and Lipopeptide are amphiphilic fabricates present in living surfaces, for the basic piece on microbial cell surfaces or passed on extracellular hydrophobic and hydrophilic moieties that present the adaptability to total between fluid stages, beginning now and for a significant length of time lessening surface and interfacial bear the surface and interface independently [1-10].They need the name property of lessening the surface and interfacial strain using close to instruments as passed on mixed surfactants . Surfactants are the incredible enhancements found in made blends and designed substances with the adaptability to hoard at the air-water interface and are typically wont to detach smooth materials from a particular media by excellence of the way that they will amass fluid dissolvability of Non-Fluid Phase Liquids (NAPLS) by diminishing their surface/interfacial suffer air–water bundles oil interfaces [6-15]. Lipoprotein and Lipopeptide are on a really essential level portrayed by their substance structure and their microbial beginning. The standard classes of Lipoprotein and Lipopeptide are glycolipids, phospholipids, polymeric biosurfactants and lipopeptides (surfactin)[15-20]. The predominant standard glycolipids are rhamnolipids, sophorolipids and trehalose lipids [21-30]. Surfactants are thoroughly utilized for present day, making, food, beautifiers and medications application regardless by a wide edge. An immense piece of those mixes are blended misleadingly and possibly cause regular and toxicology issues because of the unmanageable and suffering nature of those substances[31-35]. With current advances in biotechnology, thought has been paid to the decision of normal great cycles for making various kinds of biosurfactants from microorganisms[35-40]. Lipoprotein and lipopeptides are produced by yeast, fungi and bacteria but most of the surfactants are produced from bacteria[25-31]. They are surface active compounds which can be produced by various microbes. This is used to reduce surface and interfacial tension between two miscible and immiscible components. Lipoprotein and lipopeptides are used in the fields such as food processing, pharmacy, petroleum refining, petrochemicals, bioprocess engineering, washing agents and many household detergents[38-39] etc. Biosurfactants are amphiphilic, containing two areas, a polar (hydrophilic) moiety and a non-polar (hydrophobic) gathering. The hydrophilic part includes mono-, oligo-, or polysaccharides, peptides or proteins while the hydrophobic moiety generally contains inundated, unsaturated and hydroxylated unsaturated fats or oily alcohols[17,36]. Biosurfactants expect various parts including expanding the surface region and bioavailability of hydrophobic water-insoluble substrates, conclusive of huge metals, lion's offer perceiving and biofilm fromation[26,36]. Differentiated and fabricated surfactants, biosurfactants have higher surface activity, cut down toxic quality, higher biodegradability and better normal comparability 27[,36]. With their high surface activity and biological similitude, biosurfactants are comprehensively used as a piece of characteristic applications, for instance, for development of oil debasement[17,36], as malignancy anticipation specialists, as antimicrobials in the magnificence care items industry[29] and as antagonistic to concretes against a couple of microorganisms and yeasts in restorative applications. In oil taking care of, a couple in any case not omnipotent oil-ruining minute living creatures make extracellular biosurfactants to engage microbial oil take-up and debasement by emulsifying the hydrocarbon[14]. Surfactants and biosurfactants can create the pseudo-dissolvability of oil partitions in water[19]. Additionally, biosurfactants can be just about as convincing as fabricated invention surfactants due to their high explicitness and their biodegradability[41]. The objective of this study is to optimize medium composition biosurfactant production of achromobacter xylos using response surface quadratic model .

2. MATERIALS AND METHODS

2.1 *Microorganism*

The achromobacter xylos used in this study was procured from Bioprocess arranging Laboratory culture gathering of the Biosciences and Biotechnology Department at K L Deemed to be University, andhrapradesh, India. The way of life was kept up in LB agar plates at 37°C and sub refined at customary reaches. Inoculums were set up by moving a loopful of culture to 100 mL of cleaned LB medium stock and kept in a rotational shaker at 200 rpm at 30°C and 35°C for 48 h. All of the made substances utilized in the appraisal are of proficient evaluation and procured from Quality-control, India.

2.2 *Experimental Design*

Verifiable upgrade for biosurfactant production was executed by central composite arrangement of RSM utilizing design ace programming. The response, biosurfactant creation was reviewed for thirty assessments. The backslide information was introduced to backslide examination to assess backslide coefficients. The assessed coefficients were introduced in Table.4 and a second sales polynomial condition (Final Equation in Terms of Coded Factors) (Eqn. 1) and Final Equation in Terms of Actual Factors (Eqn.2) for biosurfactant production was made by utilizing the coefficients.

$$Y = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_4 X_4 + \alpha_{11} X_1^2 + \alpha_{22} X_2^2 + \alpha_{33} X_3^2 + \alpha_{44} X_4^2 + \alpha_{12} X_1 X_2 + \alpha_{13} X_1 X_3 + \alpha_{14} X_1 X_4 + \alpha_{23} X_2 X_3 + \alpha_{24} X_2 X_4 + \alpha_{34} X_3 X_4 \quad (1)$$

3. RESULTS AND DISCUSSIONS

3.1 *Response Surface Optimization*

Genuine upgrade for biosurfactant creation was executed by central composite arrangement of Response surface model utilizing Design ace programming. The response, biosurfactant creation was reviewed for thirty assessments. The correlation between the coded values of the input variables and the actual values was defined in equation described elsewhere[28-35]. The backslide information was introduced to backslide examination to assess backslide coefficients. The assessed coefficients were introduced in Table.3 and a second sales polynomial condition (Final Equation in Terms of Coded Factors) (Eqn. 1) and Final Equation in Terms of Actual Factors (Eqn.2) for biosurfactant creation was made by utilizing the coefficients.

$$production = 7.92 - 2.90 * A + 3.56 * B - 0.23 * C + 6.87 * A * B - 1.21 * A * C + 3.00 * B * C - 3.23 * A^2 - 3.70 * B^2 + 1.36 * C^2 \quad (2)$$

3.2 *Final Equation In Terms Of Actual Factors*

$$production = 34.12819 - 981.01361 * CaCl_2 - 15.01904 * KH_2PO_4 - 36.66723 * (NH_4)_2PO_4 + 54995.50320 * CaCl_2 * KH_2PO_4 - 6445.42533 * CaCl_2 * (NH_4)_2PO_4 + 32.00489 *$$

KH_2PO_4 * $(NH_4)_2PO_4$ - 1.29374 E + 007 * $(CaCl_2)^2$ - 59.24709 * $(KH_2PO_4)^2$ + 9.65593 * $((NH_4)_2PO_4)^2$ (3)

3.3 ANOVA Analysis

The adequacy of the model was checked by assessment of distinction (ANOVA) and the outcomes appeared in Table 2. The Model F-assessment of 2.79 instigates the model is basic. There is just a 2.92% possibility that a "Model F-Value" this enormous could occur because of unsettling influence. High appraisal of F-test for backslide showing that the model is fit well and would have the option to clarify the grouping saw in biosurfactant center with the organized degrees of components. Probability regard ($p<0.0500$) is generally used to check the quantifiable imperativeness of the cutoff points. Results tended to in Table 1 clarified that the individual impact of $CaCl_2$ (A), $CaCl_2$*KH_2PO_4 (AB), KH_2PO_4*$(NH_4)_2PO_4$ (BC) and square impact of $KH_2PO_4^2$ (B^2) and $(NH_4)_2PO_4^2$ (C^2) were discovered in the creation of biosurfactants. R^2 regard was seen as 0.7527 which showed that the model was fitted for 75.27% of biosurfactant creation. These results showed that the model picked can acceptably explain the straight effects and square effects of the components decided for the biosurfactant creation. Response surface methodology (RSM) has been effectively employed to reduce the production cost of biosurfactants through the selection of balanced proportions of the constituents of the culture medium and the optimization of culture conditions[15-25]

Table 1: RSQM with experimental values of lipoprotein and lipopeptides biosurfactant produced from contaminated soil

Std	Run	Factor 1 A:$CaCl_2$ g/L	Factor 2 B:KH_2PO_4 g/L	Factor 3 C:$(NH_4)_2PO_4$ g/L	Response 1 Surface Tension mN/m	Response 2 Production g/L
16	1	0.0015	0.99	1.25	70.25	10.53
14	2	0.0018	0.86	1.35	56.32	7.17
3	3	0.0013	0.77	1.48	41.15	10.32
8	4	0.0014	0.93	0.95	65.42	7.76
6	5	0.0012	0.86	1.13	57.36	8.04
4	6	0.0016	0.99	0.95	79.35	7.64
18	7	0.0013	1.00	0.84	57.52	3.08
1	8	0.0017	0.94	1.08	70.36	9.79
2	9	0.0013	0.77	1.00	62.34	8.32
17	10	0.0012	0.72	1.19	59.21	7.37
19	11	0.0010	0.90	0.87	49.11	3.26
20	12	0.0014	0.68	1.29	58.64	8.73
9	13	0.0018	0.82	0.94	64.68	7.98
15	14	0.0012	0.95	1.11	59.58	4.34
5	15	0.0011	0.83	0.77	59.08	8.87
7	16	0.0014	0.75	1.20	54.31	8.15
10	17	0.0013	0.83	0.78	67.24	7.37
11	18	0.0012	0.59	0.76	58.85	9.33
13	19	0.0015	0.66	0.97	54.88	7.59
12	20	0.0017	0.84	1.03	61.25	6.98

Table 2: ANOVA statistics for Lipoprotein and Lipopeptide production from contaminated soil

Source	Sum of squares	df	Mean Square	F - value	P - value	Significance
Model	59.01	9	6.56	3.38	0.0355	significant
A-$CaCl_2$	7.93	1	7.93	4.09	0.0706	
B-KH_2PO_4	9.20	1	9.20	4.74	0.0544	
C-$(NH_4)_2PO_4$	0.12	1	0.12	0.061	0.8094	
AB	12.84	1	12.84	6.62	0.0277	
AC	0.81	1	0.81	0.42	0.5334	
BC	5.84	1	5.84	3.01	0.1132	
A^2	5.27	1	5.27	2.72	0.1302	
B^2	8.12	1	8.12	4.19	0.0678	
C^2	2.36	1	2.36	1.22	0.2958	
Residual	19.39	10	1.94			
Cor Total	78.40	19				

df=Degrees of freedom,F-Value=F-Test value,P-Value=P test value

The "Model F-regard" of 3.38 construes the model is immense. There is simply 3.55 % chance that a "Model F-regard" this immense could happen due to upheaval. Assessments of "Prob > F" under 0.0500 show model terms are basic. For the present circumstance AB are immense model terms. Characteristics more critical than 0.1000 show the model terms are not gigantic. In case there are various unimportant model terms (excluding those expected to help hierarchy), model lessening may improve your model

Table 3: Coefficients of Lipoprotein and Lipopeptide production from contaminated soil			
Std.Dev.	**1.39**	**R-Squared**	**0.7527**
Mean	7.63	Adj R-Squared	0.5302
C.V.	18.25	Pred R-Square	0.8259
PRESS	143.15	Adeq Precision	7.356

Std.Dev.=Standard deviation, R-Squared=R^2, C.V.=Variant coefficient

A negative "Pred R-Squared" deduces that the overall mean is an unrivaled marker of your response than the current model. "Adeq Precision" checks the sign to racket extent. An extent more essential than 4 is desirable.Your extent of 7.356 shows an adequate sign. This model can be used to investigate the arrangement space.

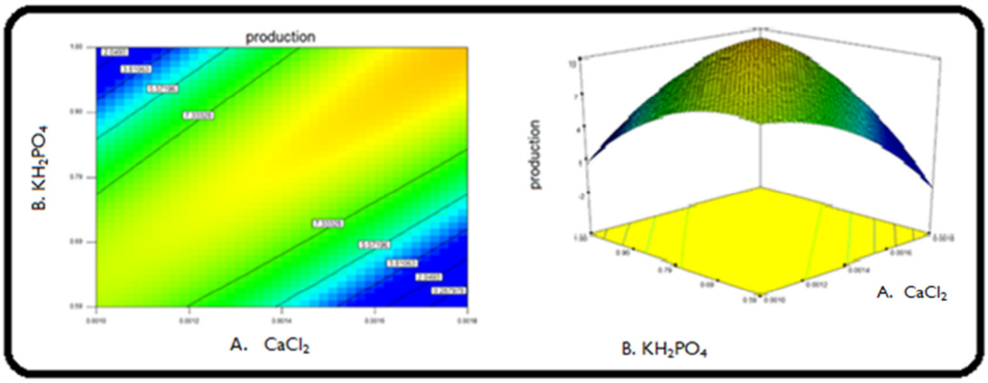

Fig 1: Effect of $CaCl_2$ (A) and KH_2PO_4 (B) on Lipopeptide and lipoprotein production

Figure 1 addresses the joint impact of $CaCl_2$ and KH_2PO_4 and most extreme biosurfactant creation (10.53 gram/L) was seen at a low degree of KH_2PO_4 (3.26 gram/L). There was a huge expansion in the item focus when agar powder fixation expanded from 0.0010, 0.90 and 0.87 g/L t announced that CaCl2 was the most reasonable carbon hotspot for biosurfactant creation by glycolipids among different carbs. A few analysts inferred that presence of yeast removed in low focus builds the biosurfactant combination. Supplementation of yeast extricate (4 g/L) in the creation medium was adequate for upgrading biosurfactant creation as the amino acids are needed for the arrangement of the glycolipids biosurfactant by *achromobacter xylos*[13-20] Lipoprotein and lipopeptides strain. Likewise announced that low degree of KH_2PO_4 enhances the biosurfactant creation. Figure 2 exhibits that increment in both $CaCl_2$ and $(NH_4)_2PO_4$ improves the biosurfactant creation. It was seen that the $(NH_4)_2PO_4$ in the medium assumes a critical part in efficiency. At the point when $CaCl_2$ focus increments from low to undeniable level, the efficiency was additionally expanded though expansion in convergence of KH_2PO_4 doesn't show any effect in the biosurfactant creation (Figure 3).

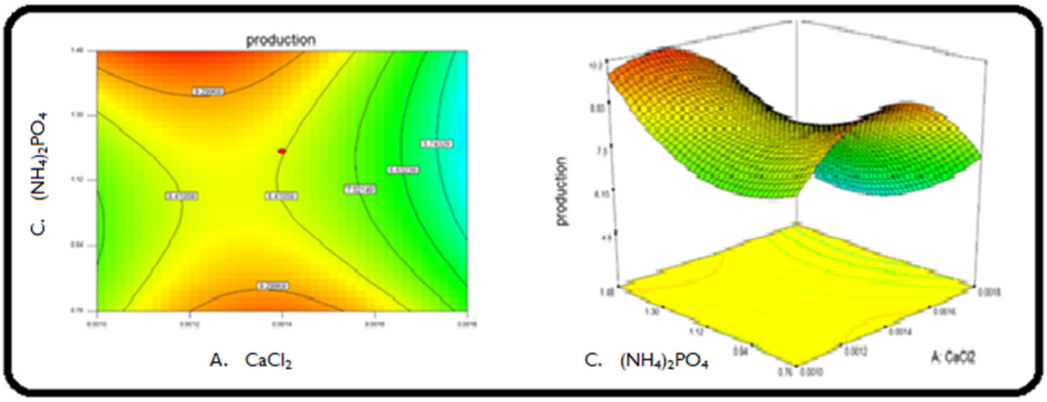

Fig 2: Effect of $CaCl_2$ (A) and $(NH_4)_2PO_4$ (C) on Lipopeptide and lipoprotein production

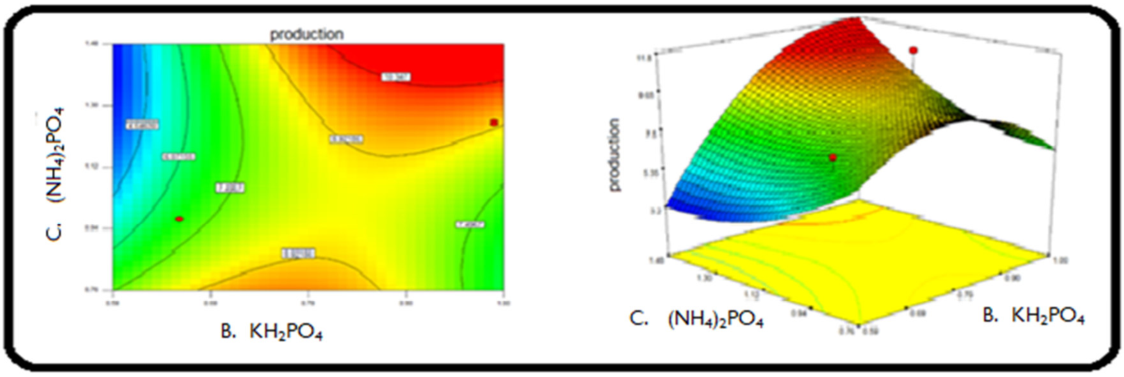

Fig 3: Effect of KH_2PO_4 (B) and $(NH_4)_2PO_4$ (C) on Lipopeptide and lipoprotein production

3.4 *Surface Tension Optimization Using Response Surface Methodology*

Measurable enhancement for biosurfactant surface strain was completed by a focal composite plan of RSM utilizing Design master programming[21-28]. The reaction, biosurfactant surface strain was assessed for twenty analyses and addressed in Table.2. The reaction information was exposed to relapse investigation to gauge relapse coefficient. The assessed coefficients were introduced in Table 3 and a second request polynomial condition (Final Equation in Terms of Coded Factors) (Eqn. 4) and Final Equation in Terms of Actual Factors (Eqn.5) for biosurfactant creation was developed by utilizing the coefficients.

Surface tension = 59.41 – 7.50 * A + 15.03 * B – 8.88 * C + 28.03 * A * B + 8.80 * A * C + 0.44 * B * C – 10.18 * A^2 – 3.67 * B^2 - 8.93 * C^2 (4)

3.5 *Final Equation In Terms Of Actual Factors*

surface tension = 121.82712 - 65821.76829 * $CaCl_2$ - 193.34793 * KH_2PO_4 + 109.31428 * $(NH_4)_2PO_4$ + 2.24248 E + 005 * $CaCl_2$ * KH_2PO_4 + 4277.10239 * $CaCl_2$ * $(NH_4)_2PO_4$ + 4.66711 * KH_2PO_4 * $(NH_4)_2PO_4$ - 4.07241 E + 007 * $(CaCl_2)^2$ - 58.77752 * $(KH_2PO_4)^2$ - 63.51848 * $((NH_4)_2PO_4)^2$ (5)

Table 4: ANOVA statistics for bio-surfactant surface tension optimization for contaminated soil

Source	Sum of squares	df	Mean Square	F - value	P - value	Significance
Model	1054.61	9	117.18	5.73	0.0058	significant
A-$CaCl_2$	53.08	1	53.08	2.60	0.1383	
B-KH_2PO_4	155.67	1	155.67	7.61	0.0202	
C-$(NH_4)_2PO_4$	181.79	1	181.79	8.89	0.0138	
AB	213.52	1	213.52	10.44	0.0090	
AC	0.36	1	0.36	0.017	0.8978	
BC	0.12	1	0.12	6.073E-003	0.9394	
A^2	52.21	1	52.21	2.55	0.1412	
B^2	8.00	1	8.00	0.39	0.5458	
C^2	102.09	1	102.09	4.99	0.0495	
Residual	204.54	10	20.45			
Cor Total	1259.16	19				

df=Degrees of freedom,F-Value=F-Test value,P-Value=P test value

The Model F-estimation of 5.73 infers the model is huge. There is just a 0.58% possibility that a "Model F-Value" this enormous could happen because of commotion. Estimates of "Prob > F" under 0.0500 demonstrate model terms are critical. For this situation B, C, AB, C^2 are huge model terms. Qualities more noteworthy than 0.1000 demonstrate the model terms are not critical. On the off chance that there are numerous immaterial model terms (not including those needed to help chain of importance), model decrease may improve your model[9-12].

Table 5: Coefficients of lipoprotein and lipopeptide production from contaminated soil

Std.Dev.	0.065	R-Squared	0.2113
Mean	0.064	Adj R-Squared	-0.4985
C.V.	102.11	Pred R-Square	0.8259
PRESS	1.351E+005	Adeq Precision	7.356

Std.Dev.=Standard deviation , R-Squared=R^2 ,C.V.=Variant coefficient

A negative "Pred R-Squared" suggests that the general mean is a preferable indicator of your reaction over the current model."Adeq Precision" gauges the sign to commotion proportion. A proportion more noteworthy than 4 is attractive. Your proportion of 9.843 shows a satisfactory sign. This model can be utilized to explore the plan space[10-13].

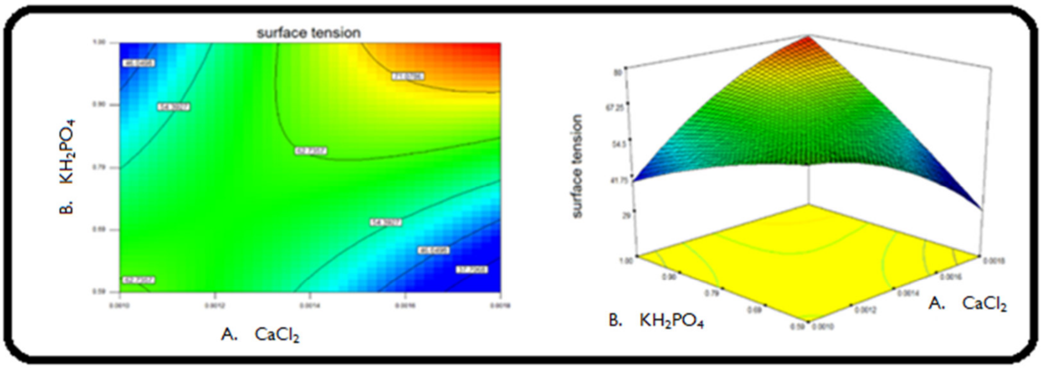

Fig 4: Effect of $CaCl_2$ (A) and KH_2PO_4 (B) on lipopeptides and lipoprotein surface tension.

Figure 4 addresses the joint impact of $CaCl_2$ and KH_2PO_4 and least surface pressure (9.9 mN/m) was seen at a low degree of KH_2PO_4 (3.53 g/L). There was a huge expansion in the item surface strain when $CaCl_2$ fixation expanded from 10 g/L to 20 g/L detailed that $CaCl_2$ was the most reasonable carbon hotspot for biosurfactant creation by glycolipid among different starches examined[25-35]. A few specialists presumed that presence of yeast separate in low focus expands the biosurfactant union. Supplementation of KH_2PO_4 (4 g/L) in the creation medium was adequate for upgrading biosurfactant creation as the amino acids are needed for the development of the glycolipids biosurfactant by Lipoprotein and lipopeptides strain. [18-35]additionally revealed that low degree of KH_2PO_4 enhances the biosurfactant creation.

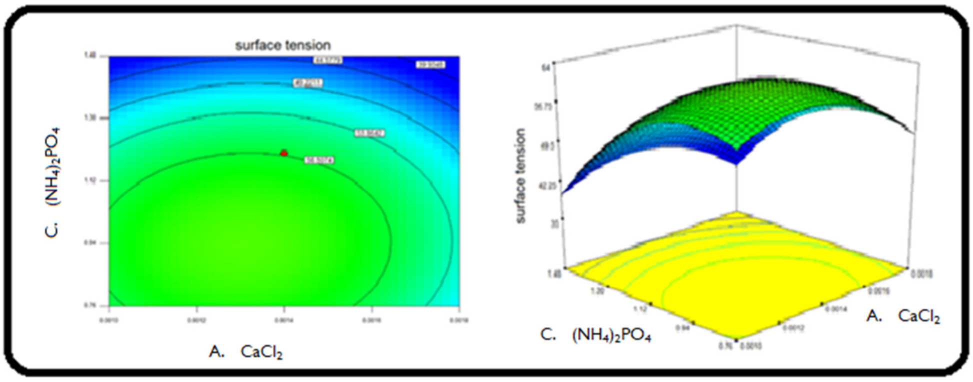

Fig 5: Effect of $CaCl_2$ (A) and $(NH_4)_2PO_4$ (C) on lipopeptides and lipoprotein surface tension.

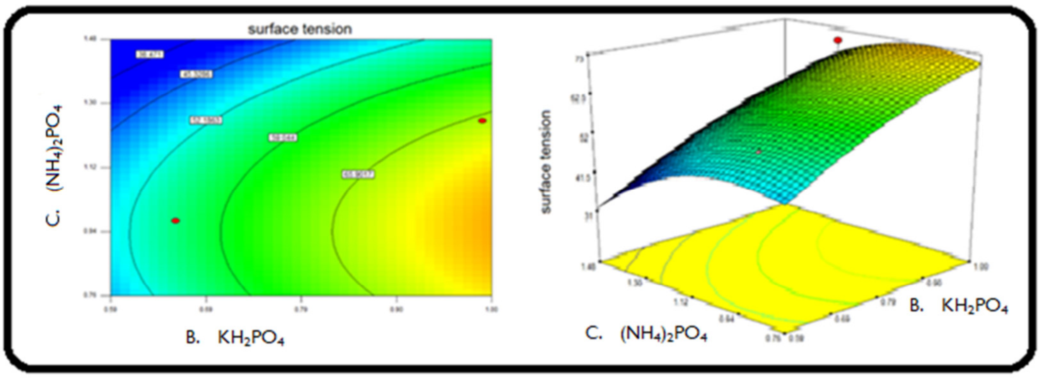

Fig 6: Effect of KH_2PO_4 (B) and $(NH_4)_2PO_4$ (C) on lipopeptides and lipoprotein surface tension.

Figure 5 showed that increment in both $CaCl_2$ and $(NH_4)_2PO_4$ improves the biosurfactant surface pressure. It was seen that the $(NH_4)_2PO_4$ in the medium assumes a huge part in diminishing biosurfactant surface pressure[18-21]. At the point when $CaCl_2$ fixation increments from low to significant level, the efficiency was additionally expanded while expansion in convergence of KH_2PO_4 doesn't show any effect in the biosurfactant surface pressure (Figure 6).

3.6 Optimization Of Medium Components For Lipopeptide and Lipoprotein Production and Surface Tension

The variables showing positive effect with confidence level above 98% ($CaCl_2$ and KH_2PO_4) and variables with negative effect above 96% ($CaCl_2$ and $(NH_4)_2PO_4$).Figure.1 explains the $CaCl_2$ highly affected on lipoprotein and lipopeptides production when compared to KH_2PO_4 (9.41 g/ml),same results observed in[22] . Figure 2 and 3 speaks 9.01 g/ml of $(NH_4)_2SO_4$ and 9.01 g/ml of KH_2 PO_4 effected on 9.86 g/ml and 9.99 g/ml of Biosurfactant production and same results reflected on[23] .Figure 4, 5 and 6 indicates 0.58 g/ml of KH_2PO_4 and 0.29 g/ml of $CaCl_2$ effected on 4.54 mN/m of biosurfactant surface tension and 10 g/ml of $(NH_4)_2SO_4$ with 11 g/ml of $FeSO_4.7H_2O$ and $MgSO_4$-60 g/L with $NaNO_3$-17g/L highly affected on 3.58 mN/m of lipoprotein and lipopeptides surface tension and the same results observed in[24-40] .

4. CONCLUSION

Response Surface Quadratic Model was widely applied to optimize the four media components for the production and surface tension of biosurfactant of *achromobacteria xylos* . Four components $CaCl_2$, $(NH_4\ )_2PO_4$,$MgSO_4$, and KH_2 PO_4) were advanced strong with focal composite course of action of RSM. Surface plots were made and in this manner the redesigned esteems got for the most creation of biosurfactant were MgSO4-60 g/L, NaNO3-17g/L, FeSO4.7H2O – 1.06g/L and KH2 PO4-0.50g/L. Support of the assessment was performed and it shows that the model was well fitted with the test results. Use of RSM enlightens the best levels for further developed creation of biosurfactants with less fundamental endeavors and affiliation impacts of the components .

5. AUTHORS CONTRIBUTION STATEMENT

Golamari Siva Reddy, Kadiyala Himavarshini, Asapu Devi Prasanna, Singavarapu Harini, Narra Sai Nikitha, Mannam Bhuvaneswari conceived of the presented idea. Golamari Siva Reddy and Kadiyala Himavarshini, Asapu Devi Prasanna, Singavarapu Harini, Narra Sai Nikitha, Mannam Bhuvaneswari developed the theory and performed the computations.G Siva Reddy verified the analytical methods. G Siva Reddy encouraged Kadiyala Himavarshini, Asapu Devi Prasanna, Singavarapu Harini, Narra Sai Nikitha, Mannam Bhuvaneswari to investigate [a design expert software for medium component analysis] and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript. All the authors read and approved the final version of the manuscript

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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