International Journal of Research Studies in Biosciences (IJRSB)
Volume 3, Issue 5, May 2015, PP 65-73
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
www.arcjournals.org

Optimization Studies for Enhancing Rhamnolipid Production *by Pseudomonas aeruginosa* RT Using Response Surface Methodology

Reshma Turbekar[1*], Nagesh Malik[2], Deepak Thakare[3]

[1, 3] Department of Microbiology, K.B.P. College, Vashi, Navi Mumbai, India.
[2] Department of Microbiology, V.E.S. College, Chembur, Mumbai, India.
**Corresponding author: (email: rturbekar@yahoo.com)*

Abstract: *Rhamnolipid production from Pseudomonas aeruginosa RT was investigated using design of experiments (DOE) methodology. The individual and interactive effects of five independent variables i.e. Molasses, Yeast extract, Potassium dihydrogen phosphate, Magnesium sulphate and Sodium chloride were studied. The central composite design and Response surface methodology have been applied for designing of experiments to evaluate the interactive effects through a full 50 factorial design. The optimum conditions were Molasses 12 g/lit, Yeast extract 2.5 g/L, Potassium dihydrogen phosphate 1.731g/L, Magnesium sulphate 1.001g/L and Sodium chloride 5.214g/L. Higher value of the regression coefficient R^2= 0.9761 indicates excellent evaluation of experimental data by second order polynomial regression model. The RSM revealed that the maximum biosurfactant production of 5.45 g/L was obtained at the above optimum conditions.*

Keywords: *Molasses, Pseudomonas aeruginosa RT, Rhamnolipid, Response surface methodology*

1. INTRODUCTION

Biosurfactants are low molecular weight surface-active compounds which are produced by bacteria, yeast and fungi. These biomolecules have an amphiphilic nature and they reduce the surface tension at the air-water interfaces and the interfacial tension at oil-water interfaces (Satpute et al. 2010). There has been increased interest in research on biosurfactants, as they are prospective candidates for many commercial applications in various industries. Biosurfactants have distinctive advantages over synthetic surfactants such as their biodegradable nature, exhibits less toxicity and have greater diversity (Kosaric N, 1992).

Cell growth and the accumulation of metabolic products are strongly subjective to composition of media such as the type of carbon source, nitrogen source, growth factors and inorganic salts (Techaoei et al., 2011). Thus, it is very challenging to search for the main factors and to optimize them for commercial processes as several parameters are involved. Due to expensive raw material, less production yield and high purification cost large scale production of biosurfactant is still at infancy (Makkar and Cameotra, 1999). Selection of inexpensive raw materials is vital to the overall economics of the process because they account for 50% of the final product cost. In spite of ongoing research it is still challenging to find the appropriate waste substrate using unconventional sources. Finding a waste with the right balance of carbohydrates and lipids to support optimal growth of microorganisms and maximum production of biosurfactant is a major problem faced by many researchers. Agro industrial wastes and Industrial wastes with high content of carbohydrates or lipids can meet the requirement for use as substrate for biosurfactant production. Recently, numerous renewable substrates, especially from industrial wastes have been extensively studied for biosurfactant production as it confers low-cost feed stocks (Joshi et al. 2008). Response surface methodology which is a collection of mathematical and statistical techniques that are useful for the modelling and analysis of problems in which a response of interest is influenced by several variables and the purpose is to optimize this response (Murthy et al. 2000).

Increasing environmental awareness during the recent years, has led to serious consideration of biosurfactants as possible alternative to synthetic surfactants, as they responsible for causing

environmental problems due to their non-biodegradable and toxic nature (George et al. 2009). The potential of biosurfactant certainly exists if economic strategies are implemented for their cost effective production (Dubey et al. 2001). In the present study, response surface methodology (RSM) was used to enhance the production of biosurfactant by *Pseudomonas aeruginosa* RT.

2. MATERIALS AND METHODS

2.1. Inoculum and Culture Conditions

Biosurfactant producing bacterium *Pseudomonas aeruginosa* RT was previously isolated from petroleum contaminated soils of Mumbai Maharashtra (data not shown). The strain was grown at 30^0C for 24 hrs. On Nutrient agar supplemented with 5% diesel and maintained at 4^0C with proper sub-culturing at an interval of 30 days. The inoculum was prepared using bacterial cells transferred from the storage culture to a test tube containing 10 ml of the nutrient broth and incubated at 37^0 C in an orbital incubator shaker (Remi RIS 24 BL) at 180 rpm. The cells were separated by centrifugation at 5,000 rpm and washed twice with saline. After second centrifugation (Remi RM-12C DX), the biomass pellet was aseptically re-suspended into saline and maintained with an optical density of $0.6x10^8$cfu/ml this was then used as inoculum for biosurfactant production.

2.2. Experimental Design and Statistical Analysis

Important media components molasses, yeast extract, potassium dihydrogen phosphate, magnesium sulphate, sodium chloride and the design of level of each tested factor in Response surface methodology (RSM) was determined by previously implemented single-factor optimization (data not shown). A central composite experimental design of 50 experiments was employed as shown in table no 2 7. (D.Montgomery, 1997). Fermentation medium was prepared by mixing the above components, the volume was adjusted up to 1,000 ml of distilled water. The pH of medium was adjusted to 7.0 using 1 M HCl and 1M NaOH and was autoclaved at 121^0C and 15 lbs pressure for 20 min, (Molasses and yeast extract were autoclaved separately and added) the medium was then inoculated with 3 ml of $0.6x10^8$ cfu/ml bacterial suspension and incubated at 30^0C for 7 days in an orbital shaker at 200 rpm. One response value Rhamnolipid yield was determined.

A mathematical model, relating the relationships among the process dependent variable and the independent variables in a second-order equation, was developed (Equation 1). The regression analysis was performed to estimate the response function as a second order polynomial.

$$\boldsymbol{Y} = \boldsymbol{\beta 0} + \boldsymbol{\beta i X i k i} = \mathbf{1} + \boldsymbol{\beta i i X i 2 k i} = \mathbf{1} + \boldsymbol{\beta i j X i k j} = \mathbf{2} \boldsymbol{X j k} - \mathbf{1} \boldsymbol{i} = \mathbf{1}, < \boldsymbol{j} \quad (1)$$

Where Y is the predicted response, βi, βj, βij are coefficients estimated from regression. They represent the linear, quadratic and interactive effects of A, B, C, D and E on response. A statistical software package Design Expert 9, was used for regression analysis of the data obtained and to estimate the coefficient of the regression equation. The equations were validated by the statistical tests called the ANOVA analysis. Design based experimental data were matched according to the second order polynomial equation. The independent variables were fitted to the second order model equation and examined for the goodness of fit. The quality of fit of the second order equation was expressed by the coefficient of determination R^2, and its statistical significance was determined by-test. The significance of each term in the equation is to estimate the goodness of fit in each case. To establish the individual and interactive effects of the test variable on the biosurfactant production response surfaces were drawn.

Table 1. *Range and levels of independent variables*

Variables		**Levels**				
g/L	**Code**	**-2.378**	**-1**	**0**	**1**	**2.378**
KH2PO4	A	0.310	1	1.5	2	2.68
$MgSo_4$	B	0.310	1	1.5	2	2.68
Molasses	C	1.864	6	9	12	16.13
Yeast extract	D	1.31	2	2.5	3	3.68
Sodium chloride	E	0.93	3	4.5	6	8.06

Table 2. *Central composite design (CCD)*

Run	A:KH_2PO_4	B:$MgSO_4$	C:Molasses	D:Yeast Extract	E:Sodium Chloride	Actual Response g/L
1	0	0	1	0	0	5.2
5	0	0	1	0	0	5.2
10	0	0	1	0	0	5.2
15	0	0	1	0	0	5.2
32	0	0	1	0	0	5.2
35	0	0	1	0	0	5.2
42	0	0	1	0	0	5.2
44	0	0	1	0	0	5.2
30	1	1	2	1	1	5.1
33	1	-1	2	1	1	4.7
24	-1	1	2	1	1	4.6
46	0	0	1	2.37841	0	4.6
14	1	-1	2	-1	1	4.4
34	0	2.37841	1	0	0	4.4
37	0	0	3.37841	0	0	4.4
16	1	1	0	1	1	4.3
48	-1	1	2	1	-1	4.3
9	0	0	1	0	2.37841	4.2
17	1	-1	2	1	-1	4.2
22	1	1	2	-1	1	4.2
47	1	1	2	1	-1	4.2
13	-1	1	2	-1	1	4.1
39	2.37841	0	1	0	0	4.1
41	-1	1	0	1	1	4.1
50	-1	-1	2	1	1	4.1
36	1	1	0	1	-1	4.0
27	1	-1	0	1	1	3.9
29	-1	-1	2	-1	1	3.9
19	1	1	0	-1	1	3.8
28	0	0	1	-2.37841	0	3.8
49	-1	1	2	-1	-1	3.8
18	-1	1	0	1	-1	3.7
20	1	-1	2	-1	-1	3.7
45	-1	1	0	-1	1	3.7
40	1	-1	0	-1	1	3.6
3	1	1	2	-1	-1	3.5
6	1	-1	0	1	-1	3.5
21	-1	-1	2	-1	-1	3.4
2	-1	-1	2	1	-1	3.2
11	-2.37841	0	1	0	0	3.2
12	-1	1	0	-1	-1	3.2
26	0	0	1	0	-2.37841	3.2
38	1	1	0	-1	-1	3.1
43	1	-1	0	-1	-1	3.1
7	0	-2.37841	1	0	0	2.9
23	0	0	-1.37841	0	0	2.9
8	-1	-1	0	-1	1	2.8
31	-1	-1	0	-1	-1	2.6
4	-1	-1	0	1	-1	2.5
25	-1	-1	0	1	1	2.5

2.3. Extraction of Rhamnolipid

The crude biosurfactant was isolated from the cell-free broth. The bacterial cells were removed from surfactant containing culture broth by centrifugation at 1062 g at 4^0C for 20min. The supernatant was precipitated overnight at 4^0C by adding concentrated HCl to achieve a final pH of 2.0 in order to

precipitate lipids and proteins. Grey white pellets formed by precipitation were collected by centrifugation at 1062 g at 4^0C for 20 min (Cao et al. 2007).

3. RESULT AND DISCUSSION

To examine the interactive effect of five various process parameters (independent variables), on biosurfactant production, a central composite design of $2^5 = 32$, 8 center points and 10 star points leading to a total of 50 experiments were performed. Equation (2) represents the mathematical model relating the biosurfactant production and the second order polynomial coefficient for each term of the equation determined through multiple regression analysis using the Design 9 the experimental and predicted values of biosurfactant production are also given in Table no 2.

R1= +4.54+0.26*a+0.36*B+0.93*C+0.16* D+0.18* E-0.17* aB-0.050*AC +0.094*AD+0.050* AE-0.10*BC+0.12*BD+0.012*BE+0.025*CD+0.056*CE-0.013*DE-0.30*a^2-0.30*B^20.30*C^ 2-0.20*D^2-0.29*E^2 (2)

Table 3. *Analysis of Variance (ANOVA) for response surface quadratic model for the biosurfactant production*

Source	Sum of squares	df	Mean square	F values	P value p>f
Model	29.98	20	1.50	59.25	< 0.0001
A-KH2PO4	1.21	1	1.21	47.83	< 0.0001
B-MgSO4	2.36	1	2.36	93.15	< 0.0001
C-Molasses	9.09	1	9.09	359.27	< 0.0001
D-yeast extract	0.46	1	0.46	18.04	0.0002
E-Sodium Chloride	0.59	1	0.59	23.24	< 0.0001
AB	0.91	1	0.91	36.02	< 0.0001
AC	0.080	1	0.080	3.16	0.0858
AD	0.28	1	0.28	11.12	0.0023
AE	0.080	1	0.080	3.16	0.0858
BC	0.32	1	0.32	12.65	0.0013
BD	0.45	1	0.45	17.84	0.0002
BE	5.000E-003	1	5.000E-003	0.20	0.6599
CD	0.020	1	0.020	0.79	0.3812
CE	0.10	1	0.10	4.00	0.0549
DE	5.000E-003	1	5.000E-003	0.20	0.6599
A^2	4.89	1	4.89	193.26	< 0.0001
B^2	4.89	1	4.89	193.26	< 0.0001
C^2	4.89	1	4.89	193.26	< 0.0001
D^2	2.21	1	2.21	87.33	< 0.0001
E^2	4.60	1	4.60	181.91	< 0.0001
Residual	0.73	29	0.025		
Lack of Fit	0.73	22	0.033		
Pure Error	0.000	7	0.000		
Cor Total	30.71	49			

Std. Dev.0.16, R^2 0.9761, Mean 3.98 Adj R^{-2} 0.9596 C.V. % 3.99 PredR^2 0.9037 PRESS 2.96, Adeq Precision 25.758

The results obtained were analyzed by ANOVA (analysis of variance) appropriate for the experimental design used and shown in Table no 3. The ANOVA of the quadratic regression model indicates the model to be significant. The Model F-value of 57.54 implied the model to be significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Model P value (Prob.>F) is very low [<0.0001]. This reiterates that the model is significant. The P values are used as a tool to check the significance of the coefficients used, which in turn are necessary to understand the pattern of the mutual interactions between the test variables. The F value and the corresponding P values, along with the coefficient estimates are given in Table no 3. The smaller the magnitude of the P, the more significant is the corresponding coefficient. Values of P less than 0.0500 indicates the model terms to be significant. The coefficient estimates and the corresponding P values along with the coefficient estimate are given in table no 3. The coefficients estimate and the corresponding P values suggests that, among the test variables used in the study, A, B, C, D, E, AB, AD, BC, BD, CE, A^2, B^2, C^2, D^2, E^2 are significant model terms. The coefficient of interaction terms

AB, BD, BC, AD and CE was found to be highly significant. The predicted R^2 of 0.9037 is in reasonable agreement with the adjusted R^2 of 0.9596. Adequate precision measures the signal to noise ratio. This model can be used to navigate the design space. The fit of the model was also expressed by the coefficient of regression R^2, which was found to be 0.9761 indicating that 97.61% the variability in the response could be explained by the model. The closer the value of *R* (correlation coefficient) to 1, the better is the correlation between the experimental and predicted values. Here the value of R^2 (0.9761) being close to 1 indicated a close agreement between the experimental results and the theoretical values predicted by the model equation. This implies that the prediction of experimental data is quite satisfactory. The Coefficient of Variation (CV) indicates the degree of precision with which the experiments are compared. The higher the value of the CV, the lower the reliability of the experiment. Here a lower value of CV (3.99) indicates greater reliability of the experiments performed. The response surface methodology was used with five process variables to evaluate their effect on the biosurfactant production. The response Eq. (2) was obtained for the biosurfactant production. Response surface plots as a function of two factors at a time, maintaining all other factors at fixed levels are more helpful in understanding both the main and the interaction effects of these two factors. The interaction effects of the variables and optimal levels of the each variable were determined by plotting the response surface graphs.

4. The Interaction Between The Variables

The graph of RSM was a 3D response surface plot consisting of response values of experimental variables (presented in Figs. 1 to 4). They can reflect the interaction between the variables (Molasses, Yeast Extract, KH_2PO_4, $MgSO_4$ and Sodium Chloride) on total rhamnolipid production.

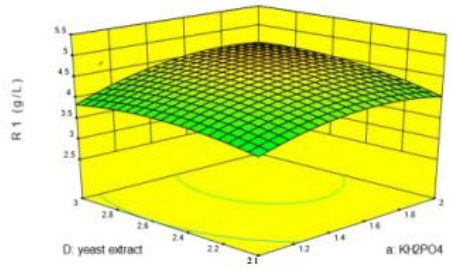

Figure 1(a)

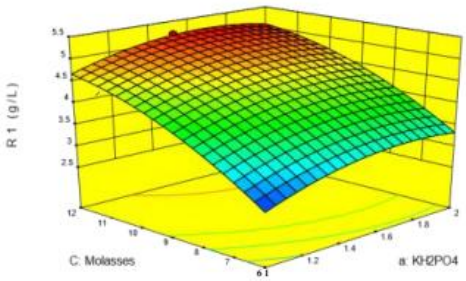

Figure 1(b)

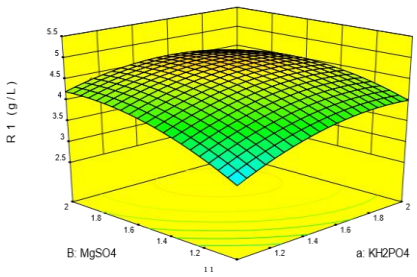

Figure 1(c)

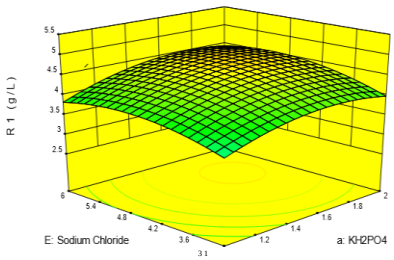

Figure 1(d)

Fig 1. *Effect of (a) Molasses and KH_2PO_4,(b) Yeast extract and KH_2PO_4,(c)$MgSO_4$ and KH_2PO_4, (d)Sodium Chloride and KH_2PO_4 on rhamnolipid production*

Figure 1 shows the effect of KH_2PO_4 with (a) Molasses; (b) Yeast Extract; (c) $MgSO_4$ and (d) Sodium Chloride on rhamnolipid production. KH_2PO_4 was found to be most significant factor that is responsible for the production of rhamnolipid in the presence of other nutrients. Interaction between molasses and KH_2PO_4 proved significant when the medium was supplemented with 12.0 g/L of molasses along with 1.7 g/L of KH_2PO_4. Three-dimensional graphs pointed a decline in rhamnolipid production level when the interaction was carried beyond optimum level of molasses and KH_2PO_4. The other interaction of KH_2PO_4 with yeast extract, $MgSO_4$ and sodium chloride was found to be non-significant as the 3D surface plot have not showed any decline or increase in the production level of rhamnolipid when both the nutrient were added at their optimum level. However the ANOVA studies showed that, the effect between $MgSO_4$ and KH_2PO_4 was found to be significant. Many works have

demonstrated that the limitation of multivalent ions and nitrogen is able to cause the overproduction of rhamnolipids. It was reported that the rhamnolipid production by *Pseudomonas aeruginosa* DSM2659 was promoted as the iron concentration in the culture media was reduced (Guerra Santos L et al. 1984).

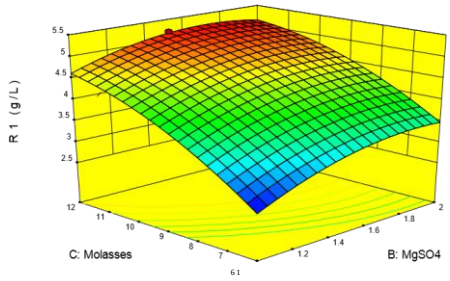

Figure 2(a)

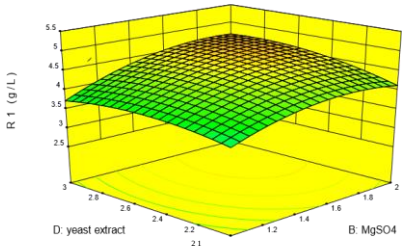

Figure 2(b)

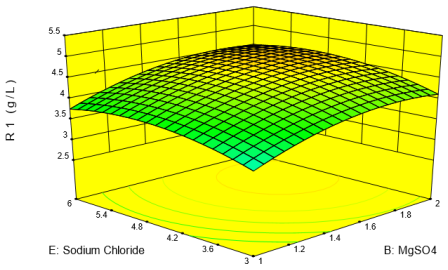

Figure 2(c)

Fig 2. *Effect of (a) Molasses and $MgSO_4$,(b) Yeast extract and $MgSO_4$,(c)Sodium Chloride and $MgSO_4$ on rhamnolipid production*

Figure 2, showed the effect of $MgSO_4$ with (a) Molasses, (b) Yeast Extract and (c) Sodium Chloride. Out of these three nutrients, again the interaction between molasses and $MgSO_4$ was observed to be significant, medium that was supplemented with 12.0 g/L along with 1.0 g/L of $MgSO_4$. Other two interactions had a least significant effect on rhamnolipid production as seen from 3D surface plots.

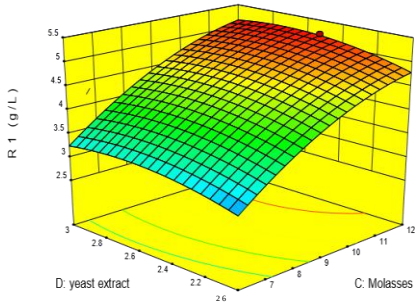

Figure 3(a)

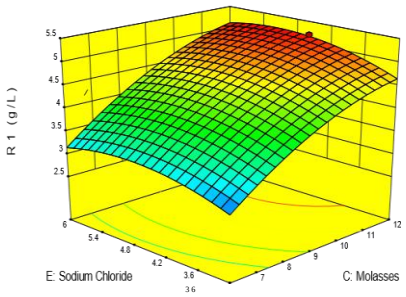

Figure 3(b)

Fig 3. *Effect of (a) Yeast extract and Molasses,(b)Sodium Chloride and Molasses on rhamnolipid production*

Figure 3 showed the effect of Molasses with (a) Yeast Extract and (B) Sodium Chloride on rhamnolipid production. Matsufuji et al. (1997) reported that a high production of rhamnolipids was achieved when *Pseudomonas aeruginosa* IFO 3924 was cultivated under nitrogen-limiting conditions at a carbon to nitrogen (C/N) ratio of 18/1.54. Santa Anna *et al.* (2002) found that a C/N ratio of 60/1 caused the overproduction of rhamnolipids by *Pseudomonas aeruginosa* PA1. These results suggest that the effect of C/N ratio on the rhamnolipid production depends on the bacterial strains. In our studies, molasses (carbon source) and yeast extract (nitrogen source) showed significant interaction on rhamnolipid production. A decline was observed, beyond the optimum level of concentrations of molasses 12.0 g/L and yeast extract 2.5 g/L. Molasses was found to be influential nutrient as compared to yeast extract when both the nutrients increased gradually in the medium. The effect on rhamnolipid production was more in case of molasses as compared to the yeast extract. The same effect in the case of molasses was also seen when studied with sodium chloride. Hence the role of carbon source plays a very important role in the production of rhamnolipid.

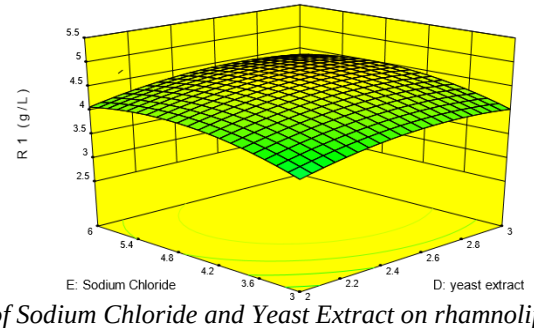

Fig4. *Effect of Sodium Chloride and Yeast Extract on rhamnolipid production*

Yateem et al. (2002) also reported that an increase in the nitrogen concentration caused a reduction of the rhamnolipid production by *Pseudomonas aeruginosa* KISR C1, but the bacterial growth was enhanced, leading to an increase in the bacterial number. **Figure 4** showed the effect of sodium chloride and yeast extract on rhamnolipid production. In this study, yeast extract had showed a very little effect on the production of rhamnolipid in the presence of other nutrients except with the molasses. It was concluded that concentration level of yeast extract 2.5 g/L was found be optimum for maximum production of rhamnolipid.

5. VALIDATION EXPERIMENTS

Validation of the experimental model was carried out by conducting the batch experiment under optimal operation conditions obtained by the regression model table no 4. The optimum conditions were Molasses 12 g/lit, Yeast extract 2.5 g/L, Potassium dihydrogen phosphate 1.731g/L, Magnesium sulphate 1.001g/L and Sodium chloride 5.214g/L. The experiments were performed in triplicates and the results are compared. The biosurfactant (5.45g/L) obtained from experiments was close to the actual response (5.241 g/L) predicted by the regression model, which demonstrated the validity of the model.

Table 4. *Validation Experiments*

Number	KH_2PO_4 g/L	$MgSO_4$ g/L	Molasses g/L	Yeast extract g/L	Sodium Chloride g/L	Predicted R1 g/L	Obtained R1 g/L
1	1.731	1.001	12.00	2.546	5.241	5.241	5.45
2	1.738	1.459	11.993	2.553	4.526	5.214	5.3
3	1.477	1.59	11.899	2.427	5.005	5.205	5.2

6. CONCLUSIONS

On the basis of Response surface methodology it was possible to select the experimental conditions that lead to maximum rhamnolipid production. By using the method of experimental factorial design and RSM analysis, it was possible to find out the optimal handling conditions which would obtain a higher rhamnolipid yield. The present findings would aid in higher rhamnolipid yield which would assist in various environmental and industrial application.

REFERENCES

[1] Satpute S.K., Banpurkar, A.G., Dhakephalkar, P.K., Banat I.M., Chopade, B.A.: Methods for investigating biosurfactants and bioemuslifiers. Crit. Rev. Biotechnol., 30, 127-144 (2010).

[2] Kosaric N.: Biosurfactants in industry. J Am Oil Chem Soc., 64, 1731–7.17 (1992).

[3] Techaoei, S. Lumyung, S., Prthumpai, W., Santiarwarm, D., and Leelapornpisid, P. Screening, characterization & stability of biosurfactant produced by Pseudomonas aeruginosa SCMU106 Isolated from soil in Nothron Thailand." Asia. J. Bio. Sci 7: 340-345 (2011).

[4] Makkar, R.S. and S.S. Cameotra: Biosurfactant production by microorganisms on unconventional carbon sources – a review. J. Surf. Det., 2,237-241 (1999).

[5] Joshi, S., Bharucha, C. and Desai, A. J.: Production of biosurfactant and antifungal compound by fermented food isolate *Bacillus subtilis* 20B, Bioresour. Technol.99, 4303- 4608 (2008).

[6] Murthy, M.S.R.C., Swaminathan, T., Rakshit, S.K. and Kosugi, Y.: Statistical optimization of lipase catalyzed hydrolysis of methyl oleate by response surface methodology. Bioproc Bioeng., 22, 35–39 (2000).

[7] George, S., Jayachandran, K.: Analysis of rhamnolipid biosurfactants produced through submerged fermentation using orange fruit peelings as sole carbon source. Appl. Biochem. Biotechnol.158, 694-705 (2000).

[8] Dubey, K., Juwarkar A.: Distillery and curd whey wastes as viable alternative sources for biosurfactant production. World J. Microbiol. Biotechnol. , 7, 61-69 (2000).

[9] D.C. Montgomery: Response Surface Methods and Other Approaches to Process Optimization. In: Design and Analysis of Experiments, D.C. Montgomery (Ed.), John Wiley & Sons, New York, USA. 427–510 (1997).

[10] Cao X.H., Cai P, Li F, Wang C.L., and Lu M.F.: Medium optimization for lipopeptide produced by *Bacillus natto* TK-1 using response surface methodology. China Biotechnol. , 27, 59-65 (2000).

[11] Guerra-Santos I.H., Kappeli O., Fiechter A.: *Pseudomonas aeruginosa* biosurfactant production in continuous culture with glucose as carbon source. Appl Enviorn Microbiol.48, 301-305 (1994).

[12] Matsufuji M, Nakata K, Yoshimoto A.: High production of rhamnolipids by *Pseudomonas aeruginosa* growing on ethanol. Biotechnology Letters.19, 1213–1215 (1997).

[13] Santa-Anna L.M., Sebastian G.V., Menezes E.P., Alves T.L.M., Santos A.S., Pereira N. Jr. and Freire, D.M.G.: Production of biosurfactants from Pseudomonas aeruginosa PA1 isolated in oil environments Brazilian Journal of Chemical Engineering., 19 (2):159-166 (2000).

[14] Yateem A., Balba, M.T., Al-Shayji, Y., Al-Awadhi, N.: Isolation and characterization of biosurfactant-producing bacteria from oil contaminated soil. Soil and Sediment Contamination. , 11(1):41-55 (2002).

AUTHORS' BIOGRAPHY

Ms. Reshma Turbekar is a Ph.D. fellow at Dept. of Microbiology, Karmaveer Bhaurao Patil College, Vashi, New Mumbai (Maharashtra). She has published 03 research papers and 01 case study. She has established Industrial collaborations for product development as a part of her Ph.D. work. She was appointed as Assistant Professor and Head Department of Biotechnology at K.B.P. College and has 4 years of teaching experience in Microbiology, Biochemistry and Cell biology. She is a recognized practical examiner and paper corrector for University Examination. She was also in charge of undergraduate research in Biotechnology and has mentored 25 students for departmental projects. She was an outstanding student and has two academic proficiencies awards. She has carried out her master's dissertation in Cancer biology at Gude lab, ACTREC, Kharghar Navi Mumbai. Presently she is a guest lecturer in Microbiology and Molecular Biology at Cistron Integrated Systems Mumbai, Maharashtra.

Dr. Nagesh Malik is Associate Prof and Head of the Department of Microbiology, V.E.S. College of Arts, Science and Commerce, Sindhi Society, Chembur, Mumbai 400071and has 32 years of teaching and 25 years of research experience. He has guided, around 40 dissertations of post-graduate students, 4 M.Sc. by research students, and is also a recognized guide for Ph.D.in Microbiology in Mumbai University. He has published 16 research papers in national and international Journals and also has one patent publication to his credit. He is a life member of several scientific organizations/societies. He has also reviewed many research papers.

Dr. Deepak Thakare is Associate Professor and Head of Department of Microbiology, Karmaveer Bhaurao Patil College, Vashi, New Mumbai (Maharashtra) .This College is permanently affiliated to the University of Mumbai. He has 35 years of teaching experience in the area of microbial biochemistry & applied microbiology and 25 years of research experience in the fields of Legume-Rhizobium Technology and applied microbiology, published 12 research papers in national & international journals. Presently 06 students are pursuing Ph.D. under his guidance, 01 student has submitted Ph.D. thesis, 02 students have completed M.Sc. (By Research) and 01 has completed M.Phil. under his guidance. He has completed one minor research project and one is ongoing. He is life member of several scientific societies like Biotech Research Society of India, Association of Microbiologists of India, Microbiologists Society, International Society of Science & Technology. He is editorial consultant of Elixir: National Journal of Multidisciplinary Research. He is also a referee for the evaluation of Ph.D.theses of various universities in Maharashtra. Presently he is working as the Chairman, Board of Studies in Microbiology, University of Mumbai and also a member of Academic Council & Faculty of Science, University of Mumbai.