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Research Article

APPLICATION OF MULTIFACTORIAL EXPERIMENTAL DESIGN FOR OPTIMIZATION OF PRODIGIOSIN PRODUCTION USING *SERRATIA MARCESCENS* MBB01, MBB02 AND MBB05

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ABSTRACT

Objective: The present study was aimed at investigating the prodigiosin production under optimized conditions with different parameters such as temperature, incubation time, substrate concentration, and inoculum size.

Methods: Response surface methodology was found to be useful in optimizing and determining interactions among process variables in prodigiosin production by applying Box–Behnken and face-centered central composite design.

Results: Prodigiosin producing *Serratia marcescens* (SM) MBB01, MBB02, and MBB05 were isolated from soil (Western Ghats Ecosystem). The significant R^2 value was 0.9666, 0.9459, and 0.9433, and the maximum experimental response for prodigiosin production was 497, 690, and 560 mg/mL, whereas the predicted value was 495.3, 706.6, and 574.8 mg/mL for SM MBB01, SM MBB02, and SM MBB05, respectively.

Conclusion: Statistically optimized conditions by Box–Behnken design found to be very significant in improved pigment production by SM MBB01, MBB02, and MBB05. The correlation between the predicted and observed values indicates the adequacy of the model.

Keywords: Prodigiosin, Serratia marcescens, RSM.

INTRODUCTION

Secondary metabolite, a low molecular weight compound is a natural product secreted by organisms, has a major impact on many aspects in our society. One of the important classes of these secondary metabolites is often referred to as biopigments. The two major sources of biopigments are plants [1] and microorganisms [2], where the pigments from microorganism favored over those from plants because of their stability, availability of the possible cultivation technology, and ecologically safe compounds in the chemical industry [3]. Microorganisms produce various pigments such as carotenoids, melanins, flavins, quinones, prodigiosins, and more specifically monascins, violacein, or indigo [4]. Prodigiosin is one of the most studied biopigments of microbial origin normally produced by *Serratia marcescens* (SM), *Pseudomonas magnesorubra*, *Vibrio psychroerythrous,* and other bacteria. SM, a Gram-negative *Enterobactericeae* is a major producer of dark red pigment called prodigiosin, which has got its attention because of Tripyrrylmethene, a natural occurring pigment [5]. Prodigiosin is synthesized as a secondary metabolite by biotypes A1, A2, and A6 of SM, and other strains of bacteria that are taxonomically unrelated [6]. Prodigiosin (5[(3-methoxy-5-pyrrol-2-ylidene-pyrrol-2-ylidene) –methyl] -2-methyl-3-pentyl-1H-pyrrole) is an alkaloid secondary metabolite with a unique tripyrrole chemical structure, first characterized from SM [7]. Prodigiosin has been known for its broad range of cytotoxic, antifungal, antibacterial, algicidal, antiprotozoal, antimalarial, immunosuppressive, anticancer, and antiproliferative activities [7-9]. At present, fabrics dyed with synthetic pigments shows increasing interest in using pigments produced from microorganisms, as they are easy, fast in growth in the cheap culture medium, independence from weather conditions, and colors of different shades [10]. In regard to its potential commercial values, there is a need to develop high throughput and cost-effective bioprocesses for prodigiosin production. The growth and pigment production of the organism are strongly influenced by culture conditions. To increase the production yield of prodigiosin, SM was investigated while varying culture conditions including temperature, pH [11], carbon and nitrogen sources [12], and NaCl concentration [13,14]. To develop a process for the maximum production of prodigiosin, standardization of the medium and culture condition is crucial. Therefore, an alternative and more efficient approach in the microbial culture system makes use of statistical experimental design methodology. The use of the statistical technique for optimizing the growth conditions can be investigated to enhance the microbial pigment production. Statistical approaches offer ideal ways for process optimization studies in biotechnology [15-17]. Using cost-effective media, formulation and optimizing at its minimum requirements for maximum prodigiosin production is extremely important in industrial scale for economic reasons. One such method is response surface methodology (RSM), a practical experiment for optimization of a single factor, while maintaining the other factors at constant levels, does not represent the combined effects of all the factors involved. Using Box and Behnken [18], a statistical experimental design methodology, all the parameters can be optimized, eliminating the limitations of a single factor optimization process. If the proposed model is adequate, as revealed by the diagnostic checking provided by an analysis of variance (ANOVA) and residual plots, contour plots can be usefully employed to study the response and locate the optimum. In the present study, the prodigiosin production was optimized with different parameters, which include temperature, incubation time, substrate concentration, and inoculum size.

METHODS

Microorganism

SM MBB01, MBB02, and MBB05 used in this study was isolated from forest soil (Western Ghats Ecosystem) and screened for its potentiality to synthesize large quantity of prodigiosin pigment. The culture was maintained on nutrient agar at 30°C and sub-cultured every 4 weeks.

Cultural conditions for prodigiosin productions

Different substrates (coconut oil, mustard oil, olive oil, peanut oil, sesame oil, black sesame powder, coconut powder, fenugreek powder, mustard powder, peanut powder, and white sesame powder) were used to supplement the carbon source at 2% concentration in distilled water, at different temperatures (27, 28, 30, 32, and 37°C), incubation

time (12, 24, 36, 48, 60, 72, 84, and 96 hrs), and inoculum size (2.5, 5.0, and 7.5%).

RSM

RSM consists of a group of empirical techniques devoted to the evaluation of relations existing between a cluster of controlled experimental factors and the measured responses according to one or more criteria [19]. Plackett-Burman and Box–Behnken were found to be very useful for the determination of relevant variables which were further optimized. These methods made it possible to consider a large number of variables and avoid repeatedly time-consuming experiments. Application of these techniques has proven helpful to optimize the type and relative amounts of the main medium components [20]. RSM uses an experimental design such as the central composite design (CCD) to fit a model by least squares technique. If the proposed model is adequate, as revealed by the diagnostic checking provided by an ANOVA and residual plots, contour plots can be usefully employed to study the response and locate the optimum. The most common experimental design used in RSM is the CCD, which has equal predictability in all directions from the center.

Screening for factors affected on prodigiosin production using Box–Behnken design

RSM using Box–Behnken design was carried out with four factors for production of pigment. Four most important factors, i.e., temperature, incubation time, substrate concentration, and inoculum size were believed to play a significant role in the prodigiosin production. The RSM using Box–Behnken design with four factors at three levels was adopted for improving prodigiosin production. A total of 30 experiments in Box–Behnken in three blocks were performed for the media optimization part of the study. The statistical software package "Design-Expert 5.9," USA was used to analyze the experimental design. All presented runs were carried out in submerged fermentation using peanut powder as substrate. The minimum and maximum ranges of variables investigated with respect to their values in the actual and coded form are listed in Table 1. The Box–Behnken experimental design used to evaluate the effect of process variables for SM MBB01, MBB02, and MBB05 on prodigiosin production is presented in Tables 2 and 3.

Table 1: Experimental range and levels of the independent variables in terms of actual and coded factors

Response	Variables	Range of levels					
		Actual	Coded	Actual	Coded	Actual	Coded
A	Temperature (°C)	28	−1	30	0	32	+1
B	Incubation time (hrs)	24	−1	36	0	48	+1
C	Substrate concentration (%)	2.5	−1	5	0	7.5	+1
D	Inoculum size (%)	2.5	−1	5	0	7.5	+1

Table 2: The Box–Behnken design for optimization of prodigiosin production by SM MBB01

Run	Coded levels			
	Temperature (°C)	Incubation time (hrs)	Substrate concentration (%)	Inoculum size (%)
1	0	0	0	0
2	1	1	0	0
3	0	0	−1	1
4	1	−1	0	0
5	−1	1	0	0
6	−1	−1	0	0
7	0	0	1	1
8	0	0	1	−1
9	0	0	−1	−1
10	0	0	0	0
11	0	−1	1	0
12	−1	0	0	1
13	1	0	0	1
14	0	0	0	0
15	0	1	−1	0
16	0	−1	−1	0
17	0	1	1	0
18	−1	0	0	−1
19	0	0	0	0
20	1	0	0	−1
21	0	1	0	1
22	1	0	−1	0
23	−1	0	−1	0
24	−1	0	1	0
25	0	1	0	−1
26	0	0	0	0
27	1	0	1	0
28	0	0	0	0
29	0	−1	0	−1
30	0	−1	0	1

SM: *Serratia marcescens*

Table 3: The Box–Behnken design for optimization of prodigiosin production by SM MBB02 and SM MBB05

Run	Coded levels			
	Temperature (°C)	Incubation time (hrs)	Substrate concentration (%)	Inoculum size (%)
1	0	0	0	0
2	1	1	0	0
3	0	0	−1	1
4	1	−1	0	0
5	−1	1	0	0
6	−1	−1	0	0
7	0	0	1	1
8	0	0	1	−1
9	0	0	−1	−1
10	0	0	0	0
11	0	−1	1	0
12	−1	0	0	1
13	1	0	0	1
14	0	0	0	0
15	0	1	−1	0
16	0	−1	−1	0
17	0	1	1	0
18	−1	0	0	−1
19	0	0	0	0
20	1	0	0	−1
21	0	1	0	1
22	1	0	−1	0
23	−1	0	−1	0
24	−1	0	1	0
25	0	1	0	−1
26	0	0	0	0
27	1	0	1	0
28	0	0	0	0
29	0	−1	0	−1
30	0	−1	0	1

SM: *Serratia marcescens*

RESULTS AND DISCUSSION

RSM consists of empirical techniques devoted to evaluate relations existing between a cluster of controlled experimental factors and measured responses according to one or more criteria [19]. RSM uses an experimental design such as the CCD to fit a model by least squares technique. If the proposed model is adequate, as revealed by the diagnostic checking provided by ANOVA and residual plots, the contour plots can be successfully employed to study the response and evaluate the optimum. The most common experimental design used in RSM is the CCD, which has equal predictability in all directions from the center. The values were based on the "one-variable-at-a-time" approach and could not explain the mutual interactions among the independent variables and guarantee the determination of optimal conditions. Therefore, the interactive effects of these factors selected as key parameters were investigated to maximize the prodigiosin production. In the present study, the respective low and high levels with the coded levels in parentheses for the factors were defined as 28 (−1) and 32 (+1) for temperature, 24 (−1) and 48 (+1) for incubation time, 2.5 (−1) and 7.5 (+1) for substrate concentration, and 2.5 (−1) and 7.5 (+1) for inoculum size were studied for prodigiosin production for SM MBB01, MBB02, and MBB05. The results obtained after face-centered CCD (FCCCD) were then analyzed by standard ANOVA, which gave the following regression equation (in terms of coded factors) of the levels of prodigiosin produced as a function of temperature (A), incubation time (B), substrate concentration (C), and inoculum size (D).

Optimization of prodigiosin production for SM MBB01 by RSM

Following the regression analysis of the experimental data, the following quadratic equations were obtained for prodigiosin production. The effects of four independent variables with the predicted and observed response are presented in Table 4.

Final equation in terms of coded factors:

Prodigiosin = + 488.333 − 12.4167 × A + 10.3333 × B + 13 × C + 9.08333 × D − 213.875 × A2 − 51 × B2 − 133.25 × C2 − 120.875 × D2 − 22.75 × A × B − 9 × A × C + 10 × A × D + 8 × B × C + 20.25 × B × D − 20.5 × C × D

Where, A, B, C, and D represents temperature, incubation time, substrate concentration, and inoculum size, respectively.

Optimization of prodigiosin production for SM MBB02 by RSM

The regression analysis of the experimental data the following quadratic equations were obtained for prodigiosin production by MBB02.

Final equation in terms of coded factors:

Prodigiosin = + 683.5 + 9.83333 × A + 25.9167 × B + 19.25 × C + 10.5 × D − 257.708 × A2 − 211.583 × B2 − 172.583 × C2 − 145.708 × D2 + 1 × A × B − 3.25 × A × C + 23.25 × A × D + 20 × B × C − 11.25 × B × D + 32.5 × C × D

Optimization of prodigiosin production for SM MBB05 by RSM

Final equation in terms of coded factors:

Prodigiosin = + 553.333 + 3.33333 × A + 35.75 × B + 33.0833 × C + 18.6667 × D − 226.167 × A2 − 155.042 × B2 − 117.542 × C2 − 89.9167× D2 − 14 × A × B + 7 × A × C + 8 × A × D − 4.5 × B × C − 11.25 × B × D + 24.25 × C × D

Practical experiments for optimizing the prodigiosin production were carried out changing each single factor while maintaining the other factors at a constant level. It shows clearly that they did not represent a combined effect of all the factors which required additionally a large number of experiments to determine an optimized culture condition. However, using statistical experimental designs such as

Table 4: Results of FCCCD using four independent variables and three center points showing observed and predicted response of prodigiosin production from SM MBB01

Run	Actual levels				Prodigiosin (mg/mL)	
	Temperature (°C)	Incubation time (hrs)	Substrate concentration (%)	Inoculum size (%)	Observed	Predicted
1	30	36	7.5	7.5	198	242.8
2	32	24	5	5	151	130.4
3	32	48	5	5	101	105.6
4	30	36	7.5	2.5	225	265.6
5	30	36	2.5	2.5	244	198.6
6	28	48	5	5	156	175.9
7	30	36	2.5	7.5	299	257.8
8	30	36	5	5	497	495.3
9	28	24	5	5	115	109.8
10	30	36	5	5	491	495.3
11	32	36	5	2.5	91	109.2
12	28	36	5	7.5	164	152.2
13	32	36	5	7.5	124	147.3
14	30	48	7.5	5	248	222.5
15	30	48	2.5	5	135	180.5
16	30	36	5	5	487	475.4
17	30	24	7.5	5	225	185.8
18	30	24	2.5	5	144	175.8
19	30	36	5	5	489	475.4
20	28	36	5	2.5	171	154.0
21	30	36	5	5	486	494.3
22	28	36	7.5	5	182	181.6
23	32	36	2.5	5	136	130.8
24	30	48	5	2.5	221	203.4
25	30	24	5	2.5	202	223.3
26	30	24	5	7.5	189	200.9
27	30	48	5	7.5	289	262.1
28	28	36	2.5	5	123	137.6
29	32	36	7.5	5	159	138.8
30	30	36	5	5	480	494.3

FCCCD: Face-centered central composite design

Plackett-Burman and Box–Behnken methodology, all the parameters could be optimized by eliminating those limitations involved in a single factor optimization process [20]. Till to date, there have been few reports on statistical optimization for the production yield-up of natural pigment [21]. In the present study, the experimental results of SM MBB01 showed maximum prodigiosin production rate of 497 mg/mL, whereas predicted value was 495.3 mg/mL (Table 4), under the condition of temperature (30°C), incubation time (36 hrs), substrate concentration (5%), and inoculum size (5%). Circular contour plot indicates that the interactions between the corresponding between the corresponding variables are significant [22]. The plots of the quadratic model with two variables kept at a constant level and

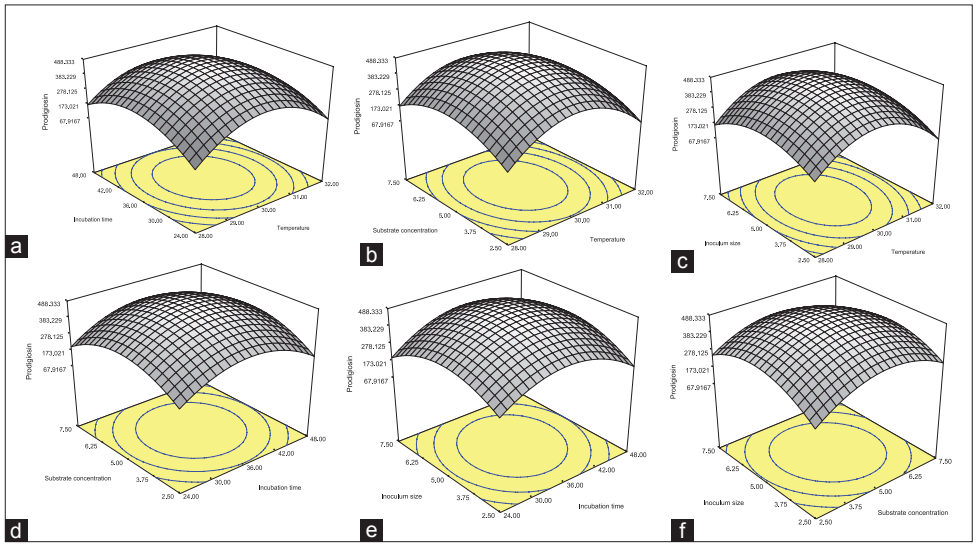

Fig. 1: Response surface curves of prodigiosin production from *Serratia marcescens* MBB01. Response surface curve for prodigiosin production, (a) Effect of temperature and incubation time, (b) effect of temperature and substrate concentration, (c) effect of temperature and inoculum size, (d) effect of incubation time and substrate concentration, (e) effect of incubation time and inoculum size, (f) effect of substrate concentration and inoculum size

Table 5: Results of FCCCD using four independent variables and three center points showing observed and predicted response of prodigiosin production from SM MBB02

Run	Actual levels				Prodigiosin (mg/mL)	
	Temperature (°C)	Incubation time (hrs)	Substrate concentration (%)	Inoculum size (%)	Observed	Predicted
1	30	36	5	5	690	706.6
2	32	48	5	5	229	274.1
3	30	36	2.5	7.5	323	347.1
4	32	24	5	5	219	220.3
5	28	48	5	5	264	252.4
6	28	24	5	5	258	202.6
7	30	36	7.5	7.5	485	450.6
8	30	36	7.5	2.5	399	364.6
9	30	36	2.5	2.5	367	391.1
10	30	36	5	5	682	706.6
11	30	24	7.5	5	213	236.0
12	28	36	5	7.5	169	220.8
13	32	36	5	7.5	249	287.0
14	30	36	5	5	679	646.8
15	30	48	2.5	5	254	249.3
16	30	24	2.5	5	299	237.5
17	30	48	7.5	5	248	327.8
18	28	36	5	2.5	266	246.3
19	30	36	5	5	688	646.8
20	32	36	5	2.5	253	219.5
21	30	48	5	7.5	455	364.9
22	32	36	2.5	5	273	260.6
23	28	36	2.5	5	204	234.4
24	28	36	7.5	5	275	279.4
25	30	48	5	2.5	385	366.4
26	30	36	5	5	687	697.0
27	32	36	7.5	5	331	292.6
28	30	36	5	5	675	697.0
29	30	24	5	2.5	210	292.1
30	30	24	5	7.5	325	335.6

FCCCD: Face-centered central composite design

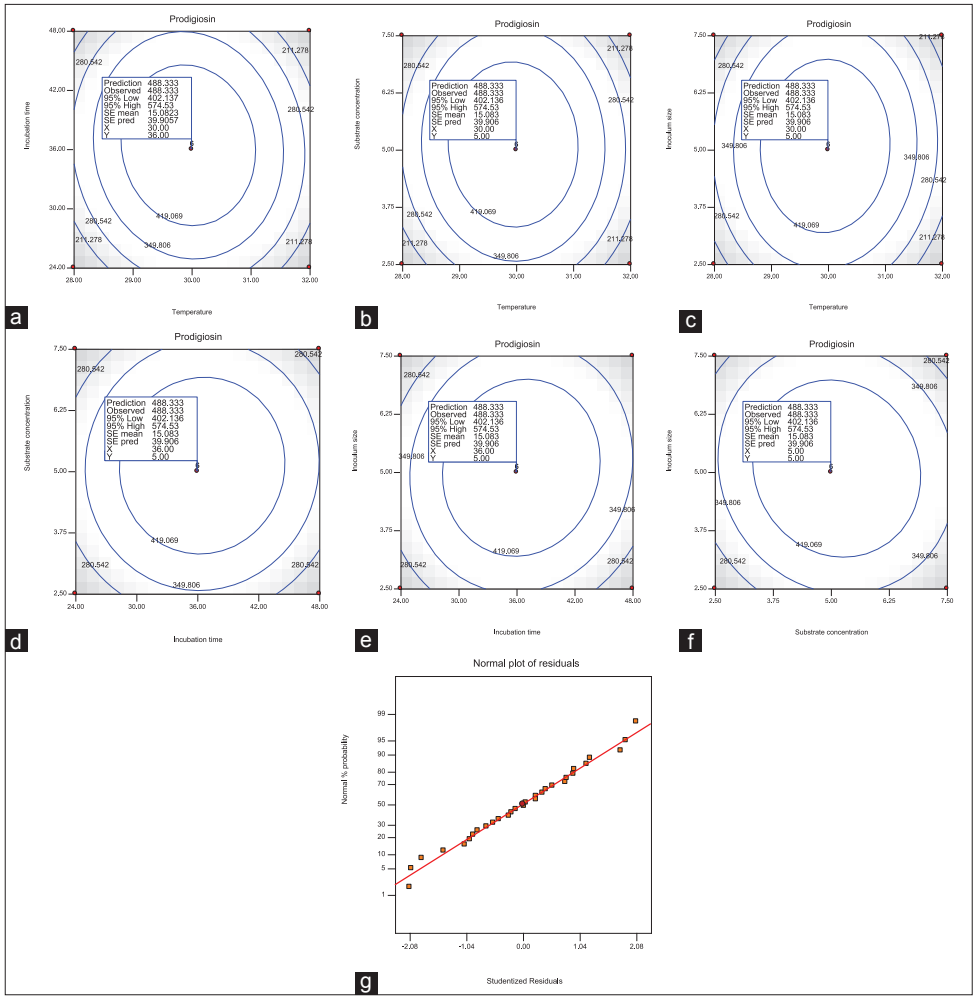

Fig. 2: Contour plot of prodigiosin production from *Serratia marcescens* MBB01. Response surface contour plot for prodigiosin production, (a) Effect of temperature and incubation time, (b) effect of temperature and substrate concentration, (c) effect of temperature and inoculum size, (d) effect of incubation time and substrate concentration, (e) effect of incubation time and inoculum size, (f) effect of substrate concentration and inoculum size, (g) model (predicted) versus experimental values in the production of prodigiosin

the other two varying with the experimental ranges are showed in Figs. 1 and 2. The experimental results of the analysis showed the values were significantly in agreement with the predicted values. The optimum conditions for the prodigiosin production from SM MBB02 was at temperature (30°C), incubation time (36 hrs), substrate concentration (5%), and inoculum size (5%) with the observed response of 690 mg/mL, and the predicted value was 706.6 mg/mL (Table 5). By analyzing the three-dimensional response surface and counter plots (Figs. 3 and 4), the optimal values of the tested variables were at temperature (30°C), incubation time (36 hrs), substrate concentration (5%), and inoculum size (5%) yielding 690 mg/mL of prodigiosin production. The maximum prodigiosin production of SM MBB05 was 560 mg/mL under the condition of temperature (30°C), incubation time (36 hrs), substrate concentration (5%), and inoculum size (5%), and the predicted value was 574.8 mg/mL (Table 6). Analysis results showed that the experimental values were significantly in agreement with the predicted values which suggested the model of the equation was satisfactory and accurate (Figs. 5 and 6). When using the optimized culture medium (sucrose - 10.0, peptone - 8.0, yeast extract - 2.0, NaCl - 10.0, Na_2SO_4 - 12, $CaCl_2$ - 1.8, $MgCl_2$ - 0.7 g/L; and H_3BO_3 - 22.0, Na_2HPO_4 - 20.0, Na_2SiO_3 - 8.0 mg/L) for a higher production yield of prodigiosin by *Hahella chejuensis* KCTC 2396, the maximum yield of prodigiosin was predicted to be 2.43 g/L while the yield obtained from the practical experiment was 2.60±0.176 g/L, 3.9 times higher than the wild type with SMB medium (0.658 g/L) while the yield of prodigiosin by *H. chejuensis* KCTC 2396 with SMB medium was 1.628 g/L [20].

Kim *et al.* [20] reported that the nutrient components were screened and those with p<0.01 were accepted as significant factors affecting the production of prodigiosin, and they found that p value of $FeCl_3 \bullet 6H_2O$, KCl, Na_2CO_3, KBr, $SrCl_2$, NaF, and NH_4NO_3 were >0.01, indicating that these seven elements are not significant factors on prodigiosin production compared with other factors. SM SM^R was investigated under modified Lysogeny broth medium to improve the prodigiosin production. However, the prodigiosin production was only 0.79 g/L [23]. Kim *et al.* [20] reported that the predicted maximum yield of prodigiosin in the optimized medium was 1.198 g/L by the Box–Behnken design, whereas the practical production was 1.495 g/L, which was three times higher than the basal medium (0.492 g/L).

The goodness of the fit of the model was checked by determination coefficient (R^2) [24]. The significance of each coefficient was determined by F value and p value. Values of the p greater than F, but <0.05 indicate that the model terms are significant. The response surface quadratic model of ANOVA for SM MBB01, MBB02, and MBB05 showed that the p<0.0001, and the model was significant. The coefficient of determination R^2=0.9666, adjusted R^2=0.9306, the coefficient of variance was 15.3472, and the predicted sum of squares PRESS was 1.409E+05. The adequate precision value was found to

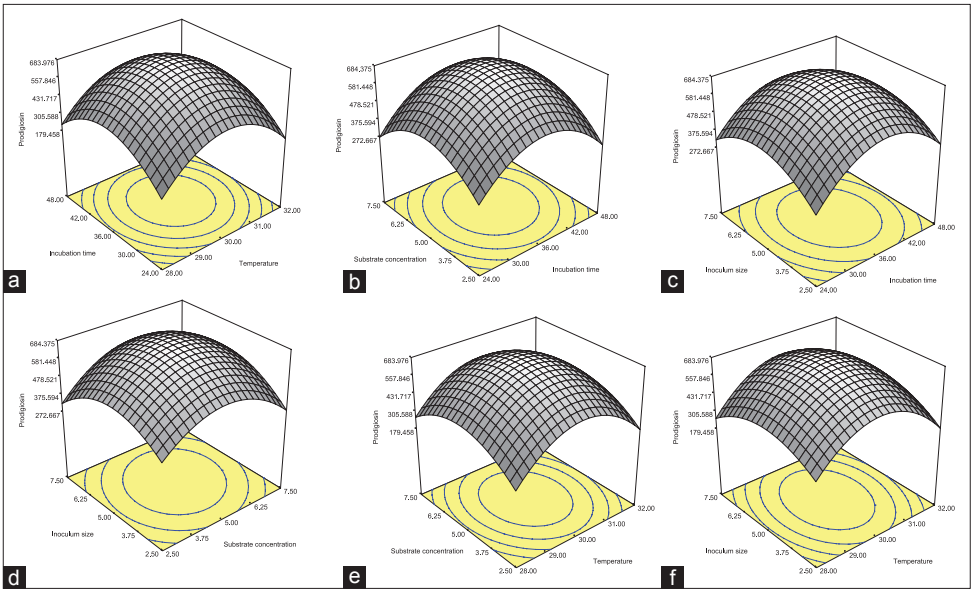

Fig. 3: Response surface curves of prodigiosin production from *Serratia marcescens* MBB02. Response surface curve for prodigiosin production, (a) Effect of temperature and incubation time, (b) effect of incubation time and substrate concentration, (c) effect of incubation time and inoculum size, (d) effect of substrate concentration and inoculum size, (e) effect of temperature and substrate concentration, (f) effect of temperature and inoculum size

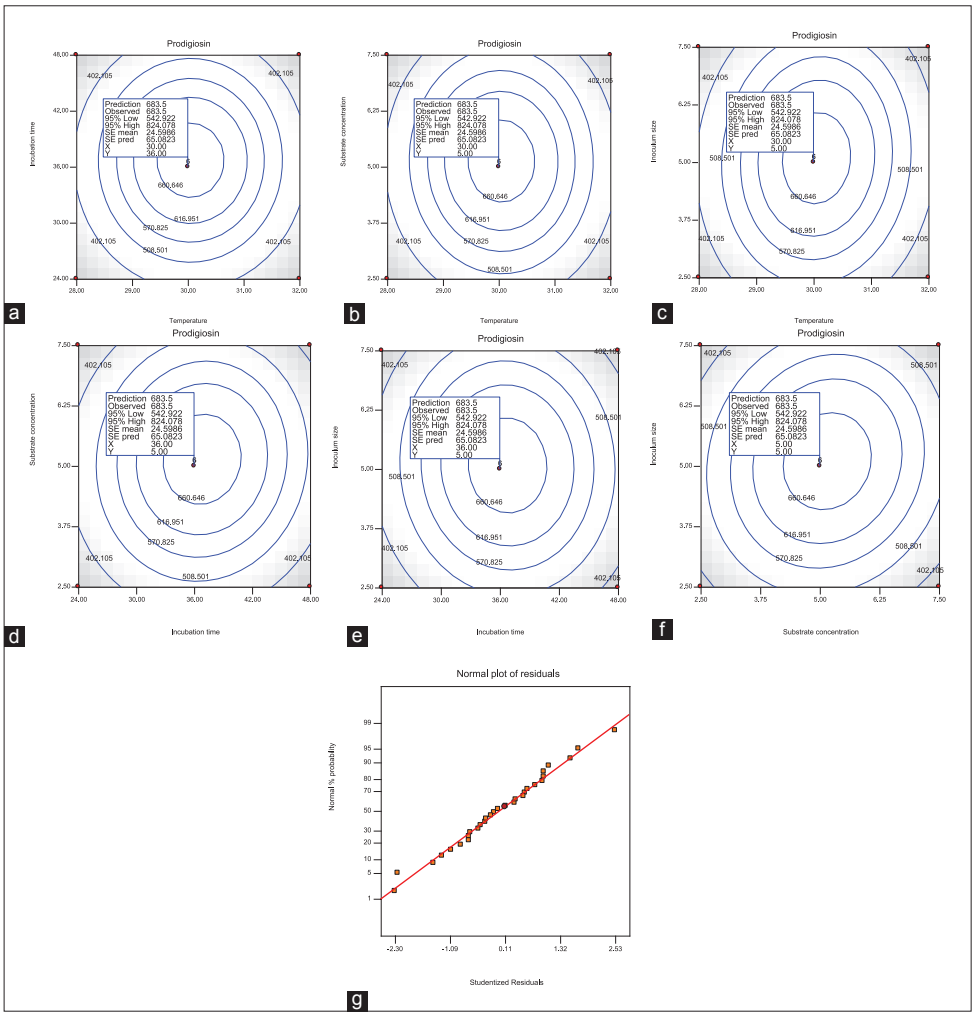

Fig. 4: Contour plot of prodigiosin production from *Serratia marcescens* MBB02. Response surface contour plot for prodigiosin production, (a) Effect of temperature and incubation time, (b) effect of temperature and substrate concentration, (c) effect of temperature and inoculum size, (d) effect of incubation time and substrate concentration, (e) effect of incubation time and inoculum size, (f) effect of substrate concentration and inoculum size, (g) model (predicted) versus experimental values in the production of prodigiosin

Table 6: Results of FCCCD using four dependent variables and three center points showing observed and predicted response of prodigiosin production from SM MBB05

Run	Actual level			Prodigiosin (mg/mL)		
	Temperature (°C)	Incubation time (hrs)	Substrate concentration (%)	Inoculum size (%)	Observed	Predicted
1	30	36	5	5	560	574.8
2	32	48	5	5	179	218.6
3	30	36	2.5	7.5	295	328.6
4	32	24	5	5	169	175.1
5	28	48	5	5	256	240.0
6	28	24	5	5	190	140.5
7	30	36	7.5	7.5	485	443.3
8	30	36	7.5	2.5	401	357.5
9	30	36	2.5	2.5	308	339.8
10	30	36	5	5	550	574.8
11	30	24	7.5	5	213	240.9
12	28	36	5	7.5	156	202.9
13	32	36	5	7.5	199	225.6
14	30	36	5	5	553	511.7
15	30	48	2.5	5	254	246.3
16	30	24	2.5	5	201	165.8
17	30	48	7.5	5	248	303.4
18	28	36	5	2.5	188	181.6
19	30	36	5	5	551	511.7
20	32	36	5	2.5	199	172.3
21	30	48	5	7.5	435	371.8
22	32	36	2.5	5	222	193.1
23	28	36	2.5	5	194	200.4
24	28	36	7.5	5	234	252.6
25	30	48	5	2.5	365	356.9
26	30	36	5	5	549	573.6
27	32	36	7.5	5	290	273.3
28	30	36	5	5	557	573.6
29	30	24	5	2.5	210	262.9
30	30	24	5	7.5	325	322.8

FCCCD: Face-centered central composite design

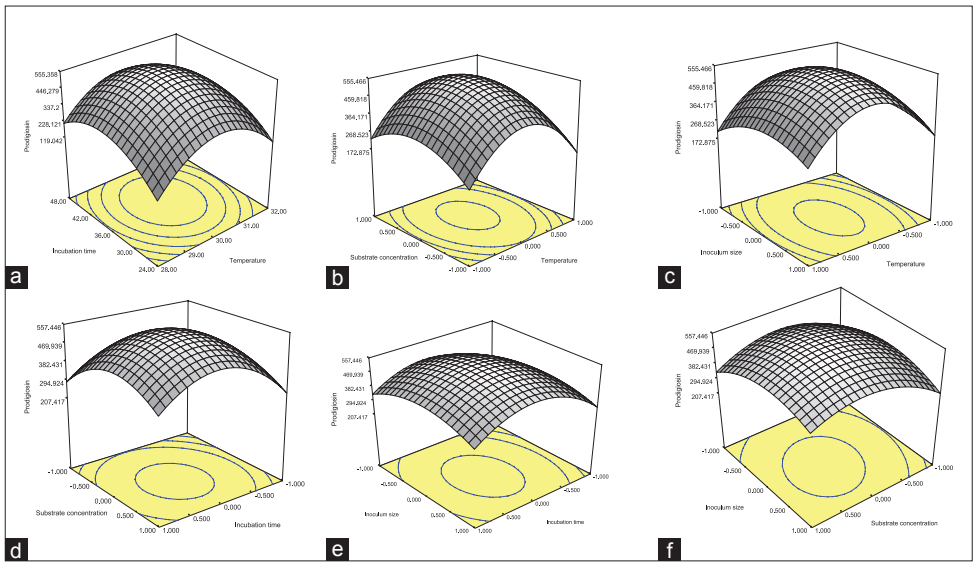

Fig. 5: Response surface curves of prodigiosin production from *Serratia marcescens* MBB05. Response surface curve for prodigiosin production, (a) Effect of temperature and incubation time, (b) effect of temperature and substrate concentration, (c) effect of temperature and inoculum size, (d) effect of incubation time and substrate concentration, (e) effect of incubation time and inoculum size, (f) effect of substrate concentration and inoculum size

be 14.0124, which indicates an adequate signal. ANOVA of FCCCD for SM MBB02 showed a high coefficient of determination R^2=0.9459, adjusted R^2=0.8876, the coefficient of variance was 16.3528, and PRESS was 3.582E+05 with the adequate precision value was 11.1125. Thus ensuring a satisfactory adjustment of the quadratic model with the experimental data, the value of the determination coefficient R^2 of SM MBB05 was found to be 0.9433, adjusted R^2=0.8822, the coefficient of variance was 15.6692, and PRESS was 2.312E+05. The adequate

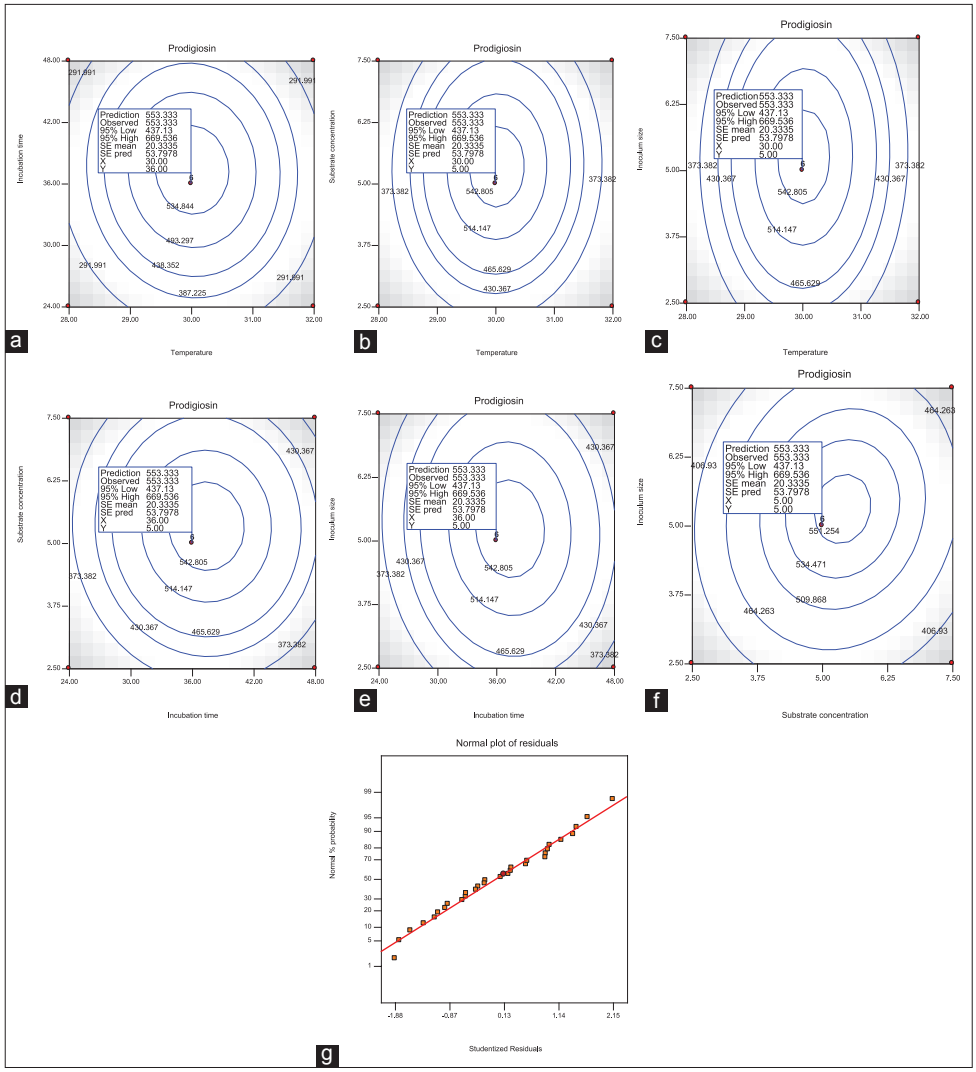

Fig. 6: Contour plot of prodigiosin production from *Serratia marcescens* MBB05. Response surface contour plot for prodigiosin production, (a) Effect of temperature and incubation time, (b) effect of temperature and substrate concentration, (c) effect of temperature and inoculum size, (d) effect of incubation time and substrate concentration, (e) effect of incubation time and inoculum size, (f) effect of substrate concentration and inoculum size, (g) model (predicted) versus experimental values in the production of prodigiosin

Table 7: ANOVA for response surface quadratic model

Source	MBB01	MBB02	MBB05
Mean	240.733	368.467	317.867
R^2	0.9666	0.9459	0.9433
Adjusted R^2	0.9306	0.8876	0.8822
Predicted R^2	0.7347	0.5893	0.5935
Coefficient of variance	15.3472	16.3528	15.6692
F value	26.8590	16.2286	15.4464
PRESS	1.409E+05	3.582E+05	2.312E+05
Adequate precision	14.0124	11.1125	11.5831

precision value was found to be 11.5831, which indicates an adequate signal (Table 7).

CONCLUSION

Box–Behnken design, for optimizing prodigiosin production by SM MBB01, MBB02, and MBB05, is effective and extremely important in industrial scale for economic. RSM using Box–Behnken design was carried out with four factors for production of pigment. Four most important factors, i.e., temperature, incubation time, substrate concentration, and inoculum size were believed to play a significant role in improving pigment production. The predicted model was verified and found to be statistically fit to apply.

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