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Statistical optimization of culture medium for yellow pigment production by *Thermomyces sp.*

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Abstract: In present study, *Thermomyces sp.* were able to produce high yield of yellow pigments screened. Pigment production by *Thermomyces sp* was optimized by employing factorial design and response surface techniques in submerged fermentation. The variables evaluated were the concentrations of, sucrose, yeast extract, ammonium sulphate, magnesium sulphate and dipotassium hydrogen phosphate having as response pigment production. One factor at-a-time method was employed for the optimization of media components. Response surface methodology (RSM) optimized these nutrient parameters for maximum yellow pigment production (1387 OD units), which resulted at 35.5 g/L sucrose 5.5 g/L yeast extract, 2.5 g/L NH_4SO_4, 0.3 g/L $MgSO_4$ and 1.0 g/L K_2HPO_4 in the medium. Response surface methodology (RSM) was further used to determine the optimum values of process variables for maximum yellow pigment production. The fit of the quadratic model was found to be significant. A significant increase in yellow pigment production was achieved using RSM.

Keywords: Nutrient parameters, Response surface methodology, *Thermomyces* sp, Yellow pigment

INTRODUCTION

There is an increased interest on natural pigments to replace some currently used synthetic dyes, since the latter have been associated with toxic effects in foods (Mapari *et al*., 2009). The pigments from microbial sources are a good alternative that could easily be produced in high yields and capability of producing different coloured pigments. Pigment producing microorganisms and microalgae are quite common in nature which includes carotenoids, melanins, flavins, quinones and more specifically monascins, violacein, phycocyanin or indigo. However, there is a long way from the Petri dish to the market place as only five productions are operated on an industrial scale. The red pigment of the fungus *Monascus* is widely used in Asia for centuries as a food colorant (Kim *et al*., 1998). New food applications, like the coloration of sausage, hams, surimi and tomato ketchup, were described (Dufossé *et al.*, 2005).

The fermentation media need to be optimized for efficient utilization of the fermentation technology. Medium optimization by one-factor-at-a-time method involves changing one variable (nutrients, pH, temperature, etc.) while fixing the others at a certain arbitrary levels (Xu *et al.*, 2003; Poorniammal *et al*., 2011). The conventional ‘‘one-factor-at-a-time’’approach is laborious and time consuming, especially for large number of variables. Moreover, it seldom guarantees the determination of optimal conditions. These limitations of a single factor optimization process can be overcome by using statistical methods. In statistical based approaches, response surface methodology (RSM) has been extensively used in fermentation media optimization (An *et al.*, 2001).

Response Surface Methodology (RSM) is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions (Xiong *et al*., 2004). It is a statistically designed experimental protocol in which several factors are simultaneously varied. In RSM, the experimental responses to design of experiments (DOE) are fitted to quadratic function. The number of successful applications of RSM suggests second order relation to reasonably approximate many of the fermentation systems (Kalil *et al.*, 2008; Sani *et al*., 2013).

The objective of the present work was to apply statistical method to optimize the medium composition for the production of yellow pigment by culturing *Thermomyces sp*. Several important factors such as carbon source, nitrogen source, phosphate, and sulphate were studied by one factor at-a-time and subsequently by RSM.

MATERIALS AND METHODS

Microorganisms and culture conditions: The microorganism used in this study is *Thermomyces sp*, which was isolated from soil. Stock cultures maintained on Potato dextrose agar slants, which contained potato extract and dextrose and sub cultivated periodically. The slants were incubated at 28 ± 2 ^{0}C for 7 days. After cultivation of 5-7 days, spores were collected with 5 mL sterilized water, and

the spore suspension corrected was used as inoculum preparation. 0.5 mL of spores suspension was inoculated in 50 mL of submerged culture medium in 250 mL Erlenmeyer flasks, whose ingredients include (g/L) Yeast extract-5, Sucrose- 30, $NaNO_3$- 3, KCl- 0.5, K_2HPO_4-1, and $MgSO_4$-1. The submerged culture medium (initial pH 6.0) was cultivated at 28 ± 2 ºC for 6-7 days in a incubator.

Optimization of fermentation medium using one-factor -at-a-time method

Effect of different carbon sources on growth and pigment production: The effect of different carbon sources on the growth and pigment production of the selected fungi were studied. In the production medium, D (+) - glucose was substituted with different carbon sources *viz.* sucrose, fructose, lactose, maltose, glycerol and soluble starch.. All carbon sources were used at 30 g/l. Sucrose at 25–60 g/l was further studied for optimum yellow pigment production.

Effect of different nitrogen sources on growth and pigment production: The nitrogen source in czapek yeast broth was replaced with other organic as well as inorganic nitrogen sources such as meat peptone, beef extract, soya peptone, and malt extract and soybean meal. The inorganic nitrogen sources screened were potassium nitrate, sodium nitrite, ammonium nitrate, ammonium chloride and ammonium sulphate. Yeast extract and ammonium sulphate were further in the range of 3-7.0g/l

Effect of trace metals on growth and pigment production: The influence of heavy metal like copper, ferrous, zinc, magnesium and manganese on the growth and pigment production was investigated. Magnesium sulphate was further optimized in the range of 0.02–0.9 g/l. for improved pigment production. The effect of phosphate salts such as potassium dihydrogen phosphate, on the production yellow pigment was studied in the concentration range of 0.3–2.5 g/l.

Optimization of chemical parameters by response surface methodology: Once the critical variables were screened, RSM was performed to optimize the screened medium components for enhanced pigment production using the central composite design-uniform precision (CCD-uniform precision). A full factorial central composite factorial design of 2^5 = 32 plus 8 centre points and (2 * 5 = 10) star points leading to a total of 50 experiments were performed. For statistical calculations, the relation between the coded values and actual values are described in table 1.

Mycelium harvesting: The mycelium after growth was harvested at the end of fermentation by filtration using cheesecloth. Cells were washed three times with distilled water to remove the adhering salts. The biomass was dried to constant weight at 50^{o}C in laboratory oven for 8 h to obtain a constant dry weight.

Pigment extraction: To the filtrate, one volume of 95% (v/v) methanol was added and kept on a rotary shaker for 30 min at 150 rpm at 35°C and was centrifuged at 5000 rpm for 15 min. The same process was repeated for removal of fungal biomass and the filtrate was filtered through a preweighed What man filter paper (47 mm). Next, the absorption spectrum was observed at 410 nm using Hitachi U-2000 spectrometer (Hitachi Ltd., Tokyo, Japan.) The absorbance values were converted to colour units (Lin and Lizuka et al., 1982).

The experimental design based on response surface methodology for the optimization of chemical parameters on pigment production is given in the Table 1.

According to central composite rotatable design for five independent factors, the total experiments to be conducted were found to be fifty. 50 experiments were performed for five variables with five levels of each variable which are shown in Table 1. Fifteen experiments starting from 1 to 50 were formulated for optimization of chemical parameters on pigment production. The base level treatment from 43- 50 was replicated eight times to calculate the reproducibility of the method and the other treatments were replicated thrice. The maximum and minimum variable levels were selected on the basis of preliminary trials. The experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The experiments were conducted with second order design so that both the first and second order models can be postulated according to the adequacy of fit. For each combination of the independent variables in the experimental design, the dependent parameters were found out (Box and Wilson, 1951; Box and Draper, 1987; Yu et al., 1997; Harry et al., 2010).

Statistical modeling of responses: Empirical statistical

Table 1. Natural levels, codes and intervals of variation of the independent variables in the design of experiments (chemical parameters).

Factors	Coded symbols	Coded levels					Interval of variation
		-α	-1	0	+1	α	
Sucrose	S	18.10	25	30	35	41.89	5
Yeast extract	Y	3.82	4.5	5.0	5.5	6.18	0.5
Ammonium sulphate	A	0.81	1.5	2.5	3.5	3.82	1.0
Magnesium sulphate	M	0.025	0.3	0.5	0.7	0.975	0.2
Dipotassium hydrogen phosphate	P	0.310	1.0	1.5	2.0	2.687	0.5

$s = \left(\frac{S-30}{25}\right)$…..(3.8), $y = \left(\frac{Y-5}{4.5}\right)$ …..(3.9), $a = \left(\frac{A-2.5}{1.5}\right)$…(3.10), $m = \left(\frac{M-0.5}{0.3}\right)$ ……(3.11), $p = \left(\frac{P-1.5}{1.0}\right)$ ……(3.12).

modeling was used to develop an appropriate approximating model between the response (*y*) and independent variables $(X_1, X_2, X_3, \ldots\ldots X_k)$.

In general the relationship is

$$Y = f(X_1, X_2, X_3, \ldots\ldots X_k) + e \quad \ldots\ldots (3.13)$$

The variables $X_1, X_2, X_3, \ldots\ldots X_k$ in the Eqn. 3.13 are usually called the natural variables, because they are expressed in OD units and biomass (g / 100 ml broth) in RSM it is convenient to transform the natural variables to coded variables $x_1, x_2, x_3, \ldots\ldots x_k$, which are dimensionless having mean zero and variance equal to 1. In terms of the coded variables, the response function (Eqn. 3.14) will be written as

$$y = f(x_1, x_2, x_3, \ldots\ldots x_k) + e \quad \ldots\ldots (3.14)$$

The function '*f*' is called the response surface. The form of the function '*f*' is unknown. The term '*e*' represents other sources of variability not accounted for in '*f*' and usually it is treated as statistical error. As the form of the response function '*f*' is unknown, it must be approximated. Polynomials are often chosen because they usually offer an adequate approximation of the true response surface. In many cases, either a first order or a second order model as shown below is used.

$$y = b_0 x_0 + \sum_{i=1}^{k} b_i x_i + e \quad \ldots\ldots (3.15)$$

$$y = b_0 x_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} b_{ij} x_i x_j + e \quad \ldots\ldots (3.16)$$

The first model (Eqn. 3.15) is likely to be appropriate when the experimenter is interested in approximating the true response surface over a relatively small region of the independent variables space in a location where there is no curvature in '*f*'. If there is a curvature in the system, then a polynomial of higher degree such as second order model (Eqn. 3.16) must be used.

To find out the effect of the independent variables on the dependent variables, the first order linear equation (Eqn. 3.17) was fitted between '*x*' and '*y*'. For optimization of the independent variables and to check the sufficiency of the experimental design, the second order non-linear regression equation (Eqn. 3.18) was fitted between dependent and independent variables.

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 \quad \ldots\ldots (3.17)$$

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{11} x_1^2 + b_{22} x_2^2 + b_{33} x_3^2 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 \quad \ldots\ldots (3.18)$$

where,

y is the response variable

b_0, b_1, b_2 and b_3 are regression coefficients of linear terms

b_{11}, b_{22}, and b_{33} are regression coefficients of quadratic terms

b_{12}, b_{13} and b_{23} are regression coefficients of cross -product terms

x_1, x_2, and x_3 are the coded values of the independent variables *X*, *viz.*, pH (X_1), temperature (X_2), and incubation time (X_3) respectively. 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 F-test. The significance of the regression coefficient was determined by p-value. The coefficients of the equation were determined by employing Design Expert software Version 7.1.6. Analysis of variance (ANOVA) for the final predictive equation was done using Design Expert software. The response surface analysis was made keeping one independent variable at middle level while changing the other two. The response surface equation was used to optimize the independent variables for the response variables such as pigment yield and biomass.

RESULTS AND DISCUSSION

Optimization of chemical parameters using one-factor -at-a-time: During the microbial fermentations, the carbon source not only acts as a major constituent for building of cellular material, but also as important energy source.

Among the carbon sources evaluated in this study, sucrose gave a maximum yield of yellow pigment. It may be due to the fact that sucrose can be easily assimilated in the metabolic pathway for biosynthesis of pigment production (Kim *et al*., 1998).

A stimulatory effect of nitrogen source on pigment formation has been reported that utilization of different nitrogen sources in fermentation had effects on microorganism growth and pigment production(Cho et al., 2002). In the present study out of 14 nitrogen sources tested for growth and pigment production, inorganic nitrogen source ammonium sulphate and organic nitrogen source yeast extract were preferred by *Thermomyces* sp. Ammonium and peptone served as good nitrogen sources that yielded better growth and pigmentation of *Thermomyces* sp (Figs 1 and 2).

It has been reported that bio-elements are one of the important factors affecting pigment production in several micro-organisms (Chen and Johns, 1993). A critical finding between pigment and metal ions was reported (Fogarty and Tobin 1996; Gunasekaran and Poorniammal, 2008). Thus, in order to investigate the effect of bio-elements on mycelial growth and pigment production, *Thermomyces* sp was cultivated in the above optimized culture medium. The maximum pigment production was achieved when magnesium sulphate (691 OD units) was supplemented (Fig. 3 and 4). The other bio-elements appeared to have no notable or detrimental effect on either mycelial growth or pigment production. The negative effect of ferrous and cobalt ions on the yellow pigment production may be explained by an indirect contribution of the metabolite to energy production in the cell. In fungal nutrition magnesium and calcium are noted as macronutrients and manganese, iron, copper and zinc as micronutrient but in case of red pigment production by *Monascus purpureus*, magnesium contribution was higher than calcium, iron and manganese. However, manganese contribution was found to be more than calcium and iron. This may be due to manganese, acting as cofactor

Table 2. Central composite rotatable design matrix of chemical parameters and their independent variables and corresponding experimental yields.

Stan-dard	Sucrose	Yeast extract	Ammonium sulphate	Magnesium sulphate	Dipotassium hydrogen phosphate	Pigment OD units	
						Observed	Predicted
1	25.00	4.50	1.50	0.30	1.0	248	296
2	35.00	4.50	1.50	0.30	1.0	877	855
3	25.00	5.50	1.50	0.30	1.0	456	511
4	35.00	5.50	1.50	0.30	1.0	1068	1066
5	25.00	4.50	2.50	0.30	1.0	476	522
6	35.00	4.50	2.50	0.30	1.0	1113	1085
7	25.00	5.50	2.50	0.30	1.0	715	742
8	35.00	5.50	2.50	0.30	1.0	1295	1301
9	25.00	4.50	1.50	0.70	1.0	101	144
10	35.00	4.50	1.50	0.70	1.0	722	722
11	25.00	5.50	1.50	0.70	1.0	338	382
12	35.00	5.50	1.50	0.70	1.0	976	955
13	25.00	4.50	2.50	0.70	1.0	319	374
14	35.00	4.50	2.50	0.70	1.0	965	956
15	25.00	5.50	2.50	0.70	1.0	565	617
16	35.00	5.50	2.50	0.70	1.0	1213	1194
17	25.00	4.50	1.50	0.30	2.0	197	252
18	35.00	4.50	1.50	0.30	2.0	832	807
19	25.00	5.50	1.50	0.30	2.0	446	486
20	35.00	5.50	1.50	0.30	2.0	1054	1037
21	25.00	4.50	2.50	0.30	2.0	418	468
22	35.00	4.50	2.50	0.30	2.0	1032	1027
23	25.00	5.50	2.50	0.30	2.0	671	708
24	35.00	5.50	2.50	0.30	2.0	1278	1262
25	25.00	4.50	1.50	0.70	2.0	65	93
26	35.00	4.50	1.50	0.70	2.0	659	667
27	25.00	5.50	1.50	0.70	2.0	289	350
28	35.00	5.50	1.50	0.70	2.0	935	920
29	25.00	4.50	2.50	0.70	2.0	278	313
30	35.00	4.50	2.50	0.70	2.0	915	891
31	25.00	5.50	2.50	0.70	2.0	520	575
32	35.00	5.50	2.50	0.70	2.0	1160	1148
33	18.11	5.00	2.0	0.50	1.50	508	263
34	41.89	5.00	2.0	0.50	1.50	1463	1610
35	30.00	3.81	2.0	0.50	1.50	425	380
36	30.00	6.19	2.0	0.50	1.50	995	942
37	30.00	5.00	0.81	0.50	1.50	444	389
38	30.00	5.00	3.19	0.50	1.50	974	931
39	30.00	5.00	2.0	0.020	1.50	836	794
40	30.00	5.00	2.0	0.980	1.50	533	478
41	30.00	5.00	2.0	0.50	0.31	765	712
42	30.00	5.00	2.0	0.50	2.69	650	605
43	30.00	5.00	2.0	0.50	1.50	704	708

Contd…..

44	30.00	5.00	2.0	0.50	1.50	693	708
45	30.00	5.00	2.0	0.50	1.50	692	708
46	30.00	5.00	2.0	0.50	1.50	724	708
47	30.00	5.00	2.0	0.50	1.50	746	708
48	30.00	5.00	2.0	0.50	1.50	752	708
49	30.00	5.00	2.0	0.50	1.50	732	708
50	30.00	5.00	2.0	0.50	1.50	689	708

Table 3. Analysis of variance (ANOVA) for optimization of chemical parameter for pigment production by *Thermomyces sp*.

Factor	Regression Co efficient	Standard error	P value
Intercept	708.57	24.82	< 0.0001
A-A	283.03	10.75	<0.0001
B-B	118.15	10.75	0.0001
C-C	113.83	10.75	0.0001
D-D	-66.41	10.75	0.0001
E-E	-22.43	10.75	0.0458

for different enzyme required for pigment biosynthesis (Makhmur and Bibhu, 2014).

Optimization of chemical parameters using central composite designs (CCD): Physical parameters pH, temperature and time were optimized using three level central composite designs (CCD). The physical parameter greatly influences the pigment production. Based on the results, chemical parameters were optimized (Chung *et al.,* 2009; Poorniammal *et al.,* 2013). To examine the combined effect of five different medium components (independent variables) on pigment production, a central composite design of 50 experiments was performed. The five independent variables (sucrose, ammonium sulphate, yeast extract, and magnesium sulphate and di-potassium hydrogen phosphate) and their concentrations at different coded and actual levels of the variables employed in the design matrix are shown in Table 1. Five level central composite design matrix and the experimental responses of the dependent variables (pigment) are listed in Table 2. Second order polynomial equation was used to correlate the independent process variables, with pigment production. The second order polynomial coefficient for each term of the equation was determined through multiple regression analysis using the Design Expert. The results were analyzed by using analysis of variance (ANOVA) suitable for the experimental design. The results are shown in tables 3 and 4. The Model F-value of 49.77 implies that the model was significant. Model F-value was calculated as ratio of mean square regression and mean square residual. Model P-value (Prob > F) was very low (0.0001). The P values were used as a tool to check the significance of each of the coefficients, which, in turn, were necessary to understand the pattern of the mutual interactions between the test variables. The smaller the magnitude of the P, the more significant was the corresponding coefficient. Values of P less than 0.05 indicated that the model terms were significant.

The coefficient estimates and the corresponding P values suggested that, among the test variables used in the study, A (Sucrose), B (yeast extract), C (Ammonium sulphate), D ($MgSO_4$), E (K_2HPO_4), A * B (sucrose * yeast extract), A * C (sucrose * ammonium sulphate), A * D (Sucrose * $MgSO_4$), A * E (Sucrose * K_2HPO_4), B * C (yeast extract * Ammonium sulphate), B * E (yeast extract * K_2HPO_4) D *E ($MgSO_4$* K_2HPO_4), C * E (Ammonium sulphate * K_2HPO_4), D * E ($MgSO_4$ * K_2HPO_4) are significant model terms. Sucrose, yeast extract, ammonium sulphate and $MgSO_4$ ($P < 0.0001$) had largest effect on pigment production, followed by magnesium sulphate and K_2HPO_4 ($P < 0.0002$). The corresponding second-order response model that was found after analysis for the regression was,

Pigment = 708.57 + 283.13 * A+ 118.15 * B + 113.83 * C - 66.41* D - 22.43 * E - 0 .06 * A * B + 0.81 * A * C + 4.62 * A * D - 0.94 * A * E +1.25 * B* C +5.69 * B * D + 4.75 * B * E + 0.94 * C * D - 2.50 * C * E -1.81 * D * E + 40.41 * A2 - 8.29 * B2 - 8.46 * C2 - 12.79 * D2 - 8.73 * E2

The fit of the model was also expressed by the coefficient of determination R^2, which was found to be 0.971, indicating that 97.10% of the variability in the response could be explained by the model. The closer the R^2 value is 1, the better the model is fit to experimental data, the less is the distance between the predicted and the observed values. Accordingly, three-dimensional graphs were generated for the pair-wise combination of the five factors, while keeping the other two at their center point levels. Graphs are given here to highlight the roles played by various factors (Fig 5). From the central point of the contour plot or from the bump of the 3D plot the optimal composition of medium components was identified. By keeping another variable at its optimal level, three-dimensional plots of two factors versus pigment production were drawn, and the corresponding contour plot was obtained. From the bump of three-dimensional plot or the central point of its respective contour plot; the optimal composition of medium components was identified. The optimal

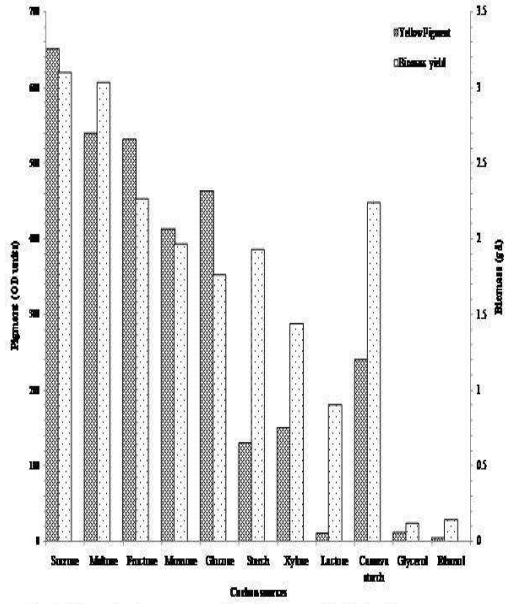

Fig. 1. *Effect of carbon source on yellow pigment production by Thermomyces sp.*

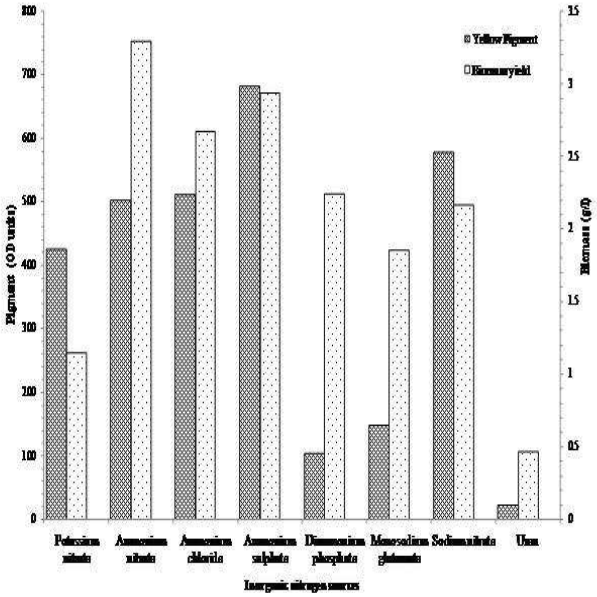

Fig. 2. *Effect of inorganic nitrogen source on yellow pigment production by Thermomyces sp.*

concentrations for the five components as obtained from the maximum point of the model were calculated to be as 35.5 g/l, 5.5g/l, 2.5 g/l, 0.3 g/l and 1.0 g/l for sucrose, yeast extract, ammonium sulphate, magnesium sulphate and K_2HPO4, respectively. By substituting levels of the factors into the regression equation, the maximum predictable response for pigment production was calculated and was experimentally verified. The maximum production of yellow pigment obtained experimentally using the optimized medium was 1301, which is in correlation with the predicted value of 1387 by the RSM regression study (Table 5).

Grape waste as a substrate for submerged fermentation of *M. purpureus* for pigment production employing factorial design. The 3 independent variables (Carbon source, peptone and monosodium glutamate) were evaluated having as response to pigment production. The result clearly showed that peptone concentrations were the most significant variable for pigment production (Choudhari and Singhal, 2008; Haisheng *et al.*, 2009; González, *et al*., 2010).

Peptone, ammonium nitrate and dipotassium hydrogen phosphate as the most significant variables for yellow pigment production by *M.anka* in submerged fermentation. The RSM optimized these nutrients for maximum yellow pigment production (Silveria *et al*., 2008; Zhou *et al*.,

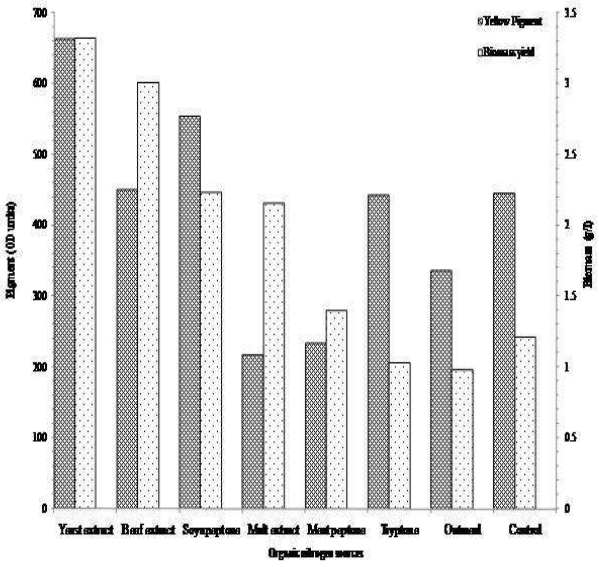

Fig. 3. *Effect of organic nitrogen source on yellow pigment production by Thermomyces sp.*

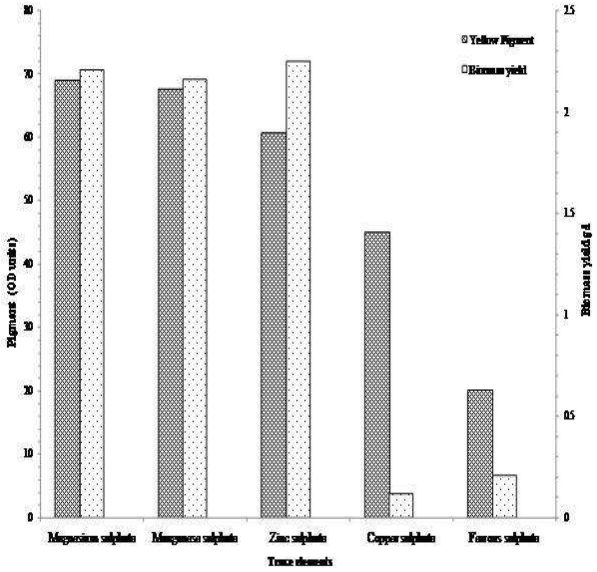

Fig. 4. *Effect of trace elements on yellow pigment production by Thermomyces sp.*

2009). According to the fitting equation, experiments under the optimized conditions, the average yellow pigment production obtained was 88.14 OD units for flask cultivation and 92.45 OD units for fermentor condition. Designing the medium is an open ended, time-consuming and laborious process involving large number of experiments. The CCD experimental design is the preliminary technique for rapid illustration of the effects of various medium constituents.

Conclusion

In the present study, the all nutrients were identified as

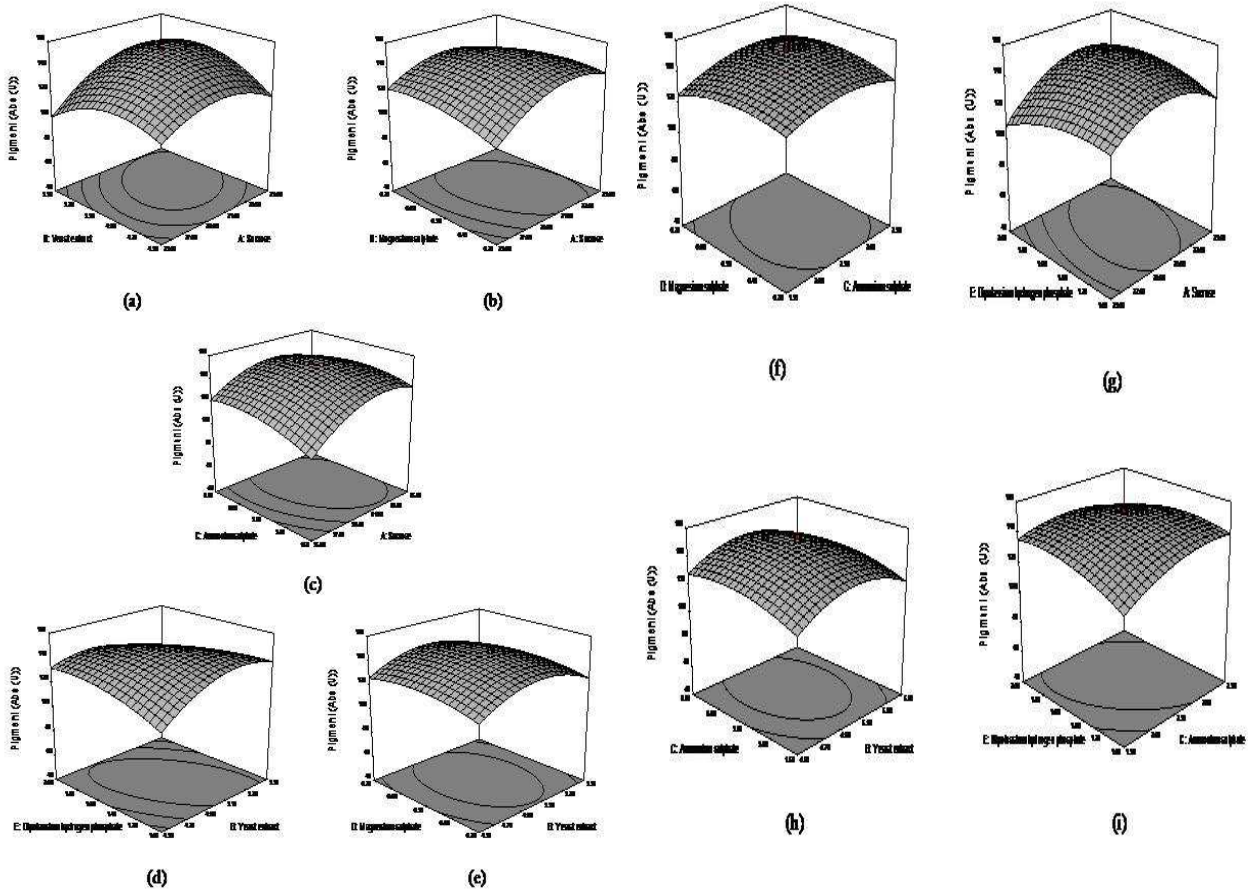

Fig. 5. *Response surface plots for the yield of yellow pigment; changing components were sucrose and yeast extract (a), sucrose and $MgSO_4$ (b), Sucrose and Ammonium sulphate (c), $MgSO_4$ and Yeast extract (d), K_2HPO_4 and yeast extract (e), K_2HPO_4 and sucrose (f), $MgSO_4$ and Ammonium sulphate (g), Ammonium sulphate and Yeast extract (h) and K_2HPO_4 and Ammonium sulphate (i).*

Table 4. Regression coefficient for optimization of chemical parameter for pigment production by *Thermomyces sp.*

Source	Sum of Squares	Degree of Freedom	Mean Square	*F*-value	Significance-*F*
Regression	4.983E+ 006	20	2.491E+006	49.77	<0.0001
Residual	1.452E+006	29	5005.33	-	-
Total Model	5.128E+006	49	-	-	-

$R^2 = 0.9717$, adjusted $R^2 = 0.9522$

Table 5. Experiment at the process conditions for maximum pigment production as predicted by model.

Depend-ent vari-ables	Independent variables yield					Pigment OD units	
	Sucrose	Yeast extract	Ammonium sulphate	Magnesium sulphate	K_2HPO_4	Observed	Predicted
Pigment (OD units)	35.00	5.50	2.50	0.30	1.0	1301	1387

most influencing components for enhancing yellow pigment production by *Thermomyces sp*. and then their optimal concentrations were obtained by using Response surface methodology. The production of yellow pigment increased to 37% compared to the unoptimized medium. In recent years, production of natural food colourants through microbial fermentation is an extensive area of investigation, since they overcome concerns of unfavorable side effects by synthetic colours. The pigment produced by *Thermomyces sp*. indicated its importance in food, textile, pharmaceutical and nutraceuticals industries

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