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Optimizing Submerged Cultivation for the Production of Red Pigments by *Monascus purpureus* on Soybean Meals using Response Surface Methodology

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Abstract

Background and objective: *Monascus purpureus* is a filamentous fungus with ability to produce pigments with therapeutic properties. Red pigments are especially used as additives, antioxidants, preservatives and substitutions for nitrites in food technology. To decrease fermentation costs, agro-industrial wastes such as soybean meals have been used as substrates. In the current study, red pigment production by *Monascus purpureus* on soybean meals was optimized.

Material and methods: In this study, red Monascus pigment production *by Monascus purpureus* ATCC 16362 was carried out under submerged fermentation using soybean meals as nitrogen sources to replace yeast extracts. Central composite design was used to assess the optimum level of soybean meal replacement (0-100%), $ZnSO_4.7H_2O$ concentration (0-0.02 g l^{-1}) and thermal stress time of spore suspension at 70°C (50-90 s). Red Monascus pigment and biomass productions were assessed as dependent responses.

Results and conclusion: The maximum production of red Monascus pigment (4.54 AU ml^{-1}) was achieved under conditions of soybean meal replacement of 79.72%, $ZnSO_4.7H_2O$ concentration of 0-0.02 g l^{-1} and thermal stress time of spore suspension of 81.89 s. The average yield of red Monascus pigment, conversion factor of biomass in red pigment $Y_{P/X}$ and cell productivity included 0.324 AU ml^{-1} day^{-1}, 1.10 AU L g^{-1} and 0.292 g l^{-1} day^{-1}, respectively. Results of the current study have demonstrated that combination of soybean meal and yeast extract as nitrogen source is beneficial for the production of red Monascus pigment by *Monascus purpureus*.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Color is one of the most important characteristics of foods. Use of synthetic dyes can cause carcinogenicity and fetal defects. Therefore, natural pigments have become popular as raw materials in food, pharmaceutical and cosmetics industries. Microbial pigments, as natural pigments (bio pigments) extracted from microorganisms, are produced by a number of bacteria, algae and fungi. Microbial pigment production is preferred over other bio-dye productions because of easy cultivations, production short times and low costs [1,2].

Of the pigment producing microorganisms, *Monascus* (*M.*) *purpureus* is important due to the production of red, yellow and orange pigments with therapeutic properties such as cancer, inflammation, diabetes and fat prevention and reduction. The *M. purpureus* pigments have been used as antioxidants, additives and preservatives in food products and as substitutions for nitrites in processing of meats. Asian countries monitored citrinin in red yeast rice and established maximum limitations for citrinin in foods for humans [3]. Singgih et al. showed that ethanol extraction of Monascus pigments decreased citrinin, which made this pigment safe for human consumption [4].

To decrease costs of fermentation in industrial scales, agricultural and industrial wastes can be used as substrates.

Agro-industrial substrates are considered for the production of secondary metabolites such as production of xanthan from date extracts [5], surfactin from rice straws [6], mycoprotein from date wastes [7], molasses, starches and fruit and vegetable wastes [8], alpha-amylase from wheat bran, rice brans and potato peels [9] and carotenoids from potato skins, mung bean husks, onion peels and pea pods [10]. Recently, studies have been carried out on use of agricultural wastes such as date wastes [11], grape wastes [12], bakery wastes [13], orange wastes [14], sugarcane bagasse [15,16] and bug damaged wheat [17] to produce Monascus pigment under submerged fermentation. Submerge fermentation for the production of red Monascus pigment can solve problems of low income, capital-intensive, time-consuming and large surface area necessity in solid-state fermentations. Moreover, this method is more appropriate for large-scale industrial production than solid-state production [18].

Several studies have been carried out for red Monascus pigment production by *M. purpureus* as a safe microorganism. Production of red Monascus pigment under submerged fermentation is affected by various parameters such as pH [11,19], zinc [20], carbon [21,22] and nitrogen sources [19,22-24], salts and environmental stresses [25].

Yeast extract, as a nitrogen source, is a beneficial substrate for Monascus pigment production [23]. However, use of other economic sources seems appropriate. Lubricated seeds are rich in proteins and used in dietary enrichments as well as biotechnological processes during fermentation. Soybean meal is a byproduct of oil seeds after lubrication and solvent extraction [26]. Soybean meal contains more than 43% of proteins [27]. Up to date, Soybean meal has been used in various fermentation processes as a substrate to produce secondary metabolites from microorganisms, including fumaric acid by *Rhizopus oryzae* [28], ethanol by *Saccharomyces cerevisiae* and *Zymomonas mobilis* [29] and Monacolin K by *M. purpureus* [30].

In nature, microorganisms are constantly affected by physical and chemical changes in their environments. However, microorganisms include defense mechanisms against a variety of environmental stresses. Numerous secondary metabolites are produced under stressful conditions. Creating environmental stresses such as high temperatures, osmotic pressures and presence of heavy metals such as Zn increases pigment productions. Therefore, pigment production by the microorganisms is increased to adapt to the new conditions [2]. This method can be used to increase production of secondary metabolites from microorganisms [31]. Although Soybean meal residues have been targets of replacing C- and N- sources [26-28], no studies have been carried out to decrease the yeast extract as nitrogen source using Soybean meal in culture media for the production of Monascus pigment during fermentation. Therefore, the aim of this study was to identify significant nutritional and physical factors such as soybean meal replacement (0-100%), $ZnSO_4.7H_2O$ concentration (0-0.02 g l^{-1}) and thermal stress time of spore suspension of 70°C (50-90 s) to produce red Monascus pigment from *M. purpureus* during submerged fermentation using response surface methodology.

2. Materials and methods

2.1. Microorganism

The *M. purpureus* ATCC 16362 was provided as live culture by the Iranian Scientific and Industrial Research Organization (IROST) (Tehran, Iran). Microorganism was cultured in potato dextrose agar (PDA) media and incubated at 30°C for 7 days. Then, culture was stored at 4°C until use [11].

2.2. Preparation of inoculum and seed culture

The yeast extract powder soluble starch media included 4 g of yeast extract, 1 g of K_2HPO_4, 0.5 g of $MgSO_4$ and 15 g of soluble starch per liter. Seed culture was prepared using 7-day-old *M. purpureus* spores. The number of spores was adjusted using hemocytometer at 10^5 (spore ml^{-1}). Spore suspension was heat stressed at 70°C with response surface design (50-90 s). These were then incubated with agitation at 120 rpm for 24-48 h at 30°C [11,25].

2.3. Media preparation and submerge fermentation

The Soybean meal was purchased from a local market and then milled to powder and sieved using 60-mesh net screen. Flavourzyme 1% (w w^{-1}) (Novozyme, Denmark) was added to the Soybean meal and mixed at 100 rpm for 24 h at 40°C. The culture media included 1 g glucose, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.1 K_2HPO_4, 0.001 g $FeSO_4.7H_2O$, 0.05 g KCl, 0.3 g $NaNO_3$, 0.4 g yeast extract (yeast extract contents varied in each treatment and SBM (0-100%) replaced it) and 0-0.002 g of $ZnSO_4.7H_2O$ per 100 ml (Table 1). Media was autoclaved at 121°C for 15 min [32,11]. Then, 10-15% (v v^{-1}) of the seed culture added to the media and incubated at 30°C for 14 days using shaker incubator (120 rpm) [11].

Table 1. Levels of the variables in central composite design ($\alpha = 1.68$)

	Range of levels				
Variables	$-\alpha$	-1	0	+1	$+\alpha$
SBMR† (%) (X_1)	0	20.27	50	79.73	100
$ZnSO_4,7H_2O$ (g l^{-1}) (X_2)	0	0.004	0.01	0.016	0.02
TST‡ (S) (X_3)	50	58.11	70	81.89	90

† Soybean meal replacement
‡ Thermal stress time

2.4. Analytical method

2.4.1. Quantitative analysis of pigment

Pigment extraction was carried out according to Kantifedaki et al. Fermented media was mixed with alcohol (70%) and transferred to ultrasonic bath (Parsunic, Iran) for 30 min at 25°C. Then, mixture was filtered using no. 1 Whatman filter papers. The supernatant was used for the red Monascus pigment estimation by measuring the absorbance at 500 nm using spectrophotometer (UNICO2100, USA) and expressing the values as AU ml^{-1} [14].

2.4.2. Biomass assessment

Filtration of the culture media was carried out using no. 1 Whatman filter papers and weighed using digital scale. Filtrates were dried at 75°C until achievement of a constant weight and weighed using digital scale [24].

2.5. Experimental design

In the present study, variables of soybean meal replacement (X_1) (0–100%), $ZnSO_4.7H_2O$ cconcentration (X_2) (0-0.02 g l^{-1}) and thermal stress time (X_3) (50-90 s) were selected for further optimization by central composite design of response surface methodology using Design Expert Software v.7.0.0. Results of the central composite design experiments for investigating effects of red Monascus pigment (Y_1) and biomass (Y_2) productions are presented in Table 2. To investigate effects of the factor and their interactions, a central composite design experiment with 14 axial points and six center points was carried out (α = 1.68). After validation of the models, the average yield of red pigment (P_M) (Eq. 1), conversion factor of biomass in red pigment ($Y_{P/X}$) (Eq. 2) and average cell productivity (P_{cells}) (Eq. 3) were calculated after 14 days of incubating [21,33].

$$P_M \text{ (AU ml}^{-1}\text{ day}^{-1}) = \frac{P_{max} - P_0}{(t_{p_{max}} - t_{p_0})} \quad \text{Eq. 1}$$

$$Y_{P/X} \text{(AU L g}^{-1}) = \frac{P_{Max} - P_0}{X_{Max} - X_0} \quad \text{Eq. 2}$$

$$P_{cells} \text{ (g l}^{-1}\text{day}^{-1}) = \frac{X_{max} - X_0}{(t - t_0)} \quad \text{Eq. 3}$$

Where, P_{max} was the maximum rate of red pigment production at time $t_{P_{Max}}$ (AU ml^{-1}), P_0 was the quantity of red pigments at t_{p0} (AU ml^{-1}), $t_{P_{Max}}$ was the time of maximum red pigment production (day^{-1}), t_0 was the initial cultivation time, X_{max} was the maximum biomass formation at time t (g l^{-1}) and X_0 was the biomass formation at time t_0 (g l^{-1}).

3. Results and discussion

3.1. Regression model

Results of red *Monascus* pigment and biomass productions were illustrated in Table 2. Red *Monascus* pigment and biomass productions varied 0.5 ±0.28 to 5 ±0.27 AU ml^{-1} and 1 ±0.11 to 9.7 ±0.43 g l^{-1}, respectively, in various combinations of the 20 treatments. Models, describing behaviors of the responses, were created by finding the best setting of variables to optimize the fermentation process. Second order models of the two responses for coded variables were calculated using Eq. 4 for red Monascus pigment and Eq. 5 for biomass productions, respectively:

$$Y_1 = (+2.66) + (0.55\ X_1) - (0.82\ X_2) - (0.46\ X_3) - (0.61\ X_2 X_3) + (0.35\ X_1^2) \quad \text{Eq. 4}$$

$$Y_2 = (+7.93) + (0.13\ X_1) - (0.55\ X_2) - (1.43\ X_3) - (1.92\ X_1 X_2) - (1.13\ X_1^2) + (0.60\ X_2^2) - (1.27\ X_3^2) \quad \text{Eq. 5}$$

Table 2. Central composite design using experimental data and predicted values

Number	Variable			Red Monascus pigment (AU ml^{-1})		Biomass production (g l^{-1})	
	SBMR (%)	$ZnSO_4 \cdot 7H_2O$ (g l^{-1})	TST (s)	Experimental	Predicted	Experimental	Predicted
1	20.27	0.004	58.11	3.2±0.08	2.92	5±0.17	4.86
2	79.73	0.004	58.11	4±0.22	4.02	9.7±0.43	8.98
3	20.27	0.016	58.11	2.3±0.12	2.51	7.78±0.28	7.61
4	79.73	0.016	58.11	3.68±.31	3.60	3.99±0.56	4.03
5	20.27	0.004	81.89	3.6±0.28	3.23	3 ±0.32	1.99
6	79.73	0.004	81.89	5±0.27	4.32	7.02±0.72	6.11
7	20.27	0.015	81.89	0.61±0.17	0.37	4.5±0.12	4.74
8	79.73	0.016	81.89	1.89±0.26	1.46	1.7±0.10	1.16
9	0	0.010	70.00	2.56±0.52	2.74	4.22±0.46	4.52
10	100	0.010	70.00	4.1±0.33	4.58	4.1±0.21	4.97
11	50	0.000	70.00	3.5±0.25	4.03	5.9±0.63	7.16
12	50	0.02	70	1.2±0.39	1.28	5.4±1.05	5.31
13	50	0.01	50	2.98±0.20	2.84	6.5±0.52	6.75
14	50	0.01	90	0.5±0.28	1.30	1±0.11	1.92
15	50	0.01	70	2.3±0.58	2.66	8.67±0.49	7.93
16	50	0.01	70	2.87±0.43	2.66	8±0.68	7.93
17	50	0.01	70	2.65±0.75	2.66	7.6±0.80	7.93
18	50	0.01	70	2.7±0.3	2.66	7±0.18	7.93
19	50	0.01	70	2.4±.29	2.66	8.7±0.16	7.93
20	50	0.01	70	3.08±0.09	2.66	7.83±0.11	7.93

SBMR= soybean meal replacement, TST= thermal stress time,

Table 3. Variance analysis of the response surface design of *Monascus purpureus* red pigment (Y_1) and biomass (Y_2) productions

	Red *Monascus* pigment (Y_1)				Biomass production (Y_2)			
Source	df	Mean squares	F-value	P-value	df	Mean squares	F-value	P-value
Model	6	3.62	18.15	0.000	7	14.60	21.12	0.000
X_1	1	4.06	20.35	0.001	1	0.25	0.35	0.566
X_2	1	9.17	45.91	0.000	1	4.12	5.96	0.031
X_3	1	2.86	14.33	0.002	1	28.10	40.66	0.000
$X_1 X_2$	-	-	-	-	1	29.64	42.89	0.000
$X_1 X_3$	-	-	-	-	-	-	-	-
$X_2 X_3$	1	2.98	14.91	0.002	-	-	-	-
X_1^2	1	1.83	9.17	0.009	1	18.30	26.48	0.000
X_2^2	-	-	-	-	1	5.21	7.53	0.017
X_3^2	-	-	-	-	1	23.35	37.78	0.000
Residual error	13	0.20	-	-	12	0.69	-	-
Lack of Fit	8	0.27	0.37	0.105	7	0.88	2.08	0.218
Pure Error	5	0.084	-	-	5	0.42	-	-
Corrected total	19	-	-	-	19	-	-	-
$R_1 = 0.89$								
R_1(adj) 0.84								
$R_2 = 0.92$								
R_2(adj) 0.88								

Where, X_1, X_2 and X_3 represented soybean meal replacement, $ZnSO_4.7H_2O$ concentration and thermal stress time, and Y_1 and Y_2 represented red Monascus pigment and biomass productions, respectively. Based on Analysis of variance test (Table 3) coefficient of determination (R^2) and adjusted coefficient of determination (R^2_{adj}) for red Monascus pigment and biomass productions were 0.89, 0.84 and 0.92, 0.88, respectively. The R^2 value seemed better between 0 and 1. The closer the R^2 was to 1, the excellent model and the more accurate predicted responses were [34]. This demonstrated good compatibility of the experimental and predicted values for red Monascus pigment and biomass productions. Red Monascus pigment and biomass production model *F*-values of respective-ly18.15 and 21.12 and *p*-value of less than 0.0001 showed that the model was statistically significant. The lack-of-fit value of red Monascus pigment and biomass productions of 0.37 and 2.08 and p-value of 0.1052 and 0.218 showed that lack of fit was not statistically significant. Lack of fit is the variation of the data around the fitted model. If the model does not fit data extremely, the value is significant. Independent variables with no significance were neglected as backward elimination regarding their *p*-values ($P \leq 0.05$) and the model equation was modified to the new fitted model.

3.2. Interaction between the affecting factors

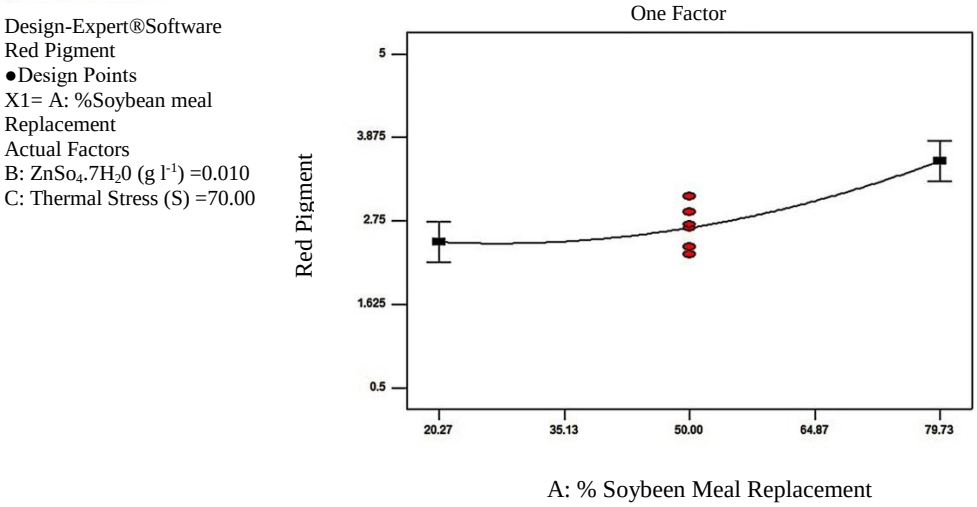
Of the three variables tested, soybean meal replacement (X_1), $ZnSO_4.7H_2O$ cconcentration (X_2) and thermal stress time (X_3) were highly significant for red Monascus pigment production, while interactions between the $ZnSO_4.7H_2O$ concentration and the thermal stress time ($X_2 X_3$) and the quadratic effects of soybean meal replacement (X_1) were significant based on the *p*-values (Table 3). Furthermore, 2D and 3D plots were created for the responses (red Monascus pigment production) at any of the two independent variables, while keeping the others at the middle point.

Based on Figure 1A, increases in soybean meal replacement resulted in increases in red Monascus pigment production. Amino acids have been reported as the most important N-sources to pigment production by *M. purpureus*. Since Soybean meal is rich in amino acids, it leads to the presence of further amine groups in the amination process of polyketide synthesis [35]. Interaction effects of various $ZnSO_4.7H_2O$ concentrations and thermal stress time on red Monascus pigment production are shown in Figure 1B. Increases in $ZnSO_4.7H_2O$ concentration led to decreases in red Monascus pigment production.

Toxicity of high zinc concentrations could be associated to the creation of magnesium deficiency, which disturbed the microbial growth and hence decreased red pigment production as growth-dependent metabolites [36,37]. The

red Monascus pigment production was further sensitive to higher levels of thermal stress time at 70°C and the red pigment content decreased with increasing thermal stress time (Figure 1B). Abrashev et al. showed that temperatures above 50°C for 10-20 min resulted in germination delay in spores of *Aspergillus niger* [38]. Disturbance in fungal metabolism leads to delay germination, resulting in decreased production of growth-dependent red pigments [37]. Based on Figure 1B, interaction effects of $ZnSO_4.7H_2O$ concentration and thermal stress time increased red pigment production using levels of both media ($ZnSO_4.H_2O$ of 0-0.01 g l^{-1} and thermal stress time of 50-70 s).

(A)



(B)

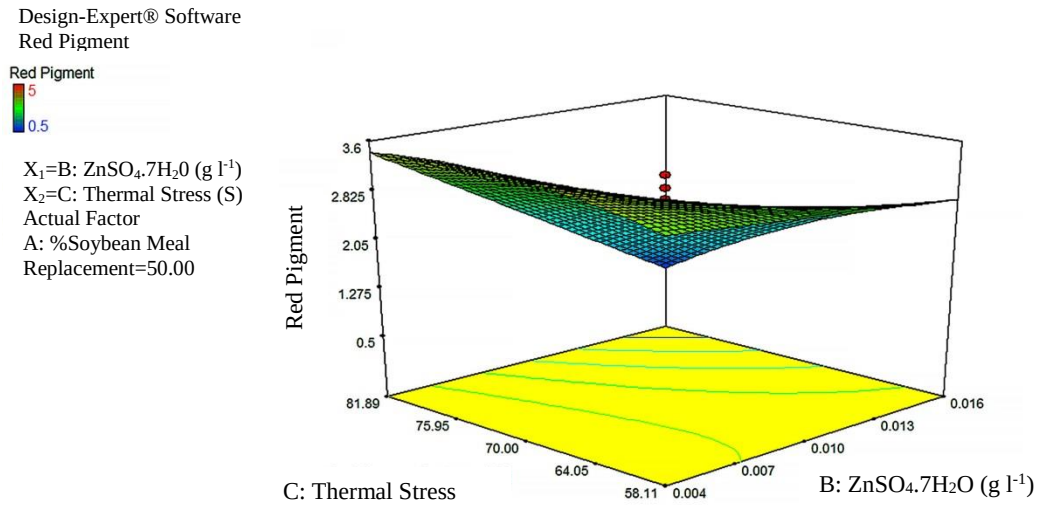
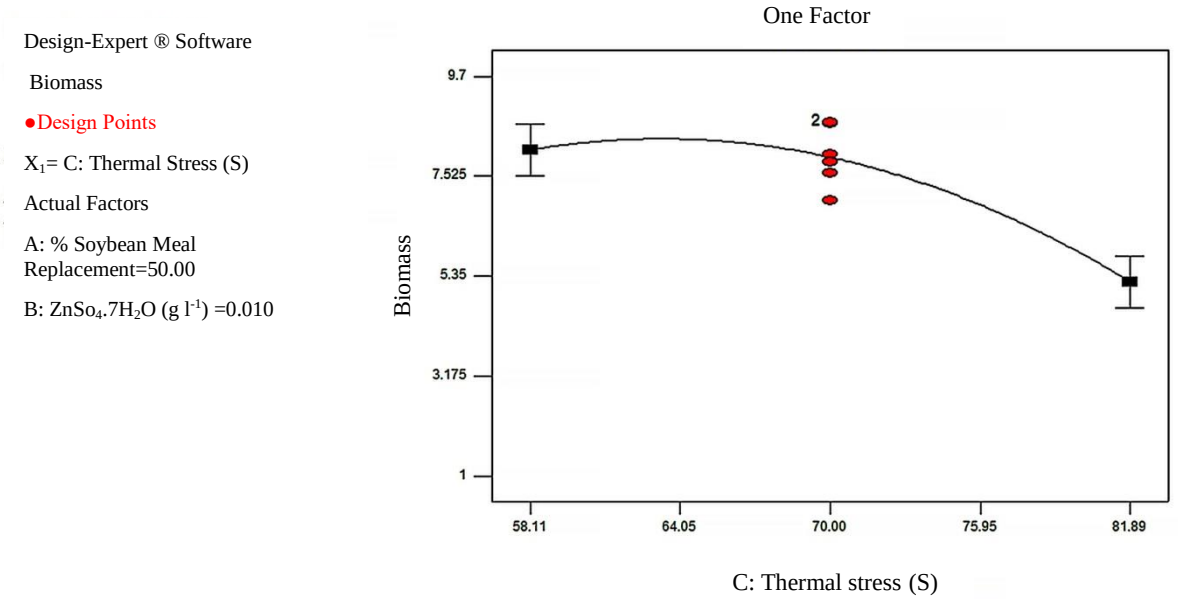


Figure 1. Effects of various levels of soybean meal replacement (A) and response surface of interaction effects between $ZnSO_4.7H_2O$ concentration and thermal stress time (B) on red pigment production by *Monascus purpureus*

In general, 2D and 3D plots were generated for the responses (biomass production) at any of the two independent variables, while keeping the others at their 0 levels. Based on Figure 2A, increases in thermal stress time led to decreases in biomass production. These were further sensitive to higher levels of thermal stress time at 70°C. Therefore, reduction process at higher levels of thermal stress time (greater than 70 s) was significant. This could be linked to delays in germination. Heat stress caused protein denaturation, resulting in polar bond weakening and exposure of hydrophobic groups. Therefore, reactive oxygen species were produced under the heat stress [39]. In nut shells, reactive oxygen species affects the apical tip growth, hyphal branching and cellular differentiation [40]. Figure 2B reveals that the interaction effects of various $ZnSO_4.7\ H_2O$ concentrations and soybean meal replacement on biomass production were significant ($P \leq 0.05$) (Table 3). Increases in soybean meal replacement of the media (0-50%) resulted in increases in biomass production up to 8.67 g l^{-1}. However, biomass production decreased by further increases in soybean meal replacement. The soybean meals contains a small quantity of zinc [41]. It could be discussed that the presence of SBM provided a sufficient quantity of zinc to stimulate the microbial growth. Bau et al. reported

that biomass production stopped with the presence of zinc at 2×10^{-3} and 3×10^{-3} M (0.57-0.86 g l^{-1}) in liquid media. In addition, the optimum concentration of $ZnSO_4.7H_2O$ for the growth of *M. purpureus* was 5×10^{-4} M (0.14 g l^{-1}) [20]. Therefore, presence of high quantities of zinc in media inhibited growth of *M. purpureus*. Gadd reported that a small quantity of zinc was essential for the growth of *M. purpureus*. However, higher quantities of zinc was toxic due to the blockage of functional groups of the enzymes [42].

(A)



(B)

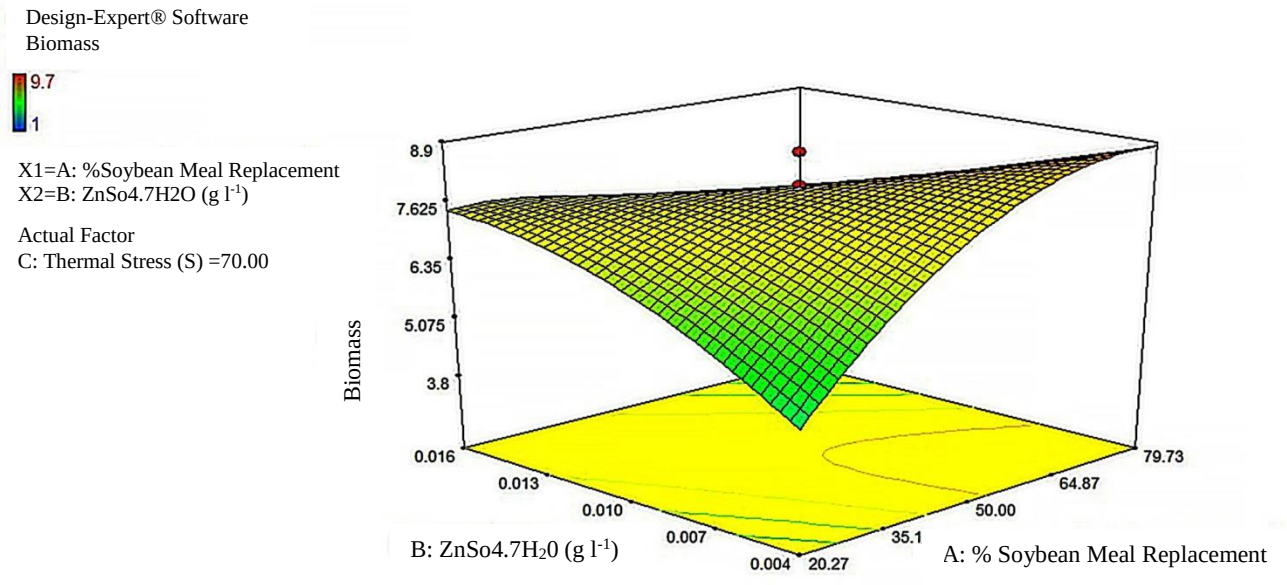


Figure 2. Effects of various levels of thermal stress time (A) and response surface of interaction effects between $ZnSO4.7H_2O$ concentration and soybean meal replacement (B) on biomass contents of *Monascus purpureus*

3.3. Validation of the model under optimized conditions

Validation was carried out under optimized conditions predicted by the model. To validate statistical model and regression equation, optimization program was used with investigation of experiment range using Design Expert Software and the following optimum conditions were achieved: soybean meal replacement of 79.72%, thermal stress time of 81.89 s and $ZnSO_4.7H_2O$ concentration of zero. Experimental responses for the red Monascus pigment and biomass productions included 4.54 AU ml^{-1} and 4.12 g l^{-1}, respectively. Experimental results were similar to the predicted value of 4.44 AU ml^{-1}. The average yield of red pigment, conversion factor of biomass in red pigment and average cell productivity of the optimized sample included 0.324 AU ml^{-1} day^{-1}, 1.10 AU L g^{-1} and 0.292 g $l^{-1}day^{-1}$, respectively. Production of the red Monascus pigment at optimized conditions was much higher than those reported by Meinicke et al. [22], Seyedin et al. [23] and Broder and Koehler [43]. Broder and Koehler (2001) produced 1.5 and 3.5 AU ml^{-1} of submerged red pigments using *M. purpureus* and yeast extracts (4 g l^{-1}) [43]. Seyedin et al. achieved red Monascus pigment at 2.05 ODU ml^{-1} using yeast extracts. In their study, K_2HPO_4 respectively included 2.75 and 1.5 g l^{-1} using response surface methodology [23].

4. Conclusion

The current study was carried out to assess effects of three important variables (soybean meal replacement, thermal stress time and $ZnSO_4.7H_2O$ concentration) on red pigment production by *M. purpureus* using response surface methodology. Results showed that use of soybean meal as a nitrogen source to replace yeast extracts increased red pigment production. Red Monascus pigment production was further sensitive to higher levels of thermal stress time at 70°C. Using selected levels of the process variables, a relatively high quantity of the red Monascus pigment was achieved with 72% soybean meal replacement and 81.89 s thermal stress time with no $ZnSO_4.7H_2O$ concentration in submerge fermentation. Under optimized conditions, quantity of the red Mp, average yield of red Monascus pigment (P_M), conversion factor of biomass in red pigments ($Y_{P/X}$) and cell productivity (P_{cells}) were assessed as 4.54 AU ml^{-1}, 0.324 AU ml^{-1} day^{-1}, 1.10 AU L g^{-1} and 0.292 g l^{-1} day^{-1}, respectively. In general, the lower application of yeast extract may result in decreases in media costs at large scales and can promote commercialization of the red Monascus pigment production from SBM. Successful replacement of yeast extract with SBM addresses gaps in other studies for costs of the recently optimized media based on use of yeast extracts for production of red Monascus pigment

5. Acknowledgements

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6. Conflict of interest

The authors declare no conflict of interest.

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بهینه سازی کشت غوطه‌وری *موناسکوس پورپورئوس* بر روی کنجاله سویا به منظور تولید رنگدانه قرمز با روش سطح پاسخ

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چکیده

سابقه و هدف: *مونا سکوس پورپورئوس* قارچی ر شته‌ای با توانایی تولید رنگدانه‌هایی دارای خواص درمانی می‌با شد. رنگدانه‌های قرمز به ویژه به عنوان افزودنی، آنتی‌اکسیدان، نگهدارنده و جایگزین نیترات در صنایع غذایی مورد استفاده قرار می‌گیرند. به منظور کاهش هزینه تخمیر، ضایعات کشاورزی_صنعتی مانند کنجاله سویا به عنوان ر شدمایه[1] مورد ا ستفاده قرار می‌گیرند. در مطالعه حا ضر، تولید رنگدانه قرمز تو سط *مونا سکوس پورپورئوس* بر روی کنجاله سویا بهینه‌سازی شد.

مواد و روش ها: در این مطالعه، تولید رنگدانه قرمز موناسکوس توسط کشت غوطه‌ور *موناسکوس پورپورئوس* ATCC 16362 با ا ستفاده از کنجاله سویا به عنوان منبع نیتروژن به جای عصاره‌های مخمر انجام شد. طراحی مرکب مرکزی به منظور ارزیابی سطح بهینه جایگزینی کنجاله سویا (۰-۱۰۰٪)، غلظت $ZnSO_4.7H_2O$ (۰-۰/۰۲ g l^{-1}) و مدت زمان تنش حرارتی سو سپانسیون ا سپور در ۷۰°C (۵۰-۹۰ s) مورد ا ستفاده قرار گرفت. تولید رنگدانه قرمز مونا سکوس و زی‌توده[2] به عنوان پاسخ‌های وابسته مورد بررسی قرار گرفتند.

یافته‌ها و نتیجه‌گیری: بیشینه تولید رنگ قرمز موناسکوس (۴/۵۴ AU ml^{-1}) تحت شرایط جایگزینی ۷۹/۷۲ درصدی کنجاله سویا، غلظت $7H_2O.ZnSO_4$ ۰-۰/۰۲ g l^{-1} و تنش حرارتی سوسپانسیون ۸۱/۸۹ ثانیه به دست آمد. متوسط راندمان رنگدانه قرمز موناسکوس (P_M)، ضریب تبدیل[3] زی‌توده در رنگدانه قرمز ($Y_{P/X}$)، و بهره‌وری سلولی به ترتیب ۰/۳۲۴ AU ml^{-1} day^{-1}، ۱/۱۰ AU L g^{-1} و ۰/۲۹۲ g l^{-1} day^{-1} بود. نتایج مطالعه حاضر نشان داده است که ترکیب کنجاله سویا و عصاره مخمر به عنوان منبع نیتروژن برای تولید رنگدانه قرمز موناسکوس توسط *موناسکوس پورپورئوس* مفید می‌باشد.

واگان کلیدی: طراحی مرکب مرکزی، *موناسکوس پورپورئوس*، رنگدانه قرمز، روش سطح پاسخ، کنجاله سویا

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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واژگان کلیدی

- طراحی مرکب مرکزی
- *موناسکوس پورپورئوس*
- رنگدانه قرمز
- روش سطح پاسخ
- کنجاله سویا

***نویسنده مسئول**

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[1] Substrates هر ماده‌ای که نیاز غذایی و بستر زیستِ ریزاندامگان‌ها را فراهم کند.

[2] Biomass
[3] Conversion factor