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Modeling Red *Monascus* Pigment Production on Date Waste Substrate Using Submerged Cultivation

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ABSTRACT: *Monascus* is one of the fungi that can be used to produce red pigments with food grades. In this study, *Monascus purpureus* ATCC 16362 was used to produce red pigments in date waste substrates, using submerged fermentation. Response surface methodology was used to optimize three significant factors of date waste sugar concentration (20–60 $g.l^{-1}$), NaCl (6–12 $g.l^{-1}$) and initial pH (6–9). The, effects of independent variables on red pigment and biomass content were assessed. Concentrations of 20 $g.l^{-1}$ date waste sugar and 6 $g.l^{-1}$ NaCl and pH 9 resulted in the maximum yield of red pigments of 6.05±0.04 $AU.ml^{-1}$ and biomass of 7.2 $g.l^{-1}$. Furthermore, substrate conversion, yield of red *Monascus* pigments on biomass and the volumetric productivity included 82%, 10.42 AU pigment g^{-1} biomass and 5.36 $g.l^{-1}.day^{-1}$, respectively. Therefore, from the results of this study, date waste can be used as a low-cost substrate for the production of red pigments in large-scale studies.

Keywords: Date Waste, *Munascus purpureus*, Red Pigment, Response Surface Methodology.

Introduction

Development of alternate ways for the production of natural pigments has been focused, recently. Natural pigments can be replaced for synthetic pigments. *Monascus* pigments (Mp) are the most important secondary metabolites of *Monascus* spp. These pigments are a mixture of azaphilones, composed of majorly red, orange and yellow pigments. Selection of the fermentation conditions that can encourage production of Mps needs a full understanding of the relevant biosynthetic pathways (Shi *et al*., 2015). Although biosynthesis of Mps still results in remedies, environmental factors such as temperature, a_W (addition of NaCl) and initial pH have been found to regulate pigment production (Babitha *et al*., 2007). Babitha *et al*. reported that *M. purpureus* LPB 97 produced further red pigments at high NaCl concentrations and 30 °C.[2] Initial pH strongly affects uptake of ionizable nitrogen sources and export of ionizable intracellular red pigments derivate during *Monascus* fermentation. However, higher pH increased red pigment biosynthesis (Yongsmith *et al*., 1993; Kang *et al*., 2013). Various agricultural

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products and byproducts such as corn cob (Velmurugan *et al*., 2011), sugarcane bagasse (Silveira *et al*., 2013), grape waste (Silveira *et al*., 2008), jackfruit seed (Babitha *et al*., 2007), corn steep liquor (Hamano and Kilikian., 2006), wheat substrate (Dominguez-Espinosa and Webb, 2003), cassava (Yongsmith, 1993), potato powder (Sharmila *et al*., 2013) and soy bean meal (Keivani *et al*., 2020) were successfully used for the production of Mps. The *Monascus* pigments may tolerate heat treatment conditions. Kinetic models for Dc of Mp under processing conditions were previously designed by Abdollahi *et al*., 2021.

Date (*Phoenix dactylifera* L.) is one of the most important crops in the Middle East countries such as Iran. More than one million tons of date fruits were produced in Iran in 2012. A large quantity of date fruits (nearly 30% of the total production) is removed from the economic cycle every year due to the damages that occur during picking, storage and production of dates (Forouzan *et al*., 2012; Elleuch *et al*., 2008). These large quantities of date wastes can beneficially be used as the potential sources of nutrients for the microorganisms in production of value-added products. Date wastes include high contents of carbohydrates, minerals and vitamins as well as low-cost substrates. Use of date wastes as substrates for the fermentation and production of red Mp by *M. purpureus* in solid state fermentation has been studied (Asghari *et al*., 2016). This study not only demonstrates feasibility of using date wastes to produce red pigments by *Monascus purpureus* ATCC 16362, but also focuses on the effects of pH and salt stress on the strain utilizing response surface methodology (RSM). These results can be used in commercial pigment production at industrial scales.

Materials and Methods

- ***Analysis of the chemical composition of date wastes***

Date wastes were purchased from Bushehr, Iran. Date waste fruits were stored at -20 °C ±1 until use. Moisture content was assessed at 105 ºC for 24 h using oven. Lipid content was assessed required Soxhlet apparatus (FOSS, Soxtec 2050, Sweden). Ash content was assessed at 550 ºC for 10 h using furnace (Heraeus, Germany). The total ash was expressed as the proportion of dry weight (%). Protein (N × 6.25) was analyzed based on the Kjeldahl (Tecator Kjeltec 1030 Analyzer, Foss, Warrington, UK) procedure (Horwitz, 2000).

- ***Preparation of the substrate***

For preparation of various concentrations of the substrate, 40 g of date wastes were weighed, cut into small pieces and mixed well in 300 ml of distilled water (DW). This was boiled for 10 min and filtered using filter papers (Whatman No. 1) to achieve clear date waste juices. Crude juices of date wastes were further diluted with DW to final concentrations of the substrate based on Table 1 (Keivani *et al*., 2020; Asghari *et al*., 2016).

- ***Preparation of fungal strains***

Monascus purpureus ATCC 16362 was provided by the Persian Type Culture Collection, Tehran, Iran. The *M. purpures* was cultured on YpSs (yeast powder soluble starch) slants at 4 °C and subcultured at 30 °C for 10 days monthly (Asghari *et al*., 2016; Baneshi *et al*., 2014).

- ***Preparation of the seed culture***

Seed culture media included waste date sugar, 20 g; yeast extract, 4 g; $NaNO_3$, 3 g; $FeSO_4.7H_2O$, 10 mg; KCl, 0.5 g;

$MgSO_4.7H_2O$, 0.5 g; and K_2HPO_4, 1 g in 1 L of DW. Ten day-old YpSs pure cultures of *M. purpureus* were used for the preparation of spore suspension. A suspension of spores was prepared by washing YpSs plates with DW; then, approximately 5×10^6 spores were inoculated into 50 ml of seed culture media using 250-ml Erlenmeyer flasks. This cultivation was carried out at 30 °C for 48 h at 120 rpm using shaking incubator (Sher 240, Sanateferdous, Iran). All treatments were carried out as per experimental design mixed (Table 1). Contents of the flasks were mixed with basal media consisted of yeast extract, 4 g; $FeSO_4.7H_2O$, 10 mg; KCl, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; and K_2HPO_4, 1 g in 1 L of DW. The seed culture (5×10^6 spore) was used to inoculate the flasks, incubating at 30 °C for 14 days at 120 rpm using rotary shaker (Keivani *et al*., 2020; Asghari *et al*., 2016).

- ***Pigment assessment***

Assessment of red Mp production was carried out by measuring absorbance maxima (505 nm) of the pigment extracts (Unico 2100 UV-vis Spectrophotometer, USA) and multiplying results by the dilution factor. Pigment yields were expressed as $AU.ml^{-1}$ clarified fermented broth (Abdollahi *et al*., 2021; Asghari *et al*., 2016).

- ***Statistical analysis***
- ***Optimization procedure using response surface methodology***

Pigment production was optimized using RSM. The results of the experimental design were analyzed using Design Expert Software v.8.0 (Stat-Ease, Minneapolis, Minnesota, USA). The pH and date waste and NaCl concentrations were optimized at 30 ˚C. Each factor in the design was studied at five levels (-1.68, -1, 0, 1 and 1.68). The experimental design with respect to their values in actual and coded is listed in Table 1. Pigment production ($AU.ml^{-1}$) was assessed in duplicate within 20 various experimental runs.

Table 1. Full experimentally response surface method with actual levels of variables

Run	Factor 1	Factor 2	Factor 3	Red pigmement ($AU\ ml^{-1}$)		Biomass (gl^{-1})
	A:Waste date sugar	B:NaCl	C:pH	Observed	Predicted	Observed
1	20	6	6	4.608	4.402	6
2	60	6	6	0.258	0.349	8.6
3	20	12	6	1.524	1.166	9
4	60	12	6	0.640	0.82	13.4
5	20	6	9	6.054	6.183	7.2
6	60	6	9	0.504	0.958	10.4
7	20	12	9	0.232	0.283	12.8
8	60	12	9	0.038	0.128	3.6
9	6.36	9	7.5	3.150	3.647	14.4
10	73.64	9	7.5	0.650	0.245	9.6
11	40	3.95	7.5	3.025	2.571	13.8
12	40	14.05	7.5	0.069	0.05	3.6
13	40	9	4.97	1.848	2.035	7.8
14	40	9	10.02	2.360	1.674	7.4
15	40	9	7.5	2.900	2.758	2.4
16	40	9	7.5	2.900	2.758	2.4
17	40	9	7.5	2.790	2.758	2.4
18	40	9	7.5	2.332	2.758	3
19	40	9	7.5	2.650	2.758	3
20	40	9	7.5	2.900	2.758	2.4

Data were fitted into the equation using multiple regression procedure. Model equation for the analysis was as Eq (1):

$$Y = \beta 0 + \Sigma \beta iXi + \Sigma \beta iiXi_2 + \Sigma \beta ijXiXj \quad (1)$$

Where, βo, βi, βii and βij respectively represented the constant, linear, quadratic effect of Xi and interaction effect between Xi and Xj for the production of red pigments. Validation experiment was carried out and the maximum production of red pigments was verified using the optimum values for variables predicted by the response optimization.

- ***Calculation of the fermentation parameters***

Substrate conversion was calculated based on the Eq. (2) as follows:

$$\Delta S\% = \frac{S_\circ - S}{S_\circ} \times 100 \quad (2)$$

Where, S_0 was the initial substrate concentration and S was the substrate concentration in the samples at each time interval. The volumetric productivity (Pp) was calculated as the ratio of maximum red pigment (Pmax, $g.l^{-1}$) to the fermentation time when the maximum concentration was achieved (tPmax, day) as follows:

$$P_P = \frac{P_{max}}{t_{P_{max}}} \left(\frac{g}{\text{L.day}}\right) \quad (3)$$

Yield of the red pigments in biomass ($Y_{P/X}$, g/g) was assessed using Eq. (4) as follows:

$$Y_{P/X} = \frac{P_{max} - P_\circ}{X_{max} - X_\circ} \left(\frac{\text{g}_{\text{pigment}}}{\text{g}_{\text{biomass}}}\right) \quad (4)$$

Where, P_0 and X_0 were respectively the initial product and cell concentration, while P_0 and X_0 were the product and cell concentration in the samples at each time interval (Keivani *et al*., 2020; Asghari *et al*., 2016).

Results and Discussion

- ***Chemical characteristic assessment of the waste dates***

The results from the chemical analysis are shown in Table 2. Elsanhoty *et al*. (2012) reported that the date fruits included moisture contents of 10–22%, proteins of 2.2–2.7%, total sugars of 62–75%, ashes of 3.5–4.2% and lipids of 0.4–0.7% on a dry weight basis. Date wastes are abundant and economical in Iran because of their inadequate texture. Date wastes are not edible and frequently disposed. Nowadays, use of these byproducts is limited for purposes such as animal feeds. Therefore, it is appropriate for the culture of microorganisms. Use of date waste substrate can be resulted in cheaper fermentation process as it does not need special treatments such as steam explosion, acid hydrolysis and enzymatic treatment to release sugars in fermentable forms (Chauhan *et al*., 2007). Dates are used for the production of high value-added components such as single cell protein, carotenoid, lactic acid, ethanol, glutamic acid and red Mp (Chauhan *et al*., 2007; Tavakkoli *et al*., 2012; Mehaia and Cheryan., 1991).

Table 2. Compositional analysis of wastes date

Component	Values(g/100g)
Moisture	60.48±0.26
Protein	1.97±0.09
Ash	1.26±0.05
Lipid	0.40±0.00
Reducing sugar	46.00±3.84

Means of three replicates ± SD

- ***Effective factors on red pigment production***

In this study, RSM was used to assess interactions between the significant factors and their optimal levels using second-

order model equation. Various levels of three-factor fractional RSM design with the observed and predicted responses for each run are summarized in Table 1. By taking red pigment production ($AU.ml^{-1}$) as the response value (Y), 20 experiments were designed; in which, 14 runs were the factorial experiments and six runs were the zero-point tests. The Zero-point tests were carried out in six replicates to estimate effects of the random errors (Jahadi *et al*., 2015). Furthermore, it shows production of red pigments ($AU.ml^{-1}$), corresponding to combined effect of all three components in specified ranges (Prajapati *et al*., 2014). Statistical significance of the second-order model equation was assessed using F-test analysis of variance (Table3), which revealed that this regression was highly significant statistically for the red pigment production ($p < 0.05$) (Bezerra., 2008). In this study A, B, C, AB, BC, A^2 and B^2 were significant model terms.

The F-value and *p*-value of model respectively included 27.61 and < 0.0001, which indicated that the model was significant. The R^2 value (multiple correlation coefficient) of the red pigment production model was 0.95, meaning a better correlation between the predicted and observed values. In fact, coefficient of variation (CV) demonstrates the degree of precision; to which, the experiment is compared (Bezerra, 2008). In the present study, a low CV (14.11) revealed that the experiment was reliable. The *p*-value of the lack-of-fit was 0.018, showing that the experimental data fitted well with the model and explaining effects of sugar date and NaCl content on the red Mp production. In the current study, values of the red pigments included 0.038–6.054 $AU.ml^{-1}$. Data were analyzed using Design Expert Software v.8 (Stat-Ease, Minneapolis, Minnesota, USA). Regression equation for the red Mp was as follows:

$$\text{Red pigment} = +2.50 - 1.11A - 1.02B + 0.048C + 1.10AB - 0.45BC - 0.27A^2 - 0.39B^2$$

Table 3. Variance analysis of the response surface design of *Monascus purpureus* red pigment (Y1) and biomass (Y2) productions

Source	Red *Monascus* Pigment				Bimass production^{-1}			
	Sum of Squares	Df	F-value	p-value	Sum of Squares	Df	F-value	p-value
Model	45.54	7	24.20	0.01	0.30	6	9.58	0.01
A-Waste date sugar	16.88	1	62.79	0.01	1.187E-003	1	0.23	0.63
B-NaCl	14.27	1	53.10	0.0`	9.908E-003	1	1.92	0.18
C-pH	0.032	1	0.12	0.73	1.335E-003	1	0.26	0.62
AB	9.73	1	0.01	0.01	-	-	-	-
BC	-	-	-	-	-	-	-	-
A^2	1.61	1	0.03	0.01	0.15	1	29.76	0.01
B^2	1.04	1	0.07	0.01	0.075	1	14.50	0.01
C^2	2.23	1	0.01	0.01	0.11	1	21.33	0.01
Residual	3.23	12	-	-	0.067	13		
Lack of Fit	2.78	7	4.48	0.06	0.041	8	0.97	0.53
Pure Error	0.44	5			0.026	5		
Cor Total	48.76	19			0.36	19		

Monascus purpureus red pigment productions (Y1): R-square= 0.94%, R-adjusted= 0.89%, R-prediction= 0.73%
Monascus purpureus biomass productions (Y2): R-square= 0.81%, R-adjusted= 0.73%, R-prediction= 0.58%

Analysis of the produced red pigments by *Monascus purpureus* ATCC 16362 is shown in Table 3. Results revealed that effects of the independent variables such as date waste sugar (A) and NaCl (B) were significant at $p < 0.05$, while effects of pH (C) were non-significant. Furthermore, effects of AB, BC, A^2 and B^2 were significant. As shown in Figure 1, red pigments increased with decreasing date sugar values. This was posssibly due to the Crab-three effect which generally occurs during fermentation at high sugar concentrations. This blocks respiratory enzymes and enhances ethanol production (De Deken, 1966). The highest value of red pigments (6.05 $AU.ml^{-1}$) in media was achieved at 20 g.l-1 of date waste sugar.

Mukherjee and Singh (2011) reported that when various concentrations of glucose used as sources of carbon to produce red pigments, the optimal concentration of glucose included 18 $g.l^{-1}$. They reported that pigment production decreased by increasing this concentration, possibly duo to aerobic fermentation metabolism. Said reported the best glucose concentration to produce pigment as 1–1.5%. The results showed that the lower and the higher levels of initial glucose included specific inverse effects on pigment production because osmotic pressure increased with increasing concentrations of glucose and hence decreased water availability (aW) for the microbial growth. In contrast, carbon source limitation stopped biomass and metabolites production (Said, 2010). Figure 2 shows effects of NaCl levels on the production of red Mp. Produced red pigments increased by increases in NaCl concentrations to 9 $g.l^{-1}$ and then decreased at higher concentrations of salt due to intensity of osmotic pressure, which decreased red pigment production. The current finding showed that decreases in pigment production might be due to the changes in glucose, NaCl and initial pH, concurrently. The results demonstrated that the red pigment production was further sensitive to salt changes. Babitha *et al*. showed further productions of the red pigments (Mps) at high salt concentrations in glucose based media. They reported that low concentrations of salt promoted growth of the fungal cultures. The results also demonstrated that the highest red pigment production occurred at 10 $g.l^{-1}$ NaCl concentration; in which, red pigment production increased from 11.86 to 20.14 $AU.g^{-1}$ (Babitha *et al*., 2007).

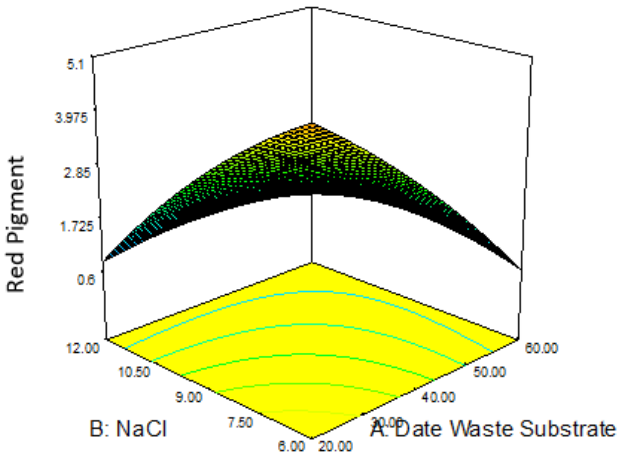

Fig. 1. Effects of date sugar and NaCl on red pigment production in submerged fermentation

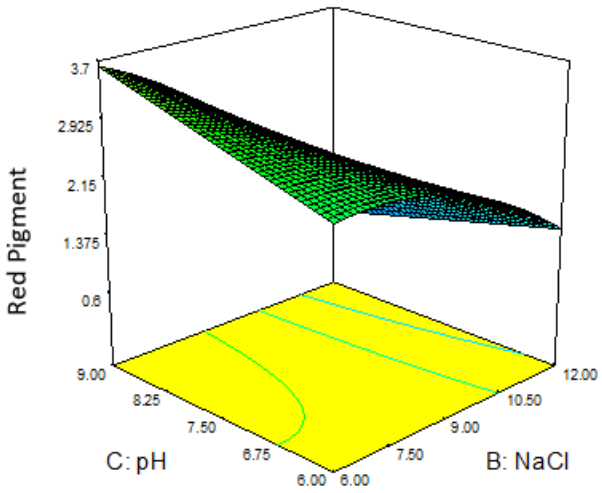

Fig. 2. Effects of NaCl and pH on red pigment production in submerged fermentation

Generally, pH is one of the most important factors to activate major enzymes in *M. purpureus* for the production of red pigments (Parajapati *et al*., 2014). The pH effects on the produced red pigments are shown in Figure 2. As shown in the figure, the Mp value increased with increasing pH. These observations are similar to those by Yongsmith *et al*. (1993) who reported that lower pH values promoted biosynthesis of the yellow pigments. However, higher pH values resulted in red pigments. Kange *et al*. (2013) reported that the export of intracellular ionizable products into extracellular broth depended on extracellular pH. They reported a relatively higher concentration of extracellular red pigments at initial pH 6. Silbir *et al*. (2019) reported that production of red pigments by *M. purpureus* at initial pH 6.5 was at its highest values of 22.25 AU500. At initial pH values 6 and 7, lower quantities of the red pigments were achieved, including 21.387 and 20.43 AU500, respectively. Moreover, they stated that the quantities of red pigments achieved in pH values 5.5 and 7.5 was lower, including 16.75 and 12.98 AU500, respectively. These results showed that production of red pigments by *M. purpureus* decreased with increasing or decreasing pH values. The optimum process variables to produce red pigments were analyzed using RSM and results were as follows: date sugar concentration of 20 $g.l^{-1}$; NaCl concentration of 6 $g.l^{-1}$ and pH 9 with the maximum red pigment production up to 6.05 $AU.ml^{-1}$. The mean value of the pigment yield was reported as 6.84 $AU.ml^{-1}$ ±0.04, which was quite close to the predicted value of 6.05 $AU.ml^{-1}$. Findings of the present study were similar to those of a study by Juzlova *et al*. Concentration of glucose should be set as less than 20 g.l-1 to prevent culture media over flow, which occurs at higher concentrations of 20 $g.l^{-1}$. Furthermore, they suggested this phenomenon changed aerobic metabolism to anaerobic one even at higher concentrations of oxygen. Additionally, it decreased the microbial growth rate and pigment production (Juzlova *et al*., 1996). Previous reports showed that the highest pigment production was achieved using potato powder (7.18 $AU.ml^{-1}$), sugarcane bagasse (3.38 $AU.ml^{-1}$), grape waste (5 $AU.ml^{-1}$) and a combination of soybean meal and yeast extract as nitrogen source (4.54

AU.ml^{-1}) (Silveira *et al*., 2008; Tavakoli *et al*., 2012). Asghari *et al*. (2016) reported that the maximum production of red *Monascus* pigments by *M. purpureus* included 5.10 AU.g^{-1}, when the proportion of date waste syrup, NaCl concentration and incubation time were 55%, 7 g.l^{-1} and 21 days in solid state fermentation, respectively.

- ***Biomass production***

As shown in Table 1, biomass production by *M. purpureus* in submerged fermentation was 2.4–14.4 g.l^{-1}. Results were assessed using Design Expert Software v.8 (Stat-Ease, Minneapolis, Minnesota, USA). The regression equation was as follows:

$$\text{Biomass} = +0.39 + 9.32A + 0.027B + 9.88C - 0.10A^2 - 0.072B^2 - 0.088C^2$$

Analysis of biomass variance is shown in Table 3. The *p*-values demonstrated that the linear effects of independent variables were non-significant ($p < 0.05$). As shown in Table 3, effects of the variables square, including waste date sugar (A), salt (B) and pH (C), were significant at $p < 0.05$. Negative sign of variables square coefficient revealed downward effects after reaching the maximum point on the reverse response variable (dry weight of biomass). As shown in Figure 3, nearly 40 g.l^{-1} increases in date sugar increased the inverse biomass content, while higher values of this variable decreased the inverse biomass content. Figure 3 shows that low salt concentrations of nearly 6–8% increased the inverse biomass content. However, further salt concentrations decreased the inverse biomass content drastically. Since the major aim of the current study was to produce the maximum pigment quantity; therefore, variables were optimized based on the maximum pigment production. In optimal levels of the variables, biomass concentration, substrate conversion, volumetric productivity and yield of the red pigments in batch fermentation of *Monascus purpureus* ATCC 16362 included 7.2 g.l^{-1}, 82%, 5.36 AU.l^{-1}.day^{-1} and 10.42 AU pigment g^{-1} biomass, respectively. The highest quantity of biomass was 14.4 g.l^{-1}. Results demonstrated that concentrations of the date sugar and salt and pH affected the maximum pigment production. However, the highest quantity of biomass was not produced. Therefore, effects of variables on biomass and pigment productions were not similar. At low salt concentrations (6–8 g.l^{-1}), fungal growth was improved while at high salt concentrations, biomass production drastically decreased.

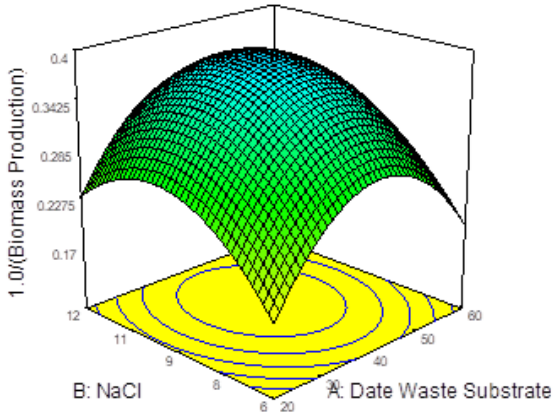

Fig. 3. Effects of date sugar and NaCl on biomass production in submerged fermentation

Finding appropriate inexpensive substrates is important, which can increase growth and red pigment production in *Monascus* species because price of the final products up to 70% depends on the primary substrates. In this study for the first time, waste dates were used as substrates to produce red pigments by *M. purpureus* in submerged cultivation. Based on the extensive studies in field of pigment production using *M. purpureus*, the most important factors to optimize include sugar and salt concentrations and pH. Factors such as nitrogen source, solutes and stirring time were reported as fixed factors (Mukherjee and Singh, 2011). The maximum quantity of pigments in culture media was achieved using a date sugar concentration of 20 $g.l^{-1}$. Results showed that decreases in date sugar concentrations included positive effects on the production of pigments. Decreased pigment production with increasing date sugar concentrations seems due to the aerobic fermentation metabolism (Miyake *et al*., 2008). Glucose concentration in culture media affected the biomass rate and pigment yield relative to biomass (Feng *et al*., 2012). Said and Hamid (2010) reported the best glucose concentration to produce pigments as 1–1.5%. While the lower and the higher glucose levels from this range included negative effects on the production of red pigments. Negative effects of high glucose contents possibly occurred because increases in biomass suppressed the pigment production. By increasing glucose concentration, osmotic pressure increased. Osmotic pressure at lower glucose concentrations of 1% decreased biomass production and a_W for fungal growth. Limited carbon resources inhibited metabolite production. Results revealed that red pigment productions decreased by increasing salt concentrations. Salinity stress was possibly due to the effects of osmotic potential inequality in the fungal cells, which inhibited biomass growth. It has been shown that low salt concentrations (6–8%) improves fungal growth. In this study, biomass drastically decreased by increasing salt concentrations. Increases in red pigments could be due to the pigment protective effects against osmotic stress (Babitha *et al*., 2007). Generally, pH is considered as a regulatory factor to produce red pigments and facilitate growth of *Monascus* spp. the maximum quantity of red pigments was achieved at pH 9, which was similar to that by Mukherjee and Singh (2011).

Conclusion

This study used date wastes as the low-cost substrates for the red Mp and biomass productions by *Monascus purpureus* ATCC 16362. Effects of the date waste sugar concentration, concentration of salt and pH on the produced red pigments and biomass in submerged fermentation were assessed. Red pigment and biomass productions ranged 0.03–6.05 $AU.ml^{-1}$ and 2.4–14.4 $g.l^{-1}$, respectively. Furthermore, the optimal conditions of independent variables to achieve the maximum quantity of red pigments included date sugar concentration of 20 $g.l^{-1}$, NaCl concentration of 6 $g.l^{-1}$and pH 9 with the maximum red pigment production up to 6.05 $AU.ml^{-1}$. The results demonstrated that production of the red pigments and biomass by *M. purpureus* increased with increasing date waste sugar concentration up to 40% and then decreased. While high concentrations of date sugar decreased biomass contents. Increases in NaCl concentrations up to 9 $g.l^{-1}$ increased production of the red pigments while decreased biomass contents. Furthermore, high pH increased quantity of the red pigments. The results have shown that date

wastes can be used as excellent inexpensive substrates to produce red pigments as well as good quantities of biomass by *M. purpureus.*

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