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Experimental Evaluation of Airlift Photobioreactor for Carotenoid Pigments Production by *Rhodotorula rubra*

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Abstract

The ability of Rhodotorula rubra in carotenoids production was enhanced to 3.98 mg g_{cell}^{-1} using methanol as a progressive carotenogenesis agent when compared with the parent cell in shake-flask experiments. Further tests on the effects of aeration rate (X_1), initial pH of the growth medium (X_2), and intensity of white-light irradiation (X_3) on the carotenogenesis activity of the cell were statistical studied by response surface methodology (RSM) in an airlift photo-bioreactor (APBR). The experimental data were modeled by second order polynomials. The ANOVA test revealed that aeration rate and light irradiation intensity were the most influential factors on cell formation and carotenoids production, respectively. Using desirability function showed that the following combination of the factors: aeration rate = 0.469 vvm, initial pH = 6.48, and light irradiation intensity = 1757.84 Lx which resulting to a maximum performance in the APBR with biomass formation of 6.69 g L^{-1}, the carotenoids yield of 8.352 mg $gcell^{-1}$, and volumetric carotenoids production rate of 14.3 mg L^{-1} day^{-1}. The predicted maximum condition was experimentally evaluated and the both responses showed less than 5% of errors.

Keywords: Operational condition, Design of experiment, Response surface methodology, Carotenoid pigment, Airlift bioreactor.

1. Introduction

Carotenoids are natural pigments that have widespread application in food industry and medicine as a colorant and/or antioxidant agent. Although plants were primary source for carotenoids preparation however, microorganisms as alternative source found more attraction in the last decade (Zoz & al. [1]). Industrial production of carotenoids with microorganisms has multiple benefits in comparison to plant production in low cost and short duration of the generation. *Rhodotorula* yeasts are among famous microorganisms that capable to produce high concentration of carotenoid pigments mainly during the stationary phase of their growth (ZOZ & al. [1]). Carotenoids in the cell have a protective role against damageable effects of light, singlet oxygen and other oxidant agents on cell membrane (ZOZ & al. [1]; SAKAKI & al. [2]). With considering the functionality of carotenoids in cell's metabolite, stimulatory conditions could be improved the yield of carotenogenesis process. Type of bioreactor and environmental conditions are important factors that influencing on the performance of the cell and thus it must be optimized in a new biotechnological process. Various bioreactors such as a stirred tank (GOKSUNGUR & al. [3]; MALISORN and SUNTORNSUK [4]) and bubble column reactors have been widely used in the literatures for the carotenoids production, but a

few study reports in airlift bioreactor (VARZAKAKOU & al. [5]). In airlift bioreactors, the fermentation medium is randomly mixed by ascending air bubbles which sparging at the bottom of the riser region in an airlift bioreactor. The difference of gas holdup between the riser and downcomer sections, creates pressure difference for circulation of liquid from the downcomer into the riser in the vessel (CAMPANI & al. [6]). In comparison with bubble column and stirred tank bioreactors, the advantages of airlift bioreactor are due to receiving energy uniformly throughout the system for the fluid to flow, simplicity of construction and low power consumption in elimination of mechanical agitation system.

Some influential variables in biological processes have interactional effects and results ability of the cells shows curvature around the optimal setting conditions. Response surface methodology (RSM) consists of mathematical and statistical techniques for development of functional relationship between the response of interest and a number of controllable input variables in a complex process (KHURI and MUKHOPADHYAY [7]). The carotenoids production by *R. rubra* in an airlift photo-bioreactor (APBR) also depends on the cultivation conditions. Although several strategies for improvement of the carotenoids production in the carotenogenesing cell have been applied in the literatures (SAKAKI & al. [2]; FRENGOVA & al. [8]; KIM & al. [9]), however to the best of our knowledge, there is no report on the optimization of carotenoids production process by the statistical analysis of the culture conditions (*R. rubra*) in an APBR. In this study, RSM as a statistically-based design of experiment (DOE) was used to study the effects of aeration rate (X_1), initial pH of the growth medium (X_2), and intensity of light irradiation (X_3) on the biomass formation (Y_1), carotenoids production yield (Y_2), and volumetric carotenoids production rate (Y_3) as the responses in an APBR operated at a batch mode.

2. Materials and Methods

All chemicals including those used for preparation of bacterial growth media were analytical grade (Merck Co.) purchased from the local suppliers. The yeast cell of *Rhodotorula rubra* (PTCC 5255) was purchased from the Persian Type Culture Collection in lyophilized form. The maintenance of the test culture was performed on the nutrient agar.

The bioreactor consisted of a glass airlift reactor with 1.5 mL capacity and the specific dimensional details presented in Figure 1. The reactor was equipped with a light irradiation system at the top and an aeration system at the bottom. Inlet and outlet gas flows in the bioreactor were passed through 0.2 µm disposable Millipore filters. Aeration to the system was provided with the use of an ordinary air diffuser supplied with air and threshold of white light was adjusted by a controller. The system's temperature was controlled through a thermostatic incubator at 30 ± 2°C. The bioreactor, all the connection tubes and cultivation media were sterilized in an autoclave (121°C for 20 min).

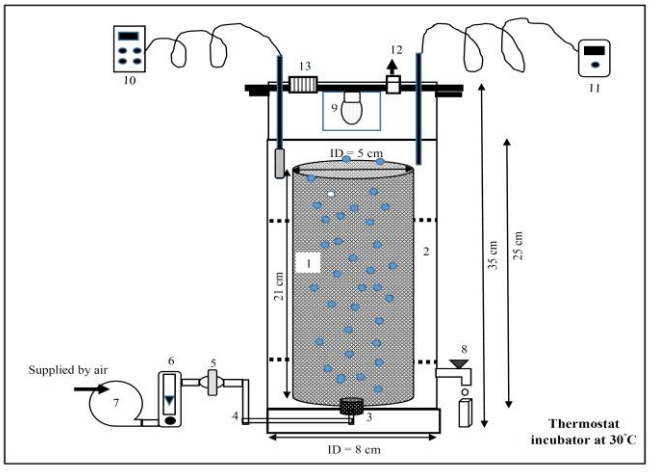

Figure 1. Schematic view of APBR used in this study. 1) Riser section, 2) downcomer section, 3) air diffuser, 4) air connecting tube, 5) disposable sterile air filter, 6) flowmeter, 7) air compressor, 8) sampling value, 9) white-LED lamp, 10) dissolved oxygen meter, 11) pH meter, 12) exhausted gas, 13) feeding gate

In each experiment, 1.0 L of nutrient cultivation medium with the following ingredients was used (g L^{-1}): glucose, 18.75; $(NH_4)_2SO_4$, 5; K_2HPO_4, 1; KH_2PO_4, 1; $MgSO_4$ $7H_2O$ 0.1, and methanol 2% (vv^{-1}). The medium was inoculated by 10% (vv^{-1}) of the 48-h grown culture of *R. rubra* on the commercial nutrient agar. It provided an initial biomass concentration about 180-200 mg L^{-1}. Suspension of the cells from nutrient agar medium was performed with 0.5% solution of TWEEN 80.

For optimization of the carotenogenesis in the airlift photo-bioreactor (APBR), three experimental factors were chosen namely, aeration rate (X_1), initial pH of the cultivation medium (X_2) and light irradiation intensity (X_3). Table 1 shows the levels of the independent variables and their corresponded coded levels used in this study. The *Design Expert* software (version 8.07) was used for experimental design, regression and graphical analyses of data.

Table 1. The independent variables and their selected levels.

Variables	Name	Coded levels of variables*		
		-1	0	+1
X_1	Aeration rate (vvm)	0.115	0.345	0.575
X_2	Initial pH of the cultivation medium	5.50	6.50	7.50
X_3	Light irradiation intensity (Lx)	200	1000	1800

*The actual levels of variables were convert to the coded values using the following transform: $X = 2x_i(x_{high} - x_{low}) / x_{high} + x_{low}$ where, X and x are coded and actual value, respectively.

At the end of the process, whole of the grown culture medium was withdrawn from the bioreactor. The biomass concentration was determined by measurement of the absorbance at 600 nm and then convert to the cell dry weight using a standard curve. Residual glucose was spectrophotometrically determined based on the DNS method after centrifugation of the culture at 10000 rpm for 5 min (MILLER [10]). The carotenoids produced in the cell were extracted according to the following method (PARK & al. [11]): the cell re-suspended by 5 mL DMSO in 50 mL tubes and left at room temperature overnight. Then, the mixture was centrifuged and DMSO phase was pipet off and kept in another tube. The sediment was mixed by 5 mL of acetone and vortexed for 2 min. Colored acetone was separated after centrifugation and combined with the stored DMSO. When the pigments were not completely extracted from the cell, the extraction process was followed by addition of a solution of DMSO and acetone (1:1) to obtain an entirely colorless cell. To separation of carotenoids, 10 mL of petroleum ether (bp 40-60^oC) was combined with the colorant stored solvents. Pooled organic phases were shacked with 5 mL saturated NaCl solution to improve the separation. All of tubes and containers used in the extraction process were coated with aluminum foil for protection from light. Also butylated hydroxytoluene (BHT) was added to all of solvents at 20 mg L^{-1} concentration.

Total carotenoids concentration on the basis of torularhodin was determined spectrophotometrically at 500 nm by extinction coefficient of 2580 (ZOZ & al. [1]). The carotenoids production yield and volumetric production rate were determined by Eq. (1) and (2), respectively:

$$\text{Yield of carotenoids production (g g}^{-1}) = \frac{P_f - P_0}{X_f - X_0} \quad (1)$$

$$\text{Carotenoids production rate (mg L}^{-1}\text{ day}^{-1}) = \frac{1}{V}\frac{P_f - P_0}{t} \quad (2)$$

where, P_0 and X_0 are carotenoids and cell biomass concentrations at zero time and P_f and X_f are the carotenoids and cell biomass concentrations at the end of the carotenogenesis process. V and t are the volume of cultivation medium and time of the cultivation, respectively.

The concentration of dissolve oxygen (DO) in the system was measured with use of a DO meter (Extech, China) having polarographic DO electrode. High performance liquid chromatography (HPLC) analysis was carried out with a reversed phase HPLC device by Knauer using NUCLEODUR C18 column, with a UV detector. The mobile phase consisted of methanol, acetonitrile and chloroform (47:47:6) with a flow rate at 1.5 mL min^{-1} and column temperature was 30°C (SCHMITZ & al. [12]).

3. Results and Conclusions

The results of shake-flask experiments presented in Figure 2 indicated that the parent cell of *R. rubra* could produce carotenoid pigments with the specific yield about 1.56 mg g_{cell}^{-1} and the production rate about 2.2 mg L^{-1} day^{-1} when grows on glucose as a sole carbon and energy source. In the literatures, some influential factors such as weak white light irradiation (Sakaki & aL. [2]), addition of mutagenesis agents for instance N-methyl-N'-nitro-N-nitrosoguanidine (FRENGOVA & al. [8]), phenol (KIM & al. [9]), and ethanol (GU & al. [13]) have been used for improvement of carotenoid production in carotenogenesing cells. In the present study, methanol was used as a progressive agent for enhancement of carotenoids production yield in *R. rubra*. The results presented in Figure 2 indicate that either presence of ethanol or methanol in the growth medium increased both yield and rate of the carotenoids production in the cells where the effect of methanol was higher than ethanol. Previously, it reported the addition of ethanol to the growth medium of red yeast *Phaffia rhodozyma* was increased the yield of carotenoids production from 1.65 to 2.65 mg g_{cell}^{-1} (GU & al. [13]). In this study, the presence of ethanol increased the yield up to 5.22 mg g_{cell}^{-1}, however the effect of methanol was higher and the carotenoids production yield reaches to 6.84 mg g_{cell}^{-1}. Ethanol as a carotenoid progressive agent limited the growth of the cell by an inhibitory role but in the methanol containing medium, growth of the cells did not limit and both yield and rate of carotenoids production were relatively high (2.29 mg L^{-1} day^{-1} in the presence of ethanol versus 3.98 mg L^{-1} day^{-1} in the presence of methanol). Methylotrophic bacteria, capable to grow on C_1 compounds, produced pink carotenoid pigments to protect themselves against powerful oxidizing agent such as singlet state oxygen when they grow on methanol as sole source of carbon and energy (VAN DIEN & al. [14]).

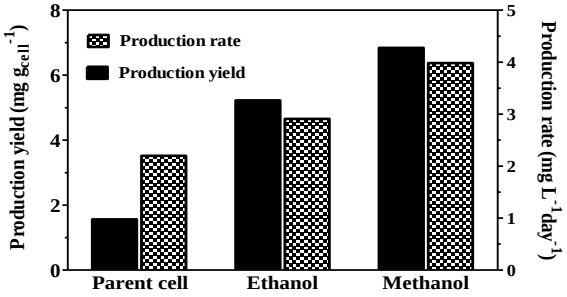

Figure 2. The carotenoids production rate and yield in the presence of ethanol, methanol and with parent cell.

A problem in development of new microbial process is achievement to the suitable environmental conditions for simultaneous occurrence of well cell growth and high carotenoids production. In the present work, a face center central composite design (FCCCD) according Table 2 was used to study the effect of the influential operational parameters in APBR. The total number of the experimental runs were twenty and the number of the repeat experiments at the center point of the design were equaled to six. In each run, the cell biomass concentration (Y_1), carotenoids yield (Y_2), and volumetric carotenoids production

rate (Y_3) as responses were experimentally determined. The results of the carotenogenesis in the APBR are presented in Table 2 where the biomass formation in the APBR was changed from 0.6 g L^{-1} in the experiment number 8 to a maximum value of 7.78 g L^{-1} in the experiment number 11. The carotenoids yield and volumetric carotenoids production rate were also varied with change the levels of the test variables.

Table 2. Arrangement of the runs on the basis of the FCCCD experimental design

Experimental run	Variables (coded)			Responses (Actual)		
Number	X_1	X_2	X_3	Y_1 (g L^{-1})	Y_2 (mg g_{cell}^{-1})	Y_3 (mg L^{-1} d^{-1})
1	-1	+1	+1	1.400	2.3805	0.925
2	0	0	0	6.270	6.3204	12.160
3	+1	-1	+1	3.047	5.3475	5.926
4	+1	-1	-1	2.571	2.0493	1.464
5	-1	0	0	3.922	5.3406	8.819
6	0	0	0	5.990	6.3687	11.568
7	-1	-1	+1	2.460	2.3943	1.636
8	-1	0	-1	0.599	0.3105	0.017
9	0	+1	0	6.560	6.4722	11.794
10	+1	0	+1	4.090	5.1129	5.809
11	0	+1	+1	6.310	7.7832	13.642
12	0	0	-1	5.926	4.7541	10.826
13	-1	0	-1	1.944	0.6279	0.339
14	+1	-1	-1	2.493	0.5658	0.392
15	0	+1	0	4.033	3.0015	3.363
16	0	0	0	6.020	6.4377	10.765
17	0	0	0	5.980	6.5274	10.843
18	0	0	0	6.110	6.5964	11.196
19	0	+1	0	3.412	2.5461	2.413
20	+1	0	0	6.120	7.3830	12.056

On the basis of the model summery statistics (Table 3), the quadratic model (Equation 3) was selected against of the linear, 2FI and cubic models for the highest predicted coefficient of determination (Pr-R^2) and lowest standard deviation (SD%) values.

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1X_2 + \beta_{13} X_1X_3 + \beta_{23} X_2X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (3)$$

where Y_i is each of the dependent variables that measured with a set of regression coefficients: β_0 as a mean, β_1, β_2, β_3 associated with each factor action, β_{12}, β_{13}, β_{23} factors interactions and β_{11}, β_{22}, β_{33} for quadratic effects.

The values of R^2 and adjusted R^2 (ad-R^2) as a measure of the adequacy of the predicted model indicate the proportion of the total variability explainable by the model which has been developed in this study. According to the R^2 values, the sample variation of 99.01% for the cell biomass formation, 99.85 for the carotenoids production yield and 99.54% for the carotenoids production rate were attributed to the test factors.

Table 3. Model summary statistics

Response	Criteria	Linear	2FI	Quadratic	Cubic
Y_1	SD%	1.98	2.16	0.27	0.22
	R^2	0.1146	0.1410	0.9901	0.9961
	Ad-R^2	-0.0492	-0.2555	0.9811	09876
	Pr-R^2	-0.5813	-5.1521	0.9282	0.6985
	PRESS	111.93	435.48	5.08	27.71
Y_2	SD%	2.26	2.47	0.14	0.10
	R^2	0.2759	0.2985	0.9985	0.9995
	Ad-R^2	0.1401	-0.0253	0.9967	0.9983
	Pr-R^2	-0.2624	-3.5112	0.9477	0.8952
	PRESS	142.38	508.82	2.89	11.82
	SD%	5.19	5.71	0.47	0.54

Y_3	R^2	0.0898	0.1055	0.9954	0.9963
	Ad-R^2	0.0808	-0.3074	0.9913	0.9884
	Pr-R^2	-0.5730	-4.6504	0.9794	0.4092
	PRESS	745.29	2677.12	9.78	279.91

Thereafter, experimental values of Y_1, Y_2 and Y_3 were treated by Equation (3) and the significance of the parameters were determined by the analysis of variance (ANOVA) (Table 4). Repeated experiments at the center point of the design are used to compute the sum error of squares ($SS_{res} = \Sigma (Y_i - \hat{Y}_i)^2$), which can be partitioned into two components, pure error (Ep) and lack-of-fit (E_{lof}) (SSE = $SS_{E\text{p}} + SS_{E\text{lof}}$). The parameters found statistically significant by the F-test statistic at the 5% confidence level (Prob > F < 0.05) remained in the simplified models as follows:

$$Y_1 = 6.17 + 0.80\,X_1 - 0.21\,X_2 + 0.38\,X_3 + 0.42\,X_1X_2 - 1.26\,X_1^2 - 2.56\,X_2^2 \quad (4)$$

$$Y_2 = 6.46 + 0.94\,X_1 - 0.25\,X_2 + 1.47\,X_3 - 0.17\,X_1X_2 + 0.50\,X_1X_3 + 0.19\,X_2X_3 - 3.79\,X_2^2 - 0.29\,X_3^2 \quad (5)$$

$$Y_3 = 11.49 + 1.39\,X_1 - 0.32\,X_2 + 1.49\,X_3 + 0.96\,X_1X_3 - 1.21\,X_1^2 - 8.76\,X_2^2 + 0.58\,X_3^2 \quad (6)$$

In statistical modeling, by removing a repressor variable, the coefficient of determination decreases and the ad-R^2, which takes the number of repressor variables into account is usually selected instead to describe the relationship. The close values of ad-R^2 and R^2 for the responses show that all of non-significant parameters removed from the above simplified models.

The response surface diagram for biomass concentration in the APBR is presented in Figure 3 to visualize the joint effects of X_1 and X_2 factors on Y_1 response. The plot presented shows an ascending and descending profile with different magnitudes for this response. In fact, *R. rubra* growth was depended on initial pH and above a certain level which corresponded to the value of 6.5, a decrease in biomass formation was observed. The relevant squared terms in the Equation (4) represents the curvature of the surface by X_1 and X_2 factors where the degree of curvature of the surface by initial pH was higher than that of aeration rate ($b_{22} > b_{11}$ in Equation 4). A comparison between the main effects indicate that the aeration rate had the highest impact on the cells formation in the APBR.

Table 4. Results of ANOVA for the models developed in this study

Source	Y_1				Y_2				Y_3			
	SS	df	F-value	p-value	SS	Df	F-value	p-value	SS	Df	F-value	p-value
Model	69.7	6	142.54	<0.0001	112.5	8	610.50	<0.0001	271.5	7	364.37	<0.0001
X_1	6.39	1	78.42	<0.0001	8.84	1	383.86	<0.0001	19.35	1	104.69	<0.0001
X_2	0.42	1	5.21	0.0399	0.63	1	27.23	0.0003	1.01	1	5.44	0.0379
X_3	1.42	1	17.47	0.0011	21.64	1	939.21	<0.0001	22.21	1	120.13	<0.0001
X_1X_2	1.42	1	17.41	0.0011	0.24	1	10.43	0.0080	-	-	-	-
X_1X_3	-	-	-	-	2.01	1	87.19	<0.0001	7.36	1	39.83	<0.0001
X_2X_3	-	-	-	-	0.30	1	13.08	0.0041	-	-	-	-
X_1^2	5.09	1	62.40	<0.0001	-	-	-	-	4.04	1	21.88	0.0005
X_2^2	20.9	1	257.10	<0.0001	45.93	1	1993.3	<0.0001	211.1	1	1142.1	<0.0001
X_3^2	-	-	-	-	0.28	1	11.98	0.0053	0.94	1	5.08	0.0436
Residual	1.06	13			0.25	11			2.22	12		
Lack of fit	0.80	8	1.97	0.2361	0.20	6	3.28	0.1069	0.70	7	0.33	0.9089
Pure error	0.26	5			0.051	5			1.52	5		
Cor total	70.79	19			112.7	19			473.7	19		

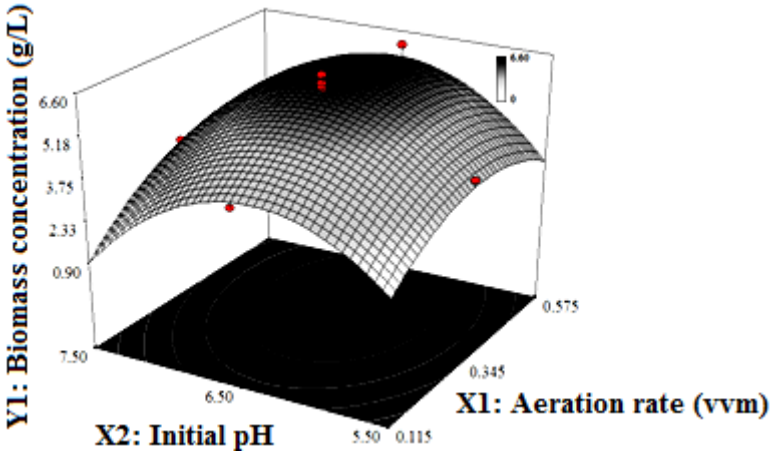

Figure 3. Surface plot of biomass concentration (Y_1) as a function of the aeration rate (X_1) and initial pH (X_2).

The response plots for carotenoids yield (Y_2) as a function of the joint effects of the test factors are illustrated in Figure 4. The yield of carotenogenesis increased linearly along X_1 lines in Figures 4A and 4B and this linear effect of X_1 on Y_2 is confirmed by Equation (5), where absence of the squared term of X_1. The influence of X_3 on Y_2 is greater that X_1 and X_2 in Figures 4. The positive effects of aeration rate and intensity of light irradiation on carotenoids production yield are presented in Equation (5). It was previously reported that the irradiation of weak white light increased torularhodin production in red yeast cells of *R. glutinis*. The *R. rubra* is an aerobic yeast and aeration facilitates the metabolites of the cell. The growth of the cells was initially increased with increase of aeration rate however, at higher aeration rates, formation of some oxidative agents such as singlet oxygen radicals result to decrease of the cell density in the APBR (Figure 3). In this case, the cell's metabolite was changed to production of antioxidant-carotenoids. As seen in Figure 4b, the altitude of the appearance yield obviously depends on both levels of aeration rate and intensity of light irradiation. Increasing of intensity of light irradiation from 200 to 1800 Lx results to enhance of the carotenoids yield from 4.26 to 6.21 mg g_{cell}^{-1} when aeration rate was increased from 0.115 to 0.575 vvm. The dependency of the carotenoids yield on X_2 and X_3 factors is presented in Figure 4C. The results showed the carotenoids yield was at the highest level when the initial pH was equal to 6.5 which corresponding to the best pH value for growth of the cell (Figure 3).

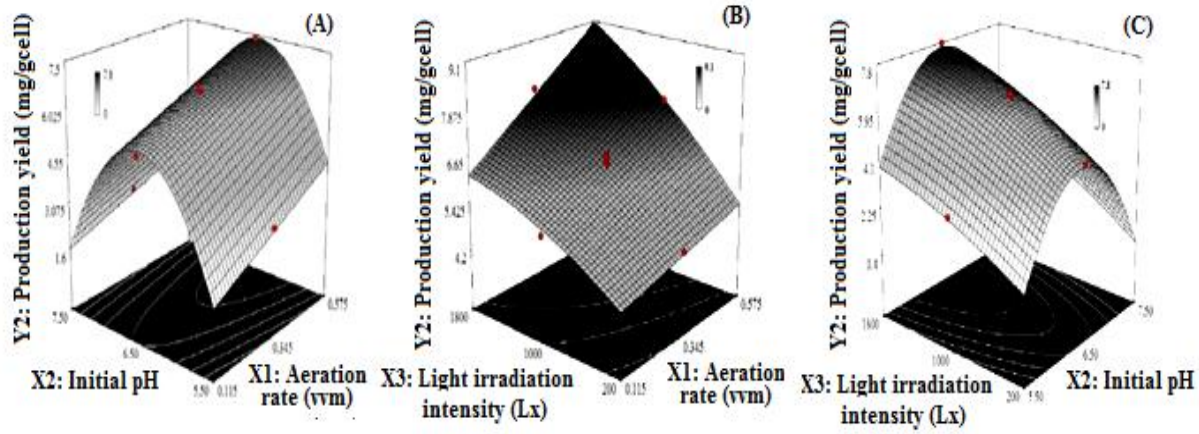

Figure 4. Surface plots for the carotenoids production yield (Y_2) as a function of X_1 and X_2 (A), X_1 and X_3 (B), and X_2 and X_3 (C).

The volumetric productivity as function of aeration rate and light intensity irradiation is illustrated in Figure 5. For achievement to the highest productivity, it is necessary to attain a more yielded cell in the APBR. Therefore, as expected before, aeration rate as main factor on cell formation and intensity of light irradiation as important parameter on carotenoids yield had an interactional effect on the carotenoid productivity in the APBR. Equation (6) predicted that the highest carotenoid productivity (14.6 mg L^{-1} day^{-1}) was obtained at the highest levels of the X_1 and X_3 parameters.

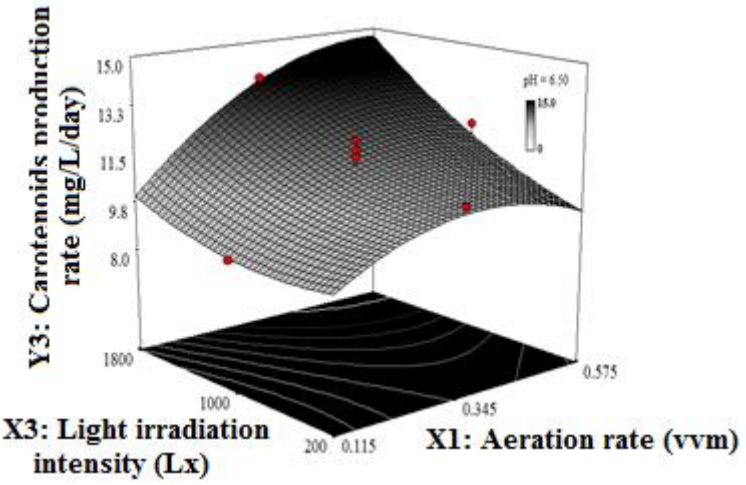

Figure 5. Surface plot for carotenoids productivity as function of aeration rate and intensity of light irradiation.

In this study, the optimal conditions were determined through the regression models obtained by RSM and the desirability function available in *Design expert* software. Desirability function is briefly defined as a function changes from zero, indicating a completely undesirable situation for the particular test factor, to 1 as the highly desirable position for the test factor. The optimization criterion used in this study was the importance of simultaneously maximum cell growth and carotenoids production when the operational factors were in studied ranges. The combination of the factors at aeration rate of 0.469 vvm, initial pH of 6.48, and light irradiation intensity of 1757.84 Lx resulted to an optimal condition for achievement to the biomass concentration of 6.69 mg L^{-1}, the carotenoids yield of 8.352 mg g_{cell}^{-1}, and volumetric carotenoids production rate of 14.3 mg L^{-1} day^{-1}. The dependency of overall desirability value on the each test factor is presented in Figure 6. The desirability

diagrams show that the highest desirability value remains constant when aeration rate is 0.34-0.50 vvm, initial pH is 6.17-6.83, and light irradiation intensity is 1570-1800 Lx.

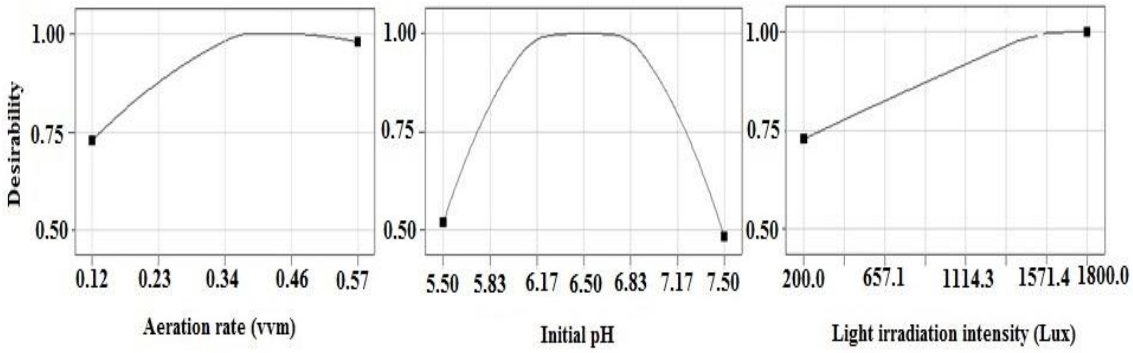

Figure 6. Dependency of the optimal condition desirability on the level of the test factors.

The accuracy of the predicted optimal condition was experimentally validated and the results are presented in Figure 7. The experimental observations were in good agreement with those values predicted from the developed models (the relatively error was 4.0% for biomass formation, 5.4% for carotenoids yield, 2.8% for volumetric carotenoids production rate). Also, a comparison of the experimental results in shake-flasks and APBR is presented in Figure 7. As a result, the cells performance was faster in the APBR when compared with shake-flask experiments where the cells growth and glucose utilization took place at higher rates. This behavior relates to effect of aeration on the aerobic metabolism of the yeast. Carotenoids, as secondary intracellular metabolites, were produced at the stationary phase that beginning after 48 h in the shake-flask and 36 h in the APBR, respectively. Concentration of the carotenoid pigment was increased during the stationary phase where the carotenoids concentration in the APBR was approximately three times higher than the shake-flask experiments. Although, the maximum total carotenoids was obtained at the end of the fourth-day in the shake-flask however, this time was decreased to three days in the APBR. Characterization of the carotenoids that produced in the APBR at the optimum condition was performed by HPLC method and using the external standard solutions. The HPLC chromatogram is presented in Figure 7. The red carotenoid pigment, torularhodin, was early eluted from the column at 4.80 min. Next, the torulene and β-carotene were eluted at 9.57 min and 12.62 min at the used condition, respectively. Torularhodin was major carotenoid pigment produced by *R. rubra* cell in the APBR. Based on the extensive studies performed elsewhere, torularhodin is the end product of the carotenoid oxidation in red yeast cells (ZOZ & al. [1]). Oxidation of the torulene toward torularhodin just performed in an oxidative cultivation conditions to protect the cell via high antioxidant role of torularhodin against membrane impairment by activated oxygen molecules.

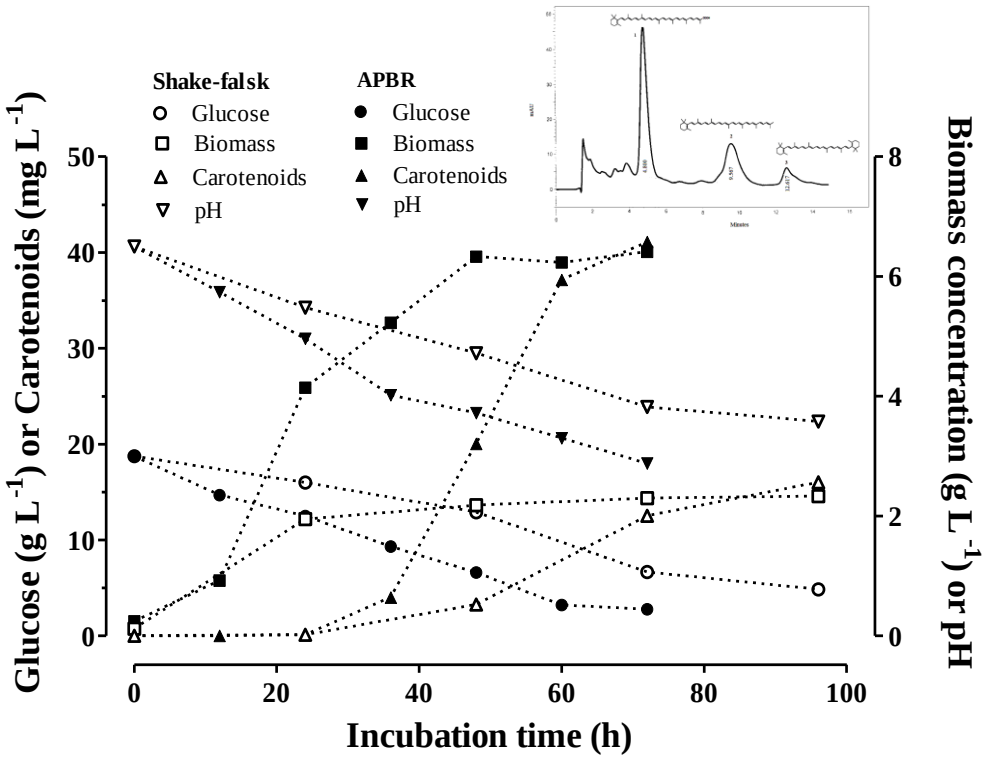

Figure 7. Comparison of carotenogenesis in *R. rubra* in the APBR and shake-flask experiments. Insert: PHLC chromatogram of the extracted pigments from the grown cell culture in the APBR.

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