ORIGINAL ARTICLE

Induction of β, ε-carotene-3, 3′-diol (lutein) production in green algae *Chlorella salina* with airlift photobioreactor: interaction of different aeration and light-related strategies

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Abstract

The impact and interaction of light irradiance strength (light intensities), lighting cycle (photoperiod), and aeration rate on biomass concentration and lutein production efficacy of the microalga *Chlorella salina* in a closed laboratory-scale airlift photobioreactor were investigated via the response surface method. Among the factors assessed, light intensity and aeration rate had significant influence on cell concentration, though a concurrent increment in light intensity noticeably decreased the lutein content. All the parameters were observed to be statistically significant. Best operating conditions for the growth of alga was evaluated to be as follows: light intensity, 200 μmol m^{-2} s^{-1}; photoperiod, 12:12 h L D; and the aeration rate, 3 lpm. These conditions could substantially enhance the microalgal growth rate (0.82 day^{-1}) and biomass production (665.89 mg). Specific lutein productivity and a recovery of 9.73 mg/L/day were achieved at a day light cycle of 16 h. According to the results of the experimental design, the optimum conditions led to a twofold increase in biomass and lutein productivity compared with unoptimized condition.

Keywords *Chlorella salina* · Lutein · Light cycle · Light intensity · Response surface methodology · Aeration

1 Introduction

Lutein is a lipophilic carotenoid that has exhibited significant nutraceutical bioactivity, and antioxidant, anti-cancer effects on wide range of biological models. For this reason, lutein has been largely consumed with a market size of nearly $150 million in the USA, and the market size is yet expanding [1]. Traditionally, lutein is produced from marigold flowers (*Tagetes erecta*); however, due to the biological and operational benefits of microalgae, including higher lutein content, flexible cultivation conditions, fast growth, and the less labor required for operation, microalgae have become an interesting alternative for lutein production [2]. Since wild microalgae harvesting may have a negative ecological impact due to over-exploitation of these natural sources, it is expected that carotenoid extraction from cultured organisms will have a dominant role to play in the coming years [3]. Despite the renewed interest in bioactive pigments, their concentrations, especially under optimal growth conditions, are often too low to make microalgae-based pigment production economically viable. Moreover, due to their simpler structure, energy is directed into photosynthesis, growth, and reproduction processes instead of the maintenance of differentiated structures [4], making microalgae organisms of interest for production of biomass and bioactive compounds.

The production of lutein in microalgae varies not only among species but also under different conditions, such as light intensity, nitrogen availability, physiological state of the

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culture, and other medium compositions. In some green microalgae (e.g., *Dunaliella salina* and *Haematococcus pluvialis*), specific stress conditions such as over-saturating light, nutrient deficiency, or high salt concentration induce the overproduction of secondary carotenoids, β-car, and astaxanthin [5–7]. Secondary pigments, including astaxanthin, β-car, and lutein, are known to play a scavenging role and prevent cell damage by reacting with reactive oxygen species (ROS) [8]. By contrast, the synthesis of primary pigments requires carbon dioxide and nutrients, such as inorganic nitrogen and phosphate in addition to light [9]. Clearly, when considering photoautotrophic microalgal cultivation, a key role is played by light availability and utilization [10]; hence, photobioreactors (PBRs) need to have a wide light-exposed surface, and a thin depth to avoid the presence of dark zones inside the culture. On the other hand, agitation during microalgae culture is needed to promote an adequate light-dark cycle regime, to increase oxygen mass transfer, and to help avoid detrimental conditions such as cell sedimentation or accumulation of oxygen in the medium [11].

For most bioreactors, the exterior surface is the only place exposed to light, called the photic zone. The rest that is unexposed to or with little light penetration is called the dark zone. Distinctive PBR geometries and designs result in varying light utilization and hydrodynamic circulation, which can affect light distribution and mixing inside the reactor. When microalgae are exposed to the light/dark zone within the PBR, the span under the light/dark zone alters the biomass production of the PBR. Air flow rate, mixing, biomass concentration, and light availability are the major parameters that influence growth superficially and productivity of microalgae in photobioreactors [12]. The mixing of the liquid in the reactor has an indirect influence on exposure of microalgae to light and dark phases [12]. High-density microalgae cultivation could be accomplished by establishing strategies to productively utilize the incident light energy. The extent of mixing is a decisive criterion that impinge light distribution and light availability in the system.

Among the physicochemical parameters that influence microalgal lutein synthesis, light intensity, CO_2, and some medium components (nitrate, manganese, and copper) were identified as the primary factors critically influencing the growth rate and accumulation of lutein [13–15]. The biosynthesis of lutein can be improved by providing optimal mode of light intensity, as the carotenoid lutein is present in the photosynthetic antenna complexes [16]. This is evident from the study of Vaquero et al. [17], which reported that sudden change in light intensity either from low to moderate level or from moderate to low level enhanced the lutein content of microalga *Coccomyxa onubensis*. However, only limited studies so far have investigated the simultaneous effect of light intensity and mixing conditions on both cellular growth and lutein production in microalgae. The objective of the present study was to evaluate culture growth of *Chlorella salina* under different growth conditions (light intensity and mixing/agitation) and medium compositions to determine optimal conditions for the production of lutein in airlift PBRs.

2 Materials and methods

Chlorella salina was obtained from Central Institute of Brackish Aquaculture (CIBA), Chennai, Tamil Nadu. The algae were cultivated in a filter-sterilized (pore size 0.2 μm) Walne's medium [18]. The cultures were pre-cultivated in a 200-mL Erlenmeyer flask containing 100 mL of medium at pH 8.0 and at 24 °C at a light intensity of 50–100 μmol photons m^{-2} s^{-1}.

2.1 Operation of photobioreactor

The microalgae were cultivated in a cylindrical airlift photobioreactor with a working volume of 8 L, which was continuously illuminated with external fluorescent light sources (TL5 light tubes, Philips) mounted on two opposite sides of the PBR, and the light intensity was measured using a quantum meter (LX 102 Lux Meter; HTC Instruments, India). The initial pH of the culture was 8.0. They were then divided into three groups grown under high light intensity (HL, 400 μmol m^{-2} s^{-1}), medium light intensity (ML, 200 μmol m^{-2} s^{-1}), and low light intensity (LL, 100 μmol m^{-2} s^{-1}), respectively. The ranges of the parameters selected in the present study are depicted in Table 1. After inoculation, operating conditions of PBR were adjusted to specified values according to the experiment (Table 2). All experiments were performed in triplicates and expressed as mean with standard deviation. Samples were collected from the PBR at set time intervals to determine the cell concentration and lutein content. The microalgal cultures were harvested upon depletion of the nitrogen source in the medium.

2.2 Determination of cell growth

Cell growth estimated as cell number per mL was determined according to Chang et al. [19]. Cell culture of 30 μL was mixed with 10 μL of Lugol solution (Sigma-Aldrich, St. Louis, MO, USA). The mixture of 10 μL was loaded to the hemocytometer (Thoma, Bad Blankenburg, Germany) and the cell number was counted under microscopy (CX 41, Olympus, Japan). Each sample was counted twice and the average was calculated as the cell number of the sample.

Cell growth was also estimated as dry weight (d. wt.) per L. A total of 100 mL of cell culture was harvested in the sampling day and subjected to centrifugation at 12,000×*g* at 4 °C for 5 min. The pellet was fixed in liquid N_2 and lyophilized at − 50 °C to obtain the dry weight.

Table 1 Experimental ranges and levels of variable

Variable	Symbol	Coded factors and levels		
		− 1	0	1
Light Intensity (μmol m^{-2} s^{-1})	X_1	100 (LL)	200 (ML)	400 (HL)
Photoperiod (h:h)	X_2	0:24	12:12	24:0
Aeration rate (lpm)	X_3	1	3	5

The relative growth rate (10^6 cells day^{-1}) was estimated as the difference in cell number between two culture times (day), $\mu = \ln (N_2/N_1)/(X_2 - X_1)$, where N_2 is the concentration of cells at time X_2 and N_1 is the concentration of cells at time X_1.

2.3 Chromatographic analysis of lutein and other carotenoids

The HPLC determination of carotenoids was modified following the previous report [20]. The sample of approximately 0.5×10^6 cells was centrifuged at 12,000×g for 5 min at 4 °C, and the supernatant was discarded. Then, 1 mL of cold 80% acetone was added to the pellet for a 2-h extraction in the darkness and then extracted again with another 1 mL of 80% acetone. The two extracts were combined and passed through the 0.2-μm nylon filter. The filtrate was loaded into a screw tube filled with N$_2$ gas and stored at − 20 °C freezer until analysis. The filtrate of 20 μL was injected into the HPLC system. The chromatographic analysis of acetone-extracted pigments was performed using a C18 column (5 mm, 150 mm × 46) on a Waters 2545 HPLC (Waters 2545, USA), at 30 °C. The mobile phase comprised of methanol/acetonitrile (90:10 V/V). The injection volume was 20 μL, the solvent flow rate was 1.2 mL min^{-1}, and the pigment detection was carried out at 445 nm [21].

2.4 Experimental design and statistical analysis

To investigate the interactive effects of the light intensity, light period, and aeration on the lutein productivity of the selected

Table 2 Design of experiments along with responses

Run	Variables			Response		
	X_1, light intensity μmol m^{-2} s^{-1}	X_2, photoperiod h	X_3, aeration rate lpm	Biomass g/L	Lutein mg/g	Lutein productivity mg
1	200	12	3	0.553	9.873	54.59
2	500.6	12	3	0.339	6.01	33.79
3	200	12	3	0.528	9.62	50.79
4	200	12	3	0.526	9.87	51.91
5	200	32.18151397	3	0.428	7.61	47.67
6	200	12	3	0.522	8.54	50.032
7	100	0	5	0.180	3.21	15.77
8	200	12	− 0.36359	0.110	1.95	10.425
9	100	24	5	0.150	2.64	15.65
10	200	12	3	0.570	9.97	55.103
11	400	24	5	0.230	4.68	25.74
12	400	0	5	0.210	3.23	17.78
13	200	12	6.363586	0.110	2.21	12.27
14	200	− 8.181513966	3	0.360	6.43	36.14
15	− 22.68	12	3	0.220	3.81	20.95
16	400	0	1	0.220	3.84	21.12
17	100	0	1	0.190	3.33	17.98
18	200	12	3	0.600	10.15	56.256
19	100	24	1	0.190	3.41	19.12
20	400	24	1	0.310	5.48	29.98

Fig. 1 Effect of different light intensities, 100 μmol m^{-2} s^{-1}, 200 μmol m^{-2} s^{-1}, and 400 μmol m^{-2} s^{-1}, on the algal biomass growth
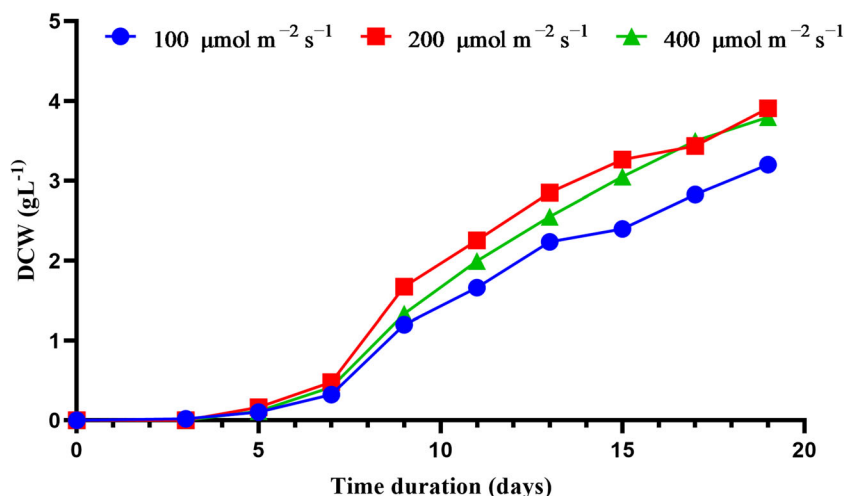
microalgal strain, central composite design (CCD) was used. In total, twenty batch experiments were designed and conducted following a response surface methodology (Table 2), which was performed in duplicate to optimize the levels of the selected variables. Data processing and calculations were carried out using a commercial statistical package, Design Expert at version 7.0.0, Stat-Ease, Minneapolis, to estimate the coefficients of the regression equation. The goodness of the fit of the model was validated using analysis of variance (ANOVA).

3 Results and discussion

3.1 Effect of the light intensity, irradiation regimes, and mixing/agitation on cell growth and biomass productivity

The selection of a specific strain with a high growth rate and high lutein productivity is crucial from a microalgal technology engineering perspective. In addition to lutein productivity, concentration of biomass, lutein content, and duration of
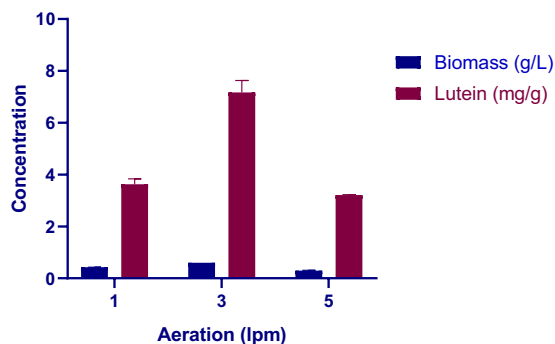

Fig. 2 Effect of different aeration rates, 1, 2, and 3 lpm, on the algal biomass growth and lutein productivity

cultivation must also be considered for fast and efficient production of lutein from algae. *Chlorella* species are well-known for their capability of lutein production. To know the significance and practical application values, the effects of different light intensities, illumination period, and aeration/mixing strategies on the cell growth and lutein production in *Chlorella salina* have further investigated in the present study.

The growth profile of *C. salina* was determined in each experiment using direct counting and dry cell weight methods. Lutein production was determined spectrophotometrically and reported as milligrams per gram of dry cell weight. The maximum values for biomass production were measured in each experiment and were shown in Fig. 1.

The difference of growth rates among the HL, ML, and LL cultures indicated that 200 μmol m^{-2} s^{-1} was more suitable for the growth of *C. salina* than other light intensities. As shown in Fig. 1, the linear growth in all aerated cultures started at 3 days and lasted for 7 to 10 days. The control showed little growth initially, and then, the cell count decreased gradually. At 200 μmol m^{-2} s^{-1}, the biomass concentration and growth rate reached maximum values of 0.82 g/L and 0.20/day, respectively. The biomass productivity was enhanced by about threefold with an increment in the intensity of light, and a maximum productivity of about 0.108 g/L/day was noticed at 200 μmol m^{-2} s^{-1}. It indicates a better availability of light inside the reactor. An increased growth rate corresponding to the light intensity was also noted in *Dunaliella tertiolecta* when it was cultivated at 100, 200, and 350 μmol photons m^{-2} s^{-1} [22].

Other than varying the light intensity, the experimentation also takes into account the photoperiod (light/dark cycle) variation. It was found that the highest biomass of 0.103 g/L/day was recorded with light-dark cycle of 12:12 h while the highest percentage of lutein production was obtained at 16:8 h for *C. salina* at 200 μmol m^{-2} s^{-1} light intensity. However, there was no significant difference in dry weight
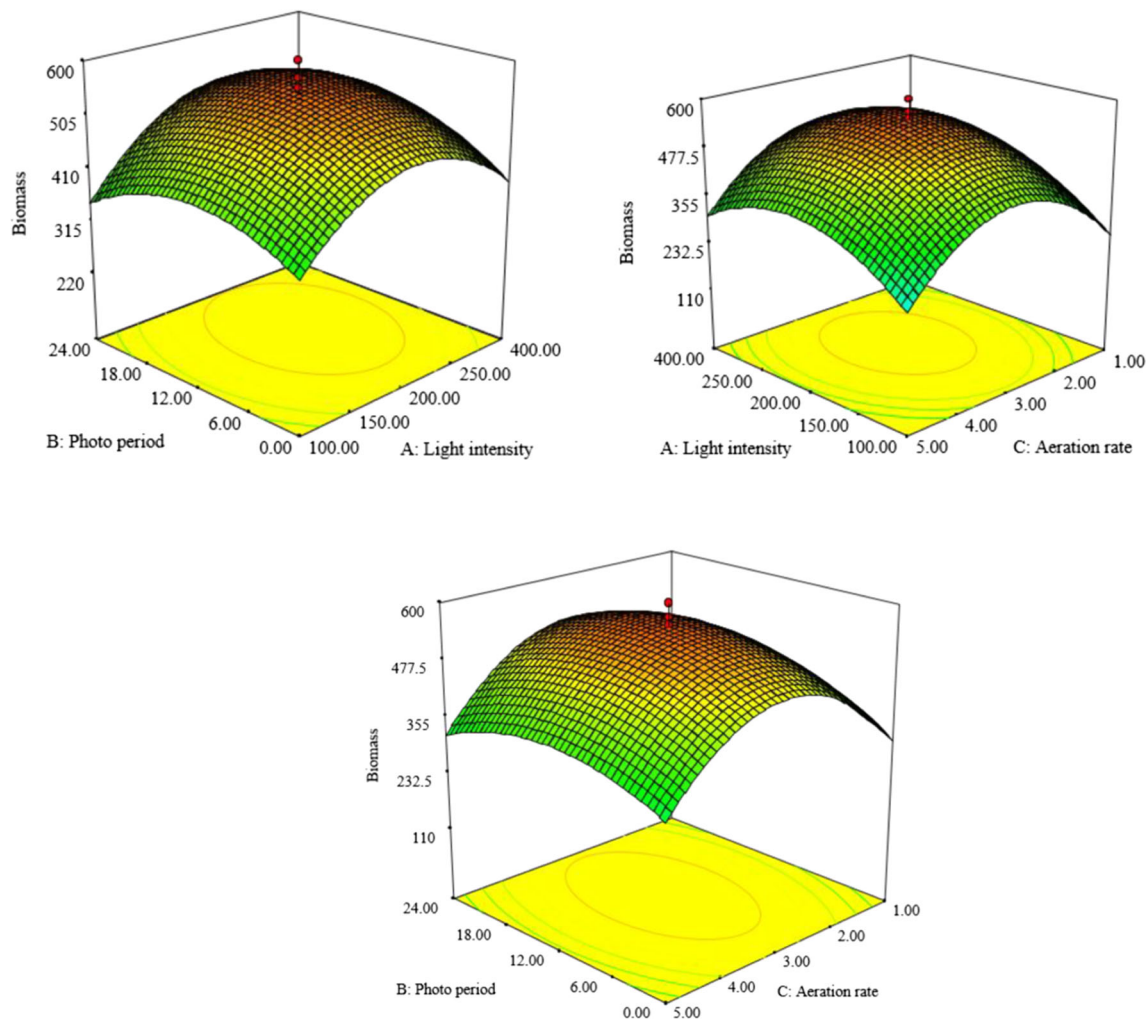
(a). The fitted RSM model for micro alga biomass concentration in the
designed irradiance strength, light hours per day and aeration rate

Fig. 3 **a** The fitted RSM model for microalga biomass concentration in the designed irradiance strength, light hours per day, and aeration rate. **b** The fitted RSM model for lutein content in the designed irradiance strength, light hours per day, and aeration rate. **c** The fitted RSM model for lutein productivity in the designed irradiance strength, light hours per day, and aeration rate

among the growth conditions. This indicates that light could alter cellular metabolism but have no effect on cell growth. For all light conditions, the biomass concentration increased with the residence time, as already reported by Martinez et al. [23] for *Scenedesmus obliquus*, as well as for other species like *Chlorella vulgaris*, *Chlorella protothecoides*, etc. [24–26].

Further, the experiment is also carried out by applying at different aeration rates: 1, 3, and 5 lpm. The initial biomass concentration was 0.08 g/L for each experiment; the biomass concentration and the growth rate were sampled at 24-h interval. With the aeration rate of 3 lpm, the culture spent 6–8 days of log phase with a specific growth rate of 0.548/day. The growth rate of *C. salina* increased from 0.426 to 0.548/day with the increase in aeration. The productivity decreased

significantly in lower flow rate because the turbulence caused by air circulation (bubbling) was insufficient to resist the auto-flocculation of the microalgae and provided poor nutrient mixing for cell cultivation [27, 28].

3.2 The effect of light intensity, irradiation regimes, and mixing/agitation on lutein productivity

The culture conditions that rendered the highest cell concentration are not the same where the highest lutein concentration was observed. With an increase in light intensity, the lutein content was increased at first, reaching the highest at 30% of DW under the light intensity of 200 $\mu mol\ m^{-2}\ s^{-1}$ which was a twofold increase of that in the dark condition. After that, as the light intensity
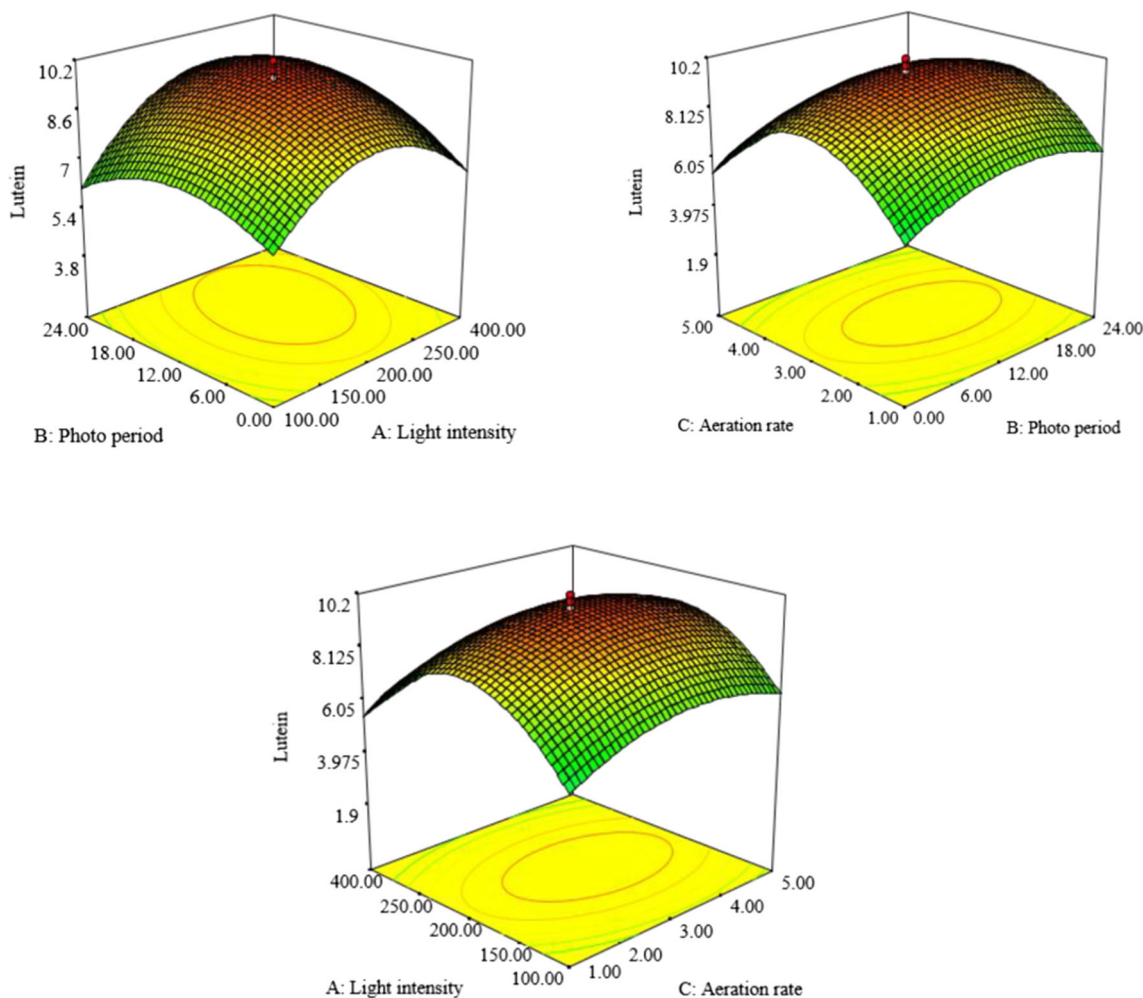
(a). The fitted RSM model for Lutein content in the designed
irradiance strength, light hours per day and aeration rate

Fig. 3 (continued)

continued to increase, the lutein content began to decrease. These results indicated that a light intensity of 200 μmol m^{-2} s^{-1} is the most suitable for lutein production. The highest lutein content obtained was 6.2 ± 0.32 mg/g, which was higher than the reported values, around 3–5 mg/g [25, 29]. At low irradiance, fewer oxygen radicals are generated, whereas under high irradiance cells are unable to utilize all the energy generated that implies a decrease in lutein for high irradiance, and this may be due to the functional group in the lutein molecule [30]. There was no coherence between growth rates, and lutein contents reveal that the most favorable conditions for growth were generally not concomitant with those with higher pigment content.

At the end of the investigation, the increase in pigment amount continued, and studies showed that light regime had an effect on lutein productivity of *C. salina*. Light had no obvious effect on lutein accumulation for the first 24 h, and the positive effect of light on lutein accumulation occurred only after 24 to 36 h. A light/dark cycle of 16:8 h shows a 1.5-fold increase in the productivity of lutein than the other experimented light/dark cycles. The specific lutein content of 9.86 mg/g yields the highest and suggests that 16:8 h is the best period for lutein accumulation. These were in agreement with the study of Mahale and Chaugule [31]; the optimal photoperiod is between 12:12 to 16:8 h for most of the cultures. These changes in pigments depending on the provided photoperiod could be related to the photo-adaptation mechanism [32].

Figure 2 shows the variation in the biomass and lutein productivity at different aeration rates. This plays crucial roles such as reducing photo-limitation or shelf-shading in high-density cultures, homogenously distributing the nutrients in the culture medium, and increasing CO_2

Table 3 Effect of stress factors on lutein production reported in the literature

Microalgae	Reactor configuration	Processing conditions	Lutein yield (mg/g)	Ref
Muriellopsis sp.	Batch (0.2 L, 4–7 days)	T: 28 °C, pH 6.5; LI: 460 μmol m^{-2} s^{-1}	5.5	[36]
	Continuous outdoor, tubular (55 L)	T: 28 °C, pH: 7; LI: continuous 200 μmol m^{-2} s^{-1}	4.3	[37]
Scenedesmus almeriensis	Continuous (2 L)	T: 30 °C, pH: 8.0; LI: 1700 μmol m^{-2} s^{-1}	5.5	[38]
	Continuous outdoor, tubular	T: 35 °C, LI: 1900 μE m^{-2} s^{-1}	4.5	[39]
Chlorella prothecoides	Batch (16 L)	T: 28 °C, pH: 6.5; LI: absence of light	4.6	[40]
Chlamydomonas acidophila	Batch (1 L)	T: 30 °C; LI: 150 μmol m^{-2} s^{-1}	3.2	[41, 42]
Chlorococcum citriforme	Batch (0.2 L)	T: 28 °C, pH: 6.5; LI: Continuous	7.2	[36]
Chlorella salina	Batch (10 L)	T: 28 °C, LI: 200 μmol m^{-2} s^{-1}	9.7	This study

dissolution and O_2 evolution [33]. In general, at low aeration rate (1 lpm), the degree of photosynthesis was low due to scarce mixing of broth. With an increase in flow rate from 1 to 3 lpm, lutein productivity improved for all light intensities. This can be attributed to better mixing at high flow rate, thus improving the rate of photosynthesis. Thus, aeration rate of 3 lpm found to be adequate for specific lutein productivity. Any further increase beyond 3 lpm reduced or had no effect on specific lutein content, indicating 3 lpm to be the optimum aeration rate for the productivity of lutein. This is because higher flow rates tend to reduce the retention time of gas bubbles and thus the utilization of CO_2 by the microalgal cells [34].

3.3 Data analysis and evaluation of the models

All experiments were designed and analyzed by RSM, a statistical technique used to design the experiments. CCD consisting of 20 sets of experiments is designed for optimizing the growth *of C. salina* with respect to biomass at incubation period of 18 days. All experiments were done in triplet form. The results of the predicted values were almost similar in comparison with the actual values of the experimental results. This model gave an optimized value of aeration rate, light intensity, and light period (LP) in respect of its growth or biomass. The results of all optimized condition of microalgae were shown in Fig. 3a, b, and c.

Final biomass concentration, lutein content, and productivity of *C. salina* cultivated under different light intensities, photoperiods, and aeration rate are shown in Table 2. It can be noted that *C. salina* had the ability to grow under all the investigated levels. The data are fitted into the quantitative surface models using a second-order polynomial regression equation [35]. Tables 3, 4, and 5

show the analysis of variance of the fitted quadratic polynomial model for biomass production, lutein production, and lutein productivity. The application of regression analysis of the experimental data using the Design Expert software (version 7.0.0; Stat-Ease, Inc., Minneapolis, MN, USA) yielded the following quadratic regression equations for biomass production (Eq. (1), lutein content (Eq. (2)), and lutein productivity (Eq. (3)).

$$\begin{aligned} \text{Biomass} = {}& 550.92 + 33.69\,X_1 + 14.23\,X_2 - 10.25\,X_3 \\ & + 17.5\,X_1\,X_2 - 5\,X_1\,A\,X_3 - 12.5\,X_3\,X_2 \\ & - 102.68\,X_1{}^2 - 62.1976\,X_2{}^2 - 162.607\,X_3{}^2 \end{aligned} \tag{1}$$

$$\begin{aligned} \text{Lutein} = {}& 9.691138 + 0.610678\,X_1 \\ & + 0.3357\,X_2 - 0.1364\,X_3 \\ & + 0.4475\,X_1\,X_2 - 0.065\,X_2\,X_3 - 0.105\,X_1\,X_3 \\ & - 1.81797\,X_1{}^2 - 1.0719\,X_2{}^2 - 2.818\,X_3{}^2 \end{aligned} \tag{2}$$

$$\begin{aligned} \text{Lutein productivity} = {}& 53.27139 + 3.492328\,X_1 \\ & + 2.726182\,X_2 - 0.74374\,X_3 \\ & + 1.975\,X_1\,X_2 - 0.2375\,X_2\,X_3 \\ & - 0.27\,X_1\,X3 - 10.133\,X_1{}^2 \\ & - 4.9948\,X_2{}^2 - 15.79\,X_3{}^2 \end{aligned} \tag{3}$$

where X_1, X_2, and X_3 are the coded terms for the three independent variables denoted as light intensity, photoperiod, and aeration rate, respectively. The p value from the ANOVA shows the significance of each variable. The smaller the p value, the more significant is the corresponding coefficient. All mutual interaction terms were found to be insignificant

Table 4 Analysis of variance (ANOVA) for the effect of the independent variables on the dependent variables and the regression coefficients, of the fitted quadratic equations obtained from experimental results. Regression coefficients, R^2, and p or probability values of four dependent variables for lutein and biomass production

Source	df	Biomass production (mg/g)				Lutein production (mg/g)			
		Sum of squares	Mean square	F value	p value, prob $> F$	Sum of squares	Mean square	F value	p value, prob $> F$
Model	9	535,093.6	59,454.84	59.70175	< 0.0001	163.7889	18.19877	52.39194	< 0.0001
X_1, light intensity	1	15,503.04	15,503.04	15.56742	0.0028	5.093023	5.093023	14.66216	0.0033
X_2, photo period	1	2766.124	2766.124	2.777612	0.1266	1.538992	1.538992	4.430561	0.0616
X_3, aeration rate	1	1435.177	1435.177	1.441137	0.2576	0.254069	0.254069	0.731431	0.4124
X_1, X_2	1	2450	2450	2.460175	0.1478	1.60205	1.60205	4.612098	0.0573
X_1, X_3	1	200	200	0.200831	0.6636	0.0338	0.0338	0.097306	0.7615
X_2, X_3	1	1250	1250	1.255191	0.2887	0.0882	0.0882	0.253917	0.6252
X_1^2	1	151,939.4	151,939.4	152.5704	< 0.0001	47.62958	47.62958	137.1195	< 0.0001
X_2^2	1	55,750.76	55,750.76	55.98229	< 0.0001	16.5604	16.5604	47.67528	< 0.0001
X_3^2	1	381,048.6	381,048.6	382.631	< 0.0001	114.4847	114.4847	329.5868	< 0.0001
Residual	10	9958.643	995.8643			3.473582	0.347358		
Lack of fit	5	5205.809	1041.162	1.095307	0.4614	1.792574	0.358515	1.066369	0.4728
Pure error	5	4752.833	950.5667			1.681008	0.336202		
Cor total	19	545,052.2				167.2625			
Std. dev.		31.55732				0.589371			
Mean		327.3				5.79315			
C.V. (%)		9.64171				10.17358			
Press		46,519.71				16.01287			
R^2		0.981729				0.979233			
Adj R^2		0.965285				0.960542			
Pred R^2		0.914651				0.904265			
Adeq precision		21.3836				19.67948			

Table 5 Analysis of variance results for the multiple regressions to predict lutein productivity

Source	Sum of squares	df	Mean square	F value	p value, prob $> F$	
Model	5075.713	9	563.9681	43.16893	< 0.0001	Significant
X_1, light intensity	166.5639	1	166.5639	12.74963	0.0051	
X_2, photo period	101.4987	1	101.4987	7.769214	0.0192	
X_3, aeration rate	7.554194	1	7.554194	0.578236	0.4646	
X_1, X_2	31.205	1	31.205	2.388586	0.1533	
X_1, X_3	0.45125	1	0.45125	0.034541	0.8563	
X_2, X_3	0.5832	1	0.5832	0.044641	0.8369	
X_1^2	1479.895	1	1479.895	113.2785	< 0.0001	
X_2^2	359.52	1	359.52	27.51945	0.0004	
X_3^2	3596.913	1	3596.913	275.3256	< 0.0001	
Residual	130.6421	10	13.06421			
Lack of fit	98.28596	5	19.65719	3.037626	0.1240	Not significant
Pure error	32.35618	5	6.471235			
Cor total	5206.355	19				
Std. dev.	3.614445		R^2		0.974907	
Mean	32.1533		Adj R^2		0.952324	
C.V. (%)	11.24129		Pred R^2		0.848374	
Press	789.4171		Adeq precision		17.97304	

in both models ($p > 0.05$). Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. R^2 (coefficient of regression) is the proportion of variation in the depended variable explained by the regression model. Adjusted R^2 is the coefficient of determination adjusted for the number of independent variables in the regression model [43]. From the analysis of variances for the experimental data (Tables 3 and 4), it showed that the R^2 value of the models were 0.98, 0.97, and 0.97 which indicating that 98% and 97% of the experimental data can be fitted with the model-predicted value. High values of R^2 obtained in for all the models indicated that they would accurately predict the relationships between the parameters. Further, the "Predicted R^2" values were in reasonable agreement with the "Adjusted R^2" which confirmed that models could be used to navigate the design space. Results acquired from the graphs by CCD indicate that light intensity and photoperiod have noticeable effects on algal biomass concentration and lutein productivity. In fact, there is a correlation between the light energy captured and carbon fixation which eventually causes variations in biomass production.

3.4 Model validation and experimental confirmation

The second-order polynomial model obtained described in Eqs. (1, (2, and (3 were utilized for response optimization by using CCD. The maximum lutein and biomass production as 10.15 mg g^{-1} and 1.15 g L^{-1}, respectively, were determined at the optimum conditions: light intensity of 200 µmol m^{-2} s^{-1}, light period of 12:12, and aeration of 3 lpm. Suggested culture conditions favored improved the production of lutein and biomass. The obtained amount of lutein and biomass production under streamlined conditions exhibited 95.89% and 97.88% productivity, respectively, showing an excellent correlation with the predicted value, and thus indicating the competence of the fitted model.

4 Conclusion

A laboratory-scale externally illuminated airlift photobioreactor was used in the present work to evaluate lutein productivity of microalga *C. salina*. Twenty sets of experiments were conducted in accordance with CCD in order to study and optimize the effect of three environmental factors on growth kinetics and lutein productivity. The optimum values were the following: light intensity, 200 µmol m^{-2} s^{-1}; photoperiod, 12:12 h, 16:8 h; and aeration rate, 3 lpm. Under these conditions, highest lutein productivity of 9.86 mg/(L day) and RC biomass concentration of 565.89 mg/(L day) were obtained. The experimental data obtained for *p* were in great concurrence with the projected data by the expressed numerical model. *C. salina* was cultivated under medium-low light intensities, and 16:8 h light supplementation yielded desirable lutein productivity.

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