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Original Article

Pigment Productions by *Spirulina platensis* as a Renewable Resource

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Abstract

Introduction: Recently, *Spirulina platensis* has scientifically become popular because of its importance as food, feed, and a natural producer of pigments with specific nutritional and functional characteristics.

Materials and Methods: In this study, the effect of various environmental factors affecting growth conditions of *Spirulina platensis*, including primary inoculation, light-dark cycle, cultivation time, Light-Emitting Diode (LED) composition, nitrogen source, carbon source, and NaCl concentration, on biomass, C-phycocyanin (C-PC), Allophycocyanin (APC) and chlorophyll-a contents were assessed using Placket-Burman Design (PBD).

Results: Results showed that out of the seven screened factors, four factors of carbon source, LED composition, light-dark cycle and NaCl concentration significantly affected biomass production (p<0.01). Among the investigated factors, nitrogen source, light-dark cycle, and NaCl concentration had significant effects on phycocyanin production (p<0.05). Results showed that cultivation time, light-dark cycle, and NaCl concentration significantly affected the production of allophycocyanin (p<0.05). Furthermore, NaCl concentration, carbon source, LED composition, cultivation time, and initial inoculation included significant effects on chlorophyll-a production (p<0.05).

Conclusions: The present study screened variables affecting biomass, phycocyanin, allophycocyanin, and chlorophyll-a production as the first step in optimizing *Spirulina platensis* growth condition. Briefly, NaCl concentration was one of the factors which had a significant impact on all responses. The dark cycle also had an effect on three dependent variables except for chlorophyll-a production.

Keywords: Chlorophyll, Pigment, Placket-Burman Design, Phycocyanin, Phycobiliprotein, *Spirulina platensis*
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Introduction

Microalgae are usually known as the most effective source of renewable mass and energy production in a waste less, environmentally pure, energy and resource saving route. Edible blue-green algae such as *Aphanizomenon*, *Nostoc*, and *Spirulina* have been used as foods for millenniums.[1] *Arthrospira platensis (Spirulina platensis)* is a photosynthetic, filamentous, multicellular blue-green microalga, which is cultivated on a large scale and processed industrially.[2] Naturally, this alga grows in several regions and especially develops at middle temperatures (25–26 °C) and alkaline pH (9.5–11) as well as shallow waters. Moreover, the alga grows in the presence of sodium bicarbonate.[3] The *S. platensis* has been used as a nutritious supplement for humans and animals because of its high nutritional and protein contents (55-70%) with perfectly balanced essential amino acids. *Spirulina* spp. include vitamins A, C and E as well as β-carotene, chlorophyll-a, minerals, omega-3 fatty acid, phycobiliproteins, and xanthophylls.[4,5] Due to the richness of these compounds, *Spirulina* spp. and its products are used in a wide variety of industries, including agriculture, food, perfume, pharmaceutical, medicine and cosmetic industries. *Spirulina* spp. include pharmacological activities such as the production of anticancer, antimicrobial, antioxidant, immunostimulant, and metal protective (prevention of heavy-metal poisoning) compounds.[6,7]

Nowadays, increased public awareness of the side effects of synthetic compounds and community preference for using natural products has resulted in the popularity of microalgae as sources of natural pigments. Various pigments such as the phycobiliproteins are found in algae. Phycocyanin is a phycobiliprotein, which is one of the essential pigments of *Spirulina* spp.[8] Phycobiliproteins are water-soluble highly fluorescent compounds with brilliant colors. These compounds are resplendent pigment complexes, including allophycocyanin (bluish-green pigments), phycocyanin (blue pigments), and phycoerythrin (red pigments). These chemicals are natural

products with non-toxic, non-carcinogenic characteristics and potentially biotechnological uses.[9] Phycocyanin is a blue pigment in cyanobacteria and two eukaryotic algal genera of *Rhodophyta* and *Cryptophyta*. It produces the blue color of several cyanobacteria, known as blue-green algae.[10] One of the common uses of phycocyanin is the use of this compound as a food pigment to replace synthetic pigments. Phycocyanin is used as a colorant in candies, chewing gums, dairy products, popsicles, sherbets, soft drinks, and cosmetics such as lipsticks and eyeliners.[10-12] Furthermore, phycocyanin includes therapeutic characteristics such as antioxidant, anti-inflammatory, anticancer, and immune enhancement. Thus, phycocyanin includes an important application as a potential medicine.[12]

Environmental factors affect the growth of algae because of their physiological requirements.[13,14] Growth and pigment accumulation in *Spirulina* spp. differ by differences in culture factors such as nitrogen source,[15,16] carbon source,[17] initial biomass concentration,[18] pH,[14,19] salt concentration,[13,19] and light.[20,21]

Generally, Plackett-Burman Design (PBD) is a highly fractionated factorial design to determine main factors among a large number. PBD is a helpful tool for the screening of process effects on product yields. It can significantly decrease the numbers of repetitive experiments and screen factors regarding their major effects and no interaction effects between various factors. In PBD for the seven factors in this study, 12 experimental trials were carried out, whereas 128 (2^7) trials must be used in other statistical designs. PBD can be used more effectively with limited explants to assess the effects of multiple factors and prioritize important factors.[22-25]

In the present study, PBD was used to screen important environmental factors, including initial inoculation (OD), light-dark cycle, cultivation time, LED composition, nitrogen source, carbon source, and NaCl concentration, on various responses such as biomass, phycobiliprotein, and chlorophyll productions in *S. platensis*. Moreover, directions for trial designs and data analyses of *in vitro* experiments were developed.

Materials and Methods

Microalgae Strain and Preculture Conditions

The microalgae strain (*S. platensis* APP1) was provided by the Microalgae Culture Collection of Tarbiat Modares University, Tehran, Iran. Inoculums of *S. platensis* was cultivated in Zarrouk medium (pH 9.8) and 250-ml glass flasks containing 150 ml of the cell suspension under sterile conditions.[26] Growth and maintenance of the culture were carried out using illuminated (150 $\mu mol.m^2.s^{-1}$) phytotron at 30 °C ± 1 under a 12/12 h light/dark cycle with mild agitation (100 rpm) for 12 days.[27]

Cultivation Conditions

Test cultures were grown in 500-ml glass flasks with 250-ml working volumes (Zarrouk medium, pH 9.8). The inoculation size was reported using a UV-visible spectrophotometer (UNICO, 2100, USA) at 560 nm. The culture was incubated at 30 °C ± 1 with mild agitation at 100 rpm with 200 $\mu mol.m^2.s^{-1}$ light intensity from 13 LEDs (6/3/4 or 3/6/4 blue /red/white) (Table 1). Shakers (Behdad, IPX0, Iran) were covered with aluminum foil to protect cultures from exposure to exterior light sources. Variables are listed in Table 1.

Carbon and Nitrogen Source Feeding

Carbon and nitrogen sources were feeded in both batch and fed-batch system. For carbon source in batch cultivation, a Date Waste Syrup (DWS) equivalent of 1 g/L of sugar was added to the culture medium only before inoculation and in fed-batch cultivation, a date syrup equivalent of 1 g/L sugar was added to the culture medium on days 0, 3 and 7 of cultivation and for nitrogen source, the urea level in batch cultivation was equivalent to 65 mg/L and was added to the culture medium only before inoculation while in fed-batch cultivation, the added urea level included 40 mg/L, which was added on days 0, 3 and 7 of cultivation.[15]

Analytical Determinations

In this study, biomass (Y_1) and pigments such as C-phycocyanin (Y_2), allophycocyanin (Y_3), and chlorophyll-a (Y_4) were assessed in *S. platensis* as response variables.

Assessment of Biomass

Biomass as Dry Weight (DW) was assessed by filtering 15 ml of the *S. platensis* suspension using pre-weighted GF/C Whatman filter papers, rinsing the filtrate well with distilled water, and culturing the biomass (g/L) after drying in an oven at 55 ºC for 24 h.[28]

Phycobiliprotein (C-phycocyanin and Allophycocyanin) Estimation

Biomass collection was carried out using centrifugation followed by washing with deionized water. Biomass was dried at 45 °C for 24 h. The C-phyocyanin was extracted by suspending a specific quantity of the dried biomass (0.1 g) in 10 ml of phosphate buffer (0.15 M, pH 7). The suspension was stored at 4 °C for 20 h. Then, the suspension was centrifuged at 13000 rpm to remove cell debris.[20] UV-visible spectrophotometer (UNICO, 2100, USA) was used for the measuring of the supernatant absorbance at 620 and 652 nm. Phosphate buffer was used as blank. Concentrations of allophycocyanin and C-phycocyanin were calculated using equations (1) and (2).[29,30]

$$\text{Allophycocyanin (g/L)} = [A_{652} - 0.208\ (A_{620})] / 5.09 \quad \text{(Eq. 1)}$$

$$\text{Phycocyanin (g/L)} = [A_{620} - 0.474\ (A_{652})] / 5.34 \quad \text{(Eq. 2)}$$

Where, A_{620} and A_{652} were the absorbance values at 620 and 652 nm, respectively.

Chlorophyll-a Estimation

Chlorophyll-a was extracted using 99.8 % (v/v) methanol. Nearly 6 ml of the culture were centrifuged at 10000 rpm for 5 min. Pellet was mixed with methanol and stored at 4 °C for 24 h under dark conditions.[31] The extract absorbance was read at wavelengths of 665.2 and 652.4 nm in dark and the pigment concentration was assessed using equation (3).[32]

$$\text{Chlorophyll-a } (\mu g/ml) = 16.72 \times A_{665.2} - 9.16 \times A_{652.4} \quad \text{(Eq. 3)}$$

Where, $A_{665.2}$ and $A_{652.4}$ were the absorbance values at 665.2 and 652.4 nm, respectively.

Statistical Analysis

The current study was carried out to investigate variables with significant effects on biomass and pigment productions in *S. platensis* using PBD. Results were analyzed using MINITAB software (version.17) and significance was reported when $p<0.05$. Table 1 demonstrates variables and their two different levels (+ as high level and – as low level). Table 2 demonstrates selected experimental factors and PBD for concluding 12 experimental trials. Each trial was carried out twice and all values were reported as mean ± SD (standard deviation).

Table 1. Various Levels of Experimental Variables Used in Production of Biomasses and Pigments in *Spirulina Platensis* Using Plackett-Burman Design

Variable	Component	Code	Unit	- Value	+ Value
X1	Initial inoculation	A	(OD)	0.4	0.6
X2	Nitrogen source (Urea)	B	mg.L^{-1}	Bach	Fed-bach
X3	Carbon source (date syrup)	C	g.L^{-1}	Bach	Fed-bach
X4	Cultivation time	D	D	11	14
X5	LED	E	LED	6 blue, 3 red, 4 white	3 blue, 6 red, 4 white
X6	Light/dark cycle	F	H	12/12	16/8
X7	NaCl	G	g.L^{-1}	0	2

X1–X7 represent various assigned variables; plus sign '+' represents high-level of variables, minus sign '-' represents low-levels of variables; OD, optical density; LED, light-emitting diode.

Table 2. Twelve-trial Plackett-Burman Design for Seven Variables with Coded Values of Results from the Screening of Factors Affecting Biomass, Phycobiliprotein and Chlorophyll a Productions

Run	Variable							Response Mean			
	A (OD)	B (mg.L^{-1})	C (g.L^{-1})	D (d)	E (LED)	F (h)	G (g.L^{-1})	Biomass (Y_1) (g.L^{-1})	C-PC (Y_2) (g.L^{-1})	APC (Y_3) (g.L^{-1})	Chlorophyll (Y_4) (µg/ml^{-1})
1	1	-1	1	-1	-1	-1	1	20.53 ± 0.19	0.80 ± 00	0.104 ± 0.03	11.20 ± 0.7
2	1	1	-1	1	-1	-1	-1	1.48 ± 0.35	0.096 ± 0.06	0.041 ± 0.02	8.33 ± 1.5
3	-1	1	1	-1	1	-1	-1	1.57 ± 0.42	0.146 ± 0.06	0.010 ± 00	9.23 ± 1.6
4	1	-1	1	1	-1	1	-1	2.78 ± 0.12	0.447 ± 0.11	0.187 ± 0.01	10.6 ± 0.17
5	1	1	-1	1	1	-1	1	1.68 ± 0.35	0.334 ± 0.08	0.145 ± 0.01	11.46 ± 0.8
6	1	1	1	-1	1	1	-1	2.04 ± 0.29	0.243 ± 0.18	0.132 ± 0.08	11.86 ± 1.9
7	-1	1	1	1	-1	1	1	3.32 ± 0.64	0.552 ± 0.02	0.207 ± 0.07	12.83 ± 0.2
8	-1	-1	1	1	1	-1	1	2.12 ± 0.68	0.336 ± 0.17	0.132 ± 0.02	11.95 ± 1.2
9	-1	-1	-1	1	1	1	-1	1.38 ± 0.16	0.714 ± 0.32	0.208 ± 0.04	9.70 ± 0.55
10	1	-1	-1	-1	1	1	1	1.58 ± 0.12	1.012 ± 0.20	0.148 ± 00	13.46 ± 0.9
11	-1	1	-1	-1	-1	1	1	2.42 ± 0.07	0.624 ± 0.09	0.153 ± 0.06	10.71 ± 0.5
12	-1	-1	-1	-1	-1	-1	-1	1.68 ± 0.12	0.564 ± 0.38	0.037 ± 0.03	7.40 ± 0.14

A, initial inoculation; B, nitrogen source; C, carbon source; D, cultivation time; E, light-emitting diode; F, light-dark cycle; G, NaCl; C-PC, C-phycocyanin; APC, allophycocyanin

Results

The current study was carried out to investigate important environmental factors that affect *S. platensis* growth conditions using the PBD. Effects of the investigated factors on response variables, including biomass (Y_1) and pigments such as C-phycocyanin (Y_2), allophycocyanin (Y_3), and chlorophyll a (Y_4), were assessed as well.

Biomass Content

Results from the assessment of biomass concentrations (Y_1) showed that trials 7 and 9 included the highest (3.32 g/L^{-1} ± 0.64) and the lowest (1.38 g/L^{-1} ± 0.16) biomass contents, respectively (Table 2). Statistical analysis is summarized in Table 3. The *p*-values were reported as significant when $p<0.05$. Results showed that only four out of seven factors, including carbon source, LED composition, light-dark cycle, and NaCl concentration, were significant ($p<0.05$). Pareto chart demonstrated that the most significant effect variable for biomass production was carbon source. Furthermore, the chart showed that the four selected factors of carbon source, LED composition, light-dark cycle, and NaCl concentration with *t*-values above the threshold (2.120) and *p*-values lower than 0.05 significantly affected the desired response (Figure 1a). Figure 1b demonstrates the normal plot of standardized effects of the factors with significant effects, displaying the vector nature of the effects. The plot indicated that the carbon source included the highest significant positive effect on biomass production. Moreover, the light-dark cycle, and NaCl concentration included significant positive effects on biomass on the right side of the response line, whereas LED composition showed a significant negative effect on biomass contents as the effect was located on the left side of the biomass production line. Linear regression coefficient (RC) (Table 4) verified these results as the RC values included 0.3437 and -0.3201 for carbon source and LED composition, respectively.

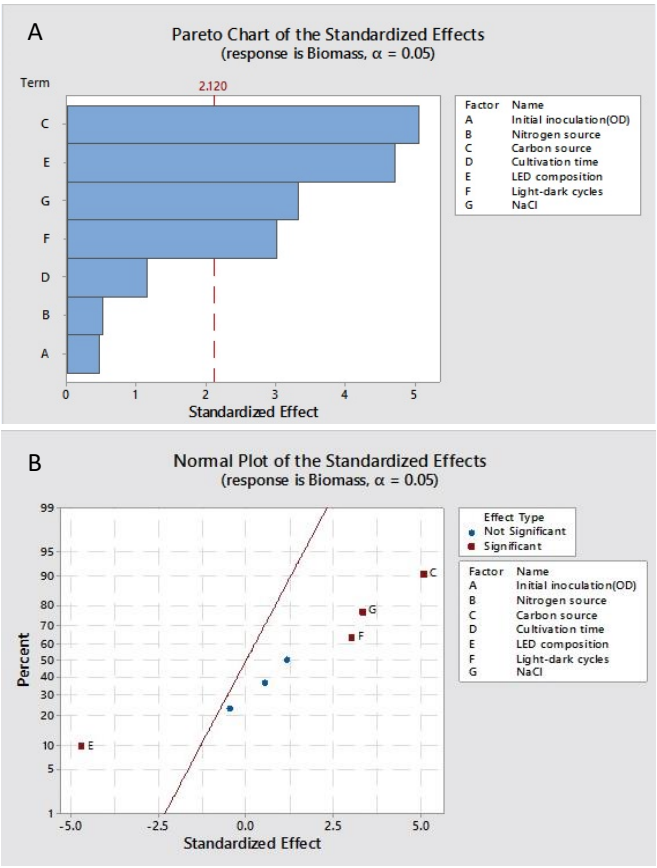

Figure 1. Pareto Chart **(A)**, and Normal Plot **(B)** for the Standardized Effects of Biomass Contents.

Phycobiliproteins Content

Two major phycobiliproteins of allophycocyanin and C-phycocyanin were characterized in *S. platensis*. In cyanobacteria, phycobiliproteins may form up to 40% of the total soluble proteins. Phycobiliprotein yields can be maximized via control or optimization of the nutrients and environmental factors.[9]

C-phycocyanin Concentration

As shown in Table 2, the highest and lowest C-phycocyanin contents (Y_2) belonged to Trials 10 and 2 (1.012 g/L^{-1} ± 0.20 and 0.096 g/L^{-1} ± 0.06), respectively. Regression analysis revealed that the nitrogen source, light-dark cycle, and NaCl concentration included significant effects on C-phycocyanin production as the *p*-values were above the selected criteria for a 95% level of confidence (Table 3). The major effects of the analyzed factors on C-phycocyanin production are graphically shown in Figure 2 based on the results from the PBD. Nitrogen source significantly affected the production of C-phycocyanin. Light-dark cycle, and NaCl concentration with *t*-values above the threshold (2.120) were significant. Based on the regression coefficient, nitrogen source included the highest significantly negative effect (RC = -0.1567) on C-phycocyanin content (Figure 2b), since the effects positioned the furthest point to the left of the response line, and NaCl concentration included the highest level of significant positive effects (RC = 0.1207) on C-phycocyanin production because

Table 3. Analysis of Variance of the Regression for Seven Factors Affecting Investigated Traits

Source		Adj. MS				*p*-value			
	DF	Biomass (Y_1)	C-PC (Y_2)	APC (Y_3)	Chlorophyll (Y_4)	biomass (Y_1)	C-PC (Y_2)	APC (Y_3)	Chlorophyll (Y_4)
Model	7	1.103	0.213	0.012	9.922	0.000	0.001	0.001	0.000
Linear	7	1.103	0.213	0.012	9.922	0.000	0.001	0.001	0.000
Initial inoculation (OD)	1	0.023	0.000	0.000	4.453	0.652	0.992	0.928	0.043
Nitrogen source	1	0.030	0.589	0.002	0.000	0.609	0.001	0.237	0.988
Carbon source	1	2.835	0.112	0.000	7.470	0.000	0.091	0.704	0.012
Cultivation time	1	0.147	0.138	0.018	0.197	0.265	0.064	0.006	0.650
LED	1	2.459	0.014	0.000	7.098	0.000	0.522	0.670	0.014
Light-dark cycle	1	1.007	0.288	0.053	15.549	0.008	0.011	0.000	0.001
NaCl	1	1.222	0.349	0.012	34.689	0.004	0.006	0.019	0.000
Error	16	0.110	0.034	0.002	0.924	-	-	-	-
Lack-of-fit	4	0.068	0.425	0.002	0.431	0.703	0.319	0.437	0.808
Pure error	12	0.124	0.032	0.002	1.089	-	-	-	-
Total	23	-	-	-	-	-	-	-	-
R-squared (%)		81.34	72.82	74.86	82.44				

DF, degrees of freedom; OD, optical density; C-PC, C-phycocyanin; APC, allophycocyanin; LED, light-emitting diode

of its position on the furthest point of the response line. The other significant factor, light-dark cycles, included positive effects on C-phycocyanin production (RC = 0.1097) (Table 4).

Allophycocyanin Concentration

Response of the PBD demonstrated that the highest and the lowest levels of allophycocyanin (Y_3) were achieved in trial 9 (0.208 g/L^{-1} ± 0.04) and 3 (0.01 g/L^{-1} ± 0), respectively (Table 2). Results of the statistical analysis showed that only three out of seven studied factors, including light-dark cycle, cultivation time, and NaCl concentration, were significant due to their *p*-values of less than 0.05 (Table 3). Data in Figure 3a represented that the most significant effective variable on the production of allophycocyanin was the light-dark cycle, followed by cultivation time, and NaCl concentration. As shown in Figure 3b, these factors included positive effects, since their effects were located on the right side of the production line. However, this plot showed non-significant effects of initial inoculation, carbon source (DWA), nitrogen

Table 4. Regression Coefficient and Corresponding *t*-value of the Desired Traits Using Plackett-Burman design

Term	Trait							
	Biomass (Y_1)		C-PC (Y_2)		APC (Y_3)		Chlorophyll (Y_4)	
	RC	*t*-value	RC	*t*-value	RC	*t*-value	RC	*t*-value
Constant	2.0493	30.16	0.4889	12.83	0.1250	14.28	10.736	54.69
Initial inoculation (OD)	-0.0313	-0.46	-0.0004	-0.01	0.0008	0.09	0.431	2.19
Nitrogen source	0.0354	0.52	-0.1567	-4.11	-0.0107	-1.23	0.005	0.02
Carbon source	0.3437	5.06	-0.0684	-1.80	0.0033	0.39	0.558	2.84
Cultivation time	0.0785	1.15	-0.0759	-1.99	0.0278	3.18	0.091	0.46
LED	-0.3201	-4.71	-0.0250	-0.65	0.0038	0.43	0.544	2.77
Light-dark cycle	0.2049	3.01	0.1097	2.88	0.0470	5.37	0.805	4.10
NaCl	0.2257	3.32	0.1207	3.17	0.0228	2.60	1.202	6.12

OD, optical density; RC, regression coefficient; C-PC, C-phycocyanin; APC, allophycocyanin; LED, light-emitting diode

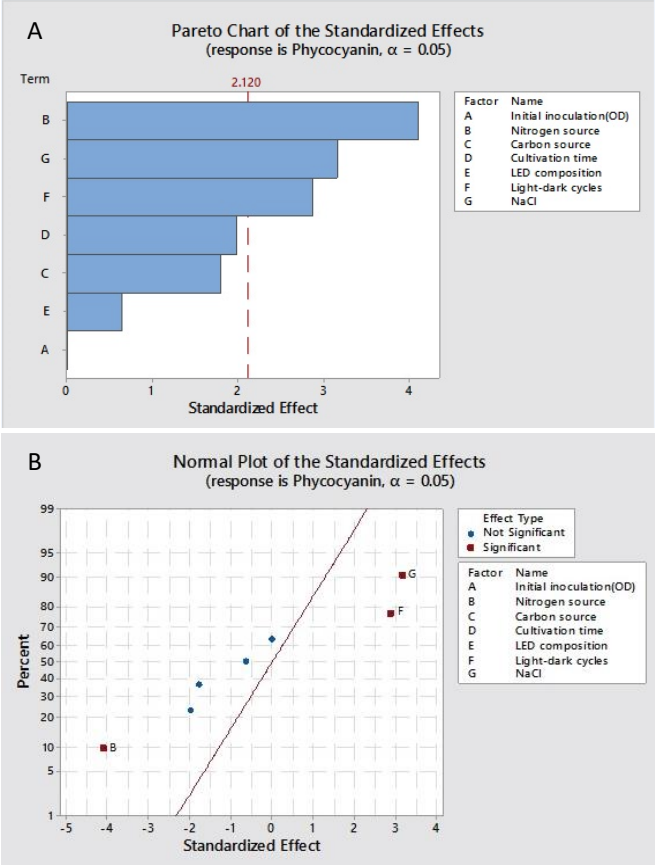

Figure 2. Pareto Chart **(A)**, and Normal Plot **(B)** for the Standardized Effects of C-phycocyanin Concentrations.

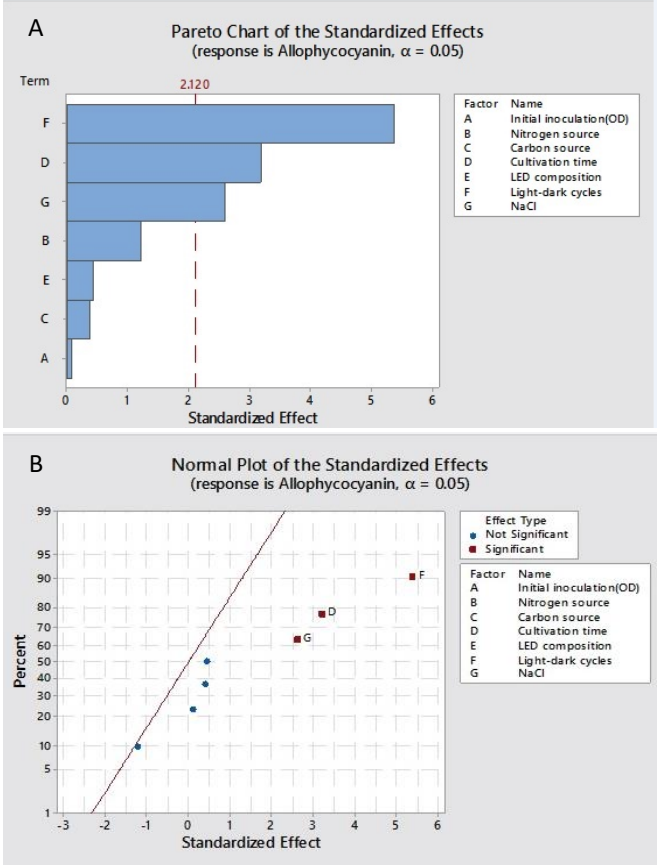

Figure 3. Pareto Chart **(A)** and Normal Plot **(B)** for the Standardized Effects of Allophycocyanin Concentrations.

source, and LED composition on the trait. The result of the regression data showed that the light-dark cycle (RC = 0.0470) included the most significant improving effect on C-phycocyanin content (p<0.05) (Table 4).

Chlorophyll-a Content

In this study, the maximum quantity of chlorophyll-a (Y_4) pigments (13.46 ± 0.9 µg/ml^{-1}) was observed in trial 10 (+ve concentration of initial inoculation, LED composition, light-dark cycles and NaCl concentration as well as -ve concentration of nitrogen source, carbon source, and cultivation time) and the minimum quantity of this pigment (7.40 µg/ml^{-1} ± 0.14) was observed in trial 12 (-ve concentration of all variables) (Table 2). The current results of the modeling study by PBD showed that factors such as NaCl concentration, light-dark cycle, carbon source, LED composition, and initial inoculation significantly affected the production of chlorophyll-a (Table 3). Based on the Pareto chart, the major effects of these factors were statistically significant (p<0.05) and the most significant effective variable on the production of chlorophyll-a included NaCl concentration (Figure 4a). The normal plot of the standardized effects of factors is shown in Figure 4b. Plot indicated that NaCl concentration included the highest level of significantly positive effects on the production of chlorophyll-a. Other significant factors included positive effects on the pigment production were located on the right side of the response line.

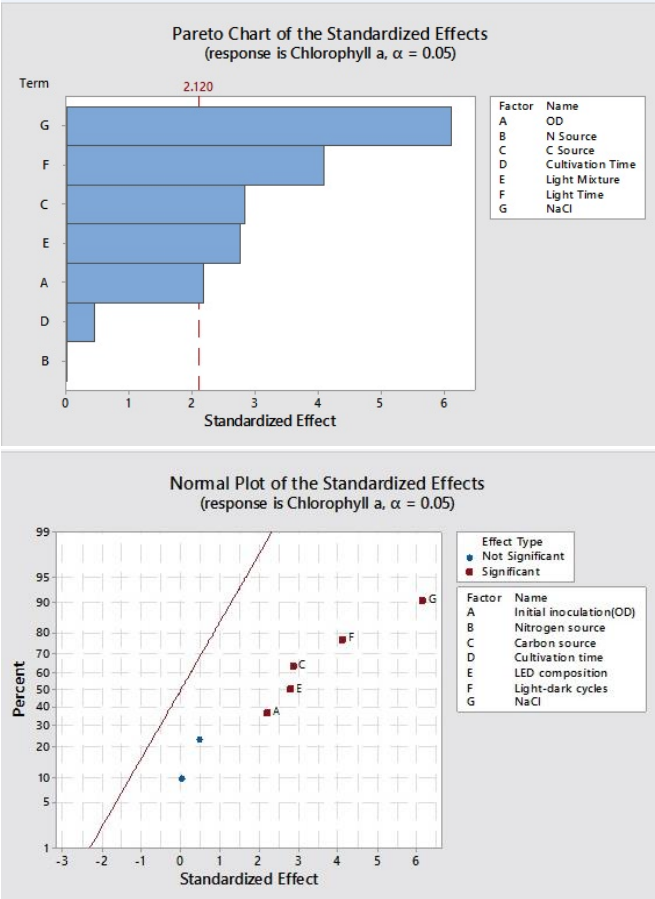

Figure 4. Pareto Chart **(A)**, and Normal Plot (B) for the Standardized Effects of Chlorophyll a Contents.

Discussion

In the growth of *Spirulina* spp., three metabolic possibilities of the cultures were seen, including autotrophic (in lights), heterotrophic (on organic substrates) and mixotrophic (simultaneously in lights and on organic substrates cultures). In the current study, the Pareto chart demonstrated that the most significant effect variable for biomass production was carbon source and LED composition, respectively. In addition, a study by Chojnacka and Noworyta showed that the highest growth rate was achieved in mixotrophic culture. Specific growth of the algae on 2.5 g/L^{-1} of glucose increased significantly with increases in light intensity up to $30W/m^2$. In mixotrophic growth, two distinctive processes were observed in the cells, photosynthesis and aerobic respiration. The first process was affected by the light intensity and the second process was affected by organic substrate concentration (glucose).[17] Molasses content in the culture medium was included as the major factor affecting the maximum biomass concentration and growth rate, although light intensity affected the two parameters after 11 days.[33] NaCl concentration with *t*-values above the threshold (2.120) and *p*-values lower than 0.05 significantly affected the biomass content (Figure 1). In line with this research, Celekli and Yavuzatmaca (2009) showed that the highest biomass yield was observed at a concentration of 1.5 g/L of NaCl at 3.495 g/L.[13]

As shown in Figure 2, the nitrogen source is the most significant factor affecting the production of C-phycocyanin. Light-dark cycle and NaCl concentration with *t*-values above the threshold on (2.120) were significant as well. Nitrogen concentration in the medium has an effect on the production of the biomass and synthesis of amino acids in proteins as well as other cellular components such as phycocyanin.[34] Urea is a nitrogen source, which is metabolized by cyanobacteria via the activity of enzymes such as urease and urea starch lyase.[35] Also, the results of studies showed that increased salinity levels in nutrient medium significantly increased phycocyanin and other soluble proteins in *S. maxima* and *S. platensis.* Pigment production was maximum under optimized NaCl concentration (2 g/L) and this concentration was most appropriate to produce phycobiliproteins.[36,37] The results of statistical analysis showed that the most significant effective variable on the production of allophycocyanin was the light-dark cycle, followed by cultivation time, and NaCl concentration. Production of C-phycocyanin, allophycocyanin, and phycoerythrin may increase under stress conditions such as carbon content, salinity, and pH in *S. platensis*.[19] It has improved with 0.4 M of NaCl (pH 7) and carbon deficiency, compared to standards medium. Maurya et al., (2014) revealed that the content of phycobiliprotein varied by factors such as light intensity, light-dark cycle, temperature, and pH in cyanobacteria. The maximum level of phycobiliprotein was recorded at pH of 8, the temperature of 35 ^oC, the light intensity of 2000 lux, and the light-dark cycle of 16:08 h. Results indicated that the production of phycobiliproteins in cyanobacteria can be optimized by regulating these factors.[38] Results demonstrated that chlorophyll-a contents varied, responding to physical factors such as agitation, light intensity, and temperature as well as chemical factors such as nutrient availability. The concentration of chlorophyll-a can be used as an indirect assay for the biomass concentration (Figure 4). Actually, chlorophyll concentration normally increases when the biomass concentration increases.[31]

Conclusion

The screening phase of research by PBD is the first step of the optimization process. Out of the seven factors in this study (initial inoculation, light-dark cycle, cultivation time, LED composition, nitrogen source, carbon source, and NaCl concentration) that affected the growth of *S. platensis* microalgae, factors affecting each response were screened using PBD. It has been shown that the replacement of low-cost nitrogen sources and the use of agricultural wastes such as date wastes in *S. platensis* culture medium can increase biomass and pigment (C-phycocyanin, allophycocyanin, and chlorophyll-a contents) productions while decreasing production costs. It is necessary to optimize and modeling growth factors of *S. platensis* using appropriate statistical methods for the scale up of this cultivation.

Authors' Contributions

SB: Collection of data, draft writing, statistical calculation; MJ: Supervising, idea, revising the manuscript; KKD: Software, revising the manuscript, data management.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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