pH, Photo, and Aeration Period Effects on *Arthrospira maxima* Growth: Taguchi Technique

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ABSTRACT: *In this study, biomass dry weight [DW (0.912 g/L)] was markedly enhanced at pH of 10 for 24 h of aeration. The maximum production of protein (14.239 mg/L) was obtained at the highest photoperiod. The highest content of PC and APC (0.129 and 0.099 mg/L, respectively) was recorded at pH of 10 for 24 h of aeration. The highest content of total chlorophyll (Ca and Cb) and carotenoids (K) (6.436, 3.421, and 2.856 mg/L) was recorded at pH of 9. The content of photosynthesis pigments (Ca, Cb, and K) was reduced by increasing pH from 9 to 10, while the high alkaline pH (10) may favor the over-production of biomass, protein, phycobiliprotein pigments (PC and APC). Moreover, A. maxima increased the accumulation of photosynthesis pigment under strong illumination and decreased the accumulation of phycobiliprotein pigments by increasing the light irradiance time from 12 to 24 h.*

KEYWORDS*: pH; Photoperiod; Aeration period; Arthrospira maxima; Taguchi.*

INTRODUCTION

Microalgae are organisms with very large biological variability [1]. Species of the genus *Spirulina* are interesting because of their ability to accumulate large amounts of phycobilioproteins (20-30%), amino acids (40%), and protein (50-70%). It contains total chlorophyll and carotenoid [2]. In general, the application of microalga has recently increased in the drug and cosmetic industries [3-5]. Moreover, microalgae are free-living in various aquatic habitats [6]. A variety of environmental factors plays a significant role in the production of biomass, pigments,

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and protein [7, 8]. Aeration is as one of the most important factors for increasing the dissolved oxygen content of culture for controlling algae growth [9]. Furthermore, light can influence the production of biomass, phycobilioproteins, photosynthesis, and protein in cyanobacterium [10]. In fact, light is as one of the most significant factors for the growth of photosynthetic microorganisms [11]. pH also has a strong impact on the functional properties and pigments of *Spirulina* [12-14]. The effect of light/dark cycles on photosynthetic activity was reported in the past five decades [15, 16].

Few studies were conducted on the cultivation of *Spirulina platensis* based on the effects of aeration [17, 18], pH [19], and light [20] for high production of biomass and pigments. The contribution effect of pH and light intensity on the productivity of biomass and protein in *Spirulina* species was proposed [13]. The influences of light irradiance and temperature on biomass and biochemical composition of microalgae were studied [7]. Moreover, the contribution effect of aeration and lighting on the biomass production and protein biosynthesis in *Spirulina Sp.* was found [13]. Furthermore, the contribution effects of pH, temperature and light on the biomass and protein productions were already discovered [21].

In this research, some experiments in the lab scale were performed for the cultivation of *A. maxima* at various conditions such as pH, photo, and aeration period to find high cell concentration, pigments and protein (under Mexico City climate). In fact, these would be useful in the optimizing growth conditions for the production of biomass, pigments, and protein. The relationship between the cultivation factors (cell optical density, pigments, and protein production) was investigated by the Taguchi method.

EXPERIMENTAL SECTION

Cultivation process

All chemicals were purchased from Merck and Sigma-Aldrich. *Arthrospira maxima* (*A. maxima*) with strain number CIB79 was obtained from National Polytechnic University (Mexico City, Mexico). The unialgal culture of *A. maxima* was grown in solid Zarrouk's media. It was then transferred in Zarrouk's broth. The medium was sterilized through an autoclave (class B) at 121 ℃ for 15 min before cultivation process. The Zarrouk's media was prepared according to a technique as mentioned in the literature [22]. The experiments were carried out in a culture chamber at the laboratory temperature of 30±2°C [23]. The contribution effects of pH, photoperiod, and aeration period (at three levels) on the biomass, pigments, and protein production were studied as shown in Table 1. The artificial illumination provided by white light fluorescent tubes (3500 lux) above the flask. The flask (with cultures) was aerated by adjusting a constant aeration with an air pump AC-9602 (RESUN, Mexico) during the growth process. During the experiments, pH was adjusted using pH meter [pH21 pH/mV meter (HANNA, America)]. The flask was daily shaken 3 times during the batch process of growth under the static conditions (non-aerated system). 50 mL of stock culture of *A. maxima* was added in 125 mL flask (which had an initial optical density of 0.3 at 674 nm) in a bath. The cultures were cultivated during 8 days of cultivation.

Chemical analysis

The biomass growth was measured by controlling an optical density at 674 nm with assisting a multi-scan spectrophotometer (Thermo SCIENTITIC, England) using a validation curve investigated in our previous work [24]. The cell walls were ruptured by the repeated five freezing and thawing cycles for phycobilioproteins isolation [25]. In addition, samples were frozen at -20 °C for 1 h then thawed at room temperature for 45 min. The samples were then frozen and thawed up to five times. Finally, they were centrifuged (velocity 14/14 R Refrigerated centrifuge, China) at 10000 rpm for 10 min at 4 ˚C where the supernatant was collected for phycobilioproteins determination. Then, the pigments concentrations of phycocyanin (PC) and allophycocyanin (APC) were calculated according to the following equations [26]:

$$\mathrm{PC}\left(\mathrm{mg/L}\ \right)\left(\mathrm{A}_{620}-0.474\,\mathrm{A}_{652}\right)/5.34 \qquad (1)$$

$$\mathrm{APC}\ (mg/\mathrm{L}^{-})=\left(\mathrm{A}_{652}-0.208\,\mathrm{A}_{620}\right)/5.09 \qquad (2)$$

where, A_{620} and A_{652} are absorbance at the wavelengths of 620 and 652 nm, respectively.

Furthermore, the amounts of chlorophylls (Ca and Cb) and carotenoids (K) pigments were measured from the pellet. The pellet was dried through an oven at 25 ℃ for 3 h. Then, it was extracted by 600 µL of an acetone (80%) and chloroform (100%) mixture (70:30 v/v) under the dim light condition. Chlorophylls (a and b) and carotenoids amounts were also measured by a spectrophotometer [LEGEND MICRO 17 spectrophotometer (Thermo Scientific, Germany)]

Table 1: Experimental design according to the Taguchi design.

Run Order[1]	pH	A[2] (h)	L[3] (h)	Run order	pH	A (h)	L (h)	Run order	pH	A (h)	L (h)
1	8	0	0	10	9	0	12	19	10	0	24
2	8	0	0	11	9	0	12	20	10	0	24
3	8	0	0	12	9	0	12	21	10	0	24
4	8	12	12	13	9	12	24	22	10	12	0
5	8	12	12	14	9	12	24	23	10	12	0
6	8	12	12	15	9	12	24	24	10	12	0
7	8	24	24	16	9	24	0	25	10	24	12
8	8	24	24	17	9	24	0	26	10	24	12
9	8	24	24	18	9	24	0	27	10	24	12

[1] Order of treatment [2]Aeration period, [3]Light photoperiod

at the wavelengths of 470, 645, and 662 nm, respectively. The chlorophylls and carotenoids pigments were calculated by the following equations [26]:

$$C_a = 11.24 \times A_{662} - 2.04 \times A_{645}\ (mg/L) \quad (3)$$

$$C_b = 20.13 \times A_{645} - 4.19 \times A_{662}\ (mg/L) \quad (4)$$

$$C_{(x+c)} = \left((1000 \times A_{470}) - (1.09C_a - 63.14C_b)\right)/214 \quad (5)$$

Protein concentration in supernatants can be measured by the Bradford's method at 595 nm [27]. Bovine serum albumin (BSA) was used as a protein concentration standard. The standard and samples were kept under the same conditions to find a valid standard curve.

Experimental design

According to Table 1, 3^3 standard orthogonal arrays (based on the Taguchi method) were designed for three various factors at three different levels.

The main objective of this research was to find the optimum conditions for maximizing the biomass, pigments, and protein concentrations. The performance of response can acceptably be measured when Signal to Noise (S/N) ratio is high as:

$$\frac{S}{N} - 10 \times \log\left(\sum_{j=1}^{N}\left(1/Y_j^2\right)/n\right) \quad (6)$$

where, j and y_j are the parameters and n is the number of trials.

RESULTS AND DISCUSSION

However, the release of pigment and protein directly is related to cell rupture but, *A. maxima* has resistant multi-layered cell walls which hardly makes the extraction process [28]. Moreover, cell rupture could be depended on pH, photoperiod, and aeration period. In this study, the culture was taken from the flask at the final day to extract biomass, pigments and protein. The results then converted to the signal to noise (SN) ratio obtained from the parameters of Taguchi method. The mean value and the signal to noise (SN) ratios were calculated according to "larger is better" parameters for each experiment as shown in Figs. 1-3. The greatest value is the target one. As shown in these figures, the calculated target values were pointed by the gray circle.

According to Fig. 1, a slight change in pH (from 8 to 10) can lead to 2-fold increase in the biomass production. Furthermore, the biomass production linearly depends on the pH. This indicates that the biomass production increased with the maximum data. On the other hand, the protein in microalgae was accumulated when it was exposure to 24h of light. The carbon dioxide of air (around 0.03% of CO_2) is bubbled into the microalgae culture after 12 h. This will limit the microalgae growth. It was also found that reduction of aeration period (A) and photoperiod (L) (below 24 h) at the maximum time at pH of 9 at the same time increase the protein production (P).

Furthermore, contrary to the biomass production, there is no a linear correlation between the production of PC and APC and light photoperiod (Fig.2).

According to Fig. 3, pH of 9 was chosen to increase photosynthesis pigments. The production of photosynthesis pigments was improved in high light at the aerated condition.

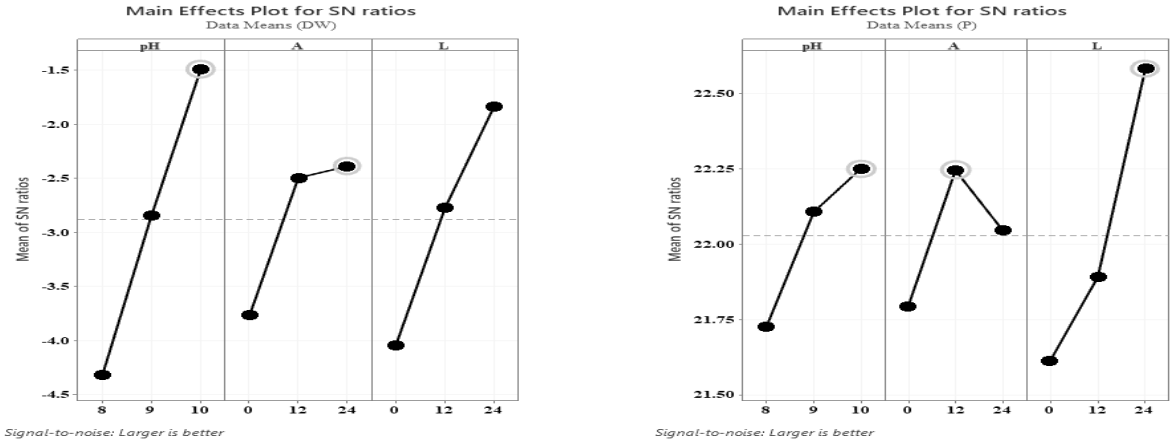

Fig. 1: Mean effects plot for means of SN ratios of biomass growth (DW) and protein (P).

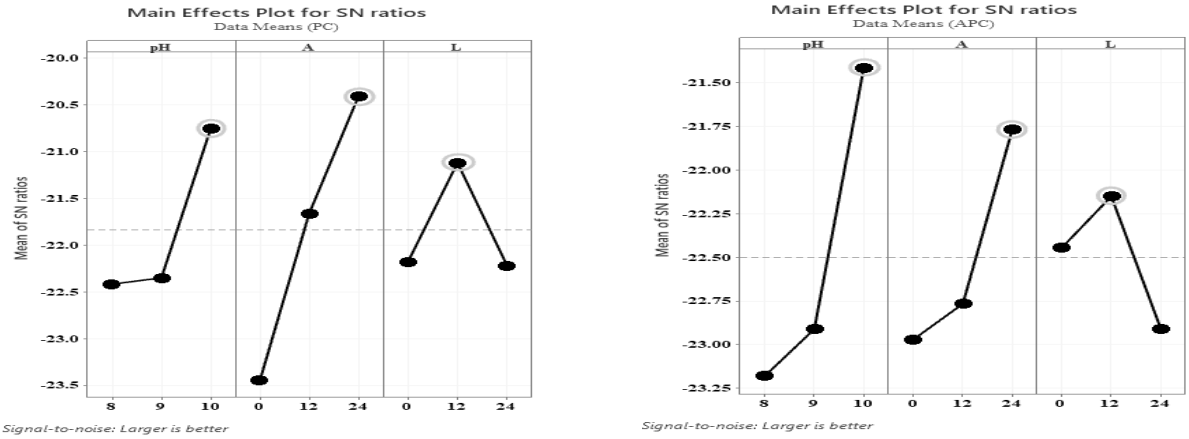

Fig. 2: Mean effects plot for means of SN ratios of phycocyanin (PC) and allophycocyanin (APC).

Fig. 4 illustrates a contour plot of pH-A-L interaction obtained from the Minitab software. The reduction of aeration period (A) and photoperiod (L) (below 24 h) at the maximum time and at pH of 9 (at the same time) increased the production of protein (P). According to this figure, the biomass production increased with the maximum data.

Fig. 5 shows that light quality (photoperiod) is the most important factor on the production of photosynthetic pigments. Furthermore, pH has a significant effect on the photosynthetic pigments extracted from *A. maxima*, as well. According to this figure, photoperiod and pH also are the most important factors on the production of biomass and protein. Furthermore, pH and aeration period have an important factor for the PC and APC production.

Effect of pH on the biomass and pigments production

pH can be considered as an important factor influencing the metabolism of microalgae and photosynthetic efficiency. The solubility of carbon dioxide in the medium can change with pH and microalgae content for the biomass growth increment [29]. In fact, the samples properly grew after 4 days of cultivation and the medium pH reached almost 10 (as steady state condition) [30]. According to the literature, carbonate (e.g. Zarrouk's medium as a source of bicarbonate ion) is a key factor for the microalgae growth [31]. Moreover, the experiment demonstrated that the pH of cultivation medium was in alkaline condition during the cultivation process [pH of 8.30 (first day of cultivation) and pH of 10.30 (last day of cultivation)] [30]. Furthermore, concentration of biomass increased with pH of medium increment [32].

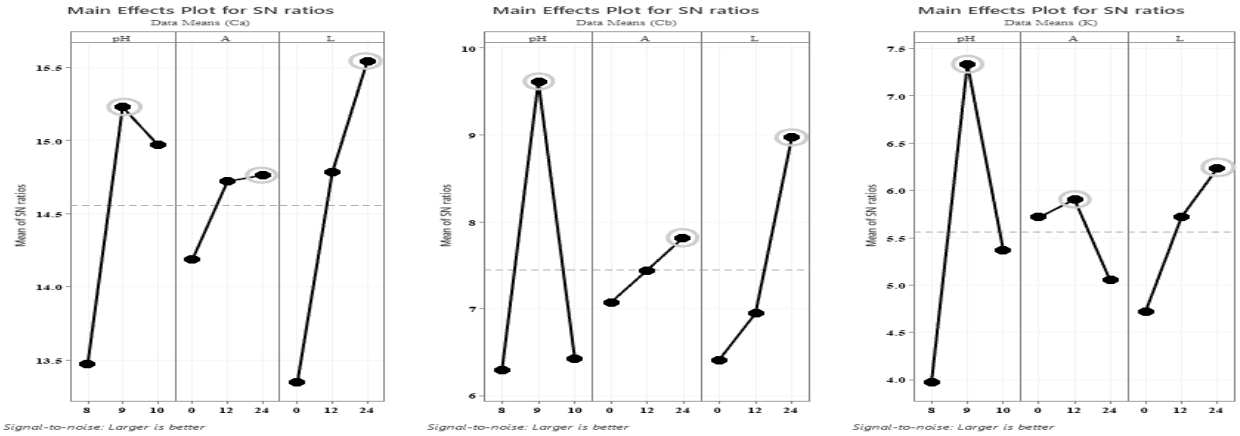

Fig. 3: Mean effects plot for means of SN ratios of photosynthetic pigments (Ca, Cb, and K).

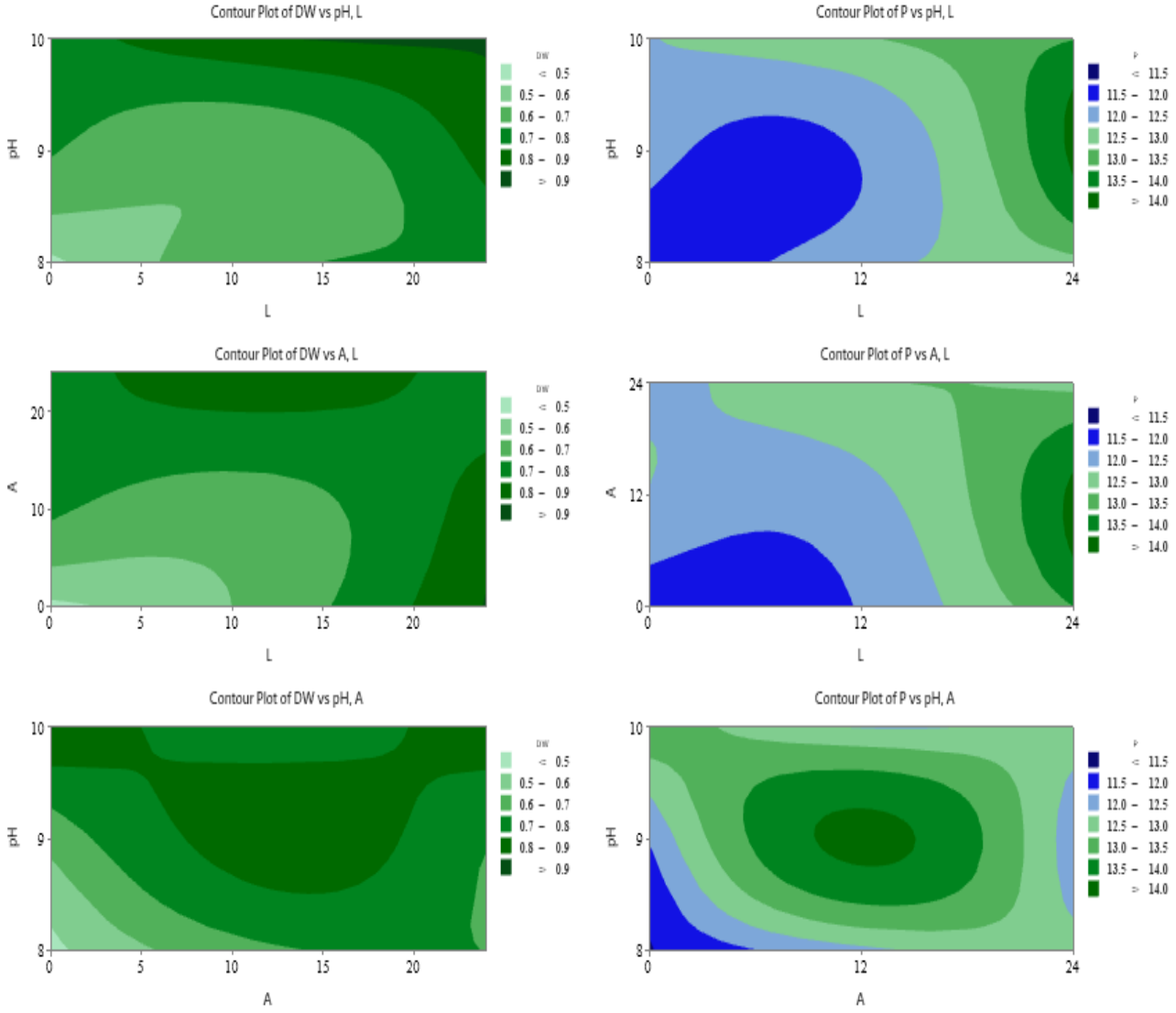

Fig. 4: Counter plot of protein versus pH, photoperiod and aeration period.

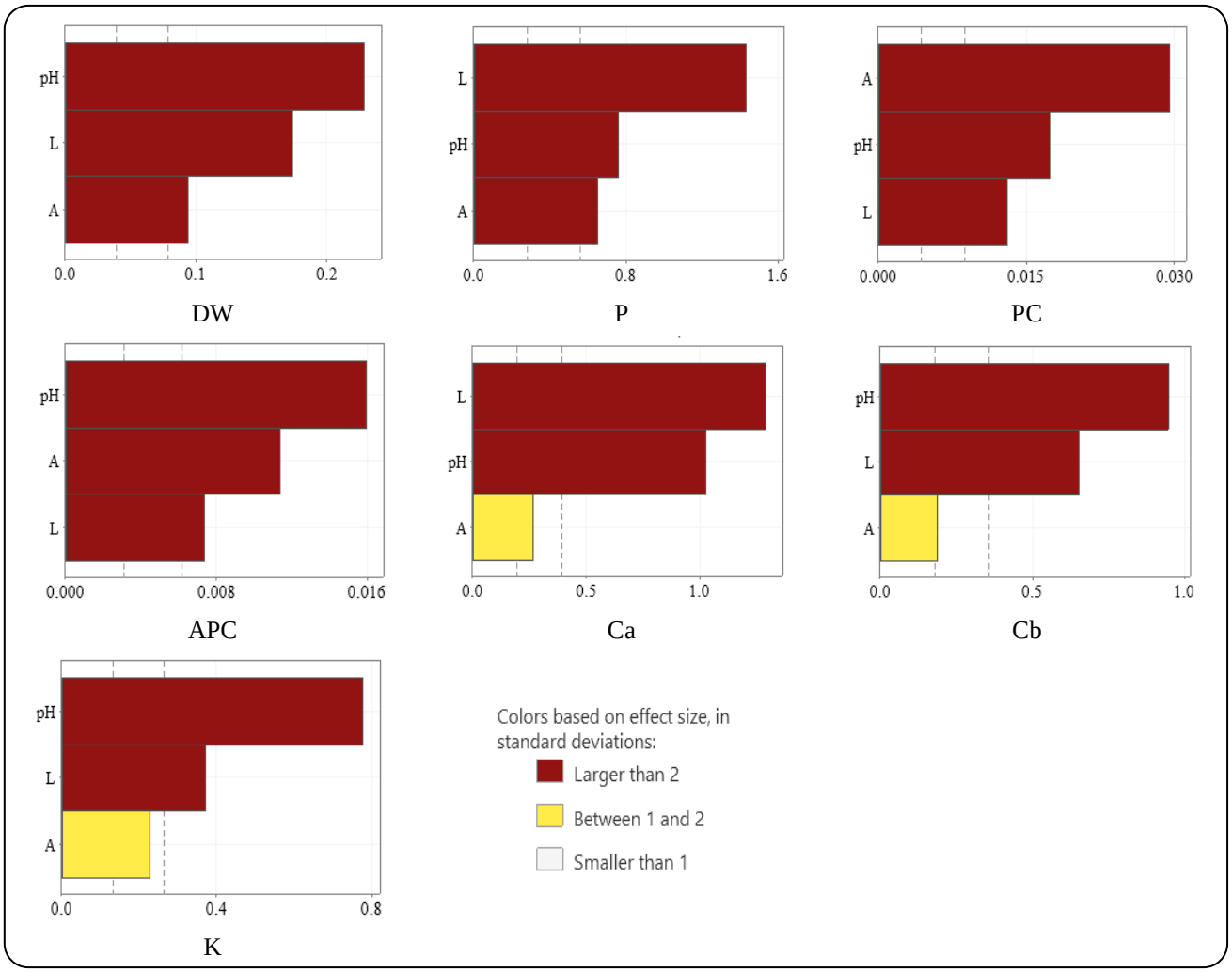

Fig. 5: Pareto effect of each response.

The difference in biomass accumulation between treatment 13 (pH=9, A12L24) and 20 (pH=10, A0L24) were about 7.7%. The present study demonstrated that the biomass concentration was significantly promoted at pH 9 and 10. The results showed the best pH for biomass production was at 10 [13] although some researchers mentioned that pHs of 9-10 are the optimum ones for the biomass production [33, 34]. 9.5 µg/mg of chlorophyll a and 64.2% of protein was reported from *A. maxima* at pH of 9andlight intensity of 5000 lux although *Spirulina* could not produce a photosynthesis pigment at pH of 10 and pH of 9 [36]. Moreover, maximum PC and APC found at the highest pH and aeration period with 12 h light at night. The results also showed that Ca, Cb and carotenoid production of all treatments were at highest amounts at pH of 9 with 24 h light which were not the best conditions for the biomass growth [16].

Effect of light photoperiod on the biomass and pigments production

Microalgae use light energy for converting carbon dioxide into carbohydrates and proteins [11]. Light photoperiod lead to some differences in biochemical composition and in pigments of microalgae [36- 38]. The results show that light photoperiod plays an important role on *A. maxima* growth. This would almost be doubled by increasing aeration period from 0 to 24 h (treatments 1 and 9). Furthermore, the carotenoids grew up fast from 0 h photoperiod (treatment 18: 1.810 mg/L) to 24 h photoperiod (treatment 14:2.856 mg/L). In fact, *A. maxima* could not synthesize overall carotenoids and chlorophyll without light. The PC and APC accumulation at pH of 10 with 12 h photoperiod rapidly increased almost two times more than that of it at pH of 8 without light. All chlorophyll a decreased from a maximum

of 6.395 (A12L24) to 5.149 (A24L0) when light photoperiod decreased from 24 h to 0 h at pH of 9 [39] although the cellular and metabolic activity of various strains of *Spirulina* was already studied at temperature of 30 to 35 ºC, and light and dark periods (12:12 h) [15]. Furthermore, few studies were conducted on the quantification of cyanobacterial proteins [15, 40]. According to the current research, strong illumination could dramatically increase accumulation of photosynthesis pigments in *A. maxima*.

Effect of aeration period on the biomass and pigments production

Biomass and pigments were chosen to investigate the oxygen consumption during the synthesizing. Furthermore, effect of aeration period on the production of protein should be interested. In fact, aeration reduces the internal phosphorus of culture and promotes the phosphorus sedimentation. Moreover, mixing (with assisting light and nutrient) is proposed to prevent the precipitation process [41]. The dissolved oxygen level in the static condition (non-aerated one) was lower than that of it in a continuous aeration (12 h or 24 h) [19]. According to the recent research, continuous aeration (24 h) in bicarbonate-enriched medium such as Zarrouk medium showed maximum removal of total inorganic carbon in the form of CO_2. However, the biomass concentration of *A. maxima* changed according to the duration of aeration from 0.466 (A0L0) to 0.711 (A24L24) at pH of 8, from 0.617 (A0L12) to 0.847 (A12L24) at pH of 9, from 0.721 (A12L0) to 0.912 (A0L24) and 0.896 (A24L0) at pH of 10 with light but, the effect of pH was more significant. A change in the aeration period from 12 to 24 h showed no significant effect on the biomass production. Moreover, further increase beyond 12 h aeration had no effect on the *A. maxima* growth. However, a change in photoperiod from 0 to 24 h showed significant changes on the biomass production but, dissolved oxygen affected the biomass production during aeration from 12 to 24 h. Moreover, the protein content widely reduced when the concentration of dissolved oxygen rose during aeration time [42]. The biomass and protein contents increased in the higher light photoperiod (24 h photoperiod) [43]. On the other hand, aeration period and pH may probably be responsible factors for the level of PC and APC pigments increment.

Taguchi approach and ANOVA analysis

According to Taguchi experimental design, the experimental data were converted to the Signal/Noise (S/N) ratio. A value with the largest S/N ratio was then used to provide the best performance. Table 2 shows the effect of each parameter on the responses.

As shown in Table 2, the level of the greatest values for each parameter indicates the test result corresponding to the best performance. In fact, the values with the highest S/N ratios give the optimum conditions. According to the previous criteria, the optimum parameters were designated as H1A3L2 for DW, H2A1L3 for PC, H1A2L3 for APC, H2A3L1 for Ca, H1A3L2 for Cb, H1A3L2 for K, and H2A3L1 for P production.

Analysis of variance (ANOVA) can be applied as a more precise technique by the Minitab 19 software to obtain the effect of each parameter (such as pH, A and L) and their significance on the responses (such as production of biomass, pigments and protein). According to Table 3, PC and APC concentrations were highly affected by photoperiod and aeration period while DW concentration was affected by all parameters however, it was less affected by the aeration period. Furthermore, all parameters were statistically investigated significant for the production of biomass, pigments and protein.

The best model was chosen based on the model validity and the highest R-squared (R^2). According to Table 4, R^2 for the proposed models were at 94.56% and 94.46% for PC and Ca, respectively. Furthermore, all R^2 data were acceptable. In order to predict the amounts of produced biomass (DW in g/L), pigments (mg/L), and protein (mg/L) as function of pH, aeration and light photoperiod, the proposed equations were obtained from Taguchi software and showed as:

$$DW = 0.73027 - 0.1125\,pH_8 - 0.0032\,pH_9 + 0.1157\,pH_{10} - 0.0584\,A_0 + 0.0232\,A_{12} + 0.0352\,A_{24} - 0.0890\,L_0 + 0.0047\,L_{12} + 0.0843\,L_{24} \quad (7)$$

$$PC = 0.082354 - 0.00584\,pH_8 - 0.00569\,pH_9 + 0.01153\,pH_{10} - 0.01493\,A_0 + 0.00041\,A_{12} + 0.01452\,A_{24} - 0.00353\,L_0 + 0.00824\,L_{12} - 0.00471\,L_{24} \quad (8)$$

$$APC = 0.075581 - 0.006033\,pH_8 - 0.003837\,pH_9 + 0.009870\,pH_{10} - 0.004371\,A_0 - 0.002605\,A_{12} + 0.006976\,A_{24} + 0.000153\,L_0 + 0.003599\,L_{12} - 0.003753\,L_{24} \quad (9)$$

Table 2: Taguchi analysis responses for signal to noise ratios.

Level	pH (H)	A (h)	L (h)
DW			
1	-4.315	-3.763	-4.039
2	-2.839	-2.494	-2.773
3	-1.491	-2.388	-1.833
Delta	2.824	1.375	2.206
Rank	1	3	2
PC			
1	-22.42	-23.45	-22.18
2	-22.35	-21.66	-21.12
3	-20.76	-20.41	-22.22
Delta	1.66	3.03	1.10
Rank	2	1	3
APC			
1	-23.18	-22.97	-22.44
2	-22.91	-22.77	-22.15
3	-21.41	-21.77	-22.91
Delta	1.77	1.20	0.76
Rank	1	2	3
Ca			
1	13.47	14.19	13.35
2	15.23	14.72	14.78
3	14.97	14.76	15.54
Delta	1.76	0.57	2.19
Rank	2	3	1
Cb			
1	6.291	7.075	6.411
2	9.613	7.441	6.947
3	6.426	7.814	8.972
Delta	3.322	0.739	2.561
Rank	1	3	2
K			
1	3.972	5.717	4.722
2	7.335	5.904	5.720
3	5.370	5.056	6.235
Delta	3.363	0.848	1.513
Rank	1	3	2
P			
1	21.73	21.79	21.61
2	22.11	22.24	21.89
3	22.25	22.05	22.58
Delta	0.52	0.45	0.97
Rank	2	3	1
Larger is better			

Table 3: Biomass, pigments, and protein concentrations according to the experimental design.

No.	pH	Period		DW	PC	APC	Ca	Cb	K	P
		A	L							
1	8	0	0	0.477	0.062	0.067	3.763	1.640	1.501	11.464
2	8	0	0	0.479	0.065	0.068	3.899	1.794	1.527	11.487
3	8	0	0	0.466	0.060	0.067	3.795	1.529	1.491	11.465
4	8	12	12	0.686	0.083	0.074	4.919	2.092	1.638	12.129
5	8	12	12	0.685	0.085	0.071	4.960	2.197	1.651	12.463
6	8	12	12	0.671	0.081	0.068	4.933	2.158	1.598	12.425
7	8	24	24	0.696	0.084	0.073	5.584	2.461	1.617	12.738
8	8	24	24	0.711	0.087	0.074	5.612	2.491	1.628	12.938
9	8	24	24	0.690	0.082	0.064	5.501	2.513	1.579	12.829
10	9	0	12	0.655	0.070	0.068	5.911	2.733	2.542	12.284
11	9	0	12	0.617	0.067	0.068	5.844	2.594	2.291	11.830
12	9	0	12	0.632	0.068	0.069	5.779	2.638	2.410	11.984
13	9	12	24	0.847	0.078	0.068	6.436	3.421	2.856	14.239
14	9	12	24	0.837	0.076	0.067	6.390	3.384	2.778	13.997
15	9	12	24	0.831	0.075	0.067	6.359	3.324	2.500	14.097
16	9	24	0	0.684	0.083	0.079	5.145	3.078	1.994	11.958
17	9	24	0	0.759	0.087	0.081	5. 165	3.162	2.038	12.391
18	9	24	0	0.682	0.087	0.078	5.137	3.033	1.810	12.259
19	10	0	24	0.912	0.073	0.079	6.074	2.673	2.001	13.658
20	10	0	24	0.912	0.073	0.078	6.094	2.659	2.091	13.808
21	10	0	24	0.897	0.071	0.077	5.909	2.590	1.885	12.990
22	10	12	0	0.739	0.090	0.082	5.272	1.815	1.809	12.591
23	10	12	0	0.721	0.086	0.079	4.652	1.802	1.714	12.195
24	10	12	0	0.765	0.090	0.080	5.543	1.791	1.728	12.650
25	10	24	12	0.896	0.118	0.099	5.646	1.932	2.050	12.964
26	10	24	12	0.895	0.129	0.098	5.977	1.994	1.817	13.095
27	10	24	12	0.876	0.116	0.097	5.558	1.879	1.699	12.793

$$Ca = 5.4021 - 0.6281pH_8 + 0.3941pH_9 + 0.2340pH_{10} - 0.1724A_0 + 0.0940A_{12} + 0.0784A_{24} - 0.6941L_0 + 0.1008L_{12} + 0.5933L_{24} \quad (10)$$

$$Cb = 2.4214 - 0.3241pH_8 + 0.6194pH_9 - 0.2952pH_{10} - 0.2388L_0 - 0.1750L_{12} + 0.4138L_{24} \quad (11)$$

$$K = 1.9349 - 0.3538pH_8 + 0.4226pH_9 - 0.0688pH_{10} + 0.0360A_0 + 0.0953A_{12} - 0.1313A_{24} - 0.2002L_0 + 0.0313L_{12} + 0.1689L_{24} \quad (12)$$

$$P = 12.6562 - 0.4410pH_8 + 0.1258pH_9 + 0.3152pH_{10} - 0.3263A_0 + 0.3198A_{12} + 0.0065A_{24} - 0.6051L_0 - 0.2156L_{12} + 0.8208L_{24} \quad (12)$$

Eqs (7-13) used to estimate the expected value under various conditions.

According to the ANOVA, The biomass production (DW) was significantly (P<0.0001) influenced by pH, photoperiod and aeration (from 46.6% to 91.2%). Furthermore, PC and APC contents were influenced by these parameters and (from 0.06 to 0.129 for PC and from 0.066 to 0.099 for APC). Moreover, the chlorophyll b was significantly (P<0.0001) influenced by all parameters in *A. maxima* treatments cultivated at various conditions (from 1.529 to 3.421 mg/L) although 3.35 mg/L was in maximum [16]. Furthermore, maximum amounts of chlorophyll (2.72 mg/L) and carotenoid (1.32 mg/L) were obtained from *Spirulina platensis* at pH of 9 after 12 days

Table 4. ANOVA for independent parameters and dependent responses

Source	DF	R^2 (%)	F-Value	P-Value
DW				
pH	2	93.05	75.34	0.000
A (h)	2		14.98	0.000
L (h)	2		43.53	0.000
PC				
pH	2	94.46	46.18	0.000
A (h)	2		100.50	0.000
L (h)	2		23.76	0.000
APC				
pH	2	92.15	69.73	0.000
A (h)	2		35.00	0.000
L (h)	2		12.70	0.000
Ca				
pH	2	94.56	70.38	0.000
A (h)	2		5.21	0.015
L (h)	2		98.25	0.000
Cb				
pH	2	90.24	70.14	0.000
A (h)	2		31.53	0.000
L (h)	2		43.27	0.000
K				
pH	2	91.16	78.44	0.000
A (h)	2		7.02	0.005
L (h)	2		17.69	0.000
P				
pH	2	90.15	17.65	0.000
A (h)	2		11.91	0.000
L (h)	2		61.94	0.000
Error	20			
Total	26			

while according to the current research these respectively were at 6.436, 2.856 mg/L from *A. maxima* at pH of 9 after 10 days. Ismail and Osman respectivelly reported maximum amounts of chlorophyll a and carotenoids at 10.6 and 2.4 mg/g DW at pH of 8.5 although they found the highest amount of phycobilioproteins at pH of 9 which is in good agreement with the current research conditions [44].

Process validation

A necessary test is requested to validate the Taguchi method. This test was conducted for DW and protein concentrations according to patterns of pH10A12L24 & pH10A24L12, and pH9A12L24 for PC & APC pigments, and photosynthesis pigments, respectively.

As shown in Table 5, the set of optimization conditions were properly validated because the error percentage was less than 10.

CONCLUSIONS

The several parameters (such as pH, photoperiod, aeration period) effects were studied on *A. maxima* (as a biomass) growth. Their effects on the production of pigments and protein were then considered. The maximum

Table 5: Confirmation test data for the set of optimization conditions.

	DW	PC	APC	Ca	Cb	K	P
pH10A12L24	0.923						
pH10A24L12		0.123	0.098				
pH9A12L24				6.413	3.401	2.811	14.208

growth of biomass was found at pH of 10 and 24 h photoperiods. The aeration (for 12 h) with artificial illumination (under white light) (for 24 h photoperiod) significantly increased the production of protein. It was concluded that *A. maxima* showed a wide range of light photoperiod from 12 h to 24 h for production of biomass and photosynthesis pigments. Moreover, high light photoperiod could assist the production of photosynthesis pigments. Furthermore, pH of 9 was more useful than pH of 10 for extraction of photosynthesis pigments.

Nomenclatures

A_{abs}	Absorbance at different wavelength
A	Aeration period
APC	Allophycocyanin
C_a	Chlorophyll a
C_b	Chlorophyll b
C_{c+x}	Carotenoid
DT	Doubling time
DW	Dried weight of biomass
H	pH
K	Carotenoids
L	Photoperiod
P	Protein
PC	Phycocyanin
R^2	Interpret R squared
vv_m	Volume of air per unit of medium per minute

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REFERENCES

[1] Hannon M., Gimpel J., Tran M., Rasala B., Mayfield S., Biofuels from Algae: Challenges and Potential, *Biofuels*, **1(5)**: 763–784 (2010).

[2] Wasmund N., Topp I., Schories D., Optimising the Storage and Extraction of Chlorophyll Samples, *Oceanologia*, **48(1)**: 125–144 (2006).

[3] Ariede M.B., Candido T.M., Jacome A.L.M., Velasco M.V.R., de Carvalho J.C.M., Baby A.R., Cosmetic Attributes of Algae - A Review, *Algal Res*, **25(2017)**: 483–487(2017).

[4] Singh S., Dwivedi V., Sanyal D., Dasgupta S., Therapeutic and Nutritional Potential of Spirulina in Combating COVID-19 Infection, *AIJR Prepr*, **49(1)**: 1–8 (2020).

[5] Blas-Valdivia V., Daniela M.D.N., Rojas-Franco P., Franco-Colín M., Mirhosseini N., Davarnejad R., Halajisani A., Tavakoli O., Cano-Europa E., Phycocyanin Prevents Acute Myocardial Infarction-Causes Oxidative Stress, Inflammation, And Cardiac Damage, *Pharm. Biol.*, **60(1)**: 755-763(2022).

[6] Whitton B.A., Potts M., The ecology of Cyanobacteria : Their Diversity in Time and Space, in: Whitton, B.A., Potts, M. (Eds.), *Kluwer Academic*, 291-316 (2000).

[7] Kumar M., Kulshreshtha J., Singh G.P., Growth and Biopigment Accumulation of Cyanobacterium Spirulina Platensis at Different Light Intensities and Temperature, *Braz. J. Microbiol.*, **42(3)**: 1128–1135 (2011).

[8] Pal R., Anshuman Gupta A.T., Impact of Environmental Factors on the Biomass Production of Spirulina in Different Conditions, *Int. J. plant Res.*, **24(2)**: 142–148(2011).

[9] Cooke, G.D., Welch, E.B., "Peterson, S., Nichols, S.A., Restoration and Management of Lakes and Reservoirs" - 3rd Edition - G., 3rd ed. Taylor and Francis, New York (2005).

[10] Danesi E.D.G., Rangel-Yagui C.O., Sato S., de Carvalho J.C.M., Growth and Content of Spirulina Platensis Biomass Chlorophyll Cultivated at Different Values of Light Intensity and Temperature Using Different Nitrogen Sources, *Braz. J. Microbiol.*, **42**: 362–373 (2011).

[11] Jorquera O., Kiperstok A., Sales E.A., Embiruçu M., Ghirardi M.L., Comparative Energy Life-Cycle Analyses of Microalgal Biomass Production in Open Ponds and Photobioreactors, *Bioresour. Technol.*, 101(4): 1406–1413(2010).

[12] Benelhadj S., Gharsallaoui A., Degraeve P., Attia H., Ghorbel D., Effect of pH on the functional Properties of Arthrospira (Spirulina) Platensis Protein Isolate, *Food Chem.*, **194**: 1056–1063(2016).

[13] Ogbonda Kemka H., Aminigo R.E., Abu G.O., Influence of Temperature and pH on biomass Production and Protein Biosynthesis in a Putative Spirulina sp., *Bioresour. Technol.*, **98(11)**: 2207–2211 (2007).

[14] Sharma G., Kumar M., Ali M.I., Jasuja N.D., Effect of Carbon Content, Salinity and pH on Spirulina Platensis for Phycocyanin, Allophycocyanin and Phycoerythrin Accumulation, *J. Microb. Biochem. Technol.*, **6(4)**: 202–206 (2014).

[15] Dautania, G.K., Singh, G.P., Role Of Light And Dark Cycle and Different Temperatures in the Regulation of Growth And Protein Expression in Oscillatoria Agardhii Strain, *Brazilian Arch. Biol. Technol.*, **57(6)**: 933–940(2014).

[16] Thi N., Nhu H., Hiep N.H., The Effect of pH , Dark - Light Cycle and Light Colour on the Chlorophyll and Carotenoid Production of Spirulina sp, *KKU Res. J.*, **19**: 190–197 (2014).

[17] Benelhadj S., Gharsallaoui A., Degraeve P., Attia H., Ghorbel D., Effect of pH on the Functional Properties of Arthrospira (Spirulina) Platensis Protein Isolate, *Food Chem.*, **194**: 1056–1063(2016).

[18] Ronda S.R., Bokka C.S., Ketineni C., Rijal B., Allu P.R., Aeration effect on Spirulina Platensis Growth and γ-Linolenic Acid Production, *Brazilian J. Microbiol.*, **43(1)**: 12–20 (2012).

[19] Abd El-Monem A.M., Gharieb M.M., Hussian A.-E.M., Doman K.M., Effect of pH on phytochemical and Antibacterial Activities of Spirulina Platensis, *Int. J. Appl. Environ. Sci.*, **13(4)**: 339–351(2018).

[20] Benyamin A.E., "Light Energy Effect on Spirulina Platensis Growth Rate", Thesis in Civil Engineering, Texas Tech University (1993).

[21] Rafiqul I M, Jalal K.C.A., Alam M.Z., Enviromental Factor for Optimisation of Spirulina Biomass in Labatory Culture, *Biotechnol. J.*, **4(1)**: 19–22 (2005).

[22] Michael A., Serapio Kyewalyanga M., Lugomela C.V., Biomass and Nutritive Value of Spirulina (Arthrospira fusiformis) Cultivated in a Cost-Effective Medium, *Ann. Microbiol.*, **69**: 1387–1395(2019).

[23] Al-homaidan A., Alhussaini M.S., Arif I.A., Shehata A., Effect of Temperature , pH and Salinity on the Growth and Protein Content of Two Species of Spirulina Isolated from Saudi Arabia, *Saudi J. Biol. Sci.*, **12(1)**: 1–13(2005).

[24] Mirhosseini N., Davarnejad R., Hallajisani A., Tavakoli O., Cano-Europa E., Nitrogen Starvation Effect Versus its Excess on the Performance of Arthrospira Maxima in Zarrouk's Medium, *IJE Transactions A: Basics*, **34(7)**: 1557-1568(2021).

[25] Sarada R., Pillai M.G., Ravishankar G.A., Phycocyanin from Spirulina sp: Influence of Processing of Biomass on Phycocyanin Yield, Analysis of Efficacy of Extraction Methods and Stability Studies on Phycocyanin, *Process Biochem.*, **34(8)**: 795–801(1999).

[26] Bennett A., Bogobad L., Complementary Chromatic Adaptation in a Filamentous Blue-Green Alga, *J. Cell Biol.*, **58(2)**: 419-435(1973).

[27] Bradford M.M., A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding, *Anal. Biochem.*, **72(1-2)**: 248–254(1976).

[28] Stewart D.E., Farmer F.H., Extraction, Identification, and Quantitation of Phycobiliprotein Pigments from Phototrophic Plankton, *Limnol. Oceanogr.*, **29(2)**: 392–397 (1984).

[29] Sun H., Zhao W., Mao X., Li Y., Wu T., Chen F., High-Value Biomass from Microalgae Production Platforms: Strategies and Progress Based on Carbon Metabolism and Energy Conversion, *Biotechnol. Biofuels*, **11(227)**: 1-23 (2018).

[30] Albert N., Wague R., Mbaïlao M., Fabienne N., Djamena N., Changes in the Physico-Chemical Properties of Spirulina Platensis from Three Production Sites in Chad, *J. Anim. Plant Sci. (JAPS)*, **13(3)**: 1811–1822(2012).

[31] Wong Y.K., Ho K.C., Lai P.K., Leung C.C., Ho Y.M., Lee O.K., Yung K.L., Leung H.M., 2013. "A Study on Algal Growth Behavior under Different Sparging Period of CO_2 Supplementation", *Benef. Uses Algal Biomass Proc. 1st Int. Conf.* (2013).

[32] Pelizer H.L., Carvalho J., Sato S., Spirulina Platensis Growth Estimation by pH Determination at Different Cultivations Conditions, *Electron. J. Biotechnol.*, **5(3)**: 251-257 (2002).

[33] Mustafa Y., Fagiri A., Salleh A., El-nagerabi S.A F., Influence of Chemical and Environmental Factors on the Growth Performance of Spirulina Platensis Strain SZ100, *J. Algal Biomass Util.*, **4(2)**: 7–15(2013).

[34] Thirumala M., Optimization of Growth of Spirulina Platensis Ln1 for Production of Carotenoids, *Int. J. Life Sci. Biotechnol. Pharma Res.*, **1(2)**: 152–157 (2012).

[35] Pandey J.P., Tiwari A., Optimization of Biomass Production of Spirulina Maxima, *J. Algal. Biomass Utln.*, **1(2)**: 20-32(2010).

[36] Khajepour, F., Hosseini, S.A., Ghorbani Nasrabadi, R., Markou, G., Effect of Light Intensity and Photoperiod on Growth and Biochemical Composition of a Local Isolate of Nostoc Calcicola, *Appl. Biochem. Biotechnol.*, **176**, 2279-2289 (2015).

[37] Khoeyi Z.A., Seyfabadi J., Ramezanpour Z., Effect of Light Intensity And Photoperiod on Biomass and Fatty Acid Composition of the Microalgae, Chlorella Vulgaris, *Aquac. Int.*, **20**: 41-49 (2012).

[38] Seyfabadi J., Ramezanpour Z., Khoeyi Z.A., Protein, Fatty Acid, and Pigment Content of Chlorella Vulgaris Under Different Light Regimes, *J. Appl. Phycol.*, **23**: 721–726 (2011).

[39] Korbee N., Figueroa F.L., Aguilera J., Effect of Light Quality on the Accumulation of Photosynthetic Pigments, Proteins and Mycosporine-Like Amino Acids in the Red Alga Porphyra Leucosticta (Bangiales, Rhodophyta), J. *Photochem. Photobiol. B Biol.*, **80(2)**: 71–78 (2005).

[40] Vijaya V., Anand N., Blue Light Enhance the Pigment Synthesis in Cyanobacterium *Anabaena ambigua* Rao (NOSTACALES), *ARPN J. Agric. Biol. Sci*, **4(3)**: 36–43 (2009).

[41] Jungo E., Visser P.M., Stroom J., Mur L.R., Artificial Mixing to Reduce Growth of the Blue-Green Alga Microcystis in Lake Nieuwe Meer, Amsterdam: An Evaluation of 7 Years of Experience, *Water Sci. Technol, Water Supply*, **1(1)**: 17–23 (2001).

[42] Torzillo G., Giovannetti L., Bocci F., Materassi R., Effect of Oxygen Concentration on the Protein Content of Spirulina Biomass, *Biotechnol. Bioeng.*, **26(9)**: 1134–1135 (1984).

[43] Priyadarshani I., Thajuddin N., Rath B., Influence of Aeration and Light on Biomass Production and Protein Content of four Species of Marine Cyanobacteria, *Int. J. Curr. Microbiol. Appl. Sci.*, **3(12)**: 173–182(2014).

[44] Ismail M.M., Osman M.E.H., Seasonal Fluctuation of Photosynthetic Pigments of Most Common Red Seaweeds Species Collected from Abu Qir, Alexandria, Egypt, *Rev. Biol. Mar. Oceanogr*, **51(3)**: 515-525 (2016).