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Optimization of the Biomass Production of *Arthrospira (Spirulina)* Using Taguchi Method

Monchai Dejsungkranont[a], Natapas Phoopat[b] and Sarote Sirisansaneeyakul[a,c,*]

[a]*Department of Biotechnology, Faculty of Agro–Industry, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand*

[b]*Institute of Food Research and Product Development, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand*

[c]*Center for Advanced Studies in Tropical Natural Resources, National Research University–Kasetsart University, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand (CASTNAR, NRU–KU, Thailand)*

Abstract: *Arthrospira* (*Spirulina*) is cyanobacteria that used as protein–rich health food for a long time. The potential health benefits of *Arthrospira* associated with antioxidant, immunomodulation, anti–virus and anti–cancer effect, which are mainly due to three bioactive constituents such as phycocyanin (a biliprotein pigment), the sulfated polysaccharide spirulan and polyunsaturated fatty acid (gamma–linolenic acid: γ–GLA). The objective of this study is to optimize the environmental growth factors for maximizing the biomass concentration and productivity of *Arthrospira* under the photoautotrophic cultivation in a microalgal culture tube. An optimization of the algal biomass production involved experiments that were statistically designed using the Taguchi method. Six factors varied at either three or two levels, which were as follows; three light intensities (klux), three initial culture pHs, two strains of the cyanobacteria, three concentrations of Zarrouk's medium (%), three rates of aeration mixed with 1–2% v/v carbon dioxide (vvm) and two temperatures (°C). The optimal conditions obtained from this study help maximizing the biomass cultivation of *Arthrospira* with using the Zarrouk's medium.

Keywords: *Arthrospira* (*Spirulina*), Biomass production, Photoautotrophic culture, Taguchi method, Environmental growth factors, Zarrouk's medium.

INTRODUCTION

Arthrospira sp. or *Spirulina* sp. is a blue–green filamentous cyanobacterium that is widely used in many countries as a dietary supplement, animal feed and pharmaceuticals due to its protein content and biochemical substances for immune system [1]. It is not only rich in proteins (50–70% of dry weight), but also has the vitamins, minerals, lipids, polysaccharides and pigments. It is reported that, *A. platensis* and its extracts are shown biological properties, such as prevent cancers, decrease blood cholesterol levels, stimulate the immunological system, reduce the nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effect of radiation [2, 3]. These properties have been attributed to different compounds such as phenolics, phycobiliproteins, carotenoids, organic acids, sulphated polysaccharide spirulan and polyunsaturated fatty acids [4-7]. For these reasons, *Arthrospira* sp. is widely used in commercial cultivation. Current production worldwide is estimated to be about 3,000 metric tons [8].

In the past, although the development process is the most widely cultured *Arthrispira* sp. But it seems, the researchers all over the world continue to focus on process developing the cultivated *Arthrospira* sp. This is to ensure higher productivity and to support food security and the health of the world's population. Nowadays, the mass production of *Arthrospira* sp. has been cultivated in large outdoor ponds under photoautotrophic growth which requires light and carbon dioxide for growth [9]. The commercial culture of *Arthrospira* sp. and others microalgae are widely used freely available sunlight and inexpensive carbon dioxide in either raceway type of open culture systems or in closed photobioreactors [10, 11]. The large–scale biomass and metabolites production by *Arthrospira* sp. depend on the various factors such as temperature [8,12-16], the light intensity [16-19], the initial culture pH [13, 16, 20], the aeration rate [19], the concentration of carbon dioxide [18-19], the carbon sources [21], the nitrogen sources [14, 17, 22], salts [19], ammonia [23] and the amount of inoculum. Furthermore, the light intensity and temperature show the most effect on the growth and metabolites production of *Arthrospira* sp. [17, 24-26]. Because these factors are directly correlated with the gross rate of photosynthesis and respiration. The earliest study of light intensity was done on *A. maxima* cultivation by Zarrouk [27]. It was found that the growth of *A. maxima* was saturated at light intensity of 25–30 klux (300–360 µmol photons m^{-2} s^{-1}). In 1997, Vonshak was reported that the growth of *A. platensis* was saturated at values of photosynthetic photon flux density (PPFD) exceeding to 150–200 µmol photons m^{-2} s^{-1} [28]. This value is highly dependent on growth conditions and correlates with

Address correspondence to this author at the Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand; Tel: +66 (0) 2562-5086; Fax +66 (0) 2579-4096:
E-mail: sarote.s@ku.ac.th

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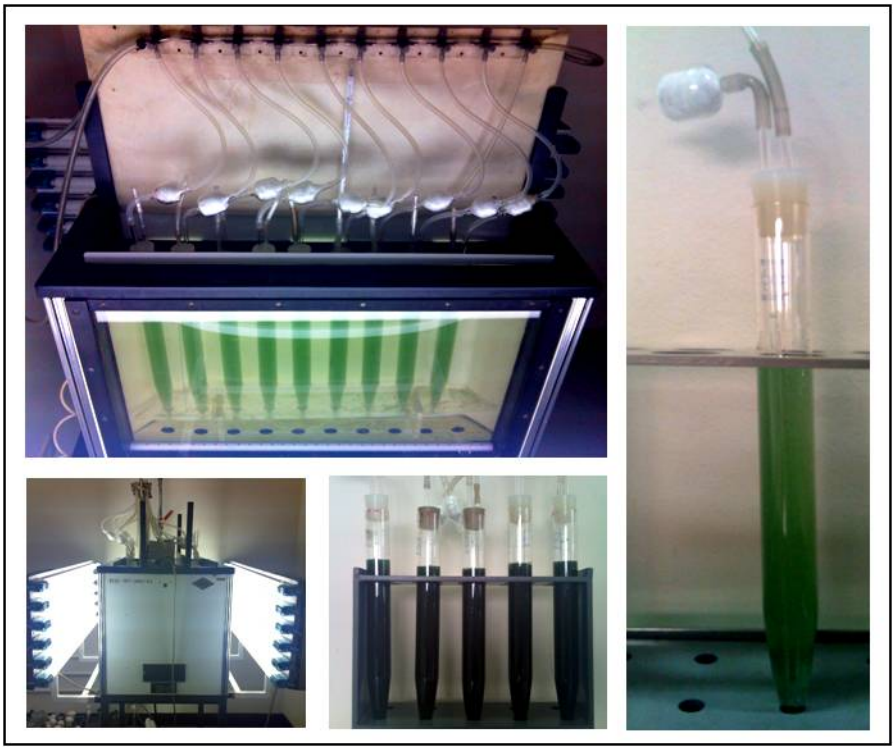


Fig. (1). A cyanobacterial culture chamber and a 200–mL culture tube**.**

the chlorophyll to biomass concentration. Temperature also was the major factor that controls the rate of photosynthesis and the growth of cyanobacteria. It shows the effect on the rate of cellular reactions, the structure of cell component, the nature of metabolism, the nutrition requirement and the composition of biomass [28]. The optimal temperature for cultivation of *Arthrospira* sp. is in the range of 30–38 °C depending on the cyanobacteria strains and cultivation condition.

A large number of research works on the optimal growth of *Arthrospira* sp. showed one or two factors at a time that have affected on the biomass production such as pH and temperature [13] or pH and phosphate regime [29] or temperature and nitrogen [14] or light and temperature [25, 30]. Having studied the available published research, we found that none of the published studies have investigated the effect of all main environment factors on the growth of *Arthrospira* sp. A few works have been studied on the optimization of three to four factors involved in the growth of *Arthrospira* sp. such as temperature, light intensity, pH and agitation [16, 31], and temperature, light intensity, incubation period and inoculum concentration [32]. Therefore, it is important to study the influence of all the factors that affect the growth of *Arthrospira* sp. at the same time. In particular, Thailand is still a quite lack of knowledge and technology that could develop the cultivation of *Arthrospira* sp.

To optimize process factors, an appropriate design of experiment was required, we were chosen Taguchi method which was the key tool for reducing the number of experiments [33-35]. It can help to optimize manufacturing processes involving multiple factors with different numbers of levels, especially fermentation processes and new food product developments, simultaneously and economically [36]. Hence the objective of the present work was to optimize the biomass production by phototrophic cultivation of *Arthrospira* sp. in culture tube with statistical design by Taguchi method, six factors, or variables, were optimized at three or two levels need only 16 experimental trials. This research is the first in the world for studying on the effects of main environmental factors critically to the growth of *Arthrospira* sp. with multiple factors at the same time. A set of optimal conditions can be established after a calculation of signal–to–noise (*S/N*) ratio and analysis of variance to produce a maximum biomass of *Arthrospira* sp. with using the Zarrouk's medium. Significant factors are the main factors and need to be tuned as the cultivation process in the laboratory, which is scaled up in the future.

MATERIALS AND METHODS

Preculture of Cyanobacteria

The *Arthrospira maxima* IFRPD 1183 and *A. platensis* IFRPD 1208 obtained from the Institute of Food Research and Product Development (IFRPD; Kasetsart University, Bangkok, Thailand) were grown in 150 mL of Zarrouk's medium [27] contained in a 200–mL culture tube (32.21 mm internal diameter, 3.89 mm wall thickness) which made of optically clear glass (Fig. **1**). An initial concentration of cyanobacteria culture was measured at 560 nm for the final absorbance of 0.2. The medium was adjusted pH to 9.0 and the culture tubes were incubated at 30°C in a chamber equipped (Fig. **1**) with six 36–W cool daylight fluorescent lamps (Philips® Co., Bangkok, Thailand) for 3 days. During the photoperiod, the light intensity at the surface of tubes was 15 klux. A diurnal cycle of a 16–h photoperiod and 8–h dark was used. The culture tubes were continuously sparged with air mixed with carbon dioxide (1–2% v/v) at a flow rate of 0.6 vvm.

Table 1. Experimental Factors and their Levels for Optimizing *Arthrospira* sp. Biomass Production

Factors	Levels		
	1	2	3
A: light intensitie (klux)	15	10	5
B: initial culture pH	9.0	9.5	10
C: strain of the cyanobacteria (IFRPD)	1183	1208	–
D: concentration of Zarrouk's medium (%)	50	75	100
E: flow rates of aeration mixed with 1–2% v/v CO_2 (vvm)	0.6	0.2	1.0
F: temperature (°C)	30	35	–

Biomass Production

Biomass production was conducted as above specified in 200–mL culture tubes (Fig. **1**). The tube containing 150 mL of the Zarrouk's medium was inoculated with the cyanobacteria preculture to obtain an initial optical density (OD) of 0.2 by measured at 560 nm with spectrophotometer (Genesys 20; Sigma–Aldrich, St. Louis, MO, USA). The distilled water was used as blank. The light intensity, the initial culture pH, the cyanobacteria strain, the concentration of Zarrouk's medium, the aeration rate, and the temperature depended on the experiments. Triplicate samples were collected every 48 h for the measurement of optical density, biomass concentration, the pH of the culture medium and the residual nitrogen in the culture medium.

Analyses

Biomass

The samples were measured by the OD at 560 nm and transformed to concentration with correspond to the standard calibration curve of *Arthrospira* sp. Biomass concentration (g L^{-1}) of *A. maxima* IFRPD 1183 and *A. platensis* IFRPD 1208 could be correlated with the OD at 560 nm by a linear equation: biomass concentration = 0.887OD (r^2 = 0.999) and biomass concentration = 0.826OD (r^2 = 0.994), respectively.

pH

The pH of the culture medium was measured using the pH meter (Metrohm 827 pH Lab meter, UK).

Nitrogen

Residual nitrogen in the culture medium was determined by brucine colorimetric method [37]. The results were converted to the concentration of nitrogen.

Parameters Calculation

The specific rates and volumetric rates of the cyanobacteria growth and nitrogen consumption were calculated after the calculation method described by Sirisansaneeyakul *et al*. [38, 39].

Experimental Design

Optimization of biomass production involved an experiment was statistically designed using the Taguchi method [40]. Six factors, or variables, were optimized at three or two levels. These factors include the light intensity, the initial culture pH, the strain of the cyanobacteria, the concentration of Zarrouk's medium (%), the rate of aeration with a gas mixture that contained air mixed with carbon dioxide (1–2% v/v) and temperature. The values, or levels, used for the six factors were shown in Table **1**. Qualitex 4 software (Nutek Inc., Bloomfield Hills, MI, USA) was used to identify the trial experimental profiles shown in Table **2**.

The objective in this study was to find the optimum cultural conditions for biomass production and productivity, the experimental data (y_i) were converted to a signal–to–noise (*S/N*) ratio [40], so the higher–the–better function was used to calculate *S/N* ratio using the following Eq. 1:

$$S/N = -\log_{10}\left(\frac{\sum\left(\frac{1}{y_i^2}\right)}{n}\right) \quad (1)$$

where y_i is the *i*th quality parameter and *n* is the number of trials [40].

The expected values of observations (Y_{opt}), i.e., the biomass concentration and the productivity of biomass, were calculated using the following Eq. 2 [41]:

$$Y_{opt} = \overline{T} + \sum(\overline{F}_i - \overline{T}) \quad (2)$$

where $\overline{T}$ and $\overline{F}_i$ are the grand averages of the *S/N* ratios and the factor averages at each factor level, respectively. The main effect was the difference between the maximum and minimum values of the factor averages at each factor level, while the percent main effect of each factor was calculated as the percentage of its main effect divided by the sum of the main effects of all the factors. Taguchi's statistical optimization has been discussed previously [42].

RESULTS AND DISCUSSION

Influence of Factors on Biomass Production (Main Effects)

The cyanobacteria were grown in Zarrouk's medium under various conditions for a period of 14 days. The results were plotted as graph and shown in the Fig. (**2**). The initial inoculum concentration was approximately adjusted to 0.2 by measuring OD at 560 nm using spectrophotometer (Genesys 20; Sigma–Aldrich, St. Louis, MO, USA). The

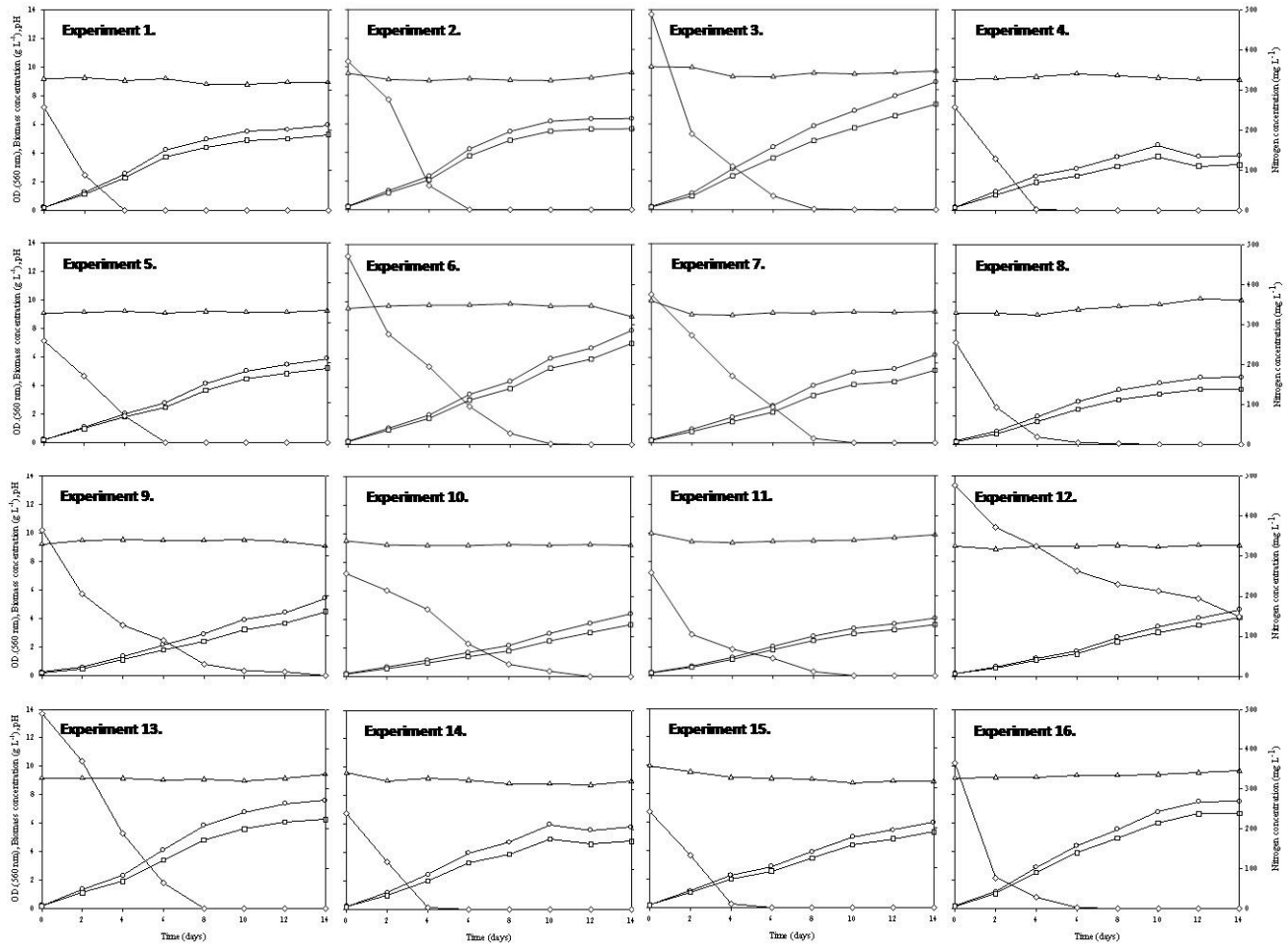


Fig. (2). Time courses of OD (○), biomass concentration (□), pH (Δ) and nitrogen concentration (◊) under difference conditions in a culture tube by photoautotrophic cultivation.

Table 2. The Factor Levels in the Experimental Design for Optimizing *Arthrospira* sp. Biomass Production

Experiment no.	Factors[a]					
	A	**B**	**C**	**D**	**E**	**F**
1	1	1	1	1	1	1
2	1	2	1	2	2	2
3	1	3	2	3	3	1
4	1	1	2	1	1	2
5	2	1	1	1	3	2
6	2	2	1	3	1	1
7	2	3	2	2	1	2
8	2	1	2	1	2	1
9	3	1	2	2	1	1
10	3	2	2	1	3	2
11	3	3	1	1	2	1
12	3	1	1	3	1	2
13	1	1	2	3	2	2
14	1	2	2	1	1	1
15	1	3	1	1	1	2
16	1	1	1	2	3	1

[a] See Table 1 for an explanation of the factors A–F

Table 3. The Specific Growth Rate, Doubling Times, Biomass Concentration and Biomass Productivity of *Arthrospira* sp. under Photoautotrophic Tube Culture by Using Taguchi Method

Exp. no.	μ (d^{-1})	t_d (d)	C_X (g-DCW L^{-1})							Q_X (g-DCW L^{-1} d^{-1})						
			1[a]	2[b]	3[c]	Ave.	SD	MSD	*S/N* Ratio[d] (dB)	1[a]	2[b]	3[c]	Ave.	SD	MSD	*S/N* Ratio[d] (dB)
1	0.50	1.40	5.09	5.48	5.29	5.29	0.19	0.04	14.45	0.35	0.38	0.37	0.37	0.01	7.52	-8.76
2	0.46	1.50	5.68	5.74	5.54	5.65	0.11	0.03	15.04	0.39	0.39	0.38	0.39	0.01	6.63	-8.21
3	0.39	1.76	7.37	7.32	7.47	7.39	0.08	0.02	17.37	0.51	0.51	0.52	0.51	0.01	3.78	-5.77
4	0.27	2.59	3.65	3.61	4.00	3.75	0.21	0.07	11.46	0.35	0.34	0.38	0.36	0.02	7.87	-8.96
5	0.35	2.01	5.31	5.10	5.27	5.23	0.11	0.04	14.36	0.37	0.35	0.36	0.36	0.01	7.73	-8.88
6	0.22	3.14	7.02	7.09	7.13	7.08	0.06	0.02	17.00	0.49	0.49	0.49	0.49	0.00	4.15	-6.18
7	0.29	2.36	5.10	5.06	5.12	5.09	0.03	0.04	14.14	0.35	0.35	0.35	0.35	0.00	8.10	-9.08
8	0.34	2.06	3.89	3.89	3.86	3.88	0.02	0.07	11.77	0.26	0.26	0.26	0.26	0.00	14.47	-11.61
9	0.21	3.26	4.42	4.47	4.55	4.48	0.06	0.05	13.03	0.30	0.31	0.31	0.31	0.00	10.67	-10.28
10	0.20	3.54	4.02	3.35	3.53	3.63	0.35	0.08	11.13	0.28	0.23	0.24	0.25	0.03	16.71	-12.23
11	0.26	2.66	3.59	3.52	3.63	3.58	0.05	0.08	11.07	0.24	0.24	0.25	0.24	0.00	17.17	-12.35
12	0.20	3.40	4.05	4.17	4.15	4.13	0.06	0.06	12.31	0.28	0.29	0.28	0.28	0.00	12.62	-11.01
13	0.38	1.85	6.16	6.29	6.36	6.27	0.10	0.03	15.94	0.43	0.44	0.44	0.43	0.01	5.32	-7.26
14	0.30	2.33	4.81	4.90	5.10	4.94	0.15	0.04	13.87	0.46	0.47	0.49	0.48	0.01	4.45	-6.48
15	0.28	2.52	5.02	5.15	5.81	5.33	0.43	0.04	14.48	0.34	0.35	0.40	0.37	0.03	7.55	-8.78
16	0.32	2.15	6.33	6.78	6.90	6.67	0.30	0.02	16.46	0.44	0.47	0.48	0.46	0.02	4.68	-6.70

[a, b c] Triplicate values, [d] Calculated using Eq. 1

results showed that the profiles of growth were mostly difference depended on the cultured conditions. The maximum values of biomass concentrations of experiments in terms of dry weight were observed between 10 and 14 days of cultivation. The growth kinetics showed different phases: a very short phase of latency indicating that the cyanobacteria were already acclimated to the culture medium [19]; an exponential phase (4–10 days) and then a stationary phase (2–4 days). The maximal specific growth rate (μ), doubling times (t_d), the biomass concentration (C_X) and the volumetric productivity (Q_X) of the various experiments at the specified conditions (Tables **1** and **2**) are shown in Table **3**.

The results obtained from experiments 3, 6, 13 and 16 showed the highest biomass concentrations of 7.39, 7.08, 6.27 and 6.67 g-DWC L^{-1}, respectively. Among these experiments, the highest productivity of biomass was found in range of 0.44–0.52 g-DWC L^{-1} d^{-1} (Table **3**). These values were obtained under the following conditions: 10–15 klux light intensity and 75–100% Zarrouk's medium, and irrespective of the initial culture pH (9.0, 9.5, 10.0), cyanobacteria strains (*A. maxima* IFRPD 1183, *A. platensis* IFRPD 1208), the flow rates of aeration mixed with 1–2% v/v CO_2 (0.2, 0.6, 1.0 vvm) and temperatures (30, 35 °C). From the results can conclude that the light intensity and the concentration of nutrients and elements (the concentration of Zarrouk's medium) likely are major factors affecting the growth of cyanobacteria. The cultures under 5 klux light intensity (experiments 9, 10, 11 and 12) showed the cultures grown slowly (Fig. **2**) and gave the lowest biomass concentrations of 4.48, 3.63, 3.58 and 4.13 g-DWC L^{-1}, respectively. Moreover, these also illustrated the lowest biomass productivities (0.24–0.31 g-DCW L^{-1} d^{-1}) (see Table **3**). These results correspond with the previous studies which demonstrated that a greater biomass is induced by stronger light intensity [43, 44]. However, if *Arthrospira* sp. got overly intensive light may be harmful from light inhibition and lowering some compositions (phycocyanin, protein and lipid), but increased carbohydrate or glycogen content [45, 46]. While the low light intensity found to support the increased accumulation of phycocyanin [47].

The experiments 1, 4, 5, 8, 10, 11, 14 and 15 were cultured under the 50% Zarrouk's medium, the concentration of nitrogen decreased rapidly and used up within 4–8 days of culture time, depending on the culture conditions (Fig. **2**). These experiments were found to be given the rather low concentration of biomass. The experiments 4, 8, 10, and 11 were showed the lower biomass production approximately 2–fold of the 3, 6, 13 and 16 experimental runs (Table **3**). From the results indicated that the overly dilution growth medium may cause the nutrients and minerals not enough on the growth, as a result, the *Arthrospira* sp. stopped growing. Therefore, the further study should determine the appropriate amount of each nutrients and minerals for growth. However, the nutrients and minerals not only influence the biomass production, but also affect the composition of the cell. In the presence of nitrogen limitation, *Arthrospira* sp. had high lipid accumulation (approximately 17.0%), especially under without sodium nitrate ($NaNO_3$) as well as other microalgae,

Table 4. Analysis of the Factors Affecting the *Arthrospira* sp. Biomass Production

Level	Factors[a]					
	A	**B**	**C**	**D**	**E**	**F**
Biomass concentration (C_X, g-DCW L^{-1})						
1	14.883	13.722	14.396	12.823	13.840	14.377
2	14.317	14.259	13.587	14.668	13.456	13.606
3	11.883	14.262	–	15.652	14.829	–
% Main effect	32.182	5.793	8.678	30.348	14.729	8.271
Biomass productivity (Q_X, g-DCW L^{-1} d^{-1})						
1	-7.618	-9.187	-8.861	-9.758	-8.695	-8.520
2	-8.937	-8.276	-8.962	-8.57	-9.859	-9.303
3	-11.472	-8.996	–	-7.559	-8.398	–
% Main effect	41.401	9.786	1.085	23.622	15.694	8.411

[a] See Table 1 for an explanation of the factors A–F

but protein is greatly reduced [22]. While the shortage of phosphorus (10 mg L^{-1} K_2HPO_4) caused a markedly decreased of biomass by *A. platensis*, but a explicitly increased of the carbohydrate content (59.64%) [48]. The case is applicably very useful for biofuel production by using the cyanobacterial biomass as substrate. Anyway, the effect of phosphorus limitation on the carbohydrate content was independent of the light intensity. The temperature is another important factor affecting biomass production and composition by cyanobacteria [13, 49]. It was reported that the cultures at 35°C were shown negative effect on biomass production but a positive effect on the production of protein, lipids and phenolics. While the cultivation under 30°C showed higher biomass production and productivity than those cultured at 35°C, but nitrogen concentrations have no effect on the protein, lipid or phenolics contents [14]. These experimental results are discussed, consistent well with this study.

The comparison of the specific growth rate (μ) and doubling times (t_d) of *Arthrospira* sp. under photoautotrophic culture in a culture tube was shown in Table **3**. The specific growth rate (μ) in the exponential phase was obtained by exponential regression and the biomass doubling time (t_d) calculated using $t_d = (\ln 2)/\mu$. The experiments 1, 2, 3, 5, 8, 13 and 16 showed the higher specific growth rates of 0.50, 0.46, 0.39, 0.35, 0.34, 0.38 and 0.32 d^{-1}, respectively. These values were obtained under cultured at the light intensity of 10–15 klux and irrespective of the initial culture pH (9.0, 9.5, 10.0), the cyanobacteria strains (*A. maxima* IFRPD 1183, *A. platensis* IFRPD 1208), the concentrations of Zarrouk's medium (50, 75 and 100%), the flow rates of aeration mixed with 1–2% v/v CO_2 (0.2, 0.6, 1.0 vvm) and temperatures (30, 35 °C). Meanwhile, the experiments 9, 10, 11 and 12 which were cultured under the light intensity of 5 klux showed the lowest specific growth rates of 0.21, 0.20, 0.26 and 0.20 d^{-1}(Table **3**), respectively.

The experimental data (Table **3**) were processed using the Qualitek–4 software with the higher–the–better attribute selected for establishing the optimum condition for the biomass production and the biomass productivities and identifying the individual factors that influenced these parameters. The percentages of main effect of each factor on the biomass production were calculated based on the statistical analysis with Qualitex 4 as shown in Table **4**. The light intensity (factor A) and the concentration of Zarrouk's medium (factor D) were two main effects on the production of biomass (32.182 and 30.348%, respectively) and the productivities of biomass (41.401 and 23.622%, respectively) (Table **4**). In addition, the flow rates of aeration mixed with 1–2% v/v CO_2 (factor E) and temperature (factor F) had the main effects of approximately 14–16% and 8.3–8.4%, respectively. The initial culture pH (factor B) had a slightly effect on the biomass production and the biomass productivities (5.793 and 9.786 %, respectively) due to the well growth of *Arthrospira* sp. in the range of pH 9–10. However, the earlier results also demonstrated that optimum pH for maximizing growth of *A. maxima* was 9–9.5 [20], which considered to be an alkalophilic organism [50]. In addition, some reports revealed that the cultures of *Arthrospira* sp. showed a wide range of optimum pH of 8–10 [51, 52]. While the cultures with pH 11 showed aggregated cells at the bottom of containers. Anyway, the cells will rapidly deteriorate when pH is changed abruptly, and this may happen in growth systems which are not well buffered [52]. Finally, the cyanobacteria strain (factor C) showed slightly affected on the productivity of biomass (main effect <2%).

Considering the percentage of main effect, the averages factor at each factor level were obtained by adding the *S/N* ratios (C_X or Q_X) into all the conditions at the level considered and then dividing by the number of data points added. The main effect was the difference between the maximum and the minimum values of the averages factor at each factor level (main effect = max−min), while the percent main effect of each factor was calculated as the percentage of its main effect divided by the sum of the main effects of all the factors; thus, percent main effect=(main effect×100)/Σ all main effects) [40].

ANOVA

Understanding of the impact of each individual factor is the key for a successful biomass production. Analysis of variance (ANOVA) was used to analyze the results of the

Table 5. Analysis of Variance (ANOVA)[a] of Factors Affecting the Production of *Arthrospira* sp. Biomass

Factors[b]	DOF	SS	Variance	*F*–Ratio	Percent *P* (%)	Confidence	Significance
Biomass concentration (C_X, g-DCW L^{-1})							
A	2	24.568	12.284	11.717	35.175	98.703	**
B	2	1.160	0.580	0.553	0.000	39.322	–
C	1	2.614	2.614	2.493	2.451	82.481	–
D	2	23.787	11.893	11.345	33.952	98.614	**
E	2	4.134	2.067	1.972	3.190	76.633	–
F	1	2.377	2.377	2.268	2.081	80.758	–
Error	5	5.241	1.048		23.151		
Total	15	63.885			100.000		
$Y_{opt,C_X} = \overline{T} + (\overline{A}_1 - \overline{T}) + (\overline{B}_3 - \overline{T}) + (\overline{C}_1 - \overline{T}) + (\overline{D}_3 - \overline{T}) + (\overline{E}_3 - \overline{T}) + (\overline{F}_1 - \overline{T})$							
Biomass productivity (Q_X, g-DCW L^{-1} d^{-1})							
A	2	39.619	19.809	20.030	55.476	99.590	***
B	2	2.247	1.123	1.136	0.397	60.800	–
C	1	0.040	0.040	0.040	0.000	15.064	–
D	2	13.525	6.762	6.838	17.019	96.291	**
E	2	5.019	2.509	2.537	4.482	82.645	–
F	1	2.453	2.453	2.481	2.159	82.395	–
Error	5	4.944	0.988		20.467		
Total	15	67.850			100.000		
$Y_{opt,Q_X} = \overline{T} + (\overline{A}_1 - \overline{T}) + (\overline{B}_2 - \overline{T}) + (\overline{C}_1 - \overline{T}) + (\overline{D}_3 - \overline{T}) + (\overline{E}_3 - \overline{T}) + (\overline{F}_1 - \overline{T})$							

***Significant at p<0.01, **significant at p<0.05, – not significant

[a] ANOVA was performed for both the average values (Avg.) and the *S/N* ratio (Table **3**)

[b] See Table 1 for an explanation of the factors A–F

experiment and to determine how much variation was contributed by each factor. The resulting of various factors from ANOVA affected on the biomass concentrations (C_X) and the biomass productivities (Q_X) were shown in Table **5**. The light intensity (factor A) and the concentration of Zarrouk's medium (factor D) showed statistically significant effects on the concentration and the productivity of biomass (p<0.05). Statistical analysis of the biomass production data revealed that among all selected the light intensity contributed the maximum impact on the biomass concentration and the biomass productivity (35.175% and 55.476%, respectively) followed by the concentration of Zarrouk's medium (33.952% and 17.019%, respectively). The initial culture pH and temperature seemly showed the least impact (0.000-2.159%) at the individual level on overall production of biomass under the selected culture conditions. This will be an advantage to culture *Arthrospira* sp. in Thailand, because the temperature is usually in the range of 30–35 °C. The presented data revealed that overall 60–70% contribution was noticed with only two selected parameters (the light intensity and the concentration of Zarrouk's medium) and the rest of 40–30% affected by other selected factors (Table **5**).

Factor Interactions and their Influence on Biomass Production

To have better insight of the biomass production, software–generated interaction effects were analyzed individually because any individual factor may interact with any or all of the other factors, creating the possibility of the presence of a large number of interactions [53]. Estimated interaction severity index (SI) of the factors on the biomass concentrations (C_X) and the biomass productivities (Q_X) under study helps to determine the influence of two individual factors at various levels of the interactions.

From Table **6**, it can be shown that the flow rates of aeration mixed with 1–2% v/v CO_2 and temperature (at levels 3 and 1; column 13) interaction had the highest interaction SI (50.61%) for the biomass concentration followed by the light intensity and the flow rates of aeration mixed with 1–2% v/v CO_2 (at levels 1 and 3; column 6) with 48.97% SI. Considering biomass productivity (data not shown), the initial culture pH and temperature (at levels 2 and 1; column 14) showed the highest interaction SI (54.01%). It was interesting to note that, the above interactions, three of the factors having least impact factor (initial culture pH (0.00–0.397%), flow rates of aeration mixed with 1–2% v/v CO_2 (3.190–6.359%)) and temperature (0.584–2.159%) was associated with stronger interaction factor.

Among all selected parameters, temperature showed the highest (50.61% and 54.01%) severity index with the flow rates of aeration mixed with 1–2% v/v CO_2 and the initial culture pH for the biomass concentration and the biomass productivity, respectively and also revealed varied interaction SI values with other components such as the

Table 6. Interaction Influence of Selected Factors on Biomass Concentration

Serial no.	Interacting Factor	Columns	SI%	Col	Opt
1	Aeration × Temp	7 × 10	50.61	13	3,1
2	Light × Aeration	1 × 7	48.97	6	1,3
3	Strain × Aeration	5 × 7	47.43	2	1,3
4	pH × Temp	4 × 10	41.2	14	2,1
5	Light × pH	1 × 4	34.23	5	2,2
6	pH × Strain	4 × 5	30.93	1	2,1
7	Zarrouk's medium × Aeration	6 × 7	30.3	1	3,3
8	Light × Strain	1 × 5	28.48	4	2,1
9	Light × Temp	1 × 10	15.28	11	1,1
10	pH × Aeration	4 × 7	11.38	3	3,3
11	Light × Zarrouk's medium	1 × 6	9.46	7	2,3
12	pH × Zarrouk's medium	4 × 6	8.33	2	3,3
13	Strain × Zarrouk's medium	5 × 6	6.95	3	2,3
14	Strain × Temp	5 × 10	4.41	15	1,1
15	Zarrouk's medium × Temp	6 × 10	2.52	12	3,1

initial culture pH (41.2%), the light intensity (15.28%), the strain of the cyanobacteria (4.41%) and the concentration of Zarrouk's medium (2.52%) for the biomass concentration. And these revealed varied interaction SI values with the initial culture pH (54.01%), the flow rates of aeration mixed with 1–2% v/v CO_2 (50.31%), the light intensity (13.74%), the strain of the cyanobacteria (6.92%) and the concentration of Zarrouk's medium (3.11%). However, the temperature also showed the lowest (2.52%) severity index with the concentration of Zarrouk's medium for the biomass concentration. Besides, the concentration of Zarrouk's medium also showed the lowest (0.88%) severity index with the initial culture pH for biomass productivity. From the above results can conclude that the influences of individual factors on biomass production have varied, while in combination; the biomass production was quite independent of the individual influence.

Optimum Conditions of Biomass Production

The equations given in Table **5** were used to estimate the expected values (Y_{opt}) of the biomass concentration (C_X, g-DCW L^{-1}) and the biomass productivity (Q_X, g-DCW L^{-1} d^{-1}) under various conditions. These have two different sets of conditions which were revealed in relation to culture the *Arthrospira* sp. The signal–to–noise (*S/N*) ratio was used in Taguchi method as the principal criterion for identifying the optimal conditions [40]. High values of the *S/N* ratio are taken to indicate optimality (Fig. **3**). Fig. (**3**) suggests that the conditions for attaining the high biomass concentration and the biomass productivities any of *Arthrospira* sp. are similar. As a result, with the optimal conditions (light intensity of 15 klux, A1; an initial pH of 10.0, B3; *A. maxima* IFRPD1183, C1; 100% Zarrouk's medium, D3; flow rates of aeration mixed with 1–2% v/v CO_2 at 1 vvm, E3; 30 °C, F1), gave the maximum biomass concentration comparing to the expected values in Table **7**. Under similar conditions, the initial culture pH of 9.5 maximized the productivity of biomass. The results were comparable to previous reported which the culture conditions of *A. platensis* at low temperature at 28–30°C and high light intensity at 180–192 μmol photons m^{-2} s^{-1} have promoted the biomass production [26, 44].

The calculation based on the Taguchi approach, with using the optimal conditions, the biomass concentration and the biomass productivity were 8.358 g-DCW L^{-1} and 0.584 g-DCW L^{-1} d^{-1}, respectively (Table **7**). Under the optimal conditions identified by the Taguchi method for maximizing the biomass production of *Arthrospira* sp. in terms of the concentration and productivity of biomass, the predicted value of the response parameter, (the biomass concentration), Y_{opt} could be calculated using Eq. 2:

$$Y_{opt,C_X} = \overline{T} + (\overline{A}_1 - \overline{T}) + (\overline{B}_3 - \overline{T}) + (\overline{C}_1 - \overline{T}) + (\overline{D}_3 - \overline{T}) + (\overline{E}_3 - \overline{T}) + (\overline{F}_1 - \overline{T})$$

$$Y_{opt,C_X} = 13.992 + (14.68 - 13.992) + (14.26 - 13.992) + (14.40 - 13.992) + (15.65 - 13.992) + (14.83 - 13.992) + (14.38 - 13.992)$$

$$Y_{opt,C_X} = 18.442dB$$

Estimate of expected results from Y_{opt}

$$Y_{opt,C_X} = -10\log(MSD) = 18.442 \quad (3)$$

$$MSD = 10^{(-18.442/10)} = 0.014315$$

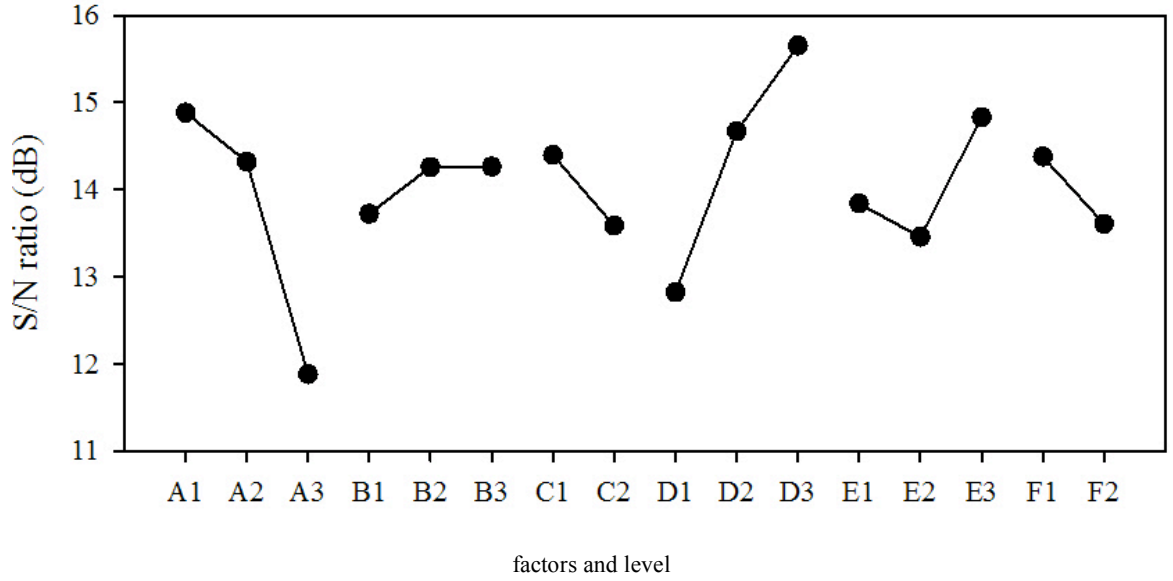
Eq. 3 produce Eq. 4:

$$MSD = \left[(1/Y_1)^2 + (1/Y_2)^2 + ... + (1/Y_n)^2 \right] / N \quad (4)$$

$$MSD = 1/(Y_{opt,C_X})^2$$

$$Y_{opt,C_X} = \sqrt{(1/MSD)}$$

(**A**)



(**B**)

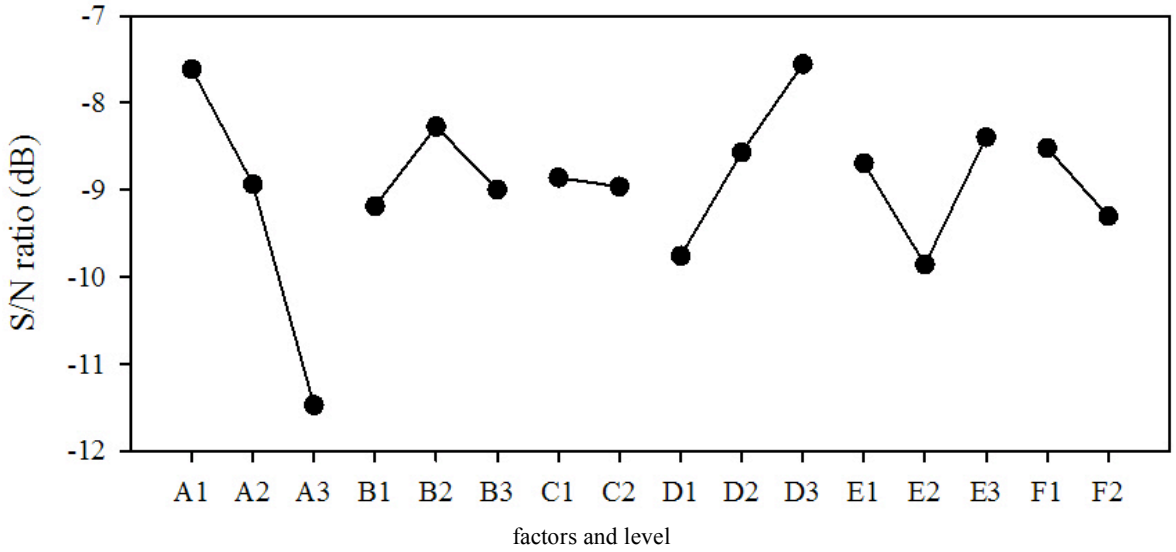


Fig. (3). Signal–to–noise (*S/N*) ratios for the various factors (A–F, Table **1**) and levels of (**A**) maximum biomass concentration and (**B**) biomass productivity. The level of each factor is shown on the X–axes as a numerical value after the factor. The level of each factor is shown on the X–axes as a numerical value after the factor. The levels were 1–3 for the factors A, B, D, E and 1–2 for the factor C and F (Table **1**). Optimal conditions are indicated by the peak values of the *S/N* ratio.

Table 7. Summary of Biomass Production from *A. Maxima* IFRD 1183

Parameter	Expected Results	Experimental Results
C_X (g-DCW L^{-1})	8.358	7.676
Q_X (g-DCW L^{-1} d^{-1})	0.584	0.535

$$Y_{opt,C_X} = \sqrt{(1/0.014315)}$$

$$Y_{opt,C_X} = 8.358 g - DCW \;\; L^{-1}$$

Confirmation testing is a necessary requirement of the Taguchi method [40, 54]. A single confirmation test was conducted for biomass production using the above identified optimum settings of the process parameters. The confirmation test results for the set of optimization condition (Table **7**) revealed a comparable predicted value with confirmation of the biomass concentration and the biomass productivity. The confirmation under these optimal

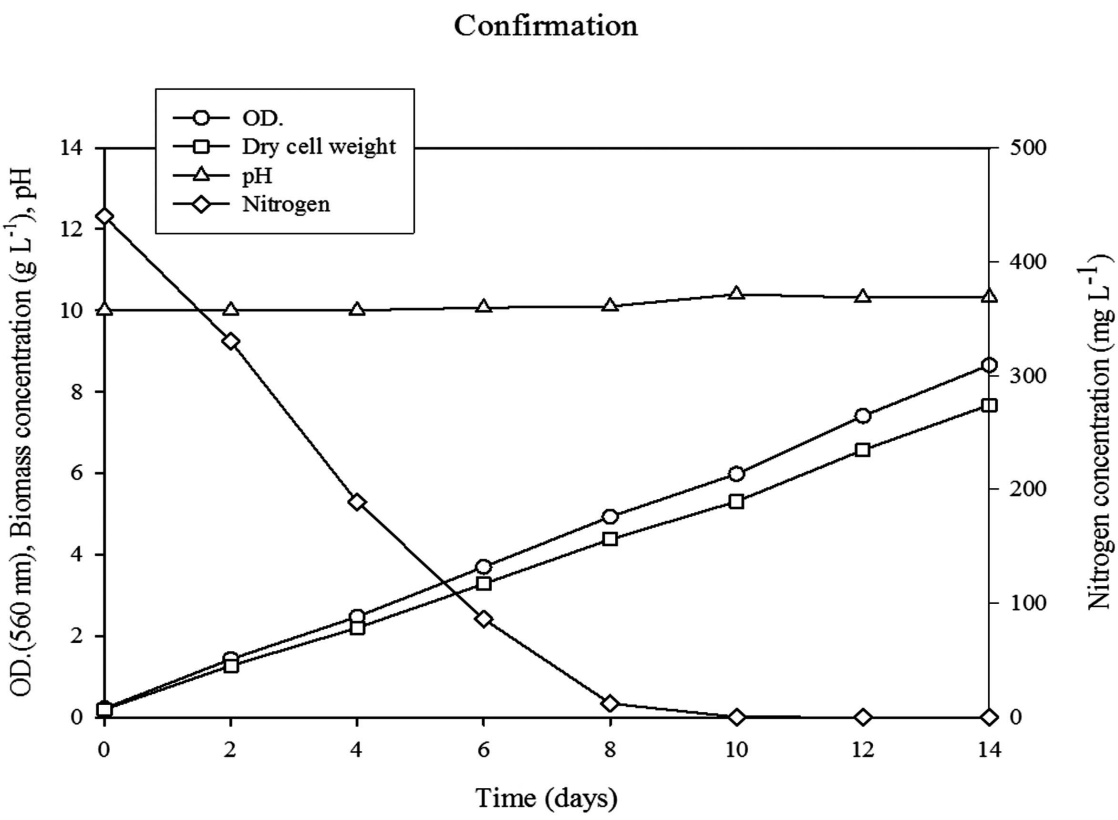


Fig. (4). Changes in biomass concentration, pH and nitrogen consumption of *A. maxima* IFRPD 1183 during tube cultivation under the optimal conditions attained by the Taguchi method (see Fig. **3**).

conditions for the production of biomass was shown in Fig. (**4**). For the prediction, the concentration and productivity of biomass were found closely to the observations (7.676 g-DCW L^{-1} and 0.535g-DCW L^{-1} d^{-1}, respectively) (Table **7**).

CONCLUSION

Taguchi method is very good tool for the optimization of biotechnological processes involving microorganism. In this study, the influence of six environmental factors on biomass production of *Arthrospira* sp. could be tested with only 16 runs with the consequent saving time and cost. A similar factorial design would have included 324 runs. The optimal conditions of biomass production were obtained as follows: 15 klux light intensity, an initial pH of 10.0, the cyanobacteria strain of *A. maxima* IFRPD1183, the concentration of Zarrouk's medium at 100%, 1 vvm flow rates of aeration mixed with 1–2% v/v CO_2 and 30 °C. The most important factors affecting the biomass production were the light intensity and the concentration of Zarrouk's medium. The expected value of optimization showed the concentration and productivity of biomass were 8.358 g-DCW L^{-1} and 0.584 g-DCW L^{-1} d^{-1}, respectively. The experimental concentration (7.676 g-DCW L^{-1}) and productivity (0.535 g-DCW L^{-1} d^{-1}) of biomass were almost similar to the prediction. In summary, this study is the first report on the use of the Taguchi method in the optimization of the culture conditions for the biomass production of *Arthrospira* sp. These growth factors obtained from the cyanobacterium *A. maxima* IFRPD1183 are very useful for the large scale production of algal biomass.

ABBREVIATIONS

C_X	=	Biomass concentration (g-DWC L^{-1})
DOF_{Factor}	=	Degree of freedom of factors
F_i	=	Averages of signal-to-noise ratio of factors at each factor level
F_{ratio}	=	*F*-ratio
n	=	Number of experiments
OD	=	Optical density
Q_X	=	Volumetric production rate of biomass (g-DCW L^{-1} d^{-1})
SI	=	Severity index
S/N	=	Signal-to-noise ratio
SS_{Error}	=	Sum of squares of error
SS_{Factor}	=	Sum of squares of factors
T	=	Grand average of signal-to-noise ratio
t	=	Time (days)
t_d	=	Doubling times (d)
y_i	=	The observed values of biomass concentrations (g-DWC L^{-1})
Y_{opt}	=	The expected values of biomass concentrations (g-DWC L^{-1}), biomass productivity (g-DCW L^{-1} d^{-1})

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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