Effect of NPK on Culture of *Arthrospira maxima* in a Reaceway Reactor Using Taguchi Methodology

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***Abstract*— Microalgae are photosynthetic microorganisms whose production has increased due to their ability to synthesize a high content of lipids, proteins, and carbohydrates. The cultivation of microalgae in open systems (raceways) generates lower operating costs. The use of inorganic fertilizers such as NPK to supplement culture can be a profitable strategy to decrease production costs. *Arthrospira maxima* is a blue spiral microalga known for its production of phycocyanin, a blue pigment with anticancer and anti-inflammatory properties. The objective of this research was to optimize culture conditions for phycocyanin production in *A. maxima* using the Taguchi methodology.**

***Keywords*—raceway, NPK, *Arthrospira*, phycocyanin**

I. Introduction

Microalgae are microscopic organisms whose production has increased due to their high content of lipids, proteins, and carbohydrates.

The cultivation of microalgae in open systems (raceways) is simple and generates lower production costs. A raceway is a pond with an enclosed flow channel. They have a central wall and a paddlewheel; the depth can oscillate between 0.25 and 0.30 m [1].

Several factors influence the growth of microalgae, including temperature, pH, nutrient availability, light intensity, and aeration [2]. In order to provide the growing medium with the nutrient requirements, the addition of fertilizers represents a cost-effective measure [3]. The use of inorganic fertilizers such as NPK for the cultivation of microalgae has been a studied strategy to reduce production costs compared to traditional commercial media [4]. According to [5] *Chlorella vulgaris* had a higher growth rate in medium supplemented with NPK compared to the traditional Chu 10 medium. *Arthrospira fusiformis* had a higher production of vitamins and minerals in medium formulated with NPK [6].

Arthrospira (commonly called Spirulina) is a blue-green, spiral microalgae that can grow in high concentrations of alkalinity and salinity [7]. It is known to produce phycocyanin (C-PC), a blue phycobiliprotein that is used as a pigment in food products and has gained increased interest for its antioxidant, anticancer, and anti-inflammatory properties [8].

The objective of this study was to evaluate an experimental matrix created under the Taguchi methodology, the culture conditions added with NPK to optimize the production of phycocyanin in A. maxima.

The Taguchi experimental design uses an orthogonal matrix that allows to reduce the number of experiments and helps to optimize the conditions for different processes that involve multiple factors with different numbers of levels [9].

II. Methodology

A. Microoganism and Culture Conditions

The *Arthrospira maxima* strain was obtained from the Environmental Remediation Laboratory of the Faculty of Agronomy, preserved at 4°C for experimentation. This strain was cultivated in Zarrouk Medium which contains (g L^{-1}): $NaHCO_3$ (16.8), K_2HPO_4 (0.5), $NaNO_3$ (2.5), K_2SO_4 (1.0), NaCl (1.0), $MgSO_4.7H_2O$ (0.2), $CaCl_2$.$2H_2O$ (0.04), $FeSO_4.7H_2O$ (0.01), EDTA (0.08); the pH was adjusted to 9.0 with NaOH. The liquid culture was kept at 26 ± 2°C, under continuous aeration and with intermittent lighting for 12 h with 1000 lux. Reaching the stationary phase of the strain, it was used for experimentation.

The NPK 18-18-18 fertilizer used, Ultrasol brand, is a water-soluble granular complex composed of 18% N, 18% P and 18% K.

B. Raceway Pilot Scale

The raceway for the experimentation is shown in Fig 1. It was made of acrylic and is 1.40 m long, 0.45 m wide and 0.25 m deep. The stirring paddlewheel has 6 blades and is powered by a geared motor. Its maximum capacity is 100 L, being operated with 80 L at 26-27 °C, under agitation for 8 h a day at 15 rpm and lighting with 2760 lux for 12 days. The control (control 2) and treatments 1 and 2 (T1* and T2*) were also cultivated with 4 LED lamps at 1080 lux to evaluate the effect of light on biomass and phycocyanin production

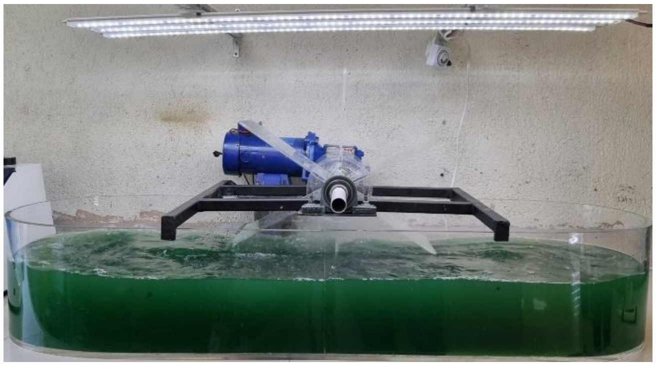

Fig. 1. Raceway reactor

C. Experimental Design

An L9 Matrix of the Taguchi experimental design was used through the Minitab software (Version 17.1.0) to evaluate the effects on the dependent variable concentration (phycocyanin concentration). The independent variables were: NPK concentration, initial inoculum concentration, photoperiod, and sodium bicarbonate concentration. Each variable was tested at three levels: low, medium and high (Table I) with 9 combinations of experiments designed by the software, which are shown in Table II.

TABLE I.

RANGE OF LEVELS OF VARIABLES USED FOR THE GROWTH OF *A. MAXIMA*

Independent variables	Factor	Low (-1)	Medium (0)	High (+1)
NPK concentration ($g L^{-1}$)	A	0.0	0.5	1.0
Photoperiod (h)	B	8	12	16
Inoculum concentration ($g L^{-1}$)	C	0.1	0.2	0.3
$NaHCO_3$ concentration ($g L^{-1}$)	D	4.2	10.5	16.8

TABLE II.

EXPERIMENTAL DESIGN FOR THE CULTURE OF A. MAXIMA

Run	A	B	C	D
1	0.0 (-1)	8 (-1)	0.1 (-1)	4.2 (-1)
2	1.0 (+1)	8 (-1)	0.3 (+1)	10.5 (0)
3	0.0 (-1)	12 (0)	0.2 (0)	10.5 (0)
4	0.5 (0)	16 (+1)	0.1 (-1)	10.5 (0)
5	1.0 (+1)	12 (0)	0.1 (-1)	16.8 (+1)
6	0.0 (-1)	16 (+1)	0.3 (+1)	16.8 (+1)
7	0.5 (0)	12 (0)	0.3 (+1)	4.2 (-1)
8	0.5 (0)	8 (-1)	0.2 (0)	16.8 (+1)
9	1.0 (+1)	16 (+1)	0.2 (0)	4.2 (-1)

D. Physicochemical Analysis

Every three days, the optical density at 680 nm was measured using an Evolution 201 spectrophotometer (Thermo Scientific, China) and cell counts with a Neubauer chamber and CxL model microscope (Labomed, USA). The pH and electrical conductivity were also measured using a STARA-2155 model potentiometer (Thermo Scientific, Indonesia) and 30 mL of sample were taken, centrifuged and filtered for the análisis of nitrate, orthophosphate and ammonium with the methodologies described in [10].

E. Biochemical Analysis

At the end of the established culture time, the biomass was harvested by filtration with a 165-wire screen mesh and dried at 35°C in a food dehydrator (Hamilton Beach model 32100). According to the methodology of [11], chlorophyll was quantified by UV-Vis spectrophotometry at 662, 645 and 470 nm using acetone for extraction. The results were calculated according to equation (1). Total lipid content was analyzed by the method described by [12], using 0.1 g of powdered microalgae subjected to sonication in a 2:1 ratio of chloroform-methanol. All analyzes were measured in triplicate. Equation (2) was used to determine the lipid percentage.

Phycocyanin was extracted by the freeze-thaw method as mentioned [13] with 4 cycles, using potassium phosphate buffer. To determine the total phycocyanin concentration (mg mL -1) equation (3) was used, described in [14].

$$Chlorophyll\ a = 11.75\ x\ A_{662} - 2.35\ x\ A_{645}. \quad (1)$$

$$Lipid\ content\ (\%) = \frac{W_L}{W_A}\ x\ 100. \quad (2)$$

$$C - PC = (A_{620} - 0.474\ x\ A_{652})/5.34 \quad (3)$$

III. RESULTS AND DISCUSSION

A. Physicochemical Analysis

As shown in Fig. 1, the pH gradually increases due to the dissociation of sodium bicarbonate. This does not affect the growth of *A. maxima* since it is an alkaliphile species [6].

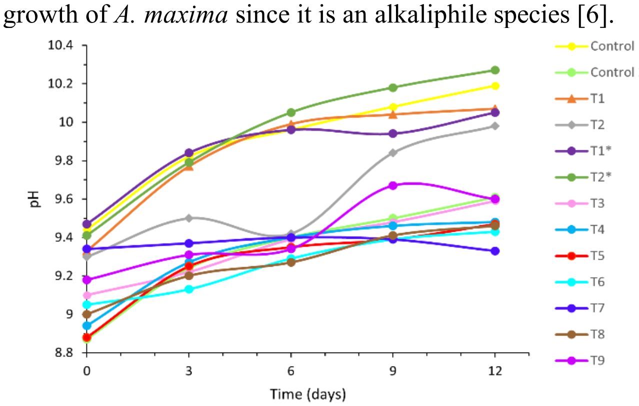

Fig. 2. Changes in pH.

The ammonium was determined in the treatments that contained NPK. According to Fig 3, the concentration decreases due to its assimilation by the microalgae.

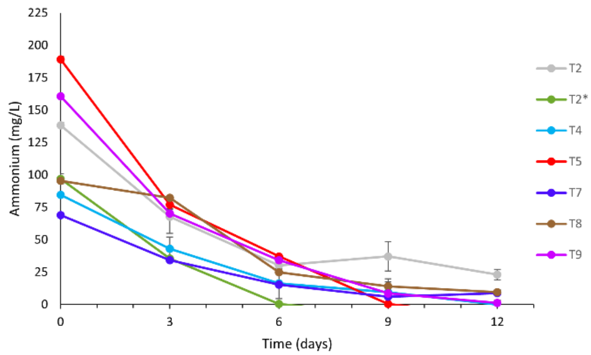

Fig. 3. Ammonium concentrations.

B. Biochemical Analysis

The Chlorophyll determination is shown in Fig. 4, it can be seen that T4 obtained the highest concentration, this treatment contained 0.5 g L^{-1} of NPK.

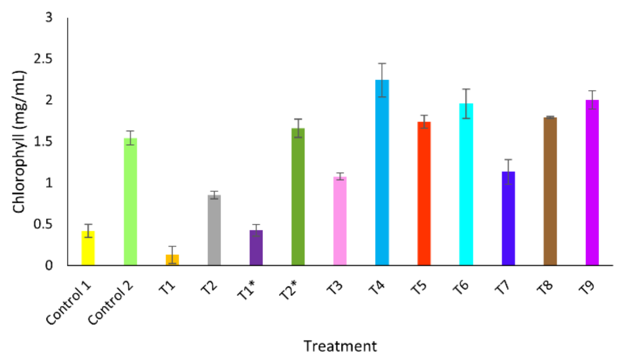

Fig. 4. Chlorophyll content.

The lipid content is shown in Fig. 5. The highest concentration was obtained in T3, which did not contain NPK. This is because nitrogen has a significant impact on lipid accumulation. These increase their concentration when nitrogen is limited [15].

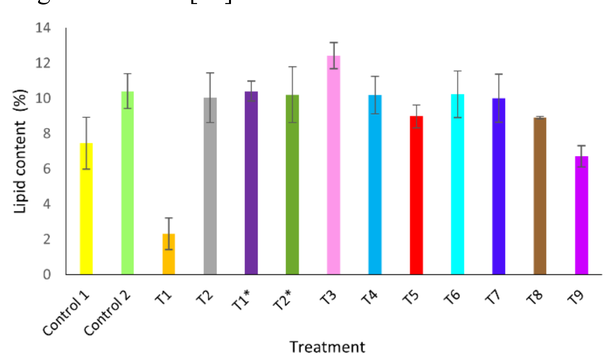

Fig. 5. Lipid content.

Fig. 6 shows the phycocyanin concentrations in the 9 treatments. Treatments 2* (1 g L^{-1} of NPK) and 6 (without NPK) were those that obtained the highest concentrations. Followed by T4 which contained 0.5 g L^{-1} of NPK.

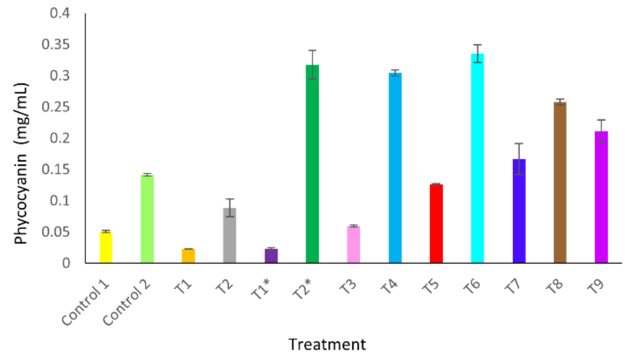

Fig. 6. Phycocyanin concentrations.

C. Analysis of Experimental Design

The principal component plot shown in Fig 7 identifies the optimal values of each variable to obtain a higher phycocyanin concentration. The NPK at a concentration of 0.5 g L^{-1}, the photoperiod of 16 hours, the inoculum and the sodium bicarbonate of 0.3 and 16.8 g L^{-1} respectively, maximize the response.

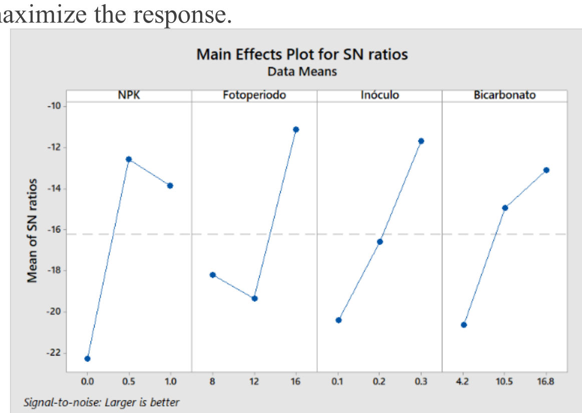

Fig. 7. Principal components plot of the signal/noise ratio when larger is better.

The analysis of variance (ANOVA) is shown in Table III.

TABLE III.

ANOVA OF PHYCOCYANIN PRODUCTION

Factor	df	SS	MS	% Contribution
NPK	2	167.259	83.6294	33.88
Photoperiod	2	119.156	59.5778	24.13
Inoculum	2	114.518	57.2591	23.19
$NaHCO_3$	2	92.808	46.4038	18.80
Total	8	493.740		100

The NPK was the factor with the greatest contribution followed by the photoperiod and the inoculum.

These conditions were reproduced except that the bicarbonate was applied at a concentration of 10.5 g L^{-1} since the component graph did not show a significant difference with respect to the highest concentration (16.8 g L^{-1}). Phycocyanin extraction was performed in the optimized run, obtaining a concentration of 0.40 mg mL^{-1}.

IV. Conclusion

Arthrospira maxima has been recognized and studied for its production of phycocyanin. Using the Taguchi methodology we evaluated the optimal conditions for their growth in a raceway reactor supplementing with NPK. The results show that using a concentration of 0.5 g L^{-1} of NPK, photoperiod of 16 hours, as well as 0.3 and 10.5 g L^{-1} of inoculum and sodium bicarbonate, respectively, are adequate to obtain a higher concentration of phycocyanin. *A. maxima* was cultivated with optimized conditions and a higher phycocyanin concentration (0.40 mg mL^{-1}) was obtained than in all the trials tested, demonstrating that NPK positively affects biomass and phycocyanin production.

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