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Improvement of Astaxanthin Production in *Coelastrum* sp. by Optimization Using Taguchi Method

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Abstract

Background and Objective: Astaxanthin is a keto-carotenoid pigment known as one of the most valuable compounds with great potentials in the market. It has widely been used in nutraceutical, pharmaceutical, cosmetics and food industries due to its strong antioxidant activity. Green microalgae seem as promising natural sources in production of astaxanthin. The aim of this study was to optimize astaxanthin production in *Coelastrum* sp. to overcome low productivity of microalgae.

Materials and Methods: This study was carried out using experimentally statistical technique and Taguchi method to find optimum conditions for maximizing production of astaxanthin in green microalgae, *Coelastrum* sp. Effects of nutritional (carbon and nitrogen) and environmental (light and salinity) factors on biomass and astaxanthin production were investigated. Experiments were carried out for light intensity (250-550 µmol photons m^{-2} s^{-1}), salinity using sodium chloride (1.0-3.0 g l^{-1}), carbon source using sodium acetate (0.5-2.0 g l^{-1}) and nitrogen source using sodium nitrate (0.1-0.3 g l^{-1}).

Results and Conclusion: Results showed that optimum conditions of astaxanthin production in *Coelastrum* sp. included 250 µmol photons m^{-2} s^{-1} of light intensity, 3 g l^{-1} salinity, 0.5 g l^{-1} carbon and 0.1 g l^{-1} nitrogen with a maximum yield of astaxanthin (14.44 mg l^{-1}), which was 2-fold higher than that before optimization. This optimization resulted in high quantities of astaxanthin production using optimization of conditions that affected production yields of astaxanthin from *Coelastrum* sp.

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1. Introduction

Astaxanthin is a member of carotenoids in xanthophyll family. It is a valuable product with high market owing to its powerful antioxidant properties in quenching free radicals [1]. It is the most common red-color pigment of marine and freshwater organisms with uses in aquacultures, pharmaceutics, cosmetics and food additives [2]. The chemical benefits as a strong antioxidant in nature are greater than other carotenoids such as β-carotene, canthaxanthin, zeaxanthin, lutein and α-tocopherol [3]. Astaxanthin can synthetically or naturally be produced from various sources. Natural astaxanthin is more efficient in scavenging free radicals with over 20-time stronger effects than synthetic astaxanthin [4]. The health benefits and high costs of astaxanthin have resulted in recent searches for natural astaxanthin. Natural astaxanthin has recently introduced to the commercial market as a food-coloring agent and a feed additive in aquaculture [5]. Furthermore, interests are rising in astaxanthin use for human consumption. The powerful antioxidative activity of astaxanthin makes it beneficial to human health. Therefore, astaxanthin

has widely been used for protection against a broad range of human diseases, including free-radical associated diseases such as oral, colon and liver cancers, cardiovascular diseases and degenerative eye diseases [6].

Natural astaxanthin can be produced from microalgae, yeasts, bacteria (*Paracoccus* sp.), fungi and shrimps [7,8]. Although various organisms are capable of producing astaxanthin, only a small number of these organisms are cultivated commercially. Microalgae represent sources with the most potency for natural astaxanthin production as it can produce a large quantity of astaxanthin [9]. *Spirulina platensis* is one of the beneficial microalgae in food biotechnology as it includes macro and micronutrients with the potency of synthesizing astaxanthin [10]. Moreover, discovery of astaxanthin in *Coelastrum* sp. can provide an alternative natural source of astaxanthin. A previous study by Tharek et al. has reported that *Coelastrum* sp. are potential strains for the production of astaxanthin from natural sources as this genus is mostly similar to *Haematococcus pluvialis* under high light-intensity and nitrogen-starvation conditions in mixotrophic cultures. This can be a potential alternative to current astaxanthin production, which further supports ability of this strain as an astaxanthin producer [11].

To accumulate higher concentrations of astaxanthin in microalgae, a two-stage cultivation method is carried out; in which, the first stage includes biomass production. This stage is followed by the second stage, which includes astaxanthin accumulation [12]. Low light-intensity was used in the first stage to promote *Coelastrum* sp. growth until an optimal cell density was reached. In the second stage, stress conditions were used to induce and increase accumulation of astaxanthin. Several nutritional and environmental factors have been reported as major effects on growth of microalgae and astaxanthin production. These factors include light radiance, nitrogen, carbon and salinity that may improve and increase production of astaxanthin [13]. Studies on *Coelastrum* sp. HA-1 showed that nitrogen limitation in culture media of this species improved production of astaxanthin [14]. Culturing *Coelastrum* cf. *pseudomicroporum* in municipal wastewater and salinity stress increased carotenoid production [15]. Improvement of astaxanthin production can be completed by optimization to maximize astaxanthin production.

Conventionally, traditional approaches have been used to maximize production of astaxanthin by changing one variable at the time while keeping other variables constant. This approach needs large sets of experimental data, which is time-consuming and leads to laborious and complex experiments as the number of factors increases [16]. For example, four parameters assessed using factorial design are necessary to carry out 81 experiments instead of nine experiments that are needed in Taguchi method. This occurs because the factorial design needs combinations of all levels within all factors. Optimization using Taguchi method has recommended a special approach using orthogonal array to test the whole parameter within less experiments. Therefore, a statistical technique of designing experiments using the Taguchi method can provide minimum experimental runs by determining the controlling factors in the experimental design [17]. Taguchi's fundamental theory works as screening filters, in which various process parameters can be studied at a time, and those with major effects can easily be identified [18]. This method has recently been used to improve biochemical methods, bioprocesses and applications in biotechnology [19]. Hsia and Yang used Taguchi method to systematically identify optimal growth conditions for the increase of algal production and growth rates of *Chlorella* sp. [20]. A study by Guo et al. reported that production of astaxanthin by *Phaffia rhodozyma* was 1.6-fold higher than that before optimization using Taguchi method [21]. Taguchi method includes several advantages as it comprises use of orthogonal arrays. In fact, orthogonal arrays is used to design experiments based on control factors and levels to achieve quality of a product with high precisions. Furthermore, it uses signal-to-noise (S/N) ratio to assess the effects by decreasing system's exposure to variance sources, resulting in excellent performances. Therefore, use of such a design can increase process robustness and hence decrease times and costs [22]. In this study, optimization information on various nutritional (carbon and nitrogen) and environmental factors (light intensity and salinity) have been provided using Taguchi method for the improvement of astaxanthin production in *Coelastrum* sp.

2. Materials and Methods

2.1 Microalgae strain and culture conditions

Green microalga *Coelastrum* sp. isolated from a sampling site at Hulu Langat River, Kuala Selangor, Malaysia, was used to assess the maximum yield of astaxanthin production by optimization using Taguchi method under various conditions. The species was cultured in AF-6 media containing $NaNO_3$, NH_4NO_3, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, Fe-citrate, Citric acid, KH_2PO_4, K_2HPO_4, trace metal solution ($FeCl_3.6H_2O$, $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$, $CoCl_2.6H_2O$, $Na_2MoO_4.2H_2O$, $Na_2EDTA.2H_2O$) and a mixture of vitamins (biotin, pyridoxine, thiamine) based on the media recipe available in Microbial Culture Collection National Institute for Environmental Studies (NIES-collection), Japan. The biomass of *Coelastrum* sp. was assessed using method of Boussiba and Vonshak and expressed as g l^{-1} [23].

In the first stage, strain was cultured in 250-ml Erlenmeyer flasks containing 100 ml of the growth media inoculated with 10% (v v^{-1}) of the inoculum and incubated at 25 °C ±1. Cultures were aeriated continuously through filtered (0.22 μm) air and illuminated at 12:12 h (light:dark) cycles using fluorescence light at standard photon flux

densities of 70 µmol photons m^{-2} s^{-1} for 14 days until desired biomass reached the exponential growth phase. Optimization of astaxanthin production was carried out in the second stage to maximize production of astaxanthin in *Coelastrum* sp. After two weeks of growth, cells were harvested by centrifugation at 2000 ×g for 10 min and resuspended in fresh culture media (pH 7) containing various parameters of nutritional (carbon and nitrogen) and environmental (light intensity and salinity) factors with continuous aeration at 1 l min^{-1}. Then, cells were collected on the first and second weeks of the optimization and further extracted for astaxanthin. The second stage was set up using Taguchi method. Experiments were carried out for light intensity (250-550 µmol photons m^{-2} s^{-1}), salinity using sodium chloride (1.0-3.0 g l^{-1}), carbon source using sodium acetate (0.5-2.0 g l^{-1}) and nitrogen source using sodium nitrate (0.1-0.3 g l^{-1}) to optimize accumulation of astaxanthin in *Coelastrum* sp.

2.2 Astaxanthin extraction

Extraction of astaxanthin was carried out using Sarada et al. method [24]. To assess astaxanthin contents, 50 ml of the microalgae cultures were centrifuged at 2000 ×g for 5 min at 4 °C. Then, samples were freeze-dried for 24 h (Eyela, FDU-1200, Tokyo). Dried biomass was treated with 5% KOH in 30% (v v^{-1}) methanol for 5 min at 70 °C and washed by resuspending in distilled water. This step was used to remove the chlorophyll when pure astaxanthin is required. The cells were then centrifuged at 2000 ×g for 10 min at 4 °C, and the supernatant was discarded. Following that, the pre-treatment of astaxanthin was done to cleave the vital bond in the cell wall of a cell to facilitate the astaxanthin extraction by adding 4N hydrochloric acid (HCl) to the cell and heated at 70 °C for 2 min, cooled and washed with distilled water. Astaxanthin extraction continues by solvent extraction with acetone and set for 1 h. Supernatant was analyzed using HPLC. All stages were carried out under dim light.

2.3 Astaxanthin analysis

Extracted astaxanthin was characterized using HPLC (Agilent Technologies 1220 Infinity LC, Germany) with a reverse phase C_{18} column (250 × 4.6 mm, 5 µm) equipped with photodiode array detector [25]. Mobile phase included an (A) acetone and (B) methanol to water ratio of 9:1 (v v^{-1}) at a flow rate of 0.8 ml min^{-1} with a column temperature of 25 °C. The injection volume included 10 µl. Absorption spectra of the pigment were shown within 250-700 nm. All peaks of carotenoid were integrated at a wavelength of 476 nm to quantify astaxanthin. Astaxanthin standard was purchased from Sigma-Aldrich, St. Louis, USA, and stored at -20 ºC. All separated carotenoids were identified by comparing retention times against astaxanthin standard.

2.4 Experimental design of Taguchi

The experiment flow chart illustrates experimental processes involved in Taguchi parameter design (Figure 1). Selection of the factors is an important parameter to improve quality of the products. In Taguchi method, factors and levels of the experiment were selected as shown in Table 1. These were set based on the concentrations of each factor to determine the optimal setting of the four parameters (carbon, nitrogen, light and salinity) in astaxanthin production. To select an appropriate orthogonal array for the experiments, degrees of freedom were computed using Minitab software v.17. Notation of L_a (b^c) was used to represent the orthogonal array; in which, a was the number of experiments, b was the number of levels for each factor and c was the number of factors [26]. In the present study, $L_9(3^4)$ was the most appropriate orthogonal array for the experiments (Table 2). An experiment included a combination of all levels of the variables that were set in the experiment. Accordingly, nine experiments were carried out to study effects of four various parameters on production of astaxanthin. These four parameters included light intensity, salinity (sodium chloride), nitrogen (sodium nitrate) and carbon (sodium acetate).

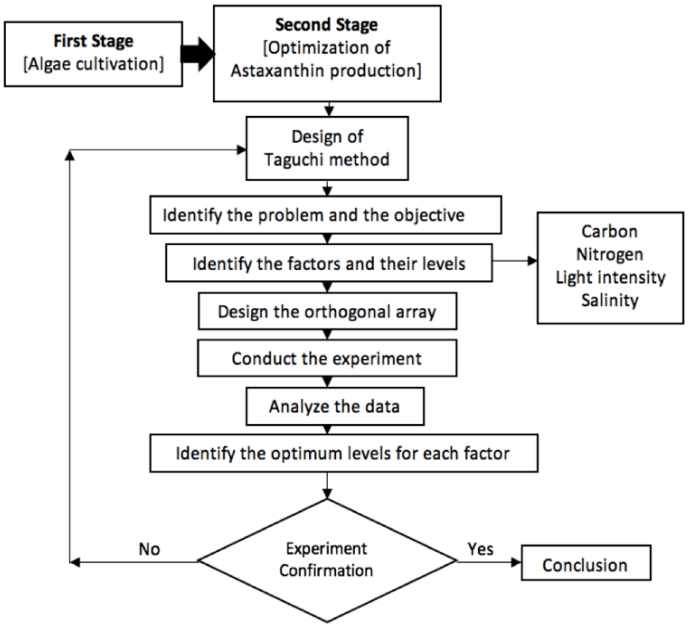

Figure 1. The experiment flow chart for design of Taguchi method

Table 1. Levels of various parameters used to maximize production of astaxanthin in *Coelastrum* sp.

Parameter	Light Intensity (µmol photon m^{-2} s^{-1})	Salinity (g l^{-1})	Nitrogen (g l^{-1})	Carbon (g l^{-1})
Level 1	250	1.0	0.1	0.5
Level 2	450	3.0	0.2	1.0
Level 3	550	6.0	0.3	2.0

Table 2. Nine experimental run using L_9 (3^4) orthogonal array for the control factors with astaxanthin yield and mean of S/N ratio using Taguchi method. Data represent an average of three replicates

Experiment No.	Parameters				Astaxanthin yield (mg l^{-1})	S/N Ratio
	Light (µmol photon m^{-2} s^{-1})	Salinity (g l^{-1})	Nitrogen (g l^{-1})	Carbon (g l^{-1})		
1	250	1.0	0.1	0.5	12.86 ±0.56	22.18
2	250	3.0	0.2	1.0	7.68 ±0.60	17.70
3	250	6.0	0.3	2.0	2.01 ±0.72	6.06
4	450	1.0	0.2	2.0	5.15 ±0.10	14.24
5	450	3.0	0.3	0.5	9.26 ±0.41	19.33
6	450	6.0	0.1	1.0	1.80 ±0.97	5.09
7	550	1.0	0.3	1.0	0.61 ±0.15	-4.31
8	550	3.0	0.1	2.0	1.30 ±0.04	2.25
9	550	6.0	0.2	0.5	0.64 ±0.16	-3.85

2.5 Statistical analysis

MINITAB-17 software was used to analyze Taguchi design. Various S/N ratio equations were available according to the objectives function. Larger-the-better characteristic was used in this experiment for the calculation of S/N ratios. Then, the mean S/N ratio was calculated to identify effects of the factors and levels on the quality of products and subsequently to prepare a response table. The S/N ratio (larger-the-better) was calculated according to the Eq.1 [27]:

Larger-the-better:

$$SN_L = -10\log[\sum_{i=1}^{n}(1/y_i^2)/n] \quad \text{Eq.1}$$

Where, the S/N ratio value was the optimal level achieved in maximum conversion for each parameter, *i* was the number of replicates, *n* was the number of repeated assessments, and *y* was the assessed value. Analysis of variance (ANOVA) was used to assess experimental errors and significance of the results. Differences were considered significant at $p \leq 0.05$. All experiments were carried out with three replications from separate cultures and values were expressed as mean ±SD (standard deviation).

3. Results and Discussion

3.1 Taguchi design analysis for the optimal astaxanthin production

The loss function in Taguchi method was used to analyze performance characteristics that were contradictory to desired target values. Signal-to-noise (S/N) ratio is commonly used for analysis of the Taguchi design as a value of the loss function. This demonstrates the optimum parameter conditions of the products. The S/N ratio derived from loss function is expressed as the ratio of signal and noise factor (uncontrollable factor) [28]. Various S/N ratio equations are available depending on objectives of the experiments. The S/N ratio analysis is divided into three performance characteristic categories as nominal-the-best, larger-the-better and smaller-the-better. The nominal-the-best approach is used when a specified value is desired and the variance occurs around the value. The smaller-the-better approach is usually needed to minimize the occurrence of undesirable product characteristics. The larger-the-better is used to maximize the product characteristics by making the system response as large as possible [29]. In this study, larger-the-better characteristic was used for the optimization of experiments to maximize responses to improve production of astaxanthin in *Coelastrum* sp. To maximize the experiment, values of the S/N ratio were assessed using larger-the-better characteristic. Based on the parameters within the highlighted levels, the mean of S/N ratios for each set of experiments was analyzed to identify effects of the factors and levels on quality of the products. Moreover, a response table was prepared to predict ranking parameters affecting astaxanthin production. Table 3 shows the factor ranking based on delta values. Delta values are the difference between the S/N ratio values within the process variables at the highest and the lowest levels of the process factors. The higher delta value of each factor is, the greater the effect on production of astaxanthin in *Coelastrum* sp. is.

Table 3. Response table for S/N ratio larger-the-better to predict ranking parameters affecting astaxanthin production. Light intensity was reported as the most affecting parameter for astaxanthin production in *Coelastrum* sp., followed by salinity, carbon and nitrogen concentrations

Level	Light	Salinity	Nitrogen	Carbon
1	15.316	10.706	9.842	12.555
2	12.889	13.096	9.366	6.162
3	-1.968	2.435	7.030	7.520
Delta	17.285	10.661	2.812	6.393
Rank	1	2	4	3

Study showed that the highest value of mean S/N was observed for light intensity and the lowest value of mean S/N was observed in nitrogen source. Thus, results stated that out of the four factors, factors with the maximum effects on astaxanthin production in *Coelastrum* sp. were affected by light intensity followed by salinity, carbon and nitrogen concentrations (Table 3). This revealed that light intensity was the most affecting parameter as it resulted in the highest

effect on astaxanthin production. Similarly, it has been reported that light is the major factor affecting growth and formation of astaxanthin [30]. A large quantity of astaxanthin was synthesized and accumulated rapidly in cells under high light conditions [13]. This possibly occurred because *Coelastrum* sp. developed a light protection mechanism to protect cells from photo-inhibition of high irradiance and hence efficiently accumulated astaxanthin as a shading pigment [31]. Figure 2. Shows linear graphs for S/N ratios corresponding to the highest S/N ratio of each control factor. The highest plot of S/N ratio suggested the best level for each of the parameters. Based on the maximal production of control factors and levels of the S/N ratio, this study revealed that the highest productivity of astaxanthin in *Coelastrum* sp. occurred at 250 µmol photons m^{-2} s^{-1} of light intensity, 3 g l^{-1} salinity, 0.5 g l^{-1} carbon and 0.1 g l^{-1} nitrogen.

Light intensity and nitrogen and carbon sources are the most essential factors in accumulating secondary carotenoids, especially astaxanthin. These have been used as primary sources for the growth and production of astaxanthin by microalgae [9]. Borowitzka et al. reported that yield of astaxanthin was improved in *Haematococcus pluvialis* by addition of 0.1 g l^{-1} of acetate under mixotrophic conditions. These conditions lead to formation of astaxanthin-containing red cysts in microalgae [32]. Del Campo et al. showed that carbon sources (acetate with nitrogen) and high light-intensity were mostly used to increase accumulation of astaxanthin in *Chlorella zofingiensis* [33]. A previous study showed that salt tolerance up to 0.2 M could increase astaxanthin yield in *Chlorella zofingiensis* with an increased rate of 60%, compared to salt-free cultures. However, further increases in salt concentrations were lethal to the cells of microalgae and decreased cell density [33]. Therefore, the study revealed that balance ratios between the nutritional and environmental factors in culture media of microalgae were important factors with great effects on carotenogenesis.

3.2 Analysis of variance

ANOVA was used to statistically evaluate significance of the four various parameters. Results of Table 4 show ANOVA for the L_9 orthogonal array. As seen in the table, light intensity (p=0.039) and salinity (p=0.044) significantly contributed to astaxanthin production ($p < 0.05$), indicating that these factors were more significant than nitrogen and carbon. The F-value in ANOVA represented its effects on production of astaxanthin. In this study, light intensity exhibited the highest F-value, which demonstrated that light intensity included the highest effects on the reaction followed by salinity, carbon and nitrogen. Therefore, the best parameter for the production of astaxanthin orderly included light intensity (250 µmol photons m^{-2} s^{-1}), salinity (3 g l^{-1}), carbon (0.5 g l^{-1}) and nitrogen (0.1 g l^{-1}). Results from ANOVA analysis were closely matched the mean of S/N ratio from Taguchi results.

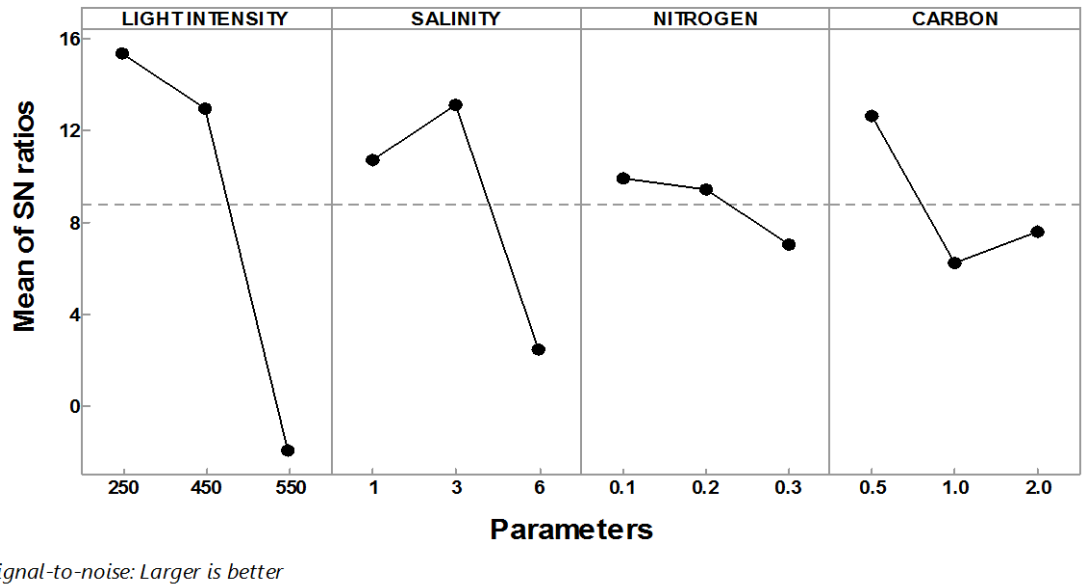

Figure 2. The S/N ratio plots for four process control factors at various levels suggesting the best level for each parameter

Table 4. Analysis of variance results for S/N ratio of the parameters involved in astaxanthin production

Parameters	Degree of Freedoms	Sum of Squares	Mean Square	F-value	P-value
Light	2	69.62	34.81	2.40	0.039
Salinity	2	43.44	21.72	1.15	0.044
Nitrogen	2	2.801	1.401	0.05	0.947
Carbon	2	40.86	20.43	1.06	0.404
Error	6	15.74			
Total	14	172.46			

3.3 Interactions within the process variables

Figure 3 shows interactions between the various parameters studied in production of astaxanthin from *Coelastrum* sp. Interactions between the two variables were assessed by keeping two other variables at their optimal levels. Interactions between the light intensity and salinity showed that astaxanthin production could be developed at the lowest level of light intensity (250 µmol photons m^{-2} s^{-1}) with the lowest value of salinity (1.0 g l^{-1}). Similarly, interactions of light intensity with nitrogen and carbon sources showed that the maximum production of astaxanthin could be achieved at the lowest level of light intensity in a minimum quantity of nitrogen (0.1 g l^{-1}) and carbon (0.5 g l^{-1}) by keeping other variables at their optimum values.

In this study, the highest level of light intensity lead to the lowest astaxanthin production. It is noteworthy that of various combinations of parametric values, the maximum production of astaxanthin could be achieved only at the lowest level of light intensity (250 µmol photons m^{-2} s^{-1}). Light energy was important to convert inorganic nutrients into proteins, carbohydrates, lipids and other secondary metabolites. However, extreme irradiance might decrease microalgae growth and thereby decrease astaxanthin accumulation [34]. By keeping light intensity and carbon concentration constant, the maximum production of astaxanthin was detected at the lowest values of salinity (1.0 g l^{-1}) and nitrogen (0.1 g l^{-1}). Fixed light intensity and salinity at the optimum levels showed that production of astaxanthin gradually increased with decreases in carbon concentration at 0.1 g l^{-1} nitrogen.

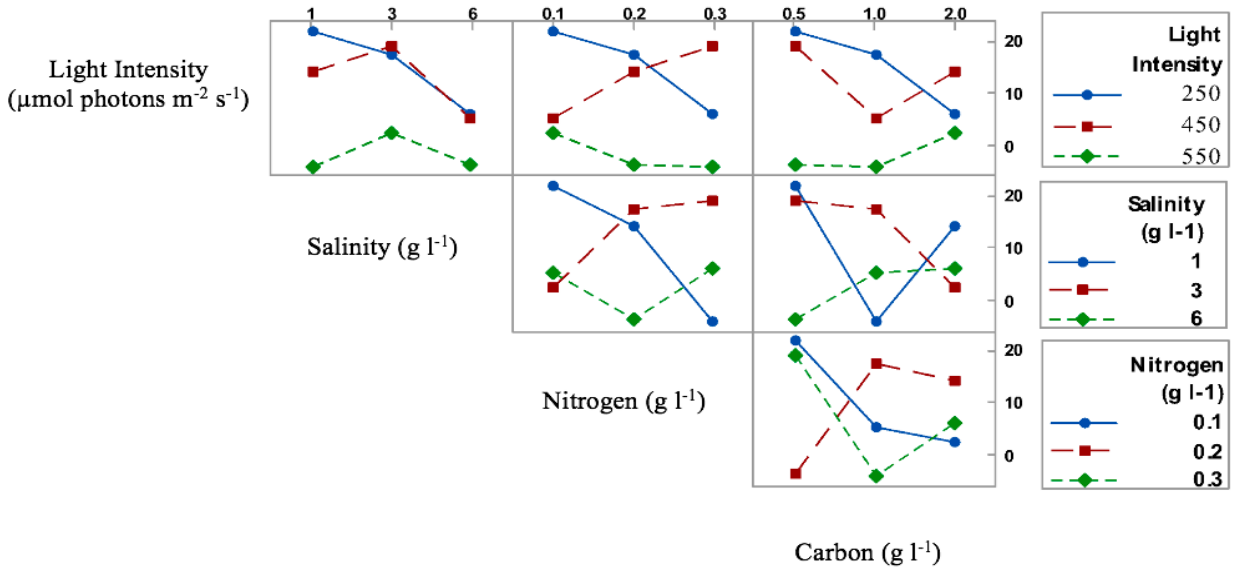

Figure 3. Interaction plot for S/N ratio between various parameters

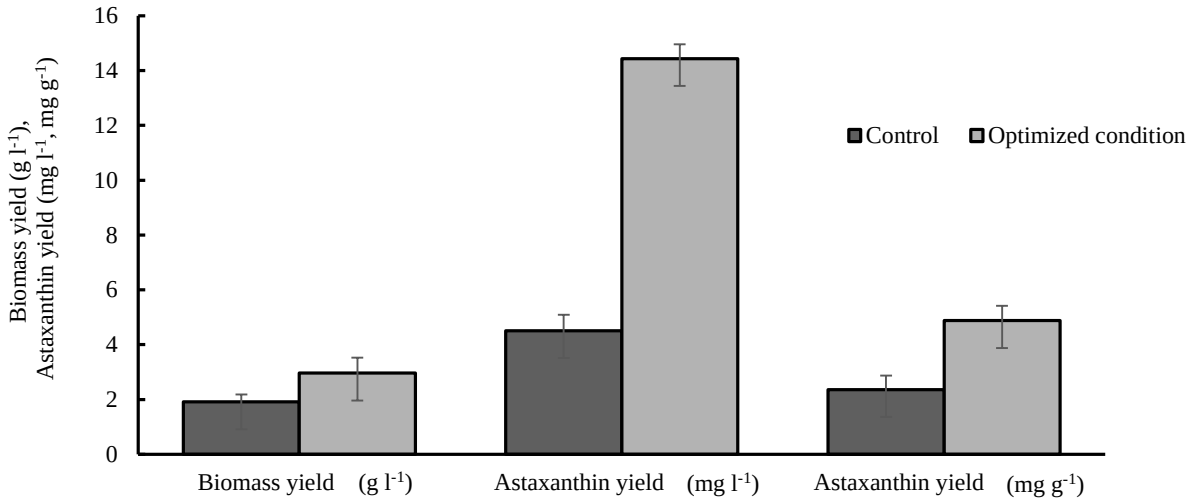

Figure 4. Astaxanthin production using optimized conditions and Taguchi optimization method, compared to non-optimized conditions. Data represent an average of three replicates and error bars indicate mean ±SD (standard deviation)

3.4 Confirmation of experiment

In this study, optimization of astaxanthin production from Taguchi method and astaxanthin production from original conditions (control) were compared to each other. Data from Fig. 4 shows that production of astaxanthin significantly increased using the optimized method (14.44 mg l^{-1} and 4.88 mg g^{-1}), which was 2-fold higher than that before optimization (4.51 mg l^{-1} and 2.36 mg g^{-1}). The biomass yield was also higher (1.6-fold) in optimized conditions, compared to controls, indicating that production of astaxanthin was linked to biomass production. Furthermore, results showed that concentration of astaxanthin in *Coelastrum* sp. was higher than that in *Haematococcus pluvialis* (8.3-10.69 mg l^{-1}) reported by Sarada et al. under NaCl stress [35]. Salinity in culture media has been reported to improve carotenogenesis in microalgae. However, increases in astaxanthin contents were achieved by a high mortality rate of microalgae cells. The microalgae growth can be significantly decreased by salt treatments; thereby, decreasing accumulation of astaxanthin [33,35]. A study by Steinbrenner and Linden showed that astaxanthin contents in *Haematococcu pluvialis* illuminated at 250 µmol photons m^{-2} s^{-1} increased up to 2.5 mg g^{-1} of microalgae dry weight, which were lower than those in the current study [36]. The high light condition can decrease microalgae growth and thereby decrease astaxanthin accumulation. To achieve the optimal biomass and astaxanthin production, combined effects with balanced ratios of all factors affecting astaxanthin accumulation need to be monitored for synthesizing astaxanthin [37]. In confirmation of these findings, combination of other factors through optimization of the culture media is an important parameter to improve astaxanthin production in maximized production of microalgae.

4. Conclusion

In this study, a statistically experimental design and Taguchi method were used to optimize production of astaxanthin in *Coelastrum* sp. with L_9 orthogonal array. Results showed that light intensity was the most affecting parameter in production of astaxanthin in *Coelastrum* sp. From this experiment, light intensity and salinity were reported as the most significant parameters affecting astaxanthin synthesis in *Coelastrum* sp. The best culture conditions for the production of astaxanthin by *Coelastrum* sp. included 250 µmol photons m^{-2} s^{-1} of light intensity, 3 g l^{-1} salinity, 0.5 g l^{-1} carbon and 0.1 g l^{-1} nitrogen. The maximum yield of astaxanthin under optimal conditions included 14.44 mg l^{-1} (4.88 mg g^{-1} of astaxanthin per biomass), which was 2-fold higher compared to non-optimized media (control). In general, results of this study have represented a successful method for optimizing conditions that affect production yields of astaxanthin from *Coelastrum* sp. Furthermore, data from this study have provided information for further production of large scales of astaxanthin to enhance bio-based economy worldwide, especially for nutraceutical and pharmaceutical industries due to the compound beneficial effects to human health.

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6. Conflict of Interest

The authors declare no conflict of interest.

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بهبود تولید آستاگزانتین در گونه *کلاستروم* با بهینه‌سازی به روش تاگوچی

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چکیده

سابقه و هدف: آستاگزانتین رنگدانه کتو-کاروتنوییدی است که به عنوان یکی از با ارزش‌ترین ترکیبات با پتانسیل زیاد در بازار شناخته شده می‌باشد. به دلیل خاصیت ضداکسایشی[1] قوی در صنایع غذا-دارو[2]، داروسازی، آرایشی و غذایی کاربرد گسترده‌ای دارد. به نظر می‌رسد ریزجلبک‌های سبز منابع طبیعی امیدبخشی در تولید آستاگزانتین باشند. هدف این مطالعه بهینه سازی تولید آستاگزانتین در گونه *کلاستروم* به منظور غلبه بر بهره‌وری پایین ریزجلبک‌ها بوده است.

مواد و روش ها: این مطالعه برای یافتن شرایط بهینه تولید آستاگزانتین در ریزجلبک‌های گونه *کلاستروم* با شیوه آماری آزمایشی و روش تاگوچی انجام شده است. اثرات عوامل تغذیه‌ای (کربن و نیتروژن) و محیطی (نور و شوری) بر زی‌توده[3] و تولید آستاگزانتین مورد بررسی قرار گرفت. آزمون‌ها شامل شدت نور (۵۵۰-۲۵۰ میکرومول فوتون در متر مربع در ثانیه)، شوری با استفاده از سدیم کلرید (۳/۰-۱/۰ گرم بر لیتر)، منبع کربن با استفاده از سدیم استات (۲/۰-۰/۵ گرم بر لیتر)، و منبع نیتروژن با استفاده از سدیم نیترات (۰/۳-۰/۱ گرم بر لیتر) بود.

یافته‌ها و نتیجه‌گیری: نتایج نشان داد شرایط بهینه تولید آستاگزانتین در گونه *کلاستروم*، شدت نور برابر ۲۵۰ میکرومول فوتون در متر مربع در ثانیه)، شوری ۳ گرم بر لیتر، کربن ۰/۵ گرم بر لیتر، و نیتروژن ۰/۱ گرم بر لیتر با بیشینه راندمان آستاگزانتین (۱۴/۴۴ گرم بر لیتر)، دو برابر بیشتر نسبت به قبل از بهینه‌سازی می‌باشد. در این تحقیق تولید مقادیر زیاد آستاگزانتین در شرایط بهینه عوامل موثر بر راندمان تولید آستاگزانتین در گونه *کلاستروم* بهینه‌سازی شد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[1] Antioxidant activity
[2] Nutraceutical
[3] Biomass