

Sampling Frequency Is a Key Factor to the Efficient Actuation of the Phytohormones in the Increase of Biomass and Macromolecules Production by *Spirulina (Arthospira)*

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Abstract

Application of microalgae in industries is limited by their low biomass production and low macromolecule content. Phytohormones are being studied to solve these problems, however is necessary to define conditions that enhance the results for this kind of cultivation strategy. The increasing of the contact time between substances and cells can be a factor of the improvement in the phytohormone uptake by microalgae. We hypothesize that by decreasing the sampling frequency for monitoring culture parameters, we would also be interfering less in the uptake of phytohormones by microalgae. Therefore, the present study aimed to investigate the influence of sampling frequency on the production of biomass and biomolecules of *Spirulina* sp. LEB 18 in cultivations supplemented with *trans*-zeatin and indole-3-acetic acid (IAA). Treatments with lower sampling frequency and supplementation of 1 mg L⁻¹ IAA enhanced biomass accumulation by 75 %, carbohydrate content by 50 %, and protein content by 30 % as compared to control experiment daily sampling. In addition, sampling every 10 days with 0,01 mg L⁻¹ IAA supplementation increased the lipid content by 51 % as compared with the experiment without phytohormone and daily sample removal. Therefore, we developed a new strategy to supplement phytohormones and improve microalgal production. This investigation presented a cultivation system efficient, which can be easy to implement in industries because of no need to change the operational aspects of the cultivation. Furthermore, the strategy will be very useful to enhance the production capacity of microalgae on a large-scale.

Introduction

Despite the potential applications of microalgae, their use in industry is limited owing to the low production capacity to their low biomass and low macromolecule content. As a result, microalgal biotechnology has focused on improving production systems in terms of efficiency, cost, and biomass composition [1]. The modification of biochemical factors, such as supplementation of phytohormones, is considered to be a promising strategy to assist in this goal [2].

Phytohormones are signaling molecules that regulate the growth and development of plants and algae [3], but also endogenous phytohormones are found in microalgae [4], performing a functional role in their physiology [5]. The principal growth phytohormones are indole-3-acetic acid (IAA), which is an auxin, and *trans*-zeatin (tZ), which is a cytokinin. They are natural phytohormones [6, 7], and different proportions of these two phytohormones determine the kind of organ that will form during plant development [8].

Although phytohormone supplementation is considered a promising strategy for improving the efficiency of microalgae cultivation, it is necessary to optimize the concentration of these substances to each kind of microalgae because small variations in concentration can result in great changes to the cells [9, 10]. In the literature, there are many relates to phytohormone supplementation in the cultivation of eukaryotic microalgae genres as *Chlorella* [11–16] and *Scenedesmus* [15, 17, 18]. Nevertheless, there are fewer studies about the use of this strategy to increase the cyanobacteria biomass [19–21], and it was found just one with *Spirulina* [22]. *Spirulina* is the most known cyanobacteria and one of the most-studied

microalgae in the world [1]. Thus, it is essential to improve the efficiency of its cultivation system. In this way, to define the adequate concentration of phytohormones to cultivate the prokaryotic microalgae can be a tool very useful.

In addition, a question yet no study is the sampling frequency of the cultures supplemented with phytohormone. It decreases in the sampling frequency could be a factor that improves the phytohormone uptake mechanism by microalga because the contact time between the substances and the cells become higher. This relation is similar to the occurred at *Dictyosphaerium pulchellum*, *Chlorella* sp., *Micractinium pusillum*, *Scenedesmus armatus*, and *Scenedesmus acutus* cultivations, when the retention time was increased from daily to ten days to improve the nitrogen uptake mechanism. More contact time allowed that nitrogen concentration incorporated into algal cells was enhanced, as well as the biomass concentration [23]. Therefore, decreasing the sampling frequency of microalgae cultivation with phytohormones could allow more contact between cells and phytohormones. In the same way, could promote an increase in the concentration of microalgal biomass.

In this study, we investigated the effect of natural phytohormones supplementation on the growth of *Spirulina*. In addition, to determine if contact time is important to improve the phytohormone uptake by microalgae, we conducted experiments trying not to interfere in this process. Thus, we propose a decrease in sampling frequency to monitoring the parameters of the cultivations. In this way, the present study aimed to investigate the influence of sampling frequency on the production of biomass and biomolecules of *Spirulina* sp. LEB 18 in cultivations supplemented with tZ and IAA.

Materials And Methods

Microalgae and cultivation conditions

Spirulina sp. LEB 18 was obtained from the Culture Collection of the Laboratory of Biochemical Engineering (LEB) at the Federal University of Rio Grande (FURG). This strain was isolated from Mangueira Lagoon (latitude 33°31' 08" S and longitude 53°22' 05" W) [24] and cultivated in Zarrouk medium [25]. The commercial phytohormones tZ and IAA (98 %; Sigma-Aldrich, Brazil) were supplemented in the cultures on day of inoculation from stock solutions. These were prepared by dissolving the phytohormones in 0.1 % (v/v) NaOH to obtain a final concentration of 0.1 mg mL⁻¹.

All experiments were performed in two replicates batch per treatment. The microalga was cultivated in 0.5 L Erlenmeyer photobioreactors (0.4 L useful volume), with an initial concentration of 0.2 g L⁻¹ (dry weight, DW); sterile distilled water was added daily to replenish evaporation. The assays were carried out in a culture chamber at 30 °C, with a 12 h light/dark photoperiod [26], illumination of approximately 70 µmol photons m⁻² s⁻¹ (promoted by fluorescent lamps), and agitation by air injection.

Primarily, to verify the effects of the supplementation in the kinetic parameters of the microalgae cultivation, an experiment was carried out for 15 days with supplementation of different concentrations

of tZ and IAA (0.01, 0.1, 1, and 10 mg L⁻¹). Sampling was performed daily (2 mL) to monitor biomass accumulation and assess kinetic parameters.

In the next set of experiments, the goal was to evaluate if decrease the sampling frequency is a successful strategy to improve the phytohormones uptake efficiency. The experiments were divided into two groups, one was 2 mL sampled daily (GD group), and the other was 5 mL sampled every ten days (G10 group). The GD group was supplemented with selected concentrations of tZ and IAA (Table 1), based on the results obtained from the previous experiments. The G10 group was supplemented with different concentrations of tZ and IAA (Table 1). In addition, these experiments also were investigated the effect of the combined supplementation between tZ and IAA in the cultivations. The proportions of tZ and IAA (Table 1) were determined from the results of biomass production and kinetic parameter analysis and were performed at the same G10 group conditions. Both groups had the cultivations were carried out for 30 days. Each group had a control assay (duplicate) without the addition of phytohormones, but which followed the sampling frequency conditions.

At the end of the experiments, the biomass was recovered from the liquid medium by centrifugation (Hitachi, Himac CR-GIII, Japan) at 9690 g at 20 °C for 20 min. The biomass was then washed with distilled water to remove salts, frozen at -80 °C, lyophilized for 48 h, and stored at -20 °C until characterization.

Evaluation of growth responses

To determine the effect of phytohormones supplementation and sampling frequency in the development of the microalgae the growth responses were monitored daily for GD group and once in 10 days for G10 group. Cell morphology was examined using an electronic optical microscope (AxioCanERc 5s Microscope camera, Zeizz, Germany). Biomass concentration was determined by measuring the optical density ($n=6$) at a wavelength of 670 nm using a spectrophotometer (Shimadzu UV/VIS UVmin-1240 spectrophotometer, Japan). For this purpose, a calibration curve of optical density versus dry weight biomass was constructed ($R^2 = 0,993$) [27].

For all cultivations, the maximum biomass concentration (X_{\max} , g L⁻¹) was determined by measuring the optical density of the cultures. Other parameters including maximum specific growth rate (μ_{\max} , d⁻¹), maximum biomass productivity (P_{\max} , mg L⁻¹ d⁻¹), and generation time (t_g , d) were calculated for the 15-day cultures. Specific growth rate was calculated by linear regression of the logarithmic growth phase obtained from the graph of ln X as a function of time (d). Maximum biomass productivity and generation time was determined using the Equations 1 and 2, where X_t is the biomass concentration (mg L⁻¹) at time t (d), X_0 is the biomass concentration (mg L⁻¹) at time t_0 (d), and μ_{\max} (d⁻¹) is the specific growth rate in the exponential growth phase.

$$P_{\max} = \frac{X_t - X_0}{t - t_0} \quad (1)$$

$$t_g = \frac{\ln(2)}{\mu_{\max}} \quad (2)$$

Quantification of macromolecules

The macromolecules were quantified in all biomass generated from the experiments conducted. The analyses were performed with lyophilized biomass stored properly. To each treatment were made four extractions replicates ($n=4$) and twelve analyses replicates ($n=12$) to the characterization analyses. The final composition was showed by mean percentage (%) of fraction macromolecule (g) in the final microalgal biomass (g) calculated to fresh weight (FW).

Lipid analysis was performed for the lyophilized biomass using the method of Marsh and Weinstein [28]. Lipids were extracted using the organic solvents chloroform and methanol (chloroform-to-methanol ratio, 1:2), colorimetrically quantified, and compared with the standard curve of tripalmitin.

For carbohydrate and protein analyses, extracts made of lyophilized biomass (5 mg) dissolved in distilled water (10 mL) was sonicated for 10 cycles (59 s on/off) using an ultrasonic probe (Cole Parmer, CPX 130, EUA) to break the cell wall and release intracellular material. Total carbohydrates were quantified using the phenol–sulfuric acid method [29] and compared to the standard curve of glucose. Protein content was quantified using the colorimetric method described by Lowry et al. [30]. The biomass was subjected to thermal and alkaline pretreatment to solubilize the proteins; and bovine serum albumin was used as the standard.

Statistical analysis

The results were compared using analysis of variance (ANOVA) followed by Fisher's test at a 95 % ($p \leq 0.05$) confidence level.

Results

Biomass concentration and kinetic parameters of 15-day cultures

The first set of analyses investigated the effect of phytohormones supplementation on the cultivations of *Spirulina* sp. LEB 18 for 15 days. Supplementation of 0.01 mg L^{-1} tZ showed significantly better kinetic parameters as compared to other concentrations of this phytohormone (Table 2). Therefore, this concentration was defined as the most promising to supplement tZ in the next set of experiments. In the IAA concentration selection, the supplementation of 0.1 mg L^{-1} was the condition with more potential to continue the assays. This supplementation showed results significantly higher than the control experiment for all parameters analyzed (Table 2).

The motivation to realized experiments with longer duration emerged through monitoring of the biomass concentration of *Spirulina* sp. LEB 18 with tZ and IAA supplementation obtained during cultured for 15 days (Fig. 1). Observing the results of this determination was possible to infer that the microalga was still growing during the last few days of cultivation because the biomass concentration was yet increasing.

Cell morphology

Phytohormones regulated the morphological development of *Spirulina* sp. LEB 18. Cultures supplemented with tZ (Fig. 2a) and IAA (Fig. 2b) was 5 mm in length, while normal trichomes of *Spirulina* exhibit a maximum length of 1 mm [31]. On the other hand, the cultivations supplemented with combinations of tZ and IAA no enhancement in microalga size (Fig. 2c), and its morphology was similar to the control experiment (Fig. 2d).

Macromolecular content

The composition of *Spirulina* biomass is an important factor governing its commercial use. Therefore, we quantified proteins, carbohydrates, and lipids present in the final biomass of *Spirulina* sp. LEB 18 supplemented with different concentrations of tZ and IAA using spectrophotometry (Table 3).

In general, IAA supplementation was more adequate than tZ to increase the content of proteins, lipids, and carbohydrates in the biomass. In G10 groups supplemented with 1 mg L⁻¹ and 10 mg L⁻¹ IAA, the protein and carbohydrate concentrations were significantly increased ($p \leq 0.05$), reaching approximately 70 % and 20 % of microalgal biomass, respectively. The lipid content in the biomass reached about 25 % in GD group supplemented with 0.1 mg L⁻¹ IAA and in G10 group supplemented with 0.01 mg L⁻¹ IAA (Table 3). The values obtained represent an increase of 30.2 % for protein, 60.1 % for carbohydrates, and 55.7 % for lipids as compared to the GD control group. When the phytohormones were supplemented together, the macromolecule content was lower than or similar to that of control group (Table 3).

Influence of sampling frequency on the production of biomass and biomolecules

The combination of phytohormone supplementation and low sampling frequency was very efficient in increasing the final biomass concentration of the cultivations. The maximum biomass concentration in G10 group supplemented with 1 mg L⁻¹ IAA was 75 % higher as compared to control experiment of GD group, which simulated the usual way of microalgae cultivation, without phytohormone supplementation and with daily sampling (Fig. 3). The final biomass concentration was 3.6 g L⁻¹ in G10 group supplemented with 1 mg L⁻¹ IAA, and only 2.1 g L⁻¹ in GD control group.

The sampling frequency alone was also a significant factor in increasing biomass concentration independent of phytohormone supplementation. This can be proved by X_{max} of the G10 control group (2.9 g L⁻¹), which was 43.3 % higher than that of GD control group (2.1 g L⁻¹) (Fig. 3). In addition, phytohormone supplementation alone could also positively influence biomass concentration independent

of sample removal frequency. Final biomass concentration of GD group supplemented with 0.01 mg L^{-1} tZ (2.6 g L^{-1}), was 24.6 % higher than that of GD control group (2.1 g L^{-1}) (Fig. 3). On the other hand, the combined supplementation between phytohormones was not successful. The supplementation of 0.1 mg L^{-1} tZ and 0.1 mg L^{-1} IAA resulted in a maximum biomass concentration equal to that in G10 control group. The combination of 0.01 mg L^{-1} tZ with 1 mg L^{-1} IAA and 1 mg L^{-1} tZ with 0.01 mg L^{-1} IAA resulted in lower biomass concentration as compared to the G10 control group.

In addition, in the results of macromolecules content there was a statistical difference ($p \leq 0.05$) between GD and G10 groups, even at same IAA concentrations, indicating that the sampling frequency influenced in the biomass composition. The supplementation of 0.1 mg L^{-1} IAA in the G10 group was 76.4 % and 108.9 % to proteins and carbohydrates, respectively, higher than to the same phytohormone concentration the GD group (Table 3). However, a contrasting result was obtained for lipid content. GD group supplemented with 0.1 mg L^{-1} IAA exhibited high lipid content (24.6 %), while G10 group supplemented with the same amount of IAA exhibited low lipid content (11.9 %). Meanwhile, the lipid content of G10 group supplemented with 0.01 mg L^{-1} IAA (23.9 %) was similar to that of GD group supplemented with 0.1 mg L^{-1} IAA.

Discussion

Our study was successful in proving that the sampling frequency is a key point to phytohormones efficiency, originating a new strategy to increase the biomass and macromolecule production of microalgae cultivation. The higher maximum biomass concentration of the G10 group compared to GD group results can be explained by the high sample removal frequency in the GD group. We hypothesize that during sampling, it is possible that a portion of the phytohormone as well as replicating cells could be withdrawn along with the sample, affecting phytohormone availability for microalgae growth.

For macromolecule content analyses there were observed different results between GD and G10 groups, even at same phytohormone concentrations, indicating that the sampling frequency influenced the results too. In our results, we demonstrated that the lipid production promoted by IAA follows a restricted phytohormone concentration range that can be added in cultivation. Supplementation of phytohormones above this concentration decreases the lipid content and increases the content of other macromolecules. This fact was observed from our approach of sampling frequency since, from it, we can let the phytohormone more or less available for the microalga consumption. Therefore, the sampling frequency study may be useful for auxiliary to enhance the content of biomass and any macromolecule in the microalgal biomass.

In addition, the results of macromolecular quantification imply that supplementation of phytohormones can be a versatile strategy to enhance the content of any macromolecule in the microalgal biomass. By simply modifying the concentration of phytohormone supplemented in the cultivation, it is possible to direct the metabolic flux towards the synthesis of one particular macromolecule. This finding corroborates the results of Yu et al. [10], who reported that carbon transport is facilitated for synthesis of

either lipids or carbohydrates depending on the concentration of phytohormone supplemented during cultivation. Nevertheless, the carbon partitioning mechanisms in microalgae are still not understood clearly.

When the phytohormones IAA and tZ were supplemented together, our results were unsatisfactory. The results of biomass concentration, macromolecular content, and the cell morphology were lower than or similar to that of the control experiment. From this, there is evidence to support the hypothesis that was no significant change in auxin–cytokinin balance, because according to Su et al. [8] is this factor that causes effects in the cells. Therefore, it is necessary to conduct new studies utilizing different proportions of tZ and IAA than that used in our study to affirm that there is no synergic action between the two phytohormones.

However, it was confirmed that the IAA and tZ supplementation influenced the *Spirulina* final biomass concentration in a dose dependent manner. Our results were showed that when the concentration of phytohormone increased, final biomass concentration also increased. Nevertheless, it looks like this trend occurs until a limited supplementation and if higher concentrations of phytohormones are added there is a reverse effect, i.e., decreased the maximum biomass concentration. This finding also reported by Saygideger and Okkay [22], who tested several concentrations of the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) in *Spirulina platensis* cultivations. The researchers noted that higher concentrations of 2,4-D inhibited the growth of the microalga. The toxic effect of these substances manifests when they are used at higher concentrations than those tolerated by the organism to which they are added [32].

There evidence to suggest that one of the causes of the increase of microalga biomass production from tZ and IAA supplementation is given by the enlargement of trichomes. We observed this phenomenon by microscopic monitoring and believed that cells appear enlarged as they are stretching considerably for dividing. This result is in agreement with the work of Park et al. [2], who evaluated the effects of IAA, gibberellic acid, kinetin, 1-triacontanol, and abscisic acid at concentrations between 0.1 to 10 ppm in *Chlamydomonas reinhardtii* strain CC124 cultivations. The authors found that treatment with all phytohormones resulted in enlargement of the cell diameter from less than 10 µm to 20 µm, and also observed increase in the biomass concentration.

Conclusion

Phytohormone supplementation combined with low sampling frequency proved to be an efficient strategy for increasing the biomass and macromolecule content for cultivating *Spirulina* sp. LEB 18.

Supplementation with 1 mg L⁻¹ IAA increased the biomass concentration by 75 %, protein content by 30 %, and carbohydrate content by 50 % as compared to the usual method of cultivation. In addition, by changing the concentration of phytohormones supplemented, it was possible to increase the production of any particular macromolecule, which demonstrates the versatility of the strategy. Our study provides

an initial basis for improving the microalgal biomass production on a larger scale utilizing an efficient and easy to implement a strategy.

Declarations

Funding

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Conflicts of interest/Competing interests

The authors confirm that there are no conflicts of interest/Competing interests.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Authors' contributions

Conceptualization: Jéssica Teixeira Silveira, Ana Priscila Centeno Rosa, and Jorge Alberto Vieira Costa; Methodology: Jéssica Teixeira Silveira and Ana Priscila Centeno Rosa; Formal analysis and investigation: Jéssica Teixeira Silveira; Writing - original draft preparation: Jéssica Teixeira Silveira; Writing - review and editing: Jéssica Teixeira Silveira, Ana Priscila Centeno Rosa, and Jorge Alberto Vieira Costa; Funding acquisition: Jorge Alberto Vieira Costa; Resources: Jorge Alberto Vieira Costa; Supervision: Ana Priscila Centeno Rosa and Jorge Alberto Vieira Costa.

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Tables

Table 1 Concentrations of trans-zeatin (tZ) and 3-indoleacetic acid (IAA) supplemented to Spirulina sp. LEB 18 cultures

Experiment	tZ (mg L^{-1})	IAA (mg L^{-1})	Preliminar	GD	G10
Control	0	0	YES	YES	YES
tZ0.01	0.01	0	YES	YES	YES
tZ0.1	0.1	0	YES	NO	YES
tZ1	1	0	YES	NO	YES
tZ10	10	0	YES	NO	YES
IAA0.01	0	0.01	YES	NO	YES
IAA0.1	0	0.1	YES	YES	YES
IAA1	0	1	YES	NO	YES
IAA10	0	10	YES	NO	YES
tZ0.01+IAA1	0.01	1	NO	NO	YES
tZ0.1+IAA0.1	0.1	0.1	NO	NO	YES
tZ1+IAA0.01	1	0.01	NO	NO	YES

Table 2 Maximum biomass concentration (X_{\max}), maximum biomass productivity (P_{\max}), maximum specific growth rate (μ_{\max}), and generation time (t_g) for 15-day Spirulina sp. LEB 18 cultures supplemented with different concentrations of trans-zeatin (tZ) and 3-indoleacetic acid (IAA)

Experiment	X_{\max} (g L ⁻¹)	P_{\max} (mg L ⁻¹ d ⁻¹)	μ_{\max} (d ⁻¹)	t_g (d)	*R ²	**Δt (d)
<i>trans</i>-Zeatin (tZ)						
Control	1.28 ^d ±0.08	76.3 ^c ±5.9	0.21 ^d ±0.01	3.28 ^d ±0.13	0.99±0.01	1-5
tZ0.01	2.15 ^a ±0.01	158.7 ^a ±5.1	0.34 ^a ≤0.01	2.03 ^a ±0.03	0.98±0.01	0-4
tZ0.1	1.76 ^b ±0.11	133.2 ^b ±25.5	0.27 ^c ±0.03	2.57 ^c ±0.25	0.98±0.01	0-4
tZ1	1.76 ^b ±0.04	143.5 ^{a,b} ±12.5	0.31 ^b ±0.01	2.22 ^b ±0.10	0.96±0.03	0-4
tZ10	1.56 ^c ±0.04	126.3 ^b ±17.8	0.29 ^c ±0.02	2.42 ^c ±0.13	0.99≤0.01	0-4
3-Indoleacetic acid (IAA)						
Control	1.28 ^b ±0.08	76.3 ^c ±5.9	0.21 ^b ±0.01	3.28 ^{b,c} ±0.13	0.99±0.01	1-5
IAA0.01	1.30 ^b ±0.05	77.5 ^c ±1.8	0.21 ^b ±0.02	3.36 ^c ±0.27	0.99≤0.01	1-5
IAA0.1	1.43 ^a ±0.05	90.7 ^a ±3.8	0.23 ^a ≤0.01	3.04 ^a ±0.06	0.99≤0.01	1-5
IAA1	1.45 ^a ±0.08	85.9 ^b ±3.8	0.23 ^a ±0.02	3.08 ^{a,b} ±0.23	0.99±0.01	1-5
IAA10	1.34 ^b ±0.03	86.6 ^{a,b} ±1.8	0.22 ^{a,b} ≤0.01	3.17 ^{a,b,c} ±0.05	0.99≤0.01	1-5

Different superscript letters in the same column correspond to a significant difference ($p \leq 0.05$) for the same phytohormone; *R²: coefficient of determination for linear regression applied to the log phase of growth; **Δt: initial time – end time of exponential growth phase. Results are presented as mean ± standard deviation (n = 6).

Table 3 Protein, carbohydrate, and lipid content of *Spirulina* sp. LEB 18 cultures supplemented with *trans*-zeatin (tZ) and 3-indoleacetic acid (IAA)

GD			
Experiment	Proteins (% w/w FW)	Carbohydrates (% w/w FW)	Lipids (% w/w FW)
Control	54.0 ^{b,C,D} ±1.2	13.3 ^{a,D,E} ±0.7	15.8 ^{b,B} ±0.3
tZ0.01	64.8 ^{a,B} ±2.1	10.9 ^{b,G} ±0.3	14.6 ^{b,B,C,D} ±0.7
IAA0.1	37.8 ^{c,I} ±1.8	9.0 ^{c,H} ±0.2	24.6 ^{a,A} ±2.6
G10			
Experiment	Proteins (% w/w FW)	Carbohydrates (% w/w FW)	Lipids (% w/w FW)
Control	52.3 ^{c,d,C,D,E,F} ±1.6	13.7 ^{d,D,E} ±0.4	13.0 ^{c,d,C,D,E} ±0.4
tZ0.01	54.6 ^{c,C} ±1.6	9.3 ^{e,f,H} ±0.3	15.0 ^{b,B,C} ±1.9
tZ0.1	54.7 ^{c,C} ±0.2	15.7 ^{c,C} ±0.1	15.5 ^{b,B} ±0.2
tZ1	51.1 ^{d,e,D,E,F} ±0.3	15.8 ^{c,C} ±1.3	15.7 ^{b,B} ±1.2
tZ10	49.4 ^{e,F,G} ±1.0	14.7 ^{c,d,C,D} ±0.9	12.8 ^{c,d,e,D,E} ±1.2
IAA0.01	53.2 ^{c,d,C,D,E} ±0.4	8.9 ^{f,H} ±0.4	23.9 ^{a,A} ±0.5
IAA0.1	66.7 ^{b,B} ±0.1	18.8 ^{b,B} ±1.7	11.9 ^{d,e,E,F} ±0.5
IAA1	70.3 ^{a,A} ±1.4	19.9 ^{a,b,A,B} ±0.3	13.0 ^{c,d,C,D,E} ±0.7
IAA10	67.7 ^{a,b,A,B} ±1.3	21.3 ^{a,A} ±0.7	14.4 ^{b,c,B,C,D} ±0.2
tZ0.01+ IAA1	43.0 ^{f,H} ±3.2	13.8 ^{d,D,E} ±0.7	11.2 ^{e,f,E,F} ±0.2
tZ0.1+IAA0.1	50.4 ^{d,e,E,F} ±0.8	13.8 ^{d,D,E} ±1.0	15.3 ^{b,B} ±0.3
tZ1+IAA0.01	36.5 ^{g,I} ±0.7	11.0 ^{e,G} ±0.1	9.9 ^{f,F} ±0.5

Different lowercase letters superscripted in the same column represent a significant difference ($p \leq 0.05$) for each response in the same group; different uppercase letters superscripted in the same column represent a significant difference ($p \leq 0.05$) for each response in comparison with all samples; *FW: fresh weight. Results are presented as mean ± standard deviation ($n = 12$).

Figures

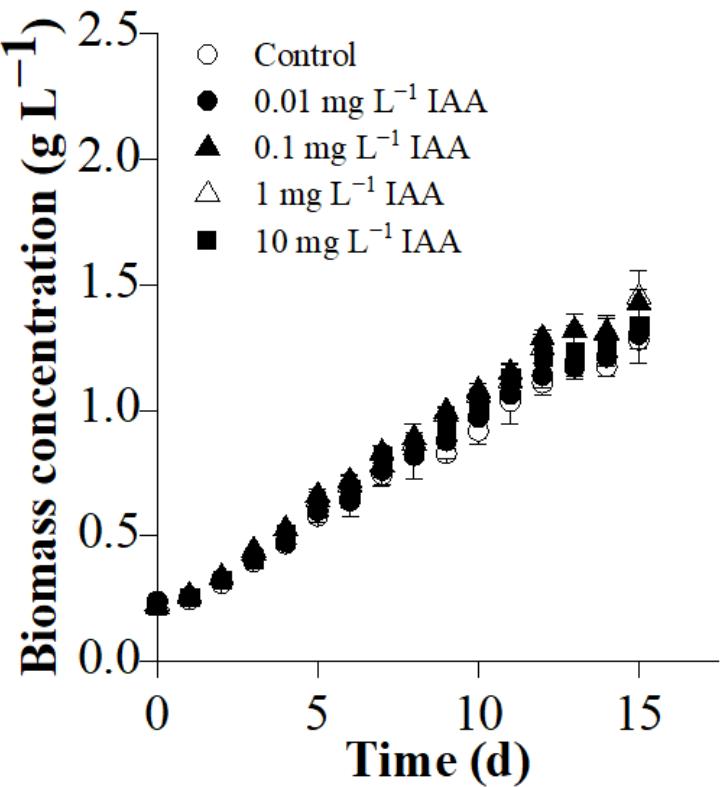
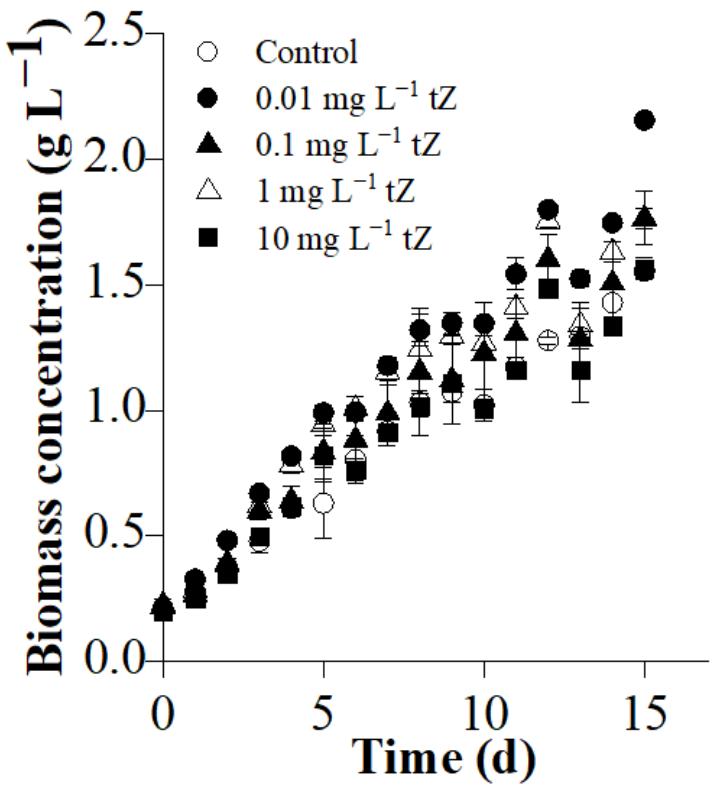


Figure 1

Biomass concentrations (DW) obtained during 15-day cultivation of *Spirulina* sp. LEB 18 cultures supplemented with (a) trans-zeatin (tZ) and (b) 3-indoleacetic acid (IAA) *Results are presented as mean \pm standard deviation ($n = 6$)

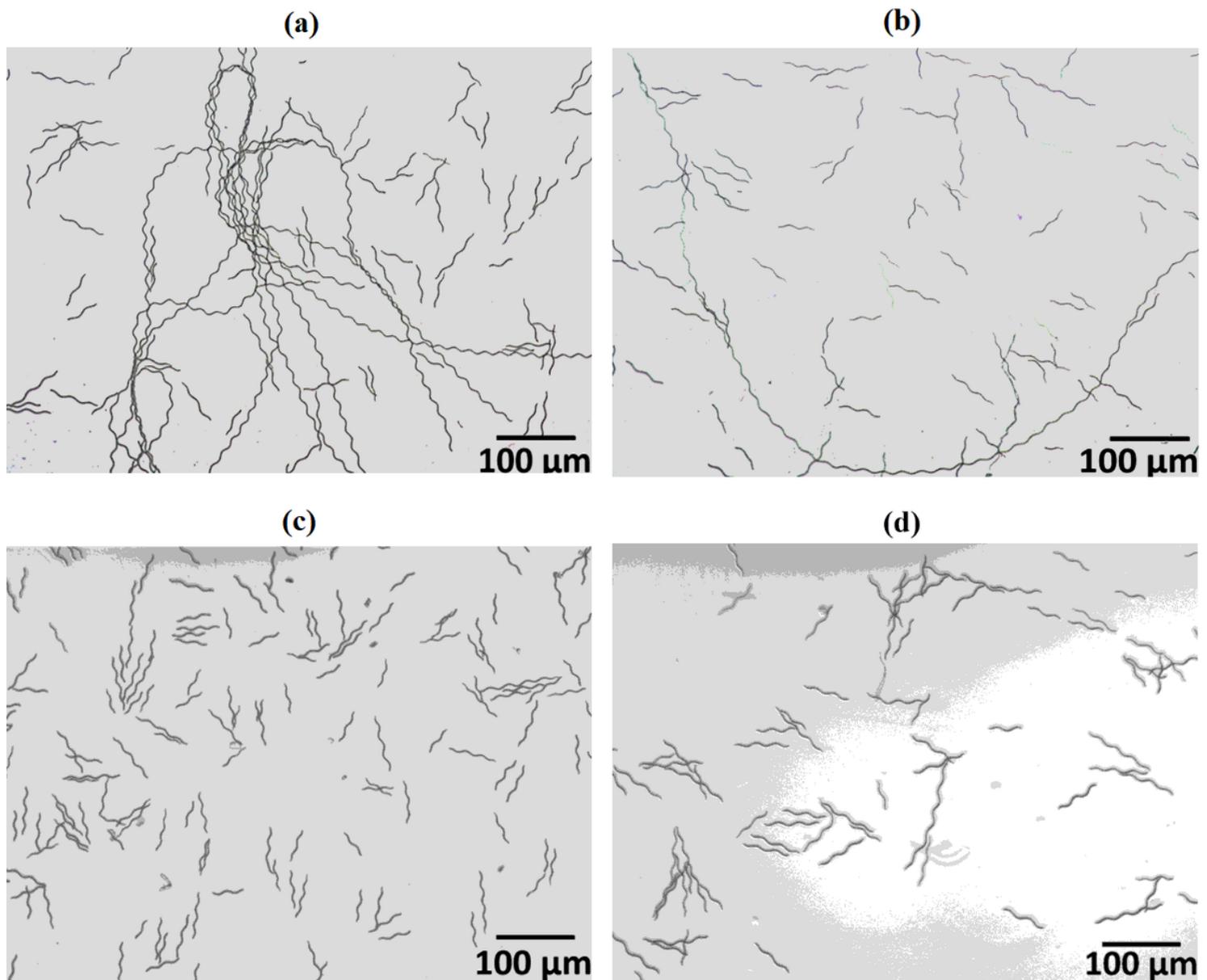


Figure 2

Effect of (a) trans-zeatin (tZ; 0.01 mg L⁻¹), (b) indole-3-acetic acid (IAA; 0.1 mg L⁻¹), and (c) tZ (0.1 mg L⁻¹) + IAA (0.1 mg L⁻¹) on the morphology of *Spirulina* sp. LEB 18; (d) is the control treatment *All micrographs are at 5x magnification

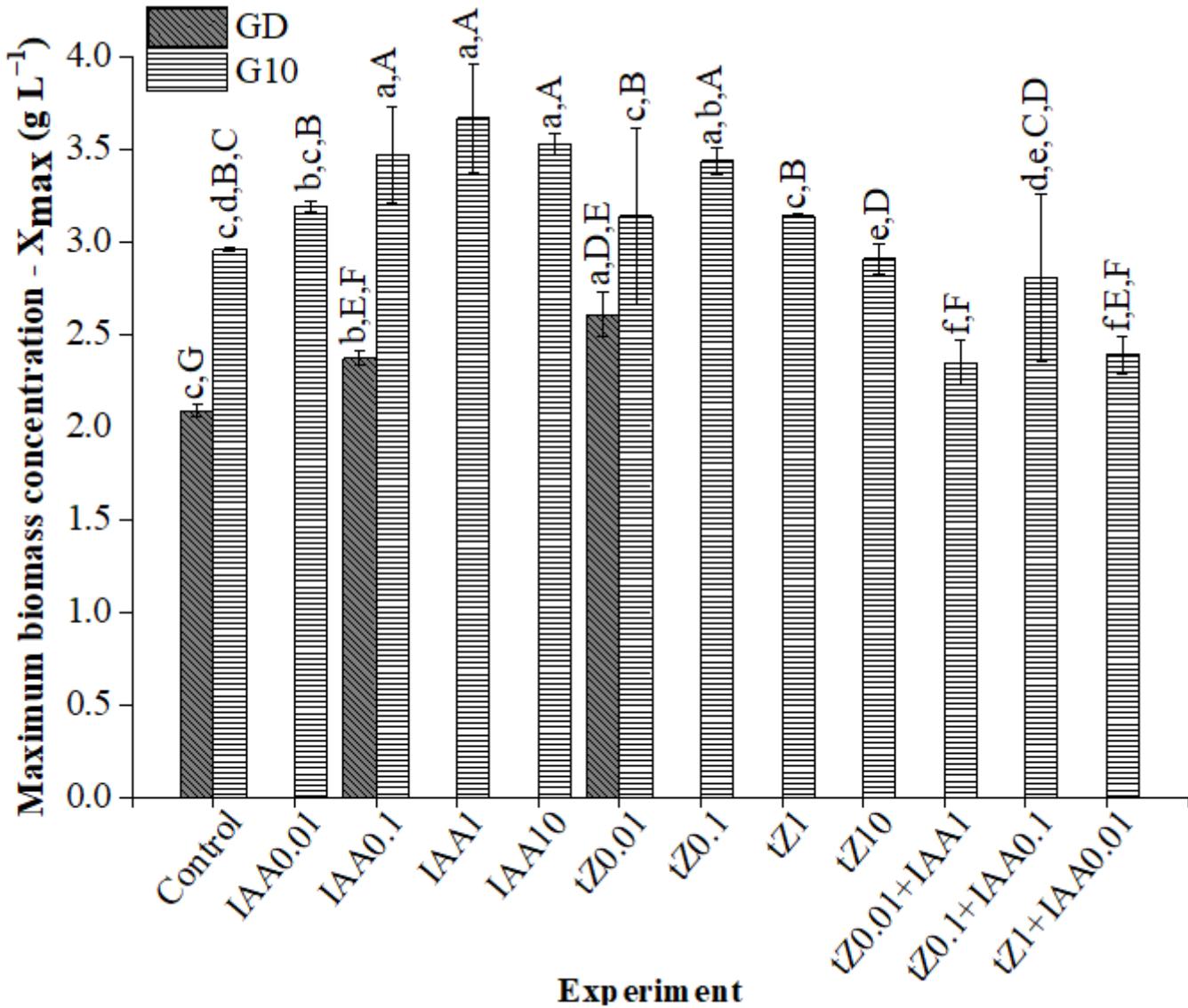


Figure 3

Maximum biomass concentration (X_{max}) for 30-day cultures of *Spirulina* sp. LEB 18 *Different lowercase letters on the same type of column represent a significant difference ($p \leq 0.05$) between experiments of the same group; different uppercase letters on the columns represent significant differences ($p \leq 0.05$) among all experiments **Results are presented as mean \pm standard deviation ($n = 6$)