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Spirulina platensis: process optimization to obtain biomass

Spirulina platensis: otimização de processo para a obtenção de biomassa

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Abstract

Spirulina platensis is a photoautotrophic mesophilic cyanobacterium. Its main sources of nutrients are nitrate, urea, and ammonium salts. *Spirulina* cultivation requires temperature, light intensity, and nutrient content control. This microalgae has been studied and used commercially due to its therapeutic and antioxidant potential. In addition, several studies have reported its ability to use CO₂, its immune activity, and use as an adjuvant nutritive factor in the treatment of obesity. The objective of this study is the production of biomass of *S. platensis* using different rates of stirring, nitrogen source, amount of micronutrients, and luminosity. A 2⁴ experimental design with the following factors: stirring (120 and 140 RPM), amount of nitrogen (1.5 and 2.5 g/L), amount of micronutrients (0,25 and 0,75 mL/L) (11 and 15 W), and luminosity was used. Fermentation was performed in a 500 mL conical flask with 250 mL of culture medium and 10% inoculum in an incubator with controlled stirring and luminosity. Fermentation was monitored using a spectrophotometer (560 nm), and each fermentation lasted 15 days. Of the parameters studied, luminosity is the one with the highest significance, followed by the amount of nitrogen and the interaction between stirring and micronutrients. Maximum production of biomass for 15 days was 2.70 g/L under the following conditions: luminosity 15W; stirring, 120 RPM; source of nitrogen, 1.5 g/L; and micronutrients, 0.75 mL/L.

Keywords: *Spirulina platensis*; human food; biomass; cyanobacteria.

Resumo

A *Spirulina platensis* é uma cianobactéria mesofílica, fotoautotrófica, que utiliza como nutrientes os nitratos, ureia e sais de amônio. O cultivo necessita de controle de temperatura, intensidade de luz e do teor de nutrientes utilizados. Esta microalga tem sido estudada pelo seu potencial nutricional, antioxidante, terapêutico e adjuvante em tratamento de obesidade. O objetivo deste trabalho foi a produção de biomassa de *S. platensis* em diferentes níveis de agitação, fonte de nitrogênio, quantidade de micronutrientes e luminosidade. No estudo desenvolvido na Universidade Guarulhos, foi realizado um planejamento experimental 2⁴ com os parâmetros: agitação, fonte de nitrogênio, quantidade de micronutrientes e luminosidade. A fermentação foi feita em erlenmeyer de 500 mL, com 200 mL de meio de cultura, e 10% de inóculo, em estufa com agitação e luminosidade controlada, por 15 dias e o acompanhamento realizado através de leitura em espectrofotômetro (560 nm). Dos parâmetros estudados o que apresentou maior significância foi a luminosidade, seguida da quantidade de fonte de nitrogênio e a interação agitação e micronutrientes. A produção máxima em biomassa por 15 dias foi de 3,24 g/L nas condições de luminosidade 15 W, agitação em 120 RPM, fonte de nitrogênio 1,5 g/L e micronutrientes em 0,75 mL/L.

Palavras-chave: *Spirulina platensis*; alimento; biomassa; cianobactéria.

1 Introduction

The blue-green algae are perhaps the oldest and most primitive forms of life on Earth. Fossilized microorganisms dating 3.5 billion years have been found. Algae have been used as human food since ancient times in various parts of the world such as China, India, Mexico, North America, Peru, Japan, and others.

The great nutritional interest for the production of algal biomass is due to its high protein content (60 to 65% in biomass of *Spirulina* sp.), high digestibility, and balanced content of some essential amino acids. Obtaining biomass of *Spirulina* sp. can be a representative example of algal production. Some production strategies include the use of

fed batch type process (for controlling nutrient) and simple batch process. *Spirulina platensis* is an African species which develops in middle high temperatures (25-26 °C), alkaline pH (9.5 to 11), and high salt concentrations ranging from 8.5 to 200 g/L; therefore, their production is relatively simple and does not require a complex culture medium and high cost, thus enabling carrying out the present study (ARRUDA et al., 2009; PELIZER et al., 2002; PELIZER; MORAES, 2009).

Arthrospira (*Spirulina*) sp., is a filamentous cyanobacterium, autotroph, also known as blue-green algae, which owes its name to the nature of its helical or spiral filaments. This microorganism grows naturally in many places, particularly in shallow water

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bodies and in the presence of sodium bicarbonate, an alkaline medium of high salinity (VONSHAK; TOMASELLI, 2000).

Some microalgae such as *Spirulina* and *Chlorella* are certified GRAS (Generally Recognized As Safe) and can be used as food without offering health risk. Since June 23rd, 1981, *Spirulina* has been legally approved by the FDA (Food and Drug Administration), which confirmed that

Spirulina is a source of protein and contains several vitamins and minerals. *Spirulina* can be legally marketed in the United States as a food as long as it is labeled accurately and contains no contaminated or adulterated substances (FOX, 1996, p. 89).

The cyanobacterium *Spirulina* has been marketed and studied because of its nutritional and therapeutic properties for the treatment of diseases such as cancer, hypercholesterolemia, and atherosclerosis. Certain compounds present in *Spirulina*, such as phenolic compounds, phycocyanin, and tocopherol may have antioxidant activity (COLLA; FURLONG; COSTA, 2007; MULITERNO et al., 2005; ANDRADE; CAMERINI; COSTA, 2008).

Nutritional studies show that these microorganisms have one of the highest protein contents ever found, high nutritional value, good digestibility, and all essential amino acids in the proportions recommended by FAO, with the exception of methionine. (AVILA-LEON et al., 2010). Some technical aspects of *Spirulina* production have advantages over those of other of photoautotrophs, such as its easy harvest from the culture medium due to its spiral shape and larger size (diameter of 10 µm and length of tens to hundreds of µm). The biomass production can vary according to the culture medium used and the conditions of the external environment. The manipulation of culture conditions can promote the biosynthesis of various compounds, and in addition, one can control these variables in order to reduce the costs of the production process (REINEHR, 2001).

Avila-Leon et al. (2012) investigated the production of single-cell proteins from *Arthrospira platensis* due to its biomass composition and ability to grow in alternative media. They evaluated the effects of different dilution rates (D) and urea concentrations (N_0) on *A. platensis* continuous culture in terms of growth, kinetic parameters, biomass composition, and nitrogen removal.

The dependence of cell growth as a function of light intensity has been observed, i.e. there is a correlation between the increase of luminous energy received and cell growth, although limited by certain enzymes produced during growth (PELIZER et al., 2002). This boundary is called light saturation point, which is 5-10 Klux for cyanobacteria (BALLONI et al., 1980). This experimental finding indicates that the maximum cell growth rate is limited by other processes besides the assimilation of carbon and nitrogen.

Since *Spirulina* sp. is autotrophic, light intensity causes the metabolites to vary its biomass according to the incidence of light, ie higher production under higher light intensities with shorter feed (BEZERRA, 2006).

Arruda et al. (2009) states that under the conditions studied, the one that yielded better results in terms of biomass production in all trials was the bioreactor containing 300 mL of culture medium Paoletti et al. modified with half of the nitrogen source (KNO_3). Working with the viability of the production of *Spirulina platensis* in a plastic reactor (polypropylene with 1 L volume), the best results in terms of biomass production was achieved in the treatment with 300 mL of medium and 15% of inoculum with an average yield of 2.32 g/L in 18 days of fermentation.

Arruda et al. (2009) investigated the viability of the production process of *Spirulina platensis* in a plastic reactor (polypropylene with 1 L volume) facilitating its transport and reducing the cost of production. The best results for the production of biomass found were with 300 mL volume of culture medium and 15% of inoculum with an average yield of 2.32 g/L in 18 days of fermentation.

Pelizer et al. (2008), determined the total protein content by the micro-Kjeldahl method and obtained an average of 49%.

Under the conditions examined, the one which yielded better results in terms of biomass production in all trials was the bioreactor containing 300 mL of culture medium modified with half of the nitrogen source (KNO_3). *S. platensis* was grown in an autotrophic culture medium with sodium bicarbonate and mixotrophic with 0.25, 0.50, 0.75, and 1.00 g/L of dried molasses (MEP) or liquid molasses (MEL); the growth was completed in 35 days. Maximum biomass concentration (2.83 g/L) and a yield of 0.098 g/L day were reaches in cultures with MEL 0.50 g/L. Cultures with MEP 0.25 g/L reached biomass concentration of 2.59 g/L and productivity of 0.066 g/L.day. It can be concluded that molasses can be used to obtain biomass of algae to help combat malnutrition (ANDRADE; COSTA 2008).

The cultivation of *S. platensis* with the addition of glucose was also studied. A 2^3 factorial design was used for the cultivation, and the variables were: the concentration of glucose (0.5 g/L and 1.0 g/L), the dilution of Zarrouk medium (50% and 75%), and illumination (1800 lux and 3000 lux). The maximum concentration achieved was 5.38 g/L with a growth rate of 0.0063 h⁻¹ and 0.5 g/L glucose, medium dilution of 75%, and illuminance of 3000 lux (MULITERNO et al., 2005).

The present study investigated the production of biomass of *S. platensis* under different rates of stirring, nitrogen source concentration, amount of micronutrients, and luminosity, as well as the influence of these culture medium factors on the production of *Spirulina platensis* focusing on biomass increase under different rates of stirring, nitrogen source, amount of micronutrients, and luminosity.

2 Materials and methods

The factors considered in this experiment were stirring, light, nitrogen content, and amount of micronutrients. Fermentation was performed in a 500 mL conical flask with 250 mL of culture medium and 10% inoculum in an incubator with controlled stirring and light. Fermentation monitoring was carried out using a spectrophotometer (560 nm), and each fermentation lasted 15 days. A 2^3 experimental design was used, and the values of each parameter studied are shown in Table 1.

All experiments were performed in duplicate. The variation in these values was used to verify the effect of the factors on the production of bioproducts.

The medium used for the fermentation is shown in Table 2. Experiments were performed in duplicate using 500 mL of each culture medium.

Table 1. Values assigned to each parameter studied for the production of *S. platensis*.

Parameters	Values	
	+	-
Stirring	140 rpm	120 rpm
Light	15 W	11 W
Nitrogen	2.5 g/L	1.5 g/L
Micronutrients	0.75 mL/L	0.25 mL/L

Table 2. Composition of mineral medium (assuming a volume of 500 mL)

	++	+-	-+	--
NaCl	0.46 mg	0.46 mg	0.46 mg	0.46 mg
Na ₂ SO ₄	0.94 mg	0.94 mg	0.94 mg	0.94 mg
K ₂ HPO ₄	0.25 mg	0.25 mg	0.25 mg	0.25 mg
Na ₂ CO ₃	4.45 mg	4.45 mg	4.45 mg	4.45 mg
NaHCO ₃	7.58 mg	7.58 mg	7.58 mg	7.58 mg
CaCl ₂ ·2H ₂ O	0.025 mg	0.025 mg	0.025 mg	0.025 mg
KNO ₃	1.25 mg	1.25 mg	0.75 mg	0.75 mg
MgSO ₄	0.13 mg	0.13 mg	0.13 mg	0.13 mg
EDTA solution	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Micronutrients	0.40 mL	0.20 mL	0.40 mL	0.20 mL

3 Results and discussion

The response of the experimental design was achieved by the absorbance read at the end of the experiment, which was converted into biomass (g/L) by curve fitting. (Equation 1). These results are shown in Table 3 and Figure 1. Since the experiments were performed in duplicate, the average of the values of each experiment was used.

$$\text{Biomass} = (\text{absorbance reading}) * 1.0955 + 0.506 \quad (1)$$

Table 3. Production of *S. platensis* under different conditions of agitation, light, amount of nitrogen and micronutrient.

Assay	Experimental design				Biomass (g/L)
	Stirring	Light	Nitrogen	Micronutrients	
1	+	+	+	+	2.3705
2	+	+	+	-	2.5738
3	+	+	-	+	2.2194
4	+	+	-	-	2.3530
5	+	-	+	+	1.1397
6	+	-	+	-	1.3085
7	+	-	-	+	1.1797
8	+	-	-	-	1.3085
9	-	+	+	+	2.4631
10	-	+	+	-	2.5524
11	-	+	-	+	2.7003
12	-	+	-	-	1.9838
13	-	-	+	+	1.2493
14	-	-	+	-	1.2641
15	-	-	-	+	1.0554
16	-	-	-	-	0.9825

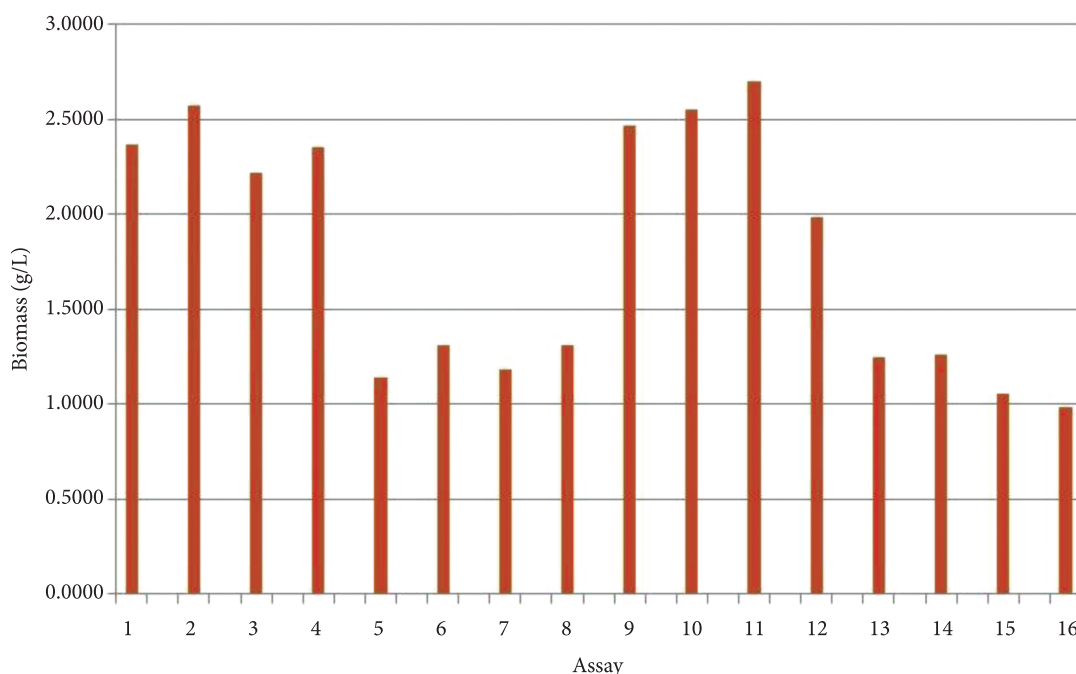


Figure 1. Production of *S. platensis* varying stirring, light, amount of nitrogen, and micronutrients.

The experimental results summarized in Table 3 were analyzed using the following software programs: Essential Regression (ER) and Essential Experimental Design (EED) for Excel 97/2000/2002/2003 (<http://www.jowerner.homepage.t-online.de/download.htm>) for effect estimation, as shown in Table 4.

The following figures show the production trends. Figure 2 shows that production increased with increased amount of light and nitrogen source. Figure 3 shows that for production increase, stirring rate and micronutrients should have opposite values, i.e., if stirring rate is increased, less amount of micronutrients should be used; and with decreased stirring rate, greater amount of nutrients should be used.

The results found are similar to those found by Muliterno et al. (2005); the maximum cellular concentration of 5.38 g.L⁻¹ and maximum specific growth rate of 0.0063 h⁻¹ were obtained with a glucose concentration of 0.5 g.L⁻¹, 50% dilution, and 1800 lux of illuminance.

Andrade and Costa (2008) found maximum biomass concentration of 2.83 g.L⁻¹ and productivity of 0.098 g.L⁻¹.day⁻¹, and these results were reached in cultures with MEL (liquid molasses) of 0.50 g.L⁻¹.

Kornfeld et al. (2003), introduced a new culture medium for the production of *Spirulina platensis* biomass based on an alkaline solution from citrus industry effluent supplemented with sources of nitrogen, phosphorus, and potassium, which

had an absorbance of 1024 and is equivalent to 5.1g.mL⁻¹ in a 5 day experiment.

The use of half of micronutrient concentration showed that biomass and absorbance values were lower when compared with those found by Andrade and Costa (2008) and Kornfeld et al. (2003), but the culture medium of these experiments were supplemented with more expensive nutrient sources.

Lacaz-Ruiz (2003) conducted an experiment to evaluate the biomass production of *Spirulina platensis* in Lacaz-Ruiz media prepared with ash and *Eucalyptus* spp. in Paoletti culture medium.

Both media were prepared using the same following culture conditions: amount of inoculum (75 mL), light intensity, reading at a wavelength of 560 nm, agitation, and temperature (33.0 ± 1.0 °C). The results of the initial and the final absorbance for the Paoletti culture medium were 0447 and 1037, respectively, and for the Lacaz-Ruiz culture medium they were 0329 and 0831, respectively, during 6 days of experiment.

Spirulina platensis cultivated for 23 days under controlled temperature (30 °C) and light intensity in three different media: Paoletti medium (control), Paoletti medium supplemented with 1 g.L⁻¹ NaCl (saline water), and medium prepared according to Paoletti culture medium with desalinator effluent; the cellular concentrations results were: 2.587, 3.545, and 4.954 g.L⁻¹ respectively, similar to those found by Volkman et al. (2008).

Table 4. Influence of each factor studied on the results obtained by experimental design.

		Biomass = b0 + b1*L + b2*N + b3*A*M					
		P value	Error	-80%	80%	t Stat	VIF
b0	1.794	5.29728 E ⁻²⁵	0.04980	1.729	1.859	36.03	b1
b1	0.608	9.87966 E ⁻¹³	0.04980	0.543	0.673	12.21	1.000
b2	0.07117	0.164	0.04980	0.00581	0.137	1.429	1.000
b3	-0.08247	0.109	0.04980	-0.148	-0.01711	-1.656	1.000

Where: L = light, N = nitrogen content, A = stirring and M = amount of micronutrients.

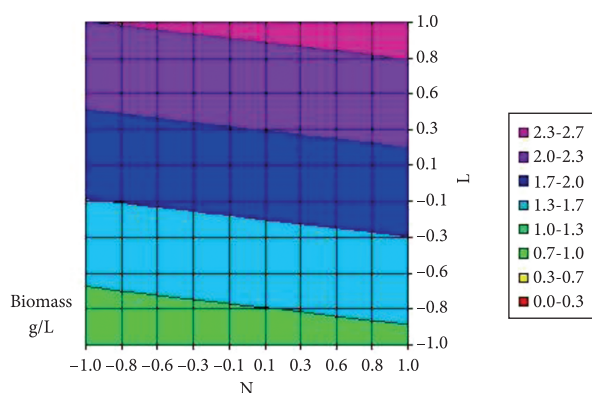


Figure 2. Interaction between the factors light (L) and quantity of nitrogen (N) for the production of *S. platensis* biomass.

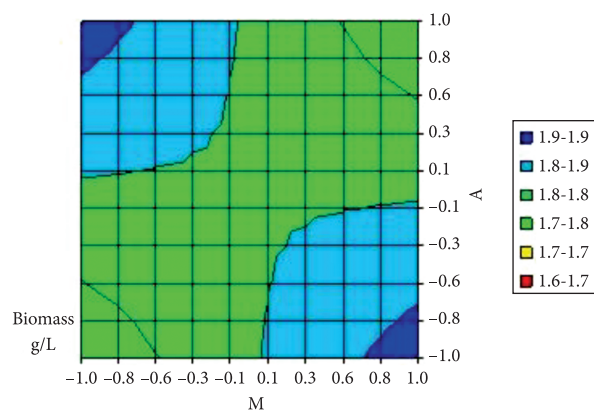


Figure 3. Interaction between the factors stirring (A) and amount of micronutrients (M) for the production of *S. platensis* biomass.

4 Conclusion

Light intensity and nitrogen source had greater influence on the production of *Spirulina platensis*.

The increase in light intensity favored the production of biomass, and this was the factor that most influenced the present study.

When 2.5 g/L of Nitrogen instead of 1.5 g/L was used, there was an increase in biomass.

Stirring rate and amount of micronutrients had no influence on the biomass in the conditions studied, but the interaction between these factors had an antagonistic effect.

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