Mixotrophic growth of *Nostoc* sp. on glucose, sucrose and sugarcane molasses for phycobiliprotein production

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ABSTRACT. Glucose, sacarose, and sugarcane molasses were tested as substrates for production of biomass and phycobiliproteins by *Nostoc* sp., varying their concentrations in relation to a mineral medium, BG11. All substrates increased the biomass and phycobiliproteins when compared with the control. Sugarcane molasses showed to be the best substrate for production of both biomass and phycobiliproteins. Greater biomass production occurred in sugarcane molasses 1.0 g L^{-1} and it was 5.7 times greater than the control. With glucose, it was in 2.5 g L^{-1} and sucrose, in 1.5 g L^{-1}, reaching 2.5 and 4.8 times greater than the control, respectively. For phycobiliproteins, the major production was in sugarcane molasses 1.0 g L^{-1}, 12.5 times greater than the control. With glucose, it was in 1.0 g L^{-1} and sucrose, in 0,5 g L^{-1}, reaching 3.0 and 4.5 times greater than the control, respectively. The *Nostoc* sp. assayed can grow mixotrophically, using glucose, sucrose, and sugarcane molasses as organic substrates, and a greater production of biomass and phycobiliproteins can be reached when compared with the autotrophic growth.

Key words: microalgae, cyanobacteria, *Nostoc* sp., mixotrophic culture, phycobiliprotein production, photoheterotrophic growth.

RESUMO. Crescimento mixotrófico de *Nostoc* sp. Glucose, sacarose e melaço de cana-de-açúcar foram testados como substratos para produção de biomassa e ficobiliproteinas. Todos os substratos aumentaram a biomassa e ficobiliproteinas em relação ao controle, meio mineral BG_{11}. Melaço de cana-de-açúcar foi o melhor substrato tanto para a produção de biomassa como de ficobiliproteinas. A maior produção de biomassa ocorreu usando melaço de cana-de-açúcar 1,0 g L^{-1} sendo 5,7 vezes maior que o controle. Com glucose foi em 2,5 g L^{-1} e sacarose 1,5 g L^{-1}, sendo 2,5 e 4,8 vezes maior que o controle, respectivamente. A maior produção de ficobiliproteinas ocorreu usando melaço de cana-de-açúcar 1,0 g L^{-1} sendo 12,5 vezes maior que o controle. Com glucose foi em 1,0 g L^{-1} e sacarose 0,5 g L^{-1}, 3,0 e 4,5 vezes maior que o controle, respectivamente. *Nostoc* sp. testado pode crescer mixotroficamente, usando glucose, sacarose e melaço de cana-de-açúcar como substratos orgânicos, uma maior produção de biomassa e ficobiliproteinas podendo ser alcançada nessas condições quando comparadas com o crescimento autotrófico.

Palavras-chave: microalgas, cianobactérias, *Nostoc* sp., cultivo mixotrófico, produção de ficobiliproteínas, crescimento fotoheterotrófico.

Introduction

Microalgae cultivation represents an efficient biological system for solar energy utilization, aiming the production of biomass and natural products with high economical value (Pinotti and Segato, 1991; Skulberg, 1994; Borowitzaka, 1999). In the case of cyanobacteria, their most striking characteristic are the presence of accessory photosynthetic pigments called phycobiliproteins, with potential use as natural food colors, pigments for cosmetics and fluorescent markers for cells and biomolecules (Oi *et al*., 1982; Silva *et al*., 1989; Sohn and Sautter, 1991; Arad and Yaron, 1992; Moreno *et al*., 1995).

The presence of organic compounds in the culture medium can interfere in the growth of cyanobacteria in many ways: being assimilated and used as carbon source, not being used or inhibiting the cell growth (Fay, 1983).

Aiming at the optimization in the production of biomass and phycobiliproteins, some researchers have explored, with success, the capability of the cyanobacteria to combine a model of autotrophic growth with a heterotrophic metabolic system, called mixotrophic.

Marquez *et al*. (1995) compared autotrophic, heterotrophic and mixotrophic growth of *Spirulina*

platensis in relation to the production of biomass and pigments at different light intensities. For all light intensities, the increase of biomass and pigments produced, in mixotrophic culture, was 1.5 to 2.0 times greater than in autotrophic culture.

Chen *et al*. (1996) used glucose and acetate to enhance cell growth and phycocianin production of *S. platensis* in batch cultures under continuous illumination. Later, Chen and Zhang (1997) showed an even higher biomass and phycocyanin production using a fed-batch system with glucose as carbon substrate.

Chojnacka and Noworyta (2004) showed that *Spirulina* sp. was able to grow photoautotrophically, heterotrophically (on glucose) and mixotrophically, and proposed kinetic models to describe the microalgal culture system.

Sugarcane molasses, a by-product of sucrose production is the most inexpensive raw material widely used for fermentation in the world. Brazil has more than 13.5 millions of hectares planted of sugarcane, being the first producer of sugar from sugarcane in the world (Rambla *et al*., 1999).

This study aimed to investigate the capability of one isolate of *Nostoc* sp. to grow in mixotrophic culture, testing glucose, sucrose and sugarcane molasses as substrates.

Material and methods

Strain and growth conditions

The cyanobacterium *Nostoc* sp. was isolated in the laboratory of Biochemistry of state University of Londrina, state of Paraná, Brazil and classified by Dra. Célia Sant'Ana, from the Botanical Institute, São Paulo, state of São Paulo, Brazil. It was grown in batch cultures in a rotatory shaker at 120 rpm and 28ºC ± 2ºC, in 250 mL erlenmeyer flasks with 50mL of medium. The illumination of 500 lux was continuous, provided by two fluorescent lamps and measured in the culture surface by a luximeter. The media were BG11 medium (Stanier *et al*., 1971) or BG11 without bicarbonate (BG 11_0) supplemented with glucose (0.5; 1.0; 1.5; 2.0; 2.5; 5.0 and 10.0 g L^{-1}), sucrose (0.5; 1.0; 1.5; 2.0 and 2.5 g L^{-1}) or sugarcane molasses (0.5; 1.0; 1.5; 2.5 and 5.0 g L^{-1} as total sugar). The sugarcane molasses were obtained from COROL (Agricultural Cooperative - Rolândia, state of Paraná, Brazil). The molasses composition in total sugar was 62.0% (w/w) and in reducing sugar, 11.4% (w/w). It was diluted to 10% (w/w) of total sugar to be used in the culture medium. All the experiments were performed in triplicate.

Biomass

The biomass was determined by dry weight measurements. The cells were centrifuged at 12,000 xg, 20 minutes, 4ºC, washed with distilled water twice and dried at 70ºC until constant weight.

Phycobiliproteins

The cell suspension obtained by centrifugation was submitted to ultrasonic cycles to break the cells, and the cell debris were subsequently removed by centrifugation at 12,000 xg, for 20 minutes at 4ºC. The supernatant was precipitated with $(NH_4)_2SO_4$ 20% saturated, kept overnight at 4ºC and centrifuged at 12,000 xg, for 20 minutes at 4ºC. The phycobiliproteins were measured in the supernatant, spectrophotometrically at 560 nm and 620 nm for determination of phycoerithrin and phycocyanin, respectively (Jorge *et al*., 1999/2000).

Carbohydrate quantification

Total carbohydrate was measured according to the phenol-sulfuric method (Dubois *et al*., 1956). Reducing sugar was measured using Somogyi-Nelson method (Nelson, 1944).

Statistical Analysis

Statistical analysis was performed by ANOVA (one way analysis of variance) applied to different concentrations of glucose, sucrose and sugarcane molasses separately, in order to test the biomass, phycoerytrin and phycocyanin productions by each substrate, with the means compared by Tukey's test (Sokal and Rohlf, 1981). The confidence level was set at 5%.

Results

First of all, the capability of *Nostoc* sp. to grow mixotrophically was evaluated using glucose as a substrate. Table 1 and Figure 1 show the effect of glucose concentration on biomass and phycobiliprotein production. When *Nostoc* sp. was grown in $BG11_0$ medium with this substrate, there was an increase in biomass with the increase in glucose concentration. The best production of biomass occurred with 2.5 g L^{-1}, being 2.5 times greater than the control. With 5.0 g L^{-1} and 10.0 g L^{-1} of glucose, occurred the complete inhibition of growth. Also phycobiliprotein production was greater in the supplemented medium, increasing until 1.0 g L^{-1}, being 3.0 times greater than the control and thereafter decreasing to the level of the control.

Table 1. Averages (n=3) of biomass, phycoerytrin and phycocyanin productions by Nostoc sp., supplemented with different concentrations of glucose.

Glucose (g L^{-1})	Biomass (g L^{-1})	Phycoerytrin Absorbance	Phycocyanin Absorbance
0.0($BG11_0$)	0.3247^{A}	0.990^{A}	0.613^{A}
0.5	$0.5010^{A, B}$	$2.130^{A, B}$	$1.467^{A, B}$
1.0	$0.5840^{A, B}$	2.633^{B}	1.897^{B}
1.5	$0.7097^{A, B}$	$2.217^{A, B}$	$1.593^{A,B}$
2.5	0.7845^{B}	1.980^{B}	1.475^{B}

Averages followed by the same capital letters in the column do not differ statistically for the Tukey's Test at 5% probability.

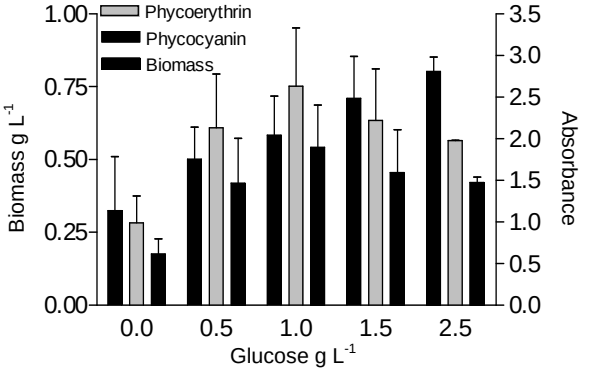

Figure 1. Effect of glucose concentration on biomass and phycobiliprotein productions by *Nostoc* sp.

The capability of *Nostoc* sp. to use sucrose was tested because sucrose is the main sugar in sugarcane molasses. Table 2 and Figure 2 show the effect of sucrose concentration on biomass and phycobiliprotein production. In relation to the growth in $BG11_0$ supplemented with sucrose, there was an increase in biomass up to 1.5 g L^{-1}, decreasing at the level of the control in 2.5 g L^{-1} of sucrose. The increase in 1.5 g L^{-1} was 4.8 times greater than the control. Phycobiliprotein production was greater in the medium containing 0.5 g L^{-1} of sucrose, reaching 4.5 times greater than the control.

Table 2. Averages (n=3) of biomass, phycoerytrin and phycocyanin productions by Nostoc sp., supplemented with different concentrations of sucrose.

Sucrose (g L^{-1})	Biomass (g L^{-1})	Phycoerytrin Absorbance	Phycocyanin Absorbance
0.0($BG11_0$)	0.2150^{A}	0.2155^{A}	0.1235^{A}
0.5	0.3350^{B}	0.8430^{B}	0.6260^{B}
1.0	0.7790^{C}	$0.4100^{A, B}$	$0.2935^{A, B}$
1.5	1.0440^{D}	$0.5990^{A, B}$	0.4250^{B}
2.5	$0.2265^{A, B}$	$0.2805^{A,}$	0.1760^{A}

Averages followed by the same capital letters in the column do not differ statistically for the Tukey's test at 5% probability.

Table 3 and Figure 3 show the effect of sugarcane molasses, given in g L^{-1} of reducing sugar, on biomass and phycobiliprotein production. With sugarcane molasses an increase was obtained in biomass in relation to the control in all concentrations. There was an increase up to 1.0 g L^{-1} remaining constant at 1.5 g L^{-1} and 2.5 g L^{-1}, then decreasing, 5.0 g L^{-1} not significantly differing from the control. The medium with 1.0 g L^{-1} of sugarcane molasses produced 5.7 times more than the control. With respect to the phycobiliproteins, they followed the production of biomass until 1.0 g L^{-1} except in greater concentrations of substrate when there was a decrease at the level of the control. The production of phycobiliproteins in 1.0 g L^{-1} was 12.5 times greater than the control.

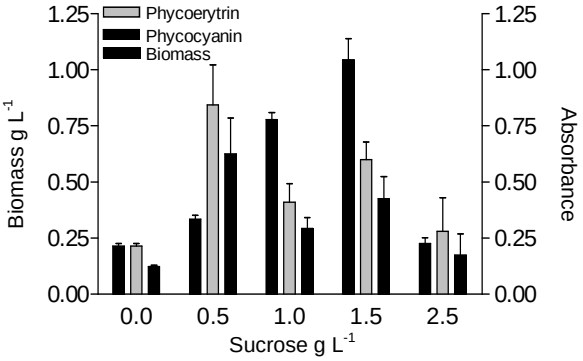

Figure 2. Effect of sucrose concentration on biomass and phycobiliprotein productions by *Nostoc* sp.

Table 3. Averages (n=3) of biomass, phycoerytrin and phycocyanin productions by Nostoc sp., supplemented with different concentrations of sugarcane molasses.

Sugarcane molasses (g L^{-1})	Biomass (g L^{-1})	Phycoerytrin Absorbance	Phycocyanin Absorbance
0.0($BG11_0$)	0.1320^{A}	0.1530^{A}	0.0850^{A}
0.5	0.5230^{B}	1.2417^{B}	0.7467^{B}
1.0	0.7517^{C}	$1.4400^{B, C}$	1.2583^{C}
1.5	$0.6660^{B, C}$	0.5380^{A}	$0.3787^{A, B}$
2.5	$0.6895^{B, C}$	$0.5845^{A, B}$	$0.3945^{A, B}$
5.0	$0.3740^{A, B}$	0.2773^{A}	0.1950^{A}

Averages followed by the same capital letters in the column do not differ statistically for the Tukey's test at 5% probability.

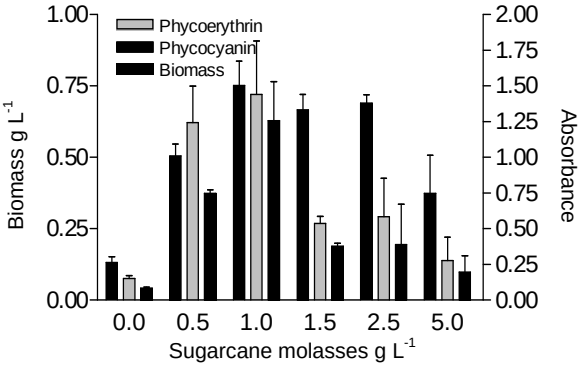

Figure 3. Effect of sugarcane molasses given in g L^{-1} of reducing sugar, on biomass and phycobiliprotein productions by *Nostoc* sp.

Discussion

Results showed that *Nostoc* sp. isolated in our laboratory can grow mixotrophically using glucose, sucrose and sugarcane molasses as substrates and a

greater production of biomass and phycobiliproteins can be reached compared with the autotrophic growth using only mineral medium.

Sugarcane molasses showed to be the best substrate for production of both biomass and phycobiliproteins when compared with glucose and sucrose. While the biomass production using sugarcane molasses, at the peak, was 5.7 times greater than the control, the biomass production using glucose and sucrose was 2.5 and 4.8 times greater than the control, respectively. For phycobiliproteins, the production in sugarcane molasses was 12.5 times greater than the control, while the production in glucose and sucrose was 3.0 and 4.5 times greater than the control, respectively. Sugarcane molasses is rich in nutrients. Besides the great concentration of carbohydrates, it has nitrogenous substances, vitamins and trace elements. Its composition varies depending on the sugarcane used for the production of sugar (Crueger and Crueger, 1989). Therefore, the greatest production when sugarcane molasses were used can be accounted to its richness of nutrients.

The strain *Nostoc* sp. used in this study showed, mainly in sugarcane molasses, a good performance when compared with another cyanobacteria reported by others. Chen *et al*. (1996), using batch cultures and varying the mineral medium from 0.0 to 10.0 g L^{-1} of glucose, obtained an increase of specific growth rate and biomass concentration of *Spirulina platensis* by addition of glucose. The highest cell concentration (2.66 g L^{-1}) was obtained at an initial glucose concentration of 2.5 g L^{-1}, nearly 2.0 times greater than the control, but for phycocianin production there was no difference in relation to the control. Blier *et al*. (1996) obtained with *Phormidium bohnery* growing on dairy anaerobic effluent, after 16 days of growth, twice the amount of the biomass produced on mineral medium, but less phycobiliproteins.

Conclusion

The results demonstrated that a mixotrophic batch culture of *Nostoc* sp. on glucose, sucrose or sugarcane molasses is suitable to enhance the production of biomass in order to produce phycobiliproteins. Sugarcane molasses, due to the greatest production of biomass and phycobiliproteins and the low cost, is a promising substrate to grow *Nostoc* sp. for this purpose, deserving future studies to optimize the growth conditions.

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