UTILIZATION OF LIGNOCELLULOSIC WASTE FOR BIOMASS AND PIGMENT PRODUCTION BY *Nostoc* sp. CACIAM 21 AS A CIRCULAR BIOECONOMY STRATEGY IN THE AMAZON

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ABSTRACT

The study aimed to supplement the cultivation of the cyanobacterium *Nostoc* sp. CACIAM 21 with different concentrations of cocoa husk hydrolysate (1%, 2.5%, and 5%) as a circular bioeconomy strategy for maximum biomass production and pigment accumulation, targeting biotechnological applications. Among the conditions tested, the maximum production of biomass (1.25 g/L), chlorophyll a (18.53 µg/mL), and total carotenoids (6.28 µg/mL) was obtained when the cultivation was supplemented with 2.5% lignocellulosic hydrolysate, demonstrating the potential of waste supplementation in the biostimulation of high-value bioproducts.

Keywords: Amazon. Bioeconomy. Cyanobacteria. Pigments. Waster.

1 INTRODUCTION

Cyanobacteria are photosynthetic microorganisms that can be used for various biotechnological purposes. These microorganisms are capable of accumulating several high-value biocompounds, such as chlorophylls and carotenoids. This diverse nutritional composition enhances the sustainable use of these microorganisms in food, health, and other sectors.[1] However, the high cost of biomass and metabolite production is primarily related to the cost of substrates, such as carbon sources. Therefore, the use of lignocellulosic residues to promote growth and biocompound production presents a potential strategy for the sustainable valorization of residual resources and cost reduction.[2]

In this context, the main objective of this study was to use a lignocellulosic residue as a carbon source to promote the accumulation of biomass and target biocompounds. For this purpose, cocoa husk hydrolysate was evaluated at different concentrations as a promising carbon source, aiming to promote the growth and accumulation of chlorophylls (Chl) and total carotenoids (Car) in the cells of the Amazonian cyanobacterium *Nostoc* sp. CACIAM 21. Our study verified, for the first time, the ability of the CACIAM 21 strain to utilize sugars derived from Amazonian lignocellulosic biomass as a circular bioeconomy strategy.

2 MATERIAL & METHODS

The cyanobacterium *Nostoc* sp. CACIAM 21 originates from the Amazonian Collection of Cyanobacteria and Microalgae (CACIAM), housed in the Laboratory of Biomolecular Technology (LTB) within the Institute of Biological Sciences (ICB) at the Federal University of Pará (UFPA). The cocoa husk was used as a carbon source to promote growth and pigment accumulation. The lignocellulosic residue was processed under maximum rotation to obtain a powder, which was subsequently sieved through a stainless steel sieve with a 35 mesh screen. The cocoa husk hydrolysate (CHH) was obtained through the acid hydrolysis of the lignocellulosic components.[3] For the cultivation of the Amazonian cyanobacterium CACIAM 21, the pH was adjusted to 7.0 with 8 N NaOH solution, and the supernatant of the hydrolysate was used to determine the sugar composition by the dinitrosalicylic acid (DNS) colorimetric method.[4] Three concentrations of CHH (1%, 2.5%, and 5%) were evaluated, and algae cells cultured with unsupplemented BG-11 were used as controls. The experiments were conducted in biological triplicates over a period of 10 days, using 250 ml erlenmeyer flasks with 100 ml of sterilized BG-11 medium, and the cultures were maintained at a temperature of 22 ± 1 °C, with a photon flux intensity of 30 µmol m^{-2} s^{-1}, under a 12:12 h photoperiod (light: dark). The harvested biomass was freeze-dried, and chlorophylls and total carotenoids were extracted with 80% acetone and quantified by UV-Visible spectrophotometer. [5] The productivity per dry biomass was determined gravimetrically. The dry weight (DW) was measured using a precision balance and calculated based on the difference between the final and initial weights, and productivity was calculated using equation 1.[6]

$$P\left(\frac{mg}{L/day}\right) = \frac{C_t - C_0}{t - t_0} \quad (1)$$

3 RESULTS & DISCUSSION

The valorization of lignocellulosic biomass residues for the production of high-value bioproducts, such as cyanobacterial biomass and biocompounds, has garnered increasing interest in recent years. This is not only because lignocellulose is a renewable source of sugars but also because it represents an abundant stream of waste, often undervalued.[7] Thus, different concentrations of cocoa husk hydrolysate were used to evaluate the growth of the Amazonian cyanobacterium *Nostoc* sp. CACIAM 21. As illustrated in Table 1, the highest concentration of biomass, productivity, and growth rate ($p < 0.05$) were observed in the experiment with 2.5% CHH, corresponding to 1.25 g/L, 120 mg/L/day, and 0.32 d^{-1}, respectively. Supplementation with 2.5% CHH improved biomass production by approximately 3 times compared to the control group. In contrast, experiments supplemented with 1% and 5% CHH did not show significant promoting effects ($p > 0.05$) on the growth of the CACIAM 21 cyanobacteria. Moreover, supplementation with 5% CHH was the most suppressive to cellular growth, resulting in only 0.36 g/L of biomass, 31.17 mg/L/day of productivity, and a growth rate of 0.20 d^{-1}. This decrease in growth may be attributed to the possible presence of inhibitors in higher concentrations, such as hydroxymethylfurfural, phenolics, and furfural.[8]

Table 1 Effects of different concentrations of cocoa husk hydrolysate on biomass production (X, g/L), biomass productivity (P, mg/L/d), and specific growth rate (μ, d^{-1}).

EXPERIMENTO	X (g/L)	P (mg/L/dia)	μ (d^{-1})
Control	0.42 ± 0.03^b	37.37 ± 1.50^b	0.21 ± 0.008^b
1%	0.49 ± 0.08^b	43.83 ± 1.76^b	0.23 ± 0.017^b
2,5%	1.25 ± 0.28^a	120.00 ± 3.50^a	0.32 ± 0.02^a
5%	0.36 ± 0.18^b	31.17 ± 1.48^b	0.20 ± 0.06^b

The provided data are mean ± standard deviation (*n=3*). Treatments sharing the same letter in the same column indicate that there is no significant difference between the values (*p>0.05*).

These results are consistent with studies reported in the literature. Using corn stover hydrolysate as a carbon source, the strains *Chlorococcum* sp., *Desmodesmo* sp., and *Chlamydomona*s debaryana demonstrated rapid growth, indicating that the utilization of lignocellulosic residues can be a viable approach for the economical production of biomass.[9] A similar trend was observed with the *Phaeodactylum* tricornutum strain, which showed a considerable increase in biomass concentration when the culture medium was supplemented with rice husk hydrolysate.[10] Similarly, *Haematococcus* pluvialis achieved excellent growth under walnut husk extract as a carbon source.[11]

It can be observed that the supplementation of the cultivation of the cyanobacterium *Nostoc* sp. CACIAM 21 with different concentrations of hydrolysate also had a significant impact on pigment accumulation (Figure 1). Among the various concentrations of hydrolysate tested, supplementation of the cultivation with 2.5% showed significant promoting effects ($p < 0.05$) on chlorophyll accumulation. The maximum concentration of chlorophyll achieved was 18.53 µg/mL, indicating an increase of approximately 43.64% compared to the control, which had a concentration of 12.90 µg/mL. In contrast, supplementation of the cultivation with 1% and 5% of hydrolysate did not show significant promoting effects ($p > 0.05$), resulting in a chlorophyll concentration of 14.07 µg/mL and 12.26 µg/mL, respectively.

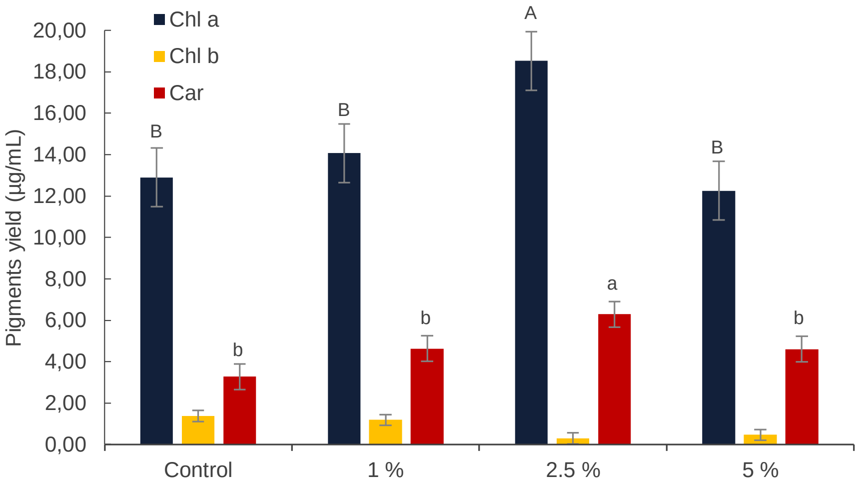

Figure 1 Effects of different concentrations of CHH on chlorophylls and carotenoids accumulation in cyanobacterium CACIAM 21. The provided data are mean ± standard deviation (*n=3*). Treatments sharing the same lowercase letter indicate no significant difference between values ($p > 0.05$). Treatments sharing the same uppercase letter indicate no significant difference ($p > 0.05$).

Similarly, when comparing the effects of supplementation with different concentrations of cocoa husk hydrolysate on the production of total carotenoids, cultures supplemented with 2.5% CHH significantly increased ($p < 0.05$) the carotenoid content in *Nostoc* sp. CACIAM 21, with an increase of 91.46% (Figure 1), corresponding to 6.28 µg/mL. In contrast, the minimum (1%) and maximum (5%) concentrations of CHH resulted in lower concentrations of carotenoids, reaching approximately 4.63 µg/mL and 4.60 µg/mL, respectively. However, no significant differences were detected in these experiments ($p > 0.05$).

Cyanobacterial pigments, such as chlorophylls and carotenoids, are essential for the development of photosynthetic microorganisms, playing important roles in light absorption and conversion into biochemical energy. Additionally, they contribute to the biotechnological potential of cyanobacterial biomass. [12,13] Chlorophyll is the most abundant pigment and, in addition to its role in energy capture, it exhibits bioactive properties such as antioxidants, antimicrobial, antitumor, anti-inflammatory, and chemoprotective effects.[14] Carotenoids play accessory functions within cells and also possess various properties, being considered excellent sources of natural antioxidants.[15] Therefore, cultivation supplementation can bring various benefits, with a maximum increase in these biocompounds. This enhancement not only improves photosynthetic efficiency and microorganism growth but also enhances the biotechnological use of biomass, making it a valuable source of bioactive compounds with multiple industrial and pharmaceutical applications.

4 CONCLUSION

This study demonstrated that cultivation supplementation with cocoa husk hydrolysate effectively stimulated the production of biomass and target bioproducts. The results indicated that the concentration of 2.5% CHH resulted in greater efficacy in promoting growth kinetics and the synthesis of chlorophylls and carotenoids. In contrast, supplementation with 1% and 5% did not show significant stimulation, resulting in lower biomass and pigment concentrations. It is relevant to note that various strategies have been widely studied to increase biomass and pigment production in cyanobacteria and reduce cultivation-related costs, including the use of lignocellulosic residues. In summary, these findings provide valuable insights for determining the optimal concentration conditions of cocoa husk hydrolysate to increase bioproduct productivity in Amazonian cyanobacteria. These compounds can be explored in various biotechnological applications, such as in the health, food, and nutraceutical industries.

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