IWA 17th SWWS AND 9th ROS
10th–14th November, 2024
Curitiba-Brazil

Evaluation of cultivation strategies in carbohydrates accumulation by microalgal biomass produced during wastewater treatment for biopolymer obtention

Assemany, P.*, Queiroz, N.*, Costa, M.*, Oliveira, O. N.* and Ensinas, A.V.**

*Environmental Engineering Department, Federal University of Lavras (*Universidade Federal de Lavras*), Campus Universitário, Lavras, MG, Brazil.

** Mechanical Engineering Department, Federal University of Lavras (*Universidade Federal de Lavras*), Campus Universitário, Lavras, MG, Brazil.

Highlights:

- Microalgae produced in wastewater were subject to cultivation strategies for carbohydrate accumulation.
- Photoperiod variation and nitrogen depletion were tested.
- Nitrogen depletion after 26 days of cultivation has led to greater total biomass production.
- Carbohydrate content did not vary among treatments, reaching a maximum of 8.4%.

Keywords: bioplastic; nitrogen; photoperiod.

INTRODUCTION

The accumulation of plastic waste in the environment and climate change are global challenges that have led to the exploration of environmentally sound and efficient technologies for socio-economic development, especially in developing countries. A promising strategy to face those problems is the production of biopolymers from algal biomass cultivated in wastewater. This approach contributes to reducing pollution in rivers and oceans and drives the circular bioeconomy by generating valued by-products from waste (López Rocha et al., 2020).

Biopolymers are polymers that can be produced by living beings, including polysaccharides (formed by the union of several monosaccharides) and peptides (the union of numerous amino acids) or acquired from raw materials from renewable sources. Among others, biopolymers from microalgae can be obtained by stimulating the accumulation of interest compounds inside the cell, i.e., polysaccharides (cellulose, hemicellulose, starch, etc.) and proteins, for further extraction of these compounds or the use of the entire rich-biomass. Various cultivation strategies are being researched to optimize the accumulation of interest compounds in microalgae. For example, controlled nutrient deficiency (Costa et al., 2018; Gifuni et al., 2018), light intensity, photoperiod, temperature, and salinity can be explored to direct algal metabolism toward the desired synthesis of polysaccharides.

Therefore, this research aimed to evaluate different strategies, i.e., photoperiod variation and nitrogen depletion, for carbohydrate accumulation by wastewater-grown microalgae biomass to biopolymer obtention. This resource-oriented sanitation solution can promote wastewater treatment plants' environmental and economic sustainability, helping sanitation universalization, especially in low-income countries.

the international water association · ABES · UFPR Universidade Federal do Paraná · inct ETEs Sustentáveis · SANEPAR

METHODOLOGY

The microalgae inoculum was produced in outdoor conditions with effluent collected in the UASB reactor from the Wastewater Treatment Plant at the Federal University of Lavras. After 20 days of autochthonous growth, the genera *Chlorell*a sp. was observed in the inoculum samples. The experiment was performed with 3 treatments: photoperiod variation (P), nitrogen depletion (N), and control (C). Each treatment was conducted in triplicate in 1L Erlenmeyer flasks containing 100 mL microalgae inoculum, 720 mL university effluent from UASB reactor, and 180 mL paint booth effluent (Braga et al., 2023). The paint booth effluent was collected from a wood and plywood furniture industry in the municipality of Guidoval, Minas Gerais, Brazil. It was used to balance the nutritional composition of the cultivation media, supplying organic carbon for microalgae growth. While P and C treatments were maintained in the incubator for 18 days, N treatment was extended for 26 days. Photoperiod was 12h for C and N, and 24, 18, and 6h during 2, 5, and 8 days, respectively, for P. All treatments were submitted to 11,000 lux, 28°C, and 3 $L.min^{-1}$ at 0.012 MPa of agitation (Boyu, SC-7500).

Total ammonia and volatile suspended solids (VSS) were determined according to APHA et al. (2012). Chlorophyll-*a* (chla-*a*) analysis was carried out using the Netherlands Norm method (Nederlands Norm (NEN), 1981) based on Nusch (1980). Biomass was harvested via gravitational separation and dried in an oven at 40°C for 24 hours. Carbohydrate content in the biomass was determined through quantitative acid hydrolysis (Hoebler et al., 1989), followed by the phenol-sulfuric acid reagent method (Dubois et al., 1956). Statistical tests for average differentiation between treatments (Shapiro-Wilk and ANOVA followed by Tukey tests) were performed using the R programming language.

RESULTS AND CONCLUSIONS

The total ammonia nitrogen concentrations at the end of the cultivation period for treatments C, P, and N were 1.5, 0.7, and 0.6 mg/L, respectively (statistically similar with $p>0.05$) (Figure 1).

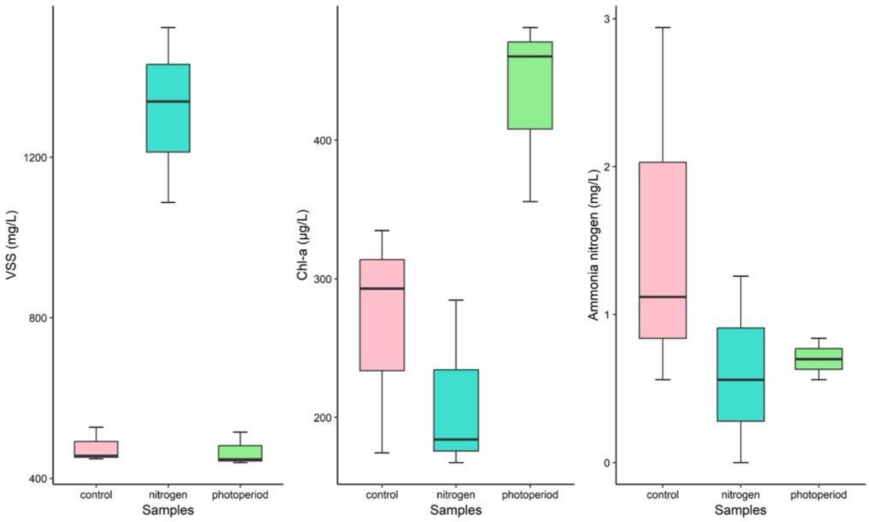

Figure 1 – Final concentrations of volatile suspended solids (a), chlorophyll-a (b), and ammonia nitrogen (c) in each cultivation strategy.

According to Table 1, cultivation conditions affected chl-*a* concentration in the biomass, with P and N presenting statistically different values of algal biomass ($p<0.05$). The samples submitted to nitrogen depletion (N) exhibited higher VSS concentrations compared to those subjected to photoperiod alterations (P) and to the control (C) ($p<0.05$). These results may indicate that 26 days of cultivation (8 days more than the other treatments) was insufficient to cause nutrient stress to the cells, leading to no total biomass death. A study with *Nannochloropsis oceanica* and *Chlorella sorokiniana* has demonstrated that nitrogen depletion can lead to increased lipid accumulation without impeding growth rates, resulting in increased biomass production (Negi et al., 2016).

Table 1- Primary (chl-*a*) and total biomass (VSS) productions and carbohydrate content for the evaluated treatments (average values and standard deviation between parenthesis).

	Chl-*a*		**VSS**		**Carbohydrate (%)**
	Chl-*a* (µg/L)	**Productivity (µg/L.d)**	**VSS (mg/L)**	**Productivity (mg/L.d)**	
Control (C)	267.4^{ab} (67.9)	14.9	477.3^{a} (35.5)	26.5	7.3
Photoperiod (P)	432.5^{a} (54.9)	24.0	467.2^{a} (34.0)	26.0	8.4
Nitrogen (N)	212.0^{b} (51.8)	8.2	1316.8^{b} (178.8)	50.6	7.9

Note: Significant at the 5% probability of error level by the Tukey test; the numbers followed by the same letter in the column did not differ statistically.

Regarding carbohydrate accumulation, both cultivation strategies increased this compound inside the cells compared to the C treatment (Table 1). However, the content was below the optimum expected for biopolymer obtention. Carbohydrate content in both conditions (photoperiod variation and nitrogen depletion) ranged from 7.9 to 8.4%, not showing a differentiation between treatments. Calculating carbohydrate productivity, the N treatment provided almost twice as much productivity as the P condition (4.0 versus 2.2 mg/L.d) due to higher total biomass production. Other studies have achieved better results when applying similar strategies. Testing N depletion, Gifuni et al. (2018) selected *Chlorella sorokiniana* as the most robust for starch production, with the highest starch content of 38% after the first day of N depletion (early depletion).

Results have shown that more studies are needed to conclude the best cultivation conditions for promoting carbohydrate accumulation by wastewater-grown microalgae biomass. Also, a detailed analysis to qualitatively characterize the carbohydrate profile and biomass structure would be relevant to conclude regarding the biomass potential for biopolymer obtention. The selected effluents may have influenced the results, as Braga et al. (2023) discussed. Also, a longer period for N starvation could be tested. On a larger scale within a wastewater treatment plant context, this strategy can be scaled up by adopting a longer hydraulic retention time in a high-rate algal pond or testing 2 phases of cultivation using a configuration of ponds in series.

ACKNOWLEDGMENTS

The authors wish to thankfully acknowledge the financial support of FAPEMIG (grant numbers PCE-00449-24 and APQ 00114/21) and CNPq (grant number 308108/2023-0).

REFERENCES

APHA, AWWA, & WEF. (2012). *Standard Methods for the Examination of Water and Wastewater* (22nd ed.). American Public Health Association (APHA).

Braga, M. Q., Assis, L. R. de, Ribeiro, V. J., Reis, M. C. F., Calijuri, M. L., & Assemany, P. P. (2023). Carbon supplementation in domestic sewage via mixing with paint booth effluent: Influence on the performance of bioremediation and algal biomass production from high-rate algal ponds. *Journal of Water Process Engineering*, *53*. https://doi.org/10.1016/j.jwpe.2023.103652

Costa, S. S., Miranda, A. L., Andrade, B. B., Assis, D. de J., Souza, C. O., de Morais, M. G., Costa, J. A. V., & Druzian, J. I. (2018). Influence of nitrogen on growth, biomass composition, production, and properties of polyhydroxyalkanoates (PHAs) by microalgae. *International Journal of Biological Macromolecules*, *116*, 552–562. https://doi.org/10.1016/j.ijbiomac.2018.05.064

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *J. Agric. Food Chem.*, *28*(3), 350–356. https://doi.org/10.1021/ac60111a017

Gifuni, I., Olivieri, G., Pollio, A., & Marzocchella, A. (2018). Identification of an industrial microalgal strain for starch production in biorefinery context: The effect of nitrogen and carbon concentration on starch accumulation. *New Biotechnology*, *41*(October 2017), 46–54. https://doi.org/10.1016/j.nbt.2017.12.003

Hoebler, C., Barry, J. L., David, A., & Delort-Laval, J. (1989). Rapid acid hydrolysis of plant cell wall polysaccharides and simplified quantitative determination of their neutral monosaccharides by gas-liquid chromatography. *Journal of Agricultural and Food Chemistry*, *37*(2), 360–367. https://doi.org/10.1021/jf00086a020

López Rocha, C. J., Álvarez-Castillo, E., Estrada Yáñez, M. R., Bengoechea, C., Guerrero, A., & Orta Ledesma, M. T. (2020). Development of bioplastics from a microalgae consortium from wastewater. *Journal of Environmental Management*, *263*. https://doi.org/10.1016/j.jenvman.2020.110353

Nederlands Norm (NEN). (1981). *NEN 6520 - Water: spectrophotometric determination of chlorophyll a content*.

Negi, S., Barry, A. N., Friedland, N., Sudasinghe, N., Subramanian, S., Pieris, S., Holguin, F. O., Dungan, B., Schaub, T., & Sayre, R. (2016). Impact of nitrogen limitation on biomass, photosynthesis, and lipid accumulation in Chlorella sorokiniana. *Journal of Applied Phycology* , *28*, 803–812. https://doi.org/10.1007/s10811-015-0652-z

Nusch, E. A. (1980). Comparison of different methods for chlorophyll and phaeopigment determination. *Archiv Fur Hydrobiologie, Beiheft Ergebnisse Der Limnologie*, *14*, 14–36.