Potential Oil Production of *Chlorella vulgaris* Microalgae Cultivated in Vinasse

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The study aimed to evaluate the potential of *Chlorella vulgaris* microalgae for oil production in treated/diluted Vinasse. The methodology involved three stages: control cultivation and vinasse 20%, 30%, and 40% concentrations (each lasting 8 days); biomass characterization; and oil extraction. The results showed that the 20% dilution had the highest production rate of chlorophyll A, chlorophyll B, and carotenoids (0.0018, 0.0002, and 0.0021125 µg.L^{-1}, respectively) and equal oil production (0.0044%) to the control culture.

Keywords: *Chlorella vulgaris*. Photosynthetic Microorganisms. Waste Treatment. Vinasse.

Microalgae have been the subject of interest for studies because they are a renewable resource and have a total capacity to provide various bioproducts, that is, substances of interest resulting from bioprocesses with economic potential and applicability in many industrial fields [1].

The controlled cultivation of microalgae has shown great promise in areas such as biodiesel production and wastewater treatment. Microalgae have several significant advantages, such as growth under simple and low-cost conditions and a highly active metabolism.

Regarding growth, many species are relatively easy to cultivate under controlled conditions and can be cultivated in photobioreactors [2], ponds, or closed systems, which allows greater flexibility and scalability in production.

We highlight that microalgae have a very high growth rate compared to other bioactive sources such as land plants. Some species of microalgae can double their biomass in a matter of hours. This rapid growth allows the large-scale production of microalgae biomass and other products derived from them in a short period, which is an essential factor for the economic viability of these processes.

Received on 15 October 2023; revised 18 December 2023.
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J Bioeng. Tech. Health 2024;7(1):31-36

Two of the main reasons that led to the choice of the species were its metabolic potential, and its ease of cultivation; that is, microalgae of this species have some significant advantages, such as growth in simple and low-cost conditions, in addition to a highly active metabolism. The present work evaluated the growth of the microalgae *Chlorella vulgaris* in treated Vinasse (diluted), oil production, and its application in the industry.

Materials and Methods

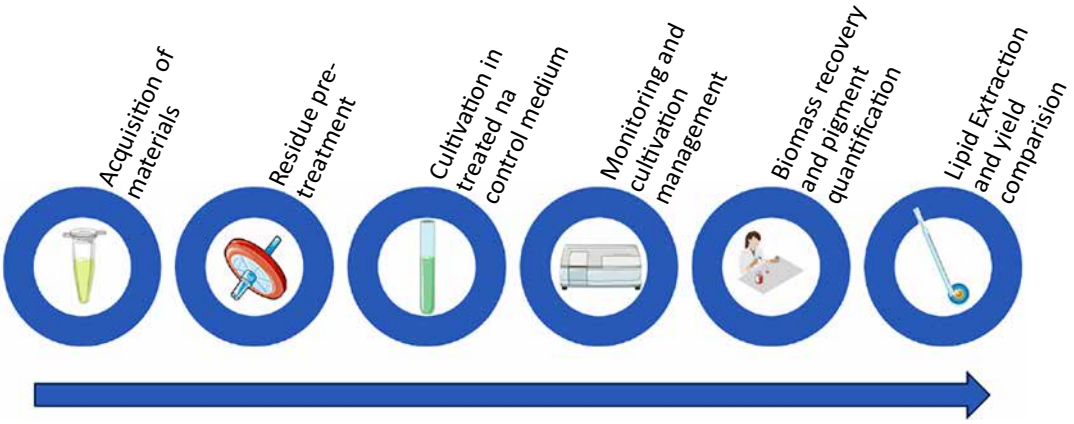
This study investigates how to reduce the oil production from *C. vulgaris*, the microalgae species chosen for the research, due to its high metabolic potential and ease of cultivation. We employed a qualitative and quantitative research approach, including experiments. In the biotechnology didactic laboratory at SENAI CIMATEC, 4 tests were performed - in duplicate, where the algal biomass of *C. vulgaris* was cultivated under stress conditions to stimulate its metabolic processes to happen with greater detectable kinetic speed [4].

A synthetic medium (BG11) was used as the standard culture medium, and alternative cultivation with Vinasse was performed for comparative analysis. Figure 1 shows the cultivation and characterization of the samples.

Acquisition of Vinasse and Microalgae

Both Vinasse and the *Chlorella vulgaris* inoculum were obtained through formal requests by

Figure 1. Cultivation and characterization of the samples.



the researchers of this study. *Chlorella vulgaris* was acquired from the Bioprospection and Biotechnology Laboratory (LabBBiotec) at the Federal University of Bahia (UFBA) in Salvador, Bahia, Brazil. Subsequently, the Vinasse was obtained from Fazenda Kiricó, a producer of artisanal cachaça (a type of Brazilian rum) located in the municipality of Mata de São João, Bahia, Brazil.

Treatment of Vinasse

The Vinasse was filtrated, starting with its mixture with smectite clay. After the settling, it underwent filtration through activated charcoal, cotton, and gauze, arranged in that order in a specially designed filter. Next, the treated Vinasse was pasteurized in a water bath at 60 °C for 30 minutes, followed by cooling (at 4 °C).

Cultination of *C. vulgaris*

For the preparation of the BG11 control culture medium, distilled water and the following proportions of reagents per 1L were used: 1.5g of $NaNO_3$; 0.04g of K_2HPO_4; 0.075g of $MgSO_4$; 0.036g of $CaCl_2$; 0.02g of Na_2CO_3; 0.006g of $C_6H_8O_7$; 0.001g of EDTA; 0.006g of $C_6H_8O_7$ xFe^{3+} γNH_3; additionally, 1mL of heavy metal solution needs to be prepared and added, with the following proportions: 0.286g/L of H_3BO_3; 0.0181g/L of MnCl; 0.022g/L of $ZnSO_4$; 0.039g/L of Na_2MoO_4; 0.0079g/L of $CuSO_2$; 0.0049g/L of $Co(NO_3)^2$ [3].

The dilution of the Vinasse in distilled water was done at different proportions (20%, 30%, and 40%) and subjected to refrigeration (4 °C) until the moment of use. The 500mL Erlenmeyer flasks were covered with cotton plugs to enable gas exchange and prevent the introduction of particles into the culture. They were then filled with 270mL of culture-covered medium (including control and alternative medium with Vinasse at 3 different dilutions) and 30mL of inoculum. Each culture was individually agitated using hoses connected to an air compressor with parameter control and monitoring for 12 hours per week (photoperiod).

Characterization of Biomass Obtained

Analysis of Cell Development by Optical Density

Optical density, also known as absorbance, is widely used in spectroscopy and biochemical analysis to quantify the concentration of a compound in a solution. This method was chosen to carry out the analyses of biomass and pigment production because it is a quantitative measure of the amount of light that a substance absorbs in a specific wavelength range of the electromagnetic spectrum, thus allowing detailed monitoring of the development of microorganisms, in this case, of the microalgae *Chlorella vulgaris* in standard medium (BG11) and different concentrations of Vinasse, in order to quantify which of these would be more advantageous in terms of growth for lipid extraction.

Chlorophyll A, Chlorophyll B, and Carotenoids Quantification

The growth of the microalgae was monitored daily by quantifying biomass and pigments (chlorophyll A, chlorophyll B, and total carotenoids) through optical density measurement using a spectrophotometer for 7 days.

The harvesting process consisted of draining the entire volume of the Erlenmeyer flasks into polyethylene buckets, previously prepared with an aluminum sulfate solution at a ratio of 2mL of flocculating solution per liter of culture. After approximately 30 minutes, the biomass separated into phases with the settling of the biomass. The excess water was then removed, leaving the biomass for the next step, where it was vacuum filtered using a system with a Kitasato flask and a porcelain funnel with filter paper, followed by drying in a kiln at 35°C for 2 days for complete drying, preparing the microalgal biomass for the lipid extraction [5].

Lipid Quantification

The lipid determination was carried out using the Bligh & Dyer method (1959). The samples were agitated until they became homogeneous. Then, the mixture was placed in a water bath on a magnetic stirring plate for 3 hours. At the end of this period, the mixture was filtered through filter paper. Next, the retained mass was discarded, and chloroform and water were added to the filtrate. Afterward, the samples were centrifuged at 1000 rpm for 3 minutes to promote the separation of the apolar (presence of lipid molecules) and polar phases [5]. After the phases were separated, the solvent was evaporated in an oven at 60 °C until completely evaporated [1].

The results were expressed as averages in graphs. A multiple linear regression was performed between the difference in measurements (dependent variable) and the mean of the measurements (independent variable) to assess similarities and differences.

Results and Discussion

Treatment of Vinasse

Unaltered Vinasse has some characteristics that are considered disadvantageous for microalgae cultivation, requiring a pre-treatment. Indeed, treating the Vinasse is necessary to reduce the organic load and turbidity and eliminate contaminants and other undesirable particles that could interfere with cellular growth and microalgal biomass production [4].

Analysis of Cell Development by Optical Density

The results showed that the cultivation of the microalga *C. vulgaris* in Vinasse diluted at 20%, 30%, and 40% was efficient and exhibited good growth compared to the control. The 20% dilution demonstrated the best growth (Figure 2). Additionally, there was unexpectedly higher growth in the control culture in the BG11 medium between days 6 and 7, surpassing the growth in the 20% diluted vinasse culture. The necessity of dilution can also be seen [7] where the dilution directly influences a specific growth, showing better results at diluted vinasse samples, also [8] displays that different concentrations of Vinasse can be used to acquire distinguished results; higher protein microalgae biomass needs equals higher Vinasse concentration; otherwise, higher carbohydrate levels mean lesser Vinasse concentration.

Analysis of Chlorophyll A, Chlorophyll B, Carotenoids' Production

We suggest that chlorophyll A (Figure 3) and chlorophyll B (Figure 4) show a significant growth of the microalgae (0.0018 and 0.0002 $\mu g.L^{-1}$, respectively). These findings support the selection of this crop for larger-scale production, aiming at extracting oil from the microalgae. It is also important to point out that, in the literature,

Figure 2. Cellular growth of the samples in different culture media over the 7 days.

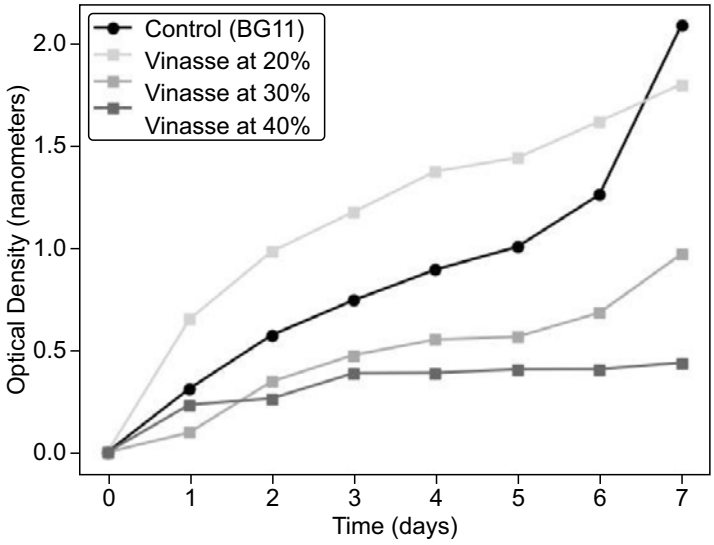


Figure 3. Production of chlorophyll A in the cultures,

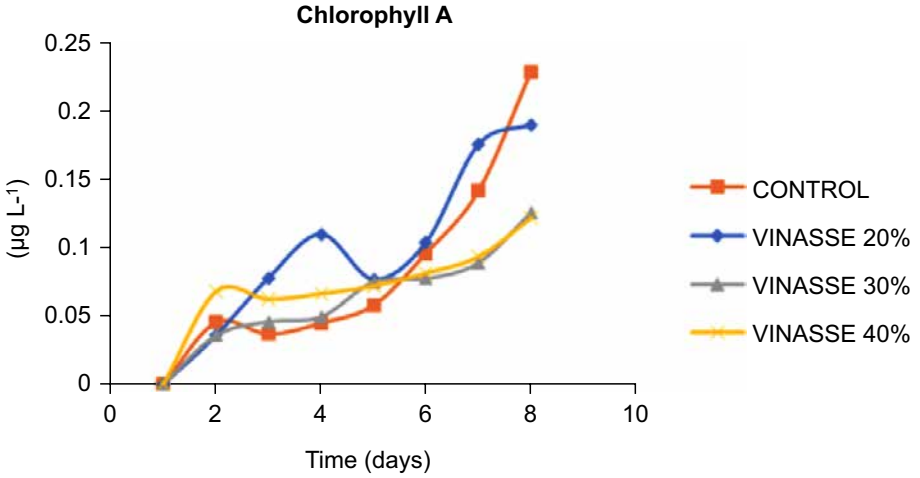
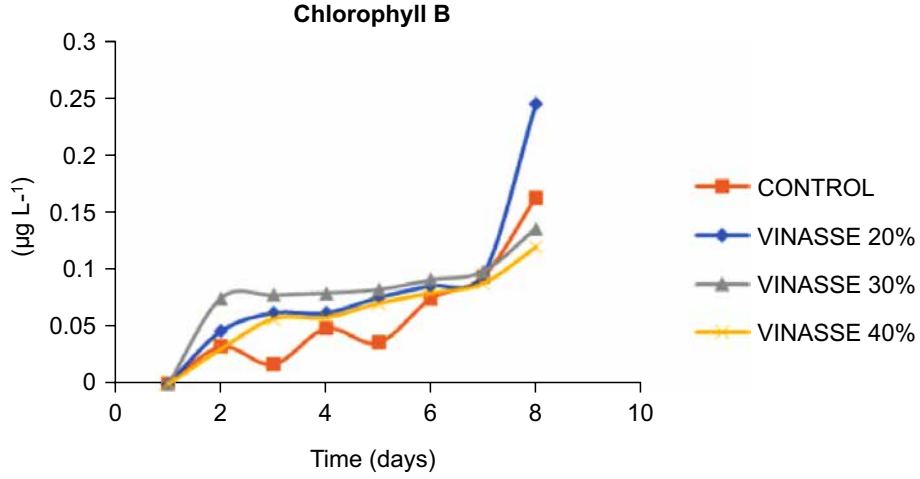


Figure 4. Production of chlorophyll B in the cultures.



the monitoring of chlorophyll A concentration is crucial in assessing microalgae growth.

Verifying the number of pigments produced is also important to evaluate the efficiency of different cultivation conditions, such as variations in light intensity, temperature, and pH, among others. This allows us to assess the best conditions for cultivating C. vulgaris and maximize its production [6]. The cultivation in Vinasse 20% also resulted in a higher production of carotenoids (0.0021125 $\mu g.L^{-1}$).

The total carotenoid production yields, observed in Figure 5, indicate that the cells cultivated in the different concentrations of Vinasse had their photosynthesis affected between days 1 and 2 of monitoring, subtly decreasing before growing again, maintaining a similar pattern during the elapsed time.

Lipid Production Rate Analysis

After the lipid extraction process of both microalgae cultures, standard (BG11 medium) and alternative (vinasse medium), it was observed that, despite the higher production from the standard culture medium, this difference, compared with Vinasse, is slight.

However, cultivation in standard medium (BG11) proved less effective in producing biomass, unlike cultivation in alternative medium (Vinasse), which was more effective.

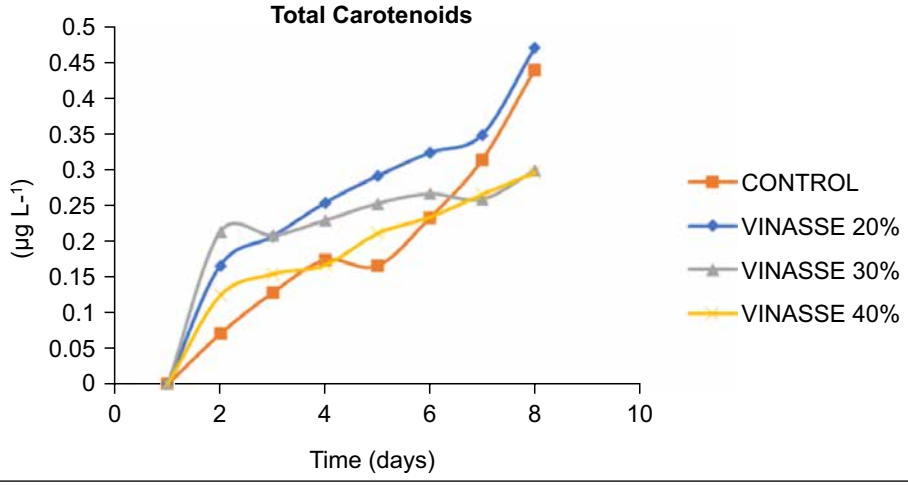
From this, it is possible to understand that cultivation in BG11 produced 34% oil while cultivation in Vinasse produced 44%.

Conclusion

This study demonstrated that the use of Vinasse treated with charcoal and smectite clay, diluted to 20%, had a positive impact on the growth of *Chlorella vulgaris* compared to synthetic medium (BG11), indicating that it provided a suitable environment for the good cellular development of the microalga. It is also possible to infer that cultivation under these conditions suggests that this medium can be a viable alternative to the traditionally chosen culture medium. This could be relevant for reducing cultivation costs by utilizing industrial byproducts, such as Vinasse, as highlighted in this study, and it can also contribute to sustainability by offering a recycling option instead of disposal.

Regarding biomass and oil production, as the dilution of Vinasse to 20% resulted in the highest production rates of chlorophyll A, chlorophyll B, and carotenoids, this indicates favorable conditions for pigment synthesis in microalgae. Furthermore, the fact that oil production was equal to the control culture suggests that using treated Vinasse did not negatively affect oil production, which is an industrially relevant compound.

Figure 5. The production of total carotenoids in each monitored culture.



This study emphasizes the potential use of *Chlorella vulgaris* cultivated in treated Vinasse as a source of biomass and oil for industrial applications. The oil produced by microalgae can be widely explored in the biofuel, cosmetics, food, supplements, and other industries.

Acknowledgments

The authors thank the researchers from LabBBiotec for donating the microalgal strains and Kiricó Farm for donating the Vinasse. This project did not receive financial support for its realization

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