# EVALUATION OF CARBON NANOPARTICLES IN MICROALGAL GROWTH: ANALYSIS OF DIFFERENT DOSAGES

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Highlights:

- The search for new technologies to enhance microalgae cultivation is necessary.
- Lower concentrations promote faster microalgal growth.
- It is possible to cultivate microalgae in media supplemented with Arbolina®.

Keywords: Microalgae; Carbon Nanoparticles; Domestic wastewater.

## INTRODUCTION

Microalgae are highly diverse and complex organisms that can be cultivated in various types of water, such as freshwater, saltwater, and wastewater (Calijuri et al., 2022). In this context, the use of microalgae in wastewater helps in the treatment process by consuming the organic matter and nutrients present, which in turn helps reduce the costs associated with biomass production (Couto et al., 2020). However, techniques to enhance microalgae cultivation are still being studied. For instance, in the review conducted by Lau et al. (2022), the interaction between nanomaterials and microalgae was analyzed, revealing that carbon nanoparticles can boost microalgal growth by increasing their light absorption capacity. These nanoparticles are capable of promoting microalgae growth and improving the efficiency of wastewater treatment due to their unique properties that influence the interaction with both the microalgae and their environment.

Arbolina®, a carbon nanoparticle-based biofertilizer composed of 47% carbon, 17% nitrogen, and 4% hydrogen, has been shown to positively impact the efficiency of agricultural product production (EMBRAPA & Krilltech Nanotecnologia, 2020). In pursuit of energy efficiency in wastewater treatment systems, this study aims to explore how the application of Arbolina® at different nanoparticle dosages can influence algal growth. Additionally, this work represents the first phase of a project focused on energy production innovation, providing valuable insights for the sustainable development of algae-based wastewater treatment, with an emphasis on increasing microalgal biomass productivity and its subsequent conversion into energy.

## METHODOLOGY

The experiment was conducted at the Laboratory of Sanitary and Environmental Engineering (LESA) at the Federal University of Viçosa (UFV). The carbon nanoparticles used in the experiment were present in the commercial compound Arbolina®, which was provided by the producing company for analysis with microalgae. The microalgae inoculum was prepared prior to the experiment, where indigenous microalgae naturally grew in a culture medium consisting of domestic wastewater. This wastewater was collected after the septic tank at the Romão dos Reis neighborhood Wastewater Treatment Plant (WWTP) in Viçosa, Minas Gerais.

Nine different dosages, each in triplicate, were tested in 600 ml flasks to evaluate microalgae growth under the effect of the compound. The Arbolina® dosages used were: 0 mg/L, 5 mg/L, 50 mg/L, 100 mg/L, 200 mg/L, 400 mg/L, 800 mg/L, 1000 mg/L, and 5000 mg/L. Dissolved oxygen (DO) concentration and pH of the medium were measured daily between 10:00 and 12:00 (from May 8, 2024, to May 17, 2024) using a Hach HQ30D/LDO probe. The flasks were photographed after each daily analysis, and digital comparisons were made to facilitate visualization of the algae growth and enable comparison between treatments. The DO production graph was created using Excel® spreadsheet software.

Microalgal growth was assessed by the increase in DO concentration and the color of the flasks, with more intense green shades indicating higher algal concentration, primarily due to the production of chlorophyll-*a*, a green pigment (Silva & Lombardi, 2020). Additionally, to confirm these observations, volatile suspended solids (VSS) analysis was performed at the end of the batch experiment.

## RESULTS AND CONCLUSIONS

Figure 1 illustrates microalgal growth and DO concentration throughout the experiment. Both DO and pH are indirect indicators of the growth and development of microalgae in the culture medium. The pH reflects the acidity or alkalinity of the solution and is associated with the metabolic activities of the organisms present (Solimeno et al., 2017). Additionally, microalgae photosynthesis increases DO concentration, which can be indirectly verified through pH data (Couto et al., 2021).

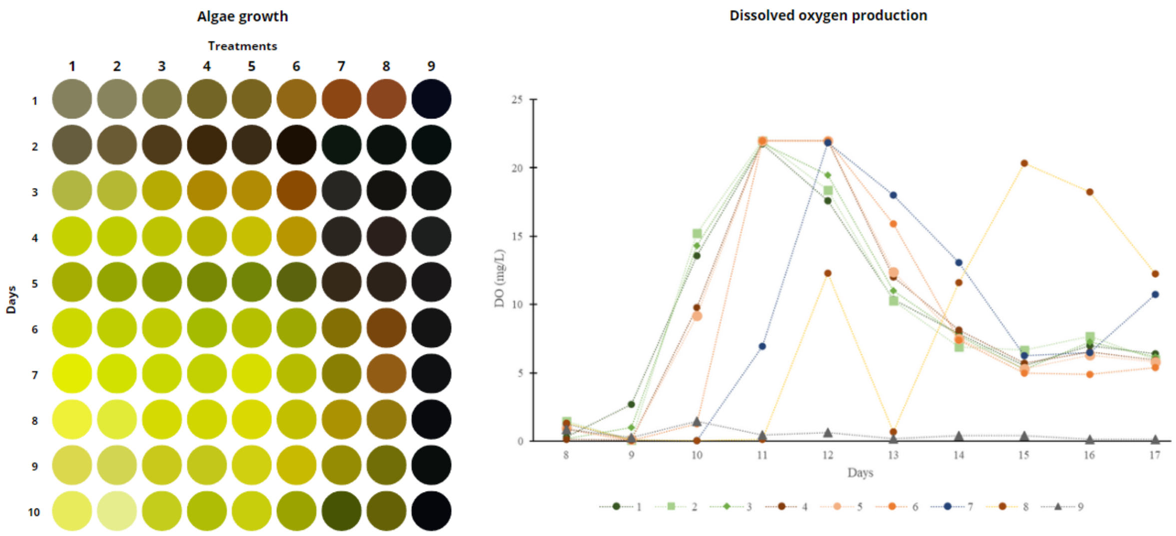


**Figure 1.** Microalgal growth (demonstrated by color intensity) and evaluation of dissolved oxygen in the culture medium.

The initial pH values were close to neutrality (1 = 6.97; 2 = 7.04; 3 = 6.87; 4 = 6.87; 5 = 6.84; 6 = 6.77; 7 = 6.48; 8 = 6.46; and 9 = 5.36). By the end of the experiment, most reached pH values close to 10 (except for treatment 9, which had a maximum value of 7.6). Changes in pH can indicate microalgal growth in the culture medium, as their activity can alter hydroxide ion concentrations (Solimeno et al., 2017).

DO concentrations (Fig. 1) started near zero (1 = 0.31 mg/L; 2 = 1.46 mg/L; 3 = 0.19 mg/L; 4 = 0.13 mg/L; 5 = 0.99 mg/L; 6 = 0.89 mg/L; 7 = 1.27 mg/L; 8 = 1.33 mg/L; and 9 = 0.90 mg/L) and reached maximum values close to 22 mg/L (except for treatment 9, which had a maximum of 1.47 mg/L). The increase in DO is primarily due to photosynthesis (Sutherland et al., 2021). It was observed that lower concentrations of Arbolina®, between 5 and 100 mg/L, reached higher DO concentrations more quickly (about four days to surpass 22 mg/L). In treatments with higher compound concentrations, such as 1000 mg/L, there was a delay in the increase of DO (about eight days to reach the same value). This delay may be associated with the initial characteristics of Arbolina®, as microalgae require an adaptation period.

In general, the final average concentration of VSS increased with the dosage of Arbolina® (1 = 151.33 mg/L; 2 = 186.00 mg/L; 3 = 157.00 mg/L; 4 = 255.33 mg/L; 5 = 273.00 mg/L; 6 = 275.00 mg/L; 7 = 439.17 mg/L; 8 = 435.00 mg/L; and 9 = 181.50 mg/L), except for treatment 9. This increase is because Arbolina® is a carbon-based compound, an essential nutrient for algal development. However, treatment 9 suggests that high doses of the compound may act as an inhibitor of algal growth, either by reducing light penetration (due to the dark color of the compound) or through toxicity, indicating an optimal dosage for algae development. Thus, the study demonstrates the feasibility of cultivating and growing algae in media supplemented with Arbolina®, considering the specific effects of different nanoparticle concentrations. Additionally, it opens up possibilities for using microalgal biotechnology in wastewater treatment, with Arbolina® acting as a potential biostimulant for biomass production.

Cultivating microalgae in wastewater offers a sustainable approach to producing valuable co-products such as biofuels while simultaneously enhancing wastewater treatment and facilitating carbon capture (Molazadeh et al., 2019). The use of Arbolina® can further enhance this process by stimulating microalgal growth, thereby increasing biomass production. This additional biomass can then be used as feedstock for hydrothermal liquefaction (HTL), a process that efficiently converts wet biomass into bio-oil, avoiding the need for energy-intensive drying (Silva et al., 2024). The HTL process not only maximizes resource recovery by converting the biomass into valuable by-products such as bio-oil, water-soluble compounds, gases, and solid residues, but also produces an aqueous phase rich in nutrients, organic acids, and cations. This nutrient-rich by-product can be reused, for instance, as a substrate for biohydrogen (bio$H_2$) production (Silva et al., 2024).

In conclusion, Arbolina® shows promise as a biostimulant for optimizing microalgae cultivation, supporting sustainable wastewater treatment, and increasing biomass for various applications, such as biofuel production.

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