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MICROALGAE BIOMASS PRODUCTION USING SWINE WASTEWATER FOR BIOFUELS PRODUCTION AND EFFLUENT BIOREMEDIATION

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Abstract. *Microalgae biomass is recognized as one of the most promising alternative sources for biofuels production, in addition to the capacity to absorb nutrients like nitrate and phosphate during cultivation, coupled with the treatment capacity of agro-industrial wastewater. The objective of this work is to contribute to the development of a microalgae biorefinery unit, through the increase of the productivity of microalgal biomass in cultures in a tubular photobioreactor with swine effluents to produce biofuels, in addition to the use of the cultures as a tool for the biological treatment of this effluent. The cultures will be carried out in a 10 L tubular photobioreactor, using the microalgae of the species Tetradesmus obliquus. Swine effluent from biodigester will be used as an alternative culture medium. The effluent bioremediation capacity and organic load reduction will be evaluated through determinations in the composition of the medium at the beginning and at the end of the cultures. The microalgal biomass composition will be evaluated to determine the potential of the species for biodiesel production. It is expected that the complex composition of the alternative culture medium and greater availability of nutrients present in the effluent will result in greater growth of microalgae, in addition to increasing the lipid content in the biomass resulting in greater productivity and potential as a raw material for biodiesel production, when compared to cultures with the synthetic medium. The present study aims to contribute to the development of a microalgae biorefinery unit, combining the increase in the productivity of biomass and lipids to produce biofuels, combined with the treatment of effluents.*

Keywords: *Microalgae, Biomass, Biorefinery, Bioremediation.*

1. INTRODUCTION

Microalgae is a term used to designate unicellular or colonial photosynthetic organisms present in aquatic or humid environments, and which, in general, do not constitute a monophyletic group, and this nomenclature defines an artificial and heterogeneous group. According to Lourenço (2006), algae are very diverse beings, mostly photosynthesizing and presenting a vegetative structure known as the thallus, whose cellular differentiation is small or null. The term algae are completely devoid of taxonomic value, as it designates organisms that are very different from each other in terms of their origin. Among algae, macroalgae (algae with macroscopic dimensions) and microalgae (algae with microscopic dimensions) are distinguished. Collectively, algae constitute a group of extreme ecological importance, since, in aquatic systems, they are the main ones responsible for primary production, thus contributing to the maintenance of biogeochemical cycles and support of the trophic chain.

Microalgae cultures are commonly carried out in two ways: in open systems, such as tanks or ponds, or in closed systems such as commonly photobioreactors (Assunção; Malcata, 2020). In general, the use of photobioreactors for the cultivation of microalgae has some advantages, among which it is possible to highlight the greater control of the conditions, since the system is closed and there is no contact with the external environment, reducing the probability of contamination. by organisms other than those cultivated and the reduction of excessive loss by evaporation, which is aggravated on hot and dry days in open crops (Xiaogang et al., 2022). Photobioreactors also have high efficiency in the production of biomass when using CO_2 injection in the medium, enhancing cell multiplication. The advance in industrial production associated with exploring the potential of microalgae involves the development of cultivation systems that can combine these characteristics. In addition, the need for development is also present in the academic sector, since the improvement of large-scale cultivation systems allows the realization of studies that bring the reality of the bench closer to the industrial one, thus favoring the scaling of the investigated processes.

Open tanks that simulate the natural habitat of microalgae are the most used due to the low cost of construction and maintenance. Three main models have been used in large-scale production, race tanks, circular ponds, and inclined tanks (Borowitzka, 1999). The expense of the construction of the race tanks is relatively low, since the system consists of a shallow trench excavated in the ground covered with plastic, and the agitation is promoted by long blades. The water level must be between 15 cm and 50 cm, lower or higher values can impair the flow and reduce turbulence. In this system, the water level requires large areas for cultivation with about 150 L m^{-2}, in addition, the cell concentration hardly exceeds 600 mg L^{-1}, the easy contamination and increasing the cost of biomass collection. Another problem is excessive evaporative loss, which is exacerbated on hot, dry days (Tredici, 2004).

Open systems can also be defined as photobioreactors, since they are reactors in which photosynthetic organisms grow and carry out their biological reactions, however, the term photobioreactor is commonly used to designate closed systems. These can be defined as culture systems for autotrophic organisms, in which light falls indirectly on the surface of the culture, having previously passed through a translucent surface. Therefore, photobioreactors limit the direct exchange of gases with the external environment, reducing contamination between the cell culture and the atmosphere (Tredici, 2004). In addition, photobioreactors occupy a much smaller area when compared to open systems and can be installed in urban areas or soils unsuitable for agriculture.

Despite having better control of cultivation conditions, photobioreactors are financially more expensive, demanding greater technological investment in the development of new structural arrangements, construction materials, and control of operations (Chisti, 2013; Gómez-Pérez et al., 2015). In this way, the viability of large-scale cultivation needs to be associated with the exploration of the potential uses of this technology, from its different perspectives.

Microalgae can be utilized in several areas as raw material in the production of bioproducts, just as biofuels, chemicals, and cosmetic materials, as well as it can be used in animal and human feeding and aquaculture. Besides, they can be a source of high-added-value molecules, like oily acids used in human food supplementation and pigment production (Spolaore, et al., 2006). Still, microalgae can be used in the treatment and production of biofertilizers, products with biostimulating and elicitor activity (Zanette, et al., 2019).

One of the biggest potential markets for microalgae is biodiesel production. Climate change and the great dependence on fossil fuels as energy sources boost more and more investment in innovative and renewable technologies as energetic alternatives, like biodiesel, bioethanol, and biohydrogen. Though, around 70% to 80% of the production cost of biofuels comes from raw material acquisition (Santos, 2016). For this reason, microalgae have a great potential to lower the cost of production due to their high productivity. In face of this context, Brazil is one of the countries that have the favorable edaphoclimatic conditions for microalgae to be one of the main sources of biofuels in the future (Ho et al., 2014).

One of the most abundant molecules in microalgae is chlorophyll, the main responsible for capturing photons in synthesizing organisms and thereby realizing photosynthesis. Chlorophyll can be used to produce chlorophyllin, which is a derivative used as a dye by the food, textile, and paper industries (Santos et al., 2021). Along with it, carotenoids are also pigments found in microalgae cells. Astaxanthin, xanthophylls, zeaxanthin, canthaxanthin, and echinenone are some examples that are exploited in nature, coloring animals like birds, fish, reptiles, and amphibians. These molecules act as well in the social dynamic of species, indicating the social level and attracting sexual mates. And, as well, one of the more important purposes: to serve as camouflage and toxicity alert.

In the industry, carotenoids are employed due to their antioxidant capacity, since that, in nature, they have the purpose of absorbing excessive luminous energy and, in that way, avoid damage to the cell and even to chlorophyll (photo-oxidation). Astaxanthin is a good example of industrial use, being applied mainly by the pharmaceutical industry due to its antioxidant properties, photo-protection, increase in immune response, and treatment of degenerative diseases. And besides carotenoids, there are other compounds of high added value, such as docosahexaenoic acid (DHA) and eicosapentaenoic (EPA), which can be commercialized for therapeutical or pharmaceutical applications, some being studied as antimicrobial compounds. Carbohydrates are also present in microalgae cells, and they have great value for presenting compounds such as B 1,3-glucan - which shows immunostimulating, antioxidant, and blood cholesterol-reducing activities - and polysaccharides that are applied in anti-adherent therapies and bacterial infections, alginate, and cellulose, which are used as emulsifiers and stabilizers in the food field (Santos et al., 2022).

Among the applications of cultivation technology, the use of microalgae in the bioremediation of urban and agro-industrial effluents has been exhaustively studied (Obaja et al., 2003; Jimenez-Perez et al., 2004; Fierro; Sanchez-Saavedra; Copalcua, 2008). Literature data show promising results in relation to the bioremediation of swine effluent and increase in the production of microalgae biomass (Kim et al., 2007; Taher, 2013). Mulbry et al. (2008) obtained biomass productivity of 24 g m^{-2} d^{-1} with nitrogen and phosphorus incorporation rates of 1.8 and 0.3 g m^{-2} d^{-1}, respectively. In addition to increasing biomass production, microalgae can reduce the organic load (COD) content, reaching a reduction of more than 65% (Jimenez-Perez et al., 2004; Dichtl; Rogge; Bauerfeld, 2007; Mulbry et al., 2008; Wang, Bluck; Van Wie, 2014).

Effluents represent a highly relevant environmental liability since the high organic load can generate eutrophication in rivers, if not properly treated. For this reason, the use of microalgae cultures as a tool for bioremediation is presented as one of the main potentialities of this technology, especially when associated with other biotechnological processes to obtain bioproducts and bioactive molecules.

To explore the potential of microalgae in obtaining bioproducts and treating effluents, one of the alternatives is to develop the concept of biorefineries. In this sense, the Center for Research and Development of Self-Sustainable Energy (NPDEAS) is a multidisciplinary research group at the Federal University of Paraná (UFPR) located in the city of Curitiba-PR, which seeks to explore various biotechnological processes, having as main activity the cultivation of microalgae in photobioreactors on an industrial scale. Initially, the group's objective was to achieve energy self-sufficiency through the production of biodiesel generated with lipids from microalgal biomass. Currently, the NPDEAS industrial plant integrates different modules and processes, aiming at obtaining different products (FIG.1).

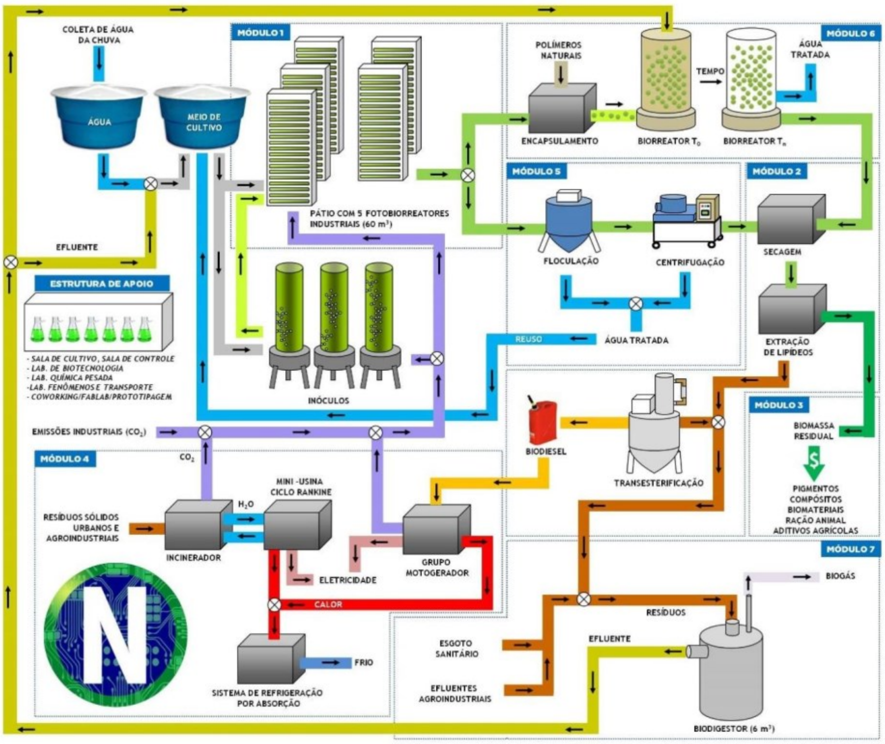

Figure 1. Schematic diagram of the production modules at NPDEAS.

Among the processes directly related to the production of biomass in scale, it is possible to highlight the cultivation of microalgae in reactors with a volume of 12 m^3, treatment of agro-industrial effluents, treatment of emissions, processing and biomass recovery, and extraction of bioproducts.

In addition to operating in an integrated manner, the units allow the development of products that meet a broad spectrum of activities in the market. The modular creation of also allows the future supply of complete systems or the realization of a demand system. Based on what was exposed in the previous paragraphs, the objective is to evaluate the biomass production of the microalgae Tetradesmus obliquus cultivated with effluent from this biodigested pig, combined with the evaluation of the bioremediation potential of the effluent from the assimilation of microalgae, thus combining the production of rich biomass into components of high commercial value with the development of a tool for treating effluents in the same biotechnological process.

2. MATERIALS AND METHODS

The microalgae *Tetradesmus obliquus* was cultivated in biodigested swine wastewater as an alternative medium for biomass production. The experiments were carried out at the Center for Research and Development of Self-Sustainable Energy (NPDEAS) of the Federal University of Paraná (UFPR). Cultures for biomass production were carried out for 14 days, in 12 L airlift photobioreactors (FIG. 2), built with transparent PVC tubes, installed in the external area of the NPDEAS, facing sunlight, and exposed to the climatic variations of the city of Curitiba – PR (Brazil). The aeration and circulation of the cultures was performed by injecting compressed air at a flow rate of 1 L min^{-1}.

Figure 2. FBR Airlift for microalgae cultivation.

The culture medium used was composed of biodigested swine manure at a concentration of 10% in tap water (v/v). The initial composition of the culture medium indicated the presence of approximately 165 mg L^{-1} of nitrogen, 30 mg L^{-1} of phosphorus and pH 6.5. The cultures were carried out in batches, with the addition of medium at the beginning of the cultivation and recovery of the entire volume at the end of the period.

To determine the effectiveness of the use of effluent in increasing the productivity of biomass, cultures were carried out with synthetic culture medium as a control (Chu, 1942), composed of inorganic salts of macro and micronutrients: $NaNO_3$ (250 mg L^{-1}), $CaCl_2.2H_2O$ (25 mg L^{-1}), $MgSO_4.7H_2O$ (75 mg L^{-1}), K_2HPO_4 (75 mg L^{-1}), KH_2PO_4 (175 mg L^{-1}), NaCl (25 mg L^{-1}), EDTA (50 mg L^{-1}), KOH (31 mg L^{-1}), $FeSO_4.7H_2O$ (5 mg L^{-1}), H_3BO_3 (11.4 mg L^{-1}), $ZnSO_4.7H_2O$ (8.8 10^{-3} mg L^{-1}), $MnCl_2.4H_2O$ (1.4 10^{-3} mg L^{-1}), $Na_2MoO_4.2H_2O$ (1.2 10^{-3} mg L^{-1}), $CuSO_4.5H_2O$ (1.6 10^{-3} mg L^{-1}), $Co(NO_3)_2.6H_2O$ (0.49 10^{-3} mg L^{-1}).

2.1 Culture growth evaluation

To determine the growth of the cultures, samples were periodically taken from each of the photobioreactors. The parameters measured were the growth of the number of cells, pH of the cultures, and dry biomass. The determination of the cell concentration of the samples was performed by optical microscopy, with a 400x magnification and the aid of a hemocytometer.

Dry biomass was determined by gravimetry after filtration of a known volume on glass microfiber filter paper (GF1), previously dried to remove moisture and with a determined weight. After filtering the sample, the papers were kept in an oven with air circulation for 24 h to dry to constant weight. The dry biomass of microalgae was determined by gravimetric analysis on a Shimadzu analytical balance, model AUW220D. Analyses to determine the growth of the cultures were performed daily with samples in triplicate to calculate the mean and standard deviation.

2.2 Nitrogen and phosphorus quantification

To determine the potential of microalgae in the bioremediation of wastewater, reductions in nitrogen and phosphorus contents in crops were evaluated. The determination of nitrogen and phosphorus consumption in crops with biodigested effluent was performed in collections spaced along the crops. The determination of nitrogen and phosphorus was performed based on the methodologies described in the Standard Methods. Both methodologies have a colorimetric principle, with readings performed in a Shimadzu UV-1601 spectrophotometer at characteristic wavelengths for each element.

3. RESULTS AND DISCUSSION

Typically, microalgae in culture have different growth phases, starting with a phase of adaptation to the medium (lag), followed by an exponential growth phase (log), a phase in which there are no large variations in cell concentration (stationary), and a decline phase, where more cell deaths than new divisions occur. Figure 3 shows the growth profiles and cell concentration of the microalgae *T. obliquus* in cultures carried out in synthetic culture medium and alternative culture medium, composed of biodigested swine effluent.

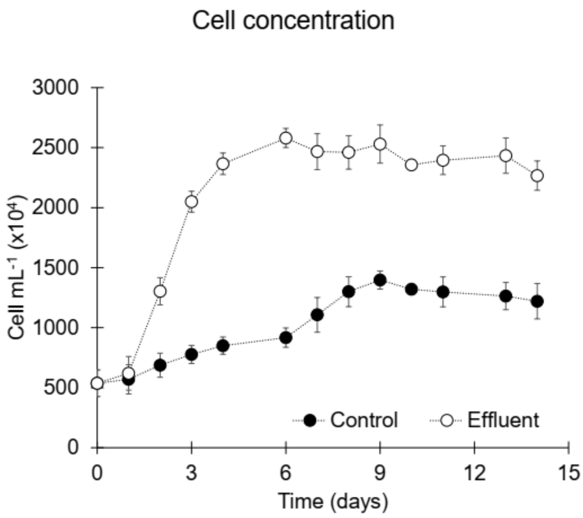

Figure 3. Cell density of *T. obliquus* cultured in FBR airlift.

Both cultures, with synthetic culture medium and with biodigested effluent, started with the same cell concentration (537 10^4 cell mL^{-1}) and presented a short adaptation phase, in the first 24 h. After this initial period, the cultures with effluent started to show accelerated growth, reaching the growth peak on the sixth day, with 2500 10^4 cell mL^{-1}, not suffering significant variations in cell concentration until the end of the period of cultivation. In comparison, the cultures with Chu medium showed slower growth, not being possible to observe an exponential growth phase, reaching a growth peak on the ninth day, with 1200 10^4 cell mL^{-1}, from that point on entering the stationary phase, with cell concentration ranging between 1000 and 1500 10^4 cell mL^{-1}.

In addition to determining cell concentration, another measure used to monitor the growth of microalgae in culture is the quantification of the biomass produced. Like cell concentration data, cultures with swine effluent showed higher biomass concentration than cultures with synthetic culture medium. The peak of biomass production was 1.66 g L^{-1} on the 13th day of cultivation with effluent, a value approximately 2.3 times higher than that observed for the control culture in the same period (FIG. 4).

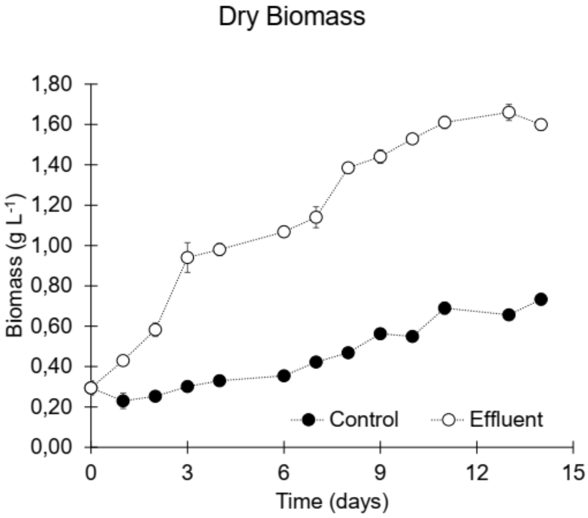

Figure 4. Biomass production of *T. obliquus* in Airlift FBR.

Although it is possible to establish a correlation between cell concentration (cell mL^{-1}) and biomass (g L^{-1}), this correlation is not absolute. Typically, cells can reduce their rates of cell division and remain metabolically active, synthesizing energy reserve materials such as carbohydrates and lipids, thus gaining mass continuously. Thus, what is observed is a growth pattern, which resembles a growth curve of microorganisms, for cell concentration data, while biomass production data describe a pattern with more linear trends.

Biomass production data corroborate the results of Selesu (2015), who evaluated the cultivation of *T. obliquus* in swine manure under different effluent treatment conditions as a culture medium and observed microalgal biomass production in the order of 1.5 g L^{-1}, in an airlift model photobioreactor. It should be noted that the higher biomass productivity recorded in crops with biodigested swine effluent can be attributed to the higher concentration and availability of nutrients. Among the main macronutrients required to produce microalgae biomass, the main ones are nitrogen and phosphorus, fundamental elements in the composition of enzymes and nucleic acids, respectively (Lourenço, 2007).

The consumption of nitrogen and phosphorus is easily observed in cultures with effluent, with both nutrients suffering a strong reduction in the first 4 days of cultivation, keeping concentrations low until the end of the period of growth and biomass production (FIG. 5).

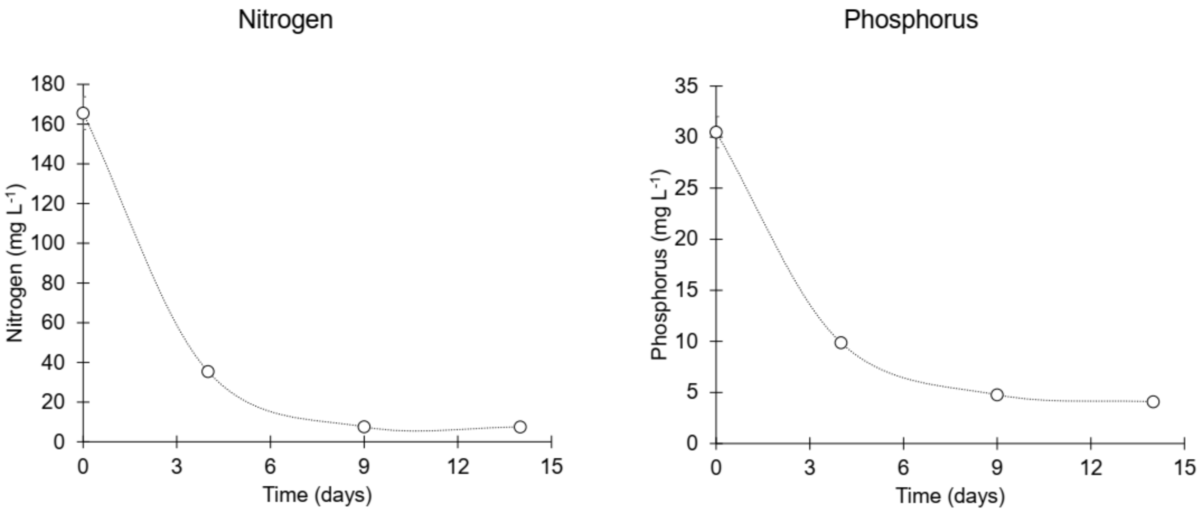

Figure 5. Nitrogen and phosphorus consumption by microalgae cultivation.

The initial nitrogen content in cultures with biodigested swine manure was approximately 160 mg L^{-1}, being metabolized during the growth and multiplication of microalgae, resulting in about 7.5 mg L^{-1} at the end of the period, which represents a reduction of approximately 95% of the total nitrogen present in the alternative culture medium. These data are compatible with those described by Taher (2013), who observed the removal of 97.1% of the total nitrogen present in swine effluent treated via microalgae cultivation. Regarding phosphorus, the initial terrors were approximately 30 mg L^{-1}, being consumed throughout the cultivation and resulting in approximately 4% at the end of 14 days, corresponding to a reduction of approximately 87%.

It is important to highlight that not only the concentrations of nitrogen and phosphorus are important to determine the growth of microalgae and biomass production but above all the balance between these components and the carbon available in the form of CO_2. Thus, it can be inferred that the C/N/P ratio in cultures with swine effluent allowed greater use of these compounds for biomass production, at the same time it resulted in greater consumption and reduction of the organic load of the effluent, reinforcing the potential of microalgae cultures as tools for wastewater bioremediation.

Finally, the levels of total lipids in the biomass produced in the synthetic culture medium and in the swine effluent were determined, in order to assess whether the production of biomass in effluent results in even greater production of oil that can be used for the production of biofuels, such as microalgae biodiesel. The biomass of both treatments showed total lipid contents of the order of 18% (m/m), which corroborates other results obtained in the NPDEAS.

4. CONCLUSIONS

The use of agro-industrial effluents to increase the production of microalgae biomass is a highly indicated strategy since the greater organic load of these materials results in greater biomass production while allowing bioremediation of what could be a liability. environmental protection in case this material was discarded in the environment and reached water bodies and water tables. Thus, integrating the production of biomass rich in compounds of high commercial value with the application of crops for environmental purposes can be a highly interesting strategy to increase the technical-economic feasibility of obtaining bioproducts from microalgae biomass, such as biofuels, for example.

5. ACKNOWLEDGEMENTS

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