INTEGRATED ENERGY RECOVERY FROM FOREST RESIDUES AND ANTIOXIDANT EVALUATION OF *TETRADESMUS OBLIQUUS* BIOMASS

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ABSTRACT

The utilisation of forestry residues as a sustainable energy source presents a viable strategy to mitigate the environmental impacts associated with fossil fuel dependency, offering a pathway to reduce greenhouse gas emissions and conserve natural resources. Additionally, exhaust gases from thermal processes provide a critical nutrient for microalgae cultivation, enhancing biomass production and contributing to sustainable development through CO_2 biofixation. This process facilitates the generation of valuable bioproducts. This study evaluates the energy potential of forestry residues derived from green pruning at UFPR as a solid fuel and investigates the antioxidant potential of microalgal biomass cultivated in a patented 12 m³ industrial photobioreactor developed by NPDEAS. The findings underscore the role of such processes in waste management, energy recovery, and the advancement of bioeconomy strategies. Spectrophotometric analysis, employing the DPPH technique at 517 nm, revealed no antioxidant activity across six tested biomass samples. Absorbance measurements ranged between 0.771 and 0.912 for samples in 60 mM DPPH at a volume of 0.2 mL, compared to a negative control absorbance of 0.758. Despite these results, the study highlights the effective use of forestry waste for CO_2 generation in microalgae cultivation systems. This integrated approach reduces atmospheric CO_2 emissions, supports microalgae growth, and enables the production of biomaterials for diverse industrial applications, including potential high-value bioactive compounds. These findings pave the way for future research into optimising the cultivation and extraction of additional valuable compounds from microalgae biomass.

1. INTRODUCTION

Green microalgae, which are unicellular, eukaryotic, and photosynthetic microorganisms, inhabit freshwater, saline, or brackish environments. These algae, belonging to the group Chlorophyta, contain chlorophyll A and B and chloroplasts, which play a crucial role in photosynthesis, as well as pyrenoids (which act as centres of carbon fixation and are involved in starch storage). Green microalgae possess unique characteristics that make them suitable for the production of pharmaceuticals, nutraceuticals, cosmetics, and biofuels, owing to their rich composition of proteins, essential amino acids, fatty acids, pigments, and other bioactive compounds (El-Monein *et al*., 2018).

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The genus *Tetradesmus*, formerly known as *Scenedesmus*, is a common green alga that includes the species *Tetradesmus obliquus* (Turpin) M.J.Wynne (formerly *Scenedesmus obliquus* (Turpin) Kützing), a microalga widely found in freshwater lakes and rivers. Known for its lipid accumulation capability, ease of cultivation, tolerance to a wide range of temperatures and pH levels, ability to remove metals, nitrogen, and phosphorus, resilience in the presence of CO_2, and high biomass production (Rörig, *et al*., 2024). This microalga stands out as a significant source of various components with potential applications in the food (functional protein nutritional products), pharmaceutical, and cosmetic industries. Studies have confirmed the antioxidant and antiviral activities of protein hydrolysates from *T. obliquus* against Coxsackie B3 virus, which can cause meningitis, myocarditis, viral pneumonia, and other systemic illnesses. Additionally, *T. obliquus* extracts and their fractions have shown promise as sources of antimicrobial and anticancer compounds. The antiproliferative activity was evidenced against human cancer cell lines MCF7 (breast), HePG2 (liver), HCT116 (colon), and HeLa (cervical adenocarcinoma). In vivo studies also indicated that *T. obliquus* may be effective in the prevention and treatment of diabetes and dyslipidaemias. Furthermore, this microalga has important applications in the production of bioethanol, biodiesel, biogas, and biohydrogen, as well as acting as a biostimulant in crop germination and growth. Various microalgae strains gain prominence due to their ease of cultivation, rapid growth, low cultivation requirements, attractive economic advantages, and high biomass productivity. Given these characteristics, *T. obliquus* presents itself as a promising and valuable source for sustainable production processes (El-Monein *et al*., 2018; Gouveia *et al*., 2021; Severo *et al*., 2024).

In addition to its extensive industrial applications and potential in sustainable production processes, *Tetradesmus obliquus* is also highly regarded for its diverse biological activities, particularly its ability to produce bioactive compounds with therapeutic potential. Notably, this microalga is a rich source of essential fatty acids (EFAs), including polyunsaturated fatty acids (PUFAs), which are critical for maintaining human health and treating various medical conditions (Piasecka *et al*. (2020). Since the human body cannot produce these fatty acids on its own, they must be acquired through diet, making this species a valuable alternative source. Additionally, it is rich in amino acids, which are fundamental for protein synthesis. Cultivating this microalga in mixotrophic conditions can significantly boost its protein yield compared to other cultivation techniques. The ability to enhance specific amino acid production through metabolic regulation presents promising opportunities for the food and feed industries, which can utilise its biomass as a key resource (Piasecka *et al*. (2020).

Protein hydrolysates derived from *Tetradesmus obliquus* have been the focus of research due to their antioxidant and antiviral properties. Studies suggest that the quality of amino acids extracted from T. obliquus cells can be enhanced through treatments with specific enzymes, such as papain and trypsin, leading to high antioxidant capacities of 41.41% and 40.62%, respectively. Moreover, both the proteins of T. obliquus and their hydrolysates have demonstrated significant antioxidant potential, reaching up to 68.23% when assessed using the ABTS radical scavenging method Afify *et al*., 2018). The DPPH and ABTS methods are regarded as effective tools for determining antioxidant activity (Marecek *et al*., 2017).

Antioxidants are essential compounds that protect cells from damage induced by free radicals, highly reactive molecules generated both by normal metabolism and environmental factors such as pollution, radiation, and smoking. These free radicals can trigger oxidative stress, a process that compromises the integrity of lipids, proteins, and DNA, leading to cellular ageing and an increased risk of chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions. By neutralising free radicals, antioxidants play a crucial preventive role, minimising oxidative damage and contributing to the maintenance of health. These compounds are found in various natural sources, including fruits, vegetables, and certain microalgae, and are widely applied in the food, pharmaceutical, and cosmetic industries. (Ferreira *et al*., 2022; Abdelrahim *et al.*, 2024).

The study of *Tetradesmus obliquus* biomass, cultivated in brewery effluent, demonstrated significant antioxidant activity, particularly under subcritical water extraction conditions at elevated temperatures. Extraction at 220°C yielded the highest total phenolic content and antioxidant activity, which inhibited 50% of DPPH radicals, indicating a greater capacity for free radical neutralisation compared to lower temperatures. Furthermore, CO_2 supplementation during microalga cultivation nearly doubled the phenolic content in the extracts, reinforcing the potential of *T. obliquus* as a promising source of bioactive compounds with high antioxidant activity, applicable across various sectors, including food, cosmetics, and pharmaceuticals (Ferreira *et al*., 2022).

Although the broad potential of microalgae is widely recognised, a crucial step for their commercialisation depends on the successful transition from laboratory-scale processes to the more challenging realm of pilot and industrial-scale operations. This transition is particularly relevant in the context of using photobioreactors (PBRs), which provide an ideal environment for cultivating microalgae. Photobioreactors offer a higher degree of

control over operational parameters, including but not limited to light, temperature, and nutrient levels, thus facilitating a more precise and scalable approach to biomass production. Additionally, they require less land space and are more productive, with shorter harvesting cycles (Costa 2018; Costa, 2023; Severo *et al*., 2024).

Thus, the implementation of photobioreactors for cultivating microalgae not only addresses the need for efficient biomass production but also aligns with efforts to reduce CO_2 emissions. The increase in CO_2 emissions has exacerbated climate change, impacting ecosystems worldwide. CO_2 biofixation by microalgae emerges as a promising solution to mitigate these effects. In addition to capturing carbon dioxide, this process results in the production of biomass rich in high-value compounds. The efficiency of CO_2 biofixation by microalgae depends on several factors, including the dissolution of CO_2 in water, its diffusion to the algal cells, and its capture during photosynthesis. In this context, the importance of operational conditions for CO_2 biofixation by microalgae becomes evident, especially in the case of the species *Tetradesmus obliquus*, which can serve as a basis for optimisations in microalgae cultivation systems aimed at carbon capture (Pchara *et al*., 2024).

The use of microalgae in wastewater treatment and heavy metal removal has been extensively investigated over the past decade, and their heavy metal accumulation has been used as biomonitors of metal pollution. Bioremediation by microalgae has been widely studied for various effluents, including municipal, agricultural, and industrial (Vladić *et al*., 2023). In addition to their carbon capture capabilities, the biomass from *Tetradesmus obliquus* also possesses significant antioxidant potential, broadening its industrial applications. This antioxidant potential of the bioproduct may be relevant for the development of nutraceutical, pharmaceutical, cosmetic, and food products, among others, offering a sustainable and effective alternative to minimise the exploitation of natural resources. The potential uses of this microalgal biomass encompass the production of biofertilisers, biostimulants, biopesticides, biopolymers, and animal feed, in addition to other conventional applications already associated with microalgae (Vladić *et al*., 2023; Rörig *et al*., 2024). The biomass produced after bioremediation treatments can be utilised as raw material in the production of renewable biofuels in the energy sector, such as alcohol, biogas, biodiesel, and biohydrogen, among other applications (Milano *et al*., 2016).

With the growing need for sustainable energy alternatives, as emphasised at the United Nations Climate Change Conference (COP 26) held in Glasgow in 2021, the importance of solutions such as bioenergy becomes even more evident (Tian *et al*., 2023). In this context, forestry residues emerge as a valuable and sustainable source for bioenergy production.

Forestry residues can be considered to include all components of trees, such as bark, needles, roots, leaves, branches, and trunks, as well as agricultural residues. All these materials can be part of the circular bioeconomy, a recently developing concept that represents the intersection of the bioeconomy and the circular economy, involving the sustainable use of forest biomass and the efficient valorisation of these resources within the production chain. These residues can often be used for the production of electricity and biomass (Carus and Dammer, 2018; Silva *et al*., 2024).

Among the sources of municipal solid waste, material resulting from the pruning and removal of urban trees faces significant challenges for more efficient disposal due to its volume, heterogeneity, and limited initiatives for uses other than combustion or composting. Municipal authorities worldwide underestimate the value of this waste for the manufacture of other products or co-products, as they ignore its composition due to the varied dimensions (length and thickness), shapes, species, and alternative uses. Many countries do not collect and disclose reliable quantitative data, nor conduct research on the generation of municipal solid waste. This lack of information creates difficulties for the proper use of the material. In Brazil, the National Policy on Solid Waste (PNRS, Law 12.305) has been implemented at the federal, state, and municipal levels since 2010. However, the national government has few comprehensive plans dedicated to the management of urban tree waste. Therefore, the PNRS requires the support of municipal and state laws to achieve maximum efficiency and applicability, also ensuring that public policies promote sustainability (Meira *et al*., 2024).

As an example, and result of the applicability of technological routes, the integration of sustainable and innovative processes aimed at the production of microalgae on an industrial scale, it is valid to consider the processes developed by the working group of the Sustainable Energy Research and Development Center (NPDEAS), located on the premises of the Federal University of Paraná (UFPR) and founded in 2008. The research conducted at NPDEAS focuses on energy sustainability by maximising the productivity of microalgae in photobioreactors for the large-scale production of biofuel and biomaterials derived from microalgal biomass. Currently, other sustainable processes aimed at eco-efficiency are also being explored by the research group. In terms of microalgae cultivation systems, NPDEAS is equipped with laboratories, compact pilot-scale tubular photobioreactors (with a volume of 12 litres) and five industrial-scale photobioreactors, each with an operational volume of 12 cubic metres, occupying an area of only 10 square metres each. Their dimensions consist of 8 metres in height, 2 metres in width, and 5 metres in length, and they use transparent polyvinyl chloride (PVC) tubes arranged to maximise the incidence of sunlight, based on compact heat

exchanger technology (Scherer, 2015; Scoculi-de-Lira *et al*., 2021).

NPDEAS has all stages of biomass processing available, from strain maintenance, harvesting, drying, extraction, and conversion into the desired final product. The first phase consists of laboratory-scale cultivation to obtain the pre-inoculum. Subsequently, production is scaled up to establish cultivation in pilot-scale photobioreactors with an industrial-scale setup (Scherer, 2015; Scoculi-de-Lira *et al*., 2021).

This process of scaling up and optimising biomass production is directly aligned with the pursuit of alternative and sustainable energy sources, which has become imperative in light of global population growth and the intensification of industrial processes. These factors have significantly increased energy demand and exacerbated environmental challenges such as climate change. To mitigate these impacts, the utilisation of solid plant waste as a sustainable energy source presents a promising alternative. The work of Scoculi-de-Lira *et al*. (2023) involved the collection and analysis of forest waste samples from UFPR to determine their energy potential. The results demonstrated the heterogeneity of these wastes, revealing a low ash content and a calorific value suitable for energy generation. This approach proves viable for reducing greenhouse gas (GHG) emissions by promoting the energy recovery of forest waste, thus contributing to global goals for reducing pollutant emissions.

Therefore, the objective of this study was to establish a sustainable procedure that integrates (1) the treatment of forest waste through incineration to generate CO_2 as a nutrient for microalgae and also to evaluate (2) the biomass obtained through cultivation in photobioreactors, characterising the potential of bioactive compounds (antioxidants) from this crude dry biomass (with the presence of lipids) and the residual biomass (after lipid extraction) from two batches produced in 2018 and 2023. The comparison between these two batches will allow for the analysis of variations in antioxidant potential over time and the identification of possible improvements in production and extraction processes. This study aims not only to optimise energy generation from forest waste but also to enhance the value of high-added-value biomaterials production, contributing to sustainable development and the circular economy. A detailed analysis of the antioxidant components present in both raw and residual biomass could offer valuable insights for industrial applications, highlighting the multifunctional potential of microalgae cultivated and sustained by forest waste incineration.

2. MATERIAL AND METHODS

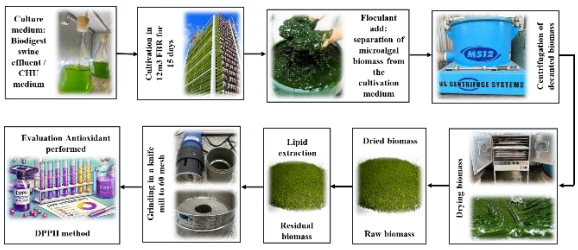
The samples were obtained from previous works by Costa (2018) and Costa (2023). To evaluate the antioxidant activity of the microalgal biomass, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method was used, adapted from the work of Huang *et al*. (2022), which is widely employed in antioxidant activity assays of plant samples. The main stages of this adapted methodology are described below.

2.1 Obtaining samples

The dried microalgal biomass was obtained from processes carried out by the NPDEAS research group. More specifically, the samples of dried biomass used for the characterisation of antioxidant potential in this work were derived from studies by Costa (2018) and Costa (2023). The microalgae were cultivated using digested swine effluent as the culture medium. The species *Tetradesmus obliquus* was grown in compact PBR's with a volume of 12 m³ (as shown in Figure 1) for 15 days. The cultures were then transferred from the PBRs to flocculation tanks. A flocculant (Tanfloc SG) was used to separate the microalgal biomass from the culture medium. The pH of the cultures was adjusted to 7 (a value within the flocculant's effective range) by adding industrial CO_2. After adding the flocculant, the flocculation tanks were stirred for 15 minutes and left to settle overnight to allow complete biomass separation. Subsequently, the decanted biomass was subjected to centrifugation (US Centrifuge System M512) at 3000 rpm and a centrifugation speed of 4 $L.min^{-1}$.

Figure 1: Industrial photobioreactor system at NPDEAS. Adapted from Scherer, 2015.

After centrifugation, the microalgae paste was evenly distributed in a tray for the drying process. Various drying methods were employed (open air drying, low pressure drying, spray drying, drum drying, freeze drying, fluidised bed drying, or drying in temperature-controlled ovens). The extraction methodology was carried out using organic solvents, and the separation of lipid compounds was performed through distillation. The preparation of the dried crude (lipid fraction) and residual (after lipid extraction) samples began with grinding in a knife mill to obtain a fine powder (60 mesh) of microalgal biomass.



Flowchart 1. Brief visual description of the processes carried out to produce and obtain the microalgal biomass extracts.

After grinding and obtaining a fine powder (60 mesh) suitable for subsequent dilution in methanol, the dry biomass samples were identified and stored in 2 ml Eppendorf tubes. The samples were obtained under the following conditions, according to Table 1:

Sample	Biomass description	Growing medium	Obtaining period
1	residual dried microalgal biomass	swine manure	2018
2	raw dried microalgal biomass – lipid fraction	swine manure	2018
3	residual dried microalgal biomass	swine manure	2023
4	raw dried microalgal biomass – lipid fraction	swine manure	2023
5	residual dried microalgal biomass	CHU	2023
6	raw dried microalgal biomass – lipid fraction	CHU	2023

These samples were prepared for subsequent analysis using the DPPH method to evaluate the antioxidant potential of the biomasses obtained under different cultivation conditions and periods.

2.2 Antioxidant Analisys – DPPH

After preparing the samples, the antioxidant analyses (DPPH) were carried out based on the experiments of Huang *et al.* (2022), as described below.

Method Validation: Mixtures of 3 mL of five methanolic solutions of DPPH (Sigma Aldrich) and the positive control, acid (+/-)-hydroxy-2,3,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) – a water-soluble analogue of vitamin E (Sigma Aldrich), were prepared. The final concentration of DPPH was fixed at 0.02 mM, with varying concentrations of Trolox (0.0075, 0.01, 0.015, 0.02, 0.03 mM). The mixtures were agitated for homogenisation and kept in the dark for 5 minutes. After this period, spectrophotometric analysis was performed, with absorbance readings taken at a wavelength of 517 nm. The experiment was carried out in triplicate. From the absorbance and concentration values, from the calibration curve, a linear relationship between Trolox concentration and absorbance was observed (R^2 = 0.99), as shown on Figure 2:

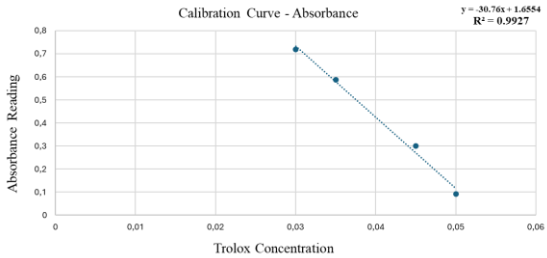
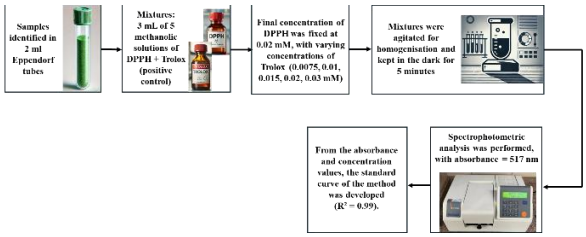


Figure 2. Calibration curve of Trolox for antioxidant activity determination using the DPPH method.

The calibration curve was constructed using different concentrations of Trolox, and the absorbance was measured at 517 nm. The procedure aims to calculate the equivalent Trolox concentration for an unknown sample. This method was developed to assess the relationship between absorbance and Trolox concentration in the presence of DPPH, maintained at a fixed concentration of 0.02 mM. Trolox is used as the antioxidant standard for this analysis.

Absorbance is measured to determine the extent to which each Trolox concentration reduces DPPH, thereby indicating antioxidant activity. Each point on the standard curve represents an absorbance measurement corresponding to a specific Trolox concentration. As the concentration of Trolox increases, a linear decrease in absorbance is observed, confirming the antioxidant effect of Trolox on DPPH.

Antioxidant Activity Assays: 100 mg of six different samples of raw and fractionated biomass residues in solid state were individually added to 100 mL of methanol. The soluble fractions were separated by filtration, and 0.2 mL of each filtrate was mixed with 2.8 mL of methanolic DPPH solution, resulting in a final DPPH concentration of 0.02 mM. Additionally, a mixture of the same DPPH concentration with 0.2 mL of a methanolic solution (1 mg/mL) of distilled biomass oil was prepared. The six samples were agitated for homogenisation and kept in the dark for 5 minutes. After this period, spectrophotometric analysis was performed, with absorbance readings taken at a wavelength of 517 nm. Any decrease in absorbance in the presence of compounds with antioxidant activity is expressed in Trolox equivalents, based on the previously described calibration curve.



Flowchart 2. Brief visual description of the processes carried out for the DPPH analyses.

3. RESULTS AND ANALYSIS

Based on the evaluation of antioxidant activity using the spectrophotometric method, no significant change was observed in the absorbance pattern of any of the six samples tested in the presence of DPPH, as shown in Table 2:

Table 2. Description of the analysed samples and their respective conditions of acquisition.

Sample	Obtaining	Volume of sample	DPPH	Absorbanc
1	(resi	0.2 mL	60	0.771
2	(raw	0.2 mL	60	0.948
3	(resi	0.2 mL	60	0.956
4	(raw	0.2 mL	60	0.953
5	(resi	0.2 mL	60	0.966
6	(raw	0.2 mL	60	0.912
Negativ	(abse	0	60	0.758

msf: methanol-soluble fraction.

Based on the observations presented in Table 2, no antioxidant activity was expressed in terms of Trolox equivalents in any of the 6 samples analysed, as there were no signs indicative of this activity under the experimental conditions evaluated. Spectrophotometric analysis was carried out using the DPPH technique, where absorbance was measured at 517 nm. The samples were prepared under the conditions described and all showed absorbance values close to the negative control, indicating the absence of compounds with antioxidant activity under the experimental conditions evaluated.

The results indicate that, under the experimental conditions evaluated, there was no significant decrease in absorbance in the presence of DPPH, suggesting that the biomass samples do not have relevant antioxidant activity when compared to the negative control.

Also, the observed increase in absorbance can be attributed to the phenomenon of Fluorescence Resonance Energy Transfer (FRET), which is particularly influenced by the greenish coloration of the samples. This greenish hue, likely resulting from the high pigment content in the microalgal biomass, contributed to significant optical interferences during spectrophotometric measurements. Such interferences are known to occur when the fluorescence emitted by donor molecules overlaps with the absorption spectrum of acceptor molecules, leading to an apparent increase in absorbance values.

The lack of significant antioxidant activity in the microalgal biomass samples cultivated under different conditions and for different lengths of time suggests the need for further research. Other methods of extracting antioxidant compounds could be explored, or tests could be carried out under different conditions to see if there is variability in antioxidant activity. Given all the processes that the biomass goes through after being inoculated and produced in the photobioreactors, analysing the fresh biomass before it is inoculated into the photobioreactors could also be considered. Further studies are considered to elucidate the antioxidant potential of microalgal biomasses under different experimental and sample conditions.

4. FINAL CONSIDERATIONS

This study has consolidated efforts to develop industrial processes that recover energy from forestry waste, demonstrating it to be an efficient fuel. This fuel can be used to generate CO_2, which is subsequently directed as a nutrient for the cultivation of microalgae in industrial photobioreactors. Additionally, spectrophotometric analysis of the resulting dried microalgal biomass samples revealed no antioxidant activities under the analysed conditions. However, the implementation of these processes not only helps to reduce CO_2 emissions into the atmosphere, but also promotes the growth of microalgae, leading to the production of biomaterials with other potentially promising activities. This approach ensures proper disposal of forestry waste without emitting pollutants into the atmosphere, contaminating the soil, or occupying physical space. Furthermore, microalgal biomass can serve as a raw material for the development of innovative

bioproducts in various industrial sectors. Thus, a potential analysis for future studies would be an evaluation of other compounds of industrial interest, such as proteins.

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