Industrial Bioresource Engineering
https://ojs.wiserpub.com/index.php/IBE/

Research Article

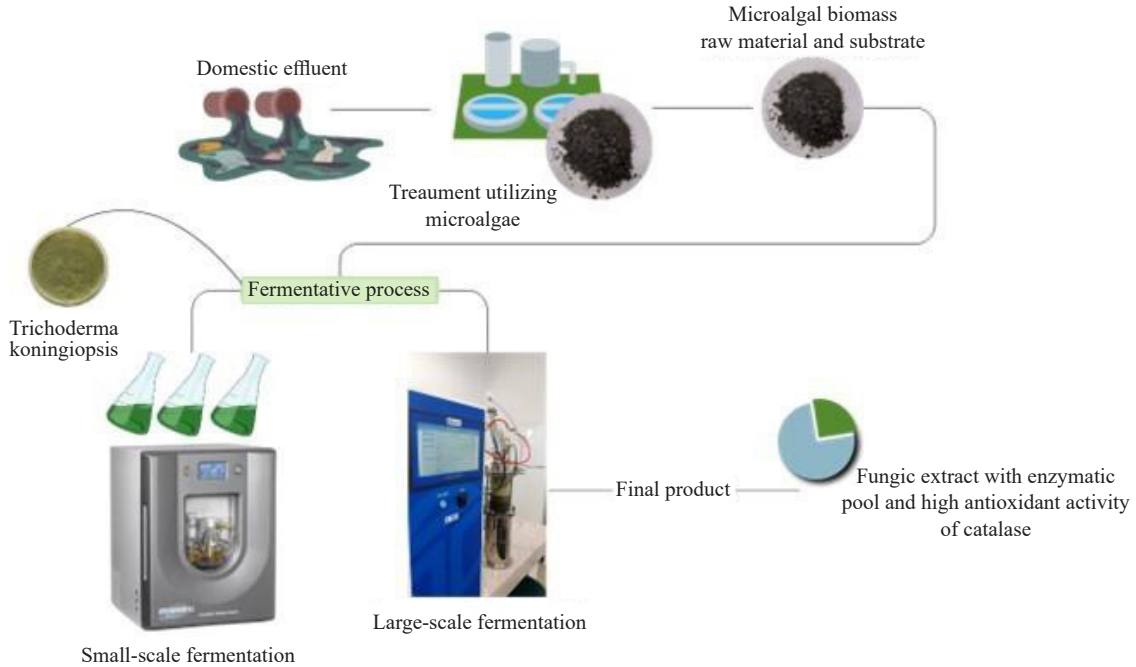
Scale-Up and Enzymatic Characterization of *Trichoderma koningiopsis* Fermented on a Microalgal Consortium from Domestic Sewage Treatment

Aline Frumi Camargo[1,3], Simone Kubeneck[2,3], Vitória Dassoler Longo[3], Larissa Capeletti Romani[3], Gustavo Henrique Ribeiro da Silva[4], Gislaine Fongaro[1], Jadiane Paola Cavaler[5,6], Helen Treichel[1,2,3*]

[1]Graduate Program in Biotechnology and Biosciences, Federal University of Santa Catarina, Florianópolis, Brazil
[2]Graduate Program in Environmental Science and Technology, Federal University of Fronteira Sul, Erechim, Brazil
[3]Laboratory of Microbiology and Bioprocess, Federal University of Fronteira Sul, Erechim, Brazil
[4]Department of Civil and Environmental Engineering, School of Engineering Bauru, São Paulo State University (UNESP), Bauru, SP, Brazil
[5]Project Analyst, Dr Biogás Company, Guarapuava, Brazil
[6]Serviço Nacional de Aprendizagem Industrial (SENAI), Concórdia, Brazil
Email: helen.treichel@uffs.edu.br

Received: 30 September 2025; **Revised:** 27 November 2025; **Accepted:** 4 December 2025

Graphical Abstract:



DOI:

Abstract: The increasing demographic growth and urbanization pose significant challenges for sanitation and pollution control. This study evaluated the feasibility of scaling up a fermentation process using microalgae cultivated in domestic sewage as a substrate and *Trichoderma koningiopsis* as the fermentative agent to produce enzymes of environmental relevance. Microalgae were grown in wastewater from the Candeia Sewage Treatment Plant (Bauru, SP, Brazil), and fermentations were carried out at both small and bench-scale, the latter conducted in an airlift bioreactor under previously optimized conditions. The methodology involved characterizing the microalgal biomass and quantifying a broad enzymatic profile. The results showed that *Trichoderma koningiopsis* effectively utilized biomass as a substrate, thereby promoting enzyme production. The transition to large-scale production was positive, maintaining significant enzyme production and validating the robustness of the process under expanded conditions. Among the enzymes evaluated (amylase, cellulase, lipase, protease, peroxidase, laccase, catalase, ascorbate peroxidase, and superoxide reductase), catalase exhibited the highest activity, reaching 7,344.83 U/mL in condition C1. These findings confirm that microalgal biomass derived from domestic wastewater is a viable resource for biotechnological applications, supporting the development of sustainable, scalable processes for producing industrial enzymes.

***Keywords*:** enzyme production, antioxidant enzymes, environmental biotechnology, wastewater valorization, bioreactor

1. Introduction

Nowadays, we are experiencing a continuous population increase. In developing countries, urbanization is growing faster than urban planning, leading to sanitation problems that can cause diseases from contaminated effluents (Iribarnegaray et al., 2018).

Conventional wastewater treatment technologies still have shortcomings, as they result in high energy consumption, carbon dioxide emissions, and the use of chemicals. Thus, it is possible to use other technologies that cause less environmental damage, such as microalgae, which can generate value-added biomass (used for pharmaceutical purposes, food supplements, health products, etc.) and remove organic carbon, nitrogen, and phosphorus. Compared to conventional treatments, microalgae-based treatments are more advantageous. In addition to the lower associated price, the effluent that reaches water bodies is oxygenated, allowing the extraction of proteins and lipids from the biomass generated while simultaneously removing phosphorus and nitrogen, among other benefits (Arbib et al., 2014). Domestic sewage treatment can be done by phycoremediation using *Chlorella vulgaris*, which can remove organic and inorganic pollutants, such as total nitrogen and phosphorus, by 71% and 67%, respectively (Gani et al., 2016).

Due to the stress caused by climate change, pollutants, and pesticides, microalgae increase their antioxidant responses and enzymatic activities, such as catalase and superoxide dismutase (Chokshi et al., 2015; Wang et al., 2020; Du et al., 2022). In addition, several studies have investigated this biomass's benefits for human health and its potential to treat diseases such as Alzheimer's and diabetes, suggesting a promising response (Odenthal et al., 2024).

The bioactive compounds in microalgae, such as proteins, carbohydrates, amino acids, and vitamins, are essential for the human diet. *Chlorella vulgaris* is widely used in this context, as its molecules exhibit strong rebiotic, antioxidant, and anticancer properties (Thiviyanathan et al., 2024). It can be used as a biofertilizer, increasing soil residual carbon and nitrogen levels and improving pH and electrical conductivity. Among pesticides, the cyanobacteria *Chlorogloea*, *Arthronema*, *Spirulina,* and *Calothrix* stand out, which, through the production of bioregulators such as indoleacetic acid, cytokinins, gibberellins, and jasmonic acid, can act in this field (Fernández et al., 2021).

In that same hand*, Trichoderma koningiopsis* is a filamentous fungus widely recognized for its capacity to secrete a broad spectrum of hydrolytic enzymes, including cellulases, xylanases, and proteases, which are essential for the bioconversion of complex biomass into fermentable sugars and bioactive compounds. While microalgae naturally produce bioactive molecules, their rigid cell walls often limit their accessibility and release. The application of *Trichoderma koningiopsis* in fermentation processes enhances the degradation of microalgal biomass, facilitating the release of intracellular content and improving the yield of enzymatic and potentially valuable metabolites. Moreover, its rapid growth, low pathogenicity, and adaptability to unconventional substrates make it an ideal candidate for integrated biotechnological systems using wastewater-derived resources (Kubeneck et al., 2025; Camargo et al., 2024).

Studying the scale-up of fermentation processes is crucial for assessing the technical and economic viability of biotechnology under conditions closer to industrial applications. Even if a process performs well at bench scale, it can

face significant challenges when scaled up to larger volumes, including changes in oxygenation profiles, mass transfer, nutrient gradients, and temperature variations. Evaluating the behavior of *Trichoderma koningiopsis* cultures under expanded conditions enables us to identify operational limitations, optimize enzyme-production parameters, and ensure reproducibility of results (Lima et al., 2022; Camargo et al., 2024). Furthermore, by using microalgal biomass from domestic sewage treatment as a fermentation substrate, the larger-scale study also helps validate the potential for reusing waste in sustainable biorefineries focused on the circular economy.

The enzyme profile resulting from the fermentation of microalgal biomass by *Trichoderma koningiopsis* aims to identify enzymes of biotechnological value, including cellulases, amylases, lipases, and antioxidant enzymes such as catalase, peroxidase, ascorbate peroxidase, and superoxide dismutase. While hydrolases degrade complex polymers and release fermentable sugars, antioxidant enzymes play a fundamental role in neutralizing Reactive Oxygen Species (ROS), particularly in environmental applications such as bioremediation, the control of oxidative stress in contaminated soils, and the development of biostimulant formulations for plants. Previous studies have shown that *Trichoderma koningiopsis* strains can express peroxidase and catalase during fermentation on different substrates, including agroindustrial waste and microalgae, indicating their adaptability and potential for multifunctional applications (Kubeneck et al., 2025; Dos Santos et al., 2025). Furthermore, the combination of microalgal biomass and fungi can promote synergy in the induction of these enzymes, thereby enhancing the antioxidant response and nutrient utilization from the substrate (Camargo et al., 2024). Thus, the expanded characterization of the enzyme profile contributes to the development of bioproducts for biorefineries and sustainable agriculture, as well as to mitigating environmental impacts (Camargo et al., 2023b).

The intertwined problems of inadequate sanitation and pollution in developing countries require a holistic approach to addressing infrastructure deficits and broader socio-economic factors. Comprehensive strategies that involve government policy, community participation, and international support are crucial to mitigating these challenges and enhancing the overall quality of life (Aranha et al., 2025). This detailed exploration underscores the importance of addressing these issues to achieve healthier, more sustainable communities and environments. This article aimed to assess the feasibility of scale-up fermentation using microalgae derived from domestic sewage treatment and the fungus *Trichoderma koningiopsis* to produce a broth rich in enzymes of environmental interest.

2. Materials and methods

2.1 *Microalgae cultivation*

The experiment was carried out during the winter season in Brazil (27 June to 2 July 2022). Ambient temperature and light intensity were obtained from the automatic meteorological station located at latitude 22°21′27.6″ S and longitude 49°01′40.8″ W (IPMet/UNESP, Bauru), with data recorded every 5 minutes. During the experimental period, the daily average light intensity ranged from 694.29 to 841.31 μmol·m^{-2}·s^{-1}, and the temperature ranged from 18.0 to 20.0 °C. The natural light: dark photoperiod averaged 11.5 : 12.5 h. The average pH values ranged from 8.90 to 9.60 throughout cultivation. The microalgae were cultivated outdoors under natural environmental conditions in a 64 L glass Flat-Panel Photobioreactor (FP-PBR) (120 cm long, 60 cm high, 10 cm wide) with a 50 L working volume of anaerobically pretreated sewage, which underwent secondary treatment in an Upflow Anaerobic Sludge Blanket (UASB) reactor. The UASB reactor operated under typical conditions for sewage treatment, including a Hydraulic Retention Time (HRT) of approximately 8 hours and a temperature maintained around 30 °C, with an organic loading rate near 2.5 kg COD/m^3·day, consistent with the parameters reported by Silva et al. (2017). This pretreatment reduced organic load and improved conditions for microalgae growth. Native microalgae inoculum was grown anaerobically using pretreated sewage from the Candeia WWTP, obtained through a phytoprospection process as established by Wilkie et al. (2011) and James et al. (2025). Although no species were isolated or purified, the consortium naturally developed from anaerobically digested wastewater. The community analysis showed that *Tetradesmus obliquus* was the dominant taxon, followed by *Cyanobium, Desmodesmus, Chlamydomonas*, and *Chlorella* spp. with lower relative abundances. The predominant species of microalgae inoculum are *Tetradesmus obliquus* (65.4%), *Cyanobium* (13.6%), *Desmodesmus* (8.1%), *Chlamydomonas* (7.4% and *Chlorella* sp. (5.4%). The total cell concentration of the inoculum was estimated at approximately 1.2 × 10^6 cells/mL using a Neubauer hemocytometer, counted under an optical microscope at 400 ×

magnification.

2.2 *Characterization of microalgae biomass*

The composition of the microalgae was analyzed according to the standard methodology of the National Renewable Energy Laboratory (NREL), which determines ash, moisture, and solid content using a muffle furnace at different temperatures, as described by NREL (Sluiter et al., 2008).

2.3 *Fungal strain*

The fungus used in this study was *Trichoderma koningiopsis* (GenBank identification code MK860714), which was isolated from the weed *Digitaria ciliares* and showed promising results for enzyme production in other studies (Bordin et al., 2018; Reichert Júnior et al., 2019; Camargo et al., 2023a; 2024).

2.4 *Small-scale fermentation*

The small-scale submerged fermentation was carried out in a 300 mL Erlenmeyer flask containing 100 mL of the proper volume. The medium consisted of 6.66 g of dry microalgal biomass, intended to supplement a previously studied and optimized synthetic fermentative medium for enzyme production (Bordin et al., 2018; Stefanski et al., 2020). The medium containing microalgal biomass was sterilized before inoculation. The Erlenmeyer flasks were inoculated with 10^6 spores/mL of the fungus *Trichoderma koningiopsis* in a fermentative medium. They were kept in an orbital shaker (New Brunswick TM, Germany) at 120 rpm for 72 h and 28 °C. Furthermore, a negative control (medium without fungal inoculation) was maintained under the same fermentation conditions to allow comparison with the inoculated samples. After fermentation, the extracts were filtered by manual pressing on synthetic fabric; the retained solid was sterilized and discarded, and the liquid permeate was centrifuged (NT 815-NovaTecnica, Brazil) at 2,000 rpm and 4 °C for 10 min. The centrifugation supernatant was used for enzyme quantification.

2.5 *Large-scale fermentation*

Preliminary small-scale fermentation tests helped to outline the scale-up process. The scale-up process was carried out in an Airlift benchtop bioreactor, model Bio-Tec-Pro-II (Tecnal, Brazil), with a proper volume of 3.0 L. Three different fermentations were performed, and operational parameters were adjusted based on studies using the same bioreactor (Camargo et al., 2024). Table 1 shows the conditions tested; all tests were performed at 28 °C for 72 hours.

Table 1. Operating conditions used in the Airlift benchtop bioreactor to study the scale-up process for obtaining an enzymatic extract from microalgae grown in domestic sewage and the fungus *Trichoderma koningiopsis*

Condition	Microalgal biomass (g)	Antifoam volume (mL) ($V_i + V_f$ = total)	Aeration (LPM)	pH*
C1	5.53	400 + 0 = 400	8	8.3
C2	11.06	0 + 16 = 16	6	7.8
C3	11.06	16 + 0 = 16	8	7.8

* The pH values showed no significant fluctuations ($p < 0.05$) during the fermentation process. The bioreactor is equipped with a sensor that takes real-time readings; from these values, it was possible to conclude that there were no pH fluctuations during the process.

The fermentations were carried out at a final volume of 2.0 L. The amount of microalgae was defined based on a previous study (Camargo et al., 2024), taking into account the moisture content of the microalgae samples (test C1) and doubling the amount in tests 2 and 3; the remainder of the liquid portion was divided between distilled water, inoculum, and antifoam (see Table 1). Dissolved oxygen was monitored to maintain it at 75% or higher. This value was determined

to keep the microorganism for enzyme production. The antifoam AFP 320 from FAXON Química was used in the tests. Table 1 shows the total amounts of antifoam used in the fermentations; the values are arranged as follows: $V_i + V_f =$ total. The initial volume values (V_i) refer to the amount of antifoam diluted in the fermentation medium. In addition, antifoam was also added during the process (V_f) under some conditions. The bioreactor was autoclaved at 120 °C for 30 min at 1 atm. After sterilization, the tests were inoculated with a suspension of 106 spores/mL of Trichoderma koningiopsis. During the tests, samples (approximately 20 mL) were collected every 24 h for enzymatic verification.

In conditions C1 and C3, at each sample withdrawal, the same volume of microalgal biomass diluted in distilled water (10% concentration) was added to the bioreactor to evaluate how the process would behave upon reintroducing the substrate over time. The collected samples and the final extract were filtered by manual pressing through a synthetic fabric; the retained solid was sterilized and discarded, and the liquid permeate was centrifuged (NT 815-NovaTecnica, Brazil) at 2,000 rpm, 4 °C for 30 min. The centrifugation supernatant was used for the subsequent steps.

2.6 *Enzyme quantification*

Some enzymes of environmental and biotechnological interest were quantified to evaluate the effects of the different tests (C1, C2, C3) performed in the bioreactor. For the determination of amylase activity, the extract was initially reacted with acetate buffer diluted in starch (100 mM, pH 5.0) at 38 °C for 10 min for subsequent quantification of Total Reducing Sugars (TRS) at 540 nm in a spectrophotometer (UV-M51, Bel Photonics, Monza, Italy) using the DNS method. Cellulase activity was evaluated by measuring the extract's reaction in acetate buffer (0.2 mM, pH 5.5) at 50 °C, followed by TRS measurement. The enzymatic activities of amylase and cellulase were calculated from the glucose standard curve and expressed as U/mL (Fuwa, 1954; Pongsawadi et al., 1987; Ghose, 1987; Miller, 1959).

Lipase activity was measured by titration according to the methodology using an emulsion composed of gum arabic (5%, v/v. Purity grade PA Dinâmica, Brazil), olive oil (10%, v/v. Gallo, Portugal) and phosphate buffer (100 mM, pH 6.0) was prepared, to which the fermented extract was added and maintained at 35 °C, 165 rpm for 32 min. The reaction was stopped with an acetone/ethanol solution (1 : 1, v/v, purity grade 99.5%, Dinâmica, Brazil) and titrated to pH 11.0 with sodium hydroxide (NaOH, 0.050 M, purity grade 98%, Dinâmica, Brazil). A control containing the extract, emulsion, and acetone/ethanol solution (for no reaction) was tested for each sample. Lipase activity was calculated using the molarity of sodium hydroxide and expressed in U/mL (Treichel et al., 2017).

To quantify the peroxidase enzyme activity, the extract was added to the reaction medium composed of phosphate buffer (5 mM, pH 5.0), guaiacol (1%, Purity grade ≥ 98.0%, Merck, Germany), hydrogen peroxide (0.08%, Purity grade 35%, Dinâmica, Brazil), and distilled water, at 35 °C for 10 min. A control sample was tested using water instead of fermented extract. The transmittance of the oxidized compounds was measured at 470 nm, and enzymatic activity was estimated by the oxidation of the substrate to tetraguaiacol and expressed in U/mL (Devaiah et al., 2009; Khan et al., 1994).

To determine laccase activity, the extract was added to a reaction medium containing 2,2′-azino-di-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), a substrate for the enzymatic reaction, at 40 °C for 4 min. A control sample was tested using water instead of fermented extract. One unit of laccase activity (U) was defined as the amount of enzyme capable of forming 1 µmol of $ABTS^+$ per minute under the reaction conditions (Hou et al., 2004).

To quantify Superoxide Dismutase (SOD), the extract was added to a reaction medium containing sodium phosphate buffer (50 mM, pH 7.8), methionine (13 mM), NBT (75 µM), EDTA (0.1 mM), riboflavin (2 µM), and distilled water. The reaction was performed for 5 minutes under light exposure using a 15 W fluorescent lamp, and absorbance was quantified in a spectrophotometer at 560 nm, with readings taken every 15 seconds for 1 minute. The reaction blank was performed in the dark. SOD activity is defined as the amount of enzyme required to inhibit 50% of NBT photoreduction (Hasan et al., 2022).

To quantify Catalase (CAT), the extract was added to a reaction medium containing potassium phosphate buffer (50 mM, pH 6.8), hydrogen peroxide (12.5 mM), and distilled water at 25 °C for 2 minutes. The absorbance of the oxidized compounds was quantified in a spectrophotometer at 240 nm for 3 minutes, taking readings every 30 seconds (Havir et al., 1987; Hasan et al., 2022).

To quantify Ascorbate Peroxidase (APx), the extract was added to a reaction medium containing potassium phosphate buffer (50 mM, pH 6.0), ascorbic acid (0.8 mM), hydrogen peroxide (1.0 mM), and distilled water at 25 °C for 2 minutes. The absorbance was quantified in a spectrophotometer at 290 nm for 1 minute, with readings taken every

15 seconds (Nakano et al., 1981; Fal et al., 2022).

2.7 *Statistical analysis*

The data obtained from the tests were analyzed using STATISTICA 8.0 Software with Analysis of Variance (ANOVA), with significance set at $p \leq 0.05$.

3. Results and discussion

3.1 *Characterization*

The microalgal biomass comprises 97.54% total solids, 2.46% moisture content, and 25.64% ash. It is a dry microalgal biomass, as reflected in its low water content. The microalgae biomass had a high solids content, which may be directly linked to the amount of organic matter available for efficient fermentation in the tests, since the microalgae served as a substrate for the fungus to ferment and produce enzymes of interest. This type of characterization is crucial for optimizing biotechnological processes and ensuring efficient enzyme production, thereby enabling us to understand the potential of biomass (Stefanski et al., 2020; Ferreira et al., 2023). The characterization shows that microalgae can potentially recover nutrients for growth from various sewage types, including domestic sewage, as it contains many organic and inorganic nutrients needed for microalgae cultivation. Thus, cultivation systems can operate at a lower cost for microalgal growth while treating sewage. In addition, by incorporating nutrients, microalgae cultivated in domestic wastewater can later replace synthetic fertilizers (Álvarez-González et al., 2022).

3.2 *Evaluation of small-scale enzyme production*

The control assay already contains enzymes, as shown in Figure 1. Microalgae are primary producers and accumulate active and bioavailable molecules (Barsanti et al., 2022). After fermentation (72 hours), enzymatic activity increased, with peroxidase activity being the most pronounced. Studies show that the fungus used, *Trichoderma koningiopsis*, is a good enzyme producer. Furthermore, this genus is widely known for colonizing various substrates under different conditions and secreting multiple hydrolytic enzymes and secondary metabolites (Nicolás et al., 2014; Reichert Júnior et al., 2019). The fungal genus *Trichoderma* is well-known for its remarkable adaptability to a wide range of environmental conditions, enabling it to colonize diverse organic and inorganic substrates, including agricultural residues, lignocellulosic biomass, and wastewater-derived materials. This ecological versatility is closely linked to its metabolic plasticity. Specifically, Trichoderma species are prolific producers of hydrolytic enzymes, such as cellulases, xylanases, proteases, and chitinases, which enable the degradation of complex biopolymers into simpler compounds that can be absorbed and utilized for growth. Furthermore, these fungi can synthesize a variety of secondary metabolites, including antibiotics, siderophores, and other bioactive compounds. These compounds contribute not only to substrate colonization and nutrient acquisition but also to the suppression of competing microorganisms, making Trichoderma a highly competitive and industrially relevant genus in biotechnological applications such as biocontrol, enzyme production, and waste valorization (Bai et al., 2023; Guo et al., 2023; Kredics et al., 2024).

The laccase enzyme was not quantified; it requires substrates with chemical inducers (e.g., aromatic and phenolic compounds), which are only detected in more complex fermentations (Camargo et al., 2023b). On the other hand, we did not detect lipase activity in any of the tests, which may be linked to factors such as the nutrient composition of the medium or the microalgae's growth phase. In addition, lipases catalyze the hydrolysis of triglycerides into fatty acids and glycerol. This process occurs between the aqueous and lipid phases, making water essential for lipase activity. As the microalgae used were dry, with a low moisture content, this may have negatively influenced the production of this enzyme, affecting various aspects, including cell growth and biochemical mechanisms (Satpati et al., 2023).

The presence of amylase in the control assay may reflect microalgae's ability to degrade carbohydrates, primarily starch. After fermentation, amylase activity increases, which can be attributed to the fungus (Camargo et al., 2024). Therefore, we understand that microalgae have potential as a fermentative substrate for enzyme production, and we expanded the scale of enzyme production accordingly; the results are presented below.

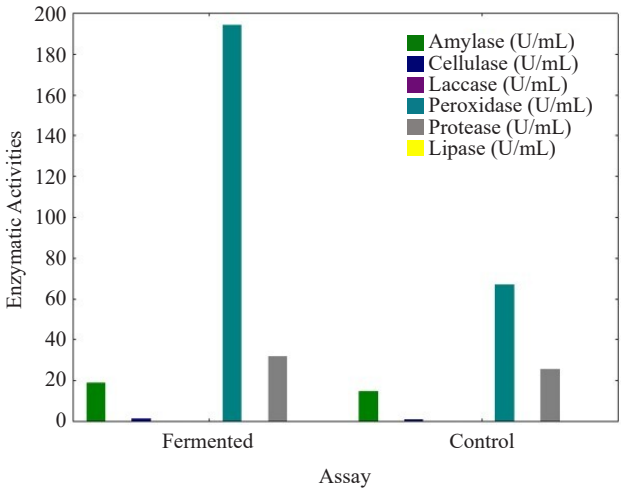
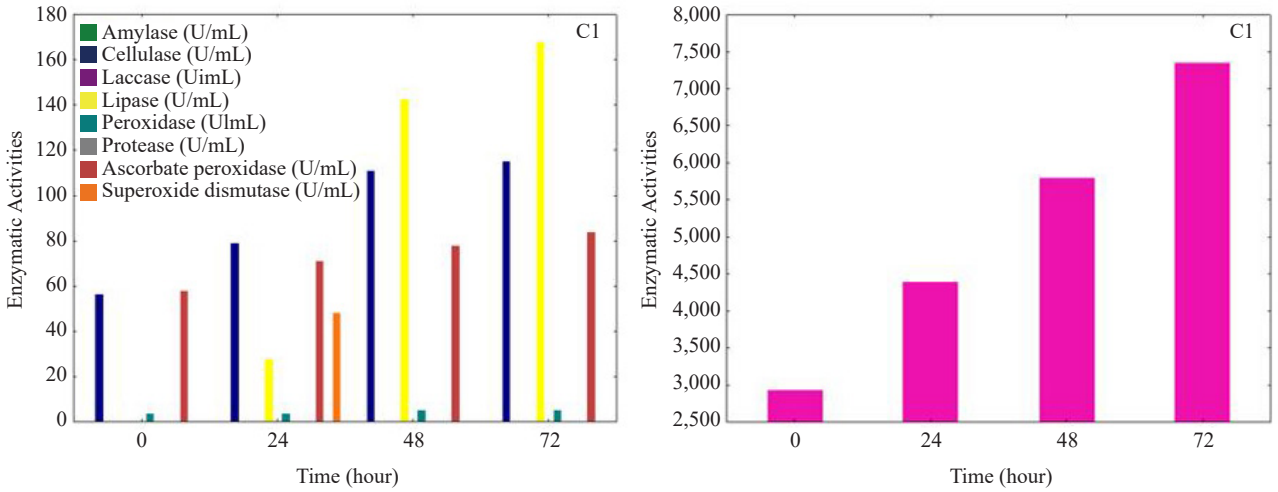


Figure 1. Results of enzymatic activity in 72 hours of small-scale fermentation. The control sample was not inoculated; the results refer only to the enzymes present in the medium

3.3 *Evaluation of scale-up enzyme production*

In fermentation processes, airlift bioreactors are commonly used because they promote foam formation in the medium; in such cases, antifoaming agents are often added to protect the equipment and the bioprocess integrity. According to previous studies, this may indicate better metabolic activity of *Trichoderma koningiopsis* (Frumi Camargo et al., 2020). However, in the tests with a greater amount of microalgal biomass (11.06 g), it was possible to reduce the volume of antifoam added (16 mL) compared to the study (Camargo et al., 2024) as foam formation is also linked to the interaction between proteins and other molecules in the medium, such as enzymes formed during fermentation (Singhania et al., 2022; Dineshkumar et al., 2020). There may have been a lower frequency of these processes, leading to less foam formation.

The first bioreactor test, C1, reproduces the best-optimized condition in the base study (Camargo et al., 2024). In this test, no foam formation was observed, unlike in previous studies. We can see that the amount of antifoam used (400 mL) was excessive. Although the conditions tested in C1 were not ideal, enzymes were produced during fermentation, as shown in Figure 2.



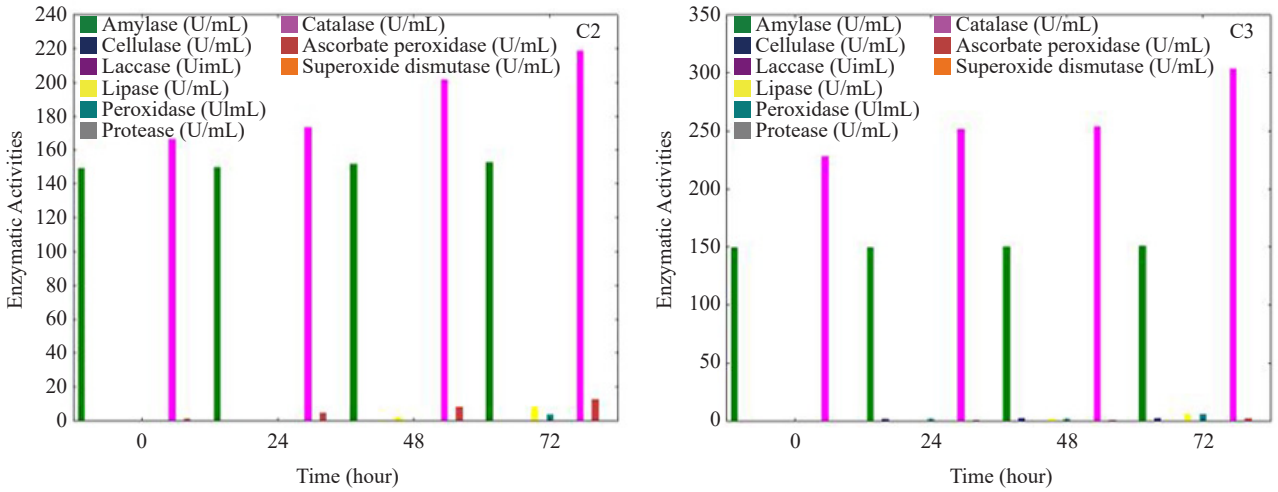


Figure 2. Results of enzymatic production present in fermentations carried out in the Airlift bioreactor under different conditions: C1, C2, and C3. The quantified enzymes are named in the legend. In condition C1, catalase activity was plotted on a separate graph for better visualization

The antifoam used in this study is a preparation of alcohols, esters, and silicone emulsions, all of which are biodegradable, an essential factor for developing the microorganism (Tiso et al., 2024). Because the antifoam volume in C1 was excessive, it may have caused stress on the fungus, leading to higher levels of lipases and cellulases, as shown in the graph in Figure 2.

Several microorganisms, including microalgae and fungi, produce extracellular cellulases that help dissolve crystalline cellulose. For this reason, the enzymes released by these microorganisms are the most suitable for large-scale use. In the same way, *Trichoderma* species are considered the best producers of cellulase for industrial use; the cost of the cellulase enzyme and its stability are essential considerations in its application. In the case of C1, the microalgae may already have contained cellulase; this can be confirmed by the presence of the enzyme at time zero. As fermentation progressed, the fungus was able to utilize microalgae and produce more cellulase (Korsa et al., 2023; Mattam et al., 2022).

The lipase enzyme appears more significantly in test C1, reaching values of 167 U/mL, while the other tests do not exceed values above 9 U/mL. In this test, 400 mL of antifoam was used. These are formed by oils, which may explain the lipase enzyme's activity and the modification of the medium's physicochemical properties (Kar et al., 2012). At the same time, studies report that alkaline conditions increase the production of this enzyme by facilitating the solubilization and hydrolysis of lipids and proteins, thereby providing easier access to the substrate (Bi et al., 2024).

As shown in Figure 2, for assay C1, the highest enzyme production occurred at 72 hours, except for superoxide dismutase, which peaked at 24 hours and showed no activity thereafter. It is worth noting that, before starting the fermentation process (time zero), enzymatic activities were already present, as shown in the graphs. These enzymes may come from microalgal biomass after heat-treatment sterilization in an autoclave. The enzyme catalase (shown in a separate graph) stands out, producing an activity of 7,344.83 U/mL. Other enzymes, such as lipase, cellulase, ascorbate, and peroxidase, showed lower activity. The enzyme laccase showed no activity, as observed in another study (Camargo et al., 2024), likely because metal inducers are required for its production. The increased enzyme production may be linked to the reintroduction of the substrate during fermentation and to maintaining dissolved oxygen. According to previous studies, this reintroduction increases enzyme activity mainly because the bioprocess is not adversely affected by depletion of nutritional sources (Dineshkumar et al., 2020; Zhan et al., 2023).

Test C2 shows higher catalase and amylase activities, with amylase remaining stable at approximately 150 U/mL throughout fermentation. In contrast, catalase peaked at the end of fermentation, reaching 218 U/mL. Enzymes such as ascorbate peroxidase, lipase, and peroxidase presented low activity in test C2. In test C3, amylase was again constant, with values of approximately 150 U/mL. At the same time, the catalase enzyme showed its peak enzymatic activity at the end of fermentation, with a value of 303.5 U/mL, similar to that in test 2.

Regarding amylase maintaining its activity during fermentations, it is known that converting microalgae into

glucose requires the enzyme α-amylase in the initial stage. It is then followed by a glucoamylase enzyme to produce glucose. The main obstacle at this stage is the binding of microalgae starch to a rigid cell wall. Therefore, a pretreatment process would be necessary to release the starch into the medium, which would later be used as a carbohydrate source for fungi (Padil et al., 2023). This may have occurred in conditions C2 and C3. After sterilization of the microalgal medium in an autoclave, the starch was released. This can be seen at time zero of the fermentations, when amylase activity was already present and persisted until the end of the fermentations.

Among all the tests, the prevalence of catalase is notable, possibly because this enzyme catalyzes the conversion of hydrogen peroxide to water (Foyer et al., 2011). Since these microalgae are used in effluent treatment and are exposed to pollutants, the activities of antioxidant enzymes (catalase, ascorbate peroxidase, and superoxide dismutase) may have increased when the microalgae absorbed the contaminants (Xiong et al., 2016), corroborating their activity in the tests. Furthermore, under stress factors (in our study, including pH conditions, antifoam presence, and air injection), the antioxidant response of microalgae may be modulated, leading to increased antioxidant enzyme production and activity (Odenthal et al., 2024).

For comparison purposes, studies of fermentation conditions using the same fungus and equipment but with different microalgae biomass demonstrated that the optimized process established in the first study (Camargo et al., 2024) can be reproduced and is an adaptable and flexible bioprocess, which is relevant since it uses residual biomass as a substrate source. Furthermore, we noticed that within 72 hours, the activities reach their peak without extending the fermentation time beyond that, which could save energy and reduce inputs added to the reactor.

Microbial broth fermented with microalgae offers a multifunctional solution—treating wastewater, reducing pollutants, and generating valuable byproducts such as biomass. This integrated technology can significantly address sanitation and pollution challenges in developing countries, ultimately improving public health, environmental sustainability, and economic development. This innovative approach holds promise for sustainable sanitation and pollution control by harnessing natural processes.

4. Conclusion

Based on the results of this study, it is evident that microalgae biomass has a diverse range of applications, and exploring its full potential is challenging. Through the fermentations studied, we realized that the fungus can utilize biomass from sewage treatment as a food source and produce a range of industrial and biotechnologically interesting enzymes.

Microalgae biomass serves as a biofactory for enzyme production, offering a promising avenue for further studies that provide insights into microalgae-fungal interactions. The results highlight the viability of microalgae biomass as a rich source for the fungus *Trichoderma koningiopsis*, which, under appropriate fermentation conditions, yields enzymes with potential applications.

This study demonstrated the feasibility of scaling up the fermentation of microalgal biomass derived from domestic sewage using Trichoderma koningiopsis as the fermenting agent. Operation in this airlift bioreactor maintained process stability and improved enzymatic performance, indicating that scaling up could be a promising alternative for industrial and environmental applications. Regarding the enzyme profile, the results revealed significant production of antioxidant enzymes, particularly catalase, as well as superoxide dismutase and peroxidase, especially since this study reduced the fermentation time (72 hours) compared to previous studies. These enzymes have high potential for applications in bioremediation processes and sustainable industrial formulations. Therefore, the studied process demonstrates not only the possibility of using microalgal biomass as a fermentation substrate but also the relevant role of the fungus in intensifying the enzyme production of environmental interest.

Acknowledgments

The authors thank the Brazilian Funding Agencies: Brazilian National Council for Scientific and Technological Development (CNPq-302484/2022-1), the support of the Bioprocess and Biotechnology for Food Research Center (Biofood), which is funded through the Research Support Foundation of Rio Grande do Sul (FAPERGS-22/2551-0000397-4,

24/2551-0001209-5), FAPERGS-FAPESP (2023 24/2551-0001945-6), Coordination of the Superior Level Staff Improvement (CAPES), Federal University of Fronteira Sul (UFFS) and Federal University of Santa Catarina (UFSC) for the financial support.

Authors contributions

AFC and HT conceived and designed the study. AFC, SK, VDL, and LCR analyzed the data, drafted, wrote, and carefully revised the manuscript. JPC, GHRS, GF, and HT critically reviewed and supervised the development of the paper. All authors reviewed and approved the final manuscript.

Funding sources

This study was supported by the Brazilian National Council for Scientific and Technological Development [CNPq-302484/2022-1] and the Coordination of the Superior Level Staff Improvement.

Conflict of interest

The authors declare no competing financial interest.

References

Aranha, S., Ferreira, M. L., Sakurai, C. A., Rocha, R. S., & Fontana, C. F. (2025). Lessons and challenges of sanitation governance in different territories of global south and north: A systematic review. *GeoJournal, 90*, 148. https://doi.org/10.1007/s10708-025-11417-2

Arbib, Z., Ruiz, J., Álvarez-Díaz, P., Garrido-Pérez, C., & Perales, J. A. (2014). Capability of different microalgae species for phytoremediation processes: Wastewater tertiary treatment, CO_2 bio-fixation, and low cost biofuels production. *Water Research, 49*, 465-474. https://doi.org/10.1016/j.watres.2013.10.036

Álvarez-González, A., Uggetti, E., Serrano, L., Gorchs, G., Ferrer, I., & Díez-Montero, R. (2022). Can microalgae grown in wastewater reduce the use of inorganic fertilizers? *Journal of Environmental Management, 323*, 116224. https://doi.org/10.1016/j.jenvman.2022.116224

Bai, B., Liu, C., Zhang, C., He, X., Wang, H., Peng, W., & Zheng, C. (2023). Trichoderma species from plant and soil: An excellent resource for biosynthesis of terpenoids with versatile bioactivities. *Journal of Advanced Research, 49*, 81-102. https://doi.org/10.1016/j.jare.2022.09.010

Barsanti, L., Birindelli, L., & Gualtieri, P. (2022). Paramylon and other bioactive molecules in micro and macroalgae. *International Journal of Molecular Sciences, 23*(15), 8301. https://doi.org/10.3390/ijms23158301

Bi, W. H., Hao, Z. H., & Butardo, V. M. (2024). Chemical pretreatment optimizes thermophilic enzyme activity in lignocellulosic and lipid-rich sesame waste composting. *International Journal of Environmental Science and Technology, 22*(6), 4007-4016. https://doi.org/10.1007/s13762-024-05869-1

Bordin, E. R., Frumi Camargo, A., Rossetto, V., Scapini, T., Modkovski, T. A., Weirich, S., Carezia, C., Barretta Franceschetti, M., Balem, A., Golunski, S. M., Galon, L. (2018). Non-toxic bioherbicides obtained from *Trichoderma koningiopsis* can be applied to the control of weeds in agriculture crops. *Industrial Biotechnology, 14*(3), 157-163. https://doi.org/10.1089/ind.2018.0007

Camargo, A. F., Dalastra, C., Ulrich, A., Scapini, T., Bonatto, C., Klanovicz, N., Michelon, W., Lerin, L., Júnior, S. L., Mossi, A. J., Tramontin, M. A. (2023a). The bioherbicidal potential of isolated fungi cultivated in microalgal biomass. *Bioprocess and Biosystems Engineering, 46*(5), 665-679. https://doi.org/10.1007/s00449-023-02852-x

Camargo, A. F., Bonatto, C., Scapini, T., Klanovicz, N., Tadioto, V., Cadamuro, R. D., Bazoti, S. F., Kubeneck, S., Michelon, W., Reichert Junior, F. W., Mossi, A. J. (2023b). Fungus-based bioherbicides on circular economy. *Bioprocess and Biosystems Engineering, 46*, 1729-1754. https://doi.org/10.1007/s00449-023-02926-w

Camargo, A. F., Kubeneck, S., Bonatto, C., Bazoti, S. F., Nerling, J. P., Klein, G. H., Michelon, W., Alves Jr, S. L.,

Mossi, A. J., Fongaro, G., Treichel, H. (2024). *Trichoderma koningiopsis* fermentation in airlift bioreactor for bioherbicide production. *Bioprocess and Biosystems Engineering, 47*(5), 651-663. https://doi.org/10.1007/s00449-024-02991-9

Chokshi, K., Pancha, I., Trivedi, K., George, B., Maurya, R., Ghosh, A., & Mishra, S. (2015). Biofuel potential of the newly isolated microalgae *Acutodesmus dimorphus* under temperature induced oxidative stress conditions. *Bioresource Technology, 180*, 162-171. https://doi.org/10.1016/j.biortech.2014.12.102

Devaiah, S. P., & Shetty, H. S. (2009). Purification of an infection-related acidic peroxidase from pearl millet seedlings. *Pesticide Biochemistry and Physiology, 94*(2-3), 119-126. https://doi.org/10.1016/j.pestbp.2009.04.010

Dineshkumar, R., & Sen, R. (2020). A sustainable perspective of microalgal biorefinery for co-production and recovery of high-value carotenoid and biofuel with CO_2 valorization. *Biofuels, Bioproducts and Biorefining, 14*(4), 879-897. https://doi.org/10.1002/bbb.2107

Dos Santos, L. B. P. R., Oliveira-Santos, N., Novais, D. P. S. D., Cruz-Magalhães, V., & Loguercio, L. L. (2025). Beneficial plants-Trichoderma interactions on host tolerance to abiotic stresses: A meta-analysis. *Frontiers in Plant Physiology, 3*, 1569221. https://doi.org/10.3389/fphgy.2025.1569221

Du, S., Meng, F., Duan, W., Liu, Q., Li, H., & Peng, X. (2022). Oxidative stress responses in two marine diatoms during acute n-butyl acrylate exposure and the toxicological evaluation with the IBRv2 index. *Ecotoxicology and Environmental Safety, 240*, 113686. https://doi.org/10.1016/j.ecoenv.2022.113686

Fal, S., Aasfar, A., Rabie, R., Smouni, A., & Arroussi, H. E. L. (2022). Salt induced oxidative stress alters physiological, biochemical and metabolomic responses of green microalga *Chlamydomonas reinhardtii*. *Heliyon, 8*(1), e08811. https://doi.org/10.1016/j.heliyon.2022.e08811

Fernández, F. G. A., Reis, A., Wijffels, R. H., Barbosa, M., Verdelho, V., & Llamas, B. (2021). The role of microalgae in the bioeconomy. *New Biotechnology, 61*, 99-107. https://doi.org/10.1016/j.nbt.2020.11.011

Ferreira, E. T., Barrochelo, S. C., de Melo, S. D. P., Araujo, T., Xavier, A. C. C., Cechin, I., & Da Silva, G. H. R. (2023). Biofertilizers from wastewater treatment as a potential source of mineral nutrients for growth of amaranth plants. *Plos One, 18*(12), e0295624. https://doi.org/10.1371/journal.pone.0295624

Foyer, C. H., & Shigeoka, S. (2011). Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiology, 155*(1), 93-100. https://doi.org/10.1104/pp.110.166181

Frumi Camargo, A., Venturin, B., Bordin, E. R., Scapini, T., Spitza Stefanski, F., Klanovicz, N., Dalastra, C., Kubeneck, S., Preczeski, K. P., Rossetto, V., Weirich, S. (2020). A low-genotoxicity bioherbicide obtained from *Trichoderma koningiopsis* fermentation in a stirred-tank bioreactor. *Industrial Biotechnology, 16*(3), 176-181. https://doi.org/10.1089/ind.2019.0024

Fuwa, H. (1954). A new method for microdetermination of amylase activity by the use of amylose as the substrate. *The Journal of Biochemistry, 41*(5), 583-603. https://doi.org/10.1093/oxfordjournals.jbchem.a126476

Gani, P., Sunar, N. M., Matias-Peralta, H., Jamaian, S. S., & Latiff, A. A. A. (2016). Effects of different culture conditions on the phycoremediation efficiency of domestic wastewater. *Journal of Environmental Chemical Engineering, 4*(4), 4744-4753. https://doi.org/10.1016/j.jece.2016.11.008

Ghose, T. K. (1987). Measurement of cellulase activities. *Pure and Applied Chemistry, 59*(2), 257-268. https://doi.org/10.1351/pac198759020257

Guo, Q., Shi, L., Wang, X., L, D., Yin, Z., Zhang, J., Ding, G., & Chen, L. (2023). Structures and biological activities of secondary metabolites from the *Trichoderma* genus (covering 2018-2022). *Journal of Agricultural and Food Chemistry, 71*(37). https://doi.org/10.1021/acs.jafc.3c02129

Hasan, M., Mokhtar, A. S., Mahmud, K., Berahim, Z., Rosli, A. M., Hamdan, H., Motmainna, Mst., & Ahmad-Hamdani, M. S. (2022). Physiological and biochemical responses of selected weed and crop species to the plant-based bioherbicide WeedLock. *Scientific Reports, 12*(1), 19602. https://doi.org/10.1038/s41598-022-24144-2

Havir, E. A., & McHale, N. A. (1987). Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiology, 84*(2), 450-455. https://doi.org/10.1104/pp.84.2.450

Hou, H., Zhou, J., Wang, J., Du, C., & Yan, B. (2004). Enhancement of laccase production by *Pleurotus ostreatus* and its use for the decolorization of anthraquinone dye. *Process Biochemistry, 39*(11), 1415-1419. https://doi.org/10.1016/S0032-9592(03)00267-X

Iribarnegaray, M. A., Rodriguez-Alvarez, M. S., Moraña, L. B., Tejerina, W. A., & Seghezzo, L. (2018). Management challenges for a more decentralized treatment and reuse of domestic wastewater in metropolitan areas. *Journal of Water, Sanitation and Hygiene for Development, 8*(1), 113-122. https://doi.org/10.2166/washdev.2017.092

Kar, T., Destain, J., Thonart, P., & Delvigne, F. (2012). Physical and physiological impacts of different foam control strategies during a process involving hydrophobic substrate for the lipase production by *Yarrowia lipolytica*. *Bioprocess and Biosystems Engineering, 35*(4), 483-492. https://doi.org/10.1007/s00449-011-0614-z

Kredics, L., Büchner, R., Balázs, D., Allaga, H., Kedves, O., Racić, G., Varga, A., Nagy, V. D., Vágvölgyi, C., & Sipos, G. (2024). Recent advances in the use of *Trichoderma*-containing multicomponent microbial inoculants for pathogen control and plant growth promotion. *World Journal of Microbiology and Biotechnology, 40*, 162. https://doi.org/10.1007/s11274-024-03965-5

Khan, A. A., & Robinson, D. S. (1994). Hydrogen donor specificity of mango isoperoxidases. *Food Chemistry, 49*(4), 407-410. https://doi.org/10.1016/0308-8146(94)90013-2

Korsa, G., Konwarh, R., Masi, C., Ayele, A., & Haile, S. (2023). Microbial cellulase production and its potential application for textile industries. *Annals of Microbiology, 73*(1), 13. https://doi.org/10.1186/s13213-023-01715-w

Kubeneck, S., Camargo, A. F., Longo, V. D., Romani, L. C., Nerling, J. P., Bazoti, S. F., Pagno, C. H., Rodrigues, E., & Treichel, H. (2025). Characterization of enzymatic and metabolic properties of a biocomposite based on *Trichoderma koningiopsis* and *Chlorella* biomass. *Biocatalysis and Agricultural Biotechnology, 65*, 103542. https://doi.org/10.1016/j.bcab.2025.103542

Lima, E. A. D., Mandelli, F., Kolling, D., Souza, J. M., Oliveira Filho, C. A. D., Silva, M. R. D., Sampaio, I. L. M., Junqueira, T. L., Chagas, M. F., Teodoro, J. C., Morais, E. R. D., & Murakami, M. T. (2022). Development of an economically competitive *Trichoderma*-based platform for enzyme production: Bioprocess optimization, pilot plant scale-up, techno-economic analysis and life cycle assessment. *Bioresource Technology, 364*, 128019. https://doi.org/10.1016/j.biortech.2022.128019

Mattam, A. J., Chaudhari, Y. B., & Velankar, H. R. (2022). Factors regulating cellulolytic gene expression in filamentous fungi: An overview. *Microbial Cell Factories, 21*(1), 44. https://doi.org/10.1186/s12934-022-01764-x

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*(3), 426-428. https://doi.org/10.1021/ac60147a030

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology, 22*(5), 867-880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Nicolás, C., Hermosa, R., Rubio, B., Mukherjee, P. K., & Monte, E. (2014). Trichoderma genes in plants for stress tolerance—Status and prospects. *Plant Science, 228*, 71-78. https://doi.org/10.1016/j.plantsci.2014.03.005

Odenthal, K., Nunes, E., Nunes, N., Fernandes, T., Fernandes, I. A., & Pinheiro de Carvalho, M. A. A. (2024). Microalgae and cyanobacteria as natural sources of antioxidant enzymes and enzyme inhibitors for Alzheimer's and diabetes. *Algal Research, 82*, 103610. https://doi.org/10.1016/j.algal.2024.103610

Padil, P. M. D., Hidayat, M., Kasiamdari, R. S., Mutamima, A., Iwamoto, K., Darmawan, M. A., & Gozan, M. (2023). Mechanism and kinetic model of microalgal enzymatic hydrolysis for prospective bioethanol conversion. *RSC Advances, 13*(31), 21403-21413. https://doi.org/10.1039/D3RA01556D

Pongsawadi, P., & Yagisawa, M. (1987). Screening and identification of a cyclomaltoxtrinv glucanotransferase-producing bacteria. *Journal of Fermentation Technology, 65*(4), 463-467. https://doi.org/10.1016/0385-6380(87)90144-0

Reichert Júnior, F. W., Scariot, M. A., Forte, C. T., Pandolfi, L., Dil, J. M., Weirich, S., Carezia, C., Mulinari, J., Mazutti, M. A., Fongaro, G., Galon, L. (2019). New perspectives for weeds control using autochthonous fungi with selective bioherbicide potential. *Heliyon, 5*(5), e01676. https://doi.org/10.1016/j.heliyon.2019.e01676

Ruas, G., Lacerda, S. F., Nantes, M. A., Serejo, M. L., Silva, G. H. R. da, & Boncz, M. Á. (2022). CO_2 addition and semicontinuous feed regime in shaded HRAP—Pathogen removal performance. *Water, 14*(24), 4047. https://doi.org/10.3390/w14244047

Ruas, A., Silva, M. R., & Oliveira, F. (2022). Effects of shading on microalgal community diversity and effluent disinfection in wastewater treatment. *Journal of Environmental Management, 310*, 114734. https://doi.org/10.1016/j.jenvman.2022.114734

Satpati, G. G., Dikshit, P. K., Mal, N., Pal, R., Sherpa, K. C., Rajak, R. C., Rather, S., Raghunathan, S., & Davoodbasha, M. (2023). A state of the art review on the co-cultivation of microalgae-fungi in wastewater for biofuel production. *Science of The Total Environment, 870*, 161828. https://doi.org/10.1016/j.scitotenv.2023.161828

Singhania, R. R., Patel, A. K., Tseng, Y.-S., Kumar, V., Chen, C.-W., Haldar, D., Saini, J. K., & Dong, C.-D. (2022). Developments in bioprocess for bacterial cellulose production. *Bioresource Technology, 344*, 126343. https://doi.org/10.1016/j.biortech.2021.126343

Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., & Scarlata, C., Templeton, D., Wolfe, J. (2008). *Determination of total solids in biomass and total dissolved solids in liquid process samples laboratory analytical procedure (LAP)*. National Renewable Energy Laboratory. http://www.nrel.gov

Stefanski, F. S., Camargo, A. F., Scapini, T., Bonatto, C., Venturin, B., Weirich, S. N., Ulkovski, C., Carezia, C., Ulrich, A., Michelon, W., Soares, H. M. (2020). Potential use of biological herbicides in a circular economy context: A sustainable approach. *Frontiers in Sustainable Food Systems, 4*, 521102. https://doi.org/10.3389/fsufs.2020.521102

Silva, R. P., Santos, T. L., & Ferreira, C. (2017). Operational parameters of UASB reactors treating domestic sewage: A review. *Water Science and Technology, 75*(8), 1836-1845. https://doi.org/10.2166/wst.2017.026

Thiviyanathan, V. A., Ker, P. J., Hoon Tang, S. G., Amin, E. P. P., Yee, W., Hannan, M. A., Jamaludin, Z., Nghiem, L. D., & Indra Mahlia, T. M. (2024). Microalgae biomass and biomolecule quantification: Optical techniques, challenges and prospects. *Renewable and Sustainable Energy Reviews, 189*, 113926. https://doi.org/10.1016/j.rser.2023.113926

Tiso, T., Demling, P., Karmainski, T., Oraby, A., Eiken, J., Liu, L., Bongartz, P., Wessling, M., Desmond, P., Schmitz, S., Weiser, S. (2024). Foam control in biotechnological processes—Challenges and opportunities. *Discover Chemical Engineering, 4*(1), 2. https://doi.org/10.1007/s43938-023-00039-0

Treichel, H., Sbardelotto, M., Venturin, B., Agnol, A. D., Mulinari, J., Golunski, S. M., Baldoni, D. B., Bevilacqua, C. B., Seminoti Jacques, R. J., Vargas, G. D., Mossi, A. J. (2017). Lipase production from a newly isolated *Aspergillus niger* by solid state fermentation using canola cake as substrate. *Current Biotechnology, 6*(4), 295-300. https://doi.org/10.2174/2211550105666151124193225

Wang, Q., Liu, W., Li, X., Wang, R., & Zhai, J. (2020). Carbamazepine toxicity and its co-metabolic removal by the cyanobacteria *Spirulina platensis*. *Science of The Total Environment, 706*, 135686. https://doi.org/10.1016/j.scitotenv.2019.135686

Xiong, J. Q., Kurade, M. B., Abou-Shanab, R. A., Ji, M. K., Choi, J., Kim, J. O., & Jeon, B. H. (2016). Biodegradation of carbamazepine using freshwater microalgae *Chlamydomonas mexicana* and *Scenedesmus obliquus* and the determination of its metabolic fate. *Bioresource Technology, 205*, 183-190. https://doi.org/10.1016/j.biortech.2016.01.038

Zhan, F., Zhou, X., Jiang, Y., Li, J., & Li, B. (2023). From an oil with "antifoaming" properties to stabilization for foam: A novel approach for establishing a long-term stable foam system. *Food Hydrocolloids, 145*, 109086. https://doi.org/10.1016/j.foodhyd.2023.109086