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Enzyme production by *Trichoderma koningiopsis* in an airlift bioreactor: potential for sustainable and circular bioproducts

Produção de enzimas por *Trichoderma koningiopsis* em um biorreator tipo *Airlift*: potencial para a obtenção de bioprodutos sustentáveis e circulares

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ABSTRACT

The growing need for sustainable solutions and the advancement of the circular economy highlight the search for more efficient processes with lower environmental impacts. In this context, this study investigated the production of an enzyme-rich fungal extract obtained from *Trichoderma koningiopsis*, a species known for its high enzyme excretion capacity, widely explored in scientific research focused on agronomic and environmental applications. The enzyme-rich fungal extract was obtained through submerged fermentation in a chemically defined culture medium, using an Airlift bioreactor, with samples collected at 24 and 48 hours for subsequent analysis of enzymatic activity. The enzymes amylase, cellulase, laccase, protease, lipase, ascorbate peroxidase, catalase, superoxide dismutase, and peroxidase were evaluated, with noteworthy results observed within the first 24 hours of fermentation: amylase (209.55 U/mL), cellulase (23.34 U/mL), protease (139.77 U/mL), peroxidase (609.55 U/mL), catalase (3,598.82 U/mL), superoxide dismutase (333.51 U/mL), and ascorbate peroxidase (53.34 U/mL). Enzymatic activity (U/mL) represented the primary parameter, and specific activity (U/mg protein) was additionally calculated to support a purity assessment. These findings demonstrate an enzyme-rich fungal extract with high biotechnological potential, offering purification and future applications in sectors such as bioprocesses, bioremediation, and other environmental solutions, thereby contributing to sustainable circular-economy strategies and supporting the future development of biocomposite-based formulations.

Keywords: antioxidant enzymes; submerged fermentation; fungal biotechnology; bioprocess scale-up; environmental biotechnology.

RESUMO

A crescente necessidade de soluções sustentáveis e o avanço da economia circular destacam a busca por processos mais eficientes e de menor impacto ambiental. Nesse contexto, este estudo investigou a produção de um extrato fúngico rico em enzimas, obtido de *Trichoderma koningiopsis*, espécie conhecida por sua elevada capacidade de excreção de enzimas e amplamente explorada em pesquisas científicas com foco em aplicações agronômicas e ambientais. O extrato fúngico rico em enzimas foi obtido por meio de fermentação submersa em meio de cultura quimicamente definido, em biorreator Airlift, com amostras coletadas em 24 e 48 horas para análise posterior da atividade enzimática. As enzimas amilase, celulase, lacase, protease, lipase, ascorbato peroxidase, catalase, superóxido dismutase e peroxidase foram avaliadas, com resultados notáveis observados nas primeiras 24 horas de fermentação: amilase (209,55 U/mL), celulase (23,34 U/mL), protease (139,77 U/mL), peroxidase (609,55 U/mL), catalase (3.598,82 U/mL), superóxido dismutase (333,51 U/mL) e ascorbato peroxidase (53,34 U/mL). A atividade enzimática (U/mL) foi o parâmetro principal, e a atividade específica (U/mg de proteína) foi calculada adicionalmente para auxiliar na avaliação da pureza. Esses resultados demonstram um extrato fúngico rico em enzimas, com alto potencial biotecnológico, oferecendo purificação e aplicações futuras em setores como bioprocessos, biorremediação e outras soluções ambientais, contribuindo, assim, para estratégias sustentáveis de economia circular e apoiando o desenvolvimento futuro de formulações à base de biocompósitos.

Palavras-chave: enzimas antioxidantes; fermentação submersa; biotecnologia fúngica; ampliação de escala de bioprocessos; biotecnologia ambiental.

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Introduction

According to industry market reports and published surveys, the specific enzymes market size was valued at USD 5.6 billion in 2023 and is expected to reach 10.7 billion USD by 2032, growing at a compound annual growth rate (CAGR) of 7.5% during the forecast period from 2024 to 2032. Microorganisms dominated the specific enzymes market, accounting for approximately 72% of the market in 2023 (GlobeNewswire, 2025).

The successful commercialization of any enzyme depends on achieving economically viable large-scale production to ensure sustainability. The introduction of scalable bioreactors for enzyme production, capable of accommodating high substrate loads under stringent aseptic conditions, represents an innovative opportunity to optimize the economic performance of the process (Ezeilo et al., 2019; Bhattacharya et al., 2024). Fungal enzymes offer an efficient, eco-friendly approach, as they are highly effective at decomposing complex organic compounds. The utilization of fungal enzymes supports global sustainability objectives by reducing greenhouse gas emissions and conserving natural resources (Zhu et al., 2022; Bibi et al., 2023; Okeke et al., 2024; Viñarta et al., 2024).

Fungi are known for their ability to produce a wide range of extracellular enzymes. Fungi utilize various enzyme secretion systems to obtain nutrients and ensure survival across a wide range of ecosystems, especially on substrates rich in complex polysaccharides. This ability of the fungi to secrete enzymes has been recognized and utilized by industry for decades. Significant investment is currently being made in the production of specific metabolites (such as enzymes) from submerged fungal cultures (Wösten, 2019). In this context, a select number of companies dominate global biotechnological enzyme production, namely Fluka, Novozymes, and Sigma-Aldrich Ltd (Gricajeva et al., 2019).

Trichoderma are fungi belonging to the ascomycete group; free-living and widely distributed in soil, mainly in association with various plant groups. This fungal genus has attracted attention due to its ability to control the growth and infection of potential plant pathogens, such as fungi and nematodes. Many *Trichoderma* species have proven economically crucial due to their production of enzymes and antibiotics, as well as their use as biocontrol agents. *Trichoderma koningiopsis* exemplifies the facts mentioned above very well (Schuster and Schmoll, 2010; Poveda, 2021; Guzmán-Guzmán et al., 2023).

Studies focusing on the potential of *Trichoderma koningiopsis* for pest and weed control in crops have been documented (Küpper et al., 2023; Bleckwedel et al., 2024). However, limited attention has been devoted to its potential as a source of enzyme-rich extracts that may support the future development of biocomposites rich in high-value-added metabolites (organic compounds, organic acids), such as enzymes, through fermentation in an airlift bioreactor. In this study, the term biocomposite is used conceptually to refer to a future formulation derived from enzyme-rich fungal extracts.

Exploring global microbial biodiversity offers numerous opportunities to discover novel enzymes with biochemical properties superior to of the existing ones. Their diversity, regiosecificity, and regioselectivity are potentially helpful for a wide range of industrial applications (Huo et al., 2017; Ranjan et al., 2018; Kubeneck et al., 2025). Biocomposites derived from fermentation processes, such as *Trichoderma spp*., can be utilized in various sectors, including biofuels, pharmaceuticals, bioplastics, animal feed, food processing, sanitation, wastewater treatment, agricultural water treatment, bioherbicides, and bioinsecticides (Robert et al., 2017; Elsayed et al., 2019; Orejuela-Escobar et al., 2021; Bibi et al., 2023; Camargo et al., 2023; 2024). Many are sustainable and ensure a circular economy.

Bioreactors are commonly used for cultivation due to the simplicity of direct spraying. The successful mass production of any bioproduct depends on the optimal and efficient design of the bioreactor (Chisti, 2000; Tamhankar et al., 2025). Variations in investment costs, operational expenditures, and productivity are significant across different types of bioreactors. The mechanically agitated stirred-tank bioreactor (STR) is the most prevalent in industry. In contrast, pneumatically agitated systems, such as simple bubble-column bioreactors (BCRs) and more advanced airlift bioreactors (ALRs), are also employed. The latter is of particular interest as it can accelerate climate action by paving the way for a circular economy (Cozma and Gavrilescu, 2012; Teli and Mathpati, 2022).

ALRs have a flexible capacity and a relatively simple design, providing dual mixing and aeration functions that offer multiple benefits. Due to the difference in gas retention between the ascending and descending tubes, a driving force is generated, inducing fluid circulation within the ALR. This type of bioreactor configuration provides high heat and mass transfer rates. These characteristics confer certain advantages to the ALR bioreactor, mainly attributed to: (I) correct contact between the gas, liquid, and solid phases; (II) low maintenance costs; (III) low energy consumption; (IV) reduced shear stress; (V) high mixing performance; and (VI) shorter reaction times. All of these benefits are less harmful to microorganisms, thus benefiting the process (Behin, 2012). They are used in a wide range of industrial applications, including chemical and wastewater treatment operations such as hydrogenation and oxidation (Sikula et al., 2007; Simcik et al., 2011).

Previous studies have demonstrated that ALRs exhibit good performance in biological applications and cell growth, as shown by Tamhankar et al. (2025). Their simple design, reduced risk of contamination, lack of moving parts, and the fact that they require no additional energy for mixing make aerobic fungal cultures good candidates for the fermentations conducted in ALR bioreactors. Furthermore, they exhibit higher heat and mass transfer rates and lower shear stress between the cells and the culture medium, thereby preventing damage to the fungal cells (Sikula et al., 2007).

All the aforementioned characteristics render the Airlift-type bioreactor preferable for cultivating anaerobic fungal cultures, primarily by enhancing yield and ensuring cell viability. The critical elements in enzyme production include the type of bioreactor used (Rahimi et al., 2018). Based on the aforementioned statements, this study employed an airlift-type bioreactor to maximize enzyme yield.

In this context, the production of an enzyme-rich biocomposite for subsequent application in new processes requires consideration of its scalability potential. The use of laboratory-scale bioreactors is crucial to maximize production, reduce costs, and make the process viable for future circular-economy applications. The current study aimed to evaluate the enzymatic yield of a biocomposite from the fungus *Trichoderma koningiopsis* via fermentation in an Airlift bioreactor, to produce high-value-added enzymes.

Material and Methods

Figure 1 presents the detailed methodology used in this study.

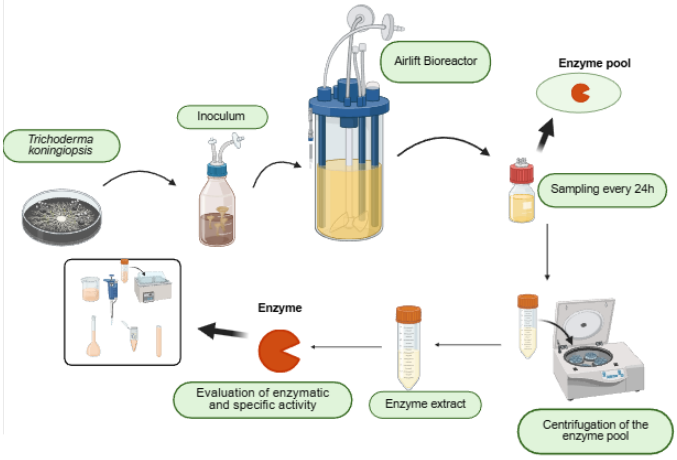

Figure 1 – Schematic design of the study.

Microorganism

The microorganism used to produce bioherbicides was the fungus *T. koningiopsis*, isolated from the weed *Digitaria horizontalis*. It was stored in the microorganism bank of the Laboratory of Agroecology at the Federal University Fronteira Sul in Erechim, Brazil. This fungus was selected due to its promising results for enzyme production in preliminary tests and previous studies by the group (Camargo et al., 2024; Kubeneck et al., 2025). The fungal strain was grown in Potato Dextrose Agar (PDA, potato infusion 4.0 g/L, infusion from 200 g potatoes); D(+) glucose 20.0 g/L; agar-agar 15.0 g/L (Merck, Germany), as previously optimized by our research group, and incubated over 7 days at 28°C (Camargo et al., 2020).

Scaling process in airlift bioreactor

The scale-up from 0.15 L to 3 L (a 20-fold increase) was performed using the methodology established in a preliminary study (Camargo et al., 2020). The culture medium consisted of: 20 g/L of glucose (anhydrous dextrose, 96% purity, Dinâmica, Brazil), 5 g/L of yeast extract powder (RM027, total nitrogen 10.50%, amino nitrogen 4.50%, sodium chloride 5.0%, Himedia, Mumbai, India), 2.5 g/L of bacteriological peptone (RM001, total nitrogen 13.50%, amino nitrogen 3.00%, sodium chloride 5.0%, Himedia) and 150 mL of antifoaming agent. An antifoaming agent was added manually during fermentation as needed and the reported volume reflects the total cumulative amount used.

Fermentation took place in a benchtop Airlift bioreactor, model Bio-Tec-Pro-II (Tecnal, Brazil), with a practical volume of 3.0 L. In this study, fermentation was conducted at a final volume of 2.5 L. Fermentation conditions were set at 75% dissolved oxygen, an airflow of 8 L/min, 28°C, and uncontrolled pH. The bioreactor was autoclaved at 120°C for 30 min at 1 atm.

After sterilization, a suspension of 10^6 spores/mL of *Trichoderma koningiopsis* was inoculated, and spore counts were performed using an automatic cell counter (Pixcel, Loccus). The initial pH of the fermentation was measured at 6.5.

The fermentation process lasted 48 hours. The final product was manually pressed through a synthetic nylon filter to remove the larger fungal cells. The retained solid was sterilized and discarded, and the liquid permeate was centrifuged (NT 815-NovaTecnica, Brazil) at 20,00 rpm and 4°C for 30 minutes. The centrifugation supernatant was used in this study and evaluated at 24-hour intervals (Camargo et al., 2024).

Enzymatic quantification

The activities of amylase, cellulose, laccase, protease, lipase, ascorbate peroxidase, catalase, superoxide dismutase, and peroxidase were quantified using the fermented extract. All determinations were conducted in triplicate.

Amylase

For the determination of the amylase activity, the extract was initially subjected to a reaction with starch diluted acetate buffer (100 mM, pH 5.0), at 38°C for 10 min, for subsequent quantification of the total reducing sugars (TRS) at 540 nm by spectrophotometry (UV M51, Bel Photonics, Monza, Italy) using the DNS method (Miller, 1959). Enzymatic activities were calculated based on the glucose standard curve and expressed as units per milliliter (U/mL).

Cellulase

Cellulase activity was assayed by measuring the reaction of the extract in acetate buffer (0.2 mM, pH 5.5) at 50°C, followed by TRS measurement. Enzymatic activities were calculated based on the glucose standard curve and expressed as units per milliliter (U/mL).

Laccase

Laccase activity was determined according to the methodology of Huo et al. (2017), using 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as the standard substrate. The reaction mixture was composed of 0.4 mL of 0.01 M ABTS, 0.2 mL of the enzyme extract, and 3.4 mL of buffer. The test tubes containing the reaction mixture were maintained in a thermostatic water bath for 4 minutes. Finally, absorbance was quantified using a spectrophotometer at 420 nm. One unit of laccase activity (U/mL) is defined as the amount

of enzyme capable of forming 0.1 µmol of ABTS per minute under the experimental conditions.

Protease

Protease enzyme activity was determined using a methodology adapted from Waghmare et al. (2015). The reaction mixture consisted of 0.1 mL of casein, 0.1 mL of the enzyme extract, and 0.5 mL of buffer. The reaction mixture was kept in an ultrathermostatic water bath for 30 min. Subsequently, the reaction was stopped by adding 0.5 mL of trichloroacetic acid. An aliquot of 0.5 mL from the initial reaction mixture was collected and mixed with 2.5 mL of sodium carbonate buffer (0.05 M) and 0.5 mL of 0.1 M Folin's reagent. The reaction mixture was kept at room temperature (20°C) for 20 to 30 minutes, after which the absorbance was measured at 660 nm using a spectrophotometer. One unit of enzymatic activity (U/mL) is defined as the amount of enzyme required to release 0.1 µg of tyrosine residue per minute under the experimental conditions.

Lipase

Lipase activity was measured by titration, as described by Treichel et al. (2017). An emulsion composed of Arabic gum (5%, v/v), olive oil (10%, v/v), and phosphate buffer (100 mM, pH 6.0) was prepared, to which the fermented extract was added. The mixture was then kept at 35°C and 165 rpm for 32 min. The reaction was stopped with an acetone/ethanol solution (1:1, v/v), then titrated to pH 11.0 with 0.050 M sodium hydroxide (NaOH). For each sample, a control sample containing the extract, emulsion, and an acetone/ethanol solution was tested (for non-reaction). Lipase activity was calculated using the molarity of sodium hydroxide and expressed as units per milliliter (U/mL).

Ascorbate peroxidase

The activity of ascorbate peroxidase was determined according to the methods of Nakano and Asada (1981) and Fal et al. (2022). The reaction medium was composed of 01.5 mL of buffer, 0.86 mL of distilled water, 0.24 mL of 0.008M ascorbic acid, 0.1 mL of enzyme extract, and 0.3 mL of 0.001M hydrogen peroxide. The reaction medium was kept in an ultrathermostatic bath for 2 minutes. Then, the absorbance was quantified using a spectrophotometer at 290 nm for 1 minute, with readings taken every 15 seconds. Activity was defined as the amount of enzymes that oxidized 1 µmol of substrate/min (U/mL).

Catalase

Catalase enzyme activity was determined according to the methods of Havir and McHale (1987) and Hasan et al. (2022). The reaction medium consisted of 1.5 mL of buffer, 0.9 mL of distilled water, 0.1 mL of enzyme extract, and 0.5 mL of 0.0125 M hydrogen peroxide. The reaction medium was kept in an ultrathermostatic bath for 2 minutes. Then, the absorbance of the oxidized compounds was quantified using a spectrophotometer at 240 nm for 3 minutes, where readings were taken every 30 seconds. The activity was determined by measuring the amount of enzyme that decomposed 1 µM of H_2O_2 (U/mL).

Superoxide dismutase

The activity of the superoxide dismutase enzyme was determined using the methodology described by Hasan et al. (2022). The reaction medium was composed of 1.5 mL of potassium phosphate buffer (0.05M; pH 6.8), 0.78 mL of 0.013M methionine, 0.225 mL of 75 µM NBT, 0.06 mL of 0.001M EDTA, 0.06 mL of 02 µM riboflavin, 0.345 mL of distilled water, and 30 µL of enzyme extract. The reaction occurred upon exposure to light from a 15 W fluorescent lamp, and absorbance was quantified using a spectrophotometer at 560 nm, with readings taken every 15 seconds for 1 minute. The reaction blank was performed in the absence of light exposure. The activity of superoxide dismutase is expressed as the enzyme required to inhibit 50% of the photoreduction of 4-nitro blue tetrazolium chloride (U/mL).

Peroxidase

To quantify peroxidase enzyme activity, the extract was added to a reaction medium composed of phosphate buffer (5 mM, pH 5.0), guaiacol (1%), hydrogen peroxide (0.08%), and distilled water, at 35°C for 10 min, according to the adapted methodology of Devaiah and Shetty (2009). A control sample was tested using water instead of the enzyme extract. The transmittance of the oxidized compounds was measured at 470 nm, and enzymatic activity was estimated by the oxidation reaction of the substrate to tetraguaiacol, expressed as units per milliliter (U/mL) (Garda-Buffon and Badiale-Furlong, 2010).

Total protein determination

Total protein concentration was determined by the Bradford method using bovine serum albumin (BSA) as a standard. Activity was determined from the construction of the standard curve (U/mg) in triplicate (Bradford, 1976). Through the use of total proteins, it was possible to quantify the specific or catalytic activity of each enzyme (U/mg of protein).

Statistical analysis

All experiments were performed in triplicate, and the results were presented as mean±sd (standard deviation). Statistical analysis of fermentation times (24 and 48 h) and enzymatic activity (U/mL) was performed using the Student t-test with 95% confidence in Statistica 10.0.

Results and Discussion

Table 1 presents the data on enzymatic activity and specific activity of the enzymes quantified during fermentation in an Airlift bioreactor for 24 and 48 hours. The results demonstrated high enzymatic production of environmental and biotechnological interest, with high added value obtained from fermentation in an Airlift bioreactor with the *Trichoderma koningiopsis* fungus. Previous studies have demonstrated high enzyme yields from Airlift bioreactors in fermentations, particularly in fungal fermentations.

Table 1 – Enzymatic (U/mL) activity of each enzyme at 24 and 48 hours of fermentation.

Enzyme	Fermentation time (h)	Activity (U/mL)*	Specific activity* (U/mg protein)
Amylase	24	29.98±2.25^{a}	210.31±2.25^{A}
	48	10.72±0.44^{b}	48,68±10,50^{B}
Cellulase	24	12.02±0.68^{c}	95,26±18,41^{C}
	48	11.45±0.12^{d}	258,59±241,14^{C}
Lipase	24	0.00±0.00^{e}	0.00±0.00^{D}
	48	2.65±0.95^{f}	72.91±88.19^{D}
Laccase	24	0.012±0.02^{g}	0.08±0.01^{E}
	48	0.020±0.02^{h}	0.08±0.02^{E}
Protease	24	20.00±6.01^{i}	138.66±33.74^{F}
	48	17.78±10.05^{i}	71.85±38.96^{F}
Peroxidase	24	70.28±3.94^{j}	494.25±59.40^{G}
	48	91.67 ±3.82^{k}	370.16±97.29^{G}
Catalase	24	664.04±16.73^{l}	4,660.79±359.10^{H}
	48	2,028.45±1,109.53^{l}	7,586.87±3,402.29^{H}
Ascorbate peroxidase	24	7.63±1.39^{m}	53.10±6.06^{I}
	48	62.74±12.27^{n}	137.67±24.78^{I}
Superoxide dismutase	24	47.72±3.10^{o}	335.81±44.06^{J}
	48	42.94±2.71^{o}	174.26±52.32^{K}

*Data are expressed as the mean of the repetitions±standard deviation. Means with different letters at the two fermentation times are significantly different at the 95% confidence level.

Fungi are reported to produce numerous enzymes, including peroxidases, monooxygenases, and hydrolases (e.g., proteases, cellulases, and lipases), allowing for the degradation of harmful organic pollutants (Mokrani et al., 2024).

It is essential to highlight that this fermentation lasted 48 hours because the enzymatic activities evaluated were higher than those reported in other studies assessing enzymatic activity from fungal fermentation (Camargo et al., 2020; Aragão et al., 2025; Kubeneck et al., 2025; Monica and Uppuluri, 2025).

Camargo et al. (2020) obtained an enzymatic pool from the fermentation of *Trichoderma koningiopsi* using the same culture medium as the current study, albeit in a stirred-tank reactor (STR). Compared with this study, higher enzymatic activities were observed when the same fermentation was performed in an airlift reactor (ALR). Another survey by Meneses et al. (2021) evaluated esterase production in stirred-tank (STR) and airlift (ALR) bioreactors, concluding that the ALR promotes better oxygen transfer than the STR. Jesus et al. (2017) concluded that the use of an STR is advantageous for processes that utilize highly viscous culture media, as it improves oxygen transfer rates. On the other hand, the ALR represents a promising alternative due to its scalability and cost- effectiveness. Furthermore, airlift bioreactors provide a greater oxygen transfer, especially for microorganisms that require such demand. Another point worth noting is that they have lower maintenance costs compared to STRs.

As shown in Table 1, the activity obtained for the amylase enzyme was 29.98 and 10.45 U/mL after 24 and 48 hours of fermentation, respectively. Statistically, the values differ; therefore, the best enzymatic yield is obtained with the shorter fermentation time. Almuhayawi et al. (2023) reported the highest amylase yield for Rhizopus stolonifer (32.61±0.89 U/mL). Ekedegba et al. (2022) after 24 hours of incubation, the following amylase enzyme activity values were observed for three fungal species: *Trichoderma viride* (7.92 U/ml), *Penicillium citrinum* (5.04 U/ml), and *Aspergillus niger* (7.00 U/ml). Blaga et al. (2022) reported 6 U/mL of amylase activity in an STR bioreactor with the fungus Aspergillus terreus.

The study by Camargo et al. (2020), which used the same culture medium and fungal strain albeit in a STR bioreactor, reported approximately 2 U/mL of amylase activity at 72 h of fermentation. This finding demonstrates that the Airlift bioreactor provides higher enzymatic activity in a shorter time for fungal strains. Due to aeration, mixing, agitation, and high airflow rates, airlift bioreactors are widely recognized for their contribution to improved productivity (Meneses et al., 2021). Furthermore, when the culture medium contains a wide variety of nutrients (proteins, starches, and fats), microorganisms can develop several mechanisms that enable the complex utilization of these nutrients, thereby increasing the production of individual enzymes (Ramamurthy et al., 2017).

For the enzyme cellulase, the values were 12.02±0.68 and 11.45±0.12 for 24 and 48 hours of fermentation, respectively. Statistics indicate that temporal variations occurred at a 95% confidence level, with the best activity (U/mL) at 24 hours. Peláez et al. (2021) obtained a cellulase production of 1.52±0.11 U/mL from the fungus *Trichoderma reesei.*

Previous studies have demonstrated that the genus *Trichoderma* is widely used in industrial cellulase production. Its genome generally comprises the fewest genes encoding cellulolytic and hemicellulolytic enzymes (Schuster and Schmoll, 2010). Furthermore, other authors report that higher glucose concentrations suppress the expression of cellulase genes, thereby reducing cellulase activity (Rai et al., 2012). The study by Camargo et al. (2020) reported a cellulase concentration of 2 U/mL after 72 hours, further highlighting the superior performance of the Airlift bioreactor for fungal fermentation in the same culture medium. In this study, the cellulase enzyme was obtained from the *Trichoderma koningiopsis* fungus, a finding that is underreported in the literature.

Table 1 demonstrates that lipase exhibits zero activity in 24 hours and 2.65 U/mL in 48 hours. Patel and Shah (2020) found that the fungi *Fusarium solani 7 F* and *Aspergillus niger 13 F* are capable of producing 5.95 U/mL of enzymatic activity for the lipase enzyme. Cesário et al. (2021) obtained an enzymatic activity of 11,007 U/mL for lipase from

the fungal strain *Aspergillus sp. F18* at 32°C and 2% oil. Souza et al. (2018) reported an enzymatic activity of 5.9 U/mL for lipase in the new strain, Preussia africana, isolated by the authors.

Alabdalall et al. (2020) explained that carbohydrates serve as monovalent carbon sources for lipase production, leading the *Aspergillus niger* to exhibit a low lipase yield in the presence of starch and sucrose. However, when fructose was used as a carbon source, lipase production increased. *Trichoderma harzianum* reached its maximum lipase activity when glucose and peptone served as carbon and nitrogen sources in its culture medium, respectively. At the same time, minimal lipase activity was observed when glucose and yeast extract were used as carbon and nitrogen sources (Kumar et al., 2023), a finding consistent with the results of this current study.

Finally, the lowest activity was observed for laccase (Table 1), at 0.012±0.02 and 0.020±0.02 after 24 and 48 hours, respectively. Table 1 shows a significant difference between both time points, with the best activity occurring after 48 hours of fermentation. Nuhu et al. (2020) reported higher laccase activity when corn cob was used as the substrate. Values of 2.23 U/mL for *Trichoderma viridae* and 2.16 U/mL for *Trichoderma harzianum*, however, after 9 days of fermentation, using a lignocellulosic substrate. Furthermore, current studies report that a longer fermentation time (longer than 6 days) is necessary for higher laccase production (Toker et al., 2021; Thrimothi et al., 2023).

The the enzyme's low activity may be a consequence of the substrate supplied to the fungus and its effectiveness. The substrate likely did not provide the nutrients required for the synthesis of this enzyme type. White-rot fungi are known to be better producers of ligninolytic enzymes, such as laccase (Zhuo and Fan, 2021). This type of fungus naturally produces laccase in response to lignin degradation (Borham et al., 2025). Furthermore, laccase is a multicopper oxidase that catalyzes the oxidation of a variety of substrates, typically including phenolic compounds and aromatic amines (Cagide and Castro-Sowinski, 2020), which were not provided to the fungus in the culture medium in this study.

For protease, no significant difference was observed between the fermentation times of 24 and 48 hours. Therefore, for obtaining proteases, only 24 hours of fermentation would be adequate to achieve good enzymatic activity, which is preferable due to the reduction in energy costs (20.00 U/mL±6.01).. Recent studies report high protease activity from fungal sources, although typically when utilizing agro-industrial lignocellulosic substrates (Chimbekujwo et al., 2020; Behera et al., 2021). Furthermore, lactose has been identified as a stimulant of protease production in Trichoderma reesei (Sun et al., 2021), a substrate absent in the cultivation medium used in this study. Additionally, Daranagama et al. (2019) observed that monosaccharides, particularly galactose, were more conducive to protease expression.

Previous studies explain that the genus *Trichoderma spp.* in their defensive actions commonly employs lytic enzymes, proteolytic enzymes, volatile metabolites, and secondary metabolites, successfully impairing the growth conditions of pathogens (Viterbo et al., 2002; Benítez et al., 2004; Eziashi et al., 2005; Reino et al., 2007). After 24 hours, protease activity declined. In general, this growth-associated protease production profile has been previously observed in other fungi. It is related to the degradation of extracellular enzymes and/or the depletion of nutrients available in the environment (Macchione et al., 2008).

The highest activity values for the antioxidant enzyme group (Table 1). A significant difference in peroxidase activity was observed between the two fermentation times, with enzyme activity (U/mL) being higher at 48 hours (91.67±3.82). For catalase, no significant difference in enzymatic activity was observed between 24 and 48 hours of fermentation. Significant differences in ascorbate peroxidase activity were observed across fermentation times, where the activity at 48 hours (62.74 U/mL±12.27) was higher than at 24 hours (7.63 U/mL±1.39). Finally, no significant difference was observed in superoxide dismutase enzymatic activity between 24 and 48 hours of fermentation. Considering the perspectives of energy and reagent economy, the shorter fermentation time would be preferable.

The fungal response to oxidative stress can explain these results. This behavior results in the generation of reactive oxygen species. To mitigate oxidative damage, fungi activate a robust antioxidant defense system. This system is essentially composed of nonenzymatic antioxidants and enzymatic components, such as superoxide dismutase, peroxidases, and catalase (Kumar and Dwivedi, 2019).

Furthermore, previous studies have demonstrated that the genus *Trichoderma* can increase the activity and content of antioxidant enzymes under salt stress (Boamah et al., 2021; Cheng et al., 2023; Sabzi-Nojadeh et al., 2024). Camargo et al. (2020) reported approximately 500 U/mL of peroxidase activity in an STR bioreactor after 120 hours of fermentation. The previous study provided high enzyme activities. However, in this study, we observed significantly higher yields of antioxidant enzymes at shorter fermentation times in an Airlift bioreactor (e.g., catalase). Some current studies report high enzymatic activity of peroxidases produced by white-rot fungi, such as *Pleurotus pulmonarius* CPG6 (Giap et al., 2022).

Zhang et al. (2019) demonstrated that *Trichoderma harzianum*, under salt stress, increased the production of antioxidant enzymes, including peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, catalase, superoxide dismutase, ascorbate peroxidase, and glutathione reductase. Asemoloye et al. (2018) observed that certain fungal strains survived the exposure to 20% crude oil. This resilience can be attributed to the production of enzymes such as laccases and peroxidases, which are involved in fungal environmental signaling in response to varying concentrations of carbon, nitrogen, and other environmental pollutants, including heavy metal ions, pesticides, and hydrocarbons.

The highest activities were observed for the enzyme catalase. Tang et al. (2024) explained that the enzyme catalase (CAT), a terminal oxidative enzyme ubiquitous to animal, plant, and microbial organisms,

constitutes a key component of the hepatoprotective enzymatic defense system. The high catalase production in fungi is attributed to their reliance on environmental organic compounds, including those derived from living organisms. As such, they must cope with severe and rapid shifts in external conditions, ranging from nutrient abundance to scarcity, temperature fluctuations, fluctuations in water availability, intense light, and the presence of competing species. These challenging conditions induce oxidative stress, which is the key reason why fungi are prolific producers of catalase and other hydrogen peroxide-degrading enzymes (antioxidants). Filamentous ascomycete fungi, such as those of the genus *Trichoderma*, possess the ability to produce various types of catalases (Kämper et al., 2006).

The quantity of an enzyme is not defined by its mass (protein), but rather by its function. This is admittedly true, as the catalytic potential, not the protein itself, is the essential characteristic of an enzyme. Even enzymes of comparable purity can exhibit significant differences in activity. A partially inactivated enzyme cannot be distinguished from an active one solely by protein analysis. The purity of an enzyme is generally expressed by its specific enzymatic activity (U/mg), that is, the enzyme units per milligram of protein in the respective enzyme extract. The higher the value, the purer the enzyme; lower values indicate impurities or partial enzymatic inactivation (Bisswanger, 2014). In this regard, Table 1 shows higher specific activities (U/mg) for most of the enzymes quantified in this study than those reported for crude enzyme extracts from other studies (Hu et al., 2023; Xu et al., 2024; Liu et al., 2025). Of particular note are certain enzymes within the important antioxidant group (catalase, superoxide dismutase, and peroxidase). It can be inferred that most enzymes in this study are of high purity, as evidenced by their high specific activities (U/mg), which suggest promising potential for future applications.

In this context, it is essential to highlight existing studies on enzymatic bioremediation (Rathore et al., 2022; Magalhães et al., 2024; Peter et al., 2024; Wang et al., 2025), as well as potential future applications for the enzymes obtained in this study. In this context, enzymes can be allies in more sustainable and practical bioremediation, in line with the principles of the circular economy. This is especially true when considering the bioremediation of emerging pollutants of global concern, such as pharmaceuticals, petroleum-derived hydrocarbons, hygiene/cosmetic products, pesticides and agrochemicals, micro and nanoplastics, among others.

Conclusions

This study demonstrated the potential of airlift bioreactor fermentation to enhance the production of high-value-added enzymes, including catalase, peroxidase, and superoxide dismutase. The Airlift bioreactor demonstrates high efficiency in fermentations with fungi that require efficient oxygenation of the medium, such as *Trichoderma koningiopsis*.

It is worth emphasizing the specific activities (U/mg) of the enzymes quantified in this study, which demonstrate high purity and are expected to yield better catalytic performance in future applications.

Moreover, future studies may focus on purifying this extract to enhance the catalytic performance of the enzymes of interest, as well as immobilization processes. Based on these results, future studies are required to investigate the application of enzyme-rich extracts or derived biocomposite formulations for bioremediation or contaminant removal, given the high levels of antioxidant enzymes produced. From a circular economy perspective, the approach proposed herein may enable the application of these low-cost enzymes across various environmental sectors.

Authors' Contributions

Amorim, M.P.: data curation; investigation; methodology; writing – original draft. **Correa**, F.S.: data curation; investigation; methodology; writing – original draft. **Longo**, V.D.: data curation; investigation; methodology; writing – original draft. **Treichel**, H.: conceptualization; data curation; formal analysis; funding acquisition; project administration; resources; software; supervision; validation; visualization; writing – review & editing.

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