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CHARLINE VENERAL

**PRODUÇÃO E APLICAÇÃO DE COMPLEXOS CELULOLÍTICOS DE *Trichoderma*
koningiopsis PARA A VALORIZAÇÃO BIOTECNOLÓGICA DE BIOMASSAS
LIGNOCELULÓSICAS DE SORGO E CAPIM-ELEFANTE**

ERECHIM

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Orientadora: Helen Treichel

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Data: 25/08/2026 13:05:10-0300
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Prof.^a Dr.^a Helen Treichel – UFFS

Orientadora



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Prof.^a Dr.^a Jadiane Paola Cavaler – SENAI

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Prof.^a Dr.^a Gean Delise Leal Pasquali – UFFS

Avaliadora

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RESUMO

A valorização de biomassas lignocelulósicas representa uma estratégia promissora para o desenvolvimento de bioprocessos sustentáveis, contribuindo para a produção de bioenergia e de produtos de maior valor agregado no contexto da bioeconomia circular. Entretanto, a elevada recalcitrância estrutural dessas biomassas limita a eficiência da hidrólise enzimática, tornando necessária a busca por sistemas enzimáticos mais eficientes e adaptados a diferentes substratos. Neste contexto, o presente estudo teve como objetivo avaliar a produção e a aplicação de complexos celulolíticos produzidos por *Trichoderma koningiopsis* durante a fermentação em estado sólido, utilizando biomassa de sorgo (*Sorghum bicolor*) e capim-elefante (*Pennisetum purpureum*) como substratos de cultivo. Também foi investigada a influência de pré-tratamentos químicos sobre a hidrólise enzimática, bem como foi comparado o desempenho dos extratos enzimáticos produzidos com uma preparação comercial de celulases. Os resultados demonstraram que a produção de enzimas foi influenciada pelas características da biomassa utilizada durante a fermentação, sendo a maior atividade celulolítica obtida em biomassa de sorgo sem pré-tratamento. Em contraste, os pré-tratamentos químicos empregados não promoveram aumento da atividade enzimática durante a hidrólise direta das biomassas. A fermentação em estado sólido também resultou na produção simultânea de proteases extracelulares, evidenciando a complexidade do sistema enzimático sintetizado por *T. koningiopsis*. Os resultados obtidos demonstram o potencial da fermentação em estado sólido para a produção de enzimas celulolíticas dependentes do substrato e reforçam a importância da otimização das condições de cultivo para obtenção de extratos enzimáticos mais eficientes e estáveis. Sob a perspectiva das Ciências Ambientais, a pesquisa contribui para a valorização sustentável de biomassas lignocelulósicas abundantes, fortalecendo estratégias de aproveitamento de recursos renováveis e o desenvolvimento de bioprocessos alinhados aos princípios da bioeconomia circular e do desenvolvimento sustentável.

Palavras-chave: biomassa lignocelulósica; fermentação em estado sólido; enzimas celulolíticas; *Trichoderma koningiopsis*; bioeconomia circular.

ABSTRACT

The valorization of lignocellulosic biomass represents a promising strategy for the development of sustainable bioprocesses, contributing to the production of bioenergy and high-value-added products within the framework of the circular bioeconomy. However, the high structural recalcitrance of these biomasses limits the efficiency of enzymatic hydrolysis, highlighting the need for more efficient enzyme systems adapted to different substrates. In this context, the present study aimed to evaluate the production and application of cellulolytic enzyme complexes produced by *Trichoderma koningiopsis* during solid-state fermentation, using sorghum (*Sorghum bicolor*) and elephant grass (*Pennisetum purpureum*) biomass as cultivation substrates. The influence of chemical pretreatments on enzymatic hydrolysis was also investigated, and the performance of the produced enzymatic extracts was compared with that of a commercial cellulase preparation. The results demonstrated that enzyme production was influenced by the characteristics of the biomass used during fermentation, with the highest cellulolytic activity being obtained from untreated sorghum biomass. In contrast, the chemical pretreatments employed did not enhance enzymatic activity during the direct hydrolysis of the biomasses. Solid-state fermentation also resulted in the simultaneous production of extracellular proteases, highlighting the complexity of the enzymatic system synthesized by *T. koningiopsis*. Overall, the findings demonstrate the potential of solid-state fermentation for producing substrate-dependent cellulolytic enzymes and reinforce the importance of optimizing cultivation conditions to obtain more efficient and stable enzymatic extracts. From an Environmental Sciences perspective, this research contributes to the sustainable valorization of abundant lignocellulosic biomasses, strengthening strategies for renewable resource utilization and the development of bioprocesses aligned with the principles of the circular bioeconomy and sustainable development.

Keywords: lignocellulosic biomass; solid-state fermentation; cellulolytic enzymes;
Trichoderma koningiopsis; circular bioeconomy.

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1. INTRODUÇÃO GERAL

A crescente demanda por processos produtivos mais sustentáveis tem impulsionado a busca por alternativas capazes de reduzir a dependência de recursos fósseis e promover o aproveitamento eficiente de biomassas renováveis. Nesse contexto, a biomassa lignocelulósica destaca-se como uma das matérias-primas mais promissoras para a produção de biocombustíveis, bioquímicos e outros produtos de maior valor agregado, devido à sua ampla disponibilidade, baixo custo e elevada capacidade de renovação (NARGOTRA et al., 2023; LIMA et al., 2024).

Entre as biomassas de interesse para sistemas de biorrefinaria destacam-se o sorgo (*Sorghum bicolor*) e o capim-elefante (*Pennisetum purpureum*), gramíneas caracterizadas pelo metabolismo fotossintético C4, elevada produtividade de matéria seca e adaptação a diferentes condições climáticas, especialmente em regiões tropicais e subtropicais. Essas características tornam ambas as espécies importantes fontes de biomassa para aplicações bioenergéticas e biotecnológicas (MORAIS et al., 2018).

Apesar desse potencial, a conversão eficiente da biomassa lignocelulósica ainda representa um importante desafio tecnológico. A parede celular vegetal apresenta uma organização estrutural complexa, formada principalmente por celulose, hemicelulose e lignina, cuja associação reduz a acessibilidade das enzimas hidrolíticas à fração celulósica e limita a eficiência dos processos de bioconversão. Essa elevada recalcitrância constitui uma das principais barreiras para o aproveitamento industrial dessas biomassas (ANDLAR et al., 2018; ZHANG et al., 2021).

Diversas estratégias têm sido propostas para superar essa limitação. Entre elas, destacam-se os pré-tratamentos físico-químicos, capazes de promover alterações estruturais na biomassa por meio da remoção parcial de lignina e hemicelulose, aumentando a exposição da celulose às enzimas hidrolíticas. Entretanto, a eficiência desses tratamentos depende das características da biomassa e das

condições operacionais empregadas, podendo ocorrer formação de compostos inibidores, aumento do consumo de reagentes e elevação dos custos do processo (ALVIRA et al., 2010; GABHANE et al., 2014; MOSIER et al., 2005).

Nesse cenário, a etapa de hidrólise enzimática assume papel fundamental na conversão da biomassa lignocelulósica. A degradação eficiente da celulose depende da ação sinérgica de diferentes enzimas, principalmente endoglucanases, exoglucanases e β -glicosidases, responsáveis pela liberação de açúcares fermentescíveis que podem ser utilizados em diferentes processos biotecnológicos (ANDLAR et al., 2018; SINGHANIA et al., 2012).

Entre os microrganismos produtores dessas enzimas, os fungos filamentosos do gênero *Trichoderma* destacam-se pela elevada capacidade de secreção extracelular e pelo amplo potencial de aplicação industrial. Espécies desse gênero são amplamente empregadas na produção de enzimas lignocelulolíticas devido à elevada eficiência na degradação de materiais vegetais e à capacidade de responder às características do substrato durante o cultivo (ANDLAR et al., 2018). Em especial, *Trichoderma koningiopsis* tem despertado interesse crescente em aplicações biotecnológicas, apresentando potencial para produção de diferentes enzimas de interesse industrial e ambiental (ULRICH et al., 2021).

A fermentação em estado sólido (FES) constitui uma estratégia particularmente promissora para a produção de enzimas lignocelulolíticas. Nesse processo, resíduos agroindustriais e biomassas vegetais atuam simultaneamente como suporte físico e fonte de carbono para o crescimento do fungo, favorecendo a produção de enzimas diretamente induzidas pelo substrato utilizado. Além de reduzir custos de produção, essa abordagem apresenta elevada eficiência para fungos filamentosos, melhor aproveitamento de resíduos agrícolas e menor geração de efluentes quando comparada aos processos fermentativos convencionais (CASTILHO et al., 2000; SINGHANIA et al., 2009; LEITE et al., 2021; LIMA et al., 2024).

Sob a perspectiva das Ciências Ambientais, a valorização biotecnológica de biomassas lignocelulósicas representa uma estratégia alinhada aos princípios da bioeconomia circular, ao estimular o aproveitamento sustentável de recursos renováveis e reduzir a dependência de insumos de origem fóssil. A produção de enzimas utilizando as próprias biomassas agrícolas como substrato de cultivo contribui para agregar valor a materiais de ampla disponibilidade, favorecendo o desenvolvimento de tecnologias ambientalmente mais sustentáveis e potencialmente aplicáveis aos sistemas de biorrefinaria e à conversão de resíduos agroindustriais.

Apesar da ampla disponibilidade de preparações comerciais de celulasas, sua eficiência pode variar em função das características estruturais de cada biomassa, uma vez que formulações padronizadas nem sempre apresentam composição enzimática adequada a diferentes substratos lignocelulósicos. Por outro lado, ainda são escassos os estudos que avaliam a produção de enzimas por *Trichoderma koningiopsis* utilizando sorgo e capim-elefante como substratos de fermentação, bem como a influência dessas biomassas sobre o perfil enzimático produzido.

Diante desse contexto, esta dissertação teve como objetivo avaliar a produção, caracterização e aplicação de complexos celulolíticos produzidos por *Trichoderma koningiopsis* em fermentação em estado sólido, utilizando biomassa de sorgo e capim-elefante como substratos de cultivo. Além da comparação com preparações comerciais, buscou-se investigar a influência de pré-tratamentos químicos sobre a hidrólise enzimática e discutir o potencial dessa estratégia para a valorização sustentável de biomassas lignocelulósicas e para o desenvolvimento de bioprocessos alinhados aos princípios da bioeconomia circular.

2. ARTIGO SUBMETIDO

Substrate-dependent cellulolytic enzyme production by *Trichoderma koningiopsis* during solid-state fermentation of sorghum and elephant grass

Abstract

Lignocellulosic biomass is an abundant renewable resource for sustainable bioprocesses, although its structural recalcitrance limits the efficiency of enzymatic conversion. This study evaluated cellulolytic enzyme production by *Trichoderma koningiopsis* using sorghum (*Sorghum bicolor*) and elephant grass (*Pennisetum purpureum*) as substrates under solid-state fermentation (SSF) and compared the performance of the obtained crude enzymatic extracts with a commercial cellulase preparation. The effects of acid and alkaline pretreatments on enzymatic hydrolysis were also investigated. Commercial cellulase preparations exhibited higher cellulolytic activities during direct hydrolysis than the laboratory-produced crude extract; however, the pretreatments employed did not improve enzymatic performance for either biomass. In contrast, SSF promoted substantially higher cellulolytic enzyme production, reaching 33.82 U g⁻¹ when *T. koningiopsis* was cultivated on untreated sorghum. High extracellular protease activities were also detected, demonstrating that substrate composition influenced not only cellulase production but also the overall extracellular enzymatic profile. The results demonstrate that sorghum and elephant grass are suitable substrates for cellulolytic enzyme production by *T. koningiopsis* and highlight solid-state fermentation as a promising strategy for enhancing substrate-dependent cellulolytic enzyme production for lignocellulosic biomass conversion.

Keywords: biomass valorization; cellulase; extracellular enzymes; lignocellulosic biomass; fungal biotechnology; bioprocess.

2.1. Introduction

Lignocellulosic biomass is one of the most abundant renewable resources available for the development of sustainable bioprocesses due to its wide availability, low cost, and potential for producing biofuels and other value-added bioproducts (NARGOTRA et al., 2023; LIMA et al., 2024). The efficient conversion of these renewable feedstocks is considered a key component of modern biorefineries and circular bioeconomy strategies, in which agricultural residues are transformed into fuels, chemicals, and biobased products through environmentally sustainable processes (NARGOTRA et al., 2023).

Among high-yield energy crops, sorghum (*Sorghum bicolor*) and elephant grass (*Pennisetum purpureum*) are particularly attractive because of their C4 photosynthetic metabolism, rapid biomass accumulation, high dry matter productivity, and adaptability to different environmental conditions (MORAIS et al., 2018). These characteristics make both species promising lignocellulosic feedstocks for second-generation bioprocesses, particularly in tropical and subtropical regions where biomass production is high.

Despite these advantages, the efficient conversion of lignocellulosic biomass remains a major technological challenge. The plant cell wall consists of cellulose microfibrils embedded within a complex matrix of hemicellulose and lignin, whose structural organization restricts enzyme accessibility and limits biomass deconstruction (ANDLAR et al., 2018; NARGOTRA et al., 2023). Consequently, the natural recalcitrance of lignocellulosic materials remains one of the principal barriers to efficient enzymatic hydrolysis and subsequent biomass conversion.

To overcome this limitation, several pretreatment strategies have been investigated to increase cellulose accessibility before enzymatic hydrolysis. Although acid and alkaline pretreatments can partially disrupt the lignin-carbohydrate complex, their effectiveness strongly depends on biomass characteristics and operating conditions, while excessive treatment severity may generate inhibitory compounds and increase process costs (ALVIRA et al., 2010). Therefore, improving both enzyme

production and enzymatic performance remains an important challenge for efficient lignocellulosic bioconversion.

Among lignocellulose-degrading microorganisms, species of the genus *Trichoderma* are widely recognized for their ability to secrete extracellular cellulolytic enzymes, including endoglucanases, exoglucanases, and β -glucosidases, which act synergistically during cellulose hydrolysis (ANDLAR et al., 2018). Beyond selecting efficient microorganisms, increasing attention has been directed toward tailoring enzyme production according to the characteristics of the target substrate. In this context, solid-state fermentation (SSF) represents an attractive strategy because lignocellulosic materials can simultaneously serve as carbon sources and cultivation substrates, promoting cellulolytic enzyme production and generating enzymatic systems potentially better adapted to the structural characteristics of the biomass (SINGHANIA et al., 2009; LEITE et al., 2021; NARGOTRA et al., 2023; LIMA et al., 2024).

Although commercial cellulase preparations are widely available, their hydrolytic performance is often limited by the structural heterogeneity of lignocellulosic biomasses, since standardized enzyme formulations are not necessarily optimized for specific substrates. Furthermore, limited information is available regarding how different lignocellulosic substrates influence the extracellular enzymatic profile of *Trichoderma koningiopsis*. Studies evaluating cellulolytic enzyme production by *T. koningiopsis* using sorghum and elephant grass as cultivation substrates remain scarce, particularly those comparing substrate-dependent enzyme production with the direct application of commercial cellulase preparations.

Therefore, this study investigated substrate-dependent cellulolytic enzyme production by *T. koningiopsis* during solid-state fermentation using sorghum and elephant grass as lignocellulosic cultivation substrates. The cellulolytic and proteolytic activities of the resulting crude enzymatic extracts were determined and compared with those of a commercial cellulase preparation. In addition, the effects of acid and alkaline pretreatments on enzymatic hydrolysis were evaluated to assess how

substrate characteristics influence enzyme performance and to investigate the potential of solid-state fermentation as a strategy for producing cellulolytic enzymes for lignocellulosic biomass conversion.

2.2. Materials and Methods

2.2.1 Biomass

Sorghum (*Sorghum bicolor* BRS 716) and elephant grass (*Pennisetum purpureum* BRS Capiáu) biomasses were supplied by Embrapa (Brazil). The materials were previously milled, ensiled, vacuum-packed, and sieved to obtain a uniform particle size, thereby minimizing mass-transfer limitations during enzymatic hydrolysis (MOSIER et al., 2005).

Before use, the biomasses were washed by suspending 10% (w/v) dry material in deionized water and maintaining the suspension under agitation at 30 °C for 5 min in a Dubnoff water bath, following an adaptation of the methodology described by Bonatto et al. (2021) and Klein et al. (2024). The washed biomasses were subsequently dried at 40 °C until constant weight, and moisture content was determined to standardize the dry biomass used in all experiments (KLEIN et al., 2024).

2.2.2 Experimental design

The experimental design evaluated the effects of biomass type (*S. bicolor* and *P. purpureum*), chemical pretreatment (untreated, acid-pretreated, and alkaline-pretreated biomass), and enzymatic system (commercial cellulase preparation, laboratory-produced cellulolytic extract, and cellulolytic extract obtained by solid-state fermentation).

Initially, untreated and chemically pretreated biomasses were subjected to enzymatic hydrolysis using either a commercial cellulase preparation or the laboratory-produced cellulolytic extract. Subsequently, cellulolytic enzymes were produced by solid-state fermentation (SSF) using lignocellulosic biomass as the cultivation substrate. The crude enzymatic extracts obtained were

evaluated for cellulolytic and proteolytic activities. All experiments were performed in biological triplicate.

2.2.3 Biomass pretreatment

Chemical pretreatments were performed to increase cellulose accessibility by partially disrupting the lignin–carbohydrate complex (ALVIRA et al., 2010).

Initially, the washed biomasses were dried at 40 °C until constant weight. Subsequently, the materials were suspended in deionized water and maintained at 50 °C under agitation (150 rpm) for 120 h according to an adaptation of the methodology proposed by Gabhane et al. (2014) and Klein et al. (2024). After treatment, the biomasses were washed with deionized water until neutral pH, dried at 40 °C, and stored until use.

Acid pretreatment was performed using 5% (v/v) sulfuric acid (H₂SO₄), whereas alkaline pretreatment was performed using 1% (w/v) sodium hydroxide (NaOH), aiming to promote hemicellulose solubilization and partial delignification, respectively (ALVIRA et al., 2010).

2.2.4 Microorganism and inoculum preparation

The filamentous fungus used in this study was *Trichoderma koningiopsis* (GenBank accession number MK860714), previously isolated from the weed *Digitaria ciliaris* and molecularly identified as described by Ulrich et al. (2021). Detailed procedures for fungal isolation, molecular identification, sequencing, and phylogenetic analysis are available in the original publication. The strain was maintained on potato dextrose agar (PDA) plates and used as the inoculum source for solid-state fermentation.

For inoculum preparation, the fungus was subcultured on PDA medium (39 g L⁻¹) and incubated at 28 °C for 7 days to allow fungal growth and sporulation. Spores were recovered by adding sterile distilled water to the plate surface and gently scraping the mycelium. The resulting suspension was filtered to

remove mycelial fragments and adjusted to a final concentration of 6.2×10^6 spores mL^{-1} , as described by Castilho et al. (2000).

2.2.5 Production of cellulolytic enzymes by solid-state fermentation

Solid-state fermentation (SSF) was performed using untreated and chemically pretreated sorghum and elephant grass biomasses as solid substrates for cellulolytic enzyme production. Each experimental unit was inoculated with the standardized spore suspension (6.2×10^6 spores mL^{-1}) and incubated at 28 °C for 96 h under approximately 90% relative humidity, conditions suitable for cellulolytic enzyme production by filamentous fungi (SINGHANIA et al., 2009).

After fermentation, extracellular enzymes were extracted with 100 mM phosphate buffer (pH 7.0), then agitated and filtered to obtain the crude enzymatic extract, which was immediately used for enzymatic activity assays.

2.2.6 Enzymatic activity assays

Cellulolytic activity was determined using the filter paper assay (FPA) as described by Ghose (1987). The reducing sugars released during the reaction were quantified by the 3,5-dinitrosalicylic acid (DNS) method (MILLER, 1959) and used to calculate cellulase activity.

Proteolytic activity was determined in the crude enzymatic extracts obtained after solid-state fermentation to evaluate the simultaneous production of extracellular proteases.

2.2.7 Enzymatic hydrolysis

Hydrolysis assays were carried out using 1% (w/v) dry biomass suspended in 0.05 mol L^{-1} sodium citrate buffer (pH 4.8). Reactions were performed at 50 °C under orbital agitation (150 rpm) for 120 h using an enzymatic loading of 50 FPU g^{-1} dry biomass (SINGHANIA et al., 2012).

The cellulolytic performances of the commercial cellulase preparation, the laboratory-produced enzymatic extract, and the enzymatic extract obtained by solid-state fermentation were comparatively evaluated.

2.2.8 Statistical analysis

All experiments were performed in biological triplicate, and the results are expressed as mean $\pm$ standard error. Data normality was assessed using the Shapiro–Wilk test, followed by one-way analysis of variance (ANOVA). When significant differences were detected, means were compared using Tukey's multiple comparison test at a significance level of $p < 0.05$.

2.3. Results and Discussion

2.3.1 Substrate-dependent production of cellulolytic and proteolytic enzymes by *Trichoderma koningiopsis*

Solid-state fermentation (SSF) successfully promoted the production of cellulolytic and proteolytic enzymes by *Trichoderma koningiopsis* using sorghum and elephant grass as lignocellulosic substrates (Table 1). Both enzymatic activities were detected under all cultivation conditions, although their production strongly depended on substrate characteristics. The highest cellulolytic activity (33.823 U g⁻¹) was obtained when the fungus was cultivated on untreated sorghum (S/N), representing an approximately seven-fold increase compared with the lowest activity observed among the evaluated treatments (4.900 U g⁻¹). Interestingly, the same cultivation condition also promoted the highest proteolytic activity (16,220.620 U g⁻¹), indicating that substrate composition influenced the overall enzymatic response of the microorganism.

Table 1. Cellulolytic and proteolytic activities of crude enzymatic extracts produced by *Trichoderma koningiopsis* during solid-state fermentation.

Sample*	Cellulolytic activity (U g ⁻¹)**	Proteolytic activity (U g ⁻¹)**
S/PT/EMP	16.09 ^b ± 0.04	9112.23 ^d ± 0.02
S/PT/TK	5.99 ^c ± 0.06	9591.23 ^d ± 0.03
S/EMP	9.60 ^c ± 0.01	10968.70 ^c ± 0.02
S/TK	8.32 ^c ± 0.01	6532.11 ^f ± 0.01
S/N	33.82 ^a ± 0.01	16220.62 ^a ± 0.04
C/PT/EMP	6.72 ^d ± 0.05	8067.33 ^e ± 0.03
C/PT/TK	4.90 ^f ± 0.03	5612.85 ^g ± 0.01
C/EMP	7.05 ^d ± 0.04	8882.43 ^d ± 0.01
C/TK	6.58 ^d ± 0.08	6655.92 ^f ± 0.01
C/N	6.63 ^d ± 0.01	13375.20 ^b ± 0.01

*S: sorghum; C: elephant grass; PT: chemically pretreated biomass; EMP: commercial cellulase preparation; TK: laboratory-produced cellulolytic extract; N: untreated biomass. **Values are expressed as mean ± standard error (n = 3). Different superscript letters within the same column indicate significant differences according to Tukey's multiple comparison test (p < 0.05).

Among all evaluated conditions, untreated sorghum clearly favored cellulase production, whereas chemically pretreated biomasses and residues remaining after prior hydrolysis exhibited considerably lower enzyme activities. The highest cellulolytic activity (33.823 U g⁻¹) suggests that preservation of the native lignocellulosic structure maintained a greater availability of structural polysaccharides capable of acting as substrates, promoting cellulase production. Filamentous fungi are known to regulate cellulase production according to substrate composition, and cellulose-derived oligosaccharides play an important role in inducing cellulolytic enzyme synthesis. Consequently,

substrates retaining higher proportions of intact cellulose and hemicellulose may stimulate enzyme production more efficiently than previously degraded materials (ANDLAR et al., 2018).

Conversely, cultivation on biomass residues previously subjected to hydrolysis resulted in substantially lower cellulolytic activities (4.900–16.095 U g⁻¹). This reduction is likely due to the partial depletion of readily available carbohydrates during the initial hydrolysis step, thereby decreasing the availability of soluble compounds involved in cellulase induction (SINGHANIA et al., 2009). Furthermore, structural modifications promoted by the chemical pretreatments may have altered substrate accessibility and fungal colonization, thereby reducing enzyme production.

Proteolytic activity followed a similar trend, with untreated sorghum also promoting the highest protease production. The simultaneous secretion of cellulases and proteases is consistent with the physiological response commonly reported for filamentous fungi cultivated under solid-state fermentation, in which extracellular proteases participate in protein turnover, nutrient acquisition, and metabolic adaptation under nutrient-limited conditions (SOUZA et al., 2015). Although elevated protease activity may compromise cellulase stability during prolonged hydrolysis, the production of protease also reflects the complexity of the extracellular enzymatic system synthesized by *T. koningiopsis*.

Interestingly, cellulase and protease production did not increase proportionally across all cultivation conditions. While untreated sorghum promoted a marked increase in cellulolytic activity, the corresponding increase in protease production was comparatively less pronounced, suggesting that substrate composition differentially influences the production of these enzyme groups. This observation indicates that the lignocellulosic substrate acts not only as a physical support for fungal growth but also as a determinant of the enzymatic profile produced during solid-state fermentation.

Overall, these findings demonstrate that solid-state fermentation enables the production of substrate-dependent cellulolytic enzymes by *T. koningiopsis*. More importantly, the results indicate that the characteristics of the cultivation substrate directly influence both the magnitude and the profile of the

extracellular enzyme production. Future optimization of this process should therefore focus on maximizing cellulase production while controlling protease secretion, thereby improving the stability and biotechnological applicability of the crude enzymatic extracts.

2.3.2 Performance of commercial and laboratory cellulolytic preparations

To further evaluate the performance of the enzymatic systems, the cellulolytic activities of a commercial cellulase preparation (EMP) and a laboratory-produced crude extract (TK) were compared using untreated and chemically pretreated sorghum and elephant grass biomasses (Table 2).

Table 2. Cellulolytic activity obtained during direct hydrolysis of sorghum and elephant grass using commercial and laboratory-produced enzymatic preparations.

Sample*	Cellulolytic activity (U mL ⁻¹)**
S/PT/EMP	8.64 ^c ± 0.09
S/PT/TK	6.63 ^d ± 0.01
S/EMP	12.23 ^b ± 0.05
S/TK	4.72 ^e ± 0.04
C/PT/EMP	6.41 ^d ± 0.08
C/PT/TK	0.49 ^g ± 0.02
C/EMP	13.91 ^a ± 0.01
C/TK	3.80 ^f ± 0.05

*S: sorghum; C: elephant grass; PT: chemically pretreated biomass; EMP: commercial cellulase preparation; TK: laboratory-produced cellulolytic extract. **Values are expressed as mean ± standard error (n = 3). Different superscript letters within the same column indicate significant differences according to Tukey's multiple comparison test (p < 0.05).

Under all evaluated conditions, the commercial cellulase preparation exhibited higher cellulolytic activities than the laboratory-produced extract. The highest activities were observed for untreated

sorghum (12.273 U mL⁻¹) and untreated elephant grass (13.909 U mL⁻¹), confirming the superior hydrolytic performance of commercial enzyme formulations. This result is expected because industrial cellulase preparations are produced under optimized fermentation conditions and contain balanced proportions of endoglucanases, exoglucanases, β -glucosidases, and accessory enzymes that maximize cellulose hydrolysis (SINGHANIA et al., 2012).

An important finding was that the chemical pretreatments employed did not improve cellulolytic activity for either enzymatic system. In both sorghum and elephant grass, untreated biomasses consistently exhibited higher activities than their acid- or alkaline-pretreated counterparts, indicating that the pretreatment conditions were insufficient to overcome the intrinsic recalcitrance of the lignocellulosic matrix (ZHANG et al., 2021).

The lowest activity was observed for the C/PT/TK treatment (0.498 U mL⁻¹), representing a marked reduction compared with untreated elephant grass (3.801 U mL⁻¹). A possible explanation is that chemical pretreatments promoted lignin redistribution or condensation reactions, increasing the non-productive adsorption of cellulases onto lignin surfaces. Such interactions reduce the concentration of free enzymes available for cellulose hydrolysis and are recognized as one of the principal limitations of enzymatic conversion of lignocellulosic biomass (ZHANG et al., 2021).

Although the commercial preparation outperformed the laboratory-produced extract during direct hydrolysis, the results obtained in the previous section provide an important complementary perspective. When *T. koningiopsis* synthesized extracellular enzymes directly on the lignocellulosic substrates through solid-state fermentation, cellulolytic activities increased substantially, particularly for untreated sorghum. This finding suggests that optimizing enzyme production according to substrate characteristics may represent a promising alternative to the direct application of non-adapted crude enzymatic extracts.

Taken together, these results demonstrate that enzyme production and enzyme application should be considered complementary rather than independent strategies. While commercial preparations

remain the most efficient option for direct hydrolysis under the evaluated conditions, solid-state fermentation enables the production of substrate-dependent cellulolytic enzyme whose characteristics are influenced by the lignocellulosic biomass used during cultivation. These findings reinforce the importance of tailoring enzyme production to the target substrate as a strategy to improve lignocellulosic bioconversion.

2.4. Conclusion

This study demonstrates that cellulolytic enzyme production by *Trichoderma koningiopsis* during solid-state fermentation is strongly influenced by the lignocellulosic substrate used for fungal cultivation. Among the evaluated conditions, untreated sorghum exhibited the highest cellulolytic activity, indicating that preserving the native structural characteristics of the biomass favors cellulolytic enzyme production. In contrast, the direct application of commercial and laboratory-produced enzymatic preparations showed limited performance under the evaluated conditions, and the chemical pretreatments employed did not significantly improve cellulolytic activity.

Solid-state fermentation also promoted the simultaneous production of extracellular proteases, demonstrating that the enzymatic profile of the crude extracts is closely associated with the physiological response of *T. koningiopsis* to the cultivation substrate. Although elevated proteolytic activity may affect cellulase stability during prolonged hydrolysis, it also reflects the complexity of the extracellular enzymatic machinery naturally produced by the fungus.

Overall, the results demonstrate that lignocellulosic grasses can serve not only as cultivation substrates for fungal growth but also as determinants of cellulolytic enzyme production by *T. koningiopsis*. These findings support the potential of substrate-dependent enzyme production through solid-state fermentation as a promising strategy for obtaining crude enzymatic extracts for lignocellulosic bioprocesses. Future studies should focus on optimizing fermentation conditions to maximize cellulase

production while controlling protease secretion, thereby improving the stability, efficiency, and applicability of these enzymatic extracts in biomass conversion processes.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used generative Artificial Intelligence (AI) tools exclusively for academic writing support, linguistic revision, and text translation. Following the AI-assisted process, the authors critically reviewed, edited, and validated the final text. The authors assume full responsibility for the originality of the manuscript, the veracity of the experimental data presented, and compliance with CNPq's scientific integrity guidelines.

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3. CONCLUSÃO GERAL

Os resultados obtidos nesta dissertação demonstraram que a produção de enzimas celulolíticas por *Trichoderma koningiopsis* em fermentação em estado sólido é influenciada pelas características da biomassa utilizada como substrato de cultivo. Entre as condições avaliadas, a biomassa de sorgo sem pré-tratamento apresentou maior potencial para induzir a produção de celulasas, evidenciando que a composição e a estrutura do substrato desempenham papel determinante na resposta metabólica do fungo e, conseqüentemente, no perfil enzimático produzido.

A comparação entre os extratos enzimáticos produzidos por fermentação em estado sólido e uma preparação comercial demonstrou que, embora os coquetéis comerciais apresentem maior desempenho na hidrólise direta da biomassa nas condições avaliadas, a produção de enzimas diretamente sobre substratos lignocelulósicos representa uma estratégia promissora para obtenção de complexos enzimáticos potencialmente mais adaptados às características da biomassa de interesse. Os resultados também evidenciaram a necessidade de otimizar as condições de cultivo visando maximizar a produção de celulasas e controlar a secreção de proteases, contribuindo para aumentar a estabilidade e a eficiência dos extratos enzimáticos produzidos.

Sob a perspectiva das Ciências Ambientais, esta pesquisa contribui para o avanço de estratégias voltadas à valorização sustentável de biomassas lignocelulósicas abundantes e amplamente disponíveis no Brasil. A utilização de sorgo e capim-elefante como substratos para a produção de enzimas agrega valor aos recursos renováveis, favorece o desenvolvimento de bioprocessos ambientalmente mais sustentáveis e amplia as possibilidades de aproveitamento de resíduos agrícolas em sistemas de biorrefinaria. Dessa forma, o trabalho fortalece a interface entre biotecnologia, bioeconomia circular e desenvolvimento sustentável, demonstrando como o conhecimento científico pode contribuir para o uso mais eficiente dos recursos naturais e para o desenvolvimento de tecnologias com potencial de aplicação em diferentes cadeias produtivas.

Além da contribuição científica representada pelo artigo resultante desta dissertação, os conhecimentos gerados fornecem subsídios para o desenvolvimento de novas pesquisas envolvendo a produção enzimática, o aproveitamento de resíduos lignocelulósicos e a otimização de processos biotecnológicos. Assim, esta dissertação reforça o papel da pesquisa em Ciências Ambientais na geração de soluções inovadoras que conciliem desenvolvimento científico, inovação tecnológica, sustentabilidade ambiental e desenvolvimento regional.

4. PERSPECTIVAS PARA TRABALHOS FUTUROS

Os resultados obtidos nesta dissertação demonstram o potencial da fermentação em estado sólido para a produção de enzimas celulolíticas por *Trichoderma koningiopsis* utilizando biomassas lignocelulósicas como substratos de cultivo. Entretanto, diversas questões permanecem abertas e representam oportunidades de aprofundamento desta linha de pesquisa.

Entre as perspectivas futuras, destaca-se a otimização das condições de fermentação visando aumentar a produção de celulasas e reduzir a secreção simultânea de proteases, favorecendo a estabilidade e o desempenho dos extratos enzimáticos. A avaliação de diferentes condições de cultivo, incluindo umidade, suplementação nutricional, granulometria da biomassa e tempo de fermentação, poderá contribuir para maximizar o potencial biotecnológico do processo.

Mostra-se também relevante a caracterização mais detalhada do complexo enzimático produzido, incluindo a determinação individual das principais enzimas lignocelulolíticas e a investigação de sua estabilidade sob diferentes condições operacionais. Esses estudos permitirão compreender, de forma mais aprofundada, os mecanismos envolvidos na degradação da biomassa.

Outra perspectiva consiste na aplicação dos extratos enzimáticos produzidos pela hidrólise de diferentes resíduos lignocelulósicos e na avaliação de sua integração a processos de produção de bioenergia e de outros bioprodutos em sistemas de biorrefinaria. A combinação entre produção enzimática, pré-tratamentos e conversão biológica poderá contribuir para ampliar o aproveitamento sustentável de biomassas agrícolas.

Por fim, espera-se que os conhecimentos gerados nesta pesquisa sirvam de base para o desenvolvimento de novos estudos voltados à valorização biotecnológica de resíduos lignocelulósicos, fortalecendo a interface entre biotecnologia, bioeconomia circular e desenvolvimento sustentável.

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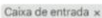
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