J Biochem Technol (2023) 14(2): 125-133
https://doi.org/10.51847/hmyfBAsNQG
ISSN: 0974-2328

Optimizing Cellulases Production by *Trichoderma atroviride* AD-130 under Submerged Fermentation using Low-Cost Substrates

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Received: 24 March 2023 / Received in revised form: 16 June 2023, Accepted: 19 June 2023, Published online: 23 June 2023

Abstract

A newly isolated strain of *Trichoderma atroviride* AD-130 was used to produce cellulases under submerged fermentation (SmF) using low-cost lignocellulosic substrates, water hyacinth, and municipal solid wastes. Although, the synthesis of β-glucosidase enzyme remains deficient in most of the *Trichoderma* spp. but the same was found considerably higher in the newly isolated fungus. Untreated and dried lignocellulosic biomasses of water hyacinth (*Eichhornia crassipes*) and municipal solid wastes were used as abundant and cost-effective substrates. *Trichoderma atroviride* AD-130 showed the highest cellulase activity on the fourth day of incubation at pH 7.0, temperature 30°C, 2% substrate concentration, and agitation speed of 180 rpm under submerged fermentation. The cellulase production decreased sharply when any of the optimized factors, whether pH, temperature, substrate concentration, or agitation speed was either increased or decreased beyond the optimized ones. Also, the poised proportion of FPase and β-*glucosidase* secreted by the cited fungus could have helped in completing the hydrolytic procedure efficiently.

Keywords: Cellulases, Submerged fermentation, *Trichoderma atroviride*, Lignocellulosic biomass, Municipal solid waste, *Eichhornia crassipes*

Introduction

Over the long-time, cellulolytic enzymes have found their widespread applications in various industrial sectors (Kuhad *et al*., 2011) such as agriculture, textile, food, biofuels, etc. Concurrently, researchers have shown keen interest in these enzymes because of their role in the bioconversion of lignocellulosic biomasses (LB) into bioethanol as it decreases our dependency on petroleum fuel, enhances the environmental quality and reduces the burden of waste management practices (Devi *et al.*, 2022). Studies have shown that low-cost lignocellulosic (LB) materials, such as wheat straw, rice straw, corn cob, and sugar cane straw, offer both environmental and socio-economic benefits (Lin, 2022). The conversion of lignocellulosic biomass (LB) to bioethanol is a three-step process: pretreatment, enzymatic hydrolysis, and fermentation. The first step is pretreatment which helps to breakdown the lignin–hemicellulose–cellulose complex of any biomass and makes it more susceptible to enzymatic hydrolysis during which cellulase and xylanase enzymes act on the cellulosic and hemicellulosic components of the LB, converting them into their respective monomeric sugars (Romero-Martínez *et al*., 2021). These glucose units can then undergo fermentation to produce bioethanol.

Cellulose is a naturally occurring polysaccharide that is the main component of plant cell walls. It is a linear, unbranched polymer of glucose subunits. Glucose subunits can be obtained by depolymerizing plant cellulose into its smaller basic building blocks with the help of cellulases, a group of enzymes that includes β-glucosidases, cellobiohydrolases, and endoglucanases (Singhania *et al*., 2010). Bioethanol, which is produced by fermenting glucose subunits, has become increasingly important in recent years as a potential alternative to petroleum-based transportation fuels (Iqbal *et al*., 2022). One of the major factors owing to which this conversion is still not commercialized is the high price of commercial cellulases available in the market (Chen *et al*., 2022).

Albeit many studies recognized the synthesis of high of cellulases from the isolation and screening of various groups of microorganisms, dwelling in a broad range of environmental niches, including cellulolytic bacteria and fungi (Balla *et al*., 2022). However, higher levels of the enzymes are found to be produced by the fungi (mainly by filamentous fungi: *Penicillium*, *Trichoderma*, and *Aspergillus* species) than those collected from yeast and bacteria (Sulyman *et al.*, 2020). Filamentous fungi, which are Generally Regarded as Safe (GRAS) strains, are the most commercially important microbes for cellulases production

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because they produce high levels of extracellular enzymes that can be easily recovered from the fermenting medium (Canassa & Baldin, 2022). Therefore, researchers are focusing on isolating and screening efficient lignocellulolytic enzymes that can produce high titers of cellulases (Duraimurugan *et al*., 2022).

Along with the isolated fungus, the choices of substrate and fermentation technology are another two important concerns that determine the success of cellulase production (Sahebzadeh *et al*., 2021). Moreover, to make the production of cellulase enzymes more economical, the exploitation of low-cost substrate as well as process optimization is of great consideration (Taiwo *et al.,* 2022). Traditionally, submerged fermentation (SmF) is used for the large-scale production of cellulases because it is easier to handle and allows for better control over parameters such as pH, temperature, and substrate concentration (Sirohi *et al.,* 2019). Therefore, envisaging the above facts, this study aimed to explore cellulases production using cost-effective feedstocks of *Eichhornia crassipes* (water hyacinth) and municipal solid waste (MSW) as substrates using newly isolated fungus *Trichoderma atroviride* AD-130 under submerged fermentation (SmF) conditions. Water hyacinth, an aquatic noxious weed capable to grow fast and having adverse impacts on aquatic life, can be seen as a potential feedstock for the production of valuable chemicals and fuels (Sindhu *et al.,* 2017). Its biomass composition ranges from 20-30% cellulose, 40- 48% hemicellulose, 13.5- 16% lignin, and low lignin content makes it more favorable for its suitability as the feedstock for bioethanol production by its commercial cultivation (Rathod *et al.,* 2018). Similarly, MSW was also used as a substrate because it is inexpensive, does not compete with food crops, and offers a waste management practice (Martau *et al.,* 2021).

Materials and Methods

Collection and Preparation of Substrates

Lignocellulosic biomass (LB), *viz., Eichhornia crassipes* (water hyacinth) was collected from the pond in Kharawar village (28.8400°N and 76.6860°E) on NH 10, Rohtak and Municipal Solid Waste (MSW) from the dumping sites for garbage in Rohtak city (28.8909°N and 76.5796°E). The biomass was collected and subsequently subjected to a thorough washing with distilled water. Following the washing process, it was carefully dried in an oven at a temperature of 70°C. Both feedstocks underwent grinding to ensure they could pass through a sieve with a mesh size of 1.0 mm. The resulting material was then stored in separate sealed plastic bags, maintaining ambient room temperature, for future use in upcoming experiments.

Inoculum Preparation

To prepare the inoculum, 20-40 mL of sterilized normal saline solution containing 0.1% (v/v) Tween-80 was added to *Trichoderma atroviride* AD-130 slants that had been cultured on potato dextrose agar (PDA) media for 5 days. After harvesting the spores with a sterile wire loop, they were vigorously shaken and a homogenous microbial suspension containing $1x10^7$ spores per one milliliter of inoculum was created.

Medium Preparation and Culture Conditions for the Cellulases Production

The study employed the submerged fermentation (SmF) technique in 250mL Erlenmeyer flasks for the production of enzymes. The flasks contained 1 to 4% (w/v) of the lignocellulosic biomass as substrate and 50mL of Mandels and Sternburg (MS) basal medium (1976). The MS medium was composed of $MnSO_4.H_2O$ (0.0016g/L), $(NH_4)_2SO_4$ (1.4g/L), $ZnSO_4.7H_2O$ (0.0014g/L), KH_2PO_4 (2.0g/L), $CaCl_2$ (0.3g/L), $MgSO_4.7H_2O$ (0.3g/L), $FeSO_4.7H_2O$ (0.005g/L), $CoCl_2$ (0.002g/L), proteose peptone (1g/L), urea (0.3g/L), Tween-80 (0.1% v/v), and glucose (0.1%; w/v). All these materials underwent autoclaving at a temperature of 121°C for 20 minutes. Following the cooling and sterilization process, the media was inoculated with 1.0 mL of *Trichoderma atroviride* AD-130. The seeded and sterilized flasks were then placed on an orbital shaker, operating at 180rpm and 30°C, for 6 days. To ensure the absence of unwanted particles and spores during the fermentation period, 1.0 mL of the culture filtrate was regularly extracted under aseptic conditions and subsequently centrifuged at 10,000 rpm for 10 minutes at 4°C. The resulting supernatant, obtained after centrifugation, served as a source of crude enzymes and was subjected to assays for cellulolytic enzymes, specifically filter paperase (FPase), carboxymethylcellulase (CMCase), and β-glucosidase (BGL).

The 'one variable at one-time' approach was utilized to optimize the culture parameters, including incubation period, pH, temperature, inducer substrate concentration, and agitation speed, to enhance the yield of cellulolytic enzymes. Specifically, for the incubation period variable, the fermentation was conducted for a total of 6 days. A series of flasks were harvested every 24 hours, beginning from the 48^{th} hour, and the resulting culture filtrate was employed for enzyme assays. The investigation of the remaining variables (pH, temperature, substrate concentration, and agitation speed) was solely conducted on the 4^{th} day, as it was determined to be the day of maximum enzyme production during the examination of the incubation period's effect.

Enzyme Assays

The method of Ghose (1987) was followed to examine the filter paperase (FPase) and carboxymethylcellulase (CMCase) activities. The activity of FPase was determined using the principle that 0.37 Filter Paper Units (FPU) of the enzyme would release 2.0 mg of glucose under standardized assay conditions. Similarly, the activity of CMCase (U/mL) was quantified based on the principle that 0.185 units of the enzyme would liberate 0.5 mg of glucose under the assay conditions. The assay for β-glucosidase followed the method outlined by Ghose and Bisaria (1987), where its activity (U/mL) was defined as the amount of enzyme that releases one micromole of p-nitrophenol per minute under standardized assay conditions.

Estimation of Reducing Sugars

The amount of reducing sugars or equivalent release due to the activity of enzymes in various assays was determined with the help

of the DNSA (3,5-dinitrosalicylic acid) method adopted by Miller (1959).

Statistical Analysis

All the experiments were performed in triplicate and the data was presented as an average mean ± standard error calculated in MS Excel.

Results and Discussion

Impact of Incubation Period on the Production of Cellulases

The incubation period is a critical factor that significantly impacts the production of cellulolytic enzymes and other metabolites. To analyze this effect, **Figure 1** showcases the production of FPase, CMCase, and BGL at regular intervals using *E. crassipes* and Municipal Solid Waste (MSW). These experiments were conducted under submerged fermentation (SmF) conditions, maintaining a pH of 5.0, a temperature of 30°C, and an agitation speed of 180 rpm.

Figure 1 demonstrates the varying activity levels (U/mL) of FPase, CMCase, and BGL on *E. crassipes*, ranging from 0.24 to 0.68, 0.17 to 0.72, and 0.09 to 0.24, respectively. Notably, the highest activity levels for FPase, CMCase, and BGL (0.68, 0.72, and 0.24U/mL, respectively) on *E. crassipes* were observed on the 4th day of the fermentation period.

Similarly, on the 4th day of the fermentation period, the highest values (U/mL) for FPase (0.92), CMCase (1.56), and BGL (0.69) on MSW were attained, as demonstrated in **Figure 1**, which showcases the production of these enzymes by *T. atroviride* AD-130. The production ranges for FPase, CMCase, and BGL on MSW were measured between 0.27 to 0.92U/mL, 0.31 to 1.56U/mL, and 0.17 to 0.69U/mL, respectively.The activity levels of BGL, FPase, and CMCase showed an upward trend with the increase in the incubation period, reaching their maximum on the 4th day after inoculation for both *E. crassipes* and MSW substrates. Subsequently, there was a gradual decline in enzyme activity. Interestingly, when comparing the two substrates, MSW exhibited higher suitability for cellulase production during submerged fermentation, as evidenced by the higher levels of all cellulolytic enzymes produced.

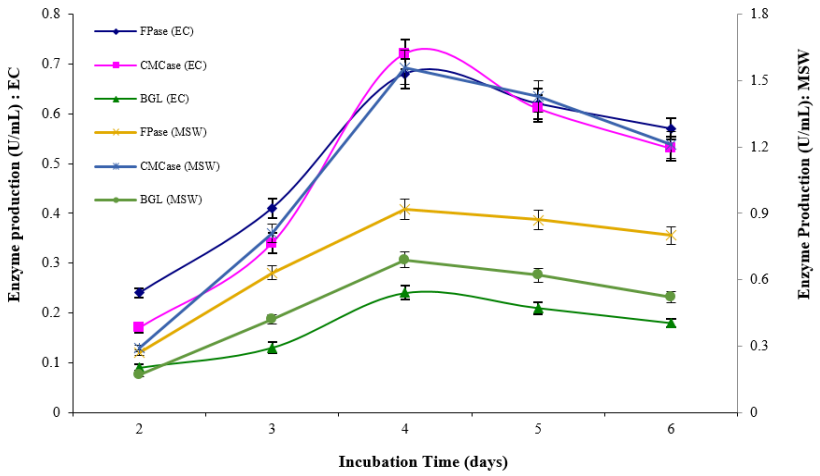


Figure 1. Effect of incubation time on cellulases production by *T. atroviride* AD-130 under SmF using *E. crassipes* (EC: 1% w/v) and MSW (1%) residues as substrate

Several studies, including those conducted by Gomathi *et al*. (2012) and Dedavid *et al*. (2009), have reported varying findings regarding the optimal incubation times for achieving maximum cellulases production in different fungi. Singh *et al*. (2009) observed that the highest activity levels of FPase and CMCase (3.2 IU/mL and 83 IU/mL, respectively) were achieved on the 5th day of incubation at a temperature of 30°C. In a separate study conducted by Sarkar and Aikat (2014), notable levels (IU/mL) of CMCase (2.31), FPase (0.261), and xylanase (48.33) were achieved after a 5-day incubation period. However, as the incubation time increased beyond 5 days, there was a significant decrease in enzyme activity. Likewise, Naher *et al*. (2021) observed the highest production of cellulases using *T. reesei* and *A. awamori* through submerged fermentation of rice straw on the 5th and 3rd days of incubation, respectively.

The observation of decreased cellulase production by *T. atroviride* AD-130 after the fourth day of incubation is not unique to this study. Similar findings have also been reported by Ogel *et al*. (2001) and Shruthi *et al*. (2020). Several factors may contribute to this decline, such as nutrient depletion in the medium (Bedade *et al*., 2017), the accumulation of cellobiose resulting during the breakdown of cellulosic material (Ojumu *et al*., 2003), or the release of phenolic compounds from residual lignin during the enzymatic hydrolysis process (Leu & Zhu, 2012).

Impact of pH on the Production of Cellulases

In this study, the influence of the initial pH of the culture medium on cellulases production by *T. atroviride* AD-130 was investigated in the context of submerged fermentation using *E. crassipes* and MSW as substrates. The pH of the culture medium was adjusted

within the range of 4 to 8 using 1N HCl/1N NaOH, and the results are presented in **Figure 2**.

Figure 2 demonstrates that the highest (U/mL) of FPase, CMCase, and BGL (0.79, 1.19, and 0.67 respectively) were attained on the fourth day of incubation at 30°C, 180 rpm, using 1.0% (w/v) *E. crassipes* biomass as the substrate, with an initial pH of 7.0. Notably, a sharp decline in cellulase production was observed at pH 8.0. Similarly, when 1.0% (w/v) MSW was used as the substrate, **Figure 2** also shows that the maximum activities (U/mL) of FPase, CMCase, and BGL were 1.14, 3.84, and 1.55 respectively, on the fourth day of incubation at pH 7.0, 30°C, and 180 rpm.

The results of the study revealed that MSW served as a more favorable carbon source for cellulase production compared to *E. crassipes*. This conclusion was based on the superior release of cellulases observed when MSW was utilized as a substrate.

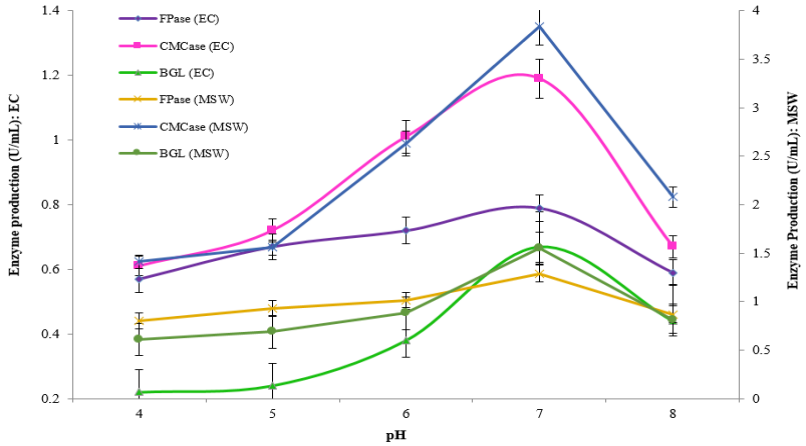


Figure 2. Effect of pH on cellulases production by *T. atroviride* AD-130 under SmF using *E. crassipes* (EC: 1% w/v) and MSW (1% w/v) residues as substrate

The results presented in this study demonstrate that the highest levels of FPase, CMCase, and BGL were achieved when *T. atroviride* AD-130 was cultivated in a medium with an initial pH of 7.0, using either *E. crassipes* or MSW residues as the substrate. The pH of the culture medium could have influenced the microorganisms' morphology, thereby affecting the extracellular secretion of enzymes (Papagianni, 2004). Additionally, the neutral pH of 7.0 may have positively influenced the stability of the microbes. Similar findings have been reported by other researchers, such as Sherief *et al*. (2010) and Karmakar and Ray (2011), who also observed maximum cellulase production within the pH range of 5.0-7.0 in different *Trichoderma* spp.

Although the optimal pH for fungal cellulases varies among species, these enzymes generally exhibit high activity over a broad pH range, typically from 3.0 to 6.0 (Grigorevski-Lima *et al*., 2013). Different studies have reported varying pH ranges for achieving maximum cellulase production by different cellulolytic fungi. For instance, Deshpande *et al*. (2009) observed significant cellulases production by *T. reesei* within the pH range of 6.0 to 8.0, while Li *et al*. (2013) found that *T. reesei* exhibited maximum FPase, CMCase, and BGL production at pH 5.0, 4.5, and 5.5, respectively.

Given the wide variation in pH requirements among cellulolytic fungal species, a pH of 7.0 was selected as the optimized standard for analyzing successive factors, such as temperature, substrate concentration, and speed of agitation.*Impact of Temperature on the Production of Cellulases*

The optimal temperature for cellulase production by *T. atroviride* AD-130 was determined by conducting experiments at different temperatures (ranging from 24°C to 37°C) using *E. crassipes* and MSW as substrates, under pH 7.0 and 180 rpm conditions. The results, depicted in **Figure 3**, demonstrate that the activity levels of FPase, CMCase, and BGL varied within the ranges of 0.23-0.79, 0.11-1.19, and 0.16-0.67U/mL, respectively, on *E. crassipes* while on MSW, the activity levels ranged from 0.34-1.29U/mL for FPase, 0.46-3.84U/mL for CMCase, and 0.29-1.55U/mL for BGL.

The cellulase production exhibited a gradual increase as the incubation temperature was raised from 24°C to 30°C. However, elevating the temperature further to 33°C or higher led to a significant decline in cellulase production. On the fourth day of submerged fermentation at 30°C, utilizing 1.0% (w/v) *E. crassipes* as the substrate, the highest levels of activity (U/mL) were observed for FPase (0.79), CMCase (1.19), and BGL (0.67). Similarly, when 1.0% (w/v) MSW was used as the substrate, the maximum activity levels (U/mL) for FPase (1.29), CMCase (3.84), and BGL (1.55) were achieved on the 4th day at 30°C.

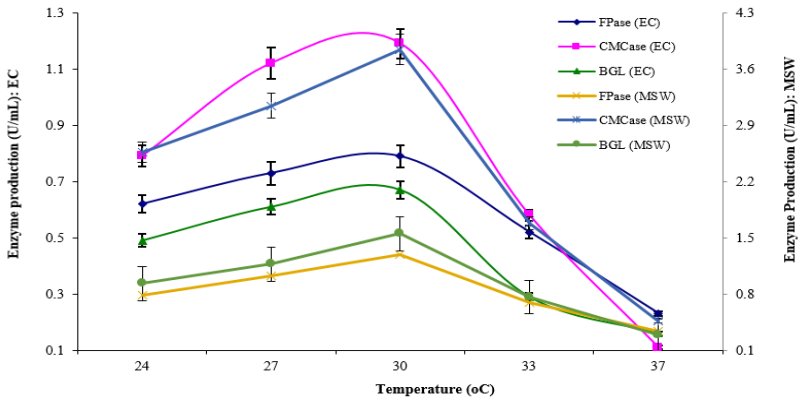


Figure 3. Effect of temperature on cellulases production by *T. atroviride* AD-130 under SmF using *E. crassipes* (EC: 1% w/v) and MSW (1% w/v) residues as substrate

Various studies, including Kang *et al*. (2004) and Jiang *et al.* (2011), have testified optimized temperature ranges of 25-30°C for generating cellulases using *Aspergillus* and *Trichoderma* spp. Furthermore, the choice of microorganism strain plays a crucial role in achieving efficient enzyme production. Various studies have highlighted different strains that demonstrate maximum cellulase production at 28°C. For instance, *Trichoderma viride* (Zhou *et al*., 2008), *Trichoderma harzianum* (Ahmed *et al*., 2009), *Trichoderma asperullum* (Bara *et al*., 2003), and *Aspergillus niger* (Kang *et al*., 2004) have all shown optimal cellulases production at the temperature of 28°C.

Gomathi *et al*. (2012) observed the highest cellulases production in *Aspergillus flavus* at 30°C, while Haiyan *et al*. (2010) found peak cellulases activity at 32°C in *Trichoderma* sp. *Trichoderma asperellum UPM1* exhibited the highest activity of β-glucosidase (0.89U/mL) at 35°C, whereas its highest CMCase and FPase activities were observed at 45°C, and similarly, *Aspergillus fumigatus* UPM2 demonstrated the highest cellulases levels (24.59U/mL of CMCase, 0.65U/mL of FPase, and 0.33U/mL of β-glucosidase) at 45°C, according to Ibrahim *et al*. (2013). However, Saqib *et al*. (2010) investigated *Aspergillus fumigatus* and reported significant cellulase production at 50°C (Macrì *et al*., 2023; Shenoy *et al*., 2023).

Thus, based on various reports by researchers, it can be concluded that the optimal production of cellulases requires consideration of both the microorganism strain and the temperature variations of the culture conditions. Although most cellulolytic fungi are best suited for mesophilic conditions, some are also stable under thermophilic conditions. Unlike the present fungal isolate *T. atroviride* AD-130, which grows best at 30°C, other fungi may exhibit better growth under thermophilic conditions.

Impact of Substrate Concentration on the Production of Cellulases

In this study, the production of cellulases using MSW and *E. crassipes* as carbon sources investigated under submerged fermentation conditions, employing *T. atroviride* AD-130 with a substrate ratio of 1:4% (w/v). The culture flasks were incubated at 30°C and 180 rpm, with the initial pH adjusted to 7.0. The highest activity levels (U/mL) of FPase (1.48), CMCase (4.56), and BGL (1.67) were achieved using 2% MSW (w/v) as the substrate **(Figure 4)**, while FPase (1.01), CMCase (1.28), and BGL (0.80) exhibited their highest activities using *E. crassipes* at a substrate concentration of 2% (w/v) **(Figure 4)**. The production of all cellulases decreased at substrate concentrations higher than 2% (w/v). Corroboratively, Deshpande *et al*. (2009) also observed a marked reduction in cellulase yield with increasing substrate concentration.

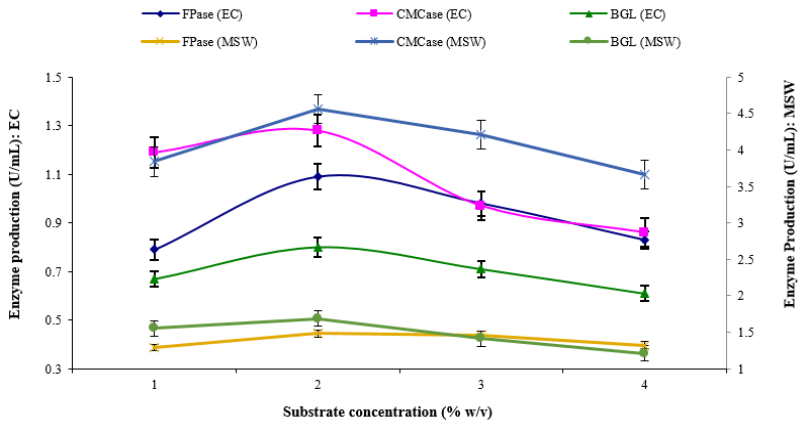


Figure 4. Effect of substrate concentration (*E. crassipes*: EC and MSW residues) on cellulases production by *T. atroviride* AD-130

In various studies, researchers have investigated the production of cellulases by different fungi using various substrates, for example, Ibrahim *et al*. (2013) found that *T. asperellum* UPM1 and *A. fumigatus* had the maximum activity at 1% substrate concentration of pretreated oil palm empty fruit branch, while Irfan *et al*. (2011) observed higher cellulases production at substrate concentrations of 1.0% for rice straw and 1.5% for wheat straw. In the study conducted by Razak *et al*. (2012), *T. asperellum* UPM1 and *A. fumigatus* UPM2 were utilized for crude cellulases production using 4% untreated palm decanter cake as a substrate. This choice was based on the lower content of cellulose and hemicellulose present in palm decanter cake. Similarly, Linggang *et al*. (2012) achieved optimal cellulase production using 1% untreated pith of the sago plant. Another study by Pothiraj *et al*. (2016) demonstrated that, after 72 hours of incubation, *T. viride* yielded maximum cellulases activity of 68.3 IU whereas *A. niger* yielded 46.3 IU at 5% substrate concentration (w/v).

The studies show that an increase in substrate concentration can lead to reduced cellulase yield due to enzyme inactivation, which is influenced by induction and repression mechanisms (Jorgersen *et al*., 2003). Cellobiose has been identified as the key inducer in β-glucosidase production, but it can also act as an inhibitor at higher concentrations (Sukumaran *et al*., 2005). Additionally, it has been observed that increasing the substrate proportion leads to a decrease in medium porosity, which in turn affects the rate of oxygen transfer (Gomathi *et al*., 2012). Therefore, considering all these factors, it can be predicted that *T. atroviride* AD-130, which yields higher cellulases production on MSW than on *E. crassipes*, can induce cellulases in a balanced proportion at a substrate concentration of 2%. This is mainly because the chemical nature of both substrates varies due to differences in their cellulose and hemicellulose composition (Dudás *et al*., 2022; Lassmann *et al*., 2022; Mahmood *et al*., 2022).

Impact of Agitation on the Production of Cellulases

In this study, the impact of agitation on cellulases production was examined in the flasks containing a substrate concentration of 2% (w/v) which were cultivated at an initial pH of 7.0 and various rotational speeds (60, 120, 180, 240, and 300 rpm) in an orbital shaker at 30°C. The results demonstrated that, on the fourth day of fermentation at 180 rpm, utilizing *E. crassipes* biomass as the carbon source resulted in a significant increase in FPase activity (1.01U/mL), CMCase activity (1.28U/mL), and BGL activity (0.80U/mL) **(Figure 5)**, while employing MSW as the substrate led to higher levels of FPase (1.48U/mL), CMCase (4.56U/mL), and BGL (1.69U/mL) production **(Figure 5)**.

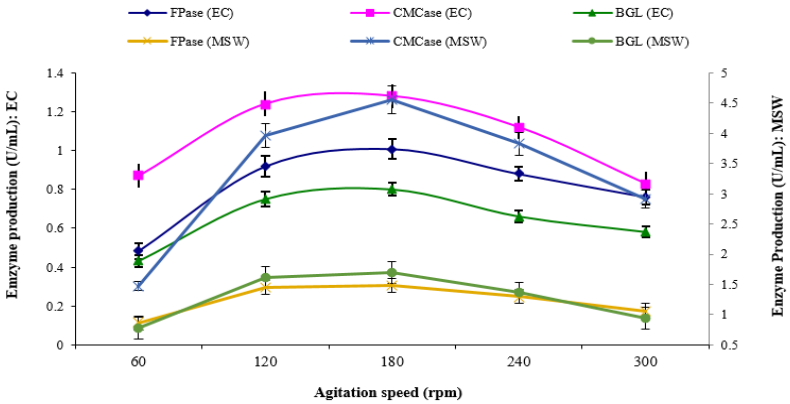


Figure 5. Effect of agitation on cellulases production by *T. atroviride* AD-130 under SmF using *E. crassipes* (EC: 2% w/v) and MSW (2 % w/v) residues as substrate

Agitation plays a dual role in enzyme production, as it promotes uniform nutrient distribution in the culture medium and provides aeration, which enhances fungal cell growth and enzyme yield (Matkar *et al*., 2013). Corroboratively, cellulase production was found to decrease significantly at much higher or lower agitation speeds. It is noteworthy that although the production of the enzyme β-glucosidase is deficient in most *Trichoderma* spp., the cited fungus produced sufficient amounts of this enzyme **(Table 1)**.

Table 1. Cellulases Production by *T. atroviride* AD-130 Using MSW and *E. crassipes* Biomasses as Carbon Source at 2% (w/v) Concentration: Submerged Fermentation Study at pH 7.0, 30°C, 180 rpm on the 4th Day of Incubation

Enzyme activity	MSW (U/mL)	*E. crassipes* (U/mL)
FPase	1.48	1.01
CMCase	4.56	1.28
BGL	1.69	0.80

Recent studies have highlighted the optimal agitation speed range of 120-200 rpm for achieving maximum cellulases production in submerged fermentation (SmF). Matkar *et al*. (2013) obtained higher cellulases yield from *A. sydowii* at 120 rpm, while Grigorevski-Lima *et al*. (2013) observed the highest activities of CMCase (1.9U/mL), FPase (0.25U/mL), and BGL (0.17U/mL) in *T. atroviride* at 200 rpm. Ibrahim *et al*. (2013) optimized SmF at 150 rpm and achieved 0.8U/mL of FPase, 24.7U/mL of CMCase, and 5.0U/mL of β-glucosidase with *T. asperellum* UPM1, and 1.7U/mL of FPase, 24.2U/mL of CMCase, and 1.1U/mL of BGL with *A. fumigatus* UPM2. Similarly, De Castro *et al*. (2010) optimized *T. harzianum* in SmF at 200 rpm, resulting in cellulases production of FPase (0.97U/mL), CMCase (0.56U/mL), and BGL (0.74U/mL).

Conclusion

In the present study, it was observed that the highest levels of FPase (1.01U/mL), CMCase (1.28U/mL), and BGL (0.80U/mL) on *E. crassipes* biomass, and FPase (1.48U/mL), CMCase (4.56U/mL), and BGL (1.69U/mL) on MSW were achieved at an agitation speed of 180 rpm. These results were obtained under submerged fermentation conditions with a substrate concentration of 2%, pH 7.0, and a temperature of 30°C after a four-day incubation period. Therefore, it can be concluded that *T. atroviride* AD-130 produced balanced amounts of all the cellulolytic extracellular enzymes, including FPase, CMCase, and BGL, enabling efficient hydrolysis of both biomasses of MSW and *Eichhornia crassipes*. Notably, although BGL production is usually deficient in most *Trichoderma* spp., the cited fungus produced sufficient amounts of all the cellulase enzymes. The proportion of FPase and BGL secreted by *T. atroviride* AD-130 may have facilitated the complete conversion of cellulose to glucose, thereby eliminating cellobiose inhibition. Therefore, *T. atroviride* AD-130 could be a suitable candidate for the commercial production of cellulases because the results indicate its potential for saccharifying lignocellulosic biomass into reducing sugars, which can then be utilized for the large-scale production of bioethanol. However, further optimization of the inoculum age, inoculum size, use of inducers (such as lactose or cellobiose), substrate particle size, and culture medium may improve cellulases production by *T. atroviride* AD-130.

Acknowledgments: All the participants wish to acknowledge the Major Research Project funded by the University Grants Commission, New Delhi.

Conflict of interest: None

Financial support: None

Ethics statement: None

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