Enhanced Cellulase Production by *Talaromyces Amestolkiae* CMIAT055 using Banana Pseudostem

Genilton S Faheina Jr
EMBRAPA Agroindustrial Tropical
Kally A Sousa
IFCE: Instituto Federal de Educacao Ciencia e Tecnologia do Ceara
Jerri E Zilli
EMBRAPA Centro Nacional de Pesquisa de Agrobiologia
Carlos Vergara
EMBRAPA Centro Nacional de Pesquisa de Agrobiologia
Gustavo A. Saavedra Pinto
EMBRAPA Agroindustrial Tropical
Rilvia Saraiva de Santiago-Aguiar (rilvia@ufc.br)
Universidade Federal do Ceara https://orcid.org/0000-0003-2513-6898

Research Article

Keywords: celulases, lignocellulosic materials, Talaromyces amestolkiae, biomass, agitation.

Posted Date: September 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-726111/v1

Abstract

Cellulases are a complex of enzymes necessary for the complete solubilization of cellulose in sugars, thus playing a key role in the natural carbon cycle through the hydrolysis of lignocellulosic structures. The aim of this study was to evaluate the increase in the capacity of *Talaromyces amestolkiae* CMIAT 055 to produce cellulases by optimizing the components of the culture medium containing banana pseudostem as an inducer, as well as in different agitation configurations in a bioreactor. Optimization was performed through statistical experimental design (Plackett-Burman and DCCR), a study of pH control in bioreactors, and a study of the agitation system by comparing impellers with different flow profiles in the liquid medium. For this purpose, a wild strain of *Talaromyces amestolkiae* CMIAT 055 was used. In the Plackett-Burman and DCCR statistical design, four components of the culture medium were significant and optimized for greater synthesis of FPase: banana pseudostem, CaCl2, KH2PO4, and urea. In bioreactors tests, these parameters were beneficial for greater enzyme activities: maintenance of pH at 5.0, use of Pitched blade impeller, and rotation speed at 300 rpm. Comparing the first test using banana pseudostem in an Erlenmeyer flask to the last fermentation process in bioreactors, it was observed that the total cellulase activity increased from 424.7 FPU/L to 2172.8 FPU/L. This fact showed that the strategies adopted in this study are a pertinent way to reduce the cost of enzyme production through the use of lignocellulosic materials.

Statement Of Novelty

The use of lignocellulosic materials in fermentation process is an emerging study. The applicability of banana pseudostem as carbon source to produce biomolecules with high added value such as cellulases, was assessed in this study. This is the first report using banana pseudostem as carbon source to produce biomolecules with high added value such as cellulases, by *Talaromyces amestolkiae* CMIAT055. The high cellulase activities were obtained after the optimization step and the results presented may be useful to researchers because the production of enzymes in an environment containing pseudostem of banana and *Talaromyces* has scarcely been reported in the literature.

Introduction

Plant materials have significant amounts of cellulose that may be used as alternative sources of carbon for a number of processes. Despite the wide availability in the environment or as agro-industrial residue, cellulose acts as a difficult polymer to be broken. Therefore, an enzymatic conversion of this material, using cellulases, stands out as a process with less environmental impact, in addition to milder operating conditions, and no formation of undesirable subproducts, compared to chemical hydrolysis [1].

In addition to the use of cellulases in fuel production processes, these enzymes also help to generate a variety of high value-added products from the breakdown of biomass, such as organic acids, amino acids, and vitamins. Just as polysaccharide fractions that can be generated from microbial biomass developed with the aid of sugars generated from the breakdown of biomass, a process in which cellulolytic enzymes play an important role [2].

The most studied and used strains in the industry for the production of cellulolytic enzymes are those of the genera *Aspergillus* and *Trichoderma* [3]. However, some studies demonstrate limitations of enzymatic activity in the enzyme complex produced by these genera, such as efficiency in narrow pH and temperature ranges [4], which reinforces the interest in cellulase synthesis research by other fungal genera.

In biotechnological processes, fungi of the genus *Talaromyces* are reported as capable of efficiently producing thermostable cellulases, presenting the advantage of carrying out the hydrolysis step at high temperatures [5]. There are cases reported in the literature in which the enzymes secreted by this genus have greater lignocellulosic saccharification abilities compared to the best-known strains, such as *Trichoderma reesei* [6, 7]. This reinforces the importance of more intense research on cellulase synthesis by *Talaromyces*, a genus in which it contains a variety of species capable of degrading cellulose through the fungus's enzymatic machinery, such as *T. amestolkiae, T. piceus* and *T. stolli* [5, 8].

Despite all the benefits derived from the use of cellulases, they still have high commercial cost and a significant reduction in price becomes fundamental in order to reduce costs in a wide variety of processes. Therefore, it is necessary to synthesize the enzymes at the lowest possible cost, since this step is primordial for the enzymatic conversion of the biomass [9]. Thus, among the strategies that can be implemented to reduce the cost of cellulolytic enzymes, are intensive selection of new microbial sources producing more stable cellulases and with higher productivity; replacement of growth medium components by lignocellulosic materials available in nature or as agro-industrial waste; accurate studies on the reactor configuration and fermentation conditions.

The compositions of the culture medium most commonly used for the production of cellulases in the submerged state were those initially suggested by Mandels, Reese and Weber [10, 11], in which pure crystalline cellulose is the main inducing component for enzyme synthesis. However, several studies suggest substitute components for pure cellulose, since a large part of the costs associated with the synthesis of cellulases fall back on the value of the raw carbohydrate material used as an inducer [12, 13]. Most studies on cellulase synthesis using alternative carbon sources and *Talaromyces* fungi report the use of glycerol, wheat bran, sorbitol, corn husk, and sorghum [5, 6, 14–16], but none with the use of banana pseudostem as a component of the culture medium.

The production of cellulases through submerged fermentation is also highly influenced by several parameters, such as nature of the cellulosic material used as an inducer, nutrient availability, pH and configuration of reactors [17]. Regarding the configuration of the bioreactors used in the production of the enzyme on larger scales, the impeller is the main part of the stirred tank reactor that determines the effects of the mixture and the energy consumed. Most of these types of equipment need maximum control of the mixing process to obtain greater productivity, which significantly affects the performance and economic efficiency of the mixing system. Consequently, studies of the types of impellers used in bioreactors become extremely relevant for increasing productivity in submerged fermentation processes [18].

Thus, the study aimed to evaluate the increase in the capacity of *T. amestolkiae* CMIAT 055 to produce cellulases by optimizing the components of the liquid culture medium containing banana pseudostem as an inducer, as well as in different agitation configurations of the stirred tank bioreactor fermentation process.

Materials And Methods

Preparation of lignocellulosic material

Banana pseudostem (BP) was kindly provided by Embrapa Agroindústria Tropical, Ceará, Brazil. The material was washed with distilled water, filtered and dried in an air circulation oven at 60 °C. Afterwards, the washed and dried biomass was ground in a Willye type mill (Fortinox, Star FT 80 model), with a 10-mesh screen attached to the equipment.

In previous studies, BP was subjected to acid-alkaline treatment in order to verify its use in cellulase synthesis. However, it presented lower enzymatic production results when compared to the untreated material. Therefore, we have chosen to use BP without any previous treatment. The cellulose, hemicellulose, lignin, and ash contents of the BP were analyzed [19] to be 83.9, 8.0, 1.8, 1.6% (w/w), respectively. The material was kept in hermetically sealed plastic bags and kept at room temperature (around 30°C).

Microorganisms

Talaromyces amestolkiae CMIAT 055, belonging to the Collection of Microorganisms of Agroindustrial Interest, of the Brazilian Agricultural Research Corporation (EMBRAPA, Ceará, Brazil) was selected in a previous step among 17 fungal strains, as the best producer of total cellulases (FPase) in submerged fermentation containing 100 mL of medium described by Mandel and Weber [11]

The microorganism was preserved in the basal medium carboxymethylcellulose agar (CMC), containing (g/L): 2.0 KH_2PO4, 0.4 $CaCl_2.2H_2O$, 0.3 $MgSO_4.7H_2O$, 0.005 $FeSO_4.7H_2O$, 0.0037 $CoCl_2.6H_2O$, 0.0016 $MnSO_4.H2O$, 0.0014 $ZnSO_4.7H_2O$, 1.4 $(NH_4)_2SO_4$,

12.0 agar, 10.0 sucrose and 10.0 CMC (pH 5.0). The inoculum was prepared by propagating the fungal spores in a medium containing wheat bran, incubated at 30°C for 7 days. After the growth period, the microorganism was extracted from the solid material with the aid of a 0.3% Tween solution (w/v). This suspension was used as an inoculum in the fermentation processes of propagation of fungus spores in the wheat bran medium.

Strain identification

We used two molecular markers (Intergenic spaces-ITS1-ITS2 region- and β-tubulin) as recommend barcoding for *Penicillium/ Talaromyces* to place this strain into a species or to reach to new one [20–22]. PCR primers and conditions of amplification for ITS1-ITS2 and β-tubulin were performed [23], and the sequencing was done with the forward primers. The sequencing edition, alignment and phylogenetic analyses were performed with MEGA software version 6.0, being the software default parameters considered. The generated sequences were deposited in DNA DataBank of Japan (DDBJ) and access numbers were included in the phylogenetic trees.

Selection of culture medium variables by Plackett Burman Design (PBD)

Plackett Burman Design was performed to evaluate the main linear effects of BP, sucrose, urea, peptone, yeast extract, (NH4)2SO4, KH2PO4, MgSO4.7H2O and CaCl2.2H2O on FPase production through 15 experimental runs (Table 1). The most relevant variables that significantly influence the synthesis of cellulase enzymes were selected using the Statistica software (10 Statsoft, Tulsa, OK, USA). The PBD design was based on the first-order polynomial model (Equation 1):

$$Y = \beta_0 + \sum \beta_i \, X_i \qquad (1)$$

Where Y is the answer (enzymatic activity), β_0 is the intercept model, β_i represents the linear coefficient and X_i is the independent variable.

The amounts attributed to carbon, BP and sucrose sources were determined through experiments in which enzymatic activities were observed at different concentrations of the solid material. The other variables were adopted according to what is reported in the literature [24, 25].

A regression analysis was performed to determine the factors influencing enzyme production. Those that were significant at levels above or below 95% ($p < 0.5$) were selected and optimized in the next step, by Central Composite Rotational Design (DCCR).

Table 1 - Plackett-Burman design generated for nine variables

Run	BP	Sucrose	Urea	Peptone	Yeast Extract	$(NH_4)_2SO_4$	KH_2PO_4	$MgSO_4.7H_2O$	$CaCl_2.2H_2O$	FPase
1	+1 (50)	-1 (5)	+1 (0.9)	-1 (0.75)	-1 (0.25)	-1 (1.4)	+1 (6)	+1 (0.9)	+1 (1.2)	793.8
2	+1 (50)	+1 (50)	-1 (0.3)	+1 (2.25)	-1 (0.25)	-1 (1.4)	-1 (2)	+1 (0.9)	+1 (1.2)	39.2
3	-1 (5)	+1 (50)	+1 (0.9)	-1 (0.75)	+1 (0.75)	-1 (1.4)	-1 (2)	-1 (0.3)	+1 (1.2)	114.5
4	+1 (50)	-1 (5)	+1 (0.9)	+1 (2.25)	-1 (0.25)	+1 (3.4)	-1 (2)	-1(0.3)	-1 (0.4)	322.8
5	+1 (50)	+1 (50)	-1 (0.3)	+1 (2.25)	+1 (0.75)	-1 (1.4)	+1 (6)	-1 (0.3)	-1 (0.4)	41.0
6	+1 (50)	+1 (50)	+1 (0.9)	-1 (0.75)	+1 (0.75)	+1 (3.4)	-1 (2)	+1 (0.9)	-1 (0.4)	102.0
7	-1 (5)	+1 (50)	+1(0.9)	+1 (2.25)	-1 (0.25)	+1 (3.4)	+1 (6)	-1 (0.3)	+1 (1.2)	104,9
8	-1 (5)	-1 (5)	+1 (0.9)	+1 (2.25)	+1 (0.75)	-1 (1.4)	+1 (6)	+1 (0.9)	-1 (0.4)	76.8
9	-1 (5)	-1 (5)	-1 (0.3)	+1 (2.25)	+1 (0.75)	+1 (3.4)	-1 (2)	+1 (0.9)	+1 (1.2)	120.1
10	+1 (50)	-1 (5)	-1 (0.3)	-1 (0.75)	+1 (0.75)	+1 (3.4)	+1 (6)	-1 (0.3)	+1 (1.2)	684.1
11	-1 (5)	+1 (50)	-1 (0.3)	-1 (0.75)	-1 (0.25)	+1 (3.4)	+1 (6)	+1 (0.9)	-1 (0.4)	127.4
12	-1 (5)	-1 (5)	-1 (0.3)	-1 (0.75)	-1 (0.25)	-1 (1.4)	-1 (2)	-1 (0.3)	-1 (0.4)	61.3
13*	0 (27.5)	0 (27.5)	0 (0.6)	0 (1.5)	0 (0.5)	0 (2.4)	0 (4)	0 (0.6)	0 (0.8)	110.8
14*	0 (27.5)	0 (27.5)	0 (0.6)	0 (1.5)	0 (0.5)	0 (2.4)	0 (4)	0 (0.6)	0 (0.8)	127.4
15*	0 (2.5)	0 (27.5)	0 (0.6)	0 (1.5)	0 (0.5)	0 (2.4)	0 (4)	0 (0.6)	0 (0.8)	140.4
BP = banana pseudostem. * Central points. FPase: FPU/L										

Optimization of culture media by response surface methodology

A 2^4 full factorial design was used, in five coded levels, including 8 axial points and 3 central points, totaling 27 experiments (Table 2). Data were generated using the Statistica 10.0 software (Statsoft, Tulsa, OK, USA).

Table 2 - CCRD matrix with coded and real values for independent variables and FPase responses.

Teste	BP*	$CaCl_2.2H_2O$	KH_2PO_4	Urea	FPase
1	-1 (20)	-1 (1.2)	-1 (3)	-1 (1.2)	442.5
2	-1 (20)	-1 (1.2)	-1 (3)	+1 (3.6)	303.9
3	-1 (20)	-1 (1.2)	+1 (9)	-1 (1.2)	590.1
4	-1 (20)	-1 (1.2)	+1 (9)	+1 (3.6)	379.5
5	-1 (20)	+1 (3.6)	-1 (3)	-1 (1.2)	706.1
6	-1 (20)	+1 (3.6)	-1 (3)	+1 (3.6)	410
7	-1 (20)	+1 (3.6)	+1 (9)	-1 (1.2)	689.5
8	-1 (20)	+1 (3.6)	+1 (9)	+1 (3.6)	277
9	+1 (60)	-1 (1.2)	-1 (3)	-1 (1.2)	90
10	+1(60)	-1 (1.2)	-1 (3)	+1 (3.6)	389.7
11	+1 (60)	-1 (1,2)	+1 (9)	-1 (1.2)	156.3
12	+1 (60)	-1 (1.2)	+1 (9)	+1 (3.6)	399.5
13	+1 (60)	+1 (3.6)	-1 (3)	-1 (1.2)	278.5
14	+1 (60)	+1 (3,6)	-1 (3)	+1 (3.6)	459.7
15	+1 (60)	+1 (3.6)	+1 (9)	-1 (1.2)	256.3
16	+1 (60)	+1 (3.6)	+1 (9)	+1 (3.6)	424.4
17	-α (0)	0 (2.4)	0 (6)	0 (2.4)	2.7
18	+α (80)	0 (2.4)	0 (6)	0 (2.4)	57
19	0 (40)	-α (0)	0 (6)	0 (2.4)	742.2
20	0 (40)	+α (4.8)	0 (6)	0 (2.4)	1032.4
21	0(40)	0 (2.4)	-α (0)	0 (2.4)	331.5
22	0 (40)	0 (2.4)	+α (12)	0 (2.4)	350.9
23	0 (40)	0 (2.4)	0 (6)	-α (0)	204.9
24	0 (40)	0 (2.4)	0 (6)	+α (4.8)	148.9
25**	0 (40)	0 (2.4)	0 (6)	0 (2.4)	162.6
26**	0 (40)	0 (2.4)	0 (6)	0 (2.4)	193.6
27**	0 (40)	0 (2.4)	0 (6)	0 (2.4)	194.3

* PB = banana pseudostem; ** Central points; [a]PB: g/L; [b]$CaCl_2$: g/L; [c]KH_2PO_4: g/L; [d]Urea: g/L; [e]FPAse: FPU/L

The range of levels of all variables was increased, if compared to the levels adopted in PBD-type planning, to study the induction of enzyme synthesis in higher amounts of components. The experimental data were fitted according to the following second order quadratic equation, according to equation 2:

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_{ii} \beta_{ii} X_{ii}^2 + \sum_{ij} \beta_{ij} X_i X_j \qquad (2)$$

Where Y represents enzyme activity; β_0 is the intercept term, constant in the model; β_i, β_{ii} and β_{ij} are, respectively, the linear, quadratic and interaction coefficients; x_i and x_j are the independent variables.

The results of the experiments were analyzed, regarding the goodness of fit of the generated models, by Analysis of Variance (ANOVA). The statistical significance of each equation obtained was evaluated using the R^2 regression coefficient and validation using the *Fisher* distribution test (F).

Fermentation in bioreactors

Fermentations were carried out in stirred tank reactors BioFlo 310 (New Brunswick, USA) with a working volume of 4 L, equipped with control and monitoring of pH, agitation, aeration, and temperature, as well as the use of baffles. In all fermentation processes, during seven days of incubation, the composition of the optimized culture medium was used in the statistical planning studies carried out in Erlenmeyer flasks, at a temperature of 30 °C.

From each experimental point, collected every 24 hours, 40 mL of fermented broth were removed. Samples were centrifuged at 9600 g for 20 min at 10°C. The precipitate was used for the analysis of fungus biomass and cellulose content with the supernatant were used for the enzymatic analysis.

Cellulase synthesis was tested through fermentation with the following configurations: without pH control, with Rushton type impeller and 150 rpm agitation; maintenance of pH at 5.0, with Rushton type impeller and 150 rpm agitation; maintenance of pH at 5.0, with Pitched Blade impeller and 150 rpm agitation; maintenance of pH at 5.0, with Pitched Blade impeller and 300 rpm agitation.

Cellulase activity determination

For the determination of cellulases activity in paper filter (FPase), we used Whatman n° 1 analytical paper as substrate in the reaction with crude enzymatic extract [26]. The supernatant obtained from the cultures (crude enzyme extract) was used as a cellulase enzyme source and mixed with a strip (50 mg, 1 x 6 cm) of Whatman nº 1 paper absorbed in 1 mL of citrate buffer of sodium (0.05 M), pH 4.8. After the incubation period, 1 hour at 50 ° C, aliquots were collected for analysis of total reducing sugars generated in the reaction.

The enzymatic activities were determined by quantifying the reducing sugars released in the reaction, using the DNS method [27], being glucose the standard. The spectrophotometer was read at 540 nm. Activities were reported as FPU/L (FPase). In all experiments, three replicates of each experiment were adopted and the results were compared through Tukey's statistical method, at 5% significance level.

Characterization of lignocellulosic material

BP was characterized in terms of the percentage of lignocellulosic in mass of fractions (cellulose, ash, extractives, hemicellulose, lignin and moisture) [28, 29].

Determination of the cellulose content and dry biomass of the fungus

The residual cellulose and dry biomass content of the cultures was measured according to the procedure described by Ahamed & Vermette [30].

Results

Screening of cellulase producers

The strain CMIAT 055 strain stood out among the others, with an enzymatic activity of 424.7 FPU/L, and 42.9% lower than the profile observed by *T. reseei* RUT C30 (743.9 FPU/L).

Morphological and molecular identification of CMIAT 055

The strain CMIAT 055 was identified by Embrapa Agrobiologia as *Talaromyces amestolkiae* and was included in the collection at the DNA Data Bank of Japan (DDBJ) Center (National Institute of Genetics Research Organization of Information and Systems, Mishima, Shizuoka, Japan). The ITS and BenA sequences of the fungus were deposited with the respective access codes KX827631 and KX827632.

Both, ITS and β-tubulin phylogeny placed CMIAT 055 strain together with others *Talaromyces* species and the closest species for both genes was *T. amestolkiae*. We also have analyzed the ITS and β-tubulin concatenated and again CMIAT 055 was placed within the species *T. amestolkiae* with high boostrap values (Figure 1). Therefore, based on molecular analyses we concluded that CMIAT 055 belongs to *T. amestolkiae* [31]. The ITS and BenA sequences of the fungus were deposited in DNA DataBank of Japan (DDBJ) with the respective access codes KX827631 and KX827632.

Optimization of the culture medium

Determination of important variables for FPase production by Plackett–Burman design

The most significant components of the culture medium in the enzymatic synthesis were selected by Plackett-Burman Design. As indicated in Table 1, total cellulase activity (FPase) ranged from 39.2 FPU/L to 793.8 FPU/L.

Relevant variables in enzyme synthesis were based on p value < 0.05 at the 95% confidence level and on Student's T-test (Table 3). The components BP (p = 0.00), $CaCl_2.2H_2O$ (p = 0.00), KH_2PO_4 (p = 0.00), urea (p = 0.01) and $(NH_4)2SO_4$ (p = 0.02) had a positive effect on increasing the enzymatic activity of FPase, while sucrose, peptone and yeast extract had a negative effect. Despite $MgSO_4.7H_2O$, having a negative effect, it was not significant in the response. As pointed out by Gunny et al. [32], parameters with negative effects suggest that, even in minimal amounts, such components could have a deleterious effect on cellulase synthesis.

Table 3 - Estimate of PBD effects for FPase activity

	Effects	Standard error	*t* (5)	*p*-value
Mean	197.767	3.830753	51.6261	0.000375*
BP (x_1)	229.65	8.565824	26.8100	0.001388*
Sucrose (x_2)	-254.983	8.565824	-29.7675	0.001127*
Urea (x_3)	73.617	8.565824	8.5942	0.013270*
Peptone (x_4)	-196.383	8.565824	-22.9264	0.001897*
Yeast Extract (x_5)	-51.817	8.565824	-6.0492	0.026256*
$(NH_4)_2SO_4$ (x_6)	55.783	8.565824	6.5123	0.022777*
KH_2PO_4 (x_7)	178.017	8.565824	20.7822	0.002307*
$MgSO_4.7H_2O$ (x_8)	-11.550	8.565824	-1.3484	0.309939
$CaCl_2.2H_2O$ (x_9)	187.550	8.565824	21.8952	0.002079*
* significant $p<0.05$; R^2 = 0.948 e R^2_{ajust} = 0.855. BP = banana pseudostem.				

Test 1 showed the highest FPAse determinations (793.8 FPU/L). In that experimental condition, the concentrations of the variables BP, urea, KH_2PO_4, $MgSO_4.7H_2O$, and $CaCl_2.2H_2O$ were kept in the upper level (+1), while the variables sucrose, peptone, yeast extract, and $(NH_4)_2SO_4$ were kept in the lower level (-1). It is relevant to point out that FPase production dropped by 95.0% in test 2 (39.2 FPU/L) when sucrose and peptone concentrations were used at higher levels and urea and KH2PO4 at lower levels. However, test 5 showed similar results (FPase 41.0 FPU/L) to test 2, performed at the highest level of KH_2PO_4.

Although researchers report that higher levels of this salt improve cellulase synthesis [33], it was not possible to observe a direct relationship between greater total cellulase synthesis and conditions in which KH_2PO_4 was present in greater amounts in the culture medium. This fact emphasizes the importance of verifying the interaction between carbon and nitrogen sources, in addition to micronutrients that contribute to the construction of the enzyme complex.

PBD demonstrated the need to maintain some components of the culture medium at higher levels, in order to enhance FPase synthesis. In contrast, the test also showed that certain components of the medium should be omitted or kept at lower levels to ensure performance of enzyme production. Thus, for the conduction of DCCR, sucrose was fixed at the lower level (5.0 g/L) and the negative factors, peptone, yeast extract, and $MgSO_4.7H_2O$ were suppressed in the composition of the medium. The $MgSO_4.7H_2O$ factor was not significant in the production of FPase. The model's robustness for FPase production was preserved, as the R^2 and R^2_{ajust} still remained high (0.947 and 0.878, respectively), even after $MgSO_4.7H_2O$ was promptly omitted from the statistical routine.

It should be noted that, although the effect of $(NH_4)2SO_4$ was positive in the production of FPase, it was decided to conduct the DCCR with only one nitrogen source (urea). The decision to remove this variable was based on a few points: the removal of (NH4)2SO4 did not compromise the accuracy of the model, as the values of R^2 and R^2_{ajust} remained equally high, with R^2 = 0.935 and R^2_{ajust} = 0.850 for FPase; the impact of the urea variable was greater in the production of enzymes and the use of urea was economically more advantageous for the enzyme production process.

Some works report similarities with the results obtained in the present study regarding the maintenance of these components in the synthesis of cellulases by submerged fermentation. Matkar et al. [25] identified the need for maintenance of $CaCl_2$ after the element is selected by Plackett-Burman. In DCCR analysis, the effect also showed that high concentrations of calcium chloride favored the production of FPase and endoglucanases by *Aspergillus*. Jung et al. [24], in studies with the fungus of the genus *Penicillium* concluded that only urea and ammonium sulfate were considered significant variables in the analysis by BPD, with endoglucanase activity 860 U/L. Fungi of this genus have genetic similarities with the genus *Talaromyces*, since this is considered a sexual metamorphosis of the genus *Penicillium* [34].

Therefore, the variables BP, $CaCl_2.2H_2O$, KH_2PO_4, and urea were selected for the DCCR, and their respective experimental ranges adjusted and expanded. Thus, the following concentrations of the variables for DCCR were established: BP from 0 to 80 g/L, $CaCl_2.2H_2O$ from 0 to 4.8 g/L, KH_2PO4 from 0 to 12 g/L, and urea from 0 to 4.8 g/L.

Central Composite Rotational Design

The previous results established the experimental conditions for the optimization step, so the DCCR included as independent variables: BP, $CaCl_2.2H_2O$, KH_2PO_4, and urea. The DCCR was composed of three replicas at the central point and eight axial points (2^4 plus the star-type configuration, totaling 27 experiments).

The purpose of conducting the DCCR was to obtain a second-order model for the production of total cellulases (response or dependent variables) as a function of the investigated variables (independent variables) (Table 2).

FPase determinations ranged from 2.7 to 1032.4 FPU/L. The little variation of the enzyme determinations at the central points used to estimate the statistical design error suggest the reproducibility of the experimental data. Test 20 showed the most suitable composition for FPase production (1032.4 FPU/L), within the experimental ranges previously established in the PBD.

There was an increase in the FPase analysis of 71.6 and 63.83%, respectively, in tests performed with 10.0 and 40.0 g/L of BP. Therefore, the need for lignocellulosic material as an inducer for cellulase synthesis was observed. At higher concentrations (test 18, 60 g/L of BP), it was harmful to FPase production, possibly due to the increase in the viscosity of the medium and compromised mass transfer during the fermentation process, as observed in a study with wheat bran by Han et al. [35]. These authors observed, in a study of cellulase production under submerged fermentation with T. *reesei*, that lignocellulosic material at higher concentrations had a negative effect on cellulase production.

It is important to highlight that test 17, whose composition of the culture medium did not contain lignocellulosic material (lower axial point), showed negligible enzymatic activity. The activity of 2.7 FPU/L for FPase was possibly related to the enzyme fractions present in the fungal spore suspension that was used as an inoculum.

Using regression analysis, second-order equations were proposed for the production of total cellulases. Only the significant independent variables ($p<0.05$) were considered in the equations:

$$FPase = 190.05 - 51.48\,x_1 - 34.31\,x_1^2 + 55.43x_2 + 180.05\,x_2^2 + 43.53\,x_3^2 + 121.87\,x_1x_4 - 31.65\,x_2x_3 - 34.56\,x_2x_4 \quad (3)$$

Where x_1, x_2, x_3 and x_4 are the coded values for the concentrations of BP, $CaCl_2.2H_2O$, KH_2PO_4 and urea, respectively. The experimental data were also submitted to Analysis of Variance (ANOVA) and the validity of the models was attested by the *F* Test (Table 4), comparing the F values of the models with the tabulated F values.

Table 4 - Analysis of variance (ANOVA) for FPase response

	Sum of square	Degrees of freedom	Mean square	F_{calc}
Regression	1354824.0	8	169353.0	40.25[a]
Residue	75739.0	18	4207.72	
Lack of fit	75084,0	16	4692.75	14.33[b]
Pure error	655.0	2	327.5	
Total	1430563.0	26		

R^2: 0.95306; R^2_{ajust}: 0.89829; $F_{0.05,\,8.18}$: 2.51; $F_{0.05,16.2}$: 19.43. [a] F_{calc} (regression/residue). [b] F_{calc} (*lack of fit*/pure error).

In the analysis of variance, only significant parameters were taken into account, in which the p value was below 0.05, and the non-significant factors were added to the lack of adjustment. The model for the production of FPase presented a highly significant correlation coefficient (R^2), managing to explain around 95.0% of the total variation around the mean. The F test for regression (40.25) showed good performance (higher than the F tabulated) and the lack of fit (14.33) was not significant. The equation 2 was used to generate the response surface graphs, to better understand the optimization of the four constituents of the culture medium (Figures 2a, 2b, and 2c).

By analyzing Figure 2a, which shows the interaction between lignocellulosic material and urea, it is evident that higher FPase values (dark red color of the graph) occurred when BP concentrations ranged from 0 to 30 g/L and urea from 0 to 1 g/L. It was also observed that FPase activity was inhibited when higher concentrations of urea were used. On the other hand, by analyzing the response surface graph of the interaction between the two salts ($CaCl_2.2H_2O$ and KH_2PO_4) (Figure 2b), it was evident that larger amounts of these components are important for cellulase expression in the culture medium. The same can be seen in Figure 2c, which showed the interaction between urea and $CaCl_2.2H_2O$.

Given the above, the concentrations of the components of the culture medium, for the next step, were defined by calculating the critical values of each independent variable. The procedure indicates which are the ideal concentrations of each component in the medium to obtain the best yield in FPase synthesis. Furthermore, this procedure was essential, since saturation of the culture medium may occur through an increase in the concentration of solid material. This trend can be seen in test 18, where the FPase activity was only 57.0 FPU/L. In this experimental condition, the concentration of BP was 80 g/L, while the concentrations of $CaCl_2$, KH_2PO_4 and urea were maintained, respectively, at 2.4, 6.0 and 2.4 g/L. Therefore, the culture medium for the bioreactor tests was defined as follows: 41.9 g/L of BP; 2.26 g/L of $CaCl_2$; 5.96 g/L of KH_2PO_4 and 2.98 g/L of urea.

However, it has not yet been possible to confirm whether there is a unique relationship between biomass concentration and enzyme expression, reinforcing the need to evaluate other process parameters, in order to study more accurately the relationship between cell growth and activity enzymatic. Zheng et al. [36], in a work on the effects of agitation in submerged fermentation for enzyme production by *Aspergillus oryzae*, concluded that the reduction in enzyme synthesis did not occur due to a decrease in the growth rate of the fungus. This conclusion was reached out after studies with other parameters, such as microorganism fragmentation and alteration of its morphology due to the configuration of the agitation system.

One of the parameters that possibly altered the synthesis of total cellulases was the acidification of the broth fermented by *T. amestolkiae* CMIAT 055, one of the causes for lower cellulase production without maintaining the pH. In 72 hours of the fermentation process, there was a sharp decrease in pH, from 4.2 to 2.6, which coincides with the lower synthesis of total cellulases, a significant decrease in the amount of biomass from 72 hours onwards, denoting a lower capacity of the microorganism to develop in crops with lower pH. Consequently, there was also a lower consumption of the substrate, since it was also evidenced by the lower fungal cell growth. The final pH of the process was 1.6 at 168 h.

In the second experiment (Figure 4), the entire fermentation process was maintained at pH 5, Rushton impeller, with no decline in the activity of both enzymes after 168 hours, in contrast to what occurred in the first test. It was also observed that the pH control provided greater consumption of cellulose, as a result of greater maintenance of microbial biomass. The degradation of cellulosic material started in the first 24 hours when there was a decrease in the amount of material by 8.24 g.

With constant pH values throughout the fermentation process, an increase in production was observed in both enzymatic analyses, with production growth until the end of the process. At 168 hours it was considered the maximum production of both enzymes with an activity of 702.9 ± 1.98 FPU/L. Li et al. [37] presented, in research on the effect of pH on the production of cellulases and morphology of the fungus *Trichoderma reesei*, results similar to those of the present work. Although the FPase activity remained increasing after 72 hours, as it is a genetically modified fungus and therefore more stable to extreme acidity conditions, the production of total cellulases was higher in the batch with pH control.

A third fermentation was carried out, with pH maintained at 5.0 throughout the process, but with a Pitched blade impeller (Figure 5), in order to verify the behavior of the microorganism's growth and cellulase synthesis using this type of stirrer. The first test was performed with 150 rpm orbital agitation. With the use of a Pitched blade impeller, greater enzyme synthesis was observed in the first 24 hours of the fermentation process, with the production of 172.1 FPU/L in the fermentation with this stirrer and 140.6 FPU/L in a stirred system with type Rushton.

In stirred tank reactors, the impellers are the main part of the reactor that determines the effects of the mixture. Therefore, the proper selection of impellers in submerged fermentation processes is extremely important to ensure higher productivity, since the impeller geometry used influences the hydrodynamics of the fermented juice, oxygen transfer, and the morphology of microorganisms [38]. The most conventional turbine used in bioreactors is the Rushton type, which has the characteristic of transmitting a radial flow to the liquid medium. However, its major disadvantage is the high degree of shear caused by the tips of the blades, accumulation of air behind the blades, as well as the flow that pushes the cultured medium to the walls of the reactor vessel [39].

The less accentuated decrease in biomass production of *T. amestolkiae* CMIAT 055 observed in fermentation conducted with *Pitched blade*, even if subtle in comparison to the use of Rushton impeller, may denote that this profile of greater oxygen dispersion has favored greater growth of the microorganism. While in the process with Rushton agitator the biomass remained

in decline until the end of the process, on the other hand, with the use of *Pitched blade*, the number of microorganisms remained close between 120 h and 168 h, which may have favored higher FPase activities (845.0 FPU/L) after the fermentation with this type of impeller is finished.

Therefore, it should be considered that all fermentation processes studied in this work remained standardized in the following configurations: a) the same culture broth volume, obeying a height standard of the reaction volume; b) obviously, the same vats configurations were used in all experiments; c) the turbines, used in pairs in all tests in bioreactors, were equidistantly fixed on the central axis, following the manufacturer's suggestion and taking into account the volume of culture medium adopted; d) the amount of insoluble solid material also remained standard in all tests.

The particularities of the culture medium used in the experiments, with insoluble particles of banana pseudostem suspended in the liquid medium, must also be considered. In general, and depending on the characteristics of the material to be mixed, the radial flow pattern can result in reduced pumpability as well as increased shear rate. Such radial flow configurations were possibly not favorable for maintaining the banana pseudostem particles in suspension homogeneously in the culture medium. Flow patterns through axial impellers are more suitable for the suspension of solids in fermentation broth [40].

Given the above, in the following experiment (Figure 6) the *Pitched blade* impeller was used, maintaining pH 5.0 throughout the fermentation process and the rotation speed at 300 rpm.

If the distribution of a substrate in a non-homogeneous way is observed in a fermentation process, especially in the use of materials that do not dissolve in the liquid medium, one of the main strategies to be adopted is to adjust the intensity of agitation. Some authors claim that cellulolytic enzymes tend to lose enzymatic activity as there is an increase in the agitation speed in the liquid medium in stirred tank bioreactors, mainly due to higher shear forces [41].

The morphology of fungus cultures is dependent on the number of shear forces applied to the culture medium. Therefore, if high rates are applied, the microbial morphology can be impaired in the formation and maintenance of hyphae, as well as causing fewer cell clusters [42]. On the other hand, this statement is dependent on the type of filamentous fungus being investigated, considering that the formation of hyphae increases the viscosity of the fermented broth and makes oxygen transfer difficult, for example, in cultures with *Trichoderma reesei* [43].

This possibly explains the fact that increasing the rotational speed from 150 rpm to 300 rpm was beneficial for obtaining higher FPase activities (2172.8 FPU/L) on 168 hours, where there were the highest enzymatic activities in the third and fourth experiments, with a 3.15-fold increase in FPase activity and 1.8-fold increase in endoglucanases production.

Conclusion

In this study, the potential of cellulase production by *T. amestolkiae* CMIAT055 was investigated, in different compositions of the culture medium and configurations of the agitation system in a bioreactor. The results showed that *T. amestolkiae* was able to produce enzymes from the cellulolytic complex using banana pseudostem, an unconventional, low-cost, and abundant source, as an untreated inducer. The analysis by DCCR showed that the optimized medium for the production of cellulases presented the following composition: 41.9 g/L of BP; 2.26 g/L of $CaCl_2$; 5.96 g/L of KH_2PO_4; and 2.98 g/L of urea. Based on the results, it is possible to conclude that the banana pseudostem presented itself as a potential source of inducing cellulose for the synthesis of cellulases by *T. amestolkiae* CMIAT 055, since it favored an enzyme production around 2.4 times higher if compared to the fermentation process using microcrystalline cellulose. In bioreactors tests, the maintenance of pH 5.0 throughout the fermentation process, as well as the use of the *Pitched Blade* impeller and rotation at 300 rpm, favored the synthesis of cellulolytic enzymes, whose maximum activities presented were 2642.5 FPU/L (FPase), on 168 hours of conducting the fermentation process.

Declarations

Acknowledgments

The authors acknowledge financial support by the CAPES (Coordination for the Improvement of Higher Education Personnel. Brazil), CNPq (National Council for Scientific and Technological Development. Brazil), and FUNCAP (Cearense Foundation for Support for Scientific and Technological Development). Also, authors would like to thank technical assistance received from Embrapa Agroindustry Tropical.

References

1. Juturu, V., Wu, J.C.: Microbial cellulases: Engineering, production and applications. Renew. Sustain. Energy Rev. 33, 188–203 (2014). https://doi.org/10.1016/j.rser.2014.01.077
2. Siqueira, J.G.W., Rodrigues, C., Vandenberghe, L.P. de S., Woiciechowski, A.L., Soccol, C.R.: Current advances in on-site cellulase production and application on lignocellulosic biomass conversion to biofuels: A review. Biomass and Bioenergy. 132, 105419 (2020). https://doi.org/10.1016/j.biombioe.2019.105419
3. Schuster, A., Schmoll, M.: Biology and biotechnology of *Trichoderma*. Appl. Microbiol. Biotechnol. 87, 787–799 (2010). https://doi.org/10.1007/s00253-010-2632-1
4. Maheshwari, R., Bharadwaj, G., Bhat, M.K.: Thermophilic fungi: their physiology and enzymes. Microbiol. Mol. Biol. Rev. 64, 461–88 (2000)
5. Orencio-Trejo, M., Torres-Granados, J., Rangel-Lara, A., Beltrán-Guerrero, E., García-Aguilar, S., Moss-Acosta, C., Valenzuela-Soto, H., De la Torre-Zavala, S., Gastelum-Arellanez, A., Martinez, A., Tiessen, A., Diaz-Mireles, E., Lozoya-Gloria, E.: Cellulase and xylanase production by the mexican strain *Talaromyces stollii* LV186 and its application in the saccharification of pretreated corn and sorghum stover. BioEnergy Res. 9, 1034–1045 (2016). https://doi.org/10.1007/s12155-016-9791-6
6. Fujii, T., Hoshino, T., Inoue, H., Yano, S.: Taxonomic revision of the cellulose-degrading fungus *Acremonium cellulolyticus* nomen nudum to *Talaromyces* based on phylogenetic analysis. FEMS Microbiol. Lett. 351, 32–41 (2014). https://doi.org/10.1111/1574-6968.12352
7. Gusakov, A. V: Alternatives to *Trichoderma reesei* in biofuel production. Trends Biotechnol. 29, 419–25 (2011). https://doi.org/10.1016/j.tibtech.2011.04.004
8. Nieto-Domínguez, M., de Eugenio, L.I., Barriuso, J., Prieto, A., Fernández de Toro, B., Canales-Mayordomo, Á., Martínez, M.J.: Novel pH-Stable glycoside hydrolase family 3 β-Xylosidase from *Talaromyces amestolkiae*: an enzyme displaying regioselective transxylosylation. Appl. Environ. Microbiol. 81, 6380–6392 (2015). https://doi.org/10.1128/AEM.01744-15
9. Srivastava, N., Srivastava, M., Mishra, P.K., Gupta, V.K., Molina, G., Rodriguez-Couto, S., Manikanta, A., Ramteke, P.W.: Applications of fungal cellulases in biofuel production: Advances and limitations. Renew. Sustain. Energy Rev. 82, 2379–2386 (2018). https://doi.org/10.1016/j.rser.2017.08.074
10. Mandels, M., Reese, E.T.: Induction of cellulase in *Trichoderma viride* as influenced by carbon sources and metals. J. Bacteriol. 73, 269–278 (1957). https://doi.org/10.1128/JB.73.2.269-278.1957
11. Mandels, Mary; Weber, J.: The production of cellulases. In: Cellulases and Their Applications. pp. 391–414. American Chemical Society (1969)
12. Adekunle, A., Orsat, V., Raghavan, V.: Lignocellulosic bioethanol: A review and design conceptualization study of production from cassava peels. Renew. Sustain. Energy Rev. 64, 518–530 (2016). https://doi.org/10.1016/j.rser.2016.06.064
13. Kuhad, R.C., Deswal, D., Sharma, S., Bhattacharya, A., Jain, K.K., Kaur, A., Pletschke, B.I., Singh, A., Karp, M.: Revisiting cellulase production and redefining current strategies based on major challenges. Renew. Sustain. Energy Rev. 55, 249–272 (2016). https://doi.org/10.1016/j.rser.2015.10.132
14. Folan, M.A., Michael, P.: The saccharifying ability of the cellulase complex of *Talaromyces emersonii* and comparison with that of other fungal species. Int. J. Biochem. 10, 505–510 (1979). https://doi.org/doi.org/10.1016/0020-711X(79)90006-5
15. Moloney, A.P., Considine, P.J., Coughlan, M.P.: Cellulose hydrolysis by the cellulases produced by *Talaromyces emersonii* when grown on different inducing substrates. Biotechnol. Bioeng. 25, 1169–1173 (1983)
16. Mallek-Fakhfakh, H., Fakhfakh, J., Masmoudi, N., Fatma, R., Gargouri, A., Belghith, H.: Agricultural wastes as substrates for β-glucosidase production by *Talaromyces thermophilus*: Role of these enzymes in enhancing waste paper saccharification.

Prep. Biochem. Biotechnol. 47, 414–423 (2017). https://doi.org/http://dx.doi.org/10.1080/10826068.2016.1252928

17. Arora, S., Rani, R., Ghosh, S.: Bioreactors in solid state fermentation technology: Design, applications and engineering aspects. J. Biotechnol. 269, 16–34 (2018). https://doi.org/10.1016/j.jbiotec.2018.01.010
18. Ghobadi, N., Ogino, C., Yamabe, K., Ohmura, N.: Characterizations of the submerged fermentation of *Aspergillus oryzae* using a Fullzone impeller in a stirred tank bioreactor. J. Biosci. Bioeng. 123, 101–108 (2017). https://doi.org/10.1016/j.jbiosc.2016.07.001
19. AOAC: Official Methods of Analysis of the Association of Official Analytical Chemists. Association Of Official Analytical Chemists Inc (1990)
20. Kornerup, Andreas; Wanscher, J.H.: Methuen Handbook of Colour. (1967)
21. Gary C. Glass, N.L.D.: Development of primer sets designed for use with the pcr to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol. 61, 1323–1330 (1995)
22. Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S.: MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30, 2725–2729 (2013). https://doi.org/10.1093/molbev/mst197
23. Visagie, C.M., Houbraken, J., Frisvad, J.C., Hong, S.-B., Klaassen, C.H.W., Perrone, G., Seifert, K.A., Varga, J., Yaguchi, T., Samson, R.A.: Identification and nomenclature of the genus *Penicillium*. Stud. Mycol. 78, 343–371 (2014). https://doi.org/10.1016/j.simyco.2014.09.001
24. Jung, D.U., Yoo, H.Y., Kim, S.B., Lee, J.H., Park, C., Kim, S.W.: Optimization of medium composition for enhanced cellulase production by mutant *Penicillium brasilianum* KUEB15 using statistical method. J. Ind. Eng. Chem. 25, 145–150 (2015). https://doi.org/10.1016/j.jiec.2014.10.026
25. Matkar, K., Chapla, D., Divecha, J., Nighojkar, A., Madamwar, D.: Production of cellulase by a newly isolated strain of *Aspergillus sydowii* and its optimization under submerged fermentation. Int. Biodeterior. Biodegradation. 78, 24–33 (2013). https://doi.org/10.1016/j.ibiod.2012.12.002
26. Ghose, T.K.: Measurement of cellulase activities. Pure Appl. Chem. 59, 257–268 (1987)
27. Miller, G.L.: Use of dinitrosalicylic acid reagent for the determination of reducing sugar. Anal. Chem. 31., 426–428 (1959)
28. Arendt, S.A., Bailey, S.J.: Section 7. Textiles 1: D76–D4391. In: Annual Book of ASTM Standards (2005)
29. TAPPI: T 222 om-02. Acid-insoluble lignin in wood and pulp., http://www.tappi.org/content/SARG/T222.pdf
30. Ahamed, A., Vermette, P.: Culture-based strategies to enhance cellulase enzyme production from *Trichoderma reesei* RUT-C30 in bioreactor culture conditions. Biochem. Eng. J. 40, 399–407 (2008). https://doi.org/10.1016/j.bej.2007.11.030
31. Yilmaz, N., Houbraken, J., Hoekstra, E.S., Frisvad, J.C., Visagie, C.M., Samson, R.A.: Delimitation and characterisation of *Talaromyces purpurogenus* and related species. Persoonia - Mol. Phylogeny Evol. Fungi. 29, 39–54 (2012). https://doi.org/10.3767/003158512X659500
32. Gunny, A.A.N., Arbain, D., Jamal, P., Gumba, R.E.: Improvement of halophilic cellulase production from locally isolated fungal strain. Saudi J. Biol. Sci. (2014). https://doi.org/10.1016/j.sjbs.2014.11.021
33. Rodríguez, M.D., Alonso Paiva, I.M., Castrillo, M.L., Zapata, P.D., Villalba, L.L.: KH2PO4 improves cellulase production of *Irpex lacteus* and *Pycnoporus sanguineus*. J. King Saud Univ. - Sci. (2018). https://doi.org/10.1016/j.jksus.2018.07.009
34. Samson, R.A., Pitt, J.I.: The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Mycologia. 73, 582 (1981). https://doi.org/10.2307/3759616
35. Han, X., Song, W., Liu, G., Li, Z., Yang, P., Qu, Y.: Improving cellulase productivity of *Penicillium oxalicum* RE-10 by repeated fed-batch fermentation strategy. Bioresour. Technol. 227, 155–163 (2017). https://doi.org/10.1016/j.biortech.2016.11.079
36. Li, Z.J., Shukla, V., Wenger, K.S., Fordyce, A.P., Pedersen, A.G., Marten, M.R.: Effects of increased impeller power in a production-scale *Aspergillus oryzae* fermentation. Biotechnol. Prog. 18, 437–444 (2002). https://doi.org/10.1021/bp020023c
37. Li, C., Yang, Z., He Can Zhang, R., Zhang, D., Chen, S., Ma, L., Zhang, R.H.C., Zhang, D., Chen, S., Ma, L.: Effect of pH on cellulase production and morphology of *Trichoderma reesei* and the application in cellulosic material hydrolysis. J. Biotechnol. 168, 470–7 (2013). https://doi.org/10.1016/j.jbiotec.2013.10.003

38. Tang, W., Pan, A., Lu, H., Xia, J., Zhuang, Y., Zhang, S., Chu, J., Noorman, H.: Improvement of glucoamylase production using axial impellers with low power consumption and homogeneous mass transfer. Biochem. Eng. J. 99, 167–176 (2015). https://doi.org/10.1016/j.bej.2015.03.025
39. Buffo, M.M.M., Corrêa, L.J.J., Esperança, M.N.N., Cruz, A.J.G.J.G., Farinas, C.S.S., Badino, A.C.C.: Influence of dual-impeller type and configuration on oxygen transfer, power consumption, and shear rate in a stirred tank bioreactor. Biochem. Eng. J. 114, 130–139 (2016). https://doi.org/10.1016/j.bej.2016.07.003
40. Villadsen, J., Nielsen, J., Lidén, G.: Scale-Up of Bioprocesses. In: Bioreaction Engineering Principles. pp. 497–546. Springer US, Boston, MA (2011)
41. Ganesh, K., Joshi, J.B., Sawant, S.B.: Cellulase deactivation in a stirred reactor. Biochem. Eng. J. 4, 137–141 (2000). https://doi.org/10.1016/S1369-703X(99)00045-5
42. Krull, R., Wucherpfennig, T., Esfandabadi, M.E., Walisko, R., Melzer, G., Hempel, D.C., Kampen, I., Kwade, A., Wittmann, C.: Characterization and control of fungal morphology for improved production performance in biotechnology. J. Biotechnol. 163, 112–123 (2013). https://doi.org/10.1016/j.jbiotec.2012.06.024
43. Gabelle, J.-C., Jourdier, E., Licht, R.B., Ben Chaabane, F., Henaut, I., Morchain, J., Augier, F.: Impact of rheology on the mass transfer coefficient during the growth phase of *Trichoderma reesei* in stirred bioreactors. Chem. Eng. Sci. 75, 408–417 (2012). https://doi.org/10.1016/j.ces.2012.03.053

Figures

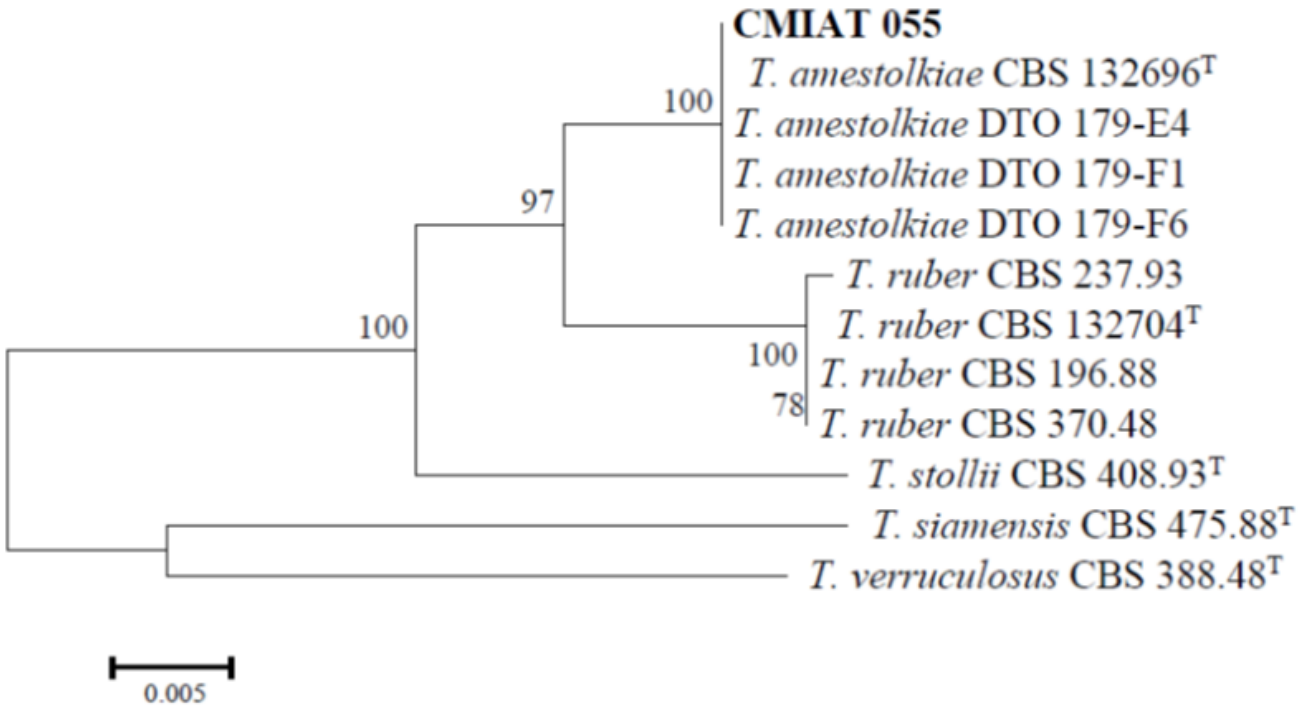


Figure 1

Neighbour-joining phylogenies of concatenated genes ITS+β-tubulin showing phylogenetic placement of CMIAT 055 (in bold). The branch numbers represent bootstrap test values (1000 replicates), where only values > 50% have been reported. T = ex type. Model selected Tamura 2-parameter.

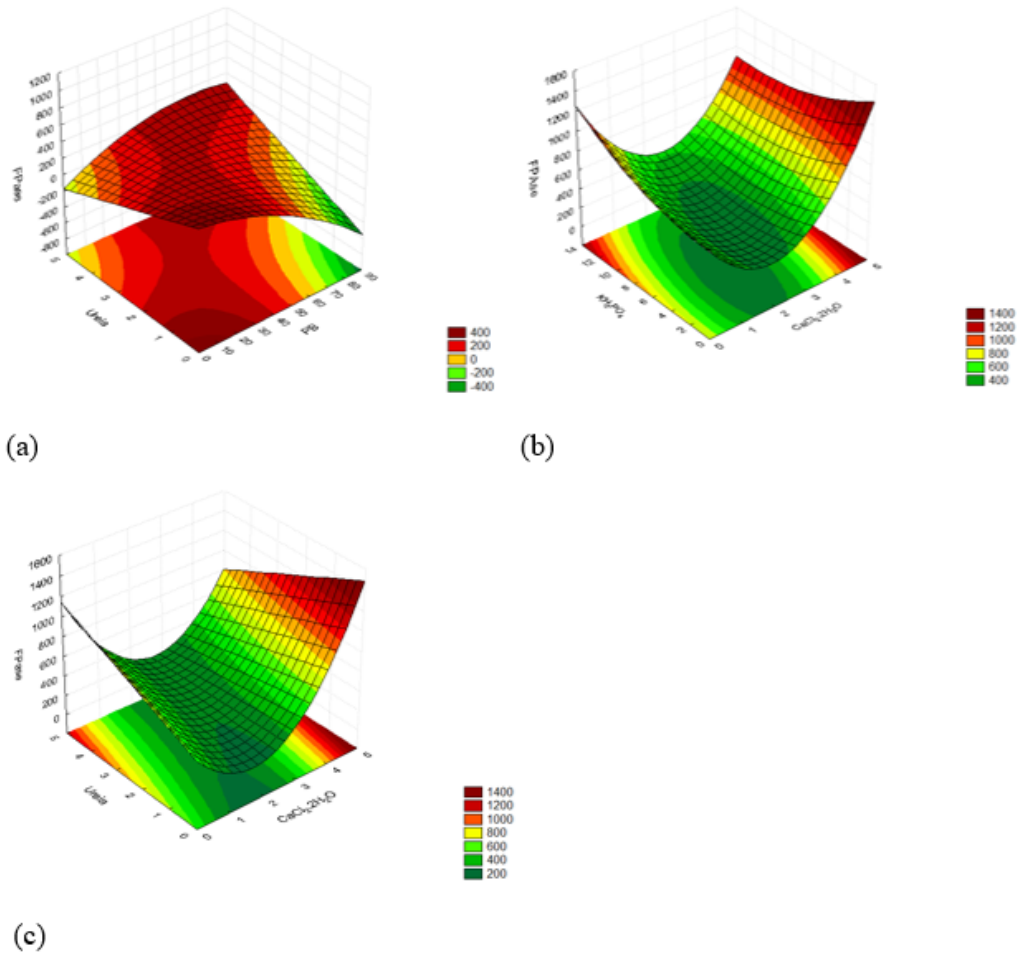
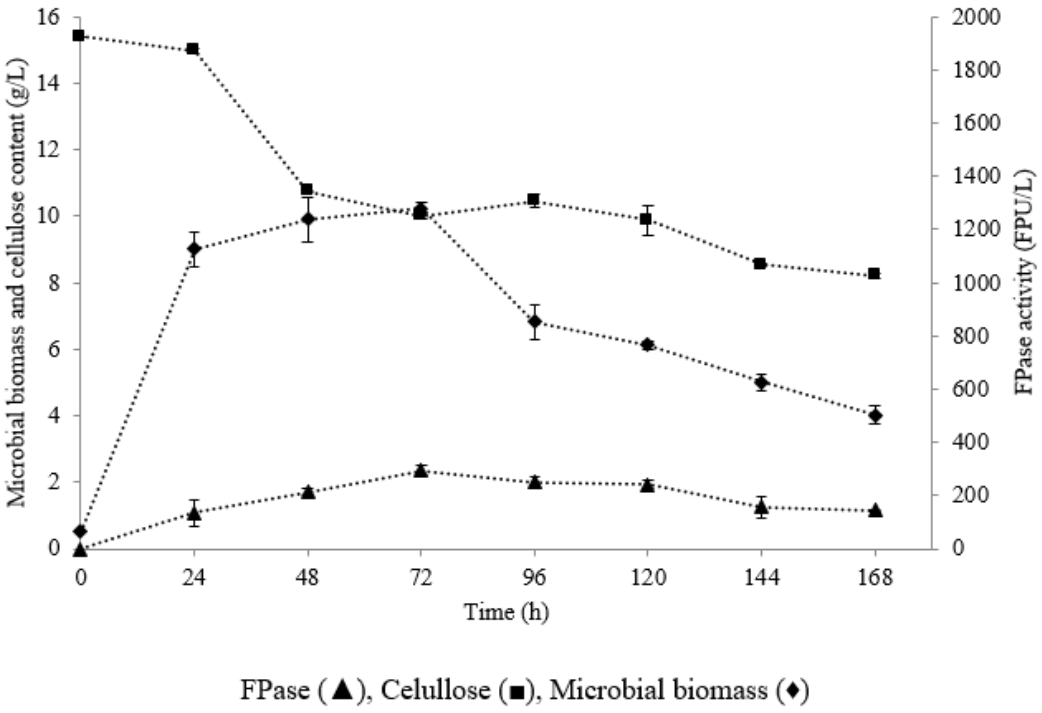


Figure 2

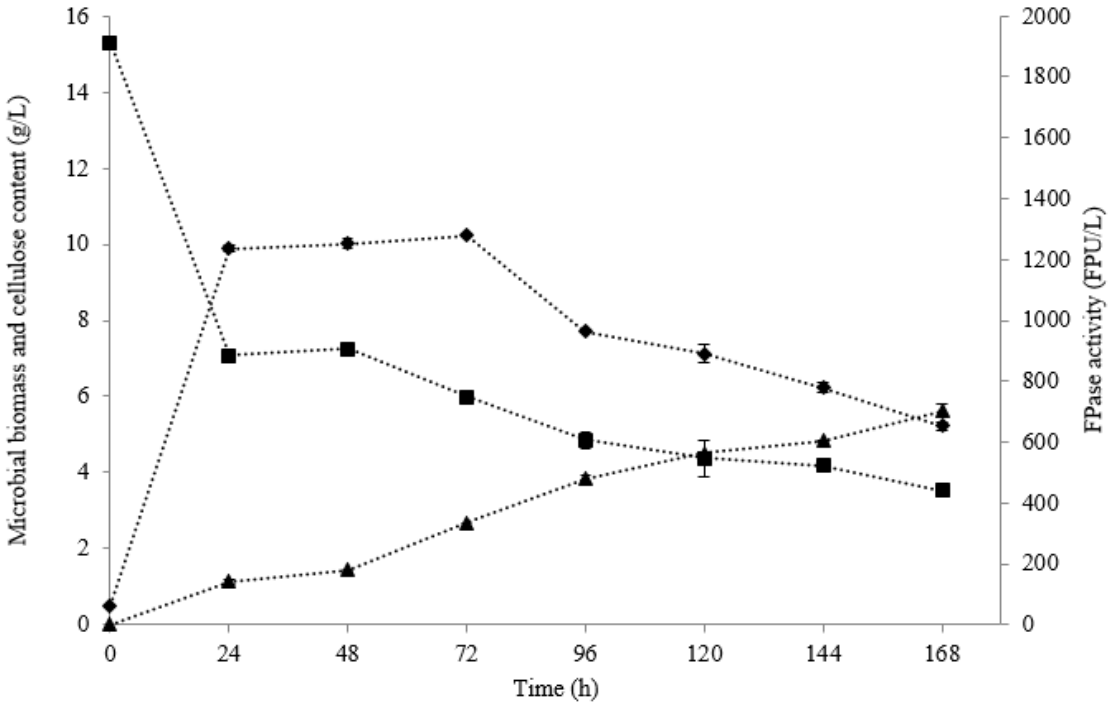
Response surface plot representing (a) FPase production as a function of BP and urea concentrations, with fixed CaCl2.2H2O (2.4 g/L) and KH2PO4 (6 g/L); (b) FPase production as a function of the concentrations of CaCl2.2H2O and KH2PO4, CP (40 g/L) and urea (2.4 g/L) fixed; (c) FPase production as a function of the concentrations of CaCl2.2H2O and urea, BP (40 g/L) and KH2PO4 (6g/L) fixed.



FPase (▲), Celullose (■), Microbial biomass (♦)

Figure 3

Cellulase production profile without pH control, with Rushton impeller



FPase (▲), Celullose (■), Microbial biomass (♦)

Figure 4

Cellulase production profile with maintenance of pH 5.0, with Rushton impeller

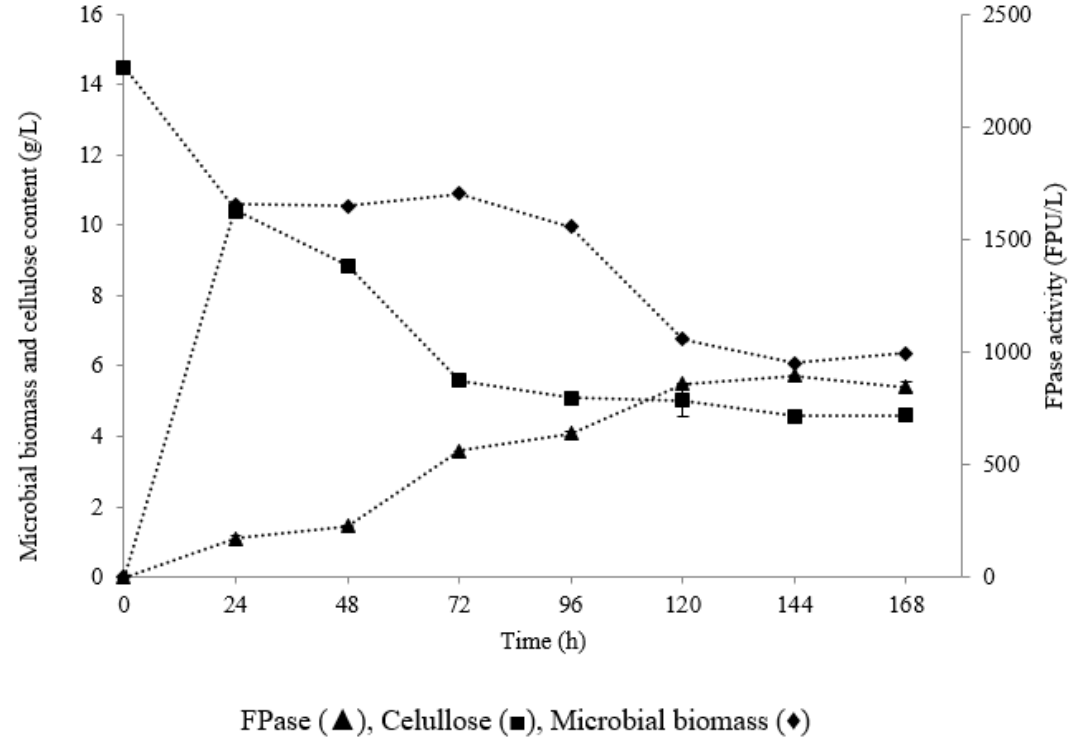


Figure 5

FPase activity profile, biomass production, and cellulose consumption in fermentation using a Pitched blade impeller, 150 rpm.

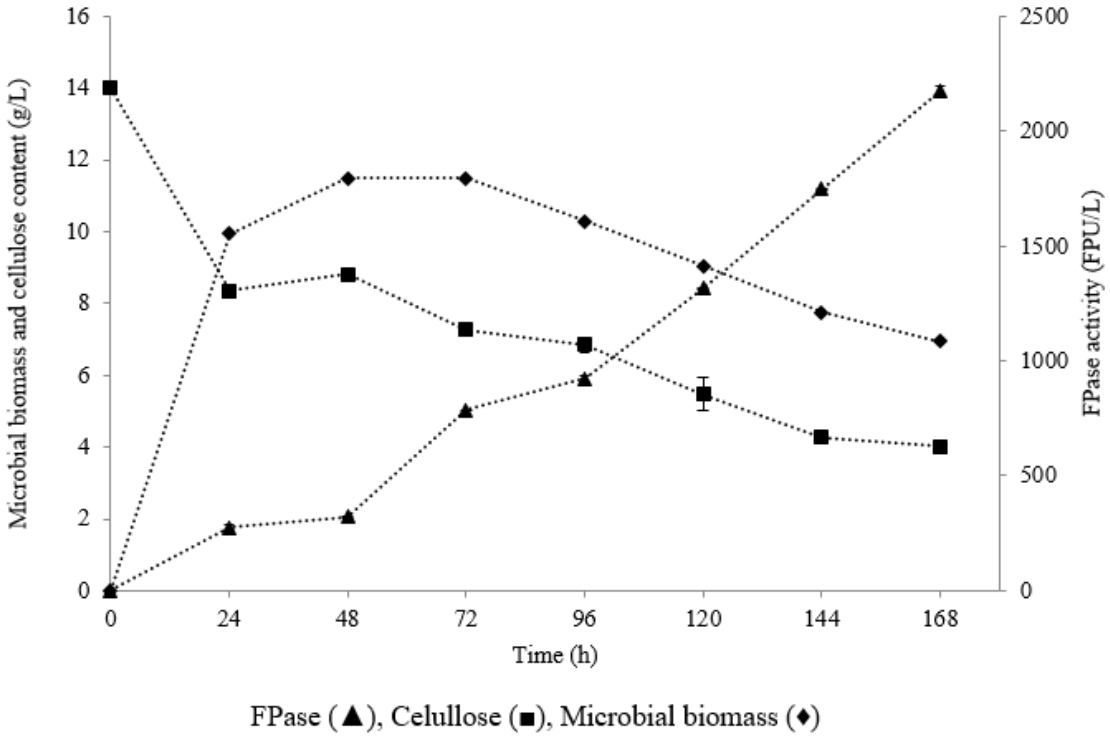


Figure 6

FPase activity profile, biomass production, and cellulose consumption in fermentation using a Pitched blade impeller, 300 rpm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalabstractWB5dejulhode2021.jpg