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Hydrolytic Potential of Cellulases from *Penicillium funiculosum* and *Trichoderma reesei* against Physico-Chemically Different Feedstocks

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Abstract

Competitive alternatives for cellulase producing organism for the development of efficient enzyme mixture should be explored to reduce the cost of commercial cellulases in the enzymatic hydrolysis of lignocellulosic biomass. Cellulolytic enzyme mixtures were produced using *Penicillium funiculosum* and *Trichoderma reesei* on ammonia treated wheat straw. Cellulolytic assay using crude extract obtained from *P. funiculosum* showed 36%, 181% and 370% higher FPase, CMCase and β-glucosidase specific activity compared to *T. reesei.* We compared enzyme mixtures of these two organisms against differently treated wheat straw, ammonia treated rice straw and bagasse. Culture supernatants obtained from both fungi showed equal hydrolytic performance among ammonia treated feedstock. However, *P. funiculosum* enzyme has released nearly 8-10% high sugar from rice straw and bagasse than *T. reesei* enzymes. Pretreatment catalysts like nitric, ammonia, caustic showed around 10% less hydrolysis using *T. reesei* enzyme extract, than *P. funiculosum* derived enzyme extracts. Here, we found that *T. reesei* with lower β-glucosidase causes decreased glucan and xylan hydrolyzing capacity than enzyme extract of P. funiculosum. However, this lower glucan yield is compensated for high cellobiose yield using *T. reesei* derived enzymes. Caustic pretreatment showed higher digestibility irrespective of enzyme source. Finally supplementation of β-glucosidase showed improvement in high glucose release and ultimately hydrolytic performance by more than 20%.

Keywords: Lignocellulosic biomass; Pretreament; Cellulase; *T. reesei; P. funiculosum*

Abbreviations: LBM: Lignocellulosic Biomass; BG: β-glucosidase; FPase: Filter Paper Activity; CMCase: Carboxymethyl Cellulose Activity; Pfu BG: B-Glucosidase Derived from *Pyrococcus furiosus*

Introduction

Lignocellulosic biomass (LBM) is an abundant source of renewable energy. It does not compete with food therefore has tremendous potential to satisfy the current demand of ethanol blending to gasoline [1,2]. LBM is composed of mainly three complex polymers namely cellulose, hemicellulose and lignin and traces of proteins [3]. Differential arrangement of these components with each other governs the biodegradability of plants [4,5]. Ethanol production from LBM requires complete conversion of cellulose and hemicellulose to fermentable monomers. For this, a well designed cocktail of endocellulases, cellobiohydrolases, xylanase and β-glucosidase is required. LBM being recalcitrant in nature requires acids and alkalis assisted pretreatment step to make it amenable to enzymatic hydrolysis using cellulases. Acid treatment facilitates hemicellulose removal and requires only cellulase to degrade cellulose, while alkali treatment retains cellulose and hemicellulose with partial removal of lignin, thus requires both cellulases and hemicellulases for hydrolysis [6].

The cellulolytic system of *Trichoderma reesei* is well established and exploited at commercial level in textile and biofuel industries. However, *T. reesei* produces low amount of extracellular β-glucosidase renders partial hydrolysis of cellulosic material [7,8]. Therefore commercial enzymes are supplemented with β-glucosidase from *Aspergillus niger* [9]. Alternatively, microbes with high β-glucosidase activity will improve sugar yields and this has been discussed widely [10]. Recently cellulases from *Penicillium* species are found to be more potent since they often display better hydrolytic performance at similar enzyme or protein loading in comparison with T. reesei enzymes [11,12]. Therefore production of cellulases using *Penicillium* species must be attempted, since it is known for balanced enzyme system with high β-glucosidase which

in turn depicts great potential of cellulases [13-15]. Though the potential of cellulase produced using *Penicillium* is higher, their protein secretion ability must be improved to replace the *T. reesei*, an undisputed king of cellulase production. Moreover, only one commercial cellulase preparation "Rovabio® Excel" from Adisseo (France) company is available using *P. funiculosum* [16] as against *T. reesei* which is exploited by many commercial companies showed scope for this study. *P. funiculosum* is not well characterized for cellulase production as compared to *T. reesei*, Therefore screening of enzymes obtained across these two strains may proved to be a viable option for establishing improved enzyme mixture. Moreover, to our best knowledge rare studies have been carried out for comparison between *T. reesei* and *P. funiculosum* for cellulases production and its saccharification potential using LBM. However, Van Wyk JPH et al. [17] showed comparative studies using paper products as a substrate for hydrolysis.

The goal of current study is to understand cellulase production using *P. funiculosum* and *T. reesei* by analyzing their hydrolytic potential against physico-chemically different LBMs. Acid and/or base catalysts were used for the preparation of substrates with diverse characteristics. The effect of supplemented β-glucosidase was also analysed for enhancing hydrolytic performance of in house produced cellulases and compared with commercial enzyme CTec2 during saccharification.

Materials and Methods

Substrate preparation for hydrolysis

Agricultural residues like wheat straw, rice straw and bagasse were obtained from the fields of Uttarakhand in Northern India (was supplied by India Glycols Ltd., Uttarakhand, India). These residues were pretreated using alkali and acid. For alkali pretreatment ammonia and caustic, while for acid pretreatment HNO3 were used at different pretreatment conditions (Table 1). The solid contents of differently pretreated substrates were evaluated according to NREL Laboratory Analytical Protocol [18]. The residual solids were rinsed with water to remove the soluble matters like lignin. Pretreated biomass was stored at 4 °C till further use.

Table 1: Preparation of substrates. Wheat straw was sieved through 200um mesh to get uniform size before chemical pretreatments. Reactions were carried out in high pressure stirred reactor provided by Amar equipment Pvt. Ltd.

Pretreament	Temperature (°C)	Pressure (Bar)	Time (minutes)	Glucose (%)	Xylose (%)	Arabinose (%)	Lignin(%)
12.5% NH_3 (AWS)	150	15-18 bar	30	67	20.5	1.04	9.80
12.5% NH_3 (ARS)	150	15-18 bar	30	61.7	22.65	3.15	10.60
12.5% NH_3 (ABG)	150	15-18 bar	30	62.67	19.22	1.15	12.60
10% NaOH (CWS)	120	3-5 bar	30	87.91	5.26	0.44	6.47
2%HNO_3 (NWS)	120	7-8 bar	30	72.00	5.04	0.00	10.32

AWS: Ammonia Treated Wheat Straw; ABG: Ammonia Treated Bagasse; ARS: Ammonia Treated Rice Straw; NWS: Nitrate Treated Wheat Straw; CWS: Caustic Treated Wheat Straw

In-house production of cellulase mixtures

The *P. funiculosum* (NCIM 1228) strain was obtained from National Collection of Industrial Microorganism, National Chemical Laboratory (NCIM-NCL), Pune, India and *T. reesei* QM6a strain was obtained from Lovely professional university (LPU), India. Strains were maintained on Potato dextrose agar (PDA; Hi Media, Mumbai, India) at 28 °C and stored on PDA slants. AWS was used as inducing substrate for the production of cellulases. 1% of AWS was dispensed into 250ml Erlenmeyer flasks, containing 100ml of mineral salt solution (Urea-0.3gm/l, $CaCl_2.2H_2O$-0.4g/l, $MgSO_4.7H_2O$-0.3g/l, $(NH_4)2\ SO_4$-1.4gm/l, KH_2SO_4-2gm/l, Peptone-1g/l, Tween 80-0.2gm/l, $FeSO47H_2O$-5mg/l, $MnSO_47H_2O$-1.6mg/l, $ZnSO_47H_2O$-1.4mg/l, $CoCl_2.6H_2O$-2mg/l. The initial pH was 4.

Flasks with mineral salt solution and carbon source were sterilized using autoclave at 121 °C, 15psi for 20 minutes after sterilization and cooling spores were inoculated from Petri plate to the flasks. The initial pH was adjusted at 4 by adding 5% H_2SO_4. Aeration was provided by shaking at 200rpm to induce enzyme production for period of 5 days. Samples were was centrifuged at 7000rpm for 15 minutes and supernatant were stored at 4 °C for analyzing FPase, CMCase, BG activity and protein concentration.

Enzyme assays

The crude enzyme mixtures obtained were assayed for FPase, CMCase and β-glucosidase activities, as described by the Ghose et al. [19]. Protein concentration was measured using Bio-Rad Protein Assay (Bio-Rad Laboratories, USA) using Bradford assay with BSA as standard. Determination of reducing sugar was done by dinitrosalicylic acid method. The xylanase were assayed using method described by Bailey M et al. [20] using birchwood xylan obtained from Sigma.

Hydrolysis using in-house produced enzyme mixtures

The crude enzyme mixtures obtained from *P. funiculosum* and *T. reesei* using ammonia treated wheat straw (AWS) were used for hydrolyzing ammonia treated wheat straw, bagasse,

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rice straw (AWS, ABG, ARS). In order to study the effect of different pretreatment, nitric and caustic treated wheat straw (NWS, CWS) were also used as a substrate for hydrolysis. Enzyme mixtures obtained here were used in duplicates to hydrolyze above five different type of pretreatment/ substrate combinations at 15FPU/g enzymes loading. To improve the hydrolytic performance, external β-glucosidase (7.5CBU/gm biomass) was added to the in-house produced cellulases. For comparative study, CTec2 (commercial cellulase preparation) was used as a control in this experiment. All the substrates were suspended in the citrate buffer to make 1% and 2.5% slurry for hydrolysis at pH 4.8. Hydrolysis was carried out using 50 °C for 24hrs. Samples were harvested and centrifuged at 8000rpm for 15 minutes.

Analytical methods

Sugar samples obtained after ASTM and enzymatic hydrolysis were analyzed with the help of high performance liquid chromatography (Agilent, India) using refractive index detector. Cellobiose, and monomers like glucose, xylose and arabinose were separated using an Aminex 87-H column (Biorad, Hercules, CA, USA) at 50 °C with 5mM H_2SO_4 as eluent at flow rate of 0.6ml/min.

Result and Discussion

Preparation of substrates

Pretreatment helps in reducing recalcitrance and complexity which facilitates augmentation in hydrolysis. Here, the composition of ammonia pretreated substrates like wheat straw (AWS), rice straw (ARS) and bagasse (ABG) is shown in the Table 1. The major components of these pretreated substrates were glucan (60-70%), followed by xylan (11 to 23%), lignin (8 to 13%) and ash 0.5 to 2%. Soluble components in all the substrates proved to be glucose, xylose and traces of arabinose. The ammonia pretreatment facilitates the partial removal of lignin and retains holocellulose (Hemicellulose + Cellulose) in intact form. This particular pretreatment allows minimal loss of sugar components. Alternatively wheat straw was pretreated with nitric acid and caustic to generate substrates with different physicochemical characteristics. Here we focused on the amenability of these substrates towards cellulases induced by one type (AWS) of substrate.

In-house lignocellulolytic enzyme production and their hydrolytic screening

T. reesei and *P. funiculosum* NCIM 1228 was grown on ammonia pretreated substrates for the production of the lignocellulolytic enzymes to use them in hydrolysis of differently pretreated lignocellulosic biomass. The concentration of substrate used was 1% on dry weight basis.

After successful enzyme production, hydrolytic performance was analyzed using enzyme extracted on different days of fermentation to find out the potential period for culture harvesting. Enzyme activities from *P. funiculosum* on 3rd day showed low β-glucosidase activity (0.02U/ml) but reasonable FPase (0.14U/ml) and high endoglucanase activity (0.47U/ml). The endoglucanase activity remained more or less stable on 5th and 7th day, however as fermentation progresses the FPase and β-glucosidase activity increased to 0.3 and 0.07 (on 5th day), 0.33 and 0.1 (on 7th day). On the other hand, *T. reesei* showed decreasing trends of activities from 3rd, 5th and 7th day for endoglucanase/ml (0.65, 0.44, and 0.37) and β-glucosidase activity (0.06, 0.03, and 0.01). However FPase activity showed highest activities on the 7th day (0.44U/ml) as compared to 3rd (0.37U/ml) and 5th day (0.29U/ml). Therefore, to compare the hydrolytic potential of enzyme extracts, enzymes derived from these organisms were used for hydrolysis of 1% biomass at enzyme loading of 15FPU/g. Increasing hydrolysis trend was observed using *P. funiculosum* enzyme extracts derived on 3rd day (38%), 7th day (44%) and highest hydrolysis was observed using enzyme extracts of 5th day (48%). This trend was exactly reverse for *T. reesei* where highest hydrolysis (35.45%) using enzyme extracts derived on 3rd day as compared to 5th day (31.58%) and 7th day (12.8%) as shown in Figure 1. Therefore, 5th day was found to be suitable based on two aspects, firstly, it showed balanced activity of FPase, endoglucanase and β-glucosidase for both organisms and secondly, it showed sufficient hydrolyzing ability using *T. reesei* and *P. funiculosum*. Furthermore this selection was screened to compare two organisms' potencies for maximum hydrolysis at similar enzyme loading using different substrates.

Table 2: Relation between xylanases and β-glucosidase. Enzyme ratios derived from *T. ressei* and *P. funiculosum.* The data presented are the mean values of two separate measurements. The standard deviation for above mentioned values is less than 10%.

Enzymes	BG: Xylanase	Highest Xylose Release	BG: FPase	Highest Glucose Release
T. reesei	01:00.0	34.50-45.83%	0.068	27.75-38.66%
P. funiculosum	01:00.0	38.00-61.46%	0.232	36.52-44.08%

β-glucosidase; FPase: Filter Paper Activity

Specific activities of the enzymes produced after 5 days of fermentation is shown in Table 2. FPase, CMCase, B-glucosidase used to determine the cellulase activity, and xylanase for hemicellulase activity. There were significant differences in the enzyme titer produced by these two organisms and it showed that different fungi responded differently to the same substrates. Similar observation also reported by Rosa Estela et al. [21]. Protein, FPase and xylanase (0.3mg/ml, 0.44FPU/ml and 72.48U/ml) produced by *T. reesei* is higher than *P. funiculosum* (0.15mg/ml, 0.30FPU/ml and 23.04U/ml). However higher endocellulase and β-glucosidase production by *P. funiculosum* (0.51CMCase/ml and 0.07CBU/ml) as compared to *T. reesei* (0.37CMCase/ml and 0.03CBU/ml) differentiate the production profile of these two organisms on the same inducing substrate. However, *T. reesei* culture showed FPase, CMCase, β-glucosidase specific activity lower than that of the *P. funiculosum*.

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Hydrolytic efficiency of the crude culture

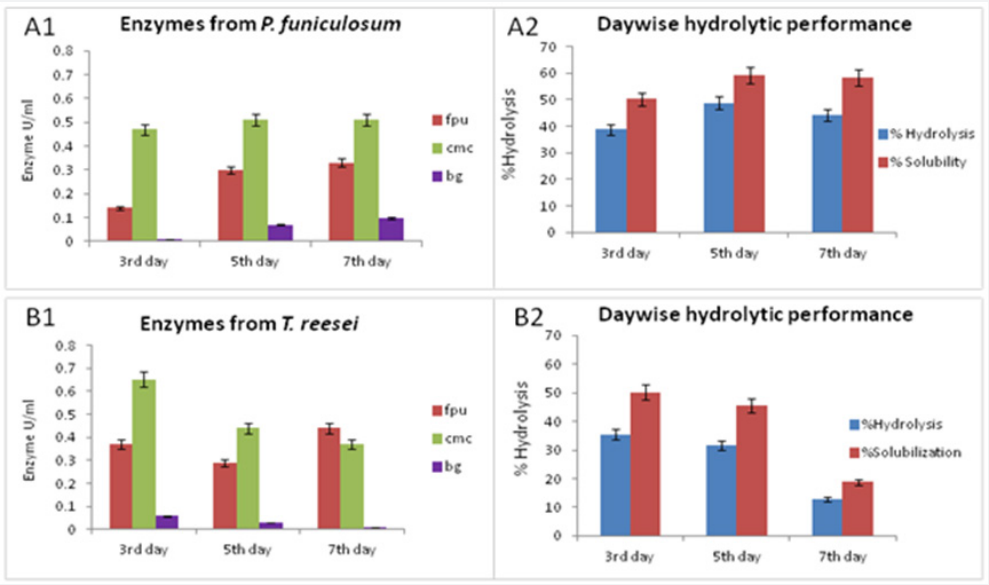

Figure 1: Hydrolytic performance of cellulase extracted at different time points. Extraction of cellulase at different time-points and their hydrolytic performance Enzyme titers (U/ml) derived from *T. reesei* and *P. funiculosum* on different days of fermentation (A1 and B1). Their hydrolytic performance showed in figure (A2 and B2). The activities presented are the mean values of two separate measurements. The standard deviation for above mentioned values is less than 10%.

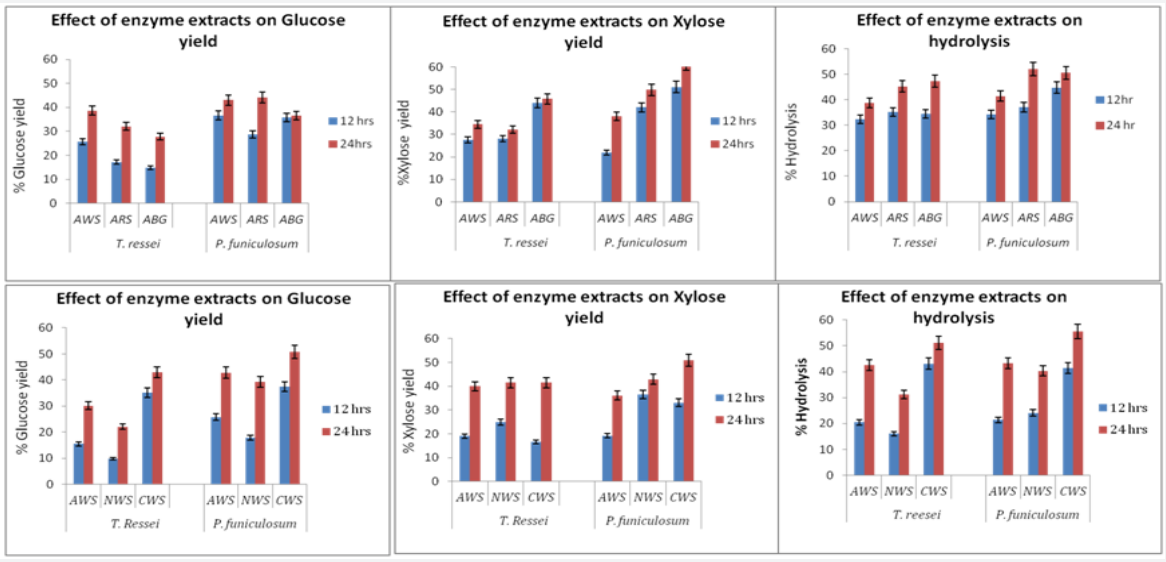

Figure 2: Hydrolytic performance of cellulases derived from fungi. Enzymatic hydrolysis of Ammonia pretreated lignocelluloses using in house cellulases Sugar production profile (A) Glucose production (B) Xylose production (C) Reducing sugar production from different feedstocks using enzyme derived from *T. reesei, P. funiculosum*. Here, A,B,C shows different feedstocks but same pretreatment and D,E,F showed same feedstocks but different pretreatment. Blue and red bar represents for glucose and xylose release after 12hrs and 24hrs.

Using ammonia pretreatment of different substrates: The hydrolytic efficiency of the culture supernatants was carried out at 1% (w/v, dry basis) with 15FPU/g enzymes loading to relate the compatibility of different enzyme source on various lignocellulosic substrates. Figure 1 gives the yields of glucose, xylose and total sugar achieved in the hydrolysis of AWS, ARS, and ABG. Yield of arabinose is not discussed much here as it contributes very less in the total sugar content of biomass. The ASTM analysis showed concentrations of glucose were similar for AWS, ARS, and ABG (67, 61 & 62%, respectively), however after hydrolysis, significant difference in glucose concentrations were observed using two different enzyme extracts. The highest levels were reached for AWS (2.88mg/ml) and lowest for the ABG (2.29mg/ml) using *P. funiculosum* though the difference was not significant. Similar trend was observed using *T. reesei* which released 2.45 & 1.74mg/ml glucose from AWS and ABG respectively. We also observed that the glucose yield obtained higher for AWS after 24hrs (38 & 43%) and lowest for ABG

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(27.75%, 36.5%) and in similar range for ARS (32 and 44%) using *T. reesei* and *P. funiculosum* enzyme mixtures. Production amount of glucose after hydrolysis is directly proportional to the ratio of β-glucosidase to FPU. *T. reesei* being low secretor of β-glucosidase [22], The BG to FPase ratio for *T. reesei* is (1:0.07), while *P. funiculosum* has higher ratio of 0.23 (Table 3). Therefore as expected after 24hrs of hydrolytic reaction, glucose yield of 44.08 and 38.66 using *P. funiculosum* and *T. reesei* enzyme mixtures respectively. The low ratio of β-glucosidase activity in the *T. reesei* culture is might be responsible for the accumulation of cellobiose (Figure 2), which is reported as strong inhibitor of exo and endocellulase during enzymatic hydrolysis of cellulose [23-25].

Table 3: Enzyme composition of crude culture extracts. Enzyme titer (U/ml) and specific activities U/mg (of protein) derived from *T. ressei* Qm9414 and *P. funiculosum*. The activities presented are the mean values of two separate measurements. The standard deviation for above mentioned values is less than 10%.

Enzyme Mixtures	FPase		Endoglucanase (CMCase)		β-glucosidase		Xylanase	
	U/ml	U/mg	U/ml	U/mg	U/ml	U/mg	U/ml	U/mg
T. reesei	0.44	1.47	0.37	1.23	0.03	0.10	72.48	241
P. funiculosum	0.30	2.0	0.51	3.46	0.07	0.47	23.04	153

BG: β-glucosidase; FPase: Filter paper activity; CMCase: Carboxymethyl Cellulose Activity; U/ml: Units/mililitre (titer); U/mg: Units Per Milligram of Protein (specific activity).

Second reason for low yield of glucose from ABG is probably due to the high content of lignin (12.6%). It is well known fact that the hydrolytic performance of cellulolytic enzymes obstructs in the presence of lignin by nonproductive bonding between lignin and cellulases [6,26,27]. Above findings are in compliance with prior findings [28-30], which stated that partial delignification improves the hydrolytic performances and hence the sugar yield. We observed slightly higher glucose yield with *P. funiculosum* enzyme mixture in case of ARS, AWS (44.09%, 43%) compared to ABG (36.5%). Similarly enzyme mixture derived from *T. reesei* showed decreased glucose yield by 11% for ABG as compared to AWS. However the overall glucose yield obtained from ARS & AWS was remained higher with all the enzyme mixtures because of its low lignin content as compared to ABG. This effect was seen prominent with *T. reesei* because of its very low β-glucosidase activity. Moreover, β-glucosidase though acts on the soluble oligosaccharides, it has high tendency to adsorb on lignin and get inhibited [31,32]. However, glucose yield was not much affected when enzyme mixture used from *P. funiculosum*. Though the glucose yield is affected when hydrolysis was performed with *T. reesei* enzyme mixture, it has been compensated for high cellobiose content in the hydrolysate obtained (Figure 3). Therefore, there was no significant difference observed in terms of total sugar yield (45-52%) when two enzyme mixtures used for hydrolysis for all three substrates (Figure 3). The glucose yield obtained here (27.75% & 36.52%) for ABG after 24hr were lower than Gottschalk et al. [33] who used culture blend obtained from *T. reesei* and A. awamori was used at 15FPU/g for the hydrolysis of pretreated bagasse and the yield obtained was 57%. This could be due to high FPU: BG content in the mixture(1:5), whereas mixtures we used in this study had much lower β-glucosidase for *P. funiculosum* (1:0.23) and *T. reesei* (1:0.07).

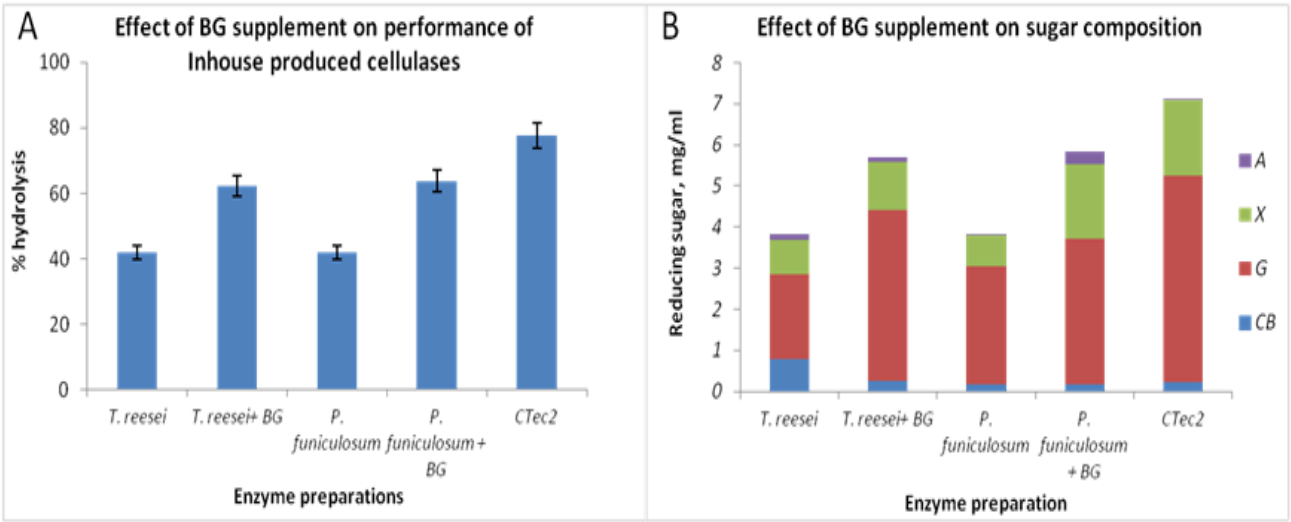

Figure 3: Hydrolytic improvement of fungal derived cellulases. Enzymatic hydrolysis of ammonia pretreated wheat straw using in house and commercial enzyme mixtures. Sugar production profile (A) effect of β-glucosidase on hydrolysis (B) effect of β-glucosidase on sugar profile using enzyme derived from *T. reesei, P. funiculosum.*

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As we have discussed above, higher glucose production in the cellulolytic reaction is possible when BG: FPase ratio is high [34]. For instance, this ratio was highest for *P. funiculosum* (1:0.23) and hence released higher glucose than *T. reesei* enzyme mixtures. On the parallel platform of BG: FPase ratio, we have observed another ratio called BG: xylanase for release of xylose from pretreated substrates. Enzyme mixture obtained from *P. funiculosum* shows high BG: xylanase ratio and release 61.46% xylose, which is higher than *T. reesei* (45.83%) as shown in the Table 3. The possible reason for this could be low BG: xylanase ratio of *T. reesei* enzyme mixture. Likewise glucose releasing study, the xylose release comparison was studied (Figure 3). We noticed that overall xylose release from ABG using *T. reesei* and *P. funiculosum* (45% and 61.46%) is remained higher and trend is followed by ARS and AWS respectively. Least xylose was released from AWS (34%, 38%) using *T. reesei*, *P. funiculosum* respectively. Although the differences were not significant this gives us idea that crude extract obtained of *P. funiculosum* has ample amount of hemicellulase activity. Regardless of initial xylan content of the substrates, higher the β-glucosidase to xylanase ratio of enzyme mixture, more effective was the xylose release. The xylose yield (61.46%) obtained after 24hr of hydrolysis here is more for ABG than 55.7% reported by Gottschalk et al. [32]. However, that can be a result of their higher FPU loading and/or the pretreatment type. Maria P et al. [35], studied the ratio of β-glucosidase to xylanase and the high ratio of β -glucosidase to xylanase improved xylose yield. The relation between the β-glucosidase and xylosidase has been discussed by Krisztina Kovacs et al. [8] where, P. brasilianum showed high ratio of β-glucosidase to FPA and β- xylosidase to FPA than F-1663/B (inhouse produced) enzyme mixture. These high ratios of enzymes improve the overall xylose and glucose yield compared to commercial cellulases like Celluclast and Novozyme mixture. Overall, the crude extract of enzyme mixture obtained from *P. funiculosum* is more potent for glucose and xylose release than *T. reesei* after 24hrs of hydrolytic reaction (Figure 2). The possible reason could be that extra β-glucosidase helps to increase xylose yield using the enzyme mixture of *P. funiculosum*. This finding is in well agreement with Kristina Kovacs et al. [8] where, they have got 9% improvement in the xylose yield when additional β-glucosidase is supplemented. Similarly additional β-glucosidase in *P. funiculosum* gives improved yield of 10% for ABG and 17% for ARS. However, in case of AWS there was similar yield using both the enzyme mixtures and this might be explained by the structural difference for xylose bonding in AWS. Though the xylanase specific activity of *P. funiculosum* derived enzyme mixture is lower than that of *T. reesei* (Table 2), the β-glucosidase to FPA and β-glucosidase to xylanase ratio is higher for former. These ratios of respective enzymes help *P. funiculosum* to release more xylose and glucose than *T. reesei*. We also observed that the higher release of xylose is compensated for lower release of glucose and vice versa for AWS, ARS and ABG using enzyme mixture. Therefore, total sugar yield for all the substrate using respective enzyme mixture remains similar (Figure 3). Current study showed connection between cellobiose accumulation and its effect on the xylose and glucose release. This links the synergistic action of xylanases, cellulases and β-glucosidase needed for increasing total soluble sugar yield in the enzymatic hydrolysis.

Using different pretreatment of wheat straw

The ASTM analysis showed glucose content was varied for AWS, ARS, and ABG (67, 75 & 88% respectively). Therefore after hydrolysis, significant difference in glucose concentrations was observed using two different enzymes extracts. Moreover, 10% caustic pretreatment for CWS, removed maximum lignin and hemicellulose and yielded comparatively pure cellulose. Nitrate pretreatment removed xylose equally compared to caustic pretreatment. These differences in physicochemical characteristics of biomass were found to be responsible for different sugar yields. CWS showed high glucose yield of 42.95% and 50.86% as compared to AWS (30.12% and 42.79%) and NWS (22.12% and 39.17%) using *T. reesei* and *P. funiculosum*. Xylose yield was similar for AWS and NWS (~38-42%) and for CWS it was slightly higher (50%) when *P. funiculosum* used for hydrolysis and this might be due to its high β-glucosidase and least lignin content of the CWS. Together it was reflected in the highest hydrolysis for CWS (51.14% and 55.46%) compared to NWS (31.15% and 40%) and AWS (42.55% and 43.21%) using *P. funiculosum* and *T. reesei* (Figure 1).

Much of work has been carried out where different enzyme sources are compared using single substrate [32,36] but our study provides insight into the two different enzyme preparation on three different substrates and pretreatments. It showed that how different substrates with the same pretreatment undergoes hydrolysis to give similar yield of sugars using different enzyme preparation. Also single substrate followed by varied pretreatment showed hydrolysis up to different extent. Therefore we have successfully showed that the pretreatment is a most important factor which decides biomass digestibility (hydrolysis) and not the only feedstock.

Improvement in the potency of the crude culture

β-glucosidase (pfuBG) from bacterial source was added in to the crude culture obtained from *P. funiculosum* and *T. reesei* to meet β-glucosidase deficiency. FPU: BG ratios were used for this study were made equal to CTec2 (1:0.5) to elucidate the efficiency of pfuBG in improving the enzymatic hydrolysis of wheat straw. In Figure 3A, when the pfuBG were mixed to the crude culture obtained from *T. reesei* and *P. funiculosum* to hydrolyze pretreated biomass at 1% substrate loading, we observed an obvious improvement in saccharifying ability.

When the ratio of FPU to BG were modified to 1:0.5 from 1:0.07 (*T. reesei*) and 1:0.23 (*P. funiculosum*) by adding pfuBG, the hydrolyzing performance towards AWS was improved by more than 21% and 22% using *T. reesei* and *P. funiculosum*

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respectively. The improved performance of these cultures was compared with CTec2, and we found that Ctec2 was more efficient and showed 77% hydrolysis against 62% by *T. reesei* and 63% by *P. funiculosum* enzyme extracts. This was probably due to presence of accessory proteins and/ or enzymes in the CTec2 could reduce the unspecific adsorption on substrate which improves the efficiency of cellulases which is in compliance with reported data of Yang B et al. [32]. Figure 2B focused light on the sugar composition obtained after hydrolysis; this study seems to be crucial for increase in total hydrolysis. Increase in glucose release showed overall hydrolysis enhancement and this was due to supplementation of β glucosidase. *T. reesei* and *P. funiculosum* showed 200% and 123% increase in glucose release. Xylose release was also found to be augmented by 141% and 240% using *T. reesei* and *P. funiculosum* respectively due to addition of β-glucosidase. The reason for this enhancement is explained in earlier section 3.3.3a.

Conclusion

In this study we have compared the cellulase and xylanase specific activities of *P. funiculosum* and *T. reesei* enzyme extracts and investigated their hydrolytic potential using physico-chemically different lignocellulosic materials. Enzyme extracts derived from *P. funiculosum* showed higher glucose yield than *T. reesei* derived enzyme, however this lower glucan yield is compensated for high cellobiose yield. Sugar profile gives idea of enzyme composition as low β glucosidase in *T. reesei* derived enzyme extracts affects glucose yield despite of having similar overall hydrolytic performance for both enzyme extracts. Pretreatment is a major factor which decides biomass digestibility as caustic pretreatment showed higher digestibility than ammonia and nitrate pretreatment irrespective of enzyme source. Lower β-glucosidase causes decreased glucan and xylan hydrolyzing capacity than enzyme extract of *P. funiculosum*. However, this lower glucan yield is compensated for high cellobiose yield using *T. reesei* derived enzymes. Finally supplementation of β-glucosidase showed improvement in glucose release and thus its hydrolytic performance.

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