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Catalytic Performance of Corn Stover Hydrolysis by a New Isolate *Penicillium* sp. ECU0913 Producing both Cellulase and Xylanase

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Abstract A fungal strain, marked as ECU0913, producing high activities of both cellulase and xylanase was newly isolated from soil sample collected near decaying straw and identified as *Penicillium* sp. based on internal transcribed spacer sequence homology. The cultivation of this fungus produced both cellulase (2.40 FPU/ml) and xylanase (241 IU/ml) on a stepwisely optimized medium at 30 °C for 144 h. The cellulase and xylanase from *Penicillium* sp. ECU0913 was stable at an ambient temperature with half-lives of 28 and 12 days, respectively. Addition of 3 M sorbitol greatly improved the thermostability of the two enzymes, with half-lives increased by 2.3 and 188-folds, respectively. Catalytic performance of the *Penicillium* cellulase and xylanase was evaluated by the hydrolysis of corn stover pretreated by steam explosion. With an enzyme dosage of 50 FPU/g dry substrate, the conversions of cellulose and hemicellulose reached 77.2% and 47.5%, respectively, without adding any accessory enzyme.

Keywords *Penicillium* sp. · Cellulase · Xylanase · Enzymatic hydrolysis · Corn stover

Introduction

Enzymatic hydrolysis of lignocellulose to produce fuel-grade ethanol was considered to be an attractive opportunity for the production of renewable, environmentally friendly biofuels [20]. However, there are several bottlenecks existed in the enzymatic hydrolysis of lignocellulose. Among these bottlenecks, the high cost of hydrolytic enzymes and the recalcitrant nature of the lignocellulosic substrate were two of the most important obstacles to the application of this bioconversion at a commercial scale [23].

Cellulase is a group of enzymes that hydrolyze the β-(1–4) linkages in cellulose. As a multicomponent enzyme system, cellulase consists of three major enzymes, including

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cellobiohydrolase (exoglucanase), endoglucanase, and β-glucosidase, which act synergistically in the hydrolysis of cellulose [14]. Compared to the cellulose, the nature of hemicellulose is more heterogeneous and the complete degradation of hemicellulose requires more complex mixture of enzymes, including endoxylanase, β-xylosidase, endomannanase, β-mannosidase, α-L-arabinofuranosidase, and α-galactosidase [34]. Therefore, a more complex and balanced mixture of enzymes, including both cellulase and xylanase, is required for efficient enzymatic hydrolysis of lignocellulose to produce fermentable reducing sugars. Filamentous fungi have demonstrated a great capability of secreting a wide range of cellulase and xylanase, with the genera *Trichoderma* and *Aspergillus* being the two most extensively studied and reviewed producers. But most strains belonging to the genera *Trichoderma* and *Aspergillus* cannot secrete a complete spectrum of cellulolytic enzymes, and the genus *Trichoderma* was famous for its high production of cellulase, while the genus *Aspergillus* was prominent in producing xylanase. Ahamed and Vermette [2, 3] have reported a mixed culture of *Trichoderma reesei* RUT-C30 and *Aspergillus niger* LMA producing 7.14 FPU/ml of cellulase, and [30] have reported a strain of *Aspergillus foetidus* MTCC 4898 producing 210 IU/ml of xylanase with negligible cellulase activity. *Penicillium* genus has a long history in the production of enzymes of biotechnological importance [35], and reports on its production of cellulase and xylanase have accumulated in recent years. As reported in former research papers, *Penicillium pinophilum* IBT 13226 [13] produced 1.70 FPU/ml of cellulase and 42 IU/ml of xylanase in a submerged culture, while *Penicillium echinulatum* 9A02S1 [6] produced 32.89 FPU/g substrate of cellulase and 10 IU/g substrate of xylanase in a solid substrate fermentation. So far, there are only a few strains known that can produce both cellulase and xylanase, including *Penicillium echinulatum* 9A02S1 [7], *Bacillus subtilis* [11], *Penicillium janthinellum* NCIM 1171 [1], *Trichoderma viride* AF93252 [21]. Therefore, it is important to find more microbial isolates with high activity of both cellulase and xylanase.

In the present study, both the cellulase and xylanase were produced at high levels by a newly isolated strain *Penicillium* sp. ECU0913, and the culture medium thereof was optimized to enhance the enzyme production. To evaluate the catalytic performance of the cellulase and xylanase produced by this strain, steam pretreated corn stover was employed as a substrate of hydrolysis with a moderate solid loading of 10% (*w*/*w*).

Materials and Methods

Microbial Strain and Cultivation Conditions

A large number of potentially cellulolytic microbes from different soil samples were isolated using a dilution-plating technique. Those isolated organisms from the preliminary screening were cultured in a liquid medium containing, per liter of tap water, 10.0 g corn cob, 2.0 g $NaNO_3$, 1.0 g peptone, 2.0 g K_2HPO_4, 3.0 g KH_2PO_4, 1.0 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2$, and 1.0 ml of Mandels trace element solution [24], at pH 5.0 and 30 °C for 120 h in a shaker of 180 rpm. The filtrate of the culture broth was used for the assay of extracellular cellulase and xylanase activities. One of the best cellulase/xylanase-producing strains, marked as ECU0913, was selected and identified as *Penicillium* sp. by nucleotide sequence analysis of the enzymatically amplified internal transcribed spacer (ITS) rDNA [19]. This strain is currently also deposited at China General Microbiological Culture Collection Center (CGMCC), Beijing, China, with an

access number of CGMCC no. 3371. The ITS sequence of this strain was submitted to Genbank with the accession number HM367681.

Optimization of Culture Conditions for Crude Enzymes Production

The optimization of medium composition and other culture conditions was carried out based on stepwise modification of the governing parameters for cellulase and xylanase production. The initial production medium consisted of, per liter of tap water, 20.0 g corn cob, 3.0 g peptone, 0.5 g yeast extract, 2.0 g K_2HPO_4, 1.5 g $(NH_4)_2SO_4$, 0.3 g $MgSO_4{\cdot}7H_2O$, 0.3 g $CaCl_2$, 1.0 ml Mandels trace element solution, and 2.0 g olive oil, pH 5.0. The effect of various carbon sources (i.e., glucose, maltose, sucrose, lactose, α-cellulose, corn cob, starch, wheat bran, wheat straw, and maltodextrin) and nitrogen sources (i.e., peptone, urea, yeast extract, sodium nitrate, ammonium sulfate, beef extract, and the various combinations of them) for the enzyme production was examined. The effect of pH was observed by varying initial pH of the production medium from 3.0 to 8.0. The effect of various additives supplemented to production medium, including Triton X-100, glycerol, Tween-80, olive oil, SDS, and EDTA, was also examined.

Assay of Enzymes Activity

The filter paper activity and carboxyl methyl cellulose activity (CMCase) of cellulase were determined according to Ghose's method [10]. The amount of reducing sugar formed was measured by the DNS method [26], using glucose as the standard. Assay mixture (1.5 ml) was consisted of 1.0 ml citrate buffer (50 mM, pH 4.8) for FPA assay or 1.0 ml 1% carboxymethyl cellulose (CMC) for CMCase assay, plus 0.5 ml suitably diluted enzyme. And for FPA assay, we added 50 mg Whatman No. 1 filter paper (1×6 cm strip) to the assay mixture too. The reaction mixture was incubated at 50 °C for 60 min. The xylanase activity was measured by the method of Bailey [4] using birchwood xylan (Sigma, USA). The reducing sugar produced was also measured by the DNS method using xylose as the standard. One unit of the enzyme activity was defined as that amount of enzyme that releases 1 μmol of reducing sugar in 1 min under the assay conditions. β-Glucosidase activity was determined using Berghem's method [5] using *p*-nitrophenyl-β-D-glucopyranoside (Sigma, USA) as substrate, and one unit of β-glucosidase activity liberates 1 μmol *p*-nitrophenol (PNP) per minute under the assay conditions.

Determination of Protein Concentration and Enzyme Stability

Protein concentrations were measured by the Lowry method [22] with BSA (bovine serum albumin) as the standard.

Thermostability of cellulase and xylanase was determined by incubating the enzyme extract in 50 mM sodium citrate buffer (pH 4.8) at 4 °C, ambient temperature or 50 °C for a certain period. For checking the effects of additives, several additives with different final concentrations were added into the mixture, including $FeCl_2$ (50 mM), KCl (50 mM), glycerol (10%), PEG 4000 (50 mM), gelatin (1%), glycine (3 M), and sorbitol (3 M), respectively. At different time interval, aliquots were taken, and the residual activities of enzymes were measured. Before activity assay, the enzyme was cooled down on ice for 5 min at the end of incubation. The half-life of the enzymes was calculated as follows:

$$\text{Half}-\text{life}\left(t_{1/2}\right) = \text{Ln}2/k_d$$

Enzymatic Hydrolysis of Steam-Pretreated Corn Stover

The corn stover was grown in Northeast Province of Jilin, China, and harvested in fall, 2007. After collection, the CS was milled, washed, air-dried, and then steam-pretreated at 200 °C, 2.0 MPa for 4 min without adding any inorganic and organic acids using StakeTech batch system (SunOpta Bioprocess, Inc., Brampton, ON, Canada) [36]. This pretreated corn stover contained approximately 40% dry solid matter (DM), and the DM contained approximately 32.6% cellulose, 26.4% hemicellulose, and 8.1% lignin, in addition to some other constituents. Enzymatic hydrolysis experiments with 100 ml of total reactant volume were carried out by loading 10 g dry pretreated corn stover. These tests were carried out in 250-ml three-neck, round-bottom flask according to the method described by NREL LAP-009 [29].

Analytical Methods

The composition of samples after enzymatic hydrolysis was analyzed using a high-performance liquid chromatography instrument (HPLC) (Agilent, USA) equipped with a refractive index detector (Agilent, USA). Cellobiose, glucose, xylose, galactose, arabinose, and mannose were separated using an Aminex HPX-87P column (Bio-Rad, Hercules, CA, USA) at 85 °C, with deionized water as eluent at a flow rate of 0.4 ml/min.

The cellulose and hemicellulose conversions were calculated as follows:

$$\text{Cellulose conversion}(\%) = \text{glucose by HPLC}(g) \times 0.9 \times 100/\text{cellulose in substrate}(g)$$

$$\text{Hemicellulose conversion}(\%) = \text{xylose by HPLC}(g) \times 0.9 \times 100/\text{hemicellulose in substrate}(g)$$

Results and Discussion

Medium Optimization for Cellulase and Xylanase Production

Eighty six potentially cellulolytic microbes, including bacteria and fungi, were isolated from soil, and among these isolates, a fungal strain marked as ECU0913 and identified as *Penicillium* sp. showed the highest cellulase activity. One of the significant differences between the initial medium and the optimized one was the nitrogen source. Table 1 shows that both the maximum cellulase activity (2.00 FPU/ml) and xylanase activity (218.5 IU/ml) were evident when 1.5% $NaNO_3$ was added. The levels of enzymes activity generally decreased when complex organic sources, such as peptone, yeast extract, and beef extract, were employed. It also indicates that this strain could produce significantly high activity even in the absence of any organic nitrogen source. These interesting results are in contrast to many other reports where fungi could usually produce higher activity of cellulase or xylanase with the addition of complex organic nitrogen sources [2, 3, 12, 27].

After a stepwise medium optimization, the cellulase production increased from 0.96 to 2.40 FPU/ml, while the xylanase production also increased from 148 to 241 IU/ml. The optimized medium consisted of, per liter of tap water, 30.0 g corn cob, 15.0 g $NaNO_3$, 2.0 g KH_2PO_4, 0.3 g $MgSO_4{\cdot}7H_2O$, 0.3 g $CaCl_2$, 1.0 ml Mandels trace element solution, and

Table 1 Effects of nitrogen sources on cellulase and xylanase production by *Penicillium* sp. ECU0913 at 30 °C under submerged fermentation

Combination of nitrogen sources (g/L)	Cellulase activity (FPU/ml)[a]	Xylanase activity (IU/ml)[a]
Control	0.20±0.05	24.51±7.31
Peptone (5.0)	1.10±0.01	136.80±0.37
Yeast extract (5.0)	1.00±0.04	130.50±8.54
Beef extract (5.0)	1.26±0.05	140.74±0.74
$NaNO_3$ (5.0)	1.29±0.10	152.81±5.94
$NaNO_3$ (15.0)	2.00±0.01	218.56±2.29
$(NH_4)_2SO_4$ (5.0)	0.50±0.05	102.66±5.57
Peptone (2.5)+yeast extract (1.0)+$(NH_4)_2SO_4$ (1.5)	1.38±0.07	140.74±5.94
Peptone (2.5)+beef extract (1.0)+$(NH_4)_2SO_4$ (1.5)	1.33±0.04	147.04±0.74

[a] Values are mean of three replicates

2.5 g Tween-80. And the culture condition was 30 °C and pH 6.0, 180 rpm, incubating for 6 days.

Fungi were considered to be the most active against the most abundantly available natural polymer. Different strains belonging to *Trichoderma* sp. have been studied most extensively in this regard. Recently, a number of studies also revealed that *Penicillium* sp. could produce relatively large quantities of cellulase and xylanase, making it an ideal organism for industrial applications [6, 13, 16]. As shown in Table 2, the cellulase production of *Penicillium* sp. ECU0913 in this work was much higher than most of the reported optimal cellulase production of fungi except *P. janthinellum* NCIM 1171, *T. reesei* Rut C30, and *Acremonium cellulolyticus*. All the high-level cellulase producing strains, including *T. reesei* Rut C30 and *Acremonium cellulolyticus*, were cultivated under submerged conditions using commercial cellulose from Sigma or Solka-floc, which is a kind of pure cellulose powder, as carbon source. These indicate, to some extent, that addition of a commercial cellulose powder in submerged fermentation can increase the cellulase production considerably in some fungi. However, this practice also leads to the higher production cost. Therefore, the discovery of microbial strains that could utilize lower-price substrates is more important and promising for industrial application.

Thermostability of Cellulase and Xylanase from *Penicillium* sp. ECU0913

Storage Stability

For industrial applications, storage of enzymes at ambient and/or refrigeration temperature for a long term without apparent loss of activity is one of the most important and desirable characteristics. Both the cellulase and the xylanase form *Penicillium* sp. ECU0913 retained full activity for 6 months when stored in deeply freezed state (−80 °C). At refrigeration temperature (4 °C), the cellulase and xylanase showed similar stability performance. No loss of cellulase and xylanase activity was detected within 2 weeks, but after 4 weeks a marginal decrease (5–10%) was found. The half-lives of cellulase and xylanase were 215 and 217 days, respectively at the refrigeration temperature. At ambient temperature (around 25 °C), no considerable decrease in cellulase activity was observed within 1 week, but at the end of second week 15% loss in cellulase activity was detected. The xylanase activity

Table 2 Comparison of lignocellulolytic enzyme production from different fungi grown on various cellulosic materials and commercial preparations

Organism	Substrate	Cellulase activity (FPU/ml)	Xylanase activity (IU/ml)	Reference
T. atroviride	Sigmacell Type 20	1.18	N. D.[a]	[15]
T. reesei Rut C30	Steam pretreated spruce	0.53	N. D.	[16]
T. reesei Rut C30	Cellulose (Sigma)	5.02	N. D.	[2, 3]
P. echinulatum	Sugarcane bagasse	0.95	1.49	[7]
P. echinulatum	CMC	0.27	3.16	[25].
P. janthinellum NCIM 1171	Wheat bran	3.40	225	[1]
P. echinulatum	Cellulose and lactose	1.50	N. D.	[28]
P. persicinum IBT 13226	Mixture of solka-floc, oat xylan and birchwood xylan	1.70	42	[13]
Acremonium cellulolyticus	Solka-floc	15.50	N. D.	[12]
Penicillium sp. ECU0913	Corn cob	2.40	241	This work

[a] N. D. not determined

began to decrease after 2 days, retaining 65% of its original activity after 1 week. At ambient temperature (around 25 °C), the half-lives of cellulase and xylanase were 28 and 12 days, respectively (Fig. 1).

Effect of Additives on Thermostability of Cellulase and Xylanase

The temperature for cellulase and xylanase assay [10, 26] was 50 °C, and this was also the temperature adopted for standard lignocellulose hydrolysis. The half-lives of cellulase and xylanase from *Penicillium* sp. ECU0913 at 50 °C were 115 and 0.45 h, respectively. To improve the thermostability of enzymes, especially that of xylanase, at 50 °C, various additives (like $FeCl_2$, KCl, glycerol, polyethylene glycol 4000 (PEG 4000), sorbitol,

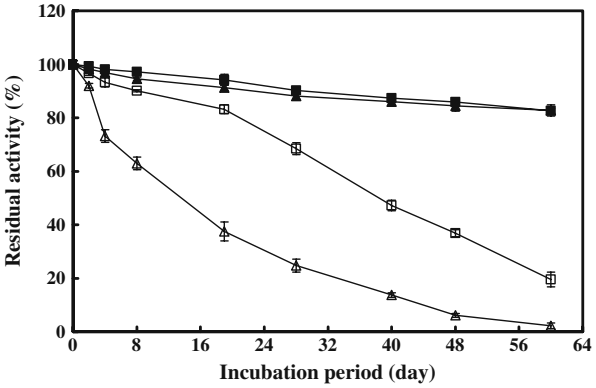

Fig. 1 Storage stability of cellulase and xylanase. Cellulase 5 FPU or xylanase 500 IU was incubated in 50-mM citrate buffer (pH 4.8) at 4 °C, and ambient temperature for different intervals and residual activity was determined. Values are mean of three replicates. Cellulase at 4 °C (*black square*) and ambient temperature (*white square*); Xylanase at 4 °C (*black triangle*) and ambient temperature (*white triangle*)

glycine, and gelatin) were added to the enzyme solution. As shown in Fig. 2, $FeCl_2$ (50 mM), KCl (50 mM), glycerol (10%), and PEG 4000 (50 mM) did not give protection to the *Penicillium* cellulase and xylanase against thermal inactivation. The addition of gelatin (1%) showed only a slightly positive effect on the thermostability of cellulase at 50 °C, but it did not have any positive effect on the stabilization of xylanase. On the contrary, glycine showed remarkable improvement in the thermostability of xylanase, but regretfully decreased the thermostability of cellulase. So neither gelatin nor glycine was the ideal protectant for the cellulase and xylanase from *Penicillium* sp. ECU0913. Figures 2 and 3 show that addition of 3 M sorbitol to the enzyme solution at 50 °C resulted in significant increases in the half-lives of cellulase and xylanase from 115 and 0.45 h to 269 and 84 h, respectively. The half-life of xylanase had been increased by 188 times, indicating a great improvement of xylanase thermostability, which was similar to the reported results [8, 9].

Enzymatic Hydrolysis of Steam-Pretreated Corn Stover

The hydrolytic performance of the enzyme preparation obtained from *Penicillium* sp. ECU0913 was evaluated using the steam-pretreated corn stover as a substrate. The concentrated enzyme preparation from *Penicillium* sp. ECU0913 contained 2.95 FPU/mg protein of filter-paper activity, 8.19 IU/mg protein of CMCase, 2.08 IU/mg protein of β-glucosidase, and 267 IU/mg protein of xylanase. The high activity of xylanase in the enzyme preparation foreshowed its good performance in lignocellulose hydrolysis, as Kumar and Wyman [18] reported that the xylanase supplementation of cellulase remarkably increased the digestion of corn stover solids. The factors that affect the enzymatic hydrolysis of pretreated corn stover, including reaction conditions (temperature, pH) and enzyme dosage were subsequently investigated in detail.

Effect of Temperature and pH on Enzymatic Hydrolysis of Corn Stover

The influence of temperature on the hydrolytic capacity of the enzymes produced by *Penicillium* sp. ECU0913 was evaluated between 40 and 55 °C using the steam-pretreated corn stover as substrate. As shown in Fig. 4, the maximum cellulose conversion (41.5%) was obtained, yielding 15.1 g/L glucose, when the hydrolytic temperature was set at 50 °C, while the maximum hemicellulose conversion (14.0%) was reached at 45 °C, yielding

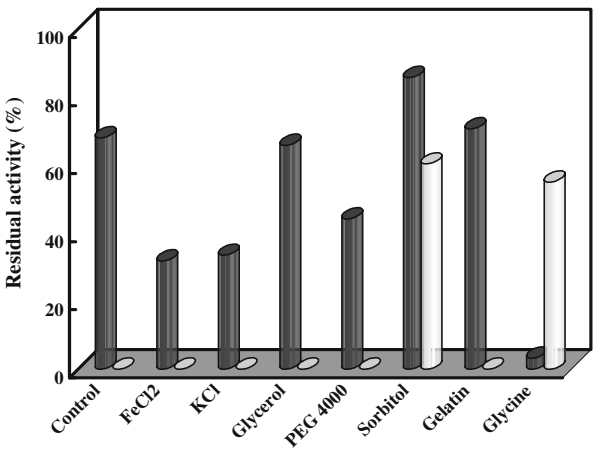

Fig. 2 Thermal stability of cellulase and xylanase at 50 °C. Assay conditions: 5 FPU of cellulase or 500 IU of xylanase was incubated in 50-mM citrate buffer (pH 4.8) in the presence of various additives for 62 h. Values are mean of three replicates. Residual cellulase activity (*black square*); Residual xylanase activity (*white square*)

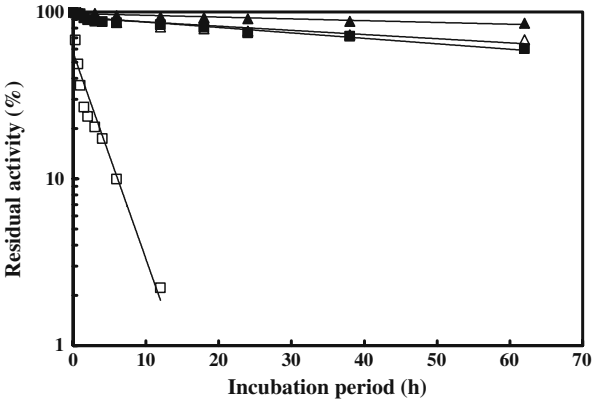

Fig. 3 The effect of 3 M sorbitol on the thermostability of cellulase and xylanase at 50 °C. Samples were withdrawn at different time intervals to estimate the residual activity. Residual cellulase activity, with addition of 3 M sorbitol solution (*black triangle*) or without any additives (*white triangle*); Residual xylanase activity, with addition of 3 M sorbitol solution (*black square*) or without any additives (*white square*)

3.7 g/L xylose. Considering the thermostability of both cellulase and xylanase, the temperature of 45 °C was chosen for the following enzymatic hydrolysis research.

The effect of pH on the hydrolysis efficiency of the produced enzymes was examined in the range of pH 3.0–8.0 at 45 °C. As shown in Fig. 5, the optimal pH for enzymatic hydrolysis of the steam-pretreated corn stover by enzymes from *Penicillium* sp. ECU0913 was found to be pH 5.0, which is consistent to that of the standard hydrolysis protocol (pH 4.8) as suggested by National Renewable Energy Laboratory (NREL). When it was at pH 5.0, both cellulose conversion and hemicellulose conversion reached the maxima (32.9% and 25.7%, respectively). The hemicellulose conversion was slightly affected when the citrate buffer was used with a pH range of 3.0–6.0. However, the hemicellulose conversion was lower when phosphate buffer was used at pH 6.0. This phenomenon meets some previous research using citrate buffer as reaction buffer for lignocellulose hydrolysis [16, 17, 25, 33].

Effect of Enzyme Dosage on Hydrolysis of Steam-Pretreated Corn Stover

To further investigate the catalytic performance of enzymes from *Penicillium* sp. ECU0913, enzymatic hydrolysis of steam-pretreated corn stover with different enzyme dosages was examined at 10% substrate concentration. As shown in Fig. 6a,b for each enzyme dosage, both the cellulose and hemicellulose conversions increased distinctly for the first 12 h, and

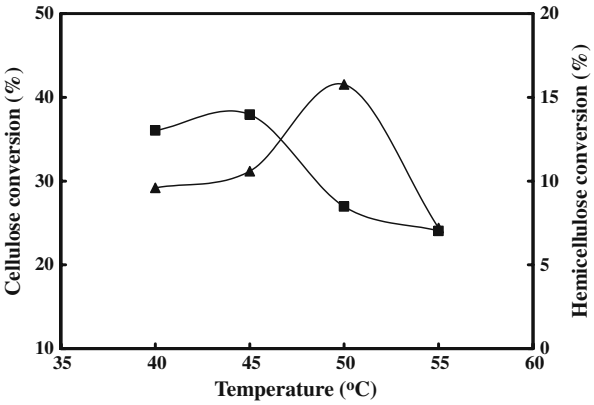

Fig. 4 Effect of temperature on lignocellulose hydrolysis. Enzymatic hydrolysis of pretreated corn stover with 10% (*w*/*v*, dry basis) solid loading and 6.5 FPU/g DM enzyme concentration at pH 4.8, shaken at 200 rpm. Cellulose conversion (*black triangle*) and hemicellulose conversion (*black square*) after 72 h were determined. *DM* dry matter of pretreated corn stover

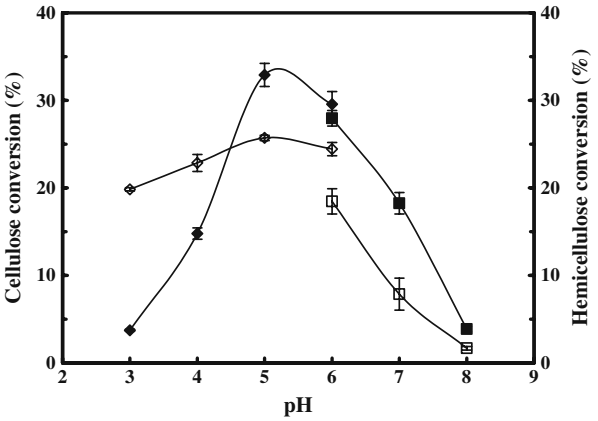

Fig. 5 Effect of pH on lignocellulose hydrolysis. Enzymatic hydrolysis of pretreated corn stover with 2% (*w/v*, dry basis) solid loading and 6.5 FPU/g DM enzyme concentration, shaken at 45 °C and 200 rpm. Cellulose conversion and hemicellulose conversion after 72 h were determined. Cellulose conversion with 50-mM citrate buffer (*white square*) and 50-mM phosphate buffer (*black square*); Hemicellulose conversion with 50-mM citrate buffer (*white diamond*) and 50-mM phosphate buffer (*white square*)

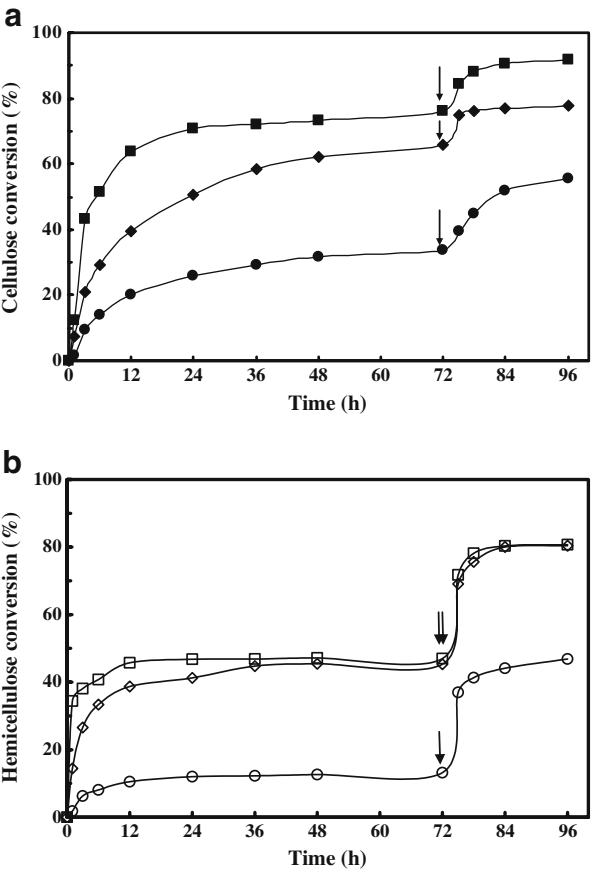

Fig. 6 Effect of enzyme dosage on lignocellulose hydrolysis. Enzymatic hydrolysis of pretreated corn stover with different enzyme dosages at 10% corn stover loading (*w/v* dry basis) was performed at 45 °C, pH 5.0 (citrate buffer), and 200 rpm (stirring). Cellulose conversions (**a**) with: 6.5 FPU/g DM (*black circle*), 20 FPU/g DM (*white square*), 50 FPU/g DM (*black square*); Hemicellulose conversion (**b**) with: 6.5 FPU/g DM (*white circle*), 20 FPU/g DM (*white diamond*), 50 FPU/g DM (*white square*), supplement of the enzyme (200 FPU) (*downwards arrow*)

then ascended slowly at 12–72 h. When the cellulase loading was increased from 6.5 to 50 FPU/g dry matter of pretreated corn stover (DM), the cellulose conversion at 72 h increased from 33.6% to 77.2%, while the hemicellulose conversion increased from 13.2% to 47.5%. The low-enzyme loading of 6.5 FPU/g DM resulted in a sharp decrease in the lignocellulose conversion and especially the decrease in the hemicellulose conversion. Figure 6b indicated that an increase of the enzyme loading up to 20 FPU/g DM can elevate the hemicellulose conversion remarkably, and further increase in enzyme dosage up to 50 FPU/g DM did not produce a corresponding increase in the hemicellulose conversion. After 72-h hydrolysis, the residual activities of lignocellulolytic enzymes in the reaction mixture were measured being 56.6%, 66.9%, 50.0%, and 7.0% of their initial activities for the filter paper activity, CMCase, β-glucosidase, and xylanase, respectively. In order to eliminate the unfavorable effect of enzyme inactivation, another 200 FPU of the enzyme was supplemented to each reaction system after 72-h hydrolysis. As shown in Fig. 6a and 6b, the lignocellulose conversion increased after the addition of more enzymes, indicating the unvoidable inactivation of enzymes, especially the hemicellulase, during the enzymatic hydrolysis process. As shown in previous researches [31], the increase of cellulase dosage to a certain extent can enhance the yield and rate of the hydrolysis, but would significantly increase the cost of the process. Considering of the enzyme cost, a reasonable enzyme dosage should be selected to reach a high lignocellulose conversion for industrial application. In this regard, further research should be taken in the future to achieve a higher lignocellulose conversion, not only enhancing the enzyme stability but also studying the mechanisms of the product inhibition, the adsorption, and desorption of cellulase onto the cellulose surface [32].

Conclusion

The present study was carried out to investigate the catalytic performance of the cellulase and xylanase produced by a newly isolated strain *Penicillium* sp. ECU0913. With the optimized nutrition sources in the submerged fermentation medium, this fungal strain produced both cellulase and xylanase with high activities towards conversion of cellulose and hemicellulose, suggesting this strain may become a new and interesting source of lignocellulose-degrading enzymes with important economic advantages. The addition of 3 M sorbitol could greatly improve the thermostability of two enzymes at 50 °C. The crude enzyme preparation from *Penicillium* sp. ECU0913 could efficiently hydrolyze the steam-pretreated corn stover at 10% concentration without adding any accessory enzyme.

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