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Full Length Research Paper

Enhanced saccharification of steam explosion pretreated corn stover by the supplementation of thermoacidophilic β-glucosidase from a newly isolated strain, *Tolypocladium cylindrosporum* syzx4

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Newly isolated fungi, strain *Tolypocladium cylindrosporum* syzx4 from rotten corn stover, can effectively produced extracellular thermoacidophilic β-glucosidase (syzx4) using agro-industrial residues. After purification and characterization, the β-glucosidase was applied to saccharify steam explosion pretreated corn stover (SCS) with commercial celluclast from *Trichoderma reesei* when compared with the β-glucosidase (Novo-188) from *Aspergillus niger* supplementation. Based on the results of the single factor tests, further studied with the response surface methodology (RSM), amid a five level four-factor central composite design (CCD), was designed for the optimal hydrolysis parameters, such as the substrate concentration, pH, temperature and the ratio of celluclast to syzx4, in order to achieve the highest saccharification yield. The substrate concentration and the ratio of celluclast to syzx4 were identified as the limiting factor for the saccharification yield. A maximum saccharification yield of 88.4% was obtained at an optimal hydrolysis condition as follows: substrate concentration (3.05%), pH (3.73), temperature (43.4°C) and the ratio of syzx4 to celluclast [0.91 (18.2 BGU/g substrate): 1 (20 FPU/g substrate)]. The results of a confirmation experiment under the optimum conditions were in agreement with model predictions and they obtained the maximum value of 88.4% saccharification yield. The results suggested that the β-glucosidase (syzx4) from *T. cylindrosporum* is a good supplementation for the production of reducing sugars from cellulosic biomass.

Key words: Saccharification, steam explosion pretreated corn stover (SCS), β-glucosidase, *Tolypocladium cylindrosporum* syzx4, hydrolysis parameters, response surface methodology (RSM).

INTRODUCTION

As the reserves of conventional energy sources decreased and human impact on global climate change increased, the development of alternatives to fossil energy is an urgent global priority (Van Maris et al., 2006; Farrell et al., 2006). The abundant renewable and inexpensive cellulosic biomass including various agricultural residues, woods, fruit and vegetable wastes, wastes from the pulp and paper industry, and herbaceous energy crops have the potential to contribute to meeting the demand for liquid fuel and other important chemicals (Edward, 2008; Shewale, 1982). In the process of enzymatic hydrolysis of cellulosic biomass, enzyme production is still the most crucial and costly step due to the large amounts of enzymes required for the hydrolysis processes and the low yields of sugars released (Lynd et al., 2002). However, the processes of enzyme production are still actively investigated (Cassman and Liska, 2007; Hong et al., 2009).

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Although there has been extensive research on cellulases science since World War II, there are still some major gaps in our understanding of the mechanism (David, 2009). All were accepted by the researchers that for the completed degradation of cellulose to simple sugars, three categories of cellulolytic enzymes are all essential with their synergistic action. Endoglucanase (EC 3.2.1.4) and exoglucanases (EC 3.2.1.91) hydrolyze the cellulosic biomass to cellobiose, which can be converted to glucose by β-glucosidase (Eriksson et al., 1990; Saha et al., 1994). β-glucosidase as a key enzyme produces glucose by cleaving cellobiose. Cellulase activity is severely inhibited by cellobiose (Han and Chen, 2008) and the competitive inhibition can be released by addition of a surplus β-glucosidase (Yan et al., 1998). Many fungal strains, such as model strains: *Trichoderma reesei* and *Phanerochaete chrysosporium* can effectively produce cellulases. Although the cellulase Celluclast used in hydrolyzing cellulosic biomass was investigated thoroughly (Arja et al., 2004), the insufficient β-glucosidase composition hurdled the effective conversion of cellulosic biomass to glucose (Saha et al., 1994). Therefore, there is a great increasing demand for the production of appropriate β-glucosidase to cellulose-degrading system for glucose and subsequently for the production of cellulosic biofuel and other fundamental biological processes (Joseph et al., 2010).

Cellulosic biofuel and chemicals production involves collection of biomass, pretreatment and saccharification, product recovery and waste treatment. Saccharification of cellulosic biomass, which decomposes the cellulosic biomass to component sugars, is the urgent step for sugar production (Marimuthu et al., 2009). After pretreatment processes reduced cellulose crystallinity and increased the porosity of the cellulosic biomass, enzymatic hydrolyzation of cellulosic biomass that replaced chemical reagents and chemical pollution was considered as an environmentally friendly method.

In this paper, a thermoacidophilic β-glucosidase-producing fungus *T. cylindrosporum* syzx4 was isolated. After fermentation, purification and characterization, β-glucosidase was applied first to saccharify SCS with commercial Celluclast from *T. reesei*, after which it was compared with β-glucosidase (Novo-188) from *Aspergillus niger* supplementation. Response surface methodology (RSM) was applied to identify the optimum conditions for maximum sugar production, and analysis was done between the relationships of a number of hydrolysis parameters in the saccharification process.

MATERIALS AND METHODS

Microorganism

In this study, a newly isolated strain syzx4 (CCTCC M 209312), which can effectively produce thermoacidophilic β-glucosidase, was isolated from rotten corn stover samples and the strain was identified as a thermophile strain of *T. cylindrosporum* by CCTCC (China Center for Type Culture Collection, Wuhan, China). The strain was maintained on potato dextrose agar (PDA) slants at 4°C and sub cultured every 2 weeks.

Cellulases

For β-glucosidase production, *T. cylindrosporum* strain syzx4 was fermented with an optimum medium by RSM. The fermentation process was carried out in a 5 L fermentor containing 2 L culture medium for 5 days at 30°C with an agitation rate of 150 rpm. The enzyme was purified to homogeneity before sulfate precipitation, diethylaminoethyl cellulose anion exchange chromatography and Sephadex G-100 gel filtration. The purified β-glucosidase (Syzzx4) was used as a supplementation in the saccharification of SCS.

Commercial cellulase from *T. reesei* QM 9414 (Celluclast) and β-glucosidase (Novo-188) from *A. niger* were purchased from Novozymes and Sigma-Aldrich Chemical Co, respectively.

Substrate and pretreatments

Corn stover and SCS were attained from a local company (Jilin Fuel Alcohol Company Ltd., China). The corn was cut into small pieces and dried to a constant weight before use. The steam explosion pretreatment was performed in a 5.0 L vessel with the pressure of 1.50 Mpa for 10 min and then dried at room temperature for enzymic hydrolysis and component analysis. The mechanical comminution pretreatment corn strove (MSC) was done according to the method of Millet et al. (1976).

Saccharification experiments of SCS

The typical saccharification experiments were carried out with 1.0 g SCS in Erlenmeyer flasks (100 mL) in 50 mL of hydrolysis mixture with antibiotics penicillin (20 ug/mL) and cellulases. The pH was adjusted necessarily with buffer and cellulases mixtures in a total volume of 50 mL. The mixture was incubated at 50°C in a rotary shaker at 150 rpm. Samples were taken and heated to 100°C immediately to denature the cellulase, then cooled at 4°C and centrifuged at 8000 rpm for 10 min. Reducing sugar was determined and the saccharification yield was calculated as follows (Hari et al., 1998):

$$\%\ Saccharification = \frac{\text{gulcose} \cdot 0.9 \cdot 100}{carbohydrate\ \text{in substrate}}$$

Single-factor experiment approach for the feasible condition of saccharification experiments

In order to attain the feasible condition for the saccharification experiments of SCS, the important hydrolysis parameters, such as reaction time (0 to 56 h), temperature (30 to 60°C), pH (2 to 6), the substrate concentration (1 to 6 %) and the activity ratio of Celluclast to syzx4 (20:5 to 20:20) [(20 FPU/g substrate) : (5 BGU/g substrate) to (20 FPU/g substrate) : (20 BGU/g substrate)] were optimized with single factor experiment.

RSM for the optimal condition of saccharification experiments

Once the feasible condition is acquainted with the hydrolysis parameters, a central composite design (CCD) with four independent variables (the substrate concentration, pH, temperature and the ratio of syzx4 to Celluclast), each of which has five levels (-2, -1, 0, 1 and 2), 24 star points and 7 replicates at the center points, were performed. The results of the CCD experiments were used to develop a second-order polynomial model to stimulate

Table 1. Saccharification yield of different cellulase mixtures with MCS and SCS.

Materials	Saccharification yield (%)	
	Celluclast+Novo-188	Celluclast+Syzx4
Comminution pretreatment corn stover (MCS)	35.5	45.7
Steam explosion pretreated corn stover (SCS)	65.2	76.4

the saccharification process. The effects of the four factors on the saccharification yield (Y) were studied by RSM.

Analytical methods

Cellulose, hemicellulose, lignin, protein and ash of SCS were determined according to the method of Goering and Vansoest (1970). Cellulase activity, as FPU (filter paper units), was determined against Whatmann No.1 filter paper with the DNS method (Miller, 1959). The β-glucosidase activity, as BGU, was determined against p-nitrophenyl-β-D-glucopyranoside (pNPG) in citrate buffer (100 mM, pH 4.5) and the liberation of p-nitrophenol was determined with absorption spectroscopy at 410 nm (Claeyssens and Aerts, 1992). One unit (U) of enzyme activity was defined as the amount of enzyme required to liberate 1 µmol of glucose or p-nitrophenol from the appropriate substrates per minute under assay conditions.

Glucose concentration was determined with a Glucose Kit purchased from Shanghai FOSUN PHARMA for the calculation of saccharification yield. Protein concentration was determined with the methods of Bradford (1976) using bovine serum albumin as a standard. SAS version 8.0 software (SAS Institute Inc., USA) and MatLab version 2008a software (Mathworks, Inc., USA) were obtained for the regression and graphical analyses of the data, respectively.

RESULTS AND DISCUSSION

Pretreatment of SCS and topical saccharification experiments

After the SCS was obtained, the contents of cellulose, hemicellulose, lignin, protein and ash were determined by the method of Van Soest. The main compositions of SCS were 56.3% cellulose, 11.6% hemicellulose, 13.3% lignin, and 18.8% ash by dry weight. When compared with the mechanical comminution pretreatment, steam explosion pretreatment increased the proportion of cellulose by 23.6% and decreased that of hemicellulose and lignin by 10.1 and 9.0%, respectively.

The topical saccharification experiments were carried out with the two kinds of cellulase mixtures and two pretreatments of corn stover, respectively. 1) Cellulast (*T. reesei*, 20 FPU/g substrate) and Novo-188 (*A. niger*, 10 BGU/g substrate); 2) Cellulast (*T. reesei*, 20 FPU/g substrate) and syzx4 (*T. cylindrosporum*, 10 BGU/g substrate). The pH was natural with 50 mM citrate buffer and celluloses mixtures in a total volume of 50 mL. After 56 h enzymatic hydrolysis, the results of saccharification yield are shown in Table 1.

Saccharification yield of SCS was much higher than that of MCS. The SCS, which increased the cellulose content, reduced the lignin content. As such, the increased surface area was considered to be an ideal material for the enhancement of enzymatic hydrolysis. Enzymic hydrolysis with supplementation of the purified β-glucosidase (syzx4) from *T. cylindrosporum* was much better than that of Novo-188 from *A. niger* with the same activity. Although the condition of typical saccharification experiments was beneficial to Novo-188 from *A. niger* (Martins et al., 2008), β-glucosidase (syzx4) made a better performance in enzymatic hydrolysis. The saccharification yield was achieved to be 76.4% with the supplementation of β-glucosidase (syzx4) and substrate of SCS. The extra 11% saccharification yield may be caused by the different kinetic parameters and inhibition of the two kinds of β-glucosidase. The pre-experiments demonstrated that the β-glucosidase from *T. cylindrosporum* had higher *Vmax* (85.23 mM/s), higher concentration tolerance of glucose *Ki* (39.5 mM) and lower *Km* (0.85 mM) than β-glucosidase (Novo-188) from *A. niger* (Eduardo et al., 2006), which caused the hydrolysis process more effectively.

Single-factor experiment approach for the saccharification experiments

Reaction time

We studied the reaction time (0 to 56 h) for the typical saccharification experiments of SCS and the results are shown in Figure 1.

The feasible reaction time was about 48 h, and extension of the reaction time to 56 h had no significant effect on saccharification of SCS. As a result of the excellent properties of syzx4, the saccharification yield with syzx4 supplementation was obtained as 75.7% on the reaction time of 36 h, which was higher than 56 h reaction with Novo-188 for that of *A. niger* supplementation.

Temperature

The feasible temperature for saccharification of SCS was about 40°C (Figure 2) instead of 50°C in typical saccharification experiments. In the range of 30 to 40°C, an obvious increase in saccharification yield was observed. A temperature of 60°C caused drastic decrease in saccharification yield. Although the syzx4 had a wild temperature of 30 to 60°C, it might be that the

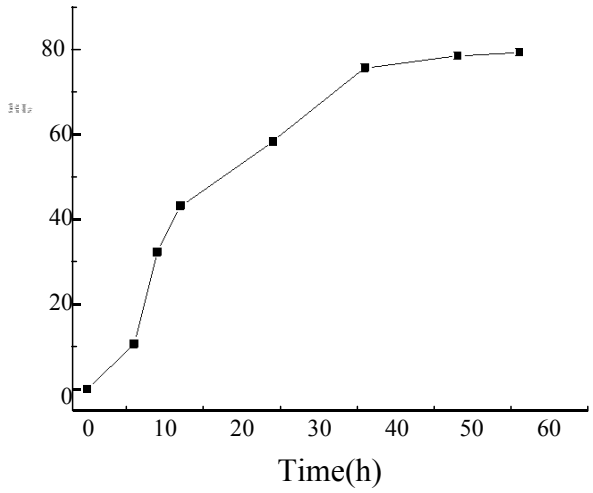

Figure 1. Effect of reaction time on saccharification. Substrate concentration (2.0%); pH nature; temperature (50°C) and the ratio of Celluclast to Syzx4 (20 FPU/g substrate: 10 BGU/g substrate).

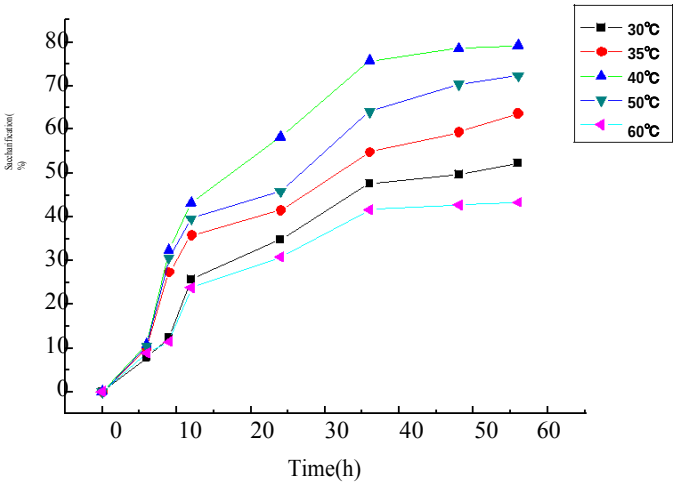

Figure 2. Effect of reaction temperature on saccharification. Reaction time (56 h); substrate concentration (2.0%); pH nature; and the ratio of Celluclast to Syzx4 (20 FPU/g substrate: 10 BGU/g substrate).

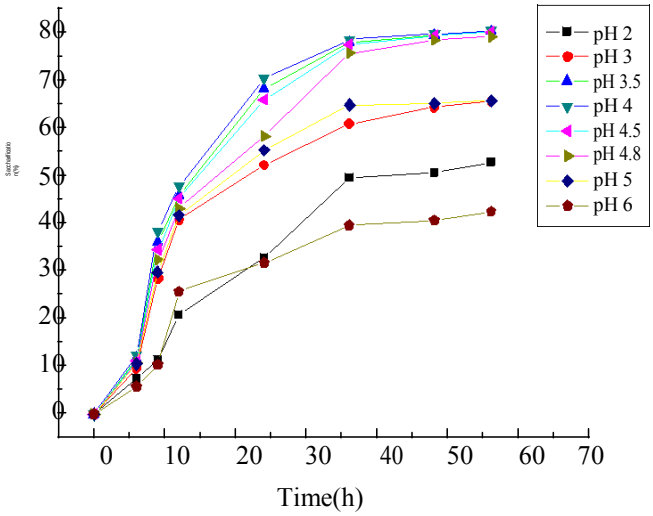

Figure 3. Effect of pH values on saccharification. Reaction time (56 h); substrate concentration (2.0%); temperature (40°C); and the ratio of Celluclast to Syzx4 (20 FPU/g substrate : 10 BGU/g substrate).

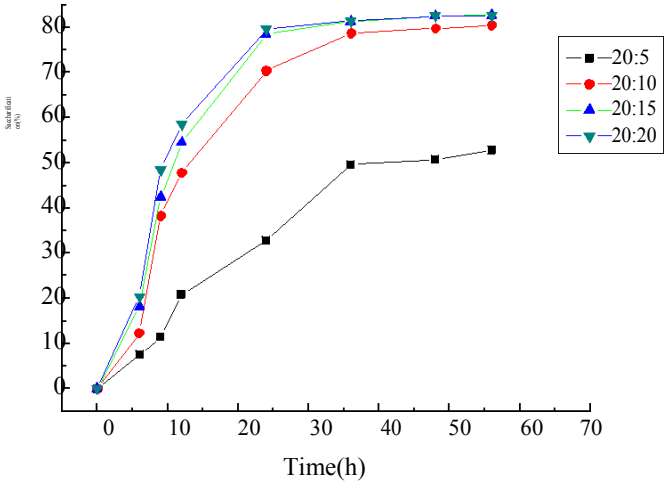

Figure 4. Effect of the ratio of Celluclast and Syzx4 on saccharification. Reaction time (56 h), pH (4.0), substrate concentration (2.0%) and temperature (40°C).

character of the cellulase (Celluclast) caused the lower saccharification yield at 60°C.

pH values

Maximum saccharification yield (80%) was reached at pH 4.0 (Figure 3) for the 48 h reaction, although pH values from 3.5 to 4.8 did not affect the saccharification yield when compared with the pH value of 6.0. The higher saccharification yield in low pH value was consistent with the properties of syzx4 and it was very useful for the ethanol production with yeast amid SCS.

The ratio of Celluclast to syzx4

During the topical saccharification experiments, the ratio of Celluclast to syzx4 was 2 (20 FPU/g substrate) : 1 (10 BGU/g substrate). In order to identify a suitable ratio of Celluclast to syzx4 for saccharification, experiments with different ratios (20:5, 20:10, 20:15 and 20:20) of Celluclast to syzx4 were performed and the suitable ratio of Celluclast to syzx4 was around 20:15 (Figure 4). Increasing the syzx4 ratio to 20:20, there was negligible

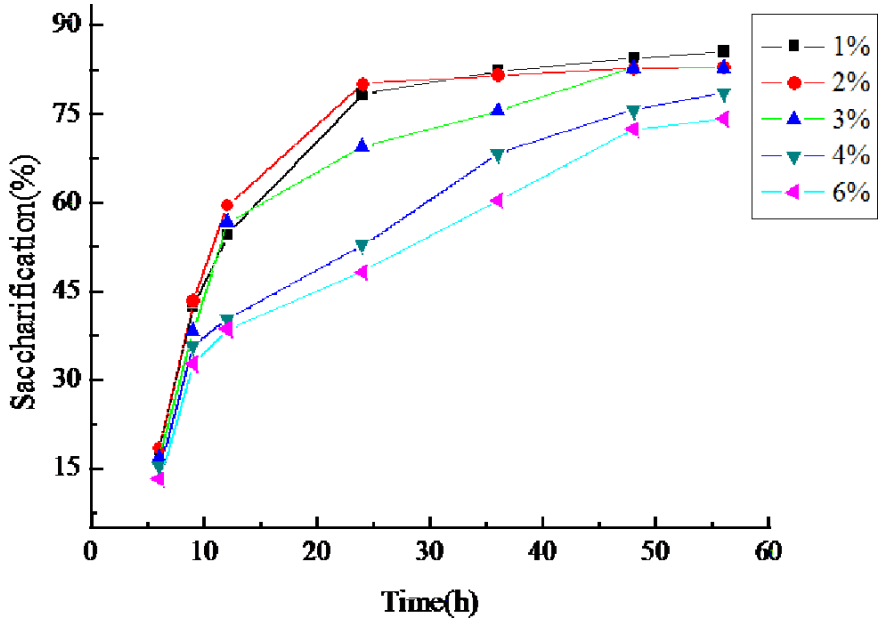

Figure 5. Effect of the substrate concentration on saccharification (%). Reaction time (56 h); ratio of Cellulast to Syzx4, 20:15 (20 FPU/g substrate : 15 BGU/g substrate); pH (4.0) and temperature (40°C).

Table 2. Maximum and minimum levels of the four variables used in CCD.

Variable	Symbol	Coded levels				
		-2	-1	0	1	2
Substrate concentration (%)	X1	1	2	3	4	5
pH	X2	3.5	3.75	4	4.25	4.5
Temperature (°C)	X3	35	40	45	50	55
Ratio of Syzx4 to Celluclast (BGU/FPU)	X4	0.5	0.625	0.75	0.875	1

increase in saccharification yield and the use of such amount of β-glucosidase (syzx4) may not be justified economically.

Substrate concentration

The results of the different (1 to 6%) concentrations of substrate on saccharification yield were determined as shown in Figure 5.

In the substrate concentration from 1 to 3% with the reaction time of 48 h, it was observed that there was no obvious change on saccharification yield. Although low substrate concentration may quickly lead a higher saccharification yield in the hydrolysis processes, they also get a lower production of reduced sugar. The feasible substrate concentration on saccharification was about 3%. An increase in substrate concentration from 4 to 6% limited the rate of hydrolysis processes, partly due to associated difficulties in mixing the reaction mixture inhibited by other chemical reagents.

RSM for the optimal condition of saccharification experiments

On the basis of the single-factor experimental results, a substrate concentration of 1 to 5%, pH of 3.5 to 4.5, temperature of 35 to 55°C and the ratio of syzx4 to Celluclast (0.5:1), were tested as conditions for optimizing the hydrolysis processes using the central composite design (CCD). The four factors with their five levels each are shown in Table 2.

Table 3 showed the actual experiments that were carried out for the four-factor-five-level central composite design (CCD) for the RSM model development. The whole design consisted of 31 experimental points, which included 24 star points and 7 center points. Seven replicates (25 to 31) at the centre of the design were used to allow an estimation of a pure error sum of squares.

The responses of the CCD were fitted with a second-order polynomial using a regression analysis in order to

Table 3. Experimental design and results of the central composite design (CCD).

Run	X1	X2	X3	X4	Saccharification yield (%)
1	-1	-1	-1	-1	53.88
2	-1	-1	-1	1	83.63
3	-1	-1	1	-1	49.08
4	-1	-1	1	1	80.72
5	-1	1	-1	-1	58.09
6	-1	1	-1	1	76.03
7	-1	1	1	-1	56.69
8	-1	1	1	1	76.52
9	1	-1	-1	-1	71.32
10	1	-1	-1	1	82.99
11	1	-1	1	-1	67.16
12	1	-1	1	1	80.70
13	1	1	-1	-1	66.98
14	1	1	-1	1	66.84
15	1	1	1	-1	66.21
16	1	1	1	1	67.95
17	-2	0	0	0	45.67
18	2	0	0	0	77.16
19	0	-2	0	0	72.64
20	0	2	0	0	71.26
21	0	0	-2	0	77.00
22	0	0	2	0	68.45
23	0	0	0	-2	64.52
24	0	0	0	2	73.41
25	0	0	0	0	83.05
26	0	0	0	0	82.75
27	0	0	0	0	82.79
28	0	0	0	0	82.85
29	0	0	0	0	83.00
30	0	0	0	0	82.85
31	0	0	0	0	82.90

attain a mathematical model that better described the relation among variables and responses. In this study, the model for the hydrolysis parameters and saccharification yield for SCS could be represented by the following equation:

Saccharification yield (%), Y = 82.88535 + 4.104274*X1 - 1.538761*X2 - 1.326197*X3 + 5.990342*X4- 5.319211*X1*X1 - 2.135962*X1*X2 + 0.157051*X1*X3 – 4.523077*X1*X4- 2.686519*X2*X2 + 0.848013*X2*X3 - 2.952628*X2*X4 - 2.492352*X3*X3+ 0.471154*X3*X4 - 3.432416*X4*X4, (1)

where Y is the saccharification yield (%); X1, X2, X3 and X4 are the substrate concentration, pH, temperature and the ratio of syzx4 to Celluclast, respectively. The significance of the second order response model was evaluated by analysis of variance (ANOVA) in Table 4.

The *Pr>F* value of the model in Table 4 was < 0.001 and the coefficient was 0.8953, which showed that the model was significant. This value indicated that the *Pr>F* value has only a 0.01% chance to occur because of noises and 89.53% of the variability in the experiments could be explained by this second order response model. All the linear terms, quadratic terms and cross product terms were significant in RSM model. The Student's t-test and p-value (Pr > |t|) were used to evaluate the significance of each coefficient of the model and the results are shown in Table 5. However, if the magnitude of the t-test is larger and the p-value is smaller, the corresponding coefficient becomes more significant (Yang et al., 2010).

It can be recognized in Table 5 that the hydrolysis parameters with the larger effects were the ratio of syzx4 to celluclast (X4), with a p-value of 0.0001 and the

Table 4. ANOVA results for the saccharification yield of SCS obtained from CCD.

Source	Degrees of freedom	Sum of squares	Mean squares	*F-value*	*Pr>F*
Model	14	3131.163	223.6545	9.771918	0.0001
Linear	4	1364.54	341.135	14.90488	0.0001
Quadratic	4	1211.353	302.8383	13.23162	0.0001
Cross Product	6	555.2695	92.54492	4.043475	0.011754
Error	16	366.1995	22.88747		
Pure Error	6	0.072133	0.012022		
Total	30	3497.362			

R^2, coefficient of determination = 0.8953.

Table 5. The results of t-test and parameter estimates.

Parameter	Estimate	Standard error	t-value	Pr > |t|
X1	4.104274	0.976547	4.202842	0.000674
X2	-1.53876	0.976547	-1.57572	0.134655
X3	-1.3262	0.976547	-1.35805	0.193292
X4	5.990342	0.976547	6.134206	0.0001
X1*X1	-5.31921	0.89464	-5.94565	0.0001
X1*X2	-2.13596	1.196021	-1.78589	0.093081
X1*X3	0.157051	1.196021	0.131311	0.897166
X1*X4	-4.52308	1.196021	-3.78177	0.001634
X2*X2	-2.68652	0.89464	-3.00291	0.008428
X2*X3	0.848013	1.196021	0.709028	0.488511
X2*X4	-2.95263	1.196021	-2.46871	0.02521
X3*X3	-2.49235	0.89464	-2.78587	0.013221
X3*X4	0.471154	1.196021	0.393934	0.69883
X4*X4	-3.43242	0.89464	-3.83665	0.001456

Substrate concentration (X1), with a p-value of 0.000674. Among the quadratic variables, X1*X1, X2*X2 and X4*X4 all had significant effects, for which p-value were 0.0001, 0.008428 and 0.001456, respectively. The coefficient of interaction between X1 and X4 had a p-value of 0.001634 and it was also significant. All the linear, quadratic and interaction relationships among X1 and X4 on the saccharification yield of SCS were statistically significant. These results agreed with the ANOVA of the RSM model which indicated that the relationship between the variables and the response was not simply linear and quadratic, but the interaction was also important.

To further understand the relationships between the hydrolysis parameters and the response, the 3D response surface curves and contour plots were obtained using Matlab R2008a by plotting the response in the Z-axis against any two variables while keeping others at its '0' level in Figure 6. In Figure 6, it was easy and convenient to understand the interactions between two variables and also to locate their optimum levels with the optimal values of the response. It can be seen in Figure 6 that the maximum response (Y) and the highest point of the response surface plots was also the center of the smallest oval in the contour plots; as such, all existed within the tested range.

The effect of X1 (substrate concentration) and X4 (ratio of syzx4 to Celluclast) on the response (Y) saccharification yield of SCS is shown in Figure 6C. It is evident that at low X1 (substrate concentration), the saccharification yield increased significantly with an increase of X4 (Ratio of syzx4 to Celluclast), but decreased smoothly at a higher level of X1 (substrate concentration). Similarly, with an increase of X4 (ratio of syzx4 to Celluclast), the saccharification yield increased smoothly at a higher level of X1 (substrate concentration) and significantly at a lower level of X1 (substrate concentration). The drastic interactions between X1 and X4 can be checked not only by the low p-value (p=0.001634, Table 5), but can also be observed easily from the response surface and contour plots. The sym-metrical response surface plots with the corresponding circular contour plots indicated that the interactions between the variables are negligible. In contrast, unsymmetrical and elliptical ones indicated that the interactions between the

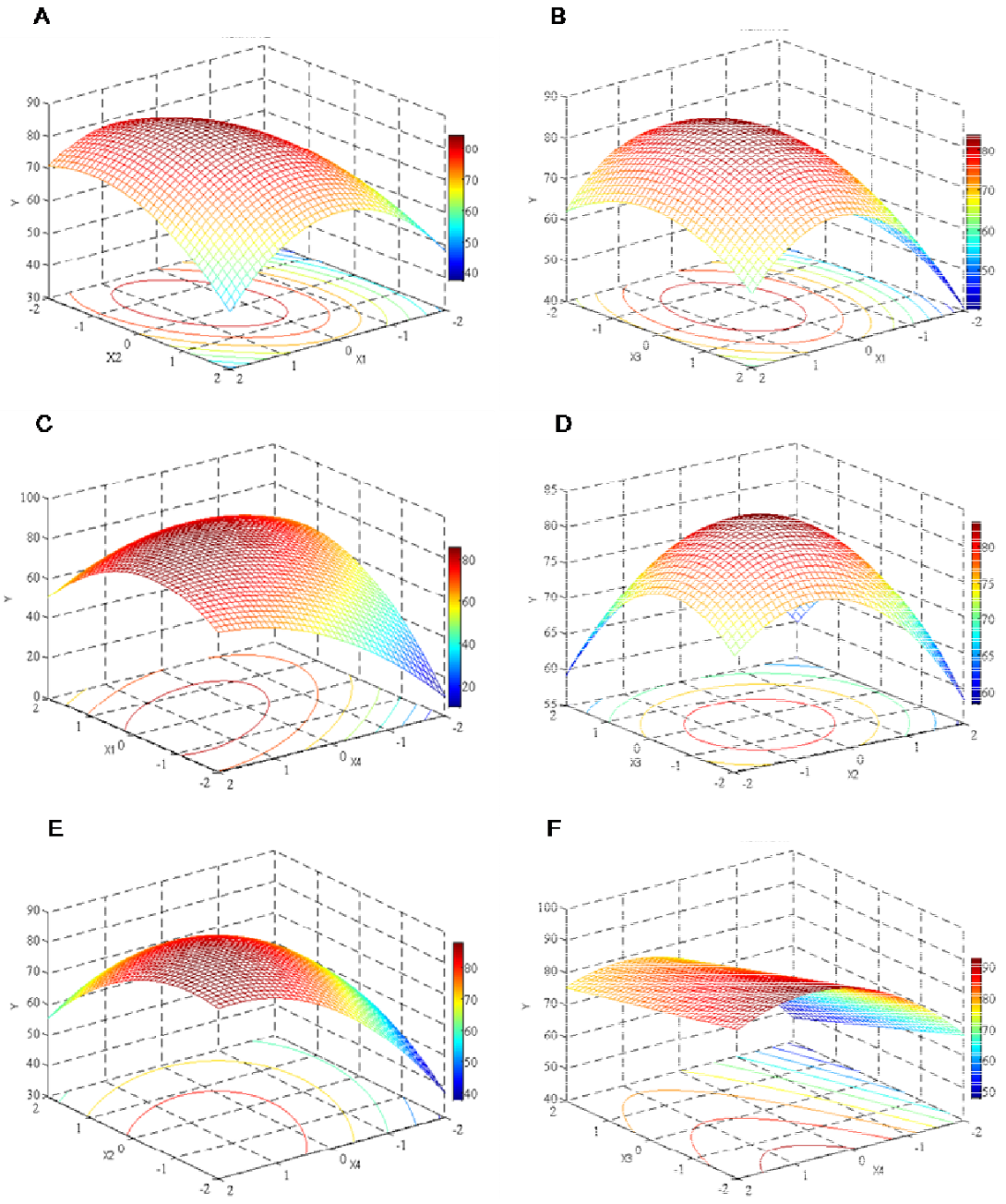

Figure 6. Response surface and contour plots showing the interaction between hydrolysis parameters and saccharification yield of SCS. (A) Interaction between X1 and X2 for Y; (B) interaction between X1 and X3 for Y; (C) interaction between X1 and X4 for Y; (D) interaction between X2 and X3 for Y; (E) interaction between X2 and X4 for Y; (F) interaction between X3 and X4 for Y. Response – Y: saccharification yield (%); Variables X1: the code of substrate concentration; X2: the code of pH; X3: the code of temperature; X4: the code of the ratio of Syzx4 to Celluclast.

corresponding variables are significant (Gu et al., 2005).

The effect of X1 (substrate concentration) and X2 (pH) on the saccharification yield of SCS is shown in Figure 6A. It can be seen from Figure 6A that at low pH value, saccharification yield steadily increased then decreased slowly with increasing substrate concentration, so as the high pH value. Although the interactions between X1 and X4 were negligible, we could conclude that a modest lower pH and higher substrate concentration were beneficial for saccharification.

By solving the fitted equation with SAS software, an optimum hydrolysis condition was found as follows: substrate concentration (3.05%), hydrolysis pH (3.7), hydrolysis temperature (43.4ºC) and ratio of syzx4 to

celluclast, 0.91:1 (18.2 BGU/ g substrate : 20FPU/ g substrate). Then the highest saccharification yield of 87.9%, predicted by the RSM model, could be obtained.

In order to confirm the predicted results of the RSM model, validated experiments, using the optimum hydrolysis condition representing this maximum point, were performed and a mean value of 88.4% was obtained, which was about 7% higher than the single-factor experiment approach. The good agreement between the predicted RSM and validated experiment showed that RSM model can be used to simulate and predict the hydrolysis condition for the saccharification of SCS. The results also suggested that the supplementation of β-glucosidase (syzx4) from *T. cylindrosporum* is a good method for the production of reducing sugars from SCS.

Conclusion

This is the first report about application of a newly isolated strain, *T. cylindrosporum* syzx4, for enzymatic hydrolysis of SCS. In this study, *T. cylindrosporum*, an efficient thermoacidophilic β-glucosidase producer, was established as an effective species for β-glucosidase supplement to commercial cellulose in saccharifying SCS to yield maximum reducing sugar levels. With the help of RSM model, the substrate concentration and the ratio of syzx4 to Celluclast and their interactions were found to be the critical factors for hydrolysis process. Optimum conditions (substrate concentration (3.05%), hydrolysis pH (3.7), hydrolysis temperature (43.4°C) and ratio of syzx4 to Celluclast, 0.91:1 (18.2 BGU/g substrate: 20FPU/ g substrate) for hydrolysis condition were identified at last with RSM model. A mean value of 88.4% saccharification yield was obtained in the validated experiments, which was 23.2% higher than that of Novo-188 from *A. niger*. The supplementation of thermoacidophilic β-glucosidase (syzx4) from *T. cylindrosporum* may be a good way to solve cellulose-degrading system problem and other industrial processes.

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