Supplementary materials S1

Materials and methods for optimization of cellulases extraction from *Aspergillus niger* HQ-1

Microorganism

Aspergillus niger HQ-1 (Accession number of ITS sequence: HQ891869), isolated from the degrading paper, was maintained at 4 °C on potato dextrose agar (PDA) slants in microbiology laboratory, School of life Sciences, Liaocheng University.

Materials

Corn stover (CS) and wheat bran were obtained from nearby farm in Liaocheng (Shandong Province, China). CSP with particle size less than 0.5 mm and wheat bran were used as main components to prepare solid medium for cellulases and xylanases production. Ammonium sulphate (AR), Potassium dihydrogen phosphate (AR), and Magnesium sulfate heptahydrate (AR) were purchased from Xilong Scientific Co., Ltd, China. 3,5-Dinitrosalicylic acid (DNS) (AR) was purchased from Tianjin Guangfu Technology Development Co., Ltd, China. Birch xylan (AR) was purchased from Sigma-Aldrich Co. (USA).

Enzymes production and activities assays

According to the methods described in our previous report [14], seed inoculum was prepared and solid-state fermentation was carried out in 250 mL Erlenmeyer flasks containing the medium with ingredients as follows: CSP 5.0 g, wheat bran 5.0 g, $(NH_4)_2SO_4$ 0.30 g, KH_2PO_4 0.06 g, $MgSO_4 \cdot 7H_2O$ 0.03 g. Prior to sterilization, initial pH and moisture content of the medium were adjusted to 4.7 and 70.3%, respectively. The medium was sterilized at 121 °C for 20 min in high-pressure steam sterilizer.

Then, the prepared seed was inoculated into the medium with 10% (v/w) of inoculum size and cultivated at 33.7 °C for 72 h in biochemical incubator.

Cellulases was extracted from solid fermented residues under the initial conditions. At first, 5.0 g solid residues were immersed with 100 mL distilled water. The mixture was shaken at 25 °C, 120 rpm for 120 min in constant temperature oscillating incubator and filtered. Finally, the filtrate was centrifuged (13,980×g) using a high speed centrifuge at 4 °C for 15 min and the clarified supernatant containing cellulases and xylanases was applied to enzymatic hydrolysis.

Total cellulase activity (filter paper activity, FPA) was assayed by incubating 0.2 mL the diluted enzymes solution with 1.8 mL sodium acetate buffer (50 mM, pH 4.8) containing rolled Whatman NO. 1 filter paper (1 cm × 6 cm, 50 mg) at 50 °C for 60 min. Xylanase activity was determined by incubating 0.2 mL the diluted enzymes solution with 1.8 mL birch xylan (Sigma) solution (1.0%, *w/v*) prepared with sodium acetate buffer (50 mM, pH 4.8) at 50 °C for 30 min. Then 1.5 mL 3,5-dinitrosalicylic acid (DNS) reagent was added and boiled for 5 min to stop the reaction. Absorbance of the appropriately diluted reaction mixtures was measured at 540 nm using ultraviolet visible photometer. One unit (U) of filter paper activity was defined as 1 μmole of glucose equivalent released per minute under the conditions described above, using a glucose standard curve. One unit (U) of xylanase activity was defined as 1 μmole of xylose equivalent released per minute under the conditions described above, using a xylose standard curve.

Methods of optimization of cellulases extraction

To obtain the crude cellulases preparation with high activities, optimization of cellulases extraction using response surface methodology (RSM) was carried out. At first, Plackett-Burman design (PBD) was applied to screening significant factors among four variables including volume of solvent,

temperature, agitation speed and extraction time. Then, the method of steepest ascent was employed to find the optimal regions of significant variables. Finally, Box-Behnken design (BBD) was used to determine the optimal values of the obtained significant factors including volume of solvent, agitation speed and extraction time. All experiments were performed in triplicate and mean values of filter paper activity (FPA) were used as response. The obtained data were analyzed by the least squares method using Minitab (14.12) statistical software package and Statistical Analysis System (SAS, 8.0) to fit the second-order polynomial model, given by the following equation in Eq. (S1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad \text{(S1)}$$

in which, Y was the predicted response, X_1, X_2 and X_3 the coded variables, β_0 intercept, β_1, β_2 and β_3 the linear coefficients, β_{11}, β_{22} and β_{33} the squared coefficients and β_{12}, β_{13} and β_{23} the interaction coefficients.

Supplementary materials S2

Results of optimization of cellulases extraction from *Aspergillus niger* HQ-1

As shown in Table S1, it was obvious that values of FPA in different trials varied from each other. Statistical analysis of PBD (Table S2) indicated that volume of solvent (P = 0.000), agitation speed (P = 0.000) and extraction time (P = 0.000) had significant effects on FPA. The three significant factors were chosen and the optimal regions of them were investigated using the steepest ascent method. Results in Table S3 indicated that FPA reached the plateau while volume of solvent, agitation speed and extraction time were 9.0 mL/g substrate, 170 rpm and 110 min, respectively. Therefore, the optimal values of the factors were ensured by using BBD (Table S4). Results in Table S5 indicated that linear terms including X_1 (P = 0.000), X_2 (P = 0.001) and X_3 (P = 0.002) and square terms including X_1^2 (P = 0.000), X_2^2 (P = 0.000) and X_3^2 (P = 0.000) had significant effects on FPA. P values of the model (P = 0.000) and lack of fit (P = 0.127) in Table S6 and high values of R^2 (99.2%) and adjusted R^2 (97.8%) in Table S5 indicated that the model was adequate to predict FPA during enzymes extraction.

According to canonical analysis, maximal filter paper activity (6.11 U/mL) could be obtained while volume of solvent, agitation speed and extraction time were 8.54 mL/g substrate, 161.7 rpm and 106.4 min, respectively. The resulting regression model was given in Eq. (S2):

$$Y = 6.05333 - 0.22500X_1 + 0.18125X_2 - 0.16225X_3 - 0.50417X_1^2 - 0.59667X_2^2 - 0.48167X_3^2 - 0.06250X_1X_2 - 0.01750X_1X_3 - 0.05500X_2X_3 \quad \text{(S2)}$$

in which, Y was predicted filter paper activity, X_1, X_2 and X_3 were codes of volume of solvent, agitation speed and extraction time, respectively.

In order to confirm the optimization results, the suggested extraction conditions (volume of solvent 8.54 mL/g substrate, agitation speed 162 rpm and extraction time 106.4 min) were performed in triplicate and average FPA was 6.13 U/mL, which was 0.33% higher than the predicted values (6.11 U/mL). Compared with initial FPA (2.02 U/mL) under unoptimized conditions [1], it was obvious that optimization results in 2.02-fold increase for FPA. In addition, the corresponding xylanase activity of the obtained enzymes solution after optimization was 889.18 U/mL.

Supplementary materials

Results of optimization of cellulases extraction from *Aspergillus niger* HQ-1

Table S1. Codes and levels of four variables and Plackett-Burman design (PBD) along with filter paper activity during optimization of cellulases extraction

Trials	A	B	C	D	FPA (U/mL)
1	1 (17.0)	-1 (20.0)	1 (120.0)	-1 (40.0)	1.45 ± 0.023
2	1	1 (30.0)	-1 (100.0)	1 (60.0)	1.56 ± 0.025
3	-1 (15.0)	1	1	-1	1.67 ± 0.026
4	1	-1	1	1	1.69 ± 0.030
5	1	1	-1	1	1.52 ± 0.024
6	1	1	1	-1	1.36 ± 0.019
7	-1	1	1	1	1.88 ± 0.035
8	-1	-1	1	1	1.94 ± 0.037
9	-1	-1	-1	1	1.71 ± 0.036
10	1	-1	-1	-1	1.23 ± 0.019
11	-1	1	-1	-1	1.34 ± 0.022
12	-1	-1	-1	-1	1.41 ± 0.024
13	0 (16.0)	0 (25.0)	0 (110.0)	0 (50.0)	1.43 ± 0.021
14	0	0	0	0	1.47 ± 0.025
15	0	0	0	0	1.44 ± 0.026

A: Volume of solvent (mL/g substrate); B: Temperature (°C); C: Agitation speed (rpm); D: Extraction time (min); FPA: Filter paper activity

Supplementary materials

Results of optimization of cellulases extraction from *Aspergillus niger* HQ-1

Table S2. Coefficients of regression for filter paper activity by *Aspergillus niger* HQ-1

Terms	Effect	Coeff	SE Coeff	*T*	*P*
Constant		1.5633	0.01023	123.76	0.000
Volume of solvent	-0.1900	-0.0950	0.01023	-7.52	0.000**
Temperature	-0.0167	-0.0083	0.01023	-0.66	0.526
Agitation speed	0.2033	0.1017	0.01023	8.05	0.000**
Extraction time	0.3067	0.1533	0.01023	12.14	0.000**

Outline criterion: 0.05; ** Significant at 1% level

Supplementary materials

Results of optimization of cellulases extraction from *Aspergillus niger* HQ-1

Table S3. Design of the steepest ascent method along with filter paper activity during optimization of cellulases extraction

Steps	Volume of solvent (mL/g substrate)	Agitation speed (rpm)	Extraction time (min)	FPA (Filter paper activity, U/mL)
1	13.0	130	70	3.15 ± 0.047
2	11.0	150	90	4.36 ± 0.052
3	9.0	170	110	6.01 ± 0.058
4	7.0	190	130	4.75 ± 0.051

Supplementary materials

Results of optimization of cellulases extraction from *Aspergillus niger* HQ-1

Table S4. Codes and levels of variables and BBD along with filter paper activity during optimization of cellulases extraction

Runs	X_1 (Volume of solvent, mL/g substrate)	X_2 (Agitation speed, rpm)	X_3 (Extraction time, min)	FPA (Filter paper activity, U/mL)
1	-1 (7.0)	-1 (150.0)	0 (110.0)	4.94 ± 0.056
2	1 (11.0)	-1	0	4.65 ± 0.058
3	-1	1 (190.0)	0	5.38 ± 0.055
4	1	1	0	4.84 ± 0.049
5	-1	0 (170.0)	-1 (90.0)	5.41 ± 0.056
6	1	0	-1	4.96 ± 0.049
7	-1	0	1 (130.0)	5.21 ± 0.063
8	1	0	1	4.69 ± 0.051
9	0 (9.0)	-1	-1	4.93 ± 0.058
10	0	1	-1	5.45 ± 0.076
11	0	-1	1	4.61 ± 0.063
12	0	1	1	4.91 ± 0.074
13	0	0	0	6.02 ± 0.079
14	0	0	0	6.05 ± 0.081
15	0	0	0	6.09 ± 0.076

Supplementary materials

Results of optimization of cellulases extraction from *Aspergillus niger* HQ-1

Table S5. Estimated regression coefficients for filter paper activity during optimization of cellulases extraction

Terms	Coefficient estimate	Standard error coefficient	*T*	*P*
Constant	6.05333	0.04354	139.015	0.000
Volume of solvent (X_1)	-0.22500	0.02667	-8.438	0.000**
Agitation speed (X_2)	0.18125	0.02667	6.797	0.001**
Extraction time (X_3)	-0.16625	0.02667	-6.235	0.002**
Volume of solvent × volume of solvent ($X_1 \times X_1$)	-0.50417	0.03925	-12.845	0.000**
Agitation speed × agitation speed ($X_2 \times X_2$)	-0.59667	0.03925	-15.202	0.000**
Extraction time × extraction time ($X_3 \times X_3$)	-0.48167	0.03925	-12.272	0.000**
Volume of solvent × agitation speed ($X_1 \times X_2$)	-0.06250	0.03771	-1.657	0.158
Volume of solvent × extraction time ($X_1 \times X_3$)	-0.01750	0.03771	-0.464	0.662
Agitation speed × extraction time ($X_2 \times X_3$)	-0.05500	0.03771	-1.458	0.205

Outline criterion: 0.05; ** Significant at 1% level; R^2: 99.2%; Adj-R^2: 97.8%

Supplementary materials

Results of optimization of cellulases extraction from *Aspergillus niger* HQ-1

Table S6. Analysis of variance (ANOVA) for the fitted quadratic polynomial model for cellulases extraction

Source	Degrees of freedom	Sum of squares	Mean square	*F*	*P*
Model	9	3.61885	0.402095	70.69	0.000
Linear	3	0.88892	0.296308	52.09	0.000**
Square	3	2.70098	0.900326	158.28	0.000**
Interaction	3	0.02895	0.009650	1.70	0.282
Residual error	5	0.02844	0.005688		
Lack of fit	3	0.02598	0.008658	7.02	0.127
Pure error	2	0.00247	0.001233		
Total	14	3.64729			

Outline criterion: 0.05; ** Significant at 1% level

Supplementary materials

Tables for optimization of alkaline hydrogen peroxide pretreatment of wheat straw

Table S7. Codes and levels of five variables and Plackett-Burman design (PBD) along with glucose and xylose yields during optimization of pretreatment

Trials	A	B	C	D	E	Y_1	Y_2
1	1	-1 (100)	1 (25)	-1 (150)	-1 (3.0)	99.18 ± 1.21	32.12 ± 0.52
2	1 (35.0)	1 (200)	-1 (10)	1 (100)	-1	84.03 ± 1.05	29.88 ± 0.68
3	-1 (25.0)	1	1	-1	1 (5.0)	92.85 ± 1.01	33.35 ± 0.54
4	1	-1	1	1	-1	96.48 ± 1.22	31.34 ± 0.49
5	1	1	-1	1	1	94.52 ± 1.13	34.09 ± 0.56
6	1	1	1	-1	1	114.18 ± 1.26	39.22 ± 0.68
7	-1	1	1	1	-1	82.68 ± 1.09	29.07 ± 0.48
8	-1	-1	1	1	1	90.41 ± 1.18	32.89 ± 0.49
9	-1	-1	-1	1	1	78.71 ± 1.03	29.72 ± 0.48
10	1	-1	-1	-1	1	101.32 ± 1.23	36.12 ± 0.53
11	-1	1	-1	-1	-1	62.76 ± 0.98	23.55 ± 0.33
12	-1	-1	-1	-1	-1	64.12 ± 0.92	23.86 ± 0.34
13	0	0	0	0	0	80.24 ± 1.12	29.41 ± 0.42
14	0	0	0	0	0	82.25 ± 1.19	30.03 ± 0.56
15	0	0	0	0	0	81.04 ± 1.15	30.22 ± 0.54

A: Pretreatment temperature (°C); B: Solid loading (g/L); C: Hydrogen peroxide concentration (g/L); D: Agitation speed (rpm); E: Pretreatment time (h); Y_1: Glucose (mg/g_{ds}); Y_2: Xylose (mg/g_{ds})

Supplementary materials

Tables for optimization of alkaline hydrogen peroxide pretreatment of wheat straw

Table S8. Design of the steepest ascent method along with glucose and xylose yields during optimization of pretreatment

Steps	x_1 (Pretreatment temperature (°C)	x_2 (Hydrogen peroxide concentration, g/L)	x_3 (Pretreatment time, h)	Y_1 (Glucose, mg/g_{ds})	Y_2 (Xylose, mg/g_{ds})
1	35.0	20	5.5	106.83 ± 1.12	36.77 ± 0.61
2	42.0	45	6.5	155.74 ± 1.52	51.06 ± 0.78
3	49.0	70	7.5	231.19 ± 2.12	90.76 ± 1.34
4	56.0	95	8.5	168.22 ± 1.94	58.75 ± 0.89
5	63.0	120	9.5	120.35 ± 1.11	39.51 ± 0.71

Supplementary materials

Tables for optimization of alkaline hydrogen peroxide pretreatment of wheat straw

Table S9. Codes and levels of variables and BBD along with glucose and xylose yields during optimization of pretreatment

Runs	x_1 (Pretreatment temperature, °C)	x_2 (Hydrogen peroxide concentration, g/L)	x_3 (Pretreatment time, h)	Y_1 (Glucose, mg/g_{ds})	Y_2 (Xylose, mg/g_{ds})
1	-1 (43.0)	-1 (55)	0 (7.5)	178.29 ± 1.95	68.55 ± 1.12
2	1 (55.0)	-1	0	183.83 ± 1.81	74.82 ± 1.05
3	-1	1 (85)	0	182.53 ± 1.91	73.71 ± 1.33
4	1	1	0	191.13 ± 2.04	77.58 ± 1.04
5	-1	0 (70)	-1 (6.9)	162.12 ± 1.66	67.25 ± 0.93
6	1	0	-1	175.59 ± 1.81	77.95 ± 1.27
7	-1	0	1 (8.1)	171.74 ± 1.76	78.66 ± 1.23
8	1	0	1	185.45 ± 1.97	80.07 ± 1.24
9	0 (49.0)	-1	-1	180.34 ± 1.79	69.41 ± 0.91
10	0	1	-1	187.78 ± 1.89	78.54 ± 1.18
11	0	-1	1	184.57 ± 1.78	78.36 ± 1.14
12	0	1	1	190.31 ± 1.93	82.84 ± 1.23
13	0	0	0	231.49 ± 2.32	91.65 ± 1.51
14	0	0	0	233.97 ± 2.42	90.92 ± 1.53
15	0	0	0	234.18 ± 2.25	90.86 ± 1.55

Supplementary materials

Tables for optimization of enzymatic hydrolysis of the pretreated wheat straw

Table S10. Codes and levels of six variables and Plackett-Burman design (PBD) along with glucose and xylose yields during optimization of enzymatic hydrolysis

Trials	A	B	C	D	E	F	Y_3	Y_4
1	1 (30.0)	-1 (50.0)	1 (7.0)	-1 (4.4)	-1 (20)	-1 (0.3)	296.44 ± 3.04	117.55 ± 1.56
2	1	1 (55.0)	-1 (3.0)	1 (4.8)	-1	-1	260.61 ± 2.71	105.91 ± 1.53
3	-1 (26.0)	1	1	-1	1 (40)	-1	291.65 ± 2.83	116.84 ± 1.89
4	1	-1	1	1	-1	1 (0.5)	295.72 ± 2.81	125.81 ± 1.21
5	1	1	-1	1	1	-1	316.25 ± 3.11	114.51 ± 1.62
6	1	1	1	-1	1	1	340.58 ± 3.58	137.96 ± 1.91
7	-1	1	1	1	-1	1	245.44 ± 2.64	100.11 ± 1.38
8	-1	-1	1	1	1	-1	301.01 ± 3.11	120.05 ± 1.88
9	-1	-1	-1	1	1	1	259.17 ± 2.55	104.15 ± 1.46
10	1	-1	-1	-1	1	1	316.18 ± 3.26	124.46 ± 2.08
11	-1	1	-1	-1	-1	1	210.58 ± 2.28	84.96 ± 1.34
12	-1	-1	-1	-1	-1	-1	210.37 ± 2.21	87.25 ± 1.26
13	0	0	0	0	0	0	262.64 ± 2.71	113.21 ± 1.72
14	0	0	0	0	0	0	262.93 ± 2.66	112.36 ± 1.78
15	0	0	0	0	0	0	260.53 ± 2.64	110.98 ± 1.69

A: Reaction time (h); B: Reaction temperature (°C); C: Enzyme loading (FPU/g_{ds}); D: Reaction pH; E: Biomass loading (g/L); F: Tween-80 concentration (%, w/v); Y_3: Glucose (mg/g_{ds}); Y_4: Xylose (mg/g_{ds})

Supplementary materials

Tables for optimization of enzymatic hydrolysis of the pretreated wheat straw

Table S11. Design of the steepest ascent method along with glucose and xylose yields during optimization of enzymatic hydrolysis

Steps	X_1 (Reaction time, h)	X_2 (Enzyme loading, FPU/g_{ds})	X_3 (Biomass loading, g/L)	Y_3 (Glucose, mg/g_{ds})	Y_4 (Xylose, mg/g_{ds})
1	28.0	6.0	50	324.46 ± 3.49	126.05 ± 1.68
2	32.0	8.0	70	416.29 ± 4.43	171.63 ± 2.39
3	36.0	10.0	90	539.44 ± 5.24	221.49 ± 2.83
4	40.0	12.0	110	490.13 ± 5.14	198.04 ± 2.32
5	44.0	14.0	130	348.54 ± 4.24	139.75 ± 2.03

Supplementary materials

Tables for optimization of enzymatic hydrolysis of the pretreated wheat straw

Table S12. Codes and levels of variables and CCD along with glucose and xylose yields during optimization of enzymatic hydrolysis

Runs	X_1 (Reaction time, h)	X_2 (Enzyme loading, FPU/g_{ds})	X_3 (Biomass loading, g/L)	Y_3 (Glucose, mg/g_{ds})	Y_4 (Xylose, mg/g_{ds})
1	-1 (32.0)	-1 (7.0)	-1 (70)	376.14 ± 3.83	160.41 ± 1.93
2	1 (40.0)	-1	-1	394.72 ± 3.91	169.31 ± 2.13
3	-1	1 (13.0)	-1	442.81 ± 4.66	168.04 ± 1.98
4	1	1	-1	464.87 ± 4.57	184.13 ± 1.92
5	-1	-1	1 (110)	364.74 ± 3.77	146.11 ± 1.83
6	1	-1	1	388.75 ± 3.93	155.67 ± 1.85
7	-1	1	1	419.39 ± 4.37	154.61 ± 2.09
8	1	1	1	448.85 ± 4.61	177.98 ± 2.17
9	-1.682 (29.3)	0 (10.0)	0 (90)	414.07 ± 4.42	174.81 ± 2.29
10	1.682 (42.7)	0	0	484.77 ± 5.23	196.61 ± 2.67
11	0 (36.0)	-1.682 (4.95)	0	346.76 ± 3.56	141.76 ± 2.18
12	0	1.682 (15.05)	0	447.02 ± 4.61	174.34 ± 2.26
13	0	0	-1.682 (56.4)	437.66 ± 4.51	164.93 ± 2.07
14	0	0	1.682 (123.6)	418.14 ± 4.31	138.93 ± 1.92
15	0	0	0	542.67 ± 5.61	224.36 ± 3.46
16	0	0	0	550.47 ± 5.68	225.18 ± 3.31
17	0	0	0	540.87 ± 5.47	221.25 ± 3.28
18	0	0	0	541.12 ± 5.31	224.68 ± 3.11
19	0	0	0	550.26 ± 5.14	221.15 ± 3.16
20	0	0	0	540.71 ± 5.24	220.38 ± 3.23