

Efficient Cellulase Production from Corn Straw by *Trichoderma Reesei* LW1 through Solid State Fermentation Process

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Abstract:

Cellulase is important enzyme required for catabolism of cellulose into smaller sugars in the animal metabolism. Cellulose is plant polysaccharides and contained higher range of sugar molecules those with help of suitable enzyme and microorganism can be used for conversion of sugar into biofuels to meet the requirements of energy. In the present study Cellulase was produced from corn straw by solid state fermentation with the help of *Trichoderma Reesei*. The corn straw was supplemented with wheat bran for supply of essential elements (N and C). The optimal experimental conditions like temperature, pH, time total water contents of substrate were selected for this study. It was observed that cellulase shows its maximum activity at when corn straw and wheat bran ratio is 1:1 (water contents ratios 1:2). The optimal temperature 28°C, pH 5.5 and time duration for maximum activity was 72 hours. Furthermore the fermented activity of FPase 119.41 U followed by CMCase 452.5 U was observed in the present study.

Keywords: *Trichoderma Reesei*, cellulase, solid state fermentation

Introduction

Corn is an important crop in all over world including People Republic of China. Total annual production of corn increased than 0.62 billion tons every year. The main uses of corn include food and feed, production of cooking oil and many others. However, corn straw is used for only for feed of animals and fire purposes in the rural areas of most of countries of the world.

Biotechnology of cellulase and hemicellulase began in early 1980s, first in animal feed followed by food applications (Chesson, 1987; Thomke *et al.*, 1980; Voragen. *et al.*, 1980, 1986). Subsequently, these enzymes were used in the textile, laundry as well as in the pulp and paper industries (Godfrey 1996; Wong and Saddler, 1992, 1993). However, since last two decades, the use of cellulases, hemicellulases and pectinases has increased considerably, especially in textile, food, brewery and wine as well as in pulp and paper industries (Godfrey and West, 1996b; Harman and Kubicek, 1998; Saddler, 1993; Uhlig, 1998). Today these enzymes account for approximately 20% of the world enzyme market (Mantyla *et al.*, 1998), mostly from *Trichoderma* and *Aspergillus* (Godfrey and West, 1996b; Uhlig, 1998).

It was reported by many authors that *Trichoderma reesei* is capable of increase the production of cellulase from substrate like corn straw and similar others. *Trichoderma. reesei* can be chosen as model fungus as it shows both the challenging rheology and a cellulase complex product that is shear- sensitive.(Weber, 2005). Cellulose, which is a polymer of glucose is the primary structural component of most plant cell walls. Although this polysaccharide is the most common carbohydrate on earth, relatively few animals are able to utilize this resource efficiently (Goodenough and. Goodenough, 1993) .Utilization of cellulose as a nutrient source requires enzymes that cleave β -1,4 glycosidic bonds between constituent sugars. The enzymes referred to as cellulases, are required to split β -1,4 glucosidic bonds in the polymer to release glucose units (Barr *et al*, 1996). In living organisms, cellulase activity represents a system of enzymes that act synergistically to degrade cellulose. The system includes endoglucanases (EC 3.2.1.4) which hydrolyse β -1,4 glucosidic bonds randomly.

Most higher animal species lack the genes that encode enzymes capable of degrading plant cell wall polysaccharides. Herbivorous vertebrates, however, have evolved digestive systems which contain symbiotic microorganisms capable of degrading these compounds (Goodenough and Goodenough, 1993). The strategies employed by invertebrates to digest cellulose, however, are less well understood.

Ethanol is an alcohol made from the fermentation of the carbohydrate or sugar fraction in biomass (plant) materials. Over 95 percent of the ethanol produced in the different countries including United States today is manufactured by dry or wet milling processes. These are relatively simple processes: corn grinding and starch separation, saccharification, fermentation and distillation.

Therefore present study was undertaken with following aim and objectives.

1. To find out cheapest source of cellulase production
2. To assess the corn straw for high production of cellulase

- Effectively utilization of *Trichoderma reesei* for production of cellulase
- Assessment of solid state fermentation for production of cellulase from corn straw

Materials and Methods

Collection of Samples

In the present experiment corn straw was used for production of cellulase. The samples of corn straw were collected from fields of Agriculture university of Hebei. Where as wheat bran (powder form) was collected from a factory working on production wheat flour. The samples of corn straw were sun dried followed by oven drying at 70 °C for overnight.



Preparation of Samples

The dried samples of corn straw were grounded into powder form and were kept in plastic bags at 4 °C for further uses. During present experiment corn straw samples were supplemented with equal amount of wheat bran.

Culture medium

(i) Media for growth of *Trichoderma. Reesei*

For the growth of fungi (*Trichoderma. Reesei*); Total of 200 g of Potato chips (without skin) were added with 1000 ml of water, boiled and filtered. Moreover, 20 g of agar and 20 g of dextrose were also added and sterilized at 121 °C for 20 minutes.

(ii)Media for cellulase

Corn straw 5 g , wheat bran 5 g, 0.2g (NH₄)₂SO₄, 0.06 g (KH₂P₃O₄) , 0.01g (MgSO₄.7H₂O) and 20 ml of H₂O was mixed together and sterilized for 1 hour at 121 °C .

(iii) Microorganisms (*Trichoderma reesei*)

Trichoderma reesei was obtained from the Microbiological Department of Science and Technology University of Tianjin. The organism was subcultured every month for used in this experiment and was stored at 4 °C .

(iv) Tests for activity of cellulase (FPase)

In order to check the activity of enzyme, 5 test tubes containing 50 mg (each) of filter paper was added with 2 ml of 0.2 M buffer of HAC- NaAC having pH 4.8 .The test tubes were kept in water bathing at 50 °C for 5 minutes. After that 0.5 ml of cellulase in each test tubes was added and keep at 50 °C for 1 h . Each test tubes was further add 3 ml of a mixture (containing 6.5g of 3,5-Dinitrosalicylic acid ,325 ml of 2 M NaOH, 37.1 ml of Glycerol with final volume 1000 ml) . Keep the test tubes in the boiling water for 5 minutes and cold down the temperature. Finally volume of test tubes was increased up to 25 ml by adding dist. water.

(v) Test for activity of cellulase (CMCase)

For second test, a 2 ml of solution (containing 1 g of CMC ,100 ml of buffer, 0.2 M HAC- NaAC) having pH 4.8 was taken in 5 test tubes and was added 2 ml of CMC- NaAC buffer. The tubes were kept in water bath at 50 °C for 5 minutes. Further added 1 ml of cellulase and warm in water bath at 50 °C for 30 minutes. Finally 3 ml of solution (in section iv) was added. The absorbance of FPase CMCase was measured at 540 nm with spectrophotometer (T6). Furthermore a calibration curve was prepared to show activity of dextrose and cellulase. Similar procedure was repeated five times to get required data which was analyzed statistically for averages and standard deviation values.

Experimental

The experiment was completed in 6 stages in which Sample of corn straw (5g) along with wheat bran (5 g) was mixed up with 2 ml bacteria culture media ($1.7-2 \times 10^6$ cell/ml)

(1) Above reaction mixture was studied for 36 to 156 hours to check proper time for maximum activity of cellulase. The activity of cellulase was checked after every 12 hours.

(2) The reaction mixture was kept at different temperature like 24, 26, 28, 30, 34, 36 for 72 hours. The activity of cellulase was checked at different stages of temperature.

(3) The reaction mixture was studied at different pH, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 to get the optimal pH on which this bacterium can produce maximum cellulase.

(4) The total contents of solid and water ratios were studied like 1:1, 1:1.5, 1:2, 1:2.5, 1:3 at 28 for 72 hours.

(5) The corn straw and wheat bran ratios was studied, like 1:4, 2:3, 1:1, 3:2, 4:1 at 28 for 72 hours were observed.

(6) Orthogonal study of three factors (Temperature, pH and duration) was carried out to select optimal condition for production of maximum amount of cellulase..

Results

Data obtained after analysis in the experimental work is given in the tables 1-6; whereas comparison of these parameters are given in Figs 1-5.

The activity of cellulase was checked after every 12 hours (Table.1). Whereas comparison of cellulase activity during different time period (Fig.1) in ascending order was studied for selection of suitable time for production of maximum cellulase activity. It was observed that cellulase show maximum activity after 72 hours of time period which was 451.9 ± 1.4 U for CMCase, followed by 111.01 ± 3.1 U for FPase (Table 1 and Fig.1).

Data in Tables 2 and 3 shows result of different temperatures and pH at time period of 72 hours. The comparison of these different degrees of temperature and pH are given in Fig.2 and 3.

The results of total water contents of substrates are at 28 for 72 when the pH was 5 was studied (Table 4). Whereas comparisons are given in Fig.4, the suitability of corn straw and wheat bran ratio is given in the table 5 and Fig.5.

The comparison of Temperature, pH and Time duration was studied (orthogonal studied) in order to create suitable condition for production of maximum activity of cellulase (Table 6).

Table 1. The effect of fermenting period for CMCase and Fpase during different intervals of time.

	CMCase	FPase
36h	74.4 \pm 4.5hF	53.9 \pm 2.4eE
48h	150.0 \pm 5.5fE	54.8 \pm 2.8deE
60h	270.0 \pm 8.9deD	65.6 \pm 3.1cDE
72h	451.9 \pm 1.4aA	111.0 \pm 3.1aA
84h	393.6 \pm 1.1bBC	80.4 \pm 4.8bBC
96h	399.3 \pm 3.9bB	54.6 \pm 3.5deE
108h	358.8 \pm 1.7cC	40.5 \pm 3.2fCD
120h	292.2 \pm 5.6dD	59.7 \pm 2.0cdB
132h	255.3 \pm 8.2eD	54.3 \pm 1.8deBC
144h	294.0 \pm 1.0dD	47.3 \pm 1.5efB
156h	114.6 \pm 1.4gE	58.5 \pm 1.9cdBC

Fig. 1. The Comparison for Fermenting period of CMCase and Fpase.

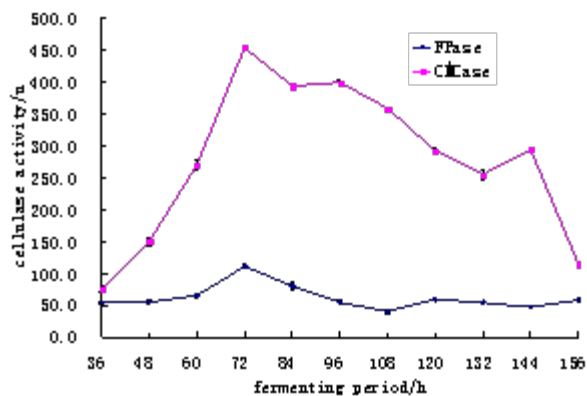


Table 2. The Effect of Temperature on Fermentation carried out by CMCase and FPase.

Temperature/°C	CMCase	FPase
24	213.8±2.0dD	53.9±3.5eD
26	393.8±3.7abAB	92.4±1.9bA
28	416.3±7.6aA	97.9±3.3aA
30	385.8±2.6abAB	94.2±0.4abA
32	367.8±1.6bB	82.2±1.8cB
34	316.3±2.2cC	82.4±1.1cB
36	242.8±1.6dD	76.4±2.2dC

Fig. 2. The Comparison in Fermenting Temperature for CMCase and FPase.

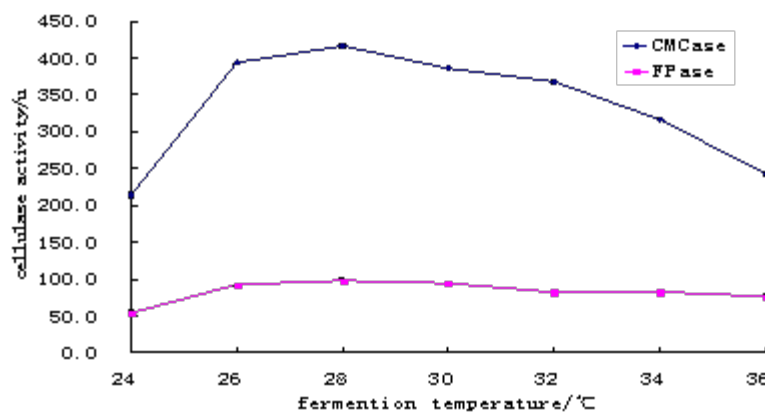


Table 3. The effect of initial pH on the activities of CMCase and FPase.

initial pH	CMCase	FPase
4.0	289.3±4.2cBC	53.2±5.1bB
4.5	318.3±2.6bcAB	53.4±2.2bB
5.0	374.8±2.2aA	70.4±3.1aA
5.5	235.8±2.5dC	56.7±1.8bB
6.0	227.3±1.6dC	51.7±1.7bB
6.5	325.8±0.1bcAB	66.9±0.7aA
7.0	339.3±3.9abAB	66.7±3.7aA

Fig.3. The Comparison in effects of initial pH on CMCase and FPase

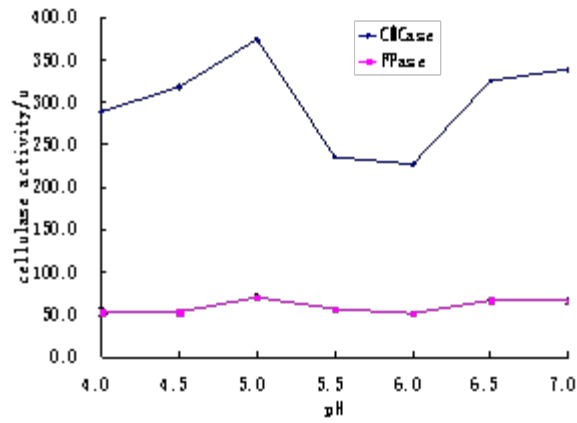


Table.4. Effects Water contents ratio of Corn straw on CMCase and FPase.

Solid:water	CMCase	FPase
1:1	198.8±1.7dC	53.9±3.4dD
2:3	212.3±4.8cC	87.1±2.2bB
1:2	288.3±1.0aA	95.7±1.8aA
2:5	263.3±1.1bB	63.7±0.4cC
1:3	115.3±2.2eD	61.9±2.6cC

Fig.4. Comparison in water contents ratio of corn straw for CMCase and FPase.

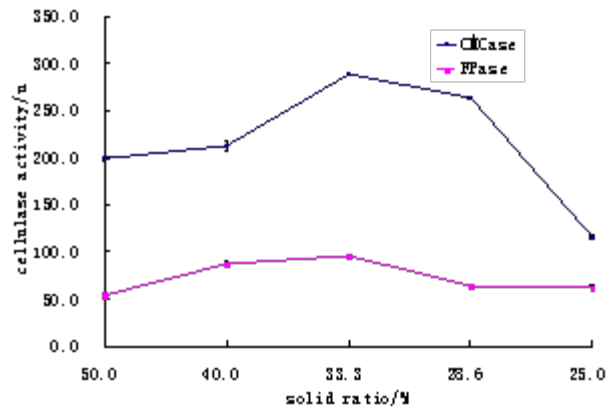


Table.5. Wheat bran and Corn straw ratios for CMCase and FPase activity.

corn straw: wheat bran	CMCase	FPase
1:4	198.3±1.7cB	85.6±2.5bAB
2:3	151.3±1.9dC	82.6±7.9bAB
1:1	286.8±1.7aA	94.6±1.5aA
3:2	234.8±3.1bB	80.9±2.4bB
4:1	216.8±5.6bcB	88.8±2.0bAB

Fig.5. Comparison between wheat bran and corn straw ratio for CMCase and FPase

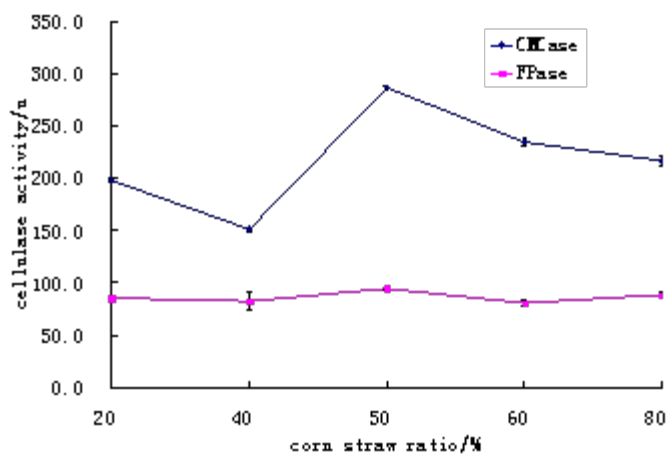


Table 6. Orthogonal Study of three Parameters for CMCase and FPase.

factors			CMCase/u	FPase/u
Temperature/°C	Time/h	pH		
26	60	5.0	124.8±2.7	55.4±0.86
26	72	5.5	198.8±3.7	74.3±2.0
26	84	6.0	82.3±4.3	69.5±1.4
28	60	5.5	161.7±2.9	90.1±4.8
28	72	6.0	237.7±4.1	81.4±1.5
28	84	5.0	181.2±6.4	63.8±6.9
30	60	6.0	130.8±2.5	62.2±3.1
30	72	5.0	123.5±2.5	73.7±1.5
30	84	5.5	195.3±3.9	61.3±1.1
28	72	5.5	452.5±2.6	119.4±1.7

Discussion

It was observed that maximum production time for cellulase is 72 hours at pH 5.0. This is the time when cellulase shows maximum activity in substrate like corn straw. The results obtained in the present study about the activity of cellulase were higher than, 5.5 U and 4.0 U cellulase activity in FPase reported by Kalogeris *et al.* (2003) and Xu *et al.* (2002).

Result showed that 28°C was suitable temperature (at pH 5) for maximum cellulase activity when other conditions are favorable (Table 2 and Fig.2). The suitable temperature found in the present study was slightly higher than 25°C at 6.7 pH reported by Wen *et al.* (2005) and 30°C at pH 4.5 reported by Yang *et al.* (2004). However, values obtained always depend on the nature of substrate used for production of cellulase.

Whereas the ratio of total solid and water contents were found 1:2 those were more reliable for maximum activity of cellulase (Table 4 and Fig. 4). The increase or decrease contents could affect enzyme activity (Chundakhadu, 1999).

It was observed that suitable ratio for corn straw and wheat bran was 1:1 and this ratio can provide sufficient cellulase activities. (Table 5 and Fig.5)

The orthogonal study of three parameters like temperature, pH and time duration shows that the most favorable condition for maximum activity of cellulase was 28 °C at pH 5.5 and time duration of this study was 72 hours (Table 6)

Conclusion

Cellulase production from corn straw with fungi like *Trichoderma reesei* through solid state fermentation is important because in this way production of cellulase can be increased, which further helps to produce cellulose. This is an important enzyme required for breakdown of polysaccharides into monosaccharides, those can further be converted into ethanol and other alcohols through fermentation process. Cellulase has a lot of industrial applications including production of food and medicines and helps to break down waste plant materials to clean up the environment.

It is therefore concluded that by using different parameters study in this experiment high yield of cellulase could be

obtained. It is expected that production of cellulase will increase manifold in future to meet the future requirements.

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