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Cellulase Production by *A. niger* on Banana Pseudostem Waste

C.A. Bhavsar and Tejomyee S. Bhalerao*

School of Environmental and Earth Sciences, North Maharashtra University,
P.B. No. 80, Jalgaon - 425 001, India.

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Cellulase is a synergistic enzyme that is used to break up cellulose into glucose or other oligosaccharide compounds. The study focuses on the production of cellulase from *A. niger*. The physico-chemical conditions such as pH, temperature, inoculum size, fermentation period, static and agitation conditions, effect of different carbon and nitrogen sources were studied and optimized for cellulase production by *A. niger*. Banana pseudostem waste proved to be the best carbon source for cellulase production and peptone proved the best nitrogen source for cellulase production. Maximum cellulase production obtained at broad pH and temperature ranges. Inoculum size 10 discs of 8 mm size, incubation of 72 h and at 35ºC fermentation temperature obtained maximum cellulase activity. Cellulase production by *A. niger* at 120 rpm found best. All these results proved applicability of cellulase enzyme produced by *A. niger* in various industrial and biotechnological applications.

Key words: Cellulase, *A. niger,* Banana pseudostem waste.

Cellulases are a group of hydrolytic enzymes and are capable of degrading lignocellulosic materials. Cellulases have wide range of applications. Active research on cellulases and related polysaccharidases began in the early 1950s, owing to their enormous potential to convert lignocellulose, the most abundant and renewable energy source on Earth, to glucose and soluble sugars (Coughlan, 1985a, b; Mandels, 1985; Reese, 1976; Reese and Mandels, 1984). Extensive basic and applied research during the 1970s and 1980s demonstrated that the enzyme-induced bio-conversion of lignocellulose to soluble sugars was rather difficult and uneconomical (Coughlan, 1985a; Ladisch *et al.*, 1983; Mandels, 1985; Ryu and Mandels, 1980). Nevertheless, continued research on cellulases, hemicellulases and Pectinases revealed their biotechnological potential in various industries, including food, brewery and wine, animal feed, textile and laundry, pulp and paper, agriculture, as well as in research and development (Bajpai, 1999; Bayer *et al.*, 1994; Beguin and Aubert, 1994; Bhat and Bhat, 1997, 1998; Gilbert and Hazlewood, 1993; Godfrey and West, 1996b; Harman and Kubicek, 1998; Lamed and Bayer, 1988; Mandels, 1985; Poutanen, 1997; Saddler, 1993; Uhlig, 1998; Viikari *et al.*, 1993; Visser *et al.*, 1992; Visser and Voragen, 1996; Wong and Saddler, 1992, 1993).

Applications of cellulases, hemicellulases and Pectinases in textile, food reported by Graham and Balnave, (1995) , brewery and wine as well as in pulp and paper industries were reported by Saddler, (1993); Godfrey and

* To whom all correspondence should be addressed.
Tel.: +91 275 2257428; Fax: +91 257 2258403
E-mail: bchetoo@gmail.com

West, (1996b); Uhlig, (1998); Harman and Kubicek, (1998). Today, these enzymes account for approximately 20% of the world enzyme market (Mantyla *et al.*, 1998), mostly from *Trichoderma* and *Aspergillus* (Uhlig, 1998; Godfrey and West, 1996b).

The proportion of cellulose in plant tissues ranges from 20 to 45% of dry weight. Much of the cellulose in nature exists as waste paper or as waste material from agricultural industry in the form of stalks, stems, and husks, reported by; Bakare M K and *et al.* (2005); Immanuel G and *et al.* (2007). And after harvest of crop the leaves are left in the field for natural degradation, which takes several months. Besides, pseudo stems of banana left behind after harvest of the fruit are generally burnt. This agro waste can be effectively utilized for production of Cellulases. Therefore our emphasis is on bioconversion on agricultural waste into useful products.

Cellulase is a synergistic enzyme that is used to break up cellulose into glucose or other oligosaccharide compounds (Chellapandi and Jani, 2008). Enzymes with better properties are expected to emerge and further benefits to both wine producers and consumers. The cellulose system in fungi is considered to comprise three hydrolytic enzymes: endo-(1,4)-_-D-glucanase (endoglucanase, endocellulase, CMCase [EC 3.2.1.4]), which cleaves linkages at random, commonly in the amorphous parts of cellulose, exo-(1,4)-_-D-glucanase (cellobiohydrolase, exocellulase, microcrystalline cellulase, avicelase [EC3.2.1.91]), which releases cellobiose from nonreducing or reducing end, generally from the crystalline parts of cellulose and glucosidase (cellobiase [EC 3.2.1.21]), which releases glucose from cellobiose and short-chain cello oligosaccharides (Bhat and Bhat, 1997).

Our study focuses on the production of cellulase from *A. niger*. The physico-chemical conditions such as pH, temperature, inoculum size, fermentation period, static and agitation conditions, effect of different nitrogen sources were studied and optimized for cellulase production by *A. niger*. This can be used in fibre rich animal feed, food, textiles and detergents and in the paper industry. Further study needs for bioconversion of cellulosic biomass using enzymes like cellulase.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade. Media and chemicals used in this study were purchased from Hi-Media.

Organism and culture condition

The experimental fungal strain was previously isolated from agricultural soil and by microscopic observation identified as *A. niger*. The culture was maintained on Czapek Dox Agar for 72 - 120 h. Czapek Dox Medium was used for fermentation.

Crude Enzyme Extraction

Cellulase was extracted from inoculated medium by filtration through Whatman's filter paper No. 1 of broth at specified intervals. The filtrate was centrifuged at 10000 rpm for 10 min at room temperature. The supernatant was carefully collected and used as crude enzyme extract. Enzyme activity is determined at regular intervals.

Cellulase Activity Assay

Cellulase activity was determined at 40°C by using banana waste as a substrate. A reactive mixture contains 0.5 ml of 1% (w/v) substrate in 0.1 M citrate buffer (pH 4.8) and 0.5 ml of culture supernatant. The mixture was incubated at 40°C for 30 min. The reducing sugar released was measured using 3,5-dinitrosalicyclic acid (DNSA) (Miller, 1959). Control was prepared with 10 min boiled enzyme. One unit of cellulase activity was expressed as the amount of enzyme required to release 1 ìmol reducing sugars per ml under the above assay condition by using glucose as a standard curve (J. Jayraman, 2006). Enzyme assay was done for each parameter optimized at regular intervals.

Parameters optimized for Cellulase production

A. niger was grown in Czapek Dox Broth medium for optimization of different parameters for cellulase production. The experiments were carried out in such a way that the parameter optimized in previous experiment was maintained at its optimum level in the further experiments.

Carbon sources

A. niger was grown in Czapek Dox Broth medium with different carbon sources viz., glucose, sucrose, banana pseudostem, chopped banana leaves, and pulp of waste paper to observe their effect on the production of Cellulases.

Nitrogen sources

A. niger was grown in Czapek Dox Broth medium with different nitrogen sources viz., peptone, ammonium sulphate and urea to check their effect on the production of Cellulases.

Inoculum size

The inoculum size was optimized by preparing the inoculum on Czapek Dox Agar plate containing *A. niger*, by using sterile cup borer of 8 mm size. The agricultural waste containing fermentation media inoculated with 5, 10, 15 and 20 discs of *A. niger* aseptically. After inoculation the flasks were incubated in orbital shaker-incubator at 25°C to 35°C at 120 rpm.

Effect of pH

Optimization was carried out by adjusting the pH ranges from 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 of the banana waste containing Czapek Dox Broth. The pH of the medium was adjusted by using 1 N HCl or 1 N NaOH. After inoculation with 10 discs of *A. niger* of 8 mm size, the flasks were kept in orbital shaker-incubator at 25°C to 35°C at 120 rpm.

Fermentation Time

Fermentation time was optimized by putting various flasks containing Czapek Dox Broth, at from 24 to 120 h, at specific temperature on to orbital shaker-incubator at 120 rpm.

Fermentation Temperature

Fermentation temperature was optimized by putting various flasks containing Czapek Dox Broth, at 25°C, 35°C, and 40°C on to orbital shaker-incubator at 120 rpm.

Static and agitated condition

There were two sets prepared, to check the effect of static and agitated condition on enzyme activity. In both sets, all the conditions (pH, temperature, inoculum size, substrate concentration) applied were kept similar. One set was put in orbital-shaker incubator at 120 rpm, while other set was kept in static condition.

RESULTS AND DISCUSSION

A. niger isolate produced cellulase on agricultural biomass and its cellulase activity is determined at different types of conditions, such as carbon sources, nitrogen sources, inoculum size, substrate concentration, pH, fermentation time, fermentation temperature, static and agitated condition.

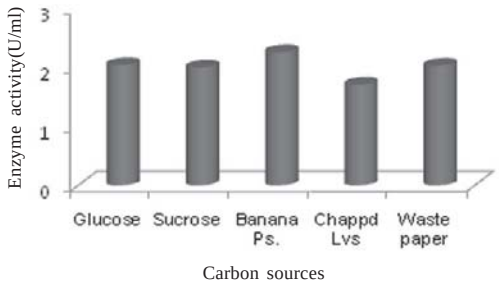

Fig. 1. Effect of carbon Sources on cellulase activity by *A. niger* (at 72h).

Effect of Carbon sources on cellulase activity

Banana pseudostem is found to be most suitable carbon source than other, which is showing 2.25 U/ml enzyme activity (Fig. 1). Sibtain Ahmed (2009) reported that use of glucose as a carbon source, gives very little enzyme activity and on the other hand higher amounts were produced when 1% CMC was used as a carbon source. Niranjane *et al.,* (2007) observed highest yields of cellulases on CMC. Similarly, Malik *et al.,* (1986) reported that negligible cellulases were produced with glucose as carbon source from *T. harzianum*.

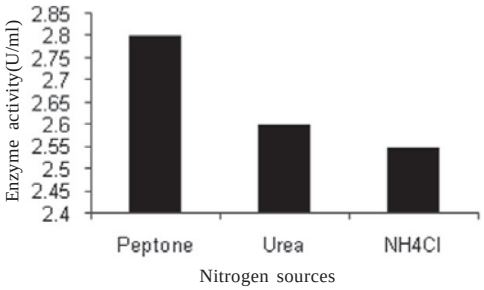

Fig. 2. Effect of nitrogen Sources on cellulase activity by *A. niger* (at 72h).

Effect of Nitrogen sources on cellulase activity

Peptone is found to be most suitable nitrogen source than urea and ammonium sulphate, which is showing 3.1 U/ml enzyme activity (Fig. 2). This result was supported by P. B. Acharya *et al.* (2008) which was obtained maximum cellulase activity at 0.125% peptone concentration. According to studies of Narasimha *et al.,* (2006),

the highest activity of cellulase obtained by urea, followed by peptone.

Table 1. Optimization of inoculum size

Inoculum Size	Cellulase activity(U/ml) 24h	48h	72h	96h	120h
5 discs	1.8	2.2	2.4	2.2	2.2
10 discs	2	2.3	2.6	2.5	2.4
15 discs	1.8	1.9	2	2	1.9
20 discs	1.6	1.9	2	1.9	1.9

Effect of inoculum size on cellulase activity

The inoculum size of 10 discs of 8 mm size gives higher cellulase activity as 2.6 Units/ml (Table 1). Acharya *et al.*, (2008) observed same result.

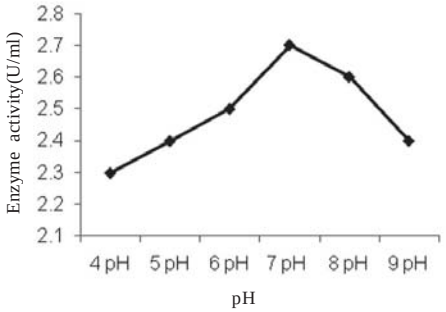

Fig. 3. Effect of pH on cellulase activity by *A. niger* (at 72h).

Effect of pH on cellulase activity

The highest cellulase activity by *A. niger* is 2.7 U/ml, obtained at pH 7.0. From the figure 3 it is cleared that cellulase activity had broad range of pH from 4.0 to 9.0. Akiba *et al.*. (1995) reported that the optimal pH for a cellulase production from *A. niger* between 6.0 and 7.0. G. Coral (2001) mentioned that at pH 4.5 and 7.5 maximum cellulase activity obtained. The Mc Cleary and *et al.* (1985) mentioned 4.0 and 4.5. as optimal pH for cellulase activity by *A. niger*.

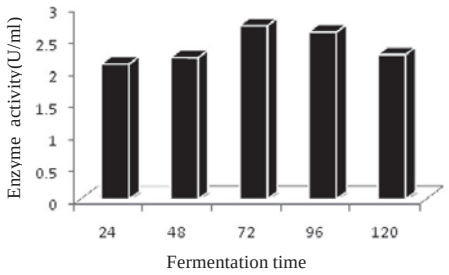

Fig. 4. Effect of fermentation time on cellulase activity by *A. niger* (at 72h).

Effect of fermentation time on cellulase activity

Highest cellulase activity 2.7 Units/ml is found at 72 hours of incubation (Fig. 4). Acharya *et al.* (2008) obtained highest cellulase activity by *A. niger* at 96 hours. Ojumu *et al.* (2003) found that the highest level of cellulase activity occurred at the 12 h of fermentation by *A. flavus*. It was noticed that a high concentration of reducing sugar was released on the 4th day of the fermentation, Khan *et al.*, (2007).

Effect of fermentation temperature on cellulase activity

Cellulase was efficiently produced by *A. niger* at temperature 35°C, activity obtained as 1.67 U/ml (Fig. 5). Coral (2002) reported 40°C as the optimum temperature for cellulase production.

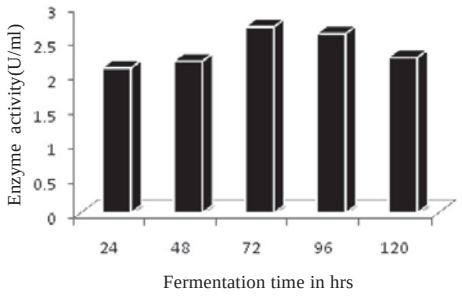

Fig. 5. Effect of different fermentation temperature on cellulase activity by *A. niger* (at 72h).

Table 2. Optimization of fermentation condition

Condition	Cellulase activity(U/ml) 24h	48h	72h	96h	120h
Static	1.5	1.6	2.2	2.1	1.9
Agitated	1.7	2.2	2.7	2.6	2.5

Effect of static and agitated conditions on cellulase activity

Agitation at rate of 120 rpm of fermentation gives highest cellulase activity than static condition. After 72 hours of incubation cellulase activity for agitated condition is 2.7 Units/ml and static condition shows activity is 2.2 Units/ml (Table 3). Maximum cellulase activity obtained to Ojumu *et al.* (2003) at 200 rpm at 35°C.

CONCLUSION

This study revealed that banana agro-waste is the best source for production of cellulase enzymes by fungal isolate *A. niger* at optimized conditions. Banana pseudostem waste proved the best carbon source for cellulase production. All parameters such as carbon source, nitrogen source, inoculum size, pH, fermentation time, fermentation temperature and static and agitated conditions are optimized for cellulase production. Cellulase production obtained at broad pH and temperature ranges. All these results proved applicability of cellulase enzyme produced by *A. niger* in various industrial and biotechnological applications. Further study needs for bioconversion of cellulosic biomass using enzymes like cellulase.

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