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Media Engineering for the Production of Cellulases by a Novel Strain *Aspergillus* sp. IICT-F 141 using Wheat Bran as Raw Material

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Fifteen different fungal strains were isolated from sawdust samples and tested for cellulase production. Out of them one fungal strain namely *Aspergillus* sp. IICT–F 141 was able to produce maximum cellulase by submerged fermentation. Wheat bran was used as a substrate for the production of cellulolytic enzymes in batch culture using shake flasks. Optimizations of fermentation medium parameters such as wheat bran particle size, concentration, pH, organic and inorganic nitrogen sources were studied to enhance the further production of cellulases. From the present optimization studies organic nitrogen sources and pH have played a major role by enhancing the celluolytic activity from 34.67 to 184.28 and 180.49 IU/ml of CMCase activities respectively. The overall increase in the production was observed to be 5 fold for both CMCase and FPase, when compared to the pre optimized conditions.

Key words: Cellulase, *Aspergillus* sp, Wheat bran, Particle size, Optimization.

Cellulose, a major polysaccharide constituent of plant cell walls, is a β-1, 4 linked linear polymer of 8000–12,000 glucose units (Saha, 2004), whose natural degradation represents an important part of the carbon cycle within the biosphere. The ability to decompose the cellulosic biomass into glucose, which in turn can be converted into other valuable chemicals and energy (Mukataka *et al.,* 1988), has made cellulases one of the most extensively investigated multicomponent enzyme systems. Cellulases are industrially important enzymes that are sold in large volumes for use in different industrial applications, for example, in starch processing, animal feed production, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper industry and textile industry (Ogel *et al.,* 2001). Cellulase-based strategies that will make the biorefinery processing more economical include: increasing commercial enzyme volumetric productivity, producing enzymes using cheaper substrates, producing enzyme preparations with greater stability for specific processes, and producing cellulases with higher specific activity on solid substrates (Percival Zhang *et al.,* 2006).

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Agro-industrial residues are generally considered the best substrates for the submerged fermentation process of cellulase production. Of all the agricultural substrates, wheat bran was found to be an ideal substrate providing all the nutrients for the synthesis of cellulases (Ikram-Ul-Haque, 1992). The soluble cello-oligosaccharides compositions in wheat bran were proved to be one of the most significant factors for cellulase production (Sun Xianyun *et al.,* 2008). Although a large number of microorganisms are capable of degrading cellulose, only a few of these microorganisms produce significant quantities of cell-free enzymes capable of completely hydrolyzing crystalline cellulose in vitro. *Curvularia, Aspergillus* and *Trichoderma* are the more potent producers of cellulase (Annapurna and Bhale Rao 1987, Kang *et al.,* 1994, Elad, 2000).

However, the high cost of cellulase production (due to use of chemicals in production) coupled with low enzyme activities limits its industrial use. Therefore, efforts are needed to economize cellulase production by media optimization and use of agro industrial renewable materials. The present study reports optimization of cellulase production by a soil isolate from soil, identified as *Aspergillus sp.* IICT – F 141, with wheat bran as a substrate.

MATERIAL AND METHODS

Isolation and screening of microorganisms

15 fungal strains were isolated from IICT garden soil and timber depot saw wood samples. All strains were maintained on the Czapek Dox agar slants at 28°C.

Inoculum preparation

The cultures were grown on Czapek Dox slants for 5 days, which were wetted by adding 10 ml of 1% Tween 80. With the help of camel hairbrush, the spores were removed and the suspension was used as an inoculum.

Fermentation procedure

A total volume of 1.0 ml of spore suspension containing 10^{6} -10^{8} spores/ml of all the 15 strains were inoculated into in 250 ml Erlenmeyer conical flask containing 100 ml of Mandels enriched medium (Chand *et al.,* 2005) individually and incubated on a orbital shaker at 28°C for 10 days. After 48 hours, every day the culture was centrifuged at 10,000×g for 10 minutes and the supernatant was analyzed for enzyme activity. Among all the 15 strains, *Aspergillus* sp. IICT – F 141 strain was used for further media optimization studies by submerged fermentation as it showed the maximum cellulase activity. The culture was maintained by regular transfer onto new slants and was stored at 4°C.

Analytical methods

Enzyme activities, such as filter paper unit (FPU) and carboxy methyl cellulose (CMC) assay were measured by the Mandels method (Mandels *et al.,* 1976). One enzyme unit (IU) was defined as the amount of cellulase, which is capable of producing one micromole (µM) of reducing sugars in one minute.

Optimization of fermentation media

Mandels enriched medium was chosen as the basic medium for optimization studies. The following parameters of fermentation medium were optimized:

To determine the effect of wheat bran particle size on cellulase production, cellulose of the basal medium was replaced by equivalent amount of different wheat bran particles. The wheat bran particles used were 710, 300, 250, 212, 180, 150, 125 µm generated by using different sieves with mesh numbers such as 25, 50, 60, 70, 80, 100, 120 respectively. Other ingredients of the medium and fermentation conditions were remained the same. During fermentation cellulase assays were carried out by CMC & FPU methods.

Crude wheat bran followed by 710 um sized wheat bran had shown the maximum cellulase activity during particle size optimization. To determine the effect of concentration of wheat bran (crude and 710 um sized particle) on cellulase production, different concentrations (1, 2, 3, 4, and 5%) of the above two were studied.

To study the effect of pH on cellulase production, pH ranging from 3 to 10 was studied.

To determine the effect of nitrogen sources on cellulase production, inorganic nitrogen sources such as NH_4Cl, $(NH_4)_2SO_4$, NH_4NO_3, KNO_3, $NaNO_3$ and organic nitrogen sources such as urea, peptone, beef extract, yeast extract, soybean meal, peanut meal, casein acid hydrolysate, casein, tryptone, and gelatin were studied.

RESULTS AND DISCUSSION

Cellulase production (24-216 h) was carried out in shake flask fermentation by all the 15 isolated strains using Mandels medium containing cellulose powder as a carbon source. All the strains had shown the maximum activity during 96 h incubation, which was shown in the table 1. Among all the strains, strain 3 had shown the maximum cellulase activity and it was identified as *Aspergillus* sp. IICT – F 141. Further optimization studies with wheat bran were carried out with this strain.

The profile of cellulase production at different time intervals by *Aspergillus* sp. was shown in the fig 1 and the maximum activity was recorded after 72 h of incubation i.e. 34.67 IU/ml of CMC activity and 9.55 IU/ml of FPU activity. Further increase in the incubation time decreased the cellulase production, which might be due to the depletion of micro and macronutrients in the fermentation medium. Depletion of nutrients effected directly on the pH value of the medium and resulted in the inactivation of the enzyme synthesis machinery with the passage of time (Nochure *et al.,* 1993). In addition, the medium components were initially more susceptible to fungal digestion and made a rapid rise in enzymes biosynthesis. But with the prolongation of cultural time, the susceptible portions were completely hydrolyzed by microorganisms, which inhibited the enzyme secretion pathways (Reese, 1977).

Particle size of wheat bran

Wheat bran is an abundantly available by-product of wheat milling industries. Among the different particle sizes of wheat bran, Crude wheat bran had shown the maximum CMC and FPU activities of 78.562 and 22.522 IU/ml respectively followed by 710 µm particle size (Table 2). Among the several factors that are important for microbial growth and enzyme production using a particular substrate, particle size was the most critical factor. Generally, smaller substrate particles provide larger surface area for microbial attack and, thus, are a desirable factor. However, too small a substrate particle may result in substrate accumulation, which may interfere with microbial respiration/ aeration, and therefore result in poor growth. In contrast, larger particles provide better respiration/aeration efficiency due to increased inter-particle space (Pandey *et al.,* 1999**).**

Concentration optimization of wheat bran (crude and 710 um particle size)

Five concentrations of crude wheat bran and 710 µm particle sized wheat bran were studied and it was found that 2% concentration of the above two had shown the maximum cellulase activity. 2% concentration of crude wheat bran had shown the 141.52 IU/ml of CMC activity and 46.73 IU/ml of FPU activity (Fig 2). 710 µm particle sized wheat bran had shown CMC and FPU activities of 130.18 and 41.93 IU/ml respectively (Fig 3). Further increase in the concentration resulted in the decreased activity.

Table 1. CMC and FPU activities of all the 15 isolated IICT strains

S. No of strain	CMC activity IU ml^{-1}	FPU activity IU ml^{-1}
1	18.81	3.23
2	12.59	2.23
3	34.67	9.55
4	21.58	6.73
5	11.51	1.97
6	8.230	1.08
7	13.19	2.68
8	27.83	7.13
9	19.00	3.55
10	1.916	0.48
11	14.12	2.57
12	7.763	1.77
13	15.60	3.50
14	5.466	0.90
15	25.87	6.81

Table 2. Effect of wheat bran particle size on cellulase production (IUml $^{-1}$)

Particle size of wheat bran (µm)	CMC activity IU ml^{-1}	FPU activity IU ml^{-1}
Crude wheat bran	78.562	22.522
710	70.149	17.777
300	59.728	15.2160
250	47.307	12.1150
212	41.323	11.3730
180	32.446	9.6900
150	24.364	6.2041
125	16.6070	2.346

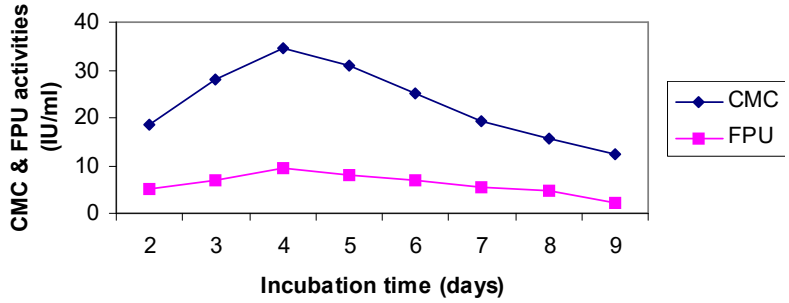


Fig. 1. Effect of incubation period on cellulase production (IUml $^{-1}$) in *Aspergillus* sp.

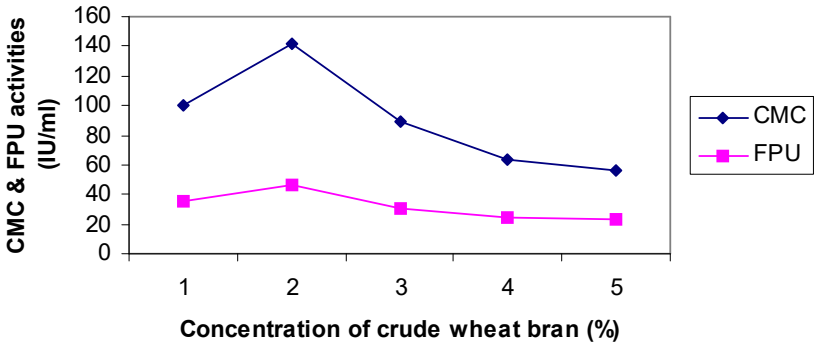


Fig. 2. Effect of crude wheat bran concentration on cellulase production (IUml $^{-1}$)

Szozodrak, reported that higher concentrations of wheat bran caused decreased enzyme activities.

pH

Among the 8 pH levels tested, pH 6.0 was optimum for cellulase production at 180.49 IU/ml of CMC and 68.63 IU/ml of FPU, followed by pH 5.0 at CMC and FPU activities of 163.366 and 39.575 IU/ml respectively (Fig 4). pH 10 had shown the least activity as alkaline pH represses the enzyme activity. pH, temperature, aeration, growth period and additives have been reported to be important parameters in optimizing cellulase production (Immanuel *et al.,* 2006). Among these, pH is of major interest (Juhasz *et al.,* 2004) as they reported a high cellobiase production in buffered medium. Zaldivar *et al.,* observed that cellulase production by *T.aureoviridae* is best if pH doesn't fall below 3.5. Pei-Jun *et al.,* reported a pH of 6.5 for optimum cellulase production by *T.koningii*.

Organic and inorganic nitrogen resources

Among the total 10 organic nitrogen sources tested, soyabean meal had shown the maximum cellulase production of 184.28 IU/ml of CMC and 53.29 IU/ml of FPU activities followed by beef extract (Fig 5). Five inorganic nitrogen sources studied by submerged fermentation of cellulase production, NH_4Cl had shown the maximum enzyme production at 143.12 IU/ml CMC and 48.284 IU/ml of FPU activities followed by KNO_3 (Fig 6). Inorganic nitrogen sources were less effective when compared with the organic nitrogen sources in the present study with this strain. Gashel, reported higher cellulase production with KNO_3 than NH_4Cl and urea. Pei-Jun *et al.,* on the other hand, reported combination of $(NH_4)_2SO_4$ and wheat bran for optimum cellulase production by *T.koningii.*

Celluase production recorded initially before optimization of fermentation medium parameters was 34.67 and 9.55 IU/ml of CMC and FPU respectively. After optimization of fermentation medium components, the production was increased up to 184.28 and 53.29 IU/ml of CMC and FPU respectively, which indicates a 5 fold increase in the CMC and FPU activities respectively (Fig 7).

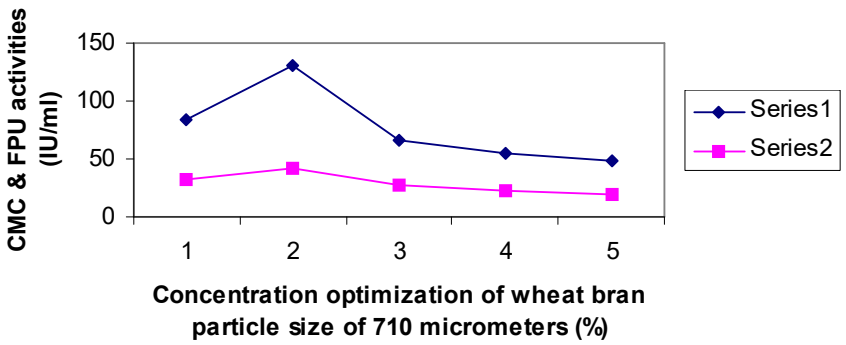


Fig. 3. Effect of 710 µm particle size concentration on cellulase production (IUml $^{-1}$)

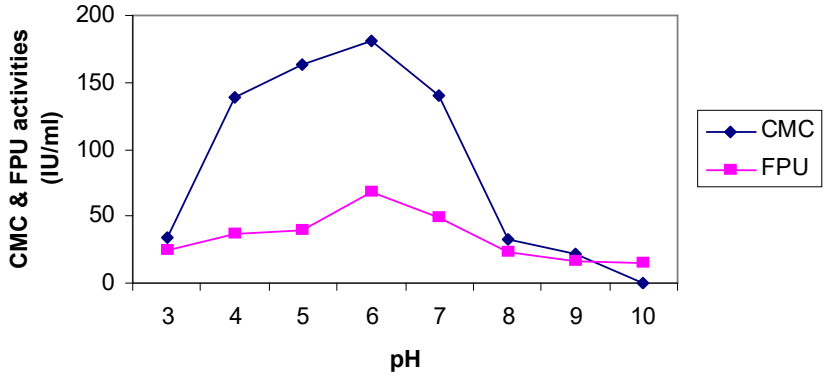
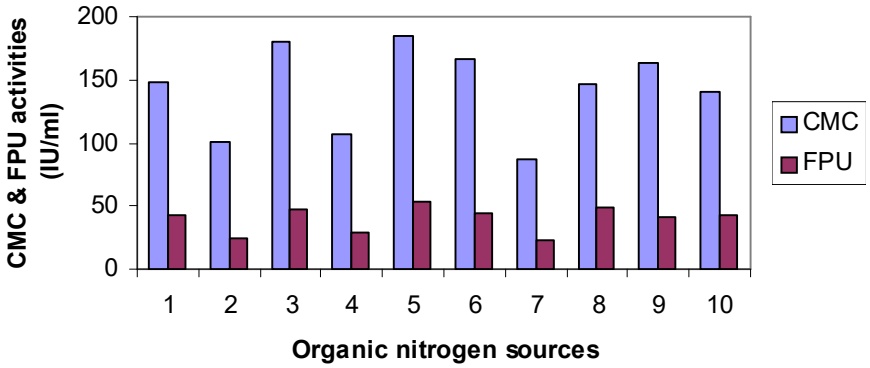


Fig. 4. Effect of pH on cellulase production (IUml^{-1}) in *Aspergillus* sp



1 urea; 2 peptone; 3 beef extract; 4 yeast extract; 5 Soyabean meal; 6 peanut meal; 7 casein acid hydrolysate; 8 casein; 9 tryptone; 10 gelatin;

Fig. 5. Effect of organic nitrogen sources on cellulase production (IUml^{-1})

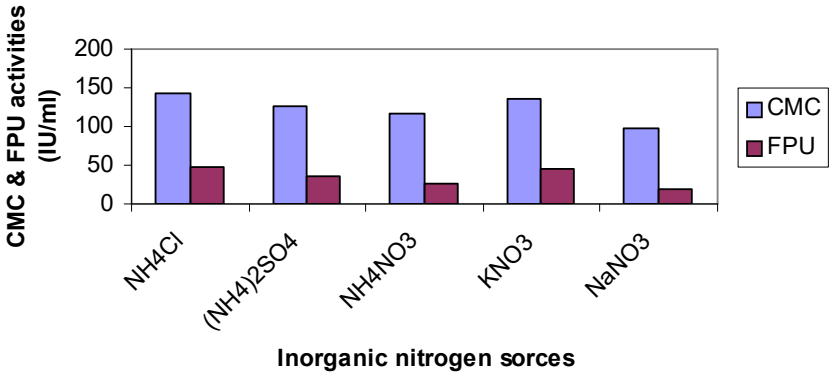
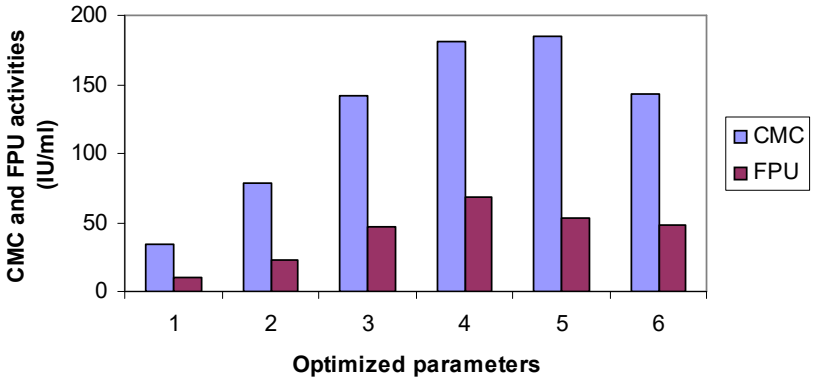


Fig. 6. Effect of inorganic nitrogen sources on cellulase production ($IUml^{-1}$)



1 incubation time; 2 particle size of wheat bran; 3 concentration of crude wheat bran; 4 pH; 5 organic nitrogen source; 6 inorganic nitrogen source;

Fig. 7. Effect of different optimized parameters on cellulase production ($IUml^{-1}$)

CONCLUSION

From the above results, it could be concluded that the best medium components for cellulolytic activity by *Aspergillus* sp. was, crude wheat bran 2%, pH 6, soyabean meal as an organic nitrogen source and NH_4Cl as an inorganic nitrogen source. Maximum cellulase production was achieved during 4^{th} day of fermentation. It was observed that wheat bran was found to be the best substrate for cellulase production by this strain and all the fermentation parameters had a significant impact on the production process. Soyabean meal and pH individually played a major role in enhancing the celluolytic activity of the isolated fungal strain.

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