Advanced Journal of Microbiology Research Vol. 2011
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Optimization of cellulase production by *Aspergillus niger* and *Tricoderma viride* using sugar cane waste

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Accepted 1 February, 2011

Sugar cane waste was used as substrates for the production of cellulase enzyme using *Aspergillus niger* and *Tricoderm aviride*. From the fermentation studies, sugar cane waste gave the best result with an enzyme activity value of (using *A. niger* and *T. aviride*) 2.7 and 2.6 IU/ml respectively. It gave the maximum enzyme activity at about the 10 days of cultivation, suggesting that is the optimum time when the enzyme may be harvested. The fermentation experiments were performed in solid stat fermentation (SSF). Incubation time and initial pH of fermentation medium watts optimized with simultaneous mixed culture. It was revealed that the sugar cane waste at pH = 4.5 and 40°C *Aspergillus niger* (37°C for *T. viride*) was the best source of carbon for the enhanced production of cellulase in the compatible culture experiments. Based on the reported results, it may be concluded that sugar cane waste can be a potential substrate for production of cellulase, incorporation of culturing *A. niger* or *T. viride.* The aim of this work is to produce cellulase from sugar cane waste and reduce the pollution.

Key words: *Aspergillus niger*, cellulase, culture, sugar cane waste.

INTRODUCTION

The biotechnology of cellulase and hemicellulases began in the early 1980s, first in animal feed and then in food applications (Chesson, 1987). Subsequently, these enzymes were used in the textile, laundry and pulp and paper industries.Today, these enzymes account for approximately 20% ofthe world enzyme market, mostly from Tricoderma and *Aspergillus*. Filamentous fungi, *Aspergillus* and *Tricoderma* species in particular, are well known and efficient producers of plant cell wall-degrading systems. These organisms consist of three classes of enzyme sendoglucanases (EC 3.2.1.4), cellobiohydrolases (EC3.2.1.91), and b-glucosidases (EC 3.2.1.21). Membersof all these classes are necessary to degrade cellulose (Bhat, 2000; Gielkens et al., 1999). Cellulose is the most abundant polymer in the biosphere with its estimated synthesis rate of 10^{10} tonnes per year (Schlesinger, 1993; Singh, 1995). Cellulose-rich plant biomass is one of the foreseeable and sustainable source of fuel, animal feedand feed stock for chemical synthesis (Bhat, 2000).

The utilization of cellulosic biomass continues to be a subject of worldwide interest in view of fast depletion of our oil reserves and food shortages (Gong et al., 1999; Kuhad et al., 1997). Cellulosic substrates hydrolysed by only one type of cellulase are categorised as follows: acid-swollen cellulose, carboxymethyl cellulose (CMC), cellulose azure, andtrinitrophenyl Cm-cellulose are hydrolysed byendoglucanases. Methylumbelliferyl-b-D-cellobiose (MUC), and p-nitrophenyl-b-D-cellobioside (pNPC) are used as substrates for the determination of exoglucanase activity, and methylumbelliferyl-b-D-glycopyranoside (MUG) and p-nitrophenyl-b-D-glycopyranoside (pNPG) are cleaved by b-glycosidases (Kuhad et al., 1997 and Han et al., 1995). It is well established that *Aspergillus niger* produces number of cellulolytic enzymes. Commercial crude cellulase preparations, derived from culture filtrates of the fungus, have been fractionated by several workers (Wolf et al., 1959; Ikeda et al., 1973b; Krishna et al., 1961; King and Smibert, 1963; Pettersson, 1963; Clarke and Stone, 1965).

Ikeda have reported theisolation of a homogeneous cellulolytic enzyme from *A. niger,* with an unusually low optimum pH (2.5).The other activities of *A. niger* have not, however, been fully characterized (Ikeda et al., 1967, 1973). However, the aim of this study was to produce

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cellulase from sugar cane waste by *A. niger* and *Tricoderma viride*. A number of microorganisms particularly fungi possessing cellulose degrading enzymes have been isolated and studied extensively (Kim et al., 2003). Cellulases are inducible enzymes which are synthesized by microorganisms during their growth on cellulosic materials (Lee and Koo, 2001). They are studied extensively due to their application in the hydrolysis of cellulose, the most abundant biopolymer and potential source of utilizable sugars, which serves as a raw material in the production of chemicals and fuel. Cellulases have a wide range of industrial applications. The main applications include textile, paper and pulp, food, animal feed, fuel and chemical industry. Additionally they can be used in waste management, pharmaceutical industry, protoplast production and genetic engineering (Bhat, 2001).

MATERIALS AND METHODS

General

All chemicals (KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4$, $CaCl_2$, $FeSO_4$, $MnSO_4$, $ZnSO_4$, $CoCl_2$, Urea, Ethanol and Methanol) used were of reagent grade (supplied by either Merck or Fluka) and used as supplied.

Substrates

The substrates used for this work is sugar cane waste it is cheap and readily available source of carbon. It was collected from sugar industry. The substrates were sundried for four days so as toreduce the moisture content.

Microorganisms

A. niger, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus wentti*, *Trichodermere esei*, *Trichoderme koninigii*, *Trichoderme linqnorum* and *T. viride* procured from biotechnology division, applied science department, University of Technology.

Inoculum preparation

The organisms were maintained as direct stock culture from which inoculums were prepared. It was grown on malt extract agar slantat 30°C for 5 days and stored at 0 to 5°C with regular sub culturing. 200 ml of the optimized medium (Solomon et al., 1999) of sample with species from a 4 day cultures wereused as inoculum prepared in a 250 ml. The inoculum was shaken continuously on an environmentcontrolledincubator shaker at 200 rpm and 35°C for 24 h before it was used for the fermentation process.

Fermentation experiment

Different fermentation runs were carried out on the batch fermentor. [KH_2PO_4 10.00 $(NH_4)_2SO_4$ 7.00, $MgSO_4$ x $7H_2O$ 0.30, $CaCl_2$ 1.50, $FeSO_4$ x $7H_2O$ 5 x10, $MnSO_4$ x H_2O 1.56 x 10, $ZnSO_4$ x $7H_2O$ 1.40 x 10, $CoCl_2$ x $6H_20$ 3.66 x 10, Urea 1.50 pH 5.00] were mixed in a fermentation vessel and sterilized with its accessories at 121°C for 15 min. After sterilization, the vessel was cooled to room temperature and the impeller shaft was coupled and filters were plugged into their receptacles. The inoculum was introduced aseptically and fermentation proceeded at agitation rate of 200 rpm and aeration maintained at 1.0 vvm. The fermentation was maintained at 35 °C for 52 h, sample of the medium was withdrawn every 4 h and the supernatant was analyzed for enzyme activity after centrifugation.

Determination of enzyme activity

The determination of enzyme activity was done by using Mandels method (Mandels, 1976). Incubation of 0.9 ml of substrate solution with enzymeextract at 45°C for 1 h, then added 1 ml of dinitrosalicylic acid (DNS) solution. The mixture was then heated at water bath for 5 min, then let it cool and then added 10 ml of distilled water. The equivalent solution was prepared by added 1 ml of DNS to 0.9 ml of substrate then added 0.1 ml of enzyme solution. The determination of reduction saccharides was done by using of Mandels method and then calculates the enzyme activity (Mandels, 1976).

Effect of temperature

For estimation of optimum temperature, the enzyme assay was carried out at seven different temperatures (20, 25, 30, 35, 40, 45 and 50°C).

Effect of pH

The best pH for enzyme activity was determined by carrying out the enzyme assay at different pH levels (3, 3.5, 4, 4.5, 5, 5.5 and 6).

RESULTS AND DISCUSSION

A total number of 8 isolates of fungi were isolated and identified; *A. niger* and *T. viride* were most predominant fungi (Figure 1).

A. niger

A. niger showed increasing trend in enzyme activity 10 days then showed a decline. At pH 4.5, the organism produced maximum enzyme (2.7 IU/ml) after 10 days of incubation followed by 2.2 and 2.1 IU/ml at pH 4.0 and 5.5 respectively (Figure 4). However, at pH 3.0 and pH 6.0 the *A. niger* showed least enzymatic activities 1.3 and 1.6 respectively (Figure 3). Production of cellulose (Cellulase production) was carried out by *A. niger*. Initially (eliminate the article) *A. niger* produced low activities of the enzyme but with the passage of time, there was gradual increase in enzyme synthesis up to 10 days. At 10th day of incubation and pH 4.5, the *A. niger* efficiently production enzyme and gave 10.42 IU/ml of cellulase that reduced to 8.3 IU/ml at 12th days of incubation. The organism exhibited same pattern on other pH levels but enzyme activities were less than those at pH 4.5. Figure 2 represents the effect of temperature on the enzyme activity of cellulase production; *A. niger* has high degree of enzymeproduction at 40°C

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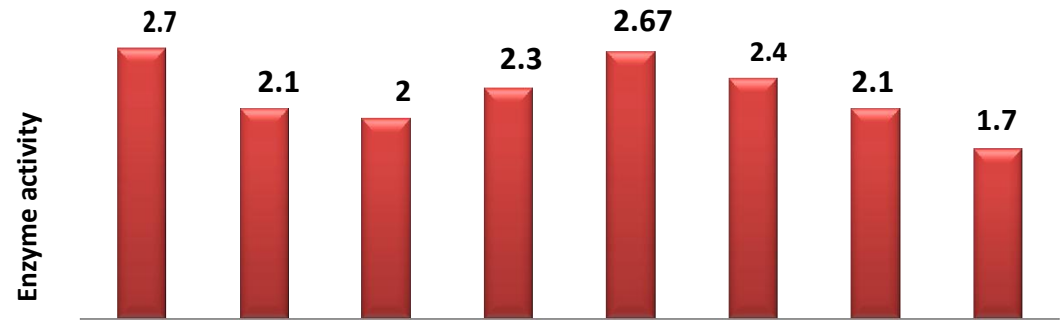

Figure 1. Effect of fungi on enzyme activity.

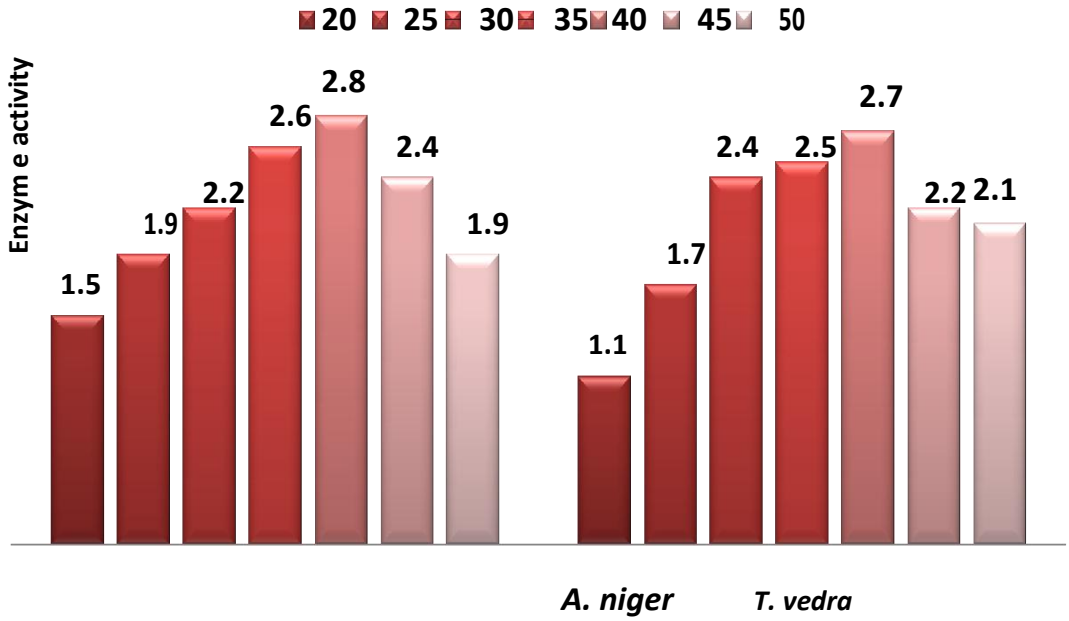

Figure 2. Effect of temperature on enzyme activity.

T. viride

T. viride showed increasing trend in enzyme activity 10 days then showed a decline. At pH 4.5, the organism produced maximum enzyme (2.6 IU/ml) after 10 days of incubation followed by 1.9 and 2.4 IU/ml at pH 4.0 and 5.5 respectively (Figure 4). However, at pH 3.0 and pH 6.0 the *T. viride* showed least enzymatic activities 1.1 and 1.8 respectively (Figure 3). The production of cellulase was carried out by *T. viride*. Initially, the *T. viride* produced low activities of the enzyme but with the passage of time, there was gradual increase in enzyme synthesis up to 10 days. At 10th day of incubation and pH 4.5, the *T. viride* efficiently production enzyme and gave 8.6 IU/ml of cellulase that reduced to 7.4 IU/ml at 12th days of incubation. The organism exhibited same pattern on other pH levels but enzyme activities were less than those at pH 4.5. Figure 2 represent the effect of

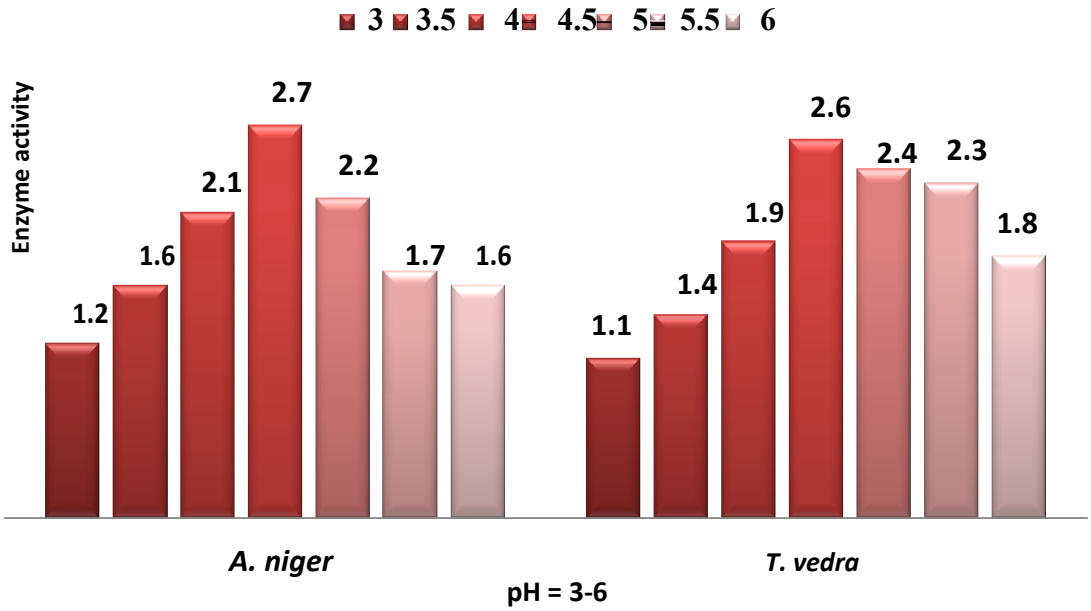

Figure 3. Effect of pH on enzyme activity.

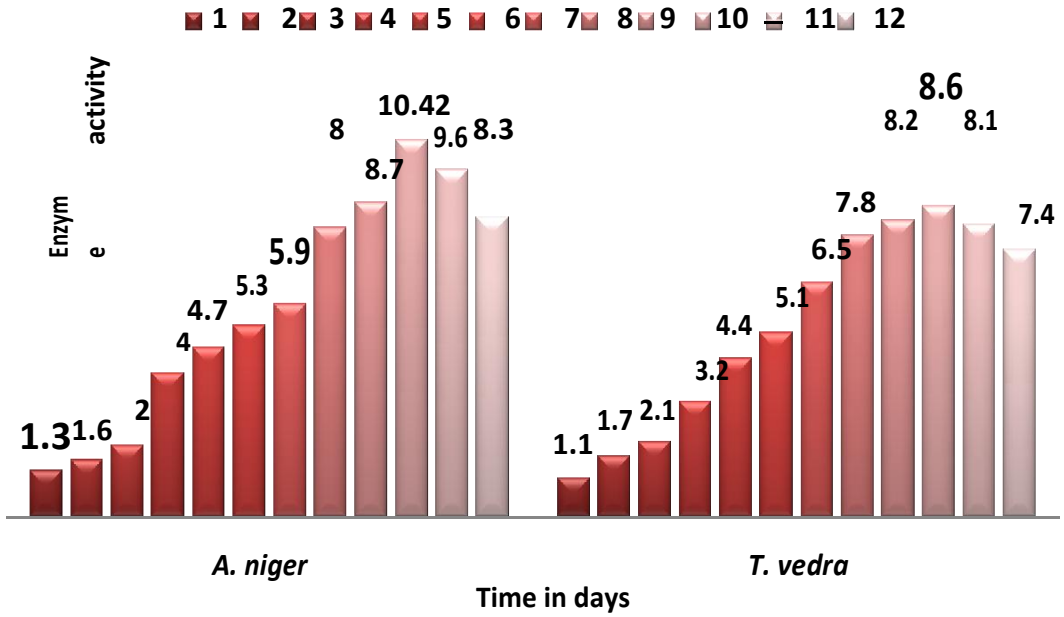

Figure 4. Effect of time on enzyme activity.

temperature on the enzyme activity of cellulase production; *T. viride* has high degree of enzyme production at 37°C.

REFERENCES

Chesson A (1987). Supplementary enzymes to improve the utilization of pigs and poultry diets, In: Haresign W ., and Cole D.J.A (eds.), Recent Advances in Animal Nutrition, London, Butterworths, pp. 71-89.

Bhat MK (2000). Cellulases and related enzymes in biotechnology, Biotechnol. Adv., 18: 355-387.

Gielkens MMC, Dekkers E, Visser J, Graaf LH (1999). Two cellobiohydrolases- encoding genes from Aspergillusniger require D-xylose and the xylanolytic transcriptional activator XlnR for their expression, Appl. Environ. Microbiol., 65(10): 4340-4345.

Schlesinger WH (1993). Biogeochemistry, an analysis of global change, Academic Press, San Diego.

Schlesinger, William. H., Biogeochemistry, an analysis of global change, Academic Press, San Diego.

Bhat MK (2000). Cellulases and related enzymes in biotechnology.

Biotech. Adv., 18: 355-383.

Gong CS, Cao NU, Du J, Tsao GT (1999). Ethanol production by renewable sources. Adv. Biochem. Eng. Biotechnol. 65: 207-241.

Kuhad RC, Singh A, Eriksson KE (1997). Microorganisms enzymes involved in the degradation of plant fiber cell walls. Adv. Biochem. Eng. Biotechnol., 57: 45-125.

Han JS, Yoo YJ, Kang HS (1995). Characterization of a bifunctional cellulase and its structural gene, J. Biol. Chem., 270: 26012-26019. Ikeda R, Yamamoto T, Funatsu M (1973b). Agric. Biol. Chem., 37: 1169-1175.

Krishna MCR, Stone BA (1961). Biochem. J., 78: 715-723.

King KW, Smibert RM (1963). Appl. Microbiol. 11: 315-319.

Pettersson G (1963). Biochim. Biophys. Acta 71: 665-667.

Clarke AE, Stone BA (1965). Biochem. J., 96: 793-801.

Ikeda R, Yamamoto T, Funatsu M (1967). Agric. Biol. Chem., 31: 1201-1209.

Ikeda R, Yamamoto T Funatsu M (1973a). Agric. Biol. Chem., 27: 1153-1159.

Solomon BO, Amigun B, Betiku E, Ojumu TV, Layokun SK (1999). African J. Biotech., 16: 61-68.

Mandels M, Andreotii RC (1976). Measurement of saccharifying cellulase. Biotechnol. Bioeng. Symp., 6: 21-23.

Kim KC, Yoo SS, Oh YA and Kim SJ (2003). Isolation and characteristics of *T. harzianum* FJ1 producing cellulases and xylanase, J. Microbial. Biotechnol., 13: 1-8.

Lee SM, Koo YM (2001). Pilot-scale production of cellulase using *T. reesei* rut C-30 in fed-batch mode. J. Microbial Biotechnol., 11: 229-233.

Bhat MK (2000). Cellulases and related enzymes in biotechnology. Biotechnol. Adv., 18: 355-383.