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Production of Carboxymethyl Cellulase from Local Isolate of *Aspergillus* Species

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Abstract

Cellulases play a pivotal role in biomass utilization. Many fungal and bacterial species produce cellulases in good amounts. Ever increasing use of cellulases in industry has led to the search for new enzymes with high activites. The production of extracellular carboxymethyl cellulase (CMCase) by a local isolate of *Aspergilus* species on wheat straw was studied in liquid state fermentation. The optimal pH and temperature for the enzyme production was found to be 4.0 and 35 ^{o}C respectively. Maximal CMCase activity was achieved with 4 % wheat straw, 0.125% cane molasses and 1.5% yeast sludge.

Key words: Cellulase, production, *Aspergilus*

Introduction

Cellulases degrade cellulose by the synergistic action of three enzyme activities; endoglucanase also called as carboxymethyl cellulase (CMCase) (endo-1,4-β-D-glucanase, EG, EC 3.2.1.4), exoglucanse (also called as cellobiohydrolase) (exo-1,4- β -D-glucanase, CBH, EC 3.2.1.91) and β-glucosidase (1,4- β -D-glucosidase, BG, EC 3.2.1.21) (Li *et al*., 2006; Gao *et al*., 2008). Researchers have strong interests in cellulases because of their applications in several industries (Jamil *et al*., 2005; Ahmed *et al*., 2005). In recent years the interest in cellulases has increased due to their application in the production of bio-energy and bio-fuel (Ahmed and Vermette, 2008; Zhou *et al*., 2008).).

Many fungal strains secrete higher amounts of cellulases such as *Trichoderma*, *Humicola*, *Aspergillus* species (Amouri and Gargouri, 2006). Cellulases from *Trichoderma* and *Aspergillus* species have been investigated in detail over the past few decades (Fang *et al*., 2008). Utilization of agro-industrial wastes for the production of enzymes from microorganisms has also been found an attractive way to resolve the environmental pollution problems. The present study describes the production of CMCase by an *Aspertillus* speices using wheat straw as a carbon source. Carboxymethyl cellulase is the main enzyme of the cellulases complex as it initiates the attack on cellulose.

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Materials and Methods

Substrate

Wheat straw was obtained from local grain market of Multan Pakistan, air dried and stored in an oven at 65 °C to a constant weight. Substrate was ground to 2 mm sieve and stored in air tight plastic jars. Analysis of the wheat straw was performed following AOAC Methods (1990).

Microorganism

The strain, *Aspergillus* species, utilized in the present study was obtained from the Dept of Plant Pathology, University of Agriculture, Faisalabad. The fungus was maintained on agar slants medium which consisted of (g/L); wheat straw 20; $CaCl_2.2H_2O$ 0.05; $MgSO_4$, 0.05; KH_2PO_4, 1.5; Urea, 3 ; Agar, 20.

Media and culture conditions

The inoculum medium consisted of (g/L); wheat straw 20; $CaCl_2.2H_2O$ 0.05; $MgSO_4$, 0.05; KH_2PO_4, 1.5; $(NH4)_2SO_4$, 2 at pH 3.5 and grown at 30 ^{o}C on an orbital shaker working at 120 rpm for 24 h. The pH of the fermentation medium was adjusted to 3.5 and temperature 30 ^{o}C for 96 h to optimize different fermentation conditions for cellulases production. Biomass was harvested by centrifugation at 10,000 rpm for 20 min at 4 ^{o}C. Resulting supernatant was tested for cellulase activity.

Effect of substrate concentration, pH, temperature, incubation time, nitrogen sources, cane molasses and yeast sludge on CMCase production

Various parameters were investigated for maximal production of CMCase from the fungal species.

CMCase activity

The CMCase was assayed in reaction mixture (1 mL) containing 1% (w/v) CMC, 1 mL of sodium acetate buffer, pH 5.0 and 60 ^{o}C. The reaction was stopped by adding 3 mL DNS reagent and absorbance was noted at 575 nm (Gadjil, 1995).

Protein Estimation

The protein content of the culture biomass was determined by Lowry method (1951).

Results and Discussion

Wheat straw was subjected to proximate analysis (AOAC, 1990) in order to find out its inherent potential value (Table 1).

Table 1: Proximate composition of wheat straw

S.No	Components	% Composition
1	Moisture content	12.4
2	Ether extract	1.8
3	Crude fibre	31.4
4	Ash	6.5
5	Crude protein	7.1
6	Nitrogen free extract (NFE)	59.2

Optimal conditions for CMCase production

Initial studies were performed in shake flasks to optimize fermentation conditions for the production of cellulase enzymes. The orbital shaker was operated at a speed of 120 rpm. Many microorganisms have been classified as cellulolytic but only few possess complete cellulase complex capable of efficient depolymerization of crystalline cellulose. In the present study different fermentation conditions were optimized for CMCase production by *Aspergillus* species to minimize production cost in order to enhance the commercial viability of cellulase production technology.

Effect of substrate concentration on CMCase production

The fungus produces cellulases extracellularly when grown on a suitable carbon source. The genes for the cellulases are induced under different carbon sources. Simple sugars cross the membranes for enzyme induction, whereas complex polysaccharides are broken down in to samller molecules that can cross the membranes. Among different substrate (wheat straw) concentrations, 4 % wheat straw supported significantly higher enzyme production (Fig 1). Gradual reduction in the enzyme production was observed when wheat straw concentration was increased or decreased.

The lignocellulosic biomass, especially agricultural wastes, is known to be an excellent carbon source for microbial enzyme production. Lignocellulosic biomass, such as wheat straw which is very abundant, cheap and easily available (Yang *et al*., 2006; Ahmed *et al*. 2010) can be used for economic production of cellulases.

Effect of pH, temperature and incubation time

CMCase activity was detected within pH range of 3-6 with maximum activity at pH 4 (Fig 2).

The temperature of the fermentation medium is one of critical factor that has profound influence on the production of end product. The temperature for the enzyme production of *Aspergillus* species was optimized. Maximum CMCase activities were achived at 35 ^{o}C (Fig. 3). Further increase in temperature resulted in decrease in cellulase production.

Time course for the enzyme production was investigated in fermentation medium with 4% wheat straw as a substrate. Maximum CMCase activity was achived after 72 h of incubation (Fig 4). Further incubation resulted in decrease in the enzyme activity.

The optimal pH for fungal cellulases varies from species to species though in most cases the optimum pH ranges from 3.0 to 6.0 (Garg and Neelakantan, 1981, Niranjane *et al*., 2007). Our results are in accordance with earlier reports. *Fusarium lini* gave maximum endoglucanase activity (6.8 IU/mL) at 30 °C (Vidya *et al*., 1984). *Trichoderma harziaunum* produced higher levels of cellulases at at 28 °C (Ahmed *et al*. 2005).

Effect of nitrogen sources $(NH_4)_2SO_4$/Urea on CMCase production

Replacement of one nitrogen source for another in the medium causes a change in protein synthesis as well as product formation. Generally, the results confirmed that urea; a low cost fertilizer, supported the maximum production of the enzyme as compared to $(NH_4)_2SO_4$ (Fig 5).

Effect of supplementation with cane molasses and yeast sludge on CMCase production

Cultivation of the *Aspergillus* species under previously optimized conditions with molasses (0.05-0.175%) showed that 0.125% molasses gave maximum productivity of CMCase (Fig 6). Addition of higher concentrations of molasses resulted in lower enzyme production.

Yeast sludege a byproduct of breing industry has attracted the attention of scientists (Sattar *et al*., 2008; Ali *et al*. 2009). Among different yeast sludge concentrations, 1.5% yeast sludge supported significantly higher CMCase production (Fig 7). Further addition of yeast sludge caused decrease in cellulase production.

Molasses, cheap by-products, are widely available from the sugar industry and consist of water, sucrose (47–50%, w/w) which is the disaccharide most easily utilized by yeast cells, 0.5–1% of nitrogen source, proteins, vitamins, amino acids, organic acids, and heavy metals (Athar *et al*. 2009; Ali *et al*. 2009). Hence it is a very attractive carbon source for cellulase production from *Aspergillus* species from economic point of view.

Conclusion

The successful use of agricultural raw materials as renewable carbon source is dependent on the development of economically feasible process for cellulase production. The abilty of a local isolate of

Aspergillus species was investigated to produce CMCase which is a key enzyme among cellulases, with wheat straw as a substrate. In order to improve the CMCase production from the fungal species grown over wheat straw, cane molasses and yeast sludge were added to the fermentation medium. Hence, 4% wheat straw, 0.125% molasses and 1.5% yeast sludge at pH 4, temperature 35 ^{o}C and 72 h of incubation were found to be optimal for CMCase production from the *Aspergillus* species. The present results demonstrate the potential of cane molasses and yeast sludge along with wheat straw as a substrate not only for the production of CMCase but also for other cellulases.

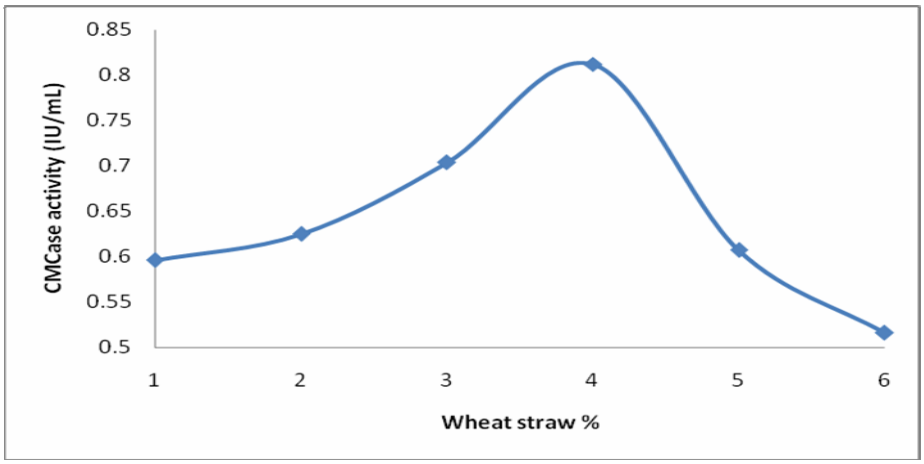

Fig 1. Effect of various susbtrate concentrations on CMCase production from *Aspergillus* species

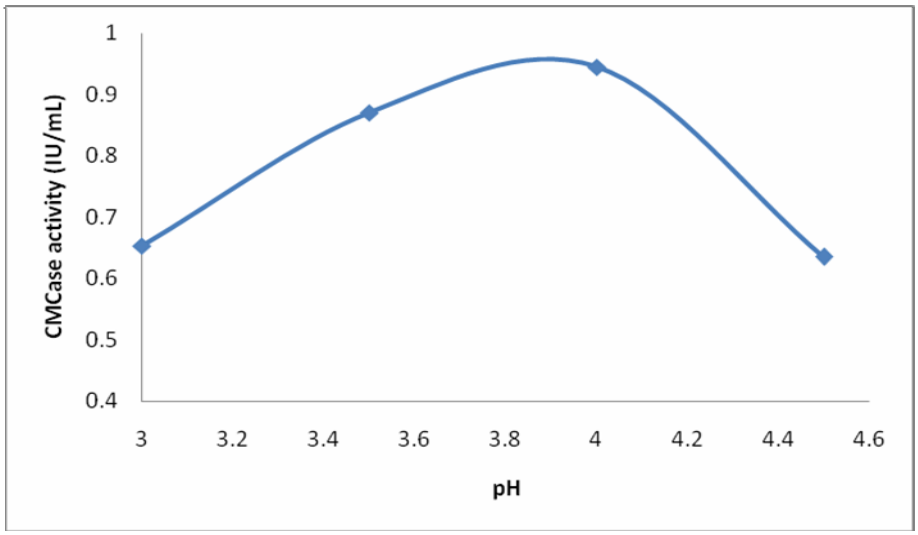

Fig 2. Effect of pH on CMCase production from *Aspergillus* species

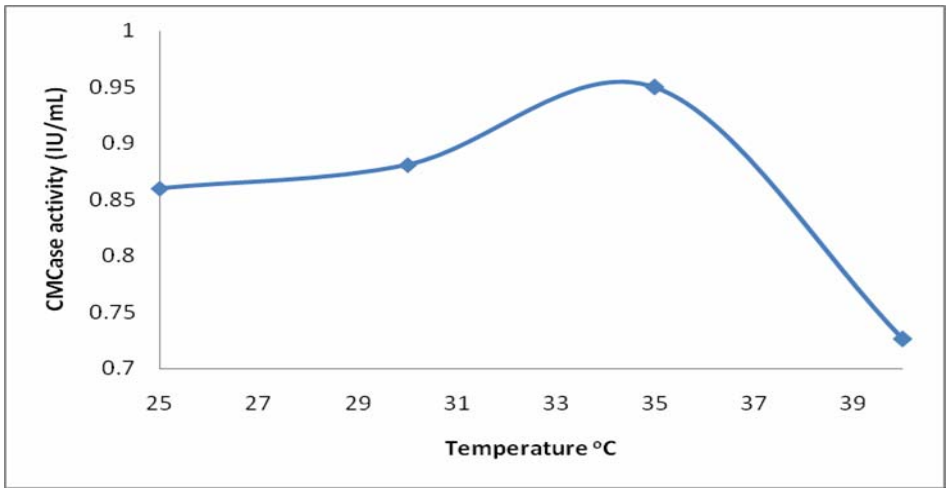

Fig 3. Effect of temperature on CMCase production from *Aspergillus* species at pH 4

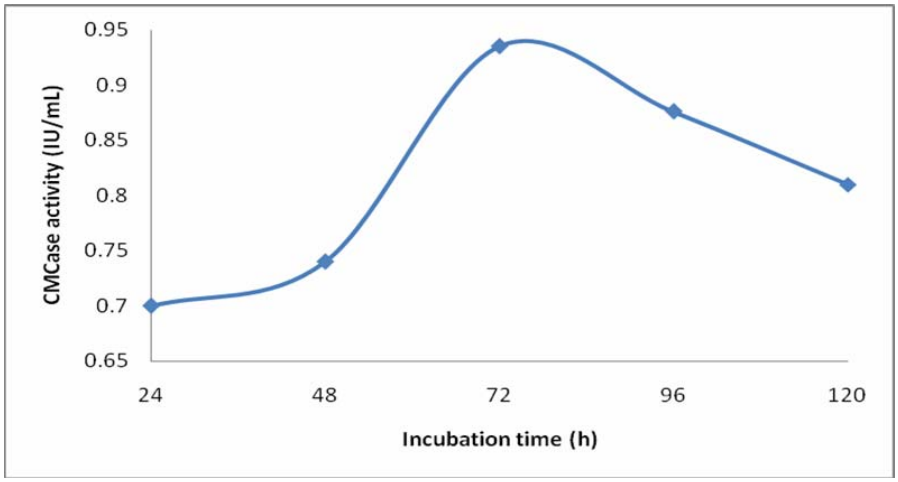

Fig 4: Time course of CMCase production from *Aspergillus* species at pH 4 and 35 °C.

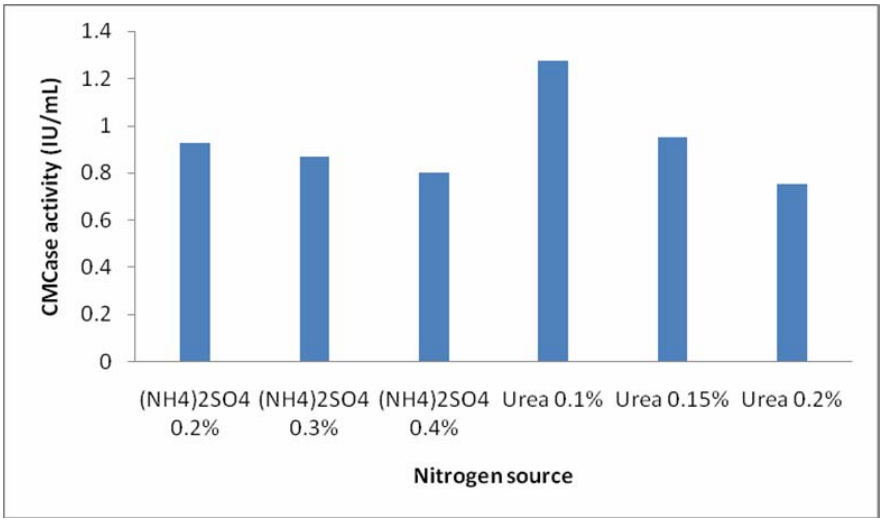

Fig 5. Effect of different concentration of $(NH_4)_2SO_4$/Urea on CMCase production from *Aspergillius* species

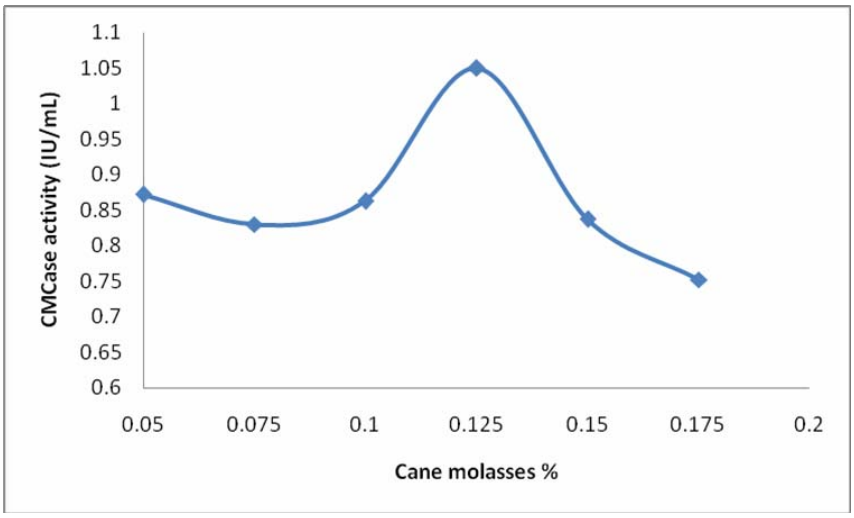

Fig 6. Influence of cane molasses on CMCase production from *Aspergillus* species

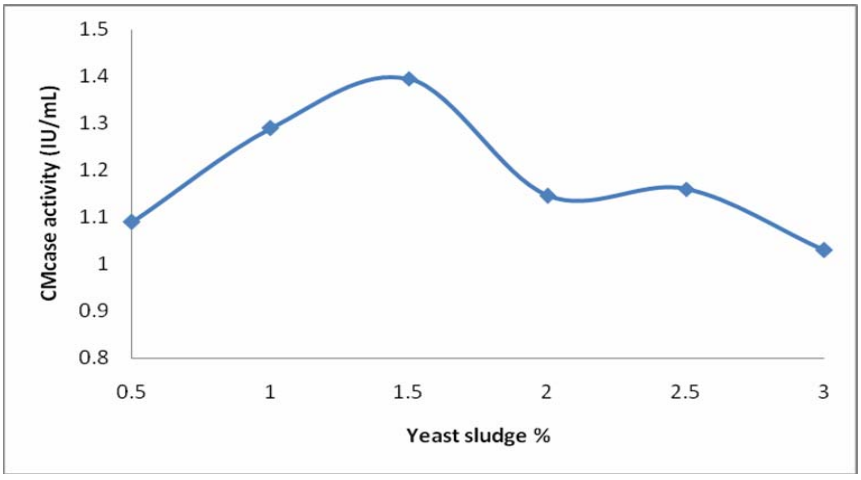

Fig 7. Influence of yeast sludge on CMCase production from *Aspergillus* species

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