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Biosynthesis of cellulase from bagasse with fungal strains

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Abstract: The biosynthesis of cellulase was optimized by comparing the activities of cellulases produced by *Trichoderma ressi M-2* and *Aspergilus nige*r M-12 on raw, NaOH and steamed treated bagasse.It was shown that the maximum units (CMC-ase 8.95 IU/ml, FP-ase 1.52 IU/ml and CMC-ase 7.71 IU/ml, FP-ase 1.31 IU/ml) were observed on steamed bagasse with *Trichoderma ressi M-4* and *Aspergillus niger*M-12.The enzyme units on CMC-ase 6.754, FP-ase 1.10 and CMC-ase 6.23, FP-ase 1.04 were observed on 2% NaOH treated bagasse while on raw bagasse , CMC-ase 5.75, FP-ase 1.02, and CMC-ase 4.92, FP-ase 0.98 were find with *Trichoderma ressi* M-4 and *Aspergillus niger* M-12.

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INTRODUCTION

The agricultural waste, which is rich with cellulose, can be exploited as cheap raw material for the production of industrially important enzymes and chemicals[1] .On the earth the cellulose is the most abundant polymer which is estimated synthesis rate of 10^{10} tones per year [2, 3, 4]. The plant biomass which is comprises on average 40 % cellulose, 23 % lignin and 33% hemi cellulose and also sustainable source of fuel, animal feed and feed stock for chemical synthesis[5].

Pakistan is fourth largest sugar cane producing country, generates annually 5.4 million tones of bagasse[6], eighty percent being used for producing fuel, the remaining bulk (2 million tones approx) containing 50-55% cellulose, can possibly be recycled and put to beneficial use instead of merely burning. The utilization of such cellulosic biomass continues to be a subject of worldwide interest in view of fast depletion of our oil reserves and food shortages[7].

In the present study raw, boiled and NaOH treated bagasse is used as substrate for cellulase production by using fungal strains in submerged fermentation at flask level.

MATERIALS AND METHODS

Organism

Trichoderma ressi M-4 and *Aspergillus niger* M-12 was procured from Microbiology Lab, Food and Biotechnology Research Centre, PCSIR Labs Complex Lahore. The both strains were grown on PDA agar slant (Oxoid) for 48 h at 30 ^{0}C. The culture were then preserved at 4 ^{0}C and further shifting on the PDA slant at the interval of 30 days to keep them viable. The pH of the medium was adjusted at 5 with 1N HCl/ NaOH before sterilization at 121 ^{o}C for 15 minutes.

Substrate preparation

The raw bagasse used in present study was obtained from Shaker Gang Sugar Mill, Jhang. The sun dried bagasse was pulverized in hammer beater mill (Japan) of 2mm mesh size. The grinded bagasse was soaked in plenty of water for 24 hours and then steamed under pressure in pressure cooker for 30 mintus.

Alkaline treatment of the bagasse

The grinded bagasse was soaked in 2% NaOH solution (1:10) at room temperature for 24 hr. The overnight soaked bagasse was autoclaved at121 ^{o}C for 15 min and four time washed with hot water to neutralized the bagasse.

Preparation of inoculum

Inoculum was prepared by transferring a 10 ml of sterilized distilled water in 48 h old slant of *Tichoderma ressi M*-4 and *Aspergillus niger* M-12. The spores were dislodged by using the sterilized inoculation needle under aseptic conditions and the spore's suspension 10^8 per ml was used to inoculate the fermentation medium for cellulase production.

Fermentation

The 100 ml of fermentation medium used for the production of cellulase was composed of 1-5 % of raw, boiled and NaOH treated bagasse, $KH_2PO_4$0.1%, $(NH_4)_2SO_4$0.2%, KCL 0.05% and $MgSO_4$, 0.05% in 250 ml Erlenmeyer flask. The pH of the medium was adjusted at 5 with 1N HCl / NaOH before sterilization at 121 ^{o}C for 15 min. The autoclaved fermentation medium was inoculated with 2 %(v/v) inoculum under aseptic conditions and incubated in water bath shaker (Eyela, Japan) at 30 ^{0}C, 150 rpm for 72 hrs. Then fermentated broth was centrifuged at 4 ^{0}C for 10 min at 10,000 rpm to get the clear solution.

Filter paper activity (FPA)

The overall cellulolytic activity was estimated by FPA method. The Mandel method was slightly modified, incubating 1.0 mL of culture supernatant

with 50 mg of filter paper (Whatman Nº 1) in 1.0 mL of 0.05 M acetate buffer (pH 5.0) at 45 ºC for 30 min [8]. One international unit of cellulase activity is the amount of enzyme that forms 1 µmol glucose (reducing sugars as glucose) per minute during the hydrolysis reaction. Reducing sugar was determined by the dinitrosalicylic acid (DNS) method[9].

CMCase activity

Carboxymethyl cellulase activity (CMCase) was estimated by incubating 0.5 mL of culture supernatant with 0.5 mL of 1% CMC in 0.05M sodium phosphate buffer, pH 7.0 at 50ºC for 15 min. The release of reducing sugars was estimated by DNS reagent[9] .One unit of enzyme activity was defined as the amount of release of one micro mole of reducing sugars equivalent to glucose under the assay conditions.

RESULT AND DISSCUSSION

In this study, bagasse was used as a substrate for cellulase enzyme in sub-merged fermentation from fungal strains. The bagasse was treated for maximum production of cellulase and it was observed that best results were obtained, on steamed 2% bagasse, which gave higher yield of CMC-ase 8.95 IU/ml) and FP-ase 1.52 IU/ml and CMC-ase 7.71 (IU/ml) and FP-ase 1.31 (IU/ml) by *Trichoderma ressi* M-4 and *Aspergillus niger* M-12 (Figures 1 & 2). The cellulase units on CMC-ase 6.754, FP-ase 1.10 and CMC-ase 6.23, FP-ase 1.04 were observed on NaOH treated bagasse and CMC-ase 5.75, FP-ase 1.02, and CMC-ase 4.92, FP-ase 0.98 on untreated bagasse were find by *Trichoderma ressi* M-4 and *Aspergillus niger* M-12 (Figures 3, 4, 5 & 6). The maximum yield was shown on the steamed bagasse was due to loose the shield formed by lignin and hemicellulose and this substrate is more acceptable for fungal strain.

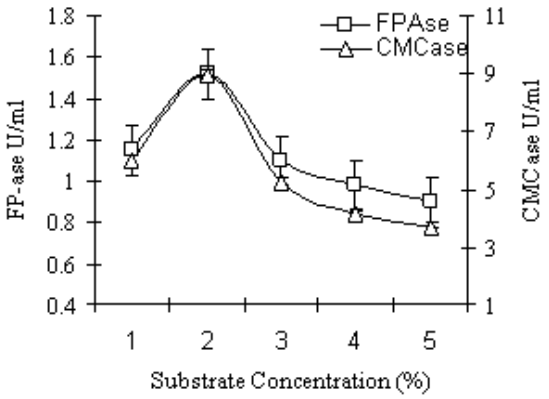

Figure 1: Cellulase production by steamed baggase by *Trichoderma ressi* M-4.

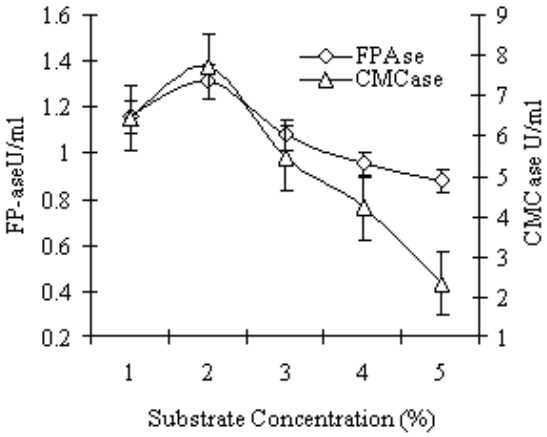

Figure 2: Cellulase production by steamed baggase by *Aspergillus niger* M-12.

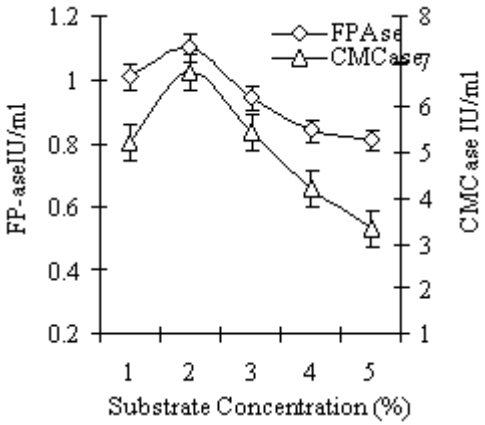

Figure 3: Cellulase production from NaOH treated baggase by *Trichoderma ressi* M-4.

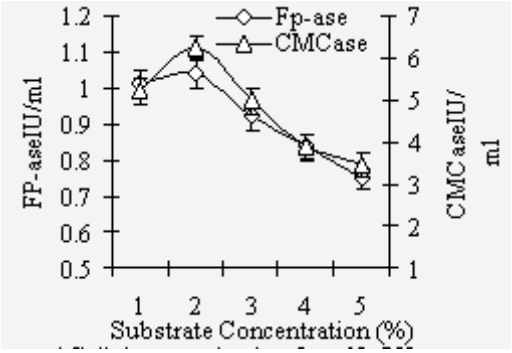

Figure 4: Cellulase production from NaOH treated baggase by *Aspergillus niger* M-12.

In the present work raw, steamed and NaOH treated bagasse is utilized as carbon source for cellulase production by *Trichoderma ressi* G-1 and *Aspergillus niger* M-2. The carbon sources induce production of cellulase, but amount was varied due

to the influence of substrate (carbon source) on the growth of cellulolytic organism[10,11].

In present study the maximum results on CMC-ase 5.14 IU/ml) and FP-ase 4.21 IU/ml and CMC-ase 4.21 IU/ml) and FP-ase 3.21 IU/ml by *Trichoderma ressi* G-1 and *Aspergillus niger* M-2 were observed in steamed bagasse.It was reported that cellulose digestibility was increased with liquid hot water[12]. The enzymatic digestibility of pretreated biomass such as corn fiber and sugarcane bagasse was increased and 80 % hemicellulose was removed[13,14].

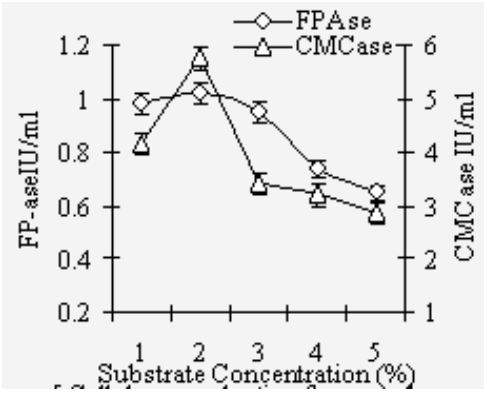

Figure 5: Cellulase production by raw baggase by *Trichoderma ressi* M-4.

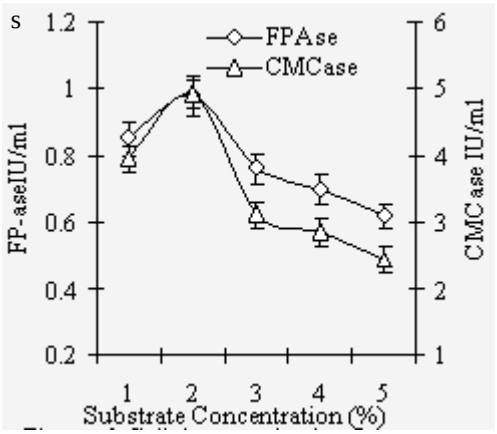

Figure 6: Cellulase production by raw baggase by *Aspergillus niger* M-12

The effect of different pretreatment methods upon various lignocellulosic wastes such as wheat bran, wheat straw, sugar cane bagasse, ground shell, rye grass straw, beet pulp, rice bran and rice husk to exposed the cellulose[15,16]. The pretreatment reduces the lignin content and crystallinity of cellulose and increase the surface area for enzyme[17]. The 1 % alkali was used to treat the bagasse for the production of cellulase enzyme and protein at pilot scale by cellulolytic mould *Aspergillus terreus* GN_1 [18]. The treated bagasse and sawdust with different acidified solvents and reported that bagasse treated gave a promising cellulase activity which was used as carbon source in fermentation medium for *Trichoderma reesei* [19]. It was reported that treated bagasse with 4% sodium hydroxide gave the best result as compared to non treated bagasse for cellulase production by *Aspergillus niger* IZ 9[20].

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