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Production of Xylanase from Corn Stover by *Arachniotus* sp.

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Abstract
Xylanase enzyme production was attempted in shake flask through fermentation of corn stover by *Arachniotus* sp. in the presence of optimum concentrations of $(NH_4)_2SO_4$, $CaCl_2$.$2H_2O$, $MgSO_4.7H_2O$, KH_2PO_4, Yeast extract and molasses (cane). Maximum production of xylanase was obtained at 48 hours from the medium containing corn stover, 7.5%; $(NH_4)_2SO_4$, 0.3%; $CaCl_2.2H_2O$, 0.05% and molasses (cane) 2.0% at pH 4 and 30°0.

Key words: *Arachniotus* sp., corn stover production

Introduction
Efficient enzymatic degradation and complete utilization of agricultural and forestry wastes for single cell protein (SCP) production by micro-organisms requires the conversion of hernicelluloses to their respective sugar components before being incorported into microbiaal biomass (Chahal *et al*., 1979). Corn stover has high fibre and low energy contents and the availability of energy is limited due to lignocellulosic bonds present in fibre (Morison, 1959). Fermentation of corn stover with *Arachniotus* sp. would not only reduce the pollutants but will also serve as vital source of energy for production of fuels. Microbial production of xylanase is preferred because of easier availability and structural stability (Biely, 1985). The present study was undertaken to find out potimum cultural conditions for maximum production of xylanse by *Arachniotus* sp. when grown on corn stover.

Materials and Methods
Organism: Pure culture of *Arachniotus* sp. was obtained from the Department of Plant Pathology, University of Agriculture, Faisalabad. The stock culture was maintained on agar-corn stover slants (Table 1).

Inoculum Preparation: The inoculum was prepared by transferring the spores of *Arachniotus* sp. from slants to autoclaved inoculum medium (Table 1). The flask was incubated on orbital shaker at pH 4.0 and 30°C for 72 h. The mature inoculum was filtered through sterilized cotton. The filtrate containing homogenous fungal spore suspension (10^6-10^7 spores/mL) was used as inoculum.

Optimum Conditions: The growth media containing corn 'stover as substrate were prepared to study different conditions like fermentation period, substrate: water ratio, conc. of $(NH_4)_2SO_4$, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, KH_2PO_4, molasses (cane) and yeast extract for the maximum production of xylanase at pH 4 and 30°C temperature. All the experiments were conducted in a sequence after one another so that the most suitable ionic concentration of one experiment was used in next one.

Table 1: Composition of sporulation and inoculum media (g/100 mL)*

Ingredients	Sporulation	Inoculum
Corn stover	5.000	10.00
$(NH_4)_2SO_4$	0.100	0.20
$CaCl_2$	0.005	0.01
$MgSO_4.7H_2O$	0.005	0.01
KH_2PO_4	0.010	0.50
Agar	20.000	-
Distilled water	To make 100 mL volume	To make 100 mL volume

*pH of the media was adjusted at 4

After each experiment the fermented broths were filtered through milipore filter and filtrates thus obtained were tested for the activity of xylanase.
Assay Method: Xylanase activity was determined by the spectrophotometric method of Miller (1959).

Results and Discussion
The production of xylanase by *Arachniotus* sp. Was optimized under following conditions and results have been given as under:

Fermentation Period: Fermentation of corn stover was carried out for different time periods viz., 24, 48, 72 and 96 hours to determine the best incubation period for the production of maximum xylanase activity. Maximum xylanase activity (0.647 IU/mL/min) was recorded after 48 h incubation. The results of present study are in line with Balakrishnan *et al*. (1992) who produced xylanase by alkalophilic bacillus grown on a wheat bran/yeast extract medium for 48 h.

Substrate: Water Ratio: Xylanase activity was determined (Table 2) in the culture supernatant of *Arachniotus* sp. grown in the medium containing different substrate:water ratios (2.5, 5, 7.5 and 10%) and it was found that 7.5 percent substrate:water ratio resulted in maximum xylanase production (0.673 IU/mL). Findings of present study are

Table 2: Effect of different levels of substrate water ratio $(NH_4)_2SO_4$, $CaCl_2.2H_20$, $MgSO_4.7H_20$, KH_2PO_4, Yeast extract and molasses (cane) on the production of xylanase by *Arachniotus* sp.

S. No.	Substrate water ratio (%)	$(NH_4)_2$ SO_4 (%)	$CaCl_2.2H_2O$ (%)	$MgSO_4.7H_2O$ (%)	KH_2PO_4 (%)	Yeast extract (%)	Molasses (cane) (%)	Enzyme activity (IU/mL)
1	2.5	-	-	-	-	-	-	0.107
2	5.0	-	-	-	-	-	-	0.404
3	7.5	-	-	-	-	-	-	0.673
4	10.5	-	-	-	-	-	-	0.630
1	7.5	0.1	-	-	-	-	-	0.93
2		0.2	-	-	-	-	-	0.963
3		0.3	-	-	-	-	-	1.095
4		0.4	-	-	-	-	-	0.962
1			0.01	-	-	-	-	1.55
2			0.05	-	-	-	-	2.21
3	7.5	0.3	0.10	-	-	-	-	1.96
4			0.50	-	-	-	-	1.74
1				0.01		-	-	2.17
2				0.015		-	-	2.43
3	7.5	0.3	0.05	0.02		-	-	3.29
4				0.025		-	-	2.92
1					0.1		-	3.50
2					0.2		-	3.43
3	7.5	0.3	0.05	0.02		0.3	-	3.32
					0.4	-	-	3.05
1						0.05	-	3.7
2	7.5	0.3	0.05	0.02		0.1	0.1	4.32
3						0.15	-	5.29
4						0.20	-	4.79
1							0.5	3.83
2	7.5 4.50	0.3	0.05	0.02		0.1	0.15	1.0
3							1.50	5.42
4							2.00	5.94

comparable with Smith and Wood (1991) who obtained optimum enzyme activity when *Aspergilus awamori* was grown on 4 percent oat spelt xylan.

$(NH_4)_2S0_4$: The xylanase activity was assayed at four concentrations of $(NH_4)_2$ SO_4 i.e. 0.1, 0.2, 0.3 and 0.4% and the results revealed that 0.3per cent $(NH_4)_2SO_4$ facilitated the maximum xylanase production (0.963 IU/mL) (Table 2). Dubeau *et al*. (1987) observed that 0.05% $(NH_4)_2SO_4$ in growth medium containing 1% xylan as a carbon source fermented with *Chaetornium cellulolyticum* maximum gave xylanase (0.92 IU/mL). The difference in xylanase activity is attributed to the difference of substrate and organsim.

$CaCl_2.2H_2O$: Xylanase production was studied at 0.01, 0.05, 0.1 and 0.5% $CaC1_2.2H_20$ alongwith potimum (7.5%) substrate: water ratio and $(NH_4)_2SO_4$ (Table 2). It was found that 0.05% $CaCl_2.2H_20$ was the best of all the levels tried. Sinha and Sengupta (1 995) also reported 0.05% as the optimum concentration of $CaCl_2.2H_20$ in growth medium of xylan fermented with *Termitomyces clypeatus* showing 4 1U/mL xylanase activity.

$MgSO_4.7H_20$: Addition of 0.01, 0.015, 0.02 and 0.025%, $MgSO_4.7H_20$ resulted in 2.17, 2.43, 3.29 and 2.92 IU/mL/min of xylanase. It was found that 0.02% $MgSO_4.7H_20$ facilitated the highest production of xylanase. Dubeau *et al*. (1987) observed 0.15 percent as the optimum concentration of $MgS0_4.7H_20$ in the growth medium containing 1 percent xylan used for xylanase production by *Chaetomium cellulolyticum*.

KH_2PO_4: To study the effect of KH_2PO_4 on xylanase production *Arachniotus* sp. was grown in basal medium containing 7.5 per cent corn stover initially adjusted at various levels of KH_2PO_4 i.e. 0.1, 0.2, 0.3 and 0.4% (Table 2). It was observed that at 0.1 % KH_2PO_4 showed maximum xylanase activity (3.53 IU/mL). Rajoka and Malik (1984) recovered maximum xylan6se activity by fermenting 1% kallar grass with *Cellulomonas flavigena* NIAB 441 after 5 days of incubation in the presence of 0.05% KH_2PO_4. The difference in optimum KH_2PO_4 level was due to the use of

kallar grass and *C. lavigena* in place of corn stover and *Arachniotus* sp. respectively.

Yeast Extract: For the maximum xylanase production different concentrations (0.05, 0.1, 0.15 and 0.2%) of yeast extract were added to the medium (Table 2). It was found that 0.15% yeast extract facilitated the maximum xylanase activity. Purkarthofer *et al*. (1993) produced maximum xylanase from xylan by *Thermomyces lanuginosus* with 1.75% yeast extract in medium. The difference may be attributed to difference of substrate and the organism.

Molasses (Cane): Xylanase production was investigated by the addition of 0.5, 1.0, 1.5 and 2.0 percent molasses (cane) alongwith optimum levels of substrate (7.5%), $(NH_4)_2SO_4$ (0.3%), $CaCl_2.2H_20$ (0.05%), $MgSO_4.7H_20$ (0.02%), KH_2PO_4 (0.1%) and yeast extract (0.15%) (Table 2). The maximum xylanase activity (5.94 IU/mL) was recorded in the medium containing 2% molasses. Smith and Wood (1991) also observed an increase in xylanase production by *A. awamori* by the addition of molasses and also recovered maximum xylanase with 2 percent molasses in the xylan growth medium.

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