PRODUCTION OF CARBOHYDRASES IN THE FERMENTATION MEDIUM OF CORNSTOVER AND ITS BIOASSAY

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ABSTRACT

A study was conducted to determine optimum conditions for maximum enzyme production by Arachniotus spp. using cornstover as substrate. Maximum FPase, CMCase, Xylanase, α-amylase and ß-amylase production were obtained in the culture supernatant after 96 hours at 32°C growth in the medium containing 6 per cent constover, 0.1 per cent KH_2PO_4, 0.005 per cent $MgSO_4.7H_2O$ and 0.0075 per cent $CaCl_2$ at pH 4. These enzymes possessed the potential to saccharify crude fibre into reducing sugars.

INTRODUCTION

Animal feed industry in Pakistan is passing through an era of shortage of conventional ingredients. The scarcity of feed ingredients challenge the nutritionists to incorporate non-conventional feed resources in the feed. Agricultural and agroindustrial wastes are abundantly available at cheaper rates but they have the problem of high fibre contents. Due to high fibre contents they cannot be incorporated in non-ruminant feed at higher levels. Microbial enzymes which are active and quite stable, have the capability to reduce fibre contents of these wastes. Ultimately these wastes can be incorporated in non-ruminant feeds at higher levels and problem of shortage of ingredients can be solved. Hence it is imperative to produce cellulytic microbial enzymes to be incorporated in the fibous poultry feed. It demands the production of cellulytic microbial enzymes.

The present study was undertaken to find out optimum cultural conditions for maximum production of carbohydrases by Arachniotus spp. grown on cornstover. Cornstover has high fibre and low energy contents. The availability of energy is limited due to lignocellulosic bonds present in fibre (Morrison, 1959). Arachniotus spp. has complete enzyme system to hydrolyse these lignocellulose into useful products.

MATERIALS AND METHODS

Organism

A fungal organism, Arachniotus spp. was used in the study. Stock culture were maintained on cornstover agar slants. Composition of cornstover agar is given in Table 1.

Table 1: Composition of sporulation and inoculation mediums for fungal culture at pH 4, 32°C incubated for 96 hours.

Ingredients	Sporulation medium quantity/ litre	Inoculum medium quantity/ litre
Cornstover	10.0 g	60.0 g
KH_2PO_4	0.001 "	1.00 "
$MgSO_4.7H_2O$	0.075 "	0.1 "
$CaCl_2$	0.001 "	0.1 "
Nitrogen Source (Poultry dropping extract)	1.000 ml	10.0 ml
Agar	20.00 g	-
Distilled water	1000.00 ml	1000.00 ml

Inoculum preparation

Fresh inoculum was prepared by transferring spores of Arachniotus spp. from the cornstover agar slants to the autoclaved inoculum medium (Table 1). The inoculated flask was incubated for 96 hours at 32°C on an orbital shaker at 120 rpm. The mycelium culture was removed by filteration. The filtrate containing homogenous suspension of fungal spores was diluted with sterile water to 10^6 - 10^7 spores/ml.

Optimum conditions

In this study cornstover was fermented for 96 hours with Arachniotus spp. under different conditions like substrate water ratio, ionic concentrations (KH_2PO_4, $MgSO_4.7H_2O$, $CaCl_2$) for optimum production of enzymes (CMCase, FPase, Xylanase, α-amylase and ß-

amylase) at pH 4 and 32°C temperature in shake medium.

Substrate water ratio (2, 4, 6 and 8) and ionic concentration of KH_2PO_4 (0.05, 0.075, 0.1, and 0.125), $MgSO_4.7H_2O$ (0.0025, 0.005, 0.0075 and 0.01) and $CaCl_2$ (0.0025, 0.005, 0.0075 and 0.01) were optimized in a series of experiments and most suitable ionic concentration of one experiment was used in next one.

After each experimental study, the broth of fermentation media was filtered through millipore filter paper. The filtrate thus obtained in each experiment was tested for the activity of different enzymes.

Enzyme Assays

Xylanase activity was determined by the method described by Millor (1959), while the FPase and CMCase activities were determined by the methods described by Gadgil *et al.* (1995) and α and ß-amylase activities were determined by the methods described by Bernfeld (1951).

RESULTS AND DISCUSSION

Substrate water ratio

FPase, CMCase, Xylanase, α-amylase and ß-amylase production were determined in the culture supernatant of *Arachniotus* spp. grown in the medium containing different concentrations (2, 4, 6 and 8%) of substrate water ratio. Optimum production of above mentioned enzymes occurred at 6% substrate water ratio as shown in Table 2.

Findings of present study are lower than those of Mousumi and Nanda (1993) who obtained 33 U mg^{-1} (0.2 IU/ml/min) Xylanase activity by using 2% jute stalk fermented with *Aspergillus syndowii*. This value is different from present value because jute stalk was found to be more fibrous and less compact. Hence more and easily accessible enzyme to pentose residue than lignocellulose present in cornstover.

Ionic concentrations

KH_2PO_4

To study the effect of KH_2PO_4 on the formation of extracellular enzymes (FPase, CMCase, Xylanase, α-amylase and ß-amylase) *Arachniotus* spp. was grown in basal medium containing 6% cornstover initially adjusted at various levels of KH_2PO_4 i.e. of 0.05, 0.075, 0.1 and 0.125% (after autoclaving) as shown in Table 2. Maximum activities of above mentioned enzymes were recorded at 0.1 % KH_2PO_4.

The results are in line with those of Jensen *et al.* (1987). They reported an optimum concentration of KH_2PO_4 (0.1%) in the growth medium containing starch as a carbon source fermented with *Thermomyces langinosus*. The extracellular amylase produced had shown 0.68 IU/ml activity. This does not agree with the value obtained in the present study. This difference is attributed to the use of insoluble fibrous substrate (cornstover) in the present study, while a soluble substrate (starch) was used by Jensen *et al.* (1987).

$MgSO_4.7H_2O$

Maximum activities of FPase, CMCase, Xylanase, α-amylase and ß-amylase produced with different concentrations (0.0025, 0.005, 0.0075 and 0.01%) of $MgSO_4.7H_2O$ along with optimum concentration of (0.1%) KH_2PO_4 and 6% substrate water ratio contained in the growth medium, as shown in Table 2. Highest activities of above mentioned enzymes were obtained when enzyme filtrate contained 0.005% $MgSO_4.7H_2O$. Findings of present study are lower with those of Mousumi and Nanda (1993). They obtained high Xylanase activity from *Aspergillus syndowii* during growth on Jute stalk lignocellulose and reported 0.2 IU/ml Xylanase activity with 0.005 % $MgSO_4.7H_2O$ after 6 days of incubation at 28°C temperature. The enzyme activity had greater value than that of the present value because Mousumi and Nanda (1993) also used trace elements in the medium alongwith $MgSO_4.7H_2O$. and KH_2PO_4. Chahal *et al.* (1987) also reported similar findings.

$CaCl_2$

Various enzyme activities of FPase, CMCase, Xylanase, α-amylase and ß-amylase were assayed by addition of 0.0025, 0.005, 0.0075 and 0.01% $CaCl_2$ in the growth medium along with optimum levels of KH_2PO_4 (0.01%), $MgSO_4.7H_2O$ (0.005%) and 6% substrate water ratio as shown in Table 2.

Maximum activities of above mentioned enzymes were obtained in the broth of fermentation medium with 0.0075% $CaCl_2$. By increasing the concentration of $CaCl_2$ the enzyme activities declined.

Results of present study are in line with those of Sinha and Sengupta (1995) who reported the optimum concentration of $CaCl_2$ (0.05%), in growth media of xylan fermented with *Termitomyces clypeatus* showed 4.8 U/ml xylanase activity. More $CaCl_2$ used in this media because xylan contain no Ca, while cornstover already has 0.54 per cent calcium. The enzyme activity value indicated that xylan was better source for Xylanase activity than cornstover.

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Table 2: Influence of different levels of substrate water ratio, KH_2PO_4, $MgSO_4.7H_2O$, $CaCl_2$ on the activity of different enzymes (IU/ml/min) by *Arachniotus* spp. in the growth medium containing cornstover.

S. #	Substrate water ratio (%)	KH_2PO_4 %	$MgSO_4.7H_2O$ %	$CaCl_2$ %	FPase	CMCase	Xylanase	ß-α-amylase	amylase
1	2	-	-	-	0.92	0.0093	0.001	1.52	3.04
2	4	-	-	-	2.13	0.077	0.003	2.60	3.90
3	6	-	-	-	3.13	0.148	0.005	3.20	6.07
4	8	-	-	-	2.23	0.073	0.003	3.00	4.20
1	6	0.05	-	-	1.16	0.117	0.0037	2.67	3.34
2	6	0.075	-	-	2.84	0.204	0.0038	2.73	4.62
3	6	0.1	-	-	4.00	0.375	0.0053	3.27	6.74
4	6	0.125	-	-	3.62	0.285	0.0042	2.80	5.20
1	6	0.1	0.0025	-	1.46	0.1852	0.0055	3.47	3.94
2	6	0.1	0.005	-	5.17	0.500	0.0065	4.60	8.00
3	6	0.1	0.0075	-	4.55	0.397	0.0058	3.94	5.42
4	6	0.1	0.01	-	3.39	0.310	0.0057	3.80	5.24
1	6	0.1	0.005	0.0025	1.80	0.273	0.0070	5.20	4.34
2	6	0.1	0.005	0.005	3.89	0.467	0.0074	5.40	5.69
3	6	0.1	0.005	0.0075	7.38	0.860	0.0080	6.60	8.67
4	6	0.1	0.005	0.01	5.85	0.585	0.0075	5.8	6.07

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