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Production of Cellulases and Hemicellulases from Wheat Bran by *Arachniotus* sp. and Their Bioassay

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Abstract

The study was carried out for the maximum production of cellulases and hemicellulases at different substrate water ratio and ionic concentration. it was observed that maximum activities of above mentioned enzymes in IU/ml/min were monitored on 30 percent substrate: water ratio with 0.2 percent KH_2P0_4 and 0.01 percent $MgSO_4.7H_20$. Maximum FPase and β-glucosidase activities were noted at 0.005 percent $CaCl_2$ whereas CMCase and xylanase showed maximum activities in he presence of 0.01 and 0.1 percent $CaCl_2$.

Introduction

The utilization of wastes from agricultural production for the production of useful products, can assest in reducing some waste management problems. In the biological systems, microorganisms are used to utilize wastes as potential energy source for the synthesis of very useful fermented products such as enzymes and biomass protein.

The enzymes like lignocellulosic in these fermented products hydrolyze lignocellulosic (LC) materials which are the most important components of renewable biomass and are currently over produced, but under utilized (Rajoka and Malik, 1997). The complete breakdown of cellulose is carried out by the cellulase complex which is found to consist of three basic components (CMCase, Avicelase and β-glucosidase) (Stutzenberger, 1990). The three enzymes act synergistically to convert cellulose into glucose.

The cellulases and hemicellulases cover a complex group of enzymes (Moo-Young, 1985). Production of these enzymes by culturing *Arachniotus* sp, on a fibrous substrate like wheat bran, would not only reduce the pollutants, but will also serve as potential source of energy and biomass protein.

The study was planned to achive the following objectives:

1. Production of cellulases and hemicellulose by *Arachniotus* sp. cultured on wheat bran substrate.
2. Optimization of substrate: water ratio as well as ionic concentrations for the maximum production of cellulase and hemicellulases in fermented biomass.
3. Bioassay of cellulases and hemicellulases thus produced.

Materials and Methods

Meat bran was dried ground and fermented by *Arachniotus* sp. as described by Mubeen (1993).

Preparation of inoculum and culture cultivation: Spores of *Arachniotus* sp. were transferred from sporulation medium agar slants to the inoculum medium (Mubeen, 1993) and incubated at 32°C in shaker for 72 hours.

Five percent inoculum was transferred to the growth media (pH14) containing wheat bran in different substrate; water itios and varying concentrations of KH_2PO_4, $MgSO_4.7H_20$ and $CaCl_2$ after sterilization by autoclaving. The inoculated growth media were incubated at 32 for 72 hours.

Optimization of conditions: Substrate: water ratio: Different concentrations of wheat bran (i.e. 10, 20. 30 and 40%) were tested to select out the best one for the production of enzymes.

Ionic concentrations: Fermentation was also carried out with varying concentrations of KH_2PO_2, (0.2, 0.4, 0.6 and 0.8%), $MgSO_4$. $7H_2O$ (0.005, 0.01, 0.05 and 0.1%) and $CaCl_2$ (0.005, 0.01, 0.05 and 0.1%) to select out the most suitable concentrations of each nutrient for the production of enzymes.

Sample harvesting: After completion of fermentation period samples were harvested by simple and millipore filtration assembly simultaneously. Extract was used as crud enzyme solution and analyzed for activity of filter paper are carboxymethyl cellulose (FPase), (CMCase) β-glucosidase, and xylanase.

Enzyme Assay: The resulting biomass was filtered and analyzed for the activities of cellulases (FPase, CMCase and β-glucosidase) (Gadgil *et al*., 1995) and Xylanase (Rajoka and Malik, 1984).

Results and Discussion

Optimization of conditions

Substrate : water ratio: It was observed that maximum activity IU/ml/min of FPase, CMCase, β-glucosidase and xylanase was obtained at 30 percent substrate to water ratio (Table 1).

The results of this experiment were supported by Chahal (1986) and Mubeen (1993). Szczodrak (1988) reported that the optimum cellulose activity was observed in the medium containing 20 percent wheat straw and higher concentrations caused a decrease in enzymes.

Table 1: Effect of varying substrate: water ratio and ionic concentration on the production of cellulases and xylanase.

					Enzymatic activities (IU/ml/min)			
		KH_2PO_4	$MgSO_4$	$CaCl_2$	Cellulose			
Experiment No.	Percent Substrate				FPase	CMCase	β-glucosidase	Xylanase
1	10	-	-	-	1.119	0.069	0.076	0.0027
	20	-	-	-	2.004	0.070	0.077	0.0041
	30	-	-	-	2.558	0.093	0.089	0.0051
	40	-	-	-	2.130	0.089	0.081	0.0044
2	30	0.2	-	-	3.910	0.420	0.398	0.0048
	30	0.4	-	-	3.019	0.365	0.347	0.0045
	30	0.6	-	-	2.583	0.340	0.316	0.0043
	30	0.8	-	-	2.016	0.301	0.284	0.0043
3	30	0.2	0.005	-	5.259	0.773	0.894	0.0084
	30	0.2	0.01	-	6.597	1.197	1.197	0.0106
	30	0.2	0.05	-	5.925	1.015	1.015	0.0097
	30	0.2	0.10	-	5.067	0.974	0.974	0.0016
4	30	0.2	0.01	0.005	9.374	1.380	4.541	0.100
	30	0.2	0.01	-	6.427	1.987	4.007	0.0109
	30	0.2	0.01	-	6.231	1.900	3.774	0.0108
	30	0.2	0.01	0.10	6.159	1.807	3.474	0.0129

Experiment were conducted in such a way that condition optimized in one experiment was maintained in the next experiment.

Effect of KH_2P0_4: It was observed that 0.2 percent KH_2PO_4 gave the best enzyme yields (Table 1) under optimum culture conditions.

Effect of $MgSO_4.7H_20$: The maximum activity of FPase, CMCase, R-glucosidase and xylanase was found in the medium containing 0.01 percent $MgSO_4.7H_20$ (Table 1). The results are in agreement with those of Illanes *et al.* (1992), Macris (1984) and Watson *et al.* (1984) who referred almost matching concentrations of $MgSO_4.7H_20$ for the optimal yield of enzymes, through microbial fermentation.

Effect of $CaCl_2$: Maximum FPase and β-glucosidase activities were observed at 0.005 percent $CaCl_2$ concentration. While maximum CMCase and xylanase activities were noted with 0.01 and 0.1 percent $CaCl_2$, in the medium respectively (Table 1). Similar results have been reported by Okeke and Paterson (1992) and Kuhad and Singh (1993) for the production of cellulases and xylanase. It was therefore, concluded that the maximum activities of cellulases and hemicellulases were produced by *Arachniotus* sp. after 72 hours were obtained with 30 percent substrate water ratio in the presence of 0.2 percent, KH_2PO_4, 0.015 percent $MgSO_4.7H_7O$ and 0.005 percent $CaCl_2$ at 32°C, pH 4.

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