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Effect of Minerals and Moisture Level on the Solid Cultures of *A. niger* and *T. viride* for Extracellular Cellulolytic and Xylanolytic Enzymes Production

[1]Muhammad Mohsin Javed, [1, 2]Tehmina Saleem Khan and [1]Ikram-ul-Haq
[1]Institute of Industrial Biotechnology, GC University, Lahore, Pakistan
[2]Institute of Molecular Sciences and Bioinformatics, Lahore, Pakistan

Abstract: Solid-state fermentation is the method of choice for generating the industrially important enzymes. Carbon containing compounds in the agricultural wastes are broken down by the microorganisms, which produce the value added commercial enzymes. Thus, the present study describes the extracellular enzymes production like carboxymethyl cellulase (CMC-ase), avicelase, β-glucosidase and xylanase by *Aspergillus niger* and *Trichoderma viride*. Agricultural wastes like wheat bran, sugarcane bagasse, corncob, dried grass and rice bran were used under solid-state fermentation conditions. It was found that both the fungal strains have potential to utilize these wastes efficiently as carbon sources and secrete the extracellular proteins possessing hydrolytic ability for the breakdown of these substrates. Culture extract analysis of both strains showed that excreted protein has CMC-ase, avicelase, β-glucosidase and xylanase activity. However, their rate and profile was somehow different, indicating the preference of these strains towards substrate degradability. Additionally, the effect of mineral salts solution with different ratios (v/w) was also investigated and compared with distilled water. Results indicated that Eggins and Pugh minerals salt solution (2:1 ratio) in the presence of corncob as carbon source was best for the bio-synthesis of extracellular hydrolytic enzymes. Moreover, CMC-ase activity 2.24 U mL^{-1} min^{-1} in the culture filtrate of *T. viride*, while avicelase (1.64 U mL^{-1} min^{-1}), β-glucosidase (4.47 U mL^{-1} min^{-1}) and xylanase (164.6 U mL^{-1} min^{-1}) were found to be more active, when *A. niger* was grown on corncob.

Key words: Substrate degradability, agricultural wastes, carboxymethyl cellulase, avicelase, β-glucosidase, xylanase

INTRODUCTION

The ability of some microorganisms to metabolize agricultural wastes makes them potentially important to take advantages from vegetable residues. Agricultural and agro-industrial wastes like sugarcane bagasse, wheat bran, rice peel, corn straw, corncob, fruit peels and seeds, effluents from paper industry and orange bagasse have increased as a result of industrialization (Pandey *et al*., 2000). These wastes have becoming a severe problem regarding space for disposal and environmental pollution. To solve these problems, the wastes should be degraded for the recycling of energy. The complete degradation of these wastes under solid-state fermentation is found to be achieved by an efficient hydrolytic enzyme system, secreted by fungi. Hence, these wastes and residues themselves represent as alternative source of substrates for the fungal growth aiming to production of a protein complex system of extracellular hydrolytic enzymes (da Silva *et al*., 2005). The role and the action mechanism of the components of this system have been the center of many studies for the last four decades. It has been established that there are a list of main hydrolytic enzymes including cellulases (exo-β-glucanase, endo-β-glucanase and β-glucosidase) and xylanases that can degrade these

Corresponding Author: Tehmina Saleem Khan, Institute of Industrial Biotechnology, GC University, Lahore, Pakistan

wastes into useful compounds. The ability of fungi to grow on a solid substrate is a function of their requirements of water activity, their capacity of adherence and penetration into the substrate and their ability to assimilate mixtures of different polysaccharides due to the complex nature of the substrates used (Mitchell, 1992). In solid-state fermentation, moisture content is a critical factor, because this variable has influence the fungal growth and bio-synthesis and secretion of different metabolites (Pandey *et al*., 2000).

Hence, the present study was aimed to utilize different agricultural wastes for the production of industrially important hydrolytic enzymes. Two efficient fungal strains *Aspergillus niger* and *Trichoderma viride* that are considered to be the best degrading species were used for this purpose. Solid-stat fermentation method was followed for the cultivation of these strains on agricultural wastes. Distilled water and Eggins and Pugh mineral salts medium (with different ratios) were used as substrate moistening agents and were compared for the production of hydrolytic enzymes. A comparison was also made between the efficiency of *A. niger* and *T. viride* for their ability to degrade and utilize agricultural wastes.

MATERIALS AND METHODS

Microorganisms and Cultural Conditions

Aspergillus niger and *Trichoderma viride* were obtained from the culture bank of Institute of Industrial Biotechnology, GC University, Lahore. These strains were re-cultured at 30±1°C in culture tubes with 4.0% Potato Dextrose Agar (PDA) medium. In the tubes containing five day old fungal growth, 5.0 mL sterilized distilled water was added to obtain conidial suspension. One milliliter this suspension (containing 3.75×10^7 conidia) was transferred to Erlenmeyer conical flasks containing 20 g Solid Medium (SM). The SM were prepared by mixing the 20 mL distilled water/E and P medium [supplemented with (g L^{-1}); KH_2PO_4 (1.0), KCl (0.5), $(NH_4)_2SO_4$ (0.5), $MgSO_4.7H_2O$ (0.2), L-asparginine (0.5), $CaCl_2$ (0.1), yeast extract (0.5) with 5.0 pH] (Eggins and Pugh, 1962) with 20 g agricultural wastes (wheat bran, sugarcane bagasse, corncob, dried grass and rice bran), separately following a manual homogenization (all the wastes were washed, dried and powdered to a diameter of 2.0 mm). After 72 h of incubation, 100 mL distilled water was added to each flask. These flasks were maintained under occasional shaking for about 1 h at room temperature. The contents in flasks were centrifuged at 8,000 rpm for 15 min and supernatant was used for analytical purposes.

Analytical Methods

Endo-β-glucanase activity determination was based on the degradation of carboxymethyl cellulose. The method used was after Wood and Bhat (1988) using glucose as standard. Avicel was used as substrate for the analysis of avicelase in the supernatant. β-Glucosidase was estimated according to the method used by Rajoka and Malik (1996). p-Nitrophenyl-β-D-glucopyranoside was used as substrate and the results were compared with the standard curve of p-nitrophenol. Xylanase in the samples was determined using xylan as substrate. The xylose was used as standard for comparison. In the estimation of all the enzymes except β-glucosidase, reducing sugars were measured according to the method as described by Miller (1959). Finally the units of all the estimated enzymes were calculated and data was further processed to statistical analysis. One unit of enzyme activity is defined as the amount of enzyme required to liberate one micromole of substrate in one minute under standard conditions.

Statistical Analysis

Treatment effects were compared after Snedecor and Cochrane (1980) using computer software Costat, cs6204W.exe by applying two-way analysis of variance (two-way ANOVA). Significance

difference among replicates has been presented as Duncan's multiple range tests in the form of probability <p> values.

RESULTS AND DISCUSSION

The present study describes the utilization of agricultural wastes for the production of extracellular hydrolytic enzymes by *Aspergillus niger* and *Trichoderma viride* under solid-state fermentation. Agricultural wastes like wheat bran, wheat straw, rice bran, rice straw, sugar cane bagasse, corncob and dried grass were used in this investigation. Distilled water and E and P medium were used as moistening agents.

The results were presented after statistical analysis by applying two-way ANOVA. Standard deviation and least significance difference among replicates were determined. After one-way ANOVA, results indicated that *A. niger* and *T. viride* secretes both cellulolytic and xylanolytic enzymes, but their rates are varied. Mitchell (1992) also stated that filamentous fungal strains like *A. niger* and *T. viride* are the best adapted micro-organisms for solid-state fermentation owing to their physiological, enzymological and bio-chemical properties. Moreover, hyphal mode of fungal growth gives them a power to penetrate into the solid substrates, which gives them major advantage over unicellular micro-organisms for the colonization of the substrate and the utilization of available nutrients. Haq *et al.* (2006) also reported that *A. niger* and *T. viride* are best source of cellulolytic and hemicellulolytic enzymes. Gawande and Kamat (1999) observed that crude culture filtrate of *Aspergillus* strains hydrolyzed various lignocellulosic materials. Results indicated that *A. niger* and *T. viride* used agricultural wastes as carbon sources in the presence of distilled water (1:1 ratio). This is because these substrates are heterogeneous water insoluble materials, which have lignocellulosic nature with different percentages (Raimbault, 1998). However, higher rate of enzymes production (CMC-ase, avicelase, β-glucosidase and xylanase) was obtained with corncob. It is because that corncob is a complete substrate with all required micro- and macro-nutrients for the growth of microorganisms. Corncob contains 90% dry matter, 9.0% net energy for growth maintenance (NEg), 3.0% crude protein (CP), 50% bypass, 36% crude fiber (CF), 39% acid detergent fiber (ADF), 88% neutral detergent fiber (NDF), 56% effective neutral detergent fiber (eNDF), 0.5% ether extract (EE), 2.0% Ash, 0.12% calcium (CA), 0.04% phosphorous (P), 0.8% potassium (K), 0.40% sulphur (S) and 5.0% Zinc (Zn).

Using corncob as a substrate, *A. niger* produced 0.586 U mL^{-1} min^{-1} CMC-ase (Table 1), 0.46 U mL^{-1} min^{-1} avicelase (Table 3), 0.71 U mL^{-1} min^{-1} β-glucosidase (Table 5) and 18.7 U mL^{-1} min^{-1} xylanase (Table 7) on corncob at 5.0% level of significance with 0.011, 0.0078,

Table 1: Effect of distilled water and different ratios (v/w) of E and P medium on the production of carboxymethyl cellulase (U mL^{-1} min^{-1}) by *A. niger* under solid-state fermentation conditions

	A. niger				
		Ratio of E and P medium			
Substrates	Dist. H_2O (1:1)	(1:1)	(2:1)	(3:1)	LSD
Wheat bran	$0.476\pm0.005_b^d$	$0.566\pm0.002_b^c$	$0.756\pm0.003_b^a$	$0.645\pm0.005_b^b$	0.029
Sugar cane bagasse	$0.410\pm0.010_d^d$	$0.486\pm0.023_d^c$	$0.659\pm0.020_d^a$	$0.570\pm0.026_{cd}^b$	0.015
Corncob	$0.586\pm0.015_a^c$	$0.678\pm0.025_a^b$	$1.995\pm0.008_a^a$	$0.673\pm0.020_a^b$	0.027
Dried grass	$0.180\pm0.010_f^b$	$0.087\pm0.003_f^d$	$0.454\pm0.005_f^a$	$0.130\pm0.010_f^c$	0.008
Rice bran	$0.086\pm0.015_g^d$	$0.512\pm0.003_c^b$	$0.246\pm0.001_g^c$	$0.560\pm0.010_d^a$	0.021
Wheat straw	$0.425\pm0.005_c^d$	$0.514\pm0.002_c^c$	$0.699\pm0.001_c^a$	$0.590\pm0.010_c^b$	0.015
Rice straw	$0.386\pm0.015_e^b$	$0.207\pm0.003_e^d$	$0.578\pm0.001_e^a$	$0.350\pm0.010_e^c$	0.017
LSD	0.011	0.023	0.013	0.026	

Superscript alphabets show significant ranges among distilled water and different ratios of E and P medium. Subscript alphabets indicate significant ranges among different agricultural by-products (Significance level increases with ascending order of alphabets and vive versa)

Table 2: Effect of distilled water and different ratios (v/w) of E and P medium on the production of carboxymethyl cellulase (U mL^{-1} min^{-1}) by *T. viride* under solid-state fermentation conditions

	T. viride				
		Ratio of E and P medium			
Substrates	Dist. H_2O (1:1)	(1:1)	(2:1)	(3:1)	LSD
Wheat bran	$0.85\pm0.010_b^c$	$1.030\pm0.023_b^b$	$1.87\pm0.002_b^a$	$0.88\pm0.010_b^c$	0.032
Sugar cane bagasse	$0.64\pm0.010_d^c$	$0.815\pm0.005_d^b$	$0.98\pm0.007_d^a$	$0.80\pm0.010_d^b$	0.152
Corncob	$0.90\pm0.010_a^d$	$1.110\pm0.035_a^b$	$2.24\pm0.018_a^a$	$0.97\pm0.010_a^c$	0.031
Dried grass	$0.35\pm0.010_f^d$	$0.412\pm0.006_f^c$	$0.85\pm0.005_e^a$	$0.49\pm0.010_f^b$	0.015
Rice bran	$0.13\pm0.003_g^d$	$0.169\pm0.001_g^c$	$0.63\pm0.002_f^a$	$0.24\pm0.011_g^b$	0.023
Wheat straw	$0.75\pm0.010_c^c$	$0.889\pm0.003_c^b$	$1.18\pm0.006_c^a$	$0.85\pm0.005_c^b$	0.045
Rice straw	$0.56\pm0.010_e^c$	$0.697\pm0.003_e^b$	$0.99\pm0.005_d^a$	$0.68\pm0.010_e^b$	0.018
LSD	0.012	0.025	0.013	0.012	

Superscript alphabets show significant ranges among distilled water and different ratios of E and P medium. Subscript alphabets indicate significant ranges among different agricultural by-products (Significance level increases with ascending order of alphabets and vive versa)

Table 3: Effect of distilled water and different ratios (v/w) of E and P medium on the production of avicelase (U mL^{-1} min^{-1}) by *A. niger* under solid-state fermentation conditions

	A. niger				
		Ratio of E and P medium			
Substrates	Dist. H_2O (1:1)	(1:1)	(2:1)	(3:1)	LSD
Wheat bran	$0.36\pm0.005_c^c$	$0.48\pm0.002_c^b$	$1.08\pm0.026_c^a$	$0.47\pm0.003_c^b$	0.021
Sugar cane bagasse	$0.37\pm0.003_b^c$	$0.51\pm0.001_b^b$	$1.13\pm0.004_b^a$	$0.51\pm0.002_b^b$	0.042
Corncob	$0.46\pm0.010_a^c$	$0.60\pm0.004_a^b$	$1.64\pm0.002_a^a$	$0.56\pm0.010_a^b$	0.521
Dried grass	$0.16\pm0.002_f^d$	$0.21\pm0.001_f^c$	$0.34\pm0.001_f^a$	$0.28\pm0.002_f^b$	0.076
Rice bran	$0.06\pm0.001_g^c$	$0.08\pm0.004_g^c$	$0.25\pm0.001_g^a$	$0.15\pm0.002_g^b$	0.012
Wheat straw	$0.27\pm0.005_d^d$	$0.33\pm0.002_d^c$	$0.96\pm0.001_d^a$	$0.43\pm0.004_d^b$	0.155
Rice straw	$0.24\pm0.001_e^c$	$0.31\pm0.002_e^b$	$0.45\pm0.001_e^a$	$0.38\pm0.002_e^b$	0.033
LSD	0.0078	0.0027	0.0172	0.0070	

Superscript alphabets show significant ranges among distilled water and different ratios of E and P medium. Subscript alphabets indicate significant ranges among different agricultural by-products (Significance level increases with ascending order of alphabets and vive versa)

Table 4: Effect of distilled water and different ratios (v/w) of E and P medium on the production of avicelase (U mL^{-1} min^{-1}) by *T. viride* under solid-state fermentation conditions

	T. viride				
		Ratio of E and P medium			
Substrates	Dist. H_2O (1:1)	(1:1)	(2:1)	(3:1)	LSD
Wheat bran	$0.68\pm0.003_b^c$	$0.81\pm0.001_b^b$	$1.12\pm0.001_b^a$	$0.81\pm0.005_b^b$	0.551
Sugar cane bagasse	$0.15\pm0.001_g^d$	$0.68\pm0.001_c^a$	$0.54\pm0.001_g^b$	$0.26\pm0.003_f^c$	0.064
Corncob	$0.77\pm0.020_a^c$	$1.00\pm0.004_a^b$	$1.42\pm0.003_a^a$	$0.83\pm0.015_a^c$	0.025
Dried grass	$0.48\pm0.010_e^b$	$0.41\pm0.002_e^c$	$0.68\pm0.001_e^a$	$0.52\pm0.001_e^b$	0.073
Rice bran	$0.65\pm0.010_c^b$	$0.68\pm0.001_c^b$	$0.95\pm0.001_c^a$	$0.79\pm0.005_c^{ab}$	0.145
Wheat straw	$0.31\pm0.003_f^c$	$0.21\pm0.002_f^d$	$0.58\pm0.002_f^b$	$0.68\pm0.002_d^a$	0.085
Rice straw	$0.53\pm0.003_d^c$	$0.58\pm0.001_d^c$	$0.74\pm0.002_d^a$	$0.68\pm0.015_d^b$	0.055
LSD	0.0140	0.0021	0.0023	0.0111	

Superscript alphabets show significant ranges among distilled water and different ratios of E and P medium. Subscript alphabets indicate significant ranges among different agricultural by-products (Significance level increases with ascending order of alphabets and vive versa)

0.0065 and 0.0391 LSD, respectively. On the other hand, *T. viride* produced 0.90 U mL^{-1} min^{-1} CMC-ase (Table 2), 0.77 U mL^{-1} min^{-1} avicelase (Table 4), 0.59 U mL^{-1} min^{-1} β-glucosidase (Table 6) and 13.4 U mL^{-1} min^{-1} xylanase (Table 8), respectively with 0.012, 0.0140, 0.005 and 0.0345 LSD. da Silva *et al*. (2005) also worked on xylanase and CMC-ase by *Thermoascus aurantiacus*

Table 5: Effect of distilled water and different ratios (v/w) of E and P medium on the production of β-glucosidase (U mL^{-1} min^{-1}) by *A. niger* under solid-state fermentation conditions

	A. niger				
		Ratio of E and P medium			
	Dist. H_2O				
Substrates	(1:1)	(1:1)	(2:1)	(3:1)	LSD
Wheat bran	$0.64\pm0.003_b^c$	$0.77\pm0.002_b^b$	$3.63\pm0.043_b^a$	$0.74\pm0.003_b^b$	0.052
Sugar cane bagasse	$0.52\pm0.005_d^c$	$0.63\pm0.002_d^b$	$0.98\pm0.001_d^a$	$0.68\pm0.003_d^b$	0.245
Corncob	$0.71\pm0.007_a^d$	$0.87\pm0.003_a^b$	$4.47\pm0.002_a^a$	$0.82\pm0.003_a^c$	0.175
Dried grass	$0.12\pm0.002_g^c$	$0.15\pm0.002_g^c$	$1.25\pm0.002_c^a$	$0.22\pm0.005_g^b$	0.086
Rice bran	$0.46\pm0.003_e^c$	$0.57\pm0.002_e^b$	$0.76\pm0.003_e^a$	$0.56\pm0.003_e^b$	0.024
Wheat straw	$0.58\pm0.002_c^c$	$0.71\pm0.002_c^b$	$1.24\pm0.001_c^a$	$0.72\pm0.003_c^b$	0.331
Rice straw	$0.34\pm0.003_f^c$	$0.41\pm0.001_f^b$	$0.68\pm0.003_f^a$	$0.44\pm0.003_f^b$	0.071
LSD	0.0065	0.0030	0.0300	0.0014	

Superscript alphabets show significant ranges among distilled water and different ratios of E and P medium. Subscript alphabets indicate significant ranges among different agricultural by-products (Significance level increases with ascending order of alphabets and vive versa)

Table 6: Effect of distilled water and different ratios (v/w) of E and P medium on the production of β-glucosidase (U mL^{-1} min^{-1}) by *T. viride* under solid-state fermentation conditions

	T. viride				
		Ratio of E and P medium			
	Dist. H_2O				
Substrates	(1:1)	(1:1)	(2:1)	(3:1)	LSD
Wheat bran	$0.48\pm0.003_b^c$	$0.58\pm0.002_b^b$	$0.84\pm0.001_b^a$	$0.59\pm0.002_b^b$	0.014
Sugar cane bagasse	$0.28\pm0.003_d^c$	$0.36\pm0.001_d^b$	$0.62\pm0.002_d^a$	$0.37\pm0.003_d^b$	0.036
Corncob	$0.59\pm0.010_a^d$	$0.72\pm0.003_a^b$	$1.12\pm0.001_a^a$	$0.65\pm0.005_a^c$	0.142
Dried grass	$0.14\pm0.003_e^c$	$0.35\pm0.001_e^b$	$0.42\pm0.002_e^a$	$0.34\pm0.003_e^b$	0.074
Rice bran	$0.36\pm0.001_c^c$	$0.43\pm0.001_c^b$	$0.64\pm0.002_c^a$	$0.46\pm0.003_c^b$	0.054
Wheat straw	$0.28\pm0.001_d^b$	$0.11\pm0.002_g^c$	$0.39\pm0.001_f^a$	$0.12\pm0.003_g^c$	0.021
Rice straw	$0.08\pm0.001_f^d$	$0.17\pm0.002_f^c$	$0.39\pm0.001_f^a$	$0.21\pm0.003_f^b$	0.068
LSD	0.0055	0.0020	0.0165	0.0037	

Superscript alphabets show significant ranges among distilled water and different ratios of E and P medium. Subscript alphabets indicate significant ranges among different agricultural by-products (Significance level increases with ascending order of alphabets and vive versa)

Table 7: Effect of distilled water and different ratios (v/w) of E and P medium on the production of xylanase (U mL^{-1} min^{-1}) by *A. niger* under solid-state fermentation conditions

	A. niger				
		Ratio of E and P medium			
	Dist. H_2O				
Substrates	(1:1)	(1:1)	(2:1)	(3:1)	LSD
Wheat bran	$15.8\pm0.015_b^d$	$19.5\pm0.020_b^c$	$157.8\pm0.030_b^a$	$28.7\pm0.036_b^b$	0.024
Sugar cane bagasse	$12.8\pm0.020_d^d$	$15.7\pm0.015_d^c$	$154.5\pm0.036_d^a$	$22.8\pm0.036_d^b$	0.055
Corncob	$18.7\pm0.040_a^d$	$23.4\pm0.015_a^c$	$164.6\pm0.015_a^a$	$29.1\pm0.036_a^b$	0.034
Dried grass	$7.45\pm0.020_f^d$	$9.55\pm0.015_f^b$	$126.7\pm0.020_f^a$	$8.48\pm0.015_f^{bc}$	0.064
Rice bran	$15.1\pm0.020_c^d$	$18.7\pm0.015_c^c$	$156.4\pm0.035_c^a$	$25.8\pm0.020_c^b$	0.027
Wheat straw	$10.7\pm0.025_e^d$	$13.1\pm0.010_e^c$	$145.7\pm0.020_e^a$	$20.7\pm0.025_e^b$	0.037
Rice straw	$3.72\pm0.020_g^d$	$4.72\pm0.002_g^c$	$112.4\pm0.037_g^a$	$5.72\pm0.015_g^b$	0.064
LSD	0.0391	0.0219	0.0281	0.0310	

Superscript alphabets show significant ranges among distilled water and different ratios of E and P medium. Subscript alphabets indicate significant ranges among different agricultural by-products (Significance level increases with ascending order of alphabets and vive versa)

in solid-state fermentation and reported that best production of these two enzymes were achieved, when corncob was used as substrate. Orgaz *et al*. (2006) reported that different plant polysaccharides are best substrates for the production of hydrolytic enzymes.

A series of experiments was performed to compare between the efficiency of distilled water and E and P medium as moistening agent. Different ratios of E and P medium were used (1:1, 2:1 and 3:1).

Table 8: Effect of distilled water and different ratios (v/w) of E and P medium on the production of xylanase (U mL^{-1} min^{-1}) by *T. viride* under solid-state fermentation conditions

	T. viride				
		Ratio of E and P medium			
Substrates	Dist. H_2O (1:1)	(1:1)	(2:1)	(3:1)	LSD
Wheat bran	$11.4\pm0.036_b^d$	$14.1\pm0.020_b^b$	$81.5\pm0.020_b^a$	$13.4\pm0.025_b^c$	0.015
Sugar cane bagasse	$8.68\pm0.015_d^d$	$11.1\pm0.020_d^b$	$61.2\pm0.020_d^a$	$10.6\pm0.015_d^c$	0.034
Corncob	$13.4\pm0.025_a^d$	$17.1\pm0.015_a^b$	$98.3\pm0.026_a^a$	$15.4\pm0.026_a^c$	0.028
Dried grass	$3.45\pm0.020_f^c$	$5.61\pm0.015_f^b$	$55.1\pm0.030_f^a$	$5.48\pm0.010_f^b$	0.054
Rice bran	$9.66\pm0.025_c^c$	$11.7\pm0.015_c^b$	$74.1\pm0.025_c^a$	$11.6\pm0.015_c^b$	0.167
Wheat straw	$7.43\pm0.020_{e+}$	$9.12\pm0.003_e^b$	$60.1\pm0.015_e^a$	$9.44\pm0.026_e^b$	0.067
Rice straw	$1.43\pm0.020_g^c$	$1.87\pm0.001_g^c$	$51.2\pm0.005_g^a$	$2.46\pm0.036_g^b$	0.055
LSD	0.0345	0.0248	0.0279	0.0284	

Superscript alphabets show significant ranges among distilled water and different ratios of E and P medium. Subscript alphabets indicate significant ranges among different agricultural by-products (Significance level increases with ascending order of alphabets and vive versa)

Data obtained was compared statistically applying two-way ANOVA. With E and P medium (1:1 ratio), *A. niger* and *T. viride* produced 0.678 and 1.110 U mL^{-1} min^{-1} CMC-ase (Table 1 and 2), 0.60 and 1.00 U mL^{-1} min^{-1} avicelase (Table 3 and 4), 0.87 and 0.72 U mL^{-1} min^{-1} β-glucosidase (Table 5 and 6) and 23.4 and 17.1 U mL^{-1} min^{-1} xylanase (Table 7 and 8). These results revealed that E and P mineral salts medium is the best moistening agent for the production of hydrolytic proteins by both *A. niger* and *T. viride* as it contains the minerals for best growth of fungal strains. These minerals provide and fulfill the basic requirements for fungal growth like phosphate, sulphate, potassium, chloride, ammonium, magnesium, calcium and nitrogen. All these are considered to be essential in fermentation process for the growth of microbial strains and ultimate production and secretion of various hydrolytic proteins. After the optimization of E and P medium as best moistening agent, moisture level analysis was made and it was observed that 2:1 ratio of E and P medium was best as higher rates of enzymes were achieved, when *A. niger* and *T. viride* were cultured with E and P medium (2:1). Whereas; lower moisture content causes reduction in solubility of nutrients of the substrate, low degree of swelling and high water tension. On the other hand, higher moisture levels can cause a reduction in enzyme yield due to stearic hindrance of the growth of the producer strain by reduction in porosity of the solid matrix, thus interfering oxygen transfer (Lonsane *et al*., 1985).

CONCLUSIONS

Cellulosic and hemi-cellulosic compounds are mainly presented in agricultural by products as these compounds are an important part. These are being replenished constantly in nature by photosynthesis, goes waste in a lion's share in the form of pre-harvest and post-harvest agricultural losses and wastes of food processing industry. These cellulosic and hemi-cellulosic wastes have an immense potential to be utilized by filamentous fungi in the presence of limited amount of moisture and some essential ions. By the utilization of these wastes, production and recovery of several products and ingredients can be achieved as these are rich materials with all essential substances. These products mainly include hydrolytic enzymes, which possess a wide spectrum of researches in industrial area like food, paper, sugar and textile industries.

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