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Production of Cellulases by Solid State Fermentation of Different Agricultural Residues Using *Humicola insolens* MTCC 1433

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A B S T R A C T

The production of cellulases is an important strategy for the development of sustainable second-generation ethanol production processes. The present study was undertaken to select cheap and efficient agricultural residue suitable for cellulase production using fungus *Humicola insolens*. Six different agricultural residues *viz*., paddy straw, soybean pod husk, sugarcane bagasse, groundnut shells, corn stalks and pigeonpea pod husk were used as substrate in the solid state fermentation medium. Among all the substrates, maximum filter paper (FPase), carboxymethyl cellulase (CMCase), cellobiase and xylanase activities of 71.28 nmol/min/gds, 227.33 nmol/min/gds, 101.02 nmol/min/gds, 1988.62 nmol/min/gds respectively were recorded at 72 hours after incubation using soybean pod husk as substrate, followed by sugarcane bagasse with 58.78 nmol/min/gds FPase, 225.55 nmol/min/gds CMCase, 96.10 nmol/min/gds cellobiase and 1727.94 nmol/min/gds xylanase activities. Soybean pod husk showed maximum cellulase activity as compared to all comparatively other residues.

Introduction

The existence of pollution problems associated with agricultural wastes, scarcity of places for their disposal, costlier treatment options and increased need to save valuable resources have necessitated the utilization and bioconversion of waste into high value industrially useful products. Presently, huge amounts of agricultural and industrial cellulosic wastes have been accumulating in environment which can be considered as "waste" and can potentially be used to produce various value added products like enzymes, biofuels, animal feeds, chemicals, etc. (Abo-state *et al.,* 2013). Cellulose has attracted worldwide attention as a renewable resource that can be converted into bio-based products and bioenergy (Li *et al.,* 2009). Cellulose is a crystalline and linear polymer of thousands of D-glucose residues linked by β-1, 4-glycosidic bonds, and is considered the most abundant and renewable biomass resource and a formidable reserve of raw material (Quiroz-castaneda and Folch-mallol, 2013). Cellulose present in the lignocellulosic biomass is considered as one of the most important reservoirs of carbon for the production of glucose, a fuel and chemical feedstock (Kalogeris *et al.,* 2003). The most

promising technology to produce ethanol from agricultural biomass is based on enzymatic hydrolysis of cellulose by cellulases (Ahamed and Vermette, 2008).

Cellulose is commonly degraded by an enzyme called cellulase. Cellulases are inducible enzymes produced chiefly by fungi, bacteria and protozoan that catalyze the hydrolysis of cellulose (Mahalakshmi and Jayalakshmi, 2016). These microorganisms can be aerobic, anaerobic, mesophilic or thermophilic (Khatiwada *et al.,* 2016). Cellulase is an important and essential enzyme used for catalyzing the depolymerization of cellulose into fermentable sugar (Li *et al.,* 2009).

Among different microbes, fungi are the most studied group of cellulose-degrading microorganisms, owing to their high protein secretion capabilities, multi-component and synergetic cellulolytic enzyme activities (Obeng *et al.,* 2017). Fungal cellulases have the potential to digest cellulose, hemicelluloses and lignin by secreting diverse set of hydrolytic and oxidative enzymes (Fatma *et al.,* 2010). The most accepted commercial microbes that are found to be highly cellulolytic are *Aspergillus fumigatus*, *A. nidulans*, *A. acculeatus*, *A. niger*, *A. oryzae* (recombinant), *Melanocorpus albomyces*, *Trichoderma koningii*, *T. viride*, *Penicillium fumiculosum*, *Talaromyces emersonii*, *Humicola grisea*, *H. insolens*, *Fusarium solani*, *Irpex lactius*, *Sclerotium rolfsii*, etc. (Imran *et al.*, 2016).

The bioconversion of cellulose to fermentable sugars requires the synergistic action of complete cellulase system comprising of 1, 4-β-D glucanglucanohydrolase (EC 3.2.1.3), 1, 4-β-D glucancellobiohydrolyase (EC 3.2.1.91) and β-D glucosidase (EC 3.2.1.21). These enzymes are commonly referred to as endoglucanase, exoglucanase and cellobiase, respectively. The endoglucanases attack randomly and cleave the cellulose chains to form glucose, cellobiose and cellotriose. The exoglucanases attack the non-reducing end of cellulose to form the cellobiose units. Finally, cellobiase converts cellobiose into D-glucose (Gupta and Verma, 2015). In addition to the three major groups of cellulase enzymes, there is also the hemicellulase system which involves xylanase (endo-1, 4- D-xylan xylanohydrolase, EC 3.2.1.8) that catalyzes the hydrolysis of xylan to produce a mixture of shorter xylo-oligosaccharides, xylose and xylobiose (Abo-state *et al.,* 2013).

The production of cellulases is a major cost factor for the nascent lignocellulosic biofuel industry. The operational cost of enzymes results from two main factors, i.e. quality of enzyme required to hydrolyze biomass and cost of each kilogram of enzyme to the end user (Ellila *et al.,* 2017). Utilization of agricultural residues as substrates can help to reduce production cost of microbial enzymes and in addition, help in the management of various agricultural residues which are generated in abundance and difficult to dispose-off (Bajaj *et al.*, 2014).

For understanding the potential of different plant-based agricultural residues available in abundance, it is necessary to develop a low-cost growth medium for fungal strain with high cellulase production. Therefore, the present study was carried out to study fungal cellulase production using different agricultural residues.

Materials and Methods

Sources of agricultural waste

The substrates used for this study included paddy straw, soybean pod husk, sugarcane bagasse, groundnut shells, corn stalks and pigeonpea pod husk. Agricultural residues

were procured from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The residues were thoroughly washed with tap water to remove soil, dust and other unwanted materials prior to sun drying. The residues were, then, milled using an electric grinder and sieved through 30 mesh screen. The powdered residues were stored in polythene bags at room temperature for subsequent studies.

Preparation of inoculum

The fungus *Humicola insolens* (MTCC 1433) was procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh and maintained on Yeast powder-soluble starch (YPSS) agar slants at 4°C. The culture was inoculated on YPSS medium at 45ºC for 5-7 days of incubation period. After the incubation period, the spores were obtained by rinsing the plates with 10 ml sterile saline water (0.9%) and the spores were collected in a sterile tube. The spore count was determined with a haemocytometer and the spore concentration of 1×10^8 spores/ml was used as inoculum for cellulase production.

Cellulase production by solid state fermentation process

In order to select the best substrate for the enzyme production, experiments were carried out in 250 ml Erlenmeyer flasks containing 2.5 g each of the substrate of 30 mesh particle size and moistened with Mandels medium in a ratio of 1:4. Mandels and Weber medium (1 litre) contained 2 g KH_2PO_4, 1.4 g $(NH_4)_2SO_4$, 1 g peptone, 0.3 g $MgSO_4.7H_2O$, 0.3 g $CaCl_2$, 5 mg $FeSO_4.7H_2O$, 1.56 mg $MnSO_4.H_2O$, 1.4 mg $ZnSO_4.7H_2O$, 2.0 mg $CoCl_2.6H_2O$ and 1 ml Tween 80 (Mandels and Weber, 1969). The pH of the medium was adjusted to 5.0. The residues were, then, subjected to steam pre-treatment by autoclaving at 121°C (15 psi) for 20 minutes and cooled before inoculation. Following the autoclaving of the flasks, 1 ml of fungal spore suspension per gram of residue was inoculated into different flasks and incubated at 45°C for 168 h.

Enzyme extraction

Enzyme extraction was carried out at an interval of 24 h for a period of 7 days. For this, 30 ml of 0.1 M chilled citrate buffer (pH 4.8) was added to each flask and the flasks were then incubated at 27°C in an orbital shaking incubator with a shaking speed of 100 rpm for 10 min. The contents were subsequently filtered through Whatman filter paper No. 1 followed by centrifugation at 10,000 rpm at 4°C for 15 min. The supernatant, thus, obtained was dialyzed against same buffer (10X diluted) and assayed for different cellulolytic enzyme activities.

Determination of enzymatic activity

The enzymatic extract was used to determinate filter paper cellulase, carboxymethyl cellulase (CMCase), cellobiase and xylanase activities.

Filter paper cellulase (FPase) activity

FPase activity of enzyme filtrate was assessed by the method of Mandels *et al.*, (1976). FPase was determined using Whatman no.1 filter paper as substrate. Filter paper strip (1cm x 6 cm) was added to a reaction mixture that contained 0.5 mL 100 mM citrate buffer (pH 4.8) and 0.5 mL enzyme preparation and incubated the mixture at 50°C for 60 min. After incubation, the amount of reducing sugars released was measured as per Nelson (1944). Control tubes were run simultaneously by using heat inactivated enzyme. The enzyme activity was expressed as nanomoles of the reducing sugars released

per min per g dry substrate (nmol/min/gds). The protein content was determined by method of Lowry *et al.,* (1951) and specific activities were expressed as nanomoles of the reducing sugars released per min per mg protein (nmol/min/mg protein).

Carboxymethyl cellulase (CMCase activity)

CMCase activity was determined by the method of Wood and Bhat (1988) in a total reaction volume of 1 ml containing 0.3 ml enzyme preparation and 0.5 ml 0.5% (w/v) medium viscosity carboxymethylcellulose solution in citrate buffer (50 mM, pH 4.8). This mixture was incubated at 50°C for 30 min. The amount of reducing sugars released was measured as per Nelson (1944). The enzyme activity was expressed as nanomoles of the reducing sugars released per min per g dry substrate (nmol/min/gds) and specific activity was expressed as nanomoles of the reducing sugars released per min per mg protein (nmol/min/mg protein).

Cellobiase activity

Cellobiase activity was measured according to the method of Toyama and Ogawa (1977). Cellobiase activity was determined in a total reaction volume of 1 ml containing 0.5 ml enzyme preparation and 0.5 ml 0.05% (w/v) cellobiose solution in citrate buffer (100 mM, pH 4.8). This mixture was incubated at 50°C for 120 min. The amount of reducing sugars was measured as per Nelson (1944). The enzyme activity was expressed as nanomoles of the reducing sugars released per min per g dry substrate (nmol/min/gds) and specific activity was expressed as nanomoles of the reducing sugars released per min per mg protein (nmol/min/mg protein).

Xylanase activity

Xylanase activity was determined as described by Bailey et al (1992). For determination of xylanase activity, 0.5 ml of substrate (1%, w/v, xylan) in citrate buffer was incubated at 50°C with 0.5 ml enzyme preparation for 10 min, and the hydrolysis product was measured by Nelson (1944). The enzyme activity was expressed as nanomoles of the xylose released per min per g dry substrate (nmol/min/gds) and specific activity was expressed as nanomoles of the xylose released per min per mg protein (nmol/min/mg protein).

Statistical analysis

The data pertaining to enzyme activities of different plant based agricultural residues using *H. insolens* were analysed using analysis of variance (ANOVA) to test the differences among treatments in factorial CRD using CPCS1 software.

Results and Discussion

In the present study, six agricultural residues, *viz*. paddy straw, soybean pod husk, sugarcane bagasse, groundnut shells, corn stalks and pigeonpea pod husk were used as substrate under solid state fermentation for cellulase production by fungi *Humicola insolens*. The filter paper, CMCase, cellobiase and xylanase activities on different substrates were determined and discussed as under.

The filter paper activity of *H. insolens* (MTCC 1433) using different agricultural residues as a substrate was studied and the results of the same have been presented in Table 1. Significantly highest mean filter paper activity of 46.40 nmol/min/gds was observed using soybean pod husk as a substrate under solid state fermentation. Significantly highest mean filter paper activity of 46.39 nmol/min/gds was observed at 72 hours after incubation, followed by mean activity of 37.11 nmol/min/gds at 96 hours after incubation. The interaction between different incubation times and

agricultural residues as substrates revealed that there were significant differences in the filter paper activity of *H. insolens*. Significantly highest filter paper activity of 71.28 nmol/min/gds was observed using soybean pod husk as substrate at 72 hours after incubation, followed by filter paper activity of 58.78 nmol/min/gds with sugarcane bagasse as substrate. Similarly, highest specific activity was observed in soybean pod husk as represented in parentheses in Table 1.

The mean CMCase activity of *H. insolens* (Table 2) using different agricultural residues varied significantly. Significantly highest mean CMCase activity of 149.91 nmol/min/gds was observed using soybean pod husk as a substrate under solid state fermentation. Significantly highest mean CMCase activity of 148.07 nmol/min/gds was observed at 72 hours after incubation, followed by mean activity of 122.43 nmol/min/gds at 96 hours after incubation. The interaction between different incubation times and agricultural residues as substrates was also significant and revealed that there were significant differences in the CMCase activity of *H. insolens*. Significantly highest CMCase activity of 227.33 nmol/min/gds was observed using soybean pod husk as substrate at 72 hours after incubation. Similarly, highest specific activity was observed in soybean pod husk as depicted in Table 2.

The cellobiase activity of *H. insolens* has been presented in Table 3. Significantly highest mean cellobiase activity of 78.09 nmol/min/gds observed using soybean pod husk as a substrate under solid state fermentation. Significantly highest mean cellobiase activity of 73.74 nmol/min/gds was observed at 72 hours after incubation, followed by mean activity of 65.17 nmol/min/gds at 96 hours after incubation. The interaction between different incubation times and agricultural residues as substrates revealed that there were significant differences in the cellobiase activity of *H. insolens*. Significantly highest cellobiase activity of 101.02 nmol/min/gds was observed using soybean pod husk as substrate at 72 hours after incubation. Similarly, highest specific activity was observed in soybean pod husk as as shown in Table 3.

Different agricultural residues were used as substrate under SSF for xylanase production and the xylanase activity of *H. insolens* (MTCC 1433) has been presented in Table 4. The results revealed that significantly highest mean xylanase activity of 1547.06 nmol/min/gds was observed using soybean pod husk as a substrate under solid state fermentation.

Significantly highest mean xylanase activity of 1418.36 nmol/min/gds was observed at 72 hours after incubation, followed by mean activity of 1263.16 nmol/min/gds at 96 hours after incubation. The interaction between different incubation times and agricultural residues as substrates revealed that there were significant differences in the xylanase activity of *H. insolens*. Significantly highest xylanase activity of 1988.62 nmol/min/gds was observed using soybean pod husk as substrate at 72 hours after incubation. Similarly, highest specific activity was observed in soybean pod husk as represented in parentheses in Table 4.

In the present study, soybean pod husk showed maximum enzyme activity as compared to other residues. Medeiros et al (2008) used different carbon sources including wheat bran, oat spelt xylan, cellulose (avicel), oat bran, banana stem and coffee spent ground as source for production of hemicellulose-degerading enzymes (β-xylanase, β-mannanase and α-arabinofuranosidase) from *H. grisea* var. *thermoidea*.

Table.1 Filter paper activity (nmol/min/gds) of standard fungus *Humicola insolens* (MTCC 1433) using different agricultural residues as substrate under solid state fermentation

Agricultural Residues	Filter paper activity (nmol/min/gds)							
	24 h	**48 h**	**72 h**	**96 h**	**120 h**	**144 h**	**168 h**	**Mean**
Paddy straw	11.33	22.02	46.82	41.72	21.51	13.31	6.02	**23.25**
	(0.76)	(1.47)	(2.82)	(2.30)	(1.32)	(0.81)	(0.39)	**(1.41)**
Soybean pod husk	37.77	47.13	71.28	52.24	46.41	39.44	30.51	**46.40**
	(10.48)	(12.29)	(17.40)	(12.37)	(10.50)	(8.33)	(5.88)	**(11.04)**
Sugarcane bagasse	16.76	30.11	58.78	50.16	42.71	32.63	27.09	**36.89**
	(4.48)	(7.30)	(12.59)	(10.06)	(7.90)	(5.54)	(4.85)	**(7.53)**
Groundnut shells	35.38	39.81	54.70	39.81	30.96	27.56	23.13	**35.91**
	(7.71)	(8.34)	(10.41)	(7.94)	(5.78)	(4.52)	(3.49)	**(6.88)**
Corn stalks	9.53	13.96	27.22	24.29	19.05	13.14	9.53	**16.67**
	(0.57)	(0.83)	(1.49)	(1.28)	(1.00)	(0.69)	(0.47)	**(0.91)**
Pigeonpea pod husk	7.06	9.98	19.57	14.45	11.13	7.04	3.91	**10.45**
	(0.49)	(0.71)	(1.28)	(0.92)	(0.63)	(0.37)	(0.21)	**(0.66)**
Mean	**19.64**	**27.17**	**46.39**	**37.11**	**28.63**	**22.19**	**16.70**	
	(4.08)	**(5.16)**	**(7.66)**	**(5.81)**	**(4.52)**	**(3.38)**	**(2.55)**	
CD (p=0.05)	**Residues (R)**		**: 0.63 (0.11)**					
	Incubation time (h)		**: 0.68 (0.11)**					
	R x h		**: 1.66 (0.28)**					

Values in parentheses are specific activities which are expressed as nmol/min/mg protein

Table.2 CMCase activity (nmol/min/gds) of standard fungus *Humicola insolens* MTCC 1433 using different agricultural residues as substrate under solid state fermentation

Agricultural residues	CMCase activity (nmol/min/gds)							
	24 h	**48 h**	**72 h**	**96 h**	**120 h**	**144 h**	**168 h**	**Mean**
Paddy straw	33.15	67.67	111.61	98.90	84.66	76.80	37.30	**72.87**
	(2.22)	(4.53)	(6.72)	(5.45)	(5.21)	(4.69)	(2.42)	**(4.46)**
Soybean pod husk	108.16	141.92	227.33	179.02	158.77	143.32	90.81	**149.91**
	(30.02)	(37.01)	(55.51)	(42.40)	(35.93)	(30.26)	(17.50)	**(35.52)**
Sugarcane bagasse	65.20	101.29	225.55	188.85	130.95	106.03	71.93	**127.11**
	(17.41)	(24.56)	(48.30)	(37.87)	(24.22)	(17.99)	(12.88)	**(26.18)**
Groundnut shells	76.37	97.29	144.21	117.49	100.05	87.38	59.67	**97.49**
	(16.68)	(20.39)	(27.44)	(23.43)	(18.66)	(14.32)	(8.99)	**(18.56)**
Corn stalks	31.47	51.39	110.45	99.46	84.70	72.38	48.07	**86.50**
	(1.87)	(3.06)	(6.05)	(5.24)	(4.45)	(3.80)	(2.39)	**(3.84)**
Pigeonpea pod husk	17.13	29.30	69.24	50.83	38.99	21.31	11.80	**34.09**
	(1.18)	(2.07)	(4.52)	(3.22)	(2.21)	(1.13)	(0.63)	**(2.14)**
Mean	**55.25**	**81.48**	**148.07**	**122.43**	**99.69**	**84.54**	**53.26**	
	(11.56)	**(15.27)**	**(24.76)**	**(19.60)**	**(15.11)**	**(12.03)**	**(7.47)**	
CD (p=0.05)	**Residues (R)**		**: 2.09 (0.44)**					
	Incubation time (h)		**: 2.26 (0.48)**					
	R x h		**: 5.53 (1.17)**					

Values in parentheses are specific activities which are expressed as nmol/min/mg protein

Table.3 Cellobiase activity (nmol/min/gds) of *Humicola insolens* MTCC 1433 using different agricultural residues as substrate under solid state fermentation

Agricultural residues	Cellobiase activity (nmol/min/g-ds)							
	24 h	**48 h**	**72 h**	**96 h**	**120 h**	**144 h**	**168h**	**Mean**
Paddy straw	37.21	41.58	60.61	57.41	41.87	21.60	13.15	**39.06**
	(2.49)	(2.78)	(3.65)	(3.16)	(2.58)	(1.32)	(0.85)	**(2.41)**
Soybean pod husk	56.31	74.18	101.02	89.69	83.08	74.37	67.95	**78.09**
	(15.63)	(19.34)	(24.66)	(21.24)	(18.80)	(15.71)	(13.09)	**(18.35)**
Sugarcane bagasse	49.69	63.37	96.10	77.88	59.88	53.54	44.27	**63.53**
	(13.27)	(15.36)	(20.58)	(15.62)	(11.07)	(9.09)	(7.93)	**(13.27)**
Groundnut shells	46.02	57.76	83.20	73.67	58.19	50.77	38.02	**58.23**
	(10.04)	(12.11)	(15.83)	(14.70)	(10.85)	(8.32)	(5.73)	**(11.08)**
Corn stalks	27.05	44.52	56.31	52.10	40.15	18.81	13.47	**36.06**
	(1.61)	(2.66)	(3.09)	(2.74)	(2.11)	(0.99)	(0.67)	**(1.98)**
Pigeonpea pod husk	14.54	25.74	45.17	40.25	35.46	17.74	8.84	**26.82**
	(1.00)	(1.82)	(2.95)	(2.55)	(2.01)	(0.94)	(0.47)	**(1.68)**
Mean	**38.47**	**51.19**	**73.74**	**65.17**	**53.11**	**39.47**	**30.95**	
	(7.34)	**(9.01)**	**(11.79)**	**(10.00)**	**(7.90)**	**(6.06)**	**(4.79)**	
CD (p=0.05)	**Residues (R)**		**: 0.36 (0.09)**					
	Incubation time (h)		**: 0.39 (0.09)**					
	R x h		**: 0.96 (0.23)**					

Values in parentheses are specific activities which are expressed as nmol/min/mg protein

Table.4 Xylanase activity (nmol/min/gds) of standard fungus *Humicola insolens* MTCC 1433 using different agricultural residues as substrate under solid state fermentation

Agricultural residues	Xylanase activity (nmol/min/g-ds)							
	24 h	**48 h**	**72 h**	**96 h**	**120 h**	**144 h**	**168 h**	**Mean**
Paddy straw	1097.34	1251.58	1471.73	1329.72	1165.58	1053.64	930.79	**1185.77**
	(73.41)	(83.77)	(88.63)	(73.27)	(71.73)	(64.32)	(60.46)	**(73.66)**
Soybean pod husk	1246.58	1494.46	1988.62	1747.80	1572.38	1476.62	1302.97	**1547.06**
	(345.92)	(389.70)	(485.47)	(413.89)	(355.81)	(311.84)	(250.98)	**(364.80)**
Sugarcane bagasse	949.37	1162.24	1727.94	1625.79	1452.86	1327.23	1189.34	**1347.83**
	(253.63)	(281.75)	(370.01)	(326.01)	(268.70)	(225.25)	(212.98)	**(276.91)**
Groundnut shells	1032.08	1336.07	1649.48	1400.72	1342.63	1220.94	1105.07	**1298.14**
	(225.29)	(280.15)	(313.90)	(279.41)	(250.39)	(200.13)	(166.62)	**(245.13)**
Corn stalks	458.73	791.47	1282.58	1165.08	1077.90	930.50	854.04	**937.19**
	(27.30)	(47.26)	(70.31)	(61.38)	(56.62)	(48.88)	(42.44)	**(50.60)**
Pigeonpea pod husk	135.06	183.86	389.81	309.84	239.75	151.51	90.55	**214.34**
	(9.30)	(13.00)	(25.48)	(19.66)	(13.62)	(8.02)	(4.80)	**(13.41)**
Mean	**819.86**	**1036.61**	**1418.36**	**1263.16**	**1141.85**	**1026.74**	**912.13**	
	(155.81)	**(182.61)**	**(225.64)**	**(195.60)**	**(169.48)**	**(143.07)**	**(123.05)**	
CD (p=0.05)	**Residues (R)**		**: 5.73 (2.06)**					
	Incubation time (h)		**: 6.18 (2.22)**					
	R x h		**: 15.15 (5.45)**					

Values in parentheses are specific activities which are expressed as nmol/min/mg protein

Coffee spent-ground was selected as the best substrate for the production of hemicellulase activity, followed by wheat bran. Delabona *et al.,* (2012) studied the cellulolytic enzymes production by *A. fumigatus* using different substrates *viz.,* wheat bran, sugarcane bagasse, soybean bran and orange peel during solid state fermentation (SSF) process. Soybean bran showed maximal endoglucanase production (160.1 IU g^{-1} of substrate), followed by wheat bran (122.8 IU g^{-1}) and a mixture of sugarcane bagasse and wheat bran (1:1) (112.6 IU g^{-1}). The highest endogluconase production for all three substrates was achieved after 72 h of incubation. Bajaj *et al.,* (2014) reported that *Sporotrichum thermophile* LAR5 fungal isolate produced significant amount of cellulase on low-cost agro-residues as substrates. Wheat bran supported maximum cellulase production (2000 IU l^{-1}) followed by maize bran (1800 IU l^{-1}) and rice husk (1600 IU l^{-1}). Borkar (2016) studied carboxymethyl cellulase enzyme production by *Myriococcum albomyces* and *H. insolens* on four different agricultural wastes *viz.,* wheat straw, corn cob, jowar straw and groundnut shell. *Humicola insolens* showed maximum CMCase activity with corn cob, wheat straw and jowar straw after 4 days of incubation, whereas *M. albomyces* showed maximum activity with corn cob and jowar straw. These fungi were able to degrade agricultural wastes at faster rate by degrading cellulose. Ellila *et al.,* (2017) developed a simple, cost efficient cellulase production process that could be employed locally at a Brazilian sugarcane biorefinery. The low cost industrial residues were used to evaluate their potential for cellulase production and among the solid residues tested, soybean hulls showed the most desirable characteristics.

Cellulase enzyme could be used for conversion of cellulose into fermentable sugars, and cellulase based bio-refinery technologies are versatile and flexible because they utilize cheaper substrates for production of enzyme (Mane *et al.,* 2007). The ability to degrade cellulose is a character distributed among a wide variety of cellulolytic fungi. Studies dealing with cellulase production by fungi using low-cost residues under solid state fermentation are going on throughout the world to enhance the production and purity of fungal cellulases.

In conclusion, based on the results of present studies, soybean pod husk was found to be the most efficient substrate for enzyme production. Soybean pod husk showed higher activities in terms of FPase, CMCase, cellobiase and xylanase after 72 hours of incubation. This study, thus, concludes that soybean pod husk could be used as a potential substrate for production of cellulase and xylanase under solid state fermentation.

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