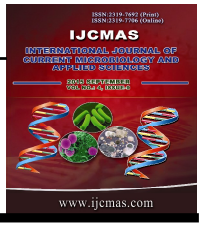
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 9 (2015) pp. 31-40**
http://www.ijcmas.com



Original Research Article

Recovery of Fermentable Sugars by Saccharolytic Fungi from Alkali Pretreated Agroresidues

T. H. Shankarappa[1]*, G. S. Geeta[2], M. J. Manju[2], B. D. Narotham Prasad[2], A. R. Alagawadi[2] and H. M. Vamadevaiah[2]

[1]Department of Agricultural Microbiology, College of Horticulture, Tamaka, Kolar, 563101, India
[2]Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad- 580 005, India
**Corresponding author email id: shankarappath@gmail.com*

Keywords

Agroresidues, Alkali Pretreatment, Cellulolytic fungi, Saccharification

A B S T R A C T

Five agro-residues namely sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk and corn stover of particle size 0.5 mm was subjected for alkali pretreatment, 3.0% NaOH for 8 hours under ambient condition followed by autoclaving at 121^0C, 15 lbs pressure for 1h. The residue obtained after the treatment was subjected for saccharification using five proven cellulolytic fungal cultures at different inoculums levels and incubation periods. The inoculation of *Trichoderma reesei* fungus at 4% under solid state fermentation had produced maximum amounts of reducing sugars and per cent saccharification in 6 days in pretreated substrates *viz*. sugarcane bagasse (173.33 mg g^{-1} and 24.64%), sugarcane tops (171.39 mg g^{-1} and 25.16%), sugarcane trash (180.50 mg g^{-1} and 26.50%), corn husk (185.84 mg g^{-1} and 26.98%) and corn stover (182.98 mg g^{-1} and 26.87%).

Introduction

The most common renewable fuel produced today is ethanol derived from corn grain (starch) and sugar cane (sucrose). It is expected that there will be limits to the supply of these raw materials in the near future; therefore, lignocellulosic biomass is seen as an attractive feed stock for future supplies of ethanol (Wyman *et al.*, 2005). The abundantly available lignocelluloses require pre-treatment for obtaining fermentable sugars and conversion of the same to ethanol. Several pretreatment methods have been used for effective delignification and recovery of cellulose and hemicellulose sugars from various substrates *viz.* physical, chemical (Shankarappa and Geeta, 2013), combination of physical and chemical (Soderstrom *et al.*, 2003) and biological pretreatments (Valmaseda *et al.*, 1991).

The hydrolysis of cellulose and hemicelluloses polysaccharides in to their respective monomers called as saccharification involves cellulolytic microorganisms or their enzymes namely,

cellulase, hemicellulase and xylanases. Several fungi are known to produce extracellular enzymes and bring about saccharification namely, *Trichoderma reesei* (Friedrich *et al.*, 1997), *T. Viridae* (Zayed and Meyer, 1996), *Aspergillus awamorii* (Friedrich *et al.*, 1997) and *Phanerochaete chrysosporium* (Koijam *et al.*, 2000).

Several feed stocks have been studied for their potentiality to yield fermentable sugars to produce ethanol by various researchers *viz*. rice straw (Karimi *et al.*, 2006), Bagasse (Singhania *et al.*, 2006), Cotton stalks, (Kerem *et al.*, 1992), Wheat straw (Zayed and Meyer, 1996), Alfalfa fibre (Sreenath *et al.*, 2001), Sugar cane leaves (Harikrishna *et al.*, 2001), sun flower hulls (Sharma *et al.*, 2004) and Corn stover (Teymouri *et al.*, 2005). Therefore, in this study, the abundantly available agroresidues which are outside the human food *viz.* sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk and corn stover were pretreated with alkali for delignification followed by biological saccharification using proven efficient cellulolytic fungi to recover fermentable sugars to be subjected for alcohol fermentation.

Materials and Methods

Five substrates *viz.,* sugarcane bagasse (procured from Malaprabha sahakari sugar factory, M. K. Hubli, Belgaum, Karnataka, sugarcane trash (Co-8014) and sugarcane tops (Co-8014) from the fields of Mr. Basavaraj, Yettinagudda, Dharwad, Karnataka and corn stover (Arjun) and corn husk (Arjun) from Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka were brought to the laboratory, chopped into small pieces, dried at 60^0C in a hot air oven for 12 h and powdered by dry milling (Willey mill) to obtain particle size of 0.5 mm (Karimi *et al.*, 2006). Five, proven cellulolytic fungal cultures *viz. Trichoderma reesei, T. Viridae, Aspergillus awamorii, A. sidowii* and *Phanerochaete chrysosporium* obtained from Department of Agricultural Microbiology, University of Agricltural Sciences, Dharwad, Karnataka were used for saccharification studies.

The substrates *viz.* Sugarcane bagasse, Sugarcane tops, Sugarcane trash, Corn husk and Corn stover of particle size 0.50 mm were pretreated with alkali NaOH at 3.0% for 8 hours under ambient condition and autoclaved at 121^0C, 15 lbs pressure for 1h. The quantity of alkali used was approximately 50 ml 10 g^{-1} dry substrate taken in 250 ml Erlenmeyer flasks, which was sufficient enough to moisten entire substrate except in case of bagasse where additional 10 ml was used (Shankarappa and Geeta, 2013). After the heat pre-treatment, substrates were washed with tap water followed by distilled water to remove the alkali content (until the pH was close to 7.0). Otherwise, the pH of the substrates was neutralized with acetic acid. The residue obtained after the treatment was dried in a hot air oven at 60^0C to constant weight. The delignified agroresidues had cellulose content of 0.633 g g^{-1} in sugarcane bagasse, 0.613 g g^{-1} in sugarcane tops, 0.613 g g^{-1} in sugarcane trash, 0.620 g g^{-1} in corn husk, and 0.613 g g^{-1} in corn stover (Shankarappa and Geeta, 2013), they were subjected for saccharification using proven cellulolytic fungal cultures.

Ten grams delignified, oven dried substrates (five) were transferred separately in to 250ml capacity Erlenmeyer flasks. Each substrate was wetted with nutrient solution (Singhania *et al.*, 2006) on the basis of its water holding capacity (approximately 5.0 ml g^{-1} dry substrate) and inoculated with each of the respective five fungal cultures at

different inoculums levels such as 2, 4 and 6% aseptically and incubated for different days *viz*. two, four, six and eight days at 30 ±1^{0}C (Valmaseda *et al.,* 1991). The fungal cultures maintained on potato dextrose agar slants were separately grown on potato dextrose broth, 50 ml in 150 ml Erlenmeyer flasks for five days on rotary shaker (150 rpm) at 30 ± 1^{0}C. The mycelia was homogenised and used as inoculums. The homogenized inoculum had 20 × 10^8 CFU per ml. After an interval of 2, 4, 6 and 8 days solid state fermentation, the growth of fungi was seized by autoclaving at 121^0 C for 15 min (Koijam *et al.,* 2000) and the samples were dried in oven at 60^0 C to constant weight and analysed for reducing sugars by DNSA method as described by Miller (1959). All the treatments were replicated three times and the data was statistically analyzed using factorial, completely randomized design. The per cent saccharification was calculated by the formula given below (Kaar and Holtzapple, 1998).

$$\% \text{ Saccharification} = \frac{\text{Reducing sugars (mg g}^{-1}) \times 0.9 \times 100}{\text{Initial cellulose (mg g}^{-1})}$$

Results and Discussion

The alkali pretreated substrates with 3.0% NaOH (8 h incubation at room temp.) followed by autoclaving at 121^{0}C (1 h) had cellulose content of 0.633 g g^{-1} sugarcane bagasse, 0.613 g g^{-1} in sugarcane tops, 0.613 g g^{-1} in sugarcane trash, 0.620 g g^{-1} in corn husk, and 0.613 g g^{-1} in corn stover. These delignified substrates were used for saccharification studies using different cellulolytic fungi (Shankarappa and Geeta, 2013).

The cellulolytic fungi *Trichoderma reesei* produced the mean maximum release of reducing sugars of 115.66, 130.74, 135.17, 135.70 and 134.86 mg g^{-1} respectively in sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk, and in corn stover which was significantly superior over other fungi. The lowest release of reducing sugars was observed in the control treatment which received no fungi inoculation (Table 1, 2, 3, 4 and 5).

The release of reducing sugars as influenced by inoculum levels indicated the mean maximum release of reducing sugars with 6.0% inoculum level to be at 94.62, 102.61, 103.22, 103.64 and 103.49 mg g^{-1} respectively for sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk, and for corn stover, 6% inoculum was on par with 4.0% inoculum level to release reducing sugars respectively in sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk, and in corn stover (91.16, 99.49, 99.96, 100.26 and 99.07 mg g^{-1}), these levels were significantly superior over 2.0% inoculum level across the cellulolytic fungi.

As regards average incubation time, incubation up to 8 days resulted in significantly highest release of reducing sugars respectively for sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk and for corn stover (122.19, 124.71, 125.04, 126.48 and 125.15 mg g^{-1}), it was found to be on par with 6 days incubation time (122.02, 120.42, 122.32, 122.89 and 125.53 mg g^{-1} respectively in sugarcane bagasse, sugarcane tops, sugarcane trash, corn husk, and in corn stover).

Incubation up to 6 days and 8 days were found to be significantly superior over 4 days and 2 days.

Table.1 Effect of different cellulolytic fungi and their various inoculum levels on release of reducing sugars from pre-treated bagasse (mg g^{-1})

Treatments (A)	Incubation time (C) (days)	Inoculums (B) 2 %	4 %	6 %	Means for treatments (A)
Trichoderma viridae	2	9.46 (01.35)	10.89 (01.55)	22.79 (03.24)	101.34
	4	63.55 (09.04)	74.03 (10.53)	98.33 (13.98)	
	6	149.81 (21.30)	162.73 (23.14)	150.59 (21.41)	
	8	152.58 (21.70)	164.87 (23.44)	156.49 (22.25)	
Trichoderma reesei	2	13.55 (01.93)	17.74 (02.52)	20.98 (02.98)	115.66
	4	83.27 (11.84)	124.00 (17.63)	137.33 (19.53)	
	6	144.79 (20.59)	173.33 (24.64)	169.44 (24.09)	
	8	174.12 (24.76)	168.00 (23.88)	161.33 (22.94)	
Aspergillus sidawia	2	17.74 (02.52)	52.60 (07.48)	89.17 (12.68)	104.89
	4	84.41 (12.00)	110.79 (15.75)	114.31 (16.25)	
	6	136.70 (19.44)	135.74 (19.30)	134.98 (19.19)	
	8	129.46 (18.40)	127.17 (18.08)	125.65 (17.86)	
Aspergillus awamori	2	32.12 (04.57)	63.74 (09.06)	78.60 (11.18)	111.17
	4	81.84 (11.64)	118.03 (16.78)	120.2 (217.09)	
	6	137.46 (19.55)	145.84 (20.73)	142.7 (92.30)	
	8	141.17 (20.07)	137.84 (19.60)	134.41 (19.11)	
Phanerochaete chrysosporium	2	9.46 (01.35)	15.46 (02.20)	20.89 (02.97)	80.45
	4	50.51 (07.18)	59.73 (08.49)	74.31 (10.56)	
	6	102.20 (14.53)	130.00 (18.48)	128.35 (18.25)	
	8	110.21 (15.67)	134.89 (19.18)	129.39 (18.39)	
Control	2	9.16 (01.30)	9.16 (01.30)	9.16 (01.30)	15.12
	4	16.83 (02.39)	16.83 (02.39)	16.83 (02.39)	
	6	17.22 (02.45)	17.22 (02.45)	17.22 (02.45)	
	8	17.27 (02.45)	17.27 (02.45)	17.27 (02.45)	
Means for inoculums (B)		**78.54**	**91.16**	**94.62**	
Incubation time (C) (days)	2	27.93			
	4	80.29			
	6	122.02			
	8	122.19			

	SEm ±	CD at 1%
Treatments (A)	1.373	5.070
Inoculums (B)	0.971	3.585
Incubation time (C) (days)	1.121	4.140
A x B	2.379	8.782
A x C	2.747	10.140
B x C	1.942	7.170
A x B x C	4.758	17.563

Figures in parentheses indicate per cent saccharification
Note: Initial cellulose content (633 mg g^{-1})

Table.2 Effect of different fungi inoculation and their inoculum levels on release of reducing sugars from pre-treated sugarcane tops (mg g^{-1})

Treatments (A)	Incubation time (C) (days)	Inoculums (B) 2 %	4 %	6 %	Means for treatments (A)
Trichoderma viridae	2	17.46 (02.56)	55.94 (08.21)	62.13 (09.12)	122.58
	4	106.89 (15.69)	128.50 (18.87)	129.84 (19.06)	
	6	155.84 (22.88)	166.50 (24.45)	148.69 (21.83)	
	8	166.13 (24.39)	172.89 (25.38)	160.12 (23.51)	
Trichoderma reesei	2	23.46 (03.44)	74.03 (10.87)	83.65 (12.28)	130.74
	4	101.08 (14.84)	130.21 (19.12)	142.26 (20.88)	
	6	167.33 (24.57)	171.39 (25.16)	166.34 (24.42)	
	8	173.73 (25.51)	173.02 (25.40)	162.33 (23.83)	
Aspergillus sidawia	2	25.08 (03.68)	75.46 (11.08)	89.36 (13.12)	113.06
	4	80.79 (11.86)	125.07 (18.36)	131.74 (19.34)	
	6	140.03 (20.56)	141.08 (20.71)	143.08 (21.01)	
	8	137.84 (20.24)	134.22 (19.71)	132.98 (19.52)	
Aspergillus awamori	2	37.46 (05.50)	77.93 (11.44)	94.03 (13.81)	116.43
	4	87.27 (12.81)	128.31 (18.84)	134.22 (19.70)	
	6	142.89 (20.98)	144.98 (21.29)	141.08 (20.71)	
	8	139.07 (20.42)	137.08 (20.13)	132.89 (19.51)	
Phanerochaete chrysosporium	2	24.89 (03.66)	15.65 (02.30)	26.79 (03.94)	74.16
	4	35.08 (05.15)	55.36 (08.13)	66.69 (09.79)	
	6	78.88 (11.58)	90.12 (13.23)	121.65 (17.86)	
	8	104.31 (15.31)	133.74 (19.64)	136.69 (20.07)	
Control	2	10.03 (01.47)	10.03 (01.47)	10.03 (01.47)	14.04
	4	14.32 (02.10)	14.32 (02.10)	14.320 (2.10)	
	6	15.89 (02.33)	15.89 (02.33)	15.89 (02.33)	
	8	15.93 (02.34)	15.93 (02.34)	15.93 (02.34)	
Means for inoculums (B)		**83.40**	**99.49**	**102.61**	
Incubation time (C) (days)	2	45.19			
	4	90.35			
	6	120.42			
	8	124.71			

	SEm ±	CD at 1%
Treatments (A)	0.607	2.241
Inoculums (B)	0.429	1.585
Incubation time (C) (days)	0.496	1.830
A x B	1.052	3.882
A x C	1.214	4.483
B x C	0.859	3.170
A x B x C	2.103	7.764

Figures in parentheses indicate per cent saccharification
Note: Initial cellulose content (613 mg g^{-1})

Table.3 Effect of different fungi inoculation and their inoculum levels on release of reducing sugars from pre-treated sugarcane trash (mg g^{-1})

Treatments (A)	Incubation time (C) (days)	Inoculums (B) 2 %	4 %	6 %	Means for treatments (A)
Trichoderma viridae	2	22.51 (03.31)	58.31 (08.56)	72.41 (10.63)	125.67
	4	108.31 (15.90)	120.41 (17.68)	123.27 (18.10)	
	6	161.27 (23.68)	171.46 (25.17)	169.65 (24.91)	
	8	167.27 (24.56)	172.89 (25.38)	160.31 (23.54)	
Trichoderma reesei	2	23.46 (03.44)	77.08 (11.32)	80.79 (11.86)	135.17
	4	111.45 (16.36)	130.60 (19.17)	142.89 (20.98)	
	6	174.07 (25.56)	180.50 (26.50)	174.03 (25.55)	
	8	175.08 (25.70)	179.08 (26.29)	173.08 (25.41)	
Aspergillus sidawia	2	23.46 (03.44)	80.98 (11.89)	89.46 (13.13)	110.15
	4	82.03 (12.04)	120.50 (17.69)	128.50 (18.87)	
	6	137.74 (20.22)	135.08 (19.83)	129.08 (18.95)	
	8	132.70 (19.48)	132.89 (19.51)	129.36 (18.99)	
Aspergillus awamori	2	36.69 (05.39)	79.17 (11.62)	95.17 (13.97)	114.67
	4	85.17 (12.50)	124.41 (18.27)	133.74 (19.63)	
	6	141.94 (20.84)	139.74 (20.52)	136.12 (19.99)	
	8	137.65 (20.21)	135.27 (19.86)	130.98 (19.23)	
Phanerochaete chrysosporium	2	8.31 (01.22)	16.31 (02.39)	25.84 (03.79)	74.79
	4	39.81 (05.85)	60.60 (08.90)	68.31 (10.03)	
	6	80.03 (11.75)	96.70 (14.20)	125.64 (18.44)	
	8	107.26 (15.75)	133.55 (19.61)	135.08 (19.83)	
Control	2	10.84 (01.59)	10.84 (01.59)	10.84 (01.59)	13.41
	4	10.50 (01.54)	10.50 (01.54)	10.50 (01.54)	
	6	16.22 (02.38)	16.22 (02.38)	16.22 (02.38)	
	8	16.08 (02.36)	16.08 (02.36)	16.08 (02.36)	
Means for inoculums (B)		**83.74**	**99.96**	**103.22**	
Incubation time (C) (days)	2	45.69			
	4	89.53			
	6	122.32			
	8	125.04			

	SEm ±	CD at 1%
Treatments (A)	0.320	1.182
Inoculums (B)	0.226	0.836
Incubation time (C) (days)	0.261	0.965
A x B	0.555	2.047
A x C	0.640	2.364
B x C	0.453	1.672
A x B x C	1.109	4.095

igures in parentheses indicate per cent saccharification
Note: Initial cellulose content (613 mg g^{-1})

Table.4 Effect of different fungi inoculation and their inoculum levels on release of reducing sugars from pre-treated corn husk (mg g^{-1})

Treatments (A)	Incubation time (C) (days)	Inoculums (B) 2 %	4 %	6 %	Means for treatments (A)
Trichoderma viridae	2	24.12 (03.50)	66.31 (09.63)	72.51 (10.53)	128.92
	4	109.46 (15.89)	122.79 (17.83)	123.17 (17.88)	
	6	168.90 (24.52)	175.08 (25.41)	171.36 (24.87)	
	8	173.65 (25.21)	173.46 (25.18)	166.22 (24.13)	
Trichoderma reesei	2	24.31 (03.53)	73.08 (10.61)	83.84 (12.17)	135.70
	4	109.50 (15.89)	132.22 (19.20)	140.50 (20.39)	
	6	177.17 (25.72)	185.84 (26.98)	173.08 (25.13)	
	8	173.65 (25.21)	180.31 (26.17)	174.89 (25.39)	
Aspergillus sidawia	2	27.74 (04.03)	75.08 (10.90)	87.27 (12.67)	107.67
	4	77.46 (11.24)	119.36 (17.33)	124.50 (18.07)	
	6	133.65 (19.40)	132.60 (19.25)	125.55 (18.23)	
	8	131.65 (19.11)	129.27 (18.77)	127.94 (18.57)	
Aspergillus awamori	2	39.74 (05.77)	77.17 (11.20)	89.46 (12.99)	112.64
	4	80.41 (11.67)	120.85 (17.54)	128.41 (18.64)	
	6	138.60 (20.12)	138.60 (20.12)	135.46 (19.66)	
	8	138.03 (20.04)	133.84 (19.43)	131.17 (19.04)	
Phanerochaete chrysosporium	2	9.46 (01.37)	16.72 (02.43)	30.79 (04.47)	78.39
	4	44.70 (06.49)	56.89 (08.26)	74.98 (10.89)	
	6	79.36 (11.52)	103.65 (15.05)	129.08 (18.74)	
	8	112.31 (16.30)	139.36 (20.23)	143.36 (20.81)	
Control	2	9.84 (01.43)	9.84 (01.43)	9.84 (01.43)	13.46
	4	13.45 (01.95)	13.45 (01.95)	13.45 (01.95)	
	6	14.69 (02.13)	14.69 (02.13)	14.69 (02.13)	
	8	15.86 (02.30)	15.86 (02.30)	15.86 (02.30)	
Means for inoculums (B)		**84.49**	**100.26**	**103.64**	
Incubation time (C) (days)	2	45.95			
	4	89.20			
	6	122.89			
	8	126.48			

	SEm ±	CD at 1%
Treatments (A)	0.257	0.950
Inoculums (B)	0.182	0.671
Incubation time (C) (days)	0.210	0.775
A x B	0.446	1.645
A x C	0.514	1.899
B x C	0.364	1.343
A x B x C	0.891	3.290

Figures in parentheses indicate per cent saccharification
Note: Initial cellulose content (620 mg g^{-1})

Table.5 Effect of different fungi inoculation and their inoculum levels on release of reducing sugars from pre-treated corn stover (mg g^{-1})

Treatments (A)	Incubation time (C) (days)	Inoculums (B) 2 %	4 %	6 %	Means for treatments (A)
Trichoderma viridae	2	23.35 (03.43)	65.46 (09.61)	69.70 (10.23)	128.24
	4	110.79 (16.27)	119.36 (17.52)	121.46 (17.83)	
	6	172.10 (25.27)	174.22 (25.58)	170.03 (24.96)	
	8	170.04 (24.97)	175.17 (25.72)	167.14 (24.54)	
Trichoderma reesei	2	25.25 (03.71)	74.22 (10.90)	79.17 (11.62)	134.86
	4	106.07 (15.57)	127.65 (18.74)	148.68 (21.83)	
	6	174.02 (25.55)	182.98 (26.87)	172.41 (25.31)	
	8	176.40 (25.90)	178.60 (26.22)	172.89 (25.38)	
Aspergillus sidawia	2	25.70 (03.77)	74.03 (10.87)	88.12 (12.94)	105.42
	4	74.50 (10.94)	114.41 (16.80)	131.74 (19.34)	
	6	126.12 (18.52)	127.74 (18.75)	127.46 (18.71)	
	8	127.84 (18.77)	125.08 (18.36)	122.31 (17.96)	
Aspergillus awamori	2	37.07 (05.44)	76.22 (11.19)	94.41 (13.86)	111.83
	4	79.70 (11.70)	121.65 (17.86)	131.93 (19.37)	
	6	138.22 (20.30)	133.93 (19.66)	132.51 (19.45)	
	8	135.36 (19.88)	132.32 (19.43)	128.60 (18.88)	
Phanerochaete chrysosporium	2	8.98 (01.32)	18.40 (02.70)	31.17 (04.58)	78.23
	4	48.03 (07.05)	55.55 (08.16)	77.17 (11.33)	
	6	81.46 (11.96)	100.70 (14.78)	126.69 (18.60)	
	8	109.84 (16.12)	140.03 (20.56)	140.69 (20.66)	
Control	2	13.46 (01.98)	13.46 (01.98)	13.46 (01.98)	15.01
	4	14.11 (02.07)	14.11 (02.07)	14.11 (02.07)	
	6	15.68 (02.30)	15.68 (02.30)	15.68 (02.30)	
	8	16.79 (02.47)	16.79 (02.47)	16.79 (02.47)	
Means for inoculums (B)		**82.78**	**99.07**	**103.49**	
Incubation time (C) (days)	2	46.20			
	4	89.50			
	6	121.53			
	8	125.15			

	SEm ±	CD at 1%
Treatments (A)	0.443	1.634
Inoculums (B)	0.313	1.155
Incubation time (C) (days)	0.361	1.334
A x B	0.767	2.830
A x C	0.885	3.267
B x C	0.626	2.310
A x B x C	1.533	5.659

Figures in parentheses indicate per cent saccharification
Note: Initial cellulose content (613 mg g^{-1})

The inoculation of 2% inoculum showed the maximum reducing sugar release in 8 days incubation period (in few cases – sugarcane bagasse and sugarcane tops with *Trichoderma reesei*) with respective substrates.

However, the inoculation of 4% and 6% inoculum levels resulted in maximum release of reducing sugars in just 6 days incubation period in the respective pretreated substrates. The release of highest amounts of reducing sugars was observed with 4% and 6% inoculum level in case of *T. reesei* in 6 days when compared to 2% inoculum in all the substrates (Table 1, 2, 3, 4 and 5). The 4% and 6% inoculation would have shortened the lag phase and favoured in the faster growth of the fungi resulting in secretion of higher amounts of cellulase

enzymes that could have saccharified the substrates in comparatively quick time (Narotham Prasad and Geeta, 2011).

Further, the inoculation of substrates with *T. reesei* fungi yielded the highest reducing sugar release as compared to other cellulolytic fungal inoculation in respective pretreated substrates, suggesting that *T. reesei* is very efficient over other fungi in terms of sugar conversion due to production of various cellulolytic enzymes (Friedrich *et al.*, 1997; Koijam *et al.*, 2000).

It was observed from the Table 1, 2, 3, 4 and 5 that the inoculation of *T. reesei* fungi at 4% under solid state fermentation had yielded the superior amounts of reducing sugars and per cent saccharification in 6 days incubation in respective pretreated substrates such as in sugarcane bagasse (173.33 mg g^{-1} and 24.64%), sugarcane tops (171.39 mg g^{-1} and 25.16%), sugarcane trash (180.50 mg g^{-1} and 26.50%), corn husk (185.84 mg g^{-1} and 26.98%) and in corn stover (182.98 mg g^{-1} and 26.87%). The use of 6% inoculums or extension of incubation of incubation period up to 8 days had not significantly increased the release of the reducing sugars.

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