Ethanol Production by *Aspergillus niger* US4MTCC9931 and *Saccharomyces cerevisiae* MTCC174 Using Different Lignocellulosic Biomass Feed Stocks

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To investigate the production of cellulases and hemicellulases from *Aspergillus niger* MTCC9931, solid state fermentation (SSF) was performed using 10 different lignocellulosic materials derived from agrowastes, *i.e*., rice straw, rice husk, wheat straw, corn cob, sugar cane bagasse, saw dust, banana stalk, *Eichhornia*, *Parthenium* stalk, and residual fruit pulp. Among these agrowastes, the maximum yield of reducing sugars (116.46 ± 2.56 g/mL) was observed with residual fruit pulp. Further, the same substrate showed the highest endoglucanase (389.1 ± 0.44 IU/g), MCC-adsorbable endoglucanase (3.4 ± 0.14 IU/g), cellulase (12.0 ± 0.13 IU/g), and FPase (4.9 ± 0.64 IU/g) activities. Sawdust showed the maximum xylanase activity (7478.0 ± 6.51 IU/g), and corncob showed maximum β-glucosidase activity (79.87 ± 1.15 IU/g). The maximum activities of all enzymes were observed at 72 h of SSF under shaking conditions. *A. niger* MTCC9931 can be concluded to be an important strain for potential applications in saccharification. The enzymatic hydrolysates of the agrowastes were used as substrates for ethanol production by *Saccharomyces cerevisiae* MTCC174. The maximum yield (35.34g/L) of ethanol was obtained when residual fruit pulp was used as a substrate. This study has further demonstrated the feasible biotechnological conversion of agrowastes to produce ethanol using both *A. niger* and *S. cerevisiae*.

Keywords: Agrowastes; Cellulases; Hemicellulases; Solid-state fermentation; Aspergillus niger

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INTRODUCTION

Lignocellulosic feedstock is considered an attractive raw material for the production of not only liquid fuels but also other chemicals. Because of its availability in large quantities and low cost, it is widely used in carbohydrate-based bio-refineries (Lynd 1989; Parisi 1989; Farrell *et al.* 2006; Gray *et al.* 2006; Herrera 2006). For bioethanol production, cellulose and hemicellulose present in the lignocellulosic biomass must be hydrolyzed with acids or enzymes to liberate fermentable sugars. During enzymatic conversion of lignocellulosic biomass to fermentable sugars, a pretreatment stage is required to break the lignin structure and partially solubilize cellulose and hemicellulose (Keller *et al.* 2003). Previous studies have demonstrated that enzymatic treatments were successful (Gubitz *et al.* 1998; Bajpai 1999). During ethanol production from lignocellulosic biomass, hydrolysis is the most problematic step. Hydrolysis of lignocellulosic biomass yields mainly glucose and xylose, and the conversion of both sugars to ethanol is imperative for the process to be economical. Hydrolysis by cellulases in combination with hemicellulases is essential for the efficient conversion of

lignocellulosic biomass to fuel (Mielenz 2001). Enzymatic hydrolysates produced by treatment with cellulases and hemicellulases have been exploited for the production of useful chemicals and solvents (Kuhad and Singh 1993). Fungi are the predominantly used organisms for the hydrolysis of lignocellulosic biomass (Bennett *et al.* 2002; Rabinovich *et al.* 2004). In this context, *Trichoderma reesei* has been the most extensively studied fungus (Goyal *et al.* 1991; Teeri *et al.* 1998).

Hydrolysis of lignocellulosic biomass is essential for the generation of fermentable sugars, which are converted to ethanol by microbes. Two methods—acid hydrolysis and enzymatic hydrolysis—are employed for this purpose. Their efficiencies vary depending upon treatment conditions, type of biomass, and the properties of hydrolytic agents. Acid hydrolysis is a mature technology; however, it has disadvantages, such as generation of hazardous acidic waste and technical difficulties in recovering sugars from acid. Enzymatic hydrolysis is more efficient and can be executed under ambient conditions without generation of any toxic waste. This method is under rapid development and has immense potential for improvement in cost and efficiency (Mishima *et al.* 2006).

In this study, the fungal strain *Aspergillus niger* US4 MTCC9931 was tested for its ability to produce cellulase and hemicellulose for the degradation of lignocellulosic biomass. Readily available agrowastes were used as substrates for this evaluation. Apart from the commonly used agrowastes substrates studied previously using other organisms, two new substrates—*Parthenium* stalk and residual fruit pulp—were studied (Tuohy *et al* 1990). Cooperative evaluation of biomass saccharification was performed with different feedstocks. Furthermore, the potential of saccharified biomass for ethanol production using *Saccharomyces cerevisiae* MTCC174 was evaluated.

EXPERIMENTAL

Microorganisms and Preparation of Inocula

A. niger MTCC9931 was isolated from dry fruit of *Emblica officinalis* (an ingredient of herbal powder Triphala), whereas *S. cerevisiae* MTCC174 was procured from Microbial Type Culture Collection (MTCC) and Gene Bank, Chandigarh, India. The *A. niger* strain was grown and maintained on potato dextrose agar (pH 5.6), whereas *S. cerevisiae* was maintained on yeast extract-peptone-dextrose agar (YEPD) slants. The *A. niger* strain was deposited at MTCC and Gene Bank, Chandigarh, India (accession numberMTCC9931). To identify the strain, a part of the large subunit 28S rRNA gene was amplified by PCR and sequenced. The sequence was deposited in NCBI public database with accession number JF521496. The fungal strain has been designated as *A. niger* US4MTCC9931 based on the phylogenetic analysis.

To prepare a fungal inoculum, 2mL of sterile distilled water containing 0.1% Tween 80 was added on the sporulated fungal hyphae grown on agar slants, and spores were dislodged into the liquid by gentle pipetting. A suspension containing 10^7spores/mL was used as an inoculum for the production of cellulase. *S. cerevisiae* was grown in YEPD broth for 12 h at 180 rpm on a rotary shaker, and the culture was used at 10% v/v as an inoculum for ethanol fermentation.

Materials

Rice straw (RS), rice husk (RH), wheat straw (WS), corn cob (CC), sugar cane bagasse (SCB), saw dust (SD), banana stalk (BS), water hyacinth leaves (WH), *Parthenium*

stalk (PR), and residual fruit pulp (RFP) were procured from rural areas around Patna, India. The material were brought to the laboratory and oven-dried overnight at 70 °C to remove residual moisture. Each material was screened to the size of less than 833 m (20 mesh) prior to pretreatment. The processed material was subsequently used for solid-state fermentation (SSF).

Production Medium

The composition of the fermentation medium was as follows (in g/L): yeast extract, 0.5; peptone, 3.0; $(NH_4)_2SO_4$, 1.5; K_2HPO_4,3.0; KH_2PO_4,4.0; $MgSO_4.7H_2O$, 0.3; $CaCl_2.7H_2O$, 0.3; traces of $ZnSO_4$, $MnSO_4$, and $FeSO_4.7H_2O$; and milled lignocellulosic biomass, 50.0. The pH of the basal medium was adjusted to 6.8. The medium was then autoclaved for 20 min at 121 °C.

SSF

Batch fermentation was conducted in 500 mL Erlenmeyer flasks. The fermentation medium was inoculated with 10^7 spores. The fermentation temperature was maintained at 28 ± 2 °C in a rotary shaker (150 rpm). Samples were taken at regular time intervals to determine the amount of reducing sugars. All experiments were conducted in triplicate.

Microbial Hydrolysis

The initial solid concentration was 5% (w/v). Samples were taken periodically and analyzed for reducing sugars. The concentrations of glucose, xylose, and arabinose after hydrolysis of 120h were determined by HPLC (Agilent Hi-Plex Ca-7.7x300 mm, 8 μm PL1170-6810, mobile phase- 100% DI H_20, flow rate- 0.6 mL/min, injection volume- 10 μL, temperature- 85 °C, detector- RI). The enzymatic digestibility was denoted as yield of reducing sugars (YRS, %) and yield of monosaccharides (YM, %). These are defined as follows,

$$\text{YRS (\%)} = W_{RS} \times 0.9 \times 100 / W_{IS} \quad (1)$$

$$\text{YM (\%)} = W_M \times 0.9 \times 100 / W_{IS} \quad (2)$$

where W_{RS} is the weight of reducing sugars produced by microbial hydrolysis; W_{IS} is the weight of initial solid; and W_M is the weight of monosaccharides (glucose, xylose, or arabinose).

Alcohol Fermentation

Ethanol production was studied using the microbial hydrolysate. The hydrolysate was sterilized by filtration and inoculated with 10% (v/v) of a 12-h old seed culture of *S. cerevisiae*. Fermentation was carried out at 28 ± 2 °C with agitation (120 rpm) for 24 h. Subsequently, the culture was maintained at stationary condition for 5 days. Samples (1 mL) were withdrawn at regular intervals and centrifuged for 10 min at 4 °C at 10000 × g.

Analytical Methods

Endoglucanase (EG) (1,4-β-D-4-glucanohydrolase, EC3.2.1.4) and xylanase (endo 1,4-β-D-xylanase, EC3.2.1.8) activities were determined using 1% carboxymethyl cellulose (CM-cellulose) and 1% birch wood xylan prepared in sodium citrate buffer (50 mM, pH 5.6), respectively. The reaction mixture containing equal amounts of suitably diluted enzyme and substrate was incubated at 50 °C for 10 and 5 min, respectively. The

reaction was stopped by addition of DNSA followed by boiling (Miller 1959). The reaction resulted in the change in color. The product was estimated by measuring absorbance at 540 nm using a spectrophotometer (Hitachi U-3210, Tokyo, Japan). The amount of released sugar was quantified using glucose and xylose standards, respectively.

Avicel-adsorbable endoglucanase (AAEG) was assayed as described by Arifoglu and Ogel (2000). The reaction mixture containing 5 mL of sodium acetate buffer (25 mM, pH 5.0), 5mL of culture supernatant, and 1.0 g of microcrystalline cellulose (Avicel, MCC) was incubated at 4 °C for 15 min. After centrifugation, the residual endoglucanase activity in supernatant was assayed by using CM-Cellulose (1%). AAEG was measured indirectly by subtracting Avicel–non-adsorbable endoglucanase activity from the total endoglucanase activity.

Total cellulase activity (FPase) was measured by using filter paper as a substrate. The reaction mixture contained small pieces of Whatman filter paper no.1 (2 × 3 mm) as described by Wood and Bhat (1988).

Avicelase/exoglucanase (1,4-β-D-glucan-4-cellobiohydrolase, CBH, EC3.2.1.91) activity was determined by using Avicel (Fisher Scientific, Waltham, MA, USA) as a substrate. The reaction mixture containing 1mL of Avicel (1%) in sodium acetate buffer and 0.5 mL of suitably diluted enzyme was incubated in a water bath for 2 h at 50 °C. The released sugar was determined as described by Miller (1959). One unit of activity was defined as the amount of enzyme releasing 1 µmol of glucose from substrate per minute.

β-glucosidase (β-D-glusido-glucanohydrolase, EC3.2.1.21) was assayed using *p*-nitrophenyl-β-D-glucopyranoside (pNPG) using a microtiter plate as described by Parry *et al*. (2001). Appropriately diluted enzyme (25 µL) was mixed with 50 µL of sodium acetate buffer (50 mM, pH 5.0). The reaction was initiated by adding 25 mL of pNPG (10 mM) and incubated at 50 °C for 30 min. The reaction was terminated by adding 100 µL of NaOH-glycine buffer (0.4 M, pH 10.8). The reaction product, yellow in color, was estimated by measuring its absorbance at 405 nm using ELISA Reader (Multiskan, Thermo, Waltham, MA, USA). Enzyme activity was expressed in International Units (IU), as the amount of enzyme, which releases 1 µmol of glucose, xylose, and p-nitrophenol in 1 min. The concentration of total proteins in enzyme extracts was determined by Lowry method (1951).

Ethanol Estimation

One mL of cell-free supernatant was mixed with 9mLof distilled water and 1mLof dichromate, and the mixture was heated for 10 min in a boiling water bath. During heating, ethanol is converted into acid, which upon reacting with dichromate, results in a color change from orange to green (Seo *et al.* 2009). The reaction product was estimated by measuring the absorbance at 600 nm on a spectrophotometer. Pure-grade ethanol was used as a standard.

Statistical Analysis

Estimation of proteins, enzyme activities, and sugars was always conducted with a minimum of three replicates. Standard deviation was calculated using the functions in available in the Excel suite (Microsoft Office 2003, Redmond, WA), and it was represented with error bars.

RESULTS AND DISCUSSION

Production of Enzymes for Biomass Hydrolysis

SSF is a well-established technology for enzyme production and provides the advantages of low cost of operation, less infrastructure requirements, ability to operate with less skilled manpower, and most importantly, the ability to use cheap agro-industrial residues and biomass as raw materials (Raimbault 1998; Pandey *et al.* 2000). Therefore, it has significant economic advantage for enzyme production (Lateef *et al.* 2012; Elegbede and Lateef 2017). In this investigation, crude cellulase preparations produced through SSF by fungi were used for hydrolysis of biomass with considerable efficiency. This is the first report on the production of the cellulases by *A. niger* US4MTCC9931 using locally available lignocellulosic agrowastes, such as PR, RH, and WH. *A. niger* US4MTCC9931 easily hydrolyzed almost all lignocellulosic materials.

The major component of lignocellulosic materials is cellulose, followed by hemicellulose and lignin. The proportions of these compounds are different among different species of plants (John *et al.* 2006; Cara *et al.* 2007; Prassad *et al.* 2007; Ruiz *et al.* 2008). This heterogeneous nature of carbon sources in plant materials plays an important role in the induction of cellulases and release of sugars during fermentation (Kaur *et al.* 2006). The production of cellulases is inducible and is affected by the nature of the substrates. Therefore, choosing the right substrate is very important for the production of optimum amount of sugars. *A. niger* US4MTCC9931 was grown on ten complex lignocellulosic carbon sources; CM-Cellulose (CMC, HIMEDIA) and Whatman filter paper no.1 were used as control substrates to determine their effect on the induction of cellulolytic enzymes. During the present investigation, RFP was the best carbon source for the production of reducing sugars (116.46 ± 2.56 g/L) and enzyme activities (Table 1). The amount of reducing sugars generated from RFP was 6.07-fold higher than that generated from CM-Cellulose (19.11 ± 1.23 g/L). The EG (389.1 ± 0.44I U/g), AAEG (3.4 ± 0.14 U/g), cellulase (12.0 ± 0.13 U/g), β-glucosidase (45.94 ± 1.15 U/g), FPase (4.90 ± 0.64 U/g), and xylanase (5511.0 ± 6.53 IU/g) activities also increased by 3.86-, 1.54-, 5.21-, 12.46-, 2.20-, and 3.88-fold, respectively, compared with the control (CMC). The highest β-glucosidase activity (79.87 ± 1.15 U/g) was observed when corncob was used as a substrate, which was 41.59-fold higher than that of the control. The highest xylanase activity (7478.0 ± 6.51 U/g) was observed when sawdust was used as a substrate, which was 5.27-fold higher than that of the control. The substrates like corncob and sugarcane bagasse also showed EG activity. Rice straw (RST) also showed noticeable AAEG activity (3.40 ± 0.15 U/g).

Since comparing the results obtained in this study with those obtained by other researchers is difficult, Table 2 compares the new strain with seven other strains. The β-glucosidase activity (79.87 ± 1.15 U/g) shown by *A. niger* US4MTCC9931was much higher than that shown by six other fungal strains; however, it was slightly less than that shown by *A. niger* KK2 (Kang *et al.* 2004). Xylanase activity, on the contrary, was much higher with this strain than that with all seven strains (Shah and Madamwar 2005). EG activity was also higher with this strain than that with six other strains, except with *T. reesei* MCG77 (Considine *et al.* 1988).

Table 1. Effect of Different Carbon Sources on the Production of Reducing Sugar, Endoglucanase, AAEG, Cellulase, β-Glucosidase, FPase, Xylanase, and Protein by *Aspergillus niger* MTCC 9931

Carbon sources	RS (gL^{-1})	EG (IUg^{-1})	AAEG (IUg^{-1})	Cellulase (IUg^{-1})	BG (IUg^{-1})	FPase (IUg^{-1})	XYL (IUg^{-1})
Rice straw	68.11 ± 1.23	189.6 ± 0.41	3.4 ± 0.15	1.4 ± 0.10	33.59 ± 1.15	1.98 ± 0.66	6456.0 ± 6.08
Wheat straw	54.00 ± 1.20	196.5 ± 0.45	2.2 ± 0.10	6.2 ± 0.11	52.23 ± 1.10	2.11 ± 0.56	6544.5 ± 6.55
Corncob	83.35 ± 1.65	358.4 ± 0.42	2.5 ± 0.13	7.2 ± 0.10	79.87 ± 1.15	3.6 ± 0.60	6793.5 ± 6.52
Sugar cane bagasse	70.76 ± 1.25	343.3 ± 0.41	2.0 ± 0.10	6.9 ± 0.15	67.98 ± 1.12	2.8 ± 0.66	6472.3 ± 6.23
Saw dust	92.12 ± 1.21	223.7 ± 0.45	0.6 ± 0.11	5.5 ± 0.12	55.07 ± 1.13	2.6 ± 0.66	7478.0 ± 6.51
Banana stalk	62.81 ± 1.35	244.9 ± 0.42	0.9 ± 0.13	3.5 ± 0.11	38.46 ± 1.11	2.4 ± 0.60	6392.5 ± 6.23
Eichhornia	67.67 ± 1.24	254.5 ± 0.40	1.1 ± 0.15	3.1 ± 0.10	58.36 ± 1.10	2.9 ± 0.62	6268.6 ± 6.42
Parthenium stalk	52.78 ± 1.20	225.2 ± 0.43	1.5 ± 0.10	4.2 ± 0.10	56.73 ± 1.14	2.7 ± 0.63	6120.5 ± 6.45
Residual fruit pulp	116.46 ± 2.56	389.1 ± 0.44	3.4 ± 0.14	12.0 ± 0.13	45.94 ± 1.15	4.9 ± 0.64	5511.0 ± 6.53
Rice husk	89.85 ± 1.05	198.0 ± 0.42	2.8 ± 0.10	2.7 ± 0.12	43.28 ± 1.12	3.9 ± 0.60	6921.0 ± 6.12
CMC	19.11 ± 1.23	107.5 ± 0.40	2.2 ± 0.12	2.3 ± 0.11	1.92 ± 1.10	2.2 ± 0.61	1417.0 ± 6.23
Filter paper	9.5 ± 1.58	82.2 ± 0.41	0.9 ± 0.11	1.1 ± 0.10	0.23 ± 1.15	2.0 ± 0.65	942.5 ± 6.52

RS: reducing sugar; EG: endoglucanase; AAEG: Avicel adsorbable endoglucanase activity; BG: β-glucosidase; XYL: xylanase

Table 2. Ethanol Production from Different Lignocellulosic Materials using SSF by *Aspergillus niger* US4MTCC9931 and *Saccharomyces cerevisiae* MTCC174

Carbon sources	RS (g/L)	Duration of incubation (h)	Ethanol (g/L)	Efficiency (RS to ethanol)%
Rice straw	68.11 ± 1.23	24	30.59 ± 0.52	44.91
Wheat Straw	54.00 ± 1.20	24	21.79 ± 0.51	40.35
Corn cob	83.35 ± 1.65	24	17.45 ± 0.58	20.93
Bagasse	70.76 ± 1.25	24	13.88 ± 0.54	19.61
Saw dust	92.12 ± 1.21	24	14.86 ± 0.50	16.13
Banana	62.81 ± 1.35	24	15.30 ± 0.52	24.35
Eichhornia	67.67 ± 1.24	24	21.98 ± 0.54	32.48
Parthenium	52.78 ± 1.20	24	22.91 ± 0.56	43.40
Fruit pulp	116.46 ± 0.56	24	35.34 ± 0.50	30.36
Rice husk	89.85 ± 1.05	24	11.58 ± 0.58	12.88

The hydrolysis of different lignocellulosic materials as well as YRS and YM depend upon the nature of the substrates. *A. niger* US4 MTCC9931 was capable of utilizing most of the agrowastes as carbon sources and produced higher amounts of cellulase enzyme and fermentable sugars. Maximum YRS (20.96%) and YM (17.55%) were observed RFP

was used as a substrate (Fig. 1). YRS and YM in sugarcane bagasse were 12.73% and 11.79%, respectively. These amounts are higher than those obtained *via* hydrolysis of untreated sugarcane bagasse (Zhao *et al.* 2009). The amount of released reducing sugar was equivalent to that obtained using *Pichia stipites* with water hyacinth as a substrate (Kumar *et al.* 2009) and higher than that obtained using *A. terreus* AV49 with groundnut shell as a substrate (Vyas *et al.* 2005) and that obtained using *A. niger* MTCC7956 with rice straw and bagasse as substrates (Kalogeris *et al*. 1999; Sukumaran *et al.* 2009).

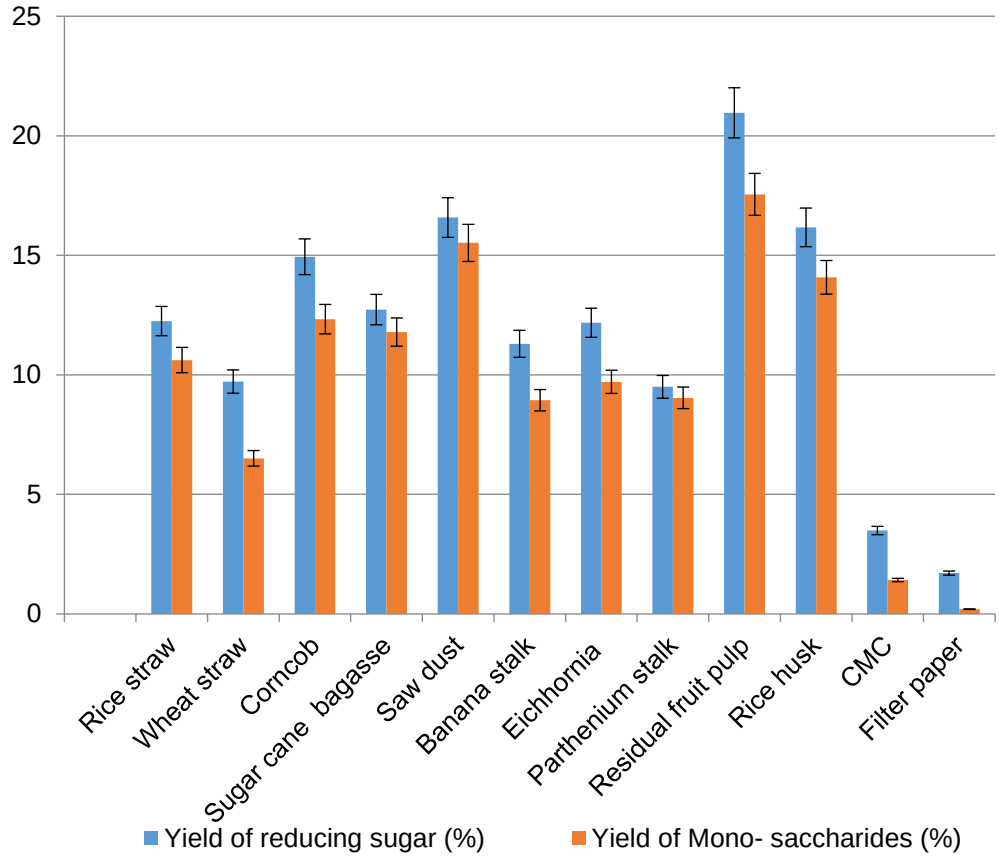

Fig. 1. YRS and YM of different lignocellulosic materials *Aspergillus niger* US4 MTCC 9931

The cost of cellulase is the primary limitation in conversion of biomass to ethanol. A strategy that can bring down the production cost of cellulase can significantly reduce the cost of bioethanol production. Therefore, microbial hydrolysates were further fermented with the use of *S. cerevisiae* MTCC174 to produce ethanol. Different types of lignocellulosic materials produced reducing sugars in the range of 52.78 to 116.46 g/L. The yields of ethanol as well as reducing sugars from ten different lignocellulosic materials are given in Table 3. The maximum ethanol yield (35.34 ± 0.50 g/L) was obtained after 24 h from the hydrolysate of RFP, and it had 11.64% reducing sugars. In rice straw hydrolysate having initial sugar concentration of 68.11%, the minimum ethanol yield (30.59 ± 0.52 g/L) was obtained. Minimum ethanol yield (22.91 ± 0.56 g/L) was obtained in *Parthenium* stalk hydrolysate having initial sugar concentration of 5.2%. The time course of ethanol production from RFP is shown in Fig. 3. The rate of ethanol production was 0.125 g/L/h for the initial 12 h and then increased to 1.418 g/L/h from 12 to 24 h. The yield of ethanol peaked at 24 h. There was no increase in the production after 24 h and the ethanol

concentration remained at about 35.34g/L. Karimi *et al*. (2006) used commercial cellulase for the hydrolysis of rice straw and three different microorganisms for the fermentation of the hydrolysate (Table 4). The yield of ethanol was 6.83 to 9.20 g/L. In contrast, the yield of ethanol by *A. niger* US4MTCC9931 was significantly much higher (30.59 g/L). It is also higher than that obtained with the strain *A. niger* MTCC7956 (25.56 g/L) (Sukumaran *et al.* 2009). Further, it is important to note that the yield of ethanol was much higher (35.34 g/L) when RFP was used as a substrate. Based on these analyses, *A. niger* US4MTCC9931 is a superior strain and can be exploited as a source of cellulase for the conversion of cellulosic wastes to ethanol.

Table 3. Enzyme Yields Production by SSF from Other Strains Grown on Lignocellulosic Biomass

Organism	Substrate	Enzyme activities (Ug^{-1})				Reference
		FP	EG	BG	XYL	
Aspergillus niger KK2	Rice straw	19	130	94	5070	Kang *et al*. (2004)
Aspergillus niger MTCC 7956	Sugar cane Bagasse	4.55	135.44	21.39		Sukumaran *et al*. (2009)
Aspergillus terreus AV49	Groundnut shell	0.36	45.1	-	-	Vyas *et al.* (2005)
Trichoderma reesei RUT C30	Wheat bran	22.8	299.55	4.5	-	Singhania *et al*. (2007)
Penicillium echinulatum *Trichoderma reesei* MCG77 *Talaromyces emersonii*	Eucalypt kraft pulp Beet pulp Wheat straw	2.7 7.7 18	153 704 265	0.31 4.6 2.9	316 7.7 350	Considine *et al.* (1988) Tuohy *et al.* (1990) Martins *et al*. (2008)
Aspergillus niger US4 MTCC 9931	Residual fruit pulp	4.9 ± 0.64	389.1 ± 0.44	45.94 ± 1.15	5511.0 ± 6.53	This work
Aspergillus niger US4 MTCC 9931	Corncob	3.6 ± 0.60	358.4 ± 0.42	79.87 ± 1.15	6793.5 ± 6.52	This work
Aspergillus niger US4 MTCC 9931	Sugar cane bagasse	2.8 ± 0.66	343.3 ± 0.41	67.98 ± 1.12	6472.3 ± 6.23	This work
Aspergillus niger US4 MTCC 9931	Saw dust	2.6 ± 0.66	223.7 ± 0.45	55.07 ± 1.13	7478.0 ± 6.51	This work

Table 4. Yield of Ethanol from Rice Straw

Hydrolysis of rice straw by	Fermentation by	Ethanol Yield (gL^{-1})	Reference
Commercial cellulase enzyme	*S. cerevisiae*	6.83	Karimi *et al.* 2006
Commercial cellulase enzyme	*Mucor indicus*	7.79	Karimi *et al.* 2006
Commercial cellulase enzyme	*Rhizopus oryzae*	9.20	Karimi *et al.* 2006
A. niger MTCC 7956	*S. cerevisiae*	25.56	Sukumaran *et al.* 2009
Aspergillus niger US4 MTCC 9931	*S. cerevisiae*	30.59	This work

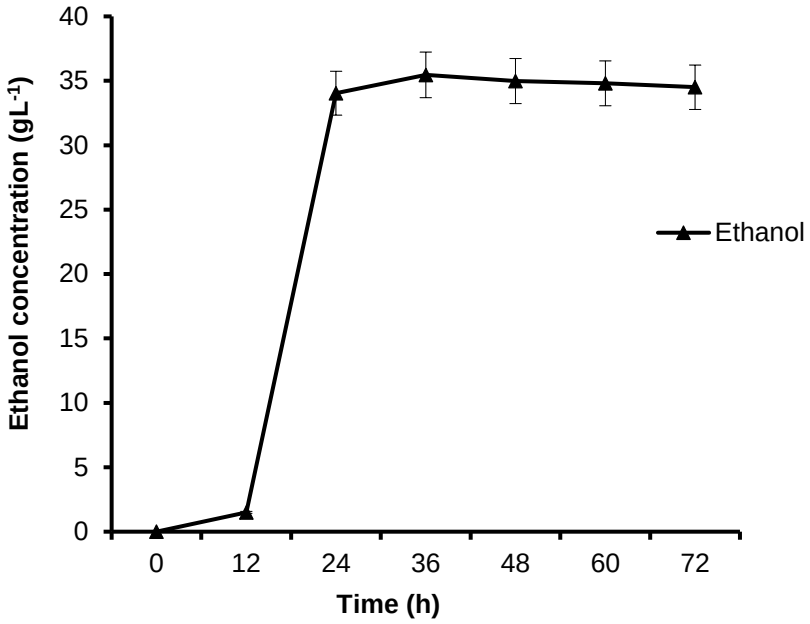

Fig. 2. Ethanol concentration profile during fermentation of RFP hydrosylate

CONCLUSIONS

1. The production of cellulases and hemicellulases by *A. niger* MTCC9931 in SSF was investigated in detail. Results of this study suggested that *A. niger* MTCC9931 is a potential microorganism for the production of cellulase and hemicellulase by using SSF. The most important observation in this study was the broad spectrum of cellulase activities in different agrowastes. Cellulase and hemicellulase produced by *A. niger* MTCC9931 were efficiently utilized for the hydrolysis of different substrates and the resultant hydrolysate was further utilized by *S. cerevisiae* for low-cost production of ethanol.
2. RFP acted as most potent LCW for bioethanol production in terms of ethanol yield % and time operation. However, rice straw appeared as one of the promising LCW for commercial bioethanol production based on its huge availability.

ACKNOWLEDGMENTS

This work was supported by a grant from the Board of Research in Nuclear Sciences, Mumbai, India. V. J. acknowledges the research fellowship from the Department of Biotechnology, New Delhi, Government of India.

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Article submitted: April 20, 2019; Peer review completed: June 23, 2019; Revised version received: August 5, 2019; Accepted: September 6, 2019; Published: September 18, 2019.
DOI: 10.15376/biores.14.4.8753-8764