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Research Article

Aspergillus fumigatus NITDGPKA3 Provides for Increased Cellulase Production

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A cellulolytic fungal strain, *Aspergillus fumigatus* NITDGPKA3, was isolated from straw retting ground. Cellulase and xylanase production by *A. fumigatus* NITDGPKA3 in submerged fermentation of rice straw was studied. The culture conditions for maximum enzyme production were found to be initial pH 4, 1% substrate concentration, temperature 30°C, incubation time 5 days, 0.2% tryptone as nitrogen source, and inoculum volumes 7% v/v (for cellulase) and 5% v/v (for xylanase). Addition of Tween 80 in fermentation broth improved xylanase production (193.58 IU/ml) much more compared to cellulase production (6.53 IU/ml). Xylanase activity found in the culture broth was approximately 50% higher compared to most of the reported data. The crude enzyme was further applied for reducing sugar production from alkali pretreated rice straw, where a dosage of 40 IU/g CMCase produced 0.522 g reducing sugar/g dry substrate after 36 hours which was higher than that in the reported literature. The high concentration of reducing sugar yield was most probably due to the extraordinarily high titer of β-glucosidase (80.1 IU/ml) found in the crude enzyme. The crude enzymes secreted by *Aspergillus fumigatus* NITDGPKA3 efficiently hydrolyzed alkali pretreated rice straw suggesting that *Aspergillus fumigatus* NITDGPKA3 is a robust microorganism.

1. Introduction

Lignocelluloses from agricultural, industrial, and forest residues can be utilized for the commercial production of useful products such as ethanol, glucose, and single cell protein. Enzymatic hydrolysis and acid hydrolysis are two methods for monomer production from complex polymer. Extracellular enzymes such as cellulase and xylanase can be produced by submerged fermentation (SmF) and by solid state fermentation (SSF). Submerged fermentation is preferred for approximately 90% of the industrial enzyme production due to less production monitoring labor, well-developed scale-up, and process control methods [1]. Production of extracellular enzymes such as cellulase and xylanase using various lignocellulosic substrates has been reported using different fungal [2–6] and bacterial strains [7]. Growth period of bacteria on lignocellulose is shorter than that of fungi and the half-baked cellulase system makes bacteria less efficient for large scale cellulase production compared to fungi [8]. *Aspergillus* species are the major agent of decomposition and decay and thus are able to produce a broad range of enzymes [9]. Cellulase production has been described for many *Aspergillus* species [10, 11]. *Aspergillus flavus* produced 1.23 IU/mL CMCase from wheat bran under submerged fermentation [12]. Prasertsan et al. [13] reported that *Aspergillus niger* ATCC 6275 produced xylanase and cellulase from palm oil mill waste under solid state and submerged fermentation. Maximum activities of cellulase and xylanase obtained were 23.8 U/g and 282.9 U/g, respectively. Jecu [14] used mixed substrate (wheat straw and wheat bran) for endoglucanase production under submerged fermentation by *Aspergillus niger,* where 11.25 IU/mL of endoglucanase was obtained. *Aspergillus heteromorphus* was grown on wheat straw under submerged fermentation, where 3.2 IU/mL filter paper activity and 83 IU/mL CMCase activity were obtained. Abo-State et al. [15] isolated *Aspergillus terreus* MAM-F23 and *Aspergillus flavus* MAM-F35 from agricultural waste and used them for cellulase production from wheat straw under solid state fermentation, where *Aspergillus terreus* MAM-F23 showed a maximum CMCase activity,

FPase activity, and avicelase activity of 385 U/mL, 113 U/mL, and 31 U/mL, respectively, and *Aspergillus flavus* MAM-F35 showed 430 U/mL CMCase, 138 U/mL FPase, and 45 U/mL avicelase activity. Besides the microorganism, cellulase and hemicellulase production is greatly influenced by media components, especially carbon and nitrogen sources, surfactants, and physical factors such as pH, temperature, and incubation period [16]. Development of a suitable medium and culture conditions is necessary in order to obtain maximum enzyme production.

The objective of the present study was reducing sugar production at a high concentration by using a low dosage of crude enzyme and a low-cost substrate. The substrate selected in the present study was rice straw, as this is an abundantly available low-cost agricultural residue in India. A cellulolytic fungus *Aspergillus fumigatus* NITDGPKA3 from straw retting ground was isolated, which produced a considerable amount of CMCase, FPase, and a high titer of xylanase enzyme under submerged fermentation. The influence of cultivation conditions on cellulase and xylanase (EC.3.2.1.8) production under submerged fermentation was explored. The crude enzyme was applied in hydrolysis of alkali pretreated rice straw, where a low concentration of enzyme produced a high amount of reducing sugar.

2. Materials and Methods

2.1. Isolation of a Cellulolytic Strain of Aspergillus fumigatus. Isolation of the cellulolytic strain of *Aspergillus fumigatus* was done by the following method. Soil sample was collected from straw retting ground at Bankura, West Bengal, India, during the summer season. Soil sample (1 g) was suspended in 20 mL of 0.85% NaCl and mixed for 10 minutes on a rotary shaker at 120 rpm. From the suspension serial dilutions (10^{-1} to 10^{-4}) were prepared. An aliquot of 50 μL of each dilution was spread on potato dextrose agar (PDA) plates and incubated for 7 days at 30°C. Ten larger fungal colonies with different morphologies were picked and subcultured on PDA to obtain pure cultures. Stock cultures were maintained on PDA slants at 4°C. Cellulase producing strains were screened by using a CMC selective medium containing 2% CMC (carboxymethylcellulose, Himedia, India), 0.2% peptone, and 1.7% agar in basal medium (0.2% $NaNO_3$, 0.05% KCl, 0.05% $MgSO_4$, 0.001% $FeSO_4$, and 0.1% K_2HPO_4). The CMC agar medium was spot-plated with spore suspension of pure culture of each fungal isolate and incubated at 28°C for 48 hours. To screen for cellulase producing fungi, the plates were flooded with Gram's iodine (2.0 g KI and 1.0 g iodine in 300 mL distilled water) for 3 to 5 minutes [17]. The plates were distained with distilled water.

Cellulolytic activity could be detected by the formation of clear zones within a blue-black background. The isolates with maximum clearing zones were selected for estimation of cellulase production capability under submerged fermentation of pretreated straw as described below.

2.2. Inoculum Preparation for Cellulase Production under Submerged Condition. The isolated and identified cellulolytic fungal culture was subcultured on Czapek modified agar medium (2% CMC, 2% peptone 0.2% $NaNO_3$, 0.05% KCl, 0.05% $MgSO_4$, 0.001% $FeSO_4$, 0.1% K_2HPO_4, and 2% agar) [30] and was incubated at 30°C. Fully sporulated plates were obtained after 6 days. The sporulated plates were flooded using 20 mL of distilled water to harvest the spores and obtain the resulting spore suspension, which was used as inoculum in subsequent experiments.

2.3. Preparation of Rice Straw. Rice straw was collected from paddy field at Bankura, India. It was air-dried, ground, and size-fractioned to 0.5 mm. Then it was pretreated with 0.5 M NaOH at 121°C for 1 hour at the ratio of 1 : 10 w/v (substrate to NaOH solution) [31]. The pretreated rice straw was washed with tap water until the pH of the filtrate became 7. The solid substrate was recovered and dried at 60°C overnight and stored at room temperature for further use.

2.4. Cellulase Production under Submerged Condition. Cellulase production was carried out in 250 mL Erlenmeyer flasks containing 0.5 g of alkali pretreated rice straw and 0.2% peptone with 50 mL basal medium discussed in Section 2.1. The pH of the medium was adjusted to 5. The flasks were autoclaved at 121°C (15 psi) for 15 minutes and thereafter cooled to room temperature and inoculated with 5% (v/v) of the inoculum containing 10^6 spores per mL and incubated at 30°C and 120 rpm for an appropriate incubation period. After incubation the culture broths were centrifuged at 8000 rpm at 4°C for 20 minutes. The crude enzyme solution was stored at 4°C for further use in enzyme assay and saccharification experiments.

2.5. Monofactorial Experiments. Cellulase and xylanase production by the isolated fungal strain was studied by varying different culture conditions. The conditions included various factors such as temperature (25°C–45°C), initial pH (3–7), incubation period (24 h–168 h), rotational speed (80–200) rpm, substrate concentration (0.5%–2%), and inoculum volume (v/v) (1%–9%). The effect of various nitrogen sources was tested by replacing peptone with 0.2% of various substrates, namely, tryptone, yeast extract, urea, ammonium nitrate, and ammonium sulfate. The concentration of the selected nitrogen source was also varied (0.1%–0.4%). In addition, the effect of Tween 80 (1–8 g/L) on enzyme production was studied.

2.6. Enzymatic Hydrolysis of Alkali Pretreated Rice Straw. Enzymatic hydrolysis of alkali pretreated rice straw was carried out at 2% (w/v) consistency in 50 mL reaction mixture of crude enzyme solution and 50 mM citrate buffer (pH 4.8). Streptomycin (40 μg/mL) and cycloheximide (30 μg/mL) were added to the mixture to prevent microbial contamination. Crude enzyme solution equivalent to CMCase activity of 40 IU/g dry substrate was added to the reaction mixture and incubated at 50°C and 120 rpm for 56 hr. Samples (0.5 mL) were withdrawn at regular intervals, centrifuged at 14000 rpm for 15 minutes, and the supernatant was analyzed for reducing sugars released.

Table 1: FPase and CMCase activities of fungal isolates. Results are presented as the mean of three replicates with standard deviation.

Strains	Enzymes	Enzyme activity (IU/mL)						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
F1	FPase	0.0045 ± 0.001	0.0123 ± 0.005	0.0213 ± 0.001	0.0412 ± 0.001	0.0376 ± 0.003	0.031 ± 0.007	0.0345 ± 0.003
	CMCase	0.233 ± 0.032	0.405 ± 0.077	0.675 ± 0.063	0.985 ± 0.106	0.90 ± 0.056	0.876 ± 0.036	0.786 ± 0.065
F2	FPase	0.0051 ± 0.001	0.0168 ± 0.001	0.0544 ± 0.006	0.0453 ± 0.004	0.0408 ± 0.001	0.0322 ± 0.002	0.0311 ± 0.004
	CMCase	0.339 ± 0.026	0.985 ± 0.012	1.265 ± 0.021	1.12 ± 0.046	1.05 ± 0.014	1.02 ± 0.056	0.834 ± 0.118
F3	FPase	0.0255 ± 0.004	0.0744 ± 0.003	0.0929 ± 0.003	0.12 ± 0.007	**0.251 ± 0.007**	0.189 ± 0.019	0.14 ± 0.024
	CMCase	0.387 ± 0.0770	0.821 ± 0.249	1.441 ± 0.147	1.715 ± 0.13	**2.305 ± 0.091**	1.77 ± 0.084	1.51 ± 0.127
F4	FPase	0.0134 ± 0.009	0.0298 ± 0.001	0.0579 ± 0.003	0.0722 ± 0.002	0.0912 ± 0.001	0.0956 ± 0.001	0.0851 ± 0.004
	CMCase	0.421 ± 0.043	0.0543 ± 0.046	0.72 ± 0.056	1.165 ± 0.12	1.34 ± 0.127	1.536 ± 0.15	1.478 ± 0.074
F5	FPase	0.0187 ± 0.003	0.0354 ± 0.004	0.0532 ± 0.004	0.0867 ± 0.001	0.0913 ± 0.001	0.143 ± 0.012	0.1165 ± 0.009
	CMCase	0.543 ± 0.009	0.886 ± 0.087	1.085 ± 0.11	1.342 ± 0.118	1.653 ± 0.087	1.942 ± 0.076	1.811 ± 0.093

2.7. Analytical Methods. The total cellulase (FPase) and carboxymethyl cellulase (CMCase) activities were determined as per the methods of the International Union of Pure and Applied Chemistry (IUPAC) Commission of Biotechnology [32].

2.7.1. CMCase. Enzyme solution (0.5 mL) was incubated with 0.5 mL of 2% carboxymethyl cellulose (Himedia, India) prepared in 0.05 M, pH 4.8 Na-citrate buffer at 50°C for 30 minutes.

2.7.2. FPase. Enzyme solution (0.5 mL) was incubated with 1 mL of 0.05 M, pH 4.8 Na-citrate buffer containing 1 cm × 6 cm (=50 mg) Whatman filter paper strip at 50°C for 60 minutes.

2.7.3. Xylanase. Enzyme solution (0.5 mL) was incubated with 0.5 mL of 2% oat spelt xylan (Himedia, India) prepared in 0.05 M, pH 4.8 Na-citrate buffer at 50°C for 10 minutes.

In all the cases, after incubation, the released reducing sugar was estimated by the DNS method with some modifications [30]. 3,5-Dinitrosalicylic acid reagent (1 mL) was added to the reaction mixture and incubated for 5 minutes in a vigorously boiling water bath. Na-K tartrate solution (1 mL) was then added to the mixtures and cooled rapidly. The reducing sugar was estimated from the absorbance measured at 540 nm using glucose (for CMCase and FPase) and xylose (for xylanase) as standard. Enzyme activities were defined in International Units (IU). One unit of enzyme activity is defined as the amount of enzyme that releases 1 μmol reducing sugars/mL/minute.

3. Results and Discussion

3.1. Screening and Identification of Cellulolytic Fungi. Twenty-seven fungal colonies were isolated from soil sample of straw retting ground. Based on their rapid growth on PDA medium (isolation medium), only ten fungal isolates were selected for screening for cellulolytic strains on CMC medium. As significant growth of all the isolates was observed within 48 hours, the CMC plates were then flooded with Gram's iodine for clearing zone measurement. Among the 10 fungal strains, only 5 strains developed significant clearing zone ranging from 1.5 cm to 4.1 cm. The five isolates were designated as F1, F2, F3, F4, and F5. The sizes of their respective clearing zones were F1: 1.5 cm, F2: 2.3 cm, F3: 4.1 cm, F4: 1.9 cm, and F5: 2.7 cm. The large zones were demonstrative of the high cellulose degrading ability and thus cellulase production capability of these five isolates. The largest clearing zone was observed for fungal strain F3 compared to the other fungal strains.

Although very little clearing zone was visible away from the colony border, evidence of extracellular activity was visible within the medium underneath the colony. Among the five screened fungal isolates, F3 showed maximum CMCase and FPase activities in submerged culture indicating its high efficiency to degrade lignocellulosic substrate and to produce the highest amount of cellulase enzymes (Table 1).

The fungal isolate F3 was identified as *Aspergillus fumigatus* by M/S. Bangalore Genei, India, through phenotypic and genotypic characterization including the results of 18S rRNA, ITS1, 5.8S rRNA, ITS2, partial 28S rRNA gene sequence, and data analysis (Figure 1). The identified strain has been tentatively designated as *Aspergillus fumigatus* NITDGPKA3. The NCBI GenBank accession number of the strain is JQ046374.

3.2. Time Course of Enzyme Production. Cellulase and xyla-nase production by *A. fumigatus* NITDGPKA3 under submerged condition was observed after only one day of incubation when 1% alkali pretreated rice straw and 0.2% peptone were the carbon and nitrogen source of the liquid culture medium, respectively (Figure 2). Maximum CMCase (2.31 IU/mL), FPase (0.261 IU/mL), and xylanase (48.33 IU/mL) were produced after 5 days of incubation. Further increase in incubation caused decrease in the enzyme production. The reduction in cellulase yield after the optimum period could be due to reasons such as depletion of nutrients, spontaneous denaturation of enzyme, or proteolytic digestion [33]. An observation of protease activity (0.073 IU/mL) in the culture broth may indicate proteolytic digestion as a reason for enzyme reduction.

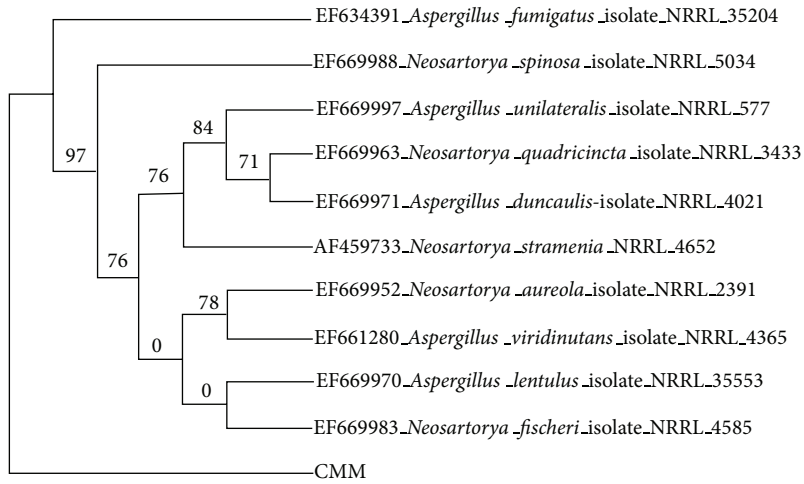

Figure 1: Characterization and phylogenetic tree of ITS sequences of *Aspergillus fumigatus* NITDGPKA3.

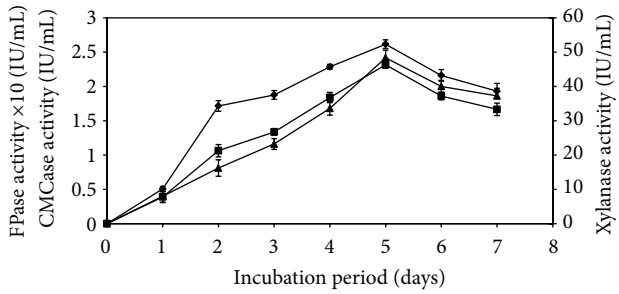

Figure 2: Effect of incubation period on CMCase, FPase, and xylanase activities. Results are presented as the mean of three replicates with standard deviation: (♦) FPase; (■) CMCase; (▲) xylanase.

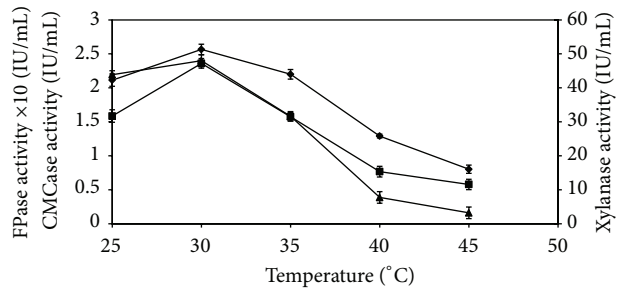

Figure 3: Effect of temperature on CMCase, FPase, and xylanase activities. Results are presented as the mean of three replicates with standard deviation: (♦) FPase; (■) CMCase; (▲) xylanase.

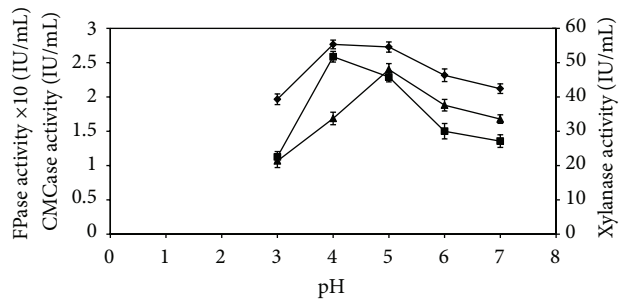

Figure 4: Effect of pH on CMCase, FPase, and xylanase activities. Results are presented as the mean of three replicates with standard deviation: (♦) FPase; (■) CMCase; (▲) xylanase.

3.3. Effect of Temperature, pH, and Rotational Speed on Cellulase Production. Temperature is an important physiological parameter that affects microbial growth. On varying the incubation temperature from 25°C to 45°C, it was observed that cellulase as well as xylanase production gradually increased from 25°C and reached maximum (CMCase 2.36 IU/mL, FPase 0.256 IU/mL, and xylanase 47.98 IU/mL) at 30°C (Figure 3). Enzyme production drastically decreased with further increase in temperature. Hanif et al. [34] also reported an increase in cellulase production by *Aspergillus niger* up to 30°C and thereafter the production of enzyme declined. Deswal et al. [9] optimized physiological parameters for cellulase production by *Fomitopsis* sp. under SSF and showed that cellulase production was maximum at 30°C.

The pH of the medium is one of the most critical environmental parameters affecting the mycelial growth and enzyme production. It also has a role in transport of various components across the cell membrane. To investigate the effect of initial pH on enzyme production, pH of the medium was varied from 3 to 7. Maximum CMCase (2.58 IU/mL) and FPase (0.276 IU/mL) production was at initial medium pH 4, whereas maximum xylanase production (47.98 IU/mL) was observed at pH 5 (Figure 4). Further increase of pH value reduced the enzyme production. The results were compared with the pH stability data of FPase, CMCase, and xylanase, where the enzymes were stable only within a pH range of 4 to 5 (data not shown). Optimum pH values ranging from 3 to 6 have been reported for many studies on cellulase production by fungi [35, 36].

As regards agitation, variation of rotational speed from 80 to 200 rpm affected enzyme production. A rotational speed

Table 2: Effect of nitrogen sources on CMCase and FPase activities. Results are presented as the mean of three replicates with standard deviation.

Source	Enzyme yield (IU/mL)		
	CMCase	FPase	Xylanase
Nitrogen source			
Peptone	2.34 ± 0.049	0.291 ± 0.006	70.06 ± 3.19
Tryptone	2.39 ± 0.028	0.446 ± 0.007	93.35 ± 4.03
Yeast extract	1.035 ± 0.062	0.29 ± 0.01	20.30 ± 3.95
Urea	0.85 ± 0.049	0.233 ± 0.012	36.80 ± 4.52
NH_4NO_3	0.629 ± 0.021	0.22 ± 0.014	36.05 ± 3.74
$(NH_4)_2SO_4$	0.531 ± 0.035	0.17 ± 0.091	45.10 ± 3.67
Tryptone (%, w/v)			
0.1	1.86 ± 0.084	0.34 ± 0.012	34.05 ± 3.32
0.2	2.37 ± 0.058	0.447 ± 0.056	93.46 ± 4.03
0.3	1.95 ± 0.084	0.343 ± 0.014	86.29 ± 3.38
0.4	1.705 ± 0.063	0.2455 ± 0.063	77.37 ± 3.68

of 150 rpm resulted in maximum CMCase (2.40 IU/mL), FPase (0.278 IU/mL), and xylanase (53.25 IU/mL) (Figure 5). Agitation rates below 150 rpm resulted in low cellulase yields. The reason may be the difficulty in maintaining sufficient dissolved oxygen (DO) level for cell growth. Higher agitation rates resulted in a slight decline in enzyme levels, which could be due to mycelial damage.

3.4. Effect of Nitrogen Sources. Cellulase production was dependent on the nature of nitrogen source in the culture medium. Various inorganic and organic nitrogen sources were tested for their effect on cellulase production. The maximum enzyme activities were with the organic nitrogen sources peptone, tryptone, and yeast extract (Table 2). Both peptone and tryptone gave considerable amounts of enzyme among the various nitrogen sources used, where tryptone resulted in significant FPase and xylanase compared to peptone. At 0.2% tryptone concentration maximum CMCase (2.39 IU/mL), FPase (0.446 IU/mL), and xylanase (93.46 IU/mL) activities were obtained. These data are in accordance with the observations of Daroit et al. [37]. However, some other studies have reported inorganic nitrogen sources resulting in higher cellulase production [38].

3.5. Effect of Inoculum Volume on Cellulase Production. Inoculum volume is an important biological factor as it ascertains biomass production in fermentation. The results showed that there was a gradual increase in cellulase as well as xylanase production with an increase in inoculum volume (Figure 6). The optimal inoculum volume for maximum cellulase (FPase 0.344 IU/mL and CMCase 2.50 IU/mL) was 7% (v/v) containing 10^6 spores per mL. In case of xylanase an inoculum volume of 5% (v/v) gave maximum production (47.98 IU/mL). Further increase in inoculum volume caused reduction in enzyme production. The decrease seen with larger inoculum volume could be due to the shortage of nutrients available for the larger biomass and faster growth of the culture.

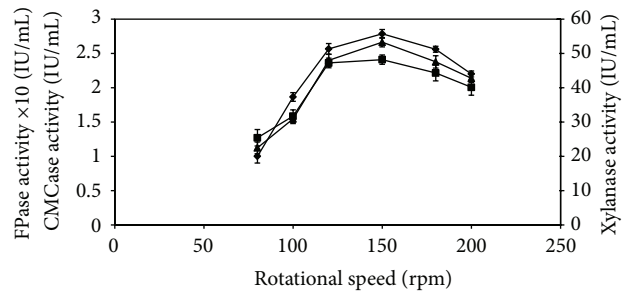

Figure 5: Effect of rotational speed on CMCase, FPase, and xylanase activities. Results are presented as the mean of three replicates with standard deviation: (♦) FPase; (■) CMCase; (▲) xylanase.

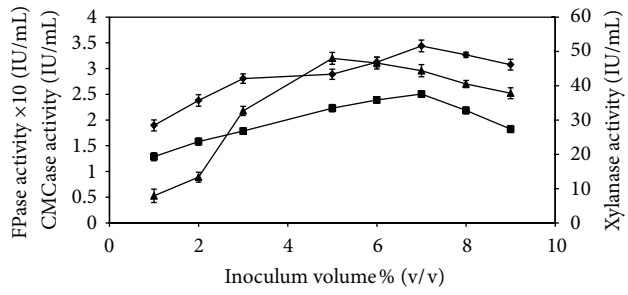

Figure 6: Effect of inoculum volume on CMCase, FPase, and xylanase activities. Results are presented as the mean of three replicates with standard deviation: (♦) FPase; (■) CMCase; (▲) xylanase.

3.6. Effect of Substrate Concentration. The level of substrate is crucial for submerged fermentation. Maximum enzyme production (FPase 0.287 IU/mL, CMCase 2.37 IU/mL, and xylanase 46.77 IU/mL) was obtained at 1% (w/v) substrate level (alkali pretreated rice straw to liquid medium ratio) (Figure 7). After optimum level the enzyme production started to reduce probably due to catabolic repression.

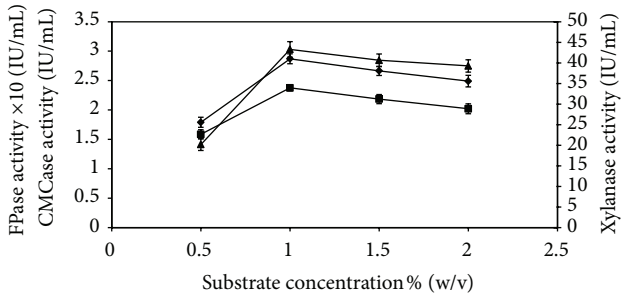

Figure 7: Effect of substrate concentration on CMCase, FPase, and xylanase activities. Results are presented as the mean of three replicates with standard deviation: (♦) FPase; (■) CMCase; (▲) xylanase.

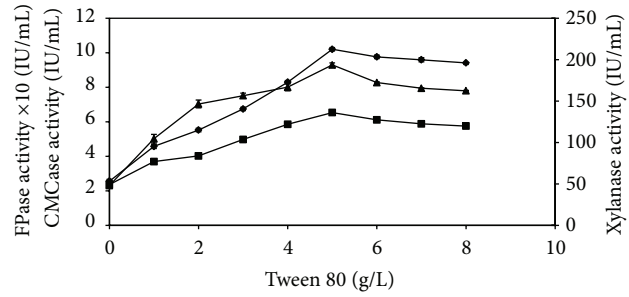

Figure 8: Effect of Tween 80 on CMCase, FPase, and xylanase activities. Results are presented as the mean of three replicates with standard deviation: (♦) FPase; (■) CMCase; (▲) xylanase.

3.7. Effect of Tween 80 on Cellulase Production. Addition of surfactant such as Tween 80 is vital for enzyme production as surfactants have been shown to enhance enzymatic conversion of lignocellulosic substrate [39]. It has been proposed that hydrophobic part of the surfactant binds to lignin and the hydrophilic part of the surfactant acts as a steric hindrance blocking the enzymes from unproductive binding with lignin which cause more enzymes available for cellulose hydrolysis [39]. Tween 80 also may be used as carbon source by *Aspergillus fumigatus* NITDGPKA3 which increases enzyme production. The results presented in Figure 8 showed that addition of Tween 80 improved xylanase production compared to cellulase production. Tween 80 concentration of 5 g/L resulted in 193.58 IU/mL xylanase, 1.02 IU/mL FPase, and 6.53 IU/mL CMCase which corresponds to the specific activity of xylanase, FPase, and CMCase to be 111.89 IU/mg, 0.589 IU/mg, and 3.77 IU/mg, respectively (protein concentration of the fermentation broth is 1.73 mg/mL). Zeng et al. [40] reported that enzyme production increased with the increase of Tween 80 concentration.

The highest values of CMCase, FPase, and xylanase obtained from the above studies were compared with the enzymes produced by the other strains of *Aspergillus fumigatus* (Table 3). The cellulase activities (CMCase and FPase) obtained were significantly higher than those in the previous reports of cellulase production by *Aspergillus fumigatus* under submerged fermentation. Xylanase activity found in the culture broth was significantly higher compared to most of the reported data. Also, none of the aforementioned reports make any mention of saccharification studies using the enzymes produced.

3.8. Biomass Saccharification. The efficacy of crude cellulase from *A. fumigatus* NITDGPKA3 in alkali pretreated rice straw hydrolysis was evaluated. The time course of enzymatic saccharification showed that the release of sugars increased with saccharification period (Figure 9). Maximum sugar yield was 0.522 g/g dry substrate obtained at 36 hours. The sugar yield was higher compared to most of the data reported in the literature (Table 4). Even though the concentration of the FPase activity obtained in the current study was lower than that reported in one of the studies using an *Aspergillus*

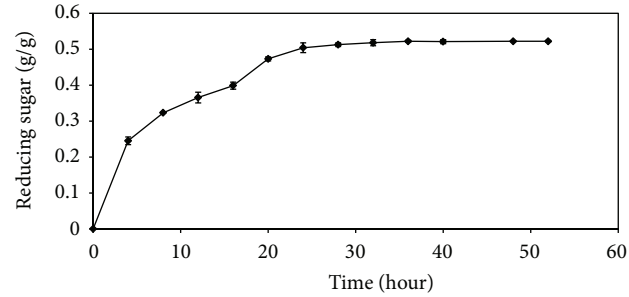

Figure 9: Enzymatic hydrolysis of alkali pretreated rice straw. Results are presented as the mean of three replicates with standard deviation.

fumigatus strain (but using a different substrate, namely, corn stover) [22], the CMCase activity in the current study was obtained at a sufficiently higher concentration than in all the mentioned studies. Compared to the aforementioned study, the yield of reducing sugar attained from the present study was also found to be higher. The high yield was even more appreciable as this result was obtained despite using a much lower concentration of crude enzyme as compared to previous studies on *Aspergillus fumigatus* (Table 4). Also, scope remains for further increase in reducing sugar production using the strain-substrate system of the present study by statistical optimization.

It is desirable that the enzyme loading be minimized as much as possible due to the high cost of cellulase [29]. The significant lowering of the requirement of crude enzyme for the saccharification process in the present study called for a look into the composition of the enzyme mixture which, on further investigation, is revealed to contain a rich amount of β-glucosidase (80.1 IU/mL). This astonishingly high titer of β-glucosidase was not found in any other reports on *Aspergillus fumigatus* as known till date (Table 3).

The lignocellulose bioconversion is a complex mechanism which requires the synergic action of three enzymes (endoglucanase, exoglucanase, and β-glucosidase). Endoglucanase (endo-1,4-D glucanohydrolase or E.C. 3.2.1.4) attacks the low crystallinity regions of the cellulose fiber, exoglucanase (1,4-b-D glucan cellobiohydrolase or E.C. 3.2.1.91) removes the cellobiase units from the free chain ends,

Table 3: Comparison of cellulase and xylanase production by *Aspergillus fumigatus* NITDGPKA3 with other strain of Aspergillus fumigatus.

Microorganism	Substrate	Enzymes				Reference
		FPase	CMCase	Xylanase	β-glucosidase	
Aspergillus fumigatus	Wheat straw	0.0089 IU/mL	—	—	0.104 IU/mL	[18]
Aspergillus fumigatus	Mixed substrate (Rice straw and wheat bran)	—	14.71 IU/g	42.7 IU/g	8.51 IU/g	[19]
Aspergillus fumigatus	Wheat straw	—	0.225 μmoL/mL	—	0.085 μmoL/mL	[20]
Aspergillus fumigatus Fresenius	Rice straw	9.73 IU/g	240.2 IU/g	2800 IU/g	470 IU/g	[21]
Aspergillus fumigatus Z5	Corn stover	144.6 IU/g	526.3 IU/g	—	—	[22]
Aspergillus fumigatus	Wheat Bran	—	—	531 U/gds	—	[23]
Aspergillus fumigatus SBS58	Wheat bran	35 IU/gds	—	608 IU/gds	—	[24]
A. fumigatus SBS62	Wheat bran	32 IU/gds	—	613 IU/gds	—	[24]
A. fumigatus SBS63	Wheat bran	20 IU/gds	—	587 IU/gds	—	[24]
Aspergillus fumigatus AR1	Wheat bran	—	—	228 IU/mL	—	[24]
Aspergillus fumigatus AR1	Xylose	—	<0.4 U/mL	135 IU/mL	—	[25]
Aspergillus fumigatus	Sawdust	0.288 U/g	5.54 U/g	—	—	[26]
Aspergillus fumigatus FBSPE-05	Sugar cane bagasse and corn steep liquor	—	365 U/L	—	—	[27]
Aspergillus fumigatus FBSPE-05	Wheat bran	—	135 U/L	—	—	[27]
Aspergillus fumigatus FBSPE-05	Sugar cane bagasse,Wheat bran	47 U/l	—	—	—	[27]
Aspergillus fumigatus NITDGPKA3	Rice straw	1.02 IU/mL~ 102 IU/g	6.53 IU/mL~ 653 IU/g	193.58 IU/mL~ 19,358 IU/g	80.1 IU/mL~ 8010 IU/g	Present study

Table 4: Comparison of enzymatic hydrolysis yield of alkali pretreated rice straw by the crude enzyme of *Aspergillus fumigatus* NITDGPKA3 with other fungi.

Enzyme source	Substrate	Enzyme load (U/g dry substrate)	Reducing sugar (g/g dry substrate)	Reference
Fomitopsis sp.RCK2010	Rice straw	25 (FPase)	0.1572 g/g dry substrate	[9]
Fomitopsis sp.RCK2010	Wheat straw	25 (FPase)	0.2141 g/g dry substrate	[9]
Aspergillus fumigatus Z5	Corn stover	250 (CMCase)	0.450 g/g dry substrate	[22]
T. reesei ZU-02	Corncob	20 (FPU)	<60 g/L	[28]
Combination of commercial enzymes (Celluclast, Novozyme 188, Viscostar 150 L, Pectinase solids)	Wheat straw	50.88 (CMCase)	0.565 g/g dry substrate	[29]
Aspergillus fumigatus NITDGPKA3	Rice straw	40 (CMCase)	0.522 g/g dry substrate~10.44 g/L	Present study

and finally cellobiose units are hydrolysed to glucose by β-glucosidase (E.C. 3.2.1.21) [41]. It has been previously reported that cellobiose accumulation may cause severe feedback inhibition of the activities of endoglucanase and exoglucanase in cellulase which results in low hydrolysis saccharification yield [29]. The feedback inhibition caused by the cellobiose accumulation can be reduced by the presence of high β-glucosidase activity in the hydrolysis system resulting in higher reducing sugar concentration.

4. Conclusion

A fungal strain *A. fumigatus* NITDGPKA3 was locally isolated. This strain produced a considerable amount of cellulase as well as xylanase from alkali pretreated rice straw in submerged fermentation. The effects of various process parameters on enzyme production were evaluated. A high titer of xylanase (193.58 IU/mL) and β-glucosidase (80.1 IU/mL) was found in the enzyme mixture. The secreted crude enzymes efficiently hydrolyzed alkali treated rice straw and produced a high level of reducing sugar (0.522 g/g dry substrate) suggesting that *Aspergillus fumigatus* NITDGPKA3 is a robust microorganism for biomass saccharification. Cellulase production by this fungus may be improved after process parameter optimization using statistical methods such as response surface methodology (RSM) which uses combinatorial interactions of culture conditions.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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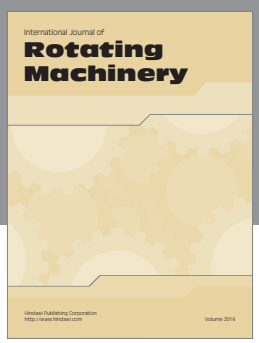

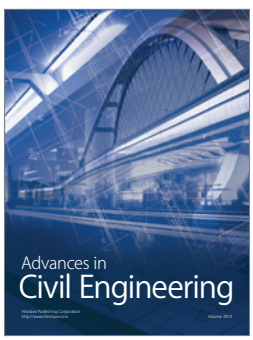

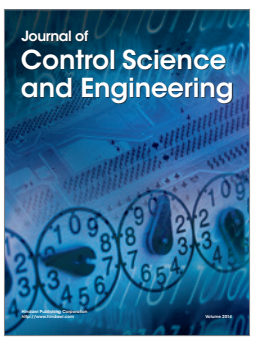

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