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ORIGINAL RESEARCH

Enhanced cellulase production from isolated fungus *Aspergillus niger* RKJP and its application in lignocellulosic saccharification for bioethanol production

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ABSTRACT
The present research work highlights the isolation and screening of newly isolated potent fungal strain as *Aspergillus niger* RKJP under solid state fermentation for production of cellulase enzyme consisting of all the three components endoglucanase, cellobiohydrase & β glucosidase. Further the studies were undertaken on the optimization of physiological and nutritional culture conditions in respect of pH , incubation time, temperature and moisture ratio (solid: liquid) for production of higher activity of cellulase enzyme consisting of endoglucanase, cellobiohydrolase & β glucosidase in synergistic ratio for complete saccharification of pretreated rice straw to fermentable sugars. Among various carbon & nitrogen sources used, wheat bran and peptone yielded higher cellulase enzyme activity. Supplementation of surfactants such as Tween- 80 with wheat bran further enhanced the production of components endoglucanase (30.14 IU/gds), β glucosidase (61.24IU/gds) and cellobiohydrase (6.13IU/gds). The cellulase enzyme thus produced was evaluated for saccharification of pretreated rice straw. The results were highly encouraging while achieving a saccharification efficiency of more than 85%.

KEY WORDS: *Aspergillus niger, Cellulase, Solid state fermentation, Wheat Bran, Saccharification, β - glucosidase*

Introduction

Cellulases are the enzyme which hydrolyse the β, 1-4 glucan linkages in cellulose and produce primary products as glucose, cellobiose and cello-oligosaccharides. These are produced by a number of microorganisms and comprise several different enzyme classifications. Three major types of cellulase enzyme are involved in the hydrolysis of native cellulose namely Cellobiohydrolase (EC 3.2.1.91), Endoglucanase ((EC 3.2.1.4) and β –glucosidase (EC 3.2.1.21) (Narra *et al.* 2012). Endo β- gluconases produce nicks in the cellulose polymer exposing reducing and non-reducing ends, cellobiohydrolases acts upon the reducing and non-reducing ends to liberate cello-oligosaccharides and cellobiose and β-glucosidase cleave the cellobiose to liberate glucose completing the hydrolysis (Sukumaran *et al.* 2005).

For lignocellulosic hydrolysis, saccharification via enzymatic means is considered to be the highly promising route. However this step is also one of the major contributors of the overall economics of ethanol cost contributing as much as upto 50% of the net production cost. (Singhania *et al.*2010). Thus one of the major challenges faced is the higher cost of cellulase enzyme used for saccharification of pretreated

lignocellulosic raw material due to higher dose of enzyme required and lower saccharification efficiency with poor of fermentable sugars i.e. glucose.

The requirement of higher levels of cellulase enzyme for productions of lignocelluloses to ethanol is due to the fact that these enzymes consist of little quantity of β –glucosidase. The low level of β –glucosidase can lead to accumulation of cellobiose resulting in the requirement of higher dose of cellulase needed to completely saccharify the lignocellulosic biomass. Therefore any cellulases enzyme consisting of cellulase system comprising of cellobiohydrolase (CBH), β, 1-4 endoglucanase (EG) and β –glucosidase component in optimum ratio acting synergistically should be an ideal enzyme to completely saccharify the crystalline cellulose to glucose for production of bioethanol from lignocellulosic waste (Kuhad *et al.*1997).

The present work aims for production of higher activity cellulase enzyme from isolated screened microbial culture consisting of all the three cellulase components in synergistic ratio utilizing the inexpensive and abundantly available lignocellulosic waste (wheat bran) as substrate. Various physiological & nutritional parameters were optimized for production of cellulase with enhanced activity containing all the three components i.e. cellobiohydrolase (CBH), endoglucanase (EG) and β –glucosidase in synergistic ratio. Further studies were undertaken using cellulase enzyme thus produced was used for saccharification of pretreated rice straw to fermentable sugars followed by its conversion into ethanol to exploit its potential on commercial scale.

Materials and Methods

Organism and Culture conditions

The fungal isolate was isolated from deteriorated apple from fruit market near Saharanpur U.P. and maintained by periodical sub culturing on potato dextrose agar (PDA) at 30^0C and stored at 4 ^{o}C.

Identification of fungus

The fungal strain got identified at the Institute of Microbial Technology (IMTECH), Chandigarh, India on the basis of internally transcribing spacers (ITS) sequencing and the culture was maintained by periodical sub culturing on PDA at 30^0C and stored at 4^oC.

Cellulase enzyme production under solid state fermentation

Crude cellulolytic enzyme production from isolated fungus was carried out in 250 ml Erlenmeyer flasks, each having 5.0 g of dry wheat bran moistened with optimized mineral salt solution (g/L): $CaNO_3$, 0.5; KH_2PO_4, 0.5; $MgSO_4$, 0.5 and pH (5) to attain the final substrate-to-moisture ratio of 1:3. The flasks were sterilized by autoclaving at 121^0C (15 psi), and thereafter cooled to room temperature and inoculated with 250μL of the spore suspension prepared in Tween-80 (0.1%, v/v) from 4 days grown old slants. The contents of the flasks were mixed well with sterilized glass rod to distribute the inoculums throughout the substrate and the inoculated flasks were incubated for different intervals in an incubator at 30^0C (Deswal *et al.* 2011).

Extraction of enzyme

The fungal fermented wheat bran was aseptically removed from flasks after an appropriate interval, suspended in 25 ml citrate phosphate buffer (.05M, pH 5) and shaken gently at 150 rpm for 45 min. The exudates were squeezed through muslin cloth for maximizing the enzyme extraction and centrifuged at 10,000 rpm at 4 ^{0}C for 10 min. The clarified supernatant thus obtained was assayed for various cellulase activities to study their production (Deswal *et al.* 2011).

Optimization of cultural and nutritional parameters

The cellulase production by the fungus was optimized following one factor at a time (OFAT) approach. The effect of various factors such as time course of fermentation (24hrs – 168 hrs), initial pH (3.0–7.0), incubation temperature (20–45^oC), substrate to moisture ratio (1:1–1:4), different carbon and nitrogen sources were tested. In addition effect of surfactants (0.1% - 0.5%) to enhance cellulase production was also investigated.

Enzymatic hydrolysis of acid treated rice straw

Saccharification of acid treated rice straw was carried out using crude enzyme in 100 ml Erlenmeyer flasks containing 5 gm acid pretreated rice straw having pore size of 1-3mm. The pretreated substrate was soaked in 50 ml of sodium citrate buffer of pH 5 and supplemented with different

dosages of enzyme ranging from 8 IU/g to 15 IU/g of dry substrate. Poly ethylene glycol (1% w/v) was also added in the reaction mixture to facilitate the enzyme action. The optimization for enzymatic hydrolysis was performed at various temperature from 30 ^{0}C- 70 ^{0}C and 150 rpm for 48 h. Sample of enzymatic hydrolysate was withdrawn after every 6 h, centrifuged at 10,000rpm for 15 min and the supernatant was analyzed for amount of fermentable sugars released. The saccharification efficiency of crude enzyme was calculated as reported elsewhere (Singh *et al.* 2011).

Analytical methods

The total cellulase cellobiohydrolase (CBH), endoglucanase (EG) and β-glucosidase activities were determined in accordance with the International Union of Pure and Applied Chemistry procedures (Ghose 1987). Cellobiohydrolase activity was assayed by measuring the release of reducing sugars in a reaction mixture containing Whatman No. 1 filter paper (1.0 * 6.0 cm , 50.0 mg) as substrate in 50 mM sodium citrate buffer (pH 4.8) at 50 ^{0}C, after 60 min. Endoglucanase (EG) activity was assayed by measuring the release of reducing sugars in a reaction mixture containing 0.5 ml of crude enzyme and 0.5 ml of 2% (w/v) of CMC solution in 50 mM sodium citrate buffer (pH 4.8) incubated at 50 ^{0}C for a period of 30 min. Reducing sugars were assayed by dinitrosalicyclic acid (DNSA) method (Miller 1959). β-Glucosidase activity was determined by assaying the release of p-nitrophenol (pNP) at 430 nm from a reaction mixture containing 1 ml p-nitrophenyl glucopyranoside (pNPG) (1 mM), 1.8 ml acetate buffer and 0.2 ml suitably diluted enzyme, incubated at 50 ^{0}C for 30 min (Wood and Bhat 1988). One unit of enzyme activity was defined as the amount of enzyme required to liberate 1 μmol of glucose or p-nitrophenol, from the appropriate substrate, per ml per min under the assay conditions.

Results

Molecular identification of potent cellulase-producing fungus

Out of the 42 fungi isolated from deteriorated fruit samples and other samples, 10 isolates showed the ability to produce cellulase on plate assay. Among these isolates, the isolate from deteriorated apple was found to exhibit the largest zone of hydrolysis on CMC agar plate isolated from selected for further studies. The identification of the fungal isolate was done by 18S rDNA sequences. The nucleotide BLAST similarity search analysis based on 18S rDNA sequence revealed that the isolate was closely related to the genus *Aspergillus niger* and the organism was termed as *Aspergillus niger* RKJP. The nucleotide accession number of 18S rDNA is KP280049 obtained from NCBI. Among well-established species of the genus *Aspergillus*, the isolate RKJP showed closest sequence identity with *Aspergillus niger* ATCC 16888 followed by *Aspergillus tubingenesis* NRRL 62644 (Fig. 1). In the absence of overall genome relatedness, chemotaxonomic data, the strain RKJP is identified as *Aspergillus niger*.

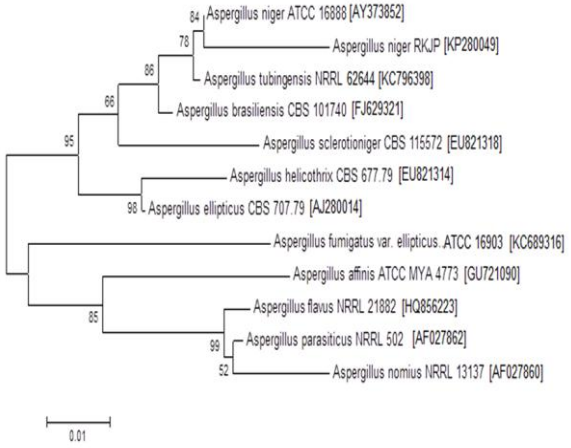

Figure 1: Phylogenetic analysis of *Aspergillus niger* RKJP based on ITS sequences available online from the NCBI database. *The tree was constructed after multiple alignments of sequence data by ClustalX. Distances and clustering with the neighbor-joining method was performed by using the Mega software package version 4.0. Bootstrap values based on 500 replications are listed as percentages at the branching points.

Time course study of cellulase production

Newly isolated fungus *Aspergillus niger* RKJP grown under Solid State Fermentation (SSF) started producing all the three cellulases on day 2nd of incubation as shown in Fig. 2. However all the three cellulase production peaked on day 3rd viz.,endoglucanase (15.04 IU/gm of dry substrate), cellobiohydrolase (3.29 IU/gm of dry substrate), β glucosidase (40.01 IU/gm of dry substrate). Further, increase in fermentation period did not favor elevation in enzyme production.

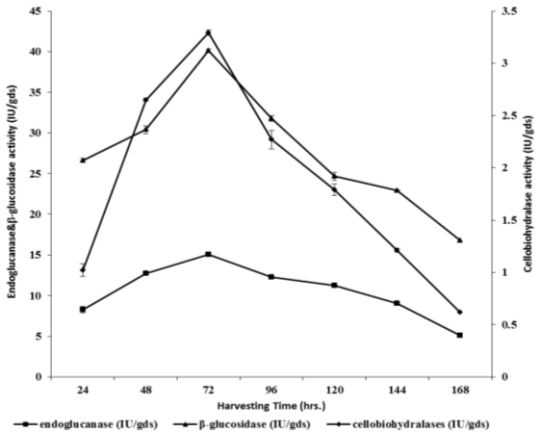

Figure 2: Time course of cellulase production by isolated fungus *Aspergillus niger* RKJP under SSF. Values of means of duplicate experiments. Errors presented here were standard deviation of duplicate experiments.

Effect of cultural and nutritional conditions

The pH of the medium is one of the most critical parameter that affects the mycelia growth and enzyme production. Many enzymatic systems and the transport of several species of enzymes across the cell membrane are influenced by initial pH. From the experiments, maximum enzyme production viz; endoglucanase (15.93 IU/gds), cellobiohydrolase (3.59IU/gds) & β- glucosidase (41.59 IU/gds) was observed at initial medium pH 5.0 during 3rd day of incubation at temperature 30 ^{0}C as shown in Table 1.

Temperature is also a critical factor in the growth of fungus under solid state fermentation. The optimization at various incubation temperatures for production of enzyme under solid state fermentation revealed that enzyme production increased from 20 ^{0}C to 30 ^{0}C during 3rd day of incubation yielding endoglucanase (20.53IU/gds), cellobiohydrolase (4.75IU/gds) & β-glucosidase (56.98IU/gds) as shown in Table 1.

Every microorganism has its own water activity for their growth in solid state fermentation. The result of this study has shown that an increase in the initial moisture ratio from 1:1 to 1:3 greatly strengthen the enzyme production of all the three cellulases (Table 1). The substrate to moisture ratio of 1:3 during 3rd day of incubation at 30 ^{0}C temperature and 5 pH resulted in maximum production of endoglucanase (20.05 IU/gds), cellobiohydrolase (4.65IU/gds) & β-glucosidase (52.45IU/gds). Any further raising the ratio resulted in falling off enzyme activity.

Various lignocellulosic wastes such as wheat bran, rice straw, wheat straw, banana fiber, and corn cobs were tested as carbon source for their effect on cellulase production. The influence of the carbon sources on cellulase production by *Aspergillus niger* RKJP was depicted in Table 2**.** Results of the study indicated among the carbon sources, wheat bran has shown the maximum production of endoglucanase (25.04 IU/gds), cellobiohydrolase (5.19 IU/gds), and β-glucosidase (55.31 IU/gds).

Different nitrogen sources had shown variable effects on cellulase production by *Aspergillus niger* RKJP (Table 2). Among various nitrogen sources, Peptone caused maximum enzyme production viz; endoglucanase (27.98IU/gds) and β glucosidase (61.99 IU/gds), while Yeast extract caused maximum cellobiohydrolase (6.01IU/gds) production.

The use of surfactants in the production of hydrolytic enzymes is well known. Such compounds probably raise the activation of enzyme by weakening the hydrophobic interactions (Helle *et al.* 1993). Tween-80 enhanced enzyme production of all three cellulase viz; endoglucanase (30.14 IU/gds), cellobiohydrolase (6.13 IU/gds) and β glucosidase (61.24IU/gds) followed by PEG-4000, Tween-20 and Tween-40 (Table 2).

Enzymatic Saccharification of pretreated rice straw using cellulase producing from *A.niger* RKJP

Time course study on enzymatic saccharification

The saccharification efficiency of the cellulase production from *A.niger* RKJP was evaluated on an acid pretreated rice straw. The time course study revealed that concentration of reducing sugars increases with increase in treatment time and the maximum yield of reducing sugars (27g/L) after enzymatic hydrolysis was achieved after treatment time of 30 h after that it did not favour any increase in hydrolysis.

Effect of temperature on enzymatic saccharification

From the results shown in Fig.3 it could be observed that increase in temperature of saccharification, yields of reducing sugars increased while increasing the temperature from 30-70 ^{0}C at interval of 10 ^{0}C. Maximum yield of

Table 1: Effect of different Physiological factors on production by isolated fungus *Aspergillus niger* RKJP under SSF**.** Values of means of duplicate experiments. Errors presented here were standard deviation of duplicate experiments.

Physiological Factors	Cellobiohydralase (IU/gds)	Endoglucanase (IU/gds)	β-glucosidase (IU/gds)
pH			
3	3.89±0.085	15.89±0.384	49.905±0.272
3.5	4.05±0.064	15.87±0.241	52.333±0.393
4	4.23±0.053	16.05±0.502	54.449±0.594
4.5	4.46±0.047	17.45±0.175	55.171±0.657
5	**3.59±0.092**	**15.93±0.714**	**41.59±0.482**
5.5	4.48±0.081	18.89±0.598	53.79±0.265
6	4.09±0.057	16.97±0.256	52.201±0.183
6.5	3.87±0.055	13.05±0.278	50.911±0.177
7	3.15±0.084	10.45±0.624	50.98±0.063
Temperature (^{0}C)			
20	3.45±0.102	12.59±.0.0234	48.03±0.492
25	4.24±0.112	16.76±0.612	52.05±0.394
30	**4.76±0.046**	**20.53±0.563**	**56.98±0.915**
35	4.38±0.084	19.8±0.437	51.389±0.28
40	3.97±0.058	18.54±0.662	48.24±0.277
Moisture ratio			
1:2	3.37 ± 0.072	16.89 ± 0.89	47.56 ± 0.83
1:2.5	4.45 ± 0.13	17.97 ±0.81	49.67 ± 0.69
1:3	**4.65 ± 0.083**	**20.05 ± 0.78**	**52.45 ± 0.75**
1:3.5	4.23 ± 0.098	18.45 ± 0.96	51.65 ± 0.83
1:4	4.05 ± 0.091	17.93 ± 0.72	44.45 ± 0.55

fermentable sugar was obtained i.e. 27g/L at temperature of 50 ^{0}C indicated that same temperature of 50 ^{0}C as optimum temperature for saccharification.

Effect of enzyme dose on enzymatic saccharification

Table 3 shows the increase in sugar yield from 21.72g/L to 27g/L with increasing enzyme load from 8 to 12 FPU g/L of substrate at optimized pH 5 and at optimized temperature 50 ^{0}C and thereafter it did not result in any significant improvement in yield of fermentable sugar.

Effect of surfactants

Hydrolysis of pretreated rice straw without surfactant yielded 23.89g/L reducing sugars and addition of nonionic surfactant PEG 4000 to the reaction mixture increased the sugar yield by 13%. A maximum reducing sugar yield 27g/L was obtained with 1% PEG.

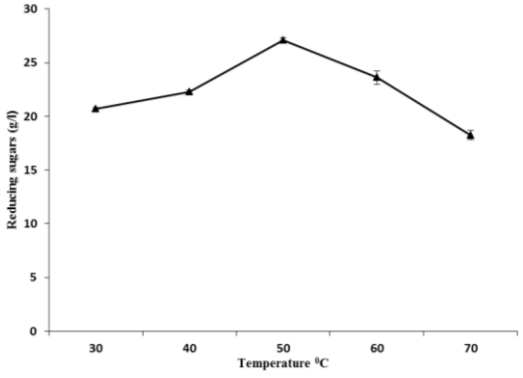

Figure 3: Effect of different temperature on enzymatic saccharification of acid pretreated rice straw. Values of means of duplicate experiments.

Table 2: Effect of supplementation of various additives on the cellulase production using *Aspergillus niger* under SSF. Values of means of duplicate experiments. Errors presented here were standard deviation of duplicate experiments.

Additives	Cellobiohydralase (IU/gds)	Endoglucanase (IU/gds)	β-glucosidase (IU/gds)
Carbon sources			
Wheat Straw	2.07± .086	8.56±0.24	51.16±0.78
Rice Straw	1.34±.059	7.56±0.42	48.2±0.19
Wheat Bran	**5.19±.090**	**25.04±0.71**	**55.31±0.49**
Corn Cobs	1.2±.063	5.78±0.39	43.46±0.17
Cellulose powder	1.98±.043	10.32±0.21	45.88±0.26
Nitrogen sources			
Beef Extract	3.78 ± 0.034	14.67±0.46	51.424±0.30
Peptone	**6.01±0.086**	**27.86±0.66**	**61.99±0.64**
Yeast Extract	5.05±0.062	19.87±0.16	51.86±0.35
Urea	4.56±0.073	17.45±0.64	48.35±0.19
Surfactant			
Tween 20	4.98 ± 0.053	20.68 ± 0.32	54.65 ±0.36
Tween 40	4.12 ±0.09	16.52 ±0.21	51.23 ±0.23
Tween 80	**6.13±0.087**	**30.14 ±0.12**	**61.24 ±0.058**
PEG 4000	5.43± 0.064	19.98 ±0.095	50.65 ±0.18

Table 3: Saccharification of pretreated rice straw at different dosage of cellulase enzyme produced from *Aspergillus niger* RKJP. Values of means of duplicate experiments. Errors presented here were standard deviation of duplicate experiments.

Enzyme dosage (IU/gm)	Concentration of fermentable sugars (g/L)	
	Initial (0 hrs.)	**Final (30 hrs.)**
1. 8 IU/gm	1.96 ± 0.001	21.72 ± 0 .78
2. 10 IU/gm	2.44 ± 0.004	25.15 ± 0 .85
3. **12 IU/gm**	**2.68 ± 0.002**	**27.03 ± 0.89**
4. 15 IU/gm	2.56 ± 0.006	26.81 ± 0.95

Discussion

We have isolated a new fungal strain, identified as *Aspergillus niger* RKJP from deteriorated fruits taken from fruit market of Saharanpur, U.P. The isolated fungal strain exhibited the potential to produce higher activity level of cellulase enzyme under solid state fermentation using mineral salt medium (MSM). During optimization of time course study we found that cellulase enzyme production peaked on day 3rd. Further, increase in fermentation period did not favour elevation in enzyme production. Our study supported the findings of Milala *et al.* 2005, who also reported maximum cellulase activity after 3 days of incubation by an isolated strain of *Aspergillus niger*. Sherif *et al.* 2010 also supported our study who isolated a strain of *Aspergillus fumigates* from rice straw & wheat bran and found maximum cellulase activity after incubation of 4 days, viz. CMCase (5.97IU/g), FPase (0.28 IU/g) & β- glucosidase (4.96IU/g) and are correlated to Longwei *et al.* 2014 study, who reported maximum cellulase activity viz; CMCase

(10.07IU/g), FPase (3.88 IU/g) & β- glucosidase (4.89IU/g) after 3 days incubation by a strain of *Trichoderma viride* from Chinese herb and after which the activity declined, might be due to the depletion of macro and micronutrients in the fermentation medium and drying of the substrate, resulted in stressing the fungal physiology that must be, inactivate secreting machinery of the enzymes The comparison with other reported studies suggested that cellulase production by the newly isolate *Aspergillus niger* RKJP is much higher. However, the exact comparison of cellulase production by different microorganisms reported in literature may not be possible because many time different laboratories have different conditions & estimate the enzyme production by different methods.

The optimization of various incubation temperatures for production of enzyme under solid state fermentation revealed that production of cellulase was maximal at 30^{o}C after 3rd of incubation. Lee *et al.* 2011 and Sohail *et al.* 2009 while studying cellulase production, also reported similar trends. Deswal *et al.* 2011 also reported an increase in cellulase production from fungus *Fomitopsis* sp. at 30 ^{o}C and thereafter the production of enzyme declined. Further increase in incubation temperature did not favor rise in enzyme production and showed a drastic declined on enzyme production because the high temperature of the medium can change membrane composition and can cause the protein catabolism and inhibition of fungal growth.
The pH of the medium is one of the most critical parameter that affects the mycelia growth and enzyme production. Many enzymatic systems and the transport of several species of enzymes across the cell membrane are influenced by initial pH.

It was noted that that production of cellulase was maximal at pH 5 and 30^{o}C after 3rd of incubation. On increasing the initial pH of the medium from 5.0 to 7.0 showed a significant reduction in the production of cellulases. The decrease in initial pH of the medium from 5.0 to 3.0 also caused a slight decrease in the endoglucanase and cellobiohydrolase production. However the results of Sohail *et al.* 2009; Dutt and Kumar 2014 and Mrudula & Murugammal 2011 correlates with our study which showed the maximum cellulase production at initial pH 4, 5.3 and 6 respectively by *Aspegillus niger* in fermentation process.In SSF the optimal moisture content depends on the requirement of microorganism, type of the substrate and the types of end products (Kalogeris *et al.* 2003). The higher enzyme production achieved with substrate to moisture ration of 1:3 in the present study is in agreement with previous findings (Deswal *et al.* 2011; Mrudula and Murugammal 2011). Low moisture level shows reduce enzyme production as it increases high water tension. Higher moisture content results in better utilization of the substrate by the microorganism as it facilitates swelling of substrate (Pandey *et al.* 2000) and according to Alam *et al.* 2005, at very higher moisture level the media become clumped and there may be poor aeration and poor growth so the enzyme production is reduced.

Extracellular enzyme production depends greatly on the composition of the medium. Various lignocellulosic wastes such as wheat bran, rice straw, wheat straw, banana fiber, and corn cobs were tested as carbon source for their effect on cellulase production. The cellulase production in the present study by isolated *Aspergillus niger* RKJP was found to be at high when grown on 5 gm of wheat bran. The yield of endoglucanase 25.04 IU/gds in the present study were higher when compared to the results in the study of Chandra *et al.* 2007, According to this study, the yields of endoglucanase by *A. niger* on wheat bran were 3.24 IU/g of substrate. The unsuitability of the lignocellulosic substrates to support enzyme production under solid state fermentation might be due to inappropriate physical properties like particle size, geometry and compactness of the substrate (Krishna *et al.*2005).

Among various nitrogen sources, it was reported that good cellulase yield can be obtained by using peptone as it is a complex nitrogen source, which has growth factors, blend of amino acids, peptides, water soluble vitamins which affect fungal growth and enzyme production. Recently similar observations have also been made in case of cellulase production that favour our findings in the present study as Gomathi *et al.* 2012 achieved maximum CMCase production by supplementing the medium with peptone as nitrogen source and wheat bran as a carbon source. Longwei *et al.* 2014 also achieved maximum FPase production by

supplementing the medium with peptone as nitrogen source with wheat bran as a carbon source.

The use of surfactants in the production of hydrolytic enzymes is well known. Such compounds probably raise the activation of enzyme by weakening the hydrophobic interactions (Helle *et al.* 1993). However this increase may be due to reduction of nonspecific and irreversible enzyme adsorption on solid state substrate (soni *et al.* 2010). The optimization of maximum enzyme production from *Aspergillus niger* RKJP revealed that maximum enzyme production viz; endoglucanasse, cellobiohydrolase and β-glucosidase was obtained at 0.2% of Tween80. Further on increasing Tween-80 concentration from 0.3- 0.5% resulted a decline in enzyme synthesis.

The present study revealed that more than 30 % increase in the enzyme production has been achieved after optimization of enzyme production by *A. niger* RKJP following One Factor At a Time (OFAT) method in comparison to the enzyme production under unoptimized medium (Endoglucanase 20.23IU/gds, Cellobiohydrolase 4.15 IU/gds and β-glucosidase 42.98IU/gds). The saccharification efficiency of the cellulase production from *A.niger* RKJP was evaluated for acid pretreated rice straw. The time course study revealed that after 30 hrs. of treatment time it did not favour any hydrolysis of reducing sugars. The decline in hydrolysis rate beyond that time point could be due to the increasing resistance of the substrate during the course of hydrolysis (Gupta *et al.* 2009).

The optimization of temperature for saccharification revealed that maximum yield of reducing sugar was obtained i.e. 27g/L of substrate at temperature of 50^{o}C. At lower enzyme load 12 FPU/gm of dry substrate reflected maximum saccharification effeiciency and thereafter it did not favor any increase in hydrolysis of sugars. This could be due to the fact that at higher enzymatic levels, steric hindrance on the cellulose surface may have occurred, there by rendering the process inefficient (Soto *et al.* 1994). These results are in accordance with the earlier findings of Das *et al.* 2013 where in it has been indicated that slight lower concentration of reducing fermentable sugars i.e. 24.9g/L only was produced during enzymatic saccharification with even higher enzyme loading of 40 IU/gm of substrate at optimum process condition i.e. temperature 50 ^{o}C and pH 5.

A maximum reducing sugar yield was obtained, supplemented with 1% PEG 4000 in the present work that are in agreement with Yao et al. 2007, who also found that a nonionic surfactant PEG increased fermentable sugars to an extent of cellulose hydrolysis.

The synergistic action of cellulase enzyme containing endoglucanase (30.14 IU/gds), cellobiohydrase (6.13 IU/gds) and β glucosidase (60.25IU/gds) in appropriate ratio led to a maximum saccharification efficiency of more than 85 % resulting in higher concentration of reducing sugars 27g/L with lower enzymatic dose 12 IU/gm of FPU (Filter paper unit) at pH 5 and 50 ^{0}C temperature, supplemented with 1% PEG- 4000 using acid treated rice straw. However earlier Sukumaran *et al.* 2009 supported our findings who found lower reducing sugar 10.98g/L only at higher enzymatic load by using acid treated rice straw and Abo –State *et al.* 2014 where saccharification efficiency was 35.12% only with lower concentration of reducing sugars 13.58g/L.

Conclusion

The cellulase enzyme thus produced under optimized conditions from the isolated fungal strain *Aspergillus niger* RKJP possess higher activity level of β,endo 1-4 gucanase (30.14 IU/gds), cellobiohydrolase(6.13 IU/gds) and β glucosidase (60.25IU/gds)) in synergetic ratio required to optimally saccharifying the lignocellulosic biomass into fermentable sugars. This has helped to achieve higher saccharification efficiency (more than 85%) requiring lesser enzyme doses of cellulase. The cellulase enzyme thus produced with higher activity level may help in improving the cost economic of the bioconversion process. Further the upscaling of the process for cellulase production of enzyme from isolated fungal strain may help to improve the process for bioconversion of lignocellulosic waste into ethanol to be cost effective making it techno economically feasible. Thus showing further may help to eradicate environmental problems caused due to burning of rice straw in paddy fields while providing an alternate source of biofuels.

Conflict of interest statement

All authors declare that they have no financial or commercial conflicts of interest.

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