



## Studies on Cellulase Production by Solid state Fermentation using Sweet Sorghum bagasse

\*<sup>1</sup>Lavudi Saida, <sup>2</sup>Harinder Singh Oberoi, <sup>3</sup>M.Laxmi Narasu

<sup>\*1</sup>Centre for Biotechnology, IST, JNT University, Hyderabad

<sup>2</sup>Central Institute of Post Harvest Engineering and Technology, Ludhiana, Punjab

\*Phone:9618528040, Email:lavudisaida@jntuh.ac.in

Received date: Nov 29<sup>th</sup> 2012, Accepted date: Dec 8<sup>th</sup> 2012, Published date: Jan 1<sup>st</sup> 2013

### Abstract:

The present study involves production of cellulolytic enzymes by a newly isolated strain of *Aspergillus niger* isolated from decaying sugarcane bagasse. Solid-state-fermentation was employed using sweet sorghum bagasse as the substrate for the production of enzyme. One factor at a time approach was used to optimize the process variables like moisture content, pH, temperature, and incubation period. A maximum of 19.2 FPU/g-ds of cellulose and 384.5 IU/g-ds of  $\beta$ -glucosidase activity of were obtained at 70% moisture content, a temperature of 30 °C, pH of 5 and incubation time of 96 h. This study showed an effective utilization of sweet sorghum bagasse for the production of cellulolytic enzymes which could be effectively used for hydrolysis to simple sugars for production of bioethanol.

**Keywords:** *Aspergillus niger*, sweet sorghum bagasse, wheat bran, Solid state fermentation, cellulase,  $\beta$ -glucosidase.

### Introduction:

Lignocellulosic waste residues obtained from energy crops, wood and agricultural residues represent the most abundant sources of renewable biomass <sup>[1]</sup>. It is estimated that the photosynthetic process produces about 150 billion tons of dry material annually with respect to carbon, of which about 50% is cellulose <sup>[2]</sup>. Cellulase enzymes are the key drivers for biomass utilization through the bioconversion of the most abundant cellulosic wastes into its monomeric forms <sup>[3]</sup>. Researchers have strong interests in cellulases because of their applications in industries such as, starch processing, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper industry, and textile industry <sup>[4]</sup>. A potential challenging area where cellulases would have a central role is the bioconversion of renewable cellulosic biomass to commodity chemicals <sup>[5]</sup>. In recent years, some important agro-industrial residues such as sugarcane bagasse, kinnow waste, wheat bran, rice straw, leaves, corn stover etc. have been used as substrates by solid state fermentation (SSF) for the production of enzymes <sup>[4,7,8]</sup>. Cost reduction of the process can be

achieved by the use of high enzyme producers and use of cheaper substrates <sup>[9]</sup>. Reduction in the production cost and improvement in cellulase yield could also be achieved using appropriate and low cost carbon and nitrogen sources in the formulation of fermentation medium <sup>[10, 11]</sup>. Thus, the use of rice straw for production of cellulases which have the potential of bioconversion of cellulosic biomass to sugars seems to be a good alternative. Microbial degradation of lignocellulosic wastes and the downstream products resulting from it is due to the concerted action of several enzymes, the most prominent of which are cellulases.

Cellulase is a multienzyme system composed of three main enzymes viz., Endoglucanase, Exoglucanase and  $\beta$ -Glucosidase which act synergistically and catalyze the hydrolysis of cellulose and cello-oligosaccharide derivatives. Cellulolytic enzymes comprise of endoglucanase (EG; 1,4- $\beta$ -D-glucan-4-glucano-hydrolases; EC3.2.1.74), exoglucanase (EC 3.2.1.91 and EC 3.2.1.74) and  $\beta$ -glucosidase (BGL; EC 3.2.1.21). Endoglucanases cleave glycosidic bonds randomly within the interior of cellulose polymer chain. Exoglucanase acts progressively on the reducing and non-reducing ends of cellulose chains, releasing cellobiose and glucose respectively as major products. The  $\beta$ -glucosidase hydrolyzes soluble cellodextrins and cellobiose to glucose <sup>[14]</sup>. In addition, auxiliary enzymes, such as, glucuronidase, acetylesterase, feruloyl esterase, xylanase,  $\beta$ -xylosidase, galactomannanase and glucomannanase attack hemicelluloses, thereby releasing pentose sugars, such as xylose and arabinose. Media for cellulase enzyme production with components like cellulose, glucose, yeast extract, peptone, urea,  $\text{KH}_2\text{PO}_4$ ,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{MgSO}_4$ ,  $\text{FeSO}_4$ ,  $\text{MnSO}_4$ ,  $\text{CoCl}_2$ ,  $\text{CaCl}_2$  etc have been reported previously <sup>[16, 17]</sup>. As large number of basal ingredients are screened and optimized in fermentation processes, experiments based on multi-factorial design are difficult, thus statistics-based experimental methods have been designed for media optimization <sup>[18, 19]</sup>.

Solid-state fermentation (SSF) holds tremendous potential for production of commercially important enzymes because of several advantages, such as

higher fermentation productivity, higher concentration of end product, higher product stability, lower catabolic repression and cultivation of microorganisms specialized for water-insoluble substrates<sup>[15]</sup>. The present study reports the optimization and validation of process parameters using one factor at a time method in SSF.

### **Materials and Method:**

#### **Materials:**

Sweet sorghum bagasse was procured from Punjab Agricultural University, Ludhiana, and Punjab, India. Wheat bran was used as co-substrate and it was procured from local wheat flour mill. The dehydrated media and media ingredients and analytical grade chemicals were obtained from Hi-Media Laboratories Pvt Ltd (Mumbai, India) and Fisher Scientific (Mumbai India), respectively. The fungal isolate used for the cellulase production was isolated from decaying sugarcane bagasse. The substrates used for enzymes assays were obtained from Sigma-Aldrich (St.Louis, MO, USA).

#### **Methodology:**

##### ***Inoculum preparation***

Fungal culture was inoculated onto PDA medium in a petri dish and after 72 hr; the spores were harvested using sterilized water. The spore count was determined with haemocytometer (Marienfeld, Germany) and the spore concentration of  $1 \times 10^8$  spores/ml was used for inoculating sweet sorghum bagasse, which was used as substrate for enzyme production purpose (Smith et al. 1996).

##### ***Enzyme Production***

Solid state fermentation using sweet sorghum bagasse and wheat bran in the ratio 5:0, 4:1, 3:2, and 1:1 was employed for production of cellulases. All the enzyme production experiments were carried out in 250-ml Erlenmeyer flasks containing 10 gm substrate. Effect of moisture content (50%, 60%, 70%, and 80%) was studied for maximum cellulase production with Mandel Weber medium<sup>[20]</sup> and effect of pH on cellulase production was determined at pH values of 3.0, 4.0, 5.0, and 6.0. pH using either 2.5 M sodium hydroxide or 2.5 M hydrochloric acid. The flasks were autoclave-sterilized for 15 min, cooled, inoculated with 1 ml fungal spore suspension and incubated in an incubator at 30 °C, 35 °C, 40 °C and 45 °C for 24 hr, 48 hr, 96 hr and 120 hr.

##### ***Enzyme extraction***

Enzyme extraction from the flasks was done by addition of a suitable volume of citrate buffer (0.1M, pH 4.8). The flasks were incubated at 30° C for 30 minutes at 125rpm in an orbital shaking BOD incubator (Macro Scientific Works Pvt. Ltd.,

Delhi) to have the complete enzyme extract in the solution form and the contents were then filtered through rough filter paper and then centrifuged at  $5000 \times g$  at 4 °C for 10 min. Supernatant was collected and analyzed for filter paper cellulase (FPase),  $\beta$ -glucosidase activities and protein concentration.

##### ***Enzyme Assays***

###### ***Filter paper activity***

Filter paper activity was used to estimate total cellulase activity and endoglucanase activity<sup>[20]</sup> in crude enzyme preparation. Filter paper activity was estimated using 1.0×6.0 cm (50 mg) Whatman no.1 filter paper strips. Filter paper strip placed in assay tube was saturated with 1 ml 0.05 M sodium citrate buffer of pH 5.0 and 0.5 ml of an appropriately diluted enzyme. These were incubated at 50 °C for 60 min. Controls were also run along with reaction mixtures. The reaction was stopped with the addition of 3 ml of 3, 5-dinitrosalicylic acid reagent in tube. The tubes were kept in boiling water bath for 5 min to develop color. Then 1ml of sodium potassium tartarate (40% w/v) was added in to the reaction mixture. The reaction mixture was diluted appropriately and was measured against a reagent blank at 540 nm in a UV-VIS spectrophotometer (PG Instruments Ltd, UK). The concentration of glucose released by enzyme was determined by comparing glucose concentration against standard curve constructed in similar way. One International unit (IU) of filter paper activity is defined as the quantity of enzyme required to liberate 1  $\mu$ mol of glucose equivalent liberated per minute of culture filtrate under standard assay conditions.

###### ***Cellobiohydrolase (CBH), $\beta$ -glucosidase, $\beta$ - xylosidases, $\alpha$ -L-arabinofuranosidase and xylan esterase activity***

EGI & CBHI activities were measured against 4-methylumbelliferyl- $\beta$ -D-lactoside (5 mM)<sup>[21]</sup>. The activity obtained without cellobiose was referred as EGI & CBHI activity, while the activity in the presence of cellobiose was considered as EGI activity, since cellobiose is a competitive inhibitor for CBH I. CBH I activity was calculated by subtracting EGI activity from EGI & CBHI activity.  $\beta$ -Glucosidase,  $\beta$ - xylosidases,  $\alpha$ -L-arabinofuranosidase and xylan esterase activities were determined using nitrophenyl  $\beta$ -D-glucopyranoside (pNPG), 4-nitrophenyl-  $\beta$ -D-xylanopyranoside, 4-nitrophenyl- $\alpha$ -L-arabinofuranoside and 4-nitrophenyl-acetate substrates, respectively (Poutanen K et al. 1987). The assay mixture containing 50  $\mu$ l of substrate and 50  $\mu$ l of appropriately diluted enzyme in sodium acetate buffer (50 mM, pH 5.0) was incubated at 50

°C for 30 min. At the end of incubation, reaction was stopped with the addition of 100 µl Glycine NaOH buffer (0.4 M, pH 10.8). The reaction mixture was analysed in ELISA reader (Multiscan Ascent, Thermo Scientific, USA) at 405nm. The concentration of p-nitrophenol released by enzyme was determined by comparing against standard curve constructed in similar way. One International unit (IU) of enzyme activity was defined as the quantity of enzyme required to liberate 1 µmol of p-nitrophenol per millilitre of crude filtrate per minute under standard assay conditions.

#### Protein estimation

Protein concentration was determined by protein dye binding method of Bradford (1976). 3ml of Bradford reagent was added to 0.1ml of crude enzyme in a test tube. Reaction mixture was incubated at 30 °C for 15 min. It was then measured against a reagent blank at 595 nm in a UV-VIS spectrophotometer (PG Instruments Ltd, UK). The concentration of protein was determined by comparing against Bovine Serum Albumin (BSA) standard curve constructed in similar way.

#### Validation studies

Validation experiments and scale-up studies were carried out in stainless steel trays of size 36 cm length × 25 cm width × 2.5 cm height. Two hundred grams substrate described previously was impregnated with 440 ml mineral solution containing optimized mineral concentrations mentioned previously in conical flasks. Flasks were autoclave-sterilized as mentioned previously for statistically designed experiments. The contents after cooling were spread uniformly up to 2 cm height in each tray and inoculated with inoculum concentration of  $1 \times 10^8$  spores/ml in the laminar flow bench using sterilized air draft. The trays were covered with muslin cloth and were incubated in the fumigated environmental chamber at optimized temperature 30°C and 95% humidity for optimized incubation period of 96 h.

### Results and discussions:

#### 1. Effect of Substrate Ratio on Cellulase production:

In this study, different ratios of carbon sources SSB and WB at 5:0, 4:1, 3:2, 1:1 were evaluated for maximum cellulase production. The maximum filter paper activities were as shown in Table 1. The maximum β-Glucosidase activities and Protein content were as shown in Tables 2&3. The substrate composed of sweet sorghum bagasse (SSB) and wheat bran (WB) in the ratio of 4:1. This substrate concentration proved to yield higher contents of enzymes F-pase, β- glucosidase, and Protein content which is shown in Tables 1,2&3:

15.8 FPU/g-ds, 344.5 IU/g-ds, and 6.2 mg/ml respectively.

Such similar types of studies were performed by using various cultures for the production of different metabolic enzymes as reported in the literature [22, 23]. Previous studies showed that supplementation of four parts of soyabean hulls with one part of wheat bran resulted in high titres of enzymes with balanced proportion of various activities [24].

**Table 1-** Filter paper activity (FPU/g-ds): *Aspergillus niger* at 35 °C, moisture 60%, pH 5.0, Substrate (Sweet sorghum baggase: wheat bran)

Subs Ratio	24 hr	48hr	72hr	96hr	120hr
5:0	2.2	2.8	4.2	7.1	6.8
4:1	5.2	6.5	9.9	15.8	14.9
3:2	5.3	7.3	10.2	16.0	15.1
1:1	4.9	9.5	10.8	14.9	14.3

**Table 2-** β-glucosidase activity (IU/ml): *Aspergillus niger* at 35 °C, moisture 60%, pH 5.0, Substrate (Sweet sorghum baggase: wheat bran)

Subs Ratio	24 hr	48hr	72hr	96hr	120hr
5:0	21.8	25.9	136.5	192.2	184.4
4:1	98.7	118.7	213.6	344.5	350.2
3:2	98.9	132.5	256.1	338.2	345.9
1:1	96.3	96.5	176.4	281.5	259.9

**Table 3-** Protein content (mg/ml): *Aspergillus niger* at 35 °C, moisture 60%, pH 5.0, Substrate (Sweet sorghum baggase:wheat bran)

Subs Ratio	24 hr	48hr	72hr	96hr	120hr
5:0	1.4	1.7	2.5	3.9	3.5
4:1	2.2	2.9	4.4	6.2	5.2
3:2	1.9	2.2	3.7	5.9	5.0
1:1	1.4	1.9	2.8	4.1	4.6

#### 2. Effect of Moisture content on cellulase production:

Effect of moisture contents 50%, 60%, 70%, and 80% were studied on cellulase production. The maximum filter paper activities were as shown in Table 4. The maximum β-glucosidase activity and protein content were as shown in Table 5&6. Similar types of observations were made in cellulolytic enzyme production [24]. Moisture content is important and SSF can be viewed as discrete solid phase in which micro-organisms

grow on the moist, discrete particles as well as inside and between them.

Maximum cellulase activity was observed at pH 4.5, moisture content of 70% and temperature of 30 °C in the study of SSF of soyabean hulls for cellulolytic enzymes production [24]. Increase in temperature beyond 34°C showed reduced cellulase activity and maximum activity exhibited around the 70% of moisture content and 33 °C.

Our results are also in agreement with the results of [25] wherein which reported that moisture content had a significant effect on cellulase production using *T. reesei* strains and maximum activity was observed at 70% of moisture content. The maximum response for β- glucosidase was observed at 70% of moisture content at pH of 4.5 and temperature of 30 °C [24]. In the present Investigation higher yields of cellulase enzyme, both FPase, β- glucosidase activities, and protein content as shown in Table 4,5&6 were found to be 17.2 FPU/g-ds, 362.2 IU/g-ds, and 7.0 mg/ml respectively at pH 5.0 , and Moisture 70% .

**Table 4-** Filter paper activity (FPU/g-ds): *Aspergillus niger* at 35 °C, pH 5.0, Substrate ratio (4:1)

Moisture level	24hr	48hr	72hr	96hr	120hr
50%	2.5	3.1	5.9	8.1	9.0
60%	3.8	6.4	9.0	14.7	15.4
70%	6.2	8.1	10.7	<u>17.2</u>	16.8
80%	4.8	5.5	8.7	14.0	14.3

**Table 5-** β- glucosidase activity (IU/g-ds): *Aspergillus niger* (M.C.Black) at 35°C, pH 5.0) substrate ratio (SSB:WB) 4:1

Moisture level	24 hr	48hr	72hr	96hr	120hr
50%	37.5	46.5	153.2	187.1	184.4
60%	75.8	123.5	224.5	348.9	350.2
70%	125.7	145.6	256.1	<u>362.2</u>	359.1
80%	98.6	102.5	192.1	226.5	203.6

**Table 6-** Protein content (mg/ml): *Aspergillus niger*(M.C.Black) at 35°C, pH 5.0, and substrate ratio (SSB:WB) 4:1

Moisture level	24 hr	48hr	72hr	96hr	120hr
50%	0.4	0.9	1.5	2.2	2.4
60%	0.6	2.1	4.9	6.6	6.7
70%	1.8	2.6	5.9	<u>7.0</u>	6.8
80%	1.5	1.9	3.6	4.9	5.1

**3. Effect of pH on cellulase production:**

The effect of pH on cellulase production was determined at pH values of 3.0, 4.0, 5.0, and 6.0. The maximum filter paper activities obtained at different pH values are shown in Table 7. The maximum β-Glucosidase activities and Protein content were obtained at different pH values are shown in Tables 8 and 9. The results presented in the Tables 7, 8&9 shows the effect of pH on enzyme production. Similar types of studies were conducted by using different microbial cultures for the production of significant enzymes in general and cellulolytic enzymes in particular as mentioned elsewhere in the literature [20]. An optimal pH with a value of 5 for cellulose production by using *Aspergillus niger* reported in literature [26]. Further increase in pH level reduced the enzyme activity at pH 6.5 [27] have also reported a similar result for other fungal cellulases. Thus, as shown in Tables 7&8, the maximum cellulase activities were observed at pH 5.0. In the present investigation maximum enzyme production observed for FPase activity, β- glucosidase activity, and Protein content are 18.9 FPU/g-ds, 355.2 IU/g-ds, and 7.8 mg/ml respectively at pH 5.0 by using cultures of *Aspergillus Niger*.

**Table 7-** Filter paper activity (FPU/g-ds): *Aspergillus niger* at 35 °C, moisture 70%, substrate ratio (SSB: WB) 4:1

pH	24 hr	48hr	72hr	96hr	120hr
3	1.2	1.8	2.8	3.2	3.0
4	4.5	7.0	10.1	16.7	16.5
5	6.8	8.9	10.9	<u>18.9</u>	18.2
6	5.8	7.6	11.1	17.1	16.9

**Table 8-** β-glucosidase activity (IU/g-ds): *Aspergillus niger* at 35°C, moisture70%, substrate ratio (SSB:WB) 4:1

pH	24 hr	48hr	72hr	96hr	120hr
3	25.7	32.5	87.2	127.4	117.8
4	78.5	108.5	200.5	332.5	315.4
5	93.5	144.7	244.7	<u>355.2</u>	344.8
6	84.5	112.5	220.5	341.2	325.8

**Table 9-** Protein (mg/ml): *Aspergillus niger* (M.C.Black) ) at 35°C, moisture 70%, substrate ratio (SSB:WB) 4:1

pH	24 hr	48hr	72hr	96hr	120hr
3	0.1	0.2	1.1	2.9	2.3
4	1.8	2.3	5.1	7.3	7.4
5	2.4	2.8	6.5	<u>7.8</u>	7.5
6	1.8	2.0	4.6	7.0	7.1

#### 4. Effect of temperature on cellulase production:

Optimization was carried out by incubating the fermentation flasks at 30, 35, 40, and 45 °C at different carbon sources SSB and WB in 4:1 ratio. The maximum filter paper activities obtained at different temperatures are shown in Table 10. The maximum  $\beta$ -glucosidase activities and Protein content obtained are shown in Table 11 and 12. Thus, as shown in Table 10, 11 & 12, maximum degradation of SSB and WB was observed at 30°C. It is reported in literature that temperature and pH have a crucial effect on cellulase production by many fungi. The positive significance of temperature on cellulase enzyme production by several *Aspergilli* was recorded with temperature optima between 28 °C – 35 °C. In the present investigation maximum enzyme production observed for both F-pase activity,  $\beta$ -glucosidase activity, and protein content are 17.5 FPU/g-ds, 359.1 IU/g-ds, and 6.9 mg/ml respectively at 30 °C by using cultures of *Aspergillus niger*.

**Table 10-** Filter paper activity (FPU/g-ds): *Aspergillus Niger* (M.C.Black)) substrate ratio (SSB: WB) 4:1, pH=5.0, moisture 70%

Temperature(°C)	24 hr	48 hr	72 hr	96 hr	120 hr
30	4.8	6.9	10.3	17.5	16.8
35	4.1	6.5	9.9	16.6	16.9
40	2.2	2.8	4.7	7.1	7.6
45	0.8	1.1	2.0	1.8	0.7

**Table 11-**  $\beta$ -Glucosidase activity (IU/g-ds): *Aspergillus niger*(M.C.Black)) substrate ratio (SSB:WB) 4:1, moisture 70%, pH =5.0

Temperature(°C)	24 hr	48hr	72hr	96hr	120hr
30	125.2	146.5	248.5	359.1	370.1
35	115.4	133.9	226.7	328.9	330.2
40	45.5	52.4	162.1	182.2	179.1
45	37.5	29.1	70.5	79.0	62.3

**Table 12-** Protein (mg/ml): *Aspergillus niger*(M.C.Black) ), substrate ratio (SSB:WB) 4:1 moisture 70%, pH =5.0

Temperature (°C)	24hr	48hr	72hr	96hr	120hr
30	2.6	2.9	5.1	6.9	6.4
35	2.4	2.2	4.6	6.5	6.5
40	0.5	1.0	1.9	2.2	2.0
45	0.1	0.2	0.9	1.1	1.1

#### Conclusion:

In the present study optimal process parameters for enzyme production by *Aspergillus niger*, were found to be pH of 5.0, moisture content of 70%, a Temperature of 30 °C, and a substrate ratio (SSB:WB)- 4:1 and Incubation time of 96h. Similar kind of work has been done by [4], on cellulase enzyme production. Work was performed on 2 levels of bagasse (2 and 5%) and it was reported that the optimal variables as mixed

substrate (RB: WB 1:4, 3:2), temperature 30°C, initial culture pH 5.5. Optimal conditions for the endoglucanase production by *T. viridae* strain was reported 97 U/ml and 95 U/ml [28].

It is reported in the literature that solutions with higher desirability gave optimum temperature of 30°C, pH of 5 and moisture content of 70% [8]. Our results are also in agreement with the observations recorded in the previous studies. In the present investigation enzyme activities under the optimized conditions for both the F-pase and  $\beta$ -glucosidase, and Protein content were maximized.

#### Acknowledgment:

Authors thankfully acknowledge the financial assistance received under the DBT project (BT/PR8488/PBD/26/68/2006) from the Department of Biotechnology, Government of India for conducting this research work.

#### References:

- [1] Lin, Y; Tanaka, S., 2006, Ethanol fermentation from biomass resources: current state and prospects. Applied Microbial Biotechnology, 69:627-642
- [2] Persson I, Tjerneld F, Hahn-Hägerdal B (1991). Fungal cellulolytic enzyme production: A review. Process Biochem. 26(2):65-74
- [3] Wen, Z., W. Liao and S. Chen, 2005. Production of cellulase/ $\beta$ -glucosidase by the mixed fungi culture of *Trichoderma reesei* and *Aspergillus phoenicis* on dairy manure. Applied Biochem. Biotechnol., 121: 93-104
- [4] Gao, J., H. Weng, D. Zhu, M. Yuan, F. Guan and Y. Xi, 2008. Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover. Bioresour. Technol., 99: 7623-7629
- [5] Zhou, J.; Wang, Y.; Chu, J.; Luo, L; Zhuang, Y.; Zhang, S., 2009, Optimization of cellulose mixture for efficient hydrolysis of steam-exploded corn stover by statistically designed experiments. Bioresource Technology, 100: 819-825.
- [6] Ibrahim, A.S.S. and A.I. El-diwany, 2007. Isolation and identification of new cellulases producing thermophilic bacteria from an Egyptian hot spring and some properties of the crude enzyme. Aust. J. Basic Applied Sci., 1: 473-478
- [7] Mojsov 2010 A Comparison of Effects of Three Commercial Pectolytic Enzyme Preparations in Red Winemaking, Int. J. Pure Appl. Sci. Technol., 1(2) (2010), pp. 127-136

- [8] Oberoi HS, Vadlani PV, Nanjundaswamy A (2011). Enhanced ethanol production from Kinnow mandarin (*Citrus reticulata*) waste via a statistically optimized simultaneous saccharification and fermentation process. *Bioresour. Technol.* 102: 1593-1601.
- [9] Kotchoni SO, Shonukan OO (2002) Regulatory mutations affecting the synthesis of cellulase. *World J Microbiol Biotechnol* 18: 487-491
- [10] Beg, Q.K., B. Bhushan, M. Kapoor and G.S. Hoondal, 2000. Enhanced production of a thermostable xylanase from *Streptomyces* sp. QG-11-3 and its application in biobleaching of eucalyptus kraft pulp. *Enzyme Microb. Technol.*, 27: 459-466
- [11] Senthilkumar, S.R., B. Ashokkumar K.C. Raj and P. Gunasekaran, 2005. Optimisation of medium composition for alkali-stable xylanase production by *Aspergillus fischeri* Fxn 1 in solid-state fermentation using central composite rotary design. *Bioresour. Technol.*, 96: 1380-1386
- [12] Oberoi et al., 2010 H.S. Oberoi, P.V. Vadlani, R.L. Madl, L. Saida, J.P. Abeykoon. Ethanol production from orange peels: two-stage hydrolysis and fermentation studies using optimized parameters through experimental design. *J. Agric. Food Chem.*, 58 (2010), pp. 3422–3429.
- [13] Maiorella BL (1985) Ethanol. In: *Comprehensive biotechnology*, vol 3. Pergamon, Oxford, pp 861–909
- [14] Ander, P., Mishra, C., Farrell, R., Eriksson, K.E., 1990. Redox interactions in lignin degradation: interaction between laccase, different peroxidases and cellobiose:quinine oxidoreductase. *J. Biotechnol.* 13, 189–198.
- [15] BENJAMIN, Sailas and PANDEY, Ashok. Isolation and characterization of three distinct forms of lipases from *Candida rugosa* produced in solid state fermentation. *Brazilian Archives of Biology and Technology*, June 2001, vol. 44, no. 2, p. 213-221
- [16] Niranjan AP, Madhou P, Stevenson TW (2007). The effect of carbohydrate carbon sources on the production of cellulase by *Phlebia gigantea*. *Enzyme Microbial. Technol.* 40: 1464-1468.
- [17] Martins LF, Kolling D, Camassola M, Dillon AJ, Ramos LP (2008) Comparison of *Penicillium echinulatum* and *Trichoderma reesei* cellulases in relation to their activity against various cellulosic substrates. *Bioresour Technol* 99: 1417-1424
- [18] Ghada AY, Mahmoud MB (2009). Improved production of endoglucanase enzyme by *Aspergillus terreus*; Application of Plackett-Burman design for optimization of process parameters. *Biotechnol.*, 8: 212-219
- [19] Rashid SS, Alam MZ, Karim MI A, Salleh HM (2009). Management of palm oil mill effluent through production of cellulases by filamentous fungi. *World J. Microbiol. Biotechnol.* 25: 2219-2226
- [20] Rashid SS, Alam MZ, Karim MIA, Salleh MH (2009). Management of palm oil mill effluent through production of cellulases by filamentous fungi. *W. J. Microbiol. Biotechnol.* 25: 2219-2226
- [21] Bailey MJ, Tahiharju J. Efficient cellulase production by *Trichoderma reesei* in continuous cultivation on lactose medium with a computer-controlled feeding strategy. *Appl Microbiol Biotechnol* 2003; 62(2–3):156–62. Sudhakar.P et al., (2011). Production of Chitinase by Solid State Fermentation from *Serratia marcescens*. *International Journal of Chem Tech Research*. Vol. 3, No. 2, pp 590-598
- [22] Sudhakar.P et al., (2011). Production of Chitinase by Solid State Fermentation from *Serratia marcescens*. *International Journal of Chem Tech Research*. Vol. 3, No. 2, pp 590-598
- [23] Brijwani K, et al., (2010). Production of cellulolytic enzyme system in mixed culture solid-state fermentation of soybean hulls supplemented with wheat bran. *Process Biochem.* 45: 120- 128
- [24] Khushal Brijwani and Praveen V. Vadlani. Solid State Fermentation of Soybean Hulls for Cellulolytic Enzymes Production
- [25] Latifian M., et al., (2007). Evaluation of culture conditions for cellulase production by two *Trichoderma reesei* mutants under solid-state fermentation conditions. *Bioresour Technol.* 98: 3634- 3637
- [26] Narasimha, G., A. Sridevi, V. Buddolla, C.M. Subhosh and R.B. Rajasekher: Nutrient effects on production of cellulolytic enzymes by *Aspergillus niger*. *Afr. J. Biotechnol.*, 5, 472-476 (2006)
- [27] Wang F, Sanz A, Brenner ML, Smith A (1993) Sucrose synthase, starch accumulation, and tomato fruit sink strength. *Plant Physiol* 101: 321–327
- [28] Kavitha Fermentative production of endoglucanase- Kinetics and Modeling. *International Journal of Engineering Science and Technology(IJEST)*

\*\*\*\*\*