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High-yield Cellulases and Xylanase Production from Sugar-cane Bagasse Pith by *Aspergillus oryzae* FK-923 Cultivated under Solid State Fermentation

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A B S T R A C T

High-yield of fungal cellulases, endo-1,4-β-D glucanases (EC3.2.1.4), exo-1,4-β-D glucanases (EC 3.2.1.91) and β-D glucosidases (EC3.2.1.21) in addition to xylanase (EC 3.2.1.8) were produced from pretreated sugar-cane bagasse pith (SCBP) by *Aspergillus oryzae* FK-923cultivated under solid state fermentation. The highest enzyme activities were 120.2, 129.2, 145.8 and 688.2 Ug^{-1} for FPase, CMCase, β -glucosidase and xylanase, respectively. The pretreatment of sugar-cane bagasse pith with sulfuric acid was more suitable for enhancing cellulases and xylanase production. Enzymes were produced under the following conditions; the moisture content of treated bagasse pith was 80%, initial pH ranged from 4.5-5.5, 4 days of incubation at 30 °C. Urea was efficient for enzyme production followed by Ammonium phosphate di basic. The pH value (5.0) was more suitable for the enzyme extracted from fermented substrate as well as solid to solvent ratio 1:15 was the optimum for enzymes elution. Among different solvents used for enzyme extraction, 0.05 citrate buffer was most efficient. The optimum pH and temperature for enzyme activity were 5.0-5.5 and 55°C.

Introduction

The biological aspects of cellulosic biomass processing become the crux of future research involving cellulases and cellulolytic microorganisms. Cellulases are being commercially produced by several industries globally and are being widely used in food, animal feed, fermentation, agriculture, pulp and paper and textile applications.

With modern biotechnology tools, novel enzymes and new enzyme applications will become available for the various industries. Biotechnological conversion of cellulosic biomass is a potentially sustainable approach to develop novel bioprocesses and products. Microbial cellulases have become the focal biocatalysts due to their complex

nature and widespread industrial applications. Cellulases are inducible enzymes synthesized by a large diversity of microorganisms, including both fungi and bacteria during their growth on cellulosic materials (Sang-Mok and Koo,2001). These microorganisms can be aerobic, anaerobic, mesophilic or thermophilic. Among them, the genera of *Clostridium, Cellulomonas, Thermomonospora, Trichoderma* and *Aspergillus* are the most extensively studied cellulase producers (Kuhad *et al.,* 2011). Nowadays, these so-called wastes are judiciously utilized to produce valuable products such as enzymes, sugars, biofuels, chemicals, cheap energy sources for fermentation, improved animal feeds and human nutrients (Kuhad *et al.,* 2011, Gupta *et al.,* 2011 and Karmakar and Ray, 2011).

Mechanistically, cellulase is a family of at least 3 groups of enzymes (Zhang *et al.,* 2006), endo-(1, 4)-β-D-glucanase (EC 3.2.1.4) exo-(1,4)-β-D-glucanase (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21). The exoglucanase (CBH) acts on the ends of the cellulose chain and releases β-cellobiose as the end product; endoglucanase (EG) randomly attacks the internal O-glycosidic bonds, resulting in glucan chains of different lengths; and the β-glycosidases act specifically on the β-cellobiose disaccharides and produce glucose (Deswal, *et al.,* 2011). Mixtures of cellulases (endoglucanases I and II) and hemicellulases have also been used for biomodification of fiber properties with the aim of improving drainage and beatability in the paper mills before or after beating of pulp (Dienes *et al.,* 2004). Cellulases have also been reported to enhance the bleach ability of softwood kraft pulp producing a final brightness score comparable to that of xylanase treatment (Singh *et al.,* 2007). Cellulases alone or in combination with xylanases are beneficial for deinking of different types of paper wastes. Most applications proposed so far use cellulases and hemicellulases for the release of ink from the fiber surface by partial hydrolysis of carbohydrate molecules. It has been postulated that improvements in dewatering and deinking of various pulps result in the peeling of the individual fibrils and bundles, which have high affinity for the surrounding water and ink particles (Kuhad *et al.,* 2010). Cellulases are the most successful enzymes used in textile wet processing, especially finishing of cellulose-based textiles, with the goal of improved .To reduce the enzyme cost in the production of fuel ethanol from lignocellulosic biomass, two aspects are widely addressed, optimization of the cellulase production and development of a more efficient cellulase-based catalysis system. Strategies for recycling and reusage of the enzymes may also be used to reduce enzymatic hydrolysis costs (Mosier *et al.,* 2005). The recovery of enzymes is largely influenced by adsorption of the enzymes onto the substrate, especially to lignin and enzyme inactivation. There are several reports where the nonspecific and irreversible adsorption of cellulase to lignin has been observed (Yang and Wyman, 2004). Cellulases, xylanases, and pectinases used for extraction and clarification of fruit and vegetable juices to increase the yield of juices (de Carvalho *et al.,* 2008). β-Glucanases and xylanases have been used in the feed of monogastric animals to hydrolyze nonstarch polysaccharides such as β-glucans and arabinoxylans. Cellulases, used as feed additives alone or with proteases, can significantly improve the quality of pork meat. Glucanases and xylanases reduce viscosity of high fiber rye and barley-based feeds in poultry and pig. These enzymes can also cause weight gain in chickens and piglets by improving digestion and absorption of feed materials (Karmakar and Ray, 2011 and Shrivastava *et*

al., 2011).The aim of the present study is to produce high yield of cellulases and xylanase from *Aspergillus oryzae* FK-923 cultivated under solid state fermentation.

Materials and Methods

Microorganism

A. oryzae FK-923 was obtained from Microbial Chemistry Department, National Research Center; Giza, Egypt. Fungus was maintained on potato dextrose agar (PDA) slants at 4°C and subcultured at monthly intervals.

Substrate

Sugar-cane bagasse pith (SCBP) was obtained from Nag Hamady Company for fiber board, Upper Egypt.

Pretreatment of Sugar-Cane Bagasse Pith

Sugar-cane bagasse pith was subjected to different pretreatments i.e. soaking for 24 h at room temperature, [1% (w/w) of each HCl, H_2SO_4 and NaOH] at 121^oC for one h.

Inoculum

Inoculum was made from three days old PDA cultures. The inoculum (containing 10^6-10^7 spores) was suspended in 0.05 M citrate buffer. The suspension (when necessary) was diluted with sterile 0.05 M citrate buffer (pH 5.0) to give a spore count within the predetermined range. One ml of inoculum was added to each 5 g of sugar-cane bagasse pith introduced in 250ml conical flask.

Enzymes Production

Enzymes production were carried out under solid state fermentation (SSF),the fermentation medium, containing 5 g of sugar-cane bagasse pith and 10 ml of nutrient solution in Erlenmeyer flasks (250 ml). The nutrient salt solution prepared for the production of enzymes contained, g/l: $(NH_4)_2SO_4$, 2.1; KH2PO4, 2.0; MgSO4.7H2O, 0.3; $CaCl_2$, 0.3; MnSO4.5H2O, 0.001; $ZnSO_4.7H_2O$,0.0014 and $CoCl_2.6H_2O$, 0.0026 to 1.0 ml/l Tween-80, all the ingredients were dissolved in 0.05 M citrate buffer pH 5.0. Sterilization was achieved by autoclaving at 121°C for 30 min. After cooling the medium was inoculated with 1ml of the fungal inoculum, followed by incubation statically at 30°C for 4 days.

Factors Affecting Enzymes Production

Effect of Solid Moisture Ratio

The effect of moisture level on both cellulases and xylanase production was tested by adjusting the sugar-cane bagasse pith moisture contents within the range of 50-90%. The liquid needed to obtain the desired moisture content was 0.05 M citrate buffer (pH 5.0) which was mixed with the substrate before sterilization. This procedure was repeated for each container as requested.

Effect of pH Value

Citrate and phosphate buffers (0.05M) were used to adjust the pH values ranging between pH 3.5 to 7.0 before sterilization. The data calculated after 96 h of incubation.

Effect of Temperature

The fermentation flasks moistened with 0.05 M sodium citrate buffer (pH 5.0) were incubated at different temperatures i.e. 25, 30, 35and 40°C for 96 h. The enzymes were eluted and the activity was assayed.

Effect of Incubation Period

Set of ten fermentation flasks under the optimum moisture level were incubated at 30°C. Two flasks were taken daily for extraction and determination of cellulases and xylanase activities.

Effect of Nitrogen Source

Different inorganic nitrogen sources i.e. ammonium phosphate, ammonium phosphate dibasic, ammonium nitrate, ammonium oxalate, .ammonium sulfate and Urea on the basis of nitrogen equivalent were used in order to determine the salt, which served as the best nitrogen source.

Extraction of Enzymes

The content of each flask was extracted with 50 ml of 0.05M citrate buffer, pH 5.0 and 0.1% Tween 80. The slurry was shaken (200 rpm) for 30 min at 25 °C. The contents were filtered through glass wool and the extract was centrifuged (2900 rpm) at 4°C for 30 min. The supernatant was placed in plastic vials and frozen until assayed. The parameters selected for this study were the solvent type and its volume.

Xylanase Activity

Xylanase activity was assayed by the method of Bailey et al. (1992). Xylan suspension was made by suspending 2.5 g birchwood xylan (Sigma) in 80 ml of 0.05 M citrate buffer, pH 5.0, 60 °C and heating until boiling. The suspension was stirred overnight and brought to a volume of 100 ml.

From the suspension, 0.9 ml was taken, pre-heated at 50 °C and incubated for 10 min with 0.1 ml diluted enzyme. The reaction was stopped by adding dinitrosalicylic acid (DNS) (Miller, 1959). Color was measured spectrophotometrically at 540 nm and compared to a standard curve of xylose. Xylanase activity was expressed in international units (U), where one unit is the amount of enzyme which produces one µmole of product (xylose) per minute under assay conditions per gram of substrate.

Cellulases Assays

Filter paper (FPase) and carboxymethylcellulase (CMCase) activities were assayed according to the method described by Mandels et al.(1974).FPase activity was determined in assay mixture contained 50 mg of Whatman No.1 strip (1 × 6 cm) in 1 ml of 0.05 M citrate buffer (pH 5) and 0.5 ml of diluted crude enzyme. The mixture was incubated at 50 °C for 30 min.

CMCase activity was determined in the assay mixture contained 0.5 ml of 1 % of carboxyl methyl cellulose (CMC) in 0.05M citrate buffer (pH 5.0) and 0.5 ml of diluted crude enzyme. The mixture was incubated at 50 °C for 30 min.

β-glucosidase activity was estimated using *p*-nitrophenol-β-D-glucoside as substrate (Kubicek, 1981).

Protein Determination

The amount of total protein was determined according to the Coomassie brilliant blue method described by Bradford (1976) using bovine serum albumin as a standard protein.

Effect of pH on Xylanase and Cellulases Activities

The activities of the crude enzymes were measured in pH range from 3.0 to 8.0 at 50°C for 10 min using 0.05 M citrate buffer and phosphate buffers.

Effect of Temperature

The precipitated crude enzymes by ammonium sulphate dissolved in 0.05M sodium citrate buffer (pH, 5.0) was incubated with the enzyme substrates at different temperatures ranged from 30 to 90°C.

Results and Discussion

Effect of Pretreatment of sugar-cane bagasse pith on cellulases and xylanase production by *A. oryzae* FK-923/

Data presented in Table (1) revealed that the positive effect of all pretreatments of (SCBP) on the enhancement of both cellulases and xylanase production by *A. oryzae* FK-923 under SSF. The pretreatment by soaking was less effective than other pretreatments.

Sulfuric acid pretreatment was more suitable for enhancing cellulases and xylanase production (69.2; 82.4; 86.6 and 480.8U/g) followed by NaOH and HCl. Pretreatment of lignocellulosic materials is required to overcome recalcitrance (Hu and Ragauskas, 2012).

The goal of pretreatment is to alter the physical features and the chemical composition structure of lignocellulosic materials. Various pretreatment technologies examined the changes in lignocellulosic structure primarily in cellulose and hemicellulose during the most commonly applied pretreatment, technologies including dilute acid pretreatment, and alkaline pretreatment (Taherzadeh and Karimi,2008).

Fawzi and Hamdy (2011) used HCl for pretreatment sugar-cane bagasse for producing CMCase by *Chaetomium cellulolyticum* NRRL 18756.

Factors Affecting Enzymes Production

Effect of Moisture Level

Both cellulases and xylanase production were increased with increasing the moisture level in sugar-cane bagasse pith Above 50%,and the highest enzymes production were observed when moisture content was 80 % and the enzymes production were (96.4; 98.2; 118.2and 625.6 U/g) for FPase, CMCase, β -glucosidase and xylanase, respectively (Table 2). It seems that the wet conditions cause swelling of the substrate and facilitates utilization of the medium by the organism. If the substrate is too moistened, the substrate porosity decreases which prevents oxygen penetration, while at very low moisture levels no growth of the organism was occurred (Fadel *et al.*, 2013). Cell growth and oxygen consumption rate increased in conjunction with an increase in moisture content (Kim *et al.*, 1985). However, the enhancement of enzyme production occurred up to a certain point and then levelled off. The initial moisture content has been identified to be the most significant factors affecting endoglucanase activity in this study.

This came in agreement with Singhania et al. (2007) and Mekala et al. (2008) findings in which they identified that the initial moisture content has affected cellulase activity in their study under SSF of wheat bran and sugar-cane bagasse, respectively by *T. reesei* RUT C-30. The levels of initial moisture content were believed to determine the level of water droplets present in between substrate particles in SSF. According to Wahid et al. (2011), the water droplets may become the carrier for nutrients and air transfer between particles and microorganism in SSF, in which, lower moisture level provided a lower degree of swelling. Whereas, higher water tension

may decreases the particle porosity, changes particle structure, promotes development of stickiness, decreases diffusion, lowers oxygen transfer or increases formation of aerial hyphae. In other words, if the initial moisture level is too low or too high, lower level of product may be produced. Therefore, initial moisture content is a very important factor to be considered in the optimization process for endoglucanase production. Liang et al. (2012) reported that 70% moisture for production cellulase from rice grass by a newly isolated strain *Aspergillus sp*. SEMCC-3.248 under solid-state fermentation.

On the other hand, Zaho et al. (2011) reported that 75% moisture was used for production of cellulase in solid-state fermentation by *T. reesei* SEMCC-3.217. Kim et al. (2014) found that 40-50% moisture was optimum for production of cellulases by *Penicillium sp*. in a solid-state fermentation of oil palm empty fruit bunch. Cellulases and xylanase yield by *A. niger* was optimal at 60% wet basis moisture content when the fungus was cultivated on pretreated cotton stalks (Fadel and Kahil.2003)**.**

Effect of Temperature

Under SSF, temperature is also a controlling factor for enzyme formation (Nagai and Nishio, 1980).The experimental organism *A. oryzae* FK-923exhibited the maximum enzymes production when incubated at 30 °C. The production of enzymes was (96.4, 98.2, 118.2 and 625.6 U/g) for FPase, CMCase, β- glucosidase and xylanase, respectively (Table 3). The temperature normally employed in SSF is in the range of 25-35ºC and it depends mostly on the growth kinetics of the microorganism used (Liang *et al.,* 2012). Nevertheless, it was observed that the activity was found to be low when incubation was carried out at 35ºC. This could not be correlated to the growth of the fungus since the total soluble protein showed a declining trend along with increase in temperature, perhaps the higher activity obtained upon incubation at 30 ºC compared to 35ºC due to the induction of some components of the cellulase system which could not be verified in the current study. Liang et al. (2012) reported that 32^{o}C most suitable for production of cellulase from rice grass by a newly isolated strain of *Aspergillus* sp. under solid-state fermentation.

On the other hand Zaho et al. (2011) reported that 28^{o}C was used for production of cellulase in solid-state fermentation by *Trichoderma reesei* SEMCC-3.217with water hyacinth. Singhania et al. (2007) found that optimum temperature was 30°C for cellulase production by *T.* reesei RUT C-30 from wheat bran. This claim has been supported by Mekala et al. (2008) in which the same optimum temperature was preferred by *T.* reesei RUT C-30 to produce cellulase from sugar-cane bagasse. The secretion of both cellulases and xylanase is reduced in *T. reesei* wild-type QM6a, but doubled in hyper secretary mutant RL-P37 when the temperature changed from 25 °C to 37°C (Suh *et al.*, 1988).

Effect of Incubation Period

Data illustrated in Table (4), indicated that the maximum enzymes activities were observed after 4 days of incubation at 30 ^{O}C (99.2, 112.8, 122.3 and 634.2 U/g) for FPase, CMCase, β-glucosidase and xylanase, respectively. Slightly decrease was noticed for the days 5 and 6 of incubation. Generally, the incubation period between 4-6 days was suitable for enzymes production. Liang et al. (2012) found that time course of the cellulase production using SSF by *Aspergillus* sp. was reached the maximum at 120 h, and then began to decrease.

Effect of Initial pH

Greater attention is given to optimizing the initial pH value of the moist solid medium (Fadel, 2000 and Fadel *et al.,* 2013).Data present in Table (5) show that the fungus expressed the highest degree of enzymes production at initial pH ranged from 4.5 to 5.5. So pH 5.5 was chosen for further studies at which the produced enzymes activity were 116.8, 129.2,148.8 and 686.2 U/g for FPase, CMCase, β- glucosidase and xylanase, respectively. The pH value of the culture medium affected the permeability of cells as well as stability of enzyme (Mase *et al.,* 1996). The effect of hydrogen ion on enzyme may be due to the stability of the enzyme at particular pH and denaturation of the enzyme proteins occur at differ pH, than optimum pH of such enzyme (Kalra and Sandhu, 1986).

Effect of Nitrogen Sources

Data presented in Table (6) show that urea and ammonium nitrate were more suitable for enzymes production by *A. oryzae* FK-923. Nitrogen is supplemented usually in organic or inorganic form and sometimes provided with the substrate itself. The use of different nitrogen sources have been shown to influence the production of cellulases and xylanase. Among those tested, NH_4NO_3 gave the highest production of cellulase by *T. reesei* under SSF (Singhania *et al.,* 2007). However, several studies have reported that organic nitrogen sources result in a higher production of cellulases than inorganic (Deswal *et al.,* 2011; Jeya *et al.,* 2010).

Extraction of Cellulases and Xylanase from Fermented Sugar-Cane Bagasse Pith

Extraction of cellulases and xylanase from fermented sugar-cane pith are important factor in the production of enzymes in solid state fermentation (Lonsane and Krishnaiah, 1992 and Chandra *et al.,* 2008).The suitable solvent is necessary, so different solvents were selected through this study (tap and distilled water, 0.05M acetate buffer (pH 5.0), and 0.05M citrate buffer (pH 5.0).Among all solvents used citrate buffer gave the highest yield of enzymes (115.6, 129.2, 144.8 and 684.4 U/g) for FPase, CMCase, β- glucosidase and xylanase, respectively(table 7).The use of many solvents to extract other enzymes recommended by many authors. Ramakrishna *et al.,* 1982 found that distilled or tap water alone or with glycerin or sodium chloride gave the highest yield in the amyloglucosidase extraction from the mold–wheat bran. While Annunziato et al. (1986) reported that the use of citrate phosphate buffer (pH 4.0) was the best solvent for elute α-galactosidase from the fermented mass. Acetate buffer (pH 5.9) was found to be more suitable for alpha-amylase extraction. Distilled water served as the best leachate in extracting FPase from the fermented bran (Chandra *et al.,* 2008).

Solid: Solvent Ratio

The volume of 0.05M citrate buffer (pH 5.0) varied from 25 to 100 ml(solid to solvent volume 1:5 to 1:20).The results of extraction are presented in table (8), at the solid to solvent ratio of 1:15, 75ml solvent was maximal for the extraction of enzymes from 5.0 g of fermented sugar-cane bagasse pith. The low recovery of enzymes might be due to the insufficient solvent volume to penetrate the fermented mass. While higher solvent to solid ratio also causes the enzymes to be more dilute in the final extract. Chandra et al. (2008) reported that distilled water achieved the maximum extraction of FPase from bran.

Table.1 Effect of Different Pretreatments on Cellulases and Xylanase Production by A. *oryzae* FK-923 after 4 Days at 30^{O}C under SSF

Enzyme activity (U/g)				Protein mg/g	Treatment
Xylanase	β-glucosidase	CMCase	FPase		
221.4	42.8	42.5	36.2	11.2	Untreated
480.8	86.6	82.4	69.2	21.2	H_2SO_4
437.3	80.5	76.4	65.8	20.5	NaOH
468.2	79.5	74.3	64.6	19.4	HCl
327.5	52.1	46.2	39.2	14,6	Soaking

Table.2 Effect of Moisture Level on Cellulases and Xylanase Production by A. *oryzae* FK-923after 4Days at 30 oC under SSF

Enzyme activity (U/g)				Protein mg/g	Moisture %
Xylanase	β-glucosidase	CMCase	FPase		
480.8	86.6	82.4	69.2	21.2	50
498.2	90.8	88.6	74.4	24.6	60
595.4	96.6	92.2	78.2	28.4	70
625.6	118.2	98.2	96.4	32.8	80
506.6	86.5	80.2	78.6	26.1	90

Table.3 Effect of Temperature on Cellulases and Xylanase Production by A. *oryzae* FK- 923 after 4Days under SSF

Enzyme activity (U/g)				Protein mg/g	Temperature ^{O}C
Xylanase	β-glucosidase	CMCase	FPase		
592.8	102.8	92.4	76.5	28.8	25
625.6	118.2	98.2	96.4	32.8	30
576.3	106.2	96.8	84.4	32.1	35
126.6	38.3	36.2	32.1	16.4	40

Table.4 Effect of Fermentation Period on Cellulases and Xylanase Production by A. FK-923 at 30°C under SSF

Enzyme activity (U/g)				Protein mg/g	Incubation period/day
Xylanase	β-glucosidase	CMCase	FPase		
23.1	0.8	15.2	14.4	6.2	1
126.6	22.2	41.2	38.5	15.8	2
416.5	78.6	71.2	68.6	26.8	3
634.2	122.3	112.8	99.2	33.2	4
620.2	118.3	104.8	96.2	33.6	5
604.2	106.2	98.6	92.8	28.6	6
506.8	84.3	77.5	76.2	22.4	7
426.8	48.6	44.2	46.9	16.4	8

Table.5 Effect of Initial pH on Cellulases and Xylanase Production by A. *oryzae* FK-923 at 30°C under SSF

Enzyme activity (U/g)				Protein mg/g	Initial pH
Xylanase	β-glucosidase	CMCase	FPase		
468.8	94.2	88.2	86.8	28.2	3.5
604.2	118.8	115.5	104.2	30.4	4.0
680.8	132.6	122.4	112.6	32.2	4.5
684.4	144.8	128.4	115.6	32.2	5.0
686.2	148.8	129.2	116.8	33.0	5.5
598.4	118.6	98.2	92.4	26.8	6.0
518.2	94.6	86.5	84.5	24.2	6.5
502.2	68.8	65.4	64.8	22.2	7.0

Table.6 Effect of Nitrogen Source on Cellulases and Xylanase Production by A. *oryzae* FK-923 at 30°C under SSF

Enzyme activity (U/g)				Protein mg/g	Nitrogen source
Xylanase	β-glucosidase	CMCase	FPase		
588.2	104.5	92.6	86.4	28.2	Ammonium phosphate
664.4	128.4	116.8	110.2	34.5	Ammonium phosphate dibasic
672.5	142.6	132.4	115.2	33.8	Ammonium nitrate
512.2	98.6	92.2	84.6	27.3	Ammonium oxalate
634.2	122.3	112.8	99.2	33.2	Ammonium sulfate
680.4	144.8	136.2	115.6	33.2	Urea

Table.7 Effect of Different Solvent on Cellulases and Xylanase Recovery from Fermented Substrate

Enzyme activity (U/g)				Protein mg/g	Eluting solvent
Xylanase	β-glucosidase	CMCase	FPase		
646.2	128.2	114.8	106.5	28.2	Tap water
654.2	132.4	115.4	110.2	29.2	Distilled water
652.4	136.5	118.4	108.6	30.6	Acetate buffer
684.4	144.8	129.2	115.6	32.2	Citrate buffer

Table.8 Effect of Solid to Solvent Volume Ratio on the Extraction of Cellulases and Xylanase from Fermented Sugar-Cane Bagasse Pith

Enzyme activity (U/g)				Protein mg/g	Solid : solvent volume
Xylanase	β-glucosidase	CMCase	FPase		
564.8	98.6	94.2	88.5	27.4	1:5
644.4	129.6	114.2	106.4	31.2	1:7:5
680.4	144.8	126.2	115.6	33.2	1:10
682.6	145.2	128,6	118.8	33.4	1:12.5
688.2	145.8	129.2	120.2	33.6	1:15
635.6	125.9	110.2	102.4	30.5	1:17.5
557.9	95.5	92.7	82.1	26.3	1:20

Fig.1 Effect of Incubation pH on the Cellulases and Xylanase Produced by A. *oryzae* FK-923 Cultivated on SCBP under SSF

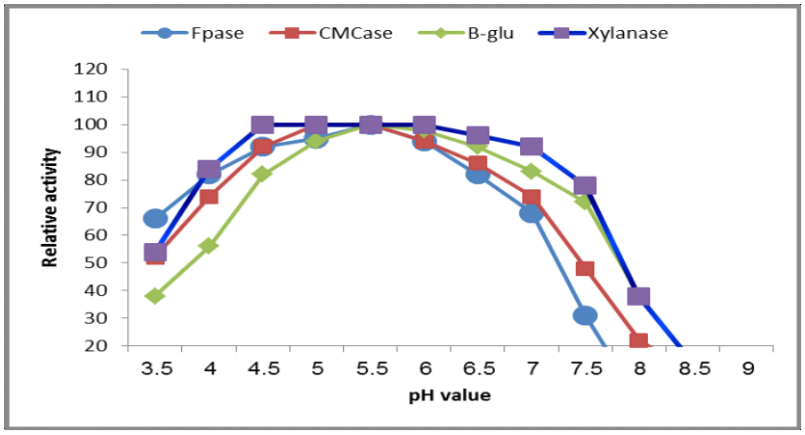
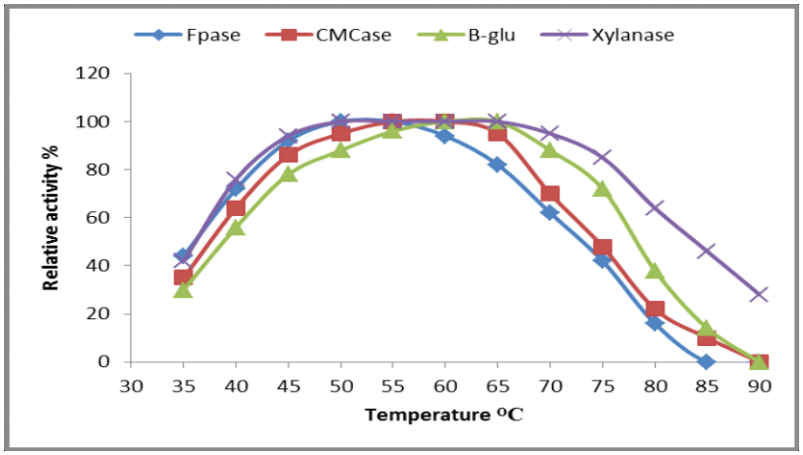


Fig.2 Effect of Incubation Temperature on the Cellulases and Xylanase Produced by A. *oryzae* FK-923 Cultivated on SCBP under SSF



Characterization of Produced Enzymes

Effect of Incubation pH on the Enzyme Activities

Data illustrated in fig. (1), proved that all tested enzymes exhibited their optimum activity at pH 5.5. The activity for all enzymes tested were stable at pH range from 5-6.The obtained results agree with that obtained for cellulase production in SSF system by different fungi i.e. *Penicillium funiculosum* (Castro, *et al.,* 2010), *Fomitopsis* sp. RCK2010 (Deswal, *et al.,* 2011) and *Trichoderma reesei* (Devendera *et al.,* 2012).

Effect of Temperature on the Enzyme Activities

The results in fig. (2) illustrated that the optimum activities were recorded at 55-60°C. Garg and Neelakantan (1982) reported that the optimum temperature for both CMCase and FPase activities were 60^{O}C and 55^{O}C, respectively when *Aspergillus terreus* GN1 was grown on alkali-treated sugar-cane bagasse. While Fadel (2000) found that the maximum activity for FPase, CMCase and β-glucosidase were 60, 55 and 65^{O}C, respectively when *Aspergillus niger* F-119 was grown under solid state fermentation.

References

Annunziato, M. E., Mahoney, R. R. and Mudgett, R. E. (1986).Production of α-galactosidase from *Aspergillus oryzae* grown in solid state culture. j. Food Sci., 51(5):1370-1371.

Bailey, M.J., Biely, P. and Poutanen, K. (1992). Interlaboratory testing of methods for assay of xylanase activity. J. Biotechnol., 23: 257-270.

Bradford, M. M. (1976).A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal. Biochem., 72: 248-254.

Castro, A. M., Carvalho, M. L. A., Leite, S. G. F. and Pereira, N. JR. (2010). Cellulases from *Penicillium funiculosum:* production, properties and application to cellulose hydrolysis. J. Indian Microbiol. Biotechnol., 37:151-158.

Chandra, M. S., Reddy, B. R. and Choi, Y. L. (2008). Optimization of extraction of FPase from the fermented bran of *Aspergillus niger* in solid state fermentation. J. Appl. Biol. Chem., 51(4): 155-159.

de Carvalho, L. M. J., de Castro, I. M. and da Silva, C. A. B.(2008).A study of retention of sugars in the process of clarification of pineapple juice (*Ananascomosus*, L. Merril) by micro- and ultra-filtration. J. Food Eng., 87 (4): 447–454.

Deswal, D., Khasa, Y. P. and Kuhad, R. C. (2011).Optimization of cellulase production by a brown rot fungus *Fomitopsis* sp. RCK2010 under Solid State Fermentation. Bioresour. Technol., 102(10): 6065–6072.

Devendra, P.M., Dhananjay, S., Durgesh, P. and Jitendra, P.M. (2012). Optimization of solid state fermentation conditions for the production of cellulase by *Trichoderma reesei*. J. Environ. Biol., 33:5-8.

Dienes, D., Egyházi, A. and Réczey, K. (2004).Treatment of recycled fiber with *Trichoderma* cellulases. Ind. Crops Prod., 20(1): 11–21.

Fadel, M. (2000). Production physiology of Cellulase and *β*- glucosidase enzymes of *Aspergillus niger* grown under solid state fermentation conditions. J. Biol. Sci., 1(5): 401 - 411.

Fadel, M., Kahil, T. and Abdel-Aziz, S. M. (2013). Farm Scaling up Biological Treatment by Solid State Fermentation to Invest Rice Straw as a Livestock Feed in Egypt. J. Basic Appl. Sci. Res., 3(12):258-263.

Fadel, M. and Kahil, T. (2003).Production and application of cellulose and hemicellulose degrading enzymes from cotton stalks by fungi in solid state fermentation. Egypt. J. Biotecnol., 16(2):79-83.

Fawzi, E. and Hamdy, H. (2011). Improvement of carboxymethyl cellulase production from *Chaetomium cellulolyticum* NRRL 18756 by mutation and optimization of solid state fermentation. Afr.J.Microbiol. Res., 5(26): 4687-4696.

Garg, S.K. and Neelakantan, S. (1982). Studies on the properties of cellulase enzyme from *Aspergillus terreus* GN1. Biotechnol. Bioeng., 24(3): 737-742.

Gupta, R., Mehta, G., Khasa, Y. P. and Kuhad, R. C. (2011). Fungal delignification of lignocellulosic biomass improves the saccharification of cellulosics .Biodegradation, 22 (4): 797–804.

Hu, F. and Ragauskas, A. (2012). Pretreatment and Lignocellulosic Chemistry. Bioenergy. Res., 5(4):1043-1066.

Jeya, M., Nguyen, N. P. T., Moon, H. J., Kim, S. H. and Lee, J. K. (2010). Conversion of woody biomass into fermentable sugars by cellulase from *Agaricus arvensis*. Bioresour. Technol., 101(22):8742–8749.

Kalra, M.K. and Sandhu D.K. (1986). Optimal production of cellulolytic enzymes and their location in *Trichoderma pseudokonigii*. Acta Biotechnol., 6 (2): 161-166.

Karmakar, M. and Ray, R. R. (2011).Current trends in research and application of microbial cellulases. Res. J. Microbiol., 6(1): 41–53.

Kim, Do-Myoung, Cho, E. J., Kim, J. W., Lee Y.W. and Chung, H.J. (2014). Production of cellulases by *Penicillium sp*. in a solid-state fermentation of oil palm empty fruit bunch Afr. J. Biotechnol., 13(1):145-155.

Kim, J. H., Hosobuchi, M., Kishimoto, M., Seki, T., Yoshida, T., Taguchi, T. and Ryu, D.D.Y. (1985).Cellulase production by a solid state culture system. Biotechnol. Bioeng., 27(10): 1445-1450.

Kubicek, C. P.(1981) . Release of carboxymethyl-cellulase and β-glucosidase from cell walls of *Tricoderma reesei*. Eur. J. Appl. Microbiol. Biotechnol., 13(4):226-231.

Kuhad, R. C., Gupta, R. and Singh A.(2011).Microbial Cellulases and Their Industrial Applications. Enzyme Res., 2011 (Article ID 280696), 10 pages.

Kuhad, R.C., Mehta, G., Gupta, R. and Sharma, K. K. (2010). Fed batch enzymatic saccharification of newspaper cellulosics improves the sugar content in the hydrolysates and eventually the ethanol fermentation by *Saccharomyces cerevisiae*. Biomass Bioenergy, 34 (8): 1189–1194. .

Liang, X., Huang, Y., Hua, D., Zhang, J., Xu, H., Li, Y. and Zhang, X. (2012).Cellulase production by *Aspergillus* sp. on rice grass (*Spartina* spp.) under solid-state fermentation. Afr. J. Microbiol. Res., 6(39): 6785-6792.

Lonsane, B. K. and Krishnaiah, M. M. (1992).Product leaching and downstream processing. In Solid substrate Cultivation, Doelle HW, Mitchell DA, and Rolz CE (eds.).pp147-153.Elsevier Science Publishers, London, UK.

Mandels M., Hontz L. and Nystrom J. (1974).Enzymatic hydrolysis of waste cellulose. Biotechnol. Bioeng., 16(11): 1471-1493.

Mase T.Y., Matsumiya, S., Mori S. and Matsura A. (1996). Purification and characterization of a novel glucoamylase *Acremimium* sp. YT.78. J. Ferment. Bioeng., 81(4): 347-350.

Mekala, N.K., Singhania, R.R., Sukumaran, R.K. and Pandey, A. (2008). Cellulase production under solid state fermentation by T*richoderma reesei* RUTC30: Statistical optimization of process parameters. Appl. Biochem. Biotechnol., 151(2-3): 122-131.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M. and Ladisch, M.(2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol., 96, (6): 673–686.

Nagai, S. and Nishio, N. (1980). Proceedings of the Second Symposium on Bioconversion and Biochemical Engineering, T. K. Ghose, Ed. (IIT, New Delhi), Vol. 1, p. 399.

Ramakrishna, S. V., Suseela, T., Ghidyal, N. P., Jaleel, S.A., Perma, P., Lonsane, B. K. and Ahmed, S. Y. (1982). Recovery of amyloglucosidase from moldy bran. Indian J. Technol.,20: 476-480.

Sang-Mok, L. and Koo, Y. M. (2001).Pilot-scale production of cellulase using *Trichoderma reesei* Rut C-30 in fed-batch mode. J. Microbiol. Biotechnol., 11(2): 229–233.

Shrivastava, B., Thakur, S., Khasa, Y. P., Gupte, A., Puniya, A. K. and Kuhad, R. C. (2011). White-rot fungal conversion of wheat straw to energy rich cattle feed. Biodegradation, 22 (4): 823–831.

Singh, A., Kuhad, R. C. and Ward, O. P. (2007). Industrial application of microbial cellulases, in Lignocellulose Biotechnology: Future Prospects, R. C. Kuhad and A. Singh, Eds., pp. 345–358, I.K. International Publishing House, New Delhi, India.

Singhania, R.R., Sukumaran, R.K. and Pandey, A. (2007).Improved cellulase production by *Trichoderma reesei* RUT C30 under SSF through process optimization. Appl. Biochem. Biotechnol., 142(1): 60-70.

Suh, D.H., Becker, T.C., Sands, J.A. and Montenecourt, B.S. (1988). Effects of temperature on xylanase secretion by *Trichoderma reesei.* Biotechnol. Bioeng., 32(6): 821-825.

Taherzadeh, M.J. and Karimi, K. (2008).Pretreatment of Ligno-cellulosic Wastes to Improve Ethanol and Biogas Production: A Review. Int. J. Mol. Sci., 9(9):1621-1651.

Wahid, M. Z. A.,Salleh, M., Yusof, F., Abdulkarim, M.I. and Alam. M.. Z. (2011). Factors affecting endoglucanase production by *Trichoderma reesei* RUT C-30 from solid state fermentation of oil palm empty fruit bunches using Plackett-Burman design. Afr. J. Biotechnol., 10(46): 9402-9409.

Yang, B. and Wyman, C. E. (2004).Effect of xylan and lignin removal by batch and flow through pretreatment on the enzymatic digestibility of corn stover cellulose. Biotechnol. Bioeng., 86 (1): 88–95.

Zhang, Y.H.P., Himmel, M. E. and Mielenz, J. R. (2006).Outlook for cellulase improvement: screening and selection strategies," Biotechnol. Adv., 24, (5):452–481.

Zhao, S., Liang, X., Hua, D., Ma, T. and Zhang, H. (2011). High yield cellulase production in solid-state fermentation by *Trichoderma reesei* SEMCC-3. 217 using water hyacinth *(Eichhornia crassipes*). Afr. J. Biotechnol., 10(50): 10178–10187.

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