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Evaluation of Fungal Xylanase and Lignin Peroxidase in Bio-bleaching of Sugar Cane Bagasse Biopulping.

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FUNGAL xylanase and lignin peroxidase enzymes were used as pretreatment for biobleaching of bagasse biopulping treated with mixed culture of *Ophiostoma piliferum* and *Ceriporiopsis subvermispora* SS- 33 at 27ºC for one week in MV medium as static culture before the pulping with propylene glycol (PG). Some agricultural wastes such as corn cobs, wheat bran and bagasse powder were used as a sole carbon source for xylanase production. The maximum production of fungal xylanase was attained after 7 days fermentation period on corn cobs medium at 30°C on rotary shake flasks at 150 rpm. The enzyme production by *Trichoderma reesie* NRRL 6156 increased 1.17 fold as compared with that obtained by *Trichoderma viride* NRRL 13034.Using 10.30 IU xylanase/g bagasse biopulp, produced by *Trichoderma reesie* NRRL 6156, for 4 h at 50ºC was the best xylanase pretreatment which reduced klason lignin% and increased the brightness % of bagasse biopulp. The solid-state HC-LN medium supplemented with tween 60 and veratryl alcohol in addition to 10 grams of bagasse pulp was the best one for lignin peroxidase production by *Phanerochaete chrvsosporium* NRRL 6361, the enzyme activity of this treatment (77.75 IU/L) was higher than that obtained using semi-solid (47.75 IU/L) and liquid (36.50 IU/L) state, after 6 days incubation period. The optimum lignin peroxidase dose, for the best biobleaching of unbleached bagasse biopulp at 37°C for 8 h was 1.54 IU/g. Using these enzyme pretreatments led to increase the brightness %, breaking length and tear factor 6.7, 18.89 and 12.7 % by xylanase bleached bagasse (XBB) and 8.94 %, 34.92 and 30.82 %, by lignin peroxidase bleached bagasse (LBB), respectively. The enzyme treatment of LBB and XBB led to decrease of chlorine consumption 40% and 26.67 % as compared to control. Scanning electron microscope (SEM) of bleached bagasse pulp clearly showed fiber that exposed to enzymes treatment had a more open surface and it becomes more accessible to subsequent bleaching agents. The biologicaly pretreatment of bagasse pulp with xylanase or

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lignin peroxidase enzymes led to increase in the crystallinty by 11.29 and 8.3 %, respectively.

Keywords: Xylanase, Lignin peroxidase, Biobleaching of bagasse, Biopulping, *Ophiostoma piliferum*, *Ceriporiopsis subvermispora*, *Phanerochaete chrvsosporium*, *Trichoderma reesie* , *Trichoderma viride* , Klason lignin.

To date, biological bleaching of pulp has been approached mainly by the use of lignolytic and hemicellulolytic enzymes. These enzymes also offer a simple approach that allows for a higher brightness ceiling to be reached (Gupta *et al.*, 2000). Application of the xylanase in the pulp bleaching process has been shown to be effective in decreasing the amount of chlorinating agents in the process and improving the brightness of the pulp (Dhillon *et al.*, 2000). Bissoon *et al.* (2002) added that the biobleaching effect induced due to xylanase pretreatment was dependent on both the enzyme and chlorine dioxide charges used.The simultaneous addition of xylanase and mannanase was more effective in pulp treatment than their sequential addition (Kansoh & Nagieb, 2004). Also, Rifaat *et al.* (2005) found that xylanase enhanced the liberation of reducing sugars, which improved pulp bleachability. Christopher *et al.* (2005) reported that the optimum pH and moisture content for xylanase production by solid state fermentation (SSF) was 7.0 and 83%, respectively. Time course experiments indicated maximum xylanase production after 4 days. The most efficient producer of xylanase was *T. lanuginosus* ATCC 46882 with 5098 U xylanase/g bagasse pulp. Also, Szendefy *et al*. (2006) found that the solid-state xylan-degrading enzymes produced under optimized conditions were 20–36% more efficient in improving the brightness of paper pulp than the commercial enzyme. Moreover, enzymatic prebleaching of kraft pulp showed 20% reduction in kappa number of the pulp without much change in viscosity and reduced the amount of chlorine by 29% without any decrease in brightness. (Rakhee & Narayan, 2007). Also, they found that xylanase pretreatment has been reported to lower bleaching chemical consumption and to result in a higher final brightness. Enzymatic bleaching is reported to result from the cleavage of bonds between lignin and carbohydrate and the opening up of the pulp structure. Also, several important enzymes, including laccases, manganese peroxidase and lignin peroxidase derived from white rot fungi were found to be responsible for the biodegradation of the lignin and the biological bleaching effect (Caixia & Yebo, 2010). The aim of this work is to evaluate the fungal xylanase and lignin peroxidase in bio-bleaching of sugar cane bagasse biopulping as well as the brightness, breaking length and tear factor.

Materials and Methods

Lignocellulosic materials

The Egyptian depithed bagasse of sugarcane (*Sacaharum officinurum*) was obtained from El- Nasr Company for Sugar and Pulp Industry at Edfu in Egypt.

Microorganisms used

White-rot fungi namely, *Ceriporiopsis subvermispora* ss - 33 and *Phanerochaete chrysosporium* NRRL 6361 and ascomycetes fungus *Ophiostoma piliferum*, in addition to *Trichoderma reesie* NRRL 6156, *Trichoderma viride*, NRRL 13034 and *Bacillus licheniformis* NRRL B- 14208 were obtained from NRRL the culture collection of Northern Regional Research Laboratory, Department of Agriculture, Peoria.

Media used

1-Peptone yeast extract agar medium (José *et al.*, 2000) was used for fungal propagation and preservation. 2-Nutrient agar medium (Difco Manual, 1977) was used for preservation of bacterial cultures.3- MV medium (Hatakka & Pirhonen, 1985) was added to the lignocellulosic materials to enhance their biodegradation by fungi. 4-PMY medium (Nobuyuki *et al.*, 1995) was used for preparation of standard inoculum of fungi.5- HC - LN medium (Nobuyuki *et al.*, 1995) was used as a basal medium for crude ligninase enzyme production by fungus. 6-Minimal medium (Medeiros *et al.*, 2002) was used as a basal medium for the production of crude xylanase enzyme by fungi and bacteria.

Standard inoculums

In solid culture, the standard inoculum of fungal strains was prepared by transferring of five disks from fungal culture plate (med. 1) to Erylenmyer flask (250 ml in volume) containing 100 ml of propagation medium. Peptone yeast extract medium (med. 1) and med. 4 were used to inoculate *Ceriporiopsis subvermispora* CZ-3 and *Ophiostoma piliferum* or *Phanerochaete chrvsosporium* NRRL 6361, respectivily. The inoculated flasks were incubated at 27 °C for former strains and for 39°C for the latter strain on rotary shaker (150 rpm) for 4- 7 days. In liquid culture, 10 ml of med. 6 was added to each slant of *Trichoderma reesie* NRRL 6156, *Trichoderma viride*, NRRL 13034 or *Bacillus licheniformis* NRRL B- 14208 to give the growth suspension, to be used as inoculate for the production of enzymes.

Bio- bleaching of bagasse biopulp

Pretreatment with xylanase

Factors affecting xylanase production: Some agricultural wastes such as corn cobs 5 %, wheat bran 5%, bagasse powder 5%, corn cobs 3% plus wheat bran 3%, corn cobs 3 % plus wheat bran 3% plus yeast extract 1 % or xylose 1% were used as a sole carbon source in med.6 for xylanase production by fungal and bacterial test strains during 1-10 days incubation period at 30^{o}C using shake flask.

Xylanase pretreatment of bagasse biopulp: In these experiments bagasse biopulp was treated with 20.61 IU/g xylanase produced by the highest selective fungi for different reaction periods ranging from 2 to 24 hr in order to identify the best period. Whereas the optimum xylanase dose for improving the bleaching of bagasse biopulp was identify by pretreatment of bagasse biopulp with different xylanase doses ranging from 4.12 to 41.22 IU/g for the suitable period

of treatment. Then klason lignin %, brightness % and degree of polymerization (DP) were determined.

Pretreatment with lignin peroxidase

Factors affecting lignin peroxidase production : Different fermentation – state systems were carried out by addition of tween 20 (0.05 W/V), or tween 60, (1mM) veratryl alcohol, tween 20 plus veratryl alcohol or tween 60 plus veratryl alcohol separately to med. 5. Bagasse pulp was added to solid and semi – solid media in concentration of 10 and 0.2g / 40 ml med. 5, respectively. The incubation of liquid and semi – solid media were carried out under submerged condition (150 rpm) at 39 °C.

Different fermentation period ranged from 1 to 10 days were tested to detect the proper time for highest lignin peroxidase production by *Phanerochaete chrvsosporium* NRRL 6361 in solid state fermentation system at 39°C using med. 5. klason lignin %, brightness % and degree of polymerization (DP) were determined.

Lignin peroxidase pretreatment of bagasse biopulp

The optimum period of biobleaching pretreatment of bagasse biopulp was identified by studying different reaction periods ranging from 2 to 24 h of 0.77IU /g bagasse biopulp at 37 °C.

The optimum lignin peroxidase dose was identified by using different crude lignin peroxidase doses ranging from 0.15 to 1.54 IU /g were used as pretreatment of biobleaching of bagasse at 37 °C for suitable reaction period in order to detect the optimum dose for this treatment. Klason lignin %, brightness % and degree of polymerization (DP) were determined at the end of reaction period.

Chemical bleaching of bagasse pulp with elemental chlorine- free (ECF) method (Bissoon *et al.*, 2002) was applied for enzyme treated and untreated pulp samples in multistage elemental chlorine- free (ECF) bleaching process using a chlorine dioxide (D_1), alkali extraction (E), chlorine dioxide (D_2). The bleaching procedure was carried out according to TAPPI test methods (1991).

Paper sheets making

Chemical analysis

Xylanase activity was determined according to the method of Michael *et al.* (1992). Ligninase activity was determined according to the method of Susan & Reetta (1993).

X-Ray diffraction measurements

A Philips X-ray diffractometer was used to record X-ray diffraction patterns of untreated and treated bleached bagasse pulp. For each measurement a disk was prepared by compressing a 0.3-gram sample under pressure of 50 Mpa. The equational diffraction patterns were measured from $2\theta = 5$ to 35 using Cu-Ka radiation at 40 KV and 25 mA.

Results and Discussion

Pretreatment with xylanase

Factors affecting xylanase production

In order to reduce the production costs of xylanase, some agricultural wastes were used as growth substrate inducer for xylanase production and optimum fermentation time was identified. Data illustrated by Fig. 1 revealed that the highest xylanase activities of all tested strains were detected on med. 6 containing 1% xylan as a sole carbon source. This result is due to the use of xylan as inducer for the production of the inducible xylanase (Seyis & Aksoz, 2005). The tested fungi gave 4.5 and 3.4 fold more xylanase activity than that obtained by the bacterial strain on this medium. At agricultural waste treatments, the highest enzyme activities by all the treated strains was obtained on med. 6 supplemented with 5% corn cobs. This may be due to their content of high quantities of pentosans released from corn cobs. Moreover *Trichoderma reesie* NRRL 6156 gave the higher xylanases activities than other strains on all different media.

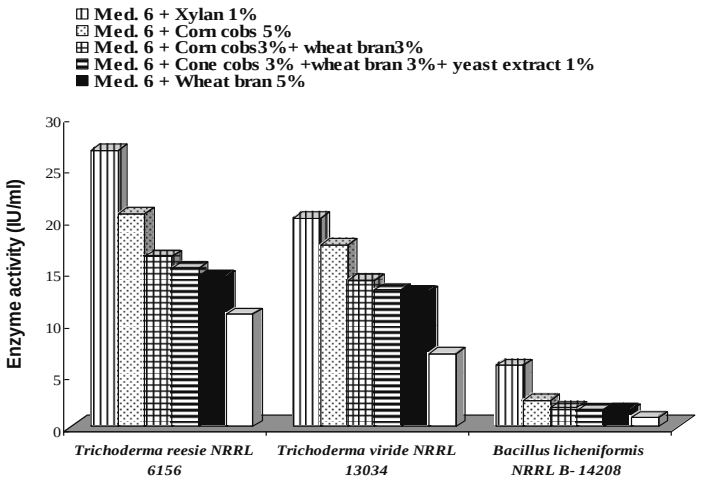

Fig. 1. Induction of xylanase by *Trichoderma reesie* NRRL 6156, *Trichoderma viride* NRRL 13034, and *Bacillus licheniformis* NRRL B- 14208 grown on different agricultural waste treatments at 30ºC for 7 days on shake flasks at 150 rpm.

The xylanase activities produced by both fungal strains were increased during fermentation period to reach the maximum values being 20.61 and 17.58 IU/ml by *Trichoderma reesie* NRRL 6156 and *Trichoderma viride* NRRL 13034, respectively after 7 days fermentation periods at 30ºC on rotary shake flasks at 150 rpm as illustrated by Fig. 2. Also, it could be noticed that the xylanase activities by *first strain* increased 1.17 fold as compared with that obtained by the second strain at this minimal medium plus corn cobs 5%. So, this strain was used for xylanase production in order to apply for bio-bleaching of bagasse pulp.

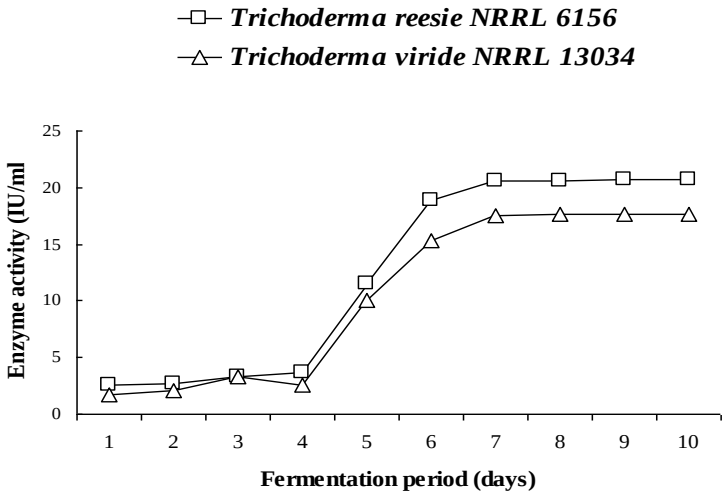

Fig. 2. Effect of fermentation period on xylanase production by *Trichoderma reesie* NRRL 6156 and *Trichoderma viride* NRRL 13034 on minimal medium (med. 6) incubated at 30 ºC on shake flasks at 150 rpm.

Xylanase pretreatment of bagasse biopulp

Results illustrated by Fig. 3 indicated that the pretreatment of bagasse bioplup with 20.61 IU/g xylanase dose for 4 h at 50ºC was the preferable time to reduce the lignin content % (Klason lignin%) and enhancement the bleachability of bagasse biopulp (brightness%) about 38.98 % and 6.19 %, respectively as compared to control (without enzyme pretreatment).

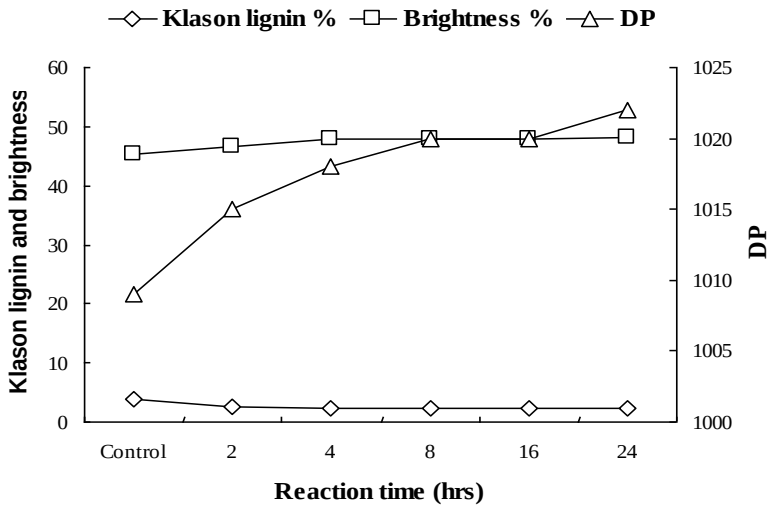

Fig. 3. Xylanase 20.61 IU/g pretreatment of bagasse biopulp at 50ºC as influenced with reaction time.

Data illustrated by Fig. 4 show that all xylanase dose pretreatment in the range of 2.14 to 41.22 unit ml^{-1} improved the values of klason lignin % and brightness % than control. The optimum figures were obtained when bagasse pulp was pretreated by 10.30 IU/g being 2.45, 48.0 % and 1015 for klason lignin %, brightness % and DP, respectively. Slight increase in DP of bagasse pulp was observed by increasing enzyme dose to 20.61 IU/g. This may be due to the removal of a portion of the low molecular weight hemicellulose fraction from pulp (Christopher *et al.*, 2005).

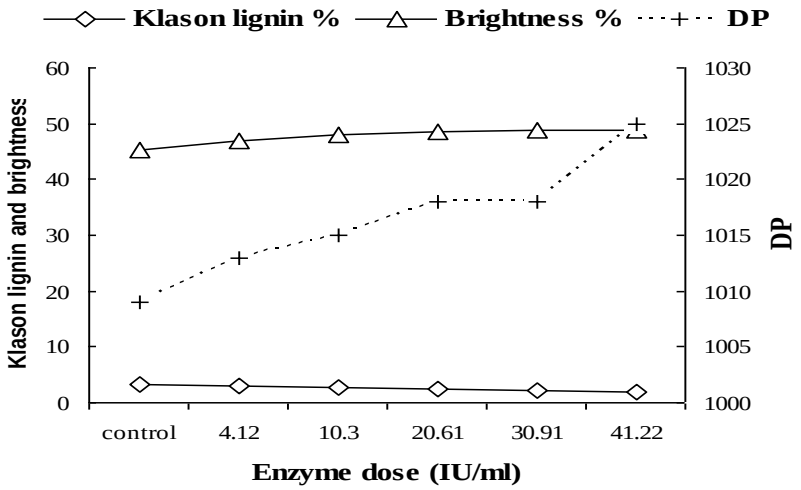

Fig. 4. Xylanase pretreatment of bagasse biopulp as influenced by xylanase doses.

Pretreatment with lignin peroxidase

Factors affecting lignin peroxidase production using different fermentation systems

Results illusterated by Fig. 5 revealed that all treatments of med. 5 supplemented with tween or veratryl alcohol recorded lignin peroxidase activity produced from the white – rot fungus *Phanerochaete chrysosporium* NRRL 6361 was higher than cultivated on med. 5 only. The solid – state fermentation was the best system for lignin peroxidase production which recorded 77.75 IU/L higher than semi – solid (47.75 IU/L) and liquid (36.5 IU/L) state fermentation system using med. 5 supplemented with tween 60 and veratryl alcohol in addition to 10g bagasse pulp in solid culture and 0.2g bagasse pulp in semi- solid culture. Whereas, adding of veratryl alcohol plus tween (20 or 60) was more obviously in semi – solid than liquid and solid fermentation. Data illustrated by Fig. 6 show that the pretreatment of 10 grams bagasse biopulp with fungal standard inoculum led to slight change in the percentage of both lignin content (expressed as klason lignin) and brightness during the first 3 days of fermentation. After this period, gradual decrease in lignin content with a gradual increase in brightness were noticed to reach the lowest and highest values being 2.05 % and 49.15 % after 6 days. No improvement was noticed by increasing fermentation period than 6 days.

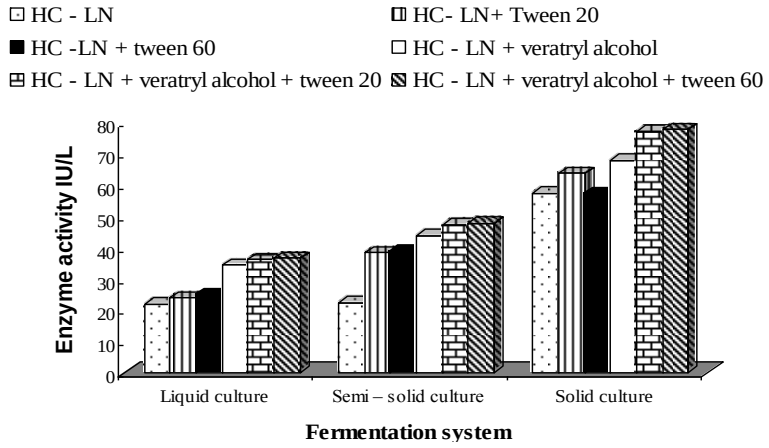

Fig. 5. Production of lignin peroxidase (IU/L) by *Phanerochaete chrvsosporium* NRRL 6361 on three states of media incubated at 39°C for 10 days.

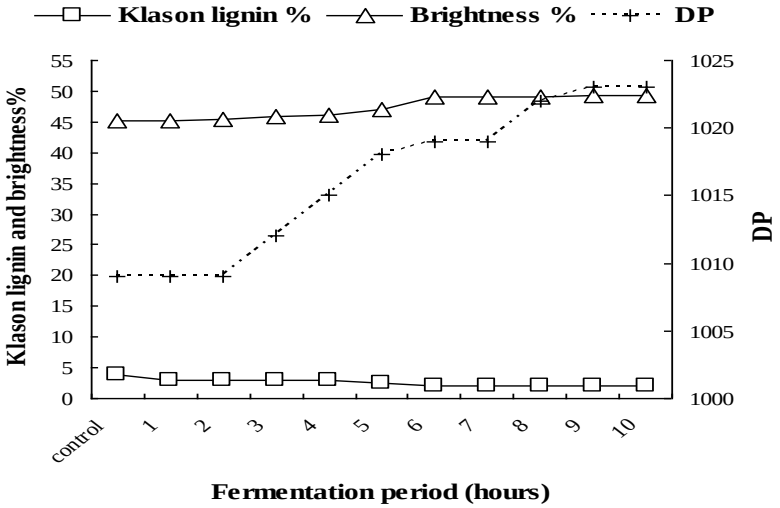

Fig. 6. Effect of fermentation period on bagasse pulp properties inoculated by *Phanerochaete chrvsosporium* NRRL 6361 in solid - state fermentation system at 39°C for different fermentation periods.

Lignin peroxidase pretreatment of bagasse biopulp

Increasing in brightness % and decreasing in klason lignin % was observed after 8 h pretreatment with 0.77 IU/g of crude lignin peroxidase produced by *Phanerochaete chrvsosporium* NRRL 6361 represent 8.62 and 48.6 %, respectively as compared to the control. With pretreatment of lignin peroxidase crude enzyme more than 8 h led to slight change in lignin % and brightness was noticed. Also, slight increase in DP was observed during incubation time till reached the maximum value after 24 h being 1025 as illustrated by Fig. 7.

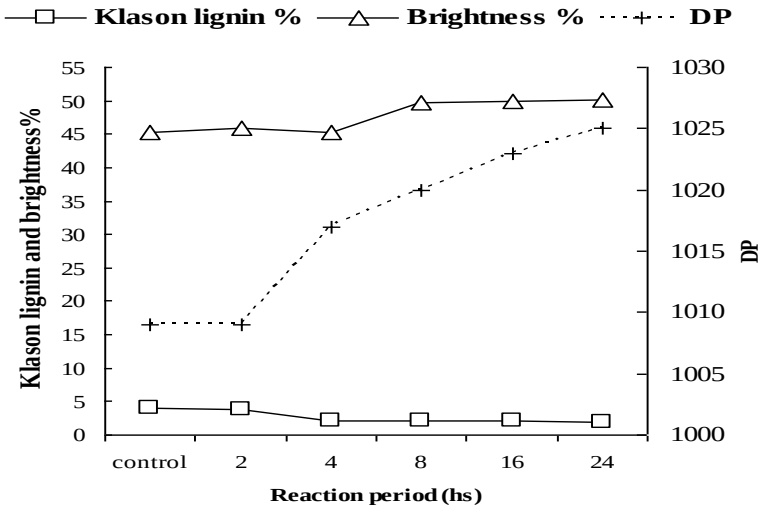

Fig. 7. Crude lignin peroxidase (0.77 IU/g) pretreatment of bagasse biopulp as influenced with reaction period.

Data illustrated by Fig. 8 show that increasing enzymatic doses led to decrease in lignin content % and increase in brightness % till reached the optimum values being 1.51 % and 51.0 % with 1.54 IU/g of enzymatic dose, respectively. So, it could be stated that this pretreatment decreased the Klason lignin from 3.95 to 1.51% and improved the bleachability of bagasse biopulp from 45.25% to 51% (brightness) as well as the degree of polymerization from 1009 to 1027.

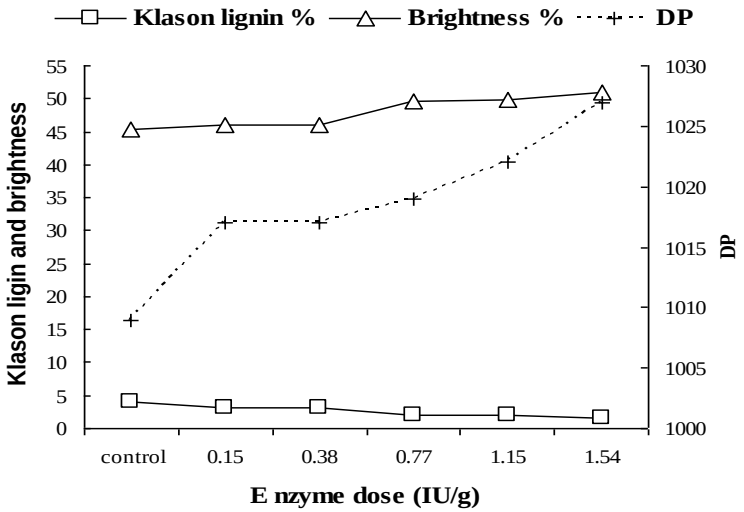

Fig. 8. Crude lignin peroxidase pretreatment of bagasse biopulp as influenced with enzyme doses.

Bleaching of enzyme pretreatment bagasse biopulp

Enzyme pretreatment bagasse biopulp was bleached by elemental chlorine-free (ECF) method and comparing their results with those obtained by bleached chemical pulp (organosolv pulp) as show in Table 1. Data clearly show that the α- cellulose % of enzyme bleached bagasse biopulp did not differ from chemical bleached bagasse pulp, whereas both pentosan % and klason lignin % were decreased to record 3.95 % and 0.82 % for xylanase bleached bagasse (XBB) and 4.62 % and 0.51 % for lignin peroxidase bleached bagasse (LBB). Also, it could be noticed that the percentage of pentosan in the former pulp was lower than the latter pulp whereas the vice versa is true for klason lignin %. The results indicate that the lowest value of klason lignin % was in LBB might be due to the activity of lignin peroxidase enzyme in this treatment. Degree of polymerization (DP) of enzyme bleached bagasse biopulp increased from 1015 in chemically bleached bagasse pulp to reach 1030 in XBB and 1020 in LBB.

TABLE 1. Chemical and physical properties of final paper sheets made from bleached bagasse pulp pretreated chemically or biologically (xylanase or lignin peroxidase) .

Properties of BPS[1]	Chemical treatment	Biological pretreatment	
	PG[2] Followed by ECF[3]	XBB[4] Followed by ECF[3]	LBB[5] Followed by ECF[3]
1- Chemical properties			
α – cellulose %	85.71	86.25	86.04
Pentosan %	6.13	3.95	4.62
Klason lignin %	1.35	0.82	0.51
DP	1015	1030	1020
2-Physical properties			
Breaking length (km)	6.3	7.49	8.5
Tear factor	83.4	94.0	109.1
Brightness %	80.5	85.9	87.7
Opacity %	92.4	93.78	93.66

[1] = Bleached paper sheets, [2] = Propylene glycol pulping
[3] = Elemental chlorine- free, [4] = Xylanase bleached bagasse,
[5] = Lignin peroxidase bleached bagasse
N.B. Minimum requirements for printing paper is 2.4 (km) Breaking length and 75% Brightness (ES: 13/2002).

With respect to properties of paper sheets made from different bleached bagasse pulp, data indicate that using enzyme pretreatment led to increase in the brightness %, breaking length and tear factor being 6.7 %, 18.89 % and 12.7 % by XBB and 8.94 %, 34.92 % and 30.82 %, by LBB, respectively, as compared to chemical treatment. Moreover, the breaking length, and brightness of LBB paper sheets increased 3.5 and 1.17 fold than the minimum requirement for standard printing paper. The corresponding figures for XBB were increased 3.12 and 1.15 fold for breaking length and brightness, respectively.

Residual active chlorine

The pulp bleaching was carried out via elemental chlorine- free (ECF) technique; using the chlorine dioxide (D_1), alkaline extraction (E) and chlorine dioxide (D_2) steps. The residual chlorine was determined after D_1 and D_2 steps for enzyme pretreated and untreated samples (Table 2). Data clearly show that the highest value of residual chlorine (g/l) and the lowest percentage of consumed chlorine were attained by enzyme pretreated bagasse pulp at different steps of chlorine bleaching. While vice versa was true for the values of untreated bagasse pulp (control). This may be due to the decrease in lignin content as a result of enzyme pretreatment. Also, it could be noticed that LBB recorded a lower consumed chlorine % and higher residual chlorine than XBB. Where the consumed chlorine % of LBB and XBB decreased about 40% and 26.67% after third step (D_2), respectively, as compared to control. This means that lignin peroxidase was more effective than xylanase as pretreatment of bagasse biobleaching.

TABLE 2. Residual chlorine (g/l) measured and consumed chlorine of the enzyme treated and untreated bagasse pulp during different bleaching steps with chlorine.

	Bleaching steps with chlorine			
	D_1		**D_2**	
Enzyme treated	**Residual chlorine g/l**	**Consumed chlorine %**	**Residual chlorine g/l**	**Consumed chlorine %**
Control•	0.15	75	0.19	68.33
XBB*	0.22	63	0.35	41.66
LBB**	0.29	51	0.43	28.33

• = without enzyme pretreatment
* = xylanase bleached bagasse Initial chlorine dose= 6 g/L
** = Lignin peroxidase bleached bagasse

Scanning electron microscopy (SEM) of bleached bagasse pulp using enzyme pretreatment

SEM studies clearly showed that the application of xylanase or lignin peroxidase caused change in the surface of the fiber. Fibers without enzyme treatment had smooth, sleek and uniform surfaces (Fig. 9-a). However, fiber that had undergone enzymes treatment had a rough surface with striations and splits, *i.e.* a more open surface (Fig. 9-b&c). This might be corroborate that enzyme treatment remove some components from the fibers and result in the alteration in fiber structure.

Determination of the crystallinty by the X – ray diffraction (XRD) technique

X – ray diffraction was used as an alternative method for pulp characterization. It is possible by this method to determine the crystallinty of the material through the relative peek intensities (Fig. 10 a,b and c). For lignocellulosics, the peak which corresponds to the crystalline portion is at approximately 26°C. The amorphous region of the sample has a peak in the 18°C.

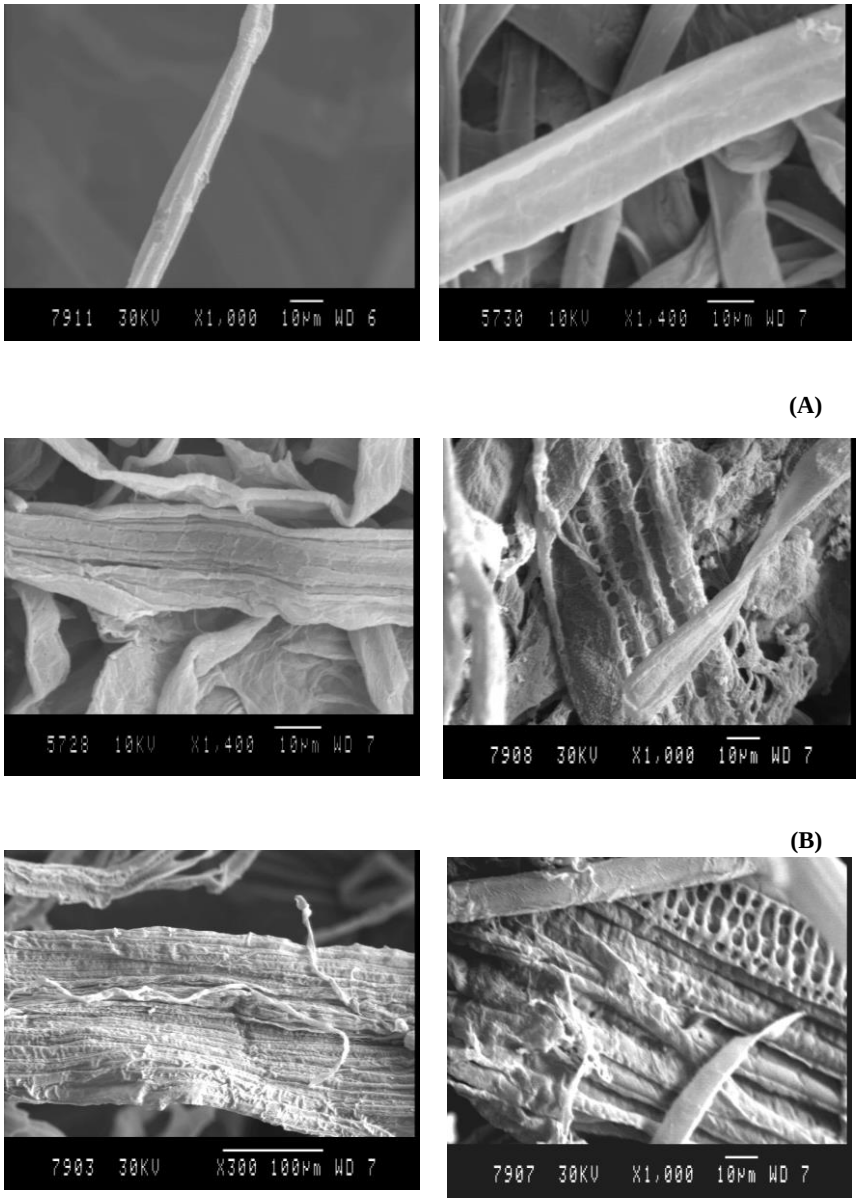

Fig. 9. Scanning electron micrographs of bleached bagasse pulp (A), xylanase pretreatment (B), lignin peroxidase pretreatment (C).

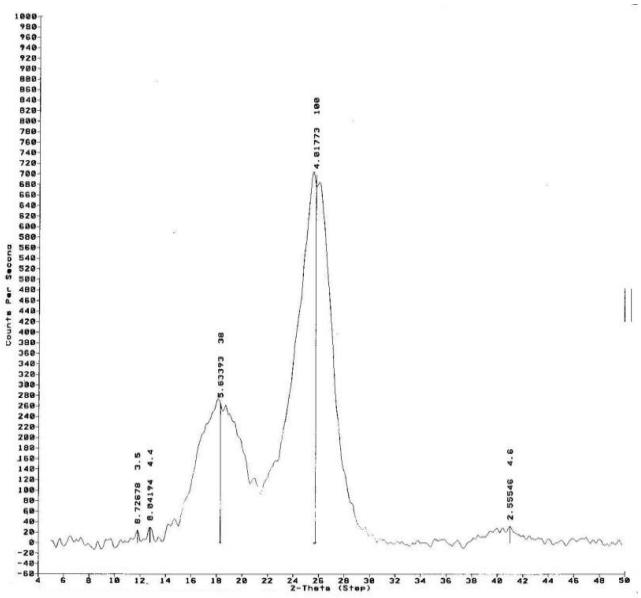

Fig. 10-a. The X–ray diffraction (XRD) for bleached bagasse pulp, without pretreatment with enzyme.

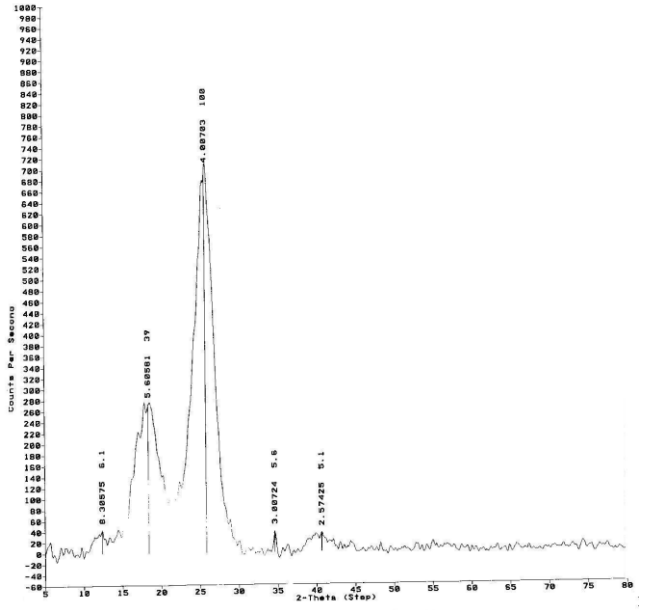

Fig. 10-b. The X – ray diffraction (XRD) for bleached bagasse pulp,with xylanase.

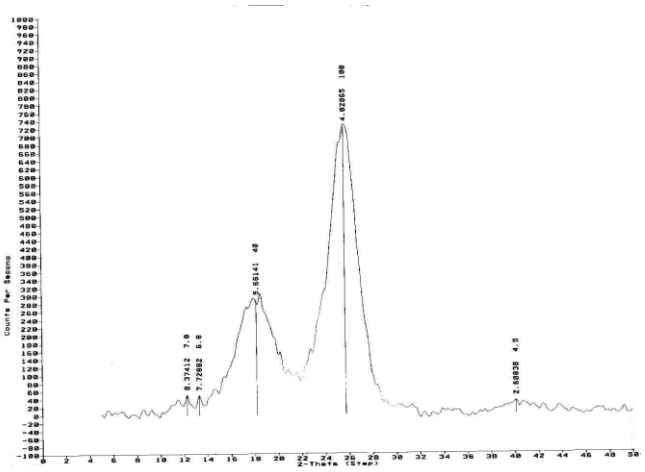

Fig. 10-c. The X – ray diffraction (XRD) for bleached bagasse pulp,with lignin peroxidase.

By this calculation, the bleached bagasse chemical pulp gave 55.4% of relative crystallinty and the biologically treated bleached bagasse pulp gave 61.66% and 60.0% for xylanase and lignin peroxidase, respectively. So, data indicate that biologically pretreatment of bagasse pulp with xylanase or lignin peroxidase enzymes led to increase in the crystallinty by 11.29 and 8.3 %, respectively.

The use of hemicellulases including xylanases for delignification in the paper industry can improve the chemical extraction of lignin from pulp leading to a significant saving of chemicals required for bleaching and to reduce the toxic chlorine compounds released into the environment (Bajpai *et al.*, 2004). The highest enzyme activities by all the treated strains was obtained on med. 6 supplemented with 5% corn cobs was used for xylanases production by seven fungal strains.

In this respect, Christopher *et al.* (2005) obtained the highest xylanase activities by seven fungal strains on corn cobs medium incubated at 28ºC on an orbital shaker at 150 rpm after 4 days. Qasim *et al.* (2000) stated that the biobleachability efficiency of cellulose-free xylanase from *Streptomyces* sp QG-11-3 was maximum after 2 h at 50ºC using a xylanase dose of 3.5 U/g at 6% pulp consistency with 23% reduction in kappa number and 5.6 fold increase in release of reducing sugars.

In similar studies, Jian *et al.* (2002) found that the kappa number and rejects of chemical pulps decreased with increase of the xylanase dosage when all other conditions were kept on constant. However, changes in the kappa number and rejects were not significant when xylanase dosage was over 4 IU/g. Therefore; the optimal xylanase dosage was 4 IU/g in enzymatic pretreatment stage.

From the aforementioned results, it could be concluded that the pretreatment of bagasse pulp by 10.30 IU/g xylanase, produced by *Trichoderma reesie* NRRL 6156, for 4 h at 50ºC was the best xylanase pretreatment which reduced klason lignin % and increased the brightness % about 38.98 and 6.19 %, respectively as compared to control.

In this respect, Ehara *et al.* (2000) reported that Tween 80 and Tween 20 exhibited several effects, such as dispersion of degraded lignin and activation of MnP that partly contributed to the brightening of hardwood kraft pulp during MnP treatment. The results suggested that Tween 80 was peroxidized by Mn (III) and that Mn (III) and lipid peroxidation of Tween 80 synergistically brightened hardwood kraft pulp.

These results are in agreement with that obtained by Kirk *et al.* (1990) who found that hardwood kraft pulp brightness increased by 15 and 30 points after 5 days of treatment with *T. versicolor* and *Ph. chrysosporium* respectively, in solid state fermentation system and the pulp kappa number decreased with increasing brightness.

Also, it could be noticed that the percentage of pentosan in the former pulp was lower than the latter pulp whereas the vice versa is true for klason lignin %. The effect of xylanase pretreatment was explained in two hypotheses. The first hypothesis is that deposits of xylan may physically entrap residual lignin on fiber surfaces. Not only would its removal facilitate the diffusion of residual lignin out of the fiber matrix, but it may also enhance the accessibility of this lignin to bleaching chemicals (Kantelinen *et al.*, 1993). The second hypothesis is : Lignin – carbohydrate linkages occur after pulping process that restrict the removal of residual lignin. Xylanase cleavage of the carbohydrate portion of lignin – xylan complexes could facilitate subsequent chemical delignification by releasing the lignin component or by reducing the overall size of macromolecules containing residual lignin (Yang & Errikson, 1992).

Hërpoel *et al.* (2002) stated that the effect of xylanase alone on kappa number reduction of chemical pulp was slight. Hemicellulose, largely composed of xylans, could be attacked by xylanase and so release a small quantity of lignin bonded to them. Whereas the laccase treatment, the chemical pulp was delignified by about 47% compared to untreated pulp. The delignification rate of the wheat straw chemical pulp was improved significantly with a two – step enzymatic treatment: xylanase treatment followed by laccase.

Nishida (2001) stated that combined fungal and chemical bleaching process could significantly reduce the total effective chlorine and the pollution load of waste liquors in comparison with the conventional bleaching process.

The total effective chlorine required to obtain a pulp of 85% brightness by chlorine-based chemical bleaching after repeated MnP treatments was reduced to 70% for hardwood kraft pulp compared to the conventional bleaching process without introducing MnP treatments.

SEM, allowed us to observe the changes in the surface of the fiber, which are very clear and visible, after enzyme treatment. Different types of enzyme act in that the "opened", *i.e.* it becomes more accessible to subsequent bleaching agents. Some enzymes even result in, or facilitate greater fibrillation. In all cases, enzymes produce changes in the surface of the fiber owing to the hydrolysis that they cause, which is in agreement with the findings of other authors, Pham *et al*. (1995), Garg *et al*. (1996) and Bustamante *et al*. (1999). So, it could be stated that increasing in the degree of crystallinty of the pulp prebleached with xylanase or lignin peroxidase, *i.e.* it decreases the amorphous region, resulting in the remaining cellulose being more crystalline.

In this respect, Roncero *et al.* (2005) stated that the crystallinty index (CrI) obtained by XRD, both enzyme treatment and oxygen delignification increased the degree of crystallinity. They effected the ratio of crystalline and amorphous regions, although in different ways by causing elimination of hemicelluloses and lignin. Also, Saad *et al.* (2008) reported that the best conditions for fungal pretreatment of sugar cane straw were 15 days with 250 mg kg^{-1} fungal mycelium per straw weight causing high lignin decomposition with a reduction of 40% in the pulping.

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تقييم إنزيمات الزيلانيز و اللجنين بيراكسيديز الفطرية على التبيض الحيوى للب مصاص القصب

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قسم الميكروبيولوجيا الزراعية- كلية الزراعة جامعة عين شمس - شبرا الخيمة و*قسم بحوث السليولوز والورق- المركز القومى للبحوث - القاهرة – مصر.

تم إستخدام بعض المخلفات الزراعية مثل قوالح الذرة وردة القمح وبودرة مصاص القصب لإنتاج الزيلانيز بواسطة *Trichoderma reesie* NRRL 6156و *Trichoderma viride* NRRL 13034 بإستخدام المزرعة المهتزة على 30°م لمدة 7 أيام . أعطى الفطر الاول كفاءة فى إنتاج الزيلانيز بمقدار 1,7ضعف الفطر الاخر فى وجود قوالح الذرة . تم معالجة اللب الحيوى لمصاصة القصب ب الزيلانيز المحضر وتم الحصول على أفضل نتائج بعد 4 ساعات عند50°م حيث سجل أكبر نقص فى نسبة اللجنين وبالتالى تحسين قابلية اللب الحيوى للتبيض مقارنة باللب الحيوى المبيض والغير معالج بالانزيم . وثبت أن إستخدام 10,3 وحدة دولية من الزيلانيز / جرام من اللب الحيوى هى أفضل جرعة . أدى نمو *Phanerochaete chrvsosporium* NRRL 6361أعلى إنتاج لانزيم اللجنين بيروكسيديز فى المزرعة الصلبة بمقارنتها بالمزرعة النصف صلبة والسائلة بعد 6 أيام و أفضل جرعة للحصول على أفضل تبيض هى 1,54وحدة دولية / جرام لاعطاء أعلى نصاعة وأقل نسبة لجنين . إن إستخدام المعالجة الاولية بانزيم الزيلانيز أدت إلى زيادة درجة النصاعة والطول القاطع ومعامل التمزق للورق المنتج بمقدار 6,7 و 18,89 و12,7 % على الترتيب فى حين إستخدام اللجنين بيروكسيديز أدى إلى الزيادة بنسبة حوالى 8,94 و 34,92 و 30,82 % على الترتيب بمقارنتها باللب غير المعامل بالانزيم . أدت هذه المعاملة إلى تقليل نسبة الكلور المستهلك أثناء عملية التبيض إلى حوالى 40 % و 26,67 % على الترتيب . وإتضح بإستخدام الميكروسكوب الإلكترونى الماسح أن إستخدام الانزيمات السابقة تسبب تغيير فى شكل سطح الألياف مقارنة باللب غير المعالج بالانزيمات . حيث كانت الالياف غير المعالجة ذات سطح ناعم ومتجانس بينما كان سطح الألياف المعالجة خشن وبه تشققات وهذا يعنى أن الألياف المعاملة بيولوجيا عرضة أكثر للكيماويات المستخدمة فى التبيض بصورة أسهل وأفضل . وأظهرت تعيين درجة البلورة بإستخدام حيود الاشعة السينية أن المعالجة بالإنزيمات المستخدمة تؤدى لزيادة درجة البلورة بمقدار 11,29% و 8,3 % على الترتيب بمقارنتها بالطرق الكيميائية .