Utilization of Waste Paper and Lignocellulosic Pulps for Production of Cellulases, β-Glucosidases and Xylanases by Penicillium Funiculosum

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ABSTRACT

In this study the production of cellulases, β-glucosidases and xylanases was investigated in two fungi obtained from NRRL *Penicillium funiculosum* 3647 and *Trichoderma viride* 6198, using different carbon source in the medium as pretreated rice straw, cotton linter and newspaper under shake-culture conditions at 30°C, *P. funiculosum* has been found to produce moderately good enzymes when growing on Dox medium supplemented with (1% w/v) pretreated rice straw and cotton linter for 4 days and had an optimum pH and temperature ranging between pH 3.5-5.0 and 50°C respectively, and air: medium 4:1, the enzyme is not stable at temperature of up to 50°C and rapidly decreased,

While *T. viride* have poor activity that was observed when grown alone.

On mixed *P. funiculosum T. viride* together through cultivation caused a significant decrease in the levels of cellulase, β-glucosidases and xylanases enzymes. It is clear that treatment of lignocellulosic by fungi enhanced the bleachability and reduced kappa no. Also degree of polymerization was highly increased. IR and scanning electron microscope were studied.

Key Words: *P. funiculosum* 3647; *T. viride* 6198; celluloses; β-glucosidase; xylanases; rice straw; cotton lint and newspaper.

INTRODUCTION

Cellulase and hemicellulase are enzymes that specifically degrade cellulose and hemicellulose, these group of enzymes includes cellulases and xylanaes. The effectiveness of cellulose and xylanase production is larglely influenced by the carbon source such as cellulose.

Pure cellulose is a linear essentially insoluble, B-1, 4-D-glucosidically linked polymer containing 8000 to 14000 glucose units forming a crystalline unit. Cellulose is degraded by the cellulase enzyme complex which consist of endoglucanase, exoglucanase and β-glucosidase that degrade cellulose into low molecular weight oligosaccharides, cellobiose and eventually glucose.

Cellulose such as solka floc is considered the best inducer for production of a well- balanced cellulase system (Ryu and Mandels, 1980), but it has estimated to be more expensive for use in industrial fermentation (Maat et al, 1992).

(Doppelbauer et al., 1987) have used lignocellulosic wastes wheat straw and waste paper for cellulase production with *T. reesei* for reduction of production costs is to use in expensive waste materials. Also (Reetta et al., 1996) reported that the endo-1, 4, β-glucanase and xylanase activities obtained with nylon-web.

Rice straw, cotton lint and newspaper are composed of three bio polymers namely cellulose, hemicellulose and lignin that are strongly associated to each other through interactions such as hydrogen bonding to form fibers, the major structural component of these waste is a long chain of glucose molecules, linked to one another primarily with β (1- 4) glycosidic bonds and the simplicity of the cellulose structure means that can be degraded (Brown and Saxena, 1996).

The potential application of cellulases with or without xylanases include the bioconversion of lignocelluloses to sugar, ethanol and other useful substances clarifying juices and wines, extracting plant oils, coffee and starch and improving the nutritional value of silage and green feed, in facilitating the bleaching of krapt pulp, it have been used to dough preparation in baking processe. (Wong and Saddler 1992; Rouau et al., 1994; Buchert et al., 1993; Frost and Moss 1987).

The aim of this work was to investigate the formation of cellulase, xylanase and β-glucosidase activities by *P. funiculosum* through optimization process and preparation of glucose, the objectives were to evaluate the effects of carbon source from waste as rice straw, cotton lint and newspaper.

Materials and Methods

Lignocellulosic materials

Waste papers used in this work were newspaper, pretreatment kraft rice straw pulp prepared by ($NAOH+NA_2S$) and cotton linter, all samples were cut into very small pieces before treatment with fungus.

Infra-red spectra

The infra-red for lignocelluloses pulp were measured as KBr disces using a Jocco FT/IR 430E spectrophotometer Japan.

Test on pulp properties

Kappa number, degree of brightness and degree of polymerization were carried out according to method of (Casey, 1981).

Kappa number is used to describe the

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Table (1):Determination of enzymes xylanase, cellulase, (FPase, CMcase) β-glucosidase using two organisms, penicillium funiculosum and trichoderma viride after 4 days growth

Organisms									Enzymes
Penicillium funiculosum			Peni + trich			Trichoderma viride			
Rice straw	Cotton lint.	News paper	Rice straw	Cotton lint.	News paper	Rice straw	Cotton lint.	News paper	
75	73	8	16	12	10	16	4	9	xylanase µg/ml
354	325	300	212	300	228	120	96	28	CMcase µg/ml
218	196	20	172	132	6	110	138	16	FPase µg/ml
160	112	92	80	100	72	82	76	9	B-glucosidase µg/ml

degree of delignification obtained in the chemical and or/enzymatic process. Degree of brightness is the percentage of diffuse reflected light at wavelength of 457 nm from a thick pad of paper, it is measure "whiteness of tested paper it was measured by Hunter Lab. Color. Difference Meter D25-2. Degree of polymerization (DP) is a function of average length of cellulose chains, and fiber length. It is one of significant factors of cellulose sample strength.

Scanning electron microscopy (SEM)

A microscopic examination was made of the progressive degradation of different lignocellulosic by fungus using model JSM-T20 from JEOI, Japan.

Microorganism

Penicillium funiculosum NRRL3647 and *Trichoderma viride* NRRL6198 were obtained from Nothern Regional Research laboratory USDA, Peoria, USA. The cultures were maintained on agar slants of malt extract. Subculturing was done after 2 weeks and the strain was stored at room temperature.

Media

Two synthetic media were used, the first one with defined chemical composition that are normally used in laboratory for academic studies on fungal physiology and biochemistry (Czapek-Dox) medium, for the fungus *P. funiculosum*, the other medium for T. viride was prepared by mixing 0.5 g $NANO_3$; 1.0 g K_2HPO_4; 0.5 g $MGSO_4$. $7H_2O$; 1.4 $MNSO_4$; 0.0014 g $ZnSO_4$. $7H_2O$; 0.01 $FeSO_4$. $7H_2O$ and 0.2 % peptone in one litre distilled H_2O.

Assay for enzymes

Assay for xylanase activity

Xylanase activity was determined by measuring the amount of the reducing sugar liberated from xylan. The reaction mixture contained 1.0 ml of the crude enzyme preparation and 1.0 ml of 1 % xylan in acetate buffer pH 5.0.

Assay for cellulase activity

Filter paper and carboxymethyl cellulase activities were determined according to (Mandels et al., 1974).

Filter paper activity (FPase)

Filter paper cellulase (FPase) was determined by incubating 1.0 ml enzymes filtrate with 1.0 ml 0.05 M acetate buffer, of pH 4.8 and 50 mg (strip 2x3 cm) of watman No. 1 filter paper for 1 hr. at 50°C. Reducing sugars were measured as glucose by oinitro Salysilic Acid (DNS) method according to (Miller, 1959).

Carboxy methyl cellulase activity (CMCase)

CMcase activity was assayed by mixing 1.0 ml of enzyme filtrate with 1.0 ml of 1% carboxy methyl cellulose (CMC) in 0.05 M acetate buffer pH 4.8, the mixture was incubated for 30 min. at 50°C, reducing sugars were measured as glucose by DNS method.

Assay β-glucosidase activity

β-glucosidase activity was measured according to (Sterenberg et al., 1977), using cellobiose as substrate, the cellobiase activity was determined by incubating 1.0 ml of enzyme filtrate and cellobiose 7.5 mM in reaction mixture in 0.05M acetate buffer at pH 4.8 for 30 min.at 50°C. Glucose released was measured by Glucostat procedure. Cellulase, β-glucosidase and xylanase are expressed in the term micrograms of glucose and xylose liberated by 1.0 ml.

Physiological studies on cellulase, β-glucosidase and xylanase

Factors affecting enzyme production

Determination of cellulases and β-glucosidase and xylanase at different incubation periods using *P. funiculosum* and *T. viride* alone and using mixed cultivation of them

The effect of incubation time on the levels of enzyme detected in culture of *P. funiculosum* and *T. viride* strains was followed daily for a 7 days period of shaking growth.

Effect of substrate concentration

P. funiculosum was chosen for further investigations as it proved to be the best fungi for enzyme formation, different concentrations of rice straw, cotton lint and newspaper ranging between 1-4 % were used in Dox medium in shake cultures flask.

Effect of aeration (air: medium)

The extent of aeration was carried by the volume of medium in the culture flasks of constant size (250 ml), the volumes of medium : air 9:1, 4:1, 3:2, 2:3 to determine the optimum ratio for enzyme formation, the enzyme activity was determined in the culture supernatants after 4 days.

Effect of initial pH value

Table (2): Effect of substrate concentration (rice straw , cotton linter and news paper) on levels of cellulase , xylanase and β- glucosidase produced by P.funiculosum

Rice straw				Cotton lint.				News paper				Enzymes
Substrate concentration												
1%	2%	3%	4%	1%	2%	3%	4%	1%	2%	3%	4%	
76	64	58	50	73	62.5	54	49	8.0	7.0	5.5	4.0	xylanase µg/ml
352	300	250	210	330	300	276	240	310	300	280	256	CMcase µg/ml
220	205	190	181	195	185	162	145	22	20	17	16	FPase µg/ml
159	130	121	100	112	97	86	64	91	82	70	54	B-glucosidase µg/ml

Table (3): Effect of aeration on the production of cellulase , xylanase and β-glucosidase enzymes by strain P.funiculosum

Rice straw				Cotton lint.				News paper				Enzymes
Aeration												
9:1	4:1	2:3	3:2	9:1	4:1	2:3	3:2	9:1	4:1	2:3	3:2	
68	75	51	13	52	73	48	18	7.5	8.5	3.3	1.5	xylanase µg/ml
335	355	120	43	300	324	134	40	270	304	129	35	CMcase µg/ml
196	218	140	53	180	196	79	45	15	21	10	2	FPase µg/ml
114	160	78	20	108	112	53	20	74	93	35	13	B-glucosidase µg/ml

This was carried out by adjusting the pH of the medium with 0.1M HCL or NaOH in the pH range between 3.5-6.0 prior to autoclaving of the medium, the experiment was carried out under standard condition.

Studies on the properties of crude cellulase, xylanase and β-glucosidase enzymes

Effect of reaction temperature on the reaction rate of the enzyme

A set of reaction tubes containing reaction mixture were incubated in water bath at different temperature ranging between 40-80°C.\

Heat stability of the enzyme

Aliquots of the crude enzyme were heated for 15 min. in water bath set at different temperatures ranging between 40-80°C prior to the enzyme assay which was carried out at 50°C.

Effect of incubation time on the activity of the enzyme

Reaction tubes containing standard reaction mixtures were incubated in a water bath set at 50°C for different periods of time ranging between 20-120 min.

RESULTS AND DISCUSSION

Determination of cellulases and β-glucosidase and xylanase at different incubation periods using *P. funiculosum* and *T. viride* alone and using mixed cultivation of them

The fungi were cultivated in shake flasks contain pretreated rice straw, cotton lint and newspaper as carbon source, samples were taken every 24 hr. and tested for filter paper activity (FPase) carboxy methyl cellulose (CMCase), β-glucosidase and xylanase. The results presented in table (1) show the maximum cellulolytic activities for each fungus after 4 days of incubation, *P. funiculosum* showed the highest activities of cellulase (FPase and CMCase) and xylanases on rice straw (354, 218 and 75 µg/ml) respectively, where as *T. viride* produced low activity than P. funiculosum and gave cellulases and xylanase (120, 110 and 16) respectively, low in β-glucosidase, i.e. the highest enzyme were found on rice straw and low on cotton lint and lowest on newspaper. In case of using mixed cultivation of *T. viride* and *P. funiculosum*, the results were much lower than those obtained by a single organism. These results not agree with (panda et al., 1983) who observed that enzyme production was enhanced by mixed culture of T. reesei and A. wenti (Trivedi and Desai, 1984) also reported that mixing of *Scytalidius lignicola* and *T. longibrachiatum* increased production of cellulases and β-glucosidase.

Effect of substrate concentration (rice straw, cotton lint and newspaper) on levels of cellulase, xylanase and β-glucosidase produced by *P. funiculosum*.

From the two previously mentioned fungi *P. funiculosum* was chosen for further investigations as it proved to be the best fungus for enzyme formation. Different amounts of rice straw, cotton lint and newspaper ranging between 1-4 % were used in shake cultures flasks. The results presented in table (2) showed the highest activity of enzymes production at 1% substrate concentration, while high amounts of substrate (2-4 %) decrease enzyme production. These results agree with (Ghahal et al., 1982) reported that maximum enzyme yields could be obtained by *T. viride* when cultured on 1% cellulosic substrate (Loowenberg, 1984) used 1% solka floc as cellulose for enzyme production by T. Reesei Gu6A. (Costa et al., 1985) reported that highest enzyme was on using 1% wheat straw by *T. harzianum*. In contrast (Gaspar et al., 1997) reported that best results were obtained with the higher

Table (4): Effect of pH on production of cellulase, xylanase and β- glucosidase enzymes by P.funiculosum

Rice straw						Cotton lint.						News paper						Enzymes
pH value																		
3.5	4.0	4.5	5.0	5.5	6.0	3.5	4.0	4.5	5.0	5.5	6.0	3.5	4.0	4.5	5.0	5.5	6.0	
76	76	75.5	74	32	9.0	74	74	73	70	28	7	8	8	8	8.0	3.5	1.5	xylanase µg/ml
355	354	356	350	94	7	326	325	324	300	32	6	304	304	303	303	42	10	CMcase µg/ml
219	219	218	213	70	4	198	196	196	194	27	13	21	22	21	21	7.5	2	FPase µg/ml
160	160	159	152	44	4	115	114	112	112	11	2.0	90	91	92	92	21	4	B-glucosidase µg/ml

Table (5): Effect of incubating temperature on the activity of cellulase, xylanase, and β-glucosidase enzymes by P. funiculosm

Rice straw					Cotton lint.					News paper					Enzymes
Temperature °C															
40	50	60	70	80	40	50	60	70	80	40	50	60	70	80	
75	87	70	8	1	74	90	40	3	0.5	8	19	7	2.5	0.3	xylanase µg/ml
290	355	210	23	5	200	325	180	4	0.7	170	300	124	4	0.6	Cmcase µg/ml
195	218	87	12	3	116	195	79	3.5	0.4	7	20	6	2	0	Fpase µg/ml
124	160	78	5	0	98	112	50	6	2	64	92	38	4	0.6	B-glucosidase µg/ml

Table (6): Effect of heating temperature on stability of cellulase, xylanase and β- glucosidase enzymes by P.funiculosum

Rice straw					Cotton lint.					News paper					Enzymes
Temperature °C															
40	50	60	70	80	40	50	60	70	80	40	50	60	70	80	
75	78	20	0.6	0.5	75	74	17	1	0.4	8	8	2	0.5	0	xylanase µg/ml
350	352	66	6.5	1.5	325	325	54	4	0.2	250	300	45	2.5	0.3	CMcase µg/ml
215	218	44	3	0	190	196	28	2	0	17.5	21	3	0	0	FPase µg/ml
151	160	34	0.3	0.5	90	112	35	1.5	0.11	81	93	26	3	0.8	B-glucosidase µg/ml

substrate concentration at 3% wheat straw by *P. canescens*, where (Natividad et al., 2000) found that the lowest substrate concentration (0.5%) give best results.

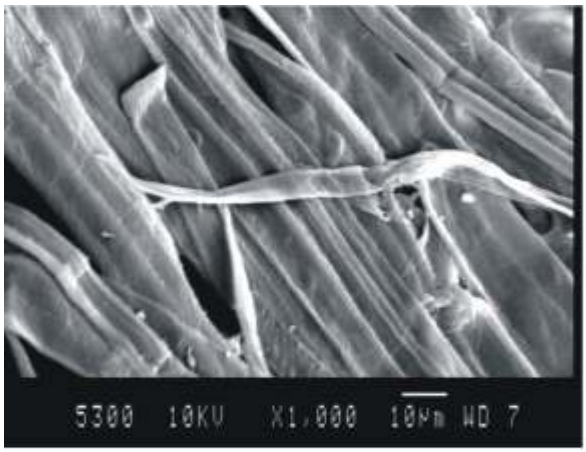
Effect of aeration in shake culture on enzyme production by *P. funiculosum*

The extent of aeration was varied by varying the volume of medium in the culture flasks of constant size (250 ml) is presented in table (3) the highest level of cellulase, xylanase and β-glucosidase enzymes were obtained in shake cultures in air: medium 4:1 after 4 days incubation. The highest activities of enzymes were obtained on rice straw than cotton lint and newspaper respectively. (Gaspar et al., 1997) reported that the smaller ratio between broth volume and volume of the shake flask give more effective mixing and mass transfers.

Effect of intial pH of medium on enzyme production by *P. funiculosum*

The pH of the growth medium is of great importance in the enzyme production, varied initial pH of the medium ranging from 3.5-6.0 were studied. The data presented in table (4) indicate that the optimum activity of enzymes were ranging between pH 3.5-5.0. The rang of pH value in enzyme system production depend on the type of fungus. (Peitersen, 1977), Kalra and (Sandhu, 1986) reported that *T. viride* and *T. Pseudoboningii A. funigatus* produce cellulase enzymes at initial pH 5.0. pH value ranging from 4.5-4.8 was favourable for cellulases production by *T. reesei* and *A. pheenicis* reported by (Duff et al., 1986). Xylanase production is highest at pH 6.0 both on cellulose and xylan based medium but endoglucanase production is to be higher at low pH about 4 (Reetta et al., 1996), while (Kristian et al., 2004) found that cellulolytic and xylanolytic activities is higher at pH 6.0-6.5. According to the obtained results the growth medium adjusted at pH 4.5 at substrate concentration 1% and aeration 4:1.

Studies on the properties of



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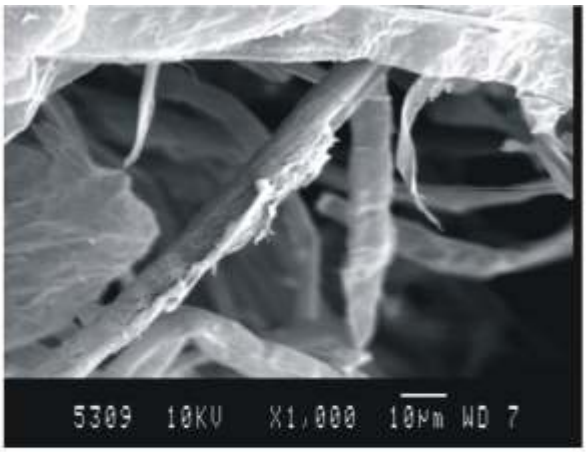

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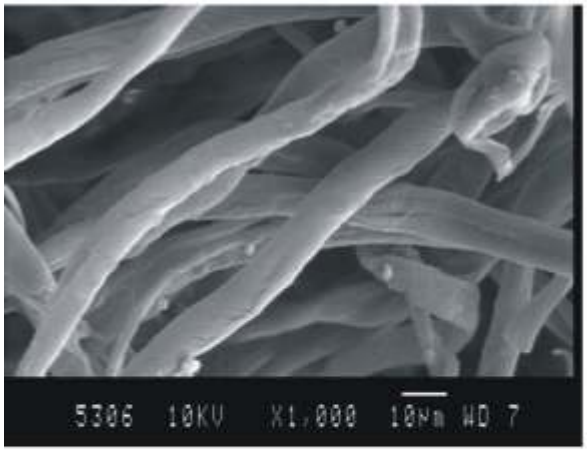

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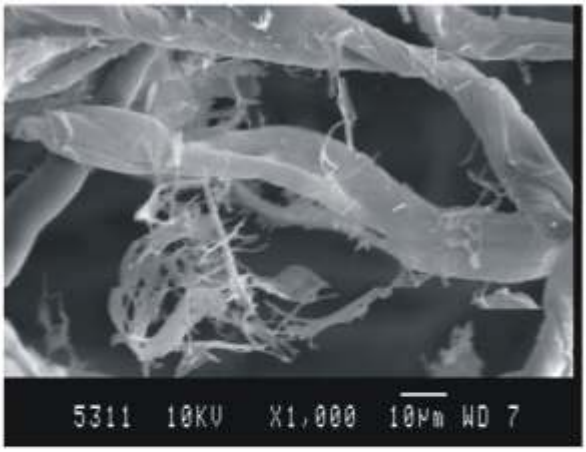

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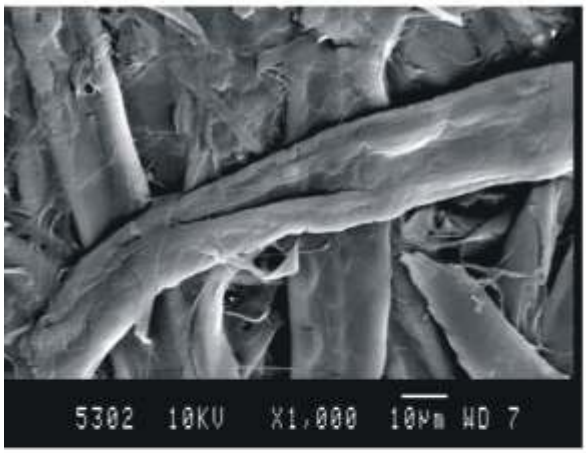

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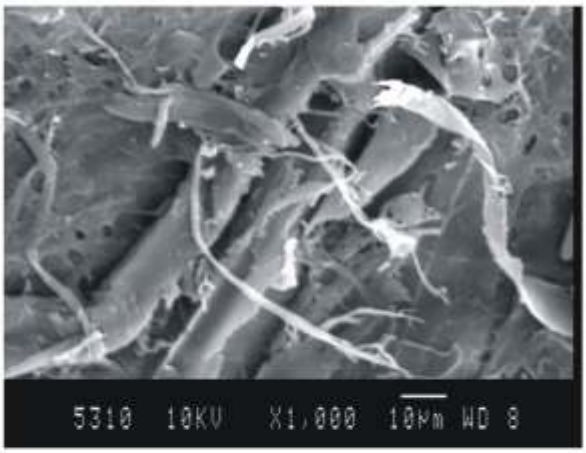

f

Fig (1) SEM photographs of rice straw, cotton linter and newspaper treated with P. funiculosum

Table (7): Reaction progress with time for the activity of cellulase, xylanase and β-glucosidase enzymes by P.funiculosum

Rice straw					Cotton lint.					News paper					Enzymes
Time (min)															
20	30	60	90	120	20	30	60	90	120	20	30	60	90	120	
74	75	75	73	68	73	73	70	65	63	7.5	8	8	7	6	xylanase µg/ml
320	350	352	345	330	320	322	325	320	305	276	284	300	290	273	Cmcase µg/ml
84	114	218	215	210	64	98	196	193	190	3	8	21	20	17	Fpase µg/ml
140	155	160	145	135	98	100	112	112	94	84	88	92	90	87	B-glucosidase µg/ml

Table (8): The effect of treatment of lignocellulose by p. funiculosum on the chemical and optical properties of the pulp

Chemical analysis	Untreated rice straw pulp	Treated rice straw	Cotton linter untreated	Cotton linter treated	News paper untreated	Treated news paper
Kappa no.	35.2	20.4	12.21	6.55	71.5	68.88
Brightness	30.2	40.5	55.7	58.3	20.5	22.3
DP	850	920	2400	2445	620	725

Table (9): IR absorption bands and their assignments

Absorption Wave number (cm^{-1})	Assignment
890	C – H deformation (amorphous cellulose for cell .1
1110	C – H stretching (C – OH)
1165	C – O – C antisymmertrical stretching (glycopyranose vibration)
1205	OH in plane bending
1280	OH Deformation
1370	Crystallinity in a mixture of cellulose I and II
1425	Measurements of the degree of crystallinity (cell. I)
1630	H – OH bond or c = c
1725	C = O stretching
2885	Unmodified measurements of crystallinity in a mixture of cellulose I and II
3450	OH stretching (H – bonded)

crude cellulase, xylanase and β-glucosidase

1- Determination of reaction temperature on the reaction rate of enzyme

This experiment determined the optimum temperature for activity of crude enzyme. A set of reaction tubes containing standard reaction mixture were presented in duplicates and incubated at different temperature ranging between 40-80°C. The result shows in table(5) that the optimal activities of xylanase, cellulase and β-glucosidase enzymes were at 50°C and decreased after that, for rice straw 87, 355, 218 and 160 µg/ml respectively, for cotton lint 90, 325, 195 and 112 µg/ml respectively.

2-Heat stability of the crude enzyme

The stability of the enzyme at various temperature was investigated and was measured as follows, small amount of the crude enzyme were heated in thin walled glass tubes for 15 min. in water bath set at different temperature ranging between 40-80°C prior to the enzyme assay which was carried out at 50°C. The high activity of enzymes were at 50°C and then decreased as shown in table (6), (Bust et al., 1995) showed that high activity at temperature 60°C and maximum stability was achieved at temperature of 55°C.

3- Reaction progress with time of the activity of the crude enzyme

Duplicates of reaction tubes containing standard reaction mixtures were incubated in a water bath set at 50°C for different periods of time ranging from 20-120 min. results show in table (7), the activity of xylanase enzyme was stable from 20-60 min. where the activity of cellulase enzyme and β-glucosidase increased reached at 60 min. then decreased.

Scanning electron microscopy (SEM) of different lignocellulosic materials.

Effect of *P. Funiculosum* pretreatment on the surface of different pulps

There may be a relation between the surface state of fibers and properties of the pulp and paper obtained. A microscopic study was carried out, using scanning electron microscopy (SEM) photographas (a) and (b) of fig. (1) show rice straw pulp and pulp-treated with respectively the fibres in the raw pulp were uniform and quite straight with smooth sleek surface, with an appearance of softness. In addition, they showed no sign of external fibrillation or formation of fibrils. On the other hand, the fibres in (b) pulp were less straight and their surface was rough, heterogeneous and striated, indicating that they were in the process of peeling, which gave rise to a morphological change in the surface of the fibre.

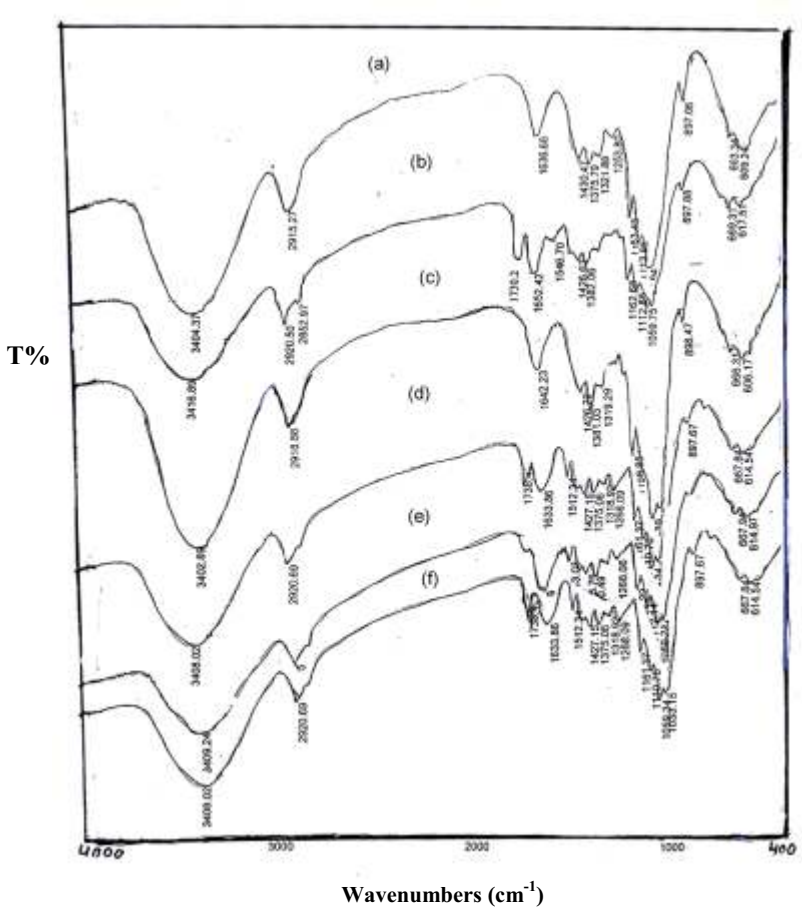

Fig. (2): IR-spectra of rice straw, cotton linters and newspaper (a, c & e) (untreated) (b, d & f) treated with *P. funiculosum.*

Grooves or cracks even appeared to have formed in certain regions of the fibres, although they were not regularly distributed, which leads us to believe that they occurred in the less crystalline regions of the fibre or where xylans had been deposited. This peeling effect also coincides with the images obtained by (Torres et al., 2000). In case of fig.(D), (F) cotton linter and newspaper treated with *P. Funiculosum* we found that more deformation and more cracks in case of cotton linter than newspaper.

Xylanase treatment is proposed to open the cell wall pores of pulps. Morphological changes such as holes cracks, flates, filaments and peeling are caused by enzyme treatment. These cracks and holes allow the diffusion of the larger lignin macromolecules as reported by other authers (Paice et al., 1995, Wang et al., 1997)

Chemical and Optical properties of lignocelluloses

Xylanase treatment improves accessibility of bleaching chemicals to the pulps, decreases diffusional resistance to outward movement of the degraded lignin fragments and allows the removal of less degraded lignin fragments from the cell wall. As result, pulps treated with xylanase show lower kappa number and higher brightness and viscosity than pulps not treated with fungus.

From table (8), it is clear that the kappa no. decreased in the following order rice straw pulp followed by cotton linters and then newspaper. The lower kappa no. the highest the brightness. We found that the gain in brightness 10.3 points in case of rice straw and 2.6 points in case of cotton linter and finally 1.8 in case of newspaper.

Fungus treatment enhance brightness which may be attributed to pulps kappa number decrease (Amany and Zenat, 2004). The brightness could increase from 2-10.3 points which means that chemical change in the subsequent bleaching stage could decreased.

It noticeable increase in the following order rice straw pulp, cotton linter and finally newspaper (table 8), it may be probably due to selective degradation of low molecular mass fraction of xylanase. Also indicate no cellulase activity a noticeable increase in the degree of polymerization was observed. This will reduce the consumption of chlorine chemicals and will reduce the energy of consumption and protect the environment from pollution.

Infra red spectra

Analysis of treated pulp by FTIR spectradata are widly used for characterization of different pulps (Faix et al 1992)

This technique is used as a tool to analyse the chemical modifications occurring in rice straw, cotton linter and newspaper.

In our work the effects of the fungus is first evaluated by comparing the FTIR spectra of control and of treated rice straw from fig.2(a) rice straw pulp (b) rice straw pulp treated with all the bands are shown in table (9), it is clear that difference between 2 spectra new bands appear at 2853 cm^{-1} unmodified measurements of crystalling this indicate in a mixture of cellulose 1 and 2 and also treated shows an absorpation peak at 1720 cm^{-1} and 1546 indicate unconjugated c=0 band could be related to new unconjugated acids present in the side chain of lignin macromolecules or to the biodegradation resistance of uronic acids and acetyl-branched structures in polyoses (Andre, et al 2000). Also the intensities of the bands 1163-1057 cm^{-1} were higher in rice straw pulp untreated than treated with fungus. It is clear from fig.(2) cotton linter untreated (c) and (d) treated with fungus that new band appeare at 1738 cm^{-1}, in case of newspaper fig.(e) and (f) is also the same we found that new peak at 1738 appear in case of treated with fungus fig.(2) also a new peak at 1319 cm^{-1} and 898 cm^{-1}.

CONCLUSIONS

- Some of the substrate of the medium and the organism that

influence the production of enzymes.

- The result confirm T. viride has low enzymes when used with/or without *P. funiculosum*, while *P. funiculosum* has moderate enzymes at substrate pretreatment rice straw, cotton linter and newspaper respectively.

- From IR spectra indicate presence of a new band at 1720 cm^{-1} and 1546 cm^{-1} due to unconjugated c=0 could be related to new unconjugated acids present in the side chain of lignin macromolecules or to the biodegradation resistance of uronic acids and acetyl-branched structures in polyoses.

- From scanning electron microscope we found that surface of lignocellulosic treated with P. funiculosum was rough, heterogeneous and striated, indicating that they were in the process of peeling, which gave rise to a morphological change in the surface of the fibre.

- Kappa no. decreased in the following order rice straw pulp followed by cotton linters and then newspaper. The lower kappa no. the highest the brightness.

- Brightness increased from 2-10.3 points.

- Xylanase improve bleaching reduce the consumption of chlorine chemicals and will reduce the energy of consumption and protect the environment from pollution.

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