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Investigation studies the effect of Microwave pretreatment for enhancing biobleaching techniques

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Abstract: The aim of this study is to improve the effect of xylanase activity on unbleached bagasse pulp. By comparing pulp treated with different temperatures of microwaves 200,300,400W for 3 minutes before treatment with xylanase enzyme &pulp treated directly by xylanase enzyme. By looking at four contagious strains *Aspergillus niger* by utilizing the same fungi *Aspergillus niger* was the superior to the next three strains delivering the high measure of xylanase enzyme. The Kappa number decreased from (39.6 to 26.5) i.e enhanced by 33% after treated by xylanase enzyme only This occurred since the xylanase my break down the lignin-carbohydrate bonds improving the extractability of solubilized lignin. Also, It was clear Kappa number decreased from 39.6 to 15,45 i.e enhanced by 60.98%and the brightness % increased from 42 to 49.3 %, from the pulp treated by microwave irradiation and xylanase enzyme. Enhanced the xylanase production Raising of temperature degree of the microwave irradiation during exposure of pulp has no effect on the Kappa number and brightness breaking length increased from 2230 to 2420.6 m, after exposure the pulp to microwave irradiation at 200W for 3minute. FTIR spectra, scanning electron microscopy helped elucidate changes in the fiber composition and morphology.
Keywords: unbleached bagasse, microwave irradiation, Strength properties; Optical properties, Kappa no. infrared and electro-microscope.

Introduction

Microorganisms were used to minimize pollution arising from pulping and bleaching processes.[1]

Lignocelluloses consist of cellulose microfibrils embedded in a matrix of hemi- cellulose and pectin with lignin deposited in the cell walls to give rigidity and strength. Lignin represents the major obstacle for the attack by microorganism penetration of enzyme into the substrate [2]. Pretreatment of the substrate is an important before solid state fermentation (SSF) to reduce lignin content, decrease crystallinity and increase the porosity to make the substrate more susceptible to the growth of the microorganism and hydroloytic action of the enzyme [3,4]. Pretreatment lignocelluloses by a dose of the chemical may be toxic to the enzyme or the fermentative microorganism and their removal is costly and complicated [5].

Physical methods such as sonication (electron beam and proton beam) and heat temperature (microwaves irradiation and γ-rays) are used as pretreatment stages of lignocellulose pulps and deinking owing to their ability of chemical consumption and enhance physical properties as well as optical properties. Microwave irradiation allows direct interaction between a heated object and an applied electromagnetic field to create volumetric and rapid heating which causes an explosion effect among particles and improves disruption of recalcitrant structure [6,7].

The combined treatment with microwave irradiation and xylanase increase brightness (4.5%), whiteness (5.9%), breaking length (27.6%), Viscosity (9.8%), burst factor (6.9). tear factor (3.8) and reduce Kappa number (40%) as compared to xylanase only for wheat bran pulp [8].

Microwave irradiation of wheat bran pulp is an economical, efficient and environment-friendly alternative to enhance substrate accessibility for microbial growth and xylanase by SSF [8].

A62.5% (W ⁄ W) $CaCl_2$ solution was used in the microwave pretreatment of corn Stover before enzymatic digestibility. The hemicelluloses digestibility reached 8.5% and the cellulose crystallinity index decreased by 13.91% compared to untreated corn Stover[9].

The association of microwave and glycerol as a pretreatment of lignocelluloses material at atmospheric pressure. It is a safe and efficient way for the sugar cane bagasse digestibility by hydrolytic enzymes from a newly isolated thermophilic fungus Myceliophtora thermopile M.7.7. [10].

The aim of this study is to improve the effect of xylanase activity on unbleached bagasse pulp. By comparing pulp treated with different temperatures of microwaves before treatment with xylanase enzyme &pulp treated directly by xylanase enzyme.

Materials and Method

Materials: unbleached bagasse from Nagy Hammed Company.

Treatment of the pulp**:** Dry unbleached bagasse pulps were placed in a sealed glass vessel in the microwave ((DOMESTIC MICROWAVE) 200- 300 and 400W for 3minute.

Fungal Strains Used**:** Among the 4 fungal strains were obtained froth culture collection of microbial chem. Dep. Nat.Res. Cent, Dokki, Giza, Egypt. They belonged to four genera& different species: namely *Aspergillus oryzae 185, A. Niger 66/200, A.oryzae DSM 186. A.Ochraceousn67/33.*

Media used**:** Peptone yeast agar medium Jose *et al.* [11] used for maintenance, propagation and growth of tested fungal cultures (Capek−Dox medium) and for fungal for fungal production of xylanase production of xylanase enzyme as we show in Table (1) For the estimation of xylanase was donned according to method adopted by Garg *et.al.* [12]

Fermentation method**:** This method by preparation of fungal inoculate the quantitative method for enzyme production, inocula of tested by adding 10ml of sterile tap water to slant culture (4−5 day old) of each fungal strain. The age of the slant culture was the day for production xylanase at 30 ^{o} C using rotary shaker at 150 rpm.The content of each experimental flask was taken daily and centrifuged at 5000 rpm for 20 min. using a cooling centrifuge Janet Ski, (k−26) The filtrates were used for analysis of the enzyme.

Papermaking:**:** The bioleached pulp tread or untreated by microwave radiation were beaten in a Jardan beater at 3.6 consistency until reached 38- 40 SR. sheets were prepared according to Tappi standard method using the sheet former of ABL orentzen and wetter (Stockholm, Sweden). After sheet forming, the papers were conditioned for 24h at 20 ^{0}C and then measured the optical properties (brightness and opacity) and breaking length [13].

Kappa number: It was calculated according to Scan-C [14].

Infrared (IR)

IR absorption of the paper samples was recorded using FI⁄IR 300 Elasco using KBr discs.

Scanning Electron Microscope (SEM)

A piece of paper covered gold from one face and the other face put on the holder, then enter in the scanning electron microscope (IEDL JXA-840A, Electron micro analyzer**).**

Results and discussion:

Xylanase using for bleaching of bagasse using four fungal strains in the table (1) show that *Aspergillus niger* produced the highest amount of xylanase when compared with the other species in amount production Garg, *et al.* [12] method. We found that various species of *Aspergilla* and *Penicillium* for extra- cellular production large amount of xylanase while the other species (*Oryzae, Flavus*) excreted moderate quantities. As we showing in the table (1) that for removal lignin by using the same fungi. *Aspergillus niger* was the better than the other three strains where producing the higher amount of xylanase.

Table (1) Effect of different incubation periods on the production of xylanase by four fungal strains

Fungal strains	**Incubation period (day)**							
	1	2	3	4	5	6	7	8
Aspergillus oryzae DSM(186)	No	no	no	weak	Mod	mod	no	No
Aspergillus oryzae(185)	No	weak	weak	mod	Mod	no	no	No
Aspergillus niger (66/200)	weak	weak	high	high	high	mod	weak	Weak
Aspergillus flavus (B/10)	weak	mod	mod	weak	weak	weak	weak	No

Levels of xylanase production Garg,*etal*.(1998): mod=moderate

Effect of xylanase alone and combined microwave irradiation with xylanase on the kappa number and brightness of bagasse pulp.

Hand-sheet of paper were prepared from unbleached bagasse, biobleached bagasse by xylanase enzyme and from pulp treated by microwave at 200,300 and 400 W for 3 minutes, then treated by enzyme.

Table (2) and fig(1) shown that the Kappa number decreased from (39.6 to 26.5) i.e enhanced by 33% after treated by xylanase enzyme This occurred since the xylanase my break down the lignin carbohydrate bonds improving the extractability of solubilized lignin. This led degraded lignin fragments allowing lignin removal from the cell wall. Also It was clear from Table (2) and Fig(1) Kappa number decreased from 39.6 to 15,45 i.e enhanced by 60.98%and the brightness % increased from 42 to 49.3 %, from the pulp treated by microwave irradiation and xylanase enzyme. This due to microwave irradiation allows direct interaction between a heated object an applied electromagnetic field to create volumetric and rapid heating which causes an explosion, off among particles and improves disruption of recalcitrant structure This led to rupture the rigid structure of the cell wall, opened the polysaccharide for microbial Attack, increased the air space for more aeration and consequently enhanced the xylanase production [12] Raising of temperature degree of the microwave irradiation during exposure of pulp has no effect on the Kappa number and brightness.

Table (2): Optical properties (Brightness and Opacity, Klason lignin and tensile index of the paper treated and untreated by microwave irradiation

	Experiment	Optical properties		Klason lignin%	Breaking length m
		Brightness%	Opacity%		
1	**Untreated paper**	42.0	97.4	21.8	1600
2	**Enzymatic paper**	49.3	96.5	14.6	2230
3	**Enzymatic paper treated by microwave irradiation at 200C**	56.9	95.6	8.5	2421.
4	**Enzymatic paper treated by microwave irradiation at 300C**	56.7	95.9	8.5	2270.
5	**Enzymatic paper treated by irradiation at 400C**	56.7	95.6	8.5	2041

Effect of xylanase alone and combined microwave irradiation with xylanase on the breaking length of the paper.

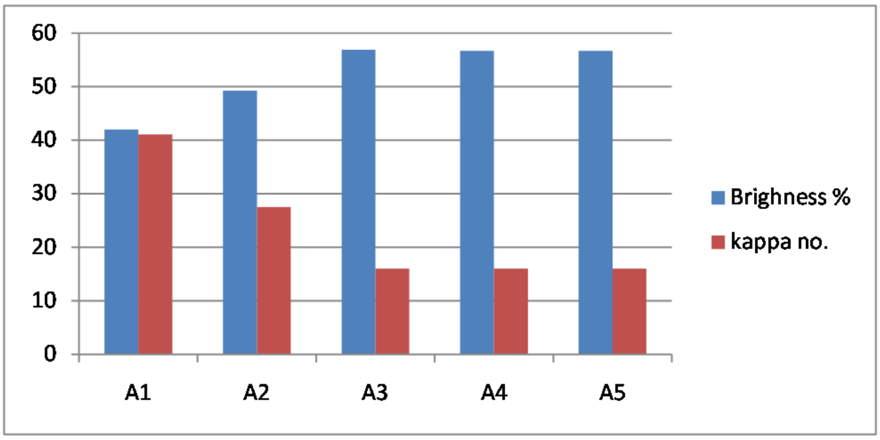

Figure (1) relation between Brightness % and Kappa no.

1. Untreated bagasse pulp
2. pulp treated with xylanase
3. pulp treated with microwave 200W + xylanase
4. pulp treated with microwave 300W + xylanase
5. pulp treated with microwave 400W + xylanase

From Table (2) we found that the breaking length of the biobleached bagasse pulp increased from 1600.4 to 223.4m. This enzymes formed threads which enter with fiber and this lead to increase the strength of paper Table (2).

Table (2) shown that the breaking length increased from 2230 to 2420.6 m. After exposure the pulp to microwave irradiation at 200°C for 3minute. Microwave irradiation enhanced the physico-mechanical properties of the bioleached pulp. Microwave irradiation is a source of efficient heating of the pulp with less energy and short time and can be applied to pulp at high consistencies. These maintain the durability of fibers and strengthen it. But the breaking length decreased clearly from 2230 to 2041.2 m after exposure the pulp to microwave irradiation at 400C. This may be attributed to the reduction of polysaccharide (carbonization indicated by IR) i.e. detoration of hemicelluloses which bond the fiber to each other and this lead to decrease the strength of the paper (8,15).

Infrared spectra

FTIR untreated bagasse pulp and the bagasse pulp treated with xylanase & irradiated with microwaves at different temperatures were observed by infrared spectra (Fig.2).

The absorption bands at 3450-2906cm^{-1}. ,assigned as a stretching due to stretching Vibration of the OHbonds of various types, meanwhile its position reveals the existence of hydrogen bonds of different strengths in cellulose, hemicellulose and lignin The bands within the 3000—3070 cm^{-1} ränge are due to the aromatic C—H stretching vibrations, while those at 2950—2870 cm-1 are assigned to the aliphatic C—H Vibration.

The 1800—1500 cm^{-1} region includes several broad bands which are not well resolved, the bands at 1750 to 1670 cm^{-1} correspond to the stretching vibrations of the C = O groups of various types namely those of the acetyl linkage (1750 cm^{-1}), and those at (1730 and 1670 cm^{-1}) are due to the ß-keto structure. The shoulder near 1630 cm"1 is due to the in-plane deformation of the water molecules, while the bands at 1610, 1590 and 1520cm^{-1} are due to Vibration of the aromatic ring.

The band at 1470 cm^{-1} is assigned to C—H bending of the pyran ring, while that at 1430 cm^{-1} is considered äs the in plane bending of the CH3O-groups. The inplane bending of the CH3-groups leads to the broad band at 1350 cm^{-1}. The two peaks at 1280 and 1240 cm^{-1} are assigned to the deformation vibrations of the —COOH and phenolic OH-groups respectively. The other types of vibrations involving the OH-groups yields the absorption bands at 1180 and 1130cm-1. However, it was stated that the first band also includes the stretching mode of the aliphatic-aryl-ether linkage.

The broad bands within the 1300—1000 cm^{-1} region would also involve some types of bending vibrations of the aromatic C—H and those of the aliphatic.

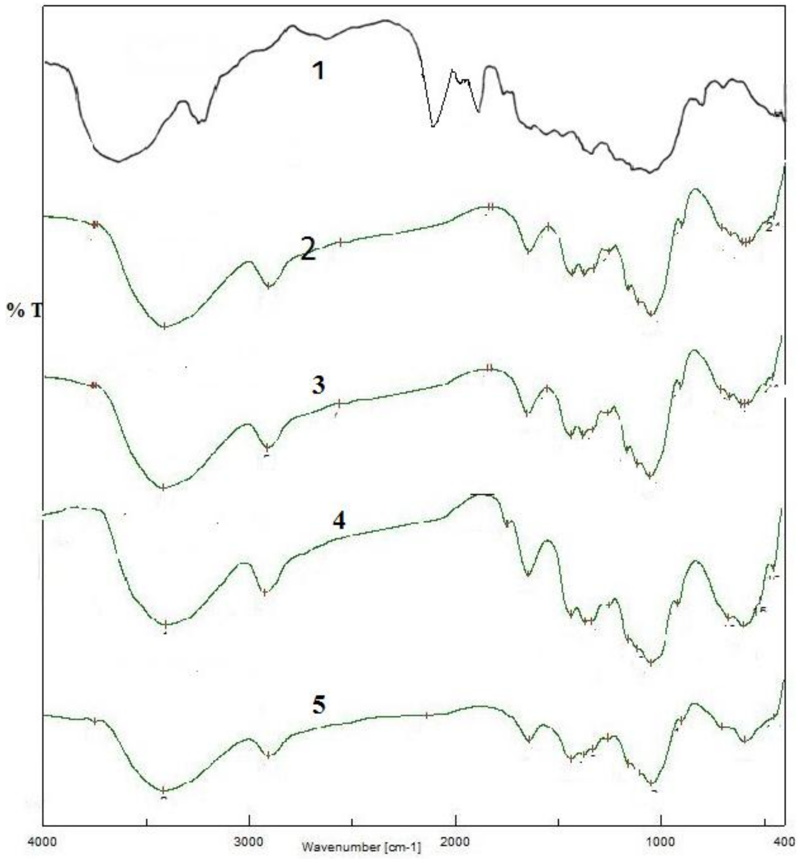

Figure (2) IR spectra of the samples

1. Untreated bagasse pulp
2. pulp treated with xylanase
3. pulp treated with microwave 200W + xylanase
4. pulp treated with microwave 300W + xylanase
5. pulp treated with microwave 400W + xylanase

There are attenuation bands attributed to lignin such as the stretching of the carbonyl group (1728 cm^{-1}), vibration of the aromatic ring (1635, 1600, 1510 cm^{-1}) [16], syringyl group (1374 cm^{-1}, Sahoo et al. [17]) and in the C-H out of plane in the p-hydroxyphenyl propane units (833 cm_1, Hoareau et al. [18]; Ciobanu et al. [19]). These alterations in the IR spectra are consistent with the lignin removal detected by chemical analysis (Table 1).

However, the biggest alteration was observed in the bands associated with cellulose and hemicellulose core structures such as those attributed to an overlap of C–O–H elongation of primary and secondary alcohols (at 980 cm^{-1}), C–O–C stretching of glycosidic linkages (at 1100 cm^{-1}), vibration of the ring C–O–C in hemicellulose (at 1050–1170 cm^{-1}) [20,21]. The band at 1170 cm^{-1} is typical of the arabinosyl side chains and its low intensity suggests that the hemicellulose was more sensitive to treatment than cellulose. These data are in accordance with Hendriks and Zeeman [22] and are corroborated by the chemical analysis which shows a decrease in the hemicellulose content.

The absorption bands at 3440 and 2906 cm^{-1}, assigned as a stretching of the hydroxyl groups present in cellulose, hemicellulose and lignin, and the axial deformation of C–H, respectively, present in whole bagasse [23] did not show significant changes.

This suggested that the general structure was kept unchanged. These data allow the inference that the microwave treatment remove lignin and hemicellulose preserving the cellulose structure.

Scanning Electron Microscope

Fig(3;1-5)show that photos of bagasse pulp treated and untreated prior and then afterward presenting to microwave irradiation at 200,300 and 400° C

The fiber is not clear in fig.3:1 untreated pulp and there are more fibers and fibrils was pronounced.Cracks and voids are present clearly in this photo. In fig 3;2 (enzymatic pulp) the strands are obviously than untreated pulp and there are little amounts of fibrils, void, and cracks [24].

In the photo of the enzymatic pulp Fig (3;3-5) enzymatic pulp exposed to microwave irradiation at 200,300 and 400 W respectively. These Figs are very similar to each other fibers are very clear. Both fibrils, voids and cracks are clearly disappeared to a large extent. it is clear that the pentosans and lignin content are higher in the bagasse pulp.

These pentosans are softened by heating, enzyme treatment which enhance adhesion of the fibers together, and has more compact structure than untreated pulp, showing a cleaner pulp with no of fibrillation or filaments between the fibers no cracks or void thus the crack does not have a straight path because it has to move around the fiber cells & ultimately stops[24]. These changes have a great effect on the surface was indicated by SEM. The fibers are high flexibility & conformability and this lead to good bonding were more visible.

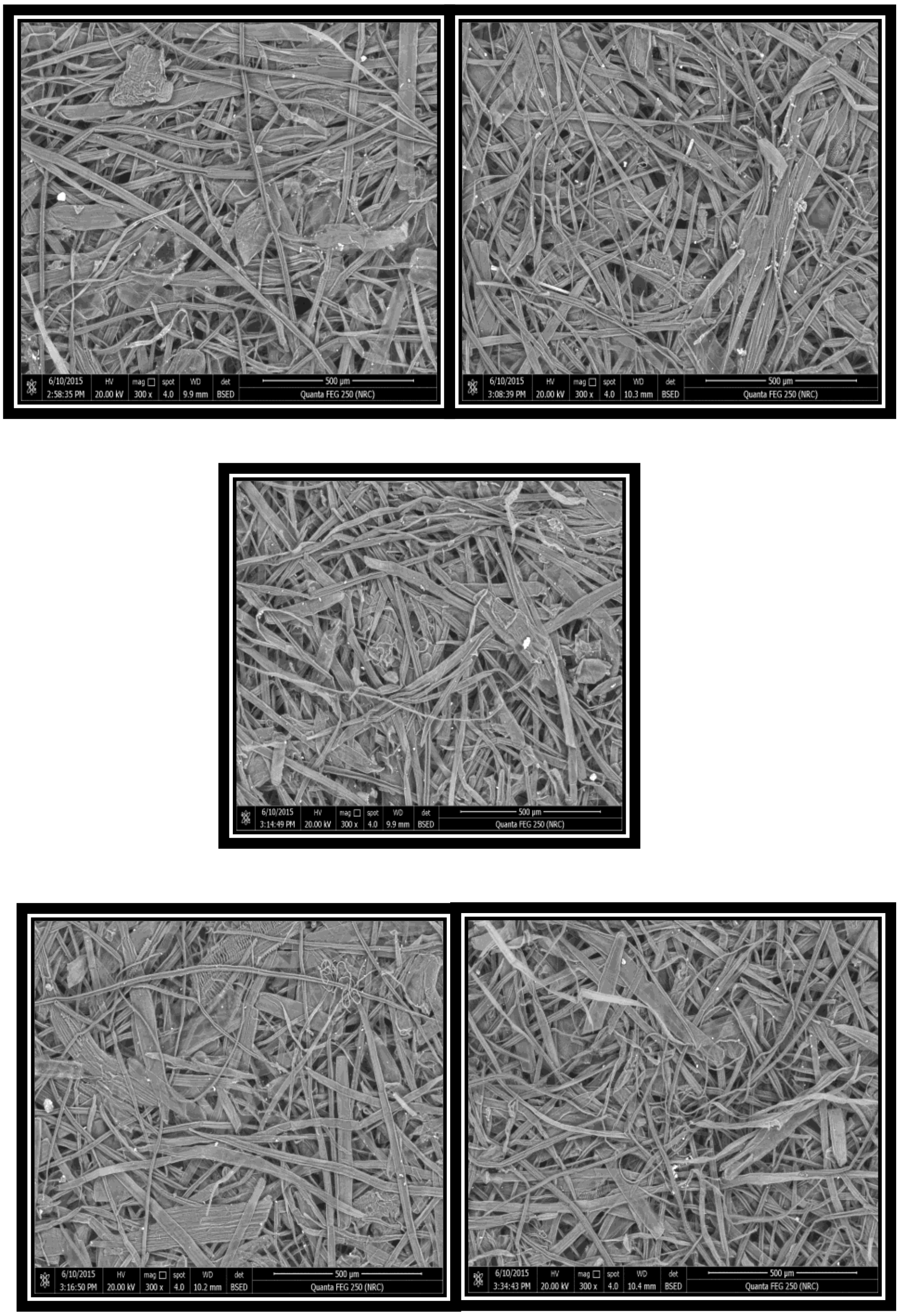

Fig. (3) Scanning electron micrographs

1. Untreated bagasse pulp
2. pulp treated with xylanase
3. pulp treated with microwave 200W+ xylanase
4. pulp treated with microwave 300W+ xylanase
5. pulp treated with microwave 400W + xylanase

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