Biochemical Pulping Of Sugarcane Bagasse Using Ceriporiopsis Subvermispora SS- 33 Fungal Influenced By Physical And Biological Pretreatments

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ABSTRACT

The physical and biological factors as well as propylene glycol (PG) were used in this work to evaluate the chemical composition of bagasse biopulping with *Ceriporiopsis subvermispora.* The best favorable conditions for bagasse pulping with PG at atmospheric pressure and on autoclave were 90 % PG concentration for two and one hours at 150°C, respectively, which gave higher pulp yield, no rejects, and lower kappa number. The extractive removal of bagasse 10-mesh size by steam extraction gave higher weight loss, APPL production and lowest kappa no. The unbleached paper sheets made from the former treatment recorded highest brightness, breaking length, and tear factor. Whereas the highest extractives loss percent was attained by biological treatment of bagasse treated by *Ophiostoma piliferum* for four weeks at 27°C on MY medium. This increased the brightness, breaking length, and tear factor of unbleached bagasse paper sheets than that obtained from steam treatment. Using both fungal *(Ceriporiopsis subvermispora* and *Ophiostoma piliferum)* strains for extractive removal and bagasse biodegradation in mixed culture as one or two stage cultivation led to improvement in the chemical pulp composition and the properties of unbleached paper sheets. Mixed fungal culture at one stage cultivation increased the unbleached paper sheets properties as brightness, breaking length and tear factor about 5.6%, 0.08 %, and 3.78 and about 3.08%, 21.41 %, and 3.69 respectively, as compared to that obtained by mixed culture at two-stage and steam extraction method respectively. Moreover, the biological fibers of hand sheets exhibit a cleaner surface, high flexibility and conformability, which would contribute to good bonding.

Keywords*: biopulping, bagasse, biodegradation, extractive removal,*

Introduction

Interest in renewable recourses for obtaining industrial product such as chemicals, pulp and paper, and fuels has given rise to an increase in research on lignin biodegradation. A rational utilization of most of lignocellulosics, especially those obtained as residues, has been pursued. Sugarcane bagasse is an agricultural waste abundant in several countries. The possibility of using bagasse pulps for the production of newsprint was studied as far back as 1950 **(Johnsrud et al, 1987)**.

Pulp production by organosolv process has been evaluated using several wood species and a broad range of organic solvents in acid or alkaline media Organosolv pulping is suitable for different. mills since such pulping is economical and pollution-free **(Messner et al, 1998)**. In addition, it yields major components of wood as valuable by-products.

One of the most important characteristics is their ability to degrade large concentrations of pitch (i.e., fatty acids and resin acids) from wood. Pitch is a major problem in pulp mill operations, resulting in pitch deposits on the paper machines and lowered quality of the finished product. Since these fungi can rapidly colonize nonsterile wood chips and remove pitch, they are ideally suited for biological pretreating of wood before pulping. The screening of blue stain fungi for their capacity to degrade pitch has identified the species Ophiostoma piliferum, with superior abilities for removing pitch from wood **(Blanchette et al, 1992)**.

Biopulping is the biological pretreatment of wood chips followed by a mechanical or chemical pulping processes. Ceriporiopsis subvermispora is one of the most suitable white-rot fungi for biopulping. Wood pretreatment with this fungus leads to a reduction of energy consumption during mechanical pulping by at least 30 %. Furthermore, a considerable improvement in pulp strength is obtained **(Akhtar, 1998).** Fungal pretreatment also provided increased delignification rate in acid sulfate and bisulfite pulping **(Messner et al, 1998)** as well as in organosolv pulping processes such as formic acid / acetone and methanol/water pulping **(Ferraz et al, 2000).** These increased delignification rates permitted preparation of biopulps in shorter cooking times, resulting in energy savings or increased digester throughput. In addition, biopulps obtained by formic acid I acetone pulping of pinus radiate biotreated with Ceriporiopsis subvermispora has

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increased tensile strength **(Ferraz et al, 2000).**

Therefore, it was found of interest to study the effect of the pretreatment with propylene glycol (PG), hot water, steam and biological treatment with Ophiostoma piliferum and Ceriporiopsis subvermispora in single or mixed culture on the improving bagasse degradation, chemical biopulping composition and paper sheets properties as brightness, breaking length and tear factor.

Materials And Methods

1- Lignocellulosic materials

The Egyptian depithed Sugarcane bagasse (Sacaharum officinurum) was obtained from El- Nasr Company for Sugar and pulp industry at Edfu. Depithing of bagasse was carried out by the dry method.

2- Microorganisms used

White-rot fungus namely, Ceriporiopsis subvermispora CZ-3, and ascomycetes fungus Ophiostoma piliferum, were obtained from NRRL the culture collection of Northern Regional Research Laboratory Department of Agriculture, Peoria, IL.

3- Media used

Peptone yeast extract agar medium (Jose et al, 2000) was used for fungal propagation and preservation of fungi. It contains of (g/l): Glucose, 20.0; Yeast extract, 2.0; Peptone, 5.0; KH_2P0_4, 1.0; $MgS0_4.5H_2O$,0.5; and Agar, 17.0; with pH adjusted to 5.0 . MV medium (Hatakka and Pirhonen, 1985) It consists of (g/l): KH_2P0_4, 2.0; $(NH4)_2.7H_20$, 2.1; $MgS0_4.5H_20$, 0.3; $CaCl_2$, 0.5; Yeast extract, 0.5; FeS04.7H20, 0.005; $MnS0_4.H_20$, 0.0015; ZnS04.H20, 0.0014; and $COCl_2.6H_20$, 0.00266 with pH adjusted to 5.0.

4- Standard inoculum

The standard inoculum of fungal strains was prepared by adding 10 ml of sterile peptone yeast extract medium (medium 1) to slant cultures (3-5-day old). Then dispensed in a conical flask (250 ml in volume) containing 100 ml of peptone yeast extract medium (medium 1). The inoculated flasks were incubated at 27 C for 7- 10 days on a rotary shaker (185 rpm). The content of these cultures was used as a standard inoculum for the current experiments (1 0 ml = 0.15 g dry weight) .

5. factors affecting bagasse biodegradation

5.1. Propylene glycol pulping

25 grams of depithed bagasse were firstly pretreated with 95% propylene glycol (PG) at liquor ratio of 1:6 (w/v) in one - liter flask and heated at 60°C for 30 minutes. The liquor was filtered, then fresh liquor of PG having different concentrations (80 -100%) and 0.29% sulfuric acid (wt/wt of liquor) was added. The pulping time and temperature varied from 1-3 hours and 140-170°C, respectively. The flasks were put in oil bath and heated under reflux for specified reaction time. After pulping, the crude pulp was filtered and washed successively with 80% aqueous acetone and water. Pulping was carried out under pressure in an autoclave using the same conditions of PG concentrations, pulping time, liquor ratio and catalyst at 150°C. After washing, the pulp was screened to remove the untreated bagasse. The yield of screened pulp and rejects were determined.

5.2. Physical treatment

5.2.1. Grinding of bagasse

Different bagasse mesh size i.e., 40, 20 and 10 mesh was used to study their effect on bagasse biodegradation by white-rot fungus Ceriporiopsis subvermispora CZ-3. Fifty grams from different bagasse mesh size were placed in 3-1iter Erylenmyer flask and autoclaved at 121°C for 15 minutes. After cooling at room temperature the sterilized bagasse were inoculated with 10 ml of the standard inoculum of white-rot fungus strain, then 100 ml of medium 2 were added to these flasks. The inoculated flasks were incubated at 27± 1 °C for 4 weeks. After incubation, fungal treated and untreated samples were harvested for determination of their weight loss and acid precipitable polymeric lignin (APPL).

5.2.2. The extractive removal

These experiments were conducted to remove the resins, fats, and waxes with two physical methods by boiling distilled water and steam using soxhlet extraction apparatus. 50 grams of bagasse were placed in an extraction thimble and extracted for 1 hour in the soxhlet apparatus with the boiling distilled water. Whereas in steam method 50g of bagasse 10 mesh size was steamed in autoclave for 1 hour. The bagasse was transferred to a Buchner funnel to wash with water. The water wash consists of covering the sample on the Buchner funnel with water and letting it stand for 10 minutes with suction off. The suction was then turned on; pulling off most of the water, and the sample was then washed three more times with the suction on. The suction was then turned off again and the same sequence repeated two more times. Thereafter the sample was air-dried. Fifty grams from extractives free bagasse was placed in 3-liter Erylenmyer flask and autoc1aved at 121°C for 20 minutes. After cooling at room temperature, the inoculation and propagation were carried out as mentioned before, the chemical analysis for APPL production and weight loss were determined at the end of incubation period.

Unbleached paper sheets were made from biopulping by Ceriporiopsis subvermispora CZ-3 treated with physically extraction treatment using 25grams of air-dried extractive-free or unextracted bagasse during the incubation and propagation procedures. At the end of incubation period, fungal treated and untreated samples were harvested for pulping with 90% propylene glycol (PG) at liquor ratio of 1:6 (w/v) at 150°C for two hrs. After pulping samples were harvested for determination of their chemical composition and pulp properties of unbleached bagasse pulp.

5.3. Biological pretreatment

5.3.1. Pretreatment of bagasse with *Ophiostoma piliferum*

This experiment was carried out to study the effect of pretreatment of bagasse with Ophiostoma piliferum on extractives content of bagasse. Fifty grams from depithed bagasse were placed in 3-liter Erylenmyer flask and autoc1aved at 121°C for 20 minutes. After cooling at room temperature the previous procedures of propagation, determination of extractives content, pulping and chemical analysis were done as mentioned before.

5.3.2. Pretreatment of bagasse with mixed culture of Ophiostoma *piliferum and Ceriporiopsis subvermispora* CZ-3

The effect of fungal mixed cultures on biodegradation of bagasse was studied in one stage and consequence cultivation in two stages. In one stage of mixed culture, the sterilized bagasse was inoculated with 10 ml of the standard inoculum of both fungal strains . Twenty-five grams of oven -

dried bagasse 10 mesh size was placed in 3-liter Erylenmyer flask and autoc1aved at 121°C for 20 minutes. The sterilized bagasse was inoculated with 10 ml of the standard inoculum of both fungal strains. But in two- stage culture, the sterilized bagasse was inoculated with 10 ml of Ophiostoma piliferum standard inoculum then inocubated at 27 ± 1°C for different incubation period (1, 2, 3, and 4. weeks) as first stage of cultivation. At the end of incubation period, it was autoc1aved at 121°C for 20 minutes then the flasks were inculated with Ceriporiopsis subvermispora CZ-3 and incubated for 4 weeks. The procedures of propagation and pulping were carried out as mentioned before.

6. Pulp and paper sheets making

The pulps were beaten for different intervals, using Jokro beater at 6% pulp consistency at a speed of 150 r.p.m., and the degree of freeness (SR) was measured by making up the mixture to 2 liters with water and disintegrated for two minutes in the standard disintegrator with the propeller running at 3000 r.p.m. The °SR was measured according to the German standard method. Paper sheets were prepared from the different pulps according to the S.C.A standard using the model S.C.A. sheet former (AB Lorentzen and Wettre). In the apparatus, sheets of 165-mm diameter (214-cm^2 surface) were formed. Then the sheets were pressed for 4 minutes using a hydraulic press. The test pressure was adjusted at five Kg/cm2. A rotating drum at 100°C made drying of the sheet for 2 hours. The sheets were placed for conditioning to 65% relative humidity and temperature 18-20°C.

7. Chemical analysis

Weight loss of treated bagasse and APPL Production were determined according to the method of Anthony et at (1986) and Crawford et at (1983) respectively. Hollocellulose, hemicellulose, a-cellulose, and extractives content were determined according to the standard method suggested by Browning (1967). With respect to paper sheets properties, Brightness was determined using a hunter- lab calorimeter. Kappa no was determined according to TAPPI standard T-236m. Degree of polymerization was determined according to SCAN- cm 15:88. Breaking length (km) was measured using a universal testing machine (Lloyd). The length of the strip of paper used was 10 cm and the width was 15 mm. The rate of cross head movement was 4 inches/min. The tear factor was determined according to the internal tearing resistance and measured by a pendulum type instrument (Elmen drof Tearing Tester).

8. Scanning electron micrography

In order to understand the effect of biological pretreatment of bagasse, the morphology of fiber surfaces after fungal treated and untreated bagasse pulp was studied by electron microscope (JXA-840A Electron Probe Micro Analyzer Scanning Electron Microscope).

Results And Discussion

1. Evaluation of pulping by propylene glycol (PG) under pressure and atmospheric pressure

In order to study the influence of the cooking temperature on pulp yield, rejects, degree of polymerization, kappa no and optical properties, laboratory trials were performed simulating best condition for the pulp production. So, the sugarcane bagasse was treated with different PG concentrations (l00, 95, and 90%) and cooking at three different temperature (150, 160, and 170°C) for different cooked period being 1, 2, and 3 hours. Because no pulping occurred at 85% PG or 140°C cooking temperature. The results of these experiments indicate to the selectivity of the lignin dissolution was practically independent of operating conditions. When concentration of PG reached a value, 100% the selectivity decreased considerably due to lignin condensation. The highest values of screened yield % were obtained by using 90% PG for one hour cooking time being 43.27, 42.14 and 45.62 at cooking temperature 150, 160 and 170°C, respectively. At this PG concentration (90%) complete removal

Table (1) : Effect of pupling time and PG concentration on chemical composition of bassage at 150°C under prssure and atmospheric pressure.

	Under pressure							At atmospheric pressure					
	Propylene glycol concentration												
		100%		95%		90%		100%		95%		90%	
Chemical Pulp composition	Pulping time (hrs)	1	2	1	2	1	2	1	2	1	2	1	2
Screebed yield%		43.48	42.52	44.06	43.43	43.51	44.94	35.88	35.51	38.11	63.35	43.27	40.07
Rejects		5.91	2.03	2.53	1.92	0.0	0.0	6.61	4.44	4.34	2.56	1.28	0.0
Hollocellulose %		81.91	81.06	82.91	80.28	89.85	81.78	88.69	90.41	89.94	92.41	92.54	93.77
□ - cellulose %		80.13	78.35	79.85	77.95	79.02	67.51	87.59	85.46	80.54	82.66	85.22	81.96
Pentosans %		11.08	10.00	10.49	9.79	9.28	9.01	12.38	11.29	11.92	9.64	10.33	9.01
Kappa no		38.65	37.52	35.84	8.11	29.33	34.85	30.56	29.16	29.05	26.0	26.11	22.45
DP		959	919	948	939	938	918	1356	1270	1321	1146	1253	1100
Brightness %		27.0	26.4	28.0	27.4	30.0	28.9	30.7	32.3	32.4	35.5	35.7	37.8

of rejects from treated bagasse occurred after 2 hours at different tested temperatures. The values of kappa no recorded the lowest figures after 2 hours cooking period, then increased by increasing the cooking period to 3 hours at different treatments of PG concentration and different temperatures. This may be due to lignin condensation. Whereas the lowest value of kappa no being 22.45 was recorded by bagasse treated with 90% PG at 150°C for 2 hours. Also the highest brightness value was attained at this treatment (not shown). So, it could be stated that the best favorable conditions for pulping with PG at atmospheric pressure was 90% for 2 hours at 150°C which gave higher pulp yield, no rejects and lower kappa no. Also, the best treatment for pulping on autoclave (under pressure), which gave higher delignification rate, high brightness (30.3%), DP slightly reduced (938), kappa no of 29.33 and no rejects obtained was at 90% PG concentration and pulping for one hour, as shown in Table (1). Comparing the effect of pulping by PG atmospheric pressure with pulping on autoclave (under pressure), it could be noticed that pulping at atmospheric pressure at 150°C for 2 hours was better than autoclave.

Some pretreatment affecting on biodegradation of bagasse by *Ceriporiopsis subvermispora* SS-33

a- Physical pretreatments

• Bagasse mesh size

Three different mesh sizes of bagasse, i.e, 10, 15, and 20 mesh were used to facilitate bagasse biodegradation by *Ceriporiopsis subvermispora* SS- 33. Data in Table (2) show that increasing the mesh size of bagasse led to increasing the percentage of weight loss and APPL to reach the maximum values being 26.29 and 2.98%, at 20 mesh respectively. Applying this treatment resulted in very week pulp after chemical pulping by propylene glycol and sulfuric acid catalyst. This may be due to severe degradation of bagasse fibrous structure and fines fibrous formation, which reduce the yield and strength of the produced pulp. No remarkable increase in both weight loss and APPL production was occurred by increasing the bagasse mesh size from 10 to 15 meshes.

Generally, it could be stated that the 10-mesh size fraction was the favorate size to obtain the best pulp. So, this size was used in the following experiments.

Table (2):
Effect of bagasse mesh size on weight loss and APPL production from bagasse treated by *Ceriporiopsis subvermispora* SS- 33 for 4 weeks.

Mesh size of bagasse	% Weight loss	% APPL
Raw bagasse	20.54	1.63
10 mesh	21.72	1.94
15 mesh	21.77	1.96
20 mesh	26.29	2.98

• The physical extractives removal

Data illustrated in Fig. (1) Indicate superiority of the steam extraction method for extractive removal from bagasse leading to increase the percentage of weight loss and APPL production by 1.81 and 1.04%, respectively, as compared with untreated bagasse. Whereas the hot-water extraction method gave approximately the similar result with that obtained by untreated bagasse.

Generally, it could be concluded that using bagasse at 10 mesh- size treated with steam for one hour were the best physical pretreatments of bagasse to remove the extractives and give proper fibers for the optimum biodegradation by *Ceriporiopsis subvermispora* SS- 33 for four weeks at 27°C. So, the effect of previous physical pretreatments of bagasse on chemical composition and properties of unbleached bagasse sheets were studied using biopulping treated with *Ceriporiopsis subvermispora* SS-33. The obtained results were compared with that obtained by untreated biopulping and chemical pulp.

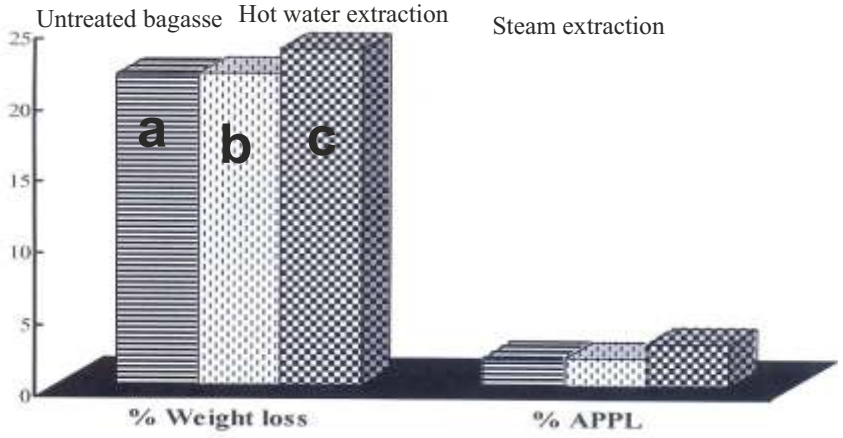


Fig. (1): Effect of some physical method of bagasse on weight loss and APPL production from bagasse treated by *Ceriporiopsis sub-vermispora* SS-33 for 4 weeks.

Data presented in Table (3) indicate that using physical pretreatment of bagasse before biopulping with *Ceriporiopsis subvermispora* SS- 33 led to increase the pulp yield of organosolv pulping process by about 1.0 and 3.38% by hot water and steam extraction methods, respectively, as compared with that produced by untreated biopulping. These results are in line with those obtained by Adilson ***et at*** **(1998)** and **ferraz *et at* (2000)** who reported that the higher screened yield of treated samples than the undecayed control indicates that *Ceriporiopsis subvermispora* SS- 33 biodegraded samples were defibrated more easily than the undecayed control, probably owing to the faster lignin removal achieved in the biotreated sample.

Also, the percentage of hollocellulose content gave the same trend of yield. The lowest kappa no was noticed of biopulping treated with steam treatment followed by hot water extraction being 15.18 and 17.58% respectively. Moreover, steam treatment gave cellulose and pentosans content lower than those obtained with hot water treatment. These results are in agreement with those obtained by **Typuk *et ai,* (2000)** and (2002) who found that the biologically treatments resulted in decrease in kappa no which were accompanied with decrease in lignin content and high value of brightness. Also biologically, treatments caused slight decrease in

cellulose compared to untreated chips.

With respect to strength and optical properties of paper hand sheets made from chemical and biopulping of bagasse, it could be noticed that the highest figures of brightness, breaking length and tear factor being 43.9 %, 4.11 km, and 56.9, respectively, were obtained by using steam extraction method.

From the previous results, it could be noticed that using the steam extraction method for extractives removal before biopulping of bagasse decreased the kappa number about 22.35 and 32.38% and increased the brightness about 7.26 and 16.14% as compared to untreated biopulping and chemical pulping, respectively. So, this treatment was applied in the further experiments. In similar studies, **Bajpai *et al* (2004)** found that treatment of depithed bagasse with different strains of C. *subvermispora* reduced the kappa number by 10-15% and increased unbleached pulp brightness by 1.1-2.0 ISO points on chemical pulping at the same alkali charge. Fungal treatment did not show in any adverse effect on the strength properties of pulp.

b- Biological pretreatment

• Effect of pretreatment with *Ophiostoma piliferum* at different incubation periods

Data illustrated in Fig. (2) show that the activity of extractives degradation, which expressed as high loss of weight and extractives content was increased by increasing the incubation period of *Ophiostoma piliferum* at 27°C on bagasse MV medium to reach the maximum values being 10.77 and 2.0 %, respectively, after 4 weeks. While the hot water and steam extraction gave loss of extractives content being 30.13 and 26.23%, respectively (not shown). Also, **Blanchette *et at* (1992) and Jose *et at* (2000)** used *Ophiostoma piliferum* to reduce pitch in wood chips and pine sapwood, respectively. The results of **Jose *et at* (2000)** indicated the potential of wood pretreatment with the selected sapstain fungi for minimizing pitch problems and decreasing effluents toxicity in pulping. The results also showed that not only does overall pitch content decrease with treatment of wood chips by the *Ophiostoma piliferum* strains tested, but also many free resin acids and fatty acids are significantly reduced. Therefore, bleach chemicals can be saved in the processing of mechanical pulps to give higher brightness paper.

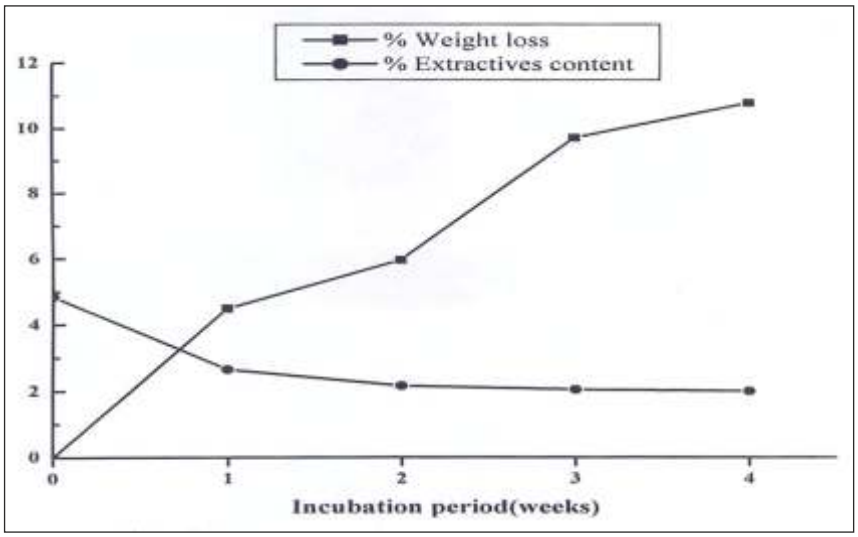


Fig. (2): Weight loss and extractives content of bagasse treated by *Ophiostoma piliferum* at different incubation period.

Table (3): Effect of physical pretreatment on the pulp composition of treated biopulping with *Ceriporiopsis subvermispora* SS- 33 at 27 °C for 4 weeks, untreated biopulping and chemical pulping and strenght and optical properties of unbleached paper sheets.

	Physical treatment			
Pulp composition	**Biopulping**	**Water extraction**	**Steatmed**	**Chemical Pulp**
Screened yield %	42.29	42.67	43.72	40.07
Hollocellulose %	95.02	96.95	97.51	93.77
? - cellulose %	80.13	80.00	79.88	81.96
Pentosans %	8.15	8.57	8.25	9.01
Kappa no	19.55	17.58	15.18	22.45
DP	985	1007	992	1100
Paper sheets properties				
Brightness %	40.93	42.52	43.9	37.8
Breaking	3.99	4.04	4.11	3.15
length(km.)				
Tear factor	55.22	56.08	56.9	53.16

The pretreatment of bagasse with *Ophiostoma piliferum* which recorded the highest extractive removal also caused slight decrease of screened yield, α - cellulose, and pentosans percentages of produced pulp than that obtained by chemical pulping as presented in Table (4). Kappa number of bagasse after fungal treatment for 4 weeks incubation was lower than that obtained by chemical pulping by 10.65 %. On the other hand, brightness, breaking length and tear factor increased with *Ophiostoma piliferum* treatment about 3.15 %, 25.4% and 4.74 %, respectively. Also, **Wall *et al* (1996)** showed that the use of *Ophiostoma piliferum* for hardwood treatment resulted in extractive degradation, improvement in chemical pulping

efficiency and reduction of kappa number up to 29 % at constant kappa number / viscosity ratio during laboratory kraft pulping of aspen wood.

• Unbleached paper sheets as influenced by mixed culture of *Ophiostoma piliferum* and *Ceriporiopsis subvermispora* SS- 33 as one stage bagasse degradation

The biodegradation of bagasse of 10 mesh size was carried out by using a mixed culture of *Ophiostoma piliferum* and *Ceriporiopsis subvermispora* SS.-33 in MV medium at 27°C for different incubation periods. Data given in Table (5) show that the α- cellulose and pentosans% contents of biopulped bagasse were decreased during incubation period of this biological pretreatment than that present in chemical pulping to reach the minimum Value at end of incubation. Causing decrease in kappa number and increase in brightness by 44.77and 21.59 %, respectively. Increasing incubation periods more than 2 weeks resulted, no remarkable decrease in kappa number or brightness was observed. Also, data indicate that using the pretreatment with mixed culture for one week gave the highest increase of screened yield%, breaking length, and tear factor being 43.21 %, 4.99 %, and 59.0, respectively, then decreased during incubation. Therefore, it is recommended to pretreatment of bagasse with mixed culture of *Ophiostoma piliferum* and *Ceriporiopsis subvermispora* SS- 33 for one week at 27 C in MV medium as static conditions to improve the pulp properties.

Table (4): Chemical composition and pulp properties of un bleached paper sheets made from bagasse treated with *Ophiostoma piliferum* for different incubation periods

	Incubation period (week)				
Pulp composition	**Chemical pulp**	**1**	**2**	**3**	**4**
Screened yield %	40.07	37.33	36.72	36.42	36.15
Hollocellulose %	93.77	93.82	93.99	94.55	94.71
□ - cellulose %	81.96	81.77	81.72	81.55	81.50
Pentosans %	9.01	8.34	8.13	8.02	8.01
Kappano	22.45	22.05	21.14	20.32	20.06
DP	1100	1099	1095	1084	1065
Paper sheets properties					
Brightness %	37.8	37.99	38.15	38.55	38.99
Breaking length(km.)	3.15	3.35	3.75	3.92	3.95
Tear factor	53.16	55.11	55.45	55.55	55.68

Table (5): Chemical composition and pulp properties of un bleached paper sheets influenced by mixed culture of *Ophiostoma piliferum* and *Ceriporiopsis subvermispora* SS- 33 as one stage bagasse degradation

	Incubation period (week)				
Pulp composition	Chemical pulp	1	2	3	4
Screened yield %	40.07	43.21	39.29	37.05	35.50
Hollocellulose %	93.77	97.68	97.72	97.91	97.93
□ - cellulose %	81.96	81.09	80.18	77.12	7.08
Pentosans %	9.01	7.56	6.61	4.51	4.11
Kappano	22.45	14.15	12.44	12.42	12.40
DP	1100	1009	1005	895	892
Paper sheets properties					
Brightness %	37.8	45.25	45.95	45.96	45.96
Breaking length(km.)	3.15	4.99	3.92	2.43	2.05
Tear factor	53.16	59	56.12	44.66	44.62

• Un bleached paper sheets as influenced by consequence two- stage degradation of bagasse by *Ophiostoma piliferum* and *Ceriporiopsis subvermispora* SS- 33.

In this experiment, bagasse was treated with *Ophiostoma piliferum* for different incubation periods (1, 2, 3, and 4 weeks) in the first stage of degradation at 27°C. Samples were taken periodically every one week and autoc1aved at 121° C for 20 minutes, then treated with *Ceriporiopsis subvermispora* SS- 33 for 4 weeks. Data of pulp composition and strength as well as optical properties of paper hand sheets are recorded in Table (6). These data indicate that the behavior of fungal strains in mixed culture as two stage of bagasse degradation was varied from that occurred in mixed culture as one stage degradation for four weeks.

Comparing the properties of paper hand sheets made from both treatments of biopulping with mixed culture, it could be stated that using both *Ophiostoma piliferum* and *Ceriporiopsis subvermispora* SS-33 as mixed culture for bagasse degradation in one stage was the best biotreatment. Where this treatment increased brightness, breaking length, and tear factor about 5.6% 0.08%, 3.78%, and about 3.08 %, 2.41 %, 3.69%, as well as 19.71 %, 58.41 %, and 11% compared to biopulping of two stage degradation, biopulping of steam extraction, and chemical pulping, respectively.

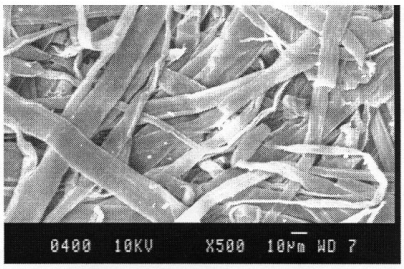
Scanning electron microscope (SEM) of unbleached bagasse pulp influenced by fungal pretreatment

Scanning electron micrographs from the pulp are shown in Photo (1). Comparing the hand sheets made from the biologically treated with mixed culture from *Ceriporiopsis subvermispora* SS- 33 and *Ophiostoma*

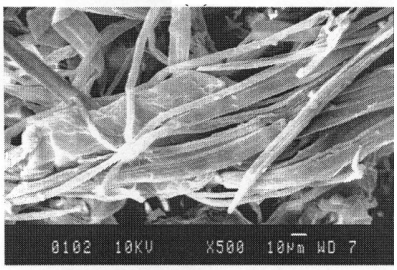
Table (6): Chemical composition and pulp properties of un bleached paper sheets with two stage of bagasse degradation by *Ophiostoma piliferum* and *Ceriporiopsis subvermispora* SS- 33 at different incubation period

		Incubation period (week)			
Pulp composition	Chemical pulp	1	2	3	4
Screened yield %	40.07	43.13	37.68	35.84	32.39
Hollocellulose %	93.77	96.25	96.61	96.85	96.85
□ - cellulose %	81.96	80.44	77.74	76.73	75.82
Pentosans %	9.01	8.15	7.77	7.23	6.55
Kappa no	22.45	17.00	14.55	1423	14.15
DP	1100	1007	899	857	845
Paper sheets properties					
Brightness %	37.8	42.85	44.77	44.65	44.88
Breaking length(km.)	3.15	4.95	2.13	1.77	1.52
Tear factor	53.16	56.85	31.53	25.23	23.03

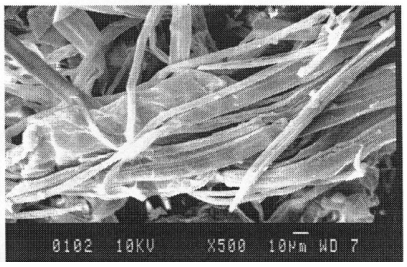
(a)



(b)



(c)



piliferum at one stage cultivation, *Ceriporiopsis subvermispora* SS- 33 in single fungal culture, and untreated chemical pulp. It could be observed that the treated fibers exhibit a cleaner surface and an apparent high flexibility and conformability, which would contribute to good bonding, were more visible in the mixed fungal culture in one stage cultivation. These results may be due to cell wall swelling, softening, and collapse of the cell structure as obtained from previous studies by **Rifaat *et al (2005).***

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