

Pulping of Sugarcane Bagasse Using *Ceriporiopsis subvermispota* SS-33 and *Ophiostoma piliferum* as a Fungal Bio-agents

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BAGASSE is an agricultural by product from sugar cane after the cane is harvested and crushed to extract the juice. The utilization of bagasse as raw material for pulp and paper industry is increasing rapidly which also increasing pollution to the environment. In renewable resource to reduce chemical pollution, white rot fungi or lignin-degrading fungi was employed to contribute to remove lignin from raw materials. The aim of this investigation, is to determine the activity of white-rot fungi on bagasse as in *in vivo* biopulping or pre-treatment by comparing the lignin content of bagasse before and after the biodegradation in different conditions. It was found that the most favorable conditions for bagasse pulping can be achieved by treatment with propylene glycol (PG) 90% under pressure for 1 h or without pressure for 2 h at the cooking temperature 150°C. The mentioned treatments gave high pulp yield, with no rejects and low kappa number. The extractive removal of bagasse (10 mesh) by steam resulted to high weight loss, acid perceptible polymeric lignin (APPL) production and low kappa number. The biological 4 weeks treatment of bagasse by *Ophiostoma piliferum* at 27°C increased the brightness, breaking length and tear factor of unbleached bagasse paper sheets, when compared with steam treatment. Using mixed culture of *Ceriporiopsis subvermispota* and *O. piliferum* either from one or two-stage cultures for extractive removal and biodegradation of bagasse, led to the improvement the chemical pulp composition and the properties of unbleached paper sheets. One stage culture treatment increased the unbleached paper sheets properties which expressed as brightness, breaking length and tear factor by 5.6%, 0.08 km and 3.78, compared with the two-stage culture treatment results, which were 3.08%, 21.41 km and 3.69, respectively. The mentioned results were more convenient when compared with steam extraction method. Scanning electron micrographs (SEM) revealed that the biological fibers of the produced paper sheets exhibit a cleaner surface, high flexibility and conformability, which would be contributed to the good bonding nature.

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In the field of chemical technology, where manufacture of a variety of products on large scale has resulted in serious effluent and hazardous waste disposal problems, the need for safer and 'environmental friendly' technologies has become imminent. This has resulted in scientists attempting to learn from natural processes, and therefore a new aspect of microbiology and biotechnology has rapidly begun to gain ground. Microorganisms perform their myriads of biochemical reactions under ambient conditions with little or no toxic and hazardous by-products. Therefore microbial enzymes alternatives to polluting chemical technologies, which began initially as an idea, have reached realistic proportions whereby they can be considered as worthwhile and practical technologies of the future. A glimmer of hope to save the environment and yet achieve the goals of chemical technology is gaining momentum in several frontier developments in this area. One such area is the pulp and paper industry where the quantities of bagasses as raw material processed are huge, as well as the use of naturally hazardous chemicals are also large in Egypt. Progress through intense research and development activities to make the technology eventually totally free from hazardous chemicals would be a cherished dream come true. In the present article, an overview is presented on the present status and future prospects on the use of white-rot fungi as effective bio-reagents to achieve biobleaching in place of poisonous chlorine compounds conventionally used to achieve pulp brightness of a high order in the manufacture of high-quality paper products.

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 (Goncalves *et al.*, 2008). Biopulping can be defined as the biological pretreatment of wood chips followed by mechanical or chemical pulping processes. *Ceriporiopsis subvermispora* is one of the most suitable white-rot fungi for the biopulping process. Wood pretreatment with such fungus leads to a reduction of energy consumption during mechanical pulping by at least 30%. Fungal pretreatment also provide an increase in delignification rate of acid sulfate and bisulfite pulping as well as in organosolv pulping processes such as formic acid/acetone and methanol/water pulping (Ferraz *et al.*, 2000a). This increased delignification rates permitted preparation of biopulps in shorter cooking times, resulting in energy saving and increased digester throughput. In addition, biopulps obtained by formic acid/acetone pulping of pinus radiata biotreatment with *Ceriporiopsis subvernispora* has increased tensile strength (Ferraz *et al.*, 2000b). Moreover, the pretreatment with the species *Ophiostoma piliferum*, had superior abilities for removing pitch from wood which produced in large concentrations during wood degradation (*i.e.*, fatty acids and resin acids), and resulting the lower quality of final product (Josefsson, 2006 and Henriksson, 2006). The aim of this work is biopulping of sugarcane bagasse after physical treatments to achieve pulp of high order in the manufacture of paper.

Materials and Methods

Bagasse source

The Egyptian depithed sugarcane (*Saccharum officinarum*) bagasse was obtained from ElNasr Company for sugar and pulp industry, Edfu, Egypt.

Fungi used

Ceriporiopsis subvernispota CZ-3 and *Ophiostoma piliferum* were obtained from the culture collection of Northern Regional Research Laboratory (NRRL), Department of Agriculture, Peoria, USA.

Media used

Peptone yeast extract medium (Jose *et al.*, 2000) was used for fungi propagation and preservation. Minimal vitamin (MV) medium (Hatakka & Pirhonen, 1985) was added to the bagasse to enhance their biodegradation by fungi.

Propylene glycol (PG) pulping

Pulping was carried out with or without pressure (using autoclave) as follows: Twenty five grams of depicted bagasse were firstly pretreated with 95% PG at liquor ratio of 1:6 (w/v) in 1 L flask then heated at 60°C for 30 min. Different concentrations of PG (80-100%) and 0.29% of sulfuric acid were added to the filtrate of the liquor. The pulping time (1-3 h) and temperatures (140-170°C) were adopted. The flasks were put in oil bath and heated under reflux for specified reaction time (1-3h). After pulping, the crude was filtered and washed successively with 80% aqueous acetone and water (Yasumitsu & Yoshihiro, 1999).

Physical factors affecting bagasse biodegradation by fungi

Mesh size

Fifty grams from different bagasse mesh size (10-20) were placed in 3 L Erlenmeyer flask, the sterile bagasse were inoculated with standard *C. subvernispota* (as a model of an effective fungus) inoculum (0.15 g CDW/10 ml) after adding 100 ml of MV medium. The weight loss and acid perceptible polymeric lignin (APPL) were determined after 4 week at 27±1.

Extractive removal

To remove the resins, fat sand waxes from bagasse, two physical methods were carried out: Boiling distilled water using soxhlet extraction apparatus and steaming by autoclave.

Fungal treatment of bagasse

After treating bagasse with the mentioned physical methods it was subjected to fungal treatments, either by single, mixed or consequence inoculum. Twenty-five grams of oven-dried bagasse (10 mesh size) were placed in 3-liter Erlenmeyer flask and autoclaved at 121°C for 20 min. For the treatment with single or mixed cultures of *O. piliferum* and *C. subvernispota*, the sterilized bagasse was inoculated with 10 ml of standard fungal inocula and incubated for 1-4 weeks at 27±1°C. Concerning the two-stage culture the sterilized bagasse was treated with *O. piliferum* for different incubation periods (1,2,3 and 4 weeks) in the first stage of degradation at 27±1°C. Samples were taken periodically every week and autoclaved at 121°C for 20 min to prevent the far effect of the

fungus, then treated with *C. subvermispora* for 4 weeks at $27\pm 1^\circ\text{C}$ as a second stage. As a control biopulping it was carried out as previously mentioned but without any physical treatments. (Xinli *et al.*, 2002 and Saad *et al.*, 2008).

Unbleached paper sheets preparation

The pulps were beaten for different time intervals using Jokro beater with 6% pulp consistency at 150 rpm. The degree of freeness ($^\circ\text{SR}$) was measured according to the standard method by rising up the mixture to 2 L with water and disintegrated for 2 min in the standard disintegrator with the propeller running of 3000 rpm. Paper sheet were prepared from different pulps according to the Standard Canadian Association (SCA) standards using the SCA sheet former. In the apparatus, sheets of 165 mm diameter (214 cm^2 surface) were formed. The sheets were then pressed for 4 min using hydraulic press. The pressure test was adjusted at 5 kgf/cm^2 (Kilogram-force per square centimeter) . The sheets were dried at 100°C for 2 h with a rotating drum and then were placed for conditioning to 65% relative humidity at $18\text{-}20^\circ\text{C}$.

Chemical analysis

Weight loss of treated bagasse and APPL were determined according to the method recommended by Anthony *et al.* (1986). Hollocellulose, α -cellulose and extractive content were determined according to the standard method suggested by Razak *et al.* (2013). The sheets properties were determined as follows: Brightness using a hunter-lab calorimeter, Kappa number according to TAPPI standard T-236, Degree of polymerization (DP) according to SCAN-CM 15:88, Tear factor according to the internal tearing resistance which was measured by a pendulum type instrument (Elmendorf Tearing Tester) and Breaking length (km) was measured using a universal testing machine (Lloyd). Available at [www.spectro.in /paper-Definition.html](http://www.spectro.in/paper-Definition.html) . Accessed on 3th March . The experiments were carried out in triplicates and the values represented in this work are the arithmetical mean of the three replicates.

Results and Discussion

The amount of sugarcane bagasse in Egypt is abundant while the utilization is still limited. One of the alternatives for bagasse utilization is as pulp material. To minimize the waste from the pulping process, the effect of using a chemical process and a biopulping process (using *O. piliferum* and *C. subvermispora* SS-33) were studied and measured by the percentage of α -cellulose content in bagasse pulp

Effect of pulping time and PG concentration on chemical bagasse composition

Data presented in Table 1 revealed that the favorable treatments were attained with 90% PG either under or without pressure at cooking temperature 150°C for 1 & 2 hour, respectively. The priority of these results was due to complete removal of rejects as well as low values of kappa number. The pulping using 90% PG without pressure for 2 h was preceded, since it gave the highest hollocellulose and brightness also the lowest figures of pentosans and kappa no which were 93.77%, 37.8%, 9.01% and 22.45, respectively. Increasing the cooking temperature to more than 150°C led to increase in kappa number by 1.25 and 1.51 fold at 160°C and 170°C , respectively.

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TABLE 1. Effect of pulping time and PG con. on chemical composition of bagasse processed at 150°C after 1.2 h under or without pressure.

	Time (h)	Under pressure						Without pressure					
		100%		95%		90%		100%		95%		90%	
		1	2	1	2	1	2	1	2	1	2	1	2
% Screened 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	0.00	0.00	6.61	4.44	4.34	2.56	1.28	0.00
% 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	76.51	87.59	85.46	80.54	83.66	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	26.00	26.11	22.45
DP(degree of polymerization)		959.00	919.00	948.00	939.00	938.00	918.00	1356.00	1270.00	1321.00	1146.00	1253.00	1100.00
% brightness		27.00	26.40	28.00	27.40	30.30	28.90	30.70	32.30	32.40	35.50	35.70	37.80

Effect of bagasse mesh size on its biodegradation by C. subvernispora

Increasing the mesh size of bagasse from 10 to 20 led to increasing in the weight loss and APPL, which were 26.29% and 2.98%, respectively (Fig.1). Therefore, the 10-mesh size fraction could be recommended as the proper size for bagasse pulping.

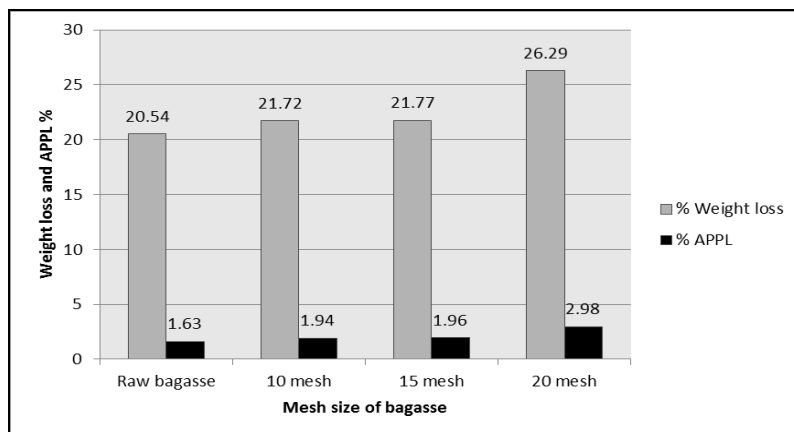


Fig.1. Effect of bagasse mesh size on weight loss & APPL after treating by *C. subvernispora*.

Effect of bagasse treatment with C. subvernispora on chemical and physical properties of unbleached paper sheet

This experiment designed to compare between the effect of *C. subvernispora* inoculation applied on untreated or physically treated bagasse (steam & boiled water), compared with chemically (PG 90%) treated bagasse and the effect on the properties of unbleached paper sheets. The illustrated data in Fig. 2 revealed that the properties of unbleached chemical pulp and its paper sheet had low quality comparing to that recorded by biopulping either by prephysical treated or bagasse biopulp. It could be stated that using steam as physical pretreatment of bagasse biopulp was superior than the other treatments, since it gave the highest figure of yield being 43.72% and lowest kappa number being 15.18. Regarding the physical properties of unbleached paper sheets, data clearly show that the highest brightness (43.9%), breaking length (4.11 km) and tear factor (56.9) were attained by steam pretreatment on bagasse biopulping using *C. subvernispora*.

Effect of bagasse treatment with O. piliferum on chemical and physical properties of unbleached paper sheet

The treatment of bagasse with *O. piliferum* recorded in Table 2 revealed that no remarkable change could be detected either in figure of α – cellulose %, pentosans % or DP. Whereas, screened yield percentage and kappa number decreased gradually during the incubation period till reached 36.15 % and 20.06 respectively, after 4 weeks, *i.e.*, reduced by 9.78 and 10.64 %, respectively, when compared with the chemical pulp treatment. Brightness % and tear factor also increased with about 3.15 % and 4.74 %, respectively.

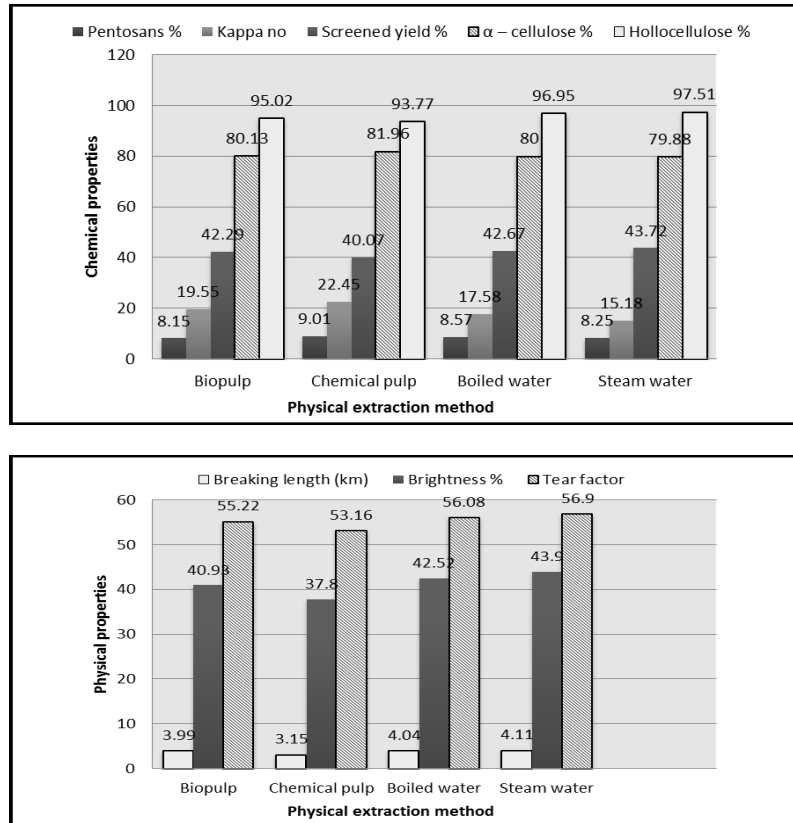


Fig. 2. Chemical and physical properties of unbleached paper sheets as influenced either by bagasse chemical pulping (PG 90%) or *C. subvermispora* biopulping.

TABLE 2. Chemical and physical properties of unbleached paper sheets made from *O. piliferum* treated bagasse.

Properties of UPS*	UPS* treated by <i>O. piliferum</i>				
	Chemical (90% PG**)	1	2	3	4 (weeks)
Screened yield %	40.07	37.33	36.72	36.42	36.15
Hollocellulose %	93.77	93.82	93.99	94.55	94.71
α-cellulose %	81.96	81.77	81.72	81.55	81.50
Pentosans %	9.01	8.34	8.13	8.02	8.01
Kappa no	22.45	22.05	21.14	20.32	20.06
DP***	1100	1099	1095	1084	1065
2- Physical 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

* = Unbleached paper sheets **=propylene glycol ***= degree of polymerization

Unbleached paper sheets of bagasse as influenced by mixed culture of O. piliferum and C. subvermispora

The biodegradation of 10 mesh size bagasse was carried out by using a mixed culture of *O. piliferum* and *C. subvermispora* in MV medium at 27°C for different incubation periods (1-4 weeks). Data given in Table 3 show that the α -cellulose and pentosans % contents of biopulped bagasse were decreased gradually during incubation period of this biological treatment than that present in chemical pulping to reach the minimum value at the end of incubation period being 77.08% and 4.11 %, respectively. On the other hand, the biological treatment by mixed fungal cultures led to loss of 5.95 and 54.38 % of α - cellulose and pentosans respectively, as compared by chemical treatment, whereas decrease in kappa number and increase in brightness by 44.77 and 21.58%, respectively were noted . Also, data indicate that using the treatment with mixed culture for one week gave the highest value of screened yield %, breaking length, and tear factor being 43.21 %, 4.99 km and 59.0 % respectively.

TABLE 3. Chemical and physical properties of unbleached paper sheets as influenced either by bagasse chemical pulping (PG 90%) or mixed culture of *O. piliferum* and *C. subvermispora* biopulping.

Properties of UPS*	UPS* treated by <i>O. piliferum</i> and <i>C. subvermispora</i>				
	Chemical (90% PG **)	1	2	3	4 (week)
Screened yield %	40.07	43.21	39.29	37.05	35.50
Hollocellulose %	93.77	97.68	97.72	97.91	97.93
α – cellulose %	81.96	81.09	80.18	77.12	77.08
Pentosans %	9.01	7.65	6.61	4.51	4.11
Kappa no	22.45	14.15	12.44	12.42	12.40
DP	1100	1009	1005	895	892
2- Physical 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

* = Unbleached paper sheets ** = Propylene glycol

Unbleached paper sheets of bagasse as influenced by consequent two-stage degradation by O. piliferum and C. subvermispora

Data of chemical and physical properties of paper hand sheets were recorded in Table 4 revealed that the highest value of breaking length and tear factor were attained when *O. piliferum* was incubated for one week as first stage of bagasse degradation followed by 4 weeks of 2nd stage inoculation. Whereas, the highest screened yield % was observed after the first week of incubation period. Moreover, α – cellulose % and pentosans % recorded the lower figures after the end in all experiments treatments than that obtained by chemical pulping treatment.

TABLE 4. Chemical and physical properties of unbleached paper sheets with two stage of bagasse degradation by *O. piliferum* and *C. subvermispora*.

Properties of UPS*	Fungus	Stage	Incubation period (week)				Chemical pulp
	O.P**	1 st	1	2	3	4	
	C.S***	2 nd	Four weeks				
1- Chemical properties							
Screened yield %			43.13	37.68	35.84	32.39	40.07
Hollocellulose %			96.25	96.61	96.85	96.85	93.77
α – cellulose %			80.44	77.74	76.73	75.82	81.96
Pentosans %%			8.15	7.77	7.23	6.55	9.01
Kappa no			17.00	14.55	14.23	14.15	22.45
DP			1007	899	857	845	1100
2- Physical properties							
Brightness %			42.85	44.77	44.65	44.88	37.8
Breaking length(km.)			4.95	2.13	1.77	1.52	3.15
Tear factor			56.85	31.53	25.23	23.03	53.16

* = Unbleached paper sheets, ** = *O. piliferum*, *** = *C. subvermispora* SS- 33

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

Scanning electron micrographs from the pulp are shown in Fig. 3. From comparing the hand sheets made from the biologically treated with mixed culture of *O. piliferum* and *C. subvermispora* SS-33 at one stage cultivation, *C. subvermispora* SS-33 in single 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. The mentioned results were more visible in the mixed one stage fungal culture treatment.

Xinli & Kokki (2002) reported that using a marine fungus, *Phlebia* sp. MG-60 and three white-rot fungi were incubated with whole sugarcane bagasse (WSB) resulted in more than 50% of lignin degraded and less than 10% of the holocellulose was lost. They predicted also that the most efficient utilization of bagasse fiber might be employed as the raw material in the pulp and paper industry after biopulping with *Phlebia* sp.

Fermentation time, fungal mycelium load and pretreatment scale were studied in the fungal treatment, and the best conditions were 15 days with 250 mg kg⁻¹ fungal mycelium per straw weight causing high lignin decomposition. At the largest scale tested (50 g straw) lignin degradation exceeded cellulose degradation by 2.4-fold (Saad *et al.*, 2008). Seven strains of *Panus tigrinus* were grown, without any additional carbon source, on sugarcane bagasse for 10 days

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in a semi-solid state fermentation. The production of ligninases by fungi were correlated with the efficiency of delignification process in bagasse after organosolv pulping with aqueous acetic acid. Analysis of decayed sugarcane bagasse by FTIR indicated a correlation between lignin modification and pulp properties in some strains, in such a way that the more the lignin modifies, the more the pulp properties improve (Adilson *et al.*, 1998).

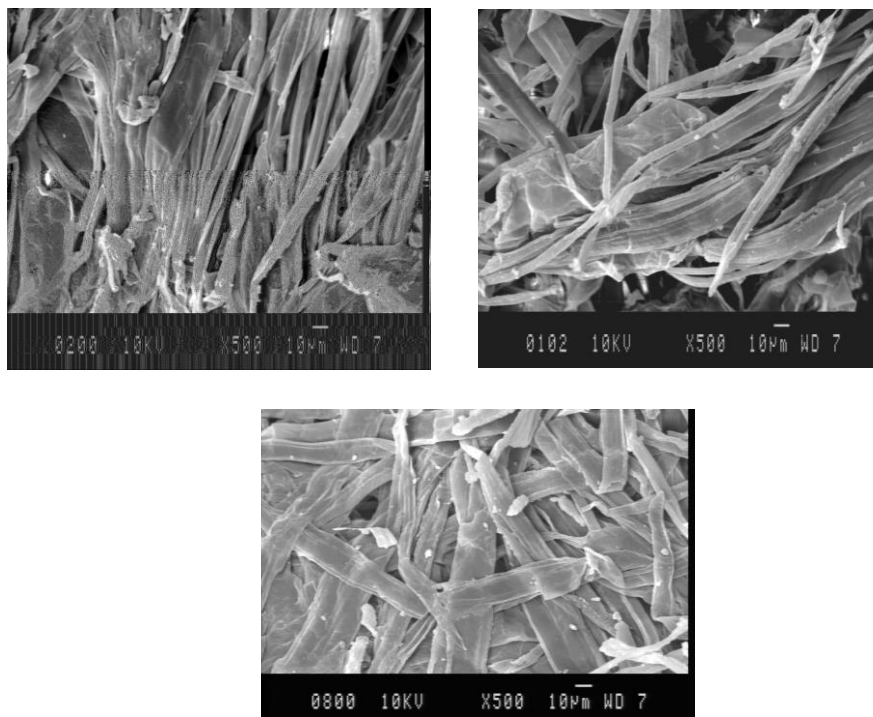


Fig. 3. Scanning electron photographs of (A): Unbleached bagasse chemical pulp (B) & (C) Bagasse treated with *O. piliferum* and *C. subvermispota*, respectively.

Antaresti *et al.* (2008), reported that in the chemical process of bagasse, the nitric acid concentration and the reaction time were varied. The pulping process was conducted at constant temperature of 102°C and the mixture was agitated at 900 rpm. The highest percentage of α -cellulose content in pulp was 79.28%, obtained at the concentration of 3.5% for a reaction time of 3.5 h. At higher concentrations and longer cooking times, the α -cellulose decreased due to the cellulose degradation. In the biopulping process, the incubation temperature was kept at 30°C. The highest percentage of α -cellulose was 65.80% obtained at an incubation time of 25 days. When compared to nitric acid pulping, biopulping is less efficient. However it has one advantage that it only uses a small amount of chemicals and, therefore, creates less pollutants.

Biopulping is a type of industrial biotechnology. It uses a type of natural fungus to convert wood chips to paper pulp. The main processes for paper production that are currently in use are mechanical and chemical. Biopulping provides a solution to the problems of chemical and mechanical paper production. It uses fungi present in natural wood decay to alter the lignin in the cell walls of the wood, which therefore "softens" the wood chips. Since the wood is already softened when it goes to the final steps to be made into paper, the remaining steps of the process require less energy and do not pollute the air since there is no need for chemicals.

Akhtar *et al.* (2000) reported that the mechanical pulping process is electrical energy intensive and results in low paper strength. Biomechanical pulping, defined as the fungal treatment of lignocellulosic materials prior to mechanical pulping, has shown at least 30% savings in electrical energy consumption, and significant improvements in paper strength properties compared to the control at a laboratory scale. In an effort to scale-up biomechanical pulping to an industrial level, 50 tons of spruce wood chips were inoculated with the best biopulping fungus in a continuous operation and stored in the form of an outdoor chip pile for 2 weeks. The pile was ventilated with conditioned air to maintain the optimum growth temperature and moisture throughout the pile paper strength properties the control and fungus-treated chips were refined through a thermomechanical pulp mill (TMP) producing lightweight coated paper. The fungal pretreatment saved 33% electrical energy and improved significantly compared to the control. Since biofibers were stronger than the conventional TMP fibers, we were able to reduce the amount of bleached softwood kraft pulp by at least 5% in the final product. Fungal pretreatment reduced brightness, but brightness was restored to the level of bleached control with 60% more hydrogen peroxide.

Results revealed that the properties of unbleached chemical pulp and its paper sheet had low quality comparing to that recorded by biopulping either by fungal treatment alone or accompanied with prephysical treatment. These results are in line with those obtained by Bajpai *et al.* (2004) who found that treatment of depithed bagasse with *Ceriporiopsis subvernisporea* reduced the kappa number by 10-15.

Comparing the properties of paper hand sheets made from both treatments of biopulping either in mixed fungal culture or separately (two stage), it could be stated that using both *Ophiostoma apiliferum* and *C. subvernisporea* SS-33 as mixed culture for bagasse degradation in one stage was the best biotreatment. Moreover, this treatment increased brightness, breaking length, and tear factor compared to biopulping by two stage degradation, biopulping of steam extraction and chemical pulping, respectively.

Scanning electron microscope images revealed 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 as one

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stage. These results may be due to cell wall swelling, softening, and collapse of the cell structure as obtained from previous studies by Gamal *et al.* (2000).

Messner & Srebotnik (1994) stated that treatment of wood chips with lignin-degrading fungi prior to pulping has been shown to have great potential for mechanical as well as chemical pulping on a laboratory scale. *C. subvermispora*, when grown on aspen or loblolly pine for 4 weeks, was found to be superior to other fungi. On aspen there was an energy savings of 47%, and an increase in burst and tear indices of 22% and 119%, respectively. With loblolly pine, energy savings amounted to 37%, while burst and tear indices increased by 41% and 54%, respectively. The weight loss was only 6%, but a decrease in optical properties had to be accepted. Wall *et al.* (1996) showed that the use of *O.piliferum* for hardwood treatment resulted in extractive degradation, improvement in chemical pulping efficiency and reduction of kappa number up to 29 %.

Messner & Srebotnik (1994) stated that information obtained by immunoelectron microscopy and differential staining led to the conclusion that the biopulping effect obtained after 2 weeks of incubation cannot be explained by the direct action of enzymes on lignin or polysaccharides. Instead, a low molecular mass agent is considered to be responsible for the biopulping effect. These results have changed the aims of biopulping from an emphasis on removing the bulk of lignin to an emphasis on a short-term process, lasting 2 weeks and yielding a low mass loss.

Hosseinpour *et al.* (2010) recorded that the chemimechanical pulping (CMP) of canola straw can be conducted using different dosages of sodium sulfite and sodium hydroxide. The results showed that, by a mild chemical pretreatment (4–12% (wt.) NaOH and 8–12% (wt.) Na₂SO₃), the pulp brightness reached almost 40% ISO and the strength properties were comparable to those of bagasse CMP and of wheat straw CMP.

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تلييب مصاص القصب بالمعالجة الفطرية باستخدام

Ceriporiopsis subvermispora SS-33,
Ophiostoma piliferum

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تم معالجة مصاص القصب بتركيزات مختلفة من البروبيلين جليكول وثبت ان 90% من المذيب اعطى اعلى حصيلة من اللب مع عدم وجود ألياف غير مطبوخة وأقل نسبة لجنين وذلك لمدة ساعتين عند 150 م تحت الضغط الجوى العادى . وبدراسة سمك الاليف التى تؤثر على التلييب باستخدام فطر *Ceriporiopsis subvermispora* كان 10 mesh يمثل الافضل سمكاً. كما تم دراسة معالجة مصاص القصب بالبخار لازالة المواد القابلة للاستخلاص وثبت ان البخار ادى الى زيادة الفقد فى الوزن وزيادة اللجنين القابل للترسب بالحامض. سجلت المعاملة الاولية لمصاص القصب بفطر *Ophiostoma piliferum* أقل حصيلة لللب ونسبة اللجنين وزيادة درجة النصاعة وأعلى معامل تمزق للورق المنتج مقارنة بالتلييب الكيمايى , كانت الخواص الميكانيكية والضونية للورق المنتج من اللب المعالج حيويًا بالمزرعة المختلطة لمدة 4 أسابيع على 27 م والمسبق معاملة بالبخار ذات قيم أعلى من خواص الورق المنتج من اللب المنتج كيميائياً من حيث درجة النصاعة ومعامل التمزق وقوة الشد . وسجلت المعاملة الاولية لمصاص القصب بمزرعة مختلطة من *Ceriporiopsis subvermispora* & *O. piliferum* فى خطوة واحدة أفضل النتائج من زيادة فى درجة النصاعة والطول القاطع ومعامل التمزق للورق بمقدار 5,6% و 0,81% و 3,78% بمقارنتها بالتلييب الحيوى على خطوتين . كما أظهرت الدراسة باستخدام الميكروسكوب الالكترونى الماسح أن الاليف المعالجة باستخدام مزرعة مختلطة من الفطرين السابقين فى خطوة واحدة كانت تتميز بسطح أفضل ومرونة وقابلية للتشكيل وترباط أفضل بين الاليف مقارنة بالمعالجات البيولوجية الاخرى المستخدمة.