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ORIGINAL PAPER

Pretreatment by *Ceriporiopsis subvesmispora* and *Phlebia subserialis* of wheat straw and its impact on subsequent soda-AQ and kraft-AQ pulping

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Abstract

Pretreatments of wheat straw with two different lignin-degrading fungal cultures - Ceriporiopsis subvesmispora and Phlebia subserialis- were carried out and their effects on soda-AQ and kraft-AQ pulping were studied. After two weeks of bio-treatment of the wheat straw, both fungus were found to be suitable for bio-chemical pulping. The fungal pretreatment decreased the kappa number by as much as 24% and the unbleached pulp brightness increased by about 7% at a constant yield. Furthermore, the bio-pretreatment increased the brightness of the fully bleached pulp by about 1 ISO% point. This can apparently reduce the amount of the chemicals used in the bleaching process. The results on the sugar analysis of the pulps showed that in terms of carbohydrate composition, there were no significant differences between the bio-treated and control pulps. However, the strength properties of the un-bleached and bleached chemical straw pulps decreased slightly after the fungal pretreatment. This is different from reported results that the strength properties of mechanical pulps were improved after the fungal pre-treatment. There was no difference of the two white-rot fungal species, Ceriporiopsis subvesmispora and Phlebia subserialis in term of their effect on the strength properties.

Keywords: Wheat straw; Biochemical pulping; *Ceriporiopsis subvesmispora*; *Phlebia subserialis*; Pulp and paper properties; D-Ep-D bleaching

Introduction

Today about 91% of the pulp and paper production in the world uses wood as the raw material, mostly in the developed countries. Environmental concern and short supply are increasing the pressure to use alternative sources for raw material in both developed and developing countries. The using of agricultural residuals in the pulp and paper industry has increased substantially. Agriculture-based fibers are particularly relevant in countries where demand for pulp and paper is increasing but the wood resources are limited [1]. Wheat straw is one of the most important agricultural residues. It is an annually renewable fiber resource that is available in abundant quantity in many regions of the world. Wheat straw could be considered as a natural fiber resource.

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Conventional pulping and bleaching methods used up to date have some disadvantages from the environmental aspects. The production of chemical pulps requires a large amount of chemicals that may have negative environmental impact. Mechanical pulping requires substantial amount of energy. Therefore, research on the development of alternative methods is still being undertaken worldwide. Thousands of strains and species of fungi have the capability to degrade lignin, cellulose or hemicelluloses. The most desirable biological systems for biopulping should have the potential to minimize the process environmental impact, reduce energy consumption and other operating costs. *Ceriporiopsis subvermispora* and *Phlebia subserialis* are rated among the most effective species for hardwood and softwood [2].

Pretreatment with lignin degrading fungi prior to pulping has been extensively studied. It was reported that, the use of some lignin degrading-fungi reduced the energy consumption by up to 30% for mechanical pulping [3, 4]. A very small decrease in brightness was seen for pulp produced from the treated wood chips and the strength properties were improved of the resulting mechanical pulping [5]. However, much less work has been done on the fungal pretreatment of agro-residues before chemical pulping [1, 6]. Earlier studies demonstrated that pre-treatment of wood chips with fungi in biochemical processes facilitated the delignification [7]. Environmental concerns made it necessary to develop new methods for reducing the use of energy, sulfur and chorine-containing compounds in the pulping process. Therefore, recently studies on bio-pulping and bio-bleaching have received much attention.

The objective of this study was to determine the nature of changes which occur in pulp and paper properties of different wheat straw chemical pulps after two different fungal-treatments. We determined and compared the hand sheets properties, pulp characteristics, acid groups content, pore volume and the amount and composition of carbohydrates remained with or without the fungal pretreatment.

Materials and methods

Fungus, inoculums preparation and bio-treatment

Straws of wheat (*Tiriticum aestivum L)* were obtained from Central Anatolia Region of Turkey and transferred to the laboratory. The straws were dried and manually chopped to pieces of 2-3 cm. The moisture, organic and inorganic material contents of the straws were determined. The white-rot fungi used in this study were *Ceriporiopsis subvermispora* and *Phlebia subserialis*, both of which are known to have good lignin degrading ability compared with various other strains [3, 8]. They were supplied by the Centre for Forest Mycology Research of the USDA Forest Products Laboratory in Madison, WI, USA.

Liquid inocula were prepared in petri dishes as described in a previous publication [9]. The spent medium in the dish containing the fungal biomass was then decanted; mycelium was washed with sterile distilled water and then blended aseptically in a Waring blender. Liquid inocula containing 0.1 $mg{\cdot}mL^{-1}$ -fragmented mycelium (dry wt.) were used for inoculation of the straws, approximately 5 mg mycelium per 1 kg of the wheat straw.

Prior to the experiment, the wheat straws were immersed in water. Corn Step Liquor (CSL) was used as a nutrient in order to lower inoculum demand [4]. The CSL utilized in this study was supplied by a local starch producer, Pendik-Nişasta (Istanbul, TURKEY). Diluted CSL were sprayed on straws (0.5 % dry w/w) before sterilization. The wet straws were steamed for 20 min for decontamination and cooled to room temperature before inoculation. Inoculated straws were introduced in a sterilized aerated static bed bioreactor. Moisture content of the wheat straw was adjusted to approximately 50 %. The straws were incubated at 27 °C for 2 weeks with humidified air (0.05 $L{\cdot}h{\cdot}L^{-1}$).

Pulping and bleaching conditions

The soda-AQ and kraft-AQ pulping processes were applied in this study. Pulping trials were carried out in a stainless-steel individual bomb reactor (2000 mL volume), containing 150 g (o.d.) straw. Prior to cooking, the straws were steamed for 20 min for decontamination. The ratio of cooking liquor to straws was 6:1. The cooking temperature was ramped to 160 °C in 60 min and maintained for 40 min. The cooking chemicals contained 12% active alkalinity (as Na_2O) and 25% sulphidity. 0.1% anthraquinone (AQ) was added for all trials. The control cooking was carried out under the same conditions without fungal inoculation of wheat straw.

Fungal treated and untreated pulp samples were bleached in a multistage elemental chlorine-free (ECF) bleaching process using a chlorine dioxide (D), alkali extraction with peroxide (Ep), chlorine dioxide (D) treatment sequence under the conditions summarized in Table 1. Bleaching trials were done in polyethylene bags placed in a water bath. The starting amount of the wheat straw soda-AQ pulps was 50 g (o.d.).

Table 1. Conditions of multistage elemental chlorine-free bleaching process

Bleaching parameters	Bleaching stages		
	D1	Ep	D2
Pulp consistency, % (w/w)	10.0	10.0	10.0
Temperature, °C	75.0	75.0	75.0
Reaction time, min	60.0	180.0	180.0
Active chlorine, % (on pulp, w/w)	3.0	-	1.0
Magnesium sulfate, % (on pulp, w/w)	-	0.5	-
Sodium hydroxide, % (on pulp, w/w)	-	1.0	0.1
Hydrogen peroxide, % (on pulp, w/w)	-	0.5	-

Analysis of the pulp

The pulp freeness was measured by following the Canadian Standard Freeness (CSF), Tappi, T-227 om-94 method. The total yield of the pulps and amount of rejects were determined according to Tappi (TAPPI T 210 cm-93) by gravimetric measurements.

Kappa numbers and viscosity were determined according to TAPPI T 236 cm-85 and TAPPI T 230 om-94 respectively. Pulps from all three replicates were mixed to obtain enough material for handsheets. Handsheets were made to determine the physical properties, including tensile index (TAPPI T 404 cm-92), bursting strength (TAPPI T 403-om-91), tearing strength (TAPPI T 414 om-88) and ISO brightness (ISO test method (ISO2469)).

The pore volume of the control and fungal pretreated pulps was determined by following the so-called size exclusion method using 3% Dextran T2000 solution. The dextran solution was added to the pulp samples and allowed to shaking for an hour. After the pulp samples were settled and the probe solution was withdrawn and filtered through a sintered-glass funnel, the concentration of the probe solution was determined refractometrically using Automatic Polarimeter (AUTOPOL III).

In order to determine acid group content, the samples were first subjected to solvent extraction using acetone (TAPPI T-264 om-88). The extracted samples were alternately soaked and rinsed two times in 0.1 N HCl for 45 min and the sample was dispersed in a 450 mL of 0.001M sodium chloride solution and titrated with 0.1 M sodium hydroxide. The alkali was added at a rate of 0.5 mL every 5 min so as to allow sufficient time for equilibrium to be reached. Following the titration, the pulp was washed and oven-dried at 105 °C. The titrations followed both potentiometrically and conductometrically.

For the sugar composition, pulp samples were subjected to acid hydrolysis, and then analyzed for the major sugars. The high performance liquid chromatography (HPLC), a Dionex model chromatography using CARBO-PAC PA-1 column, was used.

Results and discussion

Table 2 showed the pulping results. Pulp yields of fungal treated and untreated wheat straws were found to be very similar to each other, which is in a good agreement with those obtained by Bajpai et al. 2004a [1] (46% yield). The kappa number of 30.9, reported by Baipai et al. (2004a) [1] was higher than that from the current study. Others also reported that the fungal pretreatment with short duration did not change the chemical pulp yield significantly [2, 10, 11, 12]. Table 2 also showed that there is no apparent difference between the soda-AQ and kraft-AQ pulps with or without fungal pretreatment with regard to their pulping yields.

Table 2. Summary of wheat straw kraft-AQ and soda-AQ pulping results with or without fungal pretreatment

Pulping methods	Pre-treatment	Total yield (%)	Kappa no	Viscosity (cP)	CSF	Residual alkali ($g \cdot L^{-1}$)	Brightness (% ISO) Unbleached pulp	Bleached pulp
Soda-AQ	Control	46.2	12.6	37.07	435	3.1	32.74	86.30
	C. subvesmispora	46.3	9.6	28.81	445	3.1	34.95	87.00
	P. subserialis	46.5	9.7	31.88	475	2.8	35.04	87.10
Kraft-AQ	Control	46.1	12.0	40.91	425	4.5	31.06	86.00
	C. subvesmispora	46.2	10.6	28.32	450	5.7	32.11	87.00
	P. subserialis	46.3	11.0	32.63	465	5.9	32.30	87.00

C. subvermispora and *P. subserialis* grew well during the incubation. Both fungus strains reduced the kappa number of the pulp when cooked at the same alkali charge in the soda-AQ and kraft-AQ processes. These results are consistent with those from various earlier studies [1, 2, 3, 13, 14, 15, 16, 17]. It can be seen in Table 2 that for soda-AQ pulping, the effect of the fungal treatment on the decrease in kappa number (from 12.6 to 9.7 and 9.6, a reduction of about 23%) was higher than that of the kraft-AQ pulping (from 12 to 10.6 and 11.0, a reduction of about 11 %).

Included in Table 2 are also the results on the effect of the fungal pretreatment on the brightness of the soda-AQ and kraft-AQ pulps. The brightness of the un-bleached wheat straw chemical pulps was higher with fungi treatment. Bajpai et al. [1, 2] and Ahmed et al. [18] also found similar results for biochemical pulping of bagasse and wheat straw. Also these results clearly showed that both the untreated and fungal-treated soda-AQ pulps had higher brightness than those of the kraft-AQ pulps and the fungal pretreatment was more effective to increase the soda-AQ pulp brightness (from 32.7% to 43.9% and 35.0% than the kraft-AQ pulp (from 31.0 to 32.1% and 32.3%).

It was reported that the fungal pretreatment of raw material increased the strength properties of the resulting mechanical pulp [5, 19]. The tear index, burst index, zero-span breaking length were higher after fungal pretreatment with *C. subvermispora*, *Phanerochaete chrysosporium* or *P. subserialis* [5, 16, 18], these beneficial effects were consistently achieved when the wood chips were inoculated with the fungi prior to the mechanical refining process. However, negative effects on the paper strength properties were observed when coarse mechanical pulp fibers were incubated with *Phanerochaete chrysosporium* before a second refining step Jong et al. [20] observed that treatment with several white and brown fungi produced a substantial decrease in the strength properties and that *Phanerochaete chrysosporium* did not have any effect on the strength properties of the handsheets while the brightness was slightly improved in a RMP process. Also Franco et al. [17] found that the strength properties of the bio-kraft pulps were similar to the control pulps. Giovannozzi et al. [12] found that the breaking length and burst index of the paper sheets were decreased after the bio-treatment of wheat straw. Table 3 showed the physical properties of the soda-AQ and kraft-AQ process with or without fungal pretreatment. It was evident that the white-rot fungi slightly decreased the strength properties of both the soda-AQ and kraft-AQ pulps. Similar results were obtained for the fully bleached pulp samples (data not shown). Besides, there was no significant difference between the pretreated soda-AQ and kraft-AQ pulps in terms of strength properties. The fungal treatment was proposed to weak the chemical bonds in the lignocellulosic material, therefore, facilitating the delignification in the pulping/bleaching processes [12]. The decrease in pulp viscosity can be seen with the bio-treatment, as shown in Table 2. *C. subvermispora* decreased the unbleached pulp viscosity by 22.3 – 30.8%, while *P. subserialis* strain decreased it by 14 – 22%.

Table 3. Strength properties of the handsheets made from wheat straw chemical pulps treated with different fungi

Pulping methods	Pre-treatment	Tensile index ($N \cdot m \cdot g^{-1}$)	Burst index ($kPa \cdot m^2 \cdot g^{-1}$)	Tear index ($mN \cdot m^2 \cdot g^{-1}$)	Bulk ($cm^3 \cdot g^{-1}$)
	Control	63.10	1.05	6.40	1.36
Soda-AQ	*C. subvermispora*	62.98	1.05	6.28	1.30
	P. subserialis	60.74	0.99	6.50	1.34
	Control	67.65	1.12	6.69	1.33
Kraft-AQ	*C. subvermispora*	64.09	0.96	6.01	1.36
	P. subserialis	61.42	0.94	6.17	1.39

The effect of fungal pretreatment on the composition of carbohydrates in the wheat straw pulps was shown in Table 4. The results were calculated in terms of the percentage of dry weight of the pulp sample, one can find that there was no significant difference with or without the fungal pretreatment. Villalba et al. [2] reached similar conclusions from pretreatment of the loblolly pine with *C. subvesmispora* for 2-week. It was proposed that the presence of lignin, physically and to some extent chemically, serves as an effective barrier to the enzymatic degradation of polysaccharides [21].

Table 4. Composition of carbohydrates in the wheat straw pulps from the soda-AQ and kraft-AQ pulping processes

Pulping methods	Pre-treatment	Glucose (%)	Xylose (%)	Arabinose (%)	Total (%)
	Control	71.7	23.2	0.24	95.1
Soda-AQ	*C,subvermispora*	71.3	22.9	0.18	94.4
	P, subserialis	71.4	23.1	0.19	94.7
	Control	72.4	24.8	0.29	97.6
Kraft-AQ	*C.subvermispora*	71.4	25.5	0.13	97.3
	P. subserialis	71.1	24.6	0.20	95.9

Table 5 showed that the biopulping treatment increased the content of acid groups bound to a cell wall. Similar results were found by Hunt et al. [22]. The effect of the *P.* subserialis strain on the weak acid content was more pronounced than the C. subvermispora strain. Katz et al. [23] reported that there was a good correlation between the formation of acidic groups content and the degree of swelling, as well as the strength properties of mechanical pulps. A higher acid group content will lead more fiber swelling, and thus higher strength properties. An improvement in the strength properties due to the bio- treatment in the mechanical pulping process was observed before [5, 19, 24, 25]. There are many factors affecting strength properties, we found in this study that the fungal pretreatment increased the acid group content of the resulting pulps while the strength properties were somewhat lower. This may be due to the decreased fiber length resulting from the fungal pretreatment.

Table 5. Acid groups contents of wheat straw kraft-AQ pulps

Pulps	Strong acid ($Meq{\cdot}kg^{-1}$)	Weak acid ($Meq{\cdot}kg^{-1}$)	Total acid ($Meq{\cdot}kg^{-1}$)
Control	152.2	131.5	283.7
After Cooking	183.2	69.1	252.3
C.subvermispora treated	166.3	149.0	315.4
P. subserialis treated	191.5	139.6	331.1

Table 6 showed the pore volume of the different treated and untreated chemical pulps. One can find that the fungal pretreatment of wheat straw slightly increased the pore volume of pulp fibers. *C. subvermispora* was more effective than *P. subserialis*. The increased pore volume would imply that the resulting pulps will be easier to be refined in the subsequent processes, thus decreasing the refining energy. Similar results for biomechanical pulping were stated before by Setliff et all. [16] and Young and Akhtar [24].

Table 6. Results on pore volume of various pulps

Pulping methods	Pre-treatment	Pore volume ($mL{\cdot}g^{-1}$)	Pore volume change (%)
Soda-AQ	Control	2.291	0.0
	C.subvesmispora	2.368	3.4
	P. subserialis	2.363	3.1
Kraft-AQ	Control	2.338	0.0
	C.subvesmispora	2.421	3.6
	P. subserialis	2.370	1.4

Conclusions

This research showed that with fungal pretreatment of the wheat straw, in the subsequent soda-AQ and kraft-AQ pulping processes the kappa number of the resulting pulps can be decreased by as much as 24% and the unbleached pulp brightness can be increased by about 7% at a constant yield. Pretreatment of the wheat straws with fungus before soda-AQ pulping process is more effective than kraft-AQ for obtaining lower kappa number and higher brightness. In addition, the pulp brightness after bleaching was about 1 point higher for the fungus pre-treated pulps than that of the untreated sample. This can reduce the chemicals used in the bleaching process. The reported benefits on the improvement of the paper strength properties after fungal pre-treatment for mechanical pulps were not reproduced with fungal pretreatment of the wheat straws.

The fungal pre-treatment of the wheat straw increased the content of free carboxylic acid groups bound to the fiber. These acid groups increased the swelling of the fibers, and thus potentially decreasing the refining energy.

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