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The Potential in Bioethanol Production From Waste Fiber Sludges in Pulp Mill-Based Biorefineries

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Abstract

Industrial production of bioethanol from fibers that are unusable for pulp production in pulp mills offers an approach to product diversification and more efficient exploitation of the raw material. In an attempt to utilize fibers flowing to the biological waste treatment, selected fiber sludges from three different pulp mills were collected, chemically analyzed, enzymatically hydrolyzed, and fermented for bioethanol production. Another aim was to produce solid residues with higher heat values than those of the original fiber sludges to gain a better fuel for combustion. The glucan content ranged between 32 and 66% of the dry matter. The lignin content varied considerably (1–25%), as did the content of wood extractives (0.2–5.8%). Hydrolysates obtained using enzymatic hydrolysis were found to be readily fermentable using *Saccharomyces cerevisiae*. Hydrolysis resulted in improved heat values compared with corresponding untreated fiber sludges. Oligomeric xylan fragments in the solid residue obtained after enzymatic hydrolysis were identified using matrix-assisted laser desorption ionization-time of flight and their potential as a new product of a pulp mill-based biorefinery is discussed.

Index Entries: Bioethanol; biorefinery; fiber sludge; lignocellulose; *Saccharomyces cerevisiae;* xylan.

Introduction

When wood is converted to pulp in kraft mills, the fiber products need to be pure. If the pulp is contaminated with impurities, some of the production instead becomes a fiber waste. Previously, some of the fiber sludge material formed was landfilled. In Sweden, it has been prohibited to landfill organic waste since 2005 and therefore the sediment from the wastewater treatment (Fig. 1) is nowadays burnt. However, waste fiber sludge efficiently binds considerable amounts of water and the heat values (HV) are generally low or even negative.

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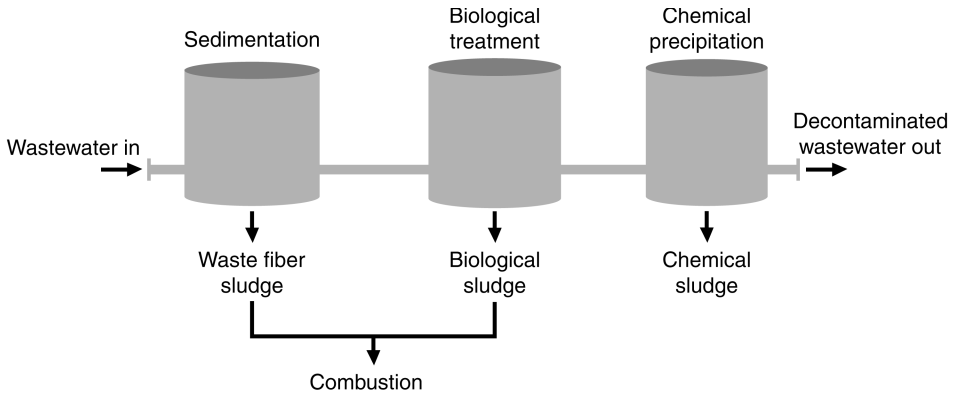

Fig. 1. Schematic flow sheet of a pulp and paper mill wastewater treatment system. Fibers from different positions in the mills enter a sedimentation basin with a stream of wastewater. The fiber sludge is collected for combustion. One mill had an addition of bark (sample 1) and one mill added biological sludge (sample 3).

A better way to utilize waste fiber sludges would be to incorporate them in a biorefinery *(1–4)*. Providing that the carbohydrate content of the material is high, it could be converted into bioethanol as a part of a pulp mill-based biorefinery. When bioethanol is made from lignocellulose using enzymatic hydrolysis, a pretreatment step is needed to make the material more accessible to the cellulases. Owing to the processes in the mill, the fiber sludges could be amenable to enzymatic hydrolysis. The chemical composition of the fiber sludge material determines its susceptibility to enzymatic hydrolysis and affects the HV of the solid material left after hydrolysis. To avoid competition for the fiber sludge between ethanol production and heat production, it would be desirable that sugars could be liberated from the fiber sludge for ethanol production without any reduction in HV, leaving the remainder for production of heat.

Samples from three selected Swedish kraft pulp mills were collected and tested to compare the properties of different waste fiber sludges and evaluate their potential as resources in pulp mill-based biorefineries. The aim of this study was to elucidate the potential of the waste fiber fractions for the generation of products with an additional value for the mill. Examples of such products are bioethanol, hemicellulose fractions including xylan, and a solid waste, which contains lignin residues and wood extractives, with improved HV.

Methods

Fiber Sludge Samples

Fiber sludge samples were collected from three Swedish kraft pulp mills (samples 1–3). The waste fiber sludges were mixtures of fibers diverted from the production through the rejects from the screen room after the pulping process, the outlets from the bleaching plant, the pulp-drying

machine, or the floor drainages. The fiber sludges were collected after the primary sedimentation basins. The samples contained varying amounts of bark residues and wood splinters. The origin of the waste fiber sludges varies from mill to mill because of the specific process solutions of the mills. The samples were selected by the mill operators and would normally have been combusted. All fiber sludge samples were washed with water to stop potential microbial growth. The samples were thereafter air-dried and homogenized by milling (Wiley laboratory mill, 40 mesh; Thomas Scientific, Swedesbaro, NJ) before the ensuing hydrolysis *(5)*.

Analysis of Fiber Sludges

The fiber sludge samples were analyzed for carbohydrates, lignin, extractives, and ash. Carbohydrates and lignin were determined through acid hydrolysis according to TAPPI Method T249 cm-85 (Technical Association of the Pulp and Paper Industry [TAPPI] Norcross, GA). The monosaccharides in the acid hydrolysate were determined using an high-performance anion exchange chromatography (HPAEC) system with an electrochemical detector (Dionex, Sunnyvale, CA) equipped with a CarboPac PA-1 column (Dionex), according to a previously described procedure *(6)*. To determine the amount of extractives, the samples were first extracted with acetone in a Soxtec apparatus (Foss Tecator AB, Höganäs, Sweden). Thereafter, the extracts were evaporated to total dryness and quantified gravimetrically (SCAN-CM 49:03 [Scandinavian Pulp, Paper and Board Testing Committee, Stockholm, Sweden]). The ash content was determined according to International Organization for Standardization (ISO) 2144:1997 (Geneva, Switzerland).

Hydrolysis of Fiber Sludges

The fiber sludge samples were hydrolyzed using the enzyme preparations Celluclast 1.5 (1500 NCU [Novo cellulase units]/g, Novozymes, Bagsvaerd, Denmark) and Novozym 188 (250 cellobiase units/g, Novozymes). The substrate concentration in the reaction mixtures was 15% (w/w) and the concentration of each of the enzyme preparations was 2% (w/w). In addition, 1% (w/w) Tween 20 was added as detergent *(7)*. The initial pH of the hydrolysis reaction mixture was 6.0. The hydrolysis was performed in sealed plastic bags in a water bath at 45°C during 48 h. After the hydrolysis, the remaining solid residue was separated from the liquid fraction (the hydrolysate) by centrifugation and filtration (GF/A, Whatman, Maidstone, UK). All hydrolysis experiments were done as duplicates.

Analysis of Fractions Obtained After Hydrolysis

The monosaccharide contents of the hydrolysates were determined using HPAEC as described under "Analysis of Fiber Sludges." The amounts of solid residue were determined gravimetrically.

Water Seizing Ability

A small sheet with a grammage of 800 g/m^2 was made. The dewatering was done during 60 s and thereafter the sample was weighed and dried at 100°C for 24 h. After the drying, the weight was determined again. The dry content after the dewatering was calculated and is hereafter referred to as the water seizing ability.

Fermentation Experiments

The fermentations were carried out using *Saccharomyces cerevisiae* (Jästbolaget AB, Rotebro, Sweden). Agar plates with yeast extract peptone dextrose (YEPD) medium (2% yeast extract, 1% peptone, 2% D-glucose, and 2% agar) were used to maintain the strain. Cultures for preparing inocula were grown in 2000-mL cotton-plugged Erlenmeyer flasks containing 1200 mL YEPD medium. The flasks were incubated with agitation at 30°C for approx 12 h. Cells were harvested in the exponential phase by centrifugation at 1500*g* and 4°C for 5 min. Thereafter, the cells were washed with a sodium chloride solution (9.0 g/L) and centrifuged as before.

To determine the dry weight of the inoculum, a 0.45 μm HA filter (Millipore Billerica, MA) was dried in a microwave oven (Husqvarna Micronett, Sweden) set at a power scale of 3 for 15 min, and thereafter placed in a desiccator. After 2 h, the filter was taken from the desiccator and weighed on an analytical scale. The yeast suspension (1.35 mL) was then filtered through the dried filter under the influence of vacuum. The filter was washed with 5 mL of water, dried as previously described, and weighed.

Before fermentation all hydrolysates were adjusted to pH 5.5 using a 5 *M* solution of NaOH. The hydrolysate sample (42.75 mL) (or, alternatively, 42.75 mL of a synthetic sugar solution in water for reference fermentations) was mixed with 0.9 mL of a nutrient solution (consisting of 50.0 g/L yeast extract, 25.0 g/L $(NH_4)_2HPO_4$, 1.25 g/L $MgSO_4 \cdot 7\ H_2O$, and 79.4 g/L $NaH_2PO_4 \cdot H_2O$) and 1.35 mL of the inoculum. The biomass concentration of the inoculum was adjusted to give an initial biomass concentration of 2.0 g/L (dry weight) in the fermentation vessel. The fermentation vessels (55-mL glass flasks) were equipped with magnetic stirrer bars and sealed with rubber stoppers with cannulas for outlet of CO_2. The vessels were then placed at 30°C in an incubator with magnetic stirring.

Analysis of Fermentations

The glucose levels during the fermentation were monitored using a glucometer (Glucometer Elite XL, Bayer, Leverkusen, Germany). Samples (0.2 mL) taken from the vessels were diluted with water (1.8 mL) and centrifuged for 5 min in a microcentrifuge (Minispin Plus, Eppendorf, Hamburg, Germany) at a speed of 14,500 rpm (14,000*g*). The supernatant was collected and stored at –20°C until analysis.

The ethanol concentration was determined using an HP 5890 Series II gas chromatograph with a flame ionization detector (Hewlett Packard, Palo Alto, CA) and a BP-20 column with a film thickness of 1.0 µm (SGE, Austin, TX). The temperature was kept at 30°C for 5 min and then raised to 180°C with a heating rate of 15°C/min. All the hydrolysate samples and the reference were fermented as duplicates.

MALDI-TOF Analysis

Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry was used to analyze the residue of sample 2. An HP G2025 A MALDI-TOF system (Hewlett Packard) was operated in positive mode with 0.1–1.3 mJ energy from the laser beam *(8)*. The matrix used for the sample was 2,5-dihydroxybenzoic acid, which was obtained from Fluka (Buchs, Switzerland).

HV Analysis

The HVs of the fiber sludge samples were determined using a bomb calorimeter (Type C110, Janke & Kunkel K.G, Staufen, Germany). A portion (0.400 g) of each sample was combusted in the calorimeter and the change in temperature was noted every 30 s until the maximum temperature was reached. The bomb calorimeter was calibrated using benzoic acid as the standard. The dry weights of the samples were determined using a moisture analyzer (Sartorius MA 50, Sartorius, Goettingen, Germany) set at a temperature of 105°C. The HV was calculated according to the formula $HV = [(\Delta T \cdot C) - E_1 - E_2 - E_3 - E_4]/m_D$, in which ΔT is the change in temperature in degrees Celsius, C is the heat capacity of the calorimeter (given in J/K), E_1 is the correction HV for the cotton thread used as ignition fuse in the calorimeter (given in J), E_2 is the correction HV for the chromium-nickel thread used for ignition of the cotton fuse (given in J), E_3 is the correction HV for the formation of nitric acid (assumed to be 40 J), E_4 is the correction HV for the formation of sulfuric acid (given in J and based on the assumed correction value 9.5 J/mg sulfur), and m_D is the dry weight of the sample (given in gram) *(9)*. The samples were analyzed twice and the HVs are presented in Table 3 as mean values with standard deviations indicated.

Results

The chemical composition of the fiber sludge samples varied considerably (Table 1). The combined glucan and mannan content varied between 34 and 67%. The xylan content varied between 8.3 and 16.9%. Lignin may have a negative effect on enzymatic hydrolysis, but it is a benefit when the HV is considered. The content of lignin in the samples varied between 1.3 and 25% (Table 1). The fraction of extractives varied between 0.2 and 5.8%. The large variation in the contents of extractives may be explained by differences in the process before the waste sedimentation. Sample 1, which

Table 1
Chemical Analysis of Fiber Sludge Samples (% weight)

Mill	Arabinan	Galactan	Glucan	Xylan	Mannan	Klason lignin	Acid-soluble lignin	Ash content	Extractives	Total
1	0.6	1.2	32.3	12.2	2.2	22.9	1.1	3.5	5.8	81.8
2	0.1	0.1	65.7	16.9	1.3	1.2	0.1	1.0	0.2	86.6
3	0.6	0.9	42.7	9.8	3.3	22.8	1.2	2.0	1.9	85.1
3 (Washed)	0.7	1.1	42.7	8.3	4.4	23.9	1.1	1.8	2.0	85.9

Table 2
Analysis of Sugars in the Hydrolysates

Hydrolysate	Sugar concentration (g/L)					Yield (%)
	Arabinose	Galactose	Glucose	Xylose	Mannose	
H1	0.0	0.2	8.2	3.1	0.8	14.0
H2	0.0	0.3	102.6	24.3	1.0	90.4
H3	0.2	0.3	20.6	4.9	1.6	27.7

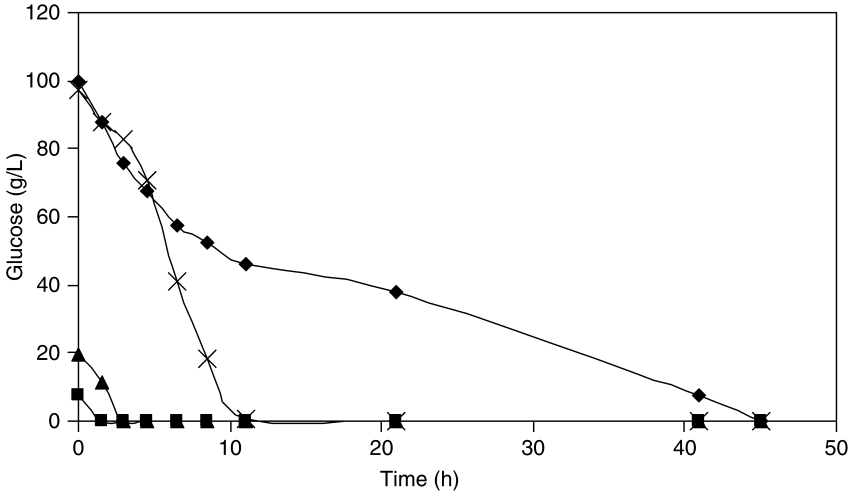

Fig. 2. Glucose consumption during fermentation of hydrolysate sample 1 (■), hydrolysate sample 2 (×), hydrolysate sample 3 (▲), and the reference fermentation (◆). The graph shows the mean values of duplicate fermentations. The standard deviation was ≤4.4%.

contained large amounts of extractives, originated from a kraft mill, which had its waste fiber sludge system integrated with a mill producing mechanical pulp fibers rich in extractives and lignin. The other measured components did not vary much and the amounts were very low (Table 1). Washing of sample 3 only resulted in minor changes (Table 1).

Hydrolysates were generated from the fiber sludge samples using enzymatic hydrolysis. The concentrations of monosaccharides in the hydrolysates are presented in Table 2. Hydrolysate 2 contained a high glucose concentration, more than 100.0 g/L. The yields of monosaccharides in the hydrolysates varied between 14 and 90%. The low conversion of carbohydrates in samples 1 and 3 can be correlated to the high lignin content. Sample 1, which showed the lowest yield, had the highest content of extractives. The yield of hexose sugars was generally low except for sample 2. This can probably be related to the high initial content of cellulose and the accessibility of the cellulose to hydrolysis by degrading enzymes. The fibers from mechanical pulping that were present in sample 1 are almost similar to those of native wood and may have had poorer accessibility for cellulose-degrading enzymes *(10)*. The ratio between glucose and xylose was almost one for samples 1 and 3, but for sample 2 the yield of glucose was higher than that of xylose. All three hydrolysate samples were readily fermented by *S. cerevisiae*. The glucose consumption rates of the hydrolysates were equal to or faster than that of the reference fermentation. All glucose in hydrolysate sample 2 was consumed within 11 h, whereas it took 45 h for the corresponding reference fermentation to consume all glucose (Fig. 2).

The offwhite solid residue obtained after enzymatic hydrolysis of sample 2 was analyzed with MALDI-TOF. The mass spectrum showed

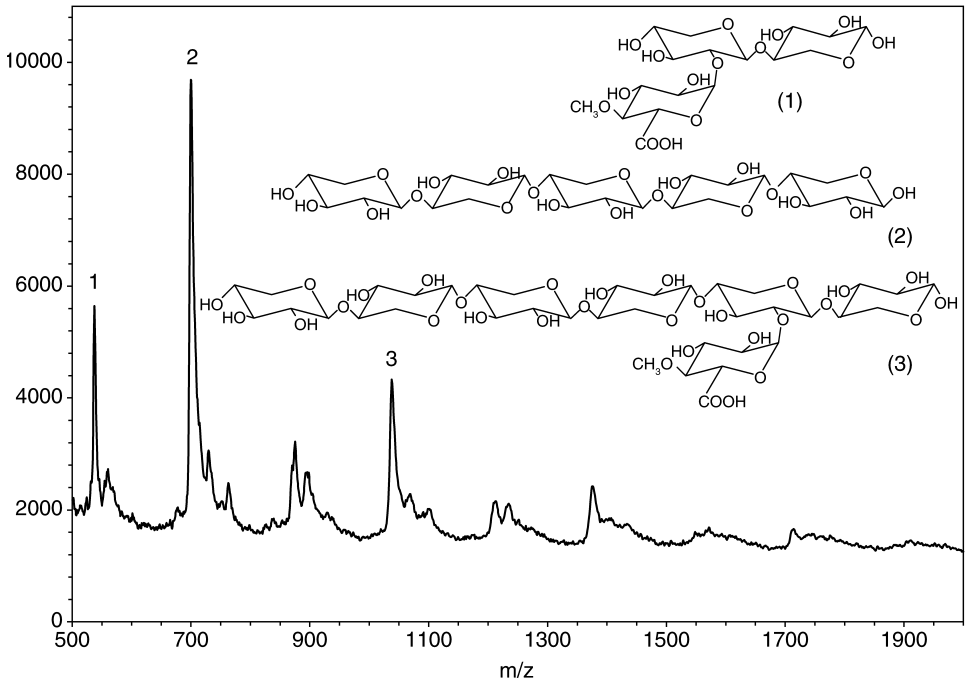

Fig. 3. Mass spectrum from the MALDI-TOF analysis. Peak 1 represents 4-*O*-methyl-glucuronic acid with two xylose units, peak 2 corresponds to a xylan fragment with five repeating pentose units, and peak 3 represents six pentose units with a 4-*O*-methyl-glucuronic acid residue (the tentative molecular structures are indicated).

peaks with a mass-to-charge ratio between 500 and 1800 (Fig. 3). The compounds representing the three major peaks were identified. Peaks 1 and 3 represent negatively charged 4-*O*-methyl-glucuronic acid residues, whereas peak 2 represents an uncharged xylan fragment consisting of five linked monomers. Peak 1 had a TOF of 11.6150 µs corresponding to a MW of 537.7, which was interpreted as 4-*O*-methylglucuronic acid-$(xylose)_2$ and a Na^+ ion. Peak 3 had a MW of 1038.9, which was interpreted as six pentose units with a 4-*O*-methyl-glucuronic acid residue and a K^+ ion. Peak 2, which matched MW 700.4, was interpreted as a xylan fragment with five repeating units and a Na^+ ion. The tentative molecular structures are indicated in Fig. 3 and the interpretations have been published previously *(8,11)*. Generally, native as well as pulped xylan fragments are much larger, but because the enzyme cocktail probably contains xylanases the reduced polymer size is not unexpected.

The HVs increased after enzymatic hydrolysis (Table 3). Although the hydrolysis of samples 2 and 3 resulted in improved HVs, they did not reach the level of sample 1. Another important parameter, which is linked to the HV, is the water seizing ability. The water seizing ability is presented in Table 4. The hydrolyzed samples had much higher dry content, which should result in better combustion efficiency.

Table 3
HVs of Fiber Sludge Samples Before and After Enzymatic Hydrolysis

Sample	HV before hydrolysis (kJ/g dry weight)	HV after hydrolysis (kJ/g dry weight)
1	20.8 ± 0.11	22.2 ± 0.19
2	17.8 ± 0.30	18.0 ± 0.36
3	18.3 ± 0.09	19.6 ± 0.12

Table 4
Dry Content of Fiber Sludge Samples Before and After Enzymatic Hydrolysis

Sample	Dry content before hydrolysis (% [w/w])	Dry content after hydrolysis (% [w/w])
1	26.2	38.7
2	31.9	43.4
3	30.0	41.8

Discussion

Lignocellulosic fibers, mainly made up of cellulose and hemicelluloses, are produced from wood at large kraft pulp mills. The annual pulp production in a modern kraft mill is often around 700,000 t or even higher. More than 1% of the production may end up as waste fibers of low value for the mill. Deposition of the waste is nowadays often connected with deposition fees or may even be prohibited. The alternative is to burn the waste fibers. However, combustion and recovery of heat is often not effective because of the inherent capacity of the carbohydrates to retain water. Current operational problems with the handling of waste fiber sludges in kraft mills can be turned into benefits in the form of new products in pulp mill-based biorefineries. The dry content and the value of the material as a solid fuel may be increased by removal of water-retaining polysaccharides by hydrolysis. Bioethanol could be produced *(12–14)* and a xylan fraction could be isolated before the residual material is used for combustion.

Waste fiber samples intended for combustion were selected in three Swedish kraft mills and used to investigate production of bioethanol, xylan, and residual material with improved HV. The chemical composition and the HVs of the samples differed considerably. Sample 2 contained only contaminated kraft pulp fibers and was easily degraded to fermentable sugars in high yield. High concentrations of fermentable sugars in the hydrolysates will lower the cost for separation of ethanol by distillation after the fermentation. Sample 1 also contained fibers from production of mechanical pulps. Sample 3 was a mixture that contained considerable amounts of bark and wood pieces. The lower convertibility

of samples 1 and 3 compared with that of sample 2 was probably because of their high lignin content. The kraft process is the most efficient way to remove lignin and wood extractives and to defibrate lignocellulosic materials. Pulp produced through refining, such as thermomechanical pulp and chemithermomechanical pulp, still contains lignin and wood extractives, especially on the fiber surfaces. The presence of such substances on the fibers strongly reduces the activities of polysaccharide-degrading enzymes. However, the negative effect of lipophilic compounds on the fiber surfaces may be reduced by addition of surfactants *(7)*, as in this study. The rapid fermentation rates observed for the hydrolysates suggest that significant amounts of inhibitory compounds were not present. The improved performance of the hydrolysate of sample 2 compared with that of the reference fermentation suggests that this hydrolysate may have contained additional nutrients that affected the yeast in a favorable way.

The HVs of the samples increased after hydrolysis. This can tentatively be explained by the increased content of lignin and extractives, because these substances are not lost during hydrolysis. Sample 2 had the lowest HV and this might be because of its low initial lignin content. The dry content is an important factor when it comes to combustion of fiber sludges. A high dry content will give a higher effective HV. All hydrolyzed samples showed significantly higher dry content compared with untreated material.

The offwhite solid residue after hydrolysis of sample 2 was analyzed using MALDI-TOF. The mass spectrum showed oligomeric xylan fragments, most of which contained 4-*O*-methyl-glucuronic acid groups. The molecular mass was low, less than 1000 Da, compared with the molecular mass of xylan found in kraft pulp fibers, which exceeds 12,000 Da *(15)*. The relatively low molecular mass was expected because the mixture of enzymes used for the hydrolysis would include xylanases. The native glucuronic acid side groups might have hindered a complete enzymatic hydrolysis to monosaccharides. Insoluble charged xylan fragments have a potential as a high-value byproduct. If isolation of a polymeric xylan is the goal, then a mixture of enzymes without xylanase activity should be chosen. As an alternative to isolation of xylan as a byproduct, xylose may be utilized for bioethanol production by pentose-fermenting microorganisms. The results show a potential in the isolation of a solid xylan fraction from a waste fiber stream. Thus, we suggest isolation of charged xylan fragments as a new product.

The xylan residue may be upgraded and used within the kraft mill. The 4-*O*-methyl-glucuronic acid groups in xylan oligomers can be converted to the corresponding unsaturated hexenuronic acid derivatives using alkali. Treatment of a 4-*O*-methyl-glucuronic acid substituted for xylan fragment in 0.5 *M* sodium hydroxide at 150°C for less than 2 h yielded one equivalent of methanol and the corresponding amount of hexenuronic acid *(16,17)*. Such hexenuronic acid substituents in kraft pulp are known as strong metal-ion chelating structures *(18)*. The hexenuronic acid groups are present in unbleached kraft pulp and bind catalytic metal ions like manganese and have

to be removed before a fully bleached pulp can be made *(19)*. The charged xylan fragments have a potential to be used as biodegradable alternatives to complexing agents like ethylenediaminetetraacetic acid and diethylenetriamine pentaacetic acid (DTPA), which are of environmental concern.

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References

1. Wyman, C. E. (2003), *Biotechnol. Prog*. **19,** 254–262.
2. Kamm, B. and Kamm, M. (2004), *Chem. Biochem. Eng. Q*. **18,** 1–6.
3. Ragauskas, A. J., Williams, C. K., Davison, B. H., et al. (2006), *Science* **311,** 484–489.
4. Ragauskas, A. J., Nagy, M., Kim, D. H., Eckert, C. A., Hallett, J. P., and Liotta, C. L. (2006), *Ind. Biotechnol.* **2,** 55–65.
5. Dahlman, O., Jacobs, A., Liljenberg, A., and Olsson, A. I. (2000), *J. Chromatogr.* **891,** 157–174.
6. Sárvári Horváth, I., Sjöde, A., Nilvebrant, N. -O., Zagorodni, A., and Jönsson, L. J. (2004), *Appl. Biochem. Biotechnol.* **114,** 525–538.
7. Eriksson, T., Börjesson, J., and Tjerneld, F. (2002), *Enzyme Microb. Technol.* **31,** 353–364.
8. Jacobs, A. (2001), *PhD Thesis*, Royal Institute of Technology, Stockholm, Sweden.
9. Österholm, L. -H. and Andersson, L. (1987), *Rapport 273: Bestämning av värmevärden för fasta bränslen,* Värmeforsk, Stockholm, Sweden.
10. Helle, S. S., Duff, S. J. B., and Cooper, D. G. (1993), *Biotechnol. Bioeng.* **42,** 611–617.
11. Jacobs, A., Larsson, P. T., and Dahlman, O. (2001), *Biomacromolecules* **2,** 894–905.
12. Jeffries, T. W. and Schartman, R. (1999), *Appl. Biochem. Biotechnol.* **77–79,** 435–444.
13. Fan, Z., South, C., Lyford, K., Munsie, J., van Walsum, P., and Lynd, L. R. (2003), *Bioprocess. Biosyst. Eng.* **26,** 93–101.
14. Kádár, Z., Szengyel, Z., and Réczey, K. (2003), *Ind. Crop. Prod.* **20,** 103–110.
15. Jacobs, A. and Dahlman, O. (2001), *Biomacromolecules* **2,** 894–905.
16. Nilvebrant, N. -O. and Reimann, A. (1996), *Xylan as a source for oxalic acid during ozone bleaching*. 4th European Workshop on Lignocellulosics and Pulp, September 8–11, 1996, Stresa, Italy.
17. Törngren, A. and Ragnar, M. (2002), *Nord. Pulp Pap. Res. J.* **17,** 179–182.
18. Devenyns, J. and Chauveheid, E. (1997), *Uronic acid and metals control*. 9th International symposium on wood and pulping chemistry (ISWPC), Montreal, Quebec, Canada, 9–12 June 1997.
19. Buchert, J., Teleman, A., Harjunpää, V., Tenkanen, M., Viikari, L., and Vuorinen, T. (1995), *Tappi J.* **78,** 125–130.