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Comparison of Diafiltration and Size-Exclusion Chromatography to Recover Hemicelluloses From Process Water From Thermomechanical Pulping of Spruce

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Abstract

Hemicelluloses constitute one of the most abundant renewable resources on earth. To increase their utilization, the isolation of hemicelluloses from industrial biomass side-streams would be beneficial. A method was investigated to isolate hemicelluloses from process water from a thermomechanical pulp mill. The method consists of three steps: removal of solids by microfiltration, preconcentration of the hemicelluloses by ultrafiltration, and purification by either size-exclusion chromatography (SEC) or diafiltration. The purpose of the final purification step is to separate hemicelluloses from small oligosaccharides, monosaccharides, and salts. The ratio between galactose, glucose, and mannose in oligo- and polysaccharides after preconcentration was 0.8: 1: 2.8, which is similar to that found in galactoglucomannan. Continuous diafiltration was performed using a composite fluoro polymer membrane with cutoff of 1000 Da. After diafiltration with four diavolumes the purity of the hemicelluloses was 77% (gram oligo- and polysaccharides/gram total dissolved solids) and the recovery was 87%. Purification by SEC was performed with 5, 20, and 40% sample loadings, respectively and a flow rate of 12 or 25 mL/min (9 or 19 cm/h). The purity of hemicelluloses after SEC was approx 82%, and the recovery was above 99%. The optimal sample load and flow rate were 20% and 25 mL/min, respectively. The process water from thermomechanical pulping of spruce is inexpensive. Thus, the recovery of hemicelluloses is not of main importance. If the purity of 77%, obtained with diafiltration, is sufficient for the utilization of the hemicelluloses, diafiltration probably offers a less expensive alternative in this application.

Index Entries: Galactoglucomannan; diafiltration; microfiltration; polysaccharides; ultrafiltration; SEC; gelfiltration.

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Introduction

Hemicelluloses and cellulose are among the most abundant renewable polymeric materials on earth. Cellulose has become one of the most widely used resources, but hemicelluloses, as such, have not the same utility in our society. However, several potential applications have been described, e.g., the production of hydrogels *(1)* and barrier films *(2,3)*, hydrolysis and fermentation to ethanol *(4)*, and as a feedstock for xylitol production *(5)*. Thus, there is significant potential for the future utilization of hemicelluloses. A prerequisite for the increased use of hemicelluloses is that cost-effective methods of extracting hemicelluloses from different raw materials will be developed.

Extraction of hemicelluloses from different raw materials has been studied since the 1950s *(6–11)*. The method used here to isolate hemicelluloses from process water from thermomechanical pulping of spruce *(Picea abis)* is membrane filtration followed by a final purification step of diafiltration or size-exclusion chromatography (SEC). Earlier studies have been performed with similar methods, e.g., membrane filtration *(10,12)* and SEC *(13,14)*.

Spruce wood is an important raw material for the Swedish pulp and paper industry. The dominating hemicellulose in spruce is *O*-acetyl galactoglucomannan (GGM). GGM is a complex, substituted heteropolysaccharide containing an *O*-acetylated β-(1→4)-linked glucomannan backbone with α-(1→6)-D-galactosyl side groups attached to some of the mannosyl units *(15,16)*. GGM is partly dissolved in the process water during thermomechanical pulping and has been isolated and shown to have good properties for production of barrier materials intended for food packaging *(3)*. The aim of this study was to compare two purification techniques, diafiltration and SEC. The purpose was to isolate the hemicelluloses from smaller-molecular-weight compounds such as small oligosaccharides, monosaccharides, and salts. The purity, recovery, and size distribution of hemicelluloses after diafiltration and SEC were studied.

Materials and Methods

Raw Material

The raw material used in this study was process water from thermomechanical pulping of spruce, from Stora Enso Kvarnsveden Mill AB, Sweden. The process water originates from the final dewatering step of the pulp, using a disc filter. The total dissolved solids (TDS) of the process water were 3.8 g/L and the pH was 4.7. The amount of hemicelluloses, defined as oligo- and polysaccharides including acetyl groups, was 1.5 g/L. The process water was stored at 4°C and 0.2 mL/L of the biocide Nalco 7647 (Nalco, Leiden, The Netherlands) was added to prevent biological activity.

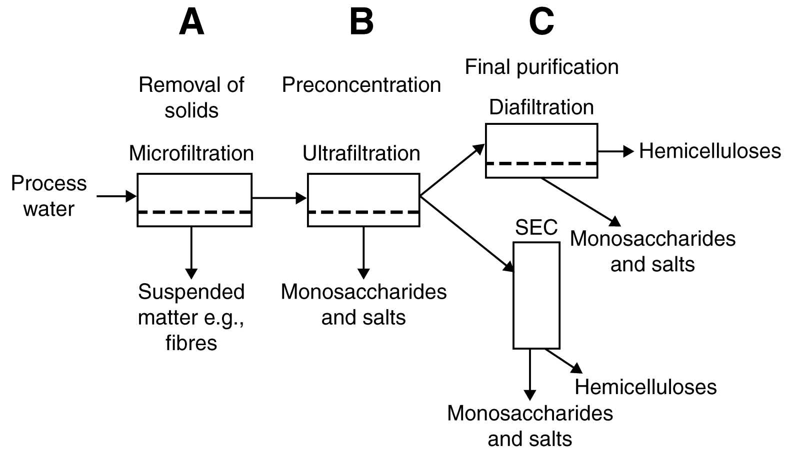

Fig. 1. Schematic illustration of the method used to isolate hemicelluloses from process water from thermomechanical pulping of spruce.

Procedure

The method used to isolate hemicelluloses from the process water involves three steps: removal of solids by microfiltration (Fig. 1A), preconcentration of hemicelluloses by ultrafiltration (Fig. 1B), and purification by diafiltration or SEC (Fig. 1C). All liquid streams were analyzed regarding oligo- and polysaccharide content and acetyl-group concentration, to determine the concentration of hemicelluloses. Monosaccharide content, ultraviolet (UV)-absorbance, TDS, ash, and conductivity were determined to evaluate the purification efficiency.

Removal of Solids

Microfiltration was performed in a vibratory shear-enhanced processing unit (series L/P, New Logic, Emeryville, CA), which is used in a wide variety of industrial separation and volume reduction (VR) processes *(17–19)*. The membrane stack consisted of 19 double-sided polytetrafluoroethylene membrane discs. The membrane pore diameter was 1 µm and the total membrane area was 1.57 m^2. The pressure was measured before and after the membrane stack. The pump was a displacement pump (G-03, Wanner Engineering Inc., Minneapolis, MN) and the permeate flow was measured with a flow meter (Promag 53, Endress+Hauser, Reinach, Switzerland).

Microfiltration was carried out at a transmembrane pressure of 200 kPa and a temperature of 22 ± 0.5°C. The vibration frequency was 49.9 Hz, which corresponds to an amplitude of 19 mm. The vibrations cause high shear forces to arise on the membrane surface and thereby prevents the formation of a filter cake, which otherwise would decrease the flux. The membranes were cleaned with 0.3 wt% Ultrasil 10 (Henkel Chemicals Ltd., Düsseldorf, Germany) at 35°C for 45 min, and thoroughly rinsed with deionized water before and after the study.

Preconcentration by Ultrafiltration and Purification by Diafiltration

Ultrafiltration and diafiltration were performed in a DDS 20 LAB module unit (Alfa Laval Corp., Lund, Sweden). The membrane stack consisted of six double-sided ETNA01PP membrane discs (Alfa Laval Corp.) made of a composite fluoro polymer with cutoff of 1000 Da. The total membrane area was 0.21 m^2 and the pressure was measured with diaphragm pressure transmitters (dTrans p02, JUMO, Fulda, Germany), before and after the membrane stack. The pump was a displacement pump (D-25, Wanner Engineering Inc.) and the circulation flow rate was measured with a rotameter. The permeate flow was measured gravimetrically with a balance (FX-3000, A&D Company Ltd., Tokyo, Japan).

The microfiltration permeate was preconcentrated by ultrafiltration to 80% VR. Four temperature-equilibrated diavolumes of deionized water were added at the same flow rate as the permeate flow during diafiltration. The number of diavolumes corresponds to the ratio between the volume of water added during diafiltration and the constant volume of feed solution in the system *(20,21)*. The average transmembrane pressure was 1.0 MPa and the temperature was 60°C during both preconcentration and diafiltration. The circulation flow rate was 5.2 L/min, which corresponds to a cross-flow velocity of 0.5 m/s. The membranes were cleaned with 0.3 wt% Ultrasil 10 at 60°C for 45 min and thoroughly rinsed with deionized water before and after the study. The pure water flux was measured at 50°C and 1.0 MPa before and after the study, and after cleaning.

Purification by SEC

SEC was performed on an INdEX 100/500 column (GE Healthcare, Uppsala, Sweden) with a diameter of 10 cm, packed with Superdex 30 chromatography media (GE Healthcare) to a height of 28 cm. Blue Dextran (GE Healthcare) was used to determine the void volume (684 mL), and acetone (Sigma-Aldrich Co, St. Louis, MO) was used to determine the total mobile volume of the column (1870 mL). Dextran T10 (GE Healthcare), maltose (Sigma-Aldrich Co.), and D-mannose (Fluka Chemie AG, Buchs, Switzerland) were used to calibrate the column. To determine the elution volumes for the standards, 6 mL of a 5 wt% solution was injected at a flow rate of 12 mL/min. The column was connected to a WellChrom high-performance liquid chromatography pump (K-1001, Knauer, Berlin, Germany), refractive index (RI) detector (K-2401, Knauer), and Filter-Photometer (K-2001, Knauer). The system was controlled with ChromGate computer software (Knauer).

SEC was performed on ultrafiltration retentate withdrawn at a VR of 80%. Six different SEC runs were performed in which the flow rates were 12 or 25 mL/min (9 or 19 cm/h) and sample volumes of 5, 20, or 40% of the total column volume were applied, respectively. The productivity of SEC is improved by increasing the sample volume or the flow rate of the mobile phase. However, these actions result in band broadening, and hence, decreased separation *(22)*. A larger sample volume contributes greatly to a larger average retention volume, because of longer time required to apply the sample. The influence of flow rate on band broadening is well described by the van Deemter equation *(23)*. The study was performed at load-limiting conditions *(22)*. Degassed Millipore water was used as eluent and the column was washed with 4000 mL 2 wt% NaOH before the first SEC run. Two fractions were collected from each SEC run and the change of fraction occurred in the valley between the first and the second peak. With non-Gaussian elution profiles it can be difficult to determine the elution volume. Therefore, the center of each peak was used to calculate the elution volume.

Analysis

The ash content and TDS were determined according to the standardized methods of the National Renewable Energy Laboratory (NREL, US Department of Energy) *(24,25)*. The absorbance at 205 nm in untreated samples was measured with a spectrophotometer (UV-160, Shimadzu, Kyoto, Japan) to indicate presence of lignin *(26)*. The conductivity was measured with a digital conductivity meter (PW9527, Philips, Amsterdam, The Netherlands) and calibrated with 0.01 *M* KCl (Merck, Darmstadt, Germany).

The monomer sugar composition and the concentration of oligo- and polysaccharides were analyzed by acid hydrolysis according to the standardized method of NREL *(27)*. Monomeric sugars were analyzed before and after acid hydrolysis and the oligo- and polysaccharide content was calculated from the difference in monosaccharide concentration before and after hydrolysis. Anhydro corrections of 0.9 and 0.88 were used for the hexoses and pentoses, respectively.

High-performance anion-exchange chromatography coupled with pulsed amperometric detection using an ED40 electrochemical detector (Dionex, Sunnyvale, CA), was used to analyze the monomeric sugars. It was equipped with a gradient pump (GP40, Dionex), an autosampler (AS50, Dionex), and a Carbo Pac PA10 guard and analytical column (Dionex). Degassed Millipore water was used as eluent at a flow rate of 1 mL/min, and the injection volume was 10 µL. D-Mannose, D-glucose, D-galactose, D-xylose, and L-arabinose (Fluka Chemie AG) were used as standards.

The acetyl content of the samples was measured by freeze-drying the filtrates and then redissolving them in 1% NaOH solution, to remove the *O*-acetyl moieties. The samples were then treated at room temperature overnight. The amount of acetic acid released was determined with high-performance liquid chromatography (GE Healthcare) using an Aminex HPX-87H column (BIO-RAD, Hercules, CA) at 65°C. Degassed 0.005 *M* H_2SO_4 was used as eluent, at a flow rate of 0.6 mL/min. Detection was performed with a RI detector (Erc - 7512, Erma Inc., Tokyo, Japan) and acetic acid (Merck) was used as standard.

Results and Discussion

Pretreatment by Microfiltration

Particles and suspended matter in the process water were removed by microfiltration. The average flux was 78 L/m^{2}h during concentration to 87% VR. The TDS were 3.8 g/L in the original process water and 3.2 g/L in the permeate. Turbidity decreased from 960 nephelometric turbidity units (NTU) in the process water to 4 NTU in the permeate. The oligo- and polysaccharide concentration including acetyl groups was 1.5 g/L in the process water and 1.3 g/L in the permeate. Hence, the recovery of oligo- and polysaccharides was 73%. A lower concentration in the permeate was expected, because oligo- and polysaccharides in or adsorbed onto the suspended matter were included in the analysis of the oligo- and polysaccharide concentration.

Preconcentration by Ultrafiltration

The average flux was 69 L/m^{2}h during preconcentration by ultrafiltration to 80% VR. The concentration of oligo- and polysaccharides including acetyl groups increased from 1.3 to 5.7 g/L and the TDS from 3.2 to 10.0 g/L in the retentate. The flux decreased from 90 to 60 L/m^{2}h. The initial flux decrease is probably because of fouling and reduction of the Zeta potential of the membrane *(28)*. Galactan, glucan, and mannan were detected in a ratio of 0.8 : 1 : 2.8 in the ultrafiltration retentate, which is similar to that found in GGM *(15,29)*. No xylan or arabinan were detected.

Purification by Diafiltration

In diafiltration the product is purified by removing permeable solutes by dilution with water. Diafiltration can be carried out in two ways: discontinuous or continuous, i.e., water is added in one batch or at the same rate as permeate is withdrawn. Continuous diafiltration was used as one of the methods to purify the hemicelluloses in the ultrafiltration retentate. If the retention is constant during diafiltration, the concentration of a solute *(i)* in the retentate can be expressed as *(21)*:

$$C_{\mathrm{i}} = C_{0,\mathrm{i}}\, e^{-V_{\mathrm{d}}(1-R_{\mathrm{i}})} \tag{1}$$

where $C_{0,\mathrm{i}}$ is the initial concentration of the solute, V_{d} is the number of diavolumes, and R_{i} is the retention of the solute. The conductivity decreased in accordance with Eq. 1 with a retention of 60%. Conductivity was used to measure the content of ions in the retentate during diafiltration, *see* Fig. 2. A small flux increase from 55 to 63 lx/m^{2}h during diafiltration implies that the retention of the oligo- and polysaccharides was high. Flux, purity, and recovery are important factors for the economy of the separation process. The purity is defined as the ratio between the

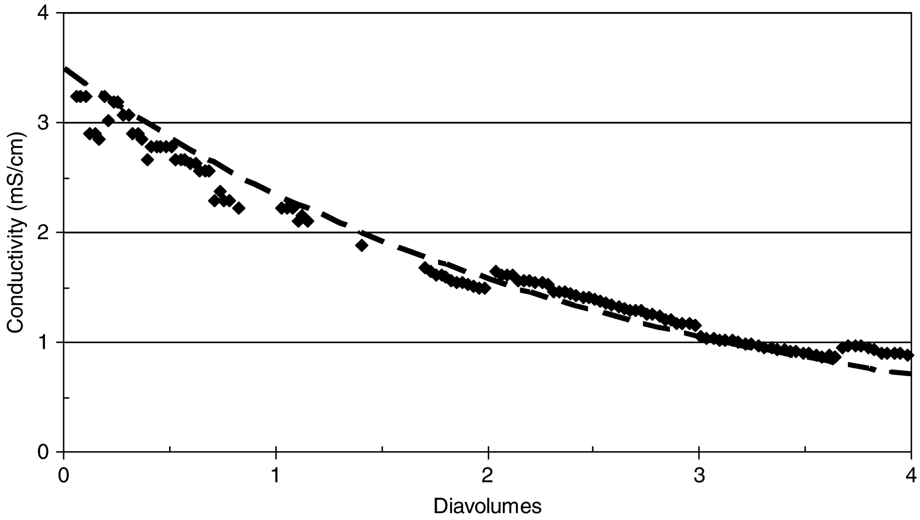

Fig. 2. Conductivity in the retentate during diafiltration of process water preconcentrated by ultrafiltration to 80% VR. The ash content in the ultrafiltration retentate was 0.2 g/g TDS. The dashed line was calculated from Eq. 1 with R = 60%.

mass of oligo- and polysaccharides and the TDS. The recovery during diafiltration can be calculated from *(17)*:

$$\text{Recovery} = \frac{m_r}{\mathrm{m_o}} = e^{-V_d \cdot (1-R_i)} \tag{2}$$

where m_r is the mass of oligo- and polysaccharides in the retentate, m_{tds} is the total mass of dissolved solids, and m_o is the original mass of oligo- and polysaccharides. Purity increased from 57 to 77% during diafiltration, but at the same time the recovery of oligo- and polysaccharides decreased by 16% compared with the ultrafiltration retentate. Figure 3 shows that the purity continued to increase with the number of diavolumes and a purer product was achieved as diafiltration progressed. However, as diafiltration proceeds, the recovery decreases. Optimal conditions are defined by 100% retention of the compound to be purified and 0% retention of contaminants. Equation 1 was used to calculate the average retention during diafiltration, presented in Table 1. The retention of oligo- and polysaccharides was high, which agrees with the small flux increase during diafiltration, whereas the retention of contaminants was smaller, but still much greater than zero.

The ash retention was constant during diafiltration and similar to the calculated retention of the conductivity (60%). The retention of lignin, on the other hand, increased during diafiltration from 42 to 85%. This was owing to the size distribution of the molecules. Both small carbohydrate degradation products and large lignin molecules and lignin bound to hemicelluloses in lignin-carbohydrate complexes *(30,31)* absorb UV-light at 205 nm. The small molecules pass through the membrane during diafiltration whereas

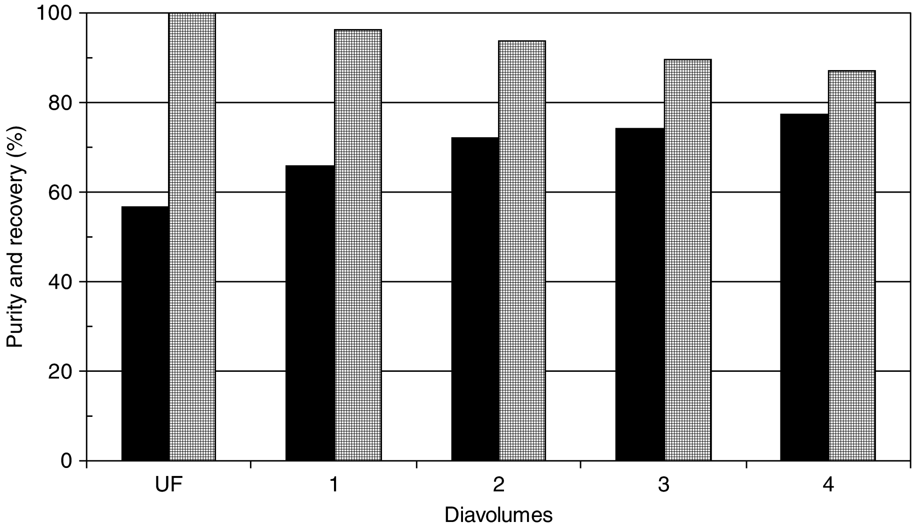

Fig. 3. Purity with respect to TDS (solid bars) and recovery of oligo- and polysaccharides (hatched bars) during diafiltration.

Table 1
Average Retention of Different Compounds During Diafiltration, Calculated With Eq. 1

	Oligo- and polysaccharides	Monosaccharides	TDS	Lignin	Ash
Retention (%)	97	38	89	63	63

the large molecules are retained, which increases the average molecular mass of the hydrophobic molecules and thus increases the retention. The pure water flux was 197 L/m^{2}h before ultrafiltration and 124 L/m^{2}h after preconcentration and diafiltration. After cleaning, the pure water flux was 204 L/m^{2}h. This flux decrease implies that the membranes are fouled during operation, but after cleaning, the original flux was restored.

Purification by SEC

SEC was used as an alternative method to purify the hemicelluloses in the ultrafiltration retentate. The influence of different flow rates and sample volumes was studied. The resolution between two solutes depends on two effects, (a) increasing separation of band centers and (b) increasing bandwidth as bands migrate along the column. The resolution of two peaks is described by:

$$R_s = \frac{\Delta V_r}{W_{av}} \tag{3}$$

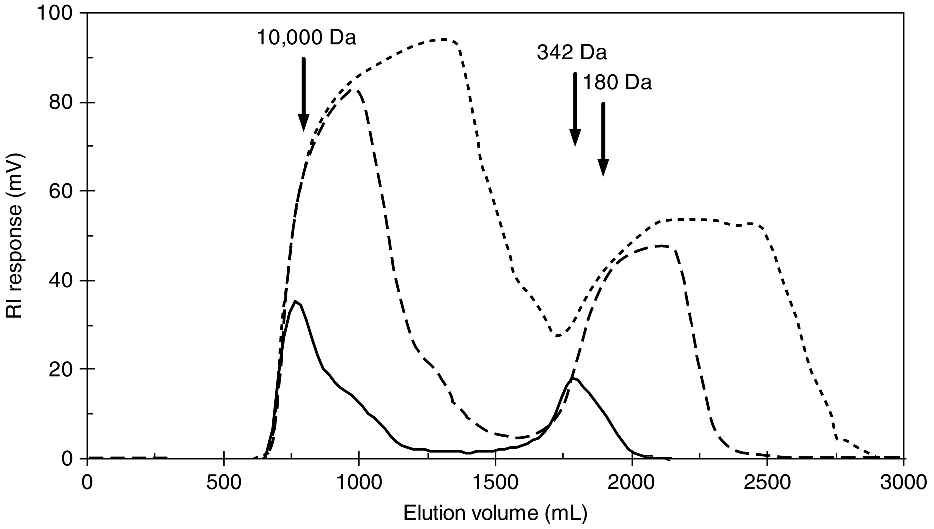

Fig. 4. RI elution profile from SEC of ultrafiltration retentate at a flow rate of 25 mL/min and sample volumes of 5% (solid line), 20% (dashed line), and 40% (dotted line) of the total column volume, which correspond to 94, 374, and 747 mL, respectively.

Table 2
Resolution and Recovery in SEC

Flow rate (mL/min)	12	12	12	25	25	25
Sample volume (%)	5	20	40	5	20	40
Resolution	1.8	1.4	0.7	1.9	1.7	0.7
Recovery (%)	>99	>99	>99	>99	>99	>99

The resolution was calculated from Eq. 3 and the recovery of GGM from Eq. 2, based on mannose residues.

where ΔV_r is the difference in elution volume between two solutes and W_{av} is the average width of the two peaks *(22,32,33)*. Therefore, the flow rate of an eluent should affect the resolution of the peaks, but no significant difference was detected at the two flow rates investigated. At flow rates higher than 25 mL/min the maximum pressure drop for the column (300 kPa) was exceeded. Thus, higher flow rates could not be studied. Larger sample volume demands a longer loading time, which leads to increased peak width. Thus, the resolution of the peaks in the chromatogram decreases with increasing sample volume, *see* Fig. 4 and. Table 2. The recovery of GGM, based on mannose residues, was above 99% and independent of sample volume. The sample volume should not be more than 20% of the total column volume, as a resolution better than 1.5 is desirable for efficient product purification *(32)*.

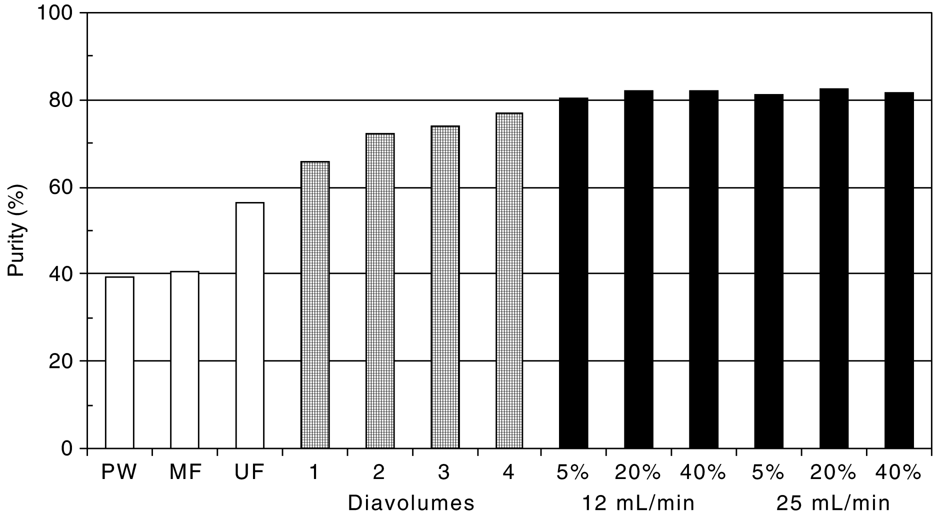

Fig. 5. Purity of oligo- and polysaccharides after primary filtration (white bars), after diafiltration (hatched bars), and after SEC (black bars). PW is the original process water, MF is after microfiltration, and UF is after preconcentration. The number of diavolumes during diafiltration and the eluent flow rate and sample volume, expressed as percent of total column volume during SEC are shown in the diagram.

Comparison Between Diafiltration and SEC

The purification efficiency during diafiltration and SEC can be compared by calculating the purity of the oligo- and polysaccharides after the removal of low-molecular-weight compounds. Figure 5 indicates that the purity is somewhat higher after SEC than after diafiltration. However, the purity could probably be further increased during diafiltration by increasing the number of diavolumes. When diafiltration is used as the final purification step, the purity of the hemicelluloses is dependent on the purity of the feed. If the feed is highly contaminated with low-molecular-weight compounds, additional diavolumes are needed to obtain a pure product. If instead, SEC is used as the purification step, the purity of the feed is not important for the purity of the product, as long as the column is not overloaded. Separation is based completely on size and is independent of the concentrations of oligo- and polysaccharides over the range studied. Measurements of the UV absorbance also indicate a slightly higher purity of samples after SEC (*see* Fig. 6: small, UV-absorbing, hydrophobic molecules are easily separated from the hemicelluloses by SEC, but because they are retained by the ultrafiltration membrane, more diavolumes are needed to achieve the same purity).

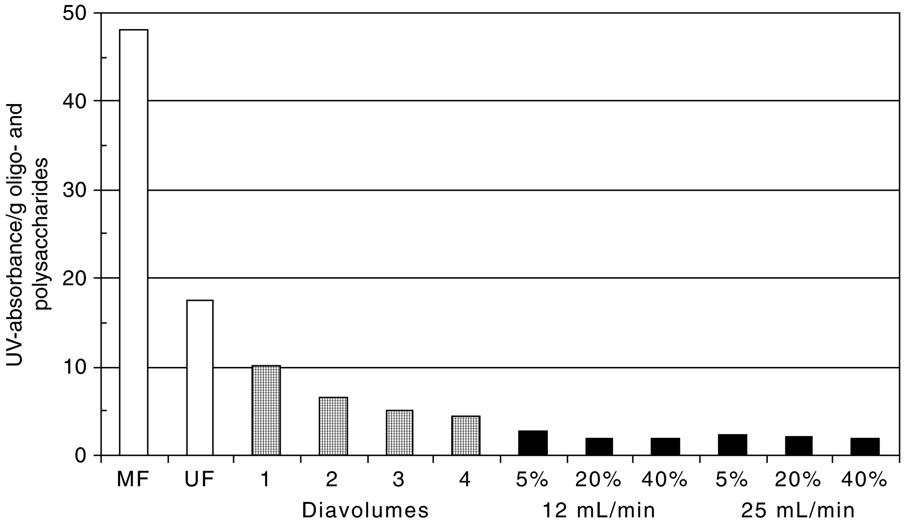

Fig. 6. The ratio between UV absorbance (205 nm) and grams of oligo- and polysaccharides before diafiltration (white bars), after diafiltration (hatched bars), and after SEC (black bars). MF is after microfiltration and UF is after preconcentration. The number of diavolumes during diafiltration and the eluent flow rate and sample volume, expressed as percent of total column volume during SEC are shown in the diagram.

Conclusions

Both purification methods have been shown to efficiently remove low-molecular-weight compounds from the process water and to have high recovery of oligo- and polysaccharides. The two separation techniques used have advantages and disadvantages. SEC gives both higher purity and recovery than diafiltration. The purity of diafiltrated material could probably be increased by adding more diavolumes or by using a membrane providing better separation between contaminants and hemicelluloses. However, increasing the number of diavolumes decreases the recovery. SEC is preferably used when the raw material is expensive and there are high demands on recovery, for example, in the purification of valuable products such as pharmaceuticals. Process water from thermomechanical pulping is an inexpensive raw material, and consequently, diafiltration is probably the most cost-efficient purification method for the extraction of hemicelluloses from this process water. Both techniques should be economically evaluated and further optimized by using different operating conditions and materials to find the most suitable procedure.

Acknowledgments

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