Carbohydrate Polymers 93 (2013) 102–108

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Enzyme-aided alkaline extraction of oligosaccharides and polymeric xylan from hardwood kraft pulp

Terhi K. Hakala*, Tiina Liitiä, Anna Suurnäkki

VTT Technical Research Centre of Finland, P.O. Box 1000, VTT, Finland

ARTICLE INFO

Article history:
Received 25 October 2011
Received in revised form 26 March 2012
Accepted 4 May 2012
Available online 11 May 2012

Keywords:
Enzyme treatment
Xylan
Xylanase
Endoglucanase
Oligosaccharide
Dissolving pulp

ABSTRACT

In this paper we describe the effect of enzyme treatments on the production of polymeric xylan, oligosaccharides and hemicellulose lean pulp by alkaline extraction of bleached hardwood kraft pulp. Enzyme treatments were carried out before one or in between two subsequent alkaline extractions by purified *Trichoderma reesei* xylanase and endoglucanase II (Cel 5a) as well as by a commercial monocomponent endoglucanase (FibreCareR). Without enzyme pre-treatment 61% and 7% of the pulp xylan was extracted in high purity in the first and second alkaline stage, respectively. Higher molecular mass xylan was obtained in the second than in the first alkaline extraction. Xylanase treatment before alkaline extraction hydrolyzed up to 12% of xylan to xylooligosaccharides. According to our results, preparation of polymeric xylan, and/or oligosaccharides as well as hemicellulose lean pulp with cellulose content of 93–94%, is possible by enzyme-aided alkaline extraction process.

1. Introduction

Currently the utilization of lignocellulose-based raw materials for novel end-uses is under vigorous investigation. The lignocellulose biorefinery research is greatly focusing on the production of biofuels and chemicals derived from abundant biomass resources. The production of several high value products such as polymeric hemicelluloses and oligosaccharides in addition to the main product could have a major impact on the economy of the biorefineries. The polymeric wood hemicelluloses, e.g. xylan and glucomannan, are interesting starting components for material applications, chemicals and liquid fuels. For example xylan as such or after modification has end-use applications in pulp and paper making, food and pharmaceutical industries (Ebringerová, Hromádková, & Heinze, 2005).

Potential value-added end-products obtained from kraft pulp, which is currently used mainly as paper grade pulp, are polymeric isolated hemicelluloses (Krogerus & Fuhrmann, 2009; Talja, Fuhrman, Krogerus, & Vähä-Nissi, 2009) and oligosaccharides (Rydlynd & Dahlman, 1997). Up to 60% of the xylan present in bleached hardwood market pulp can be isolated by alkaline extraction followed by precipitation and ultrafiltration (Talja et al., 2009). Xylan can also be isolated from the kraft cooking liquor (Dahlman, Tomani, Axegård, Lundqvist, & Lindgren, 2007) or from pulp prior to bleaching. Cold caustic extraction was used by Gomes, Colodette, Barbosa, and Oliveira (2011) to remove over 60% of xylan from unbleached eucalyptus kraft pulp. In comparison to sulphite pulps higher molecular weight xylans can be obtained from kraft pulp (Janzon, Saake, & Puls, 2008).

Xylooligosaccharides (XO) are already available especially on the Asian markets for use as food ingredients to stimulate the growth of beneficial bacteria in the intestinal tract (Vázquez, Alonso, Domínguez, & Parajo, 2000). XOs are produced from xylan rich feed-stocks from agriculture such as corn cobs and hulls but also wood based raw materials have been considered (Moure, Gullón, Domínguez, & Parajo, 2006). XOs can be manufactured by restricted acid hydrolysis, enzymatic hydrolysis, or hydrothermal treatment either directly or after fractionation of the feedstock (Vázquez et al., 2000). The advantage of enzymatic hydrolysis over acid hydrolysis is specificity and thus, although the process is slower, XOs with desired degree of polymerization (DP) are obtained without the formation of monosaccharides or furfural (Akipnar, Erdogan, Bakir, & Yilmaz, 2010).

With hydrolytic enzymes i.e. hemicellulases and cellulases different carbohydrate components from lignocellulosic raw materials and pulps can be selectively degraded. The selectivity of enzymatic treatment makes it an interesting process step when designing new process concepts. Several enzyme applications have been described for pulp and paper industry processes (Viikari, Suurnäkki, Grönqvist, Raaska, & Ragauskas, 2009). For example, up-grading hardwood kraft pulps into dissolving pulps has been successful by combining alkaline extraction and enzymatic

* Corresponding author. Tel.: +358 20 722 7417; fax: +358 20 722 7071.
E-mail address: Terhi.Hakala@VTT.fi (T.K. Hakala).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.05.013

Table 1
Enzyme activities and protein content of the used enzyme preparates.

	Xylanase (nkat/ml)	HEC[a] (nkat/ml)	Protein (mg/ml)
Trichoderma reesei, xylanase, pI 9	31 000	ND[b]	3.2
Trichoderma reesei, EG II (Cel5a)	11.5	6886	7.5
Humicola insolens, EG V	ND	1520	12.0

[a] HEC: hydroxyethylcellulose.
[b] ND: not detected.

treatment steps (Ibarra, Köpcke, & Ek, 2009; Köpcke, Ibarra, Larsson, & Ek, 2010). Paper grade birch kraft can be up-graded into dissolving grade pulps by two subsequent alkali extraction steps to decrease pulp xylan content followed by endoglucanase treatment to increase pulp reactivity (Köpcke et al., 2010). Similar results have been obtained with eucalyptus kraft pulp by xylanase-alkaline extraction-endoglucanase sequence (Ibarra et al., 2009). The utilization of the alkaline extract obtained from such process as a source of xylan to produce xylose has also been considered (Hyatt, Fengl, Edgar, & Alvarz-Wright, 1998).

Enzymatic treatments have separately been shown to have potential for the production of oligosaccharides from agricultural residues and for upgrading paper grade pulp into dissolving grade pulp. Based on the results by Talja et al. (2009), Ibarra et al. (2009) and Köpcke et al. (2010), the combination of alkaline extraction and enzymatic treatments causes formation of filtrates that contain 10–20% of the initial pulp carbohydrates. In order to make the process economically and environmentally feasible, these filtrates need to be exploited. However, so far the effect of the enzyme treatments on the DP of the carbohydrates present in these filtrates is not known. The possibility to obtain polymeric xylan and XOs as well as hemicellulose poor pulp from hardwood kraft pulp was evaluated in this study. The emphasis was on characterization of the effect of enzyme treatment on alkaline extraction and the effect on the molecular weight distribution of the extracted xylan as well as clarifying the possibility to isolate oligosaccharides from the pulp filtrates. In addition, the effect of enzyme treatments on the molecular weight distribution of kraft pulp polymers was clarified. Combination of xylanase or endoglucanase treatment and alkaline extraction of xylan were carried out and mass balance of the overall process was calculated.

2. Materials and methods

2.1. Materials

Enzyme treatments were carried out with xylanase (pI 9) purified from *Trichoderma reesei* culture filtrates as described by Tenkanen, Puls, and Poutanen (1992). Endoglucanase treatments were carried out with endoglucanase II (Cel5A) purified from *T. reesei* culture filtrate (Pere, Siika-aho, Buchert, & Viikari, 1995) (*Tr.* EG II) and with commercial endoglucanase product FibreCareR (Novozymes AS), which contains EG V from *Humicola insolens* (*Hi.* EG V). Xylanase and endoglucanase activities were determined as described by Pere et al. (1995) and the activities were expressed as katals. One nanokatal (nkat) of enzyme catalyzes the release of 1 nmol of reducing sugars from the substrate polymer (birch xylan for xylanase and hydroxyethylcellulose (HEC) for endoglucanase) in 1 s. Protein content of the enzyme preparates was determined with BIORAD protein assay. Xylanase and endoglucanase (HEC) activities and the protein content of the used enzyme preparates are presented in Table 1.

Bleached commercial hardwood kraft pulp (Södra Gold Birch Z) was utilized as raw material. Before enzyme treatments or alkaline extractions dry pulp was soaked overnight in water and disintegrated with Lorentz & Wettre pulp disintegrator in 60 g batches at 0.2% consistency for 30 000 revolutions.

2.2. Enzyme treatments

Enzyme treatments of pulp were carried out prior to one or in between two subsequent alkaline extraction steps. Pulp pH was adjusted to 5 with 0.5 M H_2SO_4. Xylanase treatment with xylanase dosage of 20 and 1000 nkat/g of dry pulp was carried out at 4% consistency, 45 °C and pH 5 for 2 h. Endoglucanase treatments with the enzyme dosage of 0.5 mg protein/g of dry pulp were carried out at 4% consistency, 45 °C and pH 5 for 2 or 24 h. Reference pulp was treated in the same way as described above for 2 h but without enzyme addition. During the enzyme treatments the pulp was mixed at 110–120 rpm. After the treatment, the pulp was heated to 90 °C for 15 min to inactivate the enzymes, and thereafter it was filtered with wire cloth and washed twice with 10 ml distilled water per g of pulp.

2.3. Alkaline extraction

Alkaline extraction of pulp was carried out as described by Talja et al. (2009) with 1 M NaOH at 5.5% consistency and at room temperature for 2 h with mixing at 70 rpm. After the alkaline extraction the pulp was filtered through wire cloth and washed thoroughly to remove the alkali. The alkaline extract contained the alkaline extract combined with the filtrate from the first washing step with 10 ml distilled water per g of pulp.

2.4. Determination of carbohydrate composition

To determine the carbohydrate composition of the pulps, pulp filtrates and extracted xylans the samples were hydrolyzed with sulphuric acid and analyzed according to Willför et al. (2009). Pulp samples were ground with Fritch pulverisette to pass 0.5 mm screen prior to acid hydrolysis. The resulting monosaccharides were determined by HPAEC with pulse amperometric detection (Dionex ICS 3000A) equipped with CarboPac PA1 column. The polysaccharide content in the samples was calculated from the corresponding monosaccharides using an anhydro correction of 0.88 for pentoses and 0.9 for hexoses. Linear oligosaccharides present in the pulp filtrates were analyzed by HPAEC with pulse amperometric detection (Dionex ICS 3000A) equipped with CarboPac PA1 column without the acid hydrolysis (Tenkanen, Makkonen, Perttula, Viikari, & Teleman, 1997). Linear XOs, xylobiose, xylotriose, xylotetraose, xylopentaose and xylohexaose (Megazyme) as well as cellobiose (Serva), cellotriose (Seikagaku), cellotetraose (Merck), cellopentaose (Seikagaku) and cellohexaose (Seikagaku) were used as standards. The mass balance of enzyme treatment and alkaline extraction was calculated as percentage of the original, un-extracted pulp.

2.5. Determination of molecular weight of extracted xylan and pulps

Molar mass measurements of xylan were performed by size-exclusion chromatography (SEC) in 0.1 M NaOH using PSS's MCX 1000 and 100 000 columns with a precolumn (0.5 ml/min, $T = 30$ °C). For pulp molar mass measurements, the extracted pulp samples were dissolved in DMAc/8% LiCl according to the solvent exchange method with ethylisocyanate derivatization (Berthold, Gustafsson, Sjöholm, & Lindstrom, 2001). The SEC measurements were performed using 2× PL gel MiniMixed A columns with a precolumn in DMAc/0.8% LiCl eluent (0.36 ml/min, $T = 80$ °C). In both cases, the