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Delignification outperforms alkaline extraction for xylan fingerprinting of oil palm empty fruit bunch

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ABSTRACT

Enzyme hydrolysed (hemi-)celluloses from oil palm empty fruit bunches (EFBs) are a source for production of bio-fuels or chemicals. In this study, after either peracetic acid delignification or alkaline extraction, EFB hemicellulose structures were described, aided by xylanase hydrolysis. Delignification of EFB facilitated the hydrolysis of EFB-xylan by a pure *endo*-β-1,4-xylanase. Up to 91% (w/w) of the non-extracted xylan in the delignified EFB was hydrolysed compared to less than 4% (w/w) of that in untreated EFB. Alkaline extraction of EFB, without prior delignification, yielded only 50% of the xylan. The xylan obtained was hydrolysed only for 40% by the *endo*-xylanase used. Hence, delignification alone outperformed alkaline extraction as pretreatment for enzymatic fingerprinting of EFB xylans. From the analysis of the oligosaccharide-fingerprint of the delignified *endo*-xylanase hydrolysed EFB xylan, the structure was proposed as acetylated 4-*O*-methylglucuronoarabinoxylan.

1. Introduction

Plant residues from agriculture, forestry and industry are accounted as a source of monosaccharides, to be released from the plant polysaccharides, for production of biofuels and chemicals (Cherubini, 2010). Oil palm empty fruit bunches (EFBs) are the empty bunches remaining after removal of the oil-rich berries and about 15.8 MT of EFBs is produced per year worldwide (Sumathi, Chai, & Mohamed, 2008). The oil palm (*Elaeis guinensis*) belongs to the Arecaceae family, which is part of the monocot class or true grasses. EFB is composed of 35% (w/w) cellulose, 25% (w/w) hemicellulose and 25% (w/w) lignin (Escarnot, Aguedo, & Paquot, 2011). Structures of EFB hemicellulose have not been described in detail. Nevertheless, based on its published carbohydrate composition, it can be assumed that the hemicellulose component is mainly composed of xylans (Sun, Fang, Mott, & Bolton, 1999). The latter is in agreement with the fact that hemicelluloses in grasses predominantly consist of glucuronoarabinoxylans (GAX). In grasses, the backbone of GAX consists of β-D-(1 → 4)-linked xylopyranosyl units that can be substituted at the *O*2, *O*3 or both positions with α-L-arabinofuranosyl residues or acetyl residues and/or at the *O*2 position with (4-*O*-methyl)-α-D-glucuronyl acid residues (Ebringerová, Hromádková, & Heinze, 2005).

Alkaline extraction of hemicelluloses is the conventional method to extract hemicelluloses from grass-type biomasses plus the addition of a characterised *endo*-xylanase to fingerprint the arabinoxylan structure from wheat bran (Gruppen, Hamer, & Voragen, 1991). For other grass-like feedstocks, such as for wheat straw, alkaline extraction showed also good xylan yields (70–90%) (Escarnot et al., 2011; Fang, Sun, Salisbury, Fowler, & Tomkinson, 1999; Sun, Wen, Ma, & Sun, 2013), followed by enzymatic fingerprinting of the extracted arabinoxylans (Van Dongen, Van Eylen, & Kabel, 2011). Sun et al. (1999) performed alkaline extraction of EFB. In that study, however, the alkaline extraction, only yielded 50% (w/w) of the total xylans present in EFBs. Apparently, EFB-xylan was more difficult to extract with the conventionally used alkaline extraction. Other lignin rich feedstocks, such as rape seed straw and wheat straw, also yield lower xylan amounts after sequential alkaline extraction

Abbreviations: EFB, empty fruit bunches; AIS, alcohol insoluble solids; DAIS, delignified alcohol insoluble solids; 1Mss, 1 M NaOH soluble solids; 4Mss, 4 M NaOH soluble solids; D1Mss, delignified 1 M NaOH soluble solids; D4Mss, delignified 4 M NaOH soluble solids; *Re*GH10, *Rasamsonia emersonii* endoxylanase from GH family 10; $\sum_{n=2}^{6} X_nAc$, acetylated *xylo*-olgosaccharides; $\sum_{n=2}^{6} X_n$, linear *xylo*-oligosaccharides; $\sum_{n=6}^{+\infty} X_n$, linear polymeric xylan; XUA_{me}, xylosyl residues substituted by a 4-*O*-methylglucuronic acid; $\sum_{n=6}^{+\infty} X_nAc$, acetylated polymeric xylan.

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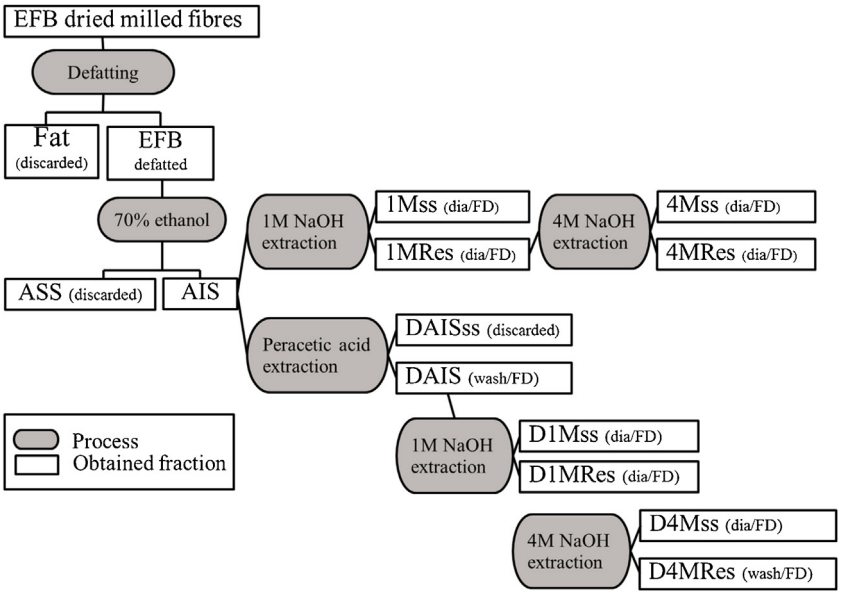

Fig. 1. Schematic overview of xylan fractionation from empty fruit bunch (dia = dialyzed, FD = freeze dried).

(Svärd, Brännvall, & Edlund, 2015; Xiao, Sun, & Sun, 2001). Various phenomena may underlie such extraction efficiencies. First, the type and distribution of substituents along the xylan backbone influence the solubility of xylan, which will influence the extraction yield. Second, the amount and type of lignin differs per feedstock and may influence the extractability of xylans. Lignin is known to covalently crosslink the plant cell wall polysaccharides (cellulose and xylan) together, thereby forming a compact network (Du et al., 2014). Delignification is reported to be performed as a pretreatment, mainly on hardwood (Bhalla et al., 2016) and often aimed at the improvement of the kraft pulping process (Li, Zhang, Hu, & Chai, 2016). The use of oxidative chemicals such peracetic acid or peroxide for the characterization of xylan in grasses is not explored well.

The present study aims at delignification prior to a representative enzymatic fingerprinting of the acetylated EFB xylan. Hereto, the EFB was delignified with peracetic acid and subsequently subjected to enzymatic fingerprinting of the EFB xylan using a pure *endo*-β-1,4-xylanase. The delignification method is compared to the conventional alkaline extraction of xylans for enzymatic fingerprinting.

2. Materials and methods

2.1. Materials used

Oil palm empty fruit bunches (EFBs) were kindly provided by Sime Darby (Kuala Lumpur, Malaysia). From a continuous palm oil crusher mill five EFB-batches of 200 g each were collected (one batch per 10 min). The batches were dried at 40 °C for 24 h. Dried EFB-batches were milled (<1 mm) in a 50 mL ball mill cuvette (Retsch, Haan, Germany) and equal amounts of all five batches were mixed to obtain the EFB-sample used. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) if not otherwise specified.

2.2. Fractionation of EFB

The EFB-sample (EFB dried milled fibres) was fractionated according to the scheme shown in Fig. 1. First, EFB (35 g) was defatted by petroleum ether using a Soxhlet-unit (Gerhardt, Bonn, Germany). The defatted EFB was dried at 20 °C overnight. Next, the material (33 g) was suspended in 70% (v/v) aqueous ethanol (1 L) and stirred at 20 °C for 1 h. After centrifugation (10000 × *g*; 15 min; 20 °C), the supernatant was discarded and the residue was again suspended in 70% (v/v) ethanol (1 L), stirred at 20 °C for 1 h and centrifuged. Again, the supernatant was discarded. The final residue was dried at 20 °C overnight, freeze dried and encoded AIS. Part of the AIS was delignified using peracetic acid (3 g peracetic acid/g dry solids; 25 °C; 24 h) as described by Kumar, Hu, Hubbell, Ragauskas, and Wyman (2013). Both the delignified fraction (DAIS) and the non-delignified AIS were sequentially extracted with 1 and 4 M NaOH, each containing 1% (w/w) $NaBH_4$, at 4 °C (24 h per extraction). After extraction, the supernatants obtained, denoted 1Mss and 4Mss, were separated from the insoluble residues (1 M and 4 MRes) by centrifugation (10000 × *g*; 15 min; 20 °C). After centrifugation, all fractions were neutralised (pH 5.0) using glacial acetic acid, dialysed (10–12 KDa cutoff, Medicell International, London, UK) against demineralised water and freeze dried. Fractions encoded as D1Mss, D4Mss and D4MRes were prepared from DAIS.

2.3. Enzyme purification

An *endo*-xylanase from *Rasamsonia emersonii* (*Re*GH10), expressed and produced in *A. niger* ISO527 as described previously (Neumüller et al., 2015), was kindly provided by DSM Biotechnology Center (Delft, The Netherlands) and purified. The enzyme containing liquid was fractionated using a Superdex 200 26/60 column (GE Healthcare, Uppsala, Sweden) eluted with 20 mM Tris HCl buffer pH 7.0 containing 0.15 M NaCl at 20 °C, over 5 column volumes at 6 mL min^{-1}. Fractions (3 mL) collected were concentrated using 10 kDa Amicon filters (Merck-Millipore, Billerica, MA, USA). *Re*GH10 containing fractions were selected based on activity towards beechwood xylan (see Enzyme hydrolysis) and SDS-page analysis (selection based on the expected molecular mass of 55 kDa). The fractions containing *Re*GH10 were pooled and again fractionated with the same column and gradient. The specific activity of the enzyme was 47.3 U mg^{-1}, which was based on the quantification of reducing ends formed over time (2 h) from wheat arabinoxylan (Megazyme, Wicklow, Ireland; 5 mg mL^{-1}) incubated at the optimum conditions of the enzyme (55 °C, pH = 4.5). The final fractions containing only *Re*GH10 were used for the EFB-xylan fingerprinting (see Enzyme hydrolysis).