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Separation of Xylose Oligomers from Autohydrolyzed *Miscanthus x giganteus* using Centrifugal Partition Chromatography

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Abstract

Autohydrolysis of cellulosic materials for saccharification generates xylose-oligosaccharides (XOS), due to the partial hydrolysis of xylan. Developing an efficient method for the separation and recovery of XOS from the prehydrolyzates would provide an excellent opportunity for the better utilization of the cellulosic material and for value-added co-product production. In this study, we investigated the use of centrifugal partition chromatography (CPC) for the fractionation of XOS from *Miscanthus x giganteus* (MxG). During autohydrolysis of miscanthus biomass at 180°C for 20 min, 63 % of xylan was converted into XOS and xylose. The ensuing XOS concentrate contained up to 30 % of XOS, which were distributed as 15.9 % xylobiose (DP2), 5.9 % xylotriose, (DP3), 5.6 % xyloetraose (DP4), 0.8 % xylopentaose (DP5) and 0.6 % xylohexaose (DP6). The XOS concentrate was further fractionated by CPC with a solvent system composed of 4:1:4 (v/v/v) butanol: methanol: water. Using CPC techniques, 230 mg (80 %) of DP2 to DP6 oligomers were fractionated from 1 g of XOS concentrate. The recoveries of individual XOS were 90.2 % DP2, 64.5 % DP3, 71.2 % DP4, 61.9 % DP5 and 68.9 % DP6. The purities of DP2 to DP6 fractions were 61.9 %, 63.2 %, 44.5 %, 31.5 % and 51.3 %, respectively. Presence of DP2 and DP3 in the CPC purified fractions was further validated by mass spectrometry analysis. The study provided information on fast recovery of individual XOS from crude biomass prehydrolyzate.

Key words: *Miscanthus x giganteus*, Autohydrolysis, XOS, Xylose oligomers, Centrifugal partition chromatography

1. Introduction

Development of cellulosic biomass for biofuels is making progress in the U.S.; according to a recent report, several commercial plants are expecting to start operation in 2014-15 (Brown and Brown, 2013). Using cellulosic biomass in fuel conversion has many benefits to the environment, energy security and rural economy (Kim and Kim, 2014). Among all bioenergy crops, *Miscanthus x giganteus* (MxG), a perennial rhizomatous grass, is a leading candidate for fuel production. MxG produces 60% more biomass than well-fertilized maize and the yield can reach 30 MT/ha/year (Somerville et al., 2010). Over 50% of the MxG cell wall consists of structural carbohydrates (cellulose and hemicellulose), which are available for biofuels and biochemicals production (Brosse et al., 2012).

The autohydrolysis involves treating biomass with hot water or steam (140 to 200°C) to open the structure of plant cell wall. At high temperatures, water acts as a weak acid and releases acetyl groups from hemicellulose, which further acidifies the reaction mixture. These hydronium ions catalyze the cleavage of glycosidic bonds within the xylan, resulting in the liberation of xylose and other sugar oligomers into the liquid phase. The sugar oligomer enriched prehydrolyzate is generally washed off and the pretreated solid residue, which is enriched in cellulose, can be further processed to ethanol (Vázquez et al., 2000).

Xylose oligomers (XOS) are indigestible carbohydrates that find application in prebiotics, since they are selectively utilized by human gut microbiota like *Bifidobacterium* and *Lactobacillus* spp., thereby resulting in health benefits to the host (Carvalho et al., 2013). XOS have the potential of becoming value added co-products of the cellulosic ethanol industry. However, direct separation of XOS from the prehydrolyzate is a complex problem (Moure et al., 2006), because in addition to XOS, there may be other monomeric and oligomeric sugars, sugar

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6 degradation products, acid solubilized lignin and inorganic compounds (Gullón et al., 2010).
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8 Multiple steps of separation including solvent extraction, vacuum evaporation, surface-
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10 adsorption, membrane separation, size exclusion chromatography and ion exchange
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12 chromatography have been reportedly used in sequential purification of XOS (Vázquez et al.,
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14 2000; Moure et al., 2006). XOS were purified from rice husk hydrolyzate using membrane
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16 concentration, *endo*-xylanase digestion, and anion exchange resins (Gullón et al., 2010).
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18 Activated carbon adsorption and ethanol elution was also used to recover XOS from MxG
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20 hydrolyzates (Chen et al., 2014). However, both Gullón et al. (2010) and Chen et al. (2014)
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22 recovered XOS mixtures, rather than purified oligomers that have a single degree of
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28 polymerization (DP).
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30 Centrifugal partition chromatography (CPC) is a separation method developed from
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32 countercurrent chromatography. This liquid-liquid extraction technology uses two immiscible
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34 solvents as the stationary and mobile phase during chromatographic purification. Separation of
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36 solutes is achieved by virtue of their difference in partition coefficients. CPC has been widely
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38 used for single step preparative separations of alkaloids and flavonoids from crude plant extracts,
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40 for pharmaceutical applications (Attoumbre et al., 2012; Jeon et al, 2012). Advantages of CPC
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42 include the ability to separate compounds that span a wide molecular weight range, such as XOS
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44 (Berthod et al., 1988). Birchwood xylan-derived XOS were previously fractionated by CPC into
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46 its individual oligomers with a tetrahydrofuran-based solvent system (Lau et al., 2011). The CPC
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48 method was further improved with a butanol-based solvent system (Lau et al., 2013). However,
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50 these CPC protocols were never tested with crude hydrolyzates that stemmed from biomass
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57 autohydrolysis reactions.
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The objective of this study was to purify XOS from crude MxG hydrolyzates, which were produced through autohydrolysis, into their individual oligomer components. It is important to note that the starting material in this study consisted of a crude complex hydrolyzate, as opposed to a semi purified xylan material (Lau et al., 2011; Lau et al., 2013; Bunnell et al., 2015). Separation and fractionation of MxG XOS could possibly provide an opportunity to produce valuable co-products from bio-refineries, for pharmaceutical and nutraceutical applications.

2. Material and methods

2.1 Raw material and chemicals

Miscanthus x giganteus (MxG) was grown at the Energy Farm, University of Illinois, Urbana, IL (40.06°N, 88.19°W). The MxG was harvested in 2011 and was dried at 49°C in a convective oven, cut and ground in a hammer mill (model MHM4, Glen Mills, Clifton, NJ) to pass through a 250 µm screen. The dried and ground MxG was stored at 4°C. The moisture content was 8.2%, as determined following the NREL-LAP-001 protocol (Sluiter et al., 2008a). Xylose (DP1), arabinose, glucose and galactose were purchased from Sigma-Aldrich (St. Louis, MO). Xylobiose (DP2), xylotriose (DP3), xyloetraose (DP4), xylopentaose (DP5), and xylohexaose (DP6) were purchased from Megazyme International (Wicklow, Ireland) and their purity was reported to be greater than 95%. High performance liquid chromatography (HPLC) grade butanol and methanol were obtained from Macron Fine Chemicals (Center Valley, PA) and EMD Chemicals (Gibbstown, NJ), respectively. Water was prepared with a Direct-Q system (Millipore, Billerica, MA). Formic acid (95%) was procured from Amresco LLC (Solon, OH).

2.2 Autohydrolysis and sample preparation

The autohydrolysis was performed in a tubular reactor inserted in a fluidized bed sand bath for fast heating (Khullar et al., 2013). The 316 stainless steel tubing (SS-T12-S-065-20, Swagelok, Chicago Fluid System Technologies, Chicago, IL) reactors had an outside diameter of 19.1 mm, a wall thickness of 1.7 mm, and a length of 104.8 mm. Reactors were capped with 19.1 mm, 316 stainless steel Swagelok caps (SS-1210-C, Swagelok) on both ends. The procedure was essentially as described by Chen et al. (2014), where the loaded reactors were immersed in a fluidized sand bath (IFB-51 Industrial Fluidized Bath, Techne Inc., Burlington, NJ) preheated to 180°C and cooled in flowing room temperature tap water. Autohydrolysis was performed at water to solid ratio of 9:1, at 180°C for 20 min. In each tubular reactor, 5 g of MxG was mixed with 45 g of water. The pretreated solids and liquids were separated by centrifugation ($2600 \times g$, 5 min) in an Eppendorf 5804R centrifuge. This procedure was repeated in six reactors for four batches and corresponding hydrolyzates were combined. The resulting volume, determined to be 420 mL, was filtered through a 0.45 μm filter membrane (Millipore, Bedford, MA) and dried in a 49°C oven, removing approximately 415 mL of water. The resulting crude dried mixture was subsequently referred to as “XOS concentrate” throughout the work.

2.3 Sugar and oligomer characterization

Xylose, glucose, arabinose, galactose and XOS (from DP2 to DP6) content in the XOS concentrate was determined using a Waters Alliance HPLC system (Model 2695, Waters Corporation, Milford, MA) equipped with SP0810 (Shodex, Kawasaki, Japan) or Bio-Rad Aminex-HPX 42A (Bio-Rad, Hercules, CA) columns for monomer and oligomer quantification, respectively. HPLC was equipped with SP-G pre-column for monomer and Micro-Guard De-ashing pre-column for oligomer determinations. Purified and 0.22 μm filtered water was used as eluent. Column temperature was 85°C. Eluent flow rate was 0.2 mL/min, and monomers or

oligomers were detected with a refractive index detector (Model 2414, Waters Corporation, Milford, MA), which was maintained at 50°C. The concentrations of xylose, glucose, arabinose, galactose and XOS (DP2 to DP6) were determined using in-house calibration curves generated from commercial standards.

The total dissolved solids and sugar content of the concentrate were determined following NREL LAP-001 (Sluiter et al., 2008a) and NREL-LAP- 014 (Sluiter et al., 2008b) protocols, respectively. Accordingly, the total yield of xylose oligomers in the XOS concentrate was determined as follows:

$$\text{Yield (\%)} = (W_h - W_i) \times 0.88 \times 100 \quad (1)$$

Where W_h was the mass fraction of xylose in the concentrate after acid hydrolysis, W_i was the initial mass fraction of xylose in the concentrate and 0.88 is the anhydro correction factor for xylose (Sluiter et al., 2008c). The total phenolic content of the concentrate was determined using the Folin & Ciocalteu's (F-C) method, based on a previously published protocol (Ainsworth and Gillespie, 2007).

2.4 Centrifugal partition chromatography

A bench scale SCPC-250 system from Armen Instruments (Dallas, TX) equipped with CherryOne Beta (C1) countercurrent chromatography control system (Chicago, IL) was used for fractionating and purifying the XOS concentrate. Samples were processed by ultrapure nitrogen at 43 psig and with an evaporative light scattering detector (ELSD) (SofTA Corp, Westminster, CO). Butanol: methanol: water solvent system in 4:1:4 (v/v/v) was used for the CPC separation (Lau et al., 2013). To prepare 2 L of the biphasic system, 889 mL of butanol was mixed with 222 mL of methanol and 889 mL of water in a separation funnel and agitated thoroughly. The upper butanol rich organic phase and the lower aqueous phase were collected separately after 4 h and

used as the mobile and stationary phases, respectively, when the CPC was operated in the ascending mode. One g of dried XOS concentrate was dissolved in 20 mL of butanol-rich phase and 8 mL of the aqueous-rich phase. The mixture was then vortexed and filtered through a 1µm PTFE syringe filter (Thermo Scientific National, Rockwood, TN) and then injected into the CPC system.

The stationary and mobile phases were loaded in the ascending mode at 500 RPM. The equilibrium was reached at retention volumes of 114 mL and 136 mL, respectively. The CPC run time was 344 min, at 2300 RPM and at a flow rate of 8.14 mL/min. The UV detector in the controller was equipped with a preparatory flow cell that was set at 254 nm for real-time monitoring. Fraction collection was started after 60 min and fractions of approximately 8.1 mL each, were collected every minute. To accomplish this task, two fraction collectors were connected in series: Foxy R1 (Teledyne Isco, Lincoln, NE) and Waters Fraction Collector III (Waters Corporation, Milford, MA). After 240 min of run time, the CPC was operated in the descending mode allowing for un-separated compounds to exit the column and be collected. All 284 CPC fractions were dried in a Savant SpeedVac Concentrator SPD 1010 (Thermo Scientific, Ashville, NC), at 7 Torr for 6 h and reconstituted in 0.5 mL of water. The reconstituted fractions were filtered through a 0.22 µm nylon syringe filter (Thermo Scientific National, Rockwood, TN) and analyzed using HPLC to determine the concentration of xylose monomer and oligomers (DP2 to DP6).

The CPC fractionation of MxG XOS was repeated twice. Fractions were consolidated based on the concentration of each xylose oligomer. The purity of consolidated samples was calculated as:

$$\% \text{ Purity} = \frac{\text{Weight of xylose oligomer}}{\text{Total weight of each fraction}} \times 100 \quad (2)$$

2.5 ESI–MS verification

Samples were dried in SpeedVac and mixed with 0.1 % formic acid solution in methanol, at 20:1 ratio and directly injected into the mass spectrometer with a syringe pump. Gas phase ions created using electrospray ionization (ESI) were analyzed with a quadrupole/time –of –flight (Q-TOF) mass analyzer (Bruker ultrTOF-Q, Bruker Daltonics Inc., Billerica, MA). The solvent (methanol) flow rate was 3 μ L/min and N₂ gas at 1 bar was used for nebulization. The temperature of the source was 180°C. Other instrument parameters were adjusted to optimize signal in the m/z range of 100-800 and the mass spectra were obtained in positive ion mode.

3. Results

3.1 Production of XOS concentrate

Miscanthus x giganteus (MxG) biomass composition, previously determined by Chen et al. (2014), was 35.9 % glucan, 19.5 % xylan, 18.5 % acid insoluble lignin, 11.3 % total extractives, 1.8 % ash and 1.1 % acid soluble lignin. Autohydrolysis of MxG at 180°C for 20 min led to 63.4 % (w/w) xylan removal from the pretreated solids. The hydrolyzates were pooled and concentrated and the resultant XOS concentrate was determined to have a total solid content of 71.2 ± 0.3 % (w/w). In previous work, MxG autohydrolysis in a 0.6 L stainless Parr reactor, at temperatures ranging from 130°C to 150°C was reported to yield 40% (w/w) of initial xylan content (El Hage et al., 2010). In a stainless tube reactor heated at 160°C for 60 min, 65% (w/w) of xylan was recovered as xylose and XOS (Ligero et al., 2011). Autohydrolysis conditions reported in the current work, provided comparable yields for the production of crude XOS concentrate.

The carbohydrate composition of the XOS concentrate, adjusted for moisture content, was determined to be 39.8 ± 2.1 % xylose, 4.4 ± 0.1 % glucose, 5.1 ± 0.1 % arabinose and 2.7 ± 0.3 % galactose. The total xylose oligomer content, determined as per equation (1), was 30.1 ± 1.0 %. Distribution of XOS (DP2 to DP6) in the concentrate varied from 0.6 to 15.9 % dry weight, as shown in Table 1 and it was determined that DP2 to DP6 constituted 95.5 % of the total XOS. The additional 4.5% may be made up of higher DPs of xylose or conjugates of xylan, arabinan, galactan and/or glucan. The total phenolic content of the concentrate was 4.1 ± 0.2 % gallic acid equivalent. Presence of phenolic compounds indicated that a certain degree of lignin degradation had occurred during the autohydrolysis reaction. It was calculated that in order to produce 1 g of the XOS concentrate, autohydrolysis of 3.7 g of MxG biomass was required. Conversely, 1 g of the concentrate used for CPC fractionation contained, 159.2 mg of DP2, 59.0 mg of DP3, 56.0 mg of DP4, 8.1 mg of DP5 and 5.6 mg of DP6.

3.2 XOS separation and purification by CPC

The XOS present in the concentrate were separated using CPC, generating 284 fractions. The ELSD chromatogram corresponding to a typical separation is presented in Figure 1; fractions corresponding to the highlighted area under the curve were consolidated. The HPLC-RID chromatograms of the original concentrate and the CPC consolidated fractions are presented in Figure 2, the average yields of XOS, calculated on a dry weight basis of the concentrate, as well as the corresponding purities are presented in Table 2. Recovery of DP2 to DP6 oligomers varied between 61.9 to 90.2 %, while purity fluctuated between 31.5 to 63.2 %. The recovery of CPC fractionated XOS was the highest for DP2 at 90.2 %, based on the original composition of XOS concentrate (Table 2). The consolidated DP2 and DP3 fractions had comparatively higher purities of 61.9% and 63.2%, respectively. The purities of CPC fractionated and consolidated

MxG XOS were calculated based on their total mass fractions (Eqn. 2). In earlier reports, purities of XOS produced from purified xylan were higher, but were calculated based on area under the (chromatogram) curve, relative to other DPs and not on a mass basis (Qing et al., 2010; Lau et al., 2013)

3.3 ESI–MS verification of CPC separated fractions

Qualitative analysis of the CPC fractionated DP2 and DP3, performed using ESI–MS, was useful to verify their identity. As evidenced in Figure 3a, the major signals in consolidated DP2 fractions were m/z 305.1 $[M+Na]^+$ and 321.1 $[M+K]^+$ which corresponded to individual pentobiose molecule bound with sodium and potassium ions. Similarly, the major signals in consolidated DP3 fractions were m/z 437.1 $[M+Na]^+$ and 453.1 $[M+K]^+$ (Figure 3b) which corresponded to individual pentotriose molecule bound with sodium and potassium ions. The ESI-MS spectrums validated that the DP2 and DP3 were isolated and were the main component in each fraction.

4. Discussion

Production of XOS for nutraceutical applications is a topic that is garnering research efforts. Low molecular weight XOS of sizes DP2 to DP5, at 70 % purity, were reportedly fermented *in vitro* as well as *in vivo*, by prebiotic organisms belonging to the *Bifidobacterium* spp. (Hopkins et al., 1998). In one particular study, 90 % of DP3 and 84 % of DP2 were preferentially utilized by *Bifidobacterium adolescentis* (Gullón et al., 2008). Similarly certain strains of *Lactobacillus* spp. were reported to preferentially utilize xylose DP2 to DP4 when compared to other oligosaccharides, during mixed acid fermentations (Kontula et al., 1998). There are several enzymatic and chemical hydrolysis methods reported for the production of

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6 XOS from lignocellulosic biomass. However, few reports are addressing fractionation practices.
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8 Cara et al. (2012), described the production and separation of glucose and XOS by gel filtration
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10 chromatography, but did not separate XOS into individual DP components. Jayapal et al. (2013),
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12 extracted hemicellulose from sugarcane bagasse and produced XOS using xylanases. They
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14 detected their product with reducing sugar and thin layer chromatography assays, with no report
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16 on fractionation. Recently Mandelli et al. (2014), reported on the production of XOS from
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18 sugarcane bagasse, detecting and quantifying their products by capillary electrophoresis and
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20 HPAEC-PAD detection; however, this group also did not attempt to purify the xylan DPs.
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25 Chen et al. (2014) originally used 10 % activated carbon adsorption followed by aqueous
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27 ethanol elution and obtained a theoretical maximum recovery of 47.9 % of XOS DP2 to DP9,
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29 from *Miscanthus x giganteus* biomass. However, fractionation and purification of DPs into
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31 individual components was not attempted. Lau et al. (2011) were the first to report the use of
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33 CPC in separation and purification of XOS from acid hydrolyzed birchwood xylan: best results
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35 were obtained for the separation of xylose DP2 to DP5. With the adoption of a 4:1:4 volumetric
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37 ratio of butanol, methanol and water system, Lau et al. (2013) recovered 12.5 mg/g xylan of
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39 81.2% pure xylobiose, 9.6 mg/g xylan of 71.0% pure xylotriose, 14.2 mg/g xylan of 62.4% pure
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41 xylotetraose and 21 mg/g xylan of 51.9% pure xylopentaose from autohydrolyzed birchwood
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43 xylan. Fractionating crude MxG hydrolyzate, containing other carbohydrates and lignin derivate,
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45 increased the complexity of separation and therefore reduced the purity of CPC fractions.
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52 Lau et al. (2013), used CPC to recover 59.1 mg/g of DP2 to DP5, from autohydrolyzed
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54 birchwood xylan, and Bunnell (2013) reported CPC recoveries of 138 mg/g of DP2 to DP6 from
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56 alkali extracted switchgrass xylan. In this study, 230 mg of xylose DP2 to DP6 were purified
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58 from 1 g of autohydrolyzed XOS concentrate, by CPC using a similar butanol- methanol-water
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6 solvent system. This work was on purifying XOS directly from a crude autohydrolyzed
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8 concentrate, illustrating that this technique can fractionate DPs from complex matrices. To our
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10 knowledge this is a first report on XOS purification from crude biomass hydrolyzates, using CPC.
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15 **5. Conclusion**

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17 XOS were purified from autohydrolyzed MxG concentrate by CPC with a solvent system
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19 composed of 4:1:4 (v/v/v) butanol, methanol and water. Through the collection of samples
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21 following elution order, 80% of DP2 to DP6 were fractionated from the original concentrate.
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23 Approximately, 90.2% DP2, 64.5% DP3, 71.2% DP4, 61.9% DP5 and 68.9% of DP6 were
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25 recovered. Purities of each oligomer fraction was 61.9% DP2, 63.3% DP3, 44.5% DP4, 31.5%
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27 DP5 and 51.3% for DP6. The DP2 and DP3 fractions were validated by electrospray ionization
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29 mass spectrometry analysis. CPC thus provides a fast and effective means of separating XOS,
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31 from biorefinery by-products, for commercial applications.
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References

- Ainsworth, E.A., Gillespie, K.M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protoc.* 2, 875–877.
- Attoumbre, J., Lesur, D., Giordanengo, P., Baltora-Rosset, S. 2012. Preparative separation of glycoalkaloids alpha-solanine and alpha-chaconine by centrifugal partition chromatography. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 908, 150–154.
- Berthod, A., Armstrong, D.W. 1988. Centrifugal Partition Chromatography. I. General Features. *J. Liq. Chromatogr.* 11, 547–566.
- Brosse, N., Dufour, A., Meng, X., Sun, Q., Ragauskas, A. 2014. Miscanthus: a fast-growing crop for biofuels and chemicals production. *Biofuels Bioprod. Biorefining.* 6, 580–598.
- Brown, T.R., Brown, R.C. 2013. A review of cellulosic biofuel commercial-scale projects in the United States. *Biofuels Bioprod. Biorefin.* 7, 235–245.
- Bunnell, K. 2013. Characterization and quantification of monomers, oligomers, and by-products from switchgrass hemicelluloses during biomass pretreatment. In: *Biological and Agricultural Engineering*, pp. 136, University of Arkansas, Fayetteville, AR.
- Bunnell, K., Lau, C.S., Lay, J.O., Gidden, J. and Carrier, D.J. 2015. Production and fractionation of xylose oligomers from switchgrass hemicelluloses using centrifugal partition chromatography. *J. Liq. Chromatogr. Relat. Technol.* 38, 801-809.

- 1
2
3
4
5
6 Cara, C., Ruiz, E., Carvalheiro, F., Moura, P., Ballesteros, I., Castroa, E., Gírio, F. 2012.
7
8 Production, purification and characterisation of oligosaccharides from olive tree pruning
9
10 autohydrolysis. Ind. Crops Prod. 40, 225–231.
11
12
13 Carvalho, A.F.A., Neto, P.d.O., da Silva, D.F., Pastore, G.M. 2013. Xylo-oligosaccharides from
14
15 lignocellulosic materials: Chemical structure, health benefits and production by chemical
16
17 and enzymatic hydrolysis. Food Res. Int. 51, 75–85.
18
19
20 Chen, M.H., Bowman, M.J., Dien, B.S., Rausch, K.D., Tumbleson, M.E., Singh, V. 2014.
21
22 Autohydrolysis of *Miscanthus x giganteus* for the production of xylooligosaccharides
23
24 (XOS): kinetics, characterization and recovery. Bioresour. Technol. 155, 359–365.
25
26
27 El Hage, R., Chrusciel, L., Desharnais, L., Brosse, N. 2010. Effect of autohydrolysis of
28
29 *Miscanthus x giganteus* on lignin structure and organosolv delignification. Bioresour.
30
31 Technol. 101, 9321–9329.
32
33
34 Gullón, P., González-Muñoz, M.J., van Gool, M.P., Schols, H.A., Hirsch, J., Ebringerová, A.,
35
36 Parajó, J.C. 2008. Assessment on the fermentability of xylooligosaccharides from rice
37
38 husks by probiotic bacteria. J. Agric. Food. Chem. 56, 7482–7487.
39
40
41 Gullón, P., González-Muñoz, M.J., van Gool, M.P., Schols, H.A., Hirsch, J., Ebringerová, A.,
42
43 Parajó, J.C. 2010. Production, refining, structural characterization and fermentability of
44
45 rice Husk Xylooligosaccharides. J. Agric. Food. Chem. 58, 3632–3641.
46
47
48 Hopkins, M.J., Cummings, J.H., Macfarlane, G.T. 1998. Inter-species differences in maximum
49
50 specific growth rates and cell yields of bifidobacteria cultured on oligosaccharides and
51
52 other simple carbohydrate sources. J. Appl. Microbiol. 85, 381–386.
53
54
55
56
57
58
59
60
61
62
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64
65

- Jayapal, N., Samanta, A.K., Kolte, A.P., Senani, S., Sridhar, M., Suresh, K.P., Sampath, K.T. 2013. Value addition to sugarcane bagasse: Xylan extraction and its process optimization for xylooligosaccharides production. *Ind. Crops Prod.* 42, 14–24.
- Jeon, J.S., Kim, S.-M., Lee, H.J., Um, B.H., Kim, H.K., Kim, C.Y. 2012. Preparative isolation and purification of prenylated isoflavanoids from *Cudrania tricuspidata* using centrifugal partition chromatography. *J. Liq. Chromatogr. Relat. Technol.* 35, 1607–1615.
- Khullar, E., Dien, B.S., Rausch, K.D., Tumbleson, M.E., Singh, V. 2013. Effect of particle size on enzymatic hydrolysis of pretreated *Miscanthus*. *Ind. Crops Prod.* 44, 11–17.
- Kim, T.H., Kim, T.H. 2014. Overview of technical barriers and implementation of cellulosic ethanol in the U.S. *Energy*. 66, 13–19.
- Kontula, P., von Wright, A., Mattila-Sandholm, T. 1998. Oat bran beta-gluco- and xylo-oligosaccharides as fermentative substrates for lactic acid bacteria. *Int. J. Food Microbiol.* 45, 163–169.
- Lau, C.S., Clausen, E.C., Lay, J.O., Gidden, J., Carrier, D.J. 2013. Separation of XOS using centrifugal partition chromatography with a butanol–methanol–water system. *J. Ind. Microbiol. Biotechnol.* 40, 51–62.
- Lau, C.S., Bunnell, K.A., Clausen, E.C., Thoma, G.J., Lay, J.O., Gidden, J., Carrier, D.J. 2011. Separation and purification of XOS using centrifugal partition chromatography. *J. Ind. Microbiol. Biotechnol.* 38, 363–370.
- Ligero, P., van der Kolk, J.C., de Vega, A., van Dam, J.E.G. 2011. Production of xylo-oligosaccharides from *Miscanthus x giganteus* by autohydrolysis. *BioResources*. 6, 4417–4429.

- Mandelli, F., Brenelli, L.B., Almeida, R.F., Goldbeck, R., Wolf, L.D., Hoffmam, Z.B., Rullera, R., Rocha, G.J.M., Mercadante, A.Z., Squin, F.M. 2014. Simultaneous production of xylooligosaccharides and antioxidant compounds from sugarcane bagasse via enzymatic hydrolysis. *Ind. Crops Prod.* 52, 770–775.
- Moure, A., Gullon, P., Dominguez, H., Parajo, J.C. 2006. Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals. *Process Biochem.* 41, 1913–1923.
- Qing, Q., Yang, B., Wyman, C.E. 2010. Xylooligomers are strong inhibitors of cellulose hydrolysis by enzymes. *Bioresour. Technol.* 101, 9624–9630.
- Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Wolfe, J. 2008a. Determination of total solids in biomass and total dissolved solids in liquid process samples National Renewable Energy Laboratory, Golden, CO.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. 2008b. Determination of sugars, byproducts, and degradation products in liquid fraction process samples National Renewable Energy Laboratory, Golden, CO.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. 2008c. Determination of structural carbohydrates and lignin in biomass. In: NREL Laboratory Analytical Procedure, National Renewable Energy Laboratory, Golden, Colorado.
- Somerville, C., Youngs, H., Taylor, C., Davis, S.C., Long, S.P. 2010. Feedstocks for lignocellulosic biofuels. *Science.* 329, 790–792.
- Vázquez, M.J., Alonso, J.L., Domínguez, H., Parajó, J.C. 2000. Xylooligosaccharides: manufacture and applications. *Trends Food Sci. Technol.* 11, 387–393.

Captions to Tables:

Table 1. Composition of autohydrolyzed XOS concentrate determined using HPLC analysis

Table 2. Yields of MxG XOS during centrifugal partition chromatography separation.

Table 1. Composition of autohydrolyzed XOS concentrate determined using HPLC analysis

Compound	% Dry wt. ^a
Xylobiose (DP2)	15.9 ± 1.5
Xylotriose (DP3)	5.9 ± 0.8
Xylotetraose (DP4)	5.6 ± 0.7
Xylopentaose (DP5)	0.8 ± 0.2
Xylohexaose (DP6)	0.6 ± 0.2
Total	28.8 ± 0.5

^a Mean and standard deviation, *n*=3

Table 2. Yields of MxG XOS during centrifugal partition chromatography separation.

Fractionated compounds	Yield of each compound ^a (mg/g)	Recovery ^b	Purity ^c
DP2-Xylobiose	143.5±6.0	90.2%	61.9±1.9
DP3-Xylotriose	38.1±5.0	64.5%	63.2±2.3
DP4-Xylotetraose	39.8±1.0	71.2%	44.5±1.9
DP5-Xylopentaose	4.9±0.1	61.9%	31.5±1.0
DP6-Xylohexaose	3.9±0.3	68.9%	51.3±1.0

^a Mean and standard deviation, *n*=2

^b % Recovery was calculated based the theoretical maximum yield

^c % Purity was calculated based on the total weight of each fraction

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6 **Figure captions**
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9 Fig. 1. ELSD chromatogram of centrifugal partition chromatography separated XOS from
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11 autohydrolyzed MxG concentrate.
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15 Fig. 2. HPLC-RID chromatogram of (a) original XOS concentrate and the consolidated XOS,
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17 (b) DP2 fraction, (c) DP3 fraction, (d) DP4 fraction, (e) DP5 fraction and (f) DP6
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19 fraction
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23 Fig. 3. ESI mass spectrum of consolidated xylose DP2 and DP3 purified from crude XOS
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25 concentrate by CPC. (a) DP2 fraction, (b) DP3 fraction
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Fig. 1

Fig. 2

Fig. 3a.

Fig. 3b.