Bioenergy: Challenges and Opportunities
International Conference and Exhibition on Bioenergy
April 6th – 9th 2008
Universidade do Minho, Guimarães, Portugal

PREBIOTIC XYLO-OLIGOSACCHARIDES AS HIGH-VALUE CO-PRODUCTS ON AN INTEGRATED BIOREFINERY APPROACH FROM LIGNOCELLULOSIC FEEDSTOCK

P. Moura[1], F. Carvalheiro[2], M.P. Esteves[3], F.M. Gírio[4]
Unidade de Fisiologia Microbiana e Bioprocessos, Dpt. Biotecnologia, INETI, Lisboa, Portugal
[1] patricia.moura@ineti.pt
[2] florbela.carvalheiro@ineti.pt
[3] paula.esteves@ineti.pt
[4] francisco.girio@ineti.pt

ABSTRACT

The present work proposes the production of prebiotic xylo-oligosaccharides (XOS) as high-value co-products of the Lignocellulose Feedstock Biorefinery concept, foreseeing potential applications on food, feed and nutraceutical industries.
Autohydrolysis was used to selectively solubilise the hemicellulosic fraction of several xylan-rich, widely available, agricultural, agro-industrial and forestry by-products: corn cobs, brewery's spent grain and *Eucalyptus* wood chips. The soluble hemicellulose-rich and the solid cellulose- and lignin-rich fractions were separated, and the crude XOS-rich hydrolysates were further purified by gel filtration chromatography. Selected fractions of purified XOS within the desired ranges of polymerization degree were characterised and their prebiotic potential was investigated in *in vitro* fermentations by bifidobacteria, lactobacilli and intestinal inocula. Parameters such as bacterial growth and XOS consumption were evaluated and compared with commercially available xylo-oligosaccharides. The differences observed were considered of relevance for the formulation of symbiotic preparations and the future design of targeted, tailor-made prebiotic xylo-oligosaccharides.

Keywords: Prebiotic, xylo-oligosaccharides, lignocellulosic feedstock, biorefinery, autohydrolysis

INTRODUCTION

One broad definition accepted for biorefinery is the sustainable processing of biomass into a spectrum of marketable products and energy. The Lignocellulose Feedstock Biorefinery (LCFBR) is based on the fractionation of lignocellulosic-rich biomass sources into three major intermediate output streams: cellulose, hemicelluloses and lignin, which can be further processed into a portfolio of bio-based end-products, chemicals, fuels and power [1].
Foreseeing potential applications on food, feed and nutraceutical industries, this study proposes the use of the hemicellulosic stream from lignocellulosic feedstock to produce prebiotic XOS, which can be looked at as potentially attractive high-value co-products of an integrated LCFBR. The inclusion of a simple and clean hydrothermal treatment on the LCFBR flow sheet envisaging the selective solubilisation of hemicelluloses from lignocellulosic feedstock makes possible the production of XOS on an economically and environmentally interesting way. When compared to commercial XOS, which are currently produced by alkaline xylan extraction followed by enzymatic hydrolysis from xylan-rich materials, XOS obtained by autohydrolysis possess the advantage of requiring exclusively water and lignocellulosic biomass as process reagents [2]. Moreover, commercial XOS consist essentially on xylobiose and xylotriose, whereas coupling autohydrolysis with a subsequent fractionation step allows the separation of XOS by degree of polymerization (DP), enabling the preparation of prebiotically interesting wider-chain XOS [3].

MATERIALS AND METHODS

Production and purification of xylo-oligosaccharides

Autohydrolysis experiments were performed with xylan-rich feedstock (corn cobs (CC) and brewery's spent grain (BSG)) in a 0.6 litre stainless steel reactor (Parr Instruments Company, Illinois, USA). Autohydrolysis was carried out under isothermal or non-isothermal conditions, the temperatures ranging between 150 and 225 ºC. The severity of the treatments was measured using the severity factor R_0 [4], which considers the effects of temperature and reaction time:

$$R_0 = \int_0^t \exp\left(\frac{T(t) - 100}{14.75}\right).dt \quad (1)$$

The crude XOS-rich hydrolysates were separated from the cellulose- and lignin-rich solid residue and characterised as described below. The purification of the crude hydrolysates was carried out by means of preparative gel filtration chromatography (GFC) on a column with Superdex 30^{TM} (Amersham Pharmacia Biotech). The separated fractions were characterised as described below. Gram-quantities of purified XOS series from *Eucalyptus* wood chips (EUC) hydrolysates were received from H. Schols (WAU University, Wageningen, the Netherlands).

Fermentation experiments

The *in vitro* fermentation experiments were carried out with strains of *Bifidobacterium* and *Lactobacillus* and with the intestinal microbiota of a weanling piglet, under anaerobic conditions, as described elsewhere [5, 6]. Short-chain and medium-chain XOS produced by autohydrolysis of the feedstock BSG, CC and EUC were used as carbon and energy source in the fermentation media. Bacterial growth was monitored and XOS consumption was determined by HPLC, as described below.

Analytical methods

Feedstock materials were analyzed for glucan, xylan, arabinan and acetyl groups after quantitative acid hydrolysis (QAH) using H_2SO_4 [7]. The acid insoluble residue was considered as Klason lignin, after correction for ash. The liquid phases obtained from autohydrolysis were analysed by HPLC for sugars, acetic acid and furan derivatives on an Aminex HPX-87H column (Bio-Rad, Hercules, USA). The oligosaccharide concentrations were determined after QAH and expressed as the increase in sugar monomers, as analysed by HPLC. The fractions obtained from GFC were characterised in terms of apparent molar mass by size exclusion chromatography, and by HPLC, after QAH. XOS standards (Megazyme Int., Ireland) were used for external calibration. The fractions of interest for the fermentation experiments were selected according to the estimated DP.

RESULTS AND DISCUSSION

Integration of XOS production on the LCFBR concept

Figure 1 shows the integration of XOS produced by autohydrolysis from lignocellulosic feedstock on the general LCFBR flow sheet. Autohydrolysis is a fractionation process based on the hydrolytic degradation of hemicelluloses by water for the selective release of soluble oligomeric structures. The operational parameters of the hydrolytic process, *e.g*. temperature and reaction time, can be optimized for maximum XOS production [5, 8]. The advantage of using only water and lignocellulosic feedstock as reagents in the autohydrolysis process enables the production of clean effluents and simultaneously the recovery of a clean cellulose- and lignin-rich solid residue. This intermediate output can be downstream processed to a multiplicity of bio-based end-products, chemicals, fuels and power, fulfilling the criteria of an LCFBR framework.

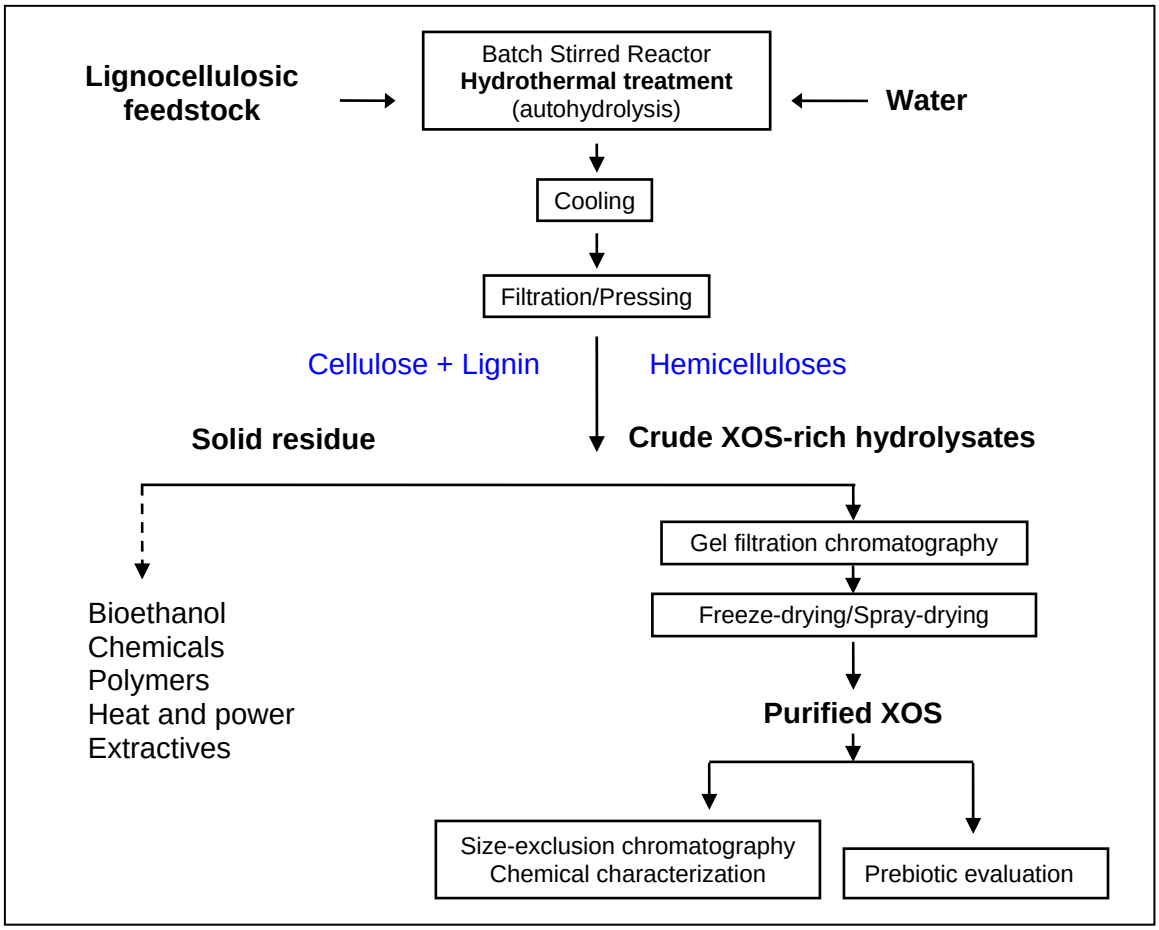

Fig. 1. Flow sheet integrating XOS production on the general LCFBR flow sheet

Feedstock composition

The chemical composition of the hydrolysates, and of XOS in particular, depends on the chemical composition of feedstock as well as on the operational conditions used to extract and/or fractionate hemicelluloses. Fig. 2 shows the chemical composition of three feedstock used in this work, BSG, CC and EUC. All the feedstock were xylan-rich materials, although with some differences in the composition of hemicellulosic components. In contrast to EUC, BSG presented the highest arabinan content but was the less acetylated feedstock. CC exhibited both the highest and lowest polysaccharide and lignin contents, respectively.

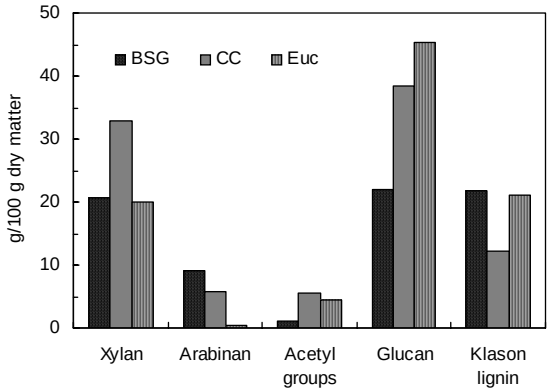

Fig. 2. Chemical composition of feedstock materials

Crude-XOS rich hydrolysates

Hydrothermal treatments enable the selective hydrolysis of hemicelluloses and, under controlled conditions, the recovery of (arabino)xylo-oligosaccharides as the main product. In order to define the operational conditions leading to the maximum recovery of XOS for each feedstock, the autohydrolysis process was optimized and the results obtained were interpreted using the severity factor (log R_0) up to 4.25.

Table 2. Composition of freeze-dried hydrolysates at the maximal XOS recovery to each feedstock [g/100 g dry matter]

	BSG	CC	EUC
(A)XOS	40.3	55.3	50.0
GlcOS	14.5	5.1	3.5
AcO	1.1	5.1	10.6
Xylose	5.2	3.4	8.0
Glucose	2.9	1.6	2.1
Arabinose	9.3	4.7	3.7
Acetic acid	2.3	1.0	2.8
Furfural	1.5	0.3	0.6
HMF	0.2	< 0.1	1.1

(A)XOS, arabinose substituted xylo-oligosaccharides; GlcOS, glucose oligomers; AcO, *O*-acetyl groups; HMF, hydroximethylfurfural

Table 2 shows the composition of crude hydrolysates obtained in the optimized conditions. For both BSG and EUC the maximum XOS recovery was obtained at 190ºC for an isothermal reaction period of 5 min (log R_0=3.73), whereas in the case of CC the best results were attained at 208ºC (log R_0=3.75) under non-isothermal conditions. The highest XOS content was obtained for CC hydrolysates. These values are 27% and 10% higher than those obtained for BSG and EUC, respectively. Conversely to BSG XOS, EUC XOS had the highest content of acetyl groups and displayed the lowest arabinose substitution degree. Total monosaccharide content in the crude liquors ranged between 9.7 and 17.4% of the total identified compounds. In all cases the concentrations of other hydrolysis by-products, *e.g.*, acetic acid, hydroximethylfurfural (HFM) and furfural, were always relatively low.

Tailoring XOS: characterization of short- and medium-chain XOS

The crude XOS-rich hydrolysates produced from BSG and CC were purified by chromatographic techniques, for the separation of XOS in fractions within the desired degree of polymerization (DP) ranges, and simultaneous elimination of low molecular weight components, such as monosaccharide, and by-products, *e.g.*, furfural and HMF.

The composition of the purified XOS selected for prebiotic evaluation is presented in Table 3. Fraction 16 of CC XOS (CC XOS F16) was mainly constituted (51% w/w) by xylotriose and xylotetraose, whereas fraction 15 of CC XOS (CC XOS F15) was mainly constituted (66% w/w) by xylotetraose, xylopentaose and xylohexaose. XOS with DP higher than 6 accounted for 4% w/w and 10% w/w of CC XOS F16 and CC XOS F15, respectively. The medium-chain XOS from BSG, CC and EUC ranged from DP 2 up to DP 25, as presented in Table 3.

Table 3. Composition of XOS produced by autohydrolysis and purified by GFC

	Short-chain XOS		Medium-chain XOS		
	CC XOS (F16)	CC XOS (F15)	BSG XOS	CC XOS	EUC XOS
DP range	2 up to >6	2 up to >6	3 - 12	2 - 14	2 - 25
(A)XOS[a]	77.5	83.9	41.1	70.9	80.7
GlcOS[a]	2.1	2.5	9.0	5.7	3.0
Arabinose[a]	1.1	ND	0.4	1.9	ND
Glucose[a]	1.1	0.6	ND	ND	0.7
Xylose[a]	1.7	0.7	1.1	1.6	0.9
Acetic acid[a]	4.7	1.6	0.1	0.4	0.1
Ara/Xyl[b]	7	4	20	2	0
AcO/Xyl[b]	13	18	12	20	43

DP, degree of polymerization; Ara, arabinose; Xyl, xylose; ND, not detected; [a], [g/100 g dry matter]; [b], [mol/100 mol]

Prebiotic properties of XOS produced by autohydrolysis

For the evaluation of the prebiotic potential of novel NDOs it is recognized that fundamental knowledge on substrate preferences of individual bacterial strains is previously necessary [9]. Bifidobacteria and lactobacilli are indigenous bacteria to the human intestinal tract and their presence is commonly associated with several health benefits, thus constituting common targets for prebiotic action [10]. Therefore, the prebiotic potential of short-chain XOS produced by autohydrolysis was assessed in *in vitro* fermentations by strains of bifidobacteria and lactobacilli, foreseeing the design of future symbiotic preparations. The maximum specific growth rates attained by *B. adolescentis* and *L. brevis* and the highest values of short-chain CC XOS consumption are assembled in Table 4. Commercial XOS were used for comparative purposes.

Table 4. Specific growth rates and consumption of short-chain CC XOS by *Bifidobacterium adolescentis* and *Lactobacillus brevis*

Strain	Carbohydrate source	Max. specific growth rate [h^{-1}]	Degree of consumption [%]
B. adolescentis	Commercial XOS	0.30	51.6
DSM 20083	CC XOS (F16)	0.27	52.9
	CC XOS (F15)	0.26	34.2
L. brevis	Commercial XOS	0.09	38.3
DSM 20054	CC XOS (F16)	0.11	31.4
	CC XOS (F15)	0.12	24.8

The results show that short-chain XOS produced by autohydrolysis of CC are capable of supporting the growth of bifidobacteria and lactobacilli strains as well as commercial XOS.

The separation of crude XOS hydrolysates by GFC (Figure 1) enables the preparation of XOS mixtures with increased molecular weight, theoretically more capable to persist throughout the gastrointestinal tract and to advantageously support a saccharolytic fermentation up to more distal compartments of the intestinal tract when compared to short-chain XOS [11]. In this study, medium-chain XOS produced from BSG, CC and EUC were used in *in vitro* fermentations by the intestinal microbiota of a weanling piglet, to assess their suitability as slower fermentable substrates.

Fig. 3 compares the consumption of medium-chain XOS and commercial short-chain XOS by the foregut microbiota. The medium-chain XOS used in this study determined a notable reduction on XOS consumption rate in the fermentations by the microbiota from the piglet's ileum, as compared to short-chain, commercially available XOS.

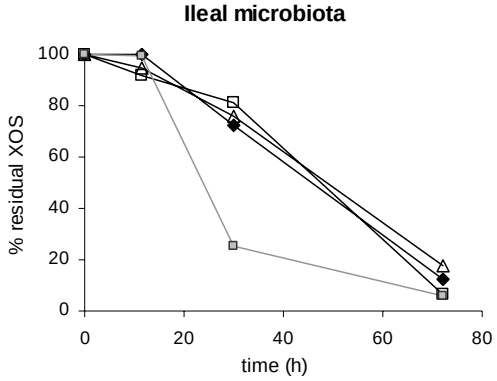

Fig. 3. Consumption of medium-chain XOS produced by autohydrolysis of BSG, CC and EUC by the ileal microbiota of a weanling piglet
(△ BSG XOS; ◆ CC XOS; □ EUC XOS; ▣ commercial XOS)

CONCLUSIONS

Autohydrolysis constitutes a promising process for the production of novel preparations of highly-valuable prebiotic xylo-oligosaccharides. The process can be advantageously integrated on the LCFBR concept and complies with the desirable sustainable use of lignocellulosic biomass.

Literature References

[1] van Ree, R, Annevelink, B (2007) "*Status Report Biorefinery*", Agrotechnology and Food Sciences Group, WAU University, Wageningen, the Netherlands

[2] Garrote, G, Domínguez, H, Parajó, JC (1999) "*Mild autohydrolysis: an environmentally friendly technology for xylooligosaccharide production from wood*", J Chem Technol Biotechnol, Vol. 74, pp 1101.

[3] van de Wiele, T, Boon, N, Possemiers, S, Jacobs, H, Verstraete, W (2007) "*Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects*", J Appl Microb, Vol. 102, pp 452.

[4] Overend, RP, Chornet, E (1987) "*Fractionation of lignocellulosics by steam-aqueous pretreatments*", Philosophical Transactions of the Royal Society of London, Vol. A321, pp 523.

[5] Moura, P, Barata, R, Carvalheiro, F, Gírio, F, Loureiro-Dias, MC, Esteves, MP (2007) "*In vitro fermentation of xylo-oligosaccharides from corn cobs autohydrolysis by Bifidobacterium and Lactobacillus strains*", LWT - Food Sci Technol, Vol. 40, pp 963.

[6] Moura, P, Cabanas, S, Lourenço, P, Gírio, F, Loureiro-Dias, MC, Esteves, MP (2008) "*In vitro fermentation of xylo-oligosaccharides by piglet intestinal microbiota",* LWT - Food Sci Technol, in press.

[7] Browning, BL in: "*Methods of Wood Chemistry*", edited by Sarkanen KV, Ludwig CH,.John Wiley & Sons, NY (1967).

[8] Carvalheiro, F, Esteves, MP, Parajó, JC, Pereira, H, Gírio, FM (2004) "*Production of oligosaccharides by autohydrolysis of brewery's spent grain*", Biores Tech, Vol. 91, pp 93.

[9] Hopkins, MJ, Cummings, JH, Macfarlane, GT (1998) "*Inter-species differences in maximum specific growth rates and cell yields of bifidobacteria cultured on oligosaccharides and other simple carbohydrate sources*", J Appl Microb, Vol. 85, pp 381.

[10] Leahy, SC, Higgins, DG, Fitzgerald, GF, van Sinderen, D (2005) "*Getting better with bifidobacteria*", J Appl Microbiol, Vol. 98, pp 1303.

[11] Rastall, RA, Maitin, V (2002) "*Prebiotics and synbiotics: towards the next generation*", Curr Opinion Biotechnol, Vol. 13, pp 490.