PRODUCTION OF XYLO-OLIGOSACCHARIDES FROM *MISCANTHUS x GIGANTEUS* BY AUTOHYDROLYSIS

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Xylo-oligosaccharides were obtained from *Miscanthus* x *giganteus*. The process was designed as a biorefinery scheme, which seeks the separation of the three main components: cellulose, hemicelluloses, and lignin. To extract the hemicelluloses, particularly xylans, in an efficient way, *Miscanthus* was subjected to autohydrolysis. The system was evaluated for the effects of temperature (160 to 200°C) and reaction time (15 to 60 min) on various parameters, reflecting the changes undergone during the process. The results showed that autohydrolysis is a suitable method for obtaining high yields of xylo-oligosaccharides, reaching values close to 65% of the dissolved xylans (based on the initial amount of potential xylose). Analysis of the process by using the severity factor (R_O) allowed for the identification of a set of time-temperature values for which the fractionation was optimal.

Keywords: Miscanthus x giganteus; Autohydrolysis; Xylo-oligosaccharides; Fractionation

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INTRODUCTION

Lignocellulosic biomass is considered to be a major source for 'green' chemicals, bio-fuels, and biobased products. Among the advantages of using lignocellulosic biomass can be mentioned that it is abundantly available around the world, non-competitive with food production, and it is a renewable and sustainable resource. Achieving more advantageous use of these natural renewable resources is a political goal in which societies are more and more immersed. This is especially the case when biomass-derived products can become competitive with fossil oil-derivatives in the short term. To meet this challenge, the biorefinery concept is receiving a renewed interest, with emphasis on using all the fractions present in plant biomass.

Biorefinery can be understood as a set of processes that enable the processing and use of each of the fractions of biomass at the highest value for energy, products, and chemicals production (Kamm et al. 2006). Among different types of biorefinery processes, the Lignocellulose Feedstock (LCF) biorefinery can contribute to valorize different woody plant biomass. The raw materials are fractionated in this process into the main components (cellulose, lignin, and hemicelluloses), which are subsequently converted into materials, chemicals, fuels, and energy (Kamm et al. 2006), each contributing to economic benefits resulting from the recovery of fractionated products

(Bachman and Riese 2006). In particular, organosolv LCF biorefineries can fractionate the biomass complex using organic solvents. Subsequently, each fraction can be processed to obtain marketable products. The purpose of these biorefineries is to maximize the value of each biomass fraction by chemical and physical transformations. In this sense, different techniques must be integrated to support optimum use of all components of plant biomass in order to increase their value.

The hemicelluloses are a mixture of non-cellulosic cell wall constituents that are the most common polysaccharides in plant biomass, next to cellulose. Hemicelluloses are more labile to chemical attack than cellulose and lignin; thus they can be separated from the vegetal tissues in an almost selective way. They consist of chains of monosaccharide units (usually xylose, mannose, and glucose) built through linkages of the type β-(1$\rightarrow$3) and β-(1$\rightarrow$4) (Ebringerová 2006). These bonds form a linear structure, from which pendant saccharide groups, such as arabinose, galactose, mannose, uronic acids, etc., are appended to the chain. The presence of esterified acetate groups is also a well known fact. Xylose is the main C5 sugar in hemicelluloses of many woody tissues, and its value added use is of great importance. Xylose is present in plants in the form of relatively short chains (xylans), alone or in combination with other monosaccharides (Fengel and Wegener 1984, Ebringerová 2006). Xylans can be transformed into xylo-oligosaccharides by chemical or enzymatic treatments. There are potential applications of xylo-oligosac-charides, focused on food, feed, and pharmaceutical industries (Ebringerová 2006, Imaizumi et al. 1991); such opportunities have motivated several studies to justify the production of the xylans.

Xylo-oligosaccharides (XOS) can be produced from biomass by autohydrolysis, a thermal treatment in which water is the only chemical added. The autohydrolysis process takes advantage of the presence of acetyl groups in biomass. These groups, at a certain temperature, release acetic acid to the reaction medium, and this released acidity then catalyzes the partial hydrolysis of xylans to produce xylo-oligosaccharides. Thus, the process causes the solubilization of most of the hemicelluloses, leaving the contents of cellulose and lignin only slightly altered. In this sense, the autohydrolysis can be considered as a pretreatment of biomass, and a preparation stage for subsequent processing. Xylo-oligosaccharides have been obtained from many different materials: cereal straw (Nabarlatz et al. 2007), nuts (Nabarlatz et al. 2004), wood (Garrote et al. 2007, Caparros et al. 2007), and other species (Aoyama and Seki 1999, Ligero et al. 2010, Alfaro et al. 2010, El Hage et al. 2010), and solid wastes (Carvalheiro et al. 2004, Fernández-Bolaños et al. 2004, Vegas et al. 2005, Moldes et al. 2007, Martínez et al. 2010).

The genus *Miscanthus*, which is native of the tropics and subtropics, was initially introduced in Europe (Denmark) from Japan in 1935 as an ornamental plant (Linde-Laursen 1993). More recently the EU has concentrated its interest in this genus due to its high biomass production, fast growth, high resistance to disease, and good adaptation to different bioclimatic sites (Lewandowski et al. 2000). *Miscanthus* are perennial rhizomatous grasses, belonging to the *Poaceae* family, with C4 photosynthesis. *Miscanthus x giganteus* is a sterile hybrid triploid between the diploid *Miscanthus sinensis* and the tetraploid *Miscanthus sacchariflorus* (Lewandowski et al. 2000).

Recently, other authors have studied the hydrothermal treatment of *Miscanthus x giganteus* (El Hague et al. 2010). However, the range of temperatures used (130 to 150°C) was lower than the present work, and therefore the required treatment times were much longer (0 to 48 h); so this work can be considered as supplementary to that already published. The aim of this work was to study the hydrothermal treatment of the stems of *M. x giganteus* for the production of the maximum amount of xylo-oligosaccharides, while the structure and composition of the solid phase is altered in order to facilitate a subsequent organosolv treatment.

MATERIALS AND METHODS

Raw Material

Miscanthus was manually collected in Galicia (NW of Spain) in spring 2008. The sample, taken from some specimens of about 3 meters high, was transferred to the laboratory, depithed, and left to dry at room temperature for a long period. Later, the stems were cut in a garden shredder and milled in a hammer mill equipped with a 0.3 mm sieve at the outlet. A final moisture equilibration period was allowed, after which a value of 9.4% moisture was measured. The milled samples were stored in polypropylene bags.

Analytical Methods

Figure 1 shows the analytical protocols performed in this work. TAPPI standards were used to determine: ash (T 211 om-02), cold water solubility (T207 cm-99), 1% NaOH solubility (T212 om-02), acid-insoluble and acid-soluble lignin (TAPPI T222-om88), and α-cellulose (T203 om-93).

Ethanol-toluene extractives were determined in an accelerated solvent extractor DIONEX. Milled samples were subjected to extraction with boiling ethanol-toluene (65:35 by volume) with full automation. A second extraction step was carried out semi-automatically by putting the organic extractive-free samples in a glass flask in a heating block with boiling water for one hour. In both cases, extractive contents were calculated from the weight difference between starting raw material and extractive-free material.

Quantitative acid hydrolysis was performed by a two-step procedure (Browning 1967) by treatment with 72% sulfuric acid: 3.75 cm^3 was added to 0.375 g of sample for 2 hours at 30°C. After that, water was added to obtain 4% sulfuric acid solution and the mixture kept at 100°C for 3 hours. From this acid hydrolysis two fractions were obtained: an acid insoluble solid fraction, that was considered as Klason lignin, after correction for the ash content (after igniting the solid at 575°C), and a liquid fraction. The liquid fraction was neutralized before HPLC determination of monosaccharides, furfural, hydroxymethylfurfural (HMF), and acetic acid.

Determination of individual sugars was performed by HPLC. The samples were diluted with water; fucose was added as internal standard, neutralized with $BaCO_3$, and filtered through a 0.45 μm membrane. A Dionex HPLC chromatograph, equipped with an anion exchange column Carbopack PA1 thermostated to 35°C and a Pulsed Amperometric Detector (PAD), were used under the conditions reported elsewhere (Gur'janov et al. 2007). HPLC chromatography also enabled the determination of other

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significant components as acetic acid, formic acid, furfural, and hydroxymethylfurfural. Uronic acids determination was performed spectrophotomet-rically using galacturonic acid as a standard for quantification (Blumenkrantz and Asboe-Hansen 1973).
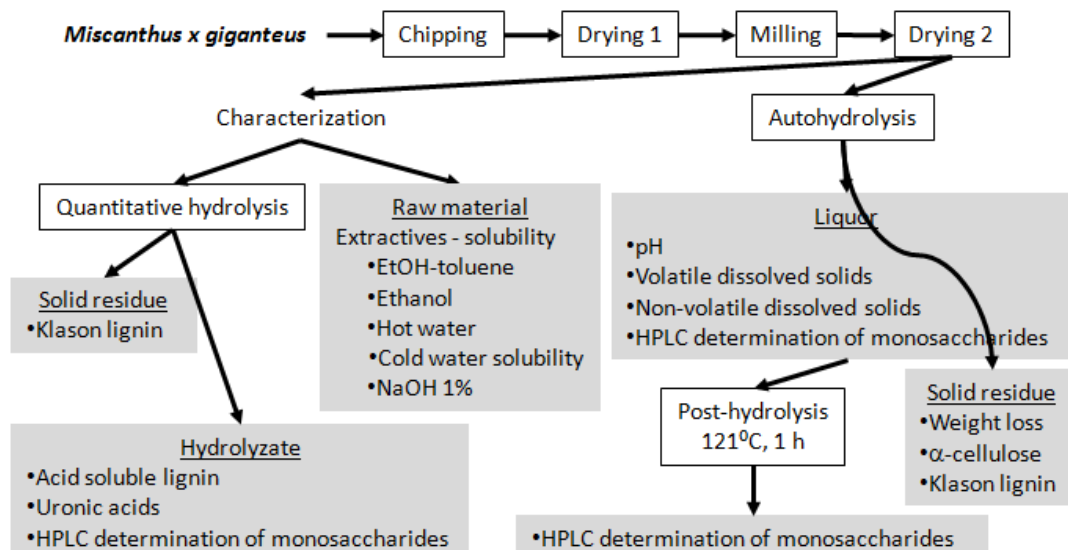

Fig. 1. Analytical protocol developed in the hydrothermal treatment of *Miscanthus x giganteus*

Hydrothermal Treatment

Autohydrolysis experiments were carried out in duplicate using cylindrical 100 cm³ stainless steel reactors submerged in an oil bath at the desired temperature. A sensor inside the reactor made it possible to know the temperature at any time. Samples were treated in duplicate during 5, 15, 30, and 60 minutes to different temperatures between 160 and 200°C. A liquid/solid ratio of 20 was used in all experiments. Figure 2 shows the typical temperature profile during the reactions.

After the reaction, the mixture was cooled, and the undissolved material was separated from liquor by filtration and the solids washed repeatedly with water.
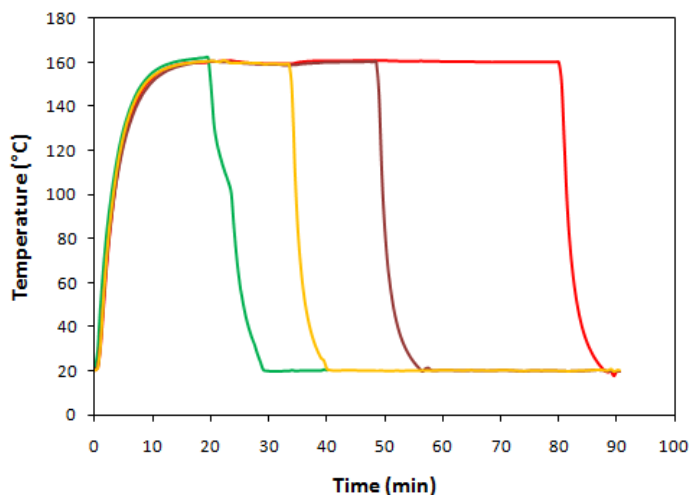

Fig. 2. Example of reactor temperature profiles

Chemical Analysis of Hydrolyzate

The combined liquid autohydrolysis fraction (liquor + washing waters) was split into two aliquots: one was submitted to determination of solubilization, as well as HPLC analysis of monosaccharides, acetic acid, formic acid, furfural, and HMF through the procedure described below. The other aliquot was subjected to quantitative acid hydrolysis with 4% sulfuric acid at 121°C for 1 hour in an autoclave. After that, the samples were neutralized prior to HPLC determination of monosaccharides, furfural, HMF, and acetic acid. The difference between the xylose amounts before and after hydrolysis was considered to be recovered xylo-oligosaccharides. In addition, selected solid residues were analyzed in order to determine the yield of dissolved carbohydrates, acid insoluble lignin, and α-cellulose.

RESULTS AND DISCUSSION

The results of chemical characterization of *Miscanthus x giganteus* are presented in Table 1. The carbohydrate distribution of the raw material shows a dominant presence of glucose and xylose. The xylan content, expressed as xylose equivalent, accounted for 14.9% of the dry weight, which is more than is found in many other non-wood species (Han 1998). Other monosaccharides as uronic acids, galactose, arabinose were present in small quantities.

Table 1. Chemical Analysis of Unextracted *Miscanthus x giganteus* Stems (g/100 g of untreated *Miscanthus*)

Ash		0.8
Solubility	Cold water	7.6
	1% NaOH	33.3
Extractives	Ethanol/toluene	2.2
	Ethanol	0.2
	Hot water	1.6
Lignin	Acid insoluble	20.8
	Acid soluble	0.9
Monosaccharides obtained after quantitative hydrolysis	Arabinose	1.1
	Xylose	14.9
	Mannose	0.0
	Galactose	0.3
	Glucose	38.0
	Uronic acids	1.2
α-cellulose		50.9

The acid-insoluble lignin content of *Miscanthus x giganteus* (20.8%) was similar to values reported previously (Ye et al. 2005; Vrije et al. 2002; Yoshida et al. 2008), and comparable with several hardwoods, but lower than most softwoods. In addition, the acid-soluble lignin (0.88%) agreed with values reported (Vrije et al. 2002). However, it should be noted that different values of composition were determined in work as compared to those previously published (El Hague et al. 2010), possibly due to differences in climate, soil, and age of the plantation.

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pH

The evolution of pH with time and temperature reflects the process of release of acetic acid into the liquor. Significant decreases in pH were observed by increasing both the treatment time and temperature, but the temperature effect was higher (Table 2). The pH of the reaction began in the range 3.2 to 3.4, depending on the temperature used, decreasing progressively with increasing treatment time and temperature. Especially important was the difference between 180 and 190°C, since in this interval the largest decrease in pH occurred, coinciding, as discussed below, with a significant increase of acetic acid in the liquor.

Table 2. Solubilized Components (on % of initial dry *Miscanthus*) Identified in the Autohydrolysis Process at Different Temperatures and Reaction Times

Time (min)	T (°C)	pH	Solubilization	Acetic acid	Furfural	HMF	Xylose	Arabinose	Glucose	Galactose
5	160	3.4	5.3	0.15	0.004	0.011	0.00	0.23	0.00	0.00
	170	3.5	7.2	0.17	0.007	0.014	0.00	0.27	0.00	0.00
	180	3.3	18.7	0.40	0.052	0.006	0.27	0.35	0.02	0.00
	190	3.0	27.7	0.48	0.123	0.019	1.22	0.41	0.09	0.06
	200	3.0	31.1	0.88	0.373	0.021	2.27	0.15	0.09	0.07
15	160	3.4	8.2	0.17	0.009	0.013	0.07	0.28	0.00	0.00
	170	3.3	15.9	0.29	0.040	0.017	0.37	0.38	0.00	0.00
	180	3.2	24.2	0.88	0.285	0.026	1.59	0.51	0.06	0.01
	190	3.0	30.2	1.43	0.738	0.036	1.88	0.43	0.13	0.11
	200	3.0	35.3	2.53	1.791	0.067	5.06	0.30	0.21	0.10
30	160	3.3	14.5	0.25	0.024	0.018	0.20	0.35	0.00	0.00
	170	3.3	22.9	0.53	0.023	0.019	1.03	0.44	0.09	0.00
	180	3.2	28.7	1.88	0.765	0.032	4.71	0.46	0.16	0.00
	190	2.9	31.2	2.57	1.973	0.079	6.19	0.30	0.24	0.14
	200	2.9	32.7	4.08	4.006	0.182	3.68	0.20	0.41	0.10
60	160	3.2	18.4	0.45	0.063	0.021	0.65	0.40	0.00	0.00
	170	3.2	26.2	1.02	0.340	0.019	2.49	0.37	0.10	0.10
	180	3.1	31.3	2.44	1.877	0.060	7.60	0.48	0.27	0.17
	190	2.9	33.3	3.69	4.061	0.200	4.71	0.36	0.65	0.17
	200	2.9	36.9	3.82	4.862	0.466	0.85	0.07	0.72	0.00

Solubilization

The percentage of dissolved material increased with time and temperature (Table 2), with a maximum dissolution value (between 31 and 37%) at 200°C. Dissolved materials can be classified into monomeric volatile compounds (furfural, HMF, acetic acid, mainly) and non-volatile (especially carbohydrates) compounds. The dissolution of volatile compounds increased for all temperatures and reaction times, and the proportion of solubilized materials was directly related to the acetic acid released. At the most extreme conditions the volatile compounds were the main portion of dissolved material (81%), while at temperatures below 190°C volatile materials solubilization remained relatively low. As expected, for higher temperatures more degradation products (furfural and HMF) were formed in the autohydrolysis liquor. Moreover, the non-volatile fraction, being composed primarily of soluble carbohydrates (Fig. 3), showed the typical concentration changes in the liquor for the production of monosaccharides (hydrolysis of hemicelluloses) and its subsequent acid-catalyzed degradation of the monosaccharides released. The maximum yields of solubilized non-volatile materials were slightly above 20% of the initial dry matter. Temperature had a great influence on the solubilization of

non-volatile compounds. At the lowest temperature the solubilization increased with time, but from $180\,^0$C degradation processes are reflected in the graph. At 200°C degradation occurred immediately after the first 5 minutes of treatment.

Monosaccharides

The dominant free monosaccharide found in the autohydrolysis liquor was xylose, with values reaching up to 6.2% (on dry untreated *Miscanthus* basis) at 190°C and 30 min (Table 2). Xylose solubilization showed a time and temperature profile parallel to the non-volatile compounds, with two distinct regions: at milder conditions, the release of xylose increased with time and temperature, while at high time and temperature values xylose degradation occurred. Other monosaccharides in the liquor were present in much lower concentrations. Arabinose was released very quickly into the liquor, but its degradation was also fast. The highest recovery (0.51% at 180°C and 15 min) only accounted for 46% of the initial amount of arabinose present in the raw material. Galactose was more resistant to attack. It was released only under more drastic conditions, although always in very small quantities (Table 2). The presence of glucose in the liquor deserves special attention. Although there are no data available on the total amount of glucose present in the hemicellulose fractions (derived from glucomannan and/or xyloglucan), it can be concluded that cellulose was not significantly degraded by the hydrothermal treatment, since under all conditions tested the solubilization of glucose was below 1%.
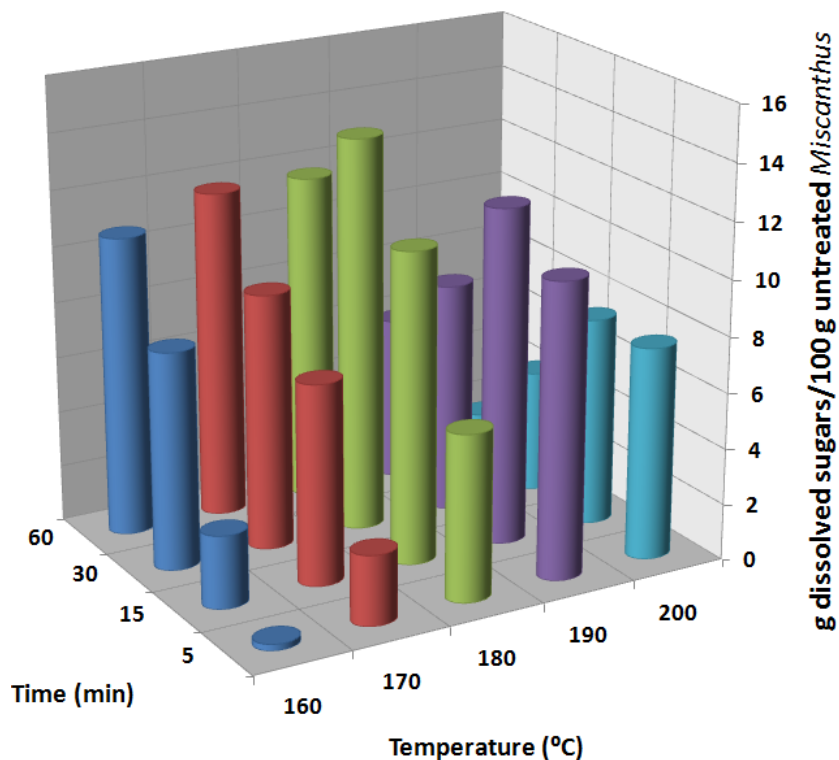

Fig. 3. Effect of time and temperature on the proportion of dissolved sugars (arabinose, galactose, glucose, xylose, and xylans) during the hydrothermal treatment of *M. x giganteus*

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Xylo-oligosaccharides (XOS)

Figure 4 shows the evolution of the solubilization of xylo-oligosaccharides as a function of time and temperature. The production of xylan oligomers reached maximum values of close to 10% of the initial dry matter (Fig. 4), representing 52.5% of the whole mass solubilized and about 65% of the initial xylose present. This is in accordance with values found by other authors treating corncobs (Vázquez et al. 2006), but significantly higher than for barley straw (43%) (Nabarlatz et al. 2007) or bamboo (55%) (Aoyama and Seki 1999). The previously published work on hydrothermal treatment of *Miscanthus* (El Hague et al. 2010) used ranges of experimental variables below used here and therefore also the proportion of dissolved XOS was lower; with the data published there, we have calculated a maximum production of 32%. Also the differences between specimens already mentioned can explain these values. Different combinations of time-temperature values produced high extraction levels, except for 200°C where, even for short treatment times, the hydrolysis of xylans was predominant. In Fig. 4 a diagonal line can be observed in a time-temperature parallel plane where, approximately, and with the exception mentioned, the production of xylo-oligosaccharides was high. Within this line the choice of the most appropriate conditions will depend on several factors but, if the dependence of the ratio (solubilization of xylans/total solubilization of components) is analyzed (Fig. 5), clarifying conclusions can be drawn. Figure 5 shows that this relationship decreased with increasing temperature and reaction time, except for 160°C. Therefore, the more selective xylans releasing corresponds to 160°C and 60 min.
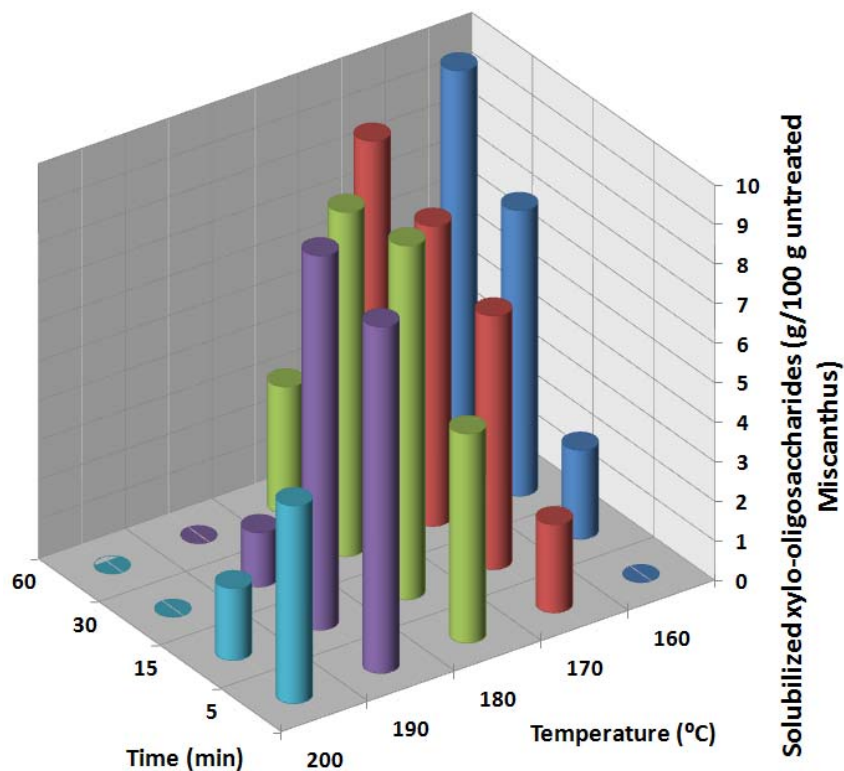

Fig. 4. Effect of time and temperature on the production of xylo-oligosaccharides in solution from hydrothermal treatment of *M. x giganteus*.

Furfural and HMF

Decomposition products of monosaccharides (furfural and HMF) were found to increase in liquors as the severity of treatment was enhanced (Table 2), reaching levels close to 5% of dry mass under the most drastic conditions. However, at 160 and 170°C only little formation of these products was observed. Combined with the conclusion from the previous paragraph, the best process conditions at 160°C and 60 min can be selected for obtaining the highest yields of xylo-oligosaccharides in solution, without degrading the raw material.
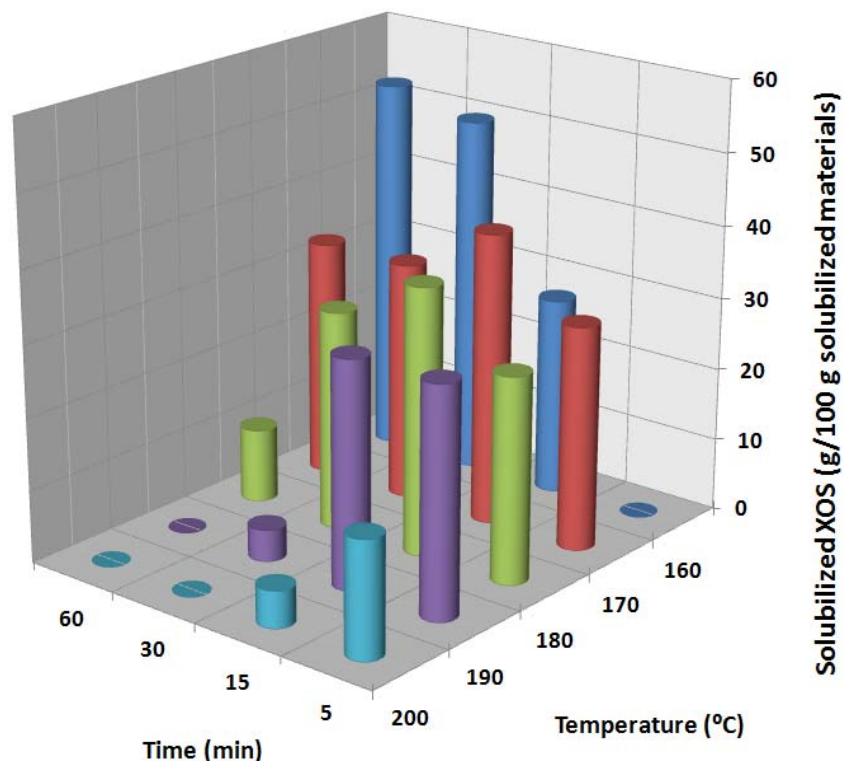

Fig. 5. Effect of time and temperature on the ratio "xylans dissolved/total solids dissolved" in the hydrothermal treatment of *M. x giganteus*

Release of xylo-oligosaccharides as a function of the severity factor

The hydrothermal treatment can also be analyzed in terms of the severity factor or reaction ordinate (R_O). The severity factor was developed to give a simplified kinetic equation for reactions where several processes take place in a complex substrate, such as the lignocellulose matrix (Abatzoglou et al. 1992). The use of the severity factor allows the observation of the combined effect of temperature and time in a single variable. R_O is defined as

$$R_O = \int_0^t e^{\frac{T - T_{ref}}{\omega}} \, dt, \tag{1}$$

where T is the reaction temperature, T_{ref} is a reference temperature (100°C), ω is an

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empirical factor depending on activation energy (from bibliography, $\omega = 14.75°C$), and t is the reaction time.

Graphical representations of the measured parameters against log R_O showed a sudden increase (solubilized volatile materials, acetic acid, furfural, HMF, galactose and glucose) or a maximum (solubilized non-volatile materials, arabinose, and xylans released) around the value of log $R_O = 3.5$, indicating that major changes occurred at that R_O value. The work by El Hague et al. (2010) found nevertheless its best results at log R_O = 4.1. Figure 6 shows the production of xylo-oligosaccharides in solution as a function of log R_O. The curves for different temperatures almost overlap and define a maximum near the mentioned value that, together with different temperature profiles, allows us to calculate for each temperature the optimal treatment time. For the lowest temperature tested (160°C) that, as discussed above, did not produce significant amounts of degradation products of sugars, the maximum corresponds to 62.5 min.
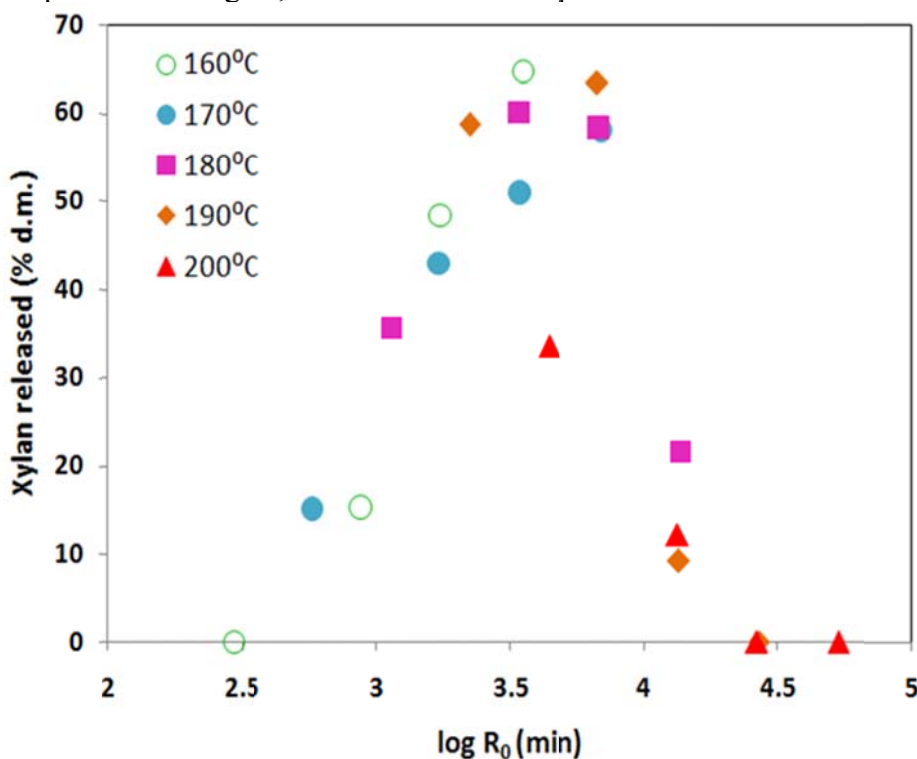

Fig. 6. Release profiles of xylans in the hydrolysis liquor as a function of severity factor.

Cellulose and Lignin Content of the Residue

The degree of dissolution of lignin and cellulose was monitored by determination of α-cellulose and acid-insoluble lignin (Klason lignin) in the solid residues obtained after the more prolonged treatments (60 min) for each of the temperatures studied. The results are shown in Table 3, which presents the percentages of α-cellulose and lignin dissolved in the treatment. As can be seen, only at 160°C, even taking into account the errors in the determination, there was virtually no dissolution of Klason lignin, which confirms, together with the discussion above, that this temperature was the most appropriate.

Recently, other work on *Miscanthus* autohydrolysis showed that, working at lower temperatures and longer times (log R_O = 4.1), lignin does suffer significant changes in its structure (El Hage et al. 2010).

As for the cellulose, the degradation was low for temperatures below 180°C, while from 190°C the deterioration is evident. As expected, the maximum deterioration (28.5% solubilization) was found at 200°C, while at 160°C cellulose did not dissolve. These last data corroborate the earlier conclusion that the optimal conditions for *Miscanthus* biomass fractionation are 160°C and 60 minutes.

Table 3. Mass Loss of α-Cellulose and Klason Lignin of *Miscanthus* after 60 Minutes Hydrothermal Treatments at Different Temperatures

Temperature (°C)	log R_O	α-cellulose	Klason lignin
160	3.5	0.0	0.0
170	3.8	0.0	6.5
180	4.1	5.2	5.8
190	4.4	12.3	6.3
200	4.7	28.5	4.9

The values are expressed as percentages of initial content of each component

CONCLUSIONS

Autohydrolysis was proven to be an efficient procedure to produce xylo-oligosaccharides in solution from *Miscanthus x giganteus*. Within the time-temperature interval tested, the treatment at 160°C and 60 min generated the highest production of xylo-oligosaccharides, without formation of significant amounts of decomposition products and no loss of cellulose and lignin, which remained in the solid in the initial amount. Therefore, the autohydrolysis of *Miscanthus x giganteus* can be a valuable procedure to produce not only high-value components, but as a method of pretreatment of the starting material in a sequential treatment scheme for a type of biorefinery.

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