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The effect of hydrogen peroxide concentration and solid loading on the fractionation of biomass in formic acid

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ABSTRACT

This study investigated the fractionation of biomass using a decomposing mixture of hydrogen peroxide-formic acid as a pretreatment for the biorefining of *Miscanthus* × *giganteus* and of sugarcane bagasse. The main parameters investigated were the hydrogen peroxide concentration (2.5, 5.0 and 7.5 wt%) and biomass loading (5.0 and 10.0 wt%). At the highest hydrogen peroxide concentration used (7.5 wt%), the energy released by the decomposition of the H_2O_2 could heat the reaction mixture up to 180 °C in a short time (6–16 min). As a result, highly delignified pulps, with lignin removal as high as 92 wt%, were obtained. This delignification process also solubilised a significant amount of pentosan (82–98 wt%) from the initial biomass feedstock, and the resulting pulp had a high cellulosic content (92 wt%). The biomass loading only affected the reaction rate of hydrogen peroxide decomposition. Various analytical methods, including Fourier transform infrared spectroscopy, and thermogravimetric and elemental analyses, characterized the lignin obtained.

1. Introduction

Lignocellulosic biomass transformation provides an attractive alternative for the replacement of fossil-based technologies for the production of fuels and chemicals. Energy crops and agricultural residues, such as *Miscanthus* × *giganteus* (Miscanthus) and sugarcane bagasse are abundant, and their utilisation can mitigate greenhouse emissions and facilitate the management of residues and of land. Pretreatment, or fractionation processes are mandatory to separate the major components (cellulose, hemicellulose, lignin) in the lignocellulosic biomass prior to their conversion. These components that contain structural carbohydrates (cellulose and hemicellulose) and aromatic compounds (lignin), can be used as feedstock in a wide range of applications, from biofuels to fine chemicals and materials (Corma, Iborra, & Velty, 2007; Lora & Glasser, 2002; Zakzeski, Bruijnincx, Jongerius, & Weckhuysen, 2010). Other minor components found in lignocellulosic materials contain waxes, esters, fatty acids, and non-structural aromatic compounds, and are generally referred to as extractives. Extractives are highly valuable not only for the production of fine chemical for the food and pharmaceutical sector, but also for the prevention of pitch accumulation during the processing of lignocellulosic materials (Prinsen, Gutiérrez, & del Río, 2012; Villaverde, De Vega, Ligero, Freire, Neto & Silvestre, 2010).

Several technologies, mostly related to applications in the pulping industry have been developed in recent decades for the pretreatment and fractionation of lignocellulosic biomass. An effective separation of the biomass components facilitates the further transformation of each of the substrates into valuable and commercially competitive products. Many of these processes involve the use of different fractionation agents in aqueous media, such as mineral acids (H_2SO_4, HCl), low molecular weight organic acids, ammonia, lime, and hydrogen peroxide (H_2O_2). Organosolv processes, on the other hand, use organic solvents, such as methanol, ethanol, acetone, and concentrated organic acids with acid additives, such as HCl. Some relevant studies are summarised in Table 1.

It has been determined that good solvents for lignin should have values close to 11 for the Hildebrand solubility parameter. Organic acids, such as acetic and formic acids, have values between 10.1 and 12.1, and these can effectively dissolve lignin at moderate temperatures (Pan & Sano, 2005). Several authors have used

Abbreviations: AHR, Acid Hydrolysis Residue; AL, Acetosolv Lignin; $C_{f,j}$, Weight percentage of substrate *j* in pulp (%-wt); $C_{i,j}$, Weight percentage of substrate *j* in raw biomass (%-wt); *j*, Substrate found in biomass (glucan, pentosan, lignin, ash, glucose); $C_{Liq,j}$, Concentration of substrate *j* in the liquid phase (mg/mL); FL, Formosolv Lignin; HHV, High heating value (MJ/kg); HUM, Humins; MISC, *Miscanthus x giganteus*; MISC-P, Pulp from Miscanthus; OSL, Organosolv Lignin; PY, Pulp yield (%-wt); SCB, Sugarcane bagasse; SCB-P, Pulp from sugarcane bagasse; V_{Liq}, Volume of liquid product (mL); W_i, Initial weight of biomass (g).

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Table 1
Pretreatment studies on the fractionation of lignocellulosic biomass.

Feedstock	Pretreatment conditions	Pulp yield (wt%)	Recovery of glucan (wt% of initial glucan)	Recovery of xylan (wt% of initial xylan)	Removal of lignin (wt% of initial lignin)	Ref.
Corn stover	195 °C, Steam 15 min	59.7	88	24	1.5	Xu, Thomsen, and Thomsen, (2009)
Corn stover	195 °C, steam, 40 g formic acid/kg stover 15 min	55.4	83	11	6.4	Xu et al. (2009)
Wheat straw	1 wt% NaOH 0.3 wt% H_2O_2 40 °C 48 h	59	100	16.8	72.5	Curreli et al. (1997)
Wheat straw	1 wt% NaOH 40 °C 24 h	47.5	86.4	23.3	66.7	Curreli et al. (1997)
Switch grass	15 wt% NH_3 120 °C 24 h	N.A.[a]	97.5	47.9	67.8	Gupta & Lee (2010)
Switch grass	15 wt% NH_3/ 5 wt% H_2O_2 120 °C 24 h	N.A.[a]	98.3	66.4	76.9	Gupta & Lee (2010)
Switch grass	1 wt% NaOH 60 °C 24 h	N.A.[a]	90.0	73.9	58.7	Gupta & Lee (2010)
Corn stover	15 wt% ammonia 170 °C MPa N_2 90 min	53.6	91.8	42.4	84.7	Kim, Kim, Sunwoo, and Lee (2003)
Bagasse	0.1 g $Ca(OH)_2$/g dry biomass 120 °C 1 h	94%	100	100	14.0	Chang, Nagwani, and Holtzapple (1998)
Miscanthus	0.65 g ethanol/g water 190 °C 60 min	N.A.[a]	93.4	10.0	66.5	Brosse, Sannigrahi, and Ragauskas, (2009)
Pinus radiate wood	98% formic acid/acetone (70/30) 150 °C 34–37 min	52.0	89.5	11.7	77.5	(Ferraz, Rodríguez, Freer, and Baeza (2000)
Beech wood	12 g wood/100 g liquor 5 g H_2O_2/100 g wood 80% formic acid First stage: 70 °C, 60 min Second stage: boiling temperature, 135 min	47.4	100.0	13.6	89.8	(Dapía, Santos, and Parajó, (2000)
Beech wood	12 g wood/100 g liquor 10 g H_2O_2/100 g wood 80% formic acid First stage: 70 °C, 60 min Second stage: boiling temperature, 300 min	44.2	90.3	8.9	91.1	Dapía et al. (2000)

[a] N.A.: Not available.

acetic and formic acids to delignify different lignocellulosic materials, and have characterised the isolated lignins (Erdocia, Prado, Corcuera, & Labidi, 2014; Ligero, Villaverde, de Vega, & Bao, 2008; Zhao, Dai, & Liu, 2009). Numerous studies have investigated the synergy of concentrated carboxylic acids with H_2O_2 to yield low residual lignin pulps (Dapía et al., 2000; Ligero, Vega, & Villaverde, 2010; Villaverde, Li, Ligero, Ek, & Vega, 2012; Zhao et al., 2009). The delignification and bleaching process known as the Milox process uses formic acid (80–85 wt%) and H_2O_2 (60–200 kg per ton of pulp) (Muurinen, 2000), and is carried out in several stages at temperatures between 60 and 100 °C for total reaction times that vary from 1.5 to 5 h (Dapía et al., 2000; Ligero et al., 2010; Zhao et al., 2009).

In a previous study carried out by the authors (Haverty, Dussan, Piterina, Leahy, & Hayes, 2012), a novel approach, using a mixture of formic acid and H_2O_2, was deployed for the fractionation of *Miscanthus* × *giganteus*. In that approach, Miscanthus was soaked in a mixture of formic acid (60–80 wt%) and H_2O_2 (2.5–7.5 wt%), and the hydrogen peroxide was subsequently decomposed by injecting a sodium hydroxide solution into the reaction mixture. The rapid peroxide decomposition accelerates the rate of delignification of the biomass, through the formation of oxidative radicals, while simultaneously supplying the energy required to fractionate the lignocellulosic structure. This approach considerably decreased the pretreatment time to a few minutes in comparison with the conventional and modified Milox processes mentioned previously, and there was no significant reduction to the quality of the lignin or in its recovery. These advantages represent a considerable potential for the application of organic solvent processes for the fractionation of lignocellulosic feedstocks and for the downstream transformation of the substrates derived (i.e. carbohydrates, lignin) through biorefining. The objective of the current study is to investigate the effects of the H_2O_2 concentration (2.5–7.5 wt%) and of biomass loading (5.0 and 10.0 wt%) on the fractionation of the lignin, the glucan (cellulose) and the pentosan (main component of hemicellulose) fractions in Miscanthus and sugarcane bagasse using the above mentioned novel methodology in a batch reactor. The lignin dissolved during this pretreatment process was isolated and characterised in order to compare the effects of process variables and its suitability for other applications, such as thermal conversion.

2. Materials and methods

2.1. Biomass feedstock

Miscanthus chips were provided by JHM Crops Ltd., Adare, Co. Limerick, Ireland. The material resulting from the same harvesting batch was sieved to a particle size less than 2 mm, thoroughly mixed to have a uniform composition, and stored in plastic containers at room temperature. Sugarcane bagasse was supplied by the Sugarcane Research Centre (Centro de Tecnologia Canavieira CTC), Piracicaba, São Paulo, Brazil. The bagasse, also with an average particle size < 2 mm was a by-product of the sugarcane processing mills. This material was thoroughly mixed, dried at room temperature, and stored in plastic containers at room temperature.

2.2. Formic acid/peroxide pretreatment

The pretreatment experiments were carried out in a stainless steel Parr Series 4550 stand reactor (2 US gal) with a magnetic stirrer (1200 rpm max.) and a temperature monitoring system. The reactor was equipped with sampling and injection ports. Biomass (150–300 g) was charged into the reactor vessel according to the respective solid loading of the experiment. The corresponding amount of solvent was prepared by mixing a certain quantity of hydrogen peroxide (30 wt% H_2O_2) and formic acid (98 wt% HCOOH) to adjust the final H_2O_2 concentration of the mixture to values between 2.5 and 7.5 wt%. The liquors were poured into the reactor and mixed at a constant rate (1000 rpm) with the solid biomass. When the material was mixed thoroughly, the reactor was sealed and temperature recording was initiated. Subsequently, 120 mL of 4 M sodium hydroxide solution was injected into the reactor to activate the decomposition of the peroxide. Samples (1-mL) were taken at different time intervals to monitor the extent of the pretreatment process. When the temperature of the mixture had reached a maximum value, the reactor was cooled to room temperature (maximum time ~3 h). The mixture of solvent and fractionated biomass was filtered through a 500-μm screen to separate the dark solution of concentrated formic acid (liquor) from the residual cellulosic pulp. The pulp was washed with formic acid (4 mL of formic acid per gram of initial biomass), and subsequently washed with water until the pH of the washing water was neutral. The liquor was stored for the isolation of the lignin. The wet pulp was dried at room temperature to a constant weight and stored for analysis of carbohydrate and lignin compositions.

The liquor from the pretreatment experiment was evaporated under vacuum at 60 °C (Laborota 4001 Rotary evaporator, Heidolph) to remove ~90 wt% of the solvent. One part of the concentrated liquor was diluted with 5 parts of deionised water to precipitate the lignin, which was isolated by centrifugation (5 °C, and 4000 rpm for 30 min, Hettich Rotina 38R, Hettich Andreas Hettich GmbH & Co.). The isolated lignin was washed with deionised water, then centrifuged under the same conditions, and subsequently dried in an air-circulation oven at 30 °C.

The effects of the process variables on the fractionation were evaluated based on the distribution of lignin, pentosan, and glucan fractions in the liquid and solid products. The amounts of these fractions in the solid product were defined as follows:

$$\text{substrate } j \text{ retained in pulp} = \frac{(C_{f,j} \times \text{PY}\%)}{C_{i,j}} \times 100 \quad (1)$$

where $C_{i,j}$ and $C_{f,j}$ correspond to the weight percentage of the substrate j (i.e. lignin, pentosan, or glucan) in the virgin biomass and in the pulp, respectively; and PY refers to the pulp yield for the treatment. PY was determined as the mass ratio between the pulp obtained after the pretreatment process and the virgin biomass on a dry weight basis before pretreatment.

The amount of substrate j removed to the liquid product was determined from the concentrations of soluble lignin, arabinose, xylose and glucose in the liquid:

$$\text{substrate } j \text{ removed to liquid} = \frac{C_{\text{Liq},j} \times V_{\text{Liq}}}{W_i \times C_{i,j}} \times 100 \quad (2)$$

where $C_{\text{Liq},j}$ corresponds to the concentration of the substrate j in the liquid, V_{Liq} the volume of the liquid product and W_i is the initial weight of biomass with C_{ij} the concentration of a particular substrate within the biomass. Based on the mass balance of each component, a fraction of substrate j that was removed to the liquid was decomposed to soluble and solid unknown products.

The carbohydrates and lignin distribution after the formic acid/peroxide treatment for a total of nine batch experiments is reported in this study, in which the two different feedstocks are combined with two different biomass loadings (5 and 10 wt%), and with three different initial hydrogen peroxide concentrations (2.5, 5.0, and 7.5 wt%). Two of the batch experimental conditions were carried out as duplicates, which reported coefficient of variations for the pulp yield and maximum temperatures lower than 8%.

2.3. Sample preparation for characterisation

Samples of raw and altered biomass and lignin were ground and sieved to a particle size of 180–850 μm. The biomass and pulp materials were subsequently extracted with ethanol (95 wt%) in a Dionex Accelerated Solvent Extractor 200 according to a modified method by the National Renewable Energy Laboratory - NREL (Sluiter, Ruiz, Scarlata, Sluiter, & Templeton, 2005).

2.4. Carbohydrates measurement

The carbohydrate and lignin contents of the raw biomass and biomass-derived materials were determined following a standard methodology described elsewhere (Haverty et al., 2012; Sluiter et al., 2008). Samples were analysed in duplicate to guarantee a variation less than 5%. An Ion Chromatograph (Dionex ICS-3000), comprising a carbohydrate column (CarboPac PA1, 4 × 250 mm), a carbohydrate guard column (CarboPac PA1, 4 × 50 mm), and an electrochemical flow cell detector was utilised for the quantification of carbohydrates (arabinose, xylose, glucose) in the hydrolysate samples derived from the standard procedure mentioned above. The analyses were carried out by isocratic elution with deionised water (1.1 mL/min) at 18 °C.

2.5. Dissolved lignin measurement

The lignin content in the liquor component of each of the samples taken throughout the experiments was determined using a UV–vis single beam spectrophotometer (Agilent HP 8452A Diode-Array Spectrophotometer) at 380 nm, using commercial organosolv lignin (Sigma Aldrich) to prepare a 6000 ppm stock solution of organosolv lignin in formic acid (98 wt%).

2.6. Characterisation of lignin

The Fourier-Transform Infrared (FT-IR) spectra of the isolated lignins were collected in a Bomem FT-IR spectrometer (ABB Bomem Inc., Canada) comprising a pyroelectric detector (DTGS) with a 4 cm^{-1} resolution in the range of 400 and 4000 cm^{-1}. The lignin samples for FT-IR analysis were prepared by mixing the dry lignin solid with potassium bromide (1:86 mass ratio).

The thermal behaviour of the lignin was investigated using a TA Instruments SDT Q600 thermogravimetric analyser. The samples (5 mg) were heated at 10 °C/min to 900 °C under an inert atmosphere of (100 mL/min STP N_2). The elemental analyses (C, H, N,

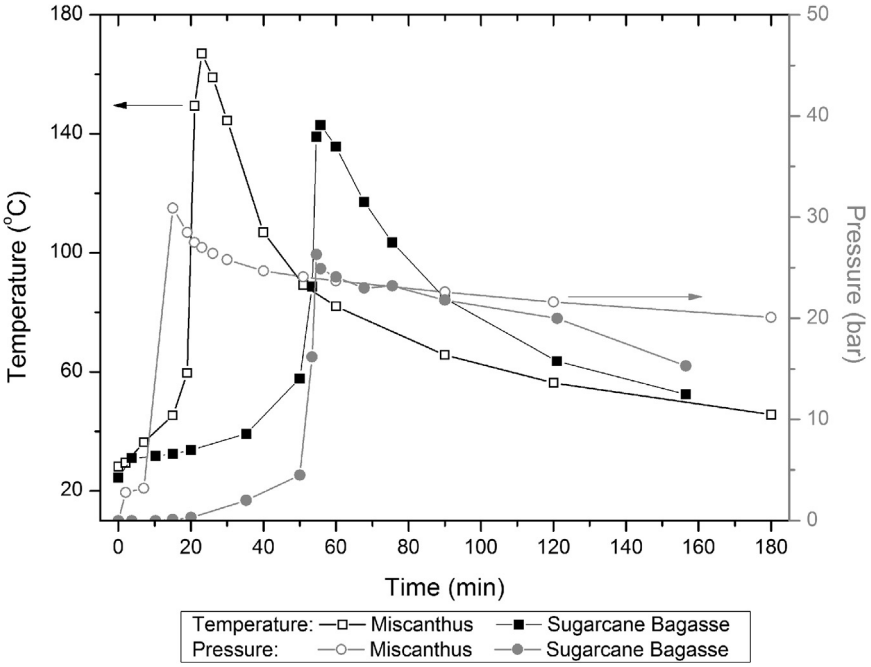

Fig. 1. Temperature and pressure profile as a function of time during the fractionation of 10 wt% miscanthus with 5.0% H_2O_2 in concentrated formic acid, and of 5 wt% sugarcane bagasse with 5.0% H_2O_2.

S) were carried out in a Vario EL Cube automatic analyser and the oxygen content was determined by difference.

3. Results and discussion

3.1. Hydrogen peroxide decomposition

As stated in our earlier study (Haverty et al., 2012), the action of the fractionation process is based on the energy released through the decomposition of the peroxide and the selectivity of the process for the delignification of the biomass. The localised effect of the addition of a base into the reactor accelerated the exothermic decomposition of H_2O_2 via the formation of hydroxyl radicals (HO•) and superoxide ions ($O_2•^-$) (Agnemo & Gellerstedt, 1979; Xiang & Lee, 2000). These radicals play an important role in breaking down the lignin and for its separation from the lignocellulosic network (Xiang & Lee, 2000). Similarly, the effect of formic acid on the dissociation of H_2O_2 and the *in situ* formation of peroxyformic acid promoted the formation of oxidative cation-like electrophiles that are known to promote the solubilisation of the lignin (Sun, Zhu, & Wang, 2000).

The increment of temperature and pressure recorded during the fractionation of 10 wt% Miscanthus with 5.0 wt% H_2O_2, and of 5 wt% sugarcane bagasse with 5.0 wt% H_2O_2 is shown in Fig. 1. The decomposition of H_2O_2 in aqueous solution liberates 1 mole of oxygen gas for every 2 mol of H_2O_2, and as a result, the pressure inside the vessel increased gradually and at a slightly faster rate than the temperature increment of the system. The observed pressure at the end of the Miscanthus fractionation with 5.0% H_2O_2 was about 20 bar, which was higher than the theoretical pressure (18 bar) calculated using the ideal gas equation of state for the total decomposition of the peroxide. This could be due to the presence of CO_2 gas arising from the decomposition reaction of peroxyformic acid formed *in situ* (Filippis, Scarsella, & Verdone, 2008).

Since the decomposition of H_2O_2 is an exothermic reaction, a gradual increase in the temperature of the reaction mixture was observed during the experiments. When the H_2O_2 was exhausted, the system had reached a maximum temperature and it then cooled to room temperature. Despite the fact that the H_2O_2 concentration was not measured throughout the experiments, the reaction time at the maximum temperature marked the completion of H_2O_2 decomposition. The reaction time at which the maximum temperature was reached is defined as the lifetime of H_2O_2.

Fig. 2 shows the maximum temperatures and the corresponding lifetimes of H_2O_2 at various initial H_2O_2 concentrations and biomass loadings. Increasing the H_2O_2 concentration gave rise to higher maximum temperatures because of the greater amount of energy that can be delivered into the system resulting from H_2O_2 decomposition. At a low concentration of H_2O_2 (2.5 wt%), the maximum temperatures observed were between 47 and 69 °C. Doubling the initial concentration of H_2O_2 to 5.0 wt% increased the maximum temperature more than 200%, to 131–162 °C, depending on the amount of biomass feedstock. Generally, the experiments using Miscanthus as the biomass feedstock gave a slightly higher maximum temperature than the experiments using sugarcane bagasse, since greater amounts of reagents were used in the experiments with Miscanthus. Increasing the H_2O_2 concentration to 7.5 wt% increased the maximum temperature to 175–187 °C. As presented in Fig. 2, it is clear that there was no significant effect of biomass loading on the maximum temperature for the experiments in which sugarcane bagasse was used.

Higher concentrations of H_2O_2 would, on the contrary, significantly shorten the lifetime of H_2O_2. At low H_2O_2 concentration (2.5 wt%), the lifetimes of H_2O_2 ranged between 97 and 195 min. Increasing the H_2O_2 concentration led to an exponential decrease in the lifetime of H_2O_2 to 4–16 min. Low biomass loading (5 wt%) of sugarcane bagasse generally gave longer lifetimes for H_2O_2. At higher H_2O_2 concentrations (5 and 7.5 wt%) and with 10 wt% biomass loading, there were no significant differences in the H_2O_2 lifetime for both feedstocks. Based on these experimental results, it can be concluded that increasing the biomass loading would increase the reaction rate of H_2O_2 decomposition. This finding is in agreement with previous studies that showed that the rate of H_2O_2 decomposition in the alkaline aqueous phase was directly proportional to the amount of wheat straw that was reacted (Gould, 1984, 1985). The implications of this finding on the delignification of the

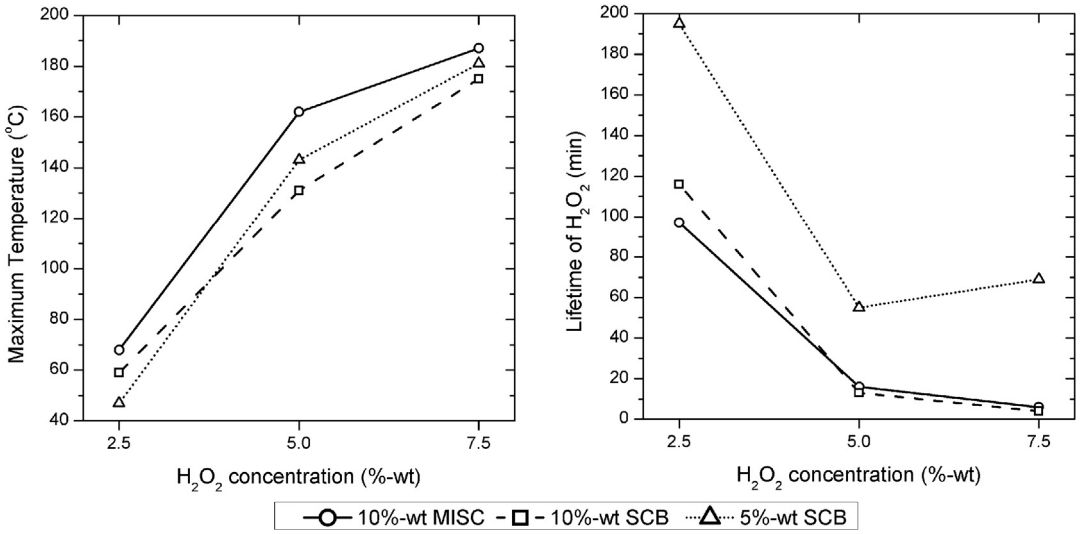

Fig. 2. Maximum temperatures and H_2O_2 lifetime observed during the fractionation of lignocellulosic biomass at various H_2O_2 concentrations and biomass loadings.

biomass and the removal of carbohydrates will be discussed in the following sections.

3.2. Characterisation of Miscanthus and sugarcane bagasse pulps

The compositions (in wt%) of virgin Miscanthus, virgin sugarcane bagasse, and the pulps obtained from various pretreatment experiments are presented in Table 2.

4. Effects of process variables on the delignification of biomass

The effects of the process variables on the distribution of the lignin after the fractionation are shown in Fig. 3. At low H_2O_2 concentrations (2.5 wt%), a significant amount of lignin (42–64 wt%) was retained in the pulp obtained after the pretreatment of either Miscanthus or sugarcane bagasse. Increasing the H_2O_2 concentration to 5 wt% significantly decreased the amount of lignin retained in the pulp to only 4–13 wt%. At higher H_2O_2 concentration, the oxidative pretreatment became more severe and the maximum temperatures were higher. These two factors were expected to lead to a more extensive delignification of the biomass. However, it was observed that increasing the H_2O_2 concentration from 5.0 to 7.5 wt% did not significantly decrease the residual lignin in the pulp, suggesting that there is an optimum H_2O_2 concentration for the delignification. Lowering the biomass loading from 10% to 5% would increase the mass ratio between H_2O_2 and biomass, and the lower biomass loading would be expected to show an enhanced delignification. However, the results revealed that a biomass loading of 5% improved only slightly (between 5% and 12%) the removal of lignin. Different types of biomass had minor effects on the lignin removal at H_2O_2 concentration of 2.5 wt%. At the same biomass loading of 10 wt%, the lignin in Miscanthus was slightly more difficult (~11%) to remove than the lignin in sugarcane bagasse, and that might be attributed to the larger particle size of Miscanthus used in this study.

The experimental results using the H_2O_2 concentration of 2.5 wt% showed that significant amounts of lignin (26–50%) were lost/decomposed, and only 8–15% of the lignin was recovered after the pretreatment. That could be attributable to the long lifetime of H_2O_2 in the medium, as was observed when low concentrations of H_2O_2 were used in the pretreatment. The solubilised lignin could, after more prolonged contact with H_2O_2, undergo further decomposition or oxidation reactions to form decomposition products or low molecular weight compounds. When the H_2O_2 concentration was increased to 5.0 wt%, significantly higher amounts of lignin (46–75%) could be transferred to the liquid compared to the results obtained from treatment with 2.5 wt% H_2O_2. Increasing the initial H_2O_2 concentration, in addition to increasing the amount of lignin removed to the liquid, also suppressed the degradation of the lignin as the result of the shorter contact of the solubilised lignin with the oxidative species. This was observed in particular for the experiment in which 7.5 wt% H_2O_2 was used, because in that case only 1–8% of lignin was lost/decomposed and 86–88% of lignin was recovered in the liquid. Decreasing the biomass loading from 10% to 5% negatively affected the amount of lignin that could be recovered in the liquid product. Lower biomass loading gave a longer lifetime for the H_2O_2, and as a result, the solubilised lignin could undergo further degradation.

5. Effects of process variables on the glucan fractionation

Fig. 4 shows the effects of process variables on the glucan fractionation. With low to moderate H_2O_2 concentrations (2.5 and 5.0 wt%), most of the glucan fraction in biomass (87–98%) was retained in the pulp of either Miscanthus or sugarcane bagasse. Due to the significantly high temperatures (175–187 °C) that were observed using 7.5 wt% H_2O_2, significant amounts of glucan were solubilised in the liquid, and only 49–70% was retained in the pulp. These results support the proposition that the cellulose or glucan is the most recalcitrant fraction of biomass, and its depolymerisation to the glucose monomer in the aqueous phase can occur only at relatively high temperatures and severe acidic conditions (Alves-Gurgel, Marabezi, Zanbom, & da Silva-Curvelo, 2011; Corma et al., 2007).

No significant effects of biomass loading on the amount of glucan retained in pulp were observed during the pretreatment using 2.5 and 5.0 wt% H_2O_2. However, using 7.5 wt% H_2O_2 and a 5 wt% biomass loading, less glucan was retained in the pulp (50%) compared to the amount retained (61%) when a 10 wt% biomass loading was used. Lower biomass loading would prolong the lifetime of H_2O_2, and result in the uptake for an extended time (70 min) by the biomass of the formic acid. As a result, a significant amount of the glucan fraction was depolymerised to its monomer or oligomers (12–15% of the initial amount of glucan), and these depolymerisation products were easily

Table 2
Compositions of virgin biomass and of pulps obtained at various conditions of formic acid/peroxide pretreatment.

No.	Biomass	Biomass loading (wt%)	H_2O_2 (wt%)	Max. temperature (°C)	Pulp yield (wt%)	Glucan (wt%)	Pentosans (wt%)	Klason lignin (wt%)	Acid soluble lignin (wt%)	Extractives (wt%)	Ash (wt%)
	Miscanthus (MISC)					40.8(±0.82)	21.5(±0.33)	21.8(±0.18)	2.0(±0.03)	1.8(±0.08)	3.9(±0.12)
1	MISC	10	2.5	68	78.9	50.5(±6.35)	19.5(±1.61)	7.5(±1.48)	3.3(±0.86)	9.5(±0.03)	1.6(±0.47)
2	MISC	10	5.0	162	52.0	82.4(±3.23)	5.3(±0.47)	3.6(±0.50)	0.8(±0.03)	4.5(±0.06)	3.3(±0.01)
3	MISC	10	7.5	187	35.0	82.8(±3.81)	1.1(±0.06)	6.7(±0.25)	0.5(±0.01)	5.0(±0.06)	3.2(±0.01)
	Sugarcane bagasse (SCB)					39.6(±3.19)	22.9(±1.66)	17.1(±0.33)	2.2(±0.01)	3.5(±0.08)	6.4(±0.19)
4	SCB	10	2.5	59	82.4	42.3(±1.97)	23.2(±0.55)	8.7(±0.37)	3.9(±0.03)	5.3(±0.91)	3.8(±0.11)
5	SCB	10	5.0	131	46.6	78.8(±1.61)	9.0(±0.17)	3.4(±0.24)	1.0(±0.01)	2.6(±0.15)	2.4(±0.00)
6	SCB	10	7.5	175	32.2	77.5(±2.02)	4.4(±0.00)	9.2(±0.29)	0.7(±0.00)	3.9(±0.29)	3.1(±0.01)
7	SCB	5	2.5	47	80.3	46.1(±0.04)	27.7(±0.54)	5.3(±0.65)	4.8(±0.10)	1.8(±0.04)	2.0(±0.29)
8	SCB	5	5.0	143	41.4	85.2(±1.16)	8.1(±0.11)	1.4(±0.29)	0.8(±0.03)	1.4(±0.07)	1.9(±0.04)
9	SCB	5	7.5	181	21.9	89.6(±1.48)	1.9(±0.01)	2.7(±0.33)	0.7(±0.01)	N.D.[a]	3.2(±0.14)

[a] N.D.: Not determined.

solubilised in the liquid product. Additionally, lowering the biomass loading would favour the dehydration to furan compounds (e.g. 5-hydroxymethylfurfural) and other by-products, when high temperatures were eventually reached (>150 °C).

It was observed that sugarcane bagasse released more glucan than Miscanthus for all the pretreatment conditions used in this study. That suggests that there is a significant difference in the glucan distribution between hemicellulose and cellulose in both feedstocks. Previous studies have shown that the hemicelluloses extracted by alkaline peroxide (0.5–3.0 wt% H_2O_2) from agricultural residues, such as sugarcane bagasse and rice straw, contain large amounts of glucan resulting from extensive degradation of the feedstocks (Sun, Sun, Sun, & Su, 2004).

6. Effects of process variables on the pentosan fractionation

The effects of process variables on the fractionation of pentosans are shown in Fig. 5. A significant amount of pentosan was retained in the pulp when 2.5 wt% H_2O_2 was used. When 10 wt% biomass (and 2.5 wt% H_2O_2) was used, 72 and 83% of the pentosans were retained in Miscanthus and bagasse, respectively. The amounts of pentosans retained in the pulp were noticeably low (11–18%) when the H_2O_2 concentration was increased to 5.0 wt%, and almost no pentosans (1–6%) were retained in the pulp when using 7.5 wt% H_2O_2. No significant losses of pentosan were observed when using 5 wt% of sugarcane bagasse. These results are in line with the maximum temperature reached in the fractionation, which strongly depends on the H_2O_2 concentration.

For all pretreatment experiments using 2.5 wt% H_2O_2, the amount of pentosan monomers (xylose and arabinose) quantified in the liquid were insignificant (1.8–5.8%), and due to the long contact with H_2O_2 most of these underwent further decomposition reactions (14–23%). The solubilisation of pentosans was at a maximum when using 5.0 wt% H_2O_2. Half of the pentosans (50–54%) were recovered as monomeric sugars in the liquid product when the experiments were commenced using 10 wt% biomass loading, and when the biomass loading was lowered to 5%, a higher amount of pentosan (76%) was found in the liquid. Increasing the concentration of H_2O_2 was observed to be detrimental to the solubilised pentosan. Significant losses (up to 94%) of the pentosans fraction were observed when 7.5 wt% H_2O_2 was used as the pretreatment agent. The pentoses were converted to furfural and to degradation products as the result of the high temperatures (175–187 °C). The compounds derived from the oxidation of lignin are likely to react with furan and furanoic compounds in what has been suggested as a Diels-Alder-type of approach (Danon, van der Aa, & de Jong, 2013).

7. Effect process variables on the minor fractions of the biomass

The experimental conditions used in this study significantly lowered the content of inorganic matter (ash) in the pulps (1–3 wt%) obtained after the pretreatment. This represents an advantage for downstream thermal processing (i.e. pyrolysis, gasification) of the pulp. Higher amounts of extractives were measured in the pulps obtained from Miscanthus and sugarcane bagasse. In the case of Miscanthus, the extractives content in the pulps were higher than those in the raw material, especially at the low H_2O_2 concentration. This can be attributed to the formylation of the hydroxyl groups in the carbohydrates of the pulp and to the presence of co-precipitated compounds (oligomers, lignin derived compounds) that are easily removed under the extraction conditions. Further investigation of the content of the non-structural

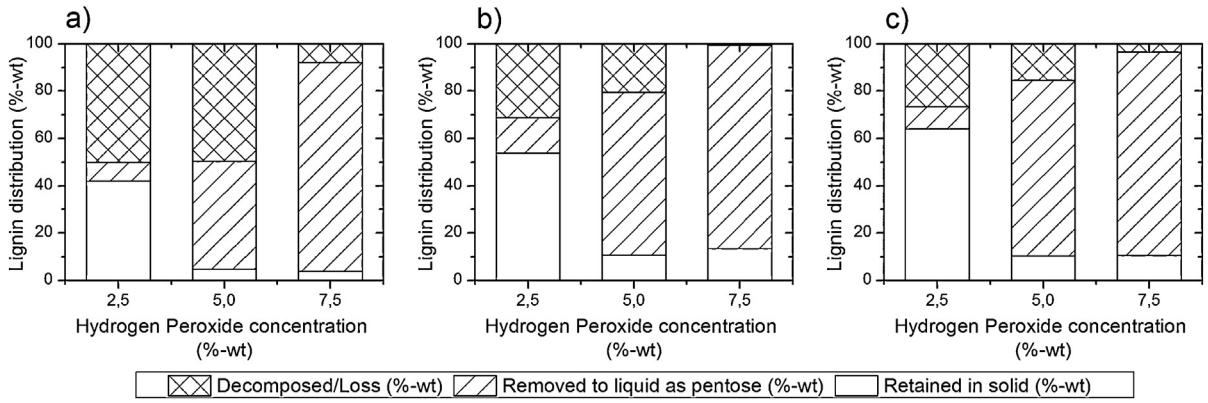

Fig. 3. Lignin distribution after the fractionation of biomass with different H_2O_2 initial concentrations: (a) 5 wt% sugarcane bagasse; (b) 10 wt% sugarcane bagasse; and (c), 10 wt% Miscanthus.

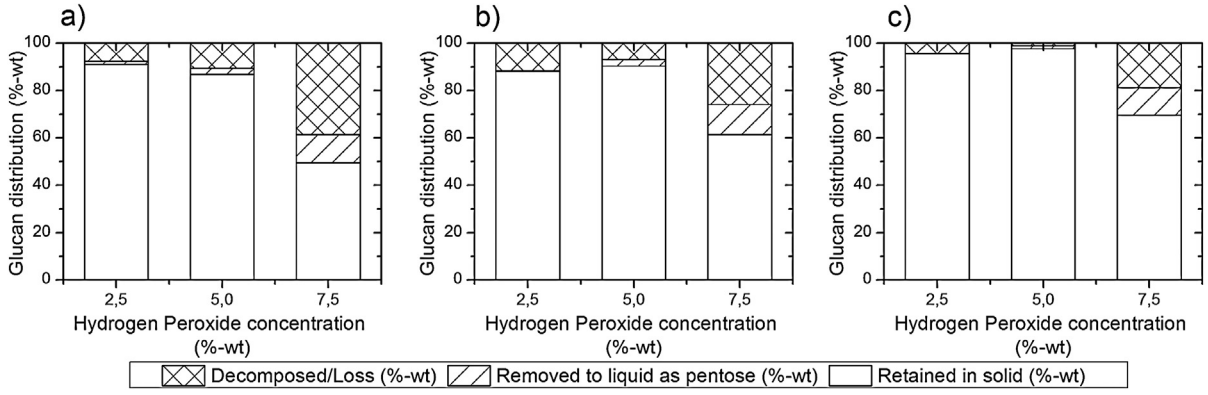

Fig. 4. Glucan distribution after the fractionation of biomass with different H_2O_2 initial concentrations: (a) 5 wt% sugarcane bagasse; (b) 10 wt% sugarcane bagasse; and (c) 10 wt% miscanthus.

extractives is required in order to determine their effect in the downstream processing and purification of the products.

7.1. Isolated lignin: elemental, thermal and FT-IR characterisation

The ultimate analysis of the raw Miscanthus and sugarcane bagasse, as well as of the lignins obtained from runs 1–3, 5, 7 and 8 is presented in Table 3. The ash content of the isolated lignins was in the order of the ash content of the raw materials, confirming that the pretreatment conditions significantly extracted the inorganic matter in the biomass. This extracted inorganic matter was likely to concentrate in the recovered lignin during the isolation. In particular, the lignins from runs 2, 5 and 6 (10 wt% biomass loading, 5.0 wt% H_2O_2, Miscanthus and bagasse, respectively; and 10 wt% bagasse, 7.5 wt% H_2O_2), seemed to have accumulated considerable amounts of ash (8.3, 11.4, and 16.0 wt%, respectively) during the pretreatment. The C, H, and O contents of the lignins obtained in this study are in agreement with other studies of organosolv treatments, in which 53–63 wt% carbon, 4–6 wt% hydrogen and 12–30 wt% oxygen contents were observed (Erdocia et al., 2014; Ligero et al., 2008). The increased H_2O_2 concentration utilised in this pretreatment led to lignins with higher carbon and lower oxygen contents; e.g. the composition of oxygen in the Miscanthus-derived lignin went from 33.5 to 27.9, when the initial H_2O_2 was increased from 2.5 to 7.5 wt%. This results from the removal of oxygen-rich compounds, such as carbohydrates.

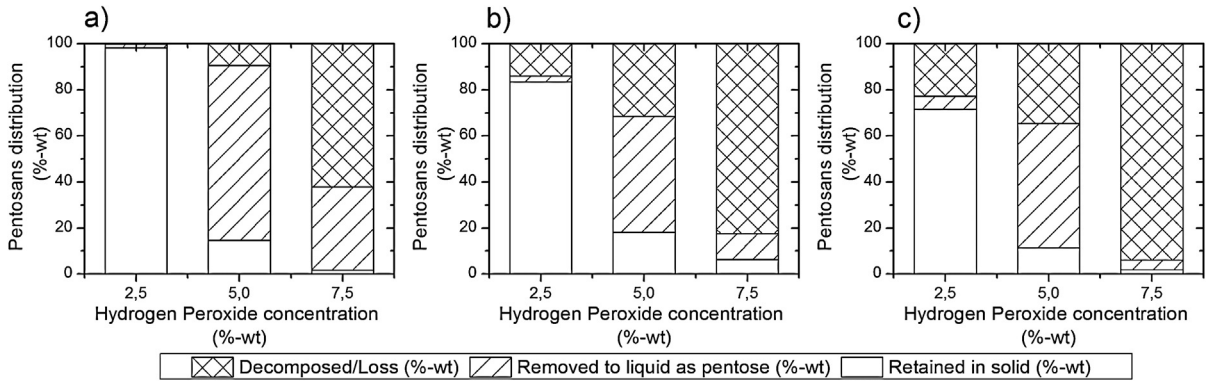

Fig. 5. Pentosan distribution after the fractionation of biomass with different H_2O_2 initial concentrations: (a) 5 wt% sugarcane bagasse; (b) 10 wt% sugarcane bagasse; and (c), 10 wt% miscanthus.

Table 3
Characterisation of raw biomass and the lignins obtained under different conditions from the formic acid/peroxide pretreatment.

Lignin fractionation conditions				Ultimate Analysis						
Run	Biomass	Biomass loading (wt%)	Hydrogen peroxide (wt%)	Ash (wt%)	C (wt%)	H (wt%)	N (wt%)	S (wt%)	O (wt%)	HHV (MJ/kg)[a]
	Raw MISC			3.9	40.6	5.3	0.5	0.1	49.6	17.9
1	MISC	10	2.5	2.4	56.7	5.2	1.8	0.4	33.5	22.1
2	MISC	10	5.0	8.3	58.6	4.4	0.9	0.4	27.4	22.7
3	MISC	10	7.5	3.3	63.1	4.8	0.8	0.1	27.9	24.6
	Raw SCB			6.4	43.6	5.5	0.3	0.5	43.7	17.0
5	SCB	10	5.0	11.4	55.1	4.4	1.0	0.3	27.8	21.4
6	SCB	10	7.5	16.0	59.2	4.3	0.7	0.1	19.7	17.3
7	SCB	5	2.5	6.3	53.6	5.5	2.5	0.1	32.0	21.4
8	SCB	5	5.0	8.4	57.6	4.9	1.9	0.1	27.1	22.7

[a] Lloyd-Davenport et al., 1980 (Friedl, Padouvas, Rotter, & Varmuza, 2005).

Fig. 6 shows the O/C and H/C atomic ratios for the lignins obtained in this study, and these are compared with other relevant materials. In general, the lignins derived from the pretreatment at higher biomass loadings had a lower hydrogen fraction (runs 1, 2, 6, 7 and 8), while the increase of the initial peroxide concentration led to a decrease of both hydrogen and oxygen (runs 1, 2, 3, 6, 7 and 8). The increased severity of the conditions during the formic acid/peroxide pretreatment corresponds with the increased heating values of the isolated lignin. In general, the hydrogen to carbon ratio was lower in the lignins obtained in this study than those obtained by Erdocia et al. (2014), in which the lignin was isolated at 130 °C with corresponding concentrated organic acids and hydrochloric acid as additives (0.2 wt%). The lignins reported by Ligero et al. (2008), obtained after formosolv and acetosolv treatments at reflux temperature and with the addition of HCl, contained lower H/C and O/C ratios than the lignins reported in this study.

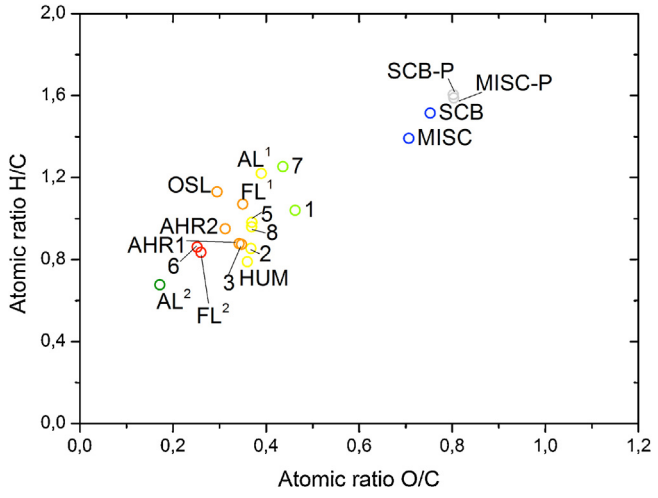

Fig. 6. O/C and H/C atomic ratios of the isolated lignins from the formic acid/peroxide pretreatment of Miscanthus and sugarcane bagasse: MISC, Miscanthus; SCB, sugarcane bagasse; MISC-P, Miscanthus pulp (5.0 wt% H_2O_2, 10 wt% biomass loading); SCB-P, sugarcane bagasse pulp (5.0 wt% H_2O_2, 10 wt% biomass loading); OSL, Organosolv lignin (Sigma-Aldrich); AL^1 and FL^1, Aceto- and Formosolv lignin from olive tree pruning (Erdocia et al., 2013); AL^2 and FL^2, Aceto- and Formosolv lignin from *Eucalyptus globulus* (Ligero et al., 2008); AHR1, acid hydrolysis residue from Miscanthus (2 wt% H_2SO_4, 200 °C, 1 h) (Girisuta et al., 2012); AHR2, acid hydrolysis residue from sugarcane bagasse (5 wt% H_2SO_4,175 °C, 1 h) (Patel, 2013); HUM, humin residue from glucose conversion (0.01 M H_2SO_4, 180 °C, 6 h) (Rasrendra et al., 2013); lignins isolated in the present study under the following conditions: (1) 10 wt% Miscanthus, 2.5 wt% H_2O_2; (2) 10 wt% Miscanthus, 5.0 wt% H_2O_2; (3) 10 wt% Miscanthus, 7.5 wt% H_2O_2; (4) 10 wt% sugarcane bagasse, 5.0 wt% H_2O_2; (5) 10 wt% sugarcane bagasse, 7.5 wt% H_2O_2; (6) 5 wt% sugarcane bagasse, 2.5 wt% H_2O_2; (7), 5 wt% miscanthus, 5.0 wt% H_2O_2.

The values of the H/C and O/C ratios of the isolated lignins have been compared with other residual materials obtained from other biomass fractionation processes. HUM corresponds to the humin-type solid residue from the conversion of glucose in acidic media at high temperature, and it is composed mainly of polymeric agglomerates of furanic compounds (Rasrendra et al., 2013). This residue is characterised by a low H/C ratio, common in compounds such as furfural and 5-hydroxymethylfurfural, which are formed from the dehydration of carbohydrates. AHR1 (Girisuta et al., 2012) and AHR2 (Patel, 2013) are residues from the acid hydrolysis of Miscanthus and sugarcane bagasse, respectively, and these are composed mainly of the native lignin in the biomass and of degradation products from the sugars and furans formed under the conditions of acid hydrolysis catalysed by sulphuric acid. These present similar ratios of oxygen, hydrogen and carbon as some of the lignins obtained in this study, suggesting that the severity of the conditions of runs 2, 3, 5 and 8 resulted in the formation of degradation products of the type formed during acid hydrolysis.

The thermal behaviour of the isolated lignins characterised using thermogravimetric (TG) analysis is shown in Fig. 7. All the samples degraded over a wide range of temperatures (160–400 °C). The DTG plots for each of the samples show at least two distinct peaks. An easily identifiable peak for lignin degradation can be observed for each sample between 300 and 330 °C (Xu, Sun, Sun, Fowler, & Baird, 2006; Yang, Yan, Chen, Lee, & Zheng, 2007). However, significant differences were found in the temperatures at which the fastest degradation of some of the lignins took place. The lignin obtained from pretreatment with 2.5 wt% H_2O_2 (10 wt% Miscanthus) shows a peak at 238 °C that can be attributed to the loss of carbohydrates co-precipitated with the lignin as the temperature range for its pyrolytic degradation is known to be around 220–315 °C (Tsujiyama & Miyamori, 2000; Yang et al., 2007). Volatilisation occurred at low temperatures (165–170 °C) in the lignins derived from Miscanthus and sugarcane bagasse (5.0 wt% H_2O_2, 10 wt% biomass loading), suggesting the liberation of higher amounts of volatiles than at other peroxide concentrations. The volatilisation observed at high temperatures (~700 °C) in the bagasse-derived lignins is attributed to the presence of inorganic compounds from the co-precipitated ash. The char formation at 900 °C was of the order of 30–35 wt%, and that is significantly lower than the char formation reported for residues of sulphuric acid hydrolysis of Miscanthus and bagasse (50–60 wt%) (Patel, 2013). The char formation observed for the Miscanthus lignin, obtained from treatment of Miscanthus with 7.5 wt% H_2O_2, was relatively higher than the char formation from the other lignins. That indicates a thermal behaviour similar to that for the hydrolysis residues, and is likely to result from the presence of sugar degradation products.

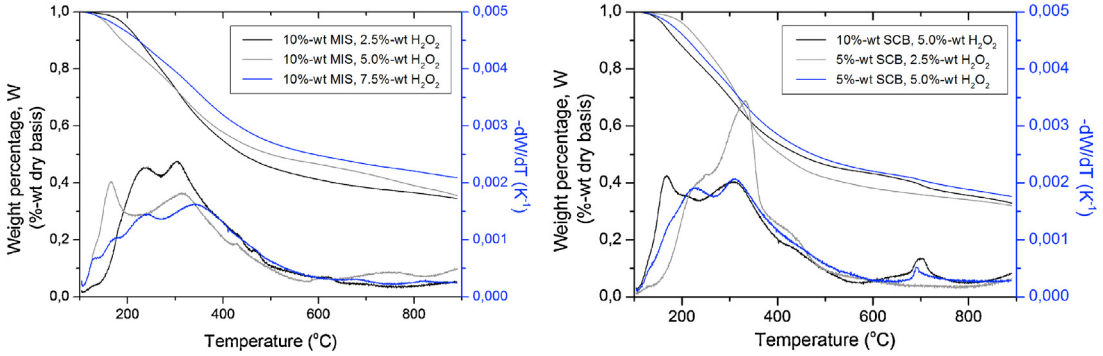

Fig. 7. TG and DTG curves for the isolated lignins obtained under different conditions from miscanthus and sugarcane bagasse.

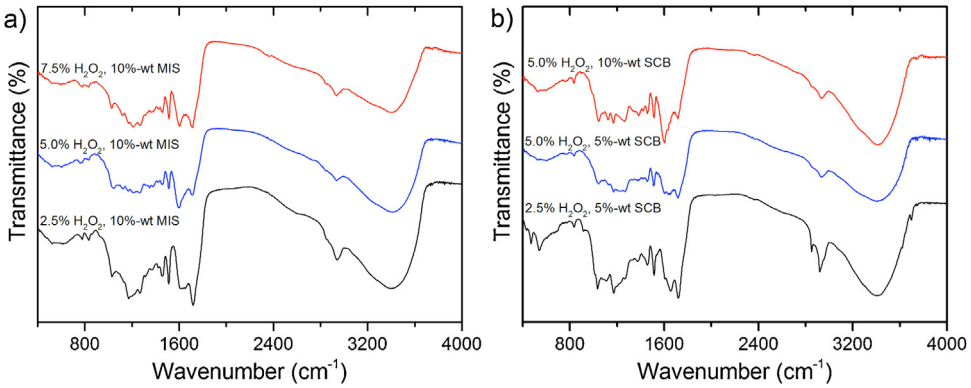

Fig. 8. FT-IR spectra of the lignin isolated from: (a) miscanthus and (b) sugarcane bagasse, obtained after the fractionation of the biomass with different hydrogen peroxide concentrations and biomass loadings.

The FT-IR spectra that compare the lignins obtained under some of the conditions of this study are presented in Fig. 8. The assignment of the absorption bands, in accordance with assignments for other studies (Lin & Dence, 1992; Zhao et al., 2009), is described in detail in Table 4. The ratio of the absorbance of each band to the absorbance at the reference band 1505–1515 cm^{-1}, which is representative of the aromatic skeletal vibrations in the lignins of the guaiacyl type, was used as the criteria for this discussion. Typical bands of absorption that are characteristic of lignin were found in the lignin samples isolated from Miscanthus and sugarcane bagasse, at 1593–1605, 1505–1515, 1266–1270, 1160–1170, 1125–1128 and 1030–1035 cm^{-1}. The band between 3412 and 3460 cm^{-1}, corresponding to the stretching of hydroxyl groups (–OH), was found in relatively lower intensities in samples prepared with higher H_2O_2 concentrations. That suggests that the fractionated lignin was oxidised to a greater extent when it was pretreated during long reaction times and highly oxidative environments (2.5 wt% H_2O_2). Similarly, the relative intensity of this band was found to be higher in the lignins obtained from sugarcane bagasse ($A\%_{3422}/A\%_{1515} = 1.25$) than in the lignins from Miscanthus ($A\%_{3422}/A\%_{1515} = 1.13$). The bands in the regions 2842–3000, 1450–1470 and 1365–1370 cm^{-1} are normally assigned to C–H

Table 4
Assignment of absorption bands in lignin samples, obtained under the different conditions of the formic acid/peroxide pretreatment from Miscanthus and sugarcane bagasse.

Band (cm^{-1})	Assignment	Band in samples (cm^{-1})	
		Lignin derived from Miscanthus	Lignin derived from sugarcane bagasse
3412–3460	O–H stretch	3404–3422	3408–3412
2842–3000	C–H stretch in–CH_2–and–CH_3	2930–2939	2917–2934
1700–1738	Unconjugated C=O stretch	1712–1720	1716
1655–1675	Conjugated C=O stretch	1651–1656	1654–1656
1593–1605	Aromatic skeletal vibrations and C=O stretch, syringyl/guaiacyl	1604–1613	1600–1608
1505–1515	Aromatic skeletal vibrations, guaiacyl > syringyl	1514–1515	1510–1514
1450–1470	C–H deformations in –CH_2– and –CH_3	1458–1460	1458–1460
1365–1370	Aliphatic C–H stretch in CH_3 (non-methoxy)	1352–1372	1372–1374
1325–1330	Syringyl ring and guaiacyl ring condensed	1314–1324	1333–1337
1266–1270	Guaiacyl ring and C=O stretch	1268	1267–1268
1221–1230	C–C, C–O and C=O stretch. Guaiacyl condensed	1215–1221	1221–1225
1166	HGS lignins	1165–1175	1169–1174
1125–1128	Aromatic C–H in plane deformation (syringyl units)	1122	1113–1126
1030–1035	Aromatic C–H in plane deformation (guaiacyl units)	1031–1041	1036–1044
833–835	C–H out-of-plane, in positions 2 and 6 of syringyl units, and all positions of p-hydroxyphenyl units	833	833–837

stretching in $-CH_2-$ and $-CH_3$ groups (not in methoxy functionalities), and were found in relatively similar ratios in all the lignins isolated from Miscanthus and from sugarcane bagasse.

The lignins obtained from low peroxide concentrations (2.5 wt%) presented higher relative absorptions in the band at 1700–1738 cm^{-1} (ascribed to the unconjugated C=O stretching) than did those lignins obtained from higher peroxide concentrations, indicating that a greater oxidation of the lignin aromatic compounds had occurred. The bands at 1593–1605 and 1125–1128 cm^{-1} are attributed to aromatic skeletal vibrations and aromatic C–H deformation in syringyl units and are present in all of the lignins isolated, confirming the presence of this type of lignin compounds.

Additionally other bands assigned to guaiacyl units (1266–1270, 1325–1330, 1221–1230, 1030–1035 cm^{-1}) were also found in the lignin samples, but in relatively lower ratios than those for the syringyl units. When comparing the general characteristics outlined through FT-IR spectra, there were no significant differences in the distribution of the absorbance bands due to the increase of the biomass loading in the lignins prepared from bagasse with 5.0 wt% H_2O_2.

8. Conclusions

The evaluation of the fractionation of Miscanthus and sugarcane bagasse in the batch reactor demonstrated the major roles that the peroxide concentration and the biomass feed played on the decomposition rate of the peroxide, and thus on the delignification/fractionation rate, as well as on the distribution of carbohydrates and lignin in the liquid and solid products. The innovative conditions under which this biomass fractionation was carried out permitted an extensive separation of the lignin and the pentosans from the lignocellulosic structure (86–92 wt%, and 82–98 wt% respectively) of Miscanthus and sugarcane bagasse, while allowing for high recovery of pentoses in the liquid product.

Additionally, the elemental compositions of the isolated lignins, as well as their thermal behaviour expressed on the basis of TG analysis, indicated the potential for the utilisation of the lignin derived from the formic acid/peroxide fractionation in other biorefining technologies, such as thermal pyrolysis.

This novel approach presents several advantages attributable to the lesser time required for fractionation, to the high recovery of carbohydrates, and to the quality of the lignin obtained. The conditions that promote the removal of the lignin from the biomass pulp also favour the hemicellulose hydrolysis, whilst avoiding an extensive hydrolysis or degradation of the cellulose. The solid product of the process described in this work is suitable for integration to other biorefining processes due to its high cellulosic content. Likewise, after separation of the lignin fraction found in the liquid product, the hemicellulosic fraction could be utilised for different applications, such as animal feed products, and the production of furan compounds. Nevertheless, as demonstrated in this study, this selectivity varies with the type of biomass and the distribution of the reactivity of the substrates in the biomass that were evaluated. In addition to this, other factors such as feedstock sourcing, as well as reactor configuration, will play major roles in the optimisation of this pretreatment approach.

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