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EXTRACTION OF HEMICELLULOSES PRIOR TO KRAFT COOKING: A STEP FOR AN INTEGRATED BIOREFINERY IN THE PULP MILL

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ABSTRACT

Two treatments, an induced autohydrolysis and an acid hydrolysis, were applied to *Eucalyptus globulus* wood chips prior to the cooking stage to extract the hemicellulosic fraction that otherwise would be dissolved in the black liquor and burnt in the recovery boiler. The obtained hydrolysates, rich in xylose, were detoxified by overliming and used for ethanolic fermentation. Impacts of each wood pretreatment on the kraft cooking process and on the quality of the produced pulp were evaluated. Both pretreatments promoted an increase in the cooking rate, but had a negative effect on pulp quality and overall yield. Autohydrolysis showed a less negative influence. However, autohydrolysates led to lower values of ethanol concentration, productivity and yield compared to the fermentation of acid hydrolysates. To get more profit from the autohydrolysates they were also submitted to secondary acid hydrolysis and vacuum evaporation processes. Overliming followed by evaporation (with a concentration factor of 3) gave better results than the inverse method. This procedure raised the fermentable sugar content and led to the production of ethanol with a concentration of ~10 g_{eth} L^{-1} (productivity of 0.23 g_{eth} L^{-1} h^{-1} and yield of 0.50 g_{eth} $g_{xyl\ eq}^{-1}$) which compares well with the results obtained with the fermentation of acid hydrolysates.

INTRODUCTION

In pulp production, nearly 50% of hemicelluloses are partly dissolved in the black liquor along with lignin. Hemicelluloses degradation products, mainly a complex mixture of sugar acids, are difficult to separate and purify from the resulting black liquor, which is burned in a recovery boiler to produce electricity and thermal energy. However, the energetic value of hemicelluloses can be better profited, as the calorific content is lower than lignin. Wood chips pretreatments can be applied prior to kraft pulping to extract the hemicellulosic fraction and use it as raw material to produce value-added coproducts by fermentation, e.g. bioethanol. The selected pre-extraction process must not jeopardize the kraft cooking and the final pulp yield and quality. Acid hydrolysis and autohydrolysis processes are commonly use to extract wood hemicelluloses. Wood acid hydrolysis extracts a higher amount of monosaccharides, which facilitate ethanolic fermentation, whilst autohydrolysis enables a less negative impact on pulp production (Yoon and van Heiningen, 2008; Mendes *et al.* 2009a; Helmerius *et al.*, 2002). Autohydrolysates are mostly composed of oligosaccharides and needs a secondary hydrolysis to convert them to fermentable monosaccharides (Sun and Cheng, 2002). Resulting hydrolysates consists predominantly of xylose and small amounts of glucose, since the Portuguese pulp and paper mills use *Eucalyptus globulus* as the main raw material. Furfural, 5-hydroxymethyl furfural and acid soluble lignin derivatives can also be found in hydrolysates composition, which may compromise the fermentation stage as their presence may inhibit the microbial metabolism (Palmqvist and Hahn-Hägerdal, 2000). *Pichia stipitis*, strongly inhibited by acid-soluble lignin (Mendes *et al.*, 2009b), was the yeast selected due to its ability to produce ethanol with high yields (up to 0.41 g g^{-1}). To remove inhibitors compounds several detoxification methods have been proposed. The inhibitors concentrations can be decreased by precipitation under certain pH values (e.g. overliming) or by vacuum evaporation process (volatile compounds), which also helps to increase the sugar content. However, it increases the concentration of non-volatile compounds as well (lignin derivatives) (Amartey and Jeffries, 1996; Martin *et al.*, 2005). Overliming was used to detoxify hemicellulosic hydrolysates to turn them into a suitable carbon and energy source for the fermentation species, due to its efficiency and low costs (Mendes *et al.*, 2009a). Our former assays showed that the fermentation of overlimed acid hydrolysates led to higher ethanol concentrations (12.3 g L^{-1}) with a productivity of 0.221 g h^{-1} L^{-1} and a yield of 0.49 g g^{-1} based on the reducing sugars consumed (Mendes *et al.*, 2009b), which compares well with the literature data (Ferrari *et al.*, 1992; Amartey and Jeffries, 1996; Telli-Okur and Eken-Saraçoglu, 2008). On the other hand, only ~2.0 g L^{-1} of ethanol was achieved in the fermentation of overlimed

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secondary autohydrolysates, with a low productivity (0.079 g h^{-1} L^{-1}), but high yield (0.47 g g^{-1}) (Mendes *et al.*, 2009a). This work shows the results obtained with autohydrolysates after being subjected to a secondary hydrolysis and a vacuum evaporation process to raise the fermentable sugars. The purpose is to achieve or overcome the ethanol amount produced in the fermentation of acid hydrolysates. Auto- and acid hydrolysis influence on kraft pulping and ethanolic fermentation results are compared to evaluate the viability of this biorefinery concept.

EXPERIMENTAL

Eucalyptus globulus wood chips (200 g, dry basis) were mixed with the extraction liquor (liquor-to-wood ratio of 4:1). Four sets of hydrolysis operating conditions were used: an autohydrolysis carried out in water at 150ºC during 120 min or 180 min (AuH120 and AuH180, respectively) and an acid hydrolysis catalysed by 0.4% (w/w) sulphuric acid at 140ºC for 120 min or 180 min (AH120 and AH180, respectively) (Mendes *et al.*, 2009a and 2009b). The extracted wood was washed with water and used to produce pulp, whilst the collected hydrolysates were used as raw material for ethanolic fermentation. A secondary hydrolysis, with 4% (w/w) H_2SO_4 at 100ºC for 180 min (Carvalho *et al.*, 2009) was performed on the autohydrolysates and the monosaccharides content increased up to twice its initial value. From now on, these hydrolysates will be referred to as secondary autohydrolysates (labelled SAuH). Original wood and extracted wood samples (by auto- and acid hydrolysis) were submitted to kraft cooking in rotary reactors as described elsewhere (Mendes *et al.*, 2009a). The corresponding unbleached pulps were submitted to the bleaching sequence, DEDED (D – chlorine dioxide stage; E – NaOH extraction stage) using a consistency of 10%, also detailed elsewhere (Mendes *et al.*, 2009a). Acid and secondary autohydrolysates were detoxified by overliming. $Ca(OH)_2$ was added until pH 10 was reached and the precipitates formed were removed by centrifugation (2500 rpm for 15 min). Sulphuric acid was then added to the hydrolysates until pH ~6.5 was achieved, followed by filtration. Detoxified hydrolysates were used as fermentation culture media. The secondary autohydrolysates were also submitted to concentration by vacuum evaporation, followed by overliming. The vacuum evaporation process was carried out in a 1 L rotary evaporator at 70ºC and 200 mbar to obtain a reducing sugar content of 30 g L^{-1} (SAuH120-B). In another set of experiments, secondary autohydrolysates were first detoxified and then concentrated by vacuum evaporation under the same conditions (SAuH120-C) to evaluate the effect of the sequence of these two steps on the sugars concentration. A higher concentration factor was also tested within this set of trials, in which a sugar content of 60 g L^{-1} was obtained (SAuH120-C'). Batch fermentations were performed in 250 mL Erlenmeyer flasks with a cotton plug, in an orbital shaker at 150 rpm and 30ºC. 150 mL of natural medium (hydrolysates) and 10 mL of fresh inoculum were used. The inocula were prepared with the same natural culture media that were intended to be used. *Pichia stiptis* cells (DSMZ, Germany) were previously adapted to hydrolysates before its application in the fermentation process. The extraction yield was measured by the dissolved solids quantification (evaporation at 105ºC of a sample of the filtrated hydrolysates). Produced pulps were characterised in terms of kappa number, hexenuronic acid content, intrinsic viscosity, reflectance and metal content described elsewhere (Mendes *et al.*, 2009a). During the fermentation stage, the reducing sugars in the hydrolysates samples were measured as xylose equivalents using the colorimetric method with dinitrosalicylic acid reagent (DNS). Yeast growth was evaluated by reading the optical density of cells suspension at 540 nm in the spectrophotometer. Ethanol and xylose concentrations were analysed by HPLC (Knauer model K 301, RI detector). Productivity, P (g L^{-1} h^{-1}), was determined dividing the maximum ethanol concentration produced by the fermentation time needed to achieve it. Ethanol yield, y (g g^{-1}), was calculated dividing the maximum ethanol concentration produced by the corresponding concentration of reducing sugars consumed. Fermentation efficiency, η (%), was measured by the ratio between the observed yield (y) and the theoretical yield (0.51 g g^{-1}).

RESULTS

The hemicelluloses extracted should not be higher than ~13% of the original wood, in order to achieve the same overall pulp yield and quality when using non pretreated wood. Acid hydrolysis conditions (AH120 and AH180) had led to extraction yields of 13.1% and 16%, respectively, whereas autohydrolysis (AuH120 and AuH180) extracted 7.5% and 12.5% of wood material, respectively. Each pre-

Table 1. Pulp properties obtained with the untreated *Eucalyptus globulus* wood chips (reference) and wood submitted to auto- and acid hydrolysis (experiments design to reach a kappa number of ~13.5) (Mendes *et al.*, 2009a)

Wood sample	Overall H-factor[a] (h)	Cooking yield (%)	Unbleached pulp viscosity (mL g^{-1})	Unbleached pulp reflectance (%)	ClO_2 consumption (%)[b]	Pulp brightness reversion (post-color number)
Reference	738	54.7	1268	45.5	4.4	0.43
Auto-hydrolysis[c]	735	51.5	1390	43.2	3.8	0.35
Acid hydrolysis[d]	404	45.7	908	40.9	4.3	0.16

(a) – Including both pre-hydrolysis and cooking stages; (b) – as active chlorine, to reach 90% ISO brightness; (c) – AuH180: 150ºC, 180min, H-factor ~500h; (d) – AH120: 140ºC, 120min, H-factor ~130h.

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Table 2. Fermentation of *Eucalyptus globulus* hydrolysates by *Pichia stipitis*

Hydrolysates treatment	Red. sugars conc.[(a)] ($g\ L^{-1}$)	Red. sugars consumed (%)	Ethanol conc. ($g\ L^{-1}$)	P ($g\ L^{-1}\ h^{-1}$)	y ($g\ g^{-1}$)	η (%)
AH180[(b)]						
Overliming	36.4	85	12.3	0.221	0.49	96
AH120[(c)]						
Overliming	34.5	87	9.6	0.199	0.32	62.7
SAuH120						
Overliming	8.5	84	2.5	0.101	0.42	82.4
Concentrated (B) overliming	19.5	88	6.0	0.218	0.42	82.4
Overliming concentrated (C)	29.0	86	9.9	0.225	0.50	98
Overliming concentrated (C')	61.0	80	7.5	0.170	0.49	96

(a) – Reducing sugars concentration after hydrolysates treatment; (b) – data from reference Mendes *et al*., 2009b; (c) – data from reference Mendes *et al*., 2009a

treatment had shown some impacts upon the kraft cooking process and some pulp properties to reach comparable kappa numbers that were studied in a previous work (Mendes *et al*., 2009a). The most relevant results are shown in **Table 1**.

A concentration of 36 g L^{-1} and 40 g L^{-1} of reducing sugars was observed in the wood acid hydrolysates AH120 and AH180, respectively. The extraction of hemicelluloses by autohydrolysis at 150ºC for 120 min (AuH120) generated 6 g L^{-1} of reducing sugars. After the secondary hydrolysis of autohydrolysates (SAuH120), the reducing sugars concentration raised to 12 g L^{-1} (a 100% increase). All hydrolysates were detoxified by overliming. However, up to 3.6 g L^{-1} of reducing sugar were lost during this treatment, corresponding to 9% and 29% decrease in the acid hydrolysates and secondary autohydrolysates, respectively. Detoxified acid hydrolysates (AH180 and AH120) and secondary hydrolysates (SAuH120-A) were fermented by adapted *Pichia stipitis*. The fermentation parameters obtained are shown in **Table 2**.

DISCUSSION AND CONCLUSION

Considering only the wood extraction yield point of view, an acid hydrolysis pretreatment is more efficient due to a greater cleavage extension of hydrogen and covalent bonds of the lignocellulosic structure. However, this pretreatment led to stronger negative effects on kraft pulping yield and pulp properties. The wood autohydrolysis is shown to be a better option as a pretreatment prior to cooking. The yield in the cooking stage decreased independently of the pre-hydrolysis nature as a result of carbohydrates removal. Even so, a smaller decrease was observed for the autohydrolysis pretreatment. The overall yield, including both pre-hydrolysis and cooking stages, was obviously lower due to the extracted material in the first one. An extra consumption of alkali, more noticed in the wood acid hydrolysis, was also observed. Yoon and van Heiningen (2008) also obtained lower total pulp yields for the kraft cooking of hot water pre-extracted pine wood compared to kraft control cooks, as well as an increase in the effective alkali consumed. A decrease in pulp cooking yield was verified by Helmerius *et al.* (2010) as well for birch wood. Table 1 shows that pulp viscosity was severely influenced by acid hydrolysis conditions. Hence, this fact results in a strength loss, which can limit pulps application. Autohydrolysis conditions promoted a slight viscosity increase. This fact can be a result of lower cellulose degradation due to the decrease in cooking time (cooking H-factor of 239 h) or to the removal of polysaccharides with low molecular weight in a higher extension than in the traditional cooking process. Minimal differences were registered by Yoon and van Heiningen (2008) between intrinsic viscosities of the pulps obtained from conventional kraft cooks and kraft cooks of hot water pre-extracted wood chips. These results suggest that the fibre strength properties will not be significantly altered by an autohydrolysis pretreatment. However, Helmerius *et al.* (2010) detected negative impacts of water extractions in some pulp properties. Table 1 shows that pre-hydrolysis step decreased the reflectance of unbleached pulps. Nevertheless, wood pre-hydrolysis promoted a decrease in the ClO_2 consumption in the conventional method used to achieve ISO brightness of 90%, regardless the initial lower reflectance. In addition, the pretreatment prevented the formation of leucochromophores, thus improving the stability of bleached pulps as shown by the lower brightness reversion values (Table 1). During detoxification procedures by overliming, a loss of 9% and 29% were observed for acid and secondary autohydrolysates. Sugar losses of 11% were registered by Hórvath *et al.* (2005) during the overliming treatment of acid hydrolysates. Martinez *et al.* (2001) verified that the sugar concentration decreased up to 17% during the detoxification of acid hydrolysates with lime. Horváth *et al.* (2008) explain the sugar losses as the consequence

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of coprecipitation of reducing sugar moieties and/or alkaline degradation catalysed by the presence of calcium ions. The monosaccharides can form the corresponding enolate species, leading to an alkali-induced sugar degradation (Horváth *et al.*, 2008). Detoxified AH180 acid hydrolysates were fermented by adapted *P. stipitis*, producing up to 12 g L^{-1} of ethanol with a productivity of 0.221 g L^{-1} h^{-1} and a yield of 0.49 g g^{-1} (Mendes *et al.*, 2009b), based on the reducing sugars consumed (Table 2). An efficiency of 96% on reducing sugars-to-ethanol conversion was determined. Lag phase on yeast growth and ethanol production have been decreased and productivity has been notably increased with the utilization of adapted cells combined with overliming treatment of hydrolysates (Mendes *et al.*, 2009b). Ferrari *et al.* (1992) produced a maximum ethanol concentration of 12.6 g L^{-1} with a production rate of 0.167 g L^{-1} h^{-1} and a yield of 0.35 g g^{-1} based on sugars consumed in the fermentation of *Eucalyptus globulus* wood hydrolysates (~40 g L^{-1} of monosaccharides) by *Pichia stipitis*. Amartey and Jeffries obtained a slightly higher ethanol concentration in the fermentation of corn cob acid hydrolysates using previous adapted *P. stipitis* cells. A concentration of 13.3 g L^{-1} of ethanol was achieved and a yield of 0.41 g g^{-1} was determined, emphasizing the combined advantages of overliming and strain adaptation (Amartey and Jeffries, 1996). Telli-Okur and Eken-Saraçoglu (2008) have obtained a lower ethanol concentration in the fermentation of sunflower seed hull hydrolysates containing a bigger amount of total reducing sugars (48 g L^{-1}). An ethanol concentration of 11 g L^{-1} was produced. The volumetric productivity and yield were 0.065 g L^{-1} h^{-1} and 0.32 g g^{-1}, respectively (Telli-Okur and Eken-Saraçoglu, 2008). In this work, a slightly lower ethanol concentration (9.6 g L^{-1}), productivity and yield were obtained in the fermentation of AH120 acid hydrolysates (Mendes *et al.*, 2009a), as shown in Table 2. The fermentation of secondary autohydrolysates (SAuH120-A) produced a lower ethanol concentration due to its small reducing sugars content, and consequently a low productivity was calculated. Nevertheless, a high efficiency on the conversion of the reducing sugars to ethanol was determined, as it can be seen in Table 2. Further studies with this hydrolysate were performed to enhance productivity by increasing the reducing sugars available for ethanol production. Untreated secondary autohydrolysate was submitted to vacuum evaporation, raising the sugar concentration to nearly 33 g L^{-1}, similar to the reducing sugars content of AH120 acid hydrolysates (36 g L^{-1} before overliming). The concentrated secondary autohydrolysates were then overlimed, after what a 13.5 g L^{-1} reducing sugars concentration was lost (a 41% decrease). Therefore, a concentration of 19.5 g L^{-1} of reducing sugars was available for the fermentation process. An ethanol concentration of 6.0 g L^{-1} was obtained with high values of productivity (0.218 g L^{-1} h^{-1}) and yield based on reducing sugars consumed (0.42 g g^{-1}). An equal sugars-to-ethanol conversion, compared to non concentrated secondary autohydrolysates, was determined (82.4%), as shown in Table 2. A second approach was tested, in which untreated secondary autohydrolysates were first overlimed followed by vacuum evaporation. After overliming, a loss of 4.7 g L^{-1} of reducing sugars was observed (a 39% decrease of the initial concentration, 12 g L^{-1}). The detoxified secondary autohydrolysate was concentrated by vacuum evaporation raising the sugar content to 29 g L^{-1} (higher value in contrast to the one observed in the process described above, 19.5 g L^{-1}). Table 2 shows that an ethanol concentration of 9.9 g L^{-1} was achieved, overcoming the ethanol concentration obtained with AH120 acid hydrolysates fermentation, as well as the volumetric productivity (0.225 g L^{-1} h^{-1}) and yield (0.50 g g^{-1}). A sugar-to-ethanol conversion of 98% was registered (Table 2). Martín *et al.* (2010) had studied the autohydrolysis of olive prunings and the ethanolic fermentation of autohydrolysates after vacuum evaporation and pH adjustment (~5) with NaOH. The authors use the yeast species *Candida tropicalis* that produced an ethanol concentration of 7.2 g per 100 g of olive prunings (nearly 12 g L^{-1}) with a yield of 0.44 g g^{-1} (Martín *et al.*, 2010). As **Figure 1** illustrates, fermentation profiles of AH120 hydrolysates and concentrated secondary autohydrolysates were very similar. No lag phase was registered on yeast growth and a small delay on ethanol production was observed.

A remaining amount of reducing sugars was not utilized by *P. stipitis*. In fact, when reducing sugars were no longer metabolised, ethanol concentration reached a stationary phase or started to decline. Since yeast cells continued to grow, it is believable that the ethanol was used as carbon and energy source for the yeast growth. A different concentration factor was tested aiming to reach double content of reducing sugars (~60 g L^{-1}). A concentration of 61 g L^{-1} was in fact obtained and a higher ethanol concentration was expected after fermentation. Contrarily, only 7.5 g L^{-1} of ethanol was achieved, with a smaller rate production (0.17 g L^{-1} h^{-1}), as presented in Table 2. A slightly lower yield and, consequently, a smaller conversion efficiency were determined, 0.49 g g^{-1} and 96%, respectively (Table 2). A lag phase on yeast growth (data not shown) was also observed. The decrease of the fermentation performance was probably due to a raise in inhibitors content above the tolerance level of toxicity of the yeast (particularly lignin and its derivatives), in spite of

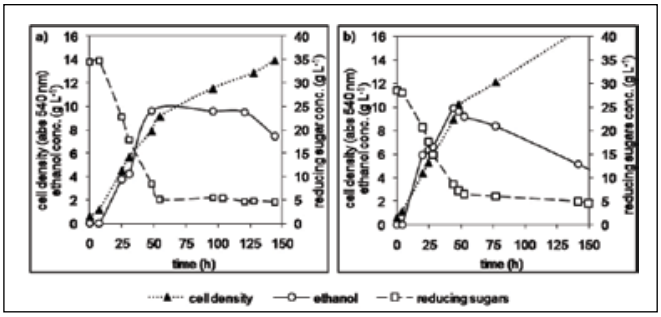

Figure 1. Yeast growth, reducing sugars consumption and ethanol production profiles in the fermentation of: a) acid hydrolysates; b) secondary autohydrolysates overlimed and concentrated (SAuH120-C)

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the detoxification step before the vacuum evaporation. The results obtained above, using the sequence overliming followed by vacuum concentration, are promising for the utilization of autohydrolysates in ethanolic fermentation processes, and combine well with the most suitable findings obtained with the extracted wood kraft pulping process. However, further studies must be performed to clarify the reasons for the observed decrease in the fermentation performance using higher concentration factors. The income with ethanol production by autohydrolysates fermentation must overcome the pulp yield losses and the energy costs required in the operating stages of the overall fermentation process. The aim of this work was to study the viability of the forest biorefinery concept, incorporating lignocellulosic biomass conversion process into an existing chemical pulp mill to produce bioethanol by fermentation. A pre-hydrolysis was applied to *Eucalyptus globulus* wood chips before kraft cooking to extract hemicelluloses. The goal was to get profit of hemicelluloses energetic value, but seeking not to jeopardise the quality of the pulp. Acid and autohydrolysis pretreatment were tested. From the pulp production perspective, wood autohydrolysis is generally a better option as a pretreatment prior to cooking because it does not compromise so harshly the quality of the final pulp. Acid hydrolysis promotes a higher monosaccharides extraction to be used in the ethanolic process and leads to better ethanol production parameters. Nonetheless, autohydrolysates can be turned into a more sustainable raw material for bioethanol production by applying a secondary hydrolysis, detoxification and vacuum evaporation. Overliming followed by evaporation (with a concentration factor of 3) gave better results than the inverse method. This procedure led to ~10 g_{eth} L^{-1} of ethanol with a productivity of 0.23 g_{eth} L^{-1} h^{-1} and a yield of 0.50 g_{eth} $g_{xyl\ eq}^{-1}$, which compares well with the results obtained with the fermentation of acid hydrolysates. ■

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