COMPARISON BETWEEN ACID HYDROLYSIS AND TWO-STEP AUTOHYDROLYSIS FOR HEMICELLULOSIC ETHANOL PRODUCTION

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The objective of this project is to develop a process for the parallel production of ethanol and cellulose in a pulp mill, by applying a prehydrolysis step to softwood chips to extract hexose sugars for their subsequent fermentation in ethanol.
Two kinds of pretreatments were compared, an acid hydrolysis and a two-step treatment composed of an autohydrolysis and a post-hydrolysis of the resulting hydrolysate. The two-step treatment allowed the limitation of the sugar degradation and subsequently the production of the inhibitors (furfural and hydroxymethylfurfural). The amount of acetic acid was however stable, compared to the amount of monomers in solution.
The effects of inhibitors, such as acetic acid, HMF and furfural, on fermentation were studied. At low concentrations, they had no detrimental effect when they were added individually and only a minor effect when they were added together. At high concentrations, acetic acid had a significant negative impact on ethanol production, whereas furfural and HMF did not impact ethanol production.

Keywords: hemicelluloses extraction, acid hydrolysis, autohydrolysis, post-hydrolysis, fermentation, inhibitors

INTRODUCTION

Decreasing the consumption of oil derivatives has become one of the main world issues nowadays. As the major part of oil is turned into transport fuels, substituting them by biofuels could be very interesting.[1] Ethanol is nowadays the only green substitute for gasoline potentially available in large quantities and its production has to increase in the future. For the time being, food resources are unfortunately the main raw material, which could have harmful fallout on food prices. That is why the ethanol of second generation produced from lignocellulosic biomass must be developed. Among a large variety of possible ways, turning galactoglucomannans (GGM), extracted from softwood chips prior to kraft pulping, into ethanol could be interesting. Hemicelluloses, and particularly GGM, are solubilized and degraded during the kraft process and burnt with the lignin, which is not highly profitable because of their low calorific value.[2] Given the fact that most of kraft mills produce an excess of energy, hemicelluloses could be valorized differently. Extracting these sugars prior to cooking to produce a sugar platform could thus be a better way of valorization of the hemicelluloses.[3]

Two main steps are necessary to produce ethanol. Firstly, a pretreatment of the wood chips needs to be done to hydrolyze and solubilize hemicelluloses into oligomers and/or monomers. Secondly, the monomers have to be fermented into ethanol after their separation from the solid fraction.

Fermentation is run by yeasts that use sugars to multiply. Ethanol and carbon dioxide are the main by-products of this reaction. There are other by-products in lower quantity, such as glycerol or acetic acid.[4] With 100 g of hexoses, a maximum theoretical yield of 51 g of ethanol is expected after fermentation (the yield of fermentation, or Gay-Lussac yield, is in this case 100%). Yeasts can be inhibited by some chemical species, among them, furans, weak acids or phenolic compounds.[5–9] Unfortunately, under the conditions of hemicellulose extraction, such products can be formed.[10–12]

In a previous work, it was shown that prehydrolysates from prehydrolysis treatment

carried out with the addition of sulfuric acid could be fermented directly into ethanol.[13] However, the overall pulp yield was significantly lower than for the control kraft process, and the final degree of polymerization of the cellulose also decreased significantly. By applying an autohydrolysis treatment, pulps with better properties could be obtained.[14] However, the drawback of the autohydrolysis stage performed at 160-170 °C was that the hydrolysate contained a majority of oligomers, which is not suitable for a direct fermentation into ethanol.[15] The objective of the present study was to compare a prehydrolysis with the addition of 1% sulfuric acid on wood with an autohydrolysis followed by a post-hydrolysis of the hydrolysate, in terms of sugars and inhibitors concentrations. The effect of the inhibitors on ethanol production was studied in a second part.

EXPERIMENTAL

Conditions of hydrolysis treatments

Industrial chips of mixed softwood species (35% Sylvestre Pine, 24% Black Pine, 18% Alep Pine, 16% Spruce, 7% Douglas fir) from a French pulp mill were used in this study. The acid hydrolysis and autohydrolysis were performed in an autoclave immersed in an oil bath. For each pretreatment, 250 g of wood (as oven dry weight) were introduced. A liquor to wood ratio of 4 was used. For the autohydrolysis, only distilled water was introduced with chips, whereas the liquor of acid hydrolysis contained 2.5 g/L of sulfuric acid (1% on dried weight wood).

For the autohydrolysis, the temperature was 170 °C, the time to temperature 30 min and time at temperature 65 min. For the acid hydrolysis, the conditions were 160 °C, time to temperature 90 min and time at temperature 120 min. The severities of the treatments can be compared by using the Combined Severity (CS). The CS is given by the following equation and takes into account the reaction time (t, in min), the temperature (T, in °C) and the pH of the hydrolysate.[16] The CS of the autohydrolysis and the acid hydrolysis were 0.38 and 2.35, respectively.

$$CS = \log_{10}\left[t\exp\left(\frac{T-100}{14.75}\right)\right] - \mathrm{pH}$$

After hydrolysis, liquor was separated from the solid fraction by filtration. Secondary hydrolysis was performed on hydrolysates resulting from the autohydrolysis under the following conditions: reaction time from 30 to 90 min, temperature from 100 to 140 °C and sulfuric acid concentration from 0.5 to 4% (w/w).

Fermentation tests

The fermentations were carried out in 100 mL flasks. 30 mL of sugar solution were introduced with 7.5 mL of a solution composed of $(NH_4)_2SO_4$ at 5 g/L and $MgSO_4$ $7H_2O$ at 1 g/L.

In the case of the study of the effect of inhibitors, they were added at that point. The pH was adjusted to 4 with sodium hydroxide at 20 g/L or citric acid at 2 g/L before adding 0.4 g of dry *Saccharomyces cerevisiae* yeasts, closing the flask and replacing air by nitrogen in the headspace. The initial concentrations of sugars and inhibitors are given in Table 1. The fermentations were run 48 hours at 30 °C under agitation (150 rpm). Samples were taken initially and after 4, 24, and 48 hours.

Analytical methods

The concentrations in monosaccharides were measured by High Pulsed Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). The column used for the separation was a CarboPac PA 10 (250*4 mm, Dionex) after a guard column (50*4 mm, Dionex). The column and the detector were in a compartment regulated at 25 °C. The eluent was composed of 2 mM KOH at a flow rate of 1 mL/min. The injection volume was 20 µL. The content of polysaccharides was measured with the same chromatograph after their hydrolysis into monosaccharides (1 mL of 24% sulfuric acid was added to 5 mL of hydrolysate and put 1 hour in an oil bath at 120 °C). Concentrations of HMF, furfural, acetic acid and ethanol were measured by HPLC. A ligand exchange column Pl Hi-Plex H (7.7* 300 mm, Varian) was used placed after a guard column (5*3 mm, Varian). The columns were in a compartment regulated at 65 °C. A refractive index detector was used at 35 °C. The eluent was sulfuric acid at 5 mM with a flow rate of 0.6 mL/min. The injection volume was 10 µL.

RESULTS AND DISCUSSION

Comparison between the effects of acid hydrolysis and two-step autohydrolysis on hydrolysate composition

The dry matter extracted from the wood by the acid hydrolysis and the autohydrolysis were 27% and 25%, respectively. The concentrations in hexoses in the hydrolysates were also higher after an acid hydrolysis (33.8 g/L) than after an autohydrolysis (24.1 g/L), as Figure 1 shows. Furthermore, the percentage of hexoses in monomeric form was 89% for the acid hydrolysis, much higher than that obtained for the autohydrolysis (13%), which is consistent with literature data.[17]

Nevertheless, the acid hydrolysis produced a significant amount of inhibitors: the concentrations in acetic acid, HMF and furfural were respectively 4.6, 3.0 and 2.7 g/L for the acid hydrolysis, compared to 1.3, 0.2 and 0.3 g/L for the autohydrolysis.

Table 1
Concentrations in monomers and inhibitors of the different synthetic solutions used for fermentation

	Sugars alone		Complete: sugars+ HMF+furfural+acetic acid		HMF		Furfural		Acetic acid	
Reference	A	B	A	B	A	B	A	B	A	B
Arabinose	2	6.2	2	6.2	2	6.2	2	6.2	2	6.2
Galactose	3.6	8.9	3.6	8.9	3.6	8.9	3.6	8.9	3.6	8.9
Glucose	6	14.2	6	14.2	6	14.2	6	14.2	6	14.2
Xylose	4.9	9.4	4.9	9.4	4.9	9.4	4.9	9.4	4.9	9.4
Mannose	13	27.6	13	27.6	13	27.6	13	27.6	13	27.6
Total hexoses	22.6	50.7	22.6	50.7	22.6	50.7	22.6	50.7	22.6	50.7
Acetic acid	0	0	4.2	10.5	0	0	0	0	4.2	10.5
HMF	0	0	2.3	7.4	2.3	7.4	0	0	0	0
furfural	0	0	2.5	7.4	0	0	2.5	7.4	0	0

A: these concentrations correspond to those obtained when applying acid hydrolysis (1% sulfuric acid on wood, liquor to wood ratio of 4); B: these concentrations correspond to a concentrated hydrolysate

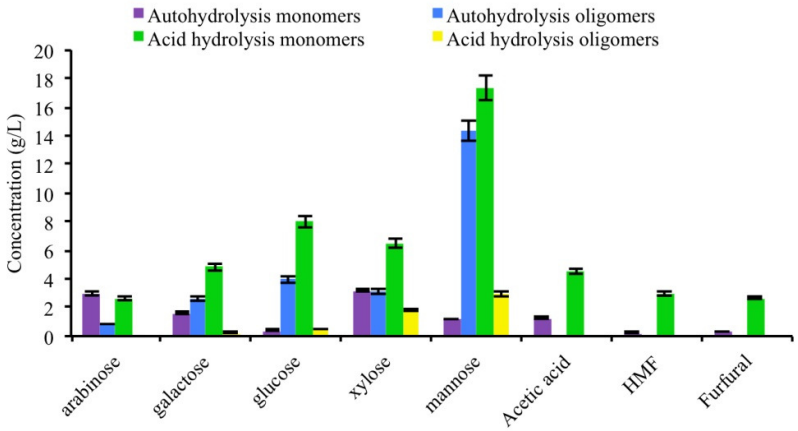

Figure 1: Concentrations of oligomers, monomers, acetic acid, HMF and furfural in hydrolysates from an autohydrolysis and an acid hydrolysis

To increase the rate in monomers after the autohydrolysis treatment, a post-acid hydrolysis was performed on the resulting hydrolysate. The temperature, time and sulfuric acid concentration of the post-hydrolysis were varied. Figure 2 shows the concentrations in hexoses, HMF, furfural and acetic acid after secondary hydrolyses run under different conditions. Two sets of conditions allowed the hydrolysis of almost all the oligomers into monomers, while minimizing the production of degradation products: 140 °C during 30 min with 0.5% of H_2SO_4 and 120 °C during 60 min with 2.25% of H_2SO_4.

The final concentrations in hexoses were 1.4 times lower than after acid hydrolysis (23.6 g/L compared to 33.1 g/L), but with concentrations in HMF and furfural respectively 4 and 2 times lower, which is due to the fact that lower temperatures were used during the secondary hydrolyses, compared to the primary acid hydrolysis on wood chips. The concentrations in acetic acid were 1.5 times lower, which means that compared to the amount of hexoses no

significant decrease was made in acetic acid concentration.

Effect of inhibitors on fermentation

The effect of three inhibitors, HMF, furfural and acetic acid was studied on synthetic media of fermentation containing sugars and inhibitors. Two ranges of saccharides and inhibitors concentrations were chosen. Firstly, the same concentrations as those measured after the hydrolysis carried out with 1% sulfuric acid. The second conditions corresponded to a concentrated hydrolysate, containing about 50 grams per liter of hexoses, but also higher quantities of inhibitors (Table 1). Indeed, for industrial application it will be necessary to have a solution concentrated in sugars to obtain a profitable distillation,[11] and increasing the sugar concentration will also result in an increase in inhibitors concentrations.

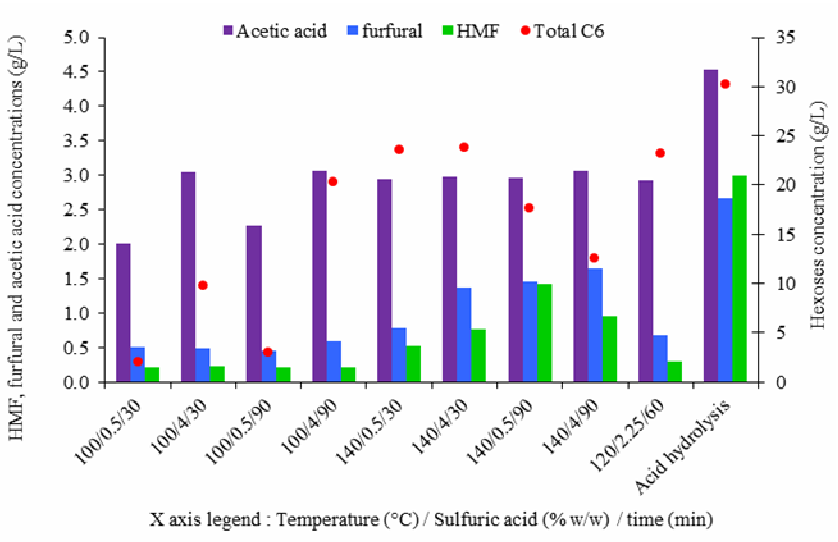

Figure 2: Concentrations in hexoses, acetic acid, furfural and HMF in hydrolysates from secondary hydrolysis applied on the hydrolysate of an autohydrolysis. Comparison with the hydrolysate from an acid hydrolysis (using 1% sulfuric acid)

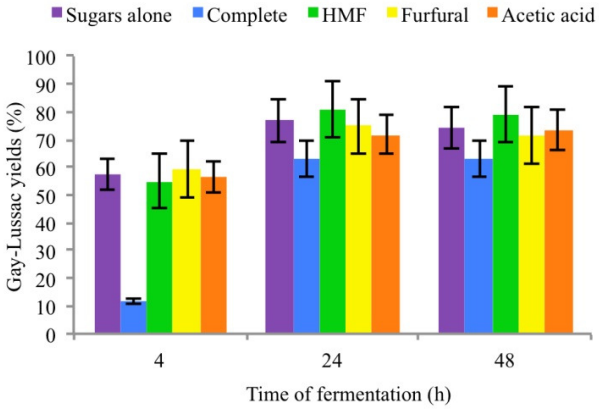

Figure 3: Gay-Lussac yields of fermentations after 4, 24 and 48 hours from low (a) and high concentrated media (b). See Table 1 for the concentration conditions

The fermentations of sugars alone, sugars with one inhibitor and sugars with the simultaneous presence of the three inhibitors were compared.

Inhibitors are known to potentially decrease the ethanol yield,[18] but can also reduce the productivity. Furfural and HMF are metabolized

by yeasts in furfuryl alcohol and 5-hydroxymethyl furfuryl alcohol.[5,19] These reactions are in competition with ethanol synthesis. Furthermore, these products and more especially HMF can increase the lag phase, which is the time needed for yeasts to accustom to their new environment.[10] The action of acetic acid, like that of other weak acids, is different. At low concentration, it can raise the ethanol production, whereas at high concentration, yeast cells cannot ensure the neutrality of their internal pH.[5]

Gay-Lussac yields of fermentations were calculated from the initial amount of hexoses after 4, 24 and 48 hours of fermentation. The yield after 4 hours gives the productivity of the fermentation before reaching the final yield. The measurement after 24 and 48 hours ensures that the final yields are reached before 24 hours, when no significant difference is observed between 24 and 48 hours. Figure 3 shows the results obtained for the low concentrations. It can be seen that the presence of HMF or furfural alone, at concentrations of 2.3-2.5 g/L had no effect after 24 h of fermentation. The same result was obtained when using a solution three times more concentrated in furfural or HMF (7.4 g/L). The addition of acetic acid at 4 g/L did not impair fermentation contrary to the case where a higher concentration, of 10.5 g/L, was used. In this latter case, the Gay-Lussac yield was 38% after 24 hours and 45% after 48 hours, to be compared with 70% at 4 g/L of acetic acid. This might be a problem for industrial fermentation of concentrated hydrolysate solution, as it will be difficult to minimize the release of acetic acid during the secondary hydrolysis, contrary to HMF and furfural, as it has been seen in the previous part. The removal of acetic acid from the hydrolysate or the development of strains that are more resistant to acetic acid could be a solution.

The fermentations led with the three inhibitors simultaneously were possible at low concentrations after 24 hours of fermentation, but a total inhibition was obtained for the experiment run with high concentrations of inhibitors due to the high concentration in acetic acid. These results were confirmed by carrying out a fermentation of “real” hydrolysates obtained from wood hydrolysis.

CONCLUSION

Autohydrolysis of softwood chips followed by a secondary hydrolysis of the hydrolysate makes it possible to obtain a solution containing only monomers, while minimizing the amount of furfural and hydroxymethyl furfural, compared to an acid hydrolysis using sulfuric acid. The amount of acetic acid was however proportional to the amount of C6 monomers. By using a liquor to wood ratio of 4, the autohydrolysis followed by a post-hydrolysis enabled to reach 24 grams per liter of C6 monomers.

The effects of inhibitors during fermentation were studied. Acetic acid appeared to be the most problematic inhibitor, especially at a concentration higher than 10 g/L, which can be reached if the hydrolysate is concentrated to more than 50 g/L of C6 sugars. The release of acetic acid is related to the amount of monomers obtained, which means that for highly concentrated sugar solutions, a partial elimination of acetic acid will be necessary to obtain a fermentable hydrolysate. Another possibility might be to use yeast strains that are more resistant to acetic acid.

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