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Effects of Dilute Acid and Steam Explosion Pretreatments on the Cellulose Structure and Kinetics of Cellulosic Fraction Hydrolysis by Dilute Acids in Lignocellulosic Materials

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ABSTRACT

This article analyzes the effects of dilute sulfuric acid and steam explosion pretreatments on the fractionation and kinetics of the dilute acid hydrolysis of the cellulosic fraction in different biomass substrates. A total of nine biomasses were analyzed: three hardwoods, two softwoods, and four types of herbaceous material. Sigmacell, a purified microcrystalline cellulosic substrate, was also studied as a reference.

It is concluded that steam explosion is a more efficient pretreatment for the fractionation of hardwood and softwood substrates with a low or moderate level of cellulose degradation. Under the experimental conditions however, the steam explosion caused a dramatic degradation of the hemicellulose sugars. The dilute acid prehydrolysis produced much less hemicellulose sugar degradation and revealed a more advantageous method, producing the subsequent acid hydrolysis of the cellulose because of its positive effect (with the exception of sweet sorghum bagasse) on the hydrolysis rate for all the substrates studied, although this seems to be primarily linked to the minimization of the neutralizing capacity of the biomasses as a consequence of the prehydrolysis step. In contrast, the steam explosion pretreatments always produce a decrease of the subsequent cellulose acid hydrolysis rates, which can be related to the significant increases of the crystallinity index values shown by the steam-exploded cellulose.

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Index Entries: Lignocellulosic biomass; cellulose; steam explosion; prehydrolysis; dilute acid hydrolysis; pretreatments; crystallinity index.

INTRODUCTION

The suitability of any treatment of lignocellulosic biomass as a previous step to the acid hydrolysis of the cellulosic fraction in biomass materials is currently linked to its effectiveness in producing the solubilization of the hemicellulose as well as to its capacity to enhance the susceptibility of the polysaccharide to the subsequent hydrolytic attack. In addition, it is very important to take into account the extent of cellulose hydrolysis and sugar degradation that occurs during the pretreatment, since these can be determinant for the economy of the hydrolytic process.

Two of the most commonly used pretreatments for lignocellulosic biomass are dilute acid at moderate temperatures (100–140°C) and steam explosion. Both cause the hydrolysis of the hemicellulose but can also produce significant levels of cellulose degradation if the process conditions are not well selected. The effects of these pretreatments, particularly of steam explosion, on the susceptibility of the cellulose to enzymatic hydrolysis, have been extensively reported (*1–5*), but much less attention has been paid to the effects on the cellulose hydrolysis with dilute acids.

The accessible surface area for the hydrolytic agent and the crystallinity in cellulose have been claimed to be the most important features influencing the kinetics of cellulose hydrolysis (*6,7*), and the former factor has a close relationship to the cell wall pore size in biomass materials (*8*). Since the biomass pore size is not a constraint for the small hydrated hydrogen ion to get the glucosidic bonds of the cellulose, crystallinity seems to be the most important factor affecting the kinetics of cellulose hydrolysis with dilute acids, and thus it is of interest to study the effects of biomass pretreatments on the cited process in order to investigate their effectiveness in modifying the susceptibility of cellulose to the ulterior acid attack.

In this context, the aim of this article is to determine the effects of both dilute acid and steam explosion pretreatments on the crystallinity index of the cellulose in different types of lignocellulosic material under comparative experimental conditions, and correlate them to the changes detected in the dilute acid hydrolysis kinetic parameters of the pretreated substrates compared to the nonpretreated ones. In addition, the ability of both pretreatments to produce the fractionation of the raw materials is also studied.

MATERIALS AND METHODS

Raw Materials

The biomass materials were the following: poplar wood, oak wood, and eucalyptus (small branches) as hardwoods; *Pinus silvestris* and *Pinus*

Table 1
Composition of the Substrates Studied

Substrate	Composition,[a] %			
	Potential glucose	Potential xylose	Lignin	Ash
Poplar wood	35.4	17.0	25.6	3.3
Oak wood	35.9	16.4	30.8	1.1
Eucalyptus (small branches)	36.0	12.2	31.1	3.6
Pine wood (*P. silvestris*)	36.1	16.1	30.3	2.4
Pine wood (*P. pinaster*)	27.8	10.0	37.8	1.1
Wheat straw	35.8	26.8	16.7	11.3
Vine shoots	32.6	15.7	23.7	10.0
Thistle biomass (*O. nervosum*)	31.1	12.2	22.1	10.0
Sweet sorghum bagasse	43.0	24.0	20.0	5.0
Sigmacell	94.8	4.0	1.1	0.5

[a] Expressed in percentage based on dry weight of biomass.

pinaster as softwood representatives; and wheat straw, vineyard residues, thistle *O. nervosum* biomass, and sweet sorghum bagasse as herbaceous materials. The composition of these substrates is indicated in Table 1. It must be mentioned that the potential glucose and xylose content of the samples can be approximately considered to be the cellulose and xylan fractions, respectively.

Pretreatments

Prior to their use, all the dried materials were milled in a hammermill using a 4-mm mesh screen. The raw materials were then subjected to different mild acid prehydrolysis and steam explosion pretreatments. In order to ensure an efficient fractionation of the biomasses, the pretreatment conditions were selected to, in general, achieve at least 80% of potential glucose hydrolysis yield with maximum potential glucose losses of about 25%.

The conditions assayed for each substrate are indicated in Table 2 (steam explosion) and Table 3 (acid prehydrolysis). The dilute acid prehydrolysis and steam explosion yields, μ, are given by the expression:

$$\mu = [(\text{biomass xylan content} - \text{pretreated solids xylan content}) / (\text{biomass xylan content})]\ 100$$

The dilute sulfuric acid pretreatment tests were performed in autoclave at 120°C, using 1000 mL Sovirel bottles filled with 50 g of dried biomass and 500 mL of the acid solution as described in previous studies (2). The residual solids were dried at 60°C and kept for subsequent hydrolytic tests. The steam explosion tests were carried out in a batchwise operated pilot plant,

Table 2
Results Obtained in the Acid Prehydrolysis at 120°C

Substrate	H_2SO_4, % vol	Residence time, min	Solid residues,[a] %		Yield,[b] %	Liquids, %	
			Potential glucose	Potential xylose		Potential glucose	Potential xylose
Poplar wood	1	120	94.9	21.6	78	3.6	50.3
Oak wood	1	120	79.2	21.8	78	5.6	62.4
Eucalyptus (small branches)	1	105	75.8	18.7	81	4.2	68.2
Pine wood (*P. silvestris*)	1	100	89.2	20.0	80	4.2	44.5
Pine wood (*P. pinaster*)	2	80	93.5	18.0	82	4.4	61.4
Wheat straw	1	100	83.7	2.4	98	4.4	40.0
Vine shoots	2	100	84.3	20.4	80	13.8	44.7
Thistle biomass (*O. nervosum*)	2	100	85.7	29.5	70	4.4	53.9
Sweet sorghum bagasse	1	120	86.5	16.3	84	n.d.	n.d.
Sigmacell	1	100	98.4	98.0	2	n.d.	n.d.

[a] Expressed in base to the initial component content in the biomasses.
[b] Expressed in base to the potential xylose hydrolyzed.
n.d.: not determined.

Table 3
Results Obtained in the Steam Explosion Treatments

Substrate	Temp, °C	Residence time, min	Solid residues,[a] % Potential glucose	Solid residues,[a] % Potential xylose	Yield,[b] %	Liquids, % Potential glucose	Liquids, % Potential xylose
Poplar wood	230	4	85.5	8.2	92	0.5	1.3
Oak wood	230	4	75.2	9.8	90	0.6	3.5
Eucalyptus (small branches)	230	2	91.7	0.0	100	2.0	10.6
Pine wood (*P. silvestris*)	210	4	87.8	9.9	90	0.4	0.1
Pine wood (*P. pinaster*)	190	8	98.9	15.0	85	1.6	3.4
Wheat straw	190	8	79.9	13.0	87	4.2	0.7
Vine shoots	190	8	83.7	20.4	80	1.3	2.8
Thistle biomass (*O. nervosum*)	210	2	73.5	9.8	90	0.7	3.0
Sweet sorghum bagasse	230	0.5	95.3	0.0	100	n.d.	n.d.
Sigmacell	230	2	90.5	70.0	30	n.d.	n.d.

[a] Expressed in base to the initial component content in the biomasses.
[b] Expressed in base to the potential xylose hydrolyzed.
n.d.: not determined.

capable of treating about 200 g dry biomass per test. The plant description and working methodology are described in a previous paper (2).

Acid Hydrolysis Treatments

The cellulose hydrolysis tests with dilute sulfuric acid were carried out in a thermostatizated silicon oil bath at 180 ± 0.5°C where 10-mL capacity glass ampules containing 200 mg of pretreated dried solids and 2 mL of the acid solution were introduced. The ampules were taken out and cooled in a fresh silicon bath at different time intervals. The values of the kinetic factors of the cellulose hydrolysis reaction (K_{1i}) were determined in each case according to the following expression:

$$K_{1i}\,(\mathrm{min}^{-1}) = [(-\,L\,C_{Ai} \,/\, C_{Aio}) \,/\, t]$$

where C_{Aio} is the initial potential glucose content (% dry basis) in the reaction media, and C_{Ai} is the potential glucose content (% dry basis) in the media at a given reaction time, t, expressed in minutes.

Analyticals

Crystallinity Index

The crystallinity index (CI) of the cellulose fractions of the substrates was measured by X-ray diffraction following Segal et al. (*9*). Prior to the X-ray measurements, the materials were broken up into fine particles (below 0.4-mm diameter), and dried at 60°C for 72 h. Then, 0.5 g samples were submitted to a pressure of 3100 kg/cm^2 in order to produce small cylindrical briquets (2.5-cm diameter) that were used for X-ray measurements. The CI values indicate the percentage of crystalline cellulose in relation to the total cellulose in the samples.

Biomass Components

The potential glucose, potential xylose, and lignin content of the untreated and pretreated substrates studied were determined by a quantitative hydrolysis method as described by Puls et al. (*10*). Xylose was measured by HPLC using a column of Aminex HPX-87P as described in a previous paper (*10*). The glucose was determined by the glucose oxidase method utilizing the commercial Boehringer-Mannheim "Gluco-quant" Kit. Ash content of the substrates was determined from 1 g of biomass samples according to the TAPPI T15m-58 standard. The lignin (Klason) content of the samples was analyzed by determining the ash content of the dried solid residues resulting from the Puls method for cellulose determination, and then calculating the weight of the ash-free residues.

The Klason lignin was then determined as the percentage of the weight of the dried ash-free residues in relation to the weight of the dried initial samples.

Table 4
Values of the Crystallinity Index (CI) of the Cellulosic Fraction of the Biomass Substrates Studied Before and After the Pretreatments Studied

Substrate	Untreated substrates	Prehydrolyzated solids	Steam-exploded solids
Poplar wood	60.0	69.0	78.0
Oak wood	53.0	62.3	77.5
Eucalyptus (small branches)	53.3	68.3	82.5
Pine wood (*P. silvestris*)	45.6	62.5	74.5
Pine wood (*P. pinaster*)	53.0	57.5	69.0
Wheat straw	58.0	64.0	76.5
Vine shoots	54.0	65.0	70.0
Thistle biomass (*O. nervosum*)	56.5	66.5	75.0
Sweet sorghum bagasse	61.0	68.5	77.5
Sigmacell	87.0	85.4	86.0

RESULTS

The raw materials were subjected to different steam explosion and prehydrolysis treatments in order to determine the most suitable conditions to produce a high level of hemicellulose hydrolysis with a small or moderate degradation of the cellulosic fraction. The main results obtained in the pretreatment tests of the substrates are shown in Tables 2 and 3. These results indicate that the highest xylan hydrolysis yields were achieved with the steam explosion pretreatments, except for the case of wheat straw and vineyard residues. The degradation of the potential glucose (cellulose) was low or moderate, not exceeding 26.5% of that contained in the original biomass. This value corresponded to the steam-exploded *O. nervosum* biomass. The xylose recovery was much lower in the case of the steam explosion pretreatments when compared to that of prehydrolysis.

The high CI value and composition of Sigmacell (Table 4) indicates a quite purified and microcrystalline cellulosic substrate. The CI obtained by this substrate is very similar to that reported by other authors, and, as indicated by Fan et al. (*11*), the difference between this value and CI = 100% could be interpreted by a certain disorder on crystalline boundaries that could be measured as amorphous cellulose. It could also be related to the small cellulose fraction degradation caused by the steam explosion treatment (*see* Table 3).

Both pretreatments, and in particular the steam explosion, caused a significant increase in cellulose fraction CI values for all the biomass materials studied (Table 4). This was not observed for Sigmacell, thus indicating that the microcrystalline cellulose structure is not affected by the pretreatments under the present test conditions.

Prehydrolyzed biomasses showed increased cellulose acid hydrolysis rate factors when compared to those of initial biomasses, with the exception of sorghum bagasse (Table 5). The opposite effect was observed for steam-exploded substrates, which showed lower cellulose acid hydrolysis rates compared to the initial substrates.

X-ray diffraction patterns of Sigmacell and eucalyptus wood before and after pretreatments are shown in Fig. 1.

DISCUSSION

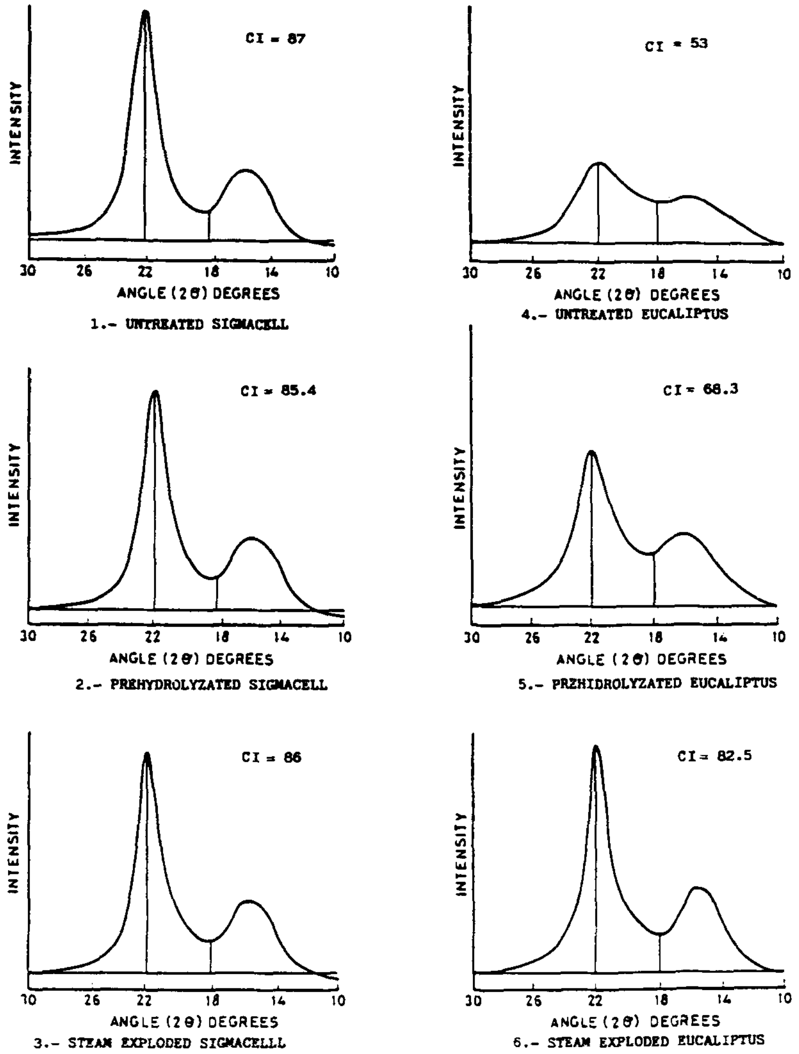
The results in Tables 2 and 3 show that the steam explosion pretreatments achieved higher level of xylan degradation than prehydrolytic ones in the case of the hardwoods and softwoods. With the exception of poplar wood, they produced similar or lower cellulose degradation. Concerning the herbaceous substrates, similar results to those described for woods have been obtained for sweet sorghum bagasse and *O. nervosum* biomasses being of interest to notice the high reticence to dilute acid hydrolysis exhibited by the xylan fraction of this biomass despite the fact that it was subjected to the most severe prehydrolysis conditions. As also occurred with poplar wood, the extent of sweet sorghum bagasse cellulose degradation was higher for the steam explosion treatment, but this was in relation to a much higher xylan hydrolysis yield. Different results were obtained for vineshoots, and, particularly, by wheat straw biomass. For the last material the acid prehydrolysis has revealed a much more efficient method to achieve, both, xylan hydrolysis and a low degradation extent of the cellulose fraction. It is difficult in the light of the results available, to give any explanation to this, but in principle, it could be in connection to the neutralizing capacity of the substrate and/or the susceptibility of its xylan fraction to the acid attack.

As already mentioned, another important aspect to be considered when the goal of the biomass pretreatments is the obtention of hemicellulose sugars for fermentation, concerns the extent of hemicellulose sugars obtained in the prehydrolyzate liquids. The steam explosion pretreatments produce very low yields of xylose in all cases studied (Tables 2 and 3), with a maximum of 10.6% of xylose recovery for the case of eucalyptus biomass. Much better results were obtained with prehydrolysis treatments, which produced xylose recoveries between 40 and 62.4%, for wheat straw and oakwood substrates, respectively. These results indicate the big constraint of steam explosion to produce both efficient biomass fractionation and high hemicellulose sugar yields.

Under some of the most severe experimental conditions, the pretreatments studied did not have a significant effect on the CI value of the microcrystalline cellulose of Sigmacell (Table 4). This suggests that the increases

Table 5
Values of the Kinetic Factors, K_{1i}, of the Cellulose Hydrolysis of the Different Substrates Studied with Dilute Sulfuric Acid at 180°C

Substrate	H_2SO_4, % vol	K_{1i} (min^{-1}) untreated substrates	K_{1i} (min^{-1}) prehydrolyzated solids	K_{1i} (min^{-1}) steam-exploded solids
Poplar wood	0.5	0.027	0.031	0.012
Oak wood	0.5	0.025	0.036	0.013
Eucalyptus (small branches)	0.5	0.024	0.033	0.013
Pine wood (*P. silvestris*)	0.5	0.020	0.033	0.015
Pine wood (*P. pinaster*)	0.5	0.022	0.036	0.020
Wheat straw	0.5	0.034	0.045	0.024
Vine shoots	1	0.056	0.083	0.045
Thistle biomass (*O. nervosum*)	1	0.031	0.064	0.014
Sweet sorghum bagasse	0.5	0.031	0.031	0.012
Sigmacell	1	0.082	0.076	0.076



1.- UNTREATED SIGMACELL

4.- UNTREATED EUCALIPTUS

2.- PREHYDROLYZATED SIGMACELL

5.- PREHIDROLYZATED EUCALIPTUS

3.- STEAM EXPLODED SIGMACELLL

6.- STEAM EXPLODED EUCALIPTUS

Fig. 1. X-ray-diffraction Patterns of Sigmacell and eucalyptus cellulose.

found in cellulose CI values of the pretreated substrates were basically caused by the acid attack on the amorphous cellulose fraction of the substrates rather than on crystalline one. The X-ray diffraction patterns shown in Fig. 1 for eucalyptus wood before and after the pretreatments also concord with this. As observed, the pretreated substrates patterns, particularly that of steam-exploded biomass, are closer to those of Sigmacell than that of the initial biomass, thus indicating that the remaining amorphous cellulose has probably become more crystalline in nature. Similar patterns were found in the rest of biomass substrates.

The increased cellulose CI values, can, on the other hand, explain the lower K_{1i} values depicted in Table 5 for dilute acid hydrolysis of steam-exploded substrates compared to those of initial biomasses. This also seems to corroborate the significant influence of the crystallinity of the cellulose fractions on the dilute acid hydrolysis kinetics rather than that of other parameters such as increased biomass pore size and available surface, and cellulose defibration. These were also found to be caused by this pretreatment, and should contribute to improve the acid hydrolysis results.

On the contrary, the K_{1i} values for the prehydrolyzated materials (Table 5) do not seem to support the values mentioned for steam-exploded substrates, since the prehydrolyzated residues showed increased values of the hydrolysis rate factors despite the higher Cl of the cellulose fractions compared to those of initial biomasses. One factor that could contribute to explain this is the much lower neutralizing capacity of the biomasses after prehydrolysis, which determines higher real acid concentrations in the cellulose hydrolysis media compared to those of the initial materials. This could be supported by the fact that the sweet sorghum bagasse, a substrate that originally has a very low neutralizing capacity owing to the sugar extraction treatment of sorghum cane with hot water, is the only biomass of those studied with the same K_{1i} values before and after the prehydrolytic treatment (*see* Table 5).

This effect was not observed for steam-exploded substrates. This could be explained by the scarce or nul susceptibility of their microcrystalline cellulose fraction to the dilute acid attack under the experimental conditions similar to the case with Sigmacell.

The results obtained reveal the greater suitability of the prehydrolytic treatments for achieving both good fractionation and hemicellulose hydrolysis results, as well as the need to consider the overall effects on biomass components and structure in order to make a proper evaluation of the suitability of a determinate pretreatment for lignocellulosic biomass.

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