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Effect of Chip Size on Steam Explosion Pretreatment of Softwood

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Abstract

Although considerable progress has been made in technology for converting lignocellulosic biomass into ethanol, substantial opportunities still exist to reduce production costs. In biomass pretreatment, reducing milling power is a technological improvement that will substantially lower production costs for ethanol. Improving sugar yield from hemicellulose hydrolysis would also reduce ethanol production costs. Thus, it would be desirable to test innovative pretreatment conditions to improve the economics by reducing electrical power of the milling stage and by optimizing pretreatment recovery of hemicellulose, as well as to enhance cellulose hydrolysis. The objective of this study was to evaluate the effect of chip size (2–5, 5–8, and 8–12 mm) on steam-explosion pretreatment (190 and 210°C, 4 and 8 min) of softwood (*Pinus pinaster*).

Index Entries: Pretreatment; steam explosion; softwood; chip size; enzymatic hydrolysis.

Introduction

Lignocellulose biomass represents a vast resource that could be used to produce fuel ethanol. Lignocellulosic materials contain carbohydrate polymers (cellulose and hemicellulose), plus lignin and smaller amounts of other materials. Agricultural residues, municipal solid waste, underutilized standing forest and logging residues from energy crops, as well as waste streams from industrial operations are examples of this renewable material.

Although progress has been made in technology for converting lignocellulosic biomass into ethanol, there are still opportunities to reduce production costs *(1,2)*. Enzymatic hydrolysis processes are advantageous because they catalyze only specific reactions; consequently, there are no

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side reactions or byproducts, and the hydrolysis can potentially be run with yields approaching 100% of theoretical *(3)*. Pretreatment is necessary to achieve reasonable rates and yields in the enzymatic hydrolysis of biomass. To open up lignocellulosic structure to microbial degradation requires expensive energy-demanding pretreatment processes. Reducing milling power is a technological improvement that will substantially lower ethanol production costs. Improving sugar yields from hemicellulose hydrolysis will also reduce ethanol production costs.

Autohydrolysis steam explosion has been recognized as a cost-effective method for pretreating lignocellulosic biomass, because no addition of external catalyst is necessary *(4–6)*. The process of autohydrolysis pretreatment efficiently increases enzymatic hydrolysis of hardwood and agriculture residues; however, it is much less effective for softwoods.

The most important variables in steam-explosion pretreatment are time, temperature, and chip size. Generally, when larger chips are used, heat transfer problems overcook the exterior (with the associated formation of inhibitors) and incompletely autohydrolyze the interior *(7)*. Consequently, prior to steam explosion, particle size must be reduced, which requires significant amounts of energy. Another problem with autohydrolysis steam explosion is the low hemicellulosic sugar yield, and its negative impact on ethanol production economics. Research efforts have concentrated on maximizing cellulose the recovery of and have failed to recognize the importance of also recovering hemicellulose, which is crucial to improve the overall economics.

Thus, it would be desirable to test innovative pretreatment conditions that improve the economics of pretreatment by reducing electrical power during milling and by optimizing the recovery of hemicellulose, as well as to enhance cellulose hydrolysis. The objective of this work was to evaluate the effect of chip size on steam-explosion pretreatment of lignocellulosic biomass. The hemicellulose recovery and enzymatic hydrolysis effectiveness of the cellulosic residue are presented for steam-explosion pretreatment of a softwood biomass (*Pinus pinaster*) using different chip sizes.

Materials and Methods

Substrates

Chipped biomass (8% moisture) from *P. pinaster* was provided by the Renewable Energy Development Center at Lubia (Soria, Spain). The chips were milled using a laboratory hammer mill. Milled material was further separated into three different fractions (2–5, 5–8, and 8–12 mm) using a portable sieve shaker.

Steam-Explosion Pretreatment

Pretreatment of raw material was performed by applying Masonite pretreatment technology. The following parameters were investigated: 190 and 210°C temperature and 4- and 8-min residence time.

Steam-explosion pretreatment was performed in a batch pilot plant described in a previous work *(8)*. The pilot plant was equipped with a 2-L reaction vessel designed to reach a maximum operating pressure of 4.12 MPa. The reactor was filled with 100 g of feedstock per batch and was then directly heated with saturated steam to the desired temperature. After the explosion, the material was recovered in a cyclone, and the wet material was cooled to about 40°C and then filtered for solid recovery. The solid fraction was analyzed for xylans, glucans, and lignin content. The carbohydrate content in the water-soluble extract was also analyzed.

Sodium Chlorite Extraction

Sodium chlorite extraction of pretreated wood was performed using methods previously described *(9)*.

Enzymatic Hydrolysis

The water-insoluble fiber and the delignified fiber after pretreatment were enzymatically hydrolyzed to determine the maximum sugar yield. The cellulolytic complex employed in enzymatic tests (Celluclast 1.5L) was a gift from NOVO Nordisk (Denmark). Cellulase enzyme loading was 15 filter paper units/g of substrate. Fungal β-glucosidase (Novozyme 188, Novo) was used to supplement the β-glucosidase activity with an enzyme loading of 12.6 IU/g of substrate. Enzymatic hydrolysis was performed at 50°C on a rotary shaker at 150 rpm for 72 h and at 20 g/L of substrate concentration.

Analytical Methods

The raw material composition was analyzed following ASTM methods *(10–12)*. Total carbohydrates were determined according to the Puls method *(13)*.

Sugar concentration was measured by high-performance liquid chromatography (HPLC) using a Hewlett-Packard 1050 equipped with a refractive index detector. The HPLC analysis was performed using an AMINEX HPX-87P carbohydrate analysis column operating at 85°C with deionized water mobile phase at a flow rate of 0.6 mL/min.

Results

Table 1 gives the composition of the raw wood. The raw materials milled to different particle sizes (2–5, 5–8, and 8–12 mm) were subjected to different steam-explosion treatments (190 and 210°C, and 4- and 8-min residence time). The goal was to determine the effect of particle size on steam-explosion pretreatment based on maximizing the cellulose recovery and conversion, and to optimize the recovery of the more labile hemicellulose.

Table 2 gives the composition of water-insoluble fiber of *P. pinaster* biomass from various pretreatment conditions for each particle size. For 2- to 5- and 5- to 8-mm particle sizes, steam explosion causes extensive

Table 1
Composition of Raw Material

Composition	Dry matter (%)
Extractives	14.2
Ash	0.9
Lignin	36.2
Glucose	32.1
Xylose	5.9
Galactose	2.9
Arabinose	2.0
Mannose	5.6

hemicellulose hydrolysis for all conditions tested. Experiments performed with large chips (8–12 mm) at mild pretreatment conditions (190°C at 4 and 8 min) showed that a significant amount of hemicellulosic sugar remained unhydrolyzed in the water-insoluble fiber. When pretreatment temperature was increased to 210°C, almost all the hemicellulose was extracted from the larger particle size. As expected, a more severe pretreatment condition resulted in higher cellulose (expressed as potential glucose) degradation. The cellulose content was higher at larger particle sizes for all conditions tested. The maximum glucose content in water-insoluble fiber (46.4%) was obtained at 210°C, 4 min, and 8- to 12-mm particle size.

Table 3 shows the sugar composition of the filtrate after pretreatment. There was increased hemicellulose degradation at higher temperature and residence time. Maximum degradation was achieved at 210°C and 8 min. However, at these conditions, less hemicellulose degradation in the filtrate was observed with larger particle sizes.

Much of the technoeconomic analysis of biomass pretreatment has emphasized the importance of three main parameters to optimize the process and ensure maximum substrate utilization: (1) recovery of cellulose in the water-insoluble fiber, (2) susceptibility of cellulose to enzymatic hydrolysis, and (3) recovery of hemicellulosic sugars in the filtrate. Figure 1 shows the cellulose recovery yield in the water-insoluble fiber for all pretreatment conditions and particle sizes tested. Yield is expressed as potential glucose in the water-insoluble fiber divided by the potential glucose in the raw material. Figure 1 shows that at 190°C, 4 and 8 min, 25% of hexoses were either destroyed or solubilized in the experiments with the small particle size used in the presented work; however, only 5% of cellulose was solubilized at higher particle sizes. The experiments at 210°C show substantial solubilization of the cellulose for 2- to 5- and 5- to 8-mm particle sizes. The cellulose recovery for these conditions increased to 80% in the larger chip size.

Figure 2 shows the enzymatic hydrolysis yields of the water-insoluble fiber, after different pretreatment conditions (expressed as a percentage of the glucose produced in the hydrolysis divided by the potential glucose)

Table 2
Sugar Composition of Water-Insoluble Fiber at Different Pretreatment Conditions and Particle Sizes

Condition								
Temperature (°C)	Time (min)	Size (mm)	Glucose (%)	Xylose (%)	Galactose (%)	Arabinose (%)	Mannose (%)	Total gravimetric recovery (%)
190	4	2–5	39.3	1.4	0.7	0	1.3	61.0
		5–8	45.1	2.0	0.7	0	1.9	67.6
		8–12	46.2	5.0	1.0	0.3	4.5	66.2
	8	2–5	40.4	1.5	0.4	0	1.3	60.7
		5–8	42.3	1.4	0.5	0	1.4	61.6
		8–12	45.3	3.8	1.2	0.2	3.5	67.0
210	4	2-5	36.1	0.9	0.5	0	1.4	53.5
		5–8	43.1	0.9	0.4	0	1.4	50.3
		8–12	46.4	0.8	0.4	0.4	0.3	55.5
	8	2–5	38.3	0.9	0	0	0.7	47.0
		5–8	43.1	0.6	0	0	0.9	45.6
		8–12	44.3	0.4	0	0	0.4	57.0

Table 3
Sugar Composition of Filtrate at Different Pretreatment Conditions and Particle Sizes

Condition							
Temperature (ºC)	Time (min)	Size (mm)	Glucose (g/100 g)	Xylose (g/100 g)	Galactose (g/100 g)	Arabinose (g/100 g)	Mannose (g/100 g)
190	4	2–5	3.0	3.2	2.9	1.1	4.5
		5–8	2.7	3.4	2.7	0.9	6.0
		8–12	1.3	2.8	1.8	1.0	3.4
	8	2–5	2.3	3.0	3.1	1.1	3.6
		5–8	2.8	3.8	2.8	1.0	5.7
		8–12	1.6	3.4	2.0	1.1	3.9
210	4	2–5	2.8	2.0	1.8	0.6	3.5
		5–8	2.7	2.1	1.7	0.6	3.7
		8–12	2.6	2.2	1.9	0.6	5.1
	8	2–5	1.3	0.8	0.7	0.3	1.1
		5–8	2.8	2.1	1.7	0.6	3.6
		8–12	3.0	2.0	1.8	0.7	4.6

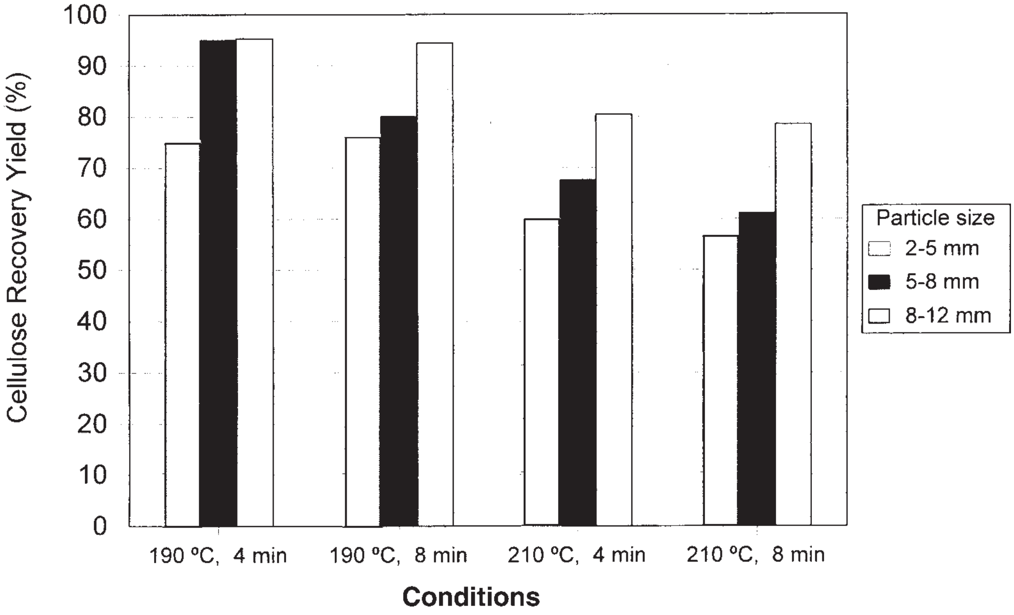

Fig. 1. Cellulose recovery yield in the water-insoluble fiber. Yield is expressed as glucose in the water-insoluble fiber (referred to original raw material) divided by the potential glucose in the raw material. Particle size: ▨, 2–5 mm; ■, 5–8 mm; □, 8–12 mm.

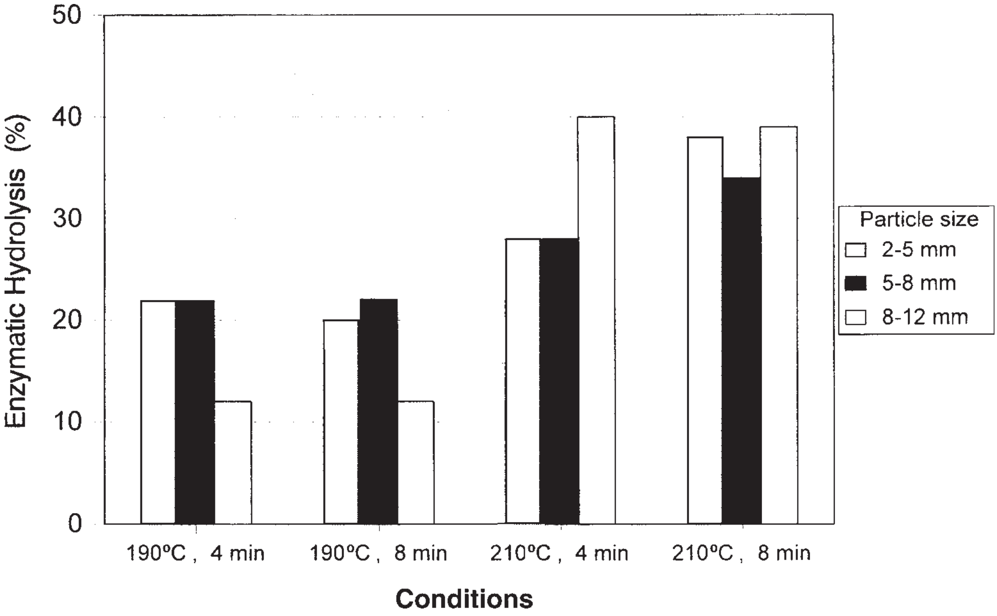

Fig. 2. Enzymatic hydrolysis yield of the water-insoluble fiber for different pretreatment conditions and particle sizes. The results are expressed as glucose obtained in the enzymatic hydrolysis divided by potential glucose in the water-insoluble fiber. Particle size: ▨, 2–5 mm; ■, 5–8 mm; □, 8–12 mm.

for each particle size. Low hydrolysis yields were obtained at 190°C and 4- and 8-min pretreatment conditions. The average enzymatic hydrolysis yields for these conditions and 2- to 5- and 5- to 8-mm particle sizes were about 22%, decreasing to 12% at larger particle sizes. Higher glucose yields in the enzymatic hydrolysis were obtained for experiments at 210°C and 8 min for all particle sizes tested. The maximum hydrolysis yield, (about 40%) was obtained with a larger particle size, under these conditions.

To improve the enzymatic digestibility of softwood used in this work (*P. pinaster*), the steam-exploded biomass was extracted with sodium chlorite to remove lignin. Table 4 gives the lignin content in the original and delignified water-insoluble fiber. Sodium chlorite is an effective delignification agent; it solubilized >70% of the lignin in the pretreated wood.

Figures 3 and 4 compare the enzymatic hydrolysis with and without delignification of the steam-exploded fibers at 190 and 210°C. As expected, lignin removal significantly improved the subsequent enzymatic hydrolysis of cellulose for all conditions tested. There were no significant differences in enzymatic digestibilities with particle size. Delignification of steam-exploded biomass under different conditions produced fibers with different digestibilities. At 190°C and 4 min, enzymatic hydrolysis yields of 87, 91, and 100% were obtained for particle sizes of 2–5, 5–8, and 8–12 mm, respectively (Fig. 4). At 190°C and 8 min, digestion yields of 100% were obtained for larger particle sizes. However, lower enzymatic digestibility of the cellulose was observed at 210°C. Enzymatic yields below 80% were obtained at this temperature for all variables (time and chip size) tested.

Figure 5 shows the hemicellulose sugars (xylose + arabinose + mannose + galactose) in the filtrate after pretreatment. The recovery yield is expressed as sugars in the filtrate divided by the potential sugars in the raw material. The recovery of hemicellulose sugars depends on the severity of the pretreatment; the more severe conditions result in more hemicellulosic sugar degradation. However, this degradation is significantly different depending on particle size. At 4 min, 210°C, and for 2- to 5- or 5- to 8-mm chip size, 50% of hemicellulose sugars was recovered. At a particle size of between 8 and 12 mm, 60% of the hemicellulosic sugars was recovered in the filtrate. The most severe condition tested in this work (210°C, 8 min) produced hemicellulose recoveries below 20% with smaller chips, and 49 and 56% with particle sizes of 5–8 and 8–12 mm, respectively.

Most conventional yeasts cannot ferment xylose and arabinose; thus, only hexoses were used to evaluate the fermentable sugars. Figure 6 shows the total yield of fermentable hexoses (glucose, mannose, and galactose) after pretreatment plus enzymatic hydrolysis. The highest yield of fermentable hexoses (20 g/100 g) was obtained for the larger chip size at 210°C and 4 min, corresponding to 50% of the theoretical yield.

Table 4
Glucose and Lignin Composition of Original and Delignified Steam-Exploded Water-Insoluble Fiber for Different Pretreatment Conditions and Particle Sizes

Condition			Original water-insoluble fiber		Delignified water-insoluble fiber	
Temperature (°C)	Time (min)	Size (mm)	Glucose (%)	Lignin (%)	Glucose (%)	Lignin (%)
190	4	2–5	39.3	55.1	78.2	16.0
		5–8	45.1	47.3	77.2	13.4
		8–12	46.2	39.6	74.4	10.1
	8	2–5	40.4	54.3	70.2	19.4
		5–8	42.3	50.6	70.1	23.1
		8–12	45.3	41.4	75.2	10.2
210	4	2-5	36.1	59.7	74.1	21.9
		5–8	43.1	53.7	81.7	14.3
		8–12	46.4	49.9	70.6	25.0
	8	2–5	38.3	58.2	65.7	29.4
		5–8	43.1	54.1	75.3	17.3
		8–12	44.3	51.3	76.5	16.2

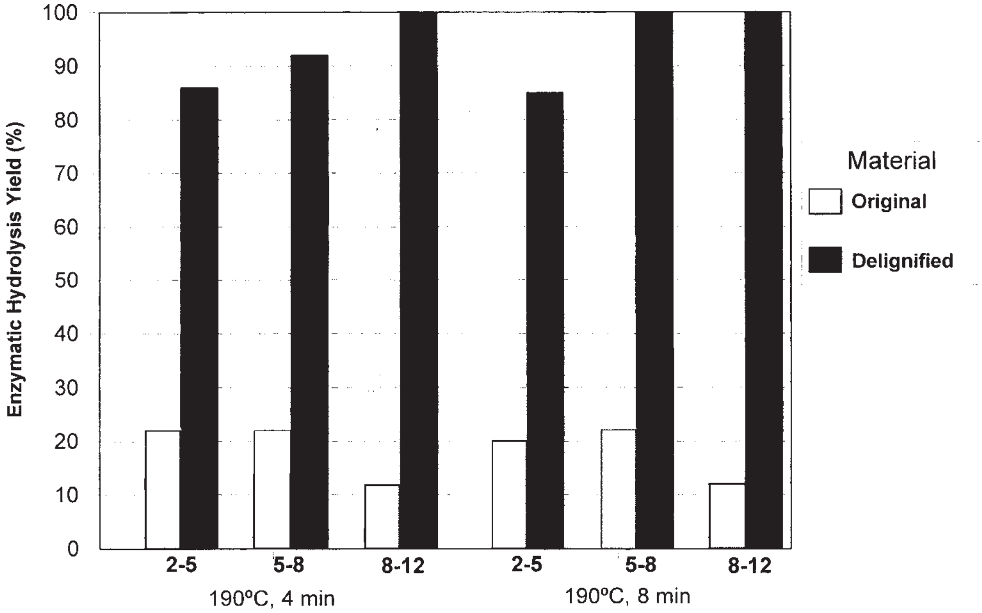

Fig. 3. Enzymatic hydrolysis yield of (▨) original and (■) delignified steam-exploded water-insoluble fiber at 190°C for different particle sizes. The results are expressed as glucose obtained in the enzymatic hydrolysis divided by potential glucose in the water-insoluble fiber.

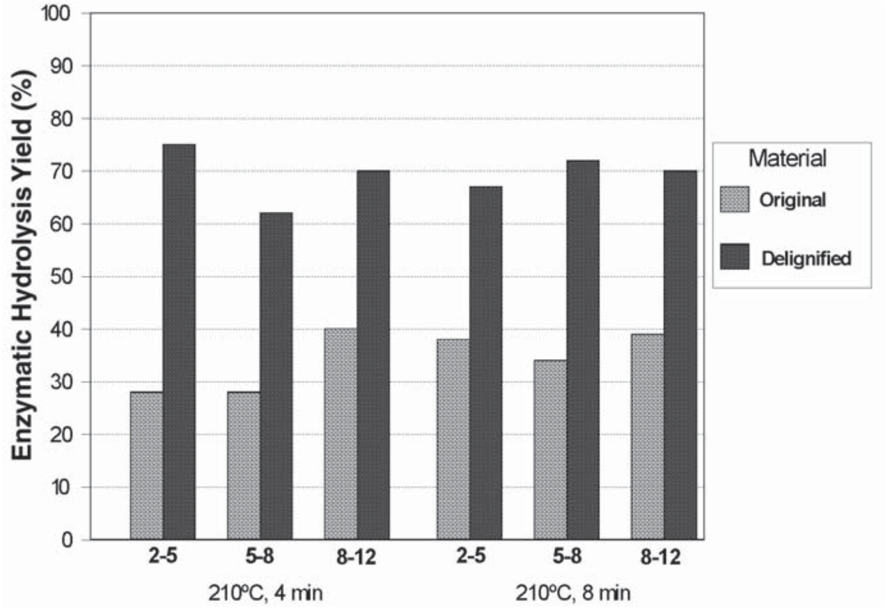

Fig. 4. Enzymatic hydrolysis yield of (▨) original and (■) delignified steam-exploded water-insoluble fiber at 210°C for different particle sizes. The results are expressed as glucose obtained in the enzymatic hydrolysis divided by potential glucose in the water-insoluble fiber.

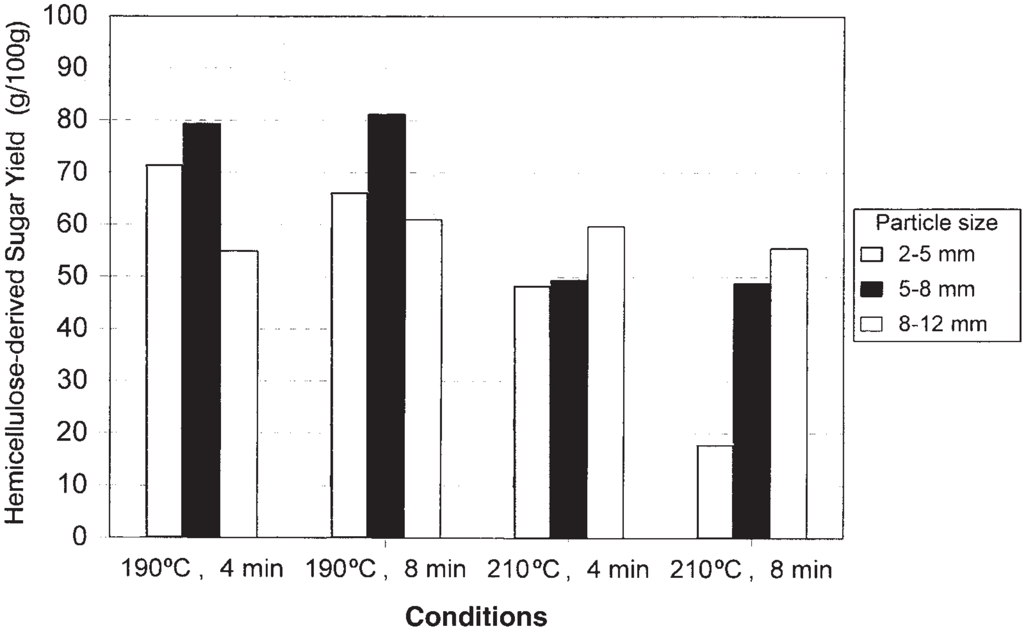

Fig. 5. Hemicellulose-derived sugar yield in the filtrate. Yield is expressed as grams of hemicellulose-derived sugars in the filtrate divided by 100 grams of hemicellulose potential sugars in the raw material. Particle size: ▨, 2–5 mm; ■, 5–8 mm; □, 8–12 mm.

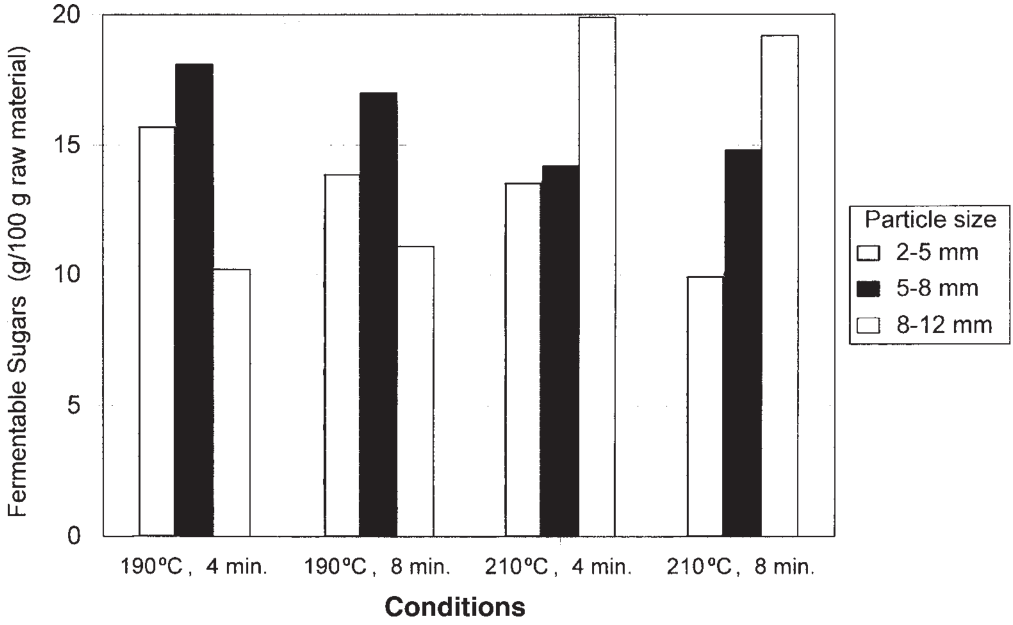

Fig. 6. Fermentable hexoses (glucose, galactose, and mannose in grams) after pretreatment plus enzymatic hydrolysis for different pretreatment conditions and particle sizes. Particle size: ▨, 2–5 mm; ■, 5–8 mm; □, 8–12 mm.

Discussion

Before efficient enzymatic hydrolysis of woody materials can occur, the substrate must first be pretreated so that the recalcitrant lignocellulosic matrix is amenable to conversion. Reduction of the wood to a size compatible with subsequent processing is the first step. Size reduction adds practically no value to the raw materials, has high energy requirements, and can significantly contribute to the total process cost. Accordingly, considerable attention should be given to select a suitable chip size, resulting from a compromise between energy consumption and good pretreatment performance *(14)*.

Softwood species require more severe steam-explosion pretreatment conditions with longer residence times or an acid catalyst *(15–18)*. Softwood hemicellulose-derived sugars are primarily mannose, glucose, and galactose with minor amounts of xylose and arabinose. These hemicellulose-derived hexoses could be combined with the glucose stream coming from the cellulose recovery, increasing the amount of sugar for fermentation. Consequently, softwood pretreatment optimization should be focused on the recovery of hemicellulose-derived hexoses, as well as on maximum cellulose recovery and enzymatic hydrolysis.

In this work the effect of the particle size on the steam-explosion pretreatment of *P. pinaster* biomass was tested. The pretreatment process was evaluated based on the recovery of cellulose in water-insoluble fiber, the effectiveness of the enzymatic hydrolysis, and the recovery of hemicellulose-derived sugars in the filtrate.

Higher cellulose recoveries are obtained at 190°C, and at more severe conditions, cellulose solubilization increases. Pretreatment at 190°C for 4 and 8 min does not improve the subsequent rate and extent of enzymatic hydrolysis. At these conditions and at larger chip sizes, less hemicellulose solubilization and lower enzymatic hydrolysis yields were obtained. On the other hand, when the temperature was increased to 210°C, at the larger particle size (8–12 mm), higher cellulose recovery and digestibility in the water-insoluble fraction was obtained. In addition, pretreatment at 210°C and 8- to 12-mm particle size permits more complete hemicellulose solubilization and less sugar degradation in the filtrate.

Brownell et al. *(7)* observed full solubilization of the pentosan fraction in 1 min using 250°C steam; however, more than 60% of pentosan was destroyed after 2 min of pretreatment. These investigators suggest that by decreasing the size chip to <6 mm, increasing incubation time, and reducing steam temperature, heat transfer heterogeneity is reduced, which can cause overcooking (degradation) of the exterior of the chips and undercooking in the center. The results obtained in this work report the maximum of hexosan yield (20 g/100 g of raw material) at 210°C, 4 min, and 8- to 12-mm particle size, corresponding to 50% of the theoretical yield. The effect of slowly heating the center of large chips—as suggested by Brownell et al. *(7)*, which results in uneven cooking of the particle—was not observed in our experiments at these pretreatment conditions and for the larger particle sizes tested.

As expected, enzymatic hydrolysis was improved by using sodium chlorite to extract lignin from the pretreated material. Figures 4 and 5 show that, although enzymatic glucose yields increased with the steam treatment severity in unextracted fibers, higher enzymatic hydrolysis yields in the delignified fibers were obtained at 190°C. Heitz et al. *(19)* investigated steam explosion of aspen wood (*Populus tremuloides*) in a Stake reactor. The treated material was delignified with NaOH. The amount of lignin recovered in the solution strongly depended on steam-treatment conditions, ranging from 5 to 90% as treatment severity increased. In these experiments, although enzymatic glucose yields increased with steam-treatment severity, delignification may, or may not, have increased enzymatic yields. Some investigators *(20,21)* have described extensive redistribution of lignin during steam-explosion pretreatment. They suggest that lignin melts as a result of steaming and subsequently forms globules, thus exposing cellulose microfibrils to enzymatic attack. Hence, the lower digestibility obtained after chlorite washing of steam-exploded biomass at 210°C could be attributed to the different redistribution of lignin during steam-explosion treatment under different conditions. This suggests that chlorite treatment, at the higher temperature conditions, deposited the unextracted lignin into accessible pores, possibly covering the surfaces of cellulose microfibrils and reducing digestibility.

From the results obtained with *P. pinaster* in this study, we can draw the following conclusions:

1. Higher cellulose recoveries at larger particle size (8–12 mm) for all pretreatment conditions tested have been obtained.
2. Pretreatment at low temperature (190°C) and residence times of 4 and 8 min resulted in low enzymatic hydrolysis yields (about 20%). At higher temperature (210°C), increased digestibilities (40%) were obtained.
3. Delignification with sodium chloride increased the enzymatic digestibility for all pretreatment conditions tested. Unexpectedly, higher enzymatic hydrolysis yields (85–100%) were obtained at a lower temperature of 190°C.
4. In general, more drastic pretreatment conditions destroyed larger amounts of hemicellulose sugars in the filtrate. This was not observed with larger particle sizes (8–12 mm), in which no significant differences in the hemicellulose recovery at different pretreatment conditions were obtained.
5. Larger steam-exploded chips (8–12 mm) produce higher cellulose and hemicellulose recoveries, as well as enzymatic digestibilities. Thus, the utilization of very small chips in steam explosion would not be desirable to optimize the effectiveness of the process and improve economy.

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