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Hydrothermal Pretreatment Conditions to Enhance Ethanol Production from Poplar Biomass

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Abstract

Pretreatment has been recognized as a key step in enzyme-based conversion processes of lignocellulose biomass to ethanol. The aim of this study is to evaluate two hydrothermal pretreatments (steam explosion and liquid hot water) to enhance ethanol production from poplar (*Populus nigra*) biomass by a simultaneous saccharification and fermentation (SSF) process. The composition of liquid and solid fractions obtained after pretreatment, enzymatic digestibility, and ethanol production of poplar biomass pretreated at different experimental conditions was analyzed. The best results were obtained in steam explosion pretreatment at 210°C and 4 min, taking into account cellulose recovery above 95%, enzymatic hydrolysis yield of about 60%, SSF yield of 60% of theoretical, and 41% xylose recovery in the liquid fraction. Large particles can be used for poplar biomass in both pretreatments, since no significant effect of particle size on enzymatic hydrolysis and SSF was obtained.

Index Entries: Poplar; ethanol; pretreatment; steam explosion; liquid hot water.

Introduction

Lignocellulose biomass represents a vast resource that could be transformed into fuel ethanol and can be produced from crops specifically grown to yield biomass for energy purposes. Among the fast growing species cultivated in short cycles for biomass production, *Populus nigra*, used in the present work as feedstock, is considered a promising energy crop for central and south Europe owing to its high yield and drought-resistant features *(1)*.

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The structure of lignocellulosic biomass is complex and consists of three main fractions (hemicellulose, cellulose, and lignin) that must be separately processed to achieve efficient conversion of carbohydrates to ethanol.

Among the different existing technologies for ethanol production, enzyme-based cellulosic biomass conversion processes offer the potential to achieve competitive prices in the longer term *(2)*. The relevance of pretreatment in such processes has been recognized for a long time *(3)*. The purpose of the pretreatment is to increase reactivity and accessibility of cellulose to enzymatic hydrolysis by removing lignin and hemicellulose and increasing the porosity of the materials. Pretreatment must meet the following requirements: improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis, avoid the degradation or loss of carbohydrates, avoid the formation of inhibitory byproducts to the subsequent hydrolysis and fermentation processes, and be cost-effective *(2)*.

Hydrothermal pretreatment refers to the use of water as liquid or vapor or both and heat to pretreat biomass. Autohydrolysis steam explosion has been proved to be an adequate method for pretreating lignocellulosic biomass, which efficiently increases enzymatic hydrolysis of hardwood and agricultural residues; however, it is less effective for softwoods. The process causes hemicellulose degradation and lignin transformation owing to high temperatures, thus increasing the potential of cellulose hydrolysis *(4)*. However, it is often found to generate inhibiting hydrolysates *(5,6)*. The factors that affect steam explosion pretreatment are residence time, temperature, chip size, and moisture content *(7)*. Optimal hemicellulose solubilization and hydrolysis can be achieved by either high temperature and short residence time (270°C, 1 min) or lower temperature and longer residence time (190°C, 10 min) *(7)*. Recent studies indicate that low temperature and long residence time are more favorable *(8)*.

Liquid hot water pretreatment, in which biomass is exposed to pressurized hot water appears to have the potential to generate reactive fiber *(9–11)*, recover most of the pentosans *(12)*, and produce hydrolysate that results in little or no inhibition of solubilized sugar fermentation *(13,14)*.

When using hydrothermal pretreatments to fractionate hardwood and agricultural byproducts, acetic acid is formed by the hydrolysis of acetyl groups in hemicellulose. High temperature stimulates the breakdown of the released sugars during pretreatment, generating decomposition products, such as furan derivatives: furfural is formed from pentose sugars and 5-hydroxymethylfurfural (HMF) from hexose sugars. The furan derivatives can, in turn, be degraded even further. HMF can be further broken down to form equimolecular amounts of levulinic and formic acids *(15)*, while furfural can be degraded to formic acid. The furan derivatives and soluble phenolic compounds from lignin will react further to form some polymeric material. The formation of furfural and HMF is undesirable because they represent a loss of fermentable sugars and may also inhibit fermentation.

In the present study different pretreatment conditions (particle size, time, and temperature) of steam explosion and liquid hot water were assayed to enhance ethanol production from poplar biomass. Pretreatment effectiveness was evaluated in terms of hemicellulose-derived sugar recovery in the liquid fraction, cellulose recovery in the solid fraction, enzymatic hydrolysis yield, and simultaneous saccharification and fermentation (SSF) yield of cellulose to ethanol.

Materials and Methods

Feed Materials

Chipped biomass (5% moisture) from *P. nigra* was supplied by The Renewable Energies Development Centre at Lubia (Soria, Spain). On average, poplar biomass contained (dry wt basis) 43.5% glucose, 17.4% xylose, 2.5% galactose, 1.7% arabinose, 2.8% mannose, 26.2% acid-insoluble lignin, 1.8% ash, and 4.1% others. The chips were milled using a laboratory hammer mill (Restsch). Milled material was further separated into two different fractions (2–5 and 12–15 mm) using a portable sieve shaker. Both fractions were submitted to hydrothermal pretreatment assays.

Pretreatment

Steam explosion pretreatment was performed by applying Masonite technology in a small batch plant described in a previous work *(16,17)*, 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 feed stock 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 filtered for solid recovery. Poplar biomass was treated at 190 and 210°C and 4- and 8-min reaction time.

Liquid hot water pretreatment was performed in a laboratory-scale stirred autoclave (model EZE-Seal; Autoclave Engineers, Erie, PA). The stainless-steel Hastelloy-C reactor has a total volume of 500 mL, with an electric heater and magnetic agitation. Cooling water was circulated through a serpentine coil to cool the reactor content at the end of each run. The amount of dry feedstock loaded corresponds to 40 g, and water was added at 1/10 (w/v) dry solid/liquid ratio. The working vessel was 400 mL. The reactor content was initially at room temperature. The heating rate was between 2.9 and 3.4°C/min *(18)*. Pretreatment was considered to be completed when the target temperature was reached, which took 48, 56, 60, 63, and 66 min for 180, 210, 220, 230, and 240°C, respectively. After pretreatment the heater was turned off, the reactor was removed from the heating jacket, and cooling water was charged through the serpentine coil. The content of the reactor cooled down to 130°C in about 2 min. The reactor was kept sealed, and the slurry was agitated until the reactor was cooled to about 40°C, and sampled.

After completion of pretreatment assays, the wet material was filtered and washed with water to recover the solid fraction. This solid fraction was analyzed for xylans, glucans, and acid-insoluble lignin content. The carbohydrate, furfural, HMF, acetic acid, formic acid, and levulinic acid content of the undiluted filtrate was also analyzed.

All pretreatment assays were performed in duplicate. To evaluate the effect of steam explosion parameters (temperature, time, and chip size), a 2^3 factorial design was applied. In liquid hot water pretreatment, temperature and chip size parameters were considered. A statistical analysis of variance was performed on data from each pretreatment using the statistical software program StatGraphics® *Plus* (Manugistics, Rockville, MD).

Microorganism and Growth Conditions

Kluyveromyces marxianus CECT 10875, a thermotolerant mutant yeast strain obtained in our laboratory *(19)*, was used in SSF experiments. Active cultures for inoculation were prepared by growing the organism on a rotary shaker at 150 rpm for 16 h at 42°C in a growth medium (initial pH = 5.5) containing: 5 g/L of yeast extract (Difco), 2 g/L of NH_4Cl, 1 g/L of KH_2PO_4, 0.3 g/L of $MgSO_4 \cdot 7\ H_2O$, and 30 g/L of glucose.

Enzymatic Hydrolysis and SSF

The washed solid fractions after both pretreatments were used as substrates for enzymatic hydrolysis and SSF experiments. Enzymatic hydrolysis was performed in 100-mL Erlenmeyer flasks, each containing 25 mL of 0.1 *M* sodium acetate buffer (pH 4.8) at 10 % (w/v) dry pretreated substrate loading at 50°C for 72 h. Enzyme loading of 15 filter paper units/g of dry pretreated substrate of Celluclast 1.5L and 12.6 IU/g of dry pretreated substrate of β-glucosidase Novozyme 188 was employed. Enzymes were a gift from Novo Nordisk (Bagsvaerd, Denmark).

SSF experiments were carried out in 100-mL Erlenmeyer flasks, each containing 25 mL of fermentation medium (growth medium described earlier), which were agitated at 150 rpm. Glucose was substituted by 10% (w/v) dry pretreated substrate concentration. The cellulolytic complex was also added as in enzymatic hydrolysis assays. In the SSF experiments, flasks were inoculated with 4% (v/v) yeast cultures (corresponding to a cell addition of 0.25 g/L), and assays were conducted at 42°C for 72 h. The pH was not controlled during enzymatic hydrolysis and SSF experiments.

Analytical Methods

The carbohydrate content of the liquid fraction after both pretreatments was determined by performing a mild acid hydrolysis (3%[v/v] H_2SO_4,120°C, and 30 min) and measuring xylose, arabinose, galactose and mannose concentration by high-performance liquid chromatography (HPLC) in a 1081B Hewlett Packard (HP) liquid chromatograph with refractive index detector. The following HPLC conditions were used: an

Aminex HPX-87P column (300 × 7.6 mm) (Bio-Rad, Hercules, CA), a temperature of 85°C, and water as eluent at 0.6 mL/min. HPLC was also used to analyze the liquid fraction for acetic, formic, and levulinic acid. Furfural and HMF content was analyzed by HPLC in an 1100 HP liquid chromatograph, equipped with a 1040A diode-array detector. The separation was performed with a Bio-Rad Aminex HPX-87H stainless steel (300 × 7.6 mm) column. The gradient elution was 82% H_2SO_4 (5 m*M*) and 18% acetonitrile. Column temperature was 55°C and flow rate was 0.3 mL/min.

Ethanol was measured by gas chromatography, using an HP 5890 Series II apparatus with a flame ionization detector and a column of Carbowax 20 M (2 m × 1.125 in.) at 85°C. Injector and detector temperature was 150°C.

National Renewable Energy Laboratory standard methods *(20,21)* were used to determine cellulose, xylans, and acid-insoluble lignin content in both raw and pretreated solid material.

Results

Steam Explosion and Liquid Hot Water Pretreatments

Poplar biomass milled to different particle sizes (2–5 and 12–15 mm) was subjected to two hydrothermal pretreatments: liquid hot water at 180, 210, 220, 230, and 240°C; and steam explosion at 190 and 210°C and 4- and 8-min residence time. The goal of our study was to evaluate pretreatment conditions (particle size, time, and temperature) on hemicellulose recovery in the liquid fraction, cellulose recovery in the solid fraction, cellulose susceptibility to enzymatic hydrolysis, and SSF yield of cellulose to ethanol.

The chemical compositions of solid and liquid fractions after liquid hot water and steam explosion pretreatments are given in Tables 1 and 2, respectively. Data represent the averages of duplicate pretreatments.

Solid recovery (expressed as solids remaining after pretreatment divided by 100 g of dry raw material) was affected by pretreatment conditions. Solid recoveries ranged from 88.7 to 60%, and 66 to 59% for liquid hot water and steam explosion pretreatments, respectively. For liquid hot water, higher solubilization was obtained at harsher conditions, and no significant dependence of chip size over the range studied was observed. A similar pattern of solid recovery decreasing at increasing steaming temperatures was found for steam explosion treatment, again showing little effect of particle sizes.

Cellulose contents (g/100 g of pretreated material) in the washed solid fractions for both pretreatments and all conditions assayed increased in relation to untreated material (39.5% cellulose). After pretreatment, cellulose content ranged from 41 to 58% and from 56 to 59% for liquid hot water and steam explosion pretreatment, respectively. The maximum cellulose content in solid fraction for LWH pretreatment (57.5%) was obtained at 220°C and large particle size (12–15mm).

Table 1
Chemical Composition of Solid and Liquid Fraction
After Liquid Hot Water Poplar Pretreatment

	180°C		210°C		220°C		230°C		240°C	
Particle sizes (mm)	2–5	12–15	2–5	12–15	2–5	12–15	2–5	12–15	2–5	12-15
Solid recovery (g/100 g dry wt) (%)	88.7	88.2	69.5	70.7	64.5	64.5	64.5	63.4	60.1	60.0
Solid fraction (%, [w/w])										
Ash	1.2	0.9	1.0	1.1	0.8	1.1	1.3	0.9	1.6	0.8
Cellulose	42.2	40.7	52.7	51.8	54.0	57.5	53.3	55.9	52.1	57.4
Hemicellulose	17.8	14.7	7.6	7.2	3.5	3.2	0.8	1.0	0.3	0.6
Lignin	25.1	28.4	34.3	30.5	37.3	32.1	38.8	36.6	40.8	36.4
Liquid fraction (g/100 g poplar biomass)										
Glucose	1.2	1.6	1.9	2.1	1.4	1.9	1.0	1.1	0.8	0.8
Xylose	1.6	1.6	10.8	8.1	3.5	3.1	0.3	0.3	0.4	0.1
Galactose	0.6	0.6	1.0	0.8	0.4	0.3	0.1	0.1	0.1	0.1
Arabinose	0.8	0.7	0.5	0.2	0.2	0.1	0.1	0.1	0.1	0.1
Mannose	0.6	0.5	1.8	1.8	1.4	1.4	0.3	0.4	0.1	0.2
Acetic acid	0.21	0.31	1.53	1.90	3.33	3.48	3.86	4.15	4.40	4.30
Formic acid	0.13	0.15	0.36	0.34	0.40	0.46	0.50	0.55	0.59	0.61
Levulinic acid	0.00	0.01	0.02	0.01	0.03	0.04	0.03	0.05	0.03	0.06
2-Furfural	0.01	0.03	0.46	0.73	1.86	2.24	1.68	2.38	2.44	2.52
5-HMF	0.01	0.01	0.08	0.17	0.37	0.53	0.50	0.93	1.16	1.29
pH	4.2	4.2	3.9	3.7	3.5	3.5	3.4	3.4	3.5	3.5

Table 2
Chemical Composition of Solid and Liquid Fraction
After Steam Explosion Poplar Pretreatment

	190°C				210°C			
	4 min		8 min		4 min		8 min	
Particle sizes (mm)	2–5	12–15	2–5	12–15	2–5	12–15	2–5	12–15
Solid recovery (g/100 g dry wt) (%)	66	66	65	64	60	62	59	61
Solid fraction (%, [w/w])								
Ash	0.9	0.8	0.9	0.9	1.2	1.1	1.1	1.1
Cellulose	57.2	57.5	57.2	59.3	57.7	58.6	55.9	59.3
Hemicellulose	8.2	8.5	7.9	8.2	5.2	4.4	4.3	3.8
Lignin	33.1	30.2	31.7	31.8	35.3	35.5	38.0	36.3
Liquid fraction (g/100 g poplar biomass)								
Glucose	1.5	1.6	1.4	1.7	1.4	1.8	1.5	1.9
Xylose	8.6	6.7	7.7	7.3	7.1	6.4	5.7	4.8
Galactose	1.1	0.9	1.0	1.0	0.8	0.7	0.8	0.7
Arabinose	0.8	0.5	0.6	0.6	0.5	0.5	0.5	0.4
Mannose	1.2	1.0	1.1	1.1	1.1	1.2	1.1	1.1
Acetic acid	1.05	0.92	1.21	1.32	2.03	2.24	2.49	2.89
Formic acid	0.35	0.33	0.25	0.35	0.43	0.46	0.43	0.53
2-Furfural	0.16	0.15	0.27	0.23	0.49	0.62	0.79	0.94
5-HMF	0.03	0.03	0.04	0.05	0.08	0.12	0.12	0.22
pH	4.1	4.0	4.0	4.0	3.9	3.8	3.8	3.7

Hemicellulose is clearly the major constituent extracted during pretreatments. In liquid hot water, at temperatures above 220°C, hemicellulosic polymer was practically dissolved from the solid (<1% hemicellulose remaining in pretreated material). Experiments performed with steam explosion at mild pretreatment conditions (190°C temperature, 4- and 8-min residence time) showed that a significant amount of hemicellulose (>8%) remained unhydrolyzed in the solid fraction. When pretreatment temperature was increased to 210°C, higher hemicellulose solubilization was obtained.

The liquid fraction of liquid hot water and steam explosion pretreated poplar biomass consisted of a mixture of hydrolyzed sugars and degradation products (e.g., carboxylic acids and furans). In liquid hot water, total hydrolyzed monomeric sugars ranged from 1.3 to 16.0 g/100 g of poplar biomass, carboxylic acids from 0.34 to 5.02 g/100 g of poplar biomass, and furans from 0.02 to 3.81 g/100 g of poplar biomass, depending on the pretreatment conditions (Table 1). The formation of carboxylic acids was detected in the liquid fraction for all pretreatment conditions. Maximum xylose content in the liquid fraction was obtained at 210°C and small particle size (10.8 g/100 g of poplar biomass), corresponding to 62% of the xylose content in the raw material. At these conditions, liquid chromatography analysis indicated that >90% of the solubilized hemicellulose appeared as oligosaccharides. At higher temperatures increased degradation of hemicellulose sugars, regardless of particle size, was obtained. Pretreatment temperatures above 220°C resulted in a complete degradation of hemicellulose-derived sugars in the filtrate.

In the liquid fraction obtained after steam explosion experiments, the amount of total hydrolyzed sugars ranged from 8.9 to 13.2 g/100 g of poplar biomass, carboxylic acids from 1.25 to 3.42 g/100 g of poplar biomass, and furans from 0.19 to 1.16 g/100 g of poplar biomass (Table 2). Levulinic acid was not detected in any steam explosion pretreatment conditions. Increased hemicellulose degradation at higher temperature and residence time was found. Particle size affected xylose content in the liquid fraction obtained after steam explosion, resulting in a slightly increased concentration at small particle size ($p < 0.05$).

With liquid hot water, pH reached a final value between 4.2 and 3.4. A strong correlation between final pretreatment temperature and final pH was not observed, although aliphatic acid content increased at higher temperatures. Thus, the constituents extracted from poplar during pretreatment apparently buffered the pH at 3.4. In experiments with steam-exploded biomass, pH values were in the range of 4.1–3.7.

To facilitate a comparison of results, Fig. 1 shows hemicellulose-derived sugars and glucose recovery in solid and liquid fractions for both pretreatments at the different conditions tested. In liquid hot water, total hemicellulose recovery, calculated as the sum of recovered hemicellulose-derived sugars in the liquid fraction and hemicellulose content in the solid fraction, ranged from 88% at 180°C to 1.5% at 240°C (Fig. 1A). Total glucose recoveries above 95% were obtained at 180, 210 and 220°C.

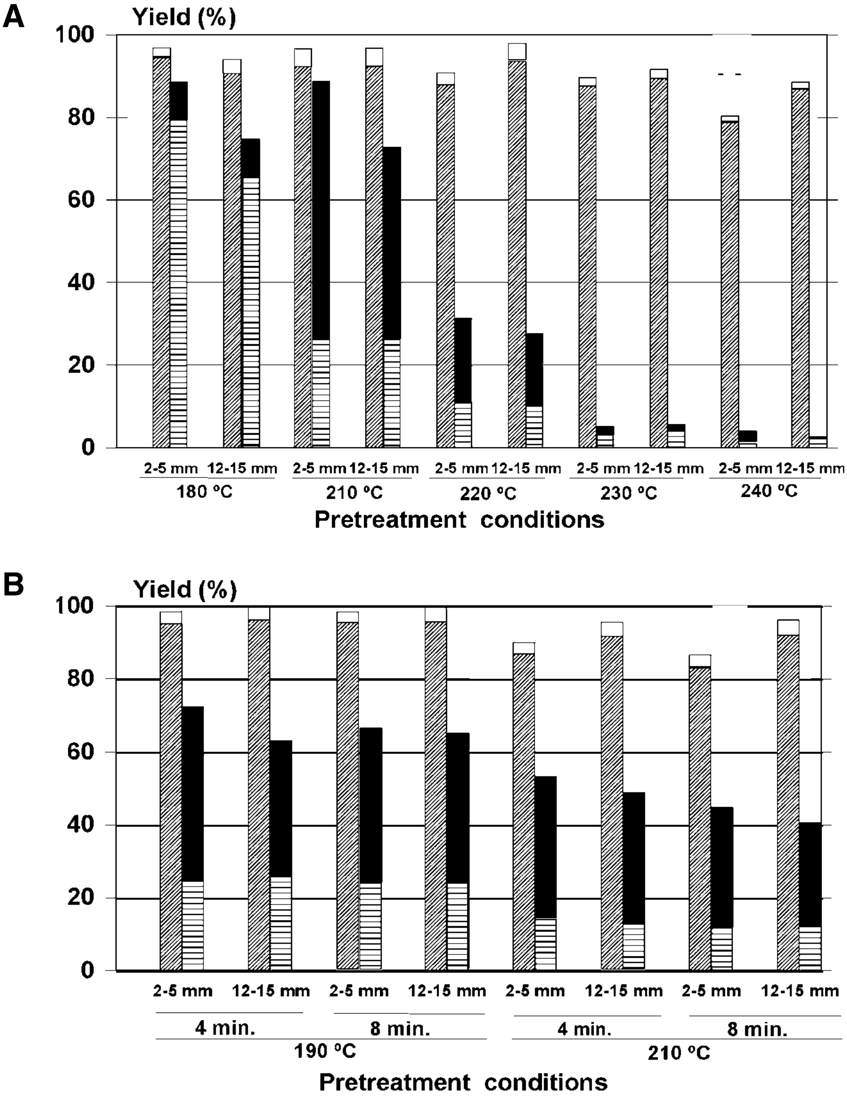

Fig. 1. Total sugar recovery yield at different pretreatment conditions. **(A)** Liquid hot water; **(B)** steam explosion. Yield is expressed as sugars in the solid or liquid fraction divided by potential sugars in the raw material. Glucose recovery in solid (▨) and liquid (☐) fractions and hemicellulose-derived sugars recovery in solid (⊟) and (■) liquid fractions.

Steam explosion pretreatment at 190°C (4 min, 2–5 mm) provided total hemicellulose recoveries close to 72%, while at 210°C (4 min, 12–15 mm) only 40% of hemicellulose was recovered (Fig. 1B). In relation to total glucose recovery, at 190°C close to 100% recovery value was obtained. At 210°C more cellulose degradation (5–15%) occurred, and this effect was smaller at large chip size. A significant interaction effect between temperature and chip size variables was found on total glucose recovery ($p < 0.05$).

Enzymatic Hydrolysis and SSF

To establish the suitability of the tested hydrothermal pretreatments to enhance the susceptibility of cellulose from poplar biomass to enzymatic attack and conversion to ethanol, enzymatic hydrolysis and SSF tests were performed.

Figure 2 illustrates enzymatic hydrolysis and SSF yields after liquid hot water and steam explosion pretreatment at different conditions. Enzymatic hydrolysis yield is expressed as a percentage of glucose produced in the hydrolysis divided by potential glucose in the pretreated material. SSF yields are presented in percentage of theoretical yield. The theoretical SSF yield is calculated by assuming that all the potential glucose in the pretreated material is available for fermentation at a fermentation yield of 0.51 g of ethanol/g of glucose.

In the case of poplar biomass subjected to liquid hot water pretreatment (Fig. 2A), enzymatic hydrolysis yield was markedly dependent on pretreatment temperature, increasing from low values of 10% at 180°C, to maximum yields of 68–70% (41–44 g of glucose/100 g of pretreated substrate) at the highest temperatures of 230–240°C. No significant differences ($p > 0.05$) in enzymatic hydrolysis yield were found at different particle sizes.

Pretreatment temperature and residence time showed significant main effects ($p < 0.05$) on enzymatic hydrolysis yield in steam-exploded materials, but no significant differences were found with particle size (Fig. 2B). Steam-exploded poplar biomass at a mild temperature of 190°C showed the lowest enzymatic hydrolysis yield (40%, equivalent to 26 g of glucose/100 g of pretreated material). Higher enzymatic hydrolysis yields of 60% (equivalent to 40 g of glucose/100 g of pretreated material) were obtained at 210°C.

SSF yields in experiments using liquid hot water pretreated materials as substrate increased as temperature rose, reaching a maximum value of 60% of theoretical yield, corresponding to a final ethanol concentration of 17 g/L at 240°C. SSF yield in steam explosion pretreated materials showed a profile similar to that of enzymatic hydrolysis yield in relation to the pretreatment parameters studied. At the lowest temperature of 190°C, values of about 40% were obtained, while at the highest temperature of 210°C, SSF yield increased up to 60%. Ethanol concentration after 72 h fermentation was about 20 g/L at the highest temperature. SSF yields obtained with pretreated biomass using a steaming temperature of 210°C reached a value similar to that obtained for liquid hot water at 240°C, about 60% of theoretical yield.

Discussion

We assessed the effectiveness of liquid hot water and steam explosion pretreatment of poplar at two different particle sizes, under several temperature and time conditions, by measuring hemicellulose and cellulose recovery, fiber susceptibility to cellulase attack, and SSF performance.

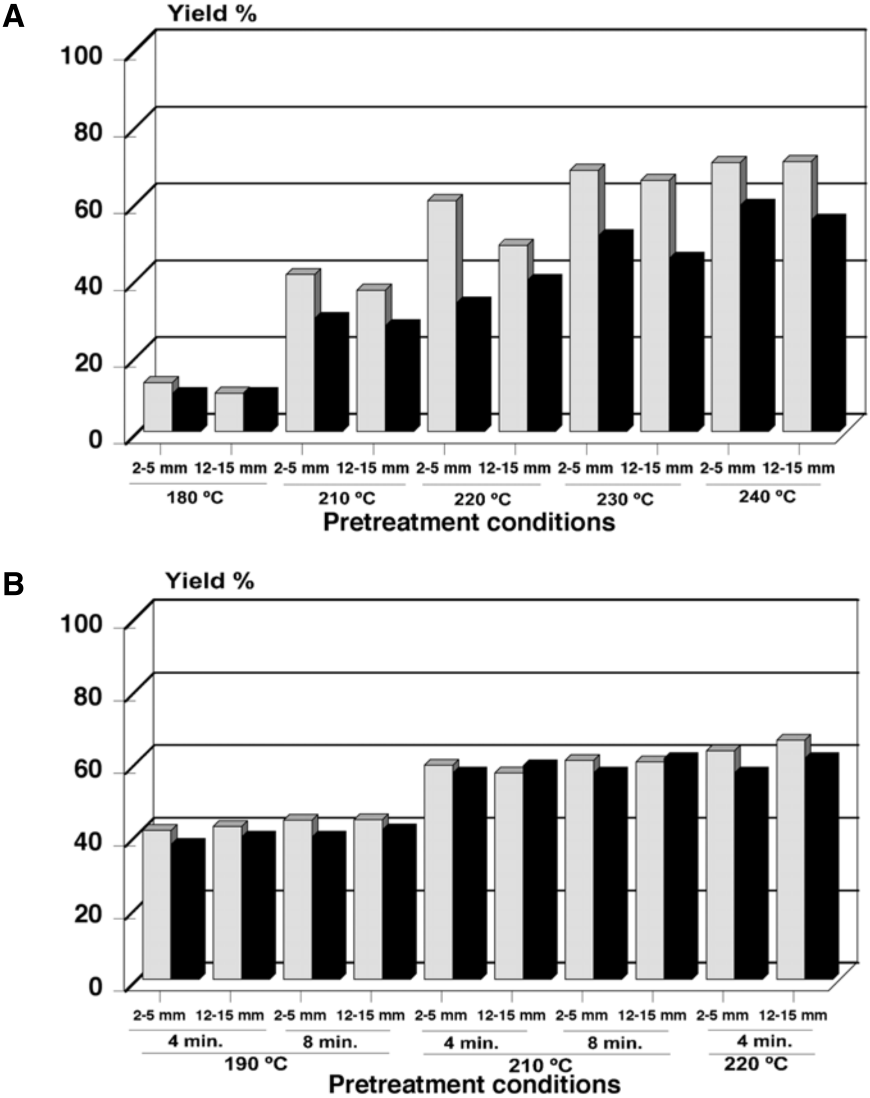

Fig. 2. Enzymatic hydrolysis yield (▨) and SSF yield (■) after **(A)** Liquid hot water and **(B)** steam explosion at different pretreatment conditions. Hydrolysis yield is expressed as glucose obtained in the enzymatic hydrolysis/potential glucose. SSF yield is expressed as percentage of theoretical yield.

In general, at the conditions tested, both hydrothermal pretreatments produced high hemicellulose solubilization from insoluble fiber. Results obtained in liquid hot water pretreatment are somewhat lower than those reported by Van Walsun et al. *(9)*. They reported extraction of 98–100% of the hemicellulose in other lignocellulosic materials, bagasse and aspen, pretreated at 220°C. However, these results were obtained in a flow-through reactor, which makes comparison difficult. The fraction of hemicellulose that is solubilized depends on the type of biomass and is associated with structure and chemical composition. Similar results of high hemicellulose solubilization were obtained by Weil et al. *(22)* when yellow poplar sawdust was pretreated by pressure cooking in water at 220–260°C.

Although hemicellulose is clearly the major constituent extracted in both liquid hot water and steam explosion pretreatments, unfortunately, good hemicellulose solubilization does not always correspond to elevated hemicellulose-derived sugar recoveries in the liquid fraction. Belkacemi et al. *(23)* proposed that hemicellulose solubilization from biomass occurred in two parallel reactions, for fast and slow solubilization. The different rate for solubilization of hemicellulose could explain the high degradation of soluble hemicellulose-derived sugars, at the same time that a considerable amount of hemicellulose still remains in the fiber.

Liquid hot water pretreatment at 180°C scarcely affected the carbohydrate composition of poplar biomass. At 210°C, a relatively high amount of hemicellulose was solubilized (76%), of which 77% was recoverable in the liquid fraction. Experiments carried out at a higher temperature of 220°C produced higher hemicellulose solubilization (90%), of which only 25% was recovered in the liquid fraction, showing high sugar degradation at this condition. If we consider that in liquid hot water reactor experiments there is only a 4-min difference in attaining 210 and 220°C, the elevated degradation occurred at 220°C can be mostly explained by the temperature effect and not to heat-up time.

Degradation products quantified in the liquid fraction can only partially explain hemicellulose losses in pretreatment. It is also possible that hemicellulose and other compounds were lost through volatilization of degradation products (e.g., furfural) and recondensation reactions *(14)*. Our finding that more material was lost in experiments run at higher liquid hot water temperature supports this hypothesis. The overall mass balance at 180°C indicates that <5% of the initial material is unaccounted for in measured products in solid or liquid fractions, while at 240°C close to 25% of the initial material is not recovered (data not shown). Reflecting the greater sugar degradation that occurs at elevated temperatures, furfural formation increased from 0.01 g/100 g of poplar at 180°C to 2.5 g/100 g of poplar at 240°C. The amounts of furfural and HMF formed are similar to those obtained by other researchers *(11)* investigating liquid hot water pretreatment of corn fiber.

According to preliminary studies (unpublished data), at 210°C (the most favorable liquid hot water conditions for hemicellulose recovery), the degradation compounds quantified (1.53 g/L of acetic acid, 0.36 g/L of formic acid, 0.46 g/L of furfural, and 0.08 g/L of HMF) in the liquid fraction (pH 3.9) do not significantly inhibit growth and fermentation of *K. marxianus*. However, the concentration of degradation compounds found in the liquid fraction at liquid hot water temperatures above 210°C (3.86 g/L of acetic acid, 0.50 g/L of formic acid, 1.68 g/L of furfural, and 0.50 g/L of HMF) inhibits *K. marxianus* growth even in synthetic media.

Severe pretreatment conditions, in which high hemicellulose solubilization is obtained, result in enhanced enzymatic digestibility. Hemicellulose solubilization and enzymatic hydrolysis yield were found to be positively correlated ($p < 0.05$) in liquid hot water pretreatment.

Weil et al. *(10)* reported 77% enzymatic hydrolysis yield using 1% (w/v) yellow poplar sawdust pretreated by liquid hot water at 240°C as substrate. In our run, at the same temperature, and using a substrate loading of 10% (w/v) liquid hot water–pretreated poplar, 70% enzymatic hydrolysis yield was obtained. Considerable degradation of hemicellulose-derived sugars was found at this condition.

SSF yield was also found to depend on pretreatment temperature for both hydrothermal treatments and was negatively correlated with hemicellulose content in the pretreated solids. The maximum SSF yield of 60% of theoretical was obtained at a liquid hot water pretreatment temperature of 240°C, a condition at which total hemicellulose-derived sugar degradation occurs. For poplar sawdust pretreated by pressure cooking at 240°C, Weil et al. *(10)* reported on SSF yield of 55% of theoretical using similar levels of enzyme loading, a 6% (w/v) solid concentration, and *Saccharomyces cerevisiae* as fermenting yeast. Our results demonstrate that *K. marxianu*s CECT 10875 is capable of attaining similar SSF yields even using a higher initial solid concentration of 10% (w/v).

At the highest temperatures tested (240 and 210°C for liquid hot water and steam explosion, respectively), higher enzymatic hydrolysis yield for LWH pretreatment (about 70%) was obtained in comparison with enzymatic hydrolysis yield in steam explosion pretreatment (60%). Thus, it was to be expected that better SSF yields in solid pretreated by liquid hot water would be found. Not only SSF yields are similar in both cases, but lower ethanol concentration was obtained in liquid hot water–pretreated solid tests. This indicates that the fermentation step and not enzymatic hydrolysis in SSF is affected in liquid hot water–pretreated samples, which is supported by the presence of residual glucose in those SSF broths when the ethanol production comes to an end.

Considering our results, the evaluation of pretreatment effectiveness should not only take into account the enhancement of cellulose hydrolysis but the overall process from cellulose to ethanol as well.

The best results were obtained when pretreating poplar biomass by steam explosion at 210°C and 4 min, taking into account cellulose recovery above 95%, enzymatic hydrolysis yield of about 60%, SSF yield of 60% of theoretical, and 41% xylose recovery in the liquid fraction.

In conclusion, our work shows that water is an effective pretreating agent that enhances enzymatic hydrolysis and SSF of cellulose to ethanol of poplar biomass, compared to untreated material. Large (12–15 mm) particle size can be used for poplar biomass substrate in both pretreatments since no significant effect of particle size on enzymatic hydrolysis and SSF was observed.

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