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Ethanol Production From Steam-Explosion Pretreated Wheat Straw

IGNACIO BALLESTEROS, Mª JOSÉ NEGRO, JOSÉ MIGUEL OLIVA,
ARACELI CABAÑAS, PALOMA MANZANARES,*
AND MERCEDES BALLESTEROS

*CIEMAT-Renewable Energies Division, Av. Complutense, 22,
28040-Madrid-Spain; E-mail: p.manzanares@ciemat.es*

Abstract

Bioconversion of cereal straw to bioethanol is becoming an attractive alternative to conventional fuel ethanol production from grains. In this work, the best operational conditions for steam-explosion pretreatment of wheat straw for ethanol production by a simultaneous saccharification and fermentation process were studied, using diluted acid [H_2SO_4 0.9 % (w/w)] and water as preimpregnation agents. Acid- or water-impregnated biomass was steam-exploded at different temperatures (160–200°C) and residence times (5, 10, and 20 min). Composition of solid and filtrate obtained after pretreatment, enzymatic digestibility and ethanol production of pretreated wheat straw at different experimental conditions was analyzed. The best pretreatment conditions to obtain high conversion yield to ethanol (approx 80% of theoretical) of cellulose-rich residue after steam-explosion were 190°C and 10 min or 200°C and 5 min, in acid-impregnated straw. However, 180°C for 10 min in acid-impregnated biomass provided the highest ethanol yield referred to raw material (140 L/t wheat straw), and sugars recovery yield in the filtrate (300 g/kg wheat straw).

Index Entries: Wheat straw; ethanol; diluted acid pretreatment; steam-explosion.

Introduction

During the past century, world energy consumption has mostly depended on the utilization of fossil fuels, which has led to harmful changes in our climate and increased the amount of greenhouse gases in the atmosphere. According to the World energy, technology and climate policy outlook report published in 2003 by the European Union *(1)*, given the continued dominance of fossil fuels, world CO_2 emissions are expected to increase more rapidly than the energy consumption (2.1%/yr on an average). In 2030, world CO_2 emissions are expected to be more than twice the level of 1990. This scenario is a clear encouragement for developing

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*Author to whom all correspondence and reprint requests should be addressed.

alternative sources that mitigate the detrimental environmental effects of fossil fuels use.

With the search for alternative renewable energy sources, bioethanol is fast becoming a viable solution, as it is a nonfossil fuel from a renewable source that may result in a cost-efficient way to reduce greenhouse gases and gasoline use in transport provided that it is produced in an efficient conversion process. Although conventional fuel ethanol is derived from grains such as corn and wheat competing as a food source for humans, the cereal industry produces vast amounts of residue that has little use today. According to Kim and Dale (2), under the 60% ground cover practice, about 354 millions of tons of wheat straw could be available globally and could produce 104 GL of bioethanol. Europe production would account for about 38% of this world bioethanol capacity. In Spain grain industry generates important amounts of wheat straw, a part of which is used as bedding straw and the remainder is burned or left on the land to fertilize the soil. Bioconversion of this residue to fuel ethanol would provide an attractive possibility to boost the development of biofuels in our country in a sustainable way.

The lignocellulosic nature of wheat straw makes the pretreatment an essential step because the physical and chemical barriers caused by the close association of main components greatly limits the suceptibility to bioprocesses such as simultaneous saccharification and fermentation (SSF). Among processes developed to pretreat lignocellulosic biomass, steam-explosion (SE) has been extensively studied and claimed as one of the most successful techniques for fractionating biomass and enhancing the accessibility of cellulose to enzymes. SE has been proved to be effective in a great variety of lignocellulosic biomass, including hardwoods (3,4), softwoods (5,6), and herbaceous residues such as corn stover (7), sugarcane bagasse (8), and wheat straw (9,10). As a way to further improve the effectiveness of SE pretreatment, the addition of an impregnation agent before pretreatment has been shown to be an effective method to increase cellulose digestibility of pretreated substrates and solubilize a significant portion of the hemicellulosic component. Preimpregnation of biomass with acid catalyst such as dilute SO_2 and H_2SO_4 has been shown to decrease both temperature and time requirements whereas achieving optima fractionation, sugar recovery, and enzymatic hydrolysis (EH) of steam-pretreated samples (11). The efficiency of dilute H_2SO_4 pretreatment in some agricultural residues as corn stover has been already demonstrated (12,13). Neverthless, few references are found about the effect of acid addition in agricultural residues as wheat straw.

In this work, the best operational conditions for SE pretreatment of wheat straw for ethanol production by a SSF process were studied, using diluted acid (H_2SO_4 0.9 % [w/w]) or water as impregnation agents before pretreatment. Acid- or water-impregnated biomass was steam-exploded at different temperatures (160–200°C) and residence times (5, 10, and 20 min). The effectiveness of SE was evaluated in terms of cellulose recovery in the water-insoluble solids (WIS) fraction, hemicellulose-derived sugar

(HS) recovery in the filtrate, and EH yield of WIS fraction. Finally, pretreated wheat straw was tested in SSF process with the thermotolerant yeast strain *Kluyveromyces marxianus* CECT 10875.

Materials and Methods

Raw Material

Wheat straw (6% moisture content) was provided by Ecocarburantes de Castilla y León (Salamanca, Spain). Biomass was coarsely crushed using a laboratory hammer mill (Retsch GmbH & Co. KG, Germany), homogenised and stored until used.

The chemical composition of raw material and WIS fraction was determined using the standard laboratory analytical procedures for biomass analysis provided by the National Renewable Energy Laboratory (Colorado) *(14)*. The chemical analysis of raw material showed the following compostion (% dry weight): cellulose, 30.2; hemicellulose, 22.3 (xylan, 18.7; arabinan, 2.8; and galactan, 0.8); acid insoluble lignin, 15.3; acid soluble lignin, 1.7; acetyl groups, 2.6; ash, 4.7; and extractives, 14.7 (total, 91.5%).

SE Pretreatment

Pretreatment assays were performed by applying Masonite technology in a 2L-SE pilot unit as described in a previous work *(3)*. Before pretreatment, wheat straw was soaked for 18 h at 45°C in 0.9% w/w diluted sulphuric acid solution or water (solid–liquid ratio: 1/10). The soaked material was vacuum filtered to approx 20% solids content and then steam-exploded. Temperature pretreatment ranged from 160°C to 200°C and time from 5 to 20 min, depending on temperature. Pretreatment experiments on wheat straw preimpregnated with water were performed at all tempertaures and selected times, except for the lowest temperature of 160°C where only acid impregnated biomass was tested. A summary of the operation conditions assayed are shown in Table 1. After pretreatment, the material was recovered in a cyclone, cooled to approx 40°C and filtered to recover two fractions: (1) the WIS fraction and (2) the filtrate or prehydrolyzate. After separating the filtrate, WIS fraction was throughly washed with water, weighted and dried at 45°C for storage. Solid recovery yield was then calculated as dry weight of WIS remaining after pretreatment referred to 100 g of raw material.

WIS fraction was analyzed for carbohydrates and acid-insoluble lignin content, and used as substrate in EH and SSF tests. Sugars, furfural, and hydroxymethylfurfural (HMF) content of the filtrate were also analyzed.

Enzymatic Hydrolysis Tests

The washed WIS fraction after pretreatment was used as substrate for EH experiments. EH tests were performed in 100-mL Erlenmeyer flasks,

Table 1
Conditions for SE Pretreatment of Wheat Straw

Temperature (°C)	Time	Impregnation agent
160	20	Catalyst[a]
170	20	H_2O
	5	Catalyst
	10	Catalyst
180	10	H_2O
	5	Catalyst
	10	Catalyst
190	10	H_2O
	5	Catalyst
	10	Catalyst
200	10	H_2O
	5	Catalyst

[a] 0.9% (w/w) H_2SO_4.

each containing 25 mL of 0.1 *M* sodium acetate buffer (pH 4.8), 10% (w/v) dry WIS loading, at 50°C for 72 h. Enzyme loading of 15 FPU/g dry WIS of Celluclast 1.5 L and 12.6 IU/g dry WIS of β-glucosidase Novozyme 188 was employed. Enzymes were a gift from Novozymes A/S (Bagsvaerd, Denmark). After EH assays completion, glucose was analyzed by HPLC as decribed earlier. Experiments were performed in duplicate. EH yield was calculated as the ratio of g glucose in the EH/100 g potential glucose in WIS. Glucose yield in the EH referred to untreated initial material was also calculated by taking into account the solid recovery yield attained in each experiment.

Microorganisms and Growth Conditions

K. marxianus CECT 10875, purchased in the Spanish collection of type cultures, was used in SSF experiments. Active cultures for inoculation were prepared by growing the organism on a rotary shaker at 150 rpm and 42°C for 16 h, in a growth medium containing (g/L): yeast extract, 5; NH_4Cl, 2; KH_2PO_4, 1; $MgSO_4 \cdot 7H_2O$, 0.3; and glucose, 30. All chemicals were from Sigma (Sigma-Aldrich Inc., St. Louis, MO).

SSF Tests

SSF experiments were carried out under no sterile conditions in 100-mL Erlenmeyer flasks, each containing 50 mL of the fermentation medium (without glucose) as described earlier, and were incubated at 150 rpm and 42°C for 72 h. No contamination was detected at the end of the experiments. The WIS fraction obtained after pretreatment was used as substrate at 10% (w/v) concentration. Enzymatic complex and loading used in SSF experiments were the same as that for EH tests. Flasks were inoculated with 0.2 g/L yeast culture and periodically analyzed for ethanol and glucose. Experiments were performed in duplicate.

SSF results are reported in percentage of the theoretical yield. The theoretical SSF yield was calculated by assuming that all the potential glucose in the WIS fraction is available for fermentation, and a fermentation yield of 0.51 g ethanol/g glucose. Results are also reported as ethanol yield based on the initial untreated material, taking into account the solid recovery yield.

Analytical Methods

The carbohydrate content of the prehydrolyzate after pretreatment was measured by performing a mild acid hydrolysis (3% [v/v] H_2SO_4, 120°C and 30 min) and measuring glucose, xylose, arabinose, galactose, and mannose concentration by HPLC in a Waters 2695 liquid chromatograph with refractive index detector. An AMINEX HPX-87P carbohydrate analysis column (Bio-Rad, Hercules, CA) operating at 85°C with deionized water as mobile-phase (0.6 mL/min) was used. Likewise, glucose concentration after completion of EH tests was measured in EH media following this method. HPLC was also used to analyze the prehydrolyzate for furfural and HMF as described previously *(3)*.

Ethanol was measured by gas chromatography, using a HP 5890 Series II apparatus equipped with an Agilent 6890 series injector, a flame ionization detector and a column of Carbowax 20 *M* at 85°C. The injector and detector temperature was maintained at 150°C.

Results and Discussion

SE Pretreatment

Results of solid recovery yield and composition of WIS fraction after SE at different process conditions are shown in Table 2. Recovery yields ranged from 42 to 60%, depending on pretreatment conditions. Higher solubilization was obtained at harsher conditions, showing great effect of residence time and acid addition on solids recovery. Regarding WIS composition, cellulose content increased in relation to untreated material (30.2%) in all conditions tested. Cellulose concentration ranged from 50% to approx 64%, depending on the pretreatment conditions. The maximum cellulose content (approx 64%) was obtained in acid-impregnated biomass at 180°C and 10 min. Higher temperatures produced somewhat lower cellulose content in pretreated materials, specially in acid-impregnated biomass. It indicates that SE at harsh conditions causes an initial breakdown of the cellulose fiber and a consequent loss of glucose in solid residue *(10)*. Acid-insoluble lignin was

Table 2
Dry Matter Recovery and Composition of Pretreated Wheat Straw (WIS Fraction) After SE at Different Process Conditions

Conditions			Solid			
Temperature (°C)	Time (min)	Impregnation agent	recovery (%)	Component (% dry WIS) Cellulose	Hemicellulose	Lignin
160	20	Acid[a]	60	50.3	15	25.4
170	10	Water	55.2	50	13.6	20.8
	5	Acid	51.8	54.1	8.6	25.5
	10	Acid	51.7	58.5	5.9	27
180	10	Water	48.6	60.2	7.5	25.9
	5	Acid	47.4	62.7	5.2	27.5
	10	Acid	46.8	63.5	1.3	32.6
190	10	Water	45.6	59.6	7.6	23.5
	5	Acid	43.4	54.8	1.8	28.3
	10	Acid	43.1	55.8	1	33.6
200	10	Water	45.1	61.9	4.5	27.9
	5	Acid	41.6	55.2	1.2	33.1
Untreated				30.2	22.3	15.3

[a] 0.9% (w/w) H_2SO_4.

considerably concentrated in comparison with untreated material (15.3%), reaching values up to 33% at the most severe conditions. Referred to raw material content, lignin losses accounted for 1–5% of initial lignin values, depending on the conditions.

As expected, results show a substantial removal of hemicelluloses during pretreatment. The most severe conditions of 180°C and 190°C for 10 min and 200°C for 5 min, in acid-impregnated biomass, led to almost complete dissolution of hemicellulose component, remaining only 1.5% in WIS fraction (<1% referred to raw material content). At lower temperature of 160°C, the hemicellulose content remaining in the solid accounted for up to 15%, corresponding to approx 50% of the content in raw material. Experiments performed in acid-impregnated biomass led to higher hemicelluloses removal in comparison with water-impregnated biomass in all temperatures tested.

Enhancing effect of acid-impregnation of biomass in solubilization of hemicelluloses during pretreatment has been previously reported in other residues as corn stover. Varga et al. (7) found that impregnation of corn stover with 0.5 and 2% (w/w) H_2SO_4 had greater effect in decreasing hemicellulose fraction in the solid residue than pretreatment temperature. SE pretreatment at 210°C, 5 min and 2% H_2SO_4 gave rise to almost complete hemicellulose removal from solid fraction. However, it is well-known that a high sugar solubilization from the solid fraction does not always correlate with good sugar recoveries in prehydrolyzate at harsh

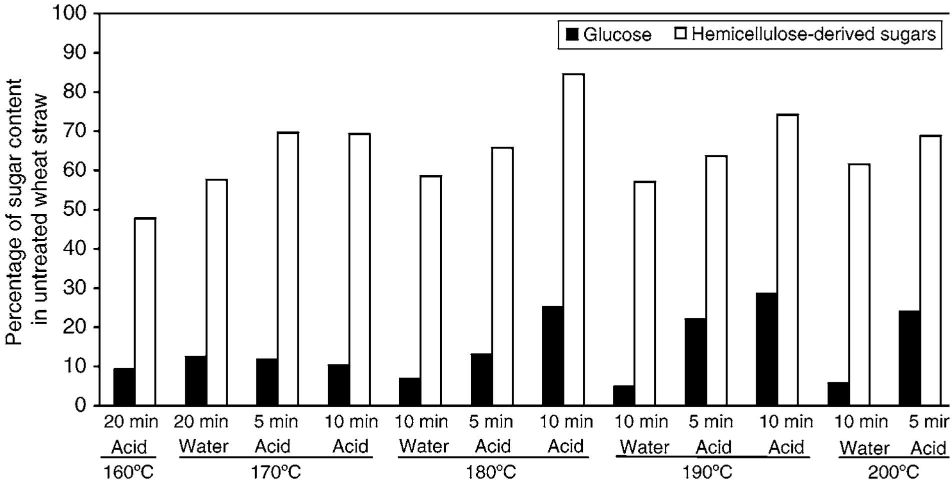

Fig. 1. Sugar recovery yield in prehydrolyzates from SE of wheat straw at different pretreatment conditions. Results reported as percentage of sugar content in untreated raw material.

pretreatment conditions, owing to sugar losses by degradation in acidic conditions *(15,16)*. So, the filtrate obtained after SE experiments must be analyzed for total sugar recovery assessment. Results of glucose and HS recovery yields in prehydrolyzate (expressed as percentage of glucose and HS content in untreated raw material) after pretreatments are shown in Fig. 1. Comparing HS recovery yields for water and acid impregnated biomass experiments, it can be observed that yields in water-impregnated biomass reached similar values (57–61%) in all temperatures tested and that the addition of acid resulted in increased values up to 65–85%, depending on temperature. A maximum HS recovery of 85% was achieved in experiments performed with acid-impregnated biomass at 180°C and 10 min. This value corresponds to 21 g/100 g raw material of which xylose is the major component (82%), followed by arabinose (13%), and low amounts of galactose (5%). Because at this condition HS content in WIS fraction only accounted for <1% of content in raw material, the remaining 15% HS must be attributed to sugar degradation. Higher temperatures resulted in decreased HS recovery yields in filtrate of approx 70%, showing increased sugar losses. However, high recovery values >90% have been obtained in other biomass residues such as corn stover at 190°C SE pretreatment of dilute-acid (1% H_2SO_4) impregnated material, but at shorter residence time of 90–110 sec *(12)*. In our study, the combination of high temperature of 190–200°C with longer times of 5–10 min in acid-impregnated biomass provided much too severe conditions for hemicellulose recovery.

Regarding glucose recovery in filtrate, acid-impregnation before pretreatment at the most severe conditions produced high glucose recovery

Table 3
Degradation Products Content and pH Values of Prehydrolyzate From SE of Wheat Straw in Different Process Conditions

Conditions					
Temperature (°C)	Time (min)	Impregnation agent	pH	HMF (g/100 g raw material)	Furfural (g/100 g raw material)
160	20	Acid[a]	1.8	0.03	0.03
170	10	Water	3.8	0.01	0.01
	5	Acid	1.8	0.04	0.02
	10	Acid	1.7	0.05	0.02
180	10	Water	3.8	0.03	0.01
	5	Acid	1.9	0.25	0.07
	10	Acid	2.0	0.32	0.07
190	10	Water	3.8	0.06	0.02
	5	Acid	1.9	0.51	0.08
	10	Acid	1.8	0.75	0.14
200	10	Water	3.5	0.04	0.16
	5	Acid	2.0	1.51	0.24

[a] 0.9% (w/w) H_2SO_4.

values from 25 to 30%, corresponding to 8–10 g glucose/100 g raw material (Fig. 1). Considering the total yield of monomers in the filtrate after pretreatment (glucose and HS), a maximum total release of about 30 g sugars/100 g raw material was found in acid-impregnated biomass at 180°C and 10 min. This value is significantly higher than that reported by Palmarola-Adrados et al. *(17)* who obtained a maximum yield of 6.4 g/100 g raw material working with starch-free wheat fiber steam-pretreated at 200°C and 10 min, without acid addition. Our results are in accordance with the fact that the addition of acid before pretreatment allows for lower pretreatment temperatures whereas achieving good recovery yields.

Concerning degradation products and pH values in prehydrolyzate (Table 3), acid-impregnation of biomass in experiments at elevated residence time gave rise to lower pH and higher values of furans, mainly HMF, in all temperatures tested. Nevertheless, the quantification of furans can hardly explain hemicellulose losses in pretreatment, particularly at 190–200°C, where high xylose degradation was found to occur. It is possible that hemicelluloses were lost through high volatilization of furfural (very low amounts were detected in all experiments) and recondensation reactions.

Enzymatic Hydrolysis and SSF

To assess the effect of different pretreatment conditions tested on the digestibility of pretreated wheat straw (WIS fraction), EH, and SSF tests were performed. EH yield, expressed as percentage of glucose released

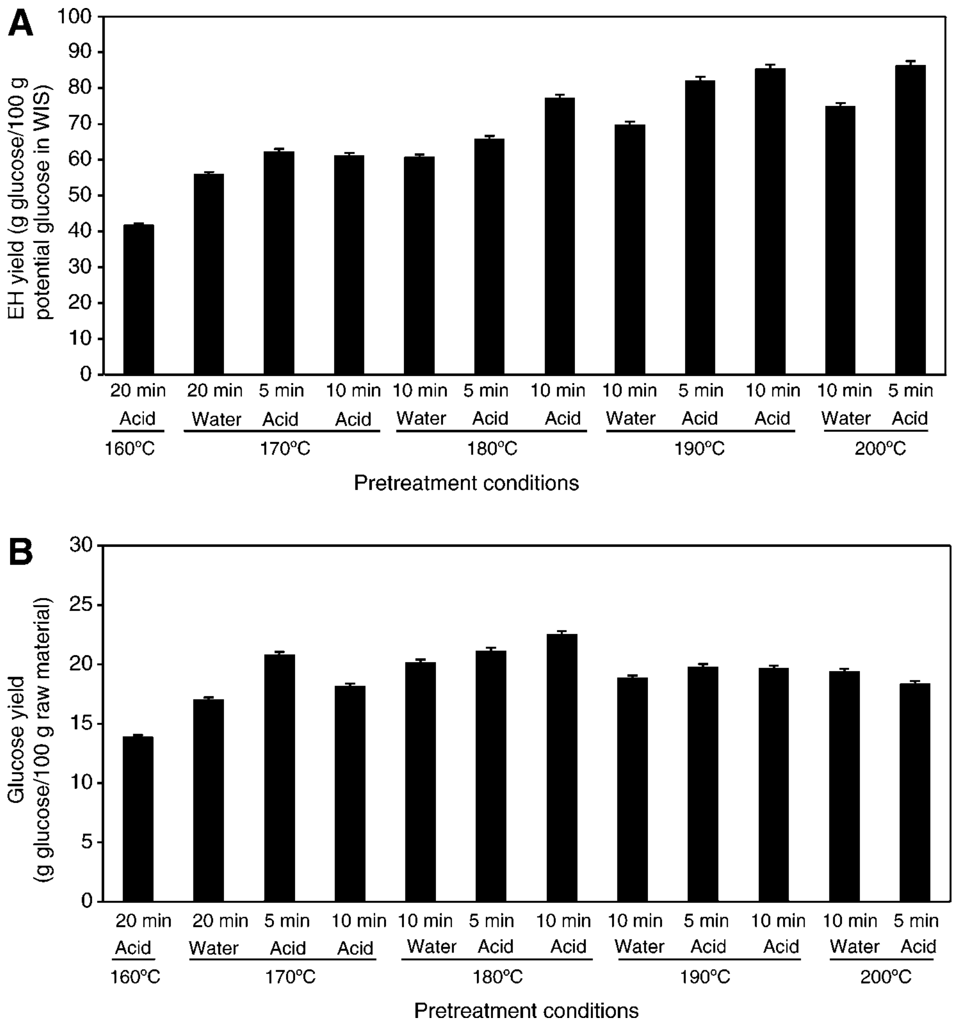

Fig. 2. Enzymatic hydrolysis yield of steam-exploded wheat straw at different pretreatment conditions, reported as g glucose obtained in EH/100 g potential glucose in WIS **(A)**, and glucose yield referred to untreated wheat straw (g/100 g raw material) **(B)**.

during hydrolysis at 72 h in relation to potential glucose in WIS fraction, together with glucose yield based on untreated raw material, are illustrated in Fig. 2. Results regarding the digestibility of pretreated straw (panel A) show that it is markedly dependent on temperature pretreatment, rising from 40% at 160°C to maximum yields of approx 85% at 190°C and 200°C in acid impregnated biomass at 10 and 5 min, respectively. It can be considered a fairly good result if we take into account that EH tests were performed at high substrate loading of 10% (w/v), which may involve end product inhibition and mixing problems. Palmarola et al. *(17)* reported increased saccharification yield of 92.4% in 190°C, 10 min pretreatment of wheat straw using similar cellulase loading of 15 FPU/g subtrate, but at 5% substrate loading. The difficulties of performing EH at high substrate loading have been also disccused by Alfani et al. *(10)* in bioconversion of steam-exploded wheat straw.

The increasing tendency of EH yield found in water-biomass samples might suggest that temperatures over 200°C could be tested to improve cellulose digestibility without acid addition. Nevertheless, experiments carried out by the authors in steam-pretreated wheat straw at 210–230°C and shorter time (data not shown) did not produce enhanced cellulose digestibilities. Beltrame et al. *(18)* working at high temperatures of 230°C in steam-exploded wheat straw did not achieve either saccharification yields exceeding 75%. Only when steam-exploded biomass was next extracted with dioxane/water was EH increased up to 93%.

In all temperatures tested, impregnation with acid resulted in higher degree of digestibility of WIS fraction compared to experiments in water-impregnated biomass. EH yields exceeded values found in water pretreated samples by 7% for experiments with acid at 170°C, and in 15–17% at higher temperatures of 180°C to 200°C in acid pretreated samples. Within acid-impregnated biomass experiments, higher residence times of 10 min also produced elevated digestibilities in comparison with 5 min experiments, except in 170°C tests, in which similar values were found among different times. The effect of harsh pretreatment conditions on enhancing the enzymatic digestibilty of pretreated substrates has been reported to be owing to a greater fraction of hemicellulose being dissolved, so allowing the enzyme to have greater access to cellulose. In experiments performed in this work, the lower HS removal from the 160°C to 180°C pretreated samples (Table 2) resulted in decreased EH yields of 40–65%, supporting the idea of incomplete hemicellulose hydrolysis lowering cellulose digestibilty. Um et al. *(13)* have described a linear relationship between cellulose digestibility and percentage of xylan removal from the solid in dilute-acid pretreated corn stover.

EH yield referred to potential glucose in WIS fraction is a unquestionable useful tool to valuate the effectiveness of pretreatment on saccharification performance. However, in establishing optimum conditions it is also valuable to calculate glucose produced by EH per gram of untreated raw material (Fig. 2B). This calculation takes into account solid recovery values in the pretreatment step and so, it provides a more representative data of overall process efficiency. In fact, in our experiments a maximum glucose yield on untreated material basis of about 23 g/100 g raw material was obtained at 180°C, 10 min in acid-impregnated biomass, a slightly higher value than that calculated for 190°C, 10 min (19 g/100 g raw material), which provided maximum EH yield reported on a WIS basis.

Figure 3 illustrates SSF yields in steam-exploded wheat straw as percentage of theoretical yield on WIS basis (panel A) and ethanol yields based on untreated raw material (panel B). Similarly to EH performance, yields reported on WIS basis increased as temperature rose, reaching maximum values of 80% of theoretical in acid-impregnated biomass at 190°C and 200°C

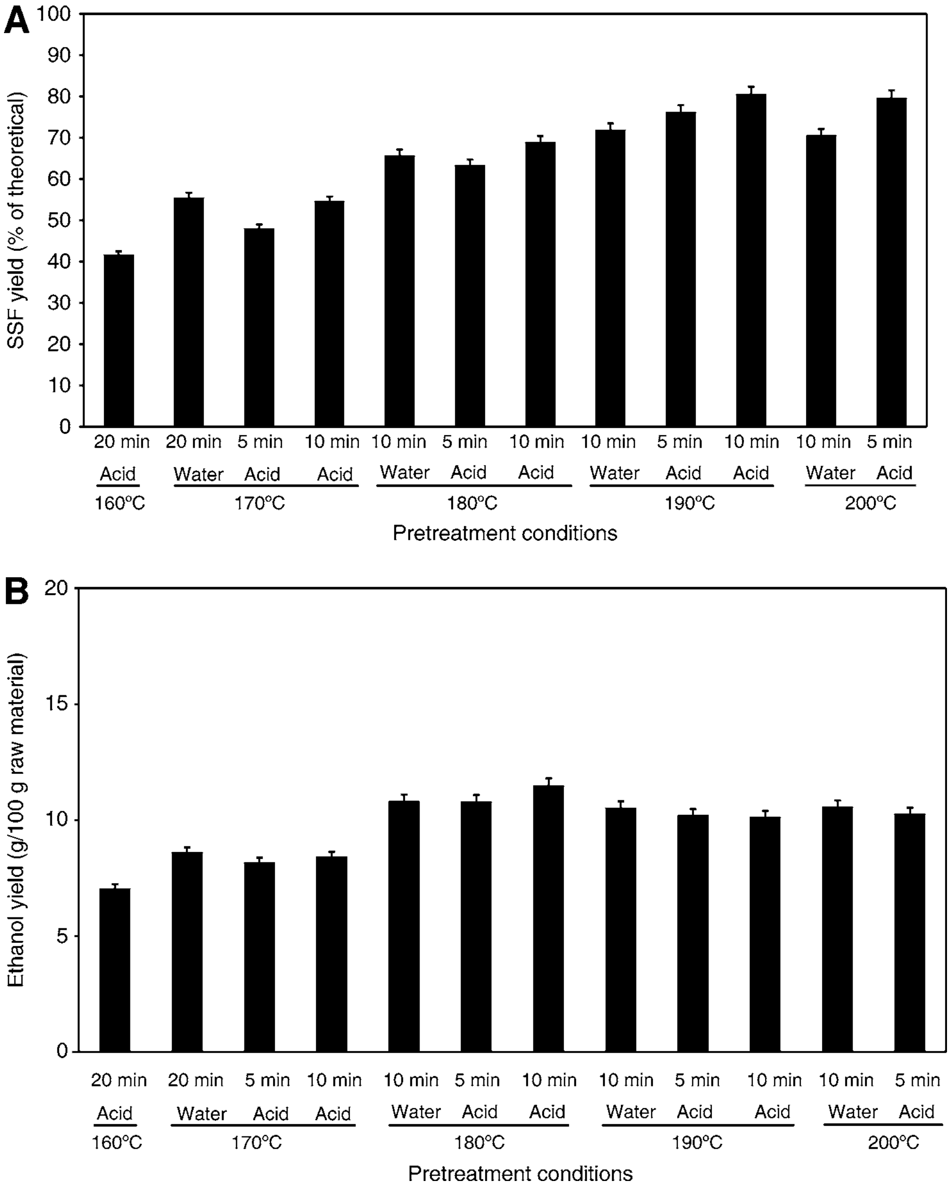

Fig. 3. SSF yield of steam-exploded wheat straw at different pretreatment conditions, calculated as the ethanol produced/potential glucose in WIS and reported as percent of theoretical **(A)**, and ethanol yield based on untreated wheat straw (g/100 g raw material) **(B)**.

during 10 and 5 min, respectively. At 180°C, values decreased up to 69% in acid-impregnated biomass pretreated for 10 min. Tucker et al. *(12)* described good SSF performance using similar cellulase and substrate loading than in our work, and *Saccharomyces cerevisiae* as fermenting yeast in WIS fraction from acid steam-pretreated corn stover at 190°C (90% SSF yield). Lowering the temperature to 180°C or 160°C resulted also in a drop in SSF of up to 65%.

However, if ethanol yields are based on untreated material, a highest value of 120 g ethanol/kg wheat straw is again found at 180°C, 10 min in acid-impregnated biomass. It is about 70% of the maximum theoretical amount of ethanol that could be attained taking into account the glucose content in raw material. At harsher conditions of 190°C and 200°C, some cellulose losses occur during pretreatment (Table 2), which result in slightly lower yields when referring to untreated material.

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

Conclusions

This study shows that dilute acid-impregnation of biomass is an efficient method to increase the EH and SSF of cellulose to ethanol of wheat straw, compared with water-impregnated biomass. Addition of acid enhances solubilization of hemicelluloses from raw material, although at high temperature and residence times tested, some release of glucose may also occur. The best pretreatment conditions to obtain high conversion yield to ethanol of cellulose-rich residue after SE were 190°C for 10 min, or 200°C during 5 min in straw impregnated with 0.9% (w/w) H_2SO_4 when reported in WIS basis. However, when calculating glucose and ethanol yields referred to initial raw material before pretreatment, 180°C for 10 min in acid-impregnated biomass gave better results than that obtained at 190°C. On the other hand, hemisugars recovery yield, considered also as a key parameter in the assessment of pretreatment efficiency was maximized in experiments performed with acid-impregnated biomass at 180°C and 10 min. So, considering that the overall utilization of the carbohydrate components in the feedstock is essential to decrease ethanol production costs, and that a lower temperature would be always advantageous since it results in saving energy cost, 180°C and 10 min would be the most adequate pretreatment conditions of acid-impregnated wheat straw for ethanol production, in the operational conditions used in this work.

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