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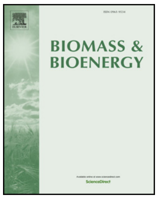

Research paper

Pretreatment of switchgrass by steam explosion in a semi-continuous pre-pilot reactor

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ABSTRACT

Switchgrass (*Panicum virgatum*) is a perennial grass highly valued as an energy crop resource for the production of bioethanol due to its high carbohydrate content, fast growth, and ability to grow in lands that cannot support crop or food production. In the present study, this biomass was submitted to steam explosion pretreatment in a semi-continuous pre-pilot reactor with the aim of obtaining a pretreated solid with high digestibility for enzymatic hydrolysis. Different conditions of temperature (170–200 °C) and residence time (5–15 min), leading to different severity factors (2.76–4.12) were used for steam explosion pretreatment, which were combined through a 2^2 central composite design. The results revealed that both variables had great influence in the process, affecting both the biomass structure and the saccharification yield, as a consequence. However, in the range of values evaluated in this study, the effect of the temperature was more prominent than the effect of the residence time. The best saccharification yield (88.3%) was obtained when using the biomass pretreated at 200 °C for 10 min. Similar result was obtained using a commercial cellulose pulp as feedstock for enzymatic hydrolysis, confirming that the best conditions for switchgrass pretreatment in the pre-pilot scale were successfully established.

1. Introduction

In the context of high demand of energy and reduction of CO_2 emissions, there is a global interest in the development of fuel production processes from renewable biomass. In fact, lignocellulosic biomass is an attractive resource for use in these processes, since it can provide environmental, economic and social benefits when compared to the production of fuels from fossil resources [1]. However, the production of cellulosic ethanol, for example, presents some important challenges to overcome. Due to the biomass recalcitrance, the introduction of an extra step, i.e., a pretreatment of the raw material, is required to make cellulose fibers more accessible to the action of enzymes during the enzymatic saccharification. Among the several alternatives of pretreatment reported in the literature, steam explosion has been one of the most commonly used, even on commercial scale, due to its efficiency to remove hemicellulose and lignin from biomass structure. In this process, the biomass is submitted to high-pressure saturated steam during a short period of time (minutes). Then, the pressure is suddenly released, causing a disruption in the cell wall structure and solubilizing mainly the hemicellulose and lignin fractions, making the cellulose fibers more available for the following step of enzymatic hydrolysis as a consequence [2–4].

Steam explosion pretreatment has already been tested for different lignocellulosic materials, including elephant grass [5], tall fescue [6], spruce bark [7], corn stalk [8], among others. In general, this process has been demonstrated to be an efficient technology for biomass pretreatment. However, the efficiency and the selectivity of this pretreatment is highly dependent on the feedstock and conditions applied, the temperature and residence time being the two main parameters affecting the results. For this reason, it is of great importance to optimize the process conditions to each lignocellulosic feedstock in order to obtain a material with improved digestibility for enzymatic hydrolysis [3,5].

In the last years, Uruguay has demonstrated a strong commitment to

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the development of technologies for the production of second-generation biofuels and, according to a recent study, by increasing the use of biofuels in the sector of transport, Uruguay has already achieved an annual reduction of 7% in greenhouse gas emissions. Among the raw materials available in the country, suitable for use on the production of second-generation biofuels, switchgrass (*Panicum virgatum*) is one of the most relevant for use on the production of cellulosic ethanol since it is an abundant perennial grass with a high carbohydrate content. In addition, switchgrass is also an attractive feedstock for use on bioethanol production since it has a fast growth, high volume of production per area, low cost of production, and ability to grow in lands that cannot support crop or food production [9,10]. Therefore, the present study is focused on the development of a process technology for ethanol production using switchgrass as a feedstock. More specifically, this study evaluated the pretreatment of switchgrass by steam explosion in a semi-continuous pre-pilot reactor able to generate between 3 and 7 kg of pretreated solid material. The effects of temperature and residence time used for pretreatment were evaluated and the conditions able to result in a solid with improved digestibility for enzymatic hydrolysis were selected.

2. Material and methods

2.1. Raw material

The switchgrass (*Panicum virgatum*) used in this study was provided by ANCAP (Uruguay). The feedstock was harvested in the Agricultural Experimental Station Mario Cassinoni, department of Paysandú, in September 2016. The biomass was dried at 40 °C and milled to an average particle size of 1 cm.

2.2. Steam explosion pretreatment

Steam explosion pretreatment was carried out in a semi-continuous pre-pilot equipment (being the *semi* due to the release of pressure every 5 s) installed at the Pilot Plant of the Technological Laboratory of Uruguay (Montevideo). The equipment (Advance Bio Systems LLC, model S1401-D2011) has an approximate capacity of 10 kg/h (depending on pretreatment conditions), a maximum working pressure of 15 bar, and a screw of variable speed to regulate residence time. Maximum working temperature was 200 °C, which was regulated according to the working pressure (as the equipment works with saturated vapor) and controlled by means of a PLC that also regulated and set different screw speeds (hopper screw, feeding screw and reactor screw) (Fig. 1).

Table 1
Chemical composition of the switchgrass used in the present study.

Component	Composition (%wt)
Cellulose	31.8
Hemicellulose	25.0
Lignin	31.2
Klason lignin	*26.9*
Soluble lignin	*4.3*
Ash	3.2
Protein	1.8
Extractives	7.4
Water extractives	*5.0*
Ethanol extractives	*2.4*

Twenty-four hours prior to the steam explosion reaction, the biomass was hydrated to 30% (w/w) with tap water at room temperature. This point was similar to the fiber-saturated point, which was considered the ideal since no extra nor incomplete hydration had taken place [11]. The hydrated biomass was then added to the pretreatment reactor and submitted to high-pressure steam under different conditions of temperature and residence time. Once the desired time lapsed, a sudden reduction of the pressure was promoted.

After steam explosion pretreatment, the biomass was filtrated using a fabric (65% polyester and 35% cotton, 180 g/m^2, 405 warp yarns in 10 cm and 194 weft yarns in 10 cm) and a press in order to separate the remaining solid from the liquid fraction. The liquid fraction was then frozen for further analysis. The solid fraction was washed three times with tap water at 60 °C in a ratio 5:1 (water:dry biomass in kg), using a portable concrete mixer during 5 min, and re-pressed. The pH of the third washing water was 5–6. Then, the solid fraction was dried at 40 °C and stored at room temperature in bags for further analyses and enzymatic saccharification.

2.3. Experimental design and severity factor calculation

Three levels of temperature (170, 185, and 200 °C), and three levels of residence time (5, 10, and 15 min), were evaluated for steam explosion pretreatment of switchgrass. Such values were combined through a 2^2 central composite design, leading to a total of 11 assays. The design included three assays in the center point to estimate the

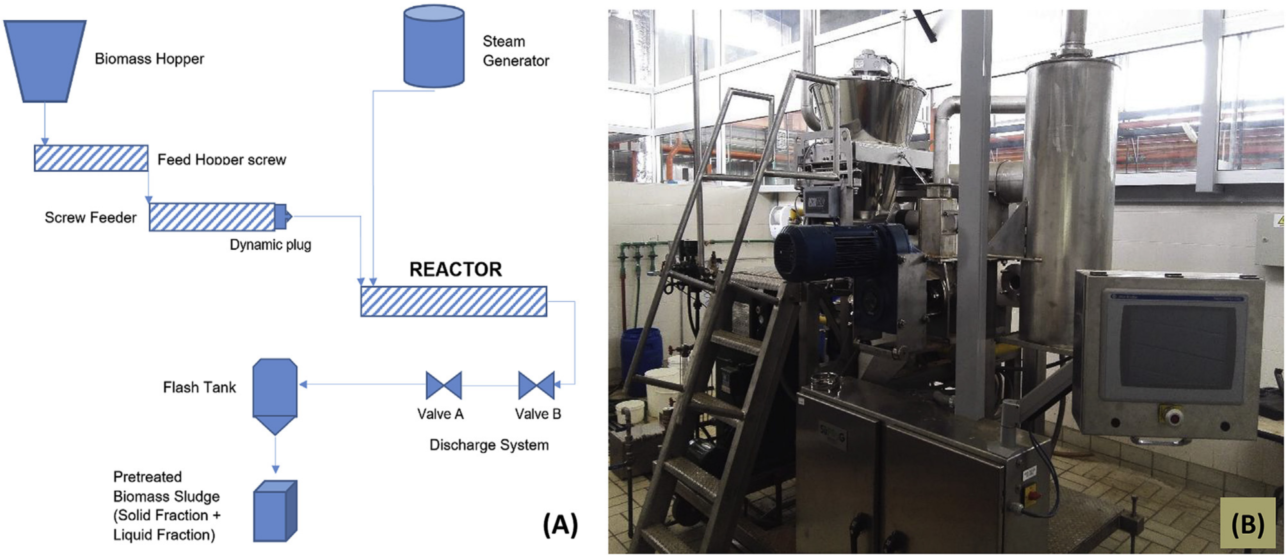

Fig. 1. Schematic representation (A) and original image (B) of the steam explosion equipment used for pretreatment of switchgrass in the present study.

Table 2
Composition of the remaining solid material obtained after steam explosion pretreatment of switchgrass under different conditions of temperature and residence time.

Run	Pretreatment variables (original and (coded) values)		Severity factor	Composition of the pretreated solid (%wt)		
	Temperature (ºC)	Residence time (min)		Cellulose	Hemicelullose	Lignin
1	170 (−1)	5 (−1)	2.76	33.8	25.5	34.8
2	170 (−1)	10 (0)	3.06	35.4	25.4	35.0
3	185 (0)	5 (−1)	3.20	36.2	22.3	36.8
4	170 (−1)	15 (+1)	3.24	34.9	27.9	36.6
5	185 (0)	10 (0)	3.50	42.1	15.5	34.7
6	185 (0)	10 (0)	3.50	44.3	15.8	38.8
7	185 (0)	10 (0)	3.50	41.2	21.0	34.4
8	200 (+1)	5 (−1)	3.64	44.4	14.0	42.2
9	185 (0)	15 (+1)	3.68	44.4	13.9	40.8
10	200 (+1)	10 (0)	3.94	49.1	5.1	45.3
11	200 (+1)	15 (+1)	4.12	50.9	2.5	50.6

Table 3
Composition of the liquid fraction obtained after steam explosion pretreatment of switchgrass under different conditions of temperature and residence time.

Run	Pretreatment variables (original and (coded) values)		Severity factor	Composition of the liquid fraction (g/L)					
	Temperature (ºC)	Residence time (min)		Glucose	Xylose	Formic Acid	Acetic Acid	HMF[a]	Furfural
1	170 (−1)	5 (−1)	2.76	0.47	0.6	1.4	1.3	nd	nd
2	170 (−1)	10 (0)	3.06	0.31	1.1	1.4	2.3	0.03	0.0
3	185 (0)	5 (−1)	3.20	0.38	1.8	2.1	2.5	0.04	0.1
4	170 (−1)	15 (+1)	3.24	0.16	0.6	0.9	1.5	0.04	0.0
5	185 (0)	10 (0)	3.50	0.27	3.3	2.9	2.9	0.05	0.2
6	185 (0)	10 (0)	3.50	0.51	4.8	3.8	4.1	0.07	0.3
7	185 (0)	10 (0)	3.50	0.12	1.2	1.2	1.3	0.03	0.1
8	200 (+1)	5 (−1)	3.64	0.18	2.9	2.1	1.7	0.07	0.2
9	185 (0)	15 (+1)	3.68	0.14	3.4	2.0	2.7	0.05	0.2
10	200 (+1)	10 (0)	3.94	0.69	15.7	4.2	6.1	0.39	1.3
11	200 (+1)	15 (+1)	4.12	2.60	20.3	7.7	10.6	0.54	2.3

[a] HMF: hydroxymethylfurfural; nd: non detected.

experimental error needed for analysis of the variance, and to verify the presence of curvature in the response surfaces. The cellulose content in the remaining solid after pretreatment and the saccharification yield were considered as responses for the experimental design. The software Statistica version 12 was used for analysis of the data.

The severity factor (*Ro*) was calculated to each combination of temperature and residence time used for steam explosion. *Ro* was calculated according to Eq. (1), where *t* is the residence time in minutes, *T* is the reaction temperature in ºC, and 14.75 is a fitted value [12].

$$R0 = t \times e^{\frac{T-100}{14.75}} \tag{1}$$

2.4. Enzymatic saccharification

Enzymatic saccharification was performed for all the solids obtained after steam explosion pretreatment. For comparison, assays were also performed using the untreated biomass and a sample of commercial Kraft cellulose pulp. For the reactions, the biomass (2% w/v) was mixed with 0.05 M sodium citrate buffer (pH 4.8) in Erlenmeyer flasks. Then, 50 FPU of enzyme (Cellic CTec2, Sigma Aldrich code SAE0020) were added to the flasks in order to initiate the reactions. The experiments were maintained in an incubator at 50 °C and 200 rpm, for 96 h. At the end of the saccharification, the samples were centrifuged (15000 rpm, 10 min) and the sugar content was determined using the dinitrosalicylic acid (DNS) method [13]. All the experiments were performed in duplicate.

2.5. Analytical methods

Cellulose, hemicellulose and lignin contents in the samples were determined according to the technical report from the National Renewable Energy Laboratory of the United States NREL/TP-510-42618 [14], being the concentration of sugars, byproducts and degradation products in the liquid samples determined as described in the technical report NREL/TP-510-42623 [15]. Ash content was determined according to the standard test method ASTM D1102-84 [16]. Protein was estimated by multiplying the nitrogen content by 6.25. Nitrogen content was determined using a CHN Analyzer Flash 2000 Organic Elemental Analyzer (ThermoScientific). Extractives were determined according to the technical report NREL/TP-510-42619 [17].

Three biomass samples were observed by scanning electron microscopy (SEM): two samples pretreated under different process conditions (the less severe and the most severe) and an untreated sample. Images were obtained by using a JEOL electron microscope model JSM-5900LV. For analysis, the dried samples were covered with a gold film and then submitted to an acceleration voltage of 20 kV. Images were obtained at 75 and 370-fold magnifications.

3. Results and discussion

3.1. Biomass composition

Chemical composition of the switchgrass used in the present study is shown in Table 1. As can be seen, the original raw material had an elevated amount of carbohydrates in the composition (approx. 57% w/w), present in the form of cellulose and hemicellulose. Cellulose was the most abundant fraction, being the amount found in this study similar to that reported by other authors [18]. Hemicellulose content was within the range of values reported to switchgrass and other grasses [5,10]; while lignin was present in higher amount, which could be explained by the natural variability of the specie, the harvesting season and storage conditions, as reported by Lindsey et al. [10]. Ash, protein and

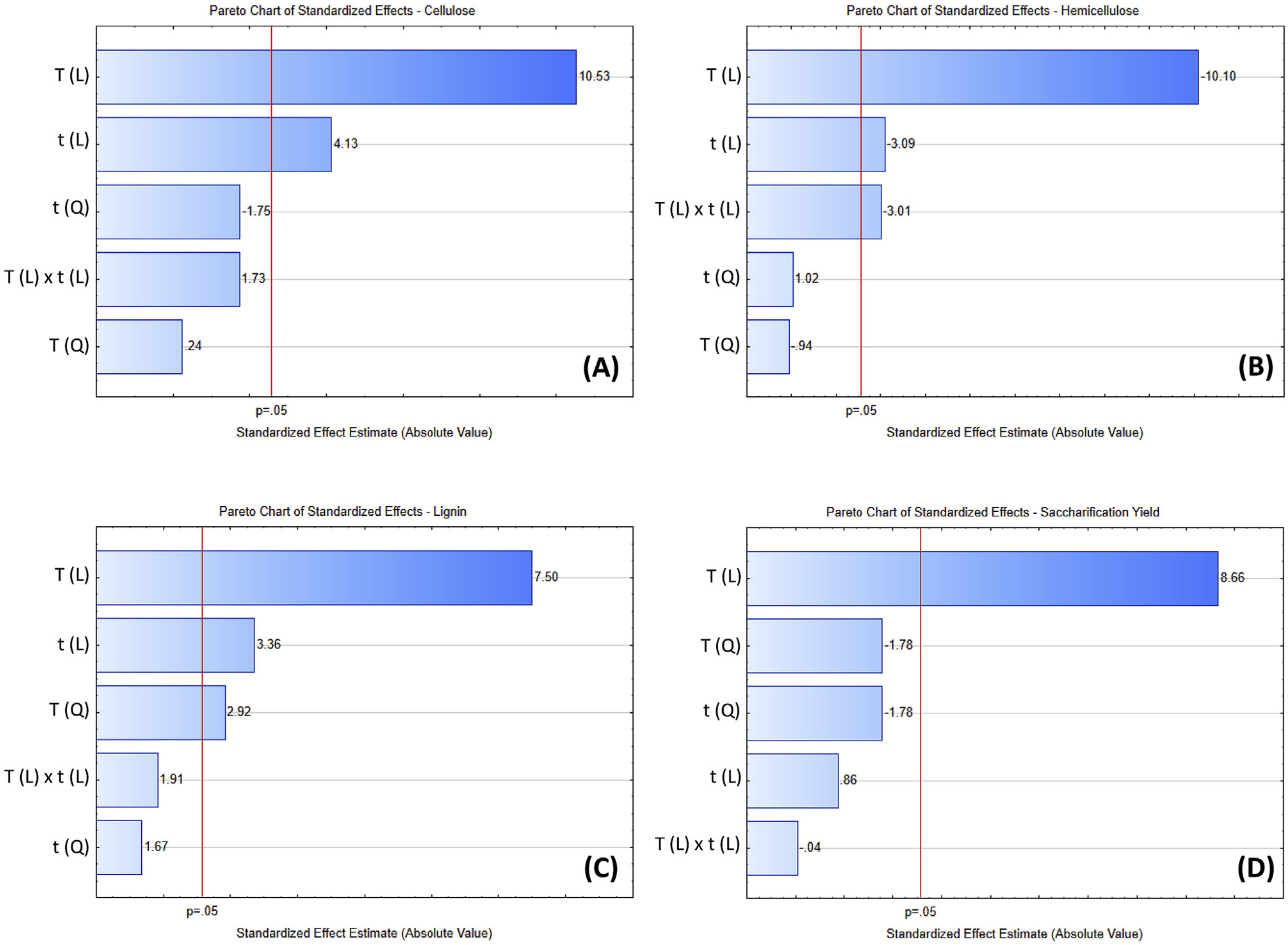

Fig. 2. Pareto chart for the effects of temperature (T) and residence time (t) and their interactions on the contents of cellulose (A), hemicellulose (B) and lignin (C) in the remaining solid material after steam explosion pretreatment, and on the saccharification yield (D) obtained using the pretreated solid materials.

Table 4
Saccharification yield obtained from the different pretreated samples of switchgrass, an untreated sample of switchgrass, and a commercial cellulose pulp sample.

Run	Pretreatment variables (original and (coded) values)		Severity factor	Saccharification yield (%)
	Temperature (°C)	Residence time (min)		
1	170 (−1)	5 (−1)	2.76	19.8
2	170 (−1)	10 (0)	3.06	26.3
3	185 (0)	5 (−1)	3.20	50.6
4	170 (−1)	15 (+1)	3.24	21.6
5	185 (0)	10 (0)	3.50	73.0
6	185 (0)	10 (0)	3.50	53.1
7	185 (0)	10 (0)	3.50	64.9
8	200 (+1)	5 (−1)	3.64	71.3
9	185 (0)	15 (+1)	3.68	64.0
10	200 (+1)	10 (0)	3.94	88.3
11	200 (+1)	15 (+1)	4.12	72.5
Untreated switchgrass				0.0
Commercial cellulose pulp				84.7

extractives contents in switchgrass were comparable to contents reported to other types of grasses and lignocellulosic biomass. Ash in switchgrass consists of inorganic elements and silica, while water and ethanol extractives are composed of a multitude of compounds, including waxes, oils, fats, resins, nonstructural sugars, chlorophyll, among others [10].

3.2. Steam explosion pretreatment

In this work, the equipment used for the steam explosion was a semi-continuous pre-pilot reactor (Fig. 1). Some advantages of this type of reactor when compared to lab-scale batch reactors include a higher production of pretreated biomass per time with less operative manipulation. However, although highly automatized, the operation of this equipment presents some challenges, among of which, the uniform biomass feeding, which in turn affects the dynamic plug to keep the pressure inside the reactor. In addition, the release of pressure at small intervals could affect the homogenous production of pretreated biomass. Nevertheless, such system and conditions are more similar to what is expected to happen in an industrial large-scale production.

Table 2 summarizes the composition of the remaining solid material obtained under the different steam explosion conditions. As expected, hemicellulose was the main fraction affected during pretreatment, the composition in the pretreated solid varying between 2.5 and 27.9% (w/w) according to the condition employed for reaction. The amount of this fraction in the pretreated solid decreased along with the increase in the reaction severity, thus increasing the relative amounts of cellulose and lignin, as a consequence. Due to the hemicellulose solubilization, higher concentration of xylose was also obtained in the liquid fraction along with the increase in the severity factor (Table 3), and a higher concentration of organic acids, mainly acetic acid, was also observed since this acid is also present in the hemicellulosic structure. On the other hand, the low concentrations of glucose and hydroxymethylfurfural (HMF) in the final liquors confirm that the reaction conditions attacked more selectively the hemicellulose than the

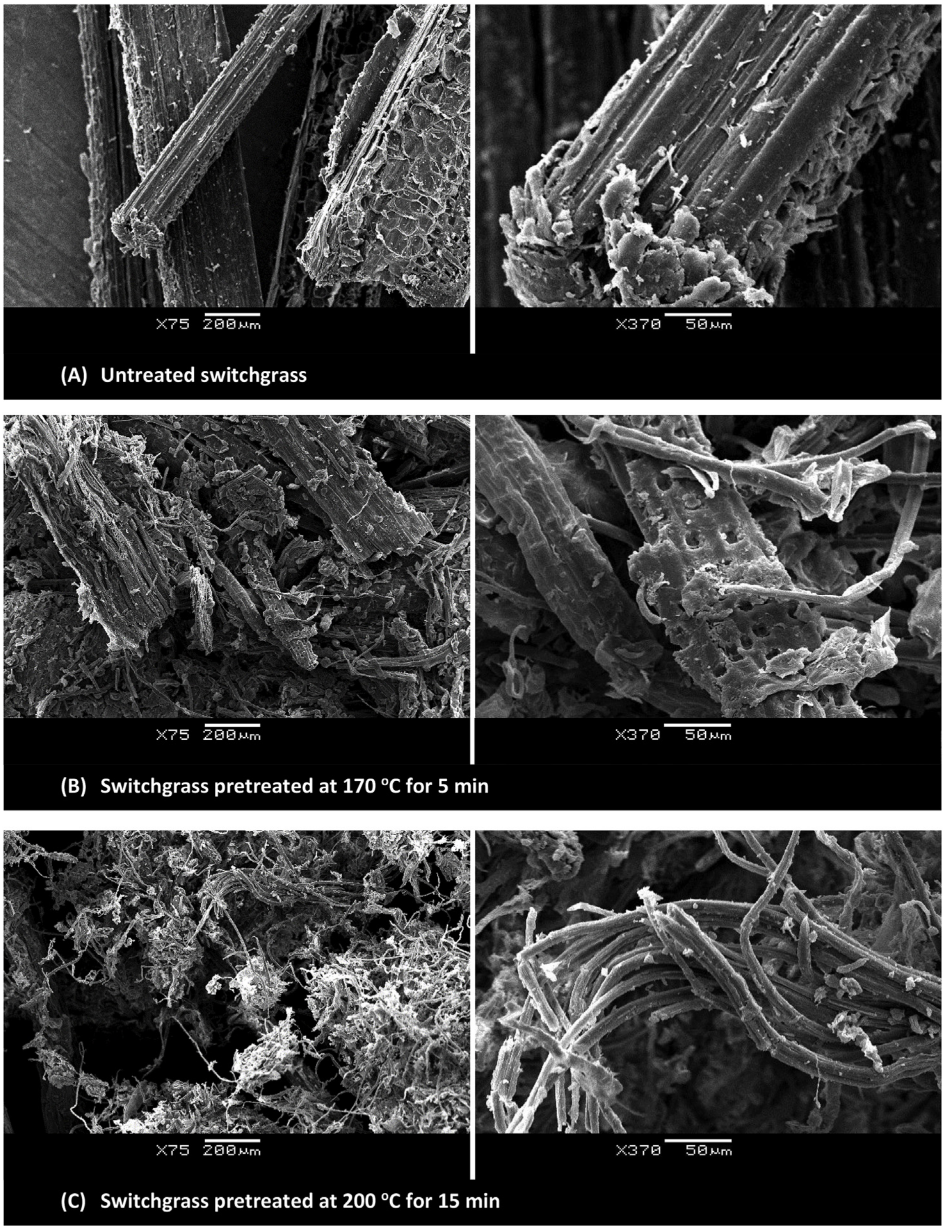

Fig. 3. SEM images obtained for switchgrass samples in the untreated form (A), pretreated by steam explosion under the less severe (B) and under the most severe (C) process conditions. Images taken at 75 and 370-fold magnifications.

cellulose present in switchgrass.

Statistical analysis of the data revealed that temperature was the most important variable affecting the results (Fig. 2). The effect of this variable was positive for cellulose and lignin responses (Fig. 2A and C) and negative for hemicellulose (Fig. 2B), revealing that more cellulose and lignin, and less hemicellulose were obtained in the solid when the temperature used for pretreatment was increased. The reaction time promoted also similar effects to the composition of the final solid but in much less intensity when compared to the temperature.

3.3. Enzymatic hydrolysis

In this step of the study, the solid fraction obtained for all the pretreatment conditions was submitted to enzymatic hydrolysis in order to release glucose for further use in fermentation process. Table 4 shows the saccharification yield obtained to each sample. Such value strongly varied according to the used sample (from 19.6 to 88.3%), confirming that the conditions used for pretreatment had great influence on the subsequent step of cellulose enzymatic hydrolysis to obtain glucose. In general, the results were better when solids pretreated under the highest values of severity were used. Statistical analysis of these data revealed that the temperature used during pretreatment had the most significant effect in this response too (Fig. 2D), confirming that the solids obtained by steam explosion under the highest temperature conditions contained more cellulose in the composition and such fibers were more available to the action of enzymes during the hydrolysis. This conclusion is supported by the results of saccharification obtained for the untreated sample of switchgrass (Table 4) and also by the images obtained by scanning electron microscopy (Fig. 3). In Fig. 3, the image obtained at 75-fold magnification (left) allows to observe the effect of the explosion on the general deconstruction of the material, meanwhile the image obtained at 370-fold magnification (right) shows a more detailed effect of the pretreatment in a fiber-level. As can be seen, under the most severe pretreatment conditions, it is evident that a better deconstruction of the biomass structure occurred, making the cellulose fibers more accessible to the enzymes (Fig. 3C). On the other

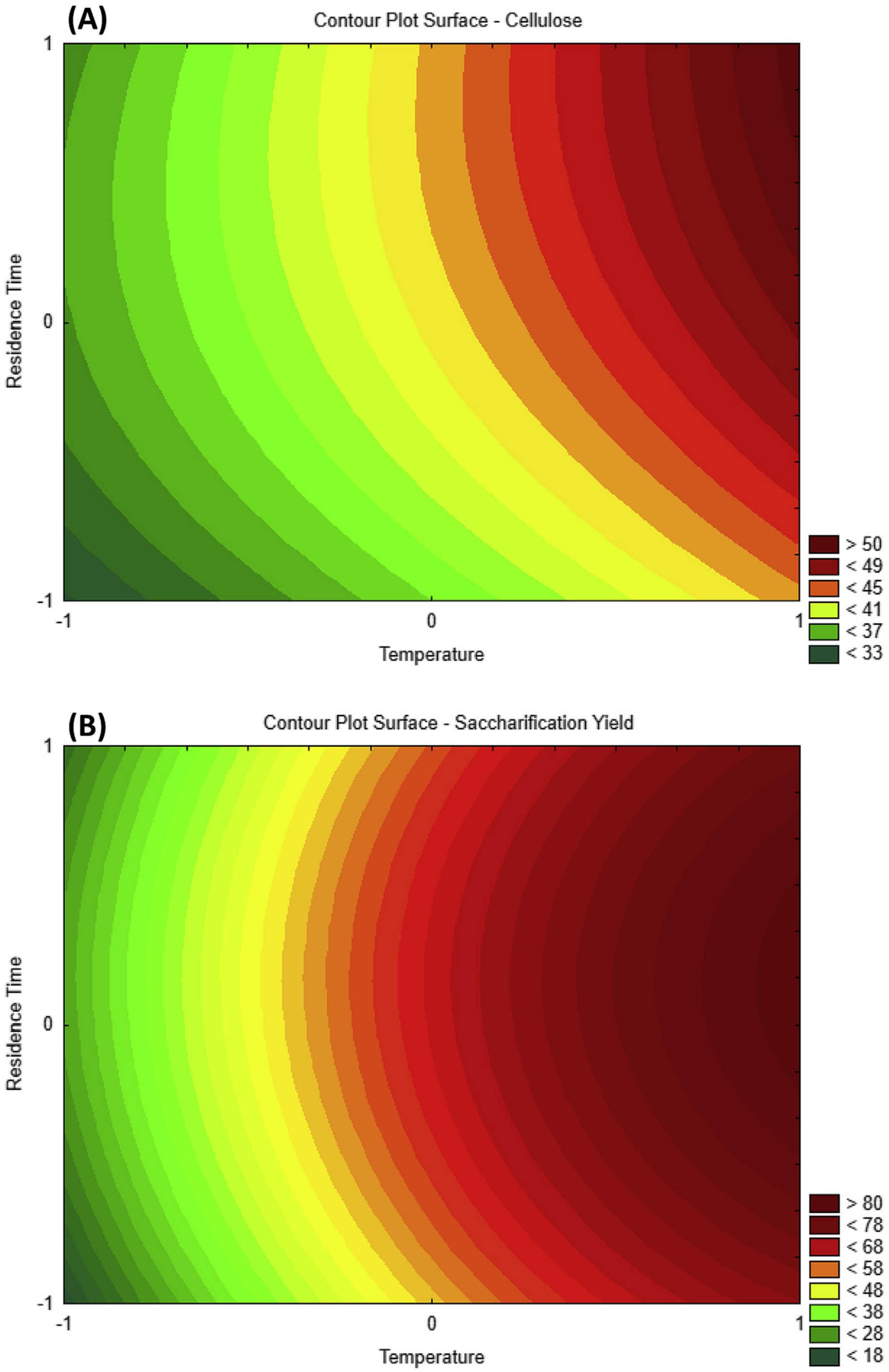

Fig. 4. Contour plots representing the variations of cellulose content in the pretreated solid material (A) and saccharification yield (B) using the solid pretreated by steam explosion under different conditions of temperature and residence time (coded values).

hand, pretreatment under the less severe conditions promoted little effects in the biomass structure, which was almost similar to the original biomass (Fig. 3A), justifying the low saccharification yield obtained for this sample.

It is important to highlight that it was not the aim of the present study to optimize the conditions used for enzymatic hydrolysis. However, in order to better understand the efficiency of the results obtained during this step, assays were also performed by submitting a commercial cellulose sample to the same conditions of enzymatic hydrolysis used for the pretreated switchgrass samples. As can be seen in Table 4, in the case of the sample pretreated with severity factor 3.94 (run number 10) it was possible to obtain a saccharification yield similar to that achieved when using a pure sample of cellulose. These results are highly relevant and demonstrate that it was possible to obtain a pretreated solid with good characteristics (high digestibility) for use in the saccharification process. In addition, when taking into account the composition of the solid obtained under these pretreatment conditions (Table 2, run 10) it is possible to conclude that it is not necessary to remove all the hemicellulose and lignin from the biomass structure to achieve efficient results of saccharification in the next step. As can be seen, the best saccharification results were obtained from a solid containing low amount of hemicellulose and high amount of lignin in the composition (45% w/w), revealing that the presence of lignin was not detrimental to the saccharification yield, but probably the lignocellulosic complexity along with the hemicellulose and lignin together was a more important factor affecting the saccharification [19,20]. Similar findings were reported by Kataria et al. [5] when studying the enzymatic saccharification of steam explosion pretreated elephant grass biomass. This is also a very important finding from an economic point of view since the use of more severe pretreatment conditions (higher temperature and/or addition of chemicals, or even an extra pretreatment step) to remove the lignin fraction from biomass can be avoided.

3.4. Optimization of pretreatment conditions

The cellulose content in the pretreated solid and the saccharification yield were considered as the main responses of the experimental design and were used in this step of the study to optimize the pretreatment conditions. As can be seen in the contour plots showed in Fig. 4, both responses had a similar profile, with the best results being achieved under the highest conditions of temperature (200 °C). By performing an analysis of variance of the experimental data and eliminating the variables with the lowest significance on the responses, the following model equations were obtained for cellulose (Eq. (2)) and saccharification yield (Eq. (3)), respectively:

$$\text{Cellulose (\%wt)} = 42.42 + 13.43\,T + 5.27t - 3.31t^2 + 2.70T \times t \quad (R^2 = 0.96) \tag{2}$$

$$\text{Saccharification yield (\%)} = 64.41 + 54.80T - 17.33T^2 - 17.33t^2 \quad (R^2 = 0.94) \tag{3}$$

The model equations (2) and (3) are able to predict the values of cellulose in the pretreated solid and saccharification yield, when using the temperature (T) and residence time (t) in the range of values studied in the present study. The high values of the coefficient R^2 demonstrates a high agreement between the experimental values and those obtained by the model equations.

Finally, the statistical analysis predicted that the highest result of saccharification yield (86.3%) can be obtained by using a solid pretreated at 209 °C for 11 min. However, this value is not statistically different from that obtained experimentally in the present study when using the solid pretreated at 200 °C for 10 min (saccharification yield of 88.3%, Table 4), revealing that the experimental result obtained under this condition is close similar to the maximum expected to be achieved through the statistical optimization. Therefore, steam explosion at 200 °C for 10 min was selected as the best condition to pretreat switchgrass by steam explosion in the semi-continuous pre-pilot reactor.

4. Conclusions

Pretreatment of switchgrass in a semi-continuous steam explosion pre-pilot reactor under selected conditions of temperature and residence time was a successful strategy to obtain a solid with high digestibility during enzymatic hydrolysis. Temperature and residence time revealed to be two important variables affecting this pretreatment and, selecting the optimal conditions (200 °C for 10 min) was very important to maximize the glucose release during enzymatic hydrolysis (saccharification yield of 88.3%). These results are very promising and contribute to the development of a technology for the production of second-generation ethanol production using switchgrass as a feedstock.

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References

[1] S.I. Mussatto, G. Dragone, Biomass pretreatment, biorefineries, and potential products for a bioeconomy development, Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery. Mussatto SI, Elsevier Inc., Waltham, MA, 2016, pp. 1–22.

[2] P. Alvira, E. Tomás-Pejó, M. Ballesteros, M. Negro, Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review, Bioresour. Technol. 101 (2010) 4851–4861.

[3] A. Duque, P. Manzanares, I. Ballesteros, M. Ballesteros, Steam explosion as lignocellulosic biomass pretreatment, Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery. Mussatto SI, Elsevier Inc., Waltham, MA, 2016, pp. 349–368.

[4] S.I. Mussatto, G. Dragone, P.M. Guimarães, J.P.A. Silva, L.M. Carneiro, I.C. Roberto, A. Vicente, L. Domingues, J.A. Teixeira, Technological trends, global market, and challenges of bio-ethanol production, Biotechnol. Adv. 28 (2010) 817–830.

[5] R. Kataria, A. Mol, E. Schulten, A. Happel, S.I. Mussatto, Bench scale steam explosion pretreatment of acid impregnated elephant grass biomass and its impacts on biomass composition, structure and hydrolysis, Ind. Crop. Prod. 106 (2017) 48–58.

[6] G. Maniet, Q. Schmetz, N. Jacquet, M. Temmerman, S. Goflot, A. Richel, Effect of steam explosion treatment on chemical composition and characteristic of organosolv fescue lignin, Ind. Crop. Prod. 99 (2017) 79–85.

[7] K. Kemppainen, J. Inkinen, J. Uusitalo, T. Nakari-Setälä, M. Siika-aho, Hot water extraction and steam explosion as pretreatments for ethanol prodcution from spruce bark, Bioresour. Technol. 117 (2012) 131–139.

[8] Y.G. Sun, Y.L. Ma, L.Q. Wang, F.Z. Wang, Q.Q. Wu, G.Y. Pan, Physicochemical properties of corn stalk after treatment using steam explosion coupled with acid or alkali, Carbohydr. Polym. 117 (2015) 486–493.

[9] G. Siri-Prieto, Switchgrass como alternativa energética en el Uruguay, Cangüé 32 (2012) 31–39.

[10] K. Lindsey, A. Johnson, K. Pyoungchung, S. Jackson, N. Labbé, Monitoring switchgrass composition to optimize harvesting periods for bioenergy and value-added products, Biomass Bioenergy 56 (2013) 29–37.

[11] W. Sui, H. Chen, Effects of water states on steam explosion of lignocellulosic biomass, Bioresour. Technol. 199 (2016) 155–163.

[12] R. Overend, E. Chornet, Fractionation of lignocellulosics by steam-aqueous pretreatments, Philos. Trans. R. Soc. London, Ser. A 321 (1987) 523–536.

[13] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31 (1959) 426–428.

[14] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, Determination of Structural Carbohydrates and Lignin in Biomass, NREL, Golden, Colorado, Revised August 2012.

[15] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples, National Renewable Energy Laboratory, Golden, Colorado, 2008.

[16] ASTM, D1102-84 Standard Test Method for Ash in Wood, ASTM International, West Conshohocken, PA, 2013.

[17] A. Sluiter, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of Extractives in Biomass, National Renewable Energy Laboratory, Golden, Colorado, 2008.

[18] Z. Hu, R. Sykes, M.F. Davis, E.C. Brummer, A.J. Ragauskas, Chemical profiles of switchgrass, Bioresour. Technol. 101 (2010) 3253–3257.

[19] S.I. Mussatto, M. Fernandes, A.M.F. Milagres, I.C. Roberto, Effect of hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent grain, Enzym. Microb. Technol. 43 (2008) 124–129.

[20] J.A. Rollin, Z. Zhu, N. Sathitsuksanoh, Y.-H. Percival Zhang, Increasing cellulose accessibility is more important than removing lignin: a comparison of cellulose solvent-based lignocellulose fractionation and soaking in aqueous ammonia, Biotechnol. Bioeng. 108 (2011) 22–30.