



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE DELLA RICERCA

Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

Combined ethanol and methane production from switchgrass (*Panicum virgatum* L.) impregnated with lime prior to steam explosion

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Capecchi, L., Galbe, M., Wallberg, O., Mattarelli, P., Barbanti, L. (2016). Combined ethanol and methane production from switchgrass (*Panicum virgatum* L.) impregnated with lime prior to steam explosion. *BIOMASS & BIOENERGY*, 90, 22-31 [10.1016/j.biombioe.2016.03.035].

Availability:

This version is available at: <https://hdl.handle.net/11585/569918> since: 2022-01-31

Published:

DOI: <http://doi.org/10.1016/j.biombioe.2016.03.035>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Lorenzo Capecchi, Mats Galbe, Ola Wallberg, Paola Mattarelli, Lorenzo Barbanti,

Combined ethanol and methane production from switchgrass (*Panicum virgatum* L.) impregnated with lime prior to steam explosion, *Biomass and Bioenergy*, Volume 90, 2016, Pages 22-31, ISSN 0961-9534,

<https://www.sciencedirect.com/science/article/pii/S0961953416300976>

The final published version is available online at:

<https://doi.org/10.1016/j.biombioe.2016.03.035>

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

Combined ethanol and methane production from switchgrass (*Panicum virgatum* L.) impregnated with lime prior to steam explosion

Lorenzo Capecchi^a, Mats Galbe^b, Ola Wallberg^b, Paola Mattarelli^a, Lorenzo Barbanti^{a,*}

^a Department of Agricultural Sciences, University of Bologna, viale Fanin 44, 40127 Bologna, Italy

^b Department of Chemical Engineering, University of Lund, P.O. Box 124, SE-221 00, Lund, Sweden

ARTICLE INFO

Article history:

Received 6 December 2014

Received in revised form

10 February 2016

Accepted 23 March 2016

Available online xxx

Keywords:

Switchgrass

Steam explosion

Lime

Simultaneous saccharification and

fermentation

Anaerobic digestion

Energy yield

ABSTRACT

Pretreatments are crucial to achieve efficient conversion of lignocellulosic biomass to soluble sugars. In this light, switchgrass was subjected to 13 pretreatments including steam explosion alone (195 °C for 5, 10 and 15 min) and after impregnation with the following catalysts: Ca(OH)₂ at low (0.4%) and high (0.7%) concentration; Ca(OH)₂ at high concentration and higher temperature (205 °C for 5, 10 and 15 min); H₂SO₄ (0.2% at 195 °C for 10 min) as reference acid catalyst before steam explosion. Enzymatic hydrolysis was carried out to assess pretreatment efficiency in both solid and liquid fraction. Thereafter, in selected pretreatments the solid fraction was subjected to simultaneous saccharification and fermentation (SSF), while the liquid fraction underwent anaerobic digestion (AD) to produce additional energy as methane. Lignin removal was lowest (12%) and highest (35%) with steam alone and 0.7% lime impregnation, respectively. In general, higher cellulose degradation and lower hemicellulose hydrolysis were observed in this study compared to others, depending on lower biomass hydration during steam explosion. Mild lime addition (0.4% at 195 °C) enhanced ethanol in SSF (+28% than steam alone), while H₂SO₄ boosted methane in AD (+110%). However, methane represented a lesser component in combined energy yield. Mild lime addition was also shown less aggressive and secured more residual solid after SSF, resulting in higher energy yield per unit raw biomass. Decreased water consumption, avoidance of toxic compounds in downstream effluents, and post process recovery of Ca(OH)₂ as CaCO₃ represent further advantages of pretreatments involving mild lime addition before steam explosion.

© 2016 Published by Elsevier Ltd.

1. Introduction

The depletion of oil reserves and the role of fossil energy in climate change provide a strong drive towards alternative energy sources. Especially in the transport sector that relies on oil products, bioethanol from lignocellulosic biomass could represent a valuable substitute for gasoline [1].

Dedicated crops have been developed for energy uses; among them, perennial grasses are preferred over annual ones for their ability to combine high biomass yields with low energy and

financial inputs [2,3]. Moreover, perennial grasses deploy a vast range of positive externalities from the environmental viewpoint: increased soil carbon sequestration and reduced nitrate leaching [2,4–6]; improved soil biological quality, and establishment of beneficial interactions with soil organisms [7,8]. Among such grasses, switchgrass (*Panicum virgatum* L.) is a promising feedstock for the production of second generation bioethanol [9], which is considered a more sustainable form of energy as it does not directly affect the food sector [9].

However, second generation bioethanol involves that lignocellulosic biomass be subjected to pretreatments for efficient fermentation [10]. Among them, steam pretreatment is one of the most frequently used [11], often in combination with an acid catalyst [12–14]. Biomass impregnation with acid catalyst prior to steam explosion has often demonstrated higher pretreatment efficiency than steam explosion alone, this latter also called autohydrolysis [14]. Sulphuric acid and sulphur dioxide have been tested as acid catalysts, using variable concentrations, temperatures and residence times. However, sulphuric compounds involve serious

Abbreviations: AD, anaerobic digestion; ANOVA, analysis of variance; FPU, filter paper units; HMF, 5-hydroxymethylfurfural; HPLC, high performance liquid chromatography; IR, infra-red; Log *R*₀, severity factor; LSD, lowest significant difference; SD, standard deviation; SSF, simultaneous saccharification and fermentation; TS, total solids; VS, volatile solids; WIS, water insoluble solids; *W*_L, residual lignin; *Y*_{ETOH}, overall ethanol yield.

* Corresponding author.

E-mail address: lorenzo.barbanti@unibo.it (L. Barbanti).

drawbacks such as acid corrosion of equipment, and the need to implement extensive processing of downstream effluents, resulting in high water consumption [15]. Thus, pretreatments without sulphur would be preferable, if they can bridge the gap with sulphur-based processing. In a biorefinery concept, the choice of a catalyst is not only important for its ability to increase the yield in final product, but also for catalyst fate [16]. This leads to the use of chemicals enhancing ethanol yield, while at the same time releasing by-products that have a market value or are easily disposable.

Lignin is the only fraction of biomass that is not converted into bioethanol [17], restricting enzymatic access to cellulose [18], which results in lower ethanol yield [19]. Compared to acids, alkaline catalysts as lime ($\text{Ca}(\text{OH})_2$) have been shown to reduce the lignin content of herbaceous biomass [20]. Lime can be easily removed before steam explosion, by carbonating the impregnation liquid with CO_2 . The resulting CaCO_3 may be recovered to be used in several applications, such as the mitigation of drought stress in tomato [21]. Hence, lime may be regarded as a by product having no negative impact, and which is cheaper and safer to handle than sodium hydroxide [22].

Saccharomyces cerevisiae is the microorganism most commonly used for the fermentation of hexoses. A process configuration involving pentose utilization is of paramount importance to increase the overall energy output and value of biomass. Recently, new engineered strains have been developed to ferment pentoses [23], although improvements are sought to bring this technology to the scale of industrial processing. At present, methane production through anaerobic digestion (AD) remains the most feasible method to exploit the residual energy content of a raw material [24]. Moreover, converting biomass into a spectrum of energy and marketable products appears a more advisable approach in the frame of biorefinery [25].

Given these premises, the aim of this work was to investigate the influence of lime impregnation before steam explosion, on ethanol and methane production from switchgrass. As reference practice, steam explosion after impregnation with sulphuric acid was also included. Time, temperature and lime concentration during pretreatment were varied, and the sugar yield determined in each case. Pretreated solid fraction was subjected to simultaneous saccharification and fermentation (SSF) at high solids loading for ethanol production. Pretreatment liquid was subjected to AD for methane production.

2. Material and methods

2.1. Origin of the raw material

Switchgrass (*P. virgatum* L.) was used as substrate in this study. The lowland cultivar "Alamo" had been seeded in 2002 at the experimental farm, University of Bologna, Cadriano (BO), Italy (44° 33' N, 11° 21' E, 32 m above sea level), on a deep alluvial soil with a clayey-loamy texture. The area features a mean annual temperature of 13.3 °C and precipitation of 700 mm, which are typical of the Mediterranean North environmental zone [26]. This is a zone with mild winter and long growing season, although precipitation is mostly concentrated in the cold semester. Switchgrass was still in full production in 2011. In that year, crop management consisted of nitrogen fertilization in the springtime (120 kg N ha⁻¹ as urea), no irrigation and no spraying for weed, pest or disease control. At the end of the growing season (October 5, 2011), switchgrass was harvested as whole plant at seed-ripening stage and chopped in ca. 20 mm pieces. Biomass samples were oven dried (40 °C) and ground to a particle size of 0.5 mm for the analysis of structural carbohydrates, lignin (Klason lignin and acid soluble lignin),

extractives, and ash [27,28].

2.2. Process configuration

After 1 h of impregnation with either water alone, alkaline ($\text{Ca}(\text{OH})_2$) or acid solution (H_2SO_4), switchgrass was subjected to steam explosion under different conditions of time and temperature, making up a total of 13 combinations (Table 1). Pretreated samples were separated into solid (slurry) and liquid fraction. The former was repeatedly washed with distilled water and subjected to enzymatic hydrolysis. Both fractions were analysed for glucose and xylose. Enzymatic hydrolysis and the subsequent analysis served to identify pretreatments that resulted in the highest glucose yields, to be selected for SSF. The corresponding liquid fractions were used in AD. Fig. 1 describes process configuration from raw biomass to final energy products, including the implementation of enzymatic hydrolysis to test pretreatment efficiency.

2.3. Pretreatments

The 13 pretreatments assessed in the experiment (Table 1) can be divided into three main groups: autohydrolysis, consisting of steam alone at increasing time (P1–P3); alkaline pretreatment, consisting of nine combinations of steam (195 and 205 °C) and lime ($\text{Ca}(\text{OH})_2$ at 0.4 or 0.7% w/w) for 5, 10 and 15 min (P4–P12); acid pretreatment (P13) using sulphuric acid (0.2% w/w) as reference catalyst, i.e. that most frequently used in steam pretreatment.

Using steam alone, the raw material (20 mm air-dried samples) was previously immersed in water for 1 h at a 20:1 water to dry weight ratio. When adding calcium hydroxide, the raw material was impregnated in an aqueous solution containing 0.4% or 0.7% $\text{Ca}(\text{OH})_2$ at a 20:1 water to dry weight ratio, and stored in a sealed bucket for 1 h. With sulphuric acid, the same procedure was followed, using a 0.2% concentration of H_2SO_4 with the same 20:1 water to dry weight ratio. In all pretreatments, after 1 h of impregnation switchgrass was dewatered in order to remove the excess solution using a 3 L capacity press (Tinkturenpressen HP5M, Fischer Maschinenfabrik GMBH, Germany), reaching a dry matter content between 50 and 60%.

Following this step, steam explosion was performed in a reactor of 10 L capacity, loaded with an amount of impregnated switchgrass corresponding to 400 g dry matter. Steam temperature and residence time were set according to each specific pretreatment (Table 1). The reactor was connected to a computer controlling process parameters and the final discharge of pretreated material into a downstream vessel.

Discharged material was then divided into two fractions:

Table 1
Experimental conditions and associated severity factor (Log R_0) in switchgrass pretreatment.

Pretreatment	$\text{Ca}(\text{OH})_2$ (% w/w)	H_2SO_4 (% w/w)	Time (min)	Temperature (°C)	Log R_0
1	–	–	5	195	3.5
2	–	–	10	195	3.8
3	–	–	15	195	4.0
4	0.4	–	5	195	3.5
5	0.4	–	10	195	3.8
6	0.4	–	15	195	4.0
7	0.7	–	5	195	3.5
8	0.7	–	10	195	3.8
9	0.7	–	15	195	4.0
10	0.7	–	5	205	3.8
11	0.7	–	10	205	4.1
12	0.7	–	15	205	4.3
13	–	0.2	10	195	3.8

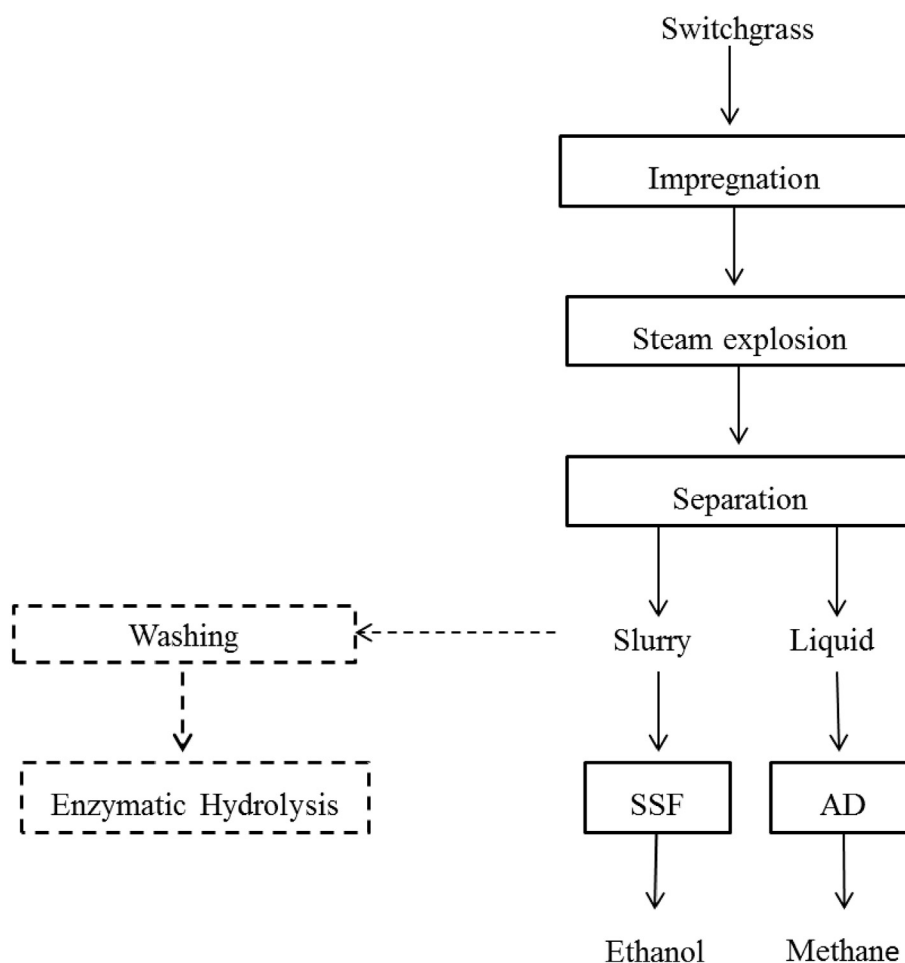


Fig. 1. Process configuration from the raw material to final ethanol and methane. Dashed graphics indicate the assessments carried out to select pretreated slurries for simultaneous saccharification and fermentation (SSF), and the corresponding liquids for anaerobic digestion (AD).

pretreatment liquid resulting from filtration through a 2.5 μm sieve, and a residual solid (slurry). The slurry was analysed for structural carbohydrates and lignin using the aforementioned methods, while pretreatment liquid was analysed for the content of total sugars (glucose, xylose and arabinose), their monomeric fractions and, by difference, the oligomeric fractions, and some inhibitors (acetic acid, formic acid, furfural and 5-hydroxymethylfurfural (HMF)), according to a U.S. National Renewable Energy Laboratory procedure [29]. In the slurry, the content of water-insoluble solids (WIS) was also determined using the method developed by Weiss *et al.* [30]. All the chemical and physical traits were analysed in duplicates.

2.4. Enzymatic hydrolysis

The slurries from the 13 pretreatments were repeatedly washed with distilled water to remove pretreatment liquid, and were subjected to enzymatic hydrolysis (Fig. 1) at a loading of 5% WIS. Hydrolysis was carried out in plastic tubes (50 mL volume) containing two steel balls to improve mixing in a rotating incubator at 100 rpm. The enzyme, CelliCTec3 (Novozymes, Bagsvaerd, Denmark), was added at an amount corresponding to 10 FPU g^{-1} WIS. Enzymatic activity was measured according to Adney and Baker [31]. Sodium acetate was used as buffer adjusted at pH 5. Hydrolysis went on for 48 h at 50 $^{\circ}\text{C}$. The pH was set manually at 5 with 10% sodium hydroxide. Following enzymatic hydrolysis, the

concentrations of glucose and xylose were determined in the slurry. All tests were conducted in duplicates.

2.5. Simultaneous saccharification and fermentation

Slurries showing the highest glucose yields during enzymatic hydrolysis, were chosen for SSF. Slurries were pressed to reach a 15% WIS content with the same procedure described previously. SSF was performed in 2 L fermenters (Infors AG, Bottmingen, Switzerland) previously sterilized at 121 $^{\circ}\text{C}$ for 20 min, using 650 g of unwashed material at 15% WIS. The pH was adjusted at 5 with 10% NaOH. Temperature in the fermenter was set at 45 $^{\circ}\text{C}$, then Cellic CTec3 (Novozymes, Bagsvaerd, Denmark) enzyme was added at 10 FPU g^{-1} WIS, and temperature was maintained at 45 $^{\circ}\text{C}$ for 20 h as a pre-hydrolysis step. Thereafter, the mixture was cooled to 35 $^{\circ}\text{C}$ and supplemented with 3 g L^{-1} of *S. cerevisiae* Ethanol Red (Lesaffre, Marq-en-Barceul, Roubaix, France) yeast, and 0.5 g L^{-1} NH_4PO_4 as nutrient source. SSF was performed at 35 $^{\circ}\text{C}$ for 96 h. Samples were taken after 2, 4, 7, 10, 12, 24, 48, 72 and 96 h, and analysed by HPLC for ethanol, monomeric sugars, acetic acid, formic acid, and sugar degradation products. All SSFs and analyses were performed in duplicates.

2.6. Anaerobic digestion

AD was performed using the method described by Hansen *et al.*

[32], to determine potential methane yield in the four pretreatment liquids corresponding to the slurries chosen for SSF. Prior to AD, the total organic carbon (TOC) content was determined in pretreatment liquids by a total carbon analyser (Shimadzu, TOC-5050A) with an auto-sampler (ASI-5000A). The carrier gas flow was set at 150 ml min⁻¹, and the working temperature was 680 °C. In parallel to this, total solids (TS) were determined drying the samples at 105 °C for 24 h, and volatile solids (VS) were determined by ashing the dried samples at 550 °C for 2 h. All analyses were conducted in duplicates.

Inoculum (active sludge) from an anaerobic digester was collected from a municipal water-treatment plant (Källbyverket, Lund, Sweden), and was maintained in mesophilic conditions (35 °C in the dark with repeated manual stirring) until the end of biogas emission. TS and VS content of the starved inoculum were determined with the same procedure used for pretreatment liquid. Thereafter, inoculum and pretreatment liquid were mixed in a 2:1 (VS/VS) ratio, to give a total 500 g broth in bottles of 1 L volume, which were kept in an incubator at 37 °C for 10 days. Anaerobic digestion was monitored using the system Yieldmaster (BlueSens[®], Herten, Germany): biogas volume was measured with precision mass flow metres (Ritter MilliGascounter[®], Bochum, Germany); methane concentration was determined with an infrared (IR) sensor, and the data were collected via BACCom units to BACVis software (BlueSens[®], Herten, Germany).

2.7. Analytical determinations

Sugars from structural carbohydrates in the raw material, slurry and pretreatment liquid were determined by HPLC equipped with a refractive index detector. Glucose, xylose, arabinose, galactose and mannose were separated using an Aminex HPX-87P column (Bio-Rad, Hercules, CA, USA) at 85 °C with a flow rate 0.5 ml min⁻¹ using water as eluent.

Ethanol, acetic acid, formic acid, furfural and HMF in pretreatment liquid were determined by HPLC with a refractive index detector, using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operating at 50 °C with a flow rate of 0.5 ml min⁻¹, using 5 mmol l⁻¹ sulphuric acid as eluent. All samples had been filtered through a filter of pore diameter 0.2 µm before analysis.

2.8. Calculations and statistical analysis

Lignin removal was calculated as proposed by Kim *et al.* [33]:

$$\text{Lignin removal} = 1 - W_L \quad (1)$$

where W_L is the fraction of residual lignin expressed as follows:

$$W_L = \frac{L \cdot Y_T}{L_0} \quad (2)$$

where L is the amount of Klason lignin in the pretreated material (g), Y_T the yield of total solids (%) determined after pretreatment, and L_0 the amount of Klason lignin in the raw material (g).

Sugar yields were calculated as percent sugar recovered after pretreatment, on the raw material basis. Specifically, glucose and xylose yields were calculated by dividing the total of each sugar determined in pretreatment liquid and washed slurry after enzymatic hydrolysis, by the total amount contained in the raw material. For each sugar, the former proportion represents pretreatment yield, while the latter is enzymatic hydrolysis yield.

Ethanol yield was calculated using the measured amounts of glucose and ethanol in the fermentation broth at the end of SSF, by the following formula:

$$Y_{\text{EtOH}} = \frac{C_{\text{EtOH}}(1 - \text{WIS}_{\text{end}}) \cdot \frac{M}{1000}}{0.51 \cdot [WIS \cdot M \cdot \sigma_{\text{glc}} + V_{\text{hyd}} \cdot C_{\text{glc}}]} \quad (3)$$

where Y_{EtOH} is the overall ethanol yield resulting from SSF (% of theoretical value); C_{EtOH} is the final concentration of ethanol (g L⁻¹); M is the total mass (g); WIS and WIS_{end} are the fractions of water insoluble solids (%) calculated at the beginning and the end of SSF, respectively; σ_{glc} is the mass fraction of glucose in pretreated fibres (g g⁻¹); V_{hyd} is the starting volume in the reactor (L); C_{glc} is the concentration of glucose at the start of SSF (g L⁻¹).

To better evaluate the effectiveness of Ca(OH)₂ as catalyst, a response surface analysis was carried out with the SigmaPlot 10 software (Systat Software Inc., Chicago, IL, USA), using Ca(OH)₂ concentration and a severity factor (Log R_0) that combines residence time and temperature, to identify optimal conditions for lignin removal. The severity factor [34] was calculated as follows:

$$\text{Log } R_0 = \text{Log} \left(t \cdot e^{\left(\frac{T - T_{\text{ref}}}{14.75} \right)} \right) \quad (4)$$

where t is the residence time (min), T pretreatment temperature (°C), and T_{ref} the reference temperature (100 °C).

The combined energy yield, i.e. ethanol from SSF, methane from AD and the amount of energy in the residual solid after SSF, was calculated per unit dry weight of the raw material, assuming 27.1, 50 and 17.4 kJ g⁻¹ energy content for ethanol, methane and solid residue, respectively [14,35].

In all traits, normal distribution and equal variance of data were controlled through the Kolmogorov–Smirnov and Bartlett tests, respectively. Data were then submitted to one way analysis of variance (ANOVA) through the CoStat 6.3 software (CoHort Software, Monterey, CA, USA). The lowest significant difference (LSD) test at $P \leq 0.05$ was used to separate means of significant traits.

3. Results and discussion

3.1. Raw material composition

On a dry weight basis, switchgrass biomass consisted of 16.0 ± 0.1% extractives, 30.3 ± 0.3% glucan, 29.0 ± 0.7% xylan, 4.2 ± 0.3% arabinan, 16.4 ± 1.3% lignin, and 2.5 ± 0.5% ash. These data are in the range of other analysis carried out on switchgrass [46,37]. However, a wide analytical range was also observed in other works, concerning extractives and ashes [10,16,38].

3.2. Pretreatment evaluation

3.2.1. Sugars and inhibitors in pretreatment liquid

Fig. 2 shows the concentration of inhibitors in pretreatment liquid: acetic acid and furfural are pentose degradation products, while formic acid and HMF are hexose degradation products. Formic acid exerts a stronger inhibition on *S. cerevisiae* [39] than acetic acid. Both acids were below the thresholds of cell death of the yeast, although concentrations above the thresholds of inhibition (6 and 4.6 g L⁻¹ for acetic and formic acid, respectively) [40] were detected in the two pretreatments conducted at high temperature (205 °C) and lime concentration (0.7%) for 10 and 15 min (P11–12). In general, even a low addition of Ca(OH)₂ (0.4%) to steam explosion enhanced the content of acetic and formic acid: 5.2 and 1.5 g L⁻¹ (average of P4–6) vs. 2.4 and 0.6 g L⁻¹ (average of P1–3), respectively. Lime concentration and temperature further augmented the level of the two respective compounds: 5.6 and 2.5 g L⁻¹ (average of P7–9); 6.2 and 4.0 g L⁻¹ (average of P10–12). Lastly, residence time

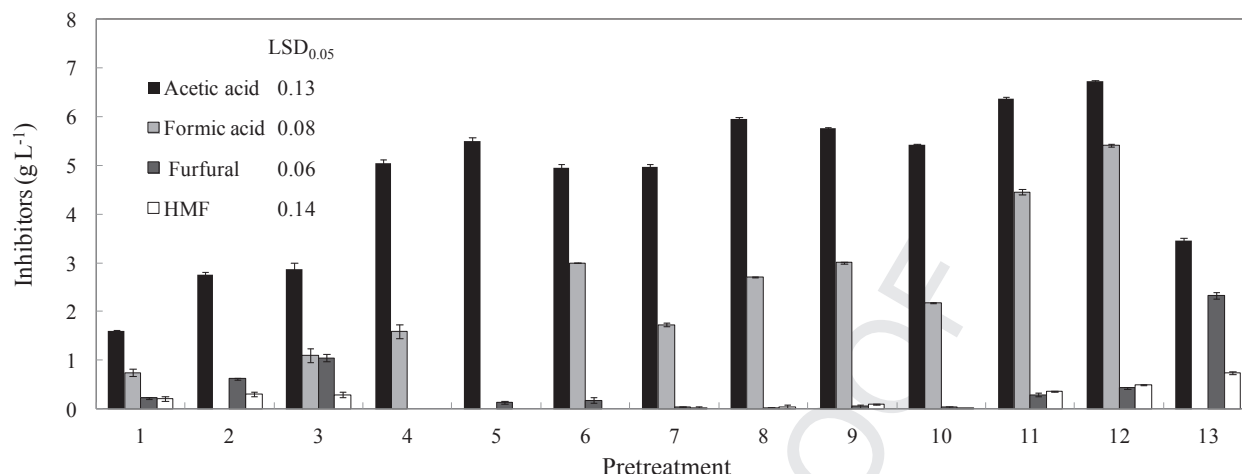


Fig. 2. Concentration of inhibitors in pretreatment liquid. Error bars show \pm SD. $LSD_{0.05}$ indicates least significant differences at $P \leq 0.05$.

only enhanced formic acid when passing from 5 to 10 min (average, 1.5 g L^{-1}) to 15 min (3.1 g L^{-1}).

In contrast to this, furfural and HMF contents were significantly increased only by acid addition to steam explosion (H_2SO_4 at 0.2%). Even so, HMF remained quite low (0.7 g L^{-1}), whereas furfural rose to a critical level (2.3 g L^{-1}). This is based on the fact that concentrations so low as $1\text{--}5 \text{ g L}^{-1}$ are acknowledged to affect fermentation, although final ethanol yield is generally uninfluenced [41].

Glucose and xylose concentration in the liquid varied with pretreatment conditions (Table 2). Glucose in its monomeric form was above detection limit only in the autohydrolysis (P1-3) and with H_2SO_4 addition (P13), which is in accordance with Balan *et al.* [42]. Glucose oligomers were always found in amounts higher than the corresponding monomer (Table 2).

Monomeric glucose concentrations with lime addition correspond to pretreatment yields (ca. 0.1%) very similar to that calculated from data of Wyman *et al.* [43] with the same catalyst. Conversely, our data with sulphuric acid corresponds to half the yield observed in the cited work [43] (3.8 vs 7.4%) with the same catalyst.

Monomeric xylose depicted a similar behaviour as glucose (Table 2): detectable amounts were only shown using autohydrolysis and, to a greater extent, acid addition. Regarding oligomeric xylose, the highest values were found using autohydrolysis

(average, 19.6 g L^{-1}). Conversely, low levels were evidenced with alkaline pretreatment under mild conditions ($195 \text{ }^\circ\text{C}$ for 5 min) (P4 and P7), and in acid addition (P13). This effect of autohydrolysis and lime in enhancing the oligomeric vs monomeric fraction of xylose is consistent with the findings of Wyman *et al.* [43].

Similar pretreatment effects were observed by Kim *et al.* [44]: high hemicellulose removal with autohydrolysis and mild acid catalysis, and strong retention ($\geq 85\%$) of initial cellulose in the solid phase. However, in the cited study [44] a higher concentration of lime was used (1 g g^{-1} of biomass), in association with lower temperature ($120 \text{ }^\circ\text{C}$), longer retention time (4 h), and higher water to solid ratio. Especially this last condition is detrimental in a perspective of full scale operation, hampering lime recovery at the end of the process. Compared to this, our study aimed for a pretreatment route compatible with lime recovery in a frame of reduced environmental impact.

3.2.2. Lignin removal

The amount of lignin removed from the raw material after pretreatment ranged between 9 and 38% (data not shown). Steam alone was least effective in removing lignin (average of P1-3, 11.5%). Supplying $\text{Ca}(\text{OH})_2$ at low concentration (0.4%) and standard temperature ($195 \text{ }^\circ\text{C}$), lignin removal increased to an average 18.1%. At high concentration (0.7%), the best delignification was achieved (34.6%). At high concentration and temperature ($205 \text{ }^\circ\text{C}$), almost the same result was obtained (30.9%). Compared to this, supplying H_2SO_4 determined a modest lignin removal (14.6%).

The overall effect of lime concentration, time and temperature, the latter two combined in the severity factor (eq. (4)), is best depicted by the plot of lignin removal in response to $\text{Ca}(\text{OH})_2$ and $\text{Log } R_0$ (Fig. 3): the alkaline catalyst played a stronger role in lignin removal, than the increase in severity. Based on this, the highest delignification occurred at high lime concentration, in combination with a moderate severity. In a previous study on sugarcane bagasse with the same pre-treatments except acid addition, a similar pattern was shown [45], although the severity factor was more adversely related to the amount of lignin removed as the likely consequence that bagasse is a post-process residue instead of a raw substrate as switchgrass.

Garlock *et al.* [46] observed a similar pattern of lignin removal in switchgrass upon the effect of multiple pretreatments. However, they obtained a stronger lignin removal (50% vs 33% in this study) with higher lime addition ($1 \text{ g Ca}(\text{OH})_2 \text{ g}^{-1}$ vs 0.125 g g^{-1} in this study) and water to solid ratio (16:1 vs 1:1), although less severe

Table 2
Sugars in pretreatment liquid.

Pretreatment	Glucose (g L^{-1})		Xylose (g L^{-1})	
	Monomer	Oligomer	Monomer	Oligomer
1	1.9	3.2	1.1	17.1
2	1.8	3.2	3.9	25.4
3	1.5	2.1	5.1	17.7
4	<i>b.d.l.</i>	2.3	0.2	6.7
5	<i>b.d.l.</i>	3.9	0.3	16.7
6	<i>b.d.l.</i>	3.7	<i>b.d.l.</i>	15.6
7	<i>b.d.l.</i>	3.1	<i>b.d.l.</i>	12.4
8	<i>b.d.l.</i>	4.6	<i>b.d.l.</i>	20.1
9	<i>b.d.l.</i>	4.3	<i>b.d.l.</i>	19.1
10	<i>b.d.l.</i>	3.9	<i>b.d.l.</i>	18.8
11	<i>b.d.l.</i>	4.4	<i>b.d.l.</i>	18.2
12	<i>b.d.l.</i>	4.2	<i>b.d.l.</i>	14.0
13	4.3	2.5	16.0	13.8
$LSD_{0.05}$	0.02	0.16	0.09	0.81

b.d.l. means below detection limit. $LSD_{0.05}$ indicates least significant differences at $P \leq 0.05$.

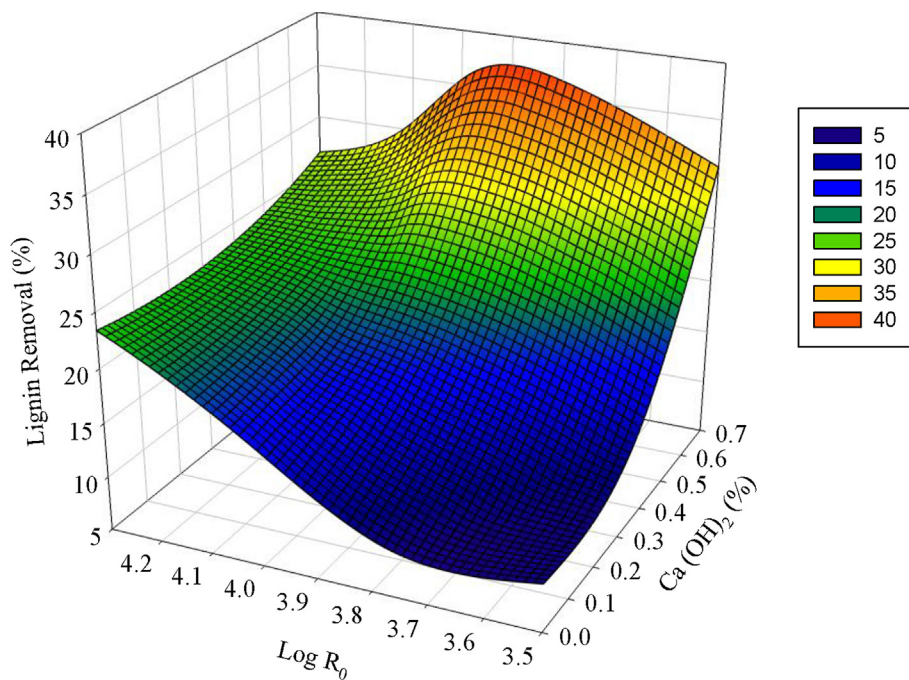


Fig. 3. Lignin removal in response to severity factor ($\text{Log } R_0$) and $\text{Ca}(\text{OH})_2$ concentration during pretreatment.

conditions were adopted ($\text{Log } R_0$ 3.0 vs 3.9). Thus, it appears that the two former factors played a major role in enhancing switchgrass delignification. However, the cost and the burden associated with higher catalyst dosage and more diluted pretreatment should be accounted for, in the perspective of full scale operation.

3.2.3. Glucose and xylose yield

Glucose and xylose yields in the slurry after enzymatic hydrolysis and in pretreatment liquid exhibit a contrasting picture between the two sugars (Figs. 4 and 5), as observed in other studies on switchgrass [38,40]. In general, glucose featured a much higher recovery in the slurry following enzymatic hydrolysis (on average 61%), than in pretreatment liquid (on average 7%). Hence, this sugar

partitioned more to the solid fraction (slurry) aimed for SSF, in accordance with its intended use. Xylose showed a more balanced yield between slurry (on average 27%) and pretreatment liquid (on average 33%). The overall yield of glucose and xylose in the two combined fractions achieved a similar share (68 and 60%) of the respective amounts of glucan and xylan contained in the raw material.

Large differences were observed among pretreatment conditions (Fig. 4). In general, the addition of alkaline catalyst did not increase the two sugars' yield, whereas the acid catalyst improved glucose recovery (in both fractions), and also xylose recovery (only in pretreatment liquid). With strong lime addition, Wyman *et al.* [43] obtained a 35% xylose release in pretreatment liquid vs. 27% in

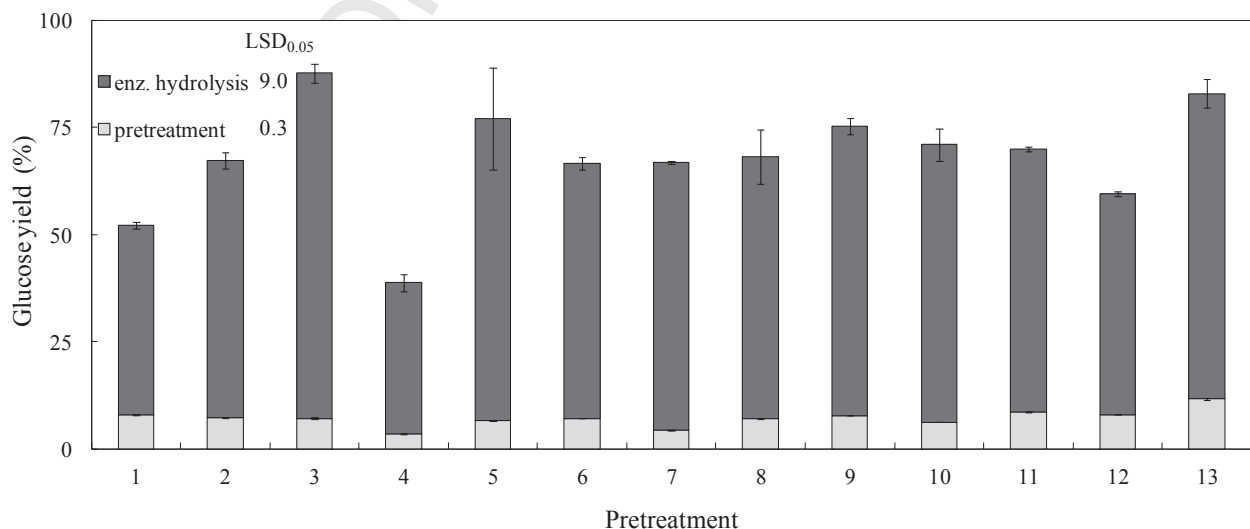


Fig. 4. Glucose (monomer and oligomers) yield in pretreatment liquid and slurry following enzymatic hydrolysis, as percentage of total glucan content in the raw material. Error bars show \pm SD. $\text{LSD}_{0.05}$ indicates least significant differences at $P \leq 0.05$.

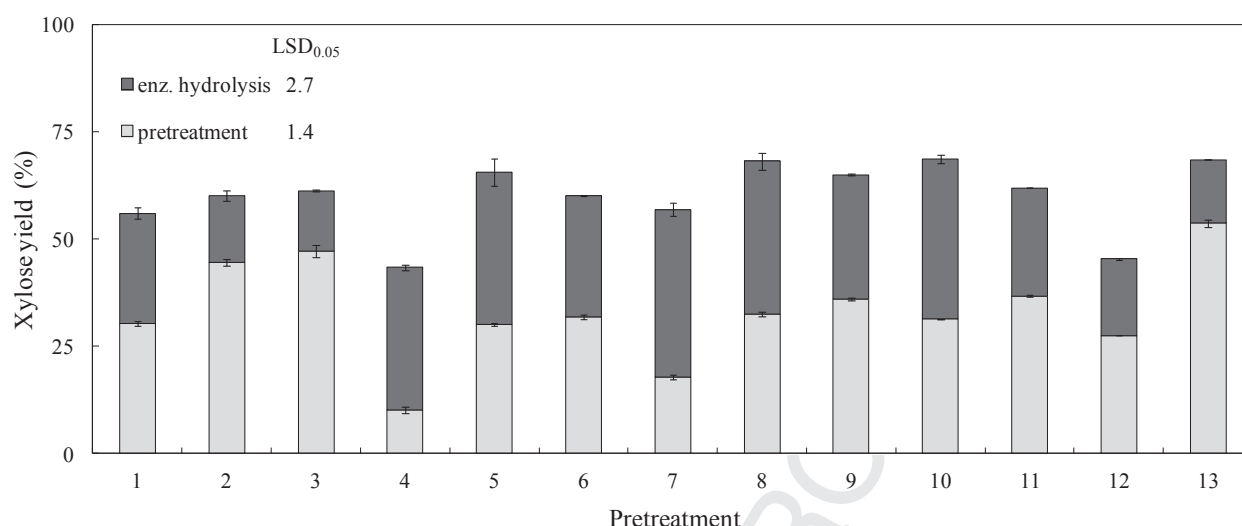


Fig. 5. Xylose (monomer and oligomers) yield in pretreatment liquid and slurry following enzymatic hydrolysis, as percentage of total xylan content in the raw material. Error bars show \pm SD. $LSD_{0.05}$ indicates least significant differences at $P \leq 0.05$.

this study, in exchange for a lower solubilisation of cellulose (1.5% vs 6.5% in this study).

Concerning the effect of acid catalyst, Wyman *et al.* [43] reported a higher xylose yield in pretreatment liquid (74% vs 53% in this study) in exchange for a lower glucose yield (7% vs. 12%), under conditions of higher acid concentration (2.5 vs 0.2%), lower severity ($\text{Log } R_0$, 2.8 vs 3.8), and higher water to solid ratio (9:1 vs 1:1) than in this study. Dien *et al.* [47] obtained the same glucose release in pretreatment liquid as in this study (11%), operating on switchgrass at an earlier stage (anthesis), i.e. potentially easier to be degraded. It appears, therefore, that in the cited work a lower severity ($\text{Log } R_0$, 2.8 vs 3.8 in this study) compensated for a much higher acid concentration (2.5 vs 0.2%) and water to solid ratio (9:1 vs 1:1) than in this study.

Increased residence time enhanced glucose enzymatic yield in autohydrolysis (P1-3) and lime at low concentration and temperature (P4-6), but not at high concentration and temperature (P7-12). A generally higher yield in glucose enzymatic hydrolysis was observed in a previous study on sugarcane bagasse [45], apparently due to the fact that bagasse is a substrate loosened by previous sugarcane processing, requiring lower pretreatment intensity than switchgrass raw biomass.

A positive effect of time was also observed in xylose pretreatment yield, including high Ca(OH)_2 concentration (P7-9). However, for this sugar increases in pretreatment yield tended to be compensated by decreases in enzymatic hydrolysis yield.

Lime did not improve enzymatic hydrolysis of the slurry, in contrast to other studies [43,46], where higher yields were evidenced for both hemicellulose and cellulose. This difference could be due to lower solid loading (1% vs 5%) associated to longer residence time (168 vs 48 h) than in this study. The two factors combined contribute to enhance the solubilisation of the solid fraction, as demonstrated by Pallapolu *et al.* [48].

In general, a higher degradation of cellulose was obtained in exchange for a lower hydrolysis of hemicellulose, with respect to other experiments on switchgrass [43,45,48]. This contrasting effect on the two fibre components is likely due to the lower moisture of switchgrass during steam explosion in this study (ca. 45% vs. 90% in the cited cases). In fact, a high water to solid ratio as in the cited works [43,46,49] facilitates biomass degradation during steam explosion. This is based on the assumption that high water

availability can better penetrate cell structure, hydrate cellulose, but especially, remove hemicellulose [20]. This, in turn, may explain the higher amount of hemicellulose hydrolysed during pretreatment with biomass at high moisture content, or under high water to solid ratio. However, massive use of water involves a proportionally higher amount of energy required for pretreatment, sugar recovery and downstream processes, resulting in a relevant drawback from several viewpoints.

3.3. Simultaneous saccharification and fermentation

Based on high glucose yields shown in enzymatic hydrolysis (Fig. 4), pretreatments 3, 5, 9 and 13 were selected for SSF at high WIS content (15%). The four slurries diverged in the amount of initial glucose (Fig. 6): 41 g L⁻¹ in P3 (steam pretreatment alone) vs. an average of 49 g L⁻¹ with alkaline (P5 and P9) or acid catalyst (P13). Despite this, glucose depletion followed the same trend during SSF: steep linear decrease from the aforementioned levels to ca. 5 g L⁻¹ in the first 10 h (average glucose consumption rate, 4.5 g L⁻¹ h⁻¹), followed by slow decrease to almost nil at the end of the process (average consumption rate, 0.05 g L⁻¹ h⁻¹).

In parallel to this, ethanol concentration increased from zero to ca. 30 g L⁻¹ in the first 20 h, settling around this figure for the rest of time. This pattern corresponds to a first-order kinetics: in fact, the four pretreatments fit this curve with very good precision ($R^2 \geq 0.95^{**}$) (function parameters not shown). However, the cumulated amount of ethanol at the end of SSF outlined statistical differences: autohydrolysis (P3) attained a ca. 10% lower ethanol (29.8 g L⁻¹) than Ca(OH)_2 addition at 0.4% (P5) (33.5 g L⁻¹). The other two pretreatments with alkaline catalyst at high dose (P9), and with acid catalyst (P13) featured an intermediate 31.5 g L⁻¹ of ethanol.

This is in contrast with enzymatic hydrolysis showing a higher glucose yield in P3 than P5 (80 vs. 70%) (Fig. 4): owing to this, the former pretreatment was expected to yield more ethanol. However, this result may be explained with a sort of alkaline detoxification associated with use of calcium hydroxide in pretreatment, resulting in a better fermentation [50].

In general, the ethanol yield obtained with lime addition ranged between 65 and 76% of the theoretical (eq. (3)) for P9 and P5, respectively. These data were slightly lower than those obtained on

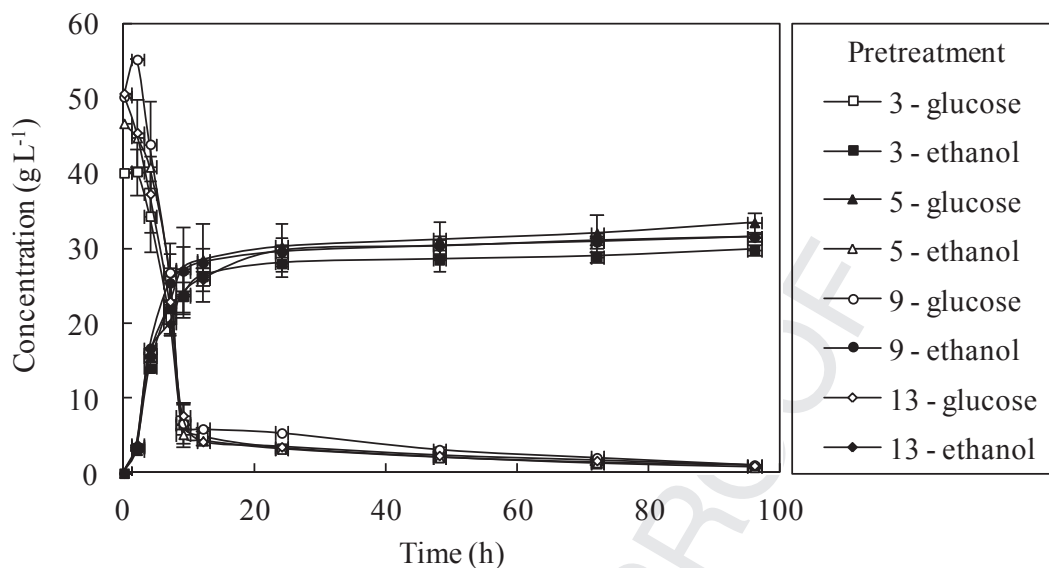


Fig. 6. Concentration of glucose (empty symbols) and ethanol (filled symbols) during the simultaneous saccharification and fermentation carried out on the slurries of four selected pretreatments. Error bars show \pm SD.

switchgrass by Chang *et al.* [20] (70–90%), which may be explained by the slightly higher enzyme loading (25 FPU g^{-1} cellulose) and moisture (WIS, ca. 5%) adopted in the cited work during SSF runs. It has already been observed [51–53] that running SSF experiments at high WIS concentration decreases percent ethanol yield on the theoretical, even though the resulting sugar concentration and subsequent ethanol concentration increase. In fact, ethanol concentrations at the end of SSF in this study (Fig. 6) were higher than those obtained in SSF's conducted at lower WIS: these latter ranged between 14 and 22 $g L^{-1}$ [20,54,55]. Final ethanol concentration significantly affects processing costs, in particular distillation [56]. This is especially true in light of the fact that an industrial titre 40 $g L^{-1}$ is indicated as threshold for profitable processing [57,58].

3.4. Anaerobic digestion

The four pretreatment liquids selected for AD consistently varied in TOC, TS and VS (Table 3). Acid catalysis (P13) attained the highest levels of TOC and TS, in accordance with a higher recovery of soluble sugars in the liquid fraction (Figs. 4 and 5). Autohydrolysis (P3) exhibited slightly lower TOC and TS, in exchange for higher VS. Lastly, lime (P5 and P9) featured the lowest TOC, TS and VS. This, too, reflects a generally low concentration of xylose in the two pretreatments with lime (Figs. 4 and 5). In fact, utilization of alkaline conditions favours the release of polymeric sugars from hemicellulose during pretreatment, compared to autohydrolysis or

Table 3
Characteristics of four selected pretreatment liquids, and methane yield after anaerobic digestion.

Pretreatment	TOC ($g L^{-1}$)	TS (%)	VS (% TS)	CH ₄ (NmL g^{-1} VS)
3	21.3	2.4	87.6	137.5
5	16.5	2.2	69.3	226.4
9	17.8	1.9	58.9	300.5
13	25.6	2.8	83.4	281.8
LSD _{0.05}	2.2	0.6	14.9	139.3

TOC, total organic carbon; TS, total solids; VS, volatile solids. LSD_{0.05} indicates least significant differences at $P \leq 0.05$.

acidic conditions. Most of these sugars are transformed into monomeric sugars only after enzymatic hydrolysis, hence they are not completely available for AD in pretreatment liquid.

AD demonstrated a much higher CH₄ output per unit VS after alkaline or acid addition to steam explosion (Table 3). In the case of lime, a dose response is also perceived. The effect of chemical pretreatment on lignocellulosic biodegradability is already acknowledged in the literature [59,60]. However, in this study such effect extends to a liquid with a low level of all inhibitors (Fig. 2), thus assumed to be easily degradable.

3.5. Combined energy yield

Combined energy yield (ethanol, methane and residual solid) best shows pretreatment effects referred to unit dry weight of switchgrass biomass (Fig. 7). Energy from ethanol increased from 3.2 $kJ g^{-1}$ in autohydrolysis (P3) to 4.2 $kJ g^{-1}$ with lime at low concentration (P5), whereas lime at high concentration (P9) and acid addition (P13) did not improve this trait with respect to P3. In contrast to this, energy from methane was remarkably increased only by acid addition (1.7 $kJ g^{-1}$ in P13 vs 0.8 $kJ g^{-1}$ in P3). Lastly, residual energy outlined the same trend as ethanol: significant increase with lime at low concentration (8.3 $kJ g^{-1}$ in P5 vs 7.2 $kJ g^{-1}$ in P3); no increase with high lime concentration (P9) and sulphuric acid (P13).

Therefore, pretreatments generally enhanced the energy output, as it concerns the two biofuels ethanol and methane. Conversely, strong pretreatments (P9 and P13) had less residual solid than pretreatments with no (P3) or low (P5) catalyst concentration, and this negatively affected the amount of residual energy. As a result, autohydrolysis and the two strong pretreatments were substantially equivalent in terms of combined energy yield (7.2, 7.2 and 7 $kJ g^{-1}$ in P3, P9 and P13, respectively), while low lime concentration (P5) was top ranking (8.3 $kJ g^{-1}$).

Although residual energy may not completely be exploited as it cannot easily be transported as a liquid (ethanol) or gaseous (methane) fuel, the fact remains that residual solid can be used for pellets, or for steam and power generation for internal uses at a power plant. Thus residual energy has to be accounted for, in the

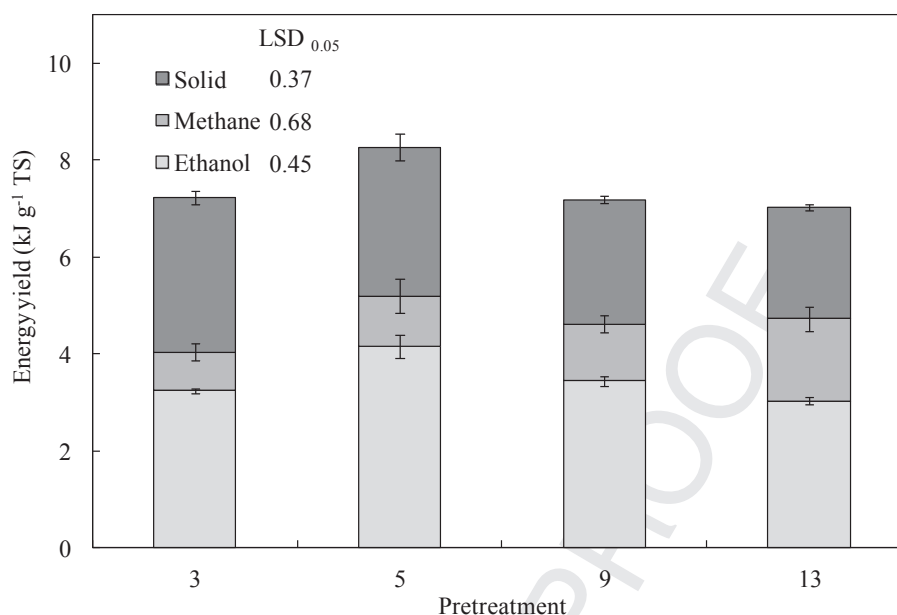


Fig. 7. Combined energy yield per unit dry weight of the raw material, in terms of ethanol from SSF, methane from AD, and energy content of the residual solid. Error bars show \pm SD. $LSD_{0.05}$ indicates least significant differences at $P \leq 0.05$.

overall product yield. Its weight on combined energy yield consistently declined from 44% in autohydrolysis to 33% in acid catalysis, further proving that mild pretreatment conditions as steam alone leave a relevant share of the total energy unexploited in the final residue.

In the literature, higher values of energy output from analogous configuration processes are reported for lignocellulosic sources as corn stover [14] and oat straw [61]. However, the cited studies evidenced a weaker benefit from pretreatments on the combined ethanol and methane: ca. +15% and +3% energy output with acid addition in the two respective sources, compared to +29% with lime addition at low concentration (P5) in this study.

4. Conclusion

The aim of this study was to compare the effects of steam explosion alone and after impregnation with calcium hydroxide or dilute sulphuric acid on switchgrass, in order to test lime as potential substitute for acid catalyst. Lime showed a great potential when ethanol was focused, whereas acid addition produced higher methane yield. The latter outcome, in association with low concentration of inhibiting compounds in pretreatment liquid, proves that restrained use of sulphuric acid may not be detrimental in steam explosion. However, low concentration of lime was less aggressive and secured more residual solid after SSF, resulting in higher energy potential per unit raw biomass.

More to this, utilization of lime favours the release of polymeric sugars from hemicellulose during pretreatment. Thus, lime impregnation could be well suited for applications where hemicellulose sugars will be used for, e.g., production of bioplastics. This could be an alternative pathway to anaerobic digestion, leading to high added value products.

The low water to solid ratio adopted in this study is the premise for reductions in the amount of water consumed during pretreatment, while the use of calcium hydroxide and its final recovery as calcium carbonate avoid to handle effluents containing toxic compounds in downstream processing, and provide a marketable by-product for agricultural applications.

Lastly, simultaneous saccharification and fermentation at high concentration of solids (15% WIS) improved previous records in final ethanol concentration. Further increases may be envisaged through augmented solids concentration (20% WIS). However, this option is responsible for lower ethanol yield on the theoretical maximum, hence potential benefits are at least partially offset.

Acknowledgements

Research carried out under the BIOSEA Project, funded by MiPAAF (Italy).

References

- [1] B. Hahn-Hagerdal, M. Galbe, M.F. Gorwa-Grauslund, G. Liden, G. Zacchi, Bioethanol – the fuel of tomorrow from the residue of today, *Trends Biotechnol.* 24 (2006) 549–556.
- [2] C. Boehemmel, I. Lewandoski, W. Claupein, Comparing annual and perennial energy cropping systems with different management intensities, *Agric. Syst.* 96 (2008) 224–236.
- [3] S. Fazio, L. Barbanti, Energy and economic assessments of bio-energy systems based on annual and perennial crops for temperate and tropical areas, *Renew. Energ* 69 (2014) 233–241.
- [4] D. Tilman, R. Socolow, R. Foley, J.A. Hill, E. Larson, L. Lynd, et al., Beneficial biofuels – the food, energy, and environmental trilemma, *Science* 325 (2009) 270–271.
- [5] C.M. Smith, M.B. David, C.A. Mitchell, M.D. Masters, K.J. Anderson-Teixera, et al., Reduced nitrogen losses after conversion of row crop agriculture to perennial biofuel crops, *J. Environ. Qual.* 42 (2013) 219–228.
- [6] F. Cattaneo, L. Barbanti, P. Gioacchini, C. Ciavatta, C. Marzadori, ¹³C abundance shows effective soil carbon sequestration in Miscanthus and giant reed compared to arable crops under Mediterranean climate, *Biol. Fertil. Soils* 50 (2014) 1121–1128.
- [7] F. Cattaneo, P. Di Gennaro, L. Barbanti, C. Giovannini, M. Labra, B. Moreno, et al., Perennial energy cropping systems affect soil enzyme activities and bacterial community structure in a South European agricultural area, *Appl. Soil Ecol.* 84 (2014) 213–222.
- [8] D.R. Keshwani, J.J. Cheng, Switchgrass for bioethanol and other value-added applications: A review, *Bioresour. Technol.* 100 (2009) 1515–1523.
- [9] S.N. Naik, V. Vaibhav, K.R. Prasant, K.D. Ajay, Production of first and second generation biofuels: A comprehensive review, *Renew. Sust. Energ Rev.* 14

- (2010) 578–597.
- [10] M. Galbe, G. Zacchi, Advances in biochemical engineering/biotechnology, in: T. Sheper (Ed.), Pretreatment of Lignocellulosic Materials for Efficient Bioethanol Production, fifth ed., Springer, London Heidelberg New York Dordrecht, 2007, pp. 41–65.
- [11] S.S. Toor, L. Rosendahl, J. Hoffmann, J.B.H. Holm-Nielsen, E.A. Ehimen, Pretreatment techniques for biofuels and biorefineries, in: Zhen Fang (Ed.), Lignocellulosic Biomass Thermal Pre-treatment with Steam, first ed., Springer, London Heidelberg New York Dordrecht, 2013, pp. 59–76.
- [12] P. Sassner, C.G. Mårtensson, M. Galbe, G. Zacchi, Steam pretreatment of H₂SO₄-impregnated salix for the production of bioethanol, *Bioresour. Technol.* 99 (1) (2008) 137–145.
- [13] C. Martín, M. Galbe, N.O. Nilvebrant, J.L. Jönsson, Comparison of the fermentability of enzymatic hydrolyzates of sugarcane bagasse pretreated by steam explosion using different impregnating agents, *Appl. Biochem. Biotechnol.* Vols (2002) 98–100.
- [14] C. Carrasco, H.M. Baudel, J. Sendelius, T. Modig, C. Roslander, M. Glabe, B. Hahn-Hagerdal, et al., SO₂-catalyzed steam pretreatment and fermentation of enzymatically hydrolyzed sugarcane bagasse, *Enzyme Microb. Tech.* 46 (2) (2010) 64–73.
- [15] P.M. Bondesson, M. Galbe, G. Zacchi, Ethanol and biogas production after steam pretreatment of corn stover with or without the addition of sulphuric acid, *Biotechnol. Biofuels* 6 (2013) 1–11.
- [16] L.P. Ramos, The chemistry involved in the steam pre-treatment of lignocellulosic materials, *Quim. Nova* 26 (2003) 863–871.
- [17] M. Thomsen, Complex media from processing of agricultural crops for microbial fermentation, *Appl. Microbiol. Biotechnol.* 68 (5) (2005) 598–606.
- [18] L.L.G. Fuentes, S.C. Rabelo, R.M. Filho, A.C. Costa, Kinetics of lime pretreatment of sugarcane bagasse to enhance enzymatic, *Appl. Biochem. Biotechnol.* 2011 (163) (2011) 612–625.
- [19] T.H. Kim, Y.Y. Lee, Pretreatment and fractionation of corn stover by ammonia recycle percolation process, *Bioresour. Technol.* 97 (2006) 224–232.
- [20] V.S. Chang, W.E. Kaar, B. Burr, M.T. Holtzapple, Simultaneous saccharification and fermentation of lime-treated biomass, *Biotechnol. Lett.* 23 (2001) 1327–1333.
- [21] C. Patané, S. La Rosa, A. Pellegrino, I. Di Silvestro, Antitraspirante su pomodoro contro stress idrico e scottature, *Inf. Agrar.* 26 (2012) 52–54.
- [22] C.E. Wyman, B.E. Dale, R.T. Elander, M. Holtzapple, M.R. Ladisch, Y.Y. Lee, Coordinated development of leading biomass pretreatment technologies, *Bioresour. Technol.* 96 (2005) 1959–1966.
- [23] A. Romani, F. Pereira, B. Johansson, L. Domingues, Metabolic engineering of *Saccharomyces cerevisiae* ethanol strains PE-2 and CAT-1 for efficient lignocellulosic fermentation, *Bioresour. Technol.* 79 (2015) 150–158.
- [24] P.M. Bondesson, M. Galbe, G. Zacchi, Comparison of energy potentials from combined ethanol and methane production using steam pretreated corn stover impregnated with acetic acid, *Biomass bioenerg.* 67 (2014) 413–424.
- [25] F. Cherubini, The biorefinery concept: Using biomass instead of oil for producing energy and chemicals, *Eng. Convers. Manag.* 51 (2010) 1412–1421.
- [26] M.J. Metzger, R.G.H. Bunce, R.H.G. Jongman, C.A. Múcher, J.W. Watkins, A climatic stratification of the environment of Europe, *Glob. Ecol. Biogeogr.* 14 (2005) 549–563.
- [27] A. Sluiter, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of Extractives in Biomass, NREL, Golden, CO, USA, 2005.
- [28] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, Determination of Structural Carbohydrates and Lignin in Biomass, NREL, Golden, CO, USA, 2008.
- [29] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples, NREL, Golden, CO, USA, 2008.
- [30] N.D. Weiss, J.J. Stickel, J.L. Wolfe, Q.A. Nguyen, A simplified method for the measurement of insoluble solids in pretreated biomass slurries, *Appl. Biochem. Biotechnol.* 162 (2010) 975–987.
- [31] B. Adney, J. Baker, Measurement of Cellulase Activities, NREL, Golden, CO, USA, 2008.
- [32] T.L. Hansen, J.E. Schmidt, I. Angelidaki, E. Marca, J.C. Jansen, H. Mosbaek, et al., Method for determination of methane potentials of solid organic waste, *Waste Manag.* 38 (2004) 393–400.
- [33] S. Kim, M.T. Holtzapple, Effect of structural features on enzyme digestibility of corn stover, *Bioresour. Technol.* 97 (2006) 583–591.
- [34] R.P. Overend, E. Chornet, Fractionation of lignocellulosics by steam-aqueous pretreatments, *Philos. Trans. R. Soc. Lond.* 321 (1987) 523–536.
- [35] S.B. McLaughlin, R. Samson, D. Bransby, A. Wiseloge, Evaluating physical, chemical, and energetic properties of perennial grasses as biofuels, Proceedings of Bioenergy '96-The Seventh National Bioenergy Conference, Nashville, TN, USA, 15–20 September 1996, 1–8.
- [36] L. Suryawati, M.R. Wilkins, D.D. Bellmer, R.L. Huhnke, N.O. Maness, I.M. Banat, Effect of hydrothermolysis process conditions on pretreated switchgrass composition and ethanol yield by SSF with *Kluyveromyces marxianus* IMB4, *Process Biochem.* 44 (2009) 540–545.
- [37] A. Isci, J.N. Himmelsbach, A.L. Pometto, D.R. Raman, R.P. Anex, Aqueous ammonia soaking of switchgrass followed by simultaneous saccharification and fermentation, *Appl. Biochem. Biotechnol.* 144 (2008) 69–77.
- [38] H. Alizadeh, F. Teymouri, T.I. Gilbert, B.E. Dale, Pretreatment of switchgrass by ammonia fiber explosion (AFEX), *Appl. Biochem. Biotechnol.* 121–124 (2005) 1133–1142.
- [39] L.J. Jönsson, E. Palmqvist, N.O. Nilvebrant, Bioconversion of lignocellulose: inhibitors and detoxification, *Biotechnol. Biofuels* 6 (2013) 1–10.
- [40] S. Larsson, E. Palmqvist, B. Hahn-Hägerdal, C. Tengborg, K. Stenberg, G. Zacchi, et al., The generation of fermentation inhibitors during dilute acid hydrolysis of softwood, *Enzyme Microb. Tech.* 24 (1999) 151–159.
- [41] L.J. Boyer, J.L. Vega, K.T. Klasson, E.C. Clausen, J.L. Gaddy, The effects of furfural on ethanol production by *Saccharomyces cerevisiae* in batch culture, *Biomass Bioenerg.* 3 (1) (1992) 41–48.
- [42] V. Balan, S. Kumar, B. Bals, S. Chundawat, M. Jin, B. Dale, Biochemical and thermochemical conversion of switchgrass to biofuels, in: A. Monti (Ed.), Switchgrass: a Valuable Crop for Energy, Springer, London Heidelberg New York Dordrecht, 2012, pp. 153–186.
- [43] C.E. Wyman, V. Balan, B.E. Dale, R.T. Elander, M. Falls, B. Hames, et al., Comparative data on effects of leading pretreatments and enzyme loadings and formulations on sugar yields from different switchgrass sources, *Bioresour. Technol.* 102 (2011) 11052–11062.
- [44] Y. Kim, N.S. Mosier, M.R. Ladish, V.R. Pallapolu, Y.Y. Lee, R. Garlock, et al., Comparative study on enzymatic digestibility of switchgrass varieties and harvests processed by leading pretreatment technologies, *Bioresour. Technol.* 102 (2011) 11089–11096.
- [45] L. Capecchi, M. Galbe, L. Barbanti, O. Wallber, Combined ethanol and methane production using steam pretreated sugarcane bagasse, *Ind. Crop Prod.* 74 (2015) 255–262.
- [46] R.J. Garlock, V. Balan, B.E. Dale, V.R. Pallapolu, Y.Y. Lee, Y. Kim, et al., Comparative material balances around pretreatment technologies for the conversion of switchgrass to soluble sugars, *Bioresour. Technol.* 102 (2011) 11063–11071.
- [47] B.S. Dien, H.-S.G. Jung, K.P. Vogel, M.D. Casler, J.F.S. Lamb, L. Iten, et al., Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass, *Biomass Bioenerg.* 30 (2006) 880–891.
- [48] V.R. Pallapolu, Y.Y. Lee, R.J. Garlock, V. Balan, B.E. Dale, Y. Kim, et al., Effects of enzyme loading and b-glucosidase supplementation on enzymatic hydrolysis of switchgrass processed by leading pretreatment technologies, *Bioresour. Technol.* 102 (2011) 11115–11120.
- [49] C. Li, B. Knierim, C. Manisseri, R. Arora, H.V. Scheller, M. Auer, et al., Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification, *Bioresour. Technol.* 101 (2010) 4900–4906.
- [50] P. Persson, J. Andersson, L. Gorton, S. Larsson, N.-O. Nilvebrant, L.J. Jönsson, Effect of different forms of alkali treatment on specific fermentation inhibitors and on fermentability of lignocellulose hydrolysates for production of fuel ethanol, *J. Agric. Food Chem.* 50 (2002) 5318–5325.
- [51] H. Jørgensen, J. Vibe-Pedersen, J. Larsen, Felby, Liquefaction of lignocellulose at high solids concentrations, *Biotechnol. Bioeng.* 96 (2007) 862–870.
- [52] A. Mohagheghi, M. Tucker, K. Grohmann, C. Wyman, High Solids Simultaneous Saccharification and fermentation of pre-treated wheat straw to ethanol, *Appl. Biochem. Biotechnol.* 33 (1992) 67–81.
- [53] E. Varga, H.B. Klinke, K. Reczey, A.B. Thomsen, High solid simultaneous saccharification and fermentation of wet oxidized corn stover to ethanol, *Biotechnol. Bioeng.* 88 (2004) 567–574.
- [54] Y.C. Chung, A. Bakalinsky, M. Penner, Enzymatic saccharification and fermentation of xylose-optimized dilute acid-treated lignocellulosics, *Appl. Biochem. Biotechnol.* 124 (2005) 947–961.
- [55] B.A. Faga, M.R. Wilkins, M.B. Banat, Ethanol production through simultaneous saccharification and fermentation of switchgrass using *Saccharomyces cerevisiae* D5A and thermotolerant *Kluyveromyces marxianus* IMB strains, *Bioresour. Technol.* 101 (2010) 2273–2279.
- [56] A. Wingren, M. Galbe, G. Zacchi, Techno-economic evaluation of producing ethanol from softwood: Comparison of SSF and SHF and identification of bottlenecks, *Biotechnol. Prog.* 19 (2003) 1109–1117.
- [57] G. Zacchi, A. Axelsson, Economic-evaluation of preconcentration in production of ethanol from dilute sugar solutions, *Biotechnol. Bioeng.* 34 (1989) 223–233.
- [58] R. Katzen, P.W. Madson, G.D. Moon, Alcohol distillation the fundamentals, in: K.A. Jacques, T.P. Lyons, D.R. Kelsall (Eds.), The Alcohol Textbook, Nottingham University Press, Nottingham (UK), 1999, pp. 103–125.
- [59] M.J. Taherzadeh, K. Karimi, Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review, *Int. J. Mol. Sci.* 9 (2008) 1621–1651.
- [60] E. Bruni, A.P. Jensen, I. Angelidaki, Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production, *Bioresour. Technol.* 101 (22) (2010) 8713–8717.
- [61] D.Y. Dereriv, S. Trobro, M.H. Momeni, H. Hansson, J. Blomqvist, V. Passoth, et al., Improved bio-energy yields via sequential ethanol fermentation and biogas digestion of steam exploded oat straw, *Bioresour. Technol.* 102 (2011) 4449–4455.