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Integrated conversion of agroindustrial residue with high pressure CO_2 within biorefinery concept

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Abstract

Sustainable production of energy and other added-value products from of biomass-derived polysaccharides is a key challenge of an efficient biorefinery facility. Most technologies for biomass processing are energy demanding and use significant amount of chemicals and catalysts. A need to develop a process which devoid of all these shortcomings associated with conventional processes in emphasized. A new approach is demonstrated for an integrated wheat straw biorefinery using a green technology, high-pressure CO_2-H_2O, to produce oligosaccharides from hemicellulose fraction and to enhance the cellulose digestibility during the enzymatic hydrolysis. Over the range of reaction conditions (130, 215, 225°C and 0 to 54 bar of CO_2), CO_2 adds value to the process by *in-situ* formation of carbonic acid that leads to higher dissolution of hemicellulose into xylo-oligosaccharides and xylose and to use of less energy in comparison with water-only technologies. Without an additional chemical catalyst, high-pressure CO_2-H_2O outperformed hydrothermal reactions and gave much higher total sugars' yield for wheat straw (as high as 84% in comparison with 67.4% with auto-hydrolysis at 10 °C higher temperature). Apart from the results obtained for valorisation of hemicellulose fraction, the both chemical and physical effect of CO_2 coupled to enzymatic hydrolysis resulted in a glucan conversion to glucose yield of 82% which consists of 26% improvement over those obtained during auto-hydrolysis. The influence of the high pressure reaction on the processed solid was examined by the spectroscopic methods (namely Scanning Electron Microscopy and Fourier Transform Infrared Spectroscopy).

The produced results suggest that supercritical CO_2-based method was found to be a very promising alternative technology allowing an integrated biomass processing within a biorefinery concept.

Introduction

Global concerns about the demand of fossil resources coupled with quick reduction of readily and economically reachable fossil feedstock as well as a security of their supply and environmental impacts has carried out a global shift of mankind to seek alternative technologies and sustainable sources of energy, materials and chemicals.[1-3] One of the ways to provide a sustainable supply chain is the use of renewable lignocellulosic biomass as a source of production of energy, materials and chemicals. Biofuels and valuable products can be obtained by depolymerisation of biomass polysaccharides such as cellulose and hemicellulose. The selectively depolymerisation typically involves a pre-treatment process, which is one of the most important step in the development of biorefineries, since it has a significant influence on the efficiency and economy of the overall process.[4,5] Several biomass pre-treatment technologies such as dilute acid,[6] alkaline hydrolysis,[7] hydrothermal processes[8-10], and high temperature solutions of various solvents including ammonia,[11] CO_2[12-15] and ionic liquids[16-19] have been successfully developed in the last years. However, the major drawbacks of these technologies are low selectivity and sugar yield or still bench scale of processes. To avoid these limitations some additional steps must be taken, which on the other hand may guide to production of undesirable products decreasing the efficiency of the process or require high investments and elevated operational costs.[17,20]

The green reagents, such as CO_2 and water, used in the valorisation of lignocellulosic residues towards valuable products make the pre-treatment processes more environmentally acceptable. Recently, the CO_2-H_2O approach at high temperature (180-210^{o}C) and pressure of CO_2 (60 bar) was demonstrated to be an interesting alternative[13] to conventional technologies principally in the comparison with dilute acid hydrolysis, since no additional chemicals were used. The high-pressure CO_2-H_2O demonstrated to be selective in the hemicellulose dissolution[21] resulting in cellulose-rich processed solid. In this process, CO_2 substituted the commonly used organic solvents and acted as green catalyst. The presence of H_2O in the medium is an advantageous, since it permits to achieve high sugar yields increasing, at the same time, an effectiveness of the pre-treatment.[22] One of the major advantages of the use of binary CO_2-H_2O system is the *in-situ* formation of carbonic acid which dissociates in two stages promoting acid-catalysed dissolution of the biomass, namely hemicellulose[13] into corresponding sugars guiding at the same time to higher cellulose enzymatic digestibility.[23] The *in-situ* formed acidic environment helps in the pre-treatment and additionally unlike in the acid-hydrolysis, the acidity of CO_2 produced medium does not represent an environmental problem since after the depressurisation the CO_2 is no longer present in the reaction environment. Besides, the chemical CO_2 effect on the pre-treatment, the CO_2 pressure (physical effect) is important because CO_2 under the high pressure, can easily penetrate small pores of recalcitrant lignocellulosic structure resulting in structural changes in feedstock. Furthermore, the synergetic attack of CO_2 and H_2O promotes fibre separation exposing the surface leading at the meanwhile to elevated enzymatic digestibility of the processed solids. The use of enzymes to catalyse cellulose conversion is considered as more green alternative[24,25] in comparison with acid hydrolysis which mostly requires two-step hydrolysis.[26]

In this study, for the first time a methodology aiming to use high pressure CO_2 assisted pre-treatment proceeded by enzymatic hydrolysis is presented. The use of green solvent such as CO_2 in the integrated approach of biomass valorisation by providing the benefits to polysaccharide conversion is presented. The high-pressure CO_2-H_2O process is employed to take advantage of influence of temperature and CO_2 presence on the biomass depolymerisation reaction and on predisposition of processed solid for enzymatic hydrolysis. This synergetic formation of value-added products such as oligosaccharides and later glucose after the enzymatic hydrolysis is a little jigsaw needed to advance towards more sustainable bioeconomy.

Materials and methods

Raw material and chemicals

Wheat straw was kindly provided by INIAV, I.P. – Estação Nacional de Melhoramento de Plantas (Elvas, Portugal). The material was milled to a particle size smaller than 1.5 mm using a knife mill (IKA® WERKE, MF 10 basic, Germany) and stored at room temperature. The moisture level in the dry wheat straw was determined and was 8% $w \cdot w^{-1}$.

CO_2 used in the high-pressure pre-treatments was purchased from Air Liquide, AlphaGaz™ gamma, Paris, France with ≥99.99% $w \cdot w^{-1}$ purity. The paper filters (Ø=150 mm, nº 1238) used for post-processing filtrations were purchased from Filter-Lab, Microchip Technology Inc., Arizona, USA. For all pre-treatment experiments and chemical analyses the following reagents were used: distilled water (18.2 MΩcm-1) produced by the PURELAB Classic Elga system, ethanol with 96% purity ($v \cdot v^{-1}$) for gas phase capturing was acquired from Carlo Erba

Group - Arese, Italy. An aqueous solution of sulphuric acid 72% (w·w^{-1}) was originated from a 96% (w·w^{-1}) sulphuric acid supplied by Panreac Química, Barcelona, Spain.

For the enzymatic assays, sodium citrate buffer 0.05 M at pH 4.8 was prepared using citric acid monohydrate (99.7% v·v^{-1} purity) acquired from VWR International Ltd. – Leicester, England. Sodium azide (99% v·v^{-1} purity) bought from Merck – Darmstadt, Germany was used to prepare 2% aqueous solution of sodium azide. Commercial enzyme cocktail of Celluclast 1.5L (cellulases from *Trichoderma reesei*) and Novozyme-188 (β-glucosidases from *Aspergillus niger*) were acquired from Sigma Co.

High pressure CO_2-H_2O approach

The reactions were carried out according to the methodology presented elsewhere.[13] The reactions were performed at non-isothermal conditions (130, 215 and 225°C) and the initial CO_2 pressure used was: 0 (water-only reaction - autohydrolysis), 15, 30, 45, 54 and 60 bar at room conditions. A 150 g of H_2O·15 g dry wheat straw^{-1} and 100 g of H_2O·10 g dry wheat straw^{-1} mixture loading were used in order to diverse the headspace and thus concentration of CO_2 in the reactor.

Analogous methodology was applied to reaction with N_2 at 54 bar at 225°C. This reaction was carried out to verify the influence of physical effect on the biomass processing and subsequent enzymatic hydrolysis.

Severity factor

In order to evaluate the effect of high-pressure CO_2-H_2O system on hydrolysis of wheat straw, a combined severity factor ($CS_{p_{CO_2}}$)[23] was applied to examine the influence of the pH value on the reaction results according to the following equation: $CS_{P_{CO_2}} = \log(R_0) - pH$. Due to the technical limitation, the acidity of the medium during the process is impossible to be measured, thus the pH was estimated according to the following expression $pH = 8.00 \times 10^{-6} \times T^2 + 0.00209 \times T - 0.216 \times \ln(P_{CO\,2}) + 3.92$, where T is temperature in °C and p_{CO_2} is the partial pressure of CO_2 expressed in atmospheres. For the calculations of partial pressure of CO_2, the Henry's constant for binary CO_2-H_2O system was used.[23] In addition, the solubility of CO_2 in water for different reaction temperatures and pressures were taken from literature[27] and predicted for 215 and 225°C using PE package.[28]

Chemical characterisation of raw material and processed solids

Wheat straw and processed solids were characterised according to the previously established method.[13] Cellulose, xylan, arabinan and acetyl groups contents were determined after treatment with a 72% (w·w^{-1}) H_2SO_4 according to the standard methods.[29] The acid insoluble residue was considered as Klason lignin after correction for the acid insoluble ash. The ash content was established at 550°C using NREL/TP-510-42622 protocol.[30]

Liquor and post-hydrolysate characterisation

The liquid phase (liquor) resulted from the high-pressure treatment was analysed as presented in the literature.[13] For the determination of total sugar content in the liquor, an acid hydrolysis procedure was applied as described in literature.[31]

Enzymatic hydrolysis

Cellulose digestibility of untreated and pre-treated washed solids were evaluated based on the NREL/TP-510-42629 protocol.[32] The cellulase activity of Celluclast® 1.5 L was 105.89 FPU·mL^{-1} [33] and the β-glucosidase activity of Novozyme-188 was 798.56 pNPGU·mL^{-1}.[34] An 1.0 mL of sample was taken at 6, 24, 48, 76 and 96 h of the digestion and heated at 90°C for 5 min in a water-bath in order to quench the hydrolysis. All assays were performed at least in triplicate.

FTIR spectroscopy

The FTIR analysis was performed according to the procedure presented elsewhere.[18] All spectra were obtained with a FTIR spectrometer Spectrum BX, Perkin Elmer, Inc. (San Jose, CA, USA). This instrument was equipped with a DTGS detector and KBr beam splitter. The operating system used was Spectrum software (Version 5.3.1, Perkin Elmer, Inc., San Jose, CA, USA). FTIR spectra were acquired in the 4000 – 400 cm^{-1} region, with a total of 64 scans and a resolution of 4 cm^{-1} with a strong apodization. These spectra were subtracted against background air spectrum and recorded as absorbance values.

Scanning electron microscope

Scanning electron microscopy (SEM) (XL30 FEG Philips) at 15 keV was used to monitor the changes in structural fibre morphology of wheat straw samples. For this study untreated and pre-treated with autohydrolysis and high-pressure CO_2-H_2O binary system processed solids were used. Samples were prepared in the following manner. Any volatile fractions of solid samples were removed by vacuum. Next, to provide the conductivity of the sample, samples were sputter-coated with gold-palladium in EMITECH k575x. The parameters of sputter current and time were set to 100 mA and 30 seconds in an inert atmosphere of argon and hydrogen.

Results and discussion

Chemical characterisation of wheat straw

The chemical analysis disclosed in the raw material (dry weight basis) was as follows: 38.8±0.1% glucan, 19.5±0.4% xylan, 2.9±0.01% arabinan, 2.7±0.03% acetyl groups, 17.6±0.1% Klason lignin, 9.7±0.03% protein and 4.5±0.1% ash. The obtained data of chemical composition are in good agreement with previous reports where analogous relation between main fractions (cellulose, hemicellulose, lignin and extractives) was found.[8,13,35]

Effect of reaction severity on the biomass processing

The liquor composition

Series of hydrolysis experiments have been carried out under variable conditions such as reaction temperature (215 and 225°C), initial pressure of CO_2 (0, 15, 30, 45 and 54 bar) at non-isothermal conditions in order to assess the efficiency and selectivity on xylan conversion to xylo-oligosaccharides (XOS). The $CS_{p_{CO_2}}$ was applied with the objective to encompass all these variables and to facilitate the comparison of the obtained data.

Both, high-pressure CO_2-H_2O and water-only reaction (autohydrolysis) resulted in liquors containing a mixture of hemicellulose constituents such as xylose and arabinose namely in oligomer form, acetic acid and furfural (the main decomposition product of pentoses) and glucose as oligosaccharide obtained from cellulose.

As it is shown in Table 1, XOS is the main compound present in liquors produced in all experiments. The amount of XOS recovered was highly dependent on the reaction conditions. When wheat straw was processed at $CS_{p_{CO_2}}$= -0.33, xylan to XOS yield of 61.7% was obtained and it corresponds to concentration of XOS as high as 11.4 $g{\cdot}L^{-1}$. Under this condition a high quality liquor, rich in pentose sugars with low amount of degradation products (1.7 $g{\cdot}L^{-1}$), was obtained. At $CS_{p_{CO_2}}$=0.19, a decrease of XOS concentration to the lowest value of 4.1 $g{\cdot}L^{-1}$ found in all experiments was detected. At this condition, an extended xylan hydrolysis (24.2% of xylose and 12.8% of furfural) coupled with the loss of XOS yield (64% lower in comparison with the best XOS yield condition) was observed. Additionally, interesting is that comparing the XOS yield at CO_2–H_2O processing at $CS_{p_{CO_2}}$=-

0.33 with the autohydrolysis $CS_{p_{CO_2}}$=0.02, a 54% higher XOS yield was produced in CO_2 coupled treatment. It may indicate that presence of CO_2 helps to promote the hydrolysis of xylan to XOS. The relation between XOS concentration and $CS_{p_{CO_2}}$ is shown in Figure 1.

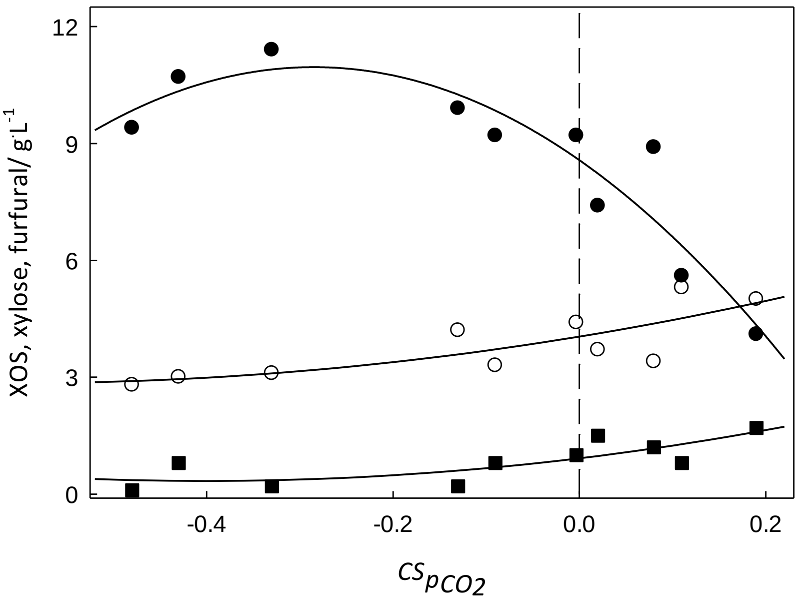

Figure 1. The XOS (●), xylose (○) and furfural (■) concentration in the function of $CS_{p_{CO_2}}$. The solid lines provided as a guide for an eye.

Xylose is the main monosaccharide present in the liquor followed by the second monosaccharide - arabinose. Under the best XOS yield condition, the concentration of released xylose and arabinose corresponds to 15% and 46.5% of initial xylan and arabinan contents, respectively. The concentration of xylose increased with the severity of the reaction up to $CS_{p_{CO_2}}$=0.11, at which a maximum concentration of 5.3 g·L^{-1} was obtained. In all other reactions at less severe conditions furfural was formed almost in undetectable concentrations as depicted in Figure 1.

Table 1. Composition of liquors (g·L^{-1}) and yields of each product present in the liquors (g·100 g^{-1} of the initial amount present in the feedstock) for all studied conditions.

Reaction conditions	High pressure H_2O		High pressure CO_2-H_2O																	
T (°C)	225		225		225		215		215		225		225		225		225		225	
$p_{CO2initial}$(bar)	0		15		15		30		54		30		30		45		45		54	
p_{final}(bar)	23.6		32.6		36.4		61.0		126.3		61.9		62.1		83.7		93.0		127.4	
$CO_{2dissolve}$/biomass(w·w^{-1})	-		0.039		0.060		0.198		0.471		0.208		0.195		0.303		0.344		0.490	
Log (R_0)	3.79		3.83		3.80		3.57		3.58		3.87		3.96		3.95		3.95		3.96	
$CS_{p_{CO_2}}$/pH[a]	0.02[b]/-		-0.48/4.31		-0.43/4.22		-0.33/3.90		-0.13/3.71		-0.09/3.96		0.00/3.96		0.08/3.87		0.11/3.84		0.19/3.77	
pH[c]	3.77		3.97		4.04		3.87		3.80		4.03		3.78		3.74		3.55		3.68	
Composition/yields	g·L^{-1}	g·100g^{-1}	g·L^{-1}	g·100g^{-1}	g·L^{-1}	g·100g^{-1}	g·L^{-1}	g·100g^{-1}	g·L^{-1}	g·100g^{-1}	g·L^{-1}	g·100g^{-1}	g·L^{-1}	g·100g^{-1}	g·L^{-1}	g·100g^{-1}	g·L^{-1}	g·100g^{-1}	g·L^{-1}	g·100g^{-1}
XOS	7.4	42.6	9.4	51.7	10.7	57.9	11.4	61.7	9.9	53.8	9.2	52.9	9.2	49.8	8.9	48.4	5.6	30.6	4.1	22.6
GlcOS	4.0	11.6	4.8	13.2	5.7	15.7	4.5	12.4	4.5	12.3	4.3	12.4	5.0	13.6	4.0	13.6	3.6	9.8	3.5	9.5
AcOS	0.4	-	0.8	-	0.6	-	2.7	-	1.9	-	2.1	-	0.6	-	0.2	-	1.7	-	1.7	-
Xylose	3.7	18.9	2.8	13.4	3.0	14.4	3.1	15.0	4.2	20.1	3.3	16.7	4.4	21.2	3.4	21.2	5.3	25.4	5.0	24.2
Arabinose	0.75	25.7	1.2	39.5	1.2	37.5	1.4	46.5	1.3	40.8	0.78	17.9	1.2	23.5	1.1	23.5	1.0	26.4	0.8	24.4
Glucose	0.8	2.1	1.2	2.9	0.8	1.9	0.9	2.2	1.0	2.6	0.7	1.8	0.9	2.3	1.2	2.3	1.1	2.7	1.2	2.9
Acetic Acid	3.5	-	3.2	-	3.0	-	2.5	-	3.3	-	3.0	-	3.5	-	3.5	-	3.6	-	4.0	-
Furfural	1.5	12.2	0.1	1.0	0.8	6.3	0.2	1.3	0.2	1.7	0.8	6.0	1.0	7.1	1.2	7.1	0.8	6.0	1.7	12.8
HMF	0.2	0.8	0.2	0.5	0.2	0.2	0.1	0.4	0.2	0.7	0.2	0.7	0.2	0.6	0.3	0.6	0.3	0.9	0.3	1.1

[a] predicted according to ref [23]; [b] calculated with measured pH value; [c] measured pH value of hydrolysate after reactions; XOS – xylo-oligosaccharides; GlcOS - gluco-oligosaccharides; AcOS - acetyl groups linked to oligosaccharides.

Gluco-oligosaccharides (GlcOS) and glucose follows similar pattern to XOS and xylose. A concentration of GlcOS tends to decrease and monomers of glucose concentration increases with reaction severity. The formation of GlcOS might have an origin in the effect of the reaction conditions on cellulose dissolution, especially in case of the amorphous cellulose which is more susceptive for hydrolysis even at milder conditions. To confirm this concept, the crystallinity index from FTIR measurements[16,36,37] was calculated. A LOI[38] is a tool to measure the degree of crystallinity of cellulosic material and is defined as a ratio of absorption bands at 1437 and 838 cm^{-1}. A band at 1437 cm^{-1}, assigned to a symmetric CH_2 bending vibration is "crystallinity band", indicating that a decrease in its intensity reflects reduction in the degree of crystallinity of the samples. The FTIR absorption band at 898 cm^{-1}, assigned to C–O–C stretching at β-(1→4)-glycosidic linkages, is an "amorphous" absorption band and an increase in its intensity happening in the amorphous samples.[37] The results obtained for untreated, and pre-treated by autohydrolysis ($CS_{p_{CO_2}}$=0.02) and with CO_2 ($CS_{p_{CO_2}}$=0.08) are given in Table 2 and Figure 2.

Table 2. The LOI index for untreated, autohydrolysis and CO_2-treated biomass.

Sample	A_{898}	A_{1437}	$LOI = \frac{A_{1437}}{A_{898}}$
untreated	0.115	0.376	3.27
autohydrolysis ($CS_{p_{CO_2}}$=0.02)	0.084	0.383	4.56
CO_2 processed ($CS_{p_{CO_2}}$=0.08)	0.079	0.311	3.94

The analysis of the produced data shows that LOI for untreated sample is 3.28, while for autohydrolysis and CO_2 pre-treated solids is 4.56 and 3.92, respectively. Surprisingly, the pre-treatment, either autohydrolysis or CO_2-assisted, increases the LOI value or in other words crystallinity. However, the close inspection of the obtained data shows that autohydrolysis removes the amorphous cellulose as the band at 898 cm^{-1} reduced by 27% in comparison with untreated biomass and 1437 cm^{-1} remains intact. The analysis of FTIR spectrum of CO_2 treated sample shows that crystallinity reduction is more extensive than in case of autohydrolysis as it reduces either amorphous cellulose or crystalline one by 31% and 17%, respectively. Therefore the LOI for both pre-treatments is higher than LOI for untreated sample while in fact crystallinity seems to be lower as in both cases amorphous cellulose was removed and in CO_2 process some portion of crystalline one was expelled as well. The FTIR results confirm that autohydrolysis is less severe process than CO_2 one and GlcOS is mostly formed from an amorphous cellulose while in case of CO_2 coupled process the amorphous cellulose is hydrolysed in the even greater extent than in autohydrolysis and crystalline cellulose is affected also allowing a progressive formation of GlcOS and glucose.

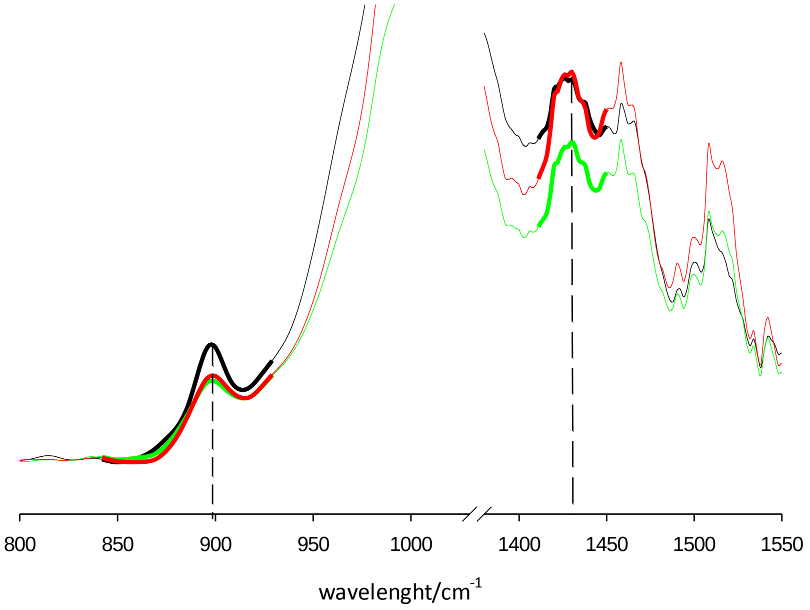

Figure 2. The FTIR spectra of untreated (black line), autohydrolysis (red line) and CO_2 processed wheat straw (green line) showing the regions for LOI determination. The adequate bands (898 and 1437 cm^{-1}) are marked by dashed lines.

The liquor obtained in the studied processes contained acetic acid in both forms: as free acetic acid and as acetyl groups bounded to OS (AcOS). As expected the maximum acetic acid (4.0 g·L^{-1}) was achieved at the severest condition while the highest AcOS concentration was obtained at moderate $CS_{p_{CO_2}}$ equals to -0.33. The mechanism of this process is analogously to previously discussed conversion of XOS to xylose. More severe conditions favour hydrolysis of AcOS to acetic acid therefore the concentration of acetic acid increases with an increase of severity of the reaction conditions. King and co-workers observed similar trend for experiments based on hydrolysis of switchgrass with carbonated water to produce carbochemicals in the range of temperature from 220 to 310°C and commensurate higher pressure (68 bar) in a semi-continuous batch flow system. The aliphatic acids (acetic and formic) were produced at levels of 3-6 g·100g^{-1} of feedstock while furfural was rapidly produced at 310°C within 10 min.[39]

The reaction conditions examined in this work permitted to produce liquors rich in oligosaccharides in a total concentration of 18.6 g·L^{-1} at $CS_{p_{CO_2}}$=-0.33. The produced solution was mostly constituted by XOS which is a major product among oligosaccharides present and corresponds to 61% of them.

One of the most important aspects examined in this work is the pH of the produced liquors. The measured and predicted pH values of hydrolysates from either autohydrolysis or high-pressure CO_2-H_2O experiments are presented in Table 1. As it can be seen, the measured pH values (after CO_2 released at room temperature) vary between 3.55 and 4.04 for experiments carried out with CO_2. The processes with CO_2 for $CS_{p_{CO_2}}$≥0.08 gave pH of hydrolysate lower than this for autohydrolysis reaction (pH=3.77). Considering that no additional amount of acetic acid was formed during these reactions, as it is shown in Table 1, it indicates that *in-situ* formed carbonic acid acidify the medium driving to the decrease of the final pH of the liquor. The obtained results are in contrast to those presented by van Walsum and co-workers, in which an addition of carbonic acid increased the pH of the liquor produced.[40] These differences can be elucidated by the fact that at reduced CO_2 pressure, the solubility of CO_2 in the aqueous phase is much lower (even one order of magnitude), therefore at these conditions the acidification of liquor by carbonic acid is practically negligible. For instance, for a reaction at 225°C and 15 bar of initial CO_2 pressure (32.6 bar final total pressure) for $CS_{p_{CO_2}}$=-0.48, the solubility of CO_2 in water is almost null (x_{CO_2}=0.0016)[28] resulting in very lower $CO_{2\text{dissolved}}$/water ratio equals to 0.039. For the contrast, the solubility of CO_2 in water for the harshest examined conditions ($CS_{p_{CO_2}}$=0.19) is x_{CO_2}=0.0196 giving 0.49 g of CO_2 dissolved in water per 1 g of biomass. This data clearly demonstrate that CO_2 pressure, and by this the severity of the reaction, plays an important role as at lesser severe conditions, reactions occur in fact in a three phase system involving solid biomass, water mostly in liquid state and gaseous phase constituted almost exclusively by CO_2. Therefore these three phase type reactions give a pH of liquors similar to pH of liquor for autohydrolysis ($CS_{p_{CO_2}}$=0.02).

Other important aspect to be scrutinised is that at the harshest conditions, degradation products with acidic characteristic are formed in the larger amount. Their presence also contributes to biomass hydrolysis and consequently guides to lower pH values of generated liquors. For example, lower pH values were found also in hydrothermal processes reported in the literature, where an extensive hydrolysis of hemicellulosic acetyl groups was found.[8,9,41] Another very important factor influencing the pH of the liquor is the composition of biomass explored. Biomass rich in high acetyl group contents may lead to a decrease of pH during reaction. A comparison of results obtained in this work with those reported in the literature[42] illustrates that the pH value of liquors from aspen wood processing with carbonic acid for $CS_{p_{CO_2}}$=0.17 is 3.95[42], and is not very different from this obtained under similar reaction conditions ($CS_{p_{CO_2}}$=0.19, pH=3.68) presented in this work. On the other hand, corn stover for $CS_{p_{CO_2}}$ close to this got in this work gives the final pH very different (pH=4.95). According to van Walsum et al., differences between the final pH can be explained by the extended autocatalytic hydrolysis of acetyl groups of aspen wood in comparison with those in corn stover.[42]

Although, the CO_2 experiments resulted in different pH of liquors, in fact the decrease of pH does not show significant effect on hemicellulose dissolution. For example, the difference of hemicellulose dissolution between all reactions with $CS_{p_{CO_2}}$≥-0.13 was only around 6%. Brunner and co-workers also found similar conclusions, where no substantial relation between hemicellulose dissolution and decrease of pH was determined.[43] Even a decrease of pH to around 2 caused by addition of sulphuric acid did not have any effect on biomass dissolution.[43]

The processed residue composition

The pH of medium produced during the process and the reaction severity not only influences the liquor composition but also affects the processed solid too. A table 3 summarises the composition and yield of the processed solid residues obtained by either autohydrolysis or CO_2-H_2O under different severity conditions. For all conditions of high-pressure CO_2-H_2O processes, the solid dissolution was high and superior to 50%. For comparison, the autohydrolysis reaction, similarly to CO_2-H_2O experiments demonstrated still high solid dissolution (46.7% of initial biomass) with hemicellulose removal close to 75%. Other important aspect is that, the processed solids suffered significant changes in composition in comparison with the raw material. The degree of biomass dissolution increases almost linearly with severity factor reaching values of hemicellulose removal up to 86.4%. This demonstrates an uncompleted hydrolysis of xylan and presence of minor amount of acetyl groups. With an increase of the severity of the reaction a faster cleavage of linkages of hemicellulose and cellulose was observed.[43] van Walsum at elevated temperatures (above 200°C) observed a similar catalytic effect of carbonic acid on pure xylan hydrolysis allowing an increase of pentoses' release and a decrease of a degree of polymerisation of xylan oligomers in comparison with autohydrolysis.[23]

The obtained data shows that cellulose, although was partially affected by either autohydrolysis or CO_2-H_2O process, is a dominant constituent of the processed solid and its relative concentration increases with the severity of the reaction because of the dissolution of hemicellulose occurs. The maximum glucan content of 74.9% in the processed solid was found at $CS_{p_{CO_2}}$=0.19. The increase of severity had minor effects on glucan dissolution, with a maximum of glucan loss of 14.3% for the most severe condition. The third principal component of biomass remained in the processed solid is lignin. The recovery of lignin in solid phase was found to be between 27.0% and 31.2%. The treatments resulted in the increase of the total amounts of lignin due to lignin condensation reactions which makes even worse digestibility of the remaining cellulose fraction.[44,45]

The aforementioned hemicellulose removal and influence on the morphology of biomass is visible and was analysed by the SEM technique. The SEM technique allowed investigating the effect of process on the ultrastructure and possible disruption of the cell walls. Figure 3 shows a SEM analysis of native wheat straw and after either autohydrolysis (225°C) or CO_2-H_2O reactions (225°C and 54 bar of CO_2).

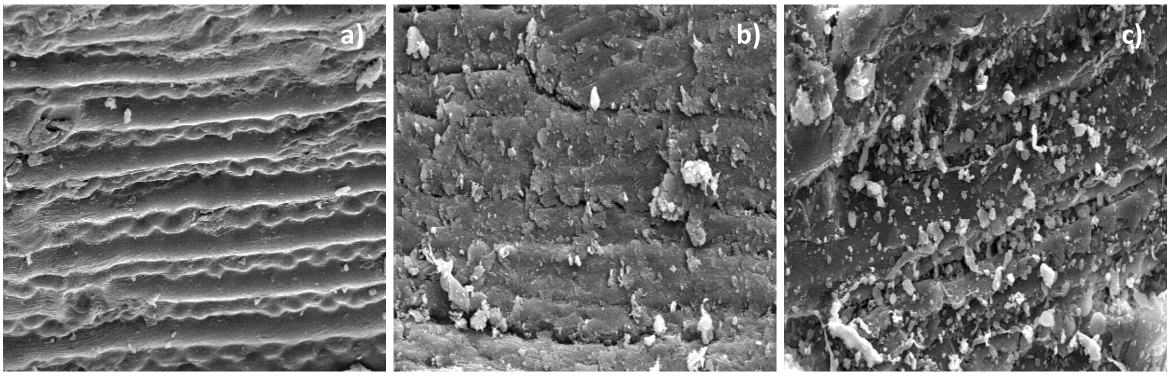

Figure 3. SEM micrographs of a) untreated wheat straw and after b) autohydrolysis and c) CO_2-H_2O reactions obtained with amplification 1200x.

Table 3. The solid phase composition (g (100 g processed solids)$^{-1}$) and solid yield (g (100 g feedstock)$^{-1}$) obtained after the examined wheat straw experiments.

Reaction conditions	High pressure H_2O	High-pressure CO_2-H_2O								
T (°C)	225	225	225	215	215	225	225	225	225	225
$CS_{p_{CO_2}}$ [a]	0.02[b]	-0.48	-0.43	-0.33	-0.13	-0.09	0.00	0.08	0.11	0.19
Solid yield	53.28	48.33	49.02	49.83	49.13	48.87	49.16	45.03	48.26	44.53
Glucan	65.85±1.15	69.93±0.17	70.11±1.0	70.62±0.46	71.26±0.81	72.15±0.52	73.83±1.06	74.39±0.73	74.63±0.30	74.88±1.71
Xylan	9.09±3.05	8.40±1.66	8.64±3.65	10.15±2.67	8.80±3.32	7.93±2.61	7.38±2.03	6.71±2.16	6.91±1.03	5.95±1.41
Arabinan	Traces	Traces	Traces	Traces	Traces	Traces	Traces	Traces	Traces	Traces
Acetyl groups	1.33±0.08	0.67±2.61	0.51±0.91	1.26±0.02	0.94±0.54	0.56±0.33	0.43±1.60	0.51±5.45	0.34±0.15	0.28±0.00
Klason lignin	31.15±0.33	27.0±0.71	27.15±0.81	28.03±2.23	28.62±4.55	28.82±0.45	29.30±1.35	29.51±1.16	29.07±1.72	27.60±4.63

[a] predicted according to ref[29]; [b] calculated with measured pH value after reaction

After both pre-treatments applied, physical changes of raw material surface were noticeable. The grinded untreated wheat straw exhibited a rigid, tight and contiguous surface while fibres of pre-treated samples have anomalous porosity and lamellar structures became fleecy. The pre-treated solids are significantly more heterogeneous in structure than untreated one. This indicates that the surface of raw material was subjected to severe conditions during both processes with the dominant effect visible in case of the CO_2 involved. These morphological changes find an explanation in the previously discussed results. The extended hemicellulose removal from middle lamella caused by the reaction conditions guided to the structural changes visible on Figure 3. In other words, the synergetic attack of CO_2 and H_2O promotes fibre separation exposing the surface leading also to an elevated enzymatic digestibility of the processed solid[46] as it is also discussed later. Furthermore, the interaction between biomass with hot liquid water and high dense CO_2 lead to increase of a diffusivity of gas into the biomass, promoting the swelling of biomass.[47] Similar conclusions regarding the effect of supercritical CO_2 on physical structure of lignocellulosic materials were presented in the literature.[12,14] Zheng et al., studied the effect of different gases such as nitrogen, helium and CO_2 and the later has demonstrated higher glucose yields from enzymatic hydrolysis of lignocellulosic materials.[48] Narayanaswamy et al., reported that supercritical CO_2 (150°C, 241 bar, 1h and moisture of 75%) had a significant effect in opening pores and exposing internal areas of corn stover.[12] On the other hand, no effect of supercritical CO_2 on switchgrass was found but probably due to rigid structure of this biomass.[12] Gao et al. discovered that reaction with supercritical CO_2 (110°C, 300 bar for 30 min and liquid/solid ratio of 1:1) promotes changes in porosity and fibres became more susceptible to enzymatic attack increasing their digestibility.[14] Contrary to the results presented in this work both referred literature studies used CO_2 explosion technique that has a great impact on biomass pore rupture. Presumably, rapid release of CO_2 leads to explosion and the effect on pores opening is more evident. Benazzi et al., reported that depressurisation rate of 50-200 $kg \cdot m^{-3} \cdot min^{-1}$ after ultrasound assisted supercritical CO_2 pre-treatment did not result in a significant increase of glucose yield obtained by enzymatic hydrolysis. It might be explained by the slow CO_2 release in comparison with CO_2 explosion pre-treatment.[49] Ferreira-Leitão and co-workers found that the CO_2-explosion pre-treatment at 205°C for 15 min resulted in less pronounced structural modifications of the material than SO_2-explosion at 190°C for 5 min which can be directly related to the combined severity of each pre-treatment.[50] Especially that the SO_2-pre-treatment resulted in more extensive hemicellulose removal equals to 68.3% in comparison with 40.5% obtained from CO_2-pre-treatment.

Enzymatic hydrolysis

To survey the influence of examined processes on the monosaccharides production, the processed solids were subject to the enzymatic hydrolysis. As it was already mentioned the enzymatic attack is affected by several factors of the pre-treatment process which are essential to make glucan much more accessible for enzymes' attack. The most important parameters influencing the rate of enzymatic hydrolysis are hemicellulose and lignin content, cellulose crystallinity, degree of polymerisation[51] as well as liquid/solid ratio. All of these factors are strongly related with the choice of pre-treatment and conditions employed.

Pressure effect

The wheat straw samples pre-treated at various CO_2 pressures at fixed temperature were hydrolysed by the addition of Cellulase and Novozyme 188 at a concentration of 60 $FPU \cdot 1g$ of $glucan^{-1}$ and 64 $pNPGU \cdot 1g$ of $glucan^{-1}$, respectively for maximum 96h. Figure 4 shows the effect of CO_2 pressure on glucose yield from glucan for the high-pressure CO_2-H_2O reaction of wheat straw performed at constant temperature (225°C). The glucose yield increased along time of hydrolysis which indicates that enzymatic digestibility of the pre-treated solids increases. The glucose yields arise to a maximum of 56.02, 57.77, 63.93, 75.39 and 82.21% at 96 h of enzymatic hydrolysis for 0, 15, 30, 45 and 54 bar of initial CO_2 pressures, respectively. For comparison, the untreated biomass showed a maximum glucose yield of 34.31% after 96h of process. The obtained data are shown in Figure 4.

The glucose yield increased by 46.8% with an increase of the pre-treatment pressure (from 0 to 54 bar of initial or from 23.6 to 127.4 bar of final total pressure). It shows that CO_2 pressure plays an important role in improving the enzymatic digestibility. Zheng et al. studied the effect of supercritical CO_2 on hydrolysis of Avicel (commercial form of cellulose). They concluded that the disruption of cellulosic structure was caused by CO_2 under supercritical conditions followed by quick depressurisation which increases the rate of enzymatic hydrolysis resulting in glucose yield around 50%.[52] Another report of Alinia et al. demonstrate that pressure changes from 80 to 120 bar in the wheat straw pre-treatment promote an increase of yield of reducing sugars. At the same time the further pressure increase above 120 bar does not change the final sugar yield.[53] Kim et al., studied the hydrolysis of aspen and southern yellow pine pre-treated at 214 and 276 bar of CO_2 and they did not observe any effect on enzymatic digestibility of both materials.[54] The conclusion taken by Kim et al. might be burdened by high lignin content present in both aspen and southern yellow that may have a negative effect of enzymatic hydrolysis. It is also important to mention that not only supercritical CO_2 affects the enzymatic hydrolysis. The CO_2 used under subcritical conditions improves the enzymatic hydrolysis also. For instances, sugarcane bagasse treated with 70 bar of CO_2 at 160°C for 60 min showed an increase of 36% in glucose yield in comparison with the pre-treatment without CO_2. Puri et al., studied the effect of steam and CO_2 under sub and supercritical condition on the cellulose hydrolysis of wheat straw.[55] The maximum glucose yield of 81% was obtained at 200°C in the range of CO_2 pressure of 34.5-138 bar.

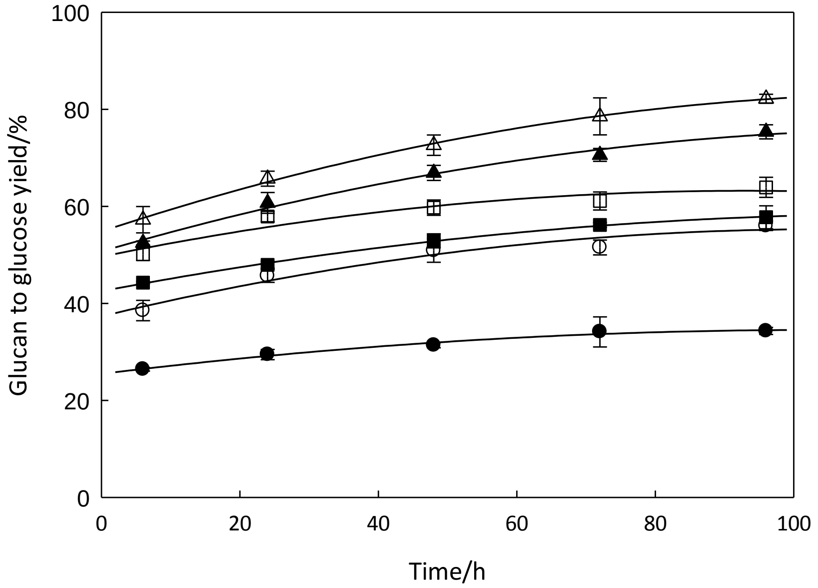

Figure 4. The glucan to glucose yield/% (with the respective error bars) for (●) untreated, (○) autohydrolysis and treated under (■) 15 bar, (□) 30 bar, (▲) 45 bar or (Δ) 54 bar of initial CO_2 pressure. The solid lines provided as guide for an eye.

The acquired results clearly show that enzymatic hydrolysis is strongly influence by chemical and physical effects of the pre-treatment. To investigate the effect of CO_2 on more favourable enzymatic hydrolysis of the processed solid, by the removal of hydrolysis inhibitors (hemicellulose) as well as by the physical cellulose structure opening, a reaction in the presence of neutral gas – nitrogen was carried out at the conditions analogous to reaction with $CS_{p_{CO_2}}$=0.19 (225°C and 54 bar of N_2). The processing of wheat straw with N_2 gave 9.8, 3.0 and 1.3 $g \cdot L^{-1}$ of XOS, xylose and furfural, respectively. At the same time the formed processed solid contained 61.2% of glucan which in the enzymatic hydrolysis process was converted to glucose giving after 72 h a 63% of glucan to glucose yield. Comparing this data with the autohydrolysis (51.6%) and analogous CO_2 process (78.6%) it can be concluded that presence of neutral gas in the headspace of the reactor and by this creation of the pressure influences positively the enzymatic digestibility of the processed solid. On the other hand, the increase of enzymatic hydrolysis from 63% for N_2 to 78.6% for CO_2 process indicates the strong chemical effect of CO_2 on the removal of hemicellulose and by this more favourable enzymatic digestibility of glucan present in the processed solid. Figure 5 depicts the influence of both effects on the enzymatic hydrolysis of processed solid produced in autohydrolysis (H_2O process), N_2 (N_2-H_2O process) and CO_2 (CO_2-H_2O process).

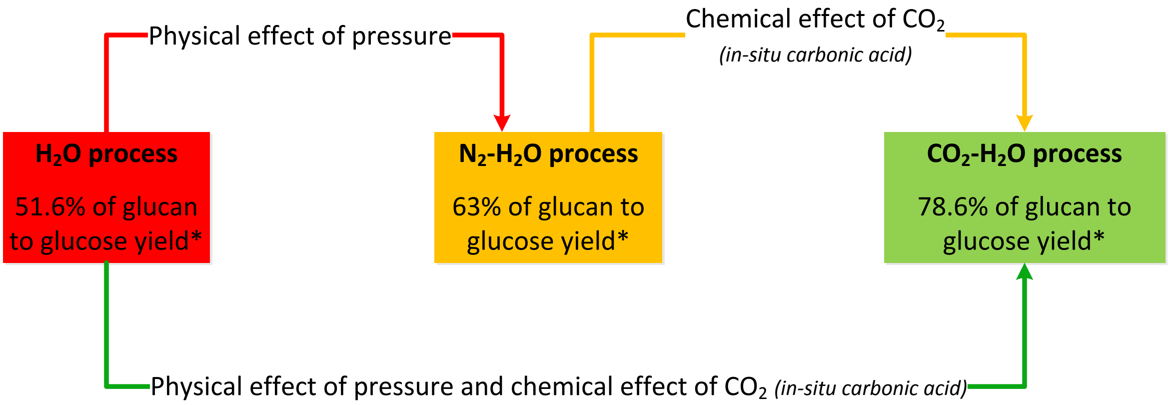

Figure 5. The schematic representation of both physical and chemical effect of high pressure processes of wheat straw valorisation. *The value of glucan to glucose yield obtained after 72 h of hydrolysis.

Temperature effect

Other variable influencing the hydrolysis of processed solid is temperature of process. In order to demonstrate the effect of temperature on high-pressure CO_2-H_2O, wheat straw was subject to reaction at three different temperatures (130, 215 and 225°C) and fixed initial CO_2 pressure (30 bar). Both 215 and 225°C temperatures were previously examined for the hemicellulosic-sugars' production while experiments at 130°C were carried out to demonstrate the influence of temperature on the enzymatic hydrolysis. The Figure 6 demonstrates this relation and maximum glucose yields for 130, 215, and 225°C were 39.97, 62.64 and 63.93%, respectively. For comparison, the enzymatic hydrolysis of untreated material after 96 h revealed a 34.31% yield of glucose.

The obtained results show that process temperature of 130°C seemed to have a low impact on a glucose yield. The obtained result can be explained by the low diffusivity of CO_2 at inferior temperatures and thus temperature as CO_2 pressure are important factors in the efficient conversion of wheat straw. Kim and Hong[52] tested the effect of scCO_2 at different temperatures (112–165°C). They found that at temperatures below 120°C and 214 bar for 60 min the pre-treatment has no significant effect on sugar yield from enzymatic hydrolysis. However, when temperature of 160°C was used, higher glucose yield was achieved. Similar conclusions were presented by Narayanaswamy et al. who reported that an increase of temperature from 120°C to 150°C led to release of 24 and 30 g glucose per 100g of dry biomass, respectively. Gao et al. also investigated the influence of temperature of rice straw pre-treatment on glucose yield from enzymatic hydrolysis. The maximum glucose yield obtained was only 32.4% at 110°C and 300 bar of CO_2 as, according to the conclusions given, the low yield may be caused by low temperature used in the experiments as hemicellulose and lignin start to dissolve under neutral conditions at 180°C.[14]

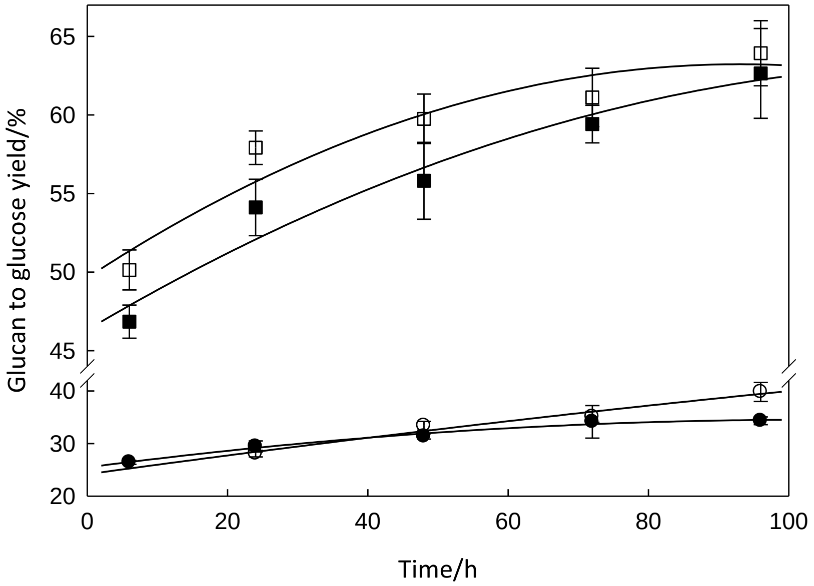

Figure 6. The glucan to glucose yield/% (with respective error bars) for (●) untreated wheat straw and treated under 30 bar of CO_2 initial pressure at (○) 130°C, (■) 215°C, (□) 225°C. The solid lines provided merely as guide for an eye.

The presented results clearly depicts that higher temperature promotes more efficient conversion of glucan to glucose. For example the increase of temperature from 130°C to 215°C gives an increase of glucose yield by more than 50% (from 39.97 to 63.93%) helping produce much monosaccharide rich solution.

Comparing the obtained data for different temperature and CO_2 pressure with those achieved by the classical methods show a more favourable result in case of reactions with CO_2. Hsu et al., investigated the effect of dilute-acid hydrolysis on enzymatic hydrolysis of rice straw and a maximum sugar yield of 83% was achieved when rice straw was pre-treated with 1% ($w \cdot w^{-1}$) of sulphuric acid with reaction time of 5 min at 180°C.[6] Also Henk and co-workers found that corn stover pre-treated with 2% ($w \cdot v^{-1}$) sulphuric acid showed a cellulose digestibility higher than 80%.[53] The found results are similar to those obtained in this work for 225°C and 54 bar of initial CO_2 pressure without additional chemical catalyst such as sulphuric acid. Considering the spent acid and consequently its neutralisation originate in gypsum which must to be eliminated making the overall process environmentally and economically unfeasible it is important to underline that CO_2 process seems to be an interesting and more environmentally friendly method of bio-waste pre-treatment enhancing the enzymatic hydrolysis of processed solid. Furthermore, dilute-acid hydrolysis requires substrate washing with water and often with alkaline solution prior enzymatic hydrolysis in order to elevate the pH to the value of optimal acidity of enzymes. Unlike acid-catalysed reactions, the CO_2-H_2O does not require additional water amounts and prevents the residue formation keeping the advantages gained from employing this technology in biomass processing.

Polysaccharide wheat straw valorisation

The valorisation of both hemicellulosic and cellulosic fractions of wheat straw polysaccharides aims to convert these inaccessible saccharides to easily transformable sugars. The integrated polysaccharide conversion to sugars in either oligomer or monomer form was analysed for all performed reactions. Figure 7 depicts the selected best case for CO_2-assisted reaction. The data for this process shows that either xylan or glucan in the integrated (pre-treatment with CO_2-H_2O and next enzymatic hydrolysis) is converted to xylose- or glucose-derived sugars with a yield of 86.3% and 83.5%, respectively. For comparison the analogous calculations for autohydrolysis process reveal yields of xylan or glucan to sugars as high as 79.6 and 61.3%, respectively. The mass balance of xylan and glucan is depicted in Figure 8.

Figure 7. The mass balance of integrated polysaccharide conversion.

Figure 8. The mass balance of integrated polysaccharide conversion for autohydrolysis process.

The obtained data confirms that CO_2-assisted autohydrolysis integrated with subsequent enzymatic hydrolysis of processed solid gives much higher total sugar yields (84.4%) than this for autohydrolysis only (67.4%).

Conclusions

This work shows the potential of high-pressure CO_2-H_2O process on both hydrolysis and pre-treatment of lignocellulosic biomass. The high-pressure CO_2-H_2O results in liquors rich in xylose oligomers which, in contrast to other valorisation methods e.g. acid hydrolysis produces monomers of xylose, can be an advantage due to the prebiotics activities of XOS. Furthermore XOS are one of the top value products, which production from null price bio-wastes favours the economy of the entire valorisation process of wheat straw.

The pre-treatment with CO_2 allows to carry out processes at lower temperatures than autohydrolysis, without losses of efficiency and additionally a hemicellulosic-sugar degradation can be avoided. The low production of undesired degradation products coupled to high xylan solubilisation yield makes this process a promising technology that can be used as a pre-treatment/hydrolysis process within biorefinery concept.

The incorporation of CO_2 into hydrothermal technologies showed to be successful, allowing to obtain hemicellulose-rich solutions and pre-treated solids highly susceptible to enzymatic hydrolysis resulting in monosaccharides-rich solutions. The presence of CO_2 in the pre-processing of biowaste helps also to increase the enzymatic hydrolysis due to the extended removal of hemicellulose and physical effect of created pressure.

The integrated valorisation of polysaccharides, using green solvent, permits to achieve an 84.7% of total sugar yield (from xylan and glucan present in the raw feedstock) in the form of mono- or oligosaccharides.

The obtained results confirm that maximal exploitation of hemicellulose fraction in the form of XOS and xylose together with glucose production during the enzymatic hydrolysis are the best approaches of wheat straw polysaccharide valorisation method. Applying the methodology exploited in this work, a significant reduction of the environmental footprint of this kind of feedstock can be achieved and the proposed method provides additional economic benefits due to the high XOS production.

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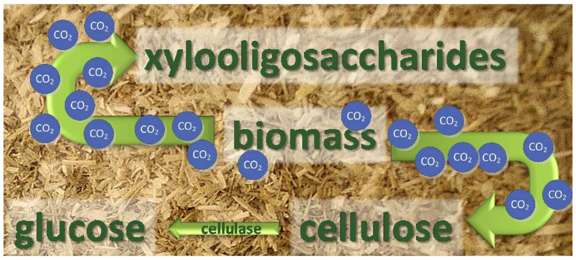

Xylooligosaccharide production with glucose formation during the enzymatic hydrolysis is the best approach of waste biomass CO_2-accompanied valorisation method.