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Sustainable Valorisation and Efficient Downstream Processing of Giant Reed by High-Pressure Carbon Dioxide Pretreatment

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This work investigated the catalytic high-pressure CO_2 pretreatment of giant reed. CO_2 is a renewable resource; its use does not generate chemical wastes and it can be easily removed and recycled. The effect of the addition of low concentrations of $FeCl_3$ (0.16 wt%) and PEG 400 (1.0 wt%) on the hemicellulose hydrolysis to xylose and xylo-oligosaccharides (XOS) is reported for the first time. Under the optimised pretreatment conditions, the xylan conversion of 82 mol% and xylose and XOS yields of 43 and 20 mol% were achieved, respectively. The solid residues obtained from different pretreatments were used as the substrate for the enzymatic hydrolysis to give glucose. The total glucose yield achieved under the optimised two-step process was 67.8 mol% with respect to the glucan units in the biomass. The results demonstrated that PEG-assisted $FeCl_3$-catalysed $scCO_2$ pretreatment can produce xylose- or XOS-rich hydrolysates and improve the enzymatic hydrolysis of biomass.

Introduction

Lignocellulosic biomasses represent strategic renewable resources for the development of innovative biorefinery processes, favouring the transition from a fossil-based economy to a bio-based one.[1] Second-generation biomass is a promising source of polysaccharides and lignin that can be converted into several value-added biobased molecules in the perspective of bioeconomy and Green Chemistry. In particular, cellulose and hemicellulose fractions, namely two of the most abundant biopolymers in the world, can be fractionated and selectively depolymerised to monosaccharides, such as glucose and xylose, respectively.[2] These reducing sugars represent a versatile industrial platform for the production of fine chemicals, solvents, fuels and materials via chemical or biological routes.[2–3] One of the main limitations of traditional lignocellulosic crops is the consumption of lands destined to the agri-food chain, causing an ethical contrast "food versus fuel".[4] A sustainable solution for this issue is represented by the exploitation of residual agro-industrial wastes or of lignocellulosic crops able to grow on marginal or polluted soils that cannot be cultivated with edible plants species. In this context, giant reed (*Arundo donax* L.) represents a strategic dedicated crop due to its several advantages with respect to other lignocellulosic biomasses. It is a rhizomatous perennial grass, thus avoiding annual soil tillage, and shows high yielding under low input management systems and irrigation, even on marginal, contaminated or underutilised lands. Moreover, giant reed removes nitrates from the soil, helping to mitigate nitrate pollution risk, and shows good resistance to any major pathogen or pest.[5] This biomass can be collected from spontaneous riparian stands, in order to maintain riverbanks and mitigate the flooding risk.[6] From a structural point of view, *A. donax* is rich in both cellulose (ca. 40 wt%) and hemicellulose (ca. 20 wt%) fractions[7] and presents high reactivity in the hydrothermal conversion, thus offering outstanding perspectives for the conversion into biochemicals and biofuels.[8]

However, similarly to all lignocellulosic biomasses, crosslinking between polysaccharides and lignin via ester and ether linkages significantly hinders the hydrolysis of hemicellulose and cellulose to valuable reducing sugars.[9] For this reason, a pretreatment step is necessary to break down crosslinking and deconstruct the crystalline matrix, thus enhancing the enzyme accessibility to the cellulose during the following hydrolysis reaction.[10] Numerous pretreatment approaches have been investigated and reported in the literature.[11] Some of them include acids,[10] bases,[12] organic solvents[13] and steam-explosion.[8a] However, these methods have several disadvantages such as the high energy costs, the corrosion of the reactor and the use of hazardous chemicals and/or large quantity of solvents, with subsequent side stream-disposal problems. Another important limitation of some of the traditional pretreatment methods is represented by the incomplete fractionation and valorisation towards sugars of the hemicellulose fraction and its undesired conversion to by-products, such as furfural and humins, due to the harsh reaction conditions adopted.[14] Thus, the complete recovery by selective

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fractionation of the hemicellulose component into xylose or xylo-oligosaccharides (XOS) to be valorised would significantly improve the economic balance of biofuels and bioproducts production. In fact, xylose and XOS can be converted to various bio-based molecules or used as animal feed.[15]

In recent years, alternative methods for biomass pretreatment were investigated. Among them, the use of high-pressure carbon dioxide has attracted much attention due to its several advantages, such as non-toxicity, high diffusion rates and low cost.[16] The use of subcritical or supercritical CO_2 does not generate chemical wastes and the solvent can be easily removed from the products and recycled by a depressurization process and eventual purification through membranes or activated carbon.[16] According to the literature,[17] the presence of water together with high-pressure CO_2 significantly improves the efficiency of the pretreatment step, favouring the cellulose digestibility by hydrolytic enzymes. In particular, H_2O acts through two mechanisms: the formation of the carbonic acid after the CO_2 solubilisation in water and the biomass swelling.[18] The carbonic acid formation is described by the following reactions:

$$CO_{2\,aq} + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

H_2CO_3 promotes the hydrolysis of hemicellulose fraction to xylose or XOS. In fact, the pH of the H_2O/CO_2 mixture can decrease up to 3, also due to the contemporary release of acetic acid by hydrolysis of acetyl groups of hemicellulose, thus creating an acidic environment that facilitates biomass hydrolysis. At the same time, fibers swelling opens the pores of the biomass to allow CO_2 penetration and exposes a larger surface area for enzymatic hydrolysis during the depressurization process.[19] The fundamental role of water in the CO_2-based pretreatment of lignocellulosic biomass was demonstrated by Kim and Hong.[20] In their study, the high-pressure CO_2 pretreatment of biomass was performed in the presence and in the absence of water, and the efficacy of the two approaches was evaluated in terms of subsequent enzymatic digestibility of the pretreated solid residue. In the enzymatic hydrolysis, the sugar yield obtained from the sample pretreated with CO_2 without water was similar to the yield value achieved in the control test, namely the enzymatic hydrolysis of the non-pretreated raw material. On the contrary, the glucose yields significantly increased using the sample pretreated with CO_2 and water. In the literature, various studies reported the lignocellulosic biomass pretreatment based on the use of high-pressure CO_2,[21] but, up to now, the application of this process technology for the valorisation of giant reed has not been investigated. Regarding other types of lignocellulosic biomasses, Narayanaswamy et al.[22] pretreated corn stover and switchgrass using supercritical carbon dioxide ($scCO_2$) under the pressure of 230–250 bar, at 100–150 °C for 1 h with different moisture contents in the range 0–75 wt%. They demonstrated that in the absence of H_2O, $scCO_2$ pretreatment was less effective. Moreover, according to the study of Gao et al.,[23] temperature plays an important role in $scCO_2$ pretreatment since that temperature values lower than 100 °C do not significantly improve the digestibility of the pretreated solid for sugars production.

The aim of this study was the optimisation of an innovative catalytic high-pressure CO_2 pretreatment of giant reed, also by the synergistic addition of very low amounts of catalyst ($FeCl_3$) and surfactant (polyethylene glycol 400), and the following enzymatic hydrolysis employing the commercial enzymatic mixture Cellic® CTec3 HS, in order to produce valuable and concentrated sugar-rich hydrolysates. To the best of our knowledge, in the literature no study reported the use of neither the homogeneous acid Iron(III) chloride nor of the surfactant PEG 400 in combination with CO_2 for the pretreatment of lignocellulosic biomasses. $FeCl_3$ catalyses the hydrolysis of the glycosidic bonds of both polysaccharides and xylo- and gluco-oligosaccharides deriving from the xylan and the glucan fractions, improving the synthesis of fermentable monosaccharides.[24] Moreover, the traditional use of this catalyst in water requires higher amount (1–3 wt%)[7,24] than the value optimised in the present study (0.16 wt%) in combination with high-pressure CO_2. The use of inorganic salts for the hydrolysis of lignocellulosic biomass is gaining significant interest in the recent years[25] since that, respect to traditional strong inorganic acids, they present several advantages such as less corrosion of reactor, recovery by precipitation, the possibility of working under mild process conditions and low inhibitors production for successive fermentative processes.[7b,24–25] Among inorganic salts, $FeCl_3$ is one of the most effective and efficient catalysts for hemicellulose and/or cellulose hydrolysis.[24,26] Moreover Fe^{3+} ions can improve the enzymatic digestibility of solid residues deriving from this pretreatment.[25a,27]

On the other hand, PEG 400 is a non-ionic surfactant that improves the lignin and humins solubility and modifies the biomass surface favouring the cellulose digestibility through the reduction of unproductive binding to the enzymes.[28] In fact, lignin is a hydrophobic aromatic polymer that shows a significant affinity for cellulase enzymes by hydrophobic, electrostatic, or hydrogen-bonding interactions, which reduce their activity. Hydrophobic interactions are the most influential on non-specific binding of cellulases onto lignin and humins surface.[29] Surfactants like PEG 400 are amphiphilic molecules that contain both hydrophilic and lipophilic groups that can reduce surface tension and help to remove hydrophobic molecules and modify the structure and surface of lignocellulosic biomass.[30] The role of PEG 400 can be explained by the modification of the structure of the biomass allowing greater accessibility to Cellic® CTec3 HS enzymes, and by the reduction of the non-productive adsorption of the cellulase enzyme on lignin due to the preferential adsorption of the surfactant onto the exposed surface of lignin.[29] The innovative combination $FeCl_3$/PEG400/CO_2 was investigated and optimised in order to maximise the sugars production and the valorisation of the renewable resource *A. donax* L. The main advantages of the proposed pretreatment approach with respect to other pretreatment systems are mainly related to: (i) the tunability of the pretreatment towards the production of xylose or xylo-oligosaccharides based on the use of PEG 400 or very low concentration of $FeCl_3$ in combination with $scCO_2$; (ii) the higher

xylose yield; (iii) the significant removal of the hemicellulose fraction in water at mild temperature in order to obtain a cellulose-rich solid residue suitable for the subsequent production of glucose-rich hydrolysates by enzymatic hydrolysis; (iv) the use of CO_2 as a green catalyst characterised by recyclability, non-flammability, non-toxicity, high diffusion rates and low cost.

Results and Discussion

Kinetics of products formation during $scCO_2$ pretreatment

Most of the literature studies based on the use of high-pressure CO_2 adopted harsh reaction conditions (high temperature and pressure) for lignocellulosic biomass pretreatment in order to maximise the biomass destructuration and the exposure of cellulosic fibers to the cellulases in the following enzymatic hydrolysis to give glucose.[17,23,31] One limitation of this approach is the absence of the hemicellulose fraction exploitation since high values of temperature favour the dehydration of xylose to furfural that cannot be valorised via fermentation and acts as a growth inhibitor for major microorganisms used as biocatalysts.[32] In the present study, in order to maximise the exploitation of both hemicellulose and cellulose fractions, a tailored $scCO_2$ pretreatment was investigated, starting from a preliminary study of the kinetics of products formation (sugars and by-products) during the process (Figure 1). A fixed temperature of 160 °C was selected according to previous studies on the selective fractionation and valorisation of the hemicellulose fraction of giant reed[7a,8b] and a proper amount of water was added into the reactor in order to obtain the biomass loading of 9 wt%. This value of solid-to-liquid ratio was selected in order to achieve xylose- and XOS-rich hydrolysates with a low concentration of by-products, i.e. furfural and humins, in the perspective of subsequent complete sugars valorisation via chemical and/or biological catalytic approaches.[33]

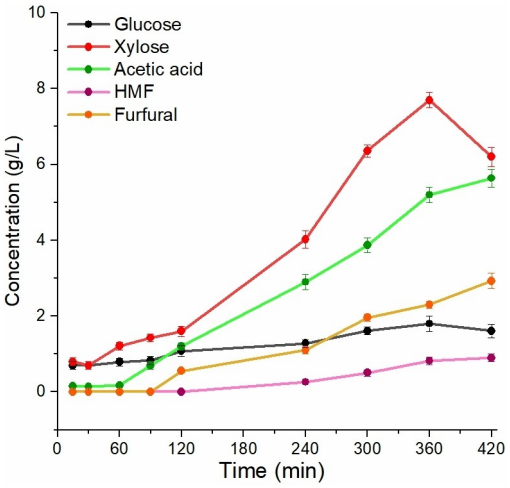

Figure 1. Kinetics of products formation during $scCO_2$ pretreatment of raw *Arundo donax* L. Reaction conditions: 160 °C, 140 bar, biomass loading 9 wt%.

As reported in Figure 1, in the first 2 h a slight increase in xylose and acetic acid concentration was observed, while a negligible formation of glucose, 5-hydroxymethylfurfural (HMF) and furfural was observed. From 2 to 6 h a significant increase in xylose, acetic acid and furfural was observed and only a low increase in glucose and HMF concentration was ascertained. This trend agreed with the selective fractionation and depolymerisation of the hemicellulose fraction to give xylose and acetic acid. The latter derives from structural acetyl groups of the hemicellulose so it can be used as an indicator of the progress of its depolymerisation.[34] Furfural and HMF derived from the acid-catalysed dehydration reaction of xylose and glucose, respectively.[2,35] The acidification of the medium is due to the formation of carbonic acid and the release of acetic acid. After 6 h a significant decrease in xylose concentration from 8 to 6 g/L was observed while furfural concentration increased from 2 to 3 g/L. Noteworthy, the formation of levulinic acid through the rehydration reaction of HMF was not observed due to the relatively mild process conditions adopted in this pretreatment. The maximum xylose concentration, namely the target product, was reached at 6 h with a yield of around 31 mol% with respect to the moles of xylan present in the unpretreated biomass and the process time for the following runs was chosen accordingly. In the literature, the reported reaction time for $scCO_2$ pretreatment of lignocellulosic biomasses ranged from 10 min up to 48 h.[16–17]

In order to optimise the high-pressure CO_2 pretreatment of giant reed, the effect of the pressure, namely subcritical and supercritical conditions, and the addition of very low amounts of acid catalyst $FeCl_3$ and/or surfactant PEG 400 was investigated according to the experimental setup reported in Table 1 and the obtained results are commented in the following paragraphs.

Study of the effect of pressure

In order to investigate the effect of the pressure on the hemicellulose fractionation and hydrolysis during the CO_2-pretreatment of giant reed, two different values of pressure at 160 °C were tested: 60 bar, corresponding to subcritical conditions, and 140 bar, corresponding to supercritical conditions. The aim was to study the effect of subcritical and supercritical CO_2 pretreatments on xylose production. In fact, most of the literature studies based on the use of high-pressure CO_2

Table 1. Summary of experimental runs. Reaction conditions: 160 °C, biomass loading 9 wt%, reaction time 6 h.

Run	Gas	Pressure [bar]	Additive [wt%]
1	CO_2	140	–
4	CO_2	140	$FeCl_3$ 0.16
6	CO_2	140	PEG 400 1.0
7	CO_2	140	$FeCl_3$ 0.16 + PEG 400 1.0
2	CO_2	60	–
3	N_2	140	–
5	N_2	140	$FeCl_3$ 0.16
8	N_2	140	$FeCl_3$ 0.16 + PEG 400 1.0

adopted harsher reaction conditions for lignocellulosic pretreatment, hampering the xylan valorisation to xylose and/or XOS and focusing only on the valorisation of the cellulose fraction. On the other hand, the present approach was focused on the two-step fractionation and hydrolysis of both hemicellulose and cellulose fractions to valuable sugars or oligosaccharides. Moreover, in order to discriminate the effect of the sole autohydrolysis, namely the effect of hydrothermal pretreatment,[36] from that of the $scCO_2$ (acidity and swelling) on the xylan fractionation/hydrolysis, a control test, i.e. a simple autohydrolysis, was performed under nitrogen atmosphere at 140 bar, 160 °C, 6 h (run 3, Table 1).

Figure 2 shows the results obtained in runs 1–3 in terms of xylan and glucan conversions and xylose, XOS, glucose and gluco-oligosaccharides (GOS) yields with respect to the moles of xylan and glucan present in the raw material, respectively.

In particular, in run 1, xylan and glucan conversions were 74.0 and 16.4 mol%, respectively, while xylose and XOS yields were 32.5 and 24.7 mol%, respectively. On the other hand, the subcritical approach (run 2) showed similar results with respect to the hydrothermal pretreatment (autohydrolysis, run 3). Water plays a fundamental role in both hydrothermal and CO_2-based pretreatments since allows the swelling of fibers and the formation of CH_3COOH from acetyl groups of hemicellulose and H_2CO_3 through the solubilisation of CO_2. The release of these acids decreases pH thus favouring the hydrolysis of glycosidic bonds of the hemicellulose and amorphous cellulose fractions to give xylose, XOS, glucose and GOS. The results obtained from hydrothermal pretreatment agreed with those reported in the literature for the same biomass[36] and for other types of lignocellulosic material.[37]

In run 2, xylan and glucan conversions were 66.1 and 11.0 mol%, respectively, while xylose and XOS yields were 19.2 and 41.1 mol%, respectively. The ineffectiveness of subcritical CO_2 pretreatment of giant reed agreed with the literature for other lignocellulosic biomasses.[34] Differently, the catalytic effect of $scCO_2$ with respect to the control test can be explained in terms of higher acidity and swelling ability, since it allowed an increase in both the conversion of xylan and, above all, the yield of xylose to the detriment of the production of XOS, whose yield decreased from 41.1 to 24.7 mol%.

The selective removal of the hemicellulose fraction through the $scCO_2$ pretreatment was confirmed by ATR-FTIR analysis (Figure 3). Figure 3 shows the comparison between the FT-IR spectra of starting biomass and the $scCO_2$ pretreated material. This last one showed the decrease of the intensity of the band at 1734 cm^{-1}, corresponding to the C=O stretching of the acetyl groups of the hemicellulose fraction. This difference confirmed the significant hemicellulose removal.[8b,36] Moreover, the same spectrum showed an increase in the intensity of the absorption bands at 1509 and 1458 cm^{-1}, assigned to the C=C stretching of benzene rings of lignin and the bending vibration of the -CH_2 groups of cellulose, respectively.[36,38] These findings agreed with the enrichment of cellulose and lignin in the pretreated biomass. Lastly, the bands at 1055 and 1034 cm^{-1} were due to C–O–C stretching of the pyranose ring in cellulose and the C–O stretching of hydroxyl and ether groups of cellulose.[8b,38]

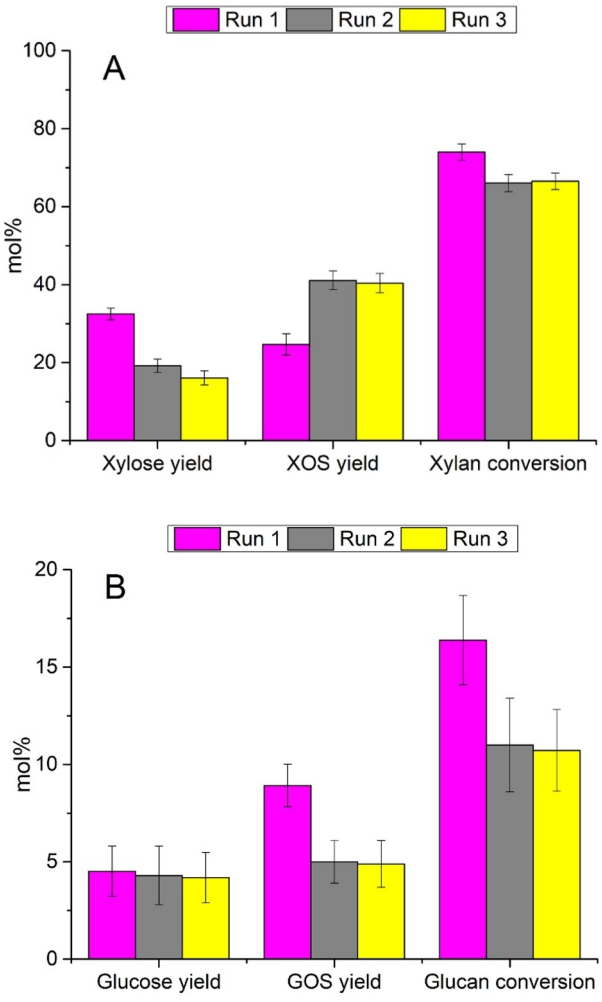

Figure 2. Comparison of the results obtained from supercritical (140 bar, run 1) and subcritical (60 bar, run 2) CO_2 pretreatment and 140 bar N_2 pretreatment (autohydrolysis, run 3) of giant reed. (A) xylose and XOS yields and xylan conversion; (B) glucose and GOS yields and glucan conversion.

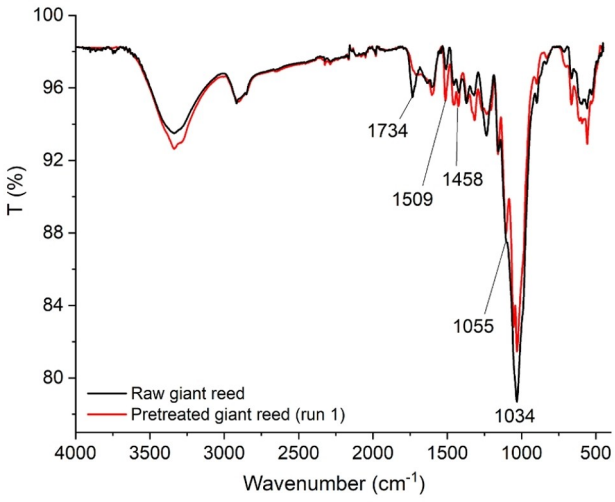

Figure 3. ATR-FTIR spectra of the raw giant reed and the solid residue recovered after $scCO_2$ pretreatment (run 1).

$FeCl_3$-catalysed $scCO_2$ pretreatment

In order to improve the sugars conversion, for the first time, the catalytic high-pressure CO_2 hydrolysis was investigated in the presence of $FeCl_3$ as homogeneous catalyst[7] (run 4, Table 1). In the last years, the use as catalysts of inorganic salts, especially metal chlorides, has significantly increased because they are efficient, safer, cheaper and more easily recoverable than mineral acids.[39]

Among metal chlorides, $FeCl_3$ is one of the most promising catalysts for the hydrolysis of polysaccharides to sugars.[24,40] Moreover, Fe^{3+} ions displayed a positive influence on the activity of the hydrolytic enzymes.[27] For these reasons, a very low amount of $FeCl_3$ (0.16 wt%) was tested, also to avoid the corrosion of the reactor. In a previous study based on the microwave-assisted $FeCl_3$-catalysed fractionation and hydrolysis of the giant reed hemicellulose to give xylose, the optimal catalyst amount was 1.6 wt% (operating at 150°C, 2.5min, biomass loading 9 wt%) namely 10-folds higher than the value adopted in the present study.[7a] Also in this case, a control test was performed under high-pressure N_2 with the same concentration of catalyst (run 5, Table 1) in order to discriminate the effect of the $FeCl_3$ and that of CO_2. Figure 4 compares the results obtained from runs 1, 4 and 5 (Table 1).

As shown in Figure 4A, the xylan conversion in the $FeCl_3$-catalysed $scCO_2$ pretreatment (74.4 mol%, run 4) was similar to that obtained in the absence of the salt (74.0 mol%, run 1), probably due to the very low amount of the catalyst. Also the xylan conversion under high-pressure N_2 was similar in runs 3 (66.5 mol%) and 5 (66.7 mol%), namely in the absence and in the presence of $FeCl_3$, confirming the negligible effect of the low concentration of the catalyst on the hemicellulose dissolution. However, the catalyst significantly affected the process selectivity. In particular, in run 4 xylose and XOS yields were 46.2 and 10.2 mol%, respectively, while in run 1 the values were 32.5 and 24.7 mol%. Therefore, $FeCl_3$ affected the selectivity of the xylan hydrolysis favouring the synthesis of monosaccharides instead of oligosaccharides. The very low amount of the homogeneous acid (0.16 wt%) in combination with $scCO_2$ allowed the achievement of a xylose yield (46.2 mol%) much higher than the value (11.2 mol%) obtained in the microwave-assisted $FeCl_3$-catalysed hydrolysis in the presence of the same amount of the catalyst.[7a] The same findings on the role of $FeCl_3$ on the selectivity of the reaction were obtained by comparing the two control tests, namely the sole hydrothermal approaches, performed under high-pressure N_2 (runs 3 and 5, Table 1). They agreed with data reported in the literature for the hydrothermal pretreatment in the presence of $FeCl_3$.[41] $FeCl_3$ catalysed the acid hydrolysis of the glycosidic bonds of XOS formed during high-pressure CO_2 pretreatment, promoting the formation of reducing sugars. In all the runs shown in Figure 4B, the glucan conversion values were quite similar and ranged from 10.1 to 16.4 mol%. The presence of the homogeneous acid catalyst slightly decreased the GOS yield and slightly increased the glucose yield. Based on the obtained results, the use of $FeCl_3$-catalysed $scCO_2$ pretreatment of giant reed helps to address the hemicellulose valorisation towards fermentable target xylose rather than XOS only slightly influencing the glucan hydrolysis.

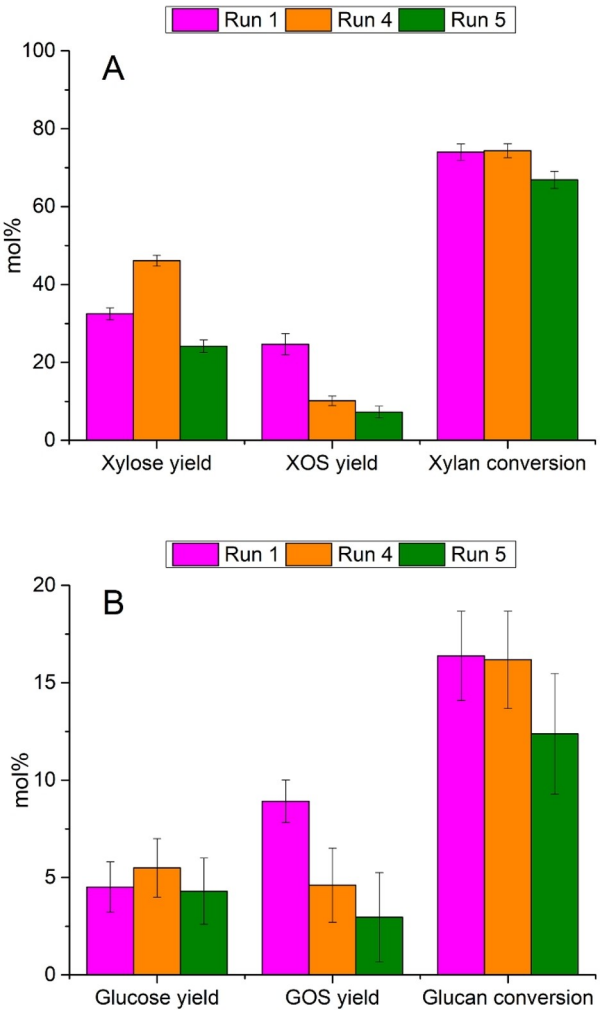

Figure 4. Comparison of the results obtained from $scCO_2$ (run 1), $FeCl_3$-catalysed $scCO_2$ (run 4) and $FeCl_3$-catalysed 140 bar N_2 (run 5) pretreatment of giant reed. (A) xylose and XOS yields and xylan conversion; (B) glucose and GOS yields and glucan conversion.

Study of the effect of surfactant PEG 400

The use of surfactants in both the pretreatment step and the following enzymatic hydrolysis reaction to enhance the biomass destructuration and improve the sugars recovery was widely reported in the literature.[42] Surfactants are amphiphilic molecules, characterised by both hydrophilic and lipophilic functional groups, that reduce surface tension and help to remove hydrophobic molecules and modify the structure and surface of biomass.

In particular, in the pretreatment step, the surfactant promotes the removal of the hemicellulose fraction from biomass by complexing the hydrophobic compounds deriving from hemicellulose and lignin, and avoiding their repolymerisation, thermal degradation and precipitation on the solid residue

surface.[28] In the present investigation, water-soluble PEG 400 was selected as a non-ionic surfactant according to previous studies[29,43] and for the first time it was employed in combination with $scCO_2$ and $FeCl_3$-catalysed $scCO_2$. Moreover, in the perspective of the valorisation of sugar-rich hydrolysates obtained from the $scCO_2$-catalysed hemicellulose hydrolysis via fermentation route, PEG 400 boosts the metabolic performance and the vitality of biocatalysts.[44] A $scCO_2$ pretreatment added with 1 wt% PEG 400 (run 6, Table 1) was performed. Figure 5 shows the results obtained in run 6 and the comparison with those obtained in $scCO_2$ pretreatment without the surfactant (run 1, Table 1). As reported in Figure 5A, the addition of PEG 400 increased the xylan conversion by 6.4 mol%, varying from 74.0 mol% (run 1) to 80.4 mol% (run 6). Differently, no significant improvement in glucan conversion was observed in the presence of the surfactant (Figure 5B). The adopted reaction conditions ensured the selective fractionation of the hemicellulose fraction, according to previous studies.[8b] Moreover, PEG 400 significantly affected the selectivity of the hemicellulose hydrolysis reaction since it favoured the formation of XOS to the detriment of xylose synthesis. The XOS yields were 24.7 and 51.4 mol% without and with PEG 400, respectively. On the contrary, the xylose yield decreased from 32.5 (run 1) to 19.4 mol% (run 6). The glucose and GOS yields were not affected by the presence of the surfactant (Figure 5B). Based on these results, it is possible to infer that PEG stabilises XOS making them less available to the acid hydrolysis of their glycosidic bonds, decreasing the synthesis of monosaccharides and increasing the XOS production.

Study of the synergistic effect of PEG 400 and $FeCl_3$

In order to investigate the eventual synergistic effect of surfactant PEG 400 and acid catalyst $FeCl_3$, the PEG-assisted $FeCl_3$-catalysed $scCO_2$ pretreatment of giant reed was carried out (run 7, Table 1). The aim was to combine the increase in the xylan conversion obtained by adding PEG 400 with the increase in xylose production due to the presence of the acid catalyst $FeCl_3$. Figure 6 shows the results obtained in run 7 and the

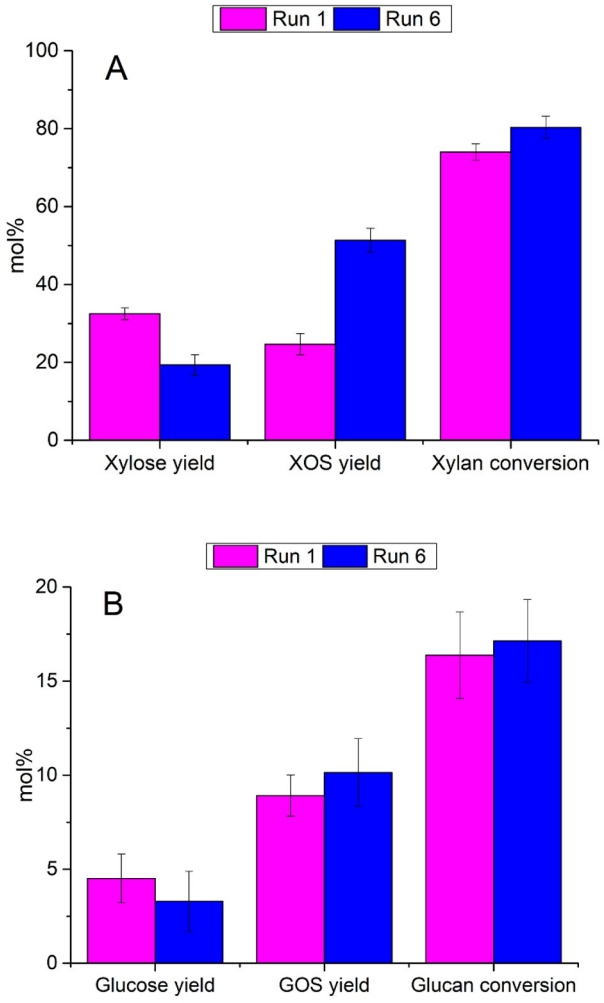

Figure 5. Comparison of the results obtained from $scCO_2$ pretreatment without (run 1) and with (run 6) surfactant PEG 400. (A) xylose and XOS yields and xylan conversion; (B) glucose and GOS yields and glucan conversion.

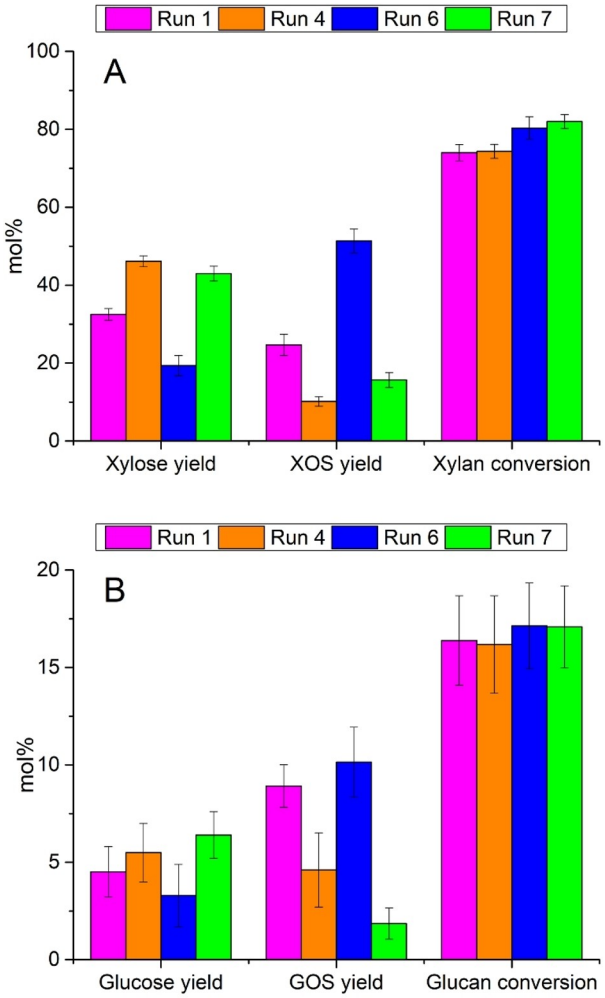

Figure 6. Comparison of the results obtained from $scCO_2$ (run 1), $FeCl_3$-$scCO_2$ (run 4), PEG-$scCO_2$ (run 6) and PEG-$FeCl_3$-$scCO_2$ (run 7) pretreatment of giant reed. (A) xylose and XOS yields and xylan conversion; (B) glucose and GOS yields and glucan conversion.

comparison with those obtained in runs 1, 4 and 6, (Table 1). In run 7 the xylan conversion was 82 mol%, namely very similar to the value (80 mol%) obtained in the presence of the sole PEG 400 (run 6) and higher than that (74.4 mol%) obtained in the presence of the sole $FeCl_3$ (run 4). The synergistic effect is better evidenced by the behaviour of product yields: XOS yield significantly decreased to 15.7 mol% while xylose yield increased up to 43.0 mol%, a value very similar to the maximum xylose yield (46.2 mol%) obtained in run 4.

By comparing runs 7 and 1, the presence of $FeCl_3$ and PEG 400 allowed to achieve higher xylan conversion and xylose yield with respect to $scCO_2$ pretreatment without them. These results confirmed the efficacy of this innovative catalytic strategy based on the use of an almost homoeopathic amount of $FeCl_3$ and PEG 400 in combination with $scCO_2$ pretreatment of giant reed. Similarly to previous runs, the glucan conversion and yield were not significantly affected due to the mild reaction conditions adopted in the present study aiming to firstly exploit the hemicellulose fraction and part of the amorphous cellulose fraction to give sugars with a low concentration of by-products.

Finally, a control test was performed under high-pressure N_2 in the presence of the same concentration of catalyst and surfactant (run 8, Table 1) in order to evaluate the role of CO_2 on the reaction behaviour in the presence of both the additives. Figure 7 shows the results obtained in run 8 and the comparison with those obtained in run 7.

The significant effect of $scCO_2$ on the xylan valorisation compared to the hot compressed water was demonstrated by the significant increase in both the conversion of xylan and the xylose and XOS yields (Figure 7A). Differently, regarding the glucan conversion, the two approaches showed almost similar results, as reported in Figure 7B. Thus, the synergistic $scCO_2$ pretreatment resulted the optimal process configuration for the selective and almost complete fractionation (82 mol%) of the xylan fraction of giant reed to give valuable xylose and XOS. The chemical composition of both the starting raw biomass and the solid residue obtained in run 7 are reported in Figure 8. The starting feedstock contained 34.7±1.4 wt% glucan, 21.9±0.7 wt% xylan, 1.9±0.1 wt% arabinan, 3.8±0.3 wt% acetyl groups, 2.7±0.1 wt% ash, 13.5±0.6 wt% extractives, 19.5±1.3 wt% acid-insoluble lignin, 2.0±0.2 wt% acid-soluble lignin. The solid residue obtained under the optimised reaction conditions was composed of 50.9±1.5 wt% glucan, 7.0±0.3 wt% xylan, 31.1±1.6 wt% acid-insoluble lignin, 3.4±0.1 wt% ash, 7.6±0.9 wt% other. All the values represent the mean, n=3, ±standard deviation. The significant removal of both the hemicellulose fraction and extractives through the pretreatment allowed the obtaining of a cellulose-rich solid residue, suitable as the substrate for the following enzymatic hydrolysis to give mainly glucose. The xylan removal to give valuable and fermentable xylose and XOS obtained under the optimised reaction conditions was higher than the values reported in the literature for other types of lignocellulosic biomasses pretreated with $scCO_2$[33,45]

In particular, Morais et al.[33a] obtained the xylan conversion of 77.4 mol% and the xylose yield of 17.1 mol% from the $scCO_2$ pretreatment of wheat straw at 215 °C, 126 bar, 1 h with a biomass loading of 9 wt%. Sohni et al.[45a] achieved the xylan conversion of 39.8 mol% from the $scCO_2$ pretreatment of oil palm biomass at 80 °C, 350 bar, 1 h with a biomass loading of 40 wt%. From the $scCO_2$ pretreatment of wheat straw, Wang et al.[45b] reached the xylan conversion of 86.4 mol% at 200 °C, 30 bar, 10 min with a biomass loading of 7.7 wt%, but the glucose and xylose yields were 3.9 and 9.0 mol%, respectively. Relvas et al.[33b] used the $scCO_2$ to selectively hydrolyse the hemicellulose fraction of wheat straw to XOS, achieving the XOS yield of 79.6 mol% at 180 °C, 50 bar (initial CO_2 pressure), 12 min and the biomass loading of 9 wt%.

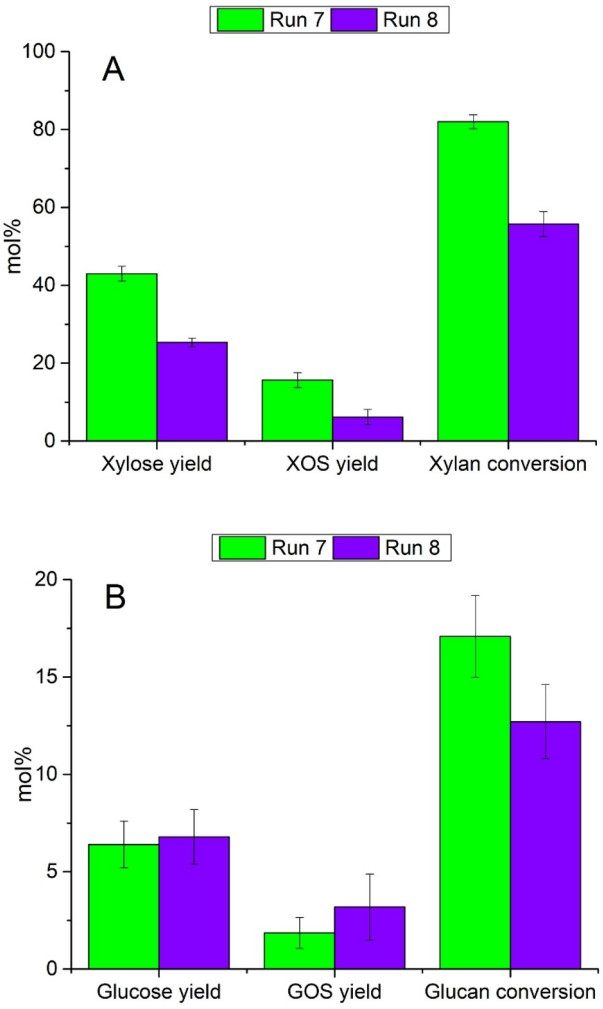

Figure 7. Comparison of the results obtained from 140 bar CO_2 (run 7) and N_2 (run 8) pretreatment in the presence of both $FeCl_3$ and PEG 400. (A) xylose and XOS yields and xylan conversion; (B) glucose and GOS yields and glucan conversion.

Enzymatic hydrolysis

In the perspective of the complete exploitation of biomass polysaccharides to give valuable second-generation sugars, the enzymatic hydrolysis of the pretreated solid residues was investigated and optimised. The use of commercial enzymes

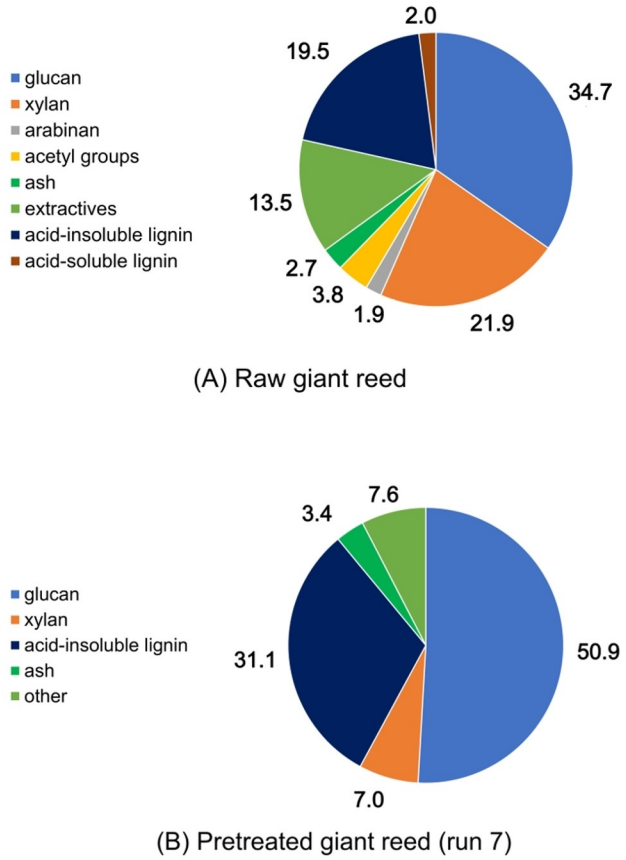

Figure 8. NREL compositional analysis of the raw giant reed (A) and the pretreated solid residue obtained from run 7 (B).

based on cellulases and hemicellulases presents some important advantages such as the high selectivity of the reaction towards glucose and xylose, the mild and sustainable reaction conditions (50 °C, pH 4.8, atmospheric pressure, use of water as solvent) and the possibility to simultaneously use various biological catalysts without any negative interactions among them. In the present work the commercial mixture Cellic® CTec 3 HS was adopted since it is one of the most efficient enzymes for lignocellulosic biomass exploitation[46] and, up to now, no study investigated its performance on the $scCO_2$ pretreated biomass. Cellic® CTec 3 HS contains cellulases, isolated from the filamentous fungus *Trichoderma reesei*, endo- and exo-cellobiohydrolases, bacterial β-glucosidase, and hemicellulases.[47] The kinetics of hydrolysis reaction was studied and the effect of both the enzyme concentration and the type of the pretreatment on the enzymatic activity was investigated.

Kinetics study of enzymatic hydrolysis in the presence of different catalyst dosages

In order to optimise the enzymatic hydrolysis of the glucan fraction of $scCO_2$ pretreated giant reed to give glucose, different catalyst amounts were adopted. In particular, 15, 30 and 60 FPU/g glucan of Cellic® CTec 3 HS were tested, and the three kinetics were compared, as shown in Figure 9.

The solid residue obtained from run 1 was used as substrate. All the enzymatic hydrolysis were performed with a biomass loading of 10 wt% in order to obtain high concentrations of sugars into the hydrolysates, making them more suitable for subsequent valorisation processes via chemical or biological routes.

After 96 h the glucose and xylose yields of 40.1 and 54.2 mol% were achieved, respectively, in the presence of 15 FPU/g glucan. Differently, the increase of the enzyme dosage to 30 or 60 FPU/g glucan improved both the glucose and xylose synthesis according to the literature,[46c,48] reaching the glucose yield of 53.0 and 56.7 mol%, respectively, and the xylose yield of 72.5 and 89.2 mol%, respectively. Based on these results, the selected enzyme concentration was 30 FPU/g glucan since no significant difference in the glucose yield was observed between 30 and 60 FPU/g glucan, thus the choice of the lower enzyme dosage reduced the process cost, increasing the economic sustainability of the proposed process. The glucose

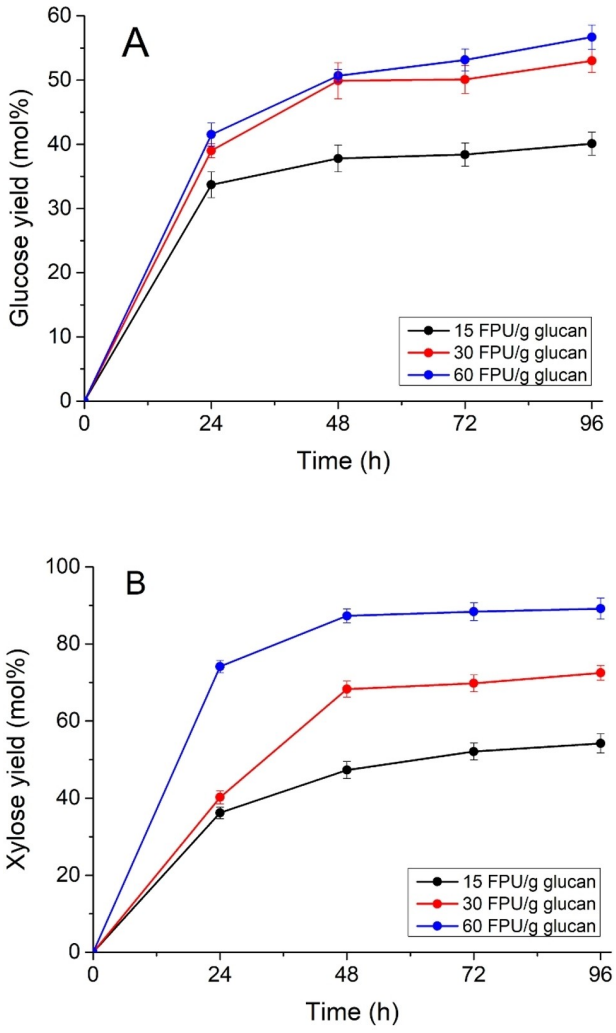

Figure 9. Kinetics of enzymatic hydrolysis of $scCO_2$ pretreated giant reed: (A) glucose yield (mol%); (B) xylose yield (mol%).

yield obtained in the presence of 30 FPU/g glucan of Cellic® CTec 3 HS from the $scCO_2$ pretreated giant reed was higher than the value (40 mol%) obtained from the enzymatic hydrolysis of microwave-assisted acid-pretreated giant reed catalysed by Cellic® CTec 2.[7b] The same enzyme dosage was adopted in all the following runs.

Enzymatic hydrolysis of pretreated solid residues

The enzymatic hydrolysis of the different solid residues was performed in order to study the effect of the various $scCO_2$ pretreatments on their digestibility to give valuable reducing sugars. In addition, a control test was performed on the unpretreated giant reed under the same reaction conditions in order to validate the effectiveness of the $scCO_2$ pretreatment for the glucan fraction exploitation. Figure 10 shows the kinetics of the enzymatic hydrolysis performed on raw giant reed (black line) and residues obtained from runs 1, 3, 4, 6 and 7, Table 1 (coloured lines). In the enzymatic hydrolysis of unpretreated biomass the glucose and xylose concentrations of 10.5 and 9.0 g/L, corresponding to 23.8 and 31.4 mol% with respect to the moles of glucan and xylan in the starting raw material, were achieved, respectively. The yield values obtained from pretreated giant reed ranged from 40 to 62 mol% for the glucose yield and from 50 to 89 mol% for the xylose yield with respect to the moles of glucan and xylan in the pretreated solid residues, confirming the fundamental role of the pretreatment step for the cellulose exploitation of giant reed. The solid residue obtained from the sole hydrothermal pretreatment (run 3, Table 1) showed a minor digestibility than those pretreated with $scCO_2$, confirming the beneficial role of the $scCO_2$ on both the destructuration of lignocellulosic matrix and the exposure of the cellulosic fibers to the enzymatic activity. In particular, the $scCO_2$ pretreatment (run 1, Table 1) allowed the production of 31.1 g/L glucose and 7.8 g/L xylose, corresponding to the yields of 53.6 and 66.9 mol%, respectively, in the following enzymatic hydrolysis. Differently, the sole hydrothermal treatment (run 3, Table 1) determined the glucose and xylose concentration of 26.8 and 8.0 g/L, corresponding to the lower yields of 40.7 and 50.1 mol%, respectively. These results agreed with those reported in the literature,[17,33a] evidencing

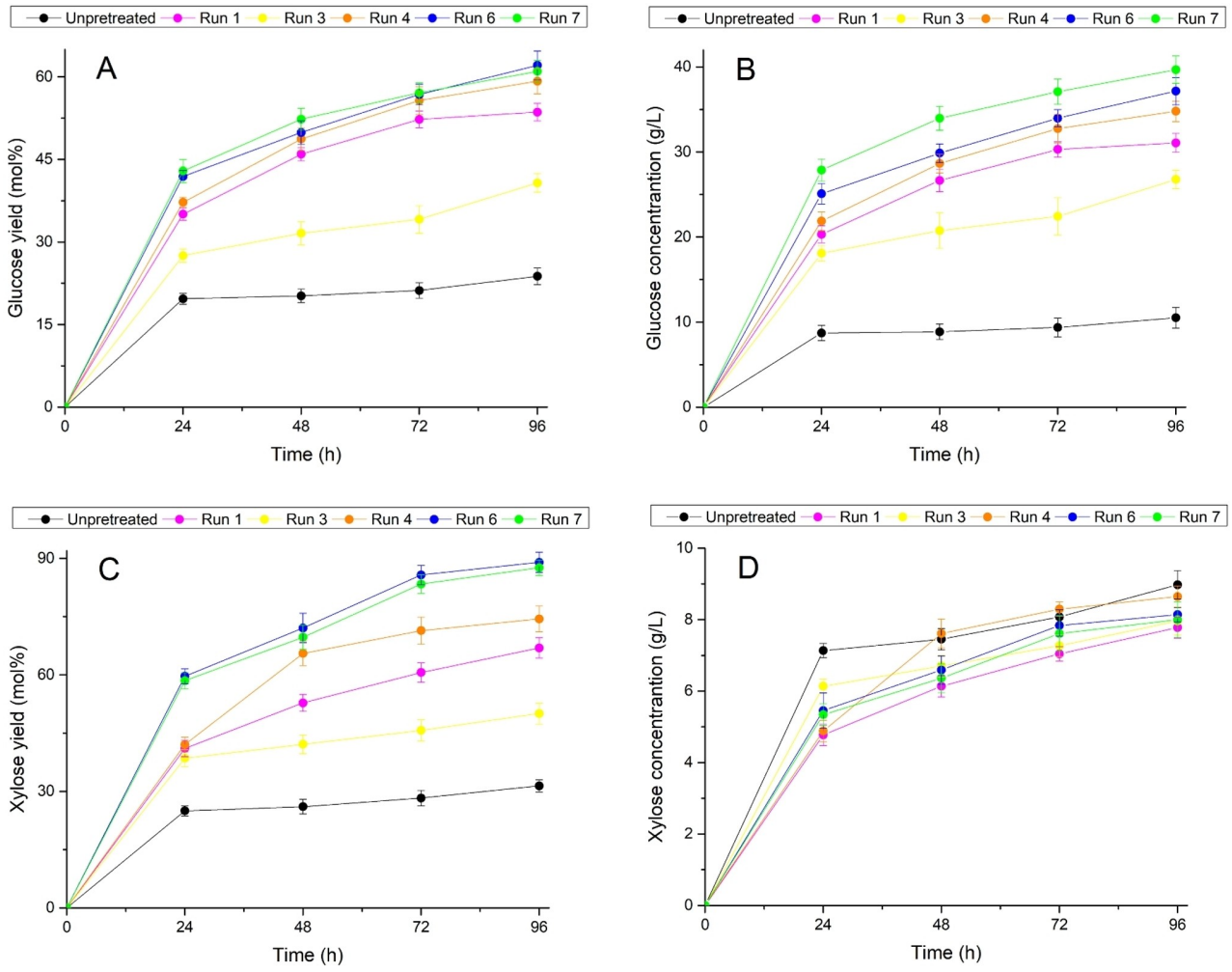

Figure 10. Kinetics of enzymatic hydrolysis of pretreated and unpretreated giant reed: (A) glucose yield; (B) glucose concentration; (C) xylose yield; (D) xylose concentration.

the beneficial effect of $scCO_2$ on the efficiency of the subsequent enzymatic hydrolysis. Regarding the $FeCl_3$-catalysed $scCO_2$ pretreatment (run 4, Table 1) and the $scCO_2$ pretreatment with PEG 400 (run 6, Table 1), they allowed the glucose production of 34.8 and 37.2 g/L, corresponding to the yields of 59.2 and 62.1 mol%, respectively, and the xylose production of 8.6 and 8.1 g/L, corresponding to the yields of 74.4 and 89.0 mol%, respectively, in the following enzymatic hydrolysis. The glucose yields were slightly higher than that obtained from the $scCO_2$ pretreatment without additives. Moreover, similar glucose and xylose yields of 61.0 mol% (39.7 g/L) and 87.6 mol% (8 g/L) were also obtained by hydrolysing the solid residue obtained with the synergistic optimal approach (run 7, Table 1). Considering all the results obtained in the pretreatment step and the enzymatic hydrolysis, the two-step process based on the synergistic PEG/$FeCl_3$/$scCO_2$ hydrolysis of the hemicellulose fraction and the subsequent enzymatic hydrolysis of the cellulose fraction with Cellic® CTec3 HS ensured the highest overall sugars production, with the total removed sugars (TRS) value of 80.1 wt% with respect to the starting raw material. The total glucose yield of 67.8 mol% achieved under the optimised two-step process was higher than the value obtained in previous studies on the exploitation of pretreated giant reed in the presence of the same dosage of Cellic® CTec2.[7b,8b,49] Moreover, regarding other types of $scCO_2$-pretreated biomasses, Sohni et al.[45a] achieved the glucose yield of 61.1 mol% from oil palm biomass by using a Cellic® CTec2 dosage of 80 FPU/g glucan, namely a very high catalyst concentration. In the present work, a very similar yield was achieved from giant reed by adopting the significantly lower enzyme dosage of 30 FPU/g glucan, thus increasing the economic sustainability of the proposed biorefinery process. Zhao et al.[19] performed the enzymatic hydrolysis of $scCO_2$ pretreated corn stover, corn cob, and sorghum stalk (process conditions of the pretreatment step: 200–250 bar CO_2, 70–80 °C, 48–60 h, 25 wt% biomass loading), achieving the glucose yield of 62.2, 45.6 and 47.2 mol%, respectively. With respect to this last study, the implemented two-step process achieved a total glucose yield (67.8 mol%) similar to that obtained from corn stover and much higher than those reached from corn cob and sorghum stalk. It is remarkable that these better performances were reached by adopting more sustainable conditions for the pretreatment step, especially in terms of CO_2 pressure (140 versus 200–250 bar) and process time (6 versus 48–60 h).

The mass balance flow diagram of the implemented two-step process under the optimised reaction conditions is reported in Figure 11. The first step allowed the significant exploitation of the hemicellulose fraction of giant reed to give xylose and XOS together with a minor amount of glucose, GOS and arabinose. This type of hydrolysate can be directly exploited via chemical or biological routes.[2] Alternatively, the purified monosaccharides and oligosaccharides can be used as animal feed[15] and for other purposes and applications, as recently reported in the literature.[50] The cellulose-rich solid residue represented a useful substrate for the production of lignocellulosic hydrolysates with a high concentration of reducing sugars (glucose and xylose). Under the optimised reaction conditions, the obtained hydrolysate contained around 50 g/L sugars (ca. 40 g/L glucose and 10 g/L xylose), representing a suitable carbon source for fermentation processes in the perspective of biological valorisation of these bio-based products.[8a,10,46c] The final solid residue, containing residual cellulose (26.3 wt% on the dry matter) and mainly lignin (49.6 wt% on the dry matter), can be valorised towards the production of levulinic and formic acid from cellulose[2,7b,8c] and aromatic compounds from lignin[40a,51] in the perspective of an integrated biorefinery scheme aiming at the complete exploitation of the renewable resource.

Conclusion

For the first time, the use of catalytic high-pressure CO_2 as a pretreatment method of giant reed was studied and optimised in order to deconstruct the lignocellulosic structure and solubilise the hemicellulose fraction in the form of oligomers (XOS) and mainly of xylose. In the pretreatment step, the combined use of supercritical CO_2 ($scCO_2$) with low amounts of $FeCl_3$ and PEG 400, allowed the removing ca. 80 wt% of the xylan and glucan contained in the starting biomass. A very low amount of inorganic salt (0.16 wt%) and surfactant (1.0 wt%) favoured the dissolution of the hemicellulose fraction to xylo-oligosaccharides and their partial hydrolysis to fermentable xylose with a xylan conversion of 86 mol% and xylose yield of 43 mol%, under the optimised process conditions. In particular, the present work demonstrated that PEG 400 in combination with $scCO_2$ favoured the xylan fractionation to oligomers, while the $FeCl_3$-catalysed $scCO_2$ favoured the xylan hydrolysis to xylose. This innovative catalytic strategy resulted in a useful versatility of the pretreatment step which appeared extremely adaptable as a function of the target use of the recovered liquid fraction. The synergistic combination of the acid with the surfactant and $scCO_2$ ensured the maximum xylan conversion and xylose yield. The solid residues obtained from $FeCl_3$-catalysed $scCO_2$, PEG 400-assisted $scCO_2$ and PEG 400-assisted $FeCl_3$-catalysed $scCO_2$ pretreatments showed good enzymatic digestibility. The enzymatic hydrolysis was optimised by using the new commercial mixture Cellic® CTec3 HS. The effect of different enzyme dosages (15, 30, 60 FPU/g glucan) on the enzymatic activity and the reaction time were investigated. Under the optimised reaction conditions (30 FPU/g glucan, 96 h, 50 °C, pH 4.8, 10 wt% biomass loading), glucose and xylose yields of around 61 and 88 mol%, respectively, were reached with respect to the glucan and xylan moles present in the solid residue deriving from the PEG 400-assisted $FeCl_3$-catalysed $scCO_2$ pretreatment. In the two-step process, total removal of sugars from the starting raw biomass of around 80 wt% was achieved. From this process layout, two valuable liquid fractions were obtained: the first one rich in xylose and XOS and the second one rich in glucose. Both these hydrolysates represent strategic feeds for subsequent valorisation steps via chemical or biological routes in order to obtain high value-added compounds from the renewable resource *Arundo donax* L.

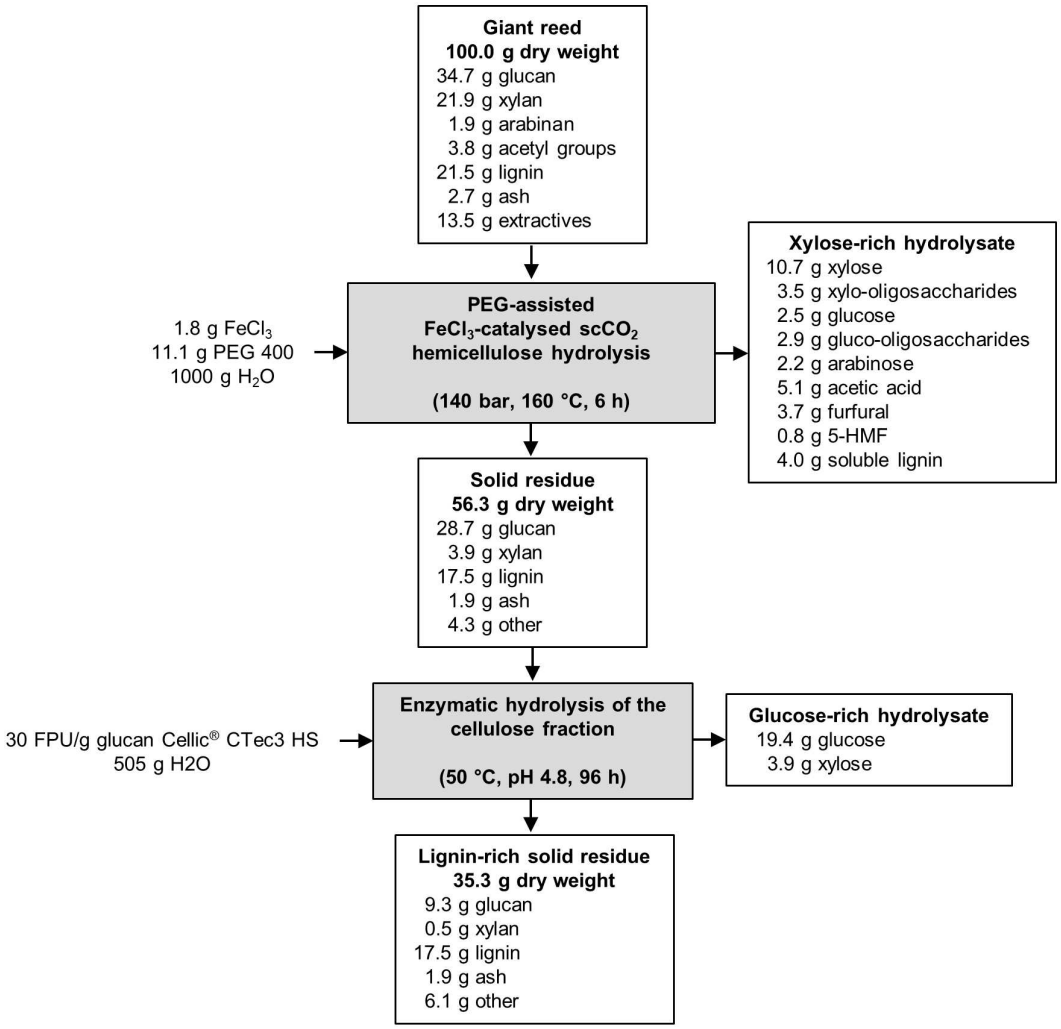

Figure 11. Mass balance flow diagram of the implemented two-step process based on the PEG 400-$FeCl_3$-$scCO_2$ hydrolysis of giant reed hemicellulose to xylose and XOS coupled with the enzymatic hydrolysis of the cellulose fraction to glucose.

Experimental Section

Raw biomass and materials

Giant reed (*Arundo donax* L.) biomass was collected from a 4-year-old plantation, routinely managed by yearly harvests, at the Centre for Agri-environmental Research "Enrico Avanzi" of the University of Pisa in San Piero a Grado (Pisa, latitude 43° 68′ N, longitude 10° 35′ E). In December 2018, giant reed biomass was harvested and then treated and characterised as previously described.[7a,8b] After the harvesting, whole culms and leaves were ground in 1.0 mm average size particles, dried at 105 °C in an oven until constant weight, and then stored in a desiccator up to their use. Chemicals of analytical purity grade were provided by Sigma-Aldrich (USA). Novozymes (Denmark) kindly provided the enzymatic mixture Cellic® CTec3 HS.

Chemical characterisation of raw biomass and residues

Analysis of the chemical composition of starting raw material, pretreated solid residues and post-hydrolysis solid residues was performed according to the standard NREL methodologies.[52] Briefly, for the quantification of hemicellulose, cellulose and lignin fractions 0.3 g of sample was resuspended in 3 mL H_2SO_4 72 wt% solution. The first hydrolysis was performed at 30 °C for 1.5 h under magnetic stirring. At the end of this reaction, the acid solution was diluted with 84 mL deionised water up to H_2SO_4 4 wt%. Then, second acid hydrolysis was performed at 121 °C for 1.5 h in an autoclave. Finally, the solid residue was filtered on a Whatman glass microfiber filter (grade GF/A), dried in an oven at 105 °C until constant weight and weighed. The liquid fraction was analysed by HPLC for sugars quantification. Each analysis was performed in triplicate. Values represent the mean, n = 3.

High-pressure carbon dioxide pretreatment

The high-pressure CO_2 pretreatment process was carried out in a 300 mL Parr autoclave. The autoclave was controlled through a Parr controller 4848. The temperature was 160 °C and it was chosen according to the literature.[7a,8b,23] In particular, under 150 °C modest hydrolysis of the hemicellulose fraction of giant reed was observed while increasing its value over 180 °C the degradation of xylose and glucose to furfural, 5-hydroxymethylfurfural, levulinic and formic acid, is promoted.[7a] The biomass loading was fixed at 9 wt% in order to obtain high concentrations of fermentable monosaccharides in the liquor. The kinetics of sugars and by-products formation was studied, and the process time was set accordingly as described

in the Results and Discussion section. Each reaction was carried out by adding in the autoclave 15 g raw biomass, 150 g water and a proper amount of solid CO_2 in order to reach the desired pressure at the working temperature. In the pretreatment runs (Table 1) characterised by the addition of sole $FeCl_3$, sole PEG 400 and their combination, 0.16 wt% $FeCl_3$ and 1.0 wt% PEG 400 were used according to the literature.[7a,29,43] After the high-pressure CO_2 pretreatment, the recovered slurry was filtered under vacuum on a Büchner funnel. The liquid fraction was analysed by HPLC in order to quantify sugars and by-products, while the solid residue was washed with deionised water, up to neutrality of pH, dried at 105 °C in an oven until constant weight, weighed and then stored in a desiccator up to its use for the enzymatic hydrolysis.

Quantification of sugars oligosaccharides in the liquor

Quantification of the oligomers of glucose (GOS) and xylose (XOS) present in the liquid fraction obtained from biomass pretreatment was performed by post-hydrolysis methodology under the same conditions of the second acid hydrolysis of the standard method for biomass characterisation, as reported in the literature.[21,36] Briefly, the liquor obtained from high-pressure CO_2 pretreatment of giant reed was added with a proper amount of H_2SO_4 96 wt% in order to reach the final concentration of 4 wt%. Then, acid hydrolysis of sugars oligomers was performed at 121 °C for 1 h in an autoclave. The monosaccharides quantification was performed by HPLC. Each analysis was performed in triplicate. Values represent the mean, n = 3. The experimental error was within 5%.

Enzymatic hydrolysis

Cellic® CTec3 HS is a recent commercial enzymatic mixture containing advanced cellulase components boosted by improved lytic polysaccharide monooxygenases, β-glucosidases and a new array of hemicellulases. This catalyst is effective on a wide variety of lignocellulosic materials for the conversion of the carbohydrates into monosaccharides and showed better hydrolytic performances with respect to previous commercial mixtures Cellic® CTec 1 and 2.[46a] The enzymatic hydrolysis of $scCO_2$-pretreated giant reed was performed in a 250 mL flask with the biomass loading of 10 wt% in order to obtain suitable glucose and xylose concentration in the perspective of their valorisation by means of fermentation processes. The values of pH, temperature and agitation speed were set at 4.8, 50 °C and 180 rpm, respectively. The 0.05 M citrate buffer solution was used as a solvent. Samples of 1 mL were withdrawn every day, cooled in ice, centrifuged at 8000 × g for 10 min, and analysed by HPLC for glucose and xylose quantification. Both enzymatic hydrolysis and HPLC analysis were carried out in triplicate and the associated error resulted within 5%. The effect of the enzyme dosage on the cellulose digestibility was assessed considering three dosages, namely 15, 30 and 60 FPU/g glucan, according to the typical range reported in the literature for the hydrolysis of lignocellulosic material.[8b,45a,53]

High-Performance Liquid Chromatography (HPLC) analysis

High-Performance Liquid Chromatography PerkinElmer Flexar Isocratic Platform equipped with a Benson 2000–0 BP-OA column (7.8 mm × 300 mm × 10 μm) and a differential refractive index detector were used for the quantification of sugars (glucose, xylose, arabinose, mannose), organic acids (acetic acid, formic acid and levulinic acid) and furan-derivatives (5-hydroxymethylfurfural, furfural). The operating conditions were already described in a previous study.[7a] Both standards concentration and samples were analysed three times and the error resulted within 3%.

Equations

The glucose and xylose yield respect to the glucan and xylan moles of the biomass (m_b), respectively, was calculated according to equations 1 and 2:

$$\text{glucose yield}\ (\text{mol}\%) = [(m_g \times 0.90)/(G_f \times m_b/100)] \times 100 \quad (1)$$

$$\text{xylose yield}\ (\text{mol}\%) = [(m_x \times 0.88)/(X_f \times m_b/100)] \times 100 \quad (2)$$

where m_g is the glucose mass, 0.90 is the ratio between the molecular weight values of the glucan monomer and the glucose, G_f is the weight percentage of glucan in the biomass, m_x is the xylose mass, 0.88 is the ratio between the molecular weight values of the xylan monomer and the xylose, X_f is the weight percentage of xylan in the biomass.

The xylo-oligosaccharides (XOS) and gluco-oligosaccharides (GOS) yield respect to the glucan and xylan moles of the starting raw material (m_r), respectively, was calculated according to equations 3 and 4:

$$\text{GOS yield}\ (\text{mol}\%) = [(m_{gp} \times 0.90)/(G_f \times m_b/100)] \times 100 \quad (3)$$

$$\text{XOS yield}\ (\text{mol}\%) = [(m_{xp} \times 0.88)/(X_f \times m_b/100)] \times 100 \quad (4)$$

where m_{gp} and m_{xp} are the net amount of glucose and xylose, respectively, produced by post-hydrolysis of the liquid fraction collected after the giant reed pretreatment.

The total removed sugars (TRS) value was calculated according to equation 5:

$$\text{TRS}\ (\text{wt}\%) = \{(m_{gu} + m_{xu} + m_{au} + m_{gos} + m_{xos} + m_{gb} + m_{xb})/[(G_f + X_f + A_f) \times m_b]\} \times 100 \quad (5)$$

where m_{gu} is the total mass of glucan units recovered as glucose in the two steps, m_{xu} is the total mass of xylan units recovered as xylose in the whole process, m_{au} is the total mass of arabinan units recovered as arabinose in the pretreatment, m_{gos} and m_{xos} are the masses of glucan and xylan units recovered as GOS and XOS, respectively, in the pretreatment, m_{gb} and m_{xb} are the glucan and xylan units weights converted to the corresponding by-products, i.e. 5-HMF and furfural, respectively, in the first step. A_f is the weight percentage of arabinan in the raw biomass.

Attenuated total reflectance Fourier transformation infrared spectroscopy (ATR-FTIR)

Attenuated total reflectance Fourier transformation infrared spectroscopy of raw and $scCO_2$-pretreated giant reed samples was performed by the Perkin–Elmer Spectrum Two spectrophotometer equipped with an attenuated total reflectance apparatus. The wavenumber ranged from 4000 to 450 cm^{-1} with a resolution of 8 cm^{-1}. 12 scans were acquired for each spectrum.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: biomass · carbon dioxide · enzymatic hydrolysis · giant reed · sustainable chemistry

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