Development of a propylene glycol-based organosolv process for biomass fractionation

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Abstract

Biomass pretreatment represents a substantial portion of the biomass processing costs and has a great impact on the economic and environmental performance of the biorefinery. Hence, improvements in pretreatment strategies are widely recognized as key to improve biorefinery competitiveness. Particularly, organosolv processes are considered very promising, but several hurdles still exist.

In this work, an innovative organosolv pretreatment, based on the use of propylene glycol (PG) as a green solvent, was developed using Eucalyptus residues, Wheat straw and Miscanthus biomass as model feedstock. The process was initially studied in batch to identify the impact of solvent content (0-100%), reaction time (up to 180 min), and temperature (120-160 ºC), both on non-catalyzed and catalyzed conditions and then intensified to semi-continuous (flow through) operation. Under the optimal conditions tested (160ºC for only 15 minutes, with 75% PG and 0.5% of sulfuric acid), it was possible to obtain xylan and lignin solubilization above 80%, accompanied by a cellulose recovery of 90% presenting an 89% digestibility. Flow-through operation mode enabled an increased lignin recovery and xylan solubilization, as well as lesser formation of degradation products, when compared to batch operation.

These results suggest the ability of the proposed process to compete with other organosolv methods, as it originates similar yields at much milder operation conditions, with potential gains in capital and operational costs.

Keywords:

propylene glycol; high-boiling solvent; organosolv pretreatment; lignocellulosic biomass; biorefinery; circular bioeconomy

1. Introduction

Biomass residues are widely available raw materials that can be favorably upgraded to various products in the biorefinery. They are composed mainly by three macromolecules (cellulose, hemicellulose and lignin) linked together in a complex structure that resists (bio)chemical conversion – biomass recalcitrance. Therefore, an initial fractionation step is required to break this structure, separating these three fractions for subsequent selective upgrade. This step, also commonly named pretreatment, has a great impact on economic and environmental performance. Hence, improvements in pretreatment steps are widely recognized as key to improve the biorefinery sustainability (1).

Currently, delignification pretreatments, such as organosolv and alkaline processes, have several disadvantages that contribute to increase the pretreatment capital and operational costs, namely, the usage of expensive and volatile solvents, corrosive media, non-environmentally friendly chemicals, high pressures, and the formation of degradation products, e.g. furfural, that inhibit the following hydrolysis and fermentation steps. Also, a key aspect determining economic viability of industrial application of all fractionation processes is the ability to fully recover and reuse the pretreatment solvent, which is still a challenging area (2).

The use of a propylene glycol (PG)-based organosolv process has the advantage of its lower vapor pressure (as opposed to ethanol and acetone organosolv processes) that allows for operation at, cheaper, near atmospheric pressure, even at moderately high temperatures. Additionally, PG is a non-toxic solvent, less flammable and hazardous than most commonly solvents in organosolv processes, e.g. ethanol and butanol (3). Furthermore, PG can be synthetized from lignocellulosic materials (4, 5), turning it into a green sustainable solvent in agreement with the circular bio-based economy strategy.

The main goal of this work is the development of a selective fractionation pretreatment for the separation of polymeric components from lignocellulosic feedstocks. Focus was given to the study of the effect of process conditions, including temperature, time and solvent composition on diversified types of feedstocks (forest and agricultural wastes and energy crop biomass). The evaluation of such performance was conducted on the basis of pretreatment

effectiveness for the selective recovery and subsequent upgrade of all biomass fractions. Pretreatment was investigated with and without catalysts and both in batch and continuous operation.

2. Material and methods

2.1. Raw material

The feedstock used in this work were Eucalyptus residues (ER), Wheat straw (WS) and Miscanthus (MS). ER were kindly provided by The Navigator Company from their paper mill in Cacia, Portugal, WS were collected in the Netherlands, and Miscanthus was kindly provided, as pellets, from Greece. ER and WS were grounded with a knife mill to particles smaller than 0.5 mm. MS was use as provided. All samples were homogenized in defined lots and stored in plastic containers, at room temperature.

2.2. Batch operation

Pretreatment experiments were carried out in 25 mL pressure tubes (ACE Glass., USA) with Teflon screw caps, using 1 g of dry biomass and a liquid-to-solid ratio of 10 (dry basis). The tubes were placed in a silicone-oil bath previously preheated to the prescribed temperature that was kept constant (±1ºC) by means of a temperature controller (IKA C-MAG HS7). Homogenization was assured by magnetic stirring. Pretreatments were carried out from 15 to 180 min, with different solvents and aqueous solutions of propylene glycol of 0-100% on weight, with or without catalyst – acid (sulfuric acid) or alkaline catalyst (sodium hydroxide) in the solvent medium with a concentration of 0-2% w/w.

Upon reaction completion the reaction mixture naturally cooled down to room temperature, after which it was filtered through previously oven-dried weighted VWR glass microfibers filters (pore size of 1.2 μm). The recovered solid fractions were washed with 20 mL of fresh solvent, and then extensively washed again with distilled water. The washed solids were oven-dried at 105ºC overnight, for solid yield determination. The dried solids were chemical characterized for determination of their structural composition and also subjected to enzymatic hydrolysis to assess their cellulose digestibility. Aliquots of the recovered liquid hydrolysates, and washing liquids (when needed), were further filtered through 0.45 μm nylon filters and analyzed by HPLC and UV spectrophotometry.

2.3. Flow-through operation

Flow-through pretreatment experiments were carried out in a Dionex™ ASE™ 150 (ThermoFisher Scientific, USA). A rigorously weighted amount of feedstock was mixed with stainless steel spheres (420 grade), and loaded into a 100 mL stainless steel cell. The selected pretreatment solvent was admitted to the extraction cell in a vertical top to bottom flow configuration. The system performed a series of 5 cycles, each consisting of: 1) quick filling of the extraction cell; 2) static period of variable time in which biomass and solvent were kept at constant temperature (140-190ºC), and pressure (~100 bar); 3) cell decompression and liquid released to a previously weighted 250 mL glass bottle. Upon pretreatment completion, extraction cell and collection bottle were allowed to naturally cool down to room temperature. The extraction cell content was unloaded and extensively washed with distilled water, under vacuum filtration, with a rapid filtration quantitative filter paper (20-25 μm. Filter-Lab), previously oven-dried and weighted for solid yield determination. The spheres were magnetically separated from the treated biomass, which was weighed and sampled for moisture content, and then frozen for posterior enzymatic digestibility analysis. The recovered liquor was centrifuged at 9600 rpm for 30 minutes to recover the precipitated solid lignin. The liquid supernatant was stored at 4ºC for posterior HPLC and UV spectrophotometry analysis. The lignin was washed four times with 40 mL deionized water, centrifuged, and then dried, overnight, at 45ºC.

2.4. Analytical methods

2.5.1. Moisture and ash content

Moisture and ash content of solid samples were determined according to standard NREL laboratory procedures (6).

2.5.3. Determination of structural carbohydrates, acetyl groups and Klason lignin

Feedstock and pretreated solids were subjected to a quantitative acid hydrolysis following NREL protocol (6), where all polysaccharides are converted into their soluble monomeric sugars and the acid insoluble residue, after correction for ash, is considered as Klason lignin. The procedure was carried out, at least, in duplicate and results were reported on a dry basis.

2.5.4. Enzymatic hydrolysis

Enzymatic digestibility of feedstock and pretreated solids was evaluated after 72 h at 50 ºC, based on the standard NREL laboratory procedure (7) using 10% (w/w cellulose) of Celli®CTec2 enzyme (kindly provided by Novozymes Europe, Denmark, presenting a titer of 199.9 FPU/mL). All assays were performed, at least, in duplicate. Proper blank assays were also prepared to account both the sugars that may arise from the enzyme solution, and the sugars derived from non-enzymatically hydrolysis of the biomass.

2.5.5. Quantification of sugar monomers and oligomers and degradation products

Analysis of pretreatment liquors and structural analysis samples were performed according to NREL laboratory procedure (8). Briefly, pretreatment liquors were directly analyzed for quantification of sugar monomers (hexoses and pentoses), aliphatic acids and furans by HPLC using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). Oligomers were indirectly quantified after quantitative acid hydrolysis, as the difference between the concentration of monomers after and before hydrolysis. HPLC analysis was performed using an Agilent 1100 Series HPLC system (Waldbronn, Germany), equipped with a RID (refractive index) detector and a DAD UV/Vis

photodiode detector set at 280 nm for furfural and 5-hydroxymethylfurfural quantification. Data analysis was performed using Agilent™ ChemStation™ software.

2.5.7 Quantification of soluble lignin

Pretreatment liquors and hydrolysates from quantitative acid hydrolysis were analyzed for soluble lignin/phenolic content following NREL protocol (6), using a Jasco 7800 UV-vis spectrophotometer (Jasco, Japan). The absorbances of the liquid samples were measured at 320 nm, after dilution with distilled water, as appropriate to obtain absorbance values below 1.0. Absorbance values were then converted to phenolic content through the Lambert-Beer Law, considering the extinction coefficients of 30 or 16 $Lg^{-1}cm^{-1}$ for ER (6) and WS (9), respectively.

3. Results and discussion

3.1. Organosolv batch operation

The initial study of the PG-organosolv process was carried out for ER and WS. In this work, only the ER data is presented and discussed, as the WS behavior presented the same trends.

Figure 1 shows the effect of PG content (PG wt%) and of the severity factor (log Ro) on the biomass solubilization of eucalyptus residues. The solubilized biomass increased with pretreatment severity, as a result of the higher reaction temperature and time with consequently higher levels of breakdown of the structural macromolecules.

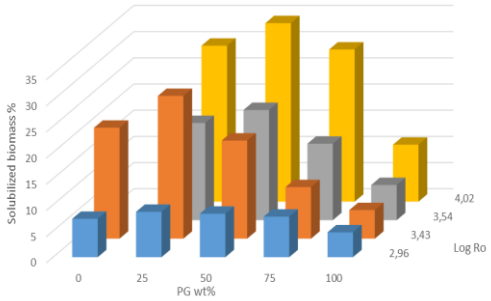

Figure 1. Effect of PG content and pretreatment severity factor on ER solubilization

On the other hand, mixtures of the organic solvent and water, always performed better than both individual solvents, clearly indicating that the organosolv approach is effective.

Figure 2 highlights that xylan is preferably solubilized when using lower PG content, as increasing water content led to an extensively increase in xylan solubilization, particularly for 3 hours of pretreatment. Arabinan solubilization was always complete (data not shown), which is explained by the fact that Arabinose residues are only present on the side-branches of the hemicellulosic structure. Delignification is improved with decreased water content up to 50%. Above this value, delignification values decreased again, as can be seen from the higher recovery of lignin in the solid phase. Glucan is always mainly retained in the solid biomass.

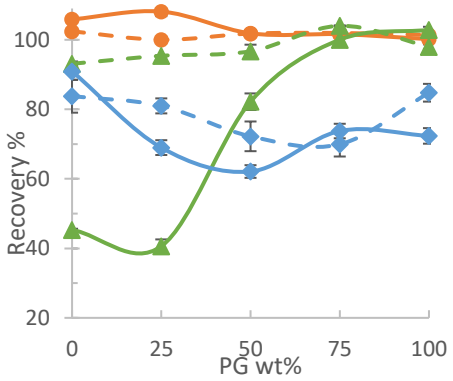

Figure 2. Recoveries of glucan (●), xylan (▲) and lignin (♦) in the solid fraction after pretreatment of ER for 1 (- - -) and 3h (—) with aqueous solutions of propylene glycol at 140ºC

A similar trend was observed at 160 ºC, but with higher yields (data not shown). For example, delignification increased from 38 to 58% for the experiment with PG 50% for 3h when temperature was raised from 140º to 160ºC, while xylan solubilization was enhanced from 18 to 57%. In fact, the better performances were obtained for pretreatments with 25-50% PG, for which the xylan and lignin solubilization were the highest. The optimal conditions occur for a severity of 4.02 (160ºC, 3h) with PG 50% (w/w), corresponding to a solid yield of 65.9%, conditions that led to a maximum delignification (58%), accompanied by a similar xylan removal (57%).

Figure 3 shows that there is a positive linear correlation between xylan and lignin solubilization meaning that, as found by other authors, there is an interdependent mechanism of xylan and lignin solubilization (10). It is worth noting that the experimental points represented by orange squares in Figure 3 do not follow the correlation obtained. These points correspond to the treatments with solutions containing low PG contents (equal or below 25%) in which lignin is poorly soluble. This explains the small values of lignin solubilization (and deviation from the trend line), because lignin has a low solubility in aqueous solutions. The positive intercept of the line in Figure 3 emphasizes that delignification occurred before xylan solubilization. This observation implies that lignin solubilization is only dependent on xylan solubilization for delignification values above a specific value (26%). This result suggests the existence of two lignin fractions in ER: one that is solubilized before the attack to xylan and

the other that is more strongly bonded to xylan because it is only removed when xylan is solubilized.

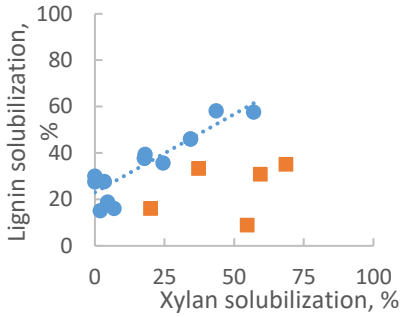

Figure 3. Correlation between lignin and xylan recovery in solid fraction obtained from the pretreatment of ER with PG (r=0.91). Orange data refers to PG content < 25%

Glucan digestibility of the solid fractions remaining after pretreatment are presented in Figure 4.

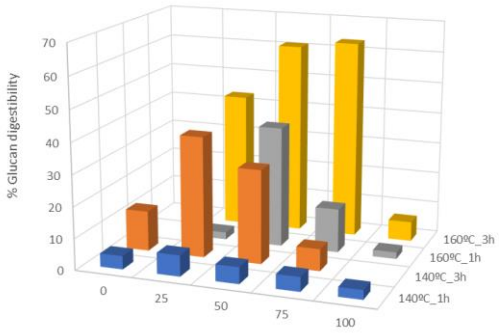

Figure 4. Effect of PG's weight percentage and pretreatment severity conditions on glucan digestibility for ER

As can be seen, a significant increase (almost 20-fold, as compared to the glucan digestibility of the feedstock, 3.5%) was achieved. The 3h pretreatments resulted always in higher digestibility than pretreatments for 1 hour, in agreement with the more extensive lignin and xylan solubilization. It was also observed that pretreatment of eucalyptus at 140ºC was practically ineffective for a reaction time of one hour, whilst being mildly effective for three hours. In fact, glucan digestibility reached a maximum of 39% for the experiment with PG25% at 140ºC for 3 hours, which is in accordance with the extension of the xylan and lignin removal observed.

When pretreatment temperature was raised to 160ºC, the digestibility increased considerably, even for reaction times of only one hour. This is correlated with the increased efficiency of the pretreatment at this temperature. Glucan digestibility reached its highest of 63% for the 3-hour treatment with PG 75%. This value reveals already some potential of the proposed pretreatment for application in the biorefinery, as it is similar to others reported in literature for similar processes, e.g. ethanol organosolv (11, 12), while presenting the advantages of a lower operation temperature and pressure and no-use of catalysts. The dependence between digestibility and xylan and lignin recoveries was properly modelled by a linear multivariable equation (Equation 1), visually represented in Figure 5, where x_1 is the xylan recovery and x_2 to the lignin recovery.

$$\%\ \text{Glucan digestibility} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 \quad \text{(Equation 1)}$$

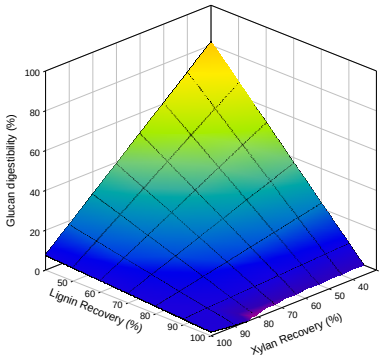

Figure 5. Glucan digestibility (%) as a function of xylan and lignin recovery for PG-organosolv pretreated ER

The mathematical regression was obtained with both significant r (0.92) and R^2 (0.85) values. Furthermore, model parameters, βi, were statistically significant at a 95% confidence level (β_0=200 ± 30, β_1=-1.8 ± 0.4, β_2=-2.1 ± 0.5, β_{12}=0.020 ± 0.006). As expected, a negative correlation was found between digestibility and both lignin and xylan recoveries, i.e. the solubilization of those fractions improves the digestibility of biomass. The model also highlights the importance of the synergistic effect of both xylan and lignin solubilization in enhancing the digestibility of treated ER.

3.2. Catalyzed batch operation

3.2.1. Catalyst selection for PG-based organosolv

The effect of (acid and alkaline) catalyst utilization on PG-organosolv was studied for ER, using sodium hydroxide and sulfuric acid, both at 1% concentration on solvent mass at 160ºc for 3 hours. Figure 6 shows that alkaline catalysis did not result in an improvement performance, as solid yields remained practically unchanged. However, it resulted in an enhanced selectivity, since the recovery of xylan was increased, while lignin was more extensively solubilized, in relation to uncatalyzed conditions. This is especially evident for PG 25%. Such result was partially expected given that lignin is

more soluble in alkaline solutions and that the reactions for xylan solubilization are acid catalyzed (13, 14).

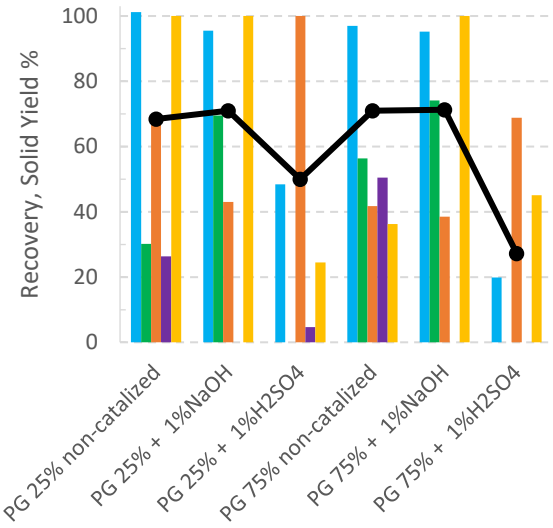

Figure 6. Solid yield (●, black line) and recoveries of glucan (▪), xylan (▪), lignin (▪), acetyl groups (▪) and ash (▪) in the solid fraction remaining after autocatalyzed and acid/alkali-catalyzed PG-organosolv pretreatment of ER

Acid catalyzed PG-organosolv had a superior performance for ER deconstruction than the alkaline pretreatment, significantly reducing experimental solid yields (when compared to un-catalyzed treatments) – from 68.4 to 50.0% for PG 25% and from 70.9 to 27.2% for PG 75%. These results are explained by an extensive hydrolysis of hemicellulose, with complete removal of xylan, but most noteworthy also the solubilization of glucan for both concentrations of PG.

Acid catalyzed treatment with PG 75% remove 80% of total glucan present in the feedstock, which represents a very effective deconstruction of the lignocellulosic structure. Recoveries of lignin in the remaining solids were always superior to those of uncatalyzed experiments – 100% for PG 25% and 68.8% for PG 75% –, since lignin is insoluble in acidic solutions. Nonetheless, decreasing solvent's water content improves the solubility of this macromolecule, which justifies the lower solid recovery for PG 75%. Like the alkaline pretreatment, the acid catalyzed PG-organosolv increased the selectivity of uncatalyzed pretreatments, but by enhancing sugar solubilization (as opposed to lignin solubilization, enhanced for alkaline organosolv).

Acetyl groups were always almost completely solubilized for either catalyzed treatments, while ash was only removed in the acid catalyzed experiments. Furthermore, acid catalyzed treatment with PG75% was able to remove a larger fraction of cellulose and so enzymatic hydrolysis might not be a required step, as acid catalyzed PG organosolv could potentially perform simultaneous hydrolysis of both the hemicellulosic and cellulosic sugars.

The analysis of the composition of hydrolysates is presented in Figure 7.

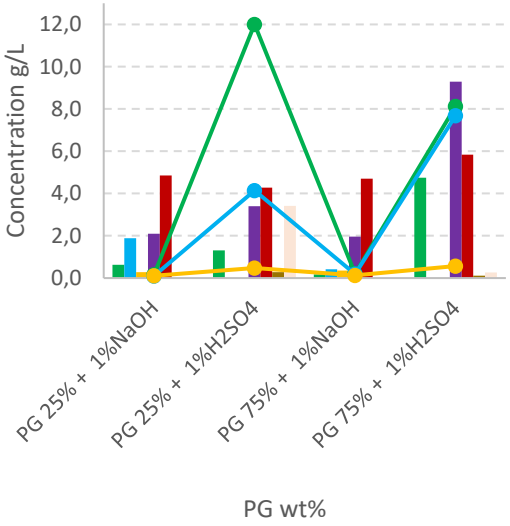

Figure 7. Concentration of sugars (● Glc, ● Xyl, ● Ara, ▪ GOS, ▪ XOS, ▪ AOS), aliphatic acids, and furans (▪ formic acid, ▪ acetic acid, ▪ HMF, ▪ furfural) in alkaline and acid catalyzed PG-organosolv ER hydrolysate

Even though glucose concentrations reached high values for the acid catalyzed treatments, 12 and 8 g/L for PG 25 and 75%, respectively, so did the concentrations of sugar degradation products, particularly formic acid and furfural. Moreover, no XOS were detected in the liquors. From mass balances, the fraction of solubilized sugars that went over degradation reactions was between 50 and 70%, which represents a very high value of sugar losses. These results revealed that the conditions of the pretreatments were too harsh, meaning that the process could benefit from a reduction of severity. Additionally, other acids could be tested.

Noteworthy, in the acid pretreatments between 16 to 37% of total initial sugars were not quantified, suggesting the existence of sugar-PG side-reactions during treatment.

3.2.2. Optimization of sulfuric acid-catalyzed PG-based organosolv

Sulfuric acid-catalyzed pretreatment was further investigated, in order to try to minimize the amounts of sugar degradation products obtained in the hydrolysates. The data is presented in Figure 8, as a function of the combined severity factor (15), defined as:

$$CS = logR_o - pH \quad \text{(Equation 2)}$$

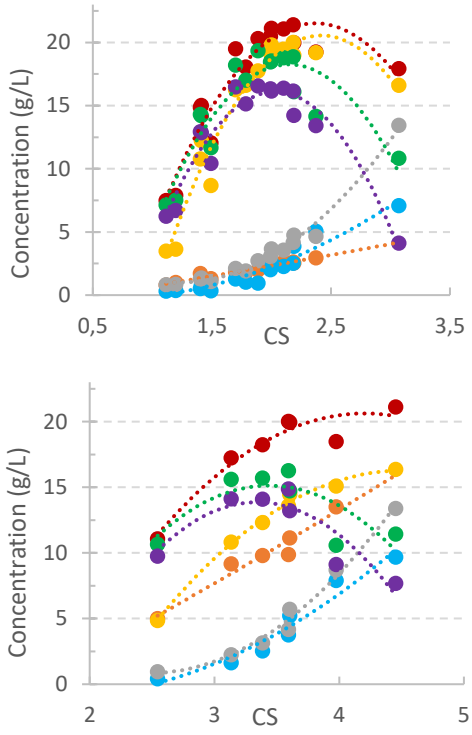

Figure 8. Hydrolysate composition (● Xyl+XOS, ● Glc+GOS, ● Total sugars, ● Total sugar monomers, ● Total degradation products, ● (Total sugars-Total degradation products), ● Phenolic content) from sulfuric acid-catalyzed PG-organosolv as a function of the combined severity factor (Top: PG25%, Bottom: PG75%)

Total xylose+XOS presented a maximum value at CS of 2 for PG25% and at 3.4 for PG75%, resulting from the counteracting effects of xylan solubilization (positive effect) and degradation of pentoses to furfural and other products (negative effect). Glucose+GOS were present in pretreatment liquors at much lower concentrations than xylose+XOS, due to the slowest hydrolysis of cellulose, when compared to hemicellulose. Nonetheless, for the harshest severity tested, glucan solubilization occurs significantly.

The difference between total and monomeric sugars, associated with the existing soluble oligomers, is considerably larger in the experiments with PG75%, while being almost null for PG25%. From this, it was concluded that the soluble sugars in the pretreatments with lower PG content were almost entirely in monomeric forms, while for the experiments with higher PG content a significant fraction was in oligomeric form, varying between 25-50% of total sugars (c.a. 5 g/L).

Figure 8 also shows that higher severity conditions led to an increase of total degradation products, as expected. Additionally, concentration of degradation products was higher for the experiments with PG content of 75% than 25%. As a result, the difference between total sugars and total degradation products was also higher for PG25% than for PG75% (19.4 vs. 16.3 g/L). This could suggest that a 25% PG content is preferably. However, the capacity of the pretreatment to remove lignin is also an important contributor to enhance the yields of enzymatic hydrolysis. Additionally, as observed in Figure 7, the phenolic content in the pretreatment with PG75% is much higher than that of PG25%. In fact, in the first case the phenolic content was between 5 and 17 g/L, while for the latter it never surpassed the 5 g/L level. It is worth noting that the phenolic content increased linearly with the combined severity factor, unlike the remaining variables.

In spite of performing better for sugar recovery, the optimum conditions of PG 25% gave solid yields around 60%, which is still a very high value, since glucan accounts for only 45.7% of ER. However, for PG75% the optimum sugar recovery corresponded already to a solid yield of about 45%, revealing a much better performance for biomass deconstruction, particularly for lignin removal. Nevertheless, the use of PG75% resulted in more degradation products. Therefore, it could be concluded that PG contents between 25 and 75%, most likely around 50% result in the optimum tradeoff between all these factors. The operational conditions that correspond to the optimum CS are: short residence times of 15 minutes, temperatures of 150-160ºC and acid concentrations of 0.5-2%. Preferably, when the lowest temperature is applied, the highest acid concentration should be used and vice-versa.

Analysis of pretreated solids resulting from three selected experiments carried out in conditions in the optimum range was performed. Results are summarized in Table I.

Table I. Solid yield, composition of pretreated solids and recovery of structural components for the optimized experiments of acid-catalyzed PG-based organosolv of ER

Experimental conditions				Solid Yield (%)	Cellulose recovery (%)	Liquid recovery/ solubilization (%)	
PG (%)	H_2SO_4 (%)	T (ºC)	Time (min)			Xylan	Lignin
75	**0.5**	**160**	**15**	48.5	90.0	82.5	83.9
50	**1.0**	**150**	**15**	54.6	91.8	83.2	64.5
50	**2.0**	**150**	**15**	50.3	96.0	100.0	68.2

Cellulose recoveries above 90% were always obtained, which is convenient for posterior enzymatic saccharification of the pretreated solids. Pretreatment at 160ºC with 75% PG and 0.5% sulfuric acid for only 15 minutes gave the best results for

xylan and lignin solubilization, 82.5 and 83.9%, respectively, with the most interesting solid yield of 48.5%. The analysis of the sugars in this solution revealed 17.3 g/L total sugars content, 71% xylose+XOS, 5% glucose+GOS yield and 1.6 g/L of degradation products in pretreatment liquid, i.e. the best sugar yields with the lowest concentration of inhibitors and hexose sugars for the experiments with 75%PG.

Table I shows that pretreatments at 150ºC and 50% PG using the same time resulted in less extensive delignification (64.5 and 68.2% for 1 and 2% acid concentration, respectively), due to lower PG content. However, the one with 2% sulfuric acid was able to completely remove hemicelluloses, resulting in a solid yield of 65.5%, not too distant from the experiment at 160ºC. Pretreatment at 150ºC with 50% PG and 2% acid for 15 minutes corresponded to a hydrolysate with 21.9 g/L of total sugars, 86.2% of xylose+XOS yield, 2 g/L degradation products, 7.8% glucose+GOS yield and a value of 0.78 for the target variable introduced above. These represent the highest values obtained for pentose yield while preserving the cellulosic fraction and also maintaining a relatively low concentration of inhibitors. However, delignification is still somewhat low, and so the option of 160ºC pretreatment seems to be the most interesting one.

It is worth noting that, the introduction of small amounts of sulfuric acid in PG organosolv allowed to reduce operation times from 3 hours to 15 minutes reducing operation costs and increasing production rate, while enhancing xylan solubilization and delignification values from about 60% to more than 80% each, at 160ºC. Moreover, solid yield was reduced from 65.9 to 48.5%, accordingly. Even though cellulose recovery in solid decreased from about 100 to 90% within the acid catalyzed process, this is still an interesting value from an economical perspective, also because the solubilized 10% glucan did not suffer degradation, but were instead recovered in the liquor as free and oligomeric glucose. Furthermore, comparison with literature, favors the present work. For example, the acid catalyzed PG organosolv performed similarly to ethanol organosolv of poplar wood (16), while allowing for a reduction of operational temperature, time and acid concentration, with the respective cost benefits. The same applies eucalyptus (12). Furthermore, application of the model of glucan digestibility developed above (Equation 1) to the acid catalyzed experiments of Table I allowed to estimate the digestibility that the acid catalyzed pretreatments would originate, reaching values of at least 80%, much higher than the maximum values of 63% for the uncatalyzed organosolv.

In conclusion, mild temperatures (150-160ºC) and short residence times (15 min) can therefore be applied in a PG-based organosolv when catalyzed with sulfuric acid to result in extensive biomass fractionation, also enabling high to enzymatic saccharification.

3.3. Flow-through operation

Flow-through operation is a valuable tool to study continuous operation, as it is difficult to mimic true continuous reactors for biomass pretreatment at lab-scale, as it can capture part of the continuous kinetic performance as described for Miscanthus (17). Figure 9, presents the data for PG-based organosolv for flow-through operation developed in this work as compared to autohydrolysis. PG-organosolv resulted in a more extensive solubilization of xylan for ER than for WS. The solid yields obtained for ER and WS follow this same trend. Glucan recoveries were similar for both feedstocks, as well as for flow-through autohydrolysis of ER, all in the range of 85-90%, an interesting value for post enzymatic hydrolysis (data not shown).

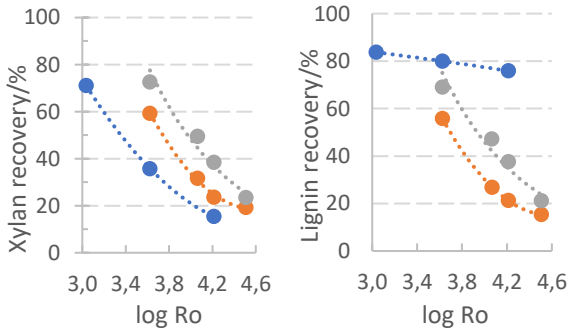

Figure 9. Solid yield and xylan recovery in the solid fraction after flow-through autohydrolysis of ER (●) and flow-through 50%PG organosolv of ER (●) and WS (●)

For ER, flow-through autohydrolysis was able to remove more xylan than the organosolv pretreatment due to increased acid potential of the solvent. The opposite was observed for lignin solubilization, given the higher lignin solubility in PG. However, while xylan solubilization became closer to similar values for both pretreatments with increased temperatures, particularly above 180ºC for which the difference is smaller than 10% (85 vs 76% xylan removal), the same was not verified for lignin in autohydrolysis as it yielded a maximum delignification plateau of 20% independently of the temperature applied. Conversely, for flow-through organosolv

delignification increased with increasing temperature, reaching a very significant maximum of 85% for 190ºC, 1h operation. Therefore, PG-organosolv has the potential to benefit from the combined effects of extensive xylan solubilization and extensive delignification associated with the presence of PG.

A literature report of a flow-through pretreatment of corn stover at 190ºC with 60:40 ethanol:water originated a xylan and lignin solubilization of 47.1 and 76.8%, respectively, while preserving 90% of cellulose in the pretreated solids (18). These results were similar to the ones of this work for wheat straw flow-through PG-organosolv (78.8% delignification and 85.6% cellulose recovery), except for xylan solubilization for which PG pretreatment considerably performed better (76.5% solubilization). In another report, Norway spruce (softwood) was delignified to 83% giving a solid yield of about 25% in a flow-through reactor with 63% ethanol-organosolv including 0.06% sulfuric acid as catalyst at 175ºC for 10 hours (19). In this work, a delignification of 73.2%, was obtained for ER at lower temperature (160ºC), shorter time (3 hours) and without the addition of catalyst. Furthermore, solid yield was of 59% instead of 25%, representing a preservation of cellulose of 88.3% against the reported value of only 67%. This comparison suggests the potential of a future continuous PG organosolv process for biomass deconstruction.

Figure 10 shows the solid lignin recoveries obtained for the flow-through pretreatments. The recovered solid lignin was found to increase with pretreatment severity, particularly above 160ºC. It was also considerably higher for organosolv than for autohydrolysis pretreatment, as discussed above. About 60% lignin was recovered as spontaneous precipitate in organosolv pretreatments at 180ºC, and for WS this reached 80% at 190ºC. These are very interesting values with potential to simplify costly and intricate post-treatment separation steps. However, the composition of the precipitated lignin must be evaluated to assess its purity. Particularly, the precipitated solid might be more of the so-called pseudo-lignin, a result of condensation reaction between lignin and sugar degradation products that form a lignin-like precipitate during pretreatment (20). Nevertheless, the observation of low concentrations of degradation products in this work, i.e. furfural concentration was always below 1g/L and HMF was not detected at all, do not predict an extensive formation of pseudo-lignin.

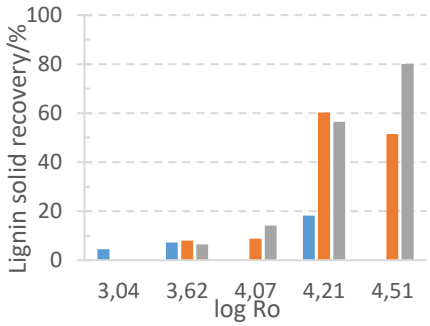

Figure 10. Recovered solid lignin (% w/w of initial total lignin content) as a function of pretreatment severity for flow-through autohydrolysis of ER (■) and flow-through 50%PG organosolv pretreatment of ER (■) and WS (■)

Additionally, PG may also be responsible for increasing lignin yields, due to PG reaction with hydroxyl groups from lignin structure, as incorporation of other alcohols into lignin structure during organosolv pretreatment has been reported (21, 22). The introduction of alcohols into the phenolic structure alters lignin solubility and reactivity, allegedly making it interesting for the production of polymers and gels (22), with a positive impact on its valorization potential.

Table II evidences the comparison of flow-through results with the ones of batch operation. Whereas for all batch experiments, the cellulose was preserved completely (more than 96%), for the flow-through experiments, cellulose recovery was always between 84 and 90%, which in spite of still being an interesting range, suggests that flow-through mode already induces some glucan removal. Advantageously, this effect appeared to be independent of pretreatment severity (as well as solvent), which allows the increase of selectivity for hemicellulose and lignin solubilization by increasing the former.

For xylan and lignin, flow-through operation was found to enhance the solubilization of both components in comparison to batch operation for comparable conditions. Flow-through autohydrolysis removed more 22% of total initial xylan in ER though not affecting its delignification. Flow-through PG-based organosolv of ER was more modest in increasing xylan solubilization (only 6 and 11% additional xylan solubilization for 160ºC 1 and 3 hours, respectively), but while enhancing delignification in 16% for 160ºC 3h. For WS, flow-through PG-based organosolv resulted in a similar enhancement of delignification, nevertheless having a stronger impact on xylan solubilization, with an additional 23 and 30% removal of total xylan of feedstock for 160ºC 1 and 3 hours, respectively.

Table II. Comparison between batch (B) and flow-through operation (FT) with respect to solid yield and recovery of lignocellulosic components in autohydrolysis and PG-based organosolv of ER and WS

Pretreatment	Solid Yield (%)		Recovery in pretreated solids					
			Glucan (%)		Xylan (%)		Lignin (%)	
	B	FT	B	FT	B	FT	B	FT
ER Autohydrolysis (H2O, 140ºC, 1h)	92.7	90.0	100.0	86.0	93.1	71.1	83.8	83.7
ER Organosolv (PG50%, 160ºC, 1h)	79.0	79.2	97.7	89.5	65.6	59.3	53.9	55.8
ER Organosolv (PG50%, 160ºC, 3h)	65.9	59.0	96.5	88.3	43.0	31.6	42.3	26.8
WS Organosolv (PG50%, 160ºC, 1h)	86.7	84.2	99.4	84.4	95.3	72.6	72.4	69.1
WS Organosolv (PG50%, 160ºC, 3h)	76.8	69.3	96.5	86.0	79.4	49.4	63.1	47.3

The apparent superior improvement of the 3-hour experiments is obviously related to the higher liquid-to-solid ratio applied (in the range of 15-16), which results from running a continuous operation for longer times (if the liquid flow is to be kept), and not necessarily from the specific dynamics of flow-through operation. However, for the 1-hour experiments the LSR was also smaller than the one applied in batch (6-7 vs. 10), which should still indicate some potential for this configuration.

Results from enzymatic hydrolysis of flow-through-pretreated solids (Figure 11) revealed higher digestibility for ER when compared to WS, for the same conditions during PG-based organosolv pretreatment. This result is in agreement with the more extensive xylan and lignin solubilization during ER pretreatment (see above), as determined by quantitative acid hydrolysis. The inversion of this tendency at the highest tested severity condition is justified by the fact xylan and lignin solubilization became similar for both feedstocks at these conditions (Figure 9), together with the less recalcitrant character of WS.

Interestingly, digestibility of ER submitted to flow-through autohydrolysis were always inferior (15 to 20% less glucose yield) than the ones of flow-through PG organosolv (for the same severity conditions), in spite of superior xylan removal and even for the conditions (log Ro below 3.8) in which autohydrolysis resulted in lower solid yields. This highlights the relevance of delignification impact on the enzymatic digestibility, which was very limited for autohydrolysis when compared to the organosolv process.

Cellulose digestibility fell always below 51% (corresponding to 180ºC, 1h) for autohydrolysis of ER at all the tested conditions. Autohydrolysis at 140ºC had practically no impact in ER digestibility.

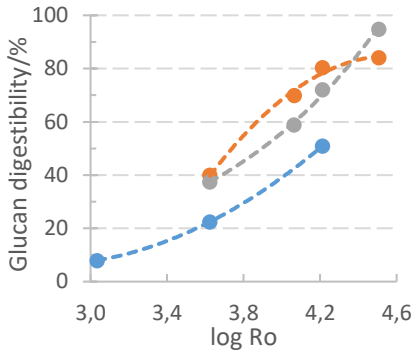

Figure 11. Glucan digestibility of the flow-through pretreated solids by autohydrolysis of ER (●) and flow-through PG-based organosolv with 50%PG of ER (●) and of WS (●)

For flow-through organosolv, however, interesting values were obtained, particularly for the harsher conditions, i.e. 1 hour at 190ºC, for which glucose yields reached 84.1% for ER and 94.8% for WS, which is very promising. The obtained digestibility for wheat straw was even much higher to the 78.8% reported for flow-through ethanol organosolv pretreatment of corn stover, at similar conditions (18).

Comparison of digestibility yields of flow-through operation with the ones of batch pretreatment had a minimal increasing impact on ER digestibility, while relatively enhancing WS susceptibility to enzymatic hydrolysis.

4. Conclusions

In this work, the development of a selective fractionation pretreatment for the separation of polymeric components from lignocellulosic biomass feedstock was pursued. Focus was given to the organosolv process as it presents the advantage of simultaneous solubilization of hemicelluloses and lignin. PG was proposed as a suitable solvent for organosolv pretreatment, which revealed similar or superior performance to the more conventional solvents, such as ethanol, while being more environmentally friendly, safer for operation, non-flammable, non-toxic, non-volatile, and reducing operational costs by allowing operation at near-atmospheric pressure. PG additionally benefits from its renewable character, as it can be produced by fermentative/chemical pathways in the biorefinery.

The effects of time, temperature and PG content on the PG-based organosolv were studied for two feedstocks – eucalyptus residues, a woody biomass, and wheat straw, an herbaceous biomass. Increasing pretreatment time and temperature was correlated with higher xylan solubilization and delig-

nification as well as increased enzymatic digestibility of pretreated solids. PG contents of about 50% also had a similar effect on those variables, benefiting from the synergistic effect of high hydrolysis potential of water fraction together with the higher lignin solubility in organic solvent. The introduction of a catalyst as a way to enhance the kinetics of deconstruction reactions was also evaluated. Alkali catalyst (sodium hydroxide) did not improve solid yield nor the digestibility level of pretreated solids, as merely resulted in a slight increase of selectivity towards delignification (as opposed to xylan solubilization). Acid catalysis of PG organosolv, however, allowed for xylan and lignin solubilization values above 80% accompanied by a cellulose recovery of 90% with an 89% digestibility, potentially, for pretreatment at 160ºC for only 15 minutes with 75%PG and 0.5% of sulfuric acid. These values suggest the ability of the proposed process to compete with other organosolv methods, as it originates similar yields at much milder operation conditions, with potential gains in capital and operational costs.

Flow-through application of PG-based pretreatment strategy was also assessed. It enabled easy spontaneous recovery of 60-80% of total lignin in feedstocks, simplifying down-stream process design for recovery of valuable products and recycle of the solvent, while reducing operational costs. Furthermore, flow-through regime allowed the removal of additional 16% of total lignin from WS and ER and of more 11% and 30% of total xylan from ER and WS, respectively, when compared to batch operation, at similar conditions. This was accompanied by an increase in cellulose digestibility. Hence, the results of this work suggest even more potential for PG-organosolv when continuous operation is pursued, as it will lead to process further intensification, with increased product yields, lower amounts of produced degradation products and simplified recovery steps.

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