

Journal Pre-proofs

Aqueous solutions of deep eutectic systems as reaction media for the saccharification and fermentation of hardwood xylan into xylitol

Aloia Romani, Eduarda S. Morais, Pedro O. Soares, Mara G. Freire, Carmen S.R. Freire, Armando J.D. Silvestre, Lucília Domingues

PII: S0960-8524(20)30796-3
DOI: <https://doi.org/10.1016/j.biortech.2020.123524>
Reference: BITE 123524

To appear in: *Bioresource Technology*

Received Date: 20 March 2020
Revised Date: 7 May 2020
Accepted Date: 9 May 2020

Please cite this article as: Romani, A., Morais, E.S., Soares, P.O., Freire, M.G., Freire, C.S.R., Silvestre, A.J.D., Domingues, L., Aqueous solutions of deep eutectic systems as reaction media for the saccharification and fermentation of hardwood xylan into xylitol, *Bioresource Technology* (2020), doi: <https://doi.org/10.1016/j.biortech.2020.123524>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Ltd.



**Aqueous solutions of deep eutectic systems as reaction media for the
saccharification and fermentation of hardwood xylan into xylitol**

Aloia Romani^{1*}, Eduarda S. Morais², Pedro O. Soares¹, Mara G. Freire², Carmen S. R.
Freire², Armando J. D. Silvestre², Lucília Domingues¹

¹CEB - Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057 Braga, (Portugal);

²CICECO—Aveiro Institute of Materials and Department of Chemistry, University of Aveiro, 3810-193 Aveiro (Portugal).

***corresponding author:** aloia@ceb.uminho.pt

Keywords: xylitol, cholinium chloride, urea, xylan, simultaneous saccharification and fermentation

Abstract

The aim of this study was to evaluate the effect of aqueous solutions of deep eutectic solvent, Cholinium Chloride:Urea ([Ch]Cl:U) at 50 wt % and 20 wt %, using different molar ratios (1:1, 2:1 and 1:2) on the enzymatic hydrolysis of xylan for xylose production and its subsequent bioconversion into xylitol using a recombinant yeast strain. The lowest xylan conversion into xylose (45 %) was obtained using 1:2 [Ch]Cl:U molar ratio. On the other hand, the 1:1 [Ch]Cl:U molar ratio, at 20 wt % in water, improved this conversion, achieving the highest xylose yield (81.4 %). The xylitol production was then optimized with [Ch]Cl:U (1:1) at 20 wt % by simultaneous saccharification and fermentation process, attaining 23.67 g/L, corresponding to 66.04 % of xylitol yield. This study reveals the possibility of using xylan solubilized in DES aqueous solutions directly for xylitol production, thus assembling a one-step process.

1. Introduction

The interest in innovative strategies to support sustainable development and growth has increased tremendously in recent years. In this vein, the United Nations (UN) outlined a new sustainability-focused development plan under the title “Transforming Our World: The 2030 Agenda for Sustainable Development”. This plan comprises several aspects spanning across all fields of human activities, many of them being related to the development of greener and more efficient chemical processes and their importance in sustainability (Assembly, 2015). In this context, the use of renewable feedstock and the replacement of conventional organic solvents by greener ones are two measures that can dramatically improve the sustainability of most chemical processes currently used in industry (Clarke et al., 2018). Simultaneously, the manufacturing of value-added products, including materials, chemicals, fuels, as well as energy from renewable resources, has emerged as an environmentally friendly and sustainable alternative to petroleum-derived products (Clark, 2007).

Within the broad scope of biorefinery, lignocellulosic feedstocks are the most promising biomass sources for chemical manufacturing (Werpy et al., 2004) and their production rate is approximately 200×10^9 tons per year. Among lignocellulosic materials, fast-growing and short-rotation hardwoods (such as eucalyptus and beech) have great importance due to their high biomass yield per hectare, which makes them extremely attractive as energy crops. Besides, these are widespread raw materials used in the cellulose pulp mill (Romaní et al., 2019). Hardwood cell wall, that accounts for about 90% of its dry weight (Kumar et al., 2009), is composed by approximately 44 % of cellulose, 20 % of hemicellulose (mainly xylan) and 27 % of lignin (Romaní et al., 2010). In a biorefinery context, cellulose is the fraction most used for biofuels production such as ethanol. Nevertheless, their implementation on large-scale is still not

economically viable. To overcome this limitation, the production of high-value products from hemicellulose (such as xylitol) coupled to biofuels is vital to improve the feasibility of lignocellulosic biorefinery (Budzianowski, 2017). On the other hand, due to the recalcitrant nature of biomass, polysaccharides conversion into monosaccharides by enzymatic hydrolysis, a key step in the conversion into value-added products, is often hampered (Romaní et al., 2010). Therefore, a pretreatment step, involving biomass fractionation into major components, is normally required (Domínguez et al., 2017). In this context, the selection of efficient processes for the biomass fractionation is determinant for the economic viability and sustainability of biorefinery processes (Soh and Eckelman, 2016).

Deep eutectic solvents (DES) are a new class of alternative solvents, initially proposed by Abbot *et al.* (2003), prepared combining hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs) thus forming eutectic mixtures with a melting point lower than those of the starting components. DES present several advantages over conventional imidazolium-based ionic liquids (ILs); notably, they are frequently of lower-cost and easier to prepare while having physicochemical properties comparable to ILs (Clarke et al., 2018; Loow et al., 2017). Furthermore, they present similar “designer solvent” characteristics (Zhang et al., 2012) given the large variety of HBAs and HBDs that can be used and respective proportions. Over the last few years, the use of DES as greener alternatives to conventional solvents for lignocellulosic biomass processing has attracted increased attention, in areas spanning from delignification (Shen et al., 2019; Soares et al., 2017 ; Alvarez-Vasco et al., 2016) to high value compounds extraction (Gullón et al., 2019) and their conversion into added value products (Morais et al. 2020).

Recently, aqueous solutions of eutectic mixtures composed of cholinium chloride and urea ([Ch]Cl:U) have been used for the extraction of xylan from *Eucalyptus globulus* wood (Morais et al., 2018). Among many other relevant applications (Zhang et al., 2014), xylan can be used as a starting material for the production of xylitol, used as sweetener in food and pharmaceutical industries (Baptista et al., 2018 and 2020). However, in most cases, an intermediate step of hydrolysis of xylan into xylose is required (Yemiş and Mazza, 2011), often achieved by chemical and enzymatic approaches (Latif and Rajoka, 2001). However, the enzymes and microorganisms required for biomass processing could be affected by DES presence, and therefore the evaluation of their effect is a critical point (Satlewal et al., 2018). Some studies have evaluated the stability of cellulases in the presence of DES and their effect on the enzymatic saccharification of cellulose (Gunny et al., 2015; Guo et al., 2018; Shen et al., 2019). Nevertheless, to the best of our knowledge, there are no previous studies about xylan saccharification and microbial conversion into value-added compounds in these solvents.

In here, an integrated strategy for the simultaneous saccharification and bioconversion of xylan has been developed for the biotechnological production of xylitol from corncob whole slurry using a *Saccharomyces cerevisiae* PE2 strain overexpressing the *GRE3* gene (codifying for an unspecific aldose reductase responsible for the conversion of xylose into xylitol), (Baptista et al., 2018).

Specifically, the aim of this work was to evaluate the enzymatic hydrolysis of solubilized xylan in [Ch]Cl:U aqueous solutions at different molar ratios (1:2, 2:1 and 1:1), and concentrations (50 and 20 wt%, respectively) and of recovered xylan after DES solubilization. A one-pot process was developed by the optimization (studied variables: xylan (wt%), glucose (g/L) and enzyme loading (g/L)) of the simultaneous

saccharification and fermentation (SSF) for the bioconversion of xylan into xylitol, with the ultimate goal of lignocellulosic biomass valorization using sustainable processes.

2. Material and Methods

2.1. Materials

Cholinium Chloride ([Ch]Cl) (Sigma, USA, 99% purity) was used as HBA and Urea (U) (Sigma, USA, $\geq 99\%$ purity) was used as HBD for the DES preparation. Xylan from beechwood, obtained from Apollo Scientific ($\geq 90\%$), was used as model compound for the solubility and bioconversion assays. YPD medium (composed by 10 g/L yeast extract (Liofilchem, Abruzzi, Italy), 20 g/L peptone (Biokar diagnostics, Beauvais, France) and 20 g/L glucose (Biochem Chemopharma, Cosne sur loire, France)) supplemented with 150 mg/L of geneticin (G418, Sigma-Aldrich, St. Louis, MO, USA) was used for yeast growth. Glucose (Biochem Chemopharma, Cosne sur loire, France $\geq 99\%$ purity), ethanol (AppliChem, Darmstadt, Germany $\geq 99\%$ purity), xylose (AppliChem, Darmstadt, Germany AppliChem, Darmstadt) and xylitol (Sigma-Aldrich, St. Louis, MO, USA AppliChem, Darmstadt) were used as standards in HPLC analysis and 0.005M sulfuric acid (Biochem Chemopharma, Cosne sur loire, France) was used as eluent.

2.2. Methods

2.2.1. Preparation of DES and xylan solutions in aqueous DES

Before DES preparation, the humidity of its precursors (cholinium chloride and urea) was measured with a Metrohm 831 Karl Fisher coulometer, with a maximum of water determined of 5.21 wt.%. [Ch]Cl and U were accurately weighted and placed in sealed glass vials, and the heated under constant stirring until a transparent liquid was formed, in order to prepare DES with 2:1, 1:1 and 1:2 molar ratios. After liquid

formation, the mixture was kept at this temperature for one hour before being allowed to return to room temperature. DES composition and stability was confirmed by ^1H NMR and ^{13}C NMR analysis, using a Bruker Avance 300 at 300.13 MHz and 75.47 MHz, respectively, deuterated water as solvent and trimethylsilyl propanoic acid (TMSP) as internal reference, as reported elsewhere (Morais et al., 2018).

For the preparation of the xylan solutions, appropriated amounts of xylan were solubilized in 20 and 50 wt% DES aqueous solutions up to a xylan concentration of 100 and 150 g/L, as shown in Table 1. These concentrations were selected taking into consideration our previous studies on the solubility of xylan in [Ch]Cl:U aqueous solutions (Morais et al., 2018). The pH of DES aqueous solutions was measured at 25.0 ± 0.01 °C using a Metrohm 827 pH meter, with an uncertainty of ± 0.01 . The density of the aqueous solutions was also measured at atmospheric pressure, and in the temperature range from 0 to 30 °C, using an automated SVM 3000 Anton Paar rotational Stabinger viscometer-densimeter (temperature uncertainty: ± 0.02 K; absolute density uncertainty: $\pm 5 \times 10^{-4}$ g/cm³).

2.2.2. Xylan precipitation and recovery

Xylan was recovered from DES aqueous solutions to evaluate DES effect in the enzymatic saccharification. Xylan was precipitated by adding the same weight of absolute ethanol to the DES solution and left under stirring at 800 rpm for 24 h. The recovered solid was then vacuum filtrated using nylon Whatman 0.45 μm pore filters. After that, the precipitated material was washed with ethanol and acetone. The recovered xylan was then put in a 40 °C ventilated oven overnight and weighted. The pristine and recovered xylan have been analyzed by NMR, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Maldi-ToF-MS) and size exclusion chromatography (SEC) as described elsewhere (Morais et al., 2018).

2.2.3. Enzymatic hydrolysis of xylan

Solubilized xylan in DES aqueous solutions, precipitated xylan from DES solution and pristine beechwood xylan samples were diluted/solubilized with 50 mmol/L citrate buffer (to reach a final xylan concentration of 100, 50 and 20 g/L) and used as substrates in enzymatic hydrolysis assays (Table 2) using commercial enzymes (CellicTec2, kindly supplied by Novozymes) at an enzyme loading of 4800 UI/g xylan. All assays were conducted in duplicate.

Xylanase activity was measured following the procedure previously described (Bailey et al., 1992) and was found to be 9764 UI/mL (expressed as $\mu\text{mol/mL}\cdot\text{min}$), corresponding to 69 mg of protein/mL quantified by Bradford method (Bradford, 1976). Enzymatic experiments were carried out in an orbital shaker incubator at 50 °C and 150 rpm. The pH was adjusted to 4.8-5 with aqueous NaOH 4 mol/L. Aliquots of the reaction media were withdrawn at desired times and analyzed by HPLC (JASCO), using a BioRad HPX-87H (300 x 7.8 mm) column, at 60 °C with 0.005 M aqueous sulfuric acid as eluent in a flow rate of 0.6 mL/min and a Knauer-IR refractive index detector. The yield of enzymatic saccharification of xylan was determined by the following equation.

$$\text{Xylose Yield} = \frac{\text{xylose concentration}}{\text{xylan concentration}} \cdot 100 \% \quad \text{equation 1}$$

2.2.4. Microorganism and inoculum preparation

The yeast strain used in this work was *Saccharomyces cerevisiae* PE-2 *GRE3* (constructed in Baptista et al., 2018). The stock cultures were maintained on YPD agar (2% of glucose, 2% of agar, 1% of yeast extract and 2 % of peptone) containing 200 mg/L of geneticin (G418) at 4 °C. For the inoculum, cells were grown in YPD liquid medium with 150 mg/L of G418 for 15 h at 30 °C. Cell suspensions were recovered by centrifugation (5 min at 3000 rpm, 4 °C) and suspended in 0.9% of NaCl. The

Simultaneous Saccharification and Fermentation (SSF) assays were inoculated with 11 g fresh yeast/L (corresponding to 5 g/L of dry yeast/L).

2.2.5. Simultaneous saccharification and fermentation for xylitol production: Box-Behnken design.

The SSF experiments were carried out in 50 mL Erlenmeyer flasks placed in an orbital shaker at 35 °C and 150 rpm. The pH of culture media was adjusted to 5 with aqueous solution of NaOH (4 mol/L) or HCl (1 mol/L). Nutrients (10 g/L of yeast extract and 20 g/L of peptone), solubilized xylan and glucose concentrate solution were sterilized separately by autoclave (121 °C, 20 min) prior to fermentation trials. Then, solubilized xylan was mixed with glucose, water and enzymes needed to achieve the conditions used in this work (as shown in Table 3).

A Box-Behnken experimental design with three replicates at the central point (total: 15 experiments) was carried out for the optimization of xylitol production by SSF process. The independent variables studied were: glucose concentration (20-60 g/L), necessary for yeast growth and metabolism, concentration of xylan (10-80 g/L) solubilized in [Ch]Cl:U aqueous solution (corresponding to concentration of 2-16 wt. %) and enzymes loadings (0.38-0.77 mg/mL) to allow the saccharification of xylan into xylose, used as substrate for xylitol bioconversion.

Aliquots were withdrawn at desired times and analyzed by HPLC. Xylitol yield was calculated using the following equation:

$$\text{Xylitol Yield} = \frac{\text{xylitol concentration}}{\text{xylan concentration}} \cdot 100 \% \quad \text{equation 2}$$

The experimental data were fitted to the following equation using a commercial software (Microsoft Excel by Microsoft, USA).

$$y_j = b_{0j} + \sum_{i=1}^2 b_{ij} x_i + \sum_{i=1}^2 \sum_{k \geq i}^2 b_{ikj} x_i x_k \quad \text{equation 3}$$

where y_j ($j=1$ to 3) is the dependent variable; x_i or x_k (i or k : 1 to 3, $k \geq i$) are the dimensionless, normalized, independent variables (glucose concentration, xylan concentration and enzyme loading), and $b_{0j} \dots b_{ikj}$ are regression coefficients calculated from experimental data by multiple regression using the least-squares method.

3. Results and Discussion

3.1. Preliminary evaluation of [Ch]Cl:U effect on enzymatic hydrolysis of xylan and yeast glucose consumption

Xylitol production from lignocellulosic biomass can be achieved through the two following processes: i) enzymatic saccharification of xylan into xylose (xylan may be hydrolysed into xylooligosaccharides by endoxylanase, which can be cleaved into xylose by xylosidase) and ii) subsequent bioconversion of xylose into xylitol by recombinant yeast, overexpressing the *GRE3* gene that codifies an unspecific aldose reductase using NADPH as co-factor able to convert xylose into xylitol (Baptista et al., 2018). With the goal of attaining insights about the DES effect on enzymes and yeast strain performance, enzymatic hydrolysis and fermentation assays were initially carried out in the presence of [Ch]Cl:U aqueous solution (1:2 molar ratio). Based on previous results, and knowing that 50 wt% of [Ch]Cl:U (1:2) can solubilize up to 321 g/L of beechwood xylan (Morais et al., 2018), 150 g/L of xylan were diluted with 50 mmol/L of citrate buffer achieving a xylan concentration of 100 g/L, 50 and 20 g/L (corresponding to 33, 17 and 7 wt% of [Ch]Cl:U, respectively).

The effect of the different DES solutions during enzymatic hydrolysis (120 h) is shown in Figure 1a. The highest xylose yield (47.9 % \pm 0.73) was achieved with a xylan

concentration of 20 g/L (7 wt% DES). Nonetheless, this yield is similar to the maximum yield ($45.1 \% \pm 0.16$) attained with 50 g/L of xylan, revealing a not significant decrease in yield with the increase in DES concentration. The inhibitory effect of DES components such as urea has been previously reported (Attri et al., 2011). Urea can cause the unfolding of enzymes leading to its inactivation (Durand et al., 2014), as it is a chaotropic agent that denatures proteins by breaking protein hydrogen bonds and by interacting preferentially with the protein surface (Attri et al., 2011). Despite xylose yields are lower than 50 % in these assays, the enzyme cocktail was able to maintain the activity at least for the first 24 h of xylan saccharification (Figure 1a), showing that this approach could be used in the next steps.

Yeast performance was also evaluated in the same conditions as the ones used for the enzymatic hydrolysis, using [Ch]Cl:U (1:2) in the following DES concentrations: 33, 17 and 7 % wt. The samples were prepared and inoculated with 11 g fresh yeast biomass/L and the results of the assays are summarized in Figure 1b. Glucose consumption by the yeast at the lowest DES concentrations (17 and 7 wt%) was similar, achieving glucose depletion in the first 24h of fermentation. On the other hand, glucose was not completely consumed in the presence of 33 wt% of [Ch]Cl:U, which suggests a negative influence in the yeast performance for this condition. Hence, the results attained for the enzymatic and yeast performance in the different [Ch]Cl:U concentrations highlight that DES aqueous solutions can be used for enzymatic hydrolysis and fermentation at concentrations below 33 wt%.

3.2. Enhancement of enzymatic hydrolysis of xylan using 20 wt% of [Ch]Cl:U aqueous solution

Although promising results were attained during the preliminary assays on enzymatic hydrolysis, it was revealed that high DES concentrations have a negative

effect on the enzyme activity. Nevertheless, [Ch]Cl:U displays a very high capability of solubilizing xylan, even at lower DES concentrations (Morais et al., 2018). Due to this solvent characteristic it is possible to have a high concentration of xylan in diluted aqueous DES solutions, which may have lower impact in the enzymatic hydrolysis process. Taking this in mind, solutions with 100 g/L of xylan in 20 wt% of DES were prepared. Moreover, and due to the possible negative effect of urea in the process, different ratios of [Ch]Cl:U were tested (Table 1), specifically (1:1), (1:2) and (2:1), and its effect was further evaluated on the enzymatic hydrolysis of xylan and on the corresponding xylose yields. These solutions were then diluted to obtain 50 and 20 g/L of xylan to evaluate the effect of the substrate concentration, percentage of [Ch]Cl:U (corresponding to 10 and 4 wt%), and molar ratio on enzymatic hydrolysis. As control, pristine xylan (without solubilization in DES) was mixed with 50 mmol/L citrate buffer to achieve the same concentrations (100, 50 and 20 g/L of xylan). Additionally, solubilized xylan in 20 wt% of [Ch]Cl:U aqueous solution (2:1 molar ratio) was precipitated with absolute ethanol and was also used as substrate in enzymatic hydrolysis assays to compare with not solubilized pristine xylan and ascertain if the DES effect in xylan structure could be beneficial for xylitol production.

Table 2 collects the operational conditions (substrates used and their concentrations) of enzymatic hydrolysis assays and the main results obtained, *viz* xylose concentration and xylose yield at 96 h. Figure 2 shows the time course of the enzymatic hydrolysis of solubilized xylan at different molar ratios of [Ch]Cl:U (results expressed as xylose yield). It is observed that the highest xylose yields were obtained using 20 g/L of solubilized xylan for all molar ratios tested. Nevertheless, improvements of 36 and 32 % in xylose yields were achieved using [Ch]Cl:U 1:1 and 2:1 molar ratios, respectively, when compared to results obtained with 1:2 molar ratio (Table 2 and Figure 2). These

results show that the molar ratio of 1:1 has the best results on xylose yields and it was thus chosen to be used in the final stage of the process optimization.

As seen in Table 2, the highest value of xylose concentration (54 g/L), in presence of DES, was achieved using 100 g/L of xylan in 20 wt. % of [Ch]Cl:U (1:1), corresponding to a xylose yield of 54%. However, the aqueous solution of 20 g/L of xylan in 4 wt.% of DES (1:1) attained a xylose yield of 81.5 %, more than 40% increase in yield when compared with the undiluted DES (Table 2). These results can be positively compared with those reported in literature, in which corncob was pretreated with [Ch]Cl:U 1:2 molar ratio at 80 °C and 115 °C for 15 h and the pretreated biomass was enzymatically saccharified obtaining 22.9 and 31.9 % of xylose yield, respectively (Procentese et al., 2015).

Particularly with DES aqueous solutions of 7 wt% (2:1) and 4 wt% (1:1) (Figure 2a and b), xylose yields were only slightly lower than the yields obtained with pristine xylan in citrate buffer as reaction media (Figure 3a) and precipitated xylan from DES aqueous solution (Figure 3b). Therefore, the enzymatic hydrolysis of xylan is not significantly hampered using these DES concentrations and molar ratios.

On the other hand, the results of enzymatic hydrolysis shown in Figure 3b reveal an improvement of 9.8 % in the xylan saccharification when comparing the pristine xylan with the precipitated xylan from the DES media. These results suggest a positive effect of the [Ch]Cl:U dissolution process over the saccharification of the polysaccharide, which might be related with the structural changes undergone during the process, namely the complete removal of 4-O-methyl- α -D-GlpcA units as demonstrated by ^1H and ^{13}C NMR and MALDI-TOF-MS, structural analyses previously reported (Morais et al., 2018). These results highlight that DES can also be used in the pretreatment of xylan, making them more accessible for enzymatic saccharification into

xylose or others. Nevertheless, to avoid costly separation stages and with the goal of achieving a one-pot process, xylan solubilized in 20 wt. % of [Ch]Cl:U molar ratio 1:1 was selected for the integrated xylitol production.

3.3. Simultaneous Saccharification and Fermentation for xylitol production

In this work, an innovative process is proposed for the biological production of xylitol from xylan by simultaneous saccharification and fermentation (SSF). The xylan is hydrolyzed by enzymes, for xylose production, while simultaneously the recombinant yeast converts the released xylose into xylitol. *S. cerevisiae* PE-2 *GRE3*, the recombinant strain used in the SSF assays, is an industrial strain isolated from first generation bioethanol plant engineered to accumulate xylitol by overexpressing the endogenous unspecific aldose reductase *GRE3* that converts xylose to xylitol (Baptista et al., 2018).

According to the optimization performed in the initial assays of this work, 20 g/L of xylan in 20 wt% of [Ch]Cl:U (1:1) were used to perform the SSF assays displayed in Figure 5. The results are displayed as the time course of xylitol produced by these SSF assays (expressed as xylitol yield). Significant differences among assays occur in xylitol production (Figure 4) depending on the conditions used (Table 3).

Results regarding glucose consumption, ethanol produced and non-consumed xylose are included in Table 4. Glucose was almost completely consumed after 20 h of SSF, except for runs 5 and 11, that had final concentrations of glucose of 14.8 and 20.8 g/L (at 30 h), respectively. Ethanol concentration varied in the range of 2.8-28 g/L (run 13 and 2, respectively). Maximum ethanol concentration (28 g/L) was achieved using 60 g/L of glucose in presence of the lowest concentration of [Ch]Cl:U (2 wt%). After

glucose depletion, ethanol was used as carbon source achieving ethanol concentrations lower than 9 g/L at 120h in all SSF assays (Table 4).

The results obtained reveal that the fermentation performance is not affected by the presence of DES in the media. This trend is contrary to a previous study in which 5 wt% DES, namely [Ch]Cl:U (1:2) and [Ch]Cl:glycerol (1:2), were used with the laboratory strain *S. cerevisiae* BY4741 and [Ch]Cl:U (1:2) displayed an inhibitory effect (Xu et al., 2018). These differences could result from differences on the robustness of laboratory and industrial strains as the robust chassis of industrial strain PE-2 evaluated in the present study, revealed higher capacity of fermentation in presence of inhibitory compounds derived from lignocellulosic pre-treatment (Pereira et al., 2014; Cunha et al., 2019). These results show the relevance of testing green solvents applicability close to process-like conditions, namely with the process yeast strain (Costa et al., 2017).

Finally, and when evaluating xylitol production, maximum xylose bioconversion into xylitol was achieved at 72 h of fermentation in almost all studied conditions. The highest xylitol concentration (25.40 g/L) was achieved at the end of the fermentation (120 h) with 20 g/L of initial glucose, 45 g/L of xylan in 9 wt% of DES solution and 0.77 mg/ml of enzyme (run 6), which translates into a yield of 56.44 g xylitol/100 g xylan. On the other hand, the highest yield (76.15 g xylitol/100 g xylan) was obtained with 60 g/L glucose, and with the lowest substrate concentration (10 g/L of xylan in 2 wt% of [Ch]Cl:U aqueous solution) and 0.58 mg/ml of enzyme (run 2).

The lowest xylitol production (4.62 g/L) and yield (5.77 g xylitol/100 g xylan) were achieved with 40 g/L, 80 g/L of xylan in 16 wt% of [Ch]Cl:U aqueous solution and 0.38 mg/mL of enzyme (run 11). These results are directly related to the enzyme sensitivity to DES (16 wt%), since xylose concentration at the end of SSF varied in the range 17.1-20.7 g/L for the assays using 80 g/L of xylan in 16 wt% of [Ch]Cl:U.

3.3. Response Surface Methodology Assessment for optimization of xylitol production

For an optimization of the conditions for the SSF assays, response surface was used. The independent variables (glucose concentration, xylan concentration and enzyme loadings) collected in Table 3 were correlated with dependent variables (xylitol concentration, xylitol yield and xylitol productivity at 48 h, shown in Table 4) following the equation 3.

Table 5 lists the regression coefficients, their significance (based on the Student's t-test), and the statistical parameters measuring the correlation (R^2) and the significance of model (based on the Fisher's F-test). Based on the results listed in Table 5, the most influential variables on SSF process for xylitol production were xylan concentration in [Ch]Cl:U aqueous solutions and enzyme loading. The variable xylan concentration in [Ch]Cl:U aqueous solutions had a negative effect in xylitol yield. This negative effect is due to the inhibitory effect of high concentrations of [Ch]Cl:U on enzymatic saccharification of xylan, as evaluated above.

Figure 5a shows the effect of glucose concentration and xylan concentration in [Ch]Cl:U aqueous solution on xylitol production fixing the enzyme loading at 0.77 mg/mL. As seen, maximal xylitol concentration was obtained for the xylan concentration < 50 g/L (corresponding to 10 wt% of [Ch]Cl:U in the fermentation medium) and glucose concentration ranged between 35 and 50 g/L. On the other hand, the decrease of xylan in [Ch]Cl:U, which corresponds to a decrease in DES concentration in the solution, as well as glucose concentration, improve the xylitol yield for the highest enzyme loading evaluated (0.77 mg/mL) (Figure 5b). The presence of [Ch]Cl:U reduces the enzymatic saccharification of xylan decreasing the substrate concentration (xylose) for the xylitol production. Moreover, the high glucose

concentration in these processes causes a catabolite repression of xylose uptake (Baptista et al., 2018), presenting the maximum xylitol yield for 20 g/L of glucose (lowest glucose concentration evaluated).

Aiming at optimizing the SSF process for xylitol production, the variables xylose concentration and yield were used as response variables for the multiple response optimization of the model. The following operational conditions maximize the concentration and yield of xylitol: glucose concentration of 20 g/L, 36.25 g/L of xylan in 7.25 wt% of ChCl:U and enzyme loading of 0.77 mg/mL. The predicted xylitol concentration and yield were 23.67 g/L and 64.83 %, respectively. To validate the model, an additional experiment was carried out under optimized conditions. The results for the validation experiment were 23.94 g/L and 66.04 % of xylitol yield, thus validating the model.

This study thus reveals that xylan in aqueous solutions of [Ch]Cl:U can be used directly for simultaneous saccharification and bioconversion into xylitol. The yields attained can be slightly hampered by high DES concentrations, but due to the outstanding capability of [Ch]Cl:U to dissolve xylan it is possible to attain good xylitol yields by simply diluting the concentrated solution. To our knowledge, this is the first work showing the use of [Ch]Cl:U solutions for the biological conversion of xylan into xylitol.

4. Conclusions

In this work, xylan solubilized in [Ch]Cl:U aqueous solutions at different molar ratios was used as substrate for xylose production, showing a significant influence of selected molar ratio. Maximum xylose yield (81 %) was attained using molar ratio 1:1. The fermentation process was not affected by the presence of [Ch]Cl:U using an

industrial *S. cerevisiae* strain. The xylitol production was optimized using xylan solubilized in [Ch]Cl:U 1:1 by SSF process, attaining a maximum xylitol yield of 66%. The results obtained pave the way for the biological process route of xylitol production from biomass applying neoteric solvents such as DES.

Acknowledgements

This study was supported by BIOVINO project (0688_BIOVINO_6_E) funded by INTERREG España - Portugal and European Regional Development Fund (ERDF), carried out at the Biomass and Bioenergy Research Infrastructure (BBRI)- LISBOA-01-0145-FEDER-022059, supported by Operational Programme for Competitiveness and Internationalization (PORTUGAL2020), by Lisbon Portugal Regional Operational Programme (Lisboa 2020) and by North Portugal Regional Operational Programme (Norte 2020) under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF) and supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte and within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MCTES. The research leading to reported results has received funding from FCT through the projects DeepBiorefinery (PTDC/AGR-TEC/1191/2014) and MultiBiorefinery (POCI-01-0145-FEDER-016403), EcoTech project (POCI-01-0145-FEDER-032206), through C. Freire Researcher contract (CEECIND/00464/2017) and E. S. Morais PhD grant (SFRH/BD/129341/2017).

References

1. Abbott, A.P., Capper, G., Davies, D.L., Rasheed, R.K., Tambyrajah, V., 2003. Novel solvent properties of choline chloride/urea mixtures. *Chem. Commun* 70–71.
2. Alvarez-Vasco, C., Ma, R., Quintero, M., Guo, M., Geleynse, S., Ramasamy, K.K., Wolcott, M., Zhang, X., 2016. Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): A source of lignin for valorization. *Green Chem.* 18, 5133–5141. <https://doi.org/10.1039/c6gc01007e>
3. Assembly, U.N.G., 2015. Transforming our world: the 2030 Agenda for Sustainable Development.
4. Attri, P., Venkatesu, P., Kumar, A., Byrne, N., 2011. A protic ionic liquid attenuates the deleterious actions of urea on α -chymotrypsin. *Phys. Chem. Chem. Phys.* 13, 17023–17026. <https://doi.org/10.1039/c1cp22195g>
5. Bailey, M.J., Biely, P., Poutanen, K., 1992. Interlaboratory testing of methods for assay of xylanase activity. *J. Biotechnol.* 23, 257–270. [https://doi.org/10.1016/0168-1656\(92\)90074-J](https://doi.org/10.1016/0168-1656(92)90074-J)
6. Baptista, S. L., Cunha, J.T., Romání, A., Domingues, L., 2018. Xylitol production from lignocellulosic whole slurry corn cob by engineered industrial *Saccharomyces cerevisiae* PE-2. *Bioresour. Technol.* 267, 481–491. <https://doi.org/https://doi.org/10.1016/j.biortech.2018.07.068>
7. Baptista, S. L., Romání, A., Domingues, L. 2020. Chapter: Biotechnological advancements, innovations and challenges for sustainable xylitol production by yeast in Book: Biotechnological advancements, innovations and challenges for sustainable xylitol production by yeast, *Encyclopedia of Mycology*, Ed. Elsevier (Accepted)
8. Budzianowski, W.M., 2017. High-value low-volume bioproducts coupled to bioenergies with potential to enhance business development of sustainable biorefineries. *Renew. Sustain. Energy Rev.* 70, 793–804. <https://doi.org/10.1016/j.rser.2016.11.260>
9. Clark, J.H., 2007. Green chemistry for the second generation biorefinery – sustainable chemical manufacturing based on biomass. *Green Chem.* 609, 603–609. <https://doi.org/10.1002/jctb>
10. Clarke, Coby J, Tu, W., Levers, O., Bro, A., Hallett, J.P., 2018. Green and Sustainable Solvents in Chemical Processes. *Chem. Rev.* 118, 747–800. <https://doi.org/10.1021/acs.chemrev.7b00571>

11. Costa, C.E., Romaní, A., Cunha, J.T., Johansson, B., Domingues, L., 2017. Integrated approach for selecting efficient *Saccharomyces cerevisiae* for industrial lignocellulosic fermentations: Importance of yeast chassis linked to process conditions. *Bioresour. Technol.* 227, 24–34. <https://doi.org/10.1016/j.biortech.2016.12.016>
12. Cunha, J.T., Romaní, A., Costa, C.E., Sá-Correia, I., Domingues, L., 2019. Molecular and physiological basis of *Saccharomyces cerevisiae* tolerance to adverse lignocellulose-based process conditions. *Appl. Microbiol. Biotechnol.* 103, 159–175. <https://doi.org/10.1007/s00253-018-9478-3>
13. Domínguez, E., Romaní, A., Domingues, L., Garrote, G., 2017. Evaluation of strategies for second generation bioethanol production from fast growing biomass *Paulownia* within a biorefinery scheme. *Appl. Energy* 187, 777–789. <https://doi.org/10.1016/j.apenergy.2016.11.114>
14. Durand, E., Lecomte, J., Baréa, B., Villeneuve, P., 2014. Towards a better understanding of how to improve lipase-catalyzed reactions using deep eutectic solvents based on choline chloride. *Eur. J. Lipid Sci. Technol.* 116, 16–23. <https://doi.org/10.1002/ejlt.201300246>
15. Gullón, B., Muñiz-Mouro, A., Lú-Chau, T.A., Moreira, M.T., Lema, J.M., Eibes, G., 2019. Green approaches for the extraction of antioxidants from eucalyptus leaves. *Ind. Crops Prod.* 138, 111473. <https://doi.org/10.1016/j.indcrop.2019.111473>
16. Gunny, A.A.N., Arbain, D., Nashef, E.M., Jamal, P., 2015. Applicability evaluation of Deep Eutectic Solvents-Cellulase system for lignocellulose hydrolysis. *Bioresour. Technol.* 181, 297–302. <https://doi.org/10.1016/j.biortech.2015.01.057>
17. Guo, Z., Ling, Z., Wang, C., Zhang, X., Xu, F., 2018. Integration of facile deep eutectic solvents pretreatment for enhanced enzymatic hydrolysis and lignin valorization from industrial xylose residue. *Bioresour. Technol.* 265, 334–339. <https://doi.org/10.1016/j.biortech.2018.06.027>
18. Kumar, P., Barrett, D.M., Delwiche, M.J., Stroeve, P., 2009. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Ind. Eng. Chem. Res.* 48, 3713–3729.
19. Latif, F., Rajoka, M.I., 2001. Production of ethanol and xylitol from corn cobs by yeasts. *Bioresour. Technol.* 77, 57–63. [https://doi.org/10.1016/S0960-8524\(00\)00134-6](https://doi.org/10.1016/S0960-8524(00)00134-6)

20. Loow, Y.L., New, E.K., Yang, G.H., Ang, L.Y., Foo, L.Y.W., Wu, T.Y., 2017. Potential use of deep eutectic solvents to facilitate lignocellulosic biomass utilization and conversion. *Cellulose* 24, 3591–3618. <https://doi.org/10.1007/s10570-017-1358-y>
21. Morais, E.S., Freire, M.G., Freire, C.S.R., Coutinho, J.A.P., Silvestre, A., 2019. Enhanced conversion of xylan into furfural using acidic deep eutectic solvents with dual solvent and catalyst behavior. *ChemSusChem* 1–8. <https://doi.org/10.1002/cssc.201902848>
22. Morais, E.S., Mendonça, P. V., Coelho, J.F.J., Freire, M.G., Freire, C.S.R., Coutinho, J.A.P., Silvestre, A.J.D., 2018. Deep Eutectic Solvent Aqueous Solutions as Efficient Media for the Solubilization of Hardwood Xylans. *ChemSusChem* 11, 753–762. <https://doi.org/10.1002/cssc.201702007>
23. Pereira, F.B., Romaní, A., Ruiz, H.A., Teixeira, J.A., Domingues, L., 2014. Industrial robust yeast isolates with great potential for fermentation of lignocellulosic biomass. *Bioresour. Technol.* 161, 192–199. <https://doi.org/10.1016/j.biortech.2014.03.043>
24. Procentese, A., Johnson, E., Orr, V., Garruto Campanile, A., Wood, J.A., Marzocchella, A., Rehm, L., 2015. Deep eutectic solvent pretreatment and subsequent saccharification of corncob. *Bioresour. Technol.* 192, 31–36. <https://doi.org/10.1016/j.biortech.2015.05.053>
25. Romaní, A., Garrote, G., Alonso, J.L., Parajó, J.C., 2010. Bioethanol production from hydrothermally pretreated *Eucalyptus globulus* wood. *Bioresour. Technol.* 101, 8706–8712. <https://doi.org/10.1016/j.biortech.2010.06.093>
26. Romaní, A., Larramendi, A., Yáñez, R., Cancela, Á., Sánchez, Á., Teixeira, J.A., Domingues, L., 2019. Valorization of *Eucalyptus nitens* bark by organosolv pretreatment for the production of advanced biofuels. *Ind. Crops Prod.* 132, 327–335. <https://doi.org/10.1016/j.indcrop.2019.02.040>
27. Sandlewal, A., Agrawal, R., Bhagia, S., Sangoro, J., Ragauskas, A.J., 2018. Natural deep eutectic solvents for lignocellulosic biomass pretreatment: Recent developments, challenges and novel opportunities. *Biotechnol. Adv.* 36, 2032–2050. <https://doi.org/10.1016/j.biotechadv.2018.08.009>
28. Shen, X.-J., When, J.-L., Mei, Q.-Q., Chen, X., Sun, D., Yuan, T.-Q., Sun, R.-C., 2019. Facile fractionation of lignocelluloses by biomass-derived deep eutectic

- solvent (DES) pretreatment for cellulose enzymatic hydrolysis and lignin valorization. *Green Chem.* 21, 275–283. <https://doi.org/10.1039/c8gc03064b>
29. Soares, B., Tavares, D.J.P., Amaral, J.L., Silvestre, A.J.D., Freire, C.S.R., Coutinho, J.A.P., 2017. Enhanced Solubility of Lignin Monomeric Model Compounds and Technical Lignins in Aqueous Solutions of Deep Eutectic Solvents. *ACS Sustain. Chem. Eng.* 5, 4056–4065. <https://doi.org/10.1021/acssuschemeng.7b00053>
30. Soh, L., Eckelman, M.J., 2016. Green solvents in biomass processing. *ACS Sustain. Chem. Eng.* 4, 5821–5837. <https://doi.org/10.1021/acssuschemeng.6b01635>
31. Werpy, T., Petersen, G., Aden, A., Bozel, J., Holladay, J., White, J., Manheim, A., 2004. Top Value Added Chemicals from Biomass Volume I — Results of Screening for Potential Candidates from Sugars and Synthesis Gas Top Value Added Chemicals.
32. Xu, F., Sun, J., Wehrs, M., Kim, K.H., Rau, S.S., Chan, A.M., Simmons, B.A., Mukhopadhyay, A., Singh, S., 2018. Biocompatible Choline-Based Deep Eutectic Solvents Enable One-Pot Production of Cellulosic Ethanol. *ACS Sustain. Chem. Eng.* 6, 8914–8919. <https://doi.org/10.1021/acssuschemeng.8b01271>
33. Yemiş, O., Mazza, G., 2011. Acid-catalyzed conversion of xylose, xylan and straw into furfural by microwave-assisted reaction. *Bioresour. Technol.* 102, 7371–7378. <https://doi.org/10.1016/j.biortech.2011.04.050>
34. Zhang, L., Yu, Han, Yu, Hong-bing, Chen, Z., Yang, L., 2014. Conversion of xylose and xylan into furfural in biorenewable choline chloride – oxalic acid deep eutectic solvent with the addition of metal chloride. *Chinese Chem. Lett.* 25, 1132–1136.
35. Zhang, Q., De Oliveira Vigier, K., Royer, S., Jérôme, F., 2012. Deep eutectic solvents: Syntheses, properties and applications. *Chem. Soc. Rev.* 41, 7108–7146. <https://doi.org/10.1039/c2cs35178a>

Figure 1. Preliminary assays using 33, 17 and 7 % wt. of [Ch]Cl:U (1:2 molar ratio) to evaluate their effect on: a) Enzymatic hydrolysis of 100, 50 and 20 g/L of solubilized xylan and b) 20 g/L of glucose consumption by *Saccharomyces cerevisiae* PE2 *GRE3* yeast strain.

Figure 2. Enzymatic hydrolysis of solubilized xylan in 20 % of [Ch]Cl:U at: a) molar ratio of 2:1; b) molar ratio of 1:1 and c) molar ratio 1:2.

Figure 3. Enzymatic hydrolysis of: a) precipitated xylan from 20% wt. of [Ch]Cl:U (1:2 molar ratio) and, b) pristine xylan

Figure 4. Simultaneous saccharification and fermentation of solubilized xylan in 20 % [Ch]Cl:U (1:1 molar ratio) (operational conditions described in Table 3).

Figure 5. Response surface of: a) Xylitol concentration (g/L) on glucose concentration (g/L) and xylan concentration in [Ch]Cl:U aqueous solutions; b) Xylitol yield (g of xylose/100 g of xylan) on glucose concentration and xylan concentration in DES aqueous solutions.

Table 1 –Cholinium Chloride:Urea ([Ch]Cl:U) DES ratios and dilutions, as well as xylan concentrations used in the present study.

Molar ratios (HBA:HBD)	DES ([Ch]Cl:U) content water (wt.%)	Xylan Concentration (g/L)
1:2	50	150
1:2	20	100
1:1	20	100

2:1

20

100

Table 2. Enzymatic hydrolysis of solubilized xylan in 20 % [Ch]Cl:U (1:2, 1:1 and 2:1 molar ratio) and precipitated xylan from 20 % [Ch]Cl:U (2:1) and pristine xylan

Operational conditions of enzymatic hydrolysis			Main results calculated at 96 h	
Substrate	Xylan concentration (g/L)	[Ch]Cl:U (wt. %)	Xylose concentration (g/L)	Xylose yield (g xylose/100 g of xylan)
Pristine xylan (as control)	100	-	70.4 ± 0.03	70.5 ± 0.03
	50	-	37.7 ± 0.32	74.7 ± 0.64
	20	-	16.0 ± 0.52	79.9 ± 0.19
Precipitated xylan from [Ch]Cl:U (molar ratio 2:1)	100	-	69.6 ± 0.62	69.6 ± 0.78
	50	-	36.5 ± 1.6	72.9 ± 1.2
	20	-	17.6 ± 0.89	87.8 ± 0.56
Solubilized xylan in [Ch]Cl:U (molar ratio 2:1)	100	20	52.0 ± 0.67	52.0 ± 0.32
	50	10	30.3 ± 1.0	60.7 ± 0.79
	20	4	15.8 ± 0.78	78.9 ± 0.48
Solubilized xylan in [Ch]Cl:U (molar ratio 1:2)	100	20	34.9 ± 1.3	34.9 ± 0.92
	50	10	24.9 ± 0.56	49.8 ± 0.43
	20	4	11.9 ± 0.78	59.6 ± 0.31
Solubilized xylan in [Ch]Cl:U (molar ratio 1:1)	100	20	54.9 ± 0.81	54.9 ± 0.96
	50	10	38.0 ± 0.45	76.0 ± 0.42
	20	4	16.3 ± 0.32	81.5 ± 1.5

Table 3. Operational conditions expressed in terms of dimensionless and dimension independent variables

Glucose concentration (g/L) (x1)	[Ch]Cl:U (1:1 molar ratio) wt. %	Xylan concentration (g/L) (x2)	Enzyme loading
----------------------------------	----------------------------------	--------------------------------	----------------

				(mg protein/mL) (x3)
run 1	40 (0)	2	10 (-1)	0.38 (-1)
run 2	60 (1)	2	10 (-1)	0.58 (0)
run 3	60 (1)	9	45 (0)	0.38 (-1)
run 4	40 (0)	9	45 (0)	0.58 (0)
run 5	60 (1)	16	80 (1)	0.58 (0)
run 6	20 (-1)	9	45 (0)	0.77 (1)
run 7	40 (0)	9	45 (0)	0.58 (0)
run 8	20 (-1)	16	80 (1)	0.58 (0)
run 9	20 (-1)	2	10 (-1)	0.58 (0)
run 10	40 (0)	16	80 (1)	0.77 (1)
run 11	40 (0)	16	80 (1)	0.38 (-1)
run 12	40 (0)	2	10 (-1)	0.77 (1)
run 13	20 (-1)	9	45 (0)	0.38 (-1)
run 14	40 (0)	9	45 (0)	0.58 (0)
run 15	60 (1)	9	45 (0)	0.77 (1)

Table 4. Main results obtained from Simultaneous Saccharification and Fermentation assays

	Glucose (g/L) at 20 h	EtOH Max (g/L)	EtOH (g/L) at 120 h	Xylose Max (g/L)	Xylose (g/L) at 120h	Xylitol (g/L) (y ₁)	Xylitol Yield (%) (y ₂)	Xylitol Productivity Qp at 48h (g/Lh) (y ₃)
run 1	9.9	15.3	0.0	5.8	0.0	6.5	64.96	0.121
run 2	0.1	28.4	8.1	5.2	0.0	7.62	76.15	0.138
run 3	1.2	22.0	8.0	9.6	0.0	19.51	43.35	0.224
run 4	8.9	17.6	8.1	12.6	8.9	24.53	54.52	0.348
run 5	32.9	15.2	9.3	18.1	16.1	13.23	16.54	0.14
run 6	0.0	7.8	0.0	10.8	0.0	25.4	56.44	0.377
run 7	2.1	14.2	0.0	9.0	0.0	23.83	52.95	0.348
run 8	6.7	9.6	0.0	20.2	19.8	12.01	15.01	0.15
run 9	0.2	3.1	0.0	5.7	0.0	6.8	68	0.116
run 10	1.2	13.1	4.2	17.1	16.8	14.96	18.69	0.183
run 11	27.5	3.9	2.6	20.7	19.3	4.62	5.77	0.024
run 12	1.9	7.8	0.0	10.0	0.0	6.55	65.53	0.127
run 13	0.0	2.8	0.0	7.0	0.0	15.72	34.93	0.298
run 14	7.4	4.9	0.0	10.7	0.0	18.71	41.57	0.222
run 15	34.1	7.5	3.3	12.3	3.5	16.64	36.97	0.175

Table 5 Regression coefficients and statistical parameters measuring the correlation and significance of the models

	Coefficients		
	y ₁ : [Xylitol]	y ₂ : Xylitol Yield	y ₃ : Qp48 (productivity)
b _{0j}	23.58	52.41	0.320
b _{1j}	-0.51	-1.79	-0.039
b _{2j}	1.95 ^a	-30.47 ^a	-0.002
b _{3j}	2.22 ^a	5.10 ^a	0.028
b _{11j}	-0.57	-0.46	-0.009
b _{22j}	-12.77	-4.67 ^b	-0.170 ^c
b _{33j}	-2.11 ^b	-5.48 ^b	-0.020
b _{12j}	0.43	1.69	-0.002
b _{13j}	-1.55 ^c	-3.44 ^c	-0.009
b _{23j}	2.02 ^b	-0.42	0.040
F	42.00	115.46	3.528
Significance	> 99	> 99	> 90
R ²	0.987	0.995	0.864

^a Coefficients significant at the 99% confidence level.

^b Coefficients significant at the 95% confidence level.

^c Coefficients significant at the 90% confidence level.

CRediT author statement

Aloia Romaní, Lucília Domingues, Armando Silvestre and Eduarda Morais:

Conceptualization. Aloia Romaní, Eduarda S. Morais and Pedro O. Soares: Writing-

Original draft preparation. Aloia Romaní, Eduarda S. Morais and Pedro O. Soares:

Tables and Figure Preparation. Aloia Romaní, Eduarda S. Morais, Pedro O. Soares,

Mara G. Freire, Carmen S. R. Freire, Armando J. D. Silvestre and Lucília Domingues:

Validation. Armando Silvestre, Mara G. Freire, Carmen S. R. Freire and Lucília

Domingues: Writing- Reviewing and Editing.

Journal Pre-proofs

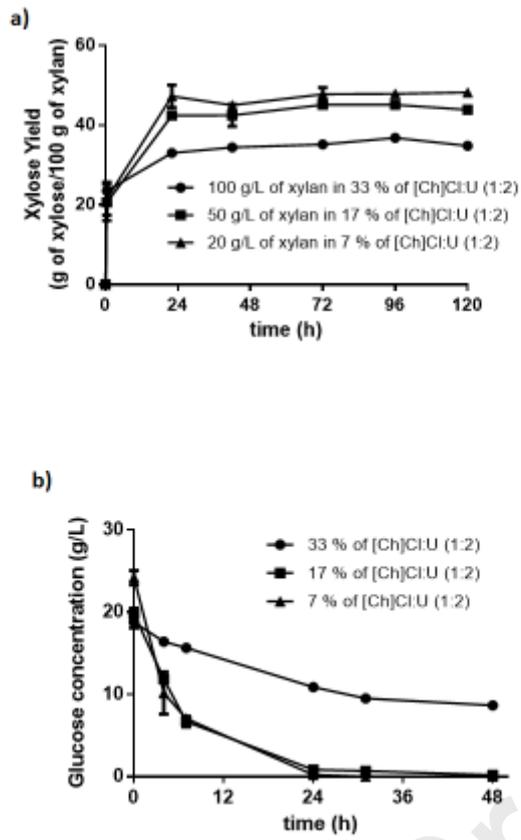


Figure 1

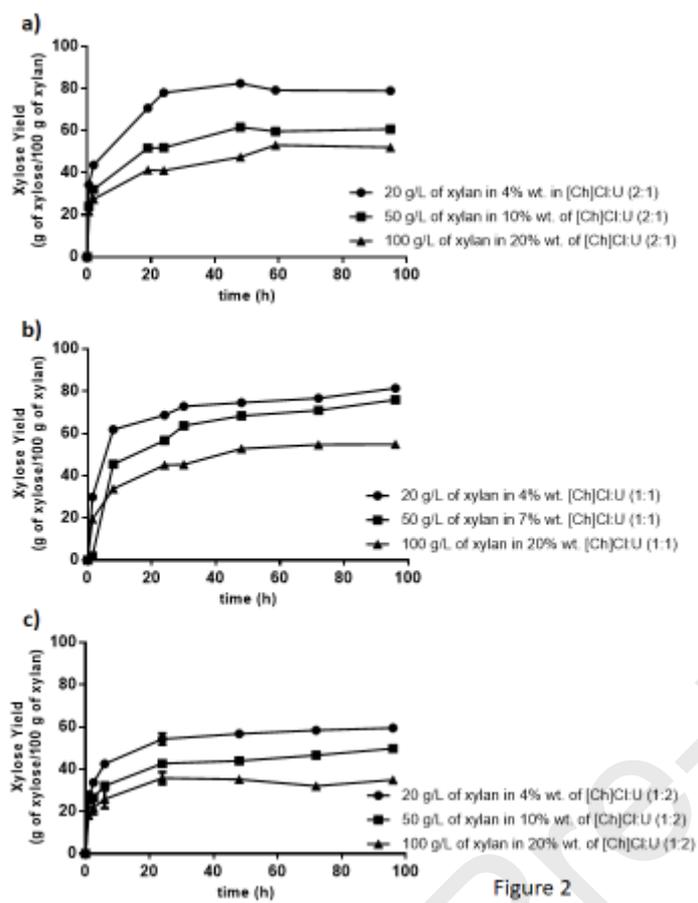


Figure 2

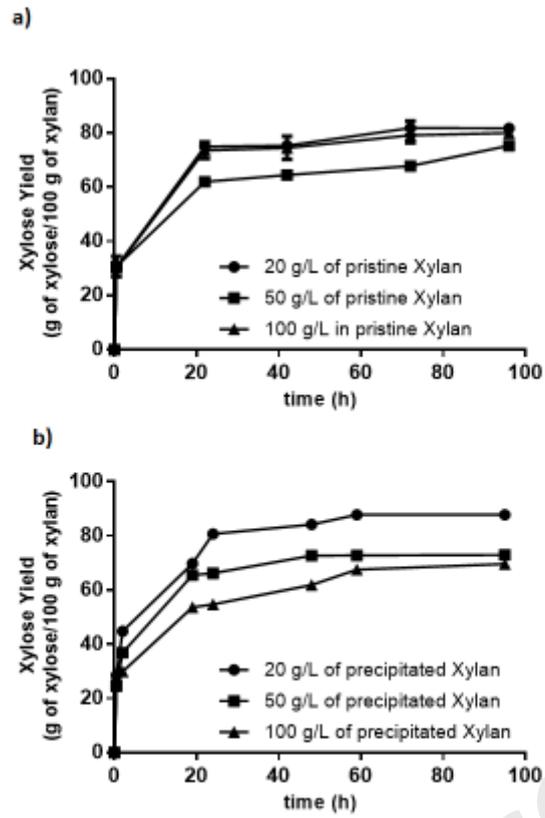


Figure 3

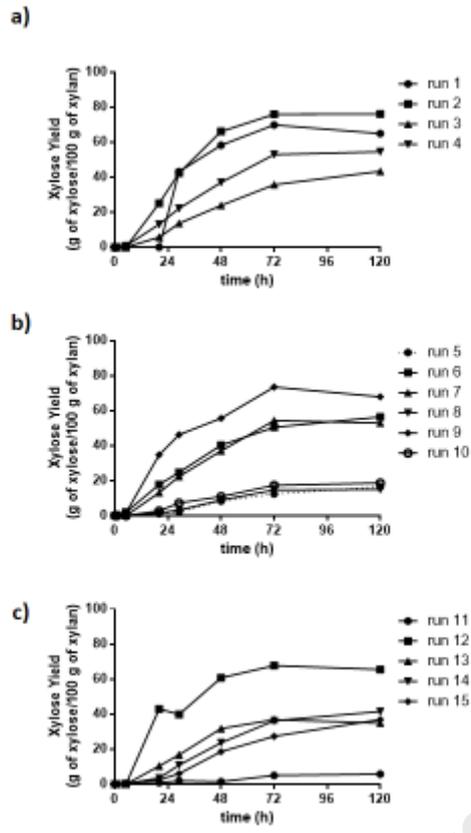


Figure 4

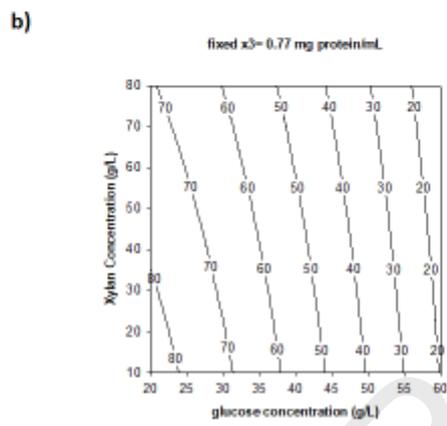
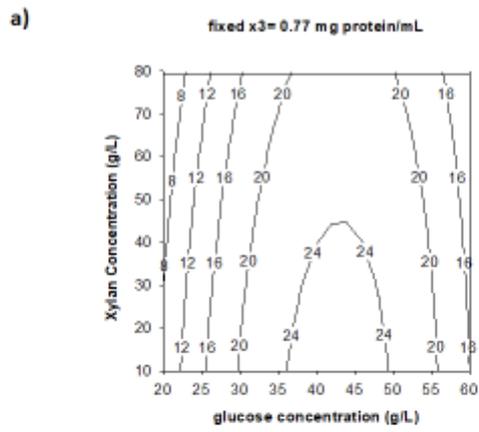
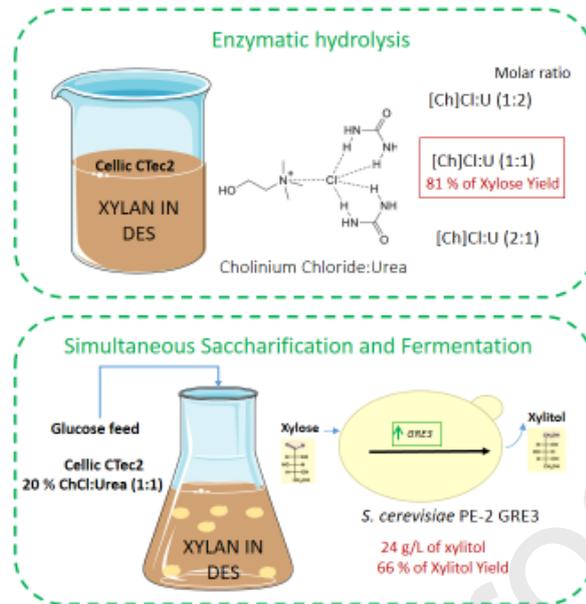


Figure 5



Deep eutectic solvent was evaluated for the development of biomass one step processing

1:2 [Ch]Cl:Urea molar ratio showed a significant decrease on xylan saccharification

81% of xylan conversion into xylose was achieved using [Ch]Cl:Urea 1:1

24 g/L of xylitol was obtained from solubilized xylan in DES by SSF

Journal Pre-proofs