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Evaluation of Miscanthus Pretreatment effect by Choline Chloride based Deep Eutectic Solvents on bioethanol production

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Abstract

This work evaluates the efficiency of three deep eutectic solvents constituted of choline chloride and urea or glycerol or ethylene glycol in the pretreatment of the miscanthus in view of extracting cellulose. Analysis of experiments shows that basicity and polarity of the hydrogen bond donor of these DESs are directly related to the miscanthus solubility. The best efficient process was found using {Choline chloride/glycerol} mixture for the pretreatment at a temperature of 373 K and a duration of about 6 hours. This may be explained by the fact that {Choline chloride/glycerol} pretreatment allows to obtain an amorphous cellulose. {Choline chloride/glycerol} was as efficiently as IL pretreatments with an ethanol production of about 72%. This study shows that Choline chloride based DESs pretreatment for biomass could be a key point to enhance the efficiency of biorefinery.

Keywords: DES pretreatment; Miscanthus; Cellulose; Choline chloride; Ethanol production

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1. Introduction

Lignocellulosic biomass appears to be an alternative to petroleum while the transformation of this feedstock into high-value compounds used in chemical industry or bioethanol is possible at an industrial scale. The complex structure of the lignocellulosic biomass requires a pretreatment to extract and separate its main constituents (Howard et al., 2003).

The classical techniques used for lignocellulosic biomass pretreatment were fully described in the literature (Haldar et al., 2016). A recent review of Haldar et al. presents the pros and cons of each approach (Haldar and Purkait, 2021). The choice of the pretreatment has a real impact on the configuration and the operating cost (Rabemanolontsoa and Saka, 2016). The technologies for the pretreatment of miscanthus reported in the literature are among others ethanol organosolv process, dilute acid treatment or alkaline treatment (Brethauer and Studer, 2015; Capolupo and Faraco, 2016; Harmsen et al., 2010). Numerous advanced methods were developed to improve the processes (Guo et al., 2019; Xu et al., 2022). The main advantages and disadvantage of the advanced methods are given in the review of (Brosse et al., 2012). Few pretreatments were studied for the valorization of Miscanthus. It was found that Miscanthus with high hemicellulose content presenting low crystallinity index is preferred for the biomass digestibility while NaOH or H_2SO_4 pretreatments have a negative effect (Xu et al., 2012). In acidi or alkali pretreatments, the cellulose degree of polymerization has also an impact on the miscanthus digestibility (Zhang et al., 2013). Then, optimal conditions for the green liquor pretreatments were determined in order to improve the saccharification or delignification processes in Miscanthus (Alam et al., 2020). More elaborated processes coupling steam explosion, alkali or acidi pretreatments and the use of surfactants were developed to enhance the enzymatic hydrolysis of the biomass (Sun et al., 2020).

In the beginning of 2000, ionic liquids (ILs) with their tunable physico-chemical properties appeared as new cleaner solvents (Passos et al., 2014). This family of solvents appears efficient

in biorefinery processes (Duque et al., 2021). Indeed, ILs possess the capacity of destroying the recalcitrant structure of the biomass and disrup the interactions between its components. Indeed, the reaction between anions and cations and the hydroxyls and hydrogen from the biomass leads to its dissolution. Dialkylimidazolium based ILs containing a chloride or acetate anion are strongly used in biomass studies. (Hassan et al., 2015) have proposed a process based on a miscanthus pretreatment methylphosphonate based ILs. Recently, (Bhatia et al., 2021) have shown that steam explosion, IL pretreatment or their combination lead to different final products. Numerous reviews on the dissolution or activation of lignin (Zubeltzu et al., 2020), cellulose (Usmani et al., 2020), bioethanol production (Vieira et al., 2020) can be found in the literature. This class of solvent has demonstrated its efficiency in the field of biomass but its main drawbacks are the viscosity, the cost and also the toxicity.

Deep Eutectic Solvents (DESs) emerged in the early of the twenty-first century. DESs have similar physico-chemical properties than ILs but it appears that this class of solvent may be less toxic and less expensive to synthetize. DESs are composed of at least one hydrogen bond acceptor and donor (Cui et al., 2021; Wu et al., 2021). Numerous DESs are based on tetraalkylammonium chloride choline chloride (ChCl) as HBA and on urea, glycerol, citric acid, carboxylic acids or phenol compounds as hydrogen-bond donors (Ma et al., 2019; Shafie et al., 2019). (Pan et al., 2017) studied the effect of the pretreatment of rice straw with the DES constituated of choline chloride and of urea. The highest solubility of isolated chemical fractions in this DES was found to be the lignin. It appears that DES-pretreatment leads to an increase of a-cellulose crystallinity index. The cholinium based DESs pretreatment allows to obtain a mixture of the three main constituents of the biomass (An et al., 2015; Kumar et al., 2016). Different procedures have been published about the separation of cellulose from biomass. The cellulose could be precipitated efficiently from DESs solution using antisolvent method (Hassan et al., 2015; Okuofu et al., 2020). Only four papers related to the use of DESs

with miscanthus were published in the literature (Chen and Wan, 2018; Guo et al., 2019; Kohli et al., 2020; Zhou et al., 2021). (Chen and Wan, 2018) developed a microwave pretreatment using DES constituted of lactic acid and choline chloride. The fast process is particularly effective for the biomass fractionation. DES pretreatment proposed by (Guo et al., 2019) improves the efficiency of the process by adding catalysts such as heteropoly acids. This approach leads to an improvement of the enzymatic digestibility, a high glucose yield and an ethanol yield of about 82 % in semi-simultaneous saccharification and fermentation process. (Kohli et al., 2020) have tested choline chloride based DESs for the microwave assisted delignification of miscanthus. Their work shows that the acidic properties of HBD forming the DESs are an important parameter governing the efficiency of delignification process of the biomass. Better results are observed with a pretreatment at 130°C during 60 minutes with the DES constituted of {choline chloride/oxalic acid}. (Zhou et al., 2021) demonstrated that the use of (choline chloride/ *p*-toluene sulfonic acid) mixture in pretreatment followed by NaOH post-treatment increases the efficiency of the enzymatic hydrolysis. Recently, it was shown that the use of heteropoly acids as catalysts in DES improves the efficiency of fractionization of lignins from miscanthus (Guo et al., 2019). The process leads to high purity lignin with good thermal stability. Recent reviews on lignocellulosic biomass pretreatments present the advantages and disadvantages to use DESs (Ab Rasid et al., 2021; Wang and Lee, 2021).

The first part of this study is devoted to the influence of the hydrogen bond donor (glycerol or ethylene glycol or urea) used in choline chloride based DESs on the miscanthus solubility. The second part of the article evaluates the efficiency of DES pretreatment of miscanthus on the extraction of cellulose. The influence of temperature, time and the components of the DES on the performance of the extraction were also evaluated. The extracted cellulose is characterized using X-ray diffraction and infrared spectra. Then, hydrolysis and fermentation were performed

on cellulose to produce biofuel. The process performances were evaluated through the ethanol conversion rate and the hydrolysis yield calculation.

2. Materials and Methods

2.1. Biomass and chemicals

Miscanthus x Gigantus (MxG) was found in Courcelles-Chaussy (France). The study was carried out using miscanthus particle size below 80 mm dried at 383.15 K during 48 h.

Ethyl alcohol absolute (99.99%) was bought from Carlo Erba. Choline chloride (98%), glycerol (99.5%), urea (99.5%) and ethylene glycol (99.8%) were purchased from Sigma Aldrich. β-glucosidase and Cellulases from T. reesei (Celluclast 1.5L) were provided by Sigma Aldrich. The Deep Eutectic Solvents (DES) were prepared by mixing choline chloride and glycerol or ethylene glycol or urea for 6 hours at 60°C (Chen and Wan, 2018; Guo et al., 2019; Kohli et al., 2020). The mole ratio of each DES and acronym are given in Table 1. Urea and Choline chloride were dried under vacuum at 323.15K during 12 hours before use.

2.2. Solubility of Miscanthus in DESs

The miscanthus solubility measurements were carried out using the apparatus and the procedure described in previous works (Hassan et al., 2013b, 2013a). A precise mass of DES was first introduced into a thermostated cell. Then, miscanthus was added in excess inside the solvent. The strongly stirred solution resulted in the formation of a dark miscanthus suspension. The presence of any solid residues was checked by the centrifugation of the solution. Microscopy was used to confirm the full dissolution.

2.3. Deep eutectic solvent pretreatment of Miscanthus

The DES pretreatment of miscanthus was carried out by adding 1 g of miscanthus in a mixture containing 20% of dimethyl sulfoxide (DMSO) and 80% of DES (Hassan et al., 2013b, 2013a,

2015). DMSO was added to reduce the viscosity of the solutions and to enhance the mass transfer. It was demonstrated that DMSO has no influence on the solubility of the biomass during the pretreatment. Indeed, DMSO is not able to dissolve the main components of miscanthus because of strong interactions and linkages whithin the biomass (Sun et al., 2009). The residue remaining in the solution was removed by filtration. After washing the clear liquors with water, a cellulose-rich extract was obtained. DES was completely removed from the residue and the extract using water. Then, both samples were dried at 373 K during 12 hours. The knowledge of the mass of the original miscanthus, M_o, of the mass of cellulose-rich extract, M_{ex} and of the mass of residue, M_r allows to calculate the recovery of miscanthus components, $Recovery_{(cell)}$, the regeneration rate, R_{reg} (%) and the miscanthus dissolution rate, R_{diss} (%) according to:

$$R_{diss}(\%) = \left(\frac{M_0 - M_r}{M_0}\right) \times 100 \quad (1)$$

$$R_{reg}(\%) = \left(\frac{M_{ex}}{M_o}\right) \times 100 \quad (2)$$

$$Recovery_{(cell)}(\%) = \left(\frac{M_{ex} \times C_{ex-cell}}{M_o \times C_{o-cell}}\right) \times 100 \quad (3)$$

Where: $C_{ex-cell}$ and C_{o-cell} are the cellulose content in the extract and the original miscanthus.

2.4. Determination of cellulose, lignin and hemicelluloses content

The content of lignin was obtained via standard method achieved by the National Renewable Energy Laboratory (NREL) (Sluiter, 2008; Tan and Lee, 2012).

The content of cellulose was also determined via standard method described in previous work (Hassan et al., 2015). A mixture of concentrated nitric acid with acetic acid (80%) in a 10:1 ratio by volume was prepared (Sun et al., 2004). A miscanthus sample of 0.10 g with 3 ml of acetic/nitric reagent were placed into conical tubes. The solution was heated at 383 K for 10

min then at 393 K for 30 min. A deionized water, 10 ml, was introduced in the cooled mixture. The solution was centrifuged at 4000 rpm for 20 min to obtain cellulose. The extracted cellulose was still washed and the centrifugation was repeated. The cellulose content was determined by gravimetric analysis. The content of hemicellulose was calculated by subtracting cellulose from holocellulose contents. Holocellulose was achieved via a method described by (Teramoto et al., 2009). According to the standard methods, the grade of lignin, cellulose and hemicellulose was calculated according to:

$$X\ grade\ (\%) = \frac{m_X}{m_{sample}} \times 100 \quad (4)$$

In equation (4), X corresponds to lignin, cellulose or hemicellulose. m_X and m_{sample} are the masses of X and the sample, respectively.

2.5. XRD and FTIR analysis

Rigaku MiniFlex II model was used for X-ray diffraction analyses of original and produced fractions. The analyses were achieved through Cu Kα1 radiation and samples were scanned at 0.2°/min. from 5 to 80 degree. ALPHA Fourier Transform model was used for the IR spectra of the original and produced fractions. The analyses were determined through an OPUS/Mentor software using a ZnSe ATR crystal prob.

2.6. Enzymatic hydrolysis process

The detailed process was fully described in previous work (Hassan et al., 2015). Enzymatic hydrolysis was carried out at 323.15 K during 3 days with the optimum conditions given in previous work (Hassan et al., 2015). To 1g of cellulose diluted in citrate Buffer (50 mM pH

4.8), 200 FBU of Celluclast 1.5 L was added. An addition of an equivalent volume of β-glucosidase were introduced in the sample. The final solution was shaked at 200 rpm. The glucose concentration in the collected samples were determined by HPLC.

The efficiency of hydrolysis process could be calculated with the following equations (Hassan et al., 2015):

$$Glucose\ hydrolysis\ efficiency\ (\%) = \frac{(\text{volume of reaction (l)} \times \text{glucose concentration (g/l)})}{(\text{mass of sample (g)} \times \text{fraction cellulose in sample})} \times 0.9 \times 100 \quad (5)$$

$$\text{Xylose}\ hydrolysis\ efficiency\ (\%) = \frac{(\text{xylose concentration (g/l)} \times \text{volume of reaction (l)})}{(\text{mass of sample (g)} \times \text{fraction hemicellulose in sample})} \times 0.88 \times 100 \quad (6)$$

2.7. Fermentation of the hydrolysates

2.7.1. Yeast and culture conditions

The method used for the fermentation of hydrolysis was described in previous work (Hassan et al., 2015). The fermentation of the different hydrolysate solutions was performed using yeast from S. cerevisiae during 72 hours.

2.7.2 Batch fermentation

The fermentation experiments were conducted under anaerobic conditions at a 1:10 ratio of inoculum: hydrolysates during 72 hr under isothermal conditions (at 30° C). Experiments were duplicated for glucose/ethanol analyses while average data were reported. The gas chromatography procedure used for the quantification of ethanol was fully described in previous work (Hassan et al., 2015).

3. Results and discussion

3.1 Miscanthus solubility in Choline Chloride Based DES

The solubility of miscanthus in choline chloride based DESs measured in a temperature range from 363 K to 403 K is presented in Fig. 1. All experimental data is listed in Table 2. The best solubility of miscanthus is found to be in {choline chloride/glycerol}. The miscanthus solubility increased with the HBD according to: urea < ethylene glycol < glycerol. Miscanthus is at least to time less soluble in {Choline chloride/urea} than in the two other DESs. The LSER parameters describing the polarity or the hydrogen bong of the solvent can be used to understand which property is directly related to a chemical process. The solubility of miscanthus in DESs increases with the hydrogen bond basicity (1.21, 0.78 and 0.84 for glycerol, ethylene glycol and urea, respectively) and polarity of the HBD (0.51, 0.4 and 0.5 glycerol, ethylene glycol and urea). Similar behaviour was observed with ionic liquids. Miscanthus solubility in DESs are of the same magnitude than those obtained in ILs. Indeed, the miscanthus solubility in ILs were found to be between 0.5 and 5 weight percent (Hassan et al., 2015; Padmanabhan et al., 2011). The biomass solubility in DESs strongly depends on the temperature and the time. Nevertheless, a compromise must be found since high temperatures degrade the cellulose and the hemicellulose. The impact of these parameters on the miscanthus solubility (<0.08 mm particle size) in DESs {CHCl/Glycerol} and {CHCl/Ethylene Glycol} is represented in Fig. 2. The rate of dissolution of miscanthus in both DESs is quite similar. The maximum solubility of miscanthus in DESs is reached after 18 hrs. At 373K, similar solubility is observed in both DESs but {CHCl/Glycerol} appears to be more efficient at 403K.

3.2. Efficiency of the DES pretreatment of miscanthus

The DES pretreatment of miscanthus was performed during 12 hrs at temperature 383 K. The cellulose was recovered using water. Table 3 presents results obtained with two DESs on the extraction process. The composition of the cellulose-rich extract given in Table 3 shows that

{CHCl/Glycerol} is more efficient than {CHCl/Ethylene Glycol} in the cellulose extraction efficiency. This agrees well with the miscanthus solubility experiments. Fig. 3 presents the impact of the temperature on the performance parameters of the DES pretreatment using {CHCl/Glycerol}. As expected, the kinetic of miscanthus dissolution increases with an increase of the pretreatment temperature. However, cellulose recovery, cellulose grade, cellulose regeneration rate first increase until they reach a maximum at about 380 K and then decrease due to the cellulose degradation (Wang et al., 2011). Fig. 4 presents the evolution of the performance parameters during the miscanthus pretreatment with {CHCl/Glycerol}. During the first six hours, the four performance parameters strongly increase. The cellulose grade increases the first 6 hours and then remains constant. After 12 hours, the dissolution rate continues to increase while the cellulose recovery and regeneration rate slightly decrease due to the beginning of the cellulose degradation. As a comparison, results may be compared with those obtained with IL pretreatment for miscanthus using the same experimental protocol (Hassan et al., 2015). The cellulose recovery with DES pretreatments is quite smaller than those with the most efficient ILs, 1.3 dimethylimidazolium methylphosphonate or 1-butyl-3-methylimidazolium chloride but the recovery of lignin and hemicellulose is similar.

Fig. 5 presents the mass balance of both DES pretreatments of 100g of miscanthus sample after the three main operations: DES pretreatment, enzymatic hydrolysis and the yeast fermentation. The {CHCl/glycerol} pretreatment allows to obtain a mixture richer in cellulose than the {CHCl/ethylene glycol} pretreatment and the content of hemicellulose and lignin is quite similar.

3.3. Cellulose-rich extract characterization

Untreated miscanthus as well as cellulose rich fractions obtained from DES pretreatment have been studied by X-ray diffraction and FTIR (see Supplementary Material). In the case of untreated miscanthus and cellulose rich fraction obtained from {CHCl/ ethylene glycol}, the two important peaks observed at 15° and 22° 2θ are attributed to the amorphous and crystalline cellulose. The {CHCl/ ethylene glycol} pretreatment seems to not affect the cellulose crystallinity degree. The spectrum of the cellulose-rich fraction obtained from {CHCl/glycerol} pretreatment indicates the presence of amorphous cellulose (Ciolacu et al., 2011; Mansikkamäki et al., 2005). Indeed, the intensities of the diffraction peaks for cellulose-rich fractions are smaller than those of the untreated miscanthus. This could be due to the fact that {CHCl/glycerol} has a stronger ability to break hydrogen bonding in the cellulose-rich fraction than {CHCl/ ethylene glycol}.

FTIR spectra of untreated miscanthus as well as cellulose rich fractions present characteristic peaks (see Supplementary Material). The broad band in the region of 3600-3000 cm^{-1} corresponds to intra and intermolecular hydrogen bonding in cellulose (Ciolacu et al., 2011). This shift of the band observed after pretreatment is mainly due the hydrogen bond breaking in cellulose to form new interaction with DES. Peaks with lower intensity are found with amorphous cellulose obtained from miscanthus pretreatment.

The shift to higher wavenumbers and the strong decrease of intensity of the peak at 2900 cm^{-1} are some characteristics of the presence of amorphous cellulose (Ciolacu et al., 2011).

For both DES pretreatments, a reduction of the intensity peaks is observed in the region 1500-899 cm^{-1}. Also, the peak at 1430 cm^{-1}, crystallinity band, was decreased in the cellulose rich fraction using {CHCl/glycerol}. The amorphous character of the cellulose rich extract can be evaluated through the peak at 898 cm^{-1}. Compared to untreated miscanthus, the peak intensity increases with {CHCl/glycerol} pretreatment while it decreases with {CHCl/ ethylene glycol} (Ciolacu et al., 2011).

3.4. Bioethanol production

The cellulose rich fraction produced from the pretreatment of miscanthus with DES was subjected to a hydrolysis and fermentation processes in order to produce bioethanol. Hydrolysis is achieved via the use of cellulose enzymes. The carbohydrate chains were broken down to simple sugars which were fermented using S. cerevisaie yeast to produce ethanol in a dilute form. Pure ethanol might be obtained via further distillation and separation techniques.

The structure of the cellulose obtained from DES pretreatment is a key parameter in hydrolysis. Indeed, crystalline cellulose tends to limit the rate of hydrolysis. In this study, cellulose rich extract obtained from {CHCl/glycerol} presents more important amorphous content than the extract produced with {CHCl/ethylene glycol}. The efficiency of the hydrolysis also depends on the lignin and hemicellulose contents in the cellulose rich fraction. Indeed, the lignin inhibits the enzymatic hydrolysis. The lignin and hemicellulose recoveries from {CHCl/glycerol} pretreatments are 17.8% and 25.9 % and from {CHCl/ethylene glycol} pretreatment 16.7% and 25 %. In this work, the crystallinity of the cellulose is the parameter governing the hydrolysis efficiency. Fig. 6 displayed the rate of hydrolysis for the original miscanthus as well as the cellulose rich extracts produced with DESs. As expected, the hydrolysis of untreated miscanthus is not effective due to high lignin content. Table 4 summarizes the hydrolysis and the fermentation products obtained from untreated miscanthus and cellulose rich fractions. The hydrolysis rate for the cellulose samples was decreased fast. Glucose hydrolysis efficiency of the cellulose rich fraction reached up to 96% with {CHCl/glycerol} mixture.

The untreated miscanthus showed poor glucose hydrolysis efficiency of 11.77%. The hydrolysis efficiency of cellulose rich fractions obtained from the treatment with {CHCl/glycerol} (96%) is higher than when they are treated with {CHCl/ethylene glycol}

(89%). The enzymatic hydrolysis of the cellulose rich extracts obtained from the pretreatment of 100g of miscanthus using {CHCl/glycerol} or {CHCl/ethylene glycol} lead to a glucose production of 27.55 g and 22.19 g, respectively (see Fig. 5). The hydrolysed hemicellulose leads to the presence of small amount of xylose.

The efficiency of the fermentation process can be evaluated through the calculation of the ethanol conversion rate (ECR) and ethanol production efficiency (EPE) using equations (7) and (8):

$$ECR\ (\%) = \left(\frac{C_e}{C_c(0)\times 0.511}\right) \times 100 \quad (7)$$

$$EPE = \frac{mass_{ethanol\ produced}(g)}{0.511\times\ (1.111\times \text{cellulose content (g)} + 1.1363 \times \text{hemicellulose content (g)})} \quad (8)$$

In equation (7), C_e and Cc(0) correspond to the concentration of ethanol in the fermentation liquid and to the concentration of sugar, glucose and xylose loaded into the system at the beginning of the fermentation.

The conversion rate values are given in table 4. A high conversion rate value was achieved, with the hydrolysates which belonged to cellulose fraction using DES, with a maximum conversion value of 85.37 %.

Ethanol production efficiency of hydrolysates from untreated miscanthus and cellulose rich fraction using {CHCl/glycerol} and {CHCl/ethylene glycol} are shown in table 4. Results are compared with those obtained from miscanthus pretreatment using ILs (Hassan et al., 2015). The most striking result is that {CHCl/glycerol} is more efficient than 1.3 dimethylimidazolium methylphosphonate. The maximum ethanol production efficiency reached up to 72.29 %. These values are of good agreement when compared to published data concerning the ethanol production efficiency values, which are in the range from 65 to 75%

(Tan et al., 2013; Yue et al., 2011, 2010). The results remain acceptable with a {CHCl/ethylene glycol} pretreatment. Then, the optimal DES pretreatments lead to high bioethanol yields up to 138.4 g/ 1 kg miscanthus (Fig. 5).

4. Conclusions

The Choline chloride based DESs pretreatment developed in this study is an effective method for the miscanthus fractionation and bioethanol production. The process conducted in optimal conditions leads to an amorphous cellulose rich fraction. The glucose hydrolysis efficiency of the cellulose rich fraction using {CHCl/glycerol}and {CHCl/ethylene glycol} are 96 and 89%, respectively. After the fermentation, the solution contains between 15 % and 18 % of ethanol. Miscanthus pretreatments with {CHCl/glycerol} and {CHCl/ethylene glycol} DES displayed high efficiency and lead to high ethanol production reached up to 138.4 and 107 g/ 1kg miscanthus respectively.

E-supplementary data for this work can be found in e-version of this paper online.

References

1.Ab Rasid, N.S., Shamjuddin, A., Abdul Rahman, A.Z., Amin, N.A.S., 2021. Recent advances in green pre-treatment methods of lignocellulosic biomass for enhanced biofuel production. Journal of Cleaner Production 321, 129038.

2. Alam, A., Wang, Youmei, Liu, F., Kang, H., Tang, S., Wang, Yanting, Cai, Q., Wang, H., Peng, H., Li, Q., Zeng, Y., Tu, Y., Xia, T., Peng, L., 2020. Modeling of optimal green liquor pretreatment for enhanced biomass saccharification and delignification by distinct alteration of wall polymer features and biomass porosity in Miscanthus. Renewable Energy 159, 1128–1138.

3. An, Y.-X., Zong, M.-H., Wu, H., Li, N., 2015. Pretreatment of lignocellulosic biomass with renewable cholinium ionic liquids: Biomass fractionation, enzymatic digestion and ionic liquid reuse. Bioresource Technology 192, 165–171.

4. Bhatia, R., Lad, J.B., Bosch, M., Bryant, D.N., Leak, D., Hallett, J.P., Franco, T.T., Gallagher, J.A., 2021. Production of oligosaccharides and biofuels from Miscanthus using combinatorial steam explosion and ionic liquid pretreatment. Bioresource Technology 323, 124625.

5. Brethauer, S., Studer, M.H., 2015. Biochemical Conversion Processes of Lignocellulosic Biomass to Fuels and Chemicals – A Review. CHIMIA International Journal for Chemistry 69, 572–581.

6. Brosse, N., Dufour, A., Meng, X., Sun, Q., Ragauskas, A., 2012. Miscanthus: a fast-growing crop for biofuels and chemicals production. Biofuels, Bioproducts and Biorefining 6, 580–598.

7. Capolupo, L., Faraco, V., 2016. Green methods of lignocellulose pretreatment for biorefinery development. Appl Microbiol Biotechnol 100, 9451–9467.

8. Chen, Z., Wan, C., 2018. Ultrafast fractionation of lignocellulosic biomass by microwave-assisted deep eutectic solvent pretreatment. Bioresource Technology 250, 532–537.

9. Ciolacu, D., Ciolacu, F., Popa, V.I., 2011. Amorphous cellulose - Structure and characterization. Cellulose Chemistry and Technology 45, 13–21.

10. Cui, Z., Enjome Djocki, A.V., Yao, J., Wu, Q., Zhang, D., Nan, S., Gao, J., Li, C., 2021. COSMO-SAC-supported evaluation of natural deep eutectic solvents for the extraction of tea polyphenols and process optimization. Journal of Molecular Liquids 328, 115406.

11. Duque, A., Álvarez, C., Doménech, P., Manzanares, P., Moreno, A.D., 2021. Advanced Bioethanol Production: From Novel Raw Materials to Integrated Biorefineries. Processes 9, 206.

12. Guo, Z., Zhang, Q., You, T., Ji, Z., Zhang, X., Qin, Y., Xu, F., 2019. Heteropoly acids enhanced neutral deep eutectic solvent pretreatment for enzymatic hydrolysis and ethanol fermentation of Miscanthus x giganteus under mild conditions. Bioresource Technology 293, 122036.

13. Haldar, D., Purkait, M.K., 2021. A review on the environment-friendly emerging techniques for pretreatment of lignocellulosic biomass: Mechanistic insight and advancements. Chemosphere 264, 128523.

14. Haldar, D., Sen, D., Gayen, K., 2016. A review on the production of fermentable sugars from lignocellulosic biomass through conventional and enzymatic route—a comparison. International Journal of Green Energy 13, 1232–1253.

15. Harmsen, P.F.H., Huijgen, W., Bermudez, L., Bakker, R., 2010. Literature review of physical and chemical pretreatment processes for lignocellulosic biomass (No. 1184). Wageningen UR - Food & Biobased Research, Wageningen.

16. Hassan, E.-S.R.E., Mutelet, F., Moïse, J.-C., 2013a. From the dissolution to the extraction of carbohydrates using ionic liquids. RSC Adv. 3, 20219.

17. Hassan, E.-S.R.E., Mutelet, F., Moïse, J.-C., Brosse, N., 2015. Pretreatment of miscanthus using 1,3-dimethyl-imidazolium methyl phosphonate (DMIMMPh) ionic liquid for glucose recovery and ethanol production. RSC Advances 5, 61455–61464.

18. Hassan, E.-S.R.E., Mutelet, F., Pontvianne, S., Moïse, J.-C., 2013b. Studies on the Dissolution of Glucose in Ionic Liquids and Extraction Using the Antisolvent Method. Environ. Sci. Technol. 47, 2809–2816.

19. Howard, R.L., Abotsi, E., Jansen, van R.E.L., Howard, S., 2003. Lignocellulose biotechnology: issues of bioconversion and enzyme production. Afr. J. Biotechnol. 2, 602–619.

20. Kohli, K., Katuwal, S., Biswas, A., Sharma, B.K., 2020. Effective delignification of lignocellulosic biomass by microwave assisted deep eutectic solvents. Bioresource Technology 303, 122897.

21. Kumar, A.K., Parikh, B.S., Pravakar, M., 2016. Natural deep eutectic solvent mediated pretreatment of rice straw: bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue. Environ Sci Pollut Res 23, 9265–9275.

22. Ma, Y., Wang, Q., Zhu, T., 2019. Comparison of hydrophilic and hydrophobic deep eutectic solvents for pretreatment determination of sulfonamides from aqueous environments. Anal. Methods 11, 5901–5909.

23. Mansikkamäki, P., Lahtinen, M., Rissanen, K., 2005. Structural Changes of Cellulose Crystallites Induced by Mercerisation in Different Solvent Systems; Determined by Powder X-ray Diffraction Method. Cellulose 12, 233–242.

24. Okuofu, S.I., Gerrano, A.S., Singh, S., Pillai, S., 2020. Deep eutectic solvent pretreatment of Bambara groundnut haulm for enhanced saccharification and bioethanol production. Biomass Conv. Bioref.

25. Padmanabhan, S., Kim, M., Blanch, H.W., Prausnitz, J.M., 2011. Solubility and rate of dissolution for Miscanthus in hydrophilic ionic liquids. Fluid Phase Equilibria 309, 89–96.

26. Pan, M., Zhao, G., Ding, C., Wu, B., Lian, Z., Lian, H., 2017. Physicochemical transformation of rice straw after pretreatment with a deep eutectic solvent of choline chloride/urea. Carbohydrate Polymers 176, 307–314.

27. Passos, H., Freire, M.G., Coutinho, J.A.P., 2014. Ionic liquid solutions as extractive solvents for value-added compounds from biomass. Green Chemistry 16, 4786–4815.

28. Rabemanolontsoa, H., Saka, S., 2016. Various pretreatments of lignocellulosics. Bioresour Technol 199, 83–91.

29. Shafie, M.H., Yusof, R., Gan, C.-Y., 2019. Synthesis of citric acid monohydrate-choline chloride based deep eutectic solvents (DES) and characterization of their physicochemical properties. Journal of Molecular Liquids 288, 111081.

30. Sluiter, A., 2008. Determination of Extractives in Biomass: Laboratory Analytical Procedure (LAP); Issue Date 7/17/2005. Technical Report 12.

31. Sun, D., Yang, Q., Wang, Yanting, Gao, H., He, M., Lin, X., Lu, J., Wang, Youmei, Kang, H., Alam, A., Tu, Y., Xia, T., Peng, L., 2020. Distinct mechanisms of enzymatic saccharification and bioethanol conversion enhancement by three surfactants under steam explosion and mild chemical pretreatments in bioenergy Miscanthus. Industrial Crops and Products 153, 112559.

32. Sun, J.X., Sun, X.F., Zhao, H., Sun, R.C., 2004. Isolation and characterization of cellulose from sugarcane bagasse. Polymer Degradation and Stability 84, 331–339.

33. Sun, N., Rahman, M., Qin, Y., Maxim, M.L., Rodríguez, H., Rogers, R.D., 2009. Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. Green Chem. 11, 646–655.

34. Tan, H.T., Lee, K.T., 2012. Understanding the impact of ionic liquid pretreatment on biomass and enzymatic hydrolysis. Chemical Engineering Journal 183, 448–458.

35. Tan, L., Tang, Y.-Q., Nishimura, H., Takei, S., Morimura, S., Kida, K., 2013. Efficient production of bioethanol from corn stover by pretreatment with a combination of sulfuric acid and sodium hydroxide. Prep Biochem Biotechnol 43, 682–695.

36. Teramoto, Y., Lee, S.-H., Endo, T., 2009. Cost reduction and feedstock diversity for sulfuric acid-free ethanol cooking of lignocellulosic biomass as a pretreatment to enzymatic saccharification. Bioresour Technol 100, 4783–4789.

37. Usmani, Z., Sharma, M., Gupta, P., Karpichev, Y., Gathergood, N., Bhat, R., Gupta, V.K., 2020. Ionic liquid based pretreatment of lignocellulosic biomass for enhanced bioconversion. Bioresource Technology 304, 123003.

38. Vieira, S., Barros, M.V., Sydney, A.C.N., Piekarski, C.M., de Francisco, A.C., Vandenberghe, L.P. de S., Sydney, E.B., 2020. Sustainability of sugarcane lignocellulosic biomass pretreatment for the production of bioethanol. Bioresource Technology 299, 122635.

39. Wang, W., Lee, D.-J., 2021. Lignocellulosic biomass pretreatment by deep eutectic solvents on lignin extraction and saccharification enhancement: A review. Bioresource Technology 339, 125587.

40. Wang, X., Li, H., Cao, Y., Tang, Q., 2011. Cellulose extraction from wood chip in an ionic liquid 1-allyl-3-methylimidazolium chloride (AmimCl). Bioresource Technology 102, 7959–7965.

41. Wu, L., Chen, Z., Li, S., Wang, L., Zhang, J., 2021. Eco-friendly and high-efficient extraction of natural antioxidants from Polygonum aviculare leaves using tailor-made deep eutectic solvents as extractants. Separation and Purification Technology 262, 118339.

42. Xu, L., Zhang, J., Zong, Q.-J., Wang, L., Xu, T., Gong, J., Liu, Z.-H., Li, B.-Z., Yuan, Y.-J., 2022. High-solid ethylenediamine pretreatment to fractionate new lignin streams from lignocellulosic biomass. Chemical Engineering Journal 427.

43. Xu, N., Zhang, W., Ren, S., Liu, F., Zhao, C., Liao, H., Xu, Z., Huang, J., Li, Q., Tu, Y., Yu, B., Wang, Y., Jiang, J., Qin, J., Peng, L., 2012. Hemicelluloses negatively affect lignocellulose crystallinity for high biomass digestibility under NaOH and H 2SO 4 pretreatments in Miscanthus. Biotechnology for Biofuels 5.

44. Yue, Z., Teater, C., Liu, Y., Maclellan, J., Liao, W., 2010. A sustainable pathway of cellulosic ethanol production integrating anaerobic digestion with biorefining. Biotechnol Bioeng 105, 1031–1039.

45. Yue, Z., Teater, C., MacLellan, J., Liu, Y., Liao, W., 2011. Development of a new bioethanol feedstock – Anaerobically digested fiber from confined dairy operations using different digestion configurations. Biomass and Bioenergy 35, 1946–1953.

46. Zhang, W., Yi, Z., Huang, J., Li, F., Hao, B., Li, M., Hong, S., Lv, Y., Sun, W., Ragauskas, A., Hu, F., Peng, J., Peng, L., 2013. Three lignocellulose features that distinctively affect biomass enzymatic digestibility under NaOH and H2SO4 pretreatments in Miscanthus. Bioresource Technology 130, 30–37.

47. Zhou, X., Huang, T., Liu, J., Gao, H., Bian, H., Wang, R., Huang, C., Sha, J., Dai, H., 2021. Recyclable deep eutectic solvent coupling sodium hydroxide post-treatment for boosting woody/herbaceous biomass conversion at mild condition. Bioresource Technology 320, 124327.

48. Zubeltzu, J., Formoso, E., Rezabal, E., 2020. Lignin solvation by ionic liquids: The role of cation. Journal of Molecular Liquids 303, 112588.

Table 1. Composition of DESs studied in this work.

Hydrogen bond acceptor	Hydrogen bond donor	Molar ratio
Choline Chloride	Urea	[1:2]
Choline Chloride	Glycerol	[1:2]
Choline Chloride	Ethylene Glycol	[1:2]

Table 2. Dissolution of Miscanthus in DESs.

Solvent	Solubility (wt%)	Temperature (K)
CHCl:glycerol	0.72	363.15
	1.07	373.15
	1.54	383.15
	2.45	393.15
	3.05	403.15
CHCl: ethylene glycol	0.85	363.15
	1.05	373.15
	1.32	383.15
	2.04	393.15
	2.56	403.15
CHCl: urea	0.50	363.15
	0.62	373.15
	0.81	383.15
	1.00	393.15
	1.20	403.15

Table 3. Influence of the solvent used in miscanthus pretreatment.

DES	Cellulose recovery %	Cellulose grade %	Hemicellulose recovery %	Hemicellulose grade %	Lignin recovery %	Lignin grade %	Others (ash & unknown products) %
ChCl/glycerol	62.2	67.5	25.9	16.8	17.8	12.8	2.9
ChCl/ethylene glycol	53.1	65.2	25.0	18.3	16.7	13.5	2.9

Table 4. Results of hydrolysis and fermentation processes.

Sample	Hydrolysis Products				Fermentation Products		
	Glucose		Xylose		Ethanol		
	Hydrolysis efficiency %	Glucose (g/l)	Hydrolysis efficiency %	Xylose (g/l)	Conversion rate (%)	Ethanol (g/l)	Production effic. (%)
Untreated miscanthus	11.77	2.68	4.02	0.56	38.05	0.63	3.13
Cell. rich from ChCl/glycerol	96.19	35.88	57.51	5.45	85.37	18.03	72.29
Cell. rich from ChCl/ethylene glycol	89.05	32.2	49.77	5.17	81.38	15.54	62.50
Cell. Rich from BMIMCl	84.72	32.53	45.52	3.10	80.85	15.72	49.03
Cell. Rich from BMIMMPh	93.98	41.88	61.18	5.02	85.13	20.40	66.75

Fig. 1. The solubility of Miscanthus in deep eutectic solvents.

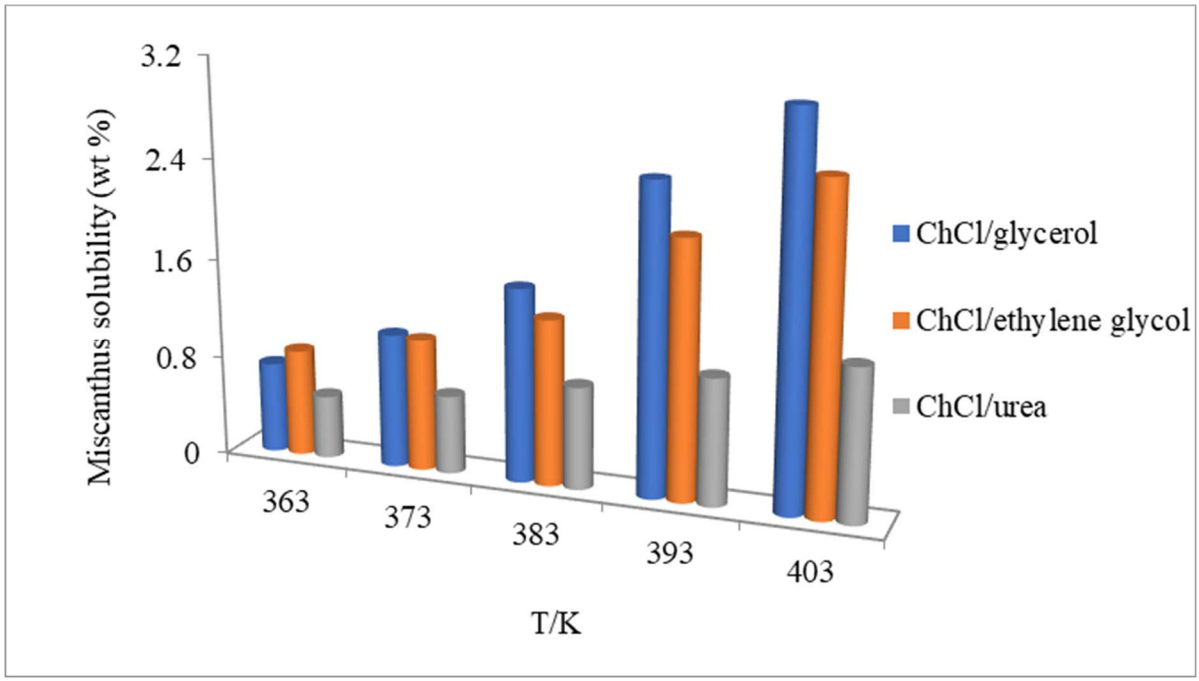


Fig. 2. Dissolution rate of miscanthus in {ChCl/glycerol} and {ChCl/ethylene glycol} at temperatures 373 and 403 K.

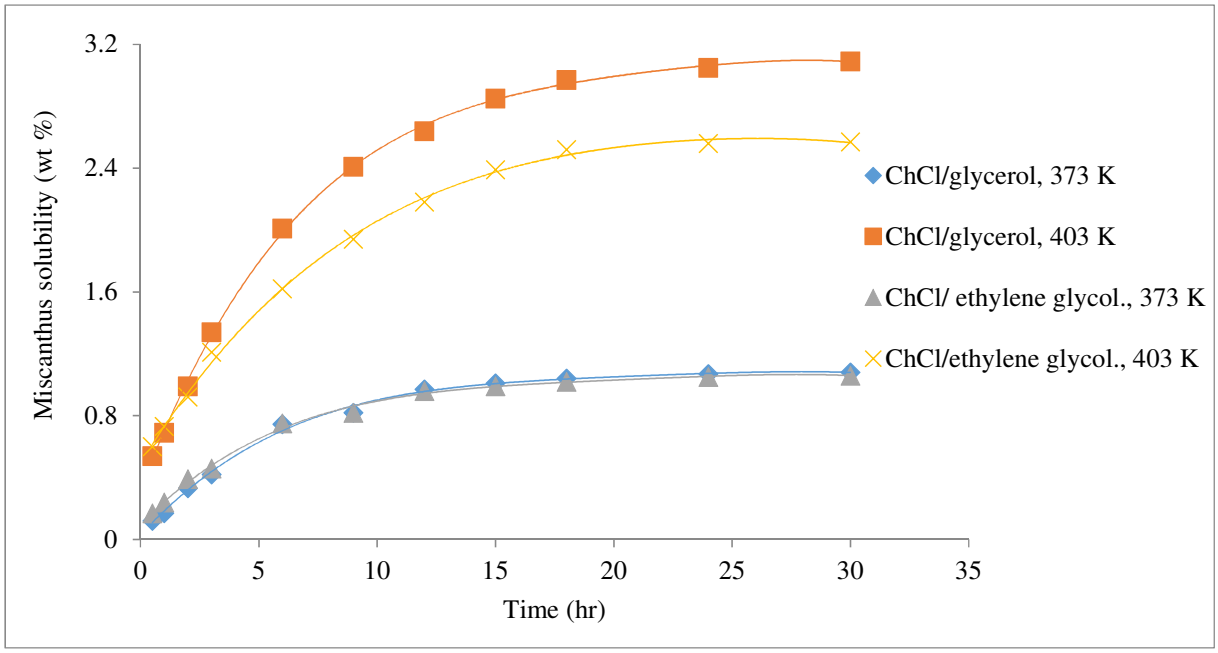


Fig. 3. Evolution of the extraction process parameters as function of the pretreatment temperature.

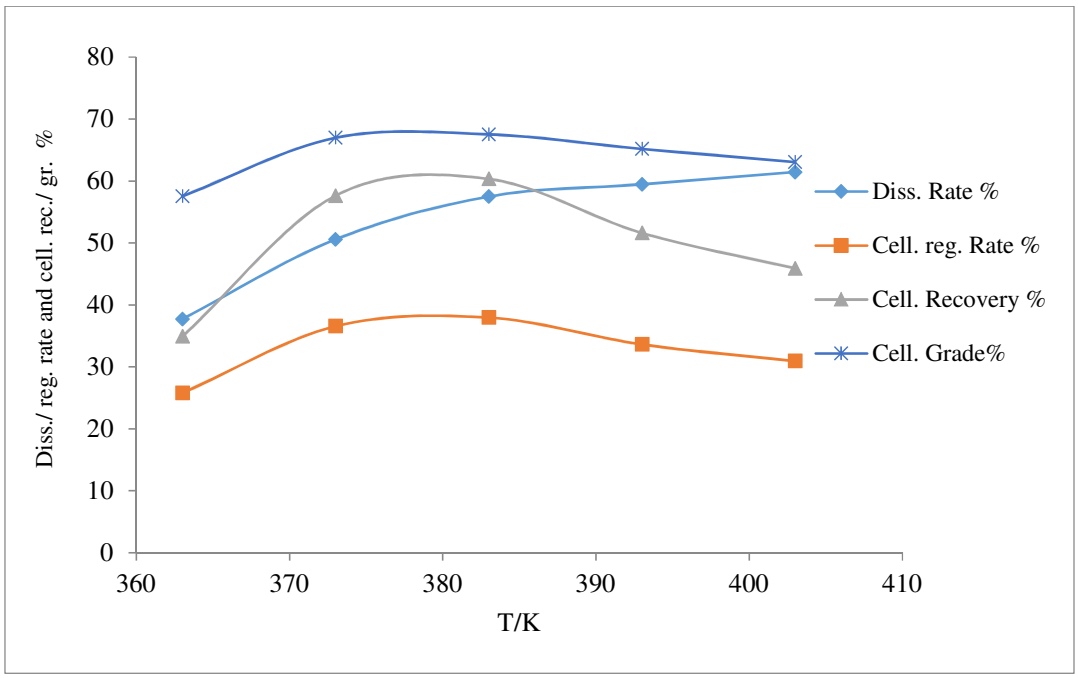


Fig. 4. Influence of pretreatment time (hr) on the extraction process parameters.

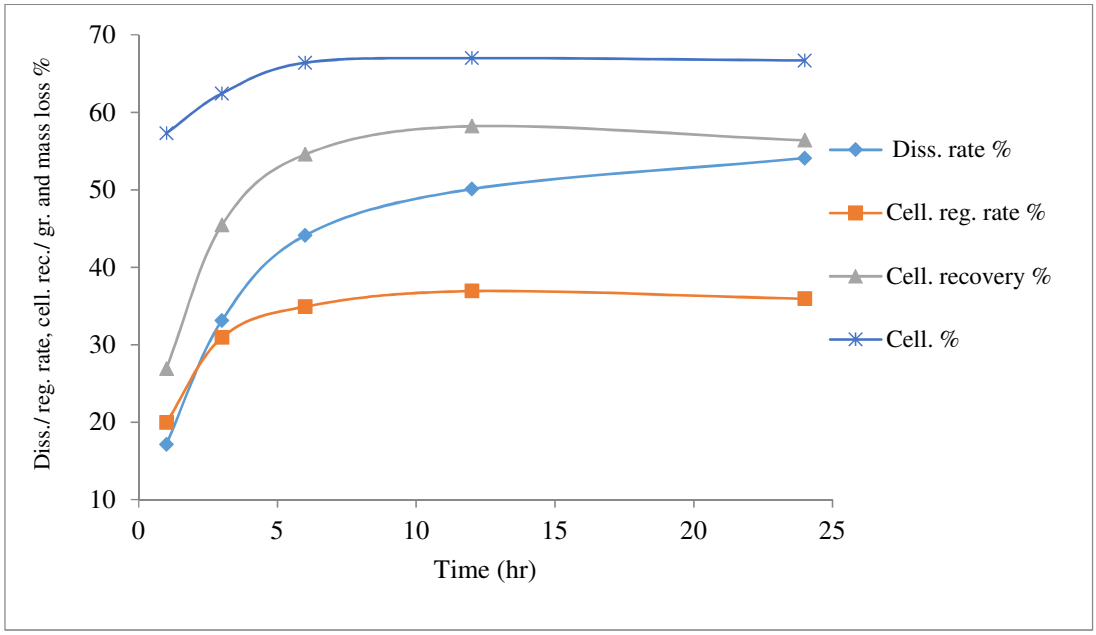


Fig. 5. Mass balance of the bioethanol production processes based on DES pretreatment.

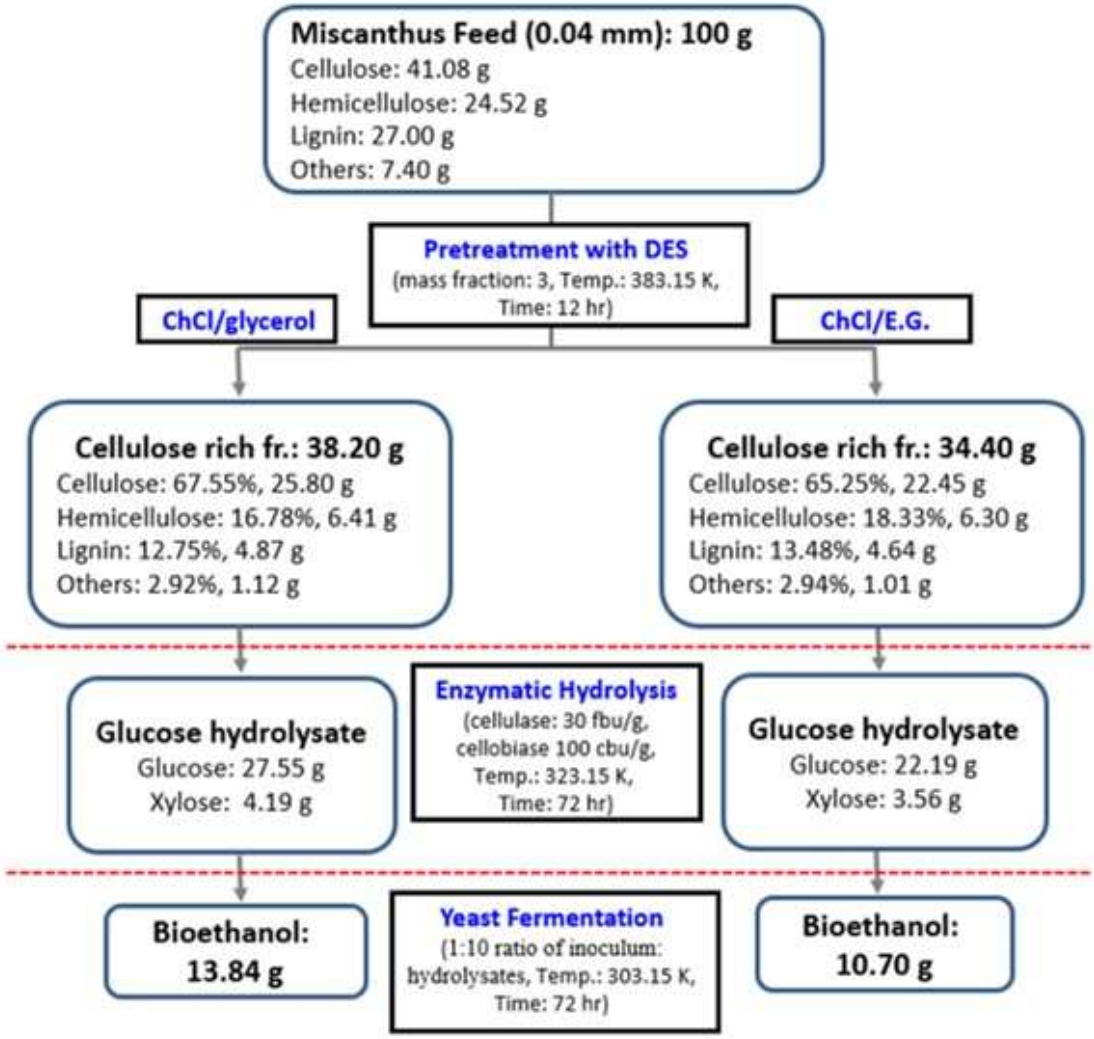
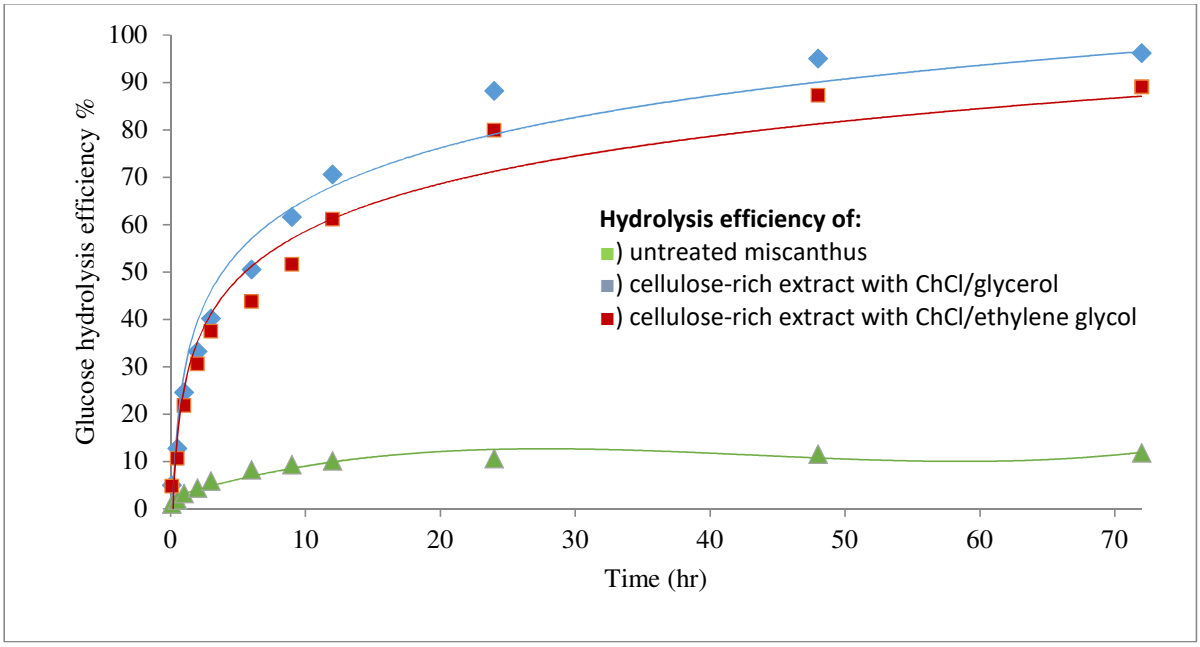
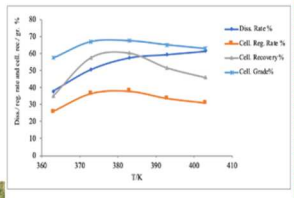
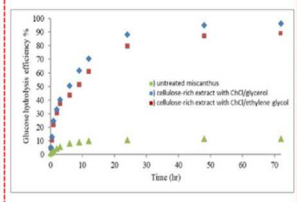


Fig. 6. Hydrolysis efficiency results.







Sample	Fermentation Products	
	Ethanol	
	Conversion rate (%)	Production effic. (%)
Untreated miscanthus	38.05	3.13
Cell. rich from ChCl/glycerol	85.37	72.29
Cell. rich from ChCl/E.G.	81.38	62.50

Original Miscanthus

Cellulose rich fraction → Glucose hydrolysate → Bioethanol → Production of 138 g Bioethanol for 1 Kg Miscanthus

Pretreatment with DES
ChCl/glycerol & ChCl/E.G.
(mass fraction: 3, Temp.: 383.15 K, Time: 12 hr)

Enzymatic Hydrolysis
(cellulase: 30 fbu/g, cellobiase 100 cbu/g , Temp.: 323.15 K, Time: 72 hr)

Yeast Fermentation
(1:10 ratio of inoculum: hydrolysates, Temp.: 303.15 K, Time: 72 hr)