energies

Article

Development of Sustainable Biorefinery Processes Applying Deep Eutectic Solvents to Agrofood Wastes

María Guadalupe Morán-Aguilar [1,2], Iván Costa-Trigo [1], Alexandra María Ramírez-Pérez [1,3], Esther de Blas [1,3], Montserrat Calderón-Santoyo [2], María Guadalupe Aguilar-Uscanga [4] and José Manuel Domínguez [1,*]

[1] Industrial Biotechnology and Environmental Engineering Group "BiotecnIA", Chemical Engineering Department, University of Vigo (Campus Ourense), 32004 Ourense, Spain; gpe.morgan@hotmail.com (M.G.M.-A.); ivan.costa.trigo@hotmail.com (I.C.-T.); alexandra@uvigo.es (A.M.R.-P.); eblas@uvigo.es (E.d.B.)
[2] Tecnológico Nacional de México/I. T. de Tepic, Integral Food Research Laboratory, Tepic C.P. 63175, Mexico; mcalderon@tepic.tecnm.mx
[3] Departamento Bioloxía Vexetal e Ciencia do Solo, Universidade de Vigo, 36310 Vigo, Spain
[4] Tecnológico Nacional de México/I. T. Veracruz, Unidad de Investigación y Desarrollo en Alimentos (UNIDA), Czda. M. A. de Quevedo Núm. 2779, Veracruz C.P. 91860, Mexico; maguilaruscanga@yahoo.com.mx
* Correspondence: jmanuel@uvigo.es; Tel.: +34-988-38-74-29

Abstract: The growing demand for renewable energies and the application of sustainable and economically viable biorefinery processes have increased the study and application of lignocellulosic biomass. However, due to lignocellulosic biomass recalcitrance hindering its efficient utilization, the pretreatment in the biorefinery is an essential stage for success in the process. Therefore, Deep Eutectic Solvent (DES) has emerged as a promising green pretreatment. During this study, the effect of choline chloride [ChCl]:glycerol and [ChCl]:urea on sugarcane bagasse and brewery bagasse is evaluated. Results have demonstrated that using [ChCl]:glycerol in SCB reduced about 80% and 15% for acid-soluble lignin and Klason lignin, respectively, and improved efficiency on saccharification yields, achieving conversions of 60, 80, and 100% for glucan, xylan, and arabinan, correspondingly. In the case of BSG saccharification yields, about 65% and 98% are attained for glucan and xylan, respectively, when [ChCl]:glycerol was employed. These results confirm the effectiveness and facility of DES pretreatment as a suitable method that can improve the biorefinery processes.

Keywords: green solvent pretreatment; lignocellulosic biomass; biorefinery process; saccharification; cellulose

Citation: Morán-Aguilar, M.G.; Costa-Trigo, I.; Ramírez-Pérez, A.M.; de Blas, E.; Calderón-Santoyo, M.; Aguilar-Uscanga, M.G.; Domínguez, J.M. Development of Sustainable Biorefinery Processes Applying Deep Eutectic Solvents to Agrofood Wastes. *Energies* **2022**, *15*, 4101. https://doi.org/10.3390/en15114101

Academic Editors: Giuliano Dragone and Solange I. Mussatto

Received: 29 April 2022
Accepted: 31 May 2022
Published: 2 June 2022

1. Introduction

Recently, a great interest in renewable sources has grown, due to the exhaustion of fossil energies and environmental problems that interfere in sustainable development [1]. Lignocellulose is considered a renewable and inexpensive resource with the ability to produce various chemical products with high added value [1]. The agroindustry, agriculture and forestry activities as well as food residues provide an annual production near 170 billion tons of biomass [2].

Lignocellulosic biomass is mainly constituted by cellulose (30–50%), hemicellulose (20–30%), and lignin (15–30%) and also pectin, proteins, ash, salts, and minerals in small amounts [2,3]. However, the amount of each compound differs depending on the type of species, material, age, and environment for growth and development [4,5].

Brewery spent grain (BSG) and sugar cane bagasse (SCB) are two outstanding examples of lignocellulosic biomass. BSG represents ~85% of all residues obtained in the brewing industry [6] with a global production of around 39 million tons per year [7]. At the same time, the annual sugarcane production is about 1.6 billion tons, being therefore the main by-product of the sugar cane industry [8]. Both BSG and SCB have a high added value, being available for conversion to other valuable products, e.g., fermentable sugars, which are the carbon source for the growth of microorganisms and enzymes production [9–12].

Nevertheless, the compact structures owing to the co-existence of cellulose, hemicellulose and lignin, via hydrogen and covalent bonds, provide resistance toward microbial and enzymatic degradation [13], preventing their easy conversion into valuable products [14]. In this regard, for a satisfactory conversion of lignocellulosic biomass into bioproducts, a pretreatment including micro and macroscopic modifications in the structure and changes in its composition is required [4].

The processes of pretreatment could be classified into physical, chemical, physicochemical, and biological, although many variations include two or more method combinations [13]. The different pretreatments applied to overcome the biomass recalcitrance are shown in Table 1. In general, the efficacy of the pretreatment is focused on a high deconstruction of biomass structure. However, the high energy consumption, aggressive chemical requirements and high operational conditions elevate environmental costs, forcing the search for new alternatives to fractionate lignocellulosic biomass [2].

Table 1. Pretreatments applied in the biomass fractionation.

Conventional Type	Pretreatment Methods	Advantages	Disadvantages
Biological	- Microorganisms (mainly fungi and bacteria)	- Low-cost - Environmentally friendly - Lesser energy requirements	- Poor success rate
Physical	- Milling and refining - Irradiation - Sonication - Mechanical - Hydrothermal - Ultrasonic	- Easy operation - Rupture the biomass structure - Combined with other processes improves hydrolysis	- High energy demand - Inefficiency in product recovery
Chemical	- Acidic pretreatment (generally with dilute HCl and H_2SO_4) - Alkali pretreatment (with solution such as NaOH, KOH or $Ca(OH)_2$) - Organosolv - Ionic Liquids	- Fast - Effective	- High energy consumption - Chemical requirements - High cost - Environmental problems - Equipment corrosion problems - Formation of toxic compounds
Physicochemical	- Microwave - Steam explosion - Liquid hot water	- Increased efficiency	

Recently, a new biomass pretreatment technique based on the use of "green solvent", as a deep eutectic solvent (DES), has received researchers' interest as an emerging technology [15]. DES is considered a lesser or non-toxic pretreatment that is biodegradable, recyclable, energy efficient, cost-effective, and available on a large scale [16]. Furthermore, DES can be obtained from natural or easily accessible compounds in a simple process (heating and mixing), making them a great potential green chemistry perspective [17].

Formerly, DESs were employed in metal processing, catalysis, extractions, analytics, electrochemistry, and as synthesis media or template [18,19]. Nevertheless, research on processing lignocellulosic biomass with DES has attracted interest focused on sustainable processes and circular biorefineries [20].

DES is composed by a hydrogen bond acceptor (HBA), generally a quaternary ammonium cation as choline chloride (ChCl), and a hydrogen bond donor (HBD), such as alcohols, amides, polysaccharides, carboxylic acids, and polyols [17]. HBA and HBD must be joined in a specific molar ratio due to the fact that the efficiency to remove lignin and to recover polysaccharides could be influenced by the ratio HBA/HBD [21].

The mechanisms of delignification in DES systems are mightily correlated with the ability of electronegative halogen anion Cl^- from ChCl to form hydrogen-bonds with hydroxy groups in lignin, leading to dissolution of aromatic compounds [22]. In the case of cellulose dissolution, DES breaks down the initial intermolecular hydrogen bonds in cellulose (β-(1,4)-glycosidic) and generates new hydrogen bonds between DES and hydroxy groups in polysaccharide [23,24]. The inter or intramolecular hydrogen bonds in cellulose can be debilitated by hydrogen bond basicity from DES, which demonstrates the ability of a solvent to be a hydrogen bond acceptor. In addition, as claimed by [25], anions with the ability of hydrogen bond accepting as Cl^-, OAc^-, $HCOO^-$, $(MeO)_2PO_2{}^-$, morpholine, or imidazole, are outstanding candidates to form DESs for cellulose dissolution.

Nevertheless, one of the factors that generate a restriction on the efficiency of DES pretreatment in biomass is its high viscosity, due to it reducing the mass transfer during the pretreatment process. Consequently, higher temperatures could be required to reduce the viscosity and enhance the transference of DES into the lignocellulosic matrix for improving delignification and hemicellulose hydrolysis [26,27].

However, severe operating conditions, such as long reaction times and extreme operating temperatures, might lead to thermal degradation of polysaccharides in furfural (a fermentation inhibitor highly undesirable in the downstream process) [28,29]. An alternative non-toxic, low-cost, and easily available solvent as compared to many chemicals is the addition of distilled water to reduce the viscosity in DES systems and enhance mass transfer while maintaining optimal operating conditions during biomass pretreatment [4,27,30].

One of the important aspects to highlight in the application of DES technology is its recyclability and reuse, because this can minimize the discharge of flows and reduce the costs of the process to a great extent, which determines the viability of the pretreatment process [31].

Chen et al. [32] reported the efficiency of switchgrass pretreatment using ChCl:glycerol up to three recycling cycles. During this study, the decrease in the yield of lignin removal and hemicellulose hydrolysis was noticed (64.50–15.27% and 53.10–25.66%, respectively). They also observed that the saccharification of the cellulose decreases from the first cycle 77.51% to 32.6% in the third recycling. This is due to impurities induced by hemicellulose and lignin degraded compounds that interfere with the DES pretreatment. For this reason, the ultrafiltration membrane has been proposed as an optimal purification and recovery mechanism for DES [33].

Shen et al. [34] fractionated *Eucalyptus* using the [ChCl]:LA eutectic mixture, reporting that up to 90% DES could be recovered and recycled. DES could maintain satisfactory pretreatment performance with saccharification from 94.3% to 73.8% after four cycles, suggesting that DES exhibited excellent recyclability in biomass pretreatment. Therefore, DES pretreatment can be considered as a low cost and recycled biorefinery process.

This work aims to study the effect of green technologies based on the use of two DESs ([ChCl]:urea and [ChCl]:glycerol) to improve the deconstruction of SCB and BSG through lignin extraction and further increment in the saccharification yield.

2. Materials and Methods

2.1. Lignocellulosic Biomass

Sugarcane bagasse (SCB) was supplied by the National Institute of Forestry Agricultural and Livestock Research (INIFAP) from Veracruz, Mexico. Brewery spent grain (BSG) was obtainable by Letra (Vila Verde, Braga, Portugal).

Lignocellulosic biomass was dried at room temperature (25–30 °C). After, it was crushed with a stainless-steel mill (SOGO, SS-111 5430 models, Sanysan Appliances SL, Valencia, Spain) and stored in propylene bags at 25 °C until further use.

2.2. Chemicals

Choline chloride (ChCl) was obtained from Alfa Aesar with a purity of >98%. It was kept in a desiccator before use to avoid moisture adsorption. The glycerol with laboratory reagent grade and urea (>99.5% of purity) were provided from Fisher Scientific (Hampton, NH, USA). All the chemical reagents used during this study were of analytical quality.

2.3. Synthesis of Deep Eutectic Solvent (DES)

The development of the DES [ChCl]:urea and [ChCl]:glycerol preparation were carried out as described by Xu et al. [2] with minor modifications. DES solution was prepared using the quaternary ammonium salt [ChCl] in a mixture with 70% (*w/v*) and adding a hydrogen bond donor, urea and glycerol with a molar ratio 1:2 (mol/mol) for each DES solution. The mixture was stirred for 30 min at 50 °C and 80 °C for [ChCl]:urea and [ChCl]:glycerol, respectively, until a colorless liquid was formed. Finally, the solutions were stored at room temperature (25 °C) until use.

2.4. DES Pretreatment

The effect of two eutectic mixtures [ChCl]:urea and [ChCl]:glycerol was evaluated, using the methodology described by Procentese et al. [35] with a solid–liquid ratio (SLR) 1:16 (*w/w*) at a reaction time of 15 h in a sand bath with magnetic stirring. The pretreatment with [ChCl]:urea was not carried out up to 140 °C, owing to the excessive increase in temperature breaking the terminal stability of [ChCl]:urea [27]. Whereas due to the viscosity presented by [ChCl]:glycerol, a reaction temperature of 160 °C was used.

Once the reaction finished, 50 mL of distilled water at room temperature (23 °C) was added to each sample to stop the reaction. Then, the sample was centrifuged (Ortoalresa, Consul 21, EBA 20, Hettich Zentrifugen, Germany) at 2755× *g* for 30 min. The supernatant was separated by decantation, and the sedimented biomass CRM (delignified carbohydrate-rich material) was dried for 24 h at 50 °C in an oven (Celsius 2007, Memmert, Schwabach, Germany) until its lignocellulosic analysis.

2.5. Lignocellulosic Analysis

The characterization of polysaccharides (glucan, xylan and arabinan) for untreated and pretreated biomass (SCB and BSG) was determined by quantitative acid hydrolysis in two stages according to the NREL/TP-510-42618 methodology described by Sluiter et al. [36]. The liquid fraction obtained for each sample was used for the analysis of structural sugars through HPLC system (Agilent model 1200, Palo Alto, CA, USA). A refractive index detector and an Aminex HPX-87H ion exclusion column (Bio Rad 147,300 × 7.8 mm, 9 μ particles) with guard column were used. The sample was eluted with 0.3 g/L of H_2SO_4 at 0.6 mL/min and 50 °C. Concentrations were quantified using calibration curves of glucose, xylose, and arabinose (g/L).

The acid-soluble lignin (ASL) quantification was carried out employing the liquid fraction of quantitative acid hydrolysis. Each sample was diluted with 4% (*w/w*) H_2SO_4 and measured at 205 nm in a UV–Vis Spectrophotometer (Libra S60-Biochrom, Cambridge, UK). The solid residue obtained after hydrolysis was oven-dried at 105 °C and considered as Klason lignin (KL) [36]. The percentage of lignin removed was calculated (Equation (1)) [37,38]:

$$\text{Lignin removal (\%)} = \left[1 - \frac{\text{Total lignin in pretreated biomass}}{\text{Total lignin in untreated biomass}} \times \text{S}\right] \times 100. \tag{1}$$

where S is the solid recovered (g) after pretreatment.

2.6. Enzymatic Saccharification

Nowadays, the transformation of lignocellulosic biomass into simple sugars (mainly glucose and xylose) by enzymatic hydrolysis is one of the green technologies used in the biorefinery concept. Therefore, during this study, the analysis of the effect of DES pretreatment in the deconstruction of SCB and BSG structures to improve the release of reducing sugars was carried out through enzymatic hydrolysis. The enzyme load used was cellulase-substrate ratio 28 FPU g^{-1} of Celluclast 1.5 L and cellobiase-cellulase ratio 13 (IU FPU^{-1}) of Novozymes, according with the methodology described by Bustos et al. [39]. Sodium citrate buffer at pH 4.8 (30 mL) was added per gram of CRM and stirred at 150 rpm and 50 °C for 72 h, using destructive flasks in triplicate. Once the reaction time was over, the enzymes were denatured in a water bath at 100 °C for 5 min. The sugar aliquots were analyzed by HPLC to calculate the glucose and xylose yields as follows:

$$\text{Glucose yield } (\%) = \left[\frac{\text{Glucose in enzymatic hydrolyzate (g)}}{\text{Initial glucan in substrate (g)} \times 1.11}\right] \times 100 \tag{2}$$

$$\text{Xylose yield } (\%) = \left[\frac{\text{Xylose in enzymatic hydrolyzate (g)}}{\text{Initial xylan in substrate (g)} \times 1.13}\right] \times 100 \tag{3}$$

2.7. Statistical Analysis

The measure of dispersion of each experiment for SCB and BSG lignocellulose composition and saccharification yield were expressed as the mean ± standard deviation of three independent experiments. Minitab 17 statistical software package (version 17.1.0) was employed.

3. Results and Discussion

3.1. Compositional Analysis

According to reported studies [10,12], SCB and BSG are predominantly composed of a glucose homopolymer, such as cellulose, heteropolymers composed of xylose, arabinose, and galactose, and to a lesser extent lignin (complex macromolecule of phenolic compounds) [8].

Table 2 shows the content of glucan, xylan, arabinan, and total lignin in the untreated SCB and BSG. The results are consistent with the extensive literature reported by various studies [10,12,37,40].

Table 2. Biomass composition before and after DES pretreatment.

			Composition (%)			**Lignin (%)**		**Lignin Remotion (%)**	
Biomass	**DES**	**Temperature**	**Glucan**	**Xylan**	**Arabinan**	**ASL**	**KL**	**ASL ***	**KL ***
SCB	Untreated	-	41.00 ± 1.53	27.80 ± 0.89	2.49 ± 1.78	3.07 ± 0.05	19.14 ± 1.23	-	-
	[ChCl]:U	140 °C	35.52 ± 2.58	31.99 ± 1.05	10.32 ± 1.69	2.80 ± 0.35	17.69 ± 1.25	2.80 ± 0.08	7.57 ± 2.21
	[ChCl]:G	160 °C	53.58 ± 5.78	19.97 ± 1.52	5.56 ± 0.78	0.75 ± 0.02	16.29 ± 2.45	75.57 ± 1.89	14.89 ± 3.65
BSG	Untreated	-	35.73 ± 0.25	17.06 ± 1.54	7.84 ± 2.54	6.37 ± 0.45	18.75 ± 1.23	-	-
	[ChCl]:U	140 °C	37.67 ± 1.47	29.53 ± 2.54	8.95 ± 0.69	2.89 ± 0.04	15.60 ± 3.21	54.63 ± 1.12	16.80 ± 3.25
	[ChCl]:G	160 °C	32.91 ± 2.85	13.94 ± 3.54	0.00 ± 0.00	1.32 ± 0.07	46.44 ± 5.35	79.27 ± 4.35	-

ChCl:U: choline chloride-urea; ChCl:G: choline chloride-glycerol; *: Based on initial composition of SCB or BSG; ASL: acid soluble lignin; KL: Klason lignin.

Table 2 also reports the results of DES pretreatment using [ChCl]:urea or [ChCl]:glycerol in both lignocellulosic materials. The effect of [ChCl]:urea at 140 °C in SCB evidenced the decrease in glucans content. According to Li et al. [41], this could be due to factors such as temperature and reaction time during the pretreatment, because temperatures above 100 °C and prolonged times promote the elimination of lignin and the conversion of a higher part of the hemicellulose (xylans and arabinans) and cellulose (glucans) into soluble products of low molecular mass, which cannot be recovered as solid material after the pretreatment.

Conversely, the DES composed of [ChCl]:glycerol increased 1.30-fold times the content of glucans after pretreatment related to the composition of the untreated SCB. However, the xylan fraction decreased, probably because of the delignification effect. In accordance with Li et al. [41], DES acts as a mild acid-base catalytic solution that tends to break the β-*O*-4 aryl ester bonds between lignin and polysaccharides, as well as ester linkages between lignin and 4-*O*-methylglucuronic acid, which is known to be randomly distributed along the xylan chains. Therefore, a majority fraction of cellulose is promoted in the pretreated material, whereas the degradation products (lignin, xylan, and arabinan) are generally dissolved in the solvent (DES). This is consistent with the results present for lignin removal using glycerol as a hydrogen bond donor, because it shows a delignification of more than 75% and ~15% for ASL and KL lignin, respectively.

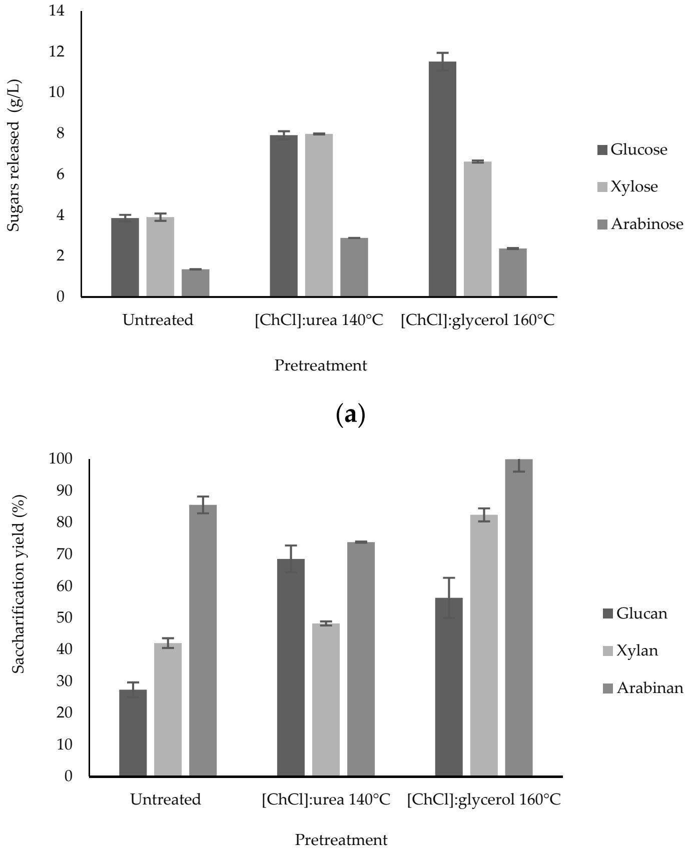
The differences observed in the composition of SCB after the pretreatment with each eutectic mixture could be related to different factors, such as the size of the alkyl chain, the viscosity, and the HBA-HBD interaction from the eutectic mixture of DES [42]. The viscosity of DES tends to increase due to the formation of hydrogen bonds between the Cl^- and the OH^+ groups from HBD, which generates a network of hydrogen bonds between the components and then increases the attractive force of the molecules and decreases the free volume of the DES, causing a smaller interaction between the solvent and the biomass [43]. However, the viscosity decreases significantly when water is added to the system (10 to 30 times depending on the HBD) [44]. According to Du et al. [45] at 25 °C, the viscosity of [ChCl]:urea hydrated with 6% (*w/w*) water was 13 times lower than the viscosity of pure [ChCl]:urea. The size of the alkyl chain in HBD influences the DES pretreatment system. This is probably owing to the formation of additional hydrogen bonds between the carboxyl group ^-COOH due to its polar character, which induces the formation of a greater number of hydrogen bonds between the carboxylic acid molecule and [$ChCl^-$], increasing the viscosity and decreasing the DES-biomass interaction [46,47]. DES formed by monocarboxylic acid as HBD is more efficient in biomass deconstruction and delignification than DES composed by dicarboxylic acid [44,48].

The transformations obtained in the composition of the BSG after the pretreatment with [ChCl]:urea and [ChCl]:glycerol are shown in Table 2. Neither of the DESs generated changes in the content of glucans. However, the use of [ChCl]:urea at 140 °C increased the content of xylan and arabinan, whereas the use of [ChCl]:glycerol at 160 °C decreased the percentage of these polymers and also generated a higher percentage of ASL delignification (80%), which could be influenced by heightening the temperature during the process (160 °C). According to Hong et al. [49], an increment of temperature promotes a minimization of viscosity in the system and enhances the diffusion between DES and biomass, which contributed to the effective interaction, improving the fractionation and recovery of lignin and polysaccharides from biomass [50]. This agrees with Guo et al. [51] using [ChCl]:1,4-butanediol with a molar ratio of 1:2 (mol/mol), SLR 1:20 (*w/v*) during 2 h of the reaction, which reported the reduction of glucan content from 92.5% to 88.7% when the temperature was incremented from 80 to 120 °C and the delignification percentage improved from 30.7% to 54%, respectively. Likewise, Chen et al. [52] pretreated herb residues of cortex albiziae with [ChCl]:*p*-coumaric acid (2:1 mol/mol) for 7 h and studied increased temperatures in a rank of 140, 160, 180 to 200 °C, obtaining a delignification percentage of 8, 10, 50, and 84%, respectively.

The results presented factors, such as the type of HBD and the reaction temperature, which influenced the pretreatment with DES, specifically in the recovery of cellulose and in the fractionation of lignin.

3.2. Effect of Enzymatic Digestibility in SCB and BSG after DES Pretreatment

The degree of release or the total yield of reducing sugars depends on the pretreatment conditions [53]. In this way, Figure 1a shows the highest concentration of total sugars after pretreatment with [ChCl]:glycerol and [ChCl]:urea of 20.52 and 18.79 g/L, respectively, about 2.25 and 2-fold times more than that obtained with the untreated SCB (9.11 g/L). Meanwhile, Figure 1b represents the saccharification yield achieved during the enzymatic hydrolysis of SCB, reaching a maximum glucan percentage conversion of 68.57 ± 4.24 and 56.29 ± 6.34, a xylose percentage conversion of 48.26 ± 0.64 and 82.47 ± 2.05, and finally an arabinan percentage conversion of 73.87 ± 0.19 and 100 ± 3.92 for [ChCl]:urea and [ChCl]:glycerol, respectively. According to the results, the pretreatment with [ChCl]:glycerol was more effective in the saccharification of hemicellulose (xylans and arabinans) than [ChCl]:urea. Less severe pretreatment conditions using [ChCl]:urea could diminish the delignification effects (Table 2).



(**a**)

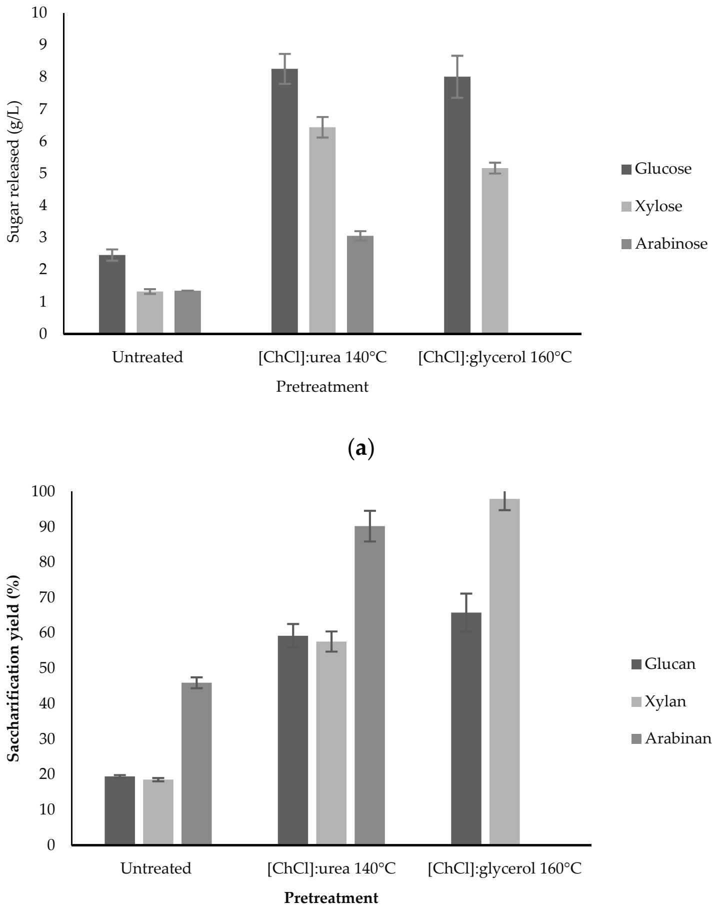
(**b**)

Figure 1. Released sugars (**a**) and saccharification yield (**b**) obtained after enzymatic hydrolysis carried out in SCB untreated and pretreated with different eutectic mixtures.

On the other hand, Figure 2a shows the release of reducing sugars from untreated and pretreated BSG with [ChCl]:urea (140 °C) and [ChCl]:glycerol (160 °C). It can be noted that the increment after the pretreatment reached a maximum concentration of 17.76 g/L and 13.18 g/L for each eutectic mixture, respectively, compared to the low value obtained using the untreated biomass (5.12 g/L).

In addition, Figure 2b represents the percentage of saccharification obtained with each DES, which achieved similar glucan conversion yields for [ChCl]:urea and [ChCl]:glycerol. However, the yield of xylan saccharification using [ChCl]:glycerol (160 °C) is close to 98%. This could be related to the maximum ASL lignin removal (80%) reported in Table 2, because it could promote a higher formation of amorphous zones in the pretreated biomass and consequently enhance the access of the enzymes to the polysaccharides [32].

The results achieved in this work were recapitulated in Table 3 along with the application of different eutectic mixtures and their efficiency after enzymatic hydrolysis. The saccharification yields vary according to the composition of the DES, the type of biomass, and the operating conditions used during the pretreatment.



(**a**)

(**b**)

Figure 2. Released sugars (**a**) and saccharification yield (**b**) obtained after enzymatic hydrolysis carried out in BSG untreated and pretreated with different eutectic mixtures.

Table 3. Saccharification yields using DES pretreatment on lignocellulosic biomass.

DES	Biomass	Operating Conditions MR (mol/mol)—T (°C)—SLR (*w*/*w*)—t (h)	Saccharification Yield (%)	References
[ChCl]:glycerol [ChCl]:urea	Corncob	1:2—150—1:16—15	91.5 glucan 95.5 xylan 58.6 glucan 31.2 xylan	[35]
[ChCl]:glycerol	Switchgrass	1:2—120—1:10—1	79.9 glucan	[32]
[ChCl]:glycerol	Brewery spent grain	1:2—150—1:32—3	94 glucan	[54]
[ChCl]:lactic acid [ChCl]:urea [ChCl]:glycerol	Oil palm empty fruit bunch	1:2—120—1:10—3	20.7 glucan and xylan mainly 20 glucan and xylan mainly 16.9 glucan and xylan mainly	[55]
[ChCl]:glycerol	Sugarcane bagasse	1:2—80—1:20—12	95.84 glucan	[37]
[ChCl]:lactic acid	Poplar sawdust	1:2—130—NR—1.5	75.8 glucan	[56]

Table 3. *Cont.*

DES	Biomass	Operating Conditions MR (mol/mol)—T (°C)—SLR (*w*/*w*)—t (h)	Saccharification Yield (%)	References
[ChCl]:glycerol	Sugarcane bagasse	1:2—160—1:16—15	56.30 glucan 82.47 xylan 100 arabinan	Present study
[ChCl]:urea		1:2—140—1:16—15	56.67 glucan 65.37 xylan 73.87 arabinan	
[ChCl]:glycerol	Brewery spent grain	1:2—160—1:16—15	65.77 glucan 97.96 xylan	
[ChCl]:urea		1:2—140—1:16—15	59.22 glucan 57.60 xylan 90.21 arabinan	

MR: molar ratio; SLR: solid liquid ratio; [ChCl]: Choline chloride, NR: not reported.

Nevertheless, low saccharification yields were exhibited using [ChCl]:urea [35,55]. This could be attributable to the feeble alkalinity (pH 9) and the elevated viscosity of the eutectic solvent, which does not allow an efficient interaction between biomass and DES [48,57].

DES with a polyalcohol, such as HBD, has a high hydrophobicity that improves the biomass fractionation and reduces the effects of corrosion responsible for the loss of polysaccharides [32]. Subsequently, employing [ChCl]:glycerol has been reported in different works, because it enhances the accessibility of enzymes during the saccharification, achieving yields between 60 and 90% [37,54].

Nonetheless, it is evidenced that the saccharification yields change according to the composition of biomass used. As mentioned by Wang and Lee [4], the xylan and lignin content are variables that hinder the saccharification of cellulose due to the crosslinking between lignin and xylan, through a strong network of covalent and hydrogen bonds with different functional groups, such as phenyl glycoside, benzyl ether, and benzyl ester [58,59].

Table 3 also indicates that temperature is one of the pretreatment variables that influence saccharification yields because it affects the degree of delignification and polysaccharide recuperation using DES pretreatment [60]. The increase in temperature reduces the viscosity of the DES system by breaking the hydrogen bonds between the components of the eutectic mixture, enhancing the diffusibility of the DES-biomass system [50].

As a result of the analysis of this work, it is important to highlight factors such as the pretreatment temperature, the reaction time, and the solid–liquid ratio, which could lead to a decrease in the saccharification yield caused by degradation of the polysaccharides during the pretreatment, the destabilization of DES, and the formation of biomass clumps that prevent immediate access to the enzymes on the substrate [61].

4. Conclusions

The evaluation of glycerol and urea as HBD in DES pretreatment demonstrated a significant effect on the physicochemical structure of biomass and saccharification yield in SCB and BSG. The participation of DES was highlighted as a promising green solvent for biomass pretreatment. In addition, the characterization of SCB and BSG after DES pretreatment demonstrated the improvement, delignification, and removal of xylan and arabinan, which led to the increase in saccharification yield because the cellulose fraction was more prone to enzymatic attack. Among the two DESs evaluated, [ChCl]:glycerol exhibited the maximum lignin extraction (~80 and 15% for ASL and KL, respectively) and increased the enzyme amenability to obtain conversion percentages of polysaccharides above 60% for both lignocellulosic residues. On the other hand, it is necessary to deepen the operating conditions, the type of eutectic mixture and the biomass used due to their importance in the further enhancement of DES pretreatment and biorefinery processes.

Author Contributions: Conceptualization, M.G.M.-A. and J.M.D.; methodology, M.G.M.-A. and I.C.-T.; formal analysis, M.G.M.-A.; investigation, M.G.M.-A.; resources, J.M.D.; writing—original draft preparation, M.G.M.-A., A.M.R.-P. and J.M.D.; writing—review and editing, M.G.M.-A., I.C.-T., A.M.R.-P., E.d.B., M.C.-S., M.G.A.-U. and J.M.D.; supervision, M.C.-S., M.G.A.-U. and J.M.D.; project administration, J.M.D.; funding acquisition, J.M.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Spanish Ministry of Science and Innovation (project PID2020-115879RB-I00) and by the Xunta de Galicia of Spain (Group with Potential for Growth GPC-ED431B 2021/23).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Xie, J.; Chen, J.; Cheng, Z.; Zhu, S.; Xu, J. Pretreatment of Pine Lignocelluloses by Recyclable Deep Eutectic Solvent for Elevated Enzymatic Saccharification and Lignin Nanoparticles Extraction. *Carbohydr. Polym.* **2021**, *269*, 118321. [CrossRef] [PubMed]
2. Xu, G.C.; Ding, J.C.; Han, R.Z.; Dong, J.J.; Ni, Y. Enhancing Cellulose Accessibility of Corn Stover by Deep Eutectic Solvent Pretreatment for Butanol Fermentation. *Bioresour. Technol.* **2016**, *203*, 364–369. [CrossRef] [PubMed]
3. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.Y.; Holtzapple, M.; Ladisch, M. Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass. *Bioresour. Technol.* **2005**, *96*, 673–686. [CrossRef]
4. Wang, W.; Lee, D.J. Lignocellulosic Biomass Pretreatment by Deep Eutectic Solvents on Lignin Extraction and Saccharification Enhancement: A Review. *Bioresour. Technol.* **2021**, *339*, 125587. [CrossRef] [PubMed]
5. Haldar, D.; Purkait, M.K. A Review on the Environment-Friendly Emerging Techniques for Pretreatment of Lignocellulosic Biomass: Mechanistic Insight and Advancements. *Chemosphere* **2021**, *264*, 128523. [CrossRef]
6. Aliyu, S.; Bala, M. Brewer's Spent Grain: A Review of Its Potentials and Applications. *Afr. J. Biotechnol.* **2011**, *10*, 324–331.
7. Mussatto, S.I. Brewer's Spent Grain: A Valuable Feedstock for Industrial Applications. *J. Sci. Food Agric.* **2014**, *94*, 1264–1275. [CrossRef]
8. Chandel, A.K.; da Silva, S.S.; Carvalho, W.; Singh, O.V. Sugarcane Bagasse and Leaves: Foreseeable Biomass of Biofuel and Bio-Products. *J. Chem. Technol. Biotechnol.* **2012**, *87*, 11–20. [CrossRef]
9. Salmon, D.N.X.; Spier, M.R.; Soccol, C.R.; de Souza Vandenberghe, L.P.; Weingartner Montibeller, V.; Bier, M.C.J.; Faraco, V. Analysis of Inducers of Xylanase and Cellulase Activities Production by *Ganoderma Applanatum* LPB MR-56. *Fungal Biol.* **2014**, *118*, 655–662. [CrossRef]
10. Outeiriño, D.; Costa-Trigo, I.; Rodríguez, A.; Pérez Guerra, N.; Domínguez, J.M. Recovery and Reuse of Ionic Liquid Cholinium Glycinate in the Treatment of Brewery Spent Grain. *Sep. Purif. Technol.* **2021**, *254*, 117651. [CrossRef]
11. Morán-Aguilar, M.G.; Santoyo, M.C.; Aguilar, J.M.D.M.G. Optimization of Cellulase and Xylanase Production by *Aspergillus niger* CECT 2700 Using Brewery Spent Grain Based on Taguchi Design. *Biomass Convers. Biorefinery* **2021**, 1–9. [CrossRef]
12. Morán-Aguilar, M.G.; Costa-Trigo, I.; Calderón-Santoyo, M.; Domínguez, J.M.; Aguilar-Uscanga, M.G. Production of Cellulases and Xylanases in Solid-State Fermentation by Different Strains of *Aspergillus niger* Using Sugarcane Bagasse and Brewery Spent Grain. *Biochem. Eng. J.* **2021**, *172*, 108060. [CrossRef]
13. Baruah, J.; Nath, B.K.; Sharma, R.; Kumar, S.; Deka, R.C.; Baruah, D.C.; Kalita, E. Recent Trends in the Pretreatment of Lignocellulosic Biomass for Value-Added Products. *Front. Energy Res.* **2018**, *6*, 1–19. [CrossRef]
14. Duarah, P.; Haldar, D.; Purkait, M.K. Technological Advancement in the Synthesis and Applications of Lignin-Based Nanoparticles Derived from Agro-Industrial Waste Residues: A Review. *Int. J. Biol. Macromol.* **2020**, *163*, 1828–1843. [CrossRef] [PubMed]
15. Lyu, H.; Zhang, J.; Zhou, J.; Shi, X.; Lv, C.; Geng, Z. A Subcritical Pretreatment Improved by Self-Produced Organic Acids to Increase Xylose Yield. *Fuel Process. Technol.* **2019**, *195*, 106148. [CrossRef]
16. Calvo-Flores, F.G.; Monteagudo-Arrebola, M.J.; Dobado, J.A.; Isac-García, J. Green and Bio-Based Solvents. *Top. Curr. Chem.* **2018**, *376*, 1–40. [CrossRef]
17. Zdanowicz, M.; Spychaj, T. Deep Eutectic Solvents for Polysaccharides Processing. A Review. *Carbohydr. Polym.* **2018**, *200*, 361–380. [CrossRef]
18. Liu, Y.; Chen, W.; Xia, Q.; Guo, B.; Wang, Q.; Liu, S.; Liu, Y.; Li, J.; Yu, H. Efficient Cleavage of Lignin–Carbohydrate Complexes and Ultrafast Extraction of Lignin Oligomers from Wood Biomass by Microwave-Assisted Treatment with Deep Eutectic Solvent. *ChemSusChem* **2017**, *10*, 1692–1700. [CrossRef]
19. Lyu, G.; Li, T.; Ji, X.; Yang, G.; Liu, Y.; Lucia, L.A.; Chen, J. Characterization of Lignin Extracted from Willow by Deep Eutectic Solvent Treatments. *Polymers* **2018**, *10*, 869. [CrossRef]

20. Wang, Y.; Kim, K.H.; Jeong, K.; Kim, N.; Yoo, C.G. Sustainable Biorefinery Processes Using Renewable Deep Eutectic Solvents. *Green Sustain. Chem.* **2021**, *27*, 100396. [CrossRef]
21. Yiin, C.L.; Quitain, A.T.; Yusup, S.; Uemura, Y.; Sasaki, M.; Kida, T. Sustainable Green Pretreatment Approach to Biomass-to-Energy Conversion Using Natural Hydro-Low-Transition-Temperature Mixtures. *Bioresour. Technol.* **2018**, *261*, 361–369. [CrossRef] [PubMed]
22. Loow, Y.-L.; Wu, T.Y.; Yang, G.H.; Ang, L.Y.; New, E.K.; Siow, L.F.; Jahim, J.M.; Mohammad, A.W.; Teoh, W.H. Deep Eutectic Solvent and Inorganic Salt Pretreatment of Lignocellulosic Biomass for Improving Xylose Recovery. *Bioresour. Technol.* **2018**, *249*, 818–825. [CrossRef] [PubMed]
23. Chen, Z.; Bai, X.; Lusi, A.; Jacoby, W.A.; Wan, C. One-Pot Selective Conversion of Lignocellulosic Biomass into Furfural and Co-Products Using Aqueous Choline Chloride/Methyl Isobutyl Ketone Biphasic Solvent System. *Bioresour. Technol.* **2019**, *289*, 121708. [CrossRef] [PubMed]
24. Morais, A.R.C.; Pinto, J.V.; Nunes, D.; Roseiro, L.B.; Oliveira, M.C.; Fortunato, E.; Bogel-Łukasik, R. Imidazole: Prospect Solvent for Lignocellulosic Biomass Fractionation and Delignification. *ACS Sustain. Chem. Eng.* **2016**, *4*, 1643–1652. [CrossRef]
25. Wang, S.; Li, H.; Xiao, L.P.; Song, G. Unraveling the Structural Transformation of Wood Lignin During Deep Eutectic Solvent Treatment. *Front. Energy Res.* **2020**, *8*, 48. [CrossRef]
26. Bubalo, M.C.; Curko, N.; Tomaševic, M.; Ganic, K.K.; Redovnikovic, I.R. Green Extraction of Grape Skin Phenolics by Using Deep Eutectic Solvents. *Food Chem.* **2016**, *200*, 159–166. [CrossRef]
27. New, E.K.; Wu, T.Y.; Tien Loong Lee, C.B.; Poon, Z.Y.; Loow, Y.L.; Wei Foo, L.Y.; Procentese, A.; Siow, L.F.; Teoh, W.H.; Nik Daud, N.N.; et al. Potential Use of Pure and Diluted Choline Chloride-Based Deep Eutectic Solvent in Delignification of Oil Palm Fronds. *Process Saf. Environ. Prot.* **2019**, *123*, 190–198. [CrossRef]
28. Loow, Y.; Wu, T.Y.; Tan, K.A.; Lim, Y.S.; Fong, L. Recent Advances in Application of Inorganic Salt Pretreatment for Transforming Lignocellulosic Biomass into Reducing Sugars. *J. Agric. Food Chem.* **2015**, *63*, 8349–8363. [CrossRef]
29. Zhang, L.; Yu, H. Conversion of Xylan and Xylose into Furfural in Biorenewable Deep Eutectic Solvent with Trivalent Metal Chloride Added. *BioResources* **2013**, *8*, 6014–6025. [CrossRef]
30. Dai, Y.; Witkamp, G.; Verpoorte, R.; Choi, Y.H. Tailoring Properties of Natural Deep Eutectic Solvents with Water to Facilitate Their Applications. *Food Chem.* **2015**, *187*, 14–19. [CrossRef]
31. Wang, Z.K.; Hong, S.; Wen, J.L.; Ma, C.Y.; Tang, L.; Jiang, H.; Chen, J.J.; Li, S.; Shen, X.J.; Yuan, T.Q. Lewis Acid-Facilitated Deep Eutectic Solvent (DES) Pretreatment for Producing High-Purity and Antioxidative Lignin. *ACS Sustain. Chem. Eng.* **2020**, *8*, 1050–1057. [CrossRef]
32. Chen, Z.; Reznicek, W.D.; Wan, C. Deep Eutectic Solvent Pretreatment Enabling Full Utilization of Switchgrass. *Bioresour. Technol.* **2018**, *263*, 40–48. [CrossRef] [PubMed]
33. Kim, M.; Kim, B.C.; Nam, K.; Choi, Y. Effect of Pretreatment Solutions and Conditions on Decomposition and Anaerobic Digestion of Lignocellulosic Biomass in Rice Straw. *Biochem. Eng. J.* **2018**, *140*, 108–114. [CrossRef]
34. Shen, X.J.; Wen, J.L.; Mei, Q.Q.; Chen, X.; Sun, D.; Yuan, T.Q.; Sun, R.C. Facile Fractionation of Lignocelluloses by Biomass-Derived Deep Eutectic Solvent (DES) Pretreatment for Cellulose Enzymatic Hydrolysis and Lignin Valorization. *Green Chem.* **2019**, *21*, 275–283. [CrossRef]
35. Procentese, A.; Johnson, E.; Orr, V.; Garruto Campanile, A.; Wood, J.A.; Marzocchella, A.; Rehmann, L. Deep Eutectic Solvent Pretreatment and Subsequent Saccharification of Corncob. *Bioresour. Technol.* **2015**, *192*, 31–36. [CrossRef]
36. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of Structural Carbohydrates and Lignin in Biomass. *Biomass Anal. Technol. Team Lab. Anal. Proced.* **2008**, *1617*, 1–16.
37. Chourasia, V.R.; Pandey, A.; Kishore, K.; Henry, R.J. Improving Enzymatic Digestibility of Sugarcane Bagasse from Different Varieties of Sugarcane Using Deep Eutectic Solvent Pretreatment. *Bioresour. Technol.* **2021**, *337*, 125480. [CrossRef]
38. Hou, X.; Feng, G.; Ye, M.; Huang, C.; Zhang, Y. Significantly Enhanced Enzymatic Hydrolysis of Rice Straw via a High-Performance Two-Stage Deep Eutectic Solvents Synergistic Pretreatment. *Bioresour. Technol.* **2017**, *238*, 139–146. [CrossRef]
39. Bustos, G.; Moldes, A.B.; Cruz, J.M. Revalorization of Hemicellulosic Trimming Vine Shoots Hydrolyzates Trough Continuous Production of Lactic Acid and Biosurfactants by *L. pentosus*. *J. Food Eng.* **2007**, *78*, 405–412. [CrossRef]
40. Templeton, D.W.; Wolfrum, E.J.; Yen, J.H.; Sharpless, K.E.; Templeton, D.W. Compositional Analysis of Biomass Reference Materials: Results from an Interlaboratory Study. *Bioenergy Res.* **2016**, *9*, 303–314. [CrossRef]
41. Li, L.; Yu, L.; Wu, Z. Delignification of Poplar Wood with Lactic-Based Deep Eutectic Solvents. *Wood Res.* **2019**, *64*, 499–514.
42. Tian, D.; Guo, Y.; Hu, J.; Yang, G.; Zhang, J.; Luo, L.; Xiao, Y. Acidic Deep Eutectic Solvents Pretreatment for Selective Lignocellulosic Biomass Fractionation with Enhanced Cellulose Reactivity. *Int. J. Biol. Macromol.* **2020**, *142*, 288–297. [CrossRef]
43. Shafie, M.H.; Yusof, R.; Gan, C. Synthesis of Citric Acid Monohydrate-Choline Chloride Based Deep Eutectic Solvents (DES) and Characterization of Their Physicochemical Properties. *J. Mol. Liq.* **2019**, *5*, 171667. [CrossRef]
44. Florindo, C.; Oliveira, F.S.; Rebelo, L.P.N.; Fernandes, A.M.; Marrucho, I.M. Insights into the Synthesis and Properties of Deep Eutectic Solvents Based on Cholinium Chloride and Carboxylic Acids. *ACS Sustain. Chem. Eng.* **2014**, *10*, 2416–2425. [CrossRef]
45. Du, C.; Zhao, B.; Chen, X.; Birbilis, N.; Yang, H. Effect of Water Presence on Choline Chloride-2urea Ionic Liquid and Coating Platings from the Hydrated Ionic Liquid. *Sci. Rep.* **2016**, *6*, 29225. [CrossRef]
46. Teles, A.R.R.; Capela, E.V.; Carmo, R.S.; Coutinho, J.A.P.; Silvestre, A.J.D.; Freire, M.G. Solvatochromic Parameters of Deep Eutectic Solvents Formed by Ammonium-Based Salts and Carboxylic Acids. *Fluid Phase Equilib.* **2017**, *448*, 15–21. [CrossRef]

47. Soares, B.; Tavares, D.J.P.; Amaral, J.L.; Silvestre, A.J.D.; Freire, C.S.R.; Coutinho, J.A.P. Enhanced Solubility of Lignin Monomeric Model Compounds and Technical Lignins in Aqueous Solutions of Deep Eutectic Solvents. *ACS Sustain. Chem. Eng.* **2017**, *5*, 4056–4065. [CrossRef]
48. Xu, H.; Peng, J.; Kong, Y.; Liu, Y.; Su, Z.; Li, B.; Song, X.; Liu, S.; Tian, W. Key Process Parameters for Deep Eutectic Solvents Pretreatment of Lignocellulosic Biomass Materials: A Review. *Bioresour. Technol.* **2020**, *310*, 123416. [CrossRef]
49. Hong, S.; Yuan, Y.; Li, P.; Zhang, K.; Lian, H.; Liimatainen, H. Enhancement of the Nanofibrillation of Birch Cellulose Pretreated with Natural Deep Eutectic Solvent. *Ind. Crops Prod.* **2020**, *154*, 112677. [CrossRef]
50. Kohli, K.; Katuwal, S.; Biswas, A.; Sharma, B.K. Effective Delignification of Lignocellulosic Biomass by Microwave Assisted Deep Eutectic Solvents. *Bioresour. Technol.* **2020**, *303*, 122897. [CrossRef]
51. Guo, H.; Chang, Y.; Lee, D.J. Enzymatic Saccharification of Lignocellulosic Biorefinery: Research Focuses. *Bioresour. Technol.* **2018**, *252*, 198–215. [CrossRef]
52. Chen, Y.; Zhang, L.; Yu, J.; Lu, Y.; Jiang, B.; Fan, Y.; Wang, Z. High-Purity Lignin Isolated from Poplar Wood Meal through Dissolving Treatment with Deep Eutectic Solvents. *R. Soc. Open Sci.* **2019**, *6*, 181757. [CrossRef] [PubMed]
53. Lin, Z.; Huang, H.; Zhang, H.; Zhang, L.; Yan, L.; Chen, J. Ball Milling Pretreatment of Corn Stover for Enhancing the Efficiency of Enzymatic Hydrolysis. *Appl. Biochem. Biotechnol.* **2010**, *162*, 1872–1880. [CrossRef]
54. Procentese, A.; Rehmann, L. Fermentable Sugar Production from a Coffee Processing By-Product after Deep Eutectic Solvent Pretreatment. *Bioresour. Technol. Rep.* **2018**, *4*, 174–180. [CrossRef]
55. Thi, S.; Lee, K.M. Comparison of Deep Eutectic Solvents (DES) on Pretreatment of Oil Palm Empty Fruit Bunch (OPEFB): Cellulose Digestibility, Structural and Morphology Changes. *Bioresour. Technol.* **2019**, *282*, 525–529. [CrossRef]
56. Su, Y.; Huang, C.; Lai, C.; Yong, Q. Green Solvent Pretreatment for Enhanced Production of Sugars and Antioxidative Lignin from Poplar. *Bioresour. Technol.* **2021**, *321*, 124471. [CrossRef] [PubMed]
57. Li, H.; Xiong, L.; Chen, X.; Li, H.; Qi, G.; Huang, C.; Luo, M.; Chen, X. Enhanced Enzymatic Hydrolysis and Acetone-Butanol-Ethanol Fermentation of Sugarcane Bagasse by Combined Diluted Acid with Oxidate Ammonolysis Pretreatment. *Bioresour. Technol.* **2017**, *228*, 257–263. [CrossRef] [PubMed]
58. Oh, Y.; Park, S.; Jung, D.; Oh, K.K.; Lee, S.H. Effect of Hydrogen Bond Donor on the Choline Chloride-Based Deep Eutectic Solvent-Mediated Extraction of Lignin from Pine Wood. *Int. J. Biol. Macromol.* **2020**, *165*, 187–197. [CrossRef]
59. Zhao, J.; Meng, Z.; Zhao, Z.; Zhao, L. Ultrasound-Assisted Deep Eutectic Solvent as Green and Efficient Media Combined with Functionalized Magnetic Multi-Walled Carbon Nanotubes as Solid-Phase Extraction to Determine Pesticide Residues in Food Products. *Food Chem.* **2020**, *310*, 125863. [CrossRef]
60. Xu, H.; Kong, Y.; Peng, J.; Song, X.; Liu, Y.; Su, Z.; Li, B. Comprehensive Analysis of Important Parameters of Choline Chloride-Based Deep Eutectic Solvent Pretreatment of Lignocellulosic Biomass. *Bioresour. Technol.* **2021**, *319*, 124209. [CrossRef]
61. Sai, Y.W.; Lee, K.M. Enhanced Cellulase Accessibility Using Acid-Based Deep Eutectic Solvent in Pretreatment of Empty Fruit Bunches. *Cellulose* **2019**, *26*, 9517–9528. [CrossRef]