Title: Natural deep eutectic solvents for lignocellulosic biomass pretreatment: Recent developments, challenges and novel opportunities

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Abstract

Conversion of lignocellulosic biomass to fuels and chemicals has attracted immense research and development around the world. Lowering recalcitrance of biomass in a cost-effective manner is a challenge to commercialize biomass-based technologies. Deep eutectic solvents (DESs) are new 'green' solvents that have a high potential for biomass processing because of their low cost, low toxicity, biodegradability, easy recycling and reuse. This article discusses the properties of DESs and recent advances in their application for lignocellulosic biomass processing. The effectiveness of DESs in hydrolyzing lignin-carbohydrate complexes, removing lignin/hemicellulose from biomass as well as their effect on biomass deconstruction, crystallinity and enzymatic digestibility have been discussed. Moreover, this review presents recent findings on the compatibility of natural DESs with enzymes and microorganisms.

Keywords: Lignocellulosic biomass; Natural deep eutectic solvents; Lignin removal; Cellulose crystallinity; Recalcitrance

Abbreviations: DES: Deep Eutectic Solvent, IL: Ionic liquid, HBA: Hydrogen bond acceptor, HBD: Hydrogen bond donor, ChCl: Choline Chloride, EG: Ethylene Glycol, TEG: Triethylene glycol, U: Urea, LA: Lactic Acid, EAC: ethylammonium chloride, HMF: 5-hydroxymethylfurfural

1. Introduction

Lignocellulosic biomass conversion to biofuels, biochemicals, and other value-added products has attracted global attention because it is a readily available, inexpensive and renewable resource (Lynd 2017; Satlewal et al., 2017). It primarily consists of polysaccharides, cellulose, and hemicellulose (50-65%), and the aromatic biopolymer, lignin (10-30%) (Agrawal et al., 2016; Wang et al., 2016). The most promising commercially viable route today for utilization of lignocellulosic biomass is fermentation of cellulose and hemicellulose sugars into ethanol. A conventional process design includes size reduction, pretreatment, enzymatic hydrolysis, fermentation, and distillation as the major process steps. Pretreatment is essential to reduce biomass recalcitrance for achieving high enzymatic hydrolysis efficiencies (Dutta et al., 2018). Sugars recovered from pretreatment and enzymatic hydrolysis can be fermented to produce ethanol or other biofuels and commodity chemicals through biochemical and thermochemical routes. Alternate process designs also exist such as combining enzymatic hydrolysis and fermentation in single step known as simultaneous saccharification and fermentation (SSF), or a one-pot process where the different processes are carried out in the same vessel, for reducing the production costs.

Deep eutectic solvents were introduced as low-cost eutectic mixtures, with physical and chemical properties comparable to ILs (Abbott et al., 2004). They are prepared by combining hydrogen bonding donors (HBDs) and hydrogen bonding acceptors (HBAs) to form eutectic mixtures. DESs are preferred over conventional ILs because they are easy to synthesize, stable, cost-competitive and typically most of them are environmental-friendly (Mbous et al., 2017). According to one estimate, the cost to synthesize a DES was only 20% of that of an IL (Xu et al., 2016b). In a similar study, Gorke et al., (2010) reported that components for DESs were ten

times less expensive than the components for ionic liquids. However, the relationship between molecular composition and the solvent properties of the resulting eutectic mixtures is not fully understood. Nevertheless, several promising DESs systems have been reported in recent literature. The numbers of publications on DESs have grown exponentially during last few years (**Figure 1a & 1b)**. It indicates their potential primarily in the areas of electrochemistry, fossil fuels, fermentation and bio-industrial chemistry, pharmaceuticals, food and feed industry and lignocellulosic biomass processing. Biocompatibility of the DESs with biomolecules i.e. nucleic acids, proteins, enzymes and microbes is one the most significant properties of DESs which has attracted recent interest for their applications in bio-pharma industries for bioorganic catalysis, biotransformation, and molecular extractions (Mbous et al., 2017). The application of DESs as an alternative to ILs in dissolving the polysaccharides (i.e. cellulose, xylose, arabinose, starch, chitin) and lignin present in biomass has attracted a vast interest of the scientific community globally to produce biofuels, value added products and commodity chemicals (Oliveira et al., 2015).

This review article focuses on properties of DESs and recent advances in their application for lignocellulosic biomass processing It begins with the current status of lignocellulosic biomass pretreatment followed by discussion on synthesis and physiochemical properties of DESs, and key findings on the effects of DES on cellulose, hemicellulose and lignin solubilization then, biomass pretreatment and changes in biomass crystallinity. The article then progresses to enzymatic hydrolysis performance of DESs pretreated solids, compatibility of DESs with enzymes and microorganisms, and recycling potential of DESs. Finally, it covers the comparison of DESs with ILs, and challenges and opportunities for furthering DESs use in lignocellulosic processing.

2. Current status of lignocellulosic biomass pretreatment

A wide array of pretreatment technologies has been evaluated in last decade for lignocellulosic biomass valorization to produce biofuels and biochemicals with high cost efficiency (Abo-Hamad et al., 2015; Agrawal et al., 2015a). These include physical (mechanical extrusion, milling, microwave, ultrasound), physicochemical (steam explosion, hot-water, wet oxidation, sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL), ammonia), biological and chemical (dilute acid, dilute alkali, ozonolysis, organosolv, ionic liquids, inorganic salts and recently deep eutectic solvents) (Singh et al., 2015). The recently published reviews discussed about these comprehensively (Capolupo and Faraco 2016; Den et al., 2018; Seidl and Goulart 2016). Albeit, plethora of pretreatment processes exist but only few of them (i.e. dilute acid, steam explosion and hydrothermal) have been demonstrated at pilot and commercial scale levels while; many are still under process intensification stages or struggling for scale up (Satlewal et al., 2018). During certain pretreatments (dilute acid, dilute alkali, organosolv, hydrothermal, chemical pulping and ionic liquids (ILs)) especially at high severity conditions, hemicelluloses and/or lignin are solubilized and degraded to form inhibitors such as hydroxymethyl furfural, furfural, hydroxy acids, aliphatic carboxylic acids. Thus, an additional step of detoxification might become inevitable to reduce enzyme or microbial toxicity for realizing high product yields (Agrawal et al., 2015b; Akinosho et al., 2015). Ionic liquids have shown high efficiency for lignin extraction, reducing cellulose crystallinity, and improving enzymatic digestibility, under mild operating conditions. However, their industrial application has been restricted by high costs, incompatibility with enzymes and microorganisms and recycling challenges (Yoo et al., 2017). Thus, multiple factors play a critical role in selecting the right pretreatment approach for biomass based upon nature of feedstock (i.e. hardwood,

softwood, agricultural residue, grass), capital and operational expenditures, energy investment, yields, efficiency and environmental sustainability. In view of this, there is still a large scope to innovate and develop novel and disruptive biomass pretreatment technologies.

DESs offer several advantages over the conventional solvents and ionic liquids yet overcome many of their drawbacks such as easy to synthesize without any purification and waste generation step at mild temperature and atmospheric pressure, renewable in nature, wide availability and cost effectiveness of its components (for example ChCl is available as chicken feed while, urea is commonly used as fertilizer), biocompatibility and biodegradability(Loow et al., 2018). DESs are widely being exploited in electrochemical and organic synthesis areas and recently huge interest has been generated for their application in biorefinery due to their unique physicochemical properties (Xing et al., 2017).

DESs were reported to dissolve and extract high-quality lignin with more than 90% purity, and nearly 60±5 % (w/w) of the total lignin present in rice straw (Kumar et al., 2016; van Osch et al., 2017), but negligible cellulose solubility was observed (Oliveira et al., 2015). In few recently published reports, selected DESs have been reported to work efficiently during biomass pretreatments such as ethylammonium chloride:ethylene glycol (EAC:EG) for oil palm trunk (OPT) fiber pretreatment with 74% glucose production (74%) (Zulkefli et al., 2017), choline chloride:oxalic acid and choline chloride:urea for rice straw to achieve a glucose yield of 90.2% (Hou et al., 2017a), choline chloride:formic acid for corn stover with a hydrolysis yield of 99% (Xu et al., 2016b). Similarly, a high glucan conversion (92% – 95%) was achieved after pretreatment of corn cob with ChCl:glycerol and ChCl:imidazole, respectively (Procentese et al., 2015). Even though DESs possess more benefits than ILs, they are still not widely used because they are relatively new in biomass processing and more research is needed for their application

(Loow et al., 2017). Next few sections in this review will provide insights about the physicochemical properties of DESs and their application in biomass processing.

3. Deep eutectic solvents and their physicochemical properties

Typically, deep eutectic solvents consist of large, non-symmetric ions that have low lattice energy and hence, low melting points (Smith et al., 2014). They are usually prepared by mixing a hydrogen bond acceptor (HBA) (such as quaternary ammonium salts) and a hydrogen bond donor (HBD) such as amides, carboxylic acids, and alcohols at moderate temperatures (60 °C to 80 °C) to form eutectic mixtures (**Figure 2)** (Sarmad et al., 2017). Hydrogen bonding results in charge delocalization between the HBA and HBD and consequently, the freezing point of the eutectic mixture is much lower as compared to the individual compounds. For an example, the melting point of a choline chloride ChCl:urea mixture (1:2) is 12 °C which is far lower than 302 °C and 133 °C for ChCl and urea, respectively (Xu et al., 2017). The thermal phase behavior of a deep eutectic solvent system prepared by mixing together the lidocaine and decanoic acid in varied composition range was evaluated by differential scanning calorimetry (Griffin et al., 2014). It clearly showed the melting transitions of crystalline solids i.e. lidocaine (Tm = 341 K or 67.85 °C) and decanoic acid (Tm = 307 K or 33.85 °C) but no crystallization or melting was observed for the lidocaine:decanoic acid mixture which remains as liquid at room temperature with a glass transition at Tg =207 K or -66.15 °C **(Figure 3)**. Since, different types of DESs exist as liquids at temperatures below 100 °C thus a suitable classification system is required for their identification as discussed in the next section. (García et al., 2015).

3.1. Classification of deep eutectic solvents

DESs have been classified based on the combinations of their chemical constituents (**Table 1**). Type I DESs have limited application in biomass processing due to the high melting

points of the non-hydrated metal halides while, Type II DESs are more viable for industrial processes because of the relatively lower costs of the hydrated metal halides (Smith et al., 2014). However, Type III DESs are the most studied due to their quick and easy preparation, non-reactivity with water, biodegradable nature and cost effectiveness (Loow et al., 2017; Smith et al., 2014). Finally, Type IV DESs incorporate the use of inorganic transition metals with urea to form eutectic mixtures, even though metal salts would not normally ionize in non-aqueous media (Loow et al., 2017; Smith et al., 2014).

Table 1. General formula for the classification of DESs (Adapted with permission from (Smith et al., 2014))

Type	**Components**	**General formula**	**Example**
I	Metal salt + organic salt	$Cat^+ X^- zMClx$; M = Zn, Sn, Fe, Al, Ga, In	$ZnCl_2$ + ChCl
II	Metal salt hydrate + organic salt	$Cat^+ X^- zMClx.yH_2O$; M = Cr, Co, Cu, Ni, Fe	$CoCl_2$.6H2O + ChCl
III	HBD + organic salt	$Cat^+ X^-$ zRZ; Z = $CONH_2$, COOH, OH	Urea + ChCl
IV	Zinc/aluminium chloride + HBD	$MClx + RZ = MCl_{x-1}{}^{+} \cdot RZ + MCl^-x_{+1}$; M = Al, Zn & Z = $CONH_2$, OH	$ZnCl_2$ + urea

Cat^+, any ammonium, phosphonium, or sulfonium cation; X, a Lewis base, generally a halide anion; Y, a Lewis or Bronsted acid; z, number of y molecules that interact with the anion

The understanding of physiochemical characteristics of DESs is essential for its industrial applications. The key properties of DESs such as freezing point, density, viscosity, surface tension and conductivity are discussed as follows:

3.2. Freezing point

Although, DESs have lower freezing point as compared to their parent compounds but a few of them such as ChCl:glucose/sucrose/inulin/fructose possess high freezing point i.e. above 80 °C and remain as solids at room temperature which restricts their mixing and mass transfer efficiency and chemical interactions with solid substrates like lignocellulosic biomass at low temperatures (Oliveira et al., 2015). However, some of the DESs have freezing point below 50 °C and remained as liquids at room temperatures and attracted wide interest as solvents in industries and biomass processing applications **(Table 2)**. In case of halide ion based DESs, charge delocalization due to hydrogen bonding with HBD leads to reduction in the freezing point. The reduction in freezing point is accompanied by disruption of crystalline structure by hydrogen bonding between quaternary ammonium salt and HBD (Domínguez de María 2014; Loow et al., 2017). Generally, the freezing point of the DESs decrease with increasing hydrogen bonding strength within the mixture (Espino et al., 2016).

3.3. Density

Most DESs are denser than water with densities in the range of 1.0 to 1.35 g/cm^3 but metallic salts based DESs like $ZnCl_2$:urea and $ZnCl_2$:ethylene glycol have high densities in the range of 1.3−1.6 g/cm^3 (García et al., 2015) (**Table 2**). The density of DESs is affected by the packing arrangement of the molecular components and testing temperature (García et al., 2015). As expected, an increase in the temperature or water content in DESs leads to lower densities (García et al., 2015; Shahbaz et al., 2011). In addition, the density also decreases with increasing alkyl chain length of DES components as well as the relative ratio of salt to HBD is increased (Chen et al., 2017; van Osch et al., 2017). Apart from this, increase in the water content of a DES molecule also results into decrease in density (García et al., 2015).

3.4. Viscosity

The viscosity of DESs is determined by their intermolecular interactions which could be influenced by numerous factors including the chemical nature of their constituents such as the type of HBD and HBA, molar ratio of HBD and HBA, temperature and water content (Smith et al., 2014). For instance, the viscosity of ChCl based DESs decreases with increasing temperature and ChCl content in certain composition ranges (Abo-Hamad et al., 2015; AlOmar et al., 2016; Smith et al., 2014). DESs with lower viscosity are desirable for industrial and biomass processing applications (Loow et al., 2017). It is generally observed that there is a linear correlation between the molar conductivity of DESs and their fluidity (reciprocal of viscosity) (Smith et al., 2014). DESs have a broad demand as replacements for conventional organic solvents because of their high stability and biodegradable nature despite poor conductivity (Li et al., 2016; Smith et al., 2014). Thus highly viscous DESs are reported to have poor conductivity which increases at elevated temperatures (**Table 2**) (Abo-Hamad et al., 2015).

3.5 Surface tension

The surface tension of DESs is highly dependent upon the dominant intermolecular forces and the type of cation (García et al., 2015; Vigier et al., 2015). It was observed that the hydroxyl group in the cation leads to higher surface tension due to their hydrogen-bonding ability (García et al., 2015; Vigier et al., 2015). Thus, surface tension of glucose-based DESs was higher than those reported for carboxylic acids-based DESs (Hayyan et al., 2013a). An increase in temperature has been reported to decrease the surface tension of DESs. This phenomenon is explained by the gain of energy in the salt, which causes the reduction of intermolecular forces (AlOmar et al., 2016).

One of the significant benefits of DESs is to fine tune its properties by precisely selecting the hydrogen bond donor and acceptor and varying their molar ratios depending upon the application (van Osch et al., 2017; Yoo et al., 2017; Zahn 2017). The preferred DESs for a biomass processing industry should possess low freezing point (< 50 °C) to remain as liquids at room temperatures with low viscosity for better mixing and heat and mass transfer efficiency. Nevertheless, both of these properties are also dependent upon temperature as well. With this basic understanding about DESs synthesis, their classification and physio-chemical properties it is now easier to understand their application in lignocellulosic biomass processing as discussed in next few sections.

Table 2. Properties of commonly used DES solvents (Adapted with permission from (Loow et al., 2017))

Hydrogen Bond Donor (HBD)	**Hydrogen Bond Acceptor (HBA)**	**Molar ratio (HBD: HBA)**	**Freezing point (°C)**	**Density (g cm^{-3})**	**Viscosity (cP)**	**Surface tension (mN m^{-1})**	**Conductivity (mS cm^{-1})**	**Reference**
Urea	ChCl	2:1	12	1.25	750 (25 °C)	52 (25 °C)	0.75 (25 °C)	(Smith et al., 2014; Zhang et al., 2012b)
Ethylene glycol	ChCl	2:1	-12.9	1.12	37 (25 °C)	49 (25 °C)	7.61 (25 °C)	(Smith et al., 2014; Zhang et al., 2012b)
Glycerol	ChCl	2:1	17.8	1.18	259 (25 °C)	55.8 (25 °C)	1.05 (25 °C)	(Smith et al., 2014; Zhang et al., 2012b)
CF_3CONH_2	ChCl	2:1	51	1.342	77 (40 °C)	–	–	(Smith et al., 2014; Zhang et al., 2012b)

ZnCl2	ChCl	2:1	–	–	85,000 (25 °C)	–	0.06 (42 °C)	(Smith et al., 2014; Zhang et al., 2012b)
Urea	ZnCl2	3.5:1	9	1.63	11,340 (25 °C)	–	0.18 (42 °C)	(Smith et al., 2014; Zhang et al., 2012b)
Imidazole	Bu_4NBr	7:3	–	–	810 (20 °C)	–	0.24 (20 °C)	(Smith et al., 2014; Zhang et al., 2012b)
Ethylene glycol	$ZnCl_2$	4:1	–	1.45	–	–	–	(Smith et al., 2014)
2,2,2-Trifluoroacetamide	ChCl	2:1	Liquid at (25 °C)	1.342	77 (40 °C)	35.9 (25 °C)	–	(Abo-Hamad et al., 2015)
Acrylic acid	ChCl	1.6:1	Liquid at (25 °C)	–	115 (22 °C)	–	–	(Abo-Hamad et al., 2015)
Glycerol	Methyltriphenylphosphonium bromide	3:1	–5.55	1.30	–	58.94 (25 °C)	0.062 (25 °C)	(Abo-Hamad et al., 2015)
Ethylene glycol	Methyltriphenylphosphonium bromide	4:1	-49.34	1.23	–	51.29 (25 °C)	1.092 (25 °C)	(Abo-Hamad et al., 2015)
Triethylene glycol	Methyltriphenylphosphonium bromide	5:1	-21	1.19	–	49.58 (25 °C)	–	(Abo-Hamad et al., 2015)
Malonic acid	ChCl	1:1	10	–	721 (25 °C)	65.7 (25 °C)	0.55 (25 °C)	(Tang and Row 2013)
1,4-Butanediol	ChCl	3:1	-32	1.06	140 (20 °C)	47.17 (25 °C)	1.64 (25 °C)	(Tang and Row 2013)
Imidazole	ChCl	7:3	56	–	15 (70 °C)	–	12 (60 °C)	(Tang and Row 2013)

Acetamide	$EtNH_3Cl$	1.5:1	–	1.041	64 (40 °C)	–	0.688 (40 °C)	(Tang and Row 2013)

4. Cellulose, hemicelluloses and lignin solubilization in DESs

Currently the major roadblock for the commercial feasibility bio-based refineries is the separation of lignin from polysaccharides at low costs for the production of fermentable sugars and other high-value products from both sugars and lignin. DESs are capable of donating and accepting protons and this characteristic enables the formation of hydrogen bonds with other compounds which enhances its solvation properties (Pandey et al., 2017). The recent advancements in solubilization of the lignocellulosic biopolymers (cellulose, hemicelluloses and lignin) in DESs are discussed here (**Table 3**).

Zhang et al., (2012a) reported that microcrystalline cellulose (i.e. Avicel PH-105) was not soluble in ChCl:urea (molar ratio 1:2) and ChCl:$ZnCl_2$ (molar ratio1:2) even after treatment at high temperature (110 °C) for a prolonged time period (12 h) however, in another report, amorphous cellulose (cotton linter pulp) was solubilized by 1.43 wt% and 2.48 wt% in ChCl:urea and ChCl:imidazole, respectively (Ren et al., 2016a; Ren et al., 2016b). Pulp solubility was further enhanced to 4.57 wt% in ChCl:imidazole by addition of 5 wt% polyethylene glycol (PEG) as co-solvents, which served as surfactant to reduce the hydrophobicity of cellulose (Ren et al., 2016a; Ren et al., 2016b; Tang et al., 2017). It showed that cellulose solubility is inversely proportional to the crystallinity of the substrate. Alike cellulose, hemicellulose was also sparingly soluble in DESs (**Table 3**).

In contrast to both cellulose and hemicellulose, DESs; especially acidic DESs i.e. lactic, malic and oxalic based DESs) were found highly effective for lignin dissolution **(Table 3)** .

Vigier et al., (2015) suggested that one of the reasons for selective solubilization of lignin over cellulose is that, both cellulose and DESs possess strong hydrogen bonding networks, and dissolving cellulose in a DES requires the two hydrogen-bond networks to be dissociated and reorganized to form a thermodynamically more stable system. However, the cohesive energy of cellulose is so strong that it may hamper its dissolution in any DES. It was also found that lignin isolated from rice straw was solubilized to a greater extent in comparison to lignin embedded in rice straw structure (in its native state) (Kumar et al., 2016). The most plausible reason for this might be the disintegration of highly cross-linked architecture of biomass and strong bonding between lignin carbohydrate complexes (LCCs) (Kumar et al., 2016).

Thus, developing and synthesizing a novel DESs having a strong capability to solubilize cellulose and hemicellulose remains a grey area. Other significant issues for the industrial application of deep eutectic solvents based biomass processing is their recyclability and thermal stability (Yoo et al., 2017). The recovery and reuse of deep eutectic solvents after biomass processing is a cost and energy intensive process. The release of trimethylamine from ChCl based solvents at high temperatures (i.e., Hoffman elimination reaction) is a detrimental component for the industrial viability of this technology. These limitations must be overcome before DESs could be broadly implemented in an industrial scale for biomass processing (Vigier et al., 2015).

Table 3. Solubility of lignin and cellulose in various deep eutectic solvents

Hydrogen bond donor	**Hydrogen bond acceptor**	**Ratio**	**T [°C]**	**Lignin [wt%]**	**Cellulose [wt%]**	**Hemicellulose [wt%]**	**Reference**
Lactic Acid	Proline	3.3 : 1	60	9	<1	<1	(Lynam et al., 2017)
Lactic Acid	Proline	2 : 1	60	7.56	0	---	(Francisco et al., 2012)
Lactic acid	Choline chloride	2 : 1	60	5.38	0.00	---	(Francisco et al., 2012)

Lactic Acid	Choline Chloride	10 : 1	60	13	<3	<5	(Lynam et al., 2017)
Lactic acid	Choline chloride	5 : 1	60	7.77	0	---	(Francisco et al., 2012)
Lactic acid	Choline chloride	10 : 1	60	11.82	0.13	---	(Francisco et al., 2012)
Lactic acid	Glycine	9 : 1	60	8.77	0.00	---	(Francisco et al., 2012)
Lactic acid	Alanine	9 : 1	60	8.47	0.00	---	(Francisco et al., 2012)
Lactic Acid	Betaine	2 : 1	60	9	<1	<1	(Lynam et al., 2017)
Lactic Acid	Betaine	2 : 1	60	12.03	0	---	(Francisco et al., 2012)
Lactic acid	Glycine	9 : 1	60	11.88	0.13	---	(Francisco et al., 2012)
Formic Acid	Choline Chloride	2 : 1	60	14	<1	<1	(Lynam et al., 2017)
Acetic Acid	Choline Chloride	2 : 1	60	12	<1	<1	(Lynam et al., 2017)
Malic acid	Proline	1 : 3	60	14.90	5.90	---	(Francisco et al., 2012)
Oxalic acid dihydrate	Choline chloride	1 : 1	60	3.62	2.5	---	(Francisco et al., 2012)
Urea	Choline chloride	2 : 1	110	---	<0.2	---	(Zhang et al., 2012a)
Zinc chloride	Choline chloride	2 : 1	110	---	<0.2	---	(Zhang et al., 2012a)
Urea	Choline chloride	2 : 1	110	---	1.43	---	(Ren et al., 2016b)
Imidazole	Choline chloride	7 : 3	110	---	2.48	---	(Ren et al., 2016b)
Ammonium thiocyanate	Choline chloride	1 : 1	110	---	0.85	---	(Ren et al., 2016b)
Caprolactum	Choline chloride	1 : 1	110	---	0.16	---	(Ren et al., 2016b)
Acetamide	Choline chloride	2 : 1	110	---	0.22	---	(Ren et al., 2016b)
Oxalic acid	Allyl triethyl ammonium chloride	1 : 1	110	---	6.48	---	(Ren et al., 2016a)

5. Biomass pretreatment by DESs

5.1. Lignin removal

Pretreatment of biomass is essential for achieving high enzymatic saccharification yields from biomass. Lignin restricts enzymatic hydrolysis of biomass by acting as a physical barrier and restricting the enzyme access and by non-productive/non-specific enzyme binding (Bhagia et al., 2016; Dumitrache et al., 2017; Li et al., 2016). Organosolv, alkali, and ionic liquids are quite effective in lignin removal, but during high severity conditions it lead to hemicellulose degradation and inhibitor formation, moreover; ILs are quite expensive in nature (Tian et al., 2017). The solubility of lignin in DESs has provided a new alternative for biomass pretreatment under mild conditions. A schematic representation of hydrogen bonding between hydroxyl groups of lignin units and chloride anions of ChCl:urea is shown in **Figure 4A.** Recently, several studies varied the ratios of hydrogen bond donor and hydrogen bond acceptor of DESs for studying their effects on biomass delignification at different temperatures (**Table 4)**. It suggested that acidic DESs have delignified with ≥ 90% lignin removal of almost all types of lignocellulosic biomass (corncob, rice straw, wheat straw, poplar, douglas fir) (Tang et al., 2017) (Zhang et al., 2016). However, a recent study showed that pretreatment with DES (ChCl:glycerol) alone was not effective with date palm residues unless a hydrothermal pretreatment was carried out to reduce date palm recalcitrance prior to the DES pretreatment (Fang et al., 2017). Hence, the efficacy varied according to the type of biomass, its inherent recalcitrant nature and physiochemical properties. Such information from the recent state of the art is indispensable for selecting the right type of DESs and pretreatment conditions and ultimately the better yields.

In a recent study, by Kim et al., (2018) a new class of renewable DESs were developed with lignin-derived phenols as HBDs and ChCl as HBA like 4-hydroxybenzyl alcohol (ChCl:HBA), catechol (ChCl:CAT), vanillin (ChCl:VAN) and p-coumaric acid (ChCl:PCA) for

delignification of switchgrass. The highest delignification of 60.8% was observed with ChCl:PCA followed by ChCl:VAN (52.5%) and ChCl:CAT (49%). In yet another recent study by Procentese et al., (2018), different agro-industrial food wastes like apple residues, potato peels, coffee silverskin, and brewer's spent grains were pretreated with two different DESs, choline chloride :glycerol and choline chloride :ethylene glycol for fermentable sugar production by enzymatic hydrolysis. Maximum delignification of 62% was observed with apple residues and minimum of 33% in potato peels (Procentese et al., 2018). They also reported that concentrations of inhibitors like hydroxymethyl furfural and furfural was lower than 0.015 g L^{-1} while, gallic acid, ferulic acid and coumaric acid were smaller than the minimum detectable value (0.1 g L^{-1}) which was lower than the typical inhibition thresholds for enzymatic hydrolysis and fermentation. Therefore, no detoxification strategy was required after DESs based biomass pretreatments (Procentese et al., 2018).

Thus, delignification and pretreatment efficiency of DESs is highly dependent upon the recalcitrant nature of biomass, selected DES and the pretreatment conditions. DESs offered a new approach of pretreating multiple feedstocks with high efficiency at mild temperatures without any significant inhibitors formation. Further, the renewable and biomass derived DESs offered another excellent opportunity to improve cost-efficiency through closed-loop biorefinery concept where, biomass derived DESs were employed for its own delignification (Kim et al., 2018).

Table 4. Biomass delignification with deep eutectic solvents

Biomass	Hydrogen bond donor	Hydrogen bond acceptor	Ratio	Temperature (°C)	Lignin removal (%)	Reference
Corncob	Imidazole	Choline chloride	2:1	115	70	(Procentese et

						al., 2015)
Corncob	Imidazole	Choline chloride	2:1	150	88	(Procentese et al., 2015)
Corncob	Urea	Choline chloride	2:1	115	24.8	(Procentese et al., 2015)
Corncob	Glycerol	Choline chloride	7:3	115	4.4	(Procentese et al., 2015)
Corncob	Lactic acid	Choline chloride	2:1	90	64.7	(Zhang et al., 2016)
Corncob	Lactic acid	Choline chloride	5:1	90	77.9	(Zhang et al., 2016)
Corncob	Lactic acid	Choline chloride	10:1	90	86.1	(Zhang et al., 2016)
Corncob	Lactic acid	Choline chloride	15:1	90	93.1	(Zhang et al., 2016)
Corncob	Glycolic acid	Choline chloride	2:1	90	56.4	(Zhang et al., 2016)
Corncob	Levulinic acid	Choline chloride	2:1	90	43.0	(Zhang et al., 2016)
Corncob	Malonic acid	Choline chloride	1:1	90	56.5	(Zhang et al., 2016)
Corncob	Glutaric acid	Choline chloride	1:1	90	34.3	(Zhang et al., 2016)
Corncob	Oxalic acid	Choline chloride	1:1	90	98.5	(Zhang et al., 2016)
Corncob	Malic acid	Choline chloride	1:1	90	22.4	(Zhang et al., 2016)
Corncob	Ethylene glycol	Choline chloride	1:1	90	87.6	(Zhang et al., 2016)
Corncob	Glycerol	Choline chloride	1:1	90	71.3	(Zhang et al., 2016)
Rice straw	Lactic acid	Choline chloride	2: 1	60	51.0	(Kuma

						r et al., 2016)
Rice straw	Lactic acid	Choline chloride	5: 1	60	60.0	(Kumar et al., 2016)
Rice straw	Lactic acid	Choline chloride	9: 1	60	59.0	(Kumar et al., 2016)
Wheat straw	Lactic acid	Choline chloride	9: 1	60	14.6	(Jablonský et al., 2015)
Wheat straw	Lactic acid	Choline chloride	10: 1	60	29.1	(Jablonský et al., 2015)
Wheat straw	Oxalic acid.2 H_2O	Choline chloride	1:1	60	57.9	(Jablonský et al., 2015)
Wheat straw	Malic acid	Choline chloride	1:1	80	21.6	(Jablonský et al., 2015)
Wheat straw	Malonic acid	Choline chloride	1:1		3.8	(Jablonský et al., 2015)
Wheat straw	Lactic acid	Choline chloride	10: 1	60	87.9	(Jablonský et al., 2015)
Corn stover	Formic acid	Choline chloride	-	130	23.8	(Xu et al., 2016b)
Poplar	Lactic acid	Betaine	2.5:1	130	52.4	(Tian et al., 2017)
Poplar	Lactic acid	Choline chloride	-	145	78	(Alvarez-Vasco et al., 2016)
Douglas fir	Lactic acid	Choline chloride	-	145	58	(Alvarez-Vasco et al., 2016)
Date Palm Residues	Glycerol	Choline chloride	2:1 – 6:1	70	Not significant	(Fang et al., 2017)

Switchgrass	4-hydroxybenzyl alcohol	Choline chloride	1:1	100	6.5	(Kim et al., 2018)
Switchgrass	Catechol	Choline chloride	1:1	100	49	(Kim et al., 2018)
Switchgrass	Vanillin	Choline chloride	2:1	100	52.5	(Kim et al., 2018)
Switchgrass	p-coumaric acid	Choline chloride	1:1	100	60.8	(Kim et al., 2018)
Apple residues	Glycerol	Choline chloride	32:1	150	62	(Procentese et al., 2018)
Potato peels	Glycerol	Choline chloride	32:1	150	33	(Procentese et al., 2018)

5.2. Hemicellulose removal

Lignin-carbohydrate complexes (LCCs), are chiefly responsible for biomass recalcitrance arising from the cross linking of lignin with carbohydrates (especially hemicellulose) via strong covalent and hydrogen bonding network with benzyl ester, benzyl ether, and phenyl glycoside functional groups (Yongzhuang et al., 2017). Thus, most of the pretreatment approaches are based upon LCCs disintegration to remove hemicellulose for enhanced enzyme accessibility and hydrolysis yields. DESs hydrolyze the LCC linkages by disrupting the existing hydrogen bonding interactions between carbohydrates and lignin and developing new and competing hydrogen bonds between the chloride ions of the DESs and hydroxyl groups present in the carbohydrates and lignin **(Figure 4B**). But the extent of hemicellulose removal depends upon the DESs and the physicochemical conditions, as discussed in the section below.

In contrast to dilute sulfuric acid pretreatment where, most of the hemicellulose (~ 80%) got hydrolyzed into soluble monomeric sugars (i.e. xylose, arabinose mannose, galactose) at temperatures above 120 °C and acidic pH (~1.5 to 2) in less than 30 min (Agrawal et al., 2015b),

but no hemicellulose hydrolysis was observed even after prolonged DESs pretreatment (ChCl:lactic acid and betaine:lactic acid having pH ~2) at 60 °C for 12 h (Kumar et al., 2016) and only ~20% reported at 120 °C for 12 h with weekly basic DES i.e. ChCl:urea (Hou et al., 2017b). However, 95.8% of the hemicellulose got hydrolyzed within 4 h at 120 °C with strongly acidic DES (ChCl:oxalic acid) (Hou et al., 2017b). Hence, elevated temperature ≥120 °C for a longer duration (≥4 h) is required to remove hemicellulose in strongly acidic DESs while, marginal hemicellulose removal observed in mildly acidic DESs. Thus, depending upon the pretreatment conditions and the type of DESs, appropriate enzyme preparation also needs to be employed. For example, hemicellulase rich enzyme preparations shall be required for xylan rich pretreatment residues and vice versa for low lignin containing residues after pretreatment.

5.3. Biomass crystallinity after DES pretreatment

Crystallinity is amongst the most discussed and widely measured parameters during pretreatment which is believed to play a critical role in bioconversion of the lignocellulosic biomass(Karimi and Taherzadeh 2016). Biomass crystallinity is a function of cellulose content as hemicellulose and lignin are amorphous in nature and their removal during pretreatment could result in increasing the apparent crystallinity of the biomass sample if the crystallinity of cellulose is not lowered appreciably by the pretreatment conditions or solvent. Thus, the focus of this section is to investigate the role of DESs pretreatment in affecting the substrate or biomass crystallinity (**Table 5)**.

The decrease in crystallinity index (CrI) has been frequently linked earlier with improved biomass conversion yields due to enhanced availability of substrate binding sites (Loow et al., 2018; Procentese et al., 2018). Some ionic liquids have been reported to efficiently decrystallize cellulose and reduce biomass crystallinity significantly, and cause complete solvation of the

whole biomass (Li et al., 2018). However, this was not always found to be true and sometimes either no relation or inverse correlation has also been observed between biomass crystallinity and conversion yields (such as dilute acid and hydrothermal pretreatments) (Agrawal et al., 2015b; Hashmi et al., 2017).

In a study by Zhang et al., (2012a), the impact of choline derived solvents for pure cellulose (Avicel) decrystallization at 110 °C for 12 h but no significant reduction in cellulose crystallinity was observed after ChCl:urea and ChCl:$ZnCl_2$ pretreatment. Procentese et al., (2015) reported that corn cobs pretreated in three different DESs (ChCl: glycerol, ChCl: urea and ChCl: imidazole) at different temperatures (80, 115, and 150 °C) efficiently removed lignin and some hemicelluloses resulting in enhanced overall crystallinity of the pretreated biomass while the crystallinity of the cellulose fraction was reduced. The crystallinity of the pretreated corn cob rose with increasing temperature from 80 °C to 150 °C. Interestingly, Nor et al., (2016) found that low crystallinity occurs at high-temperature pretreatment of oil palm with ChCl:urea (2:1 molar ratio, 110 ºC for 1 h) as compared to native oil palm while high crystallinity is evident at a relatively lower temperature (80 ºC for 1 h). This was attributed to the hydrolysis of para-crystalline cellulose whereas using a relatively low pretreatment temperature only amorphous hemicellulose and lignin were removed while crystalline cellulose remained intact (Nor et al., 2016). Thus, the overall crystallinity of the biomass generally increased after DESs pretreatment due to the removal of amorphous hemicellulose as well as lignin.

Table 5. Crystallinity index (CrI) of lignocellulosic biomass after DES pretreatment

Biomass	**Pretreatment**	**CrI**	**Reference**
Corn cob	Untreated	30.07	(Procentese et al., 2015)
Corn cob	ChCl glycerol, 150 °C	44.81	(Procentese et al., 2015)

Corn cob	ChCl urea, 115 °C	36.54	(Procentese et al., 2015)
Corn cob	ChCl imidazole, 115 °C	40.08	(Procentese et al., 2015)
Corn cob	ChCl imidazole, 150 °C	49.22	(Procentese et al., 2015)
Corn cob	Untreated	31.6	(Zhang et al., 2016)
Corn cob	ChCl lactic acid, 90 °C	38.6	(Zhang et al., 2016)
Corn cob	ChCl Glycolic acid, 90 °C	30.8	(Zhang et al., 2016)
Corn cob	ChCl Levulinic acid, 90 °C	32	(Zhang et al., 2016)
Corn cob	ChCl Malonic acid, 90 °C	29.5	(Zhang et al., 2016)
Corn cob	ChCl Glutaric acid, 90 °C	30.8	(Zhang et al., 2016)
Corn cob	ChCl Oxalic acid, 90 °C	31.6	(Zhang et al., 2016)
Corn cob	ChCl Malic acid, 90 °C	31.7	(Zhang et al., 2016)
Corn stover	Untreated	31.1	(Xu et al., 2016b)
Corn stover	ChCl formic acid, 130 °C	57.2	(Xu et al., 2016b)
Rice straw	Untreated	37.9	(Hou et al., 2012)
Rice straw	Cholinium lysine, 90 °C	62.8	(Hou et al., 2012)
Rice straw	Cholinium glycine, 90 °C	65.4	(Hou et al., 2012)
Rice straw	Cholinium serine, 90 °C	68.9	(Hou et al., 2012)
Oil palm empty fruit bunch	Untreated	38.27	(Nor et al., 2016)
Oil palm empty fruit bunch	ChCl urea, 110 ºC	34.99	(Nor et al., 2016)
Oil palm empty fruit bunch	ChCl urea, 80 ºC	39.23	(Nor et al., 2016)
Date palm residues	Untreated	27.44 (1.01)[a]	(Fang et al., 2017)
Date palm residues	ChCl glycerol, 70 ºC	31.89 (0.91) [a]	(Fang et al., 2017)
Date palm residues	Hydrothermal pretreatment followed by ChCl glycerol, 70 ºC	33.57 (0.76) [a]	(Fang et al., 2017)

[a] Crystallinity considering glucan content(Fang et al., 2017)

5.4. Enzymatic hydrolysis and DES pretreatment

The sections above have provided thorough insights of the physicochemical nature of DESs, mechanism of biomass pretreatment, and how it affects the biomass composition, biopolymers solubility (i.e. lignin, hemicellulose and cellulose) and crystallinity. The primary objectives of any lignocellulosic biomass pretreatment approach is to reduce its recalcitrance with subsequent increase in the fermentable sugar yields via enzymatic hydrolysis. Here, current status of bioconversion of different biomass via DESs pretreatment and enzymatic hydrolysis discussed **(Table 6)**. It showed that enzymatic hydrolysis yields varied significantly depending upon the type of biomass/substrate and DES, pretreatment temperature, molar ratio of hydrogen bond donor and acceptor. A few examples depicting the role of each one of them is discussed below in brief for more clarity.

5.4.1. Type of biomass and DES

In a systematic study, Jablonský et al., (2015) suggested that the increase in hydrolysis yields after DESs pretreatments was primarily due to the disruption of crystalline cellulose and delignification. A recent study compared the enzymatic hydrolysis yields of kraft dissolving eucalyptus pulp, cellulose, wheat straw and spruce saw dust before and after pretreatment with three DESs ChCl:boric acid (5:2), ChCl:glycerol (1:1) and betaine:glycerol (1:1) (Wahlstrom et al., 2016). It showed that prior to pretreatment maximum hydrolysis yields obtained with kraft dissolving eucalyptus pulp (62%) followed by cellulose (MCC) (49%), native wheat straw (18%) and spruce saw dust (8%) (**Table 6**). Although, DES pretreatment improved the enzymatic hydrolysis yields of all the substrates but still the trends remained the same, obtaining the

maximum hydrolysis yields with dissolving pulp (~100%) followed by cellulose (~65%), wheat straw (33%) and only marginal increase with saw dust (**Table 6**). This study showed that mild DESs pretreatment were effective with agricultural residues (i.e. wheat straw, corn cob, switchgrass etc.) and other low recalcitrant substrates (i.e. amorphous cellulose, kraft pulp etc.) but more research is still needed to develop and demonstrate the utility of DESs for other highly recalcitrant woody biomass such as spruce saw dust, date palm etc. Another finding of this study was better efficiency of acidic DES (ChCl:boric acid (5:2)) in comparison of glycerol based DESs (i.e. ChCl:glycerol (1:1) and betaine glycerol (1:1)). In another critical study, different ChCl:acid based DESs (listed here in the order of decreasing acidity; formic acid (high acidic strength with pKa 3.75) > lactic acid (pKa 3.86) > acetic acid (least acidic strength pKa 4.75)) were compared on a single feedstock (pine residues) for delignification and enzymatic hydrolysis yields (Lynam et al., 2017). This study revealed that highest lignin solubility (14% w/w) and hydrolysis yields (70%) were obtained with ChCl:formic acid in comparison to others. Similarly, 57.9% delignification of wheat straw achieved by highly acidic ChCl:oxalic (pKa 1.2) as compared to ChCl:lactic acid (Jablonský et al., 2015). Hence, DESs with strong acidity were found to be more effective for lignin solubilization and enzymatic hydrolysis yields.

5.4.2. Effect of temperature

The effect of temperature on pretreatment and enzymatic hydrolysis was evaluated by Procentese et al., (2015), they reported that hydrolysis yields of corncob enhanced from 39.9% to 91.5% with an increase in pretreatment temperature from 80 °C to 150 °C by using ChCl:glycerol as a DES. Similarly, hydrolysis yields improved from 51% to 58.6% with an increase in pretreatment temperature with ChCl:urea, however, no significant increase with temperature was observed with ChCl:imidazole as it worked equally well (92.3%) even at lower

temperature of 80 °C (**Table 6**) . In a similar study, Zhang et al., (2016) also reported that high delignification and enzymatic hydrolysis yields were observed with the increase in pretreatment temperature from 70 °C to 110 °C but this increase in hydrolysis yields (77.8–79.7%) was marginal after reaching 90 °C. Thus, generally enzymatic digestibility improved with an increase in temperature from 80 °C to 150 °C.

5.4.3. Molar ratio of HBA/HBD

Many of the reports cited here showed that acidity of DESs play a critical role in biomass pretreatment and delignification efficiency and generally the yields improve with increasing acidity. For example, the lignin solubility improved with the increase in the acid content of ChCl:lactic acid from a molar ratio of 1:1 to 1:9 when a synthetic blend of cellulose and lignin was used as a substrate (Francisco et al., 2012), similarly higher lignin solubilization i.e. 51% to 60% was observed with increased acid ratio from 1:2 to 1:5, respectively using rice straw as a substrate (Kumar et al., 2016). In another recent study, increasing the molar ratio of ChCl:lactic acid from 2:1 to 15:1 improved the lignin extraction (64.7–93.1%) of corncob but no significant increase in enzymatic hydrolysis yield takes place (79.1–83.5%). Moreover, 70% lignin removal from corn cob was sufficient for achieving the optimum hydrolysis yield (Zhang et al., 2016). This value of 70% for lignin removal is in accordance with the recent finding that about 65-70% of lignin in biomass is easier to remove if lignin re-deposition is prevented (Bhagia et al., 2016). Moreover, previous reports indicated that complete removal of lignin is not necessary to achieve better enzymatic hydrolysis (Fu et al., 2010; Hou et al., 2013). Fang et al., (2017) found that liquid hot water pretreatment followed by ChCl:glycerol pretreatment (70 °C for 6 h) of date palm residues had 1.7 times higher enzymatic digestibility as compared to liquid hot water pretreatment only. There was no significant increase in hydrolysis with ChCl:glycerol

pretreatment of date palm residues. It was suggested that removal of lignin and xylan by DES were responsible for the enhancement of enzymatic digestibility rather than lowering the cellulose crystallinity (Fang et al., 2017).

Recently, an important study by Kim et al., (2018) demonstrated that novel DESs developed from biomass derived lignin phenolics were as effective as other DESs produced by using acids (oxalic acid, levulinic acid, malonic acid, etc.), alcohols (glycerol, ethylene glycol, etc.), and amines (urea) in improving the enzymatic hydrolysis of switchgrass. As shown in **Table 6**, the maximum glucose yields of 85.7 % and 79.8% were observed with ChCl:p-coumaric acid and ChCl:vanillin, respectively while; the lowest efficiency (32%) was observed with ChCl:4-hydroxybenzyl alcohol as no substantial delignification was observed after pretreatment with it thus, enzyme accessibility was significantly reduced.

Table 6. Enzymatic hydrolysis efficiency after DES pretreatment

Substrate/Biomass	**DES**	**Pretreatment conditions**	**Enzymatic hydrolysis efficiency**	**Reference**
Rice husk	Nil	50 °C, 0.5 h	0.2 mM	(Gunny et al., 2015)
Rice husk	ChCl ethylene glycol	115 °C, 3 h	0.7 mM	(Gunny et al., 2015)
Corncob	Untreated	---	32.8%	(Procentese et al., 2015)
Corncob	ChCl glycerol	80 °C, 15 h	39.9%	(Procentese et al., 2015)
Corncob	ChCl glycerol	115 °C, 15 h	79.1%	(Procentese et al., 2015)
Corncob	ChCl glycerol	150 °C, 15 h	91.5%	(Procentese et al., 2015)
Corncob	ChCl urea	80 °C, 15 h	51%	(Procentese et al., 2015)
Corncob	ChCl urea	115 °C, 15 h	58.6%	(Procentese et al., 2015)
Corncob	ChCl imidazole	80 °C, 15 h	92.3%	(Procentese et al., 2015)
Corncob	ChCl imidazole	115 °C, 15 h	94%	(Procentese et al., 2015)

Corncob	ChCl imidazole	150 °C, 15 h	94.6	(Procentese et al., 2015)
Corncob	Untreated	---	22.1	(Zhang et al., 2016)
Corncob	ChCl lactic acid	90 °C,24 h	83.5%	(Zhang et al., 2016)
Corncob	ChCl glycolic acid	90 °C,24 h	67.3%	(Zhang et al., 2016)
Corncob	ChCl levulinic acid	90 °C,24 h	62%	(Zhang et al., 2016)
Corncob	ChCl malonic acid	90 °C,24 h	61.5%	(Zhang et al., 2016)
Corncob	ChCl glutaric acid	90 °C,24 h	40.7%	(Zhang et al., 2016)
Corncob	ChCl oxalic acid	90 °C,24 h	45.2%	(Zhang et al., 2016)
Corncob	ChCl malic acid	90 °C,24 h	37.4%	(Zhang et al., 2016)
Corncob	ChCl ethylene glycol	90 °C, 24 h	85.3%	(Zhang et al., 2016)
Corncob	ChCl glycerol	90 °C, 24 h	96.4%	(Zhang et al., 2016)
Corn stover	ChCl formic acid	130 °C, 3 h	99%	(Zhang et al., 2016)
Rice straw	ChCl lactic acid	60 °C, 12 h	36%	(Kumar et al., 2016)
Oil palm trunk	Nil	---	25%	(Zulkefli et al., 2017)
Oil palm trunk	Ethylammonium chloride ethylene glycol	100 °C, 48 h	74%	(Zulkefli et al., 2017)
Pine	Untreated	---	10%	(Lynam et al., 2017)
Pine	ChCl formic acid	155 °C, 2 h	70%	(Lynam et al., 2017)
Microcrystalline cellulose	Untreated	---	49%	(Wahlstrom et al., 2016)
Microcrystalline cellulose	ChCl betaine	80 °C, 24 h	49%	(Wahlstrom et al., 2016)
Microcrystalline cellulose	ChCl glycerol	80 °C, 24 h	~65%	(Wahlstrom et al., 2016)
Microcrystalline cellulose	Betaine glycerol	80 °C, 24 h	~65%	(Wahlstrom et al., 2016)
Eucalyptus dissolving pulp	Untreated	---	62%	(Wahlstrom et al., 2016)
Eucalyptus	ChCl glycerol	80 °C, 24 h	~100%	(Wahlstrom

dissolving pulp				et al., 2016)
Eucalyptus dissolving pulp	ChCl betaine	80 °C, 24 h	~100%	(Wahlstrom et al., 2016)
Eucalyptus dissolving pulp	Betaine glycerol	80 °C, 24 h	~100%	(Wahlstrom et al., 2016)
Wheat straw	Untreated	---	18%	(Wahlstrom et al., 2016)
Wheat straw	ChCl betaine	80 °C, 24 h	33%	(Wahlstrom et al., 2016)
Wheat straw	ChCl glycerol	80 °C, 24 h	<20%	(Wahlstrom et al., 2016)
Wheat straw	Betaine glycerol	80 °C, 24 h	<20%	(Wahlstrom et al., 2016)
Spruce saw dust	Untreated	---	8%	(Wahlstrom et al., 2016)
Spruce saw dust	ChCl glycerol	80 °C, 24 h	<20%	(Wahlstrom et al., 2016)
Spruce saw dust	ChCl betaine	80 °C, 24 h	<20%	(Wahlstrom et al., 2016)
Spruce saw dust	Betaine glycerol	80 °C, 24 h	<20%	(Wahlstrom et al., 2016)
Switchgrass	ChCl 4-hydroxybenzyl alcohol	160°C, 3h	32%	(Kim et al., 2018)
Switchgrass	ChCl catechol	160°C, 3h	77%	(Kim et al., 2018)
Switchgrass	ChCl vanillin	160°C, 3h	79.8%	(Kim et al., 2018)
Switchgrass	ChCl p-coumaric acid	160°C, 3h	85.7%	(Kim et al., 2018)

6. Compatibility of DES with enzymes and microorganisms

The two most popular process designs for biomass to ethanol production are pretreatment followed by separate hydrolysis and fermentation (SHF), or simultaneous saccharification (hydrolysis) and fermentation (SSF). If the pretreatment solvent is toxic to enzymes and microbes, it will need to be removed from pretreated solids by extensive washing or by other methods for achieving high yields by SHF or SSF. On the other hand, some studies that used ionic liquid pretreatment performed a one-pot process in which the three stages are carried out in

the same vessel. Thus, biocompatibility is critical concern for one-pot processes where pretreatment slurry undergoes hydrolysis and fermentation. Since many ionic liquids can cause high enzyme inhibition and cytotoxicity, recent works developed IL systems to address these issues (Liszka et al., 2016). A single-pot process design containing pretreatment, enzymatic hydrolysis and fermentation was made possible through use of aqueous choline-based ILs that allowed near 75% ethanol yield and over 40 g/L ethanol titer from fed batch process at high 30-34% solids loading of corn stover but at high enzyme loading of 20 mg/g glucan (Xu et al., 2016a). It is known that constituents of DES, like choline chloride and urea, have the ability to inactivate proteins (Gorke et al., 2008). Remarkably, when combined to form a DES, inactivation can be reduced several-fold. This was observed in the case of lipase-catalyzed transesterification where conversions with certain DESs like, choline chloride:(glycerol or urea), were comparable to that in toluene indicating excellent stability of certain lipases in DES (Gorke et al., 2008).

Some recent studies have evaluated the impact of DES on cellulase activity. Gunny et al., (2015) incubated cellulase from *Aspergillus sp.* with choline chloride based DES having glycerol or ethylene glycol or malonic acid as the hydrogen bond donors (HBD) in 1:2 ratio for 48 h. Results showed 10% reduction in filter paper activity after 48 h in control (i.e. without any DESs) whereas, 60% loss of relative filter paper activity was observed within 24 h in the presence of DES containing malonic acid (**Figure 5**). In contrast, only marginal reduction in cellulase activity was observed with glycerol or ethylene glycol containing DES. Hydrolysis of Avicel with cellulase resulted in only minor decrease of ~1 mM in the glucose concentration with 10% v/v choline chloride:(glycerol or ethylene glycol). This study proved that malonic acid is highly inhibitory to cellulase as compared to the other two HBDs (glycerol or ethylene glycol).

Wahlstrom et al., (2016) recently studied the effect of high concentrations of DESs on enzyme activity and hydrolysis yields for feasibility of a single-pot biomass deconstruction process. For enzyme activity, they purified and tested cellobiohydrolase Cel7A, endoglucanases Cel5A and Cel7B, and xylanase Xyn11 from *T. reesei* individually in three DES at 85% concentration at pH 5.0 in citrate buffer and at 50 °C with choline chloride:boric acid (5:2), choline chloride:glycerol (1:1), and betaine:glycerol (1:1). The popular cellulose solubilizing ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac) was also included in this study. This ionic liquid was highly inhibitory as the activity of all enzymes dropped to nearly zero in about 4 h whereas, choline chloride:glycerol (1: 1) was mild inhibitory to enzymes which might be expected because glycerol is considered an enzyme stabilizer (Agrawal et al., 2017b). The betaine:glycerol (1:1) appeared to stabilize the enzymes after an initial (24 h to 72 h) decline in activity. In fact, residual enzyme activity of the two endoglucanases was significantly higher after 144 h in betaine:glycerol than in buffered solution without any DES. However, choline chloride:boric acid (5: 2) was highly inhibitory amongst all of the DESs evaluated here, with complete loss of enzymatic activity occurred within 48 h. This study showed that, not only the glycerol component but also the hydrogen bond acceptor played a significant role in affecting the enzyme stability and betaine acted as more enzyme-compatible than choline chloride (Wahlstrom et al., 2016). On the other hand, enzymes can be engineered to increase tolerance of such unconventional solvents. Lehmann et al. (2012) developed a high-throughput assay based on a fluorescent cellobiose substrate for directed evolution of DES tolerant endoglucanase (CelA2). They discovered cellulase variants that had 23-fold higher cellulolytic activity in high ionic strength mediums such as DES, ionic liquid or NaCl due to activation of a salt bridge (Lehmann et al., 2014).

There are few studies on compatibility of DESs with microbes. Hayyan et al., (2013c) performed filter paper diffusion assay for 24 h on four common bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, for toxicity caused by choline chloride based DES having HBDs as glycerol, ethylene glycol, triethylene glycol, and urea, as well as the pure components that formed these DES. Their work showed that none of the bacteria were inhibited by the four DES or their pure components. In their other study (Hayyan et al., 2013b), phosphonium-based DES (methyltriphenylphosphonium bromide) with glycerol, ethylene glycol, and triethylene glycol were studied for inhibition using the same assay in similar conditions on the same four bacteria. In this case, DES with ethylene glycol and triethylene glycol HBDs inhibited all four bacteria. DES with glycerol HBD showed a zone of inhibition only with *Pseudomonas aeuriginosa*. In one study, baker's yeast was used as a whole cell biocatalyst in different mixtures of water with DES (choline chloride: glycerol) (Maugeri and Domínguez de María 2014). By replacing 100% water with 20% water in choline chloride: glycerol (1:2) led to 95% excess of (R) enantiomer than 95% excess of (S) enantiomer of ethyl 3-hydroxybutyrate catalyzed by baker's yeast possibly due to inhibition of S-enantioselective oxidoreductases . Yeast was active in 50% of this DES at long reaction times (more than 200 h)(Maugeri and Domínguez de María 2014).

These early studies on this topic suggest that certain DESs are biocompatible with enzymes, bacteria and yeast. However, there are reports where high concentration of DESs are toxic to them and for a single-pot processes it might be desirable to dilute the pretreatment slurry to a level at which the DES does not greatly affect sugar yields. Further work is essential in this area of research for better understanding and biotechnological innovations.

7. Recycling of DESs

Recycling and reuse of DESs are one of the major advantages for its application in low cost-high volume industrial applications like biomass processing (van Osch et al., 2017). DESs are considered to be recycled more readily than ILs because their synthesis/regeneration does not involve any chemical reactions and only it involves formation or rupturing the hydrogen bonding network that binds these components (HBD and HBA) (Xu et al., 2017).

The recycling of DESs in pretreatments studies has been rarely investigated but broadly acknowledged as a pressing research need for the commercial viability of the biomass based biorefinery. Recently, Kim et al., (2018) have evaluated the recovery, reuse and efficacy of the DESs during pretreatment of switchgrass. They have used an easy approach to recycle DESs by separation of residual lignin through pressurized ultrafiltration of liquid obtained after pretreatment of switchgrass and DES recovery by using a rotary evaporator to recycle ethanol and water used during the process and the recovered DES was reused for the next biomass pretreatment (**Figure 6A & 6B**). The mass balance analysis showed that ~95% of DES was recovered during each recycle without losing its efficiency for 3 successive cycles (Kim et al., 2018). We can certainly take guidance from other academic and industry studies that have utilized DESs and recycled them in differing fields, for example Jeong et al., (2015) utilized a ternary DES composed of glycerol, l-proline, and sucrose in 9:4:1 molar ratio to extract ginseng saponins from white ginseng. A solid phase extraction strategy based on HLB cartridges was used to recover the ginsenosides from the DES extracts. After extraction, lyophilization was carried out to recover and reuse the regenerated solvent up to three times. The regenerated DES was stable and only a slight reduction in the extraction efficiency was observed (the recycled DES had efficiency of 91.9%, 85.4% and 82.6% after first, second and third reuses, respectively). Lobo et al., (2012b) synthesized N-aryl phthalimide derivatives from phthalic

anhydride and primary aromatic amines by using two DESs (ChCl:urea and ChCl:malonic acid). After filtration of the reaction mixture solid product (N-aryl phthalimide) was separated and ChCl:urea was recovered and reused simply by evaporating the water. In case of the other reaction mixtures where DES (ChCl:malonic acid) was added as a catalyst in methanol, the filtrate obtained after separation of the solid product was subjected to removal of methanol by vacuum distillation and DES was recovered. Both of the recovered DESs had no significant decrease in their catalytic activity even after five times recycling. Recycling and reuse of DES was studied for 5-HMF synthesis by fructose dehydration in a biphasic system consisting of DES and organic phase (Zuo et al., 2017). Here both 5-HMF and ChCl were first extracted *in situ* by acetonitrile and later ChCl was crystallized by cooling to room temperature. The recycled ChCl was reused for 5 successive reactions without any loss of catalytic activity. Similarly, tetrabutyl ammonium chloride:polyethylene glycol was recycled four times by washing with organic solvent (diethyl ether) without losing its activity and stability after fuel desulfurization (Li et al., 2013a). Although there is scant information on DESs recycled and reused after biomass processing applications, there are a few recycling reports available in fuel and chemical processing industries. Different strategies published recently for DESs recycling are summarized in **Table 7**. It shows how DESs recycling is dependent upon their physicochemical properties, reaction conditions, and product characteristics.

Table 7. DESs application and strategies for their recycling and reuse.

S.No.	DES	Application	Method of Recycling	Recycling/ Recovery and reuse	Reference
1	Choline chloride 4-hydroxybenzyl alcohol , Choline	Switchgrass pretreatment for improved enzymatic saccharification	Ultrafiltration followed by evaporation	Three times recycling without any substantial loss in efficiency	(Kim et al., 2018)

	chloride catechol, Choline chloride vanillin, Choline chloride p-coumaric acid				
2	Ethaline 200	electrodeposition of metals	Nanofiltration	Five-fold concentration	(Haerens et al., 2010)
3	Choline chloride:urea	N-alkylation of aromatic primary amines	Biphasic extraction with immiscible organic ethyl acetate followed by drying in vacuum	Five times recycling with a slight decrease in the catalytic activity of DES	(Singh et al., 2011)
4		Nucleophilic substitution chemistry	DES was recovered from the aqueous layer of extraction at the end of the reaction, with care taken to remove the HCN generated in these reactions	Four times recycling, yield decreased from 89% to 73% after 4 cycles	(Sanap and Shankarling 2014)
5		Synthesis of dithiocarbamates	Water extraction	DES recycled several times with modest decrease in activity	(Azizi and Gholibegloo 2012)
6		Epoxide reaction (fixation of carbon dioxide with propylene oxide)	Simple filtration of the catalyst	DES retained the same levels of activity after 5 times recycling	(Zhu et al., 2007)
7		Halogenation (bromination of 1-aminoanthra-9,10-quinone)	Extraction with water followed by evaporation at 80°C under Vacuum	5 times recycling with no loss in activity	(Phadtare and Shankarling 2010)

8	Single-pot synthesis of coumarin styryl dyes	Separation of the DES with water, followed by concentration in vacuum	NR	(Phadtare et al., 2013)
9	Knoevenagel condensation (salicyl aldehydes with Meldrum's acid and other active methylenes)	Water extraction/concentration method	NR	(N. et al., 2011)
10	Synthesis of cinnamic acid via base-catalyzed reaction	Water extraction/concentration method	Four times recycling with little loss in activity	(Pawar et al., 2011)
11	Paal-Knorr synthesis of pyrroles and furans (synthesis of heteroaromatics via carbonyl condensation reactions)	Extraction of the products with ether and then brief drying in vacuum	Several times with little loss in activity	(Handy and Lavender 2013)
12	Pictet-Spengler reaction (synthesis of a wide range of β-carbolines)	Extracted using ether and brief drying in vacuum	Several times with minimal loss of activity	(Handy and Wright 2014)
13	Conversion of aldehydes to bis(indolyl)methanes	Extracted using ether and brief drying vacuum	Five times recycling with little loss in activity	(Handy and Westbrook 2014)

14		Synthesis of oxazoles and thiazoles	Products were extracted with methylene chloride	Five times recycling with no significant decrease in reaction yield	(Singh et al., 2013)
15		Thiazole synthesis	Via separation with water and concentration of the aqueous layer	DES could be recycled several times with minimal loss in activity	(Lobo et al., 2012c)
16		Multicomponent reactions for synthesizing spirocyclic products	Extraction with water followed by concentration in vacuum	NR	(Azizi et al., 2014)
17		Rapid preparation of α-aminoacyl amide derivatives via Ugi reaction (multicomponent reaction)	Extraction with water followed by concentration in vacuum	Three times recycling with slight drop in product yield	(Azizi et al., 2013)
18		Diels-Alder reactions	Decantation of the non-polar or less polar product layer to recover the DES	NR	(Yin et al., 2005)
19		Nucleophilic substitution (ring opening of epoxides with a wide range of nucleophiles, including thiols, anilines, methanol)	Extraction by diethyl ether	Three times recycling with only a modest loss in activity	(Azizi and Batebi 2012)
20	Choline chloride: $ZnCl_2$	Nucleophilic substitution with nucleophiles including anilines, amines, sulfonamides and	Extraction with water and then dried in vacuum	Four times recycling with no loss in activity	(Zhu et al., 2011)

		1,3-dicarbonyl compounds			
21	Choline chloride: $ZnCl_2$	Ketalization of carbonyls using 2,2-dimethyl-1,3-propanediol	DES could be readily recycled following separation of the product via extraction with ether.	NR	(Duan et al., 2006)
22	Choline chloride: $SnCl_2$	Preparation of either N-formylanilines or N-N'-diarylamidines starting from anilines	Simple extraction with ethyl acetate	NR	(Azizi et al., 2012)
23	Choline chloride: $ZnCl_2$	Kabachnik-Fields reaction of aldehydes, anilines, and phosphites carbonyl condensation reactions	Extraction using MTBE (Methyl Tertiary Butyl Ether) and drying in vacuum to recycle DES	Five times recycling with slight loss in activity, i.e., from 98% to 86%	(Disale et al., 2012)
24	Choline chloride: $SnCl_2$ and Choline chloride: $ZnCl_2$	Synthesis of bis(indolyl)methanes (bim)	Extraction with ether or ethyl acetate	NR	(Azizi and Manocheri 2012)
25	Choline chloride: $ZnCl_2$	Fisher indole synthesis	Sublimation	Three times recycling with reduction in activity from 91% to 72% and 34%	(Calderon Morales et al., 2004)
26	Choline chloride: tosic acid	Elimination of alcohols to afford alkenes and the transesterification of esters	Decantation of the alkene	Four times recycling with no loss in activity	(Handy 2015)
27	Choline chloride: tosic acid	Transesterifications of various vegetable oils (corn, soy, and canola)	NR	Four times recycling with a drop in efficiency from 85% to 50%	(Handy 2015)

28	Choline chloride: oxalic acid	dehydration of the carbohydrate inulin to form 5-hydromethylfurfural (HMF)	simple phase separation	Multiple times recycling	(Hu et al., 2009)
29	Combination of various ammonium salts and tosic acid	Fischer esterification of carboxylic acids.	DES could be readily restored by simple dehydration in vacuum and then reused	Eight times recycling with only a modest loss in activity	(De Santi et al., 2012)
30	Choline chloride: oxalic acid	Formation of bis(indolyl)methanes (bim) from aldehydes and indoles (carbonyl condensation type reactions)	Evaporation of the aqueous layer	Several times with little loss of activity	(Yadav and Shankarling 2014)
31	Tartaric acid: dimethylurea	Fischer indole synthesis	DES recovered from the aqueous layer via concentration in vacuum	Recycled three times with minimal loss of activity	(Gore et al., 2012)
32	Citric acid: dimethylurea	Synthesis of 1,8-dioxo-dodecahydroxanthenes.	DES recovered from the aqueous layer via concentration in vacuum	Six times recycling with little loss of activity	(Li et al., 2013b)
33	Choline chloride: malonic acid	Synthesis of 2,3-dihydroquinazolin-4(1H)-one derivatives	By recovery from the aqueous layer	Recycled several times with little loss of activity	(Lobo et al., 2012a)
34	Carbohydrate-derived DES	Rh-catalyzed hydrogenations and Pd-catalyzed Suzuki reactions (Cross-coupling)	NR	Two times recycling with considerable loss in reaction yields (94% to 66%)	(Imperato et al., 2005; Imperato et al., 2006)
35	Choline chloride:glycer	Synthesis of organolithium and	NR	NR	(Vidal et

	ol	Grignard chemistry (Organometallics)			al., 2014)
36	GPS-5 (composed of glycerol, l-proline, and sucrose at 9:4:1)	Extraction of polar ginseng saponins from white ginseng	Lyophilization of the aqueous solution of DES produced during the recovery of extracted compounds	Three times with extraction efficiencies of the DESs being 91.9%, 85.4%, and 82.6%, respectively	(Jeong et al., 2015)
37	Choline chloride: tetramethyl ammonium chloride (TMAC), tetrabutyl ammonium chloride (TBAC) were chosen as typical hydrogen bond acceptor (HBA), and malonic acid (MA), glycerol (Gl), tetraethylene glycerol (TEG), ethylene glycol (EG), polyethylene glycol (PEG), propionate (Pr), as hydrogen bond donor (HBD)	Fuels desulfurization	Washing with organic solvents, such as diethyl ether	Five times recycling with 99.48% extraction efficiency	(Li et al., 2013a)
38	Choline chloride: urea & Choline chloride: malonic acid	Synthesis of N-aryl phthalimide derivatives from phthalic anhydride and primary aromatic	DES ChCl: urea was recovered by simply evaporating water from the reaction mass after filtration of the solid product while for ChCl:	Five-time recycling with no loss in activity	(Lobo et al., 2012a)

	amines	malonic acid, the filtrate obtained after separation of solid product was subjected to removal of methanol by distillation under vacuum

NR: Not Reported

8. Comparison of DESs and ionic liquids

Both ionic liquids and deep eutectic solvents are considered as innovative solvents having the potential to transform the lignocellulosic biorefining to a green and sustainable industry (Lores et al., 2017; Tang et al., 2017). Both of these solvents offer several advantages over conventional solvents because of their versatility and industrially relevant physio-chemical properties which could be customized by rationally selecting its constituents (van Osch et al., 2017). ILs are salts composed of an organic cation and an organic/inorganic anion, with melting temperatures below 100°C and are often liquid at room temperature (Raj et al., 2016; Singh et al., 2015; Yoo et al., 2017). In comparison to ILs, DESs are novel solvents and research for their application in biomass processing is still in its nascent stages. Although, DESs and ILs share common characteristics but it is often claimed that DESs might offer several advantages over ILs. A comparison of both ILs and DESs at different parameters is summarized in **Table 8**.

Table 8. A Comparison of deep eutectic solvents and ionic liquids (Agrawal et al., 2017a; Loow et al., 2017; Lynam et al., 2017; Tang et al., 2017; van Osch et al., 2017; Yoo et al., 2017)

S.No.	Parameter	Ionic liquids (ILs)	Deep Eutectic Solvents (DESs)
1	Synthesis	Tedious synthesis with multi-step reactions and purification	Easy in preparation without any chemical reaction and purification step
2	Thermal stability	High stability and decomposition above 300°C to 430°C temperature depending upon the anion	Less stable than ILs with decomposition temperature about 200°C or below

3	Density	Low densities of most of the ILs (0.8-1.6 g cm^{-3})	High density (>1 g cm^{-3}). Hydrophobic DESs denser than hydrophilic
4	Viscosity	Low viscosity (10 mPa s to 726 mPa s)	High viscosity (>100 mPa s) and in some cases reach up to10,000 mPa s
5	Toxicity	Recalcitrant, poor biodegradability and toxicity increases with the increase in cation alkyl chain	Nontoxic, biodegradable and considered as 'green'solvents
6	Solubility	Solubilize cellulose and hemicellulose (up to 25 wt%) and lignin (up to 80 wt%) efficiently	DESs can solubilize lignin (up to 25 wt%) efficiently but cellulose and hemicellulose is sparingly soluble (<2 wt%)
7	Recycling	Difficult to recycle	Easy to recycle and reuse as compared to ILs
8	Cost	Expensive in nature	Less expensive than ILs

9. Challenges and Opportunities

Research in synthesis of DESs and their industrial applications is still in its infancy, with the first paper on the subject only published in 2001 (Smith et al., 2014). However, a significant surge in the number of research articles on this subject has been seen during the last decade with more than 1000 articles published in 2016-2017 (based on Sci-Finder data). DESs are considered as 'green' solvents that offer many advantages like ease of synthesis without any need of solvent and purification, low cost, biodegradability, and non-toxicity. They may solubilize high amounts of lignin from biomass but little cellulose and hemicellulose. Thus, DESs might play a critical role in selective solubilization and removal of lignin from biomass while keeping cellulose and hemicellulose intact for further processing with minimal losses of sugars. Biomass pretreatment with selected DESs (ChCl:oxalic acid) has been shown to remove more than 90% of lignin under

mild temperature and pressure with high saccharification yields (Procentese et al., 2015; Zhang et al., 2016). One disadvantage of DESs can be their higher viscosity, however, there is lack of understanding on this issue relevant to lignocellulosic biomass (van Osch et al., 2017). Future research in this area shall provide deeper insights for developing tailored DESs with low viscosity and high thermal stability suitable for wide industrial applications. Preliminary evidence suggests that they are relatively easy to recycle and maintain their catalytic activity. Life cycle analysis and techno-economical analysis needs to be carried out for DES pretreatment of lignocellulosic biomass. DESs need to be manufactured at an industrial scale for availability as low-cost green solvents. These recent findings suggest that deep eutectic solvents are promising alternatives to conventional solvents for upgrading lignocellulosic biomass.

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Author Contributions

AS and AJR conceived this study. AS and RA prepared the framework and wrote the first draft of all sections except biocompatibility. SB wrote the section on biocompatibility. JS participated in the critical analysis of the data and conclusions. AJR provided mentorship, planning and execution. All authors critically reviewed this manuscript. All authors read and approved the final article.

Competing Interests

Authors declare they have no competing interests.

Figure Captions

Figure 1. Phase characteristics of Lidocaine-Decanoic acid mixtures illustrating the formation of deep eutectic mixtures.

Figure 2. Analysis of recent literature available on DESs via SciFinder. A) Number of publications per year; B) distribution of number of publications under different sections.

Figure 3. Typical structures of hydrogen bond donors (HBDs) and bond acceptors (HBAs) for DES synthesis (Adapted with permission from (Xu et al., 2017)).

Figure 4. Schematic representation of reaction between DES (ChCl:Urea) and lignin (A) and; lignin carbohydrate complexes (B) (Adapted with permission from (Yongzhuang et al., 2017).

Figure 5. Cellulase activity in the presence of DESs with HBA as choline chloride and HBD as ethylene glycol (EG), glycerol (GLY) or malonic acid (MA) at various concentrations after (A) 24 h and (B) 48 h incubation times (Adapted with permission from (Gunny et al., 2015)).

Figure 6. DES pretreatment and recycling. A) Schematic process flow diagram for DES based biorefinery and B) Pictorial representation of i) DES components before reagent preparation; ii) DES after preparation; iii) rice straw pretreatment with DES; iv) lignin precipitate; v) recovered DES (Adapted with permission from (Kim et al., 2018; Mohd et al., 2017)).

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Fig. 1A

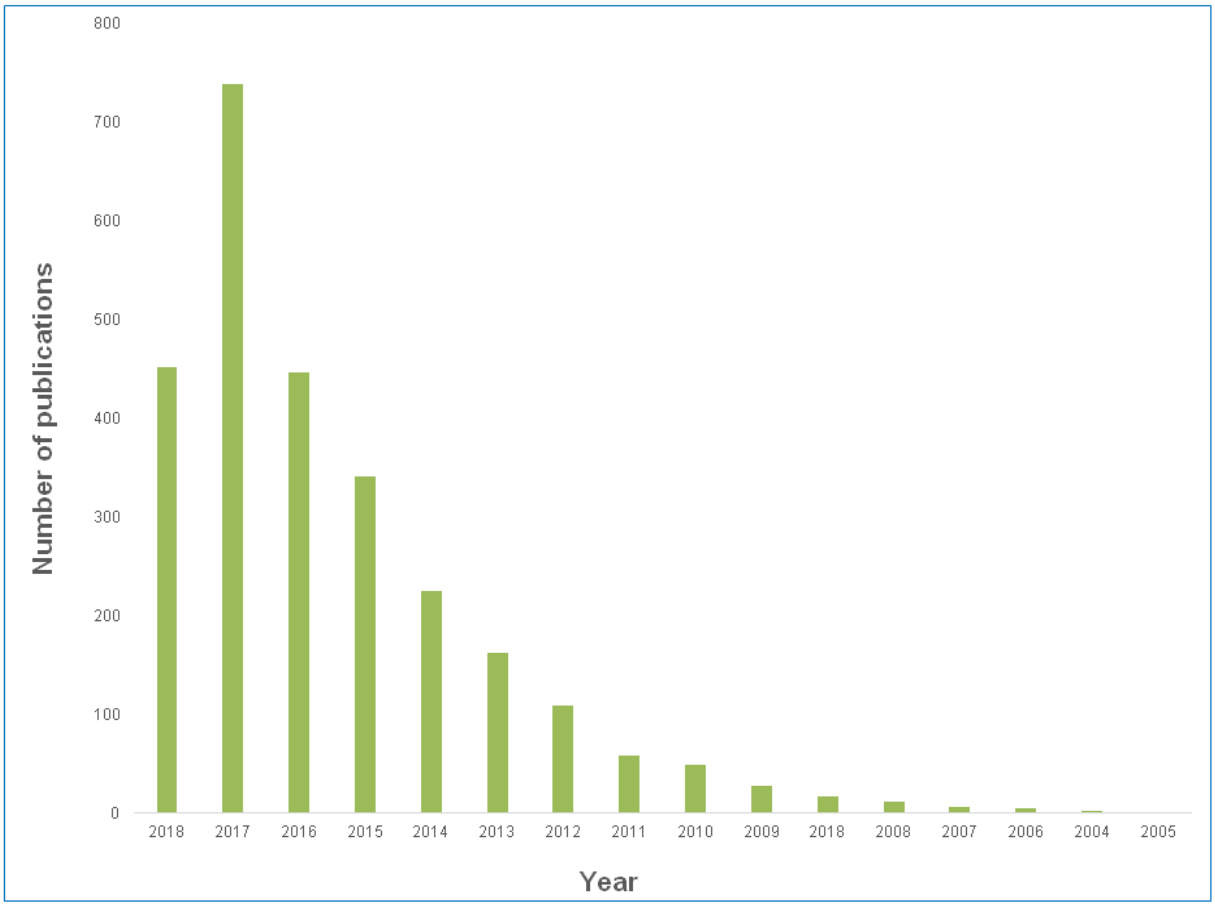
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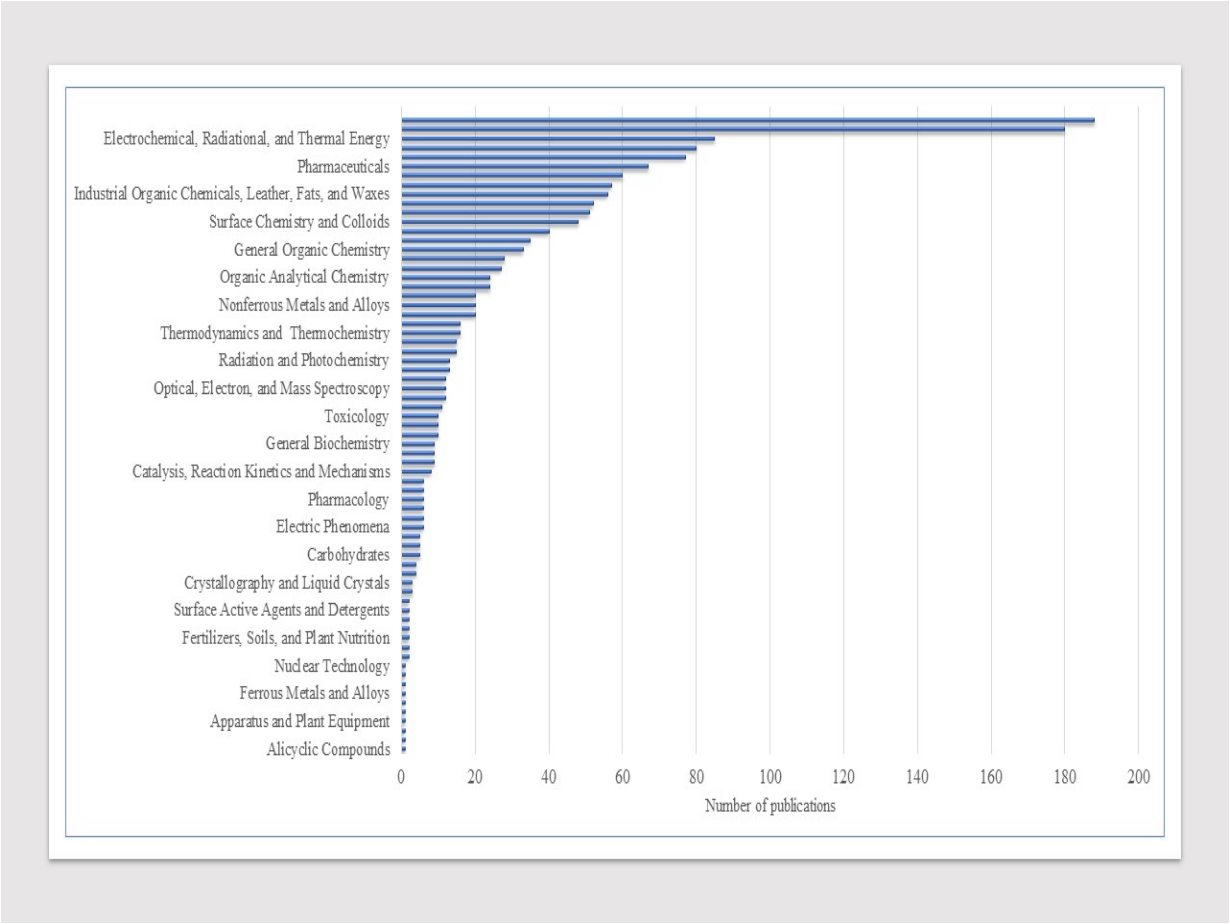
Fig. 1B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Pharmaceuticals
Industrial Organic Chemicals, Leather, Fats, and Waxes
Surface Chemistry and Colloids
General Organic Chemistry
Organic Analytical Chemistry
Nonferrous Metals and Alloys
Thermodynamics and Thermochemistry
Radiation and Photochemistry
Optical, Electron, and Mass Spectroscopy
Toxicology
General Biochemistry
Catalysis, Reaction Kinetics and Mechanisms
Pharmacology
Electric Phenomena
Carbohydrates
Crystallography and Liquid Crystals
Surface Active Agents and Detergents
Fertilizers, Soils, and Plant Nutrition
Nuclear Technology
Ferrous Metals and Alloys
Apparatus and Plant Equipment
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200
Number of publications

Fig. 2.

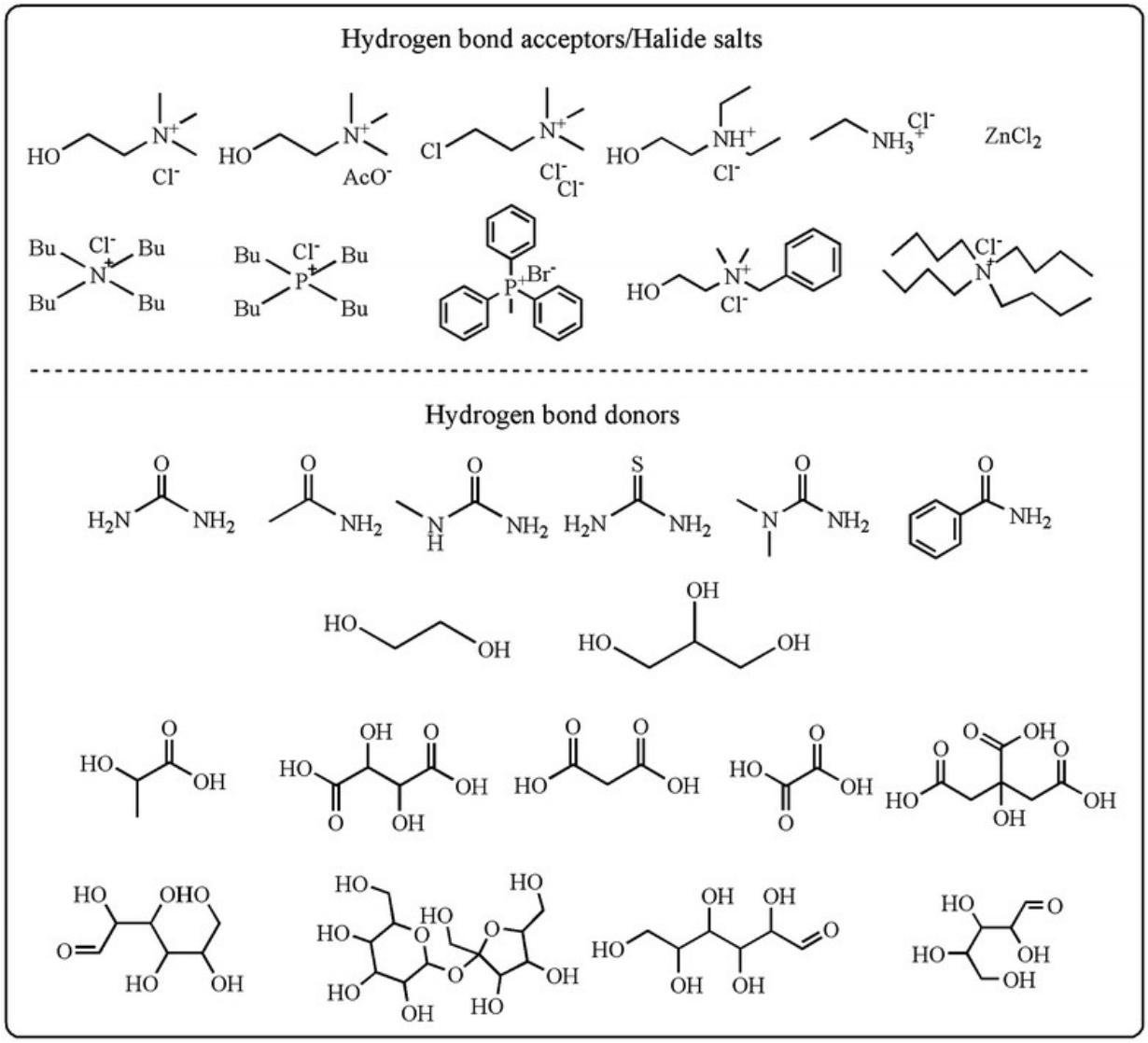
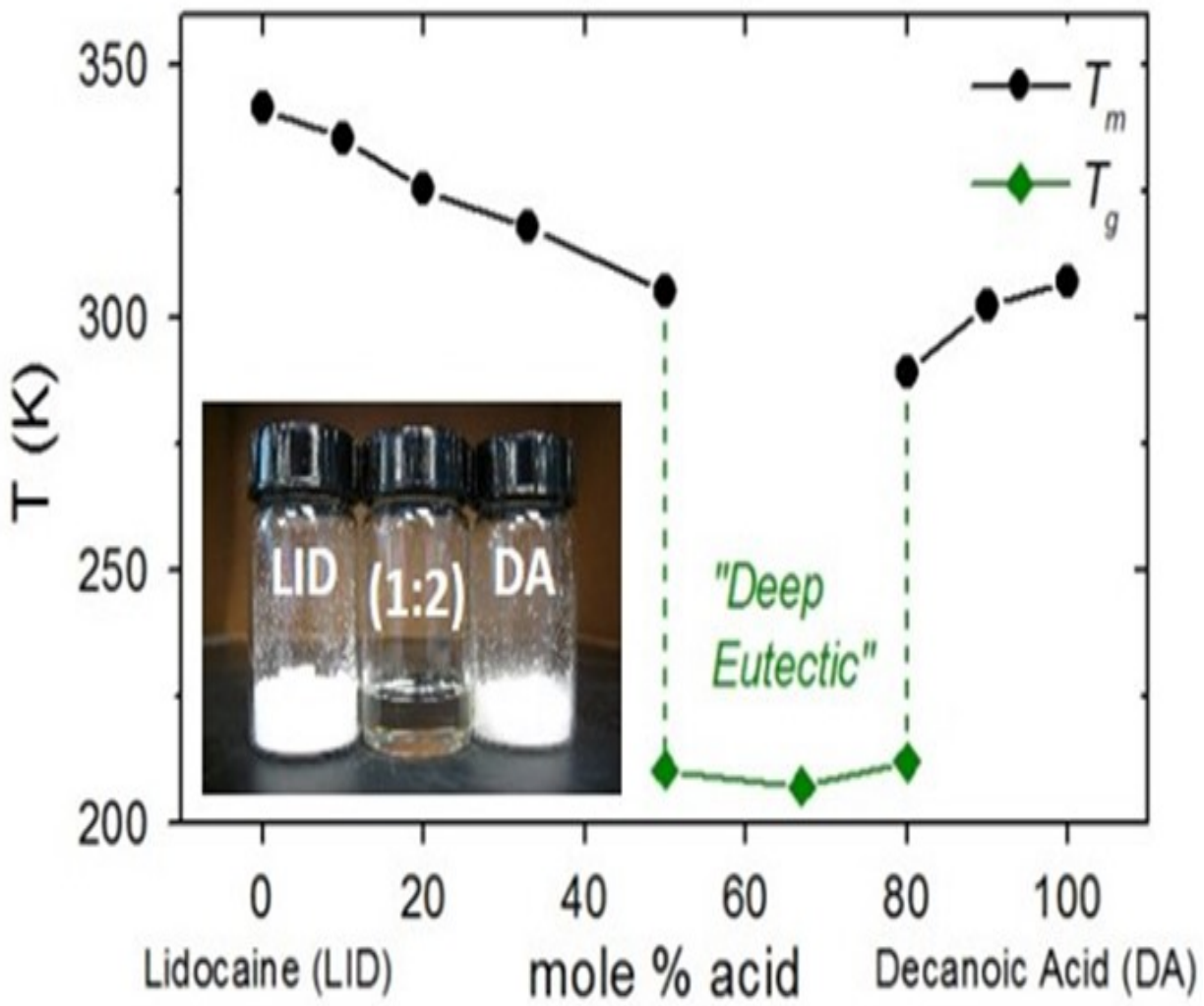

Hydrogen bond acceptors/Halide salts
Hydrogen bond donors

Fig. 3


350
300
250
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T (K)
T_m
T_g
LID
(1:2)
DA
"Deep
Eutectic"
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40
60
80
100
Lidocaine (LID)
mole % acid
Decanoic Acid (DA)

Fig. 4A

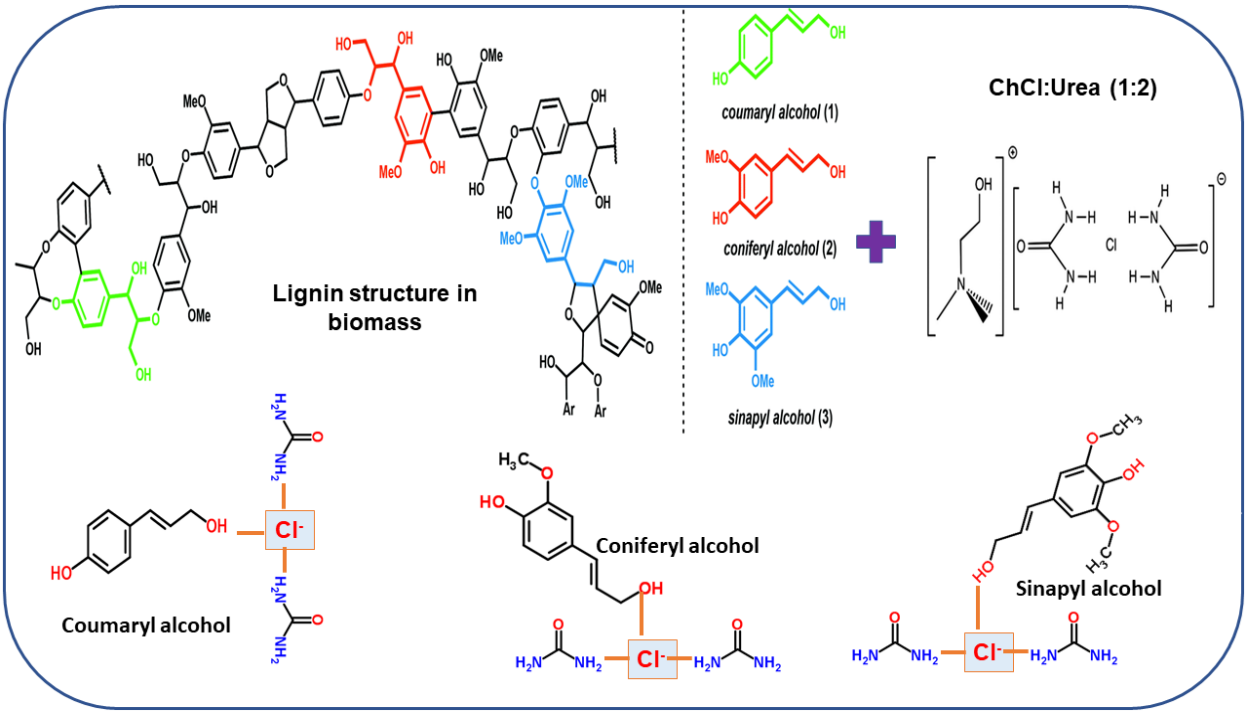

Lignin structure in biomass
coumaryl alcohol (1)
coniferyl alcohol (2)
sinapyl alcohol (3)
ChCl:Urea (1:2)
Coumaryl alcohol
Coniferyl alcohol
Sinapyl alcohol

Fig. 4B

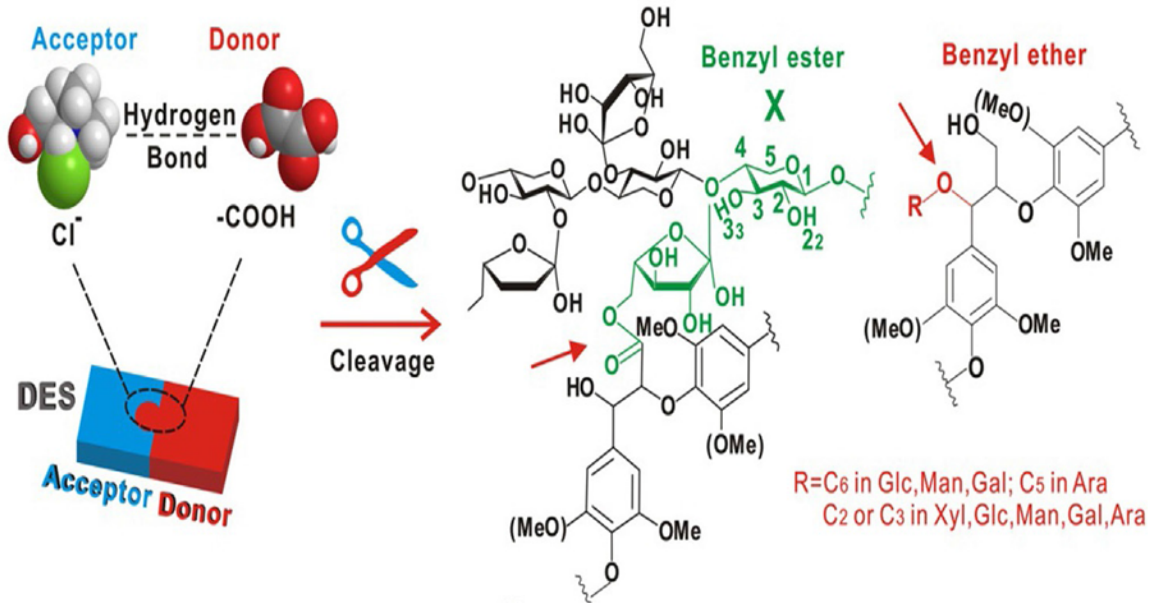
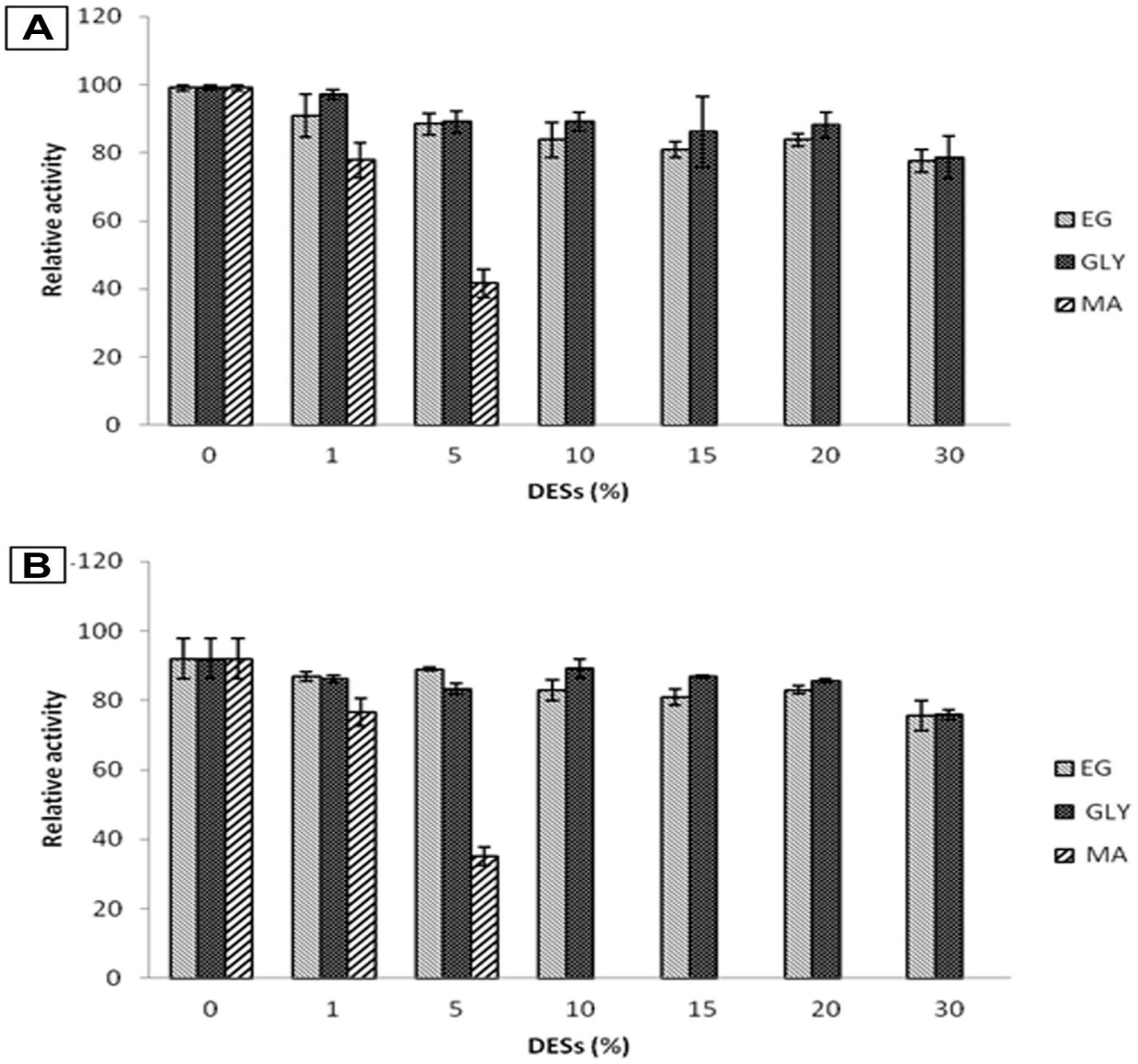

Acceptor
Donor
Hydrogen Bond
Cl-
-COOH
DES
Acceptor Donor
Cleavage
Benzyl ester
Benzyl ether
R=C6 in Glc,Man,Gal; C5 in Ara
C2 or C3 in Xyl,Glc,Man,Gal,Ara

Fig. 5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Relative activity
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Fig 6A

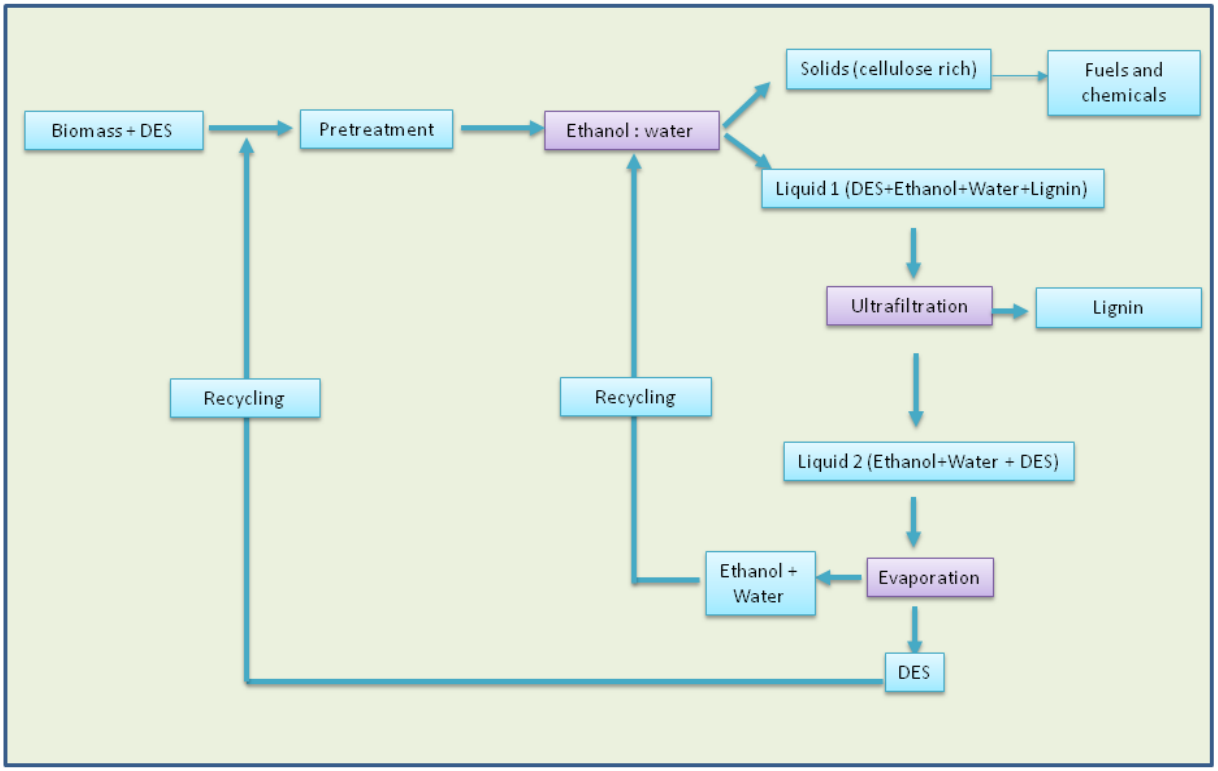
Biomass + DES
Pretreatment
Ethanol : water
Solids (cellulose rich)
Fuels and chemicals
Liquid 1 (DES+Ethanol+Water+Lignin)
Ultrafiltration
Lignin
Recycling
Recycling
Liquid 2 (Ethanol+Water + DES)
Ethanol + Water
Evaporation
DES

Fig 6B