Deep eutectic solvents in the pretreatment of feedstock for efficient fractionation of polysaccharides: current status and future prospects

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Abstract

The effective and efficient valorization of polysaccharide-rich feedstocks is undisputedly a key factor in the quest for a more sustainable planet and a biobased economy. Several covalent and non-covalent interactions within the biomacromolecules define the complex, intricate, and rigid structure of lignocellulose and related biomasses. However, none of the current methods to disrupt the complex interactions of feedstocks and enhance their hydrolysis for subsequent valorization are highly selective and efficient enough, hence the need for new methodologies in this respect. Deep eutectic solvents (DES) are special types of ionic liquids, which are cheaper, less toxic, and more biocompatible than other ionic liquids. They have been identified as important green solvents and functional fluids with immense potential for catalytic transformation of polysaccharide-rich biomass into a diverse range of products. The unique properties of DES enable them to effectively dissolve and convert biomass into valuable end products. This review paper sheds light on the application of DES in the deconstruction and fractionation of different feedstocks, particularly in the pretreatment step. In this regard, it covers the recent advancements in the applications of various DES in the valorization of biomass and its major components (i.e., cellulose, xylan, chitin, starch, pectin) with special emphasis on the relationship between the inherent properties of DES and their dissolution potential. Focus is also placed on the toxicity, biocompatibility, and reusability of DES. Finally, this article addresses the current and potential issues in the industrial application of DES, with a special focus on future opportunities.

Keywords Deep eutectic solvents · Polysaccharides · Biomass fractionation · Biocompatibility · Reusability

1 Introduction

Green chemistry, which is also referred to as sustainable chemistry, is an area of science and engineering, focused on the design and application of products and processes that eliminate or reduce the use and generation of hazardous substances. It is a relatively new concept in chemistry with the main objective to achieve cleaner, safer, and energy-efficient chemical processes. Hence, a significant area of green chemistry has also been dedicated to the discovery and application of new and alternative solvent systems for the efficient dissolution of difficult to dissolve and structurally delicate molecules to increase their functionality. In this regard, the use of deep eutectic solvents as green solvent/catalyst systems in different chemical processes has been a subject of intense research in the last decade.

Deep eutectic solvents (DES) are regarded as a special class of green solvents as a result of their unique properties such as high dissolution power, remarkable biocompatibility, low production cost, low volatility, relatively simple methods of synthesis, and significant stability. DES are also regarded as a subclass of ionic liquids (ILs) as they share a lot of comparable characteristics. However, compared to ILs they are far cheaper and more easily synthesized due to the lower cost of their raw materials; they are also less toxic and more biodegradable [1]. Hence, DES are currently attracting widespread interest as better alternatives to conventional ILs, and they are progressively replacing several hazardous

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and volatile organic compounds in many fields of science. In addition, the wide array of various compounds that can be selected for DES preparation has led to the full tailoring of their relevant properties as solvents. However, this wide variety of constituent compounds that can be combined to form DES has made the environmental friendliness of DES a matter of conjecture; for instance, DES have been shown to include salts of heavy metals which are well-known pollutants.

Chemically, DES are binary or ternary mixtures made up of a minimum of one hydrogen bond donor (HBD) and one hydrogen bond acceptor (HBA), strongly associated with each other through hydrogen bond interactions [2]. Consequently, the resulting DES solvents from the mixture have been noted to have lower melting points compared to that of their components [2, 3]. However, it has been noted that not all DES exhibit distinct melting points; some groups of DES rather exhibit glass transition temperatures and are referred to as low-transition-temperature mixtures [4].

DES are mainly classified based on the nature of the complexing agent used [5]. DES type 1 are formed from metal-Clx compounds (e.g., AlCl, $FeCl_2$, $ZnCl_2$) and quaternary ammonium salts. Examples of type 1 DES include the relatively well-known chloroaluminate/imidazolium salt melts and the ones formed with imidazolium salts and various metal halides that include AgCl, $CdCl_2$, $CuCl_2$, CuCl, $FeCl_2$, LiCl, $SnCl_2$, and $ZnCl_2$ [6]. Type 2 DES, however, are made up of hydrated metal halides and choline chloride. The popularity of type 2 DES is enhanced by their relatively low cost and inherent insensitivity to air or moisture [7, 8]. On the other hand, type 3 DES are formed from choline chloride and hydrogen bond donors including amides, carboxylic acids, and alcohols. They are particularly of interest because they can easily solvate a wide range of transition metal species, including chlorides and oxides [9, 10]. The last group of DES, type 4, is synthesized by incorporating a range of transition metals into ambient temperature eutectics based on organic cations centered on ammonium, phosphonium, and sulfonium moieties [5].

DES have been found valuable in various applications, both on an industrial and laboratory scale, due to their various inherent desirable properties. One of the major fields where the application of DES, like other ILs, has been most recorded includes analytical method development, extraction processes, isolation/fractionation of target compounds, purification of fuels, removal of environmental contaminants, and separation of azeotropes Pena-Pereira and Namieśnik [11]. DES, especially the hydrophilic DES, have been very useful in developing effective and efficient alternative technologies in the desulfurization and denitrification of fossil fuels [12]. Hydrophobic DES have also found wide applications, such as in water purification technologies, as they have been demonstrated to effectively remove micropollutants

from water. Their usefulness in water treatment is based on their low viscosity, even when in contact with water, enhancing mass transfer between them and the aqueous phase [13]. As a result, they have been widely used in extraction technologies to remove alkali and transition metallic ions such as $CoCl_2$, $CuCl_2$, $FeCl_2$, KCl, LiCl, $MnCl_2$, NaCl, $NiCl_2$, and $ZnCl_2$ from aqueous environments [14]. DES have also been noted to have potential as alternative reaction media for biotransformations including whole-cell and enzyme biocatalysis involving cellulase, lipase, and protease [15] and enhancing activity, regioselectivity, and stability in the process [16]. Hence, the application of DES in amidation, epoxidation, esterification, and transesterification reactions with various types of biocatalysts has enabled the synthesis of many important industrial products which include biodiesels, fatty acyl ethanolamides, sugar esters, phenolipids, etc. Recently, DES have also shown to be highly effective in the solubilization and functionalization of biomacromolecules including chitin, DNA, guar gum, spider silk, ε-poly-l-lysine, and polyhydroxybutyrate [17].

DES have been identified as potential key players in the exploration of both nonrenewable and renewable energies being very useful as cleaning agents for fossil fuels and in several steps during the production of alternative fuels. Various DES have been used in the area of alternative energy production, especially in capacitors [18], electrolytes for energy storage devices, and solar cells [19], as well as important components in fuel cells [20]. DES are also important in the biodiesel production industry, for removing residual glycerol, as efficient solvents and/or catalyst in esterification reactions [21, 22]. However, the functionality of DES in transforming lignocellulosic biomass into valuable energy products has been an area of major interest in recent times. The usefulness of DES has been mainly demonstrated in biomass processing, especially in the pretreatment of lignocellulose, conversion of sugars, and in biodiesel production. In biomass pretreatment, DES have been used as delignification agents owing to their well-known ability to dissolve phenolic compounds [23, 24]. Furthermore, DES pretreatment has been shown to enhance the extraction of energy-rich carbohydrate polymers, especially cellulose [25, 26] and xylan [27, 28] as well as their downstream treatments and processing. Hence, it is believed that the increased use of DES will circumvent the different operational stress conditions in the paper and pulp as well as the biofuel industries, as highlighted previously [29].

Although the field of DES is a relatively new area of research, various articles reviewing different sections of the field have been published. Recently, the extraction of chitin, chitosan [30], and phenolic compounds including lignin [31, 32] using DES was the focus of some review articles. Against this backdrop, this article takes a holistic look, for the first time, at the potential and current applications of

DES in the pretreatment of various polysaccharides including lignocellulosic biomass, pectinolytic substances as well as chitin and its derivatives. This paper also elaborates some physicochemical properties of DES by shedding light on their most desirable properties as well as their toxicity profile. Furthermore, the biocompatibility of DES with cells and enzymes as well as the current applications of these solvents in the pretreatment, modification, and extraction of various carbohydrate polymers is also discussed. Finally, the recyclability and reusability of DES, which has endeared it to the scientists, were also highlighted while charting a future course for their application in the bioconversion of lignocellulosic feedstock.

2 Physicochemical properties of deep eutectic solvents

DES are chemically tailorable solvents since they can be designed by properly combining various quaternary ammonium salts with various HBDs. Thus, task-specific DES with various physicochemical properties such as conductivity, freezing point, viscosity, and pH can be prepared. Hence, maximizing the prospective usefulness and applications of DES, especially in the pretreatment and treatment of lignocellulosic biomass, depends largely on the observation, understanding, and interpretation of their physicochemical properties. In this regard, the general physicochemical properties of DES are described and discussed below.

2.1 Conductivity

Conductivity is regarded as an indication of dissolved chemicals in a solution; hence, it is believed that the more dissolved ionic species existing and mobile within the solution, the greater the conductivity. It is a very important solvent parameter, especially regarding the control, design, and optimization of electrochemical processes [33]. DES have been noted to have lower conductivity when compared to other ionic liquids and other molecular solvents. This feature has been posited to be due to the large size of the ions and relatively free volume in the DES ionic systems (Smith et al., 2014). Furthermore, the relationship between conductivity and viscosity of DES has been demonstrated in many studies as both phenomena could be explained largely using Arrhenius equations [34]. It has been shown experimentally that conductivity is inversely proportional to viscosity for the simple reason that the motion level of the ions within the DES is driven by the solvent's consistency [35]. In the same vein, a positive linear correlation also exists between the molar conductivity of ionic liquids and the fluidity, which is the reciprocal of viscosity [36]. Thus, due to the relatively high viscosity of DES, they consequently display low

conductivity, and this low conductivity observed in DES is similar to the conductivity in the traditional ILs. The size of the DES components has been observed to affect the conductivity in DES, as small molecules are believed to have low electrical conductivity. For example, glycol-type DES were shown to have higher electrical conductivity than acid type DES [34]. It was also observed that the more polar, organic acid-based DES display higher conductivities compared to other DES [37]. DES conductivity is also known to increase with the increase in temperature, which was explained by decreased viscosity and the increased mobility of ionic species at increased temperature and hole mobility [38, 39]. Furthermore, the weight ratio of the salt, and hence the HBA, in the DES structure has been shown to affect the conductivity of the solvents positively. In this regard, in ethylene glycol (EG)-based DES, choline chloride EG had higher conductivity (7160 μs/cm) when compared to N,N-diethylethanolammnium chloride-:EG (5270 μs/cm) [35]. For some DES, especially the ternary natural DES, a relationship between the conductivity and water content has also been observed. With very low water content, an increase in conductivity was observed, but the conductivity began to reduce with increasing water content, thus obeying Gaussian theory [40, 41]. Although many studies have been able to fit the dependence of DES conductivity on temperature into the Arrhenius behavioral model, many subsequent studies have since demonstrated that the Vogel–Fulcher–Tammann (VFT) equation explains it better for most DES [37, 42]. Hence, it is safe to assert that the VFT equation gives an adequate representation of this phenomenon which indicates that the ion-conducting motions are governed by the glassy dynamics [34, 43].

2.2 Density

Density is considered a very important factor with solvents process design and is significantly affected by temperature and pressure effects. Most DES have been shown to have densities greater than water, with their density values ranging from 1.0 to 1.35 g cm^{-3} at room temperature [44]. Various factors have been highlighted in different studies to be responsible for the densities of DES. Most of these studies were based on wet-lab experiments, molecular modelling, and statistical analyses of large datasets [5, 45, 46]. The nature of the HBD has been identified as one of the major factors that affect DES density [47]. It was demonstrated that the molecular characteristics of the donor and the molar ratio at which the DES is formed significantly affect the density of the DES and its temperature variation [5]. In addition, it was also demonstrated that the density of DES varies with metallic salt components, as well as the salt to HBD ratio [48]. These effects are particularly more pronounced in type 4 DES, as they have been noted to have higher densities

than other classes, with ranges between 1.3 and 1.63 g cm^{-3} [38]. This significant difference may be attributed to a different molecular organization or packing of the DES, and the phenomenon may be explained by the hole theory. This theory assumes that on melting, ionic substances contain empty spaces (holes) that emerge from thermally generated fluctuations in local density [49]. These holes are usually of random location and size and undergo constant flux [35]. As expected, the density of DES decreases with increasing temperature, resulting from the increase in activity and molecular mobility, which result in the increased molar volume of the solution, consequently reducing the density [50].

2.3 Melting point

As highlighted earlier, DES are produced from the mixture of two solids capable of generating a new liquid phase by self-association through hydrogen bonding. A lower melting point generally characterizes this resulting phase compared to that of individual constituents. For example, when ChCl and urea are mixed in a molar ratio of 1 to 2, the melting point of the resulting eutectic solvent is 121 °C, which is significantly lower than that of ChCl (302 °C) and urea (131 °C). The significant depression of the melting point is a result of the interaction between the halide anion from ChCl and the HBD component, which is urea in this case [38]. The charge delocalization occurring via the hydrogen bonding between the HBA and HBD and donor moieties is responsible for decreasing the melting point of the mixture, relative to that of the individual components [51]. Hence, the difference in the melting point at the eutectic composition of a binary mixture of A and B, relative to that of a theoretical ideal DES mixture, is directly proportional to the magnitude of the interaction between the 2 components; thus, the larger the interaction, the larger will be the difference [5]. It was observed that the majority of DES reported so far have melting points below 150 °C; however, a melting point of below 50 °C for DES is more desirable as it will facilitate their use in many analytical and industrial processes [52]. Although a wide range of amides have been used in combination with ChCl to synthesize DES with a melting point less than 50 °C, it is important to note that there are relatively fewer DES that are liquid at room temperature [5].

The choice of HBD has been proposed as an important factor in the production of DES with low melting points. HBDs with the ability to form stronger hydrogen interactions with ChCl are believed to form liquid DES at room temperature; some of these include urea and 2,2,2-trifluoroacetamide [53]. Furthermore, the nature of the organic salt, organic salt/HBD molar ratio, as well as the anion of choline-derived salts, has been noted to affect the melting/freezing points of the corresponding DES [54]. For example, when urea is the HBD and mixed with different ammonium salts in a ratio

of 2:1, the produced DES were observed to display varying melting points ranging between 38 and 131 °C. Similarly, for instance, the melting point of a choline salt-urea derived DES was noted to decrease in the order $F > NO_3 > Cl > BF_4$ [55].

2.4 Polarity

The polarity of a solvent is a term typically used to define the capacity of a solvent for solvating dissolved charged or neutral (apolar or dipolar) species. Since polarity is the main indicator of a solvent's strength, most solvents are usually grouped based on their ability to dissolve polar and/or charged species [56]. Polarity is generally a solubilization property, and it has been observed to be directly proportional to the intermolecular attractions. However, the phenomenon has been very difficult to define and express quantitatively as no single macroscopic physical parameter could entirely account for the various solute–solvent interactions at the molecular level [57]. However, the most common approaches for assessing DES polarity are based on solvatochromic optical spectroscopic responses of several UV–Vis absorbance as well as molecular fluorescence probes [58]. Recently, an inverse relationship was observed between the polarity and pH of some PEGylated DES, prompting the author to propose the quantification of DES polarity through pH measurement [59].

It has been demonstrated in various studies that the chemical structure and the nature of the HBA and the HBD in DES definitely control their inherent dipolarity/polarizability [57, 58]. Results from photoinduced charge-transfer solvatochromic probes revealed the major effect of H-bonding ability in addition to other solute–solvent interactions present within DES as it was demonstrated that ethaline and glyceline DES had more polarity than the other evaluated DES [58]. This was attributed to the presence of OH-groups on 1,2-ethanediol and glycerol, which were the H-bond donors used to prepare ethaline and glyceline, respectively [58]. The polarity of acid-based DES was also found to be higher than that of amide- and ammonium-based DES in a previous study, and it was posited that the observation was probably a result of the polar carboxyl groups in acids [59]. In addition, the effect of dilution on DES polarity has also been shown. It was demonstrated that the addition of 25% water to different DES, including ChCl/triethylene glycol and ChCl/polyethylene glycol 200, increased their polarity and molar transition energy [60]. However, it was revealed that unlike in most other ILS where the dipolarity/polarizability is uniformly high, irrespective of cations and anions' structures, the molecular structure of the hydrogen bond acceptor has a significant effect on DES dipolarity/polarizability [57]. Recently, polarity

has been demonstrated as a factor that affects the extraction performance of DES, as the parameter was noted to directly impact the capacity of DES to dissolve target solutes [61]. However, DES polarity was previously shown to have no significant effect on the catalytic activity of incubated enzymes [62].

2.5 Surface tension

Surface tension is considered as the measure of the energy needed to increase a liquid's surface area by a unit area. It is ascribed to the liquid's intermolecular attractive forces, which results in cohesive tension that decreases the surface area of the liquid's interface with other phases in contact with the liquid. Various industrial processes such as fluid flow, mixing, and separations require accurate estimation of surface tension in unit design and operations [50]. Specifically, surface tension has been noted to determine the suitability of DES in interfacial processes in which mass transfer occurs [63]. The surface tension of DES depends largely on the composition as well as the temperature of DES mixtures. It was observed especially in glycerol- and phosphonium-based DES that an increase in temperature reduces the surface tension cohesive forces attributed to the increase in the thermal activity and kinetic energy of the DES molecules [50, 63, 64].

Different studies have also highlighted the effect of the DES components and their interactions on the surface tension. In this regard, it has been demonstrated that both the HBD and HBA have significant effects on the surface tension of DES. For instance, the DES prepared from ammonium salts (tetra-n-butylammonium bromide and ChCl) with ethylene glycol as HBDs have almost equal surface tension as glycerol-based DES and amines-based DES prepared from phosphonium salts [50]. Similarly, sugar-based DES are also within the range of surface tension of phosphonium-based DES, such as methyltriphenylphosphonium bromide:EG and benzyltriphenylphosphonium chloride:EG [65, 66]. Furthermore, within the same family of DES with similar constituents, the higher molecular weight DES displayed higher surface tension [64]. The presence of crystal water in the DES salt component has also been shown to decrease the surface tension of DES; however, surface tension has also been observed to increase when the water mole fraction was increased [67]. Statistical and computational methods have also been found useful in lieu of wet-lab experiments in predicting the DES surface method. In a previous study, a percentage error of only 2.57% was observed between the experimental and predicted method, hence confirming the efficiency of the methods in predicting DES surface tension at varying temperatures [68].

2.6 Viscosity

Owing to their potential applications as green solvents and catalysts, DES with low viscosity are more desirable and applicable in the industry. Generally, it has been observed that the viscosities of DES are majorly affected by factors that include the chemical nature of their components (nature of ammonium salts and HBDs, molar ratio, etc.), the free volume, and the temperature as well as the water content [69]. For instance, the viscosity of ChCl-based DES largely depends on the type of the HBD; in this regard, ChCl/EG (1: 4) DES exhibits the lowest viscosity (19 cP at 201C). The use of derived sugars, such as xylitol, sorbitol, or carboxylic acids such as malonic acid, leads to high viscosities [38]. The viscosity of most eutectic mixtures has been established to be a function of the temperature. Like other ILs, DES viscosity is inversely proportional to the temperature, which is in accordance with Arrhenius-like behavior [70]. Hence, it has been noted that the viscosities of DES are typically much higher than those of most common molecular solvents. Therefore, the hole theory can also be employed to design DES with relatively low viscosities. For example, it was noted specifically that using small cations or fluorinated hydrogen bond donors may lead to the formation of DES with low viscosity [5]. It was also noted that the viscosity of binary eutectic mixtures is essentially governed by hydrogen bonds, van der Waals, and electrostatic interactions. Furthermore, a significantly linear relationship has been observed between the molar conductivity of DES and their viscosity when measured in terms of fluidity [36]. On the molecular level, the high viscosity of DES is typically a result of an extensive hydrogen bond network between the individual components, restricting the mobility of free species within the DES [5]. Recently, using mathematical models, a correlation was developed based on a large data bank, including 553 data points, from 112 different DES. The statistical analysis of the model results showed that the correlation was significantly accurate, unbiased, and displayed a normal distribution behavior [71].

3 Toxicity of deep eutectic solvents

An ideal green solvent ought to meet important requirements such as affordability, biodegradability, recyclability, sustainability, and, more importantly, non-toxicity [72]. It has been agreed that DES display some of these required properties as well as excellent applicability and potential in a wide range of processes and reactions. However, their total acceptability is limited, especially in biological and industrial applications, due to the lack of toxicological data. Although several reports have highlighted the green characteristics of DES, the earliest of those conclusions were made based on the

toxicity profile of the individual components that make up the DES. However, these assumptions are not scientifically justified, as studies have always shown that DES usually display different and special properties that neither of their components possess. Hence, it is important to consider the synergistic effects that may result from the components of the DES through their physical or chemical interactions. Generally, as expected, different investigations have shown that natural deep eutectic solvents (NADES) are less toxic than other DES and other ILs [73]. NADES are the class of DES whose constituents are primary metabolites such as amino acids, organic acids, sugars, and choline derivatives; examples of NADES are ChCl:xylose, ChCl:tartaric acid, ChCl:citric acid, citric acid:glucose, etc. It has been suggested that the charge delocalization occurring through hydrogen bonding within the components of DES is expected to make it more toxic compared to the individual components since chemicals with delocalized charges are usually more toxic than the ones with localized charges [74].

As pointed out previously, DES may offer a "greener" alternative to other traditional ILs, but they cannot be considered absolutely "green.. It was proposed by Smith and Abbott [5] that some DES should be inherently non-toxic because of their benign constituents which have been documented to exhibit little or no toxicities. Hence, based on this "constituent effect" premise, types 1, 2, and 4 DES which contain metal salts with their innate toxicity, are more likely to be toxic. In contrast, type 3 DES is typically made up of various amides and polyols such as erythritol, ethylene glycol, fructose, glycerol, and urea, which exhibit low inherent toxicity which is expected to be less toxic [5]. However, subsequent studies have also suggested that the composition of DES may influence the cytotoxicity and selectivity of DES by varying the component salts-HBD and their molar ratio [75, 76]. Furthermore, studies have also demonstrated that DES have lower vapor pressures than most molecular solvents which reduce the rate of emission into the atmosphere; however, they are partially miscible with water which could lead to them inevitably ending up in the different aquatic environments [77, 78]. However, it was noted that lots of conflicting results had been reported concerning DES toxicity. Many of these reports have highlighted the evaluation of DES toxicity using a model microorganism, especially bacteria and fungi, as well as model plants and animals, including invertebrate and vertebrate animals (Fig. 1).

The toxicity of phosphonium-based DES against various bacteria, both Gram-positive and negative, showed no toxic effects on the studied microbes, including *Bacillus subtilis*, *Escherichia coli Pseudomonas aeruginosa*, and *Staphylococcus aureus* [79]. However, various cholinium-based DES were antibacterial and toxic against *Vibrio fischeri* cells in a subsequent study [80]. Recent results showed the non-toxic effect of choline chloride-based DES on *E. coli* at concentrations up to 450 mM; however, at concentrations above 600 mM, the DES becomes toxic, causing complete inhibition of cell growth [81]. However, the observed toxicity at increased concentration was mainly attributed to the high acidification of the growth media due to DES hydrolysis during cellular growth [81].

The effects of DES on yeasts are considered imperative as yeast cells are key factors in the processing of saccharified biomass into biofuels via alcohol fermentation as well as in other industrial processes such as winemaking. Hence, investigations made so far have shown that DES exhibited little or no toxicity on yeast cells [82, 83]. One such study showed that sugar- and alcohol-based DES displayed no toxicity on oenological yeast; the cells were further shown to be tolerable to DES when compared to conventional solvents such as glycerol and DMSO [83]. In addition, *Saccharomyces cerevisiae* was demonstrated to efficiently utilize sugars for ethanol production in DES medium [84].

Similarly, the toxicity profile of various DES against different model fungi has also been evaluated. In this regard, the toxicity of various DES was investigated on four different fungi, including *Aspergillus niger*, *Candida cylindracea*, *Lentinus tigrinus*, *and Phanerochaete chrysosporium* [85]. It was observed that the toxicity of the studied DES against the fungi varied according to their composition, with ChCl:ZnCl$_2$ showing the highest toxicity followed by the acidic group (malonic acid and p-toluenesulfonic acid) [85]. Previously, the same author had established the anti-fungal activity of some DES on *Aspergillus niger* and showed that type 1 (organic salts and metal salt) DES were the most toxic, while type 3 DES displayed the least toxicity, which is in agreement with Smith and Abbott [5]. The hydrogen bonding or complexation after DES formation proved to affect their toxicity level towards organisms even after dissolving in an aqueous solution [76].

Toxicity studies of different DES using multicellular organisms and cell lines have also shown varying results. Results on phytotoxicity showed that the tested DES are non-toxic with seed germination with EC$_{50}$ values higher than 5000 mg/L [86]. However, cholinium-based DES comprising choline acetate or choline chloride as the salt acetamide and ethylene glycol, glycerol, and urea as the HBD were shown to be toxic against garlic plants causing the deformation of the nucleus and cellular disintegration/collapse in some cases [87]. Various results from non-toxicity to moderate toxicity to high toxicity have been reported using animal models. However, most of the results point out that the cytotoxicity and selectivity of the DES on the living cells can be influenced by the composition of the DES by varying the salt/HBD combination and the molar ratio [75]. It was shown that different ammonium-based DES using in vitro cell lines and in vivo animal models did not elicit any DNA damage, but the DES were believed to have
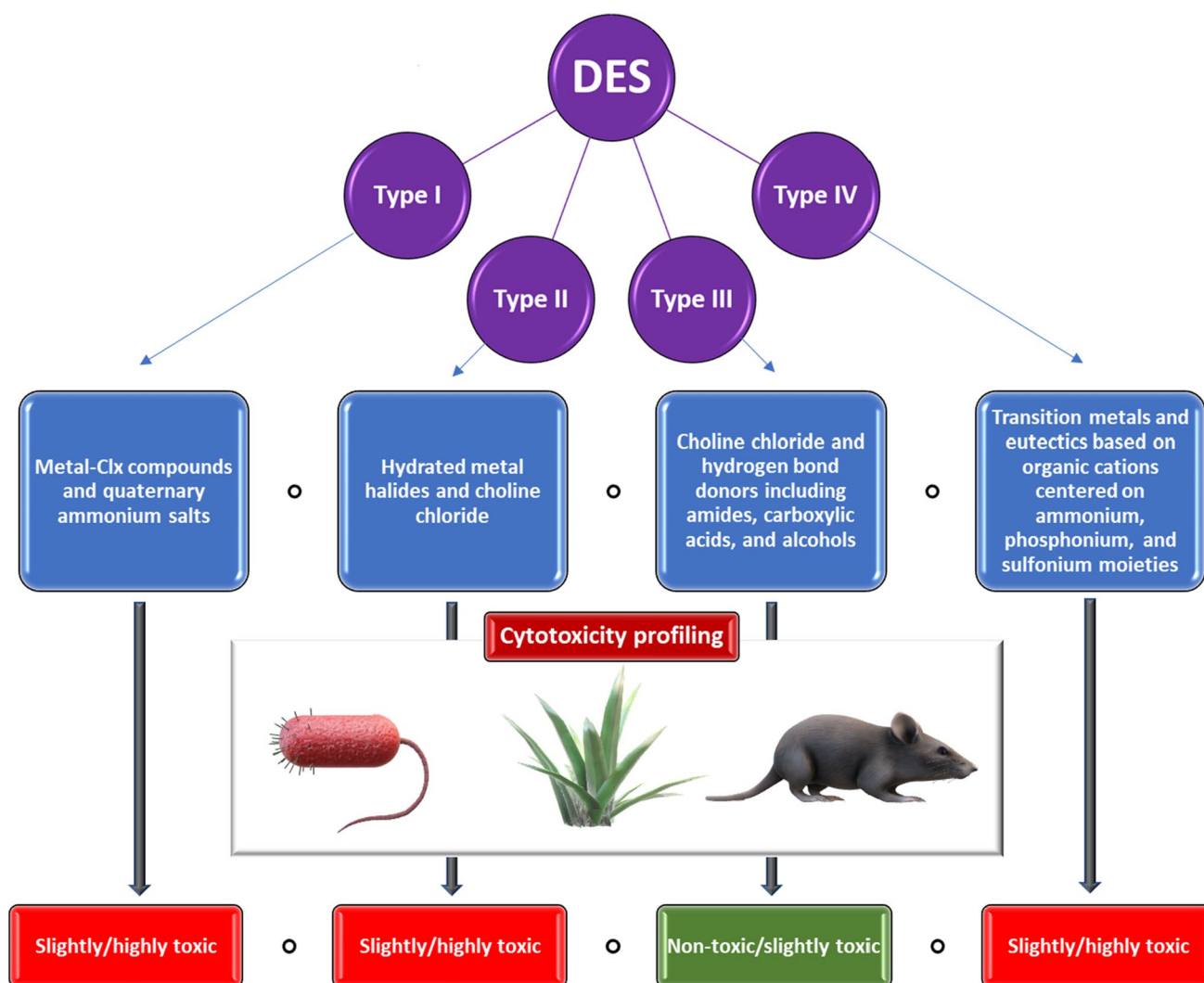
Fig. 1 Types of DES and their cytotoxicity profiling

enhanced reactive oxygen species (ROS) production in the studied cells; however, these DES showed less cytotoxicity compared with conventional ILs [75]. Very low toxicity was shown for choline chloride:glucose and choline chloride:glycerol ($EC_{50} > 10$ mM for fish and human cell line, respectively), while moderate toxicity was displayed by choline chloride:oxalic acid (EC_{50} value 1.64 mM and 4.19 mM for fish and human cell line, respectively) [86]. The study further established important roles played by HBDs with regard to DES cytotoxicity [73].

Different mechanisms have since been proposed for the observed toxicity of DES against various living cells. For example, it was proposed that the major route of toxicity was through free radical generation, eventual oxidative stress, and the eventual apoptosis of the cells [73]. In addition, the interaction of DES with cellular membrane has also been posited as a possible mechanism. It is believed that

DES may in some cases penetrate the cells or may even aggregate on the cell surface, resulting in a disruption of the cell wall, irreversible damages to the cellular enzymes and other proteins, and eventual cell death [88, 89]. However, in some cases, the toxicity observed with some DES has been ascribed to other possible reasons besides the biochemistry of the solvents. For instance, the cytotoxicity of phosphonium-based DES in brine shrimps was believed to be partly due to a lack of oxygen or the restriction in the mobility of shrimps due to the high viscosity of DES [90]. Hence, with regard to the aforestated, it was suggested that in cases where the toxicity of the DES components is higher than that of the resultant DES, purification of the DES to remove the constituents would lead to reduced toxicity [91]. In addition, a new direction in the synthesis of DES is proposed, with particular focus on the use of natural and less harmful synthons or substrates, such as amino acids which are expected

to show very low toxicity to living cells and the environment in general [92, 93].

4 Biocompatibility of DES

Most of the conventional solvents and reagents used in biomass pretreatment have been incompatible with downstream processing, such as enzymatic saccharification and microbial fermentation, mainly because of the different pH requirements, the toxicity of the chemicals, and undesirable by-products generated from the pretreatment process [84]. For instance, pretreatment of biomass usually involves the use of acidic or basic conditions to disrupt the plant cell wall and/or decrystallize cellulose for enhanced enzyme accessibility, and these conditions have been recognized with numerous disadvantages [94]. However, the advent DES has presented a promising option in biomass pretreatment due to their numerous advantages over other ILs and solvents. In addition to typical features of DES, such as low vapor pressure, low cost, low toxicity, biodegradation, and revived capacity, the remarkable biocompatibility of DES with industrial microorganisms and enzymes has also been identified as a huge advantage [32]. In this regard, DES have been shown many times to have little or no negative effects on the enzymes or microbes used in biomass processing, even after the adjustment of pH. Together, all of these remarkable properties are believed to allow for an integrated one-pot biomass-to-biofuel bioconversion process which can combine pretreatment, saccharification, and fermentation in the same vessel [95]. Usually, DES are synthesized from natural, renewable, and non-toxic precursors, giving rise to a large selection of biocompatible and biodegradable DES [96]. For example, choline chloride, which is unarguably the most widely used precursor for DES preparation, has demonstrated high biocompatibility with a vitamin B complex derived from cellular metabolites and has found application as a food additive [97]. As a result of their relatively high compatibility, DES have been used in many other industrial processes such as bioactive compound extraction, biorefinery, electrochemical applications, and especially lignocellulose dissolution [5, 13]. Furthermore, various studies have concluded that DES typically simplify and reduce the harshness of pretreatment processes without compromising the expected product yield [95, 98].

The constituent compounds and the cellular organization of source organisms for the biomass, especially in plants, have been identified as important factors in determining the biocompatibility of DES [57]. Hence, various results have been recorded in different plant studies due to differences in cell wall composition which may or may not favor the interaction of DES with proteins and phospholipids. Although water is the most used solvent since time immemorial, it is usually effective in extracting polar and hydrophilic bioactive compounds. However, DES and other eutectic mixtures have demonstrated huge effectiveness in extracting a wide range of bioactive extractions from natural matrices, most often plants because of the interest in their use in pharmaceutical, cosmetic, agrochemical, and food industries. These bioactive metabolites include alkaloids, catechins, essential oils, flavonoids, xanthones, saponins, etc. [99, 100]. It was also posited that some DES could act as osmoprotectant matrix, in which cellular activities may occur relatively unhindered in harsh environments such as cryogenic temperatures or the total absence of water [101]. Hence, DES can serve as alternative reaction media to water in biochemical processes mimicking metabolites and lipids of the cellular environment while at the same time preserving the protein structure and enhancing the actions of cofactors [2].

Thus, many studies have demonstrated clear evidence that DES are biocompatible with numerous enzymes, both in crude and purified states as well as whole-cell microorganisms, supporting biocatalysis and bioconversion under relatively mild conditions of pH, pressure, and temperature. The evaluation of choline-based DES as solvents for yeast-mediated reduction of ethyl 3-oxobutanoate showed that the DES were biocompatible with baker's yeasts and had immense potential in yeast-mediated bioreduction [102]. Subsequently, it was also demonstrated that various DES were effective in corn stover pretreatment and that they were biocompatible with the yeast used in the subsequent alcoholic fermentation. Furthermore, the DES demonstrated to not inhibit the growth of the yeast, and the level of inhibitors generated was below the reported toxicity thresholds [84]. Earlier studies have also established the very low, and sometimes undetectable levels of inhibitors, such as acetic acid, furfural, and hydroxymethylfurfural, when DES served as the pretreatment solvent; furthermore, the observed levels were way below than the suppression amounts for common yeasts [103, 104]. Other significant results have also been recorded for whole-cell biocatalysis with DES. These include the enantioselectivity inversion in yeast-mediated bioreduction of aliphatic and aromatic ketones [105], enhanced bacteria biotransformations [106], as well as improved overall yield of multistep synthetic processes [107].

Different studies have also evaluated the impact and compatibility of microbial enzymes with DES with results ranging from positive to negative [2, 108]. Ethylene glycol-based DES and glycerol-based DES (10% v/v) have been shown to retain above 90% of the original activity of *Aspergillus* sp. cellulase [108]. Furthermore, the study showed that both the DES system displayed higher glucose production and lower energy consumption than the alkali solvent system. Even at higher concentrations, DES are biocompatible with various biomass-degrading enzymes. In this regard, concentrated

solutions (85% w/w) of three DES (choline chloride:boric acid in the molar ratio 5:2, choline chloride:glycerol 1:1, and betaine:glycerol 1: 1) were shown to have various degrees of biocompatibility on *Trichoderma reesei* cellulases and xylanase. While betaine:glycerol displayed the highest biocompatibility, the choline chloride:glycerol DES displayed mild inhibition, while choline chloride:boric acid (5:2) was highly against the enzymes. Thus, these studies highlight the different effects of the glycerol component as well as the HBAs in the DES biocompatibility [109]. Moreover, many components of various DES have been shown in various studies to inhibit and inactivate different enzymes and proteins. For example, boric acid has been shown to inhibit urease activity [110], while urea inhibited various biomass-degrading enzymes such as cellulase [111] and laccase [112]. In addition, there exists the probability that when combined with other benign compounds to form a DES, the inhibitory effect can be reduced several-fold [113].

5 Deep eutectic solvents for the pretreatment, modification, and extraction of polysaccharides from different feedstocks

Polysaccharide extraction from various biomasses and their multiple valorizable applications have been well documented. Pretreatments such as steam, hydrothermal, extrusion, dilute acid, and ammonia-based methods have all been shown to improve biomass digestibility and polysaccharide recovery [114]. However, environmental and economic concerns on the use of traditional chemical methods for polysaccharide extraction have necessitated the need to look at alternative solutions that are cleaner and less harmful to the environment [115]. DES is a class of green solvents with the potential to selectively solubilize a range of polysaccharides, which offers a great alternative to traditional chemical methods and ionic liquids, due to their cost-effective synthesis, non-/low toxicity, and biodegradable properties [116]. Interestingly in the last decade, the use of DES for isolation and extraction of numerous polysaccharides such as cellulose, xylan, chitin, starch, and pectin have been thoroughly studied which corroborates the tremendous potential of DES for polysaccharide processing [117, 118].

5.1 Cellulose and xylan

Lignocellulosic biomass, a popular by-product of the agriculture and forestry sectors, has enormous potential as a source of valuable products. Lignocellulosic materials, which are made up of cellulose, hemicelluloses, and lignin, form a complex matrix that resists depolymerization [119, 120]. Depending on the sources, lignocellulosic biomass can

contain up to 50–60% cellulose, 25–35% hemicellulose, and 10–25% lignin by weight. However, the complex structure formed by lignin, on the other hand, makes accessibility to cellulose and hemicellulose even more critical [116, 117, 121]. Recently, the use of DES has offered selective solubilization and separation of cellulose, lignin, and hemicellulosic fractions, while DES-pretreated biomass yielded elevated concentration of monomeric sugars after enzymatic treatment due to enhanced accessibility to cellulosic and hemicellulosic fractions (Table 1).

For example, three acidic DES (acetic acid-, formic acid-, and lactic acid-choline chloride) were compared for their ability to selectively extract poplar wood lignin and enhance cellulose reactivity [125]. A high lignin selectivity of 6.3–7.9 was obtained with choline chloride/acetic acid treatment at 130 °C for 3 h. The treatment showed the highest delignification of 76.5% with a maximum cellulose yield of 85.4%. In another study, the effects of DES made from choline chloride in combination with lactic acid, oxalic acid, potassium hydroxide, or urea on the fractionation of beech (*Fagus sylvatica*) wood sawdust were investigated. Choline chloride in combination with oxalic acid or potassium hydroxide effectively dissolved beech wood polymers and fractionated cellulose and lignin. Within the temperature range of 60 to 80 °C and reaction times of 4 to 6 h, an optimal biomass loading of 2.5% was efficient for DES fractionation [133]. An underutilized and under-researched yet promising lignocellulosic feedstock of Bambara groundnut haulm was pretreated with five different DES [126]. The pretreatment with choline chloride/lactic acid at 100 °C for 1 h supported the removal of 54.5% hemicellulose and 60.7% lignin, subsequently yielding 25.48% cellulose along with a sugar recovery of 94.8%.

Another approach was employed for the recalcitrant date palm residues, where DES treatments were moderately ineffective. A hydrothermal pretreatment (200 °C for 10 min) prior to DES treatment on date palm residues enhanced the functioning of choline chloride/glycerol [130]. This approach has approximately doubled the enzymatic digestibility of date palm residues with elevated xylan (25%) and lignin (22%) removal. Similarly, oil palm empty fruit bunch was pretreated with three different DES at high temperature (120 °C) for 3 h. The pretreatment with choline chloride/lactic acid showed high delignification potential and hemicellulose removal capacity which subsequently yielded the highest reducing sugars of 20.7% [131]. In another study, oil palm empty fruit bunch pretreated with choline chloride/urea at 110 °C for 1 h resulted in the highest glucose yield of 66.33 mg/ml. However, this treatment failed to effectively remove lignin and hemicellulosic fractions, while not much increment was observed in sugars when compared with the control treatment [136]. Interestingly, oil palm trunk fiber pretreated with ethylammonium chloride/ethylene glycol at

Table 1 DES used for cellulose and xylan extraction

DES used	Molar ratio	Remarks	Reference
Cellulose			
CA/glycolic acid	1:1	Kraft cellulose treated with choline chloride/levulinic acid showed 100% deligni-fication with 86% cellulose and 14% hemicellulose yield	[122]
CC/imidazole			
CC/levulinic acid			
CC/1,2-propanediol	1:1	Choline chloride/glycerol pretreated rice straw released 226.7 g/L reducing sugars with a saccharification efficiency of 87.1%	[123]
CC/citric acid	1:1		
CC/ethylene glycol	1:1		
CC/glycerol	1:1		
CC/lactic acid	1:1		
CC/malic acid	1:5; 1:9		
CC/malonic acid	1:1; 1:2		
CC/oxalic acid	1:1		
CC/tartaric acid	1:1		
CC/urea	1:1		
CC/acetic acid	1:2	Choline chloride/formic acid pretreated corn stover released 17.0 g/L of glucose with a yield of 99%	[124]
CC/citric acid	1:1		
CC/formic acid	1:2		
CC/glycerol	1:2		
CC/malonic acid	1:1		
CC/oxalic acid	1:1		
CC/urea	1:2		
CC/acetic acid	1:2	Poplar wood shavings pretreated with choline chloride/acetic acid resulted in the highest delignification (76.5%) and yielded 85.4% of cellulose, 6.9% of hemicel-lulose, and 9.5% of lignin	[125]
CC/formic acid			
CC/lactic acid			
CC/acetic acid	1:2	Bambara groundnut haulm pretreated with choline chloride/lactic acid showed the highest hemicellulose (54.5%) and lignin (60.7%) removal with an optimum sugar recovery of 94.8%	[126]
CC/formic acid	1:2		
CC/glycerol	1:2		
CC/imidazole	1:2		
CC/lactic acid	3:7		
CC/urea	1:2		
CC/ethylene glycol	1:2	Ethylammonium chloride/ethylene glycol pretreated oil palm trunk fiber resulted in the removal of 42% lignin and 83% hemicellulose and achieved the highest glucose production of 74%	[127]
CC/glycerol			
CC/urea			
EAC/ethylene glycol			
EAC/glycerol			
CC/ethylene glycol	1:2	Corncob biomass pretreated with choline chloride/lactic acid showed removal of 95.5% lignin with 77.8% of glucose yield	[128]
CC/glutaric acid	1:1		
CC/glycerol	1:2		
CC/glycolic acid	1:2		
CC/lactic acid	1:2; 1:5; 1:10; 1:15		
CC/levulinic acid	1:2		
CC/malic acid	1:1		
CC/malonic acid	1:1		
CC/oxalic acid	1:1		
CC/ethylene glycol	1:1	Rice straw pretreated with lactic acid/guanidine HCl showed the highest cellulose digestibility of 80.3% comprising of cellulose (62.0%), xylan (4.5%), acid-insol-uble-lignin (14.5%), and acid-soluble-lignin (0.40%)	[129]
CC/formamide			
CC/glycerol			
CC/guanidine HCl			
CC/urea			
CC/xylitol			
Lac/ethylene glycol			
Lac/formamide			
Lac/glycerol			
Lac/guanidine HCl			
Lac/urea			
Lac/xylitol			
CC/glycerol	1:2	Choline chloride/imidazole pretreated corncob residues released 76% of ferment-able sugars (86% glucose and 63% xylose)	[103]
CC/imidazole	3:7		
CC/urea	1:2		

Table 1 (continued)

DES used	Molar ratio	Remarks	Reference
CC/glycerol	1:2 1:3 1:6	Date palm residues pretreated with choline chloride/glycerol showed elevated removal of both xylans (25%) and lignin (22%) with enhanced enzymatic digestibility of biomass	[130]
CC/glycerol CC/lactic acid CC/urea	1:2	Choline chloride/lactic acid pretreated oil palm empty fruit bunch released highest glucose yield of 20.7%	[131]
CC/lactic acid	1:1; 1:3; 1:5	Choline chloride/lactic acid pretreated rice straw removed and fractionated 30% to 35% of lignin and 70% of xylan with 60 to 70% glucose yields	[132]
CC/lactic acid CC/oxalic acid CC/potassium hydroxide CC/urea	1:2 1:1; 1:2 1:4 1:2	The mixtures of choline chloride with oxalic acid or potassium hydroxide were found to dissolve beech wood polymers more effectively compared to choline chloride with lactic acid or urea	[133]
CC/levulinic acid Am/levulinic acid Ba/levulinic acid	1:2	Moso bamboo pretreated with choline chloride/levulinic acid released the highest glucose (79.07%) with the highest lignin removal of (8.15%) and hemicellulose (6.43%)	[134]
CC/malic acid CC/malonic acid CC/oxalic acid CC/succinic acid CC/tartaric acid	1:2	Choline chloride/succinic acid pretreated moso bamboo showed 44.8% delignification with the yield of 93.8% glucan and 86.4% of xylan	[135]
CC/urea	1:2	Oil palm empty fruit bunch pretreated with choline chloride/urea at 110 °C for 1 h released the highest glucose of 66.3 mg/ml	[136]
CC/urea	1:2	Rice straw and its fractions pretreated with choline chloride/urea at 130 °C and 4 h showed separation capacity in the order of acid-insoluble-lignin (22.87%) > amorphous cellulose and hemicellulose (16.71%) > α-cellulose (9.60%)	[137]
K$_2$CO$_3$/glycerol	1:4 1:5 1:6 1:7	Paddy straw pretreated with potassium carbonate/glycerol resulted in the removal of lignin and hemicellulose and showed an elevated level of the cellulosic fraction	[138]
Xylan			
Bet/lactic acid CC/acetic acid CC/formic acid CC/lactic acid Pro/lactic acid	1:2 1:2 1:2 1:10 1:3.3	Loblolly pine pretreated with choline chloride/ lactic acid extracted < 5% of xylan	[139]
CC/acetic acid CC/urea	1:2 1:1; 1:2	Beech wood xylan pretreated with 1:2 choline chloride/urea solubilized 328 g/L of xylan with the highest recovery yield of 90%	[27]
CC/formic acid	1:2	Wheat straw biomass pretreated with choline chloride/formic acid solubilized almost 90% of the initial xylan from the solid	[140]
CC/glycerol CC/oxalic acid (dihydrate) CC/urea	1:1	Wood lignocellulose pretreated with choline chloride/oxalic acid (dihydrate) showed good solubility of xylan at room temperature and complete solubilization at 80 °C	[141]
CC/lactic acid	1:3	Rice straw biomass pretreated with choline chloride/lactic acid recovered 78.8% of xylan	[142]

Bet, betaine; *CA*, choline acetate; *CC*, choline chloride; *EAC*, ethylammonium chloride; *Lac*, lactic acid; *Pro*, proline

100 °C for 48 h showed excellent delignification potential (42%) and hemicellulose removal (83%) and achieved the highest biomass of 58% which subsequently yielded 74% glucose [127].

The effectiveness of levulinic acid-based DES was evaluated in combination with acetamide, betaine, and choline chloride as hydrogen bonding acceptors for the pretreatment of moso bamboo [134]. Bamboo biomass pretreated with choline chloride/levulinic acid at 120 °C for 2 h yielded maximum glucose (79.07%) with the highest lignin and hemicellulose removal of 8.15% and 6.43%, respectively. However, a better delignification of moso bamboo biomass was observed when DES pretreatments were assisted with microwave irradiation [135]. Out of the five kinds of dicarboxylic acids-based DES studied, choline chloride/succinic acid pretreated moso biomass with a 10 min microwave

treatment (600 W, 160 °C) showed the highest delignification of 44.8%, yielding 93.8% glucan and 86.4% xylan, respectively.

Deconstruction of rice straw was evaluated by pretreating with DES formed using choline chloride and different hydrogen bond donors [123]. A highly specific delignification potential was observed for choline chloride/glycerol pretreated rice straw, without altering cellulosic and hemicellulosic fractions, which subsequently released 226.7 g/L reducing sugars, with a saccharification efficiency of 87.1%. Rice straw was also pretreated with other DES, where pretreatment with lactic acid/guanidine HCl showed good delignification potential and hemicellulose removal, with a cellulose recovery of 62% and cellulose digestibility of 80.3% [129]. In addition, the effectiveness of different molar concentrations of choline chloride/lactic acid was evaluated for the pretreatment of rice straw which efficiently fractionated 30 to 35% lignin and 70% xylan and yielded 60 to 70% of glucose [132]. In a different study, rice straw and its fractions pretreated with choline chloride/urea at 130 °C for 4 h showed a higher selective solubility of lignin with the increased crystallinity index of rice straw residue and α-cellulose [137].

Seven DES with various hydrogen donors were synthesized and used to pretreat corn stover biomass. Choline chloride/formic acid pretreatment at 130 °C for 2 h efficiently removed hemicellulose (66.2%) and lignin (23.8%), with a solid yield of 69.4% and 47.9% cellulose [124]. Choline chloride-based DES (with glycerol, imidazole or urea) were applied on corncob biomass for enhanced digestibility [103]. Choline chloride/imidazole pretreated biomass at 80 °C for 15 h efficiently reduced lignin content with improved cellulose and hemicellulosic fractions, yielding 76% of fermentable sugars (86% glucose and 63% xylose). Interestingly, in another study, out of the nine DES evaluated, corncob biomass pretreated with choline chloride/lactic acid at 110 °C for 24 h showed excellent delignification potential and removed 98.5% lignin, with a residue recovery of 47% and glucose yield of 77.8% [128].

The main hemicellulose constituent, xylan, is a non-crystalline complex polysaccharide consisting of a backbone of 1,4-linked β-D-xylopyranosyl units substituted with acetyl, arabinosyl, and glucuronosyl side chains [119]. The use of DES for improved xylan recovery has also been evaluated. For example, loblolly pine biomass was pretreated with five different acidic DES [139]. The biomass pretreated with choline chloride/lactic acid at 60 °C for 20 min showed good delignification potential (13%) with < 5% xylan solubilization. Similarly, rice straw pretreated with choline chloride/lactic acid at 120 ℃ for 3 h improved xylan digestibility (78.8%) and showed the highest xylose recovery of 25.7% [142]. In another study, one acidic (acetic acid) and one basic (urea) DES were synthesized with choline chloride

for the pretreatment of beech wood xylan [27]. Choline chloride/urea pretreated biomass solubilized 328 g/L xylan with the highest recovery yield of 90%. It was noted that the fractionation efficiency of lignocellulosic biomass could be improved with microwave irradiation. For example, choline chloride/oxalic acid pretreatment of wood lignocellulosic biomass with microwave assistance (80 °C, 800 W for 3 min) showed enhanced xylan recovery [141]. Similarly, wheat straw biomass pretreated with an acidic DES, choline chloride/formic acid combined with microwave, solubilized almost 90% of the initial xylan from the biomass [140].

Though the fractionation potential of DES is solely dependent on the nature of the biomass, most of the time, acidic DES such as choline chloride with lactic acid, levulinic acid, acetic acid, oxalic acid, or formic acid showed remarkable delignification potential with improved recovery of cellulosic and hemicellulosic fractions; however, the DES formed with neutral glycerol also showed prominent results in lignocellulosic fractionation [116, 118]. Although acidic DES systems are advantageous for lignin fractionation, the acidic conditions used tend to cause severe hemicellulose degradation. Interestingly, in recent years, two-stage pretreatment, which includes hemicellulose pre-extraction followed by lignin extraction using various solvent systems, has been developed to maximize the product revenue from lignocellulose [125]. However, using aqueous DES will be beneficial in this regard and should be studied in detail for better hemicellulosic extraction [143]. In addition to this, longer pretreatment times in industrial processes usually resulted in lower production efficiency and the generation of higher amounts of undesired products [118, 144]. As a result, it would be preferable to choose the pretreatment time based on a fine balance of energy consumption and pretreatment efficiency.

5.2 Chitin

Chitin is a linear polysaccharide made up of (1-4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose monomers structurally identical to cellulose, with the acetamide group (-NHCOCH$_3$) at the C-2 position and is the most abundant polysaccharide after cellulose. It is ubiquitous and is the primary structural component that gives support to the exoskeletons of crustaceans, insects, and fungal cell walls [145]. The chitin and its derivative chitosan have tremendous applications in medical industry for wound care dressing and in the agriculture industry for the control of arthropod pests and fungal pathogens [146]. DES-mediated chitin extraction has recently piqued people's interest, and some of the reports are presented in Table 2.

DES systems based on choline chloride with different neutral, acidic, or basic compounds were evaluated for their efficacy to fractionate chitin from shrimp shell powder

Table 2 DES used for chitin, starch, and pectin extraction

DES used	Molar ratio	Remarks	Reference
Chitin			
Bet/butyric acid Bet/glycerol Bet/lactic acid Bet/oxalic acid Bet/urea CC/butyric acid CC/glycerol CC/lactic acid CC/oxalic acid CC/urea	1:1	Skimmed black soldier fly (insect: *Hermetia illucens*) prepupae powder pretreated with betaine/urea yielded 26.71% chitin	[147]
Bet/lactic acid Bet/thiourea Bet/urea CC/lactic acid CC/thiourea CC/urea	1:2	White button mushroom biomass (*Agaricus bisporus*) pretreated with betaine/urea produced the highest yields of chitin (28.3%) with reasonably low protein and mineral contents	[148]
BetHCl/urea CC/ethylene glycol CC/glycerol CC/urea	1:2	Shrimp shell powder pretreated with choline chloride/urea extracted superior quality chitin	[149]
CC/1,4-butanediol CC/1,6-hexanediol CC/citric acid CC/ethylene glycol CC/glycerol CC/lactic acid CC/maleic anhydride CC/malic acid CC/malonic acid CC/oxalic acid CC/propylene glycol CC/tartaric acid CC/thiourea CC/urea	1:2	Shrimp shell (*Marsupenaeus japonicus*) powder pretreated with choline chloride/malonic acid yielded 19.41% of chitin	[150]
CC/citric acid CC/DL-malic acid CC/D-malic acid CC/L-lactic acid CC/L-malic acid	1:2; 2:1 1:1; 1:2; 1:3; 1:4; 1:5 1:2 1:2 1:2; 2:1	Red shrimp shell powder (*Solenocera crassicornis*) pretreated with choline chloride/DL-malic acid (1:2) resulted in superior quality chitin with 98.6% purity	[151]
CC/citric acid CC/lactic acid CC/malonic acid CC/urea	1:1 1:1 1:1 1:2	Shrimp (*Pandalus borealis*) shell powder pretreated with choline chloride/lactic acid yielded chitin with <90% yield with purity higher than 98%	[152]
CC/glycerol CC/malonic acid CC/thiourea CC/urea	1:2 1:2 1:1 1:2	Lobster shell powder pretreated with choline chloride/malonic acid yielded 20.63% of chitin	[153]
CC/lactic acid CC/levulinic acid CC/malic acid CC/malonic acid	1:1 1:2 1:1 1:2	Lobster shell powder pretreated with choline chloride–malonic acid yielded superior quality chitin with 22.21% yield	[154]
CC/malic acid	1:1	Shrimp shell powder pretreated with choline chloride/malic acid extracted superior quality chitin	[155]

[150]. For example, choline chloride/malonic acid pretreated shell powder at 80 °C for 2 h yielded 19.41% pure chitin. In another study, out of the five different DES evaluated, choline chloride/DL-malic acid pretreatment at 130 °C for 3 h resulted in the extraction of superior quality chitin from red shrimp shell powder with 98.6% purity and 13.2% yield

Table 2 (continued)

DES used	Molar ratio	Remarks	Reference
Starch			
Ala/lactic acid	1:1; 1:9	Analytical grade starch pretreated with 1:1 glycine/malic acid dissolved 7.65% of starch	[156]
Ala/malic acid	1:1		
Ala/oxalic acid (anhydrous)	1:1		
Ala/oxalic acid (dihydrate)	1:1; 1:2		
Bet/lactic acid	1:1; 1:2		
Bet/malic acid	1:1		
Bet/oxalic acid (anhydrous)	1:1		
Bet/oxalic acid (dihydrate)	1:1		
CC/lactic acid	1:1.2; 1.2:1; 1.4:1; 2:1		
CC/malic acid	2:1; 1.2:1; 1:2		
CC/nicotinic acid	1:1		
CC/oxalic acid (anhydrous)	1:1.5; 1.5:1		
CC/oxalic acid (dihydrate)	1:1		
Gly/lactic acid	1:1; 1:9		
Gly/malic acid	1:1		
Gly/oxalic acid (anhydrous)	1:1		
Gly/oxalic acid (dihydrate)	1:1; 1:2; 1:3		
His/lactic acid	1:2; 1:5; 1:9		
His/malic acid	1:1; 1:2		
His/oxalic acid (anhydrous)	1:1; 1:2; 1:3; 1:4		
His/oxalic acid (dihydrate)	1:1		
Nic/malic acid	1:9		
Nic/oxalic acid (dihydrate)	1:9		
Pro/lactic acid	2:1; 1:1; 1:4		
Pro/malic acid	1:1; 1:3; 3:1		
Pro/nicotinic acid	1:1		
Pro/oxalic acid (anhydrous)	1:1; 1:1.5		
Pro/oxalic acid (dihydrate)	1:1		
$CaCl_2$/urea	1:4	Corn starch pretreated with choline chloride/oxalic acid or choline chloride/$ZnCl_2$ solubilized 5–10% of starch concentration	[157]
CC/citric acid	2:3		
CC/oxalic acid	2:1		
CC/urea	1:1		
CC/zinc chloride	2:3		
CC/citric acid	2:1	Potato starch pretreated with choline chloride/urea dissolved 5% of starch	[158]
CC/succinic acid	1:1		
CC/urea	1:2		
CC/imidazole	3:7; 2:3	Potato starch pretreated with 3:7 choline chloride/imidazole dissolved 20% starch	[159]
CC/urea	1:2		
Cit/imidazole	3:7		
Glycerol/imidazole	3:7; 1:1		
Mal/imidazole	3:7		
Sucrose/L-malic acid/water	1:1:1; 1:1:2; 1:1:10; 1:2:10; 1:3:10	Empty fruit bunches pretreated with 1:3:10 sucrose/L-malic acid/water dissolved 2.25% starch	[160]
Sucrose/monosodium glutamate/water	3:1:1; 3:1:2; 3:1:10		
CC/lactic acid	1:2	Apple pomace (*Malus domestica* var. *Kermerrien*) pretreated with choline chloride/lactic acid extracted the highest concentration of starch	[161]
CC/oxalic acid			
CC/urea			
Pectin			
Bet/citric acid	3:1	*Myrciaria cauliflora* (jaboticaba pomace) by-products pretreated with citric acid/glucose/water extracted maximum concentration of pectin	[162]
CC/citric acid	1:1		
CC/malic acid	1:1		
CC/propylene glycol	1:2		
Cit/glucose/water	1:1:3		
Cit/propylene glycol	1:1		

Table 2 (continued)

DES used	Molar ratio	Remarks	Reference
CC/citric acid	1:1	*Citrus grandis* (pomelo) peels pretreated with choline chloride/	[163]
CC/fructose	5:2	malonic acid and choline chloride/glucose/water yielded 94%	
CC/fructose/H_2O	5:2:5	pectin	
CC/glucose	5:2		
CC/glucose/H_2O	5:2:5		
CC/glycerol/H_2O	1:2:1		
CC/malic acid	1:1		
CC/malonic acid	1:1		
CC/oxalic acid	1:1		
CC/sucrose/H_2O	4:1:4		
CC/citric acid (monohydrate)	3:1; 2:1; 1:1; 1:2; 1:3	*Averrhoa bilimbi* biomass pretreated with 1:1 choline chloride/citric acid extracted 14.44% of pectin	[164]
CC/lactic acid	1:2	Apple pomace (*Malus domestica* var. *Kermerrien*) pretreated with	[161]
CC/oxalic acid		choline chloride/lactic acid facilitated the highest extraction of hot	
CC/urea		water-soluble pectin (19.1%)	
Lac/glucose/water	6:1:6; 5:1:3	Pomelo peel pretreated with lactic acid/glucose/water with a ratio	[165]
Lac/glucose	5:1	of 6:1:6 yielded the highest pectin yield of 23.04%	
Lac/glycine/water	3:1:3		
Lac/glycine	9:1		

Ala, alanine; *Bet*, betaine; *CC*, choline chloride; *Cit*, citric acid; *Gly*, glycine; *His*, histidine; *Lac*, lactic acid; *Mal*, malic acid; *Nic*, nicotinic acid; Pro, proline

[151]. Similarly, DES of citric acid, lactic acid, malonic acid, or urea with choline chloride was used to pretreat the shrimp shell powder, where choline chloride/lactic acid DES yielded ~90% chitin with more than 98% purity [152].

For the extraction of chitin from lobster shell powder, four DES of choline chloride synthesized with glycerol, malonic acid, thiourea, or urea were assessed. The shell powder pretreated with choline chloride/malonic acid at 50 °C for 2 h yielded 20.63% chitin, which was higher than chemically prepared chitin with a yield of 16.53% [153]. Similarly, another study reported the efficacy of choline chloride/malonic acid (50 °C for 2 h) for the effective extraction of chitin from lobster shell powder with a yield of 22.21% and 93% purity [154].

For the preparation of insect chitin, DES-mediated selective deproteinization and demineralization was investigated. Betaine and choline chloride in combination with butyric acid, glycerol, lactic acid, oxalic acid, or urea were synthesized and used for pretreatment. Skimmed black soldier fly prepupae powder pretreated with betaine/urea with pretreatment conditions of 50 °C for 2 h yielded 26.71% chitin with 83.29% purity [147]. In contrast, in a study on white button mushroom biomass, pretreatment with betaine/urea with ultrasonic assistance produced the highest yields of chitin (28.3%) with reasonably low protein and mineral contents [148].

DES-mediated chitin extraction, unlike lignocellulosic pretreatment, produced better results at lower temperatures and time conditions. However, higher extractability in acidic DES may be a related feature in lignocellulosic and chitin-rich biomass pretreatment, while malic and malonic acid showed prominent results in chitin extraction. However, chitin from insect and mushroom biomass, which was successfully extracted using DES of betaine/urea, was a contradiction to this.

5.3 Starch

Starch is a mixture of branched amylopectin and nearly linear amylose which are composed of anhydroglucopyranose units [166]. Starch is commonly used in the food and other industrial sectors such as pharmaceuticals, paper, textiles and packaging [116]. However, not much research has been conducted on the DES-mediated extraction of starch from crude biomass. Moreover, due to the poor solubility of starch in water, only a few DES have been used to solubilize starch for their potential application in various industries (Table 2).

A range of DES with different molar concentrations was synthesized and evaluated for the solubilization of analytical grade starch, where glycine/malic acid (1:1) mediated pretreatment dissolved 7.65% starch [156]. Similarly, corn starch pretreated with either choline chloride/oxalic acid or choline chloride/$ZnCl_2$ solubilized around 5 to 10% starch [157]. In contrast, potato starch pretreated with choline chloride/urea dissolved approximately 5% starch [158]. In another study, imidazole combined with choline chloride, citric acid, glycerol, or malic acid was evaluated to solubilize potato starch. It was observed that choline chloride/imidazole with a 3:7 molar concentration achieved the highest starch solubilization of 20% [159]. The efficacy of sugar

(sucrose) with malic acid or monosodium glutamate was evaluated to pretreat empty fruit bunches for starch extraction. The biomass pretreated with 1:3:10 sucrose/L-malic acid/water dissolved 2.25% starch [160]. Recently, it was observed that apple pomace pretreated with choline chloride/lactic acid at 80 °C for 1 h facilitated the highest starch extraction [161]. However, the lack of ample reports on the extraction of starches by DES indicates a void in this area and stresses the need for further research.

5.4 Pectin

Pectin is a complex heteropolysaccharide with a backbone primarily composed of -(1,4)-linked D-galacturonic acid with varying degrees of esterification. Pectin is used as an emulsifier, gelling agent, thickener, and stabilizer in the food industry, as well as in new applications such as the synthesis of edible films and coatings [165]. Lately, DES have been used in pectin extraction and proved to be the best among the available methods (Table 2).

The industrial blue Brazilian berry by-product (jaboticaba pomace) was pretreated with six different DES, where biomass pretreated with citric acid/glucose/water at 80 °C for 2.5 h extracted pectin with a yield of 27.3%. The yield achieved was three times higher than that of citric acid solution (9.7%), which is widely used in this regard [162]. In another study, choline chloride in combination with different acids and sugars was evaluated for the pretreatment of pomelo peels [163]. The biomass pretreated in a sonication bath with choline chloride/malonic acid at 80 °C for 1 h yielded pectin (94%) with 52% degree of esterification. The high pectin yields obtained could be linked to the nature of the biomass and the improved DES efficacy due to ultrasonication. In another study, pomelo peel pretreated with lactic acid/glucose/water with a molar ratio of 6:1:6 at 88 °C for 141 min resulted in the highest pectin yield of 23.04% [165].

Averrhoa bilimbi biomass pretreated with 1:1 molar concentration of choline chloride/citric acid at 80 °C for 2.5 h yielded 14.44% pectin [164]. Similarly, in another study, apple pomace pretreated with choline chloride/lactic acid at 80 °C for 2 h facilitated the highest extraction of hot water-soluble pectin (19.1%) [161]. Interestingly, pectin extraction using DES was achieved at lower temperatures and time durations. In contrast, it has followed the same pattern as cellulose and chitin extraction protocols, where the maximum extraction was achieved using acidic DES.

5.5 Other polysaccharides

Besides the fractionation of abundant polysaccharides such as cellulose, xylan, starch, and chitin, the use of DES for the extraction of various other polysaccharides such as

k-carrageenan, alginate, and fucoidan have also been studied recently (Table 3).

Numerous DES were synthesized to fractionate polysaccharides from *Camellia oleifera* seed cake [168]. The biomass pretreated with choline chloride/ethylene glycol at 55 °C for 35 min with ultrasound assistance gave a maximum polysaccharide extraction yield of 121.9 mg/g with a recovery of 84.92% and purity of 77.64%. In another study, *Kappaphycus alvarezii* (seaweed) biomass pretreated with 10% hydrated choline chloride/glycerol at 90 °C for 1 h resulted in the highest k-carrageenan extraction of 60.25% [167]. Similarly, *Sargassum horneri* (seaweed) biomass pretreated with a molar concentration of 1:2 choline chloride/1,2-propanediol at 70 °C for 30 min with ultrasound assistance yielded 11.31% polysaccharide recovery [170]. Another seaweed, *Saccharina japonica*, biomass pretreated with choline chloride/glycerol at a temperature of 150° C and a pressure of 19.85 bar resulted in high yields of alginate (28.12%) and fucoidan (14.93%) [169].

Nineteen different DES were evaluated for the fractionation of *Dendrobium officinale* biomass to extract the polysaccharides. The biomass pretreated with 1:6 choline chloride/glycerol at 55 °C for 90 min yielded two novel polysaccharides with a molecular weight of 297,911 Da and 30,400 Da, primarily composed of D-glucose and D-mannose with the ratio of 2.2:1 and 3.7:1, respectively [171]. In another study, *Fucus vesiculosus* biomass pretreated with choline chloride/1,4-butanediol with microwave-assisted pretreatment conditions of 168 °C for 35 min showed maximum polysaccharide yields 116.33 mg/g [172]. Similarly, *Dioscorea opposita* (Chinese yam) biomass pretreated with 1:4 choline chloride/1,4-butanediol (94 °C and 44.74 min) with ultrasound assistance extracted 15.98% polysaccharides [173]. Six DES comprising of choline chloride in combination with citric acid, ethylene glycol, glycerol, imidazole, oxalic acid, or urea were synthesized and evaluated to pretreat an edible fungus *Poria cocos* [174]. The biomass pretreated with 1:2 molar ratio of choline chloride/oxalic acid at 100 °C for 15 min extracted 46.24% polysaccharides. In another study, out of the nine DES synthesized with different phenol derivatives, *Ganoderma lucidum* biomass pretreated with ethanolamine/o-cresol at 60 °C for 50 min produced a maximum polysaccharide extraction yield of 92.35 mg/g [175]. It was noted that alcohol-based DES were efficient in extracting low-molecular-weight polysaccharides, in contrast to high-molecular-weight polysaccharides such as cellulose, xylan, starch, and chitin, which showed good extractability with acidic DES.

Table 3 DES used for polysaccharide extraction

DES used	Molar ratio	Remarks	Reference
10% HCC/ethylene glycol 10% HCC/glycerol 10% HCC/urea CC/ethylene glycol CC/glycerol CC/urea	1:2	*Kappaphycus alvarezii* (seaweed) biomass pretreated with 10% hydrated choline chloride/glycerol extracted the highest k-carrageenan of 60.25%	[167]
Bet/ethylene Glycol Bet/glycerol Bet/L-lactic acid Bet/urea CC/citric acid CC/DL-malic acid CC/ethylene glycol CC/glycerol CC/L-lactic acid CC/urea Gly/L-lactic acid L-Pro/DL-malic acid L-Pro/D-sorbitol L-Pro/glycerol L-Pro/L-lactic acid L-Pro/urea	1:2 1:2 1:1 1:2 2:1 1:1 1:2 1:2 1:1 1:2 1:3 1:1 1:1 1:2; 5:2 1:1 1:2	*Camellia oleifera* seed cake pretreated with choline chloride/ethylene glycol gave a maximum polysaccharide extraction yield of 121.9 mg/g	[168]
CC/1,2-propanediol CC/1,3-butanediol CC/1,4-butanediol CC/ethylene glycol CC/glycerol CC/propanedioic acid CC/urea	1:2	*Saccharina japonica* (seaweed) biomass pretreated with choline chloride/glycerol extracted a high yield of alginate (28.12%) and fucoidan (14.93%)	[169]
CC/1,2-propanediol	1:1; 1:2; 1:3; 1:4; 1:5	*Sargassum horneri* (seaweed) biomass pretreated with 1:2 choline chloride/1,2-propanediol yielded 11.31% polysaccharide recovery	[170]
CC/1,4-butanediol CC/citric acid CC/D-sorbitol CC/ethylene glycol CC/glycerol CC/lactic acid CC/levulinic acid CC/malic acid CC/malonic acid CC/oxalic acid CC/p-toluenesulfonic acid CC/tartaric acid CC/triethylene glycol CC/urea CC/xylitol Lac/acetamide Lac/sodium acetate ZnCl$_2$/acetamide ZnCl$_2$/urea	1:4 1:1 1:1 1:2 1:1; 1:2; 1:4; 1:6; 1:8 1:2 1:2 1:1 1:1 1:1 1:1 2:1 1:4 1:2 1:1 3:1 3:1 1:4 1:3.5	*Dendrobium officinale* biomass pretreated with 1:6 choline chloride/glycerol-based enzyme-assisted extraction yielded two novel polysaccharides with a molecular weight of 297,911 Da and 30,400 Da composed of D-glucose and D-mannose with the ratio of 2.2:1 and 3.7:1, respectively	[171]
CC/1,4-butanediol CC/citric acid CC/DL-malic acid CC/ethylene glycol CC/glycerol CC/lactic acid CC/L-proline/DL-malic acid CC/propylene glycol/urea CC/urea	1:5 1:1 1:1 1:2 1:1 1:2 1:1:1 1:1:1 1:2	*Fucus vesiculosus* (bladder wrack) pretreated with 1:5 choline chloride/1,4-butanediol possessed the optimal extraction efficiency for polysaccharides with the maximum yields of 116.33 mg/g	[172]

Table 3 (continued)

DES used	Molar ratio	Remarks	Reference
CC/1,4-butanediol	2:1; 1:1; 1:2; 1:3; 1:4; 1:5	*Dioscorea opposita* (Chinese yam) biomass pretreated with 1:4 choline chloride/1,4-butanediol extracted 15.98% of Chinese yam polysaccharides	[173]
CC/1,6-hexanediol	1:1		
CC/ethylene glycol	1:1		
CC/glycerol	1:1		
CC/citric acid (monohydrate)	1:2	*Poria cocos* biomass pretreated with 1:2 choline chloride/oxalic acid extracted 46.24% of polysaccharides	[174]
CC/ethylene glycol	1:2		
CC/glycerol	1:2		
CC/imidazole	3:7		
CC/oxalic acid	2:1; 1.5:1; 1:1; 1:1.5: 1:2		
CC/urea	1:2		
DETH/m-cresol	1:1	*Ganoderma lucidum* pretreated with ethanolamine/o-cresol showed a maximum extraction yield of 92.35 mg/g	[175]
DETH/o-cresol			
DETH/p-cresol			
ETH/m-cresol			
ETH/o-cresol			
ETH/p-cresol			
TETH/m-cresol			
TETH/o-cresol			
TETH/p-cresol			

10% HCC, 10% hydrated choline chloride; *Bet*, betaine; *CC*, choline chloride; *Cit*, citric acid; *DETH*, diethanolamine; *ETH*, ethanolamine; *Gly*, glycine; *Lac*, lactic acid; *Pro*, proline; *TETH*, triethanolamine

6 Reusability and regeneration of deep eutectic solvents

Pretreatment of biomass for polysaccharide recovery by conventional methods is economical than the DES pretreatments, which limit their commercial application. On the other hand, DES are reusable like ILs, which contributes to the overall cost-effectiveness of the operation [176]. Owing to these properties, DES could pave the way for environmentally sustainable and effective pretreatment processes. Furthermore, the following DES characteristics attest its viability in various applications: (a) broad pH tunability, (b) convenient and selective extraction of materials, (c) dissolution of a varied range of materials, (d) effective recycling, and (e) physicochemical properties similar to conventional ionic liquids, at a lower cost and with less environmental impact [38]. However, it is critical to assess the recyclability of DES to improve the cost-effectiveness of the pretreatment and expand its applicability. Previous reports suggest that DES is easily recyclable using simple methods such as vacuum distillation or freeze-drying [177, 178]. However, small amounts of DES used in laboratory-scale operations are difficult to recover completely, but in large-scale operations, enhanced recovery is possible [133]. Several experiments were carried out in this regard to determine the impact of recovered DES and the extent to which it could be reused.

In one study, almost all molar concentrations of choline chloride/lactic acid DES could be recovered with a yield of ~90% [132]. Rice straw pretreatment with recovered DES was repeated for five cycles which resulted in reduced delignification capacity of DES from 64.8 to 46.4% after 5 cycles. However, xylan removal increased steadily (67.9–73.3%), while the recovery yields of carbohydrate-rich materials gradually increased from 53 to 64%, with glucose yield increased from 60 to 70% [132]. In another study, moso bamboo biomass pretreated with levulinic acid-based DES in combination with acetamide, betaine, and choline chloride as hydrogen bonding acceptors showed good glucose conversion yield (56.44%, 48.90%, and 79.07%, respectively) [134]. After the re-purification and second pretreatment of the respective DES on raw material, the glucose conversion yield dropped, respectively, to 40.52%, 40.67%, and 63.46%. While in the third pretreatment, the enzymatic conversion yield was further decreased to 24.05%, 35.94%, and 38.80%, respectively.

Efforts were made to recycle and reuse choline chloride/DL-malic acid for the pretreatment of shrimp shell powder to extract chitin [151]. Around 70% of DES recovery was obtained in the first and second recycling experiments, which increased up to 97.6% in the third experiment and decreased steadily thereafter. The purity of the O-malate chitin obtained with recycled DES was, on the other hand, preserved at around 97%, with yield increased from 13.2 to 36.6% (from 1 to 5 cycles), while the degree of substitution of the O-malate chitin decreased from 0.46 to 0.18 [151]. While in another study, all the DES showed 50–70% of reusability at the end of the fifth cycle [149]. However, the DES was too viscous to be reused further due to the presence of proteins and other impurities.

While studying the extraction of xylan from different sources, the recovered DES were analyzed by ^{1}H and/or ^{13}C NMR, where the nature of recovered DES well resembled freshly synthesized DES and could be reused for the further extraction of xylans [27, 141]. As recycled DES was used to pretreat poplar wood flour, a 12.8% lignin fraction (which accounts for 66.3% of the lignin in poplar wood flour) and 46.3% cellulose residue were recovered [141]. In contrast, another study confirmed that at least two recycling cycles could be completed without significantly reducing xylan solubility with less than 5% decrease [27].

In another report, the recovery efficiency for ethanolamine/o-cresol showed a very slight decrease (< 5%) after the fifth recycling of DES, where a small amount of o-cresol dissolved in the ethanolamine solution is responsible for the loss of o-cresol. The extraction yield (92.35 mg/g) and recovery yield (88.09%) of *Ganoderma lucidum* polysaccharides were found to be stable even at the fifth cycle [175]. Similarly, the regenerated DES of choline chloride/oxalic acid was then reused for the extraction of *Poria cocos* polysaccharides. These recycling experiments were repeated six times under optimized conditions which yielded 46.23%, 42.13%, 42.29%, 40.33%, 39.61%, and 38.40% of *Poria cocos* polysaccharides recovery, respectively. However, after the sixth recycling, the yield of polysaccharides decreased significantly owing to the loss of DES extractability [174].

7 Challenges of deep eutectic solvent application in feedstock pretreatment

The emergence of DES systems for the pretreatment of feedstock is a relatively new topic; however, it is exceptionally exploited in the last two decades owing to its numerous characteristic features. DES is termed green solvents due to its low cost, simple synthesis protocols, selective separation, non-toxic nature, biodegradability, and reusability [179]. However, DES pretreatments are often very complex, where multiple variables are responsible for the yield of the product of interest, including the composition of the feedstock, structural properties of DES, and the reaction conditions [118]. The most important application of DES includes the pretreatment of lignocellulosic biomass for the effective solubilization of lignin from the biomass with little effects on the cellulosic and hemicellulosic fractions both by qualitative and quantitative means. Similarly, DES plays a critical role in the selective separation of other polysaccharides from different feedstocks. Irrespective of these distinguishing characteristics, industrial applications of DES for feedstock treatment and polysaccharide extraction are still in their infancy, and the following associated challenges need to be addressed to enhance future applications [179]:

- A major disadvantage of DES is their high viscosity, limiting their applicability severely. Hence, a deeper insight is desired to develop a less viscous DES with high thermal stability, suitable for comprehensive functionality.
- Understanding the impact of DES structure and physicochemical properties for selective fractionation of polysaccharides.
- The effect of tailor-made DES compositions, their different molar ratios on intermolecular hydrogen bonding in DES systems and feedstocks, as well as the final quality of the DES-pretreated polysaccharides should be thoroughly investigated.
- Synergistic effects of different DES pretreatment and reaction conditions, such as biomass particle size, reaction temperature, reaction time, and solid-to-liquid ratio, should be considered.
- Process control variables should be optimized based on the specified raw material, tailor-made DES, and pretreatment reaction conditions.
- Combining custom DES with various other technologies (such as high-pressure, hydrothermal, instant controlled pressure drop, microwave, pulsed electric field, sub- and supercritical processing, and ultrasound.) for improved polysaccharide recovery should be investigated.
- DES recovery and reuse should be thoroughly researched to ensure its long-term viability.

8 Conclusion

Our planet and the ecosystem are severely constrained by the overburden of chemicals and toxic substances used injudiciously in many industrial sectors. Green chemistry, which is gaining traction as an environmentally sustainable model, creates processes that remove or reduce the input and output of harmful compounds. In this instance, finding a new generation of environmentally friendly solvents to replace conventional chemicals used in the manufacturing processes is a sustainable solution. Deep eutectic solvents have recently been proposed as ideal solvents for green chemistry applications. DES is widely used as a solvent or separation medium in the extraction of polysaccharides, enabling the macromolecular structure of the polysaccharides to be retained. Based on the literature, it can be concluded that the acidic DES are most efficient in extracting almost all of the polysaccharides including cellulose, hemicellulose, chitin, starch, and pectin. However, the time and temperature requirement for extracting cellulose and hemicellulose are slightly higher than chitin, starch, and pectin which could be easily extracted at a relatively lower time and temperature conditions. Alternatively, low-molecular-weight polysaccharides could be effectively fractionated using alcohol-based DES. In the coming years, DES are expected to play a more significant role in

the technical areas of polysaccharide extraction. However, the option of DES in biomass pretreatment is still very dispersed, given the large variety of DES available in organic biomass fractionation. Furthermore, the optimal DES type, its aqueous combinations, and process parameters for DES pretreatment remain uncertain. However, DES have a lot of potential for being implemented and integrated into biorefinery platforms to achieve more sustainable biomass valorization. In this regard, more focused research is critical in improving DES applicability and, as a result, the design of solvents with high selectivity, better extraction performance, and longer reusability.

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