GREEN SOLVENTS FOR LIGNOCELLULOSE PRETREATMENT AND ENZYMATIC SACCHARIFICATION

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Abstract
Development of biorefinery platform asks for efficient, cost saving, and green processes for lignocellulosic biomass saccharification. Novel biomass pretreatments aimed at lignin dissolution and reduction of cellulose crystallinity are currently studied. Among these, processes based on Deep Eutectic Solvents (DESs) draw scientific and industrial research’s attention.
The present work reviews main results available in the literature on the recent findings on DES pretreatment of lignocellulosic biomass. Moreover, some open issues related with process optimization and integration of pretreatment and enzymatic hydrolysis for fermentable sugar recovery are discussed.

Introduction
Pretreatment step of lignocellulosic feedstock is mandatory in a biorefinery platform. It is characterized by the two following processes:

- physical/chemical pretreatment to remove the lignin and to increase the cellulose and hemicellulose surface area;
- enzymatic hydrolysis to convert cellulose and hemicellulose into monomeric sugars (mainly glucose and xylose).

The pretreatment process must be efficient, cheap, environmental friendly and it must produce low concentration of inhibitors for the subsequent fermentation process (e.g. furfural, hydroxymethyl furfural, ferulic acid) [1]. The main investigated physical/chemical pretreatments are: steam explosion, pyrolysis and acid or basic hydrolysis. These processes are characterized by severe pretreatment conditions (e.g., high temperature and pressure) [2]. The subsequent enzymatic hydrolysis of the pretreated biomass is characterized by low temperature (40-50°C) and pressure (1 atm) and almost neutral pH. For this reason the implementation of the sequence including firstly the physical/chemical pretreatment and secondly the enzymatic hydrolysis of biomass is characterized by several issues (Fig.1).

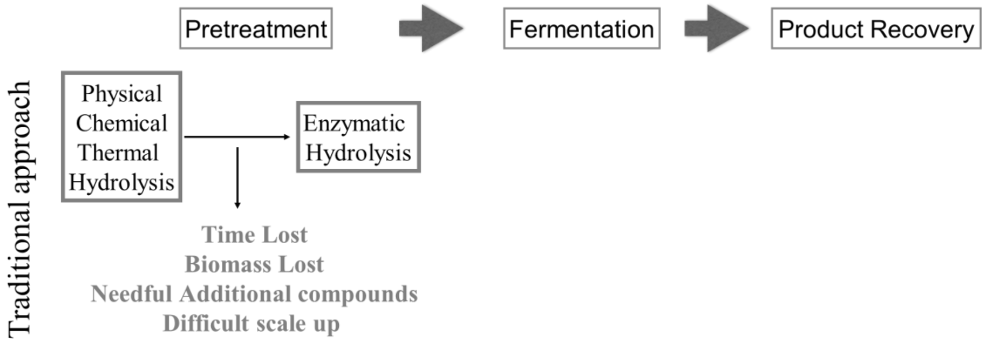

Figure 1. Main issues related to the conventional Two Steps Pretreatment (TSP) in a biorefinery platform.

First of all, time and solvent spending intermediate steps are required to decrease the temperature and pressure adopted in the first pretreatment as well as to neutralize the pH whenever the pretreatment is carried out at strongly acidic or alkaline conditions. The most common pretreatments are also characterized by production of fermentation inhibitors [3] and for this reason an additional detoxification step of the final product (hydrolysate) is mandatory. All these aspects have to be taken into account when the conventional physical/chemical pretreatments are used, so that scale up of the TSP may result difficult. For this reason, studies referring to pretreatments acting at mild conditions are required in order to develop a novel approach based on the integration of lignin dissolution and cellulose hydrolysis steps and aimed at time and operating costs saving.

Biomass pretreatment with Deep Eutectic Solvents

Green solvents offers the opportunity to develop novel processes for lignin dissolution at mild conditions. In the recent years, various studies focused on ionic liquids (ILs) and their potential advantages for the pretreatment of lignocellulosic biomass [4]. ILs act on lignocellulose by disrupting the inter- and intra-molecular hydrogen bonds of cellulose and replacing them by hydrogen bonding between the IL anion and the carbohydrate hydroxyls [5]. Although these solvents are able to solubilize lignocellulose without significant production of inhibitors, their use on large scale is hindered by several issues: i) costs; ii) toxicity; iv) high viscosity.

Among ionic fluids, Deep Eutectic Solvents (DESs) have been recently proposed as alternative. DES is a fluid composed of two or three cheap and safe components that are able of self-association to form an eutectic mixture. Two salts with a high melting point are mixed to obtained a liquid phase with a melting point lower than that of each individual component [6]. DESs exhibit physical and chemical properties similar to ILs and are much environmentally friendly and cheaper than ILs [8]. Costs of DES component reported so far are: ~ 65 $US/Kg for choline chloride, ~ 20 $US/Kg for urea, ~ 35 $US/Kg for glycerol vs. ~ 240 $US/Kg for

95% pure 1-butyl-3-methylimidazoliumchloride (one of the most used IL). For these reasons, more recently, DESs have been proposed as green solvent for lignocellulose biomass dissolution [8]. Table 1 shows results from different studies regarding the DES pretreatment optimization. The optimal temperature ranges between 60-150°C for all investigated DES couples [9-13] and the process time can notably change depending on DES composition.

Table 1. DES pretreatment optimization reported in literature.

Biomass	**Lignin (%)**	**Operating conditions**				**Optimal conditions**	**Ref.**
		DES	**Reaction time (h)**	**Temperature (°C)**	**Solid/solvent ratio ($g\ g^{-1}$)**		
Corncob	17	Glycerol: ChCl	16	80-150	1:16	Glycerol Ch-Cl 150°C	[9]
		Imidazol: ChCl					
		Urea: ChCl					
Oil Palm Empty Fruit Bunch (OPEFB)	10	Urea: ChCl	1-4	80-110	Not reported	1h 110°C	[10]
Corncob	17	Lactic acid: ChCl	24	70-110	1:20	24h 90°C	[11]
			1.5-36	90	1:20		
Cornstover	17.3	Formic acid: ChCl	2	90-130	1:20	3h 130°C 1:20 ($g\ g^{-1}$)	[12]
			0.1-3	90	1:20		
			3	90	1:10-1:20		
Rice straw	9	Lactic acid: ChCl	12	60	1:10-1:20	12h 60°C 1:20	[13]

Studies reported in Table 1 showed sugar yields ranging between 23 and 99 $g_{glucose}/g_{glucan}$. These results are quietly satisfactory when compared with those related to the conventional pretreatments [2]. On the basis of these scenario, further studies can be focused on the selection of optimal DES composition in order to minimize pretreatment temperature. This opportunity is offered by the wide spectrum of ionic species that may form a DES. The procedure adopted to optimize DES pretreatment of lignocellulose includes extensive biomass washing prior to enzymatic hydrolysis [9-13]. In order to approach at integration of DES pretreatment and enzymatic hydrolysis, the intermediate washing step should be avoided. This option is strictly relate do the feasibility of the process at large scale and requires that cellulase activity and stability in the presence of DES is verified. A literature analysis referring to the cellulase activity and stability in the presence of DES has been reported in the following section.

Enzymatic saccharification of DES pretreated biomass

The analysis of the literature showed that few studies addressed the cellulase activity and stability in the presence of DES and the possibility to perform process integration. Gunny and co-workers [14] studied the stability of cellulase in the presence of three different DES couples: Choline Chloride-Glycerol, Choline Chloride- Ethylene glycol, Choline Chloride-Malonic acid. The enzyme stability in DES was measured by incubating cellulases from *Aspergillus sp.* in citrate buffer (50 mM, pH 4.8) at different concentrations of DESs (between 1and 30% v/v) at 30 °C. The stability of cellulase activity was studied after a period of 24 and 48h. Results showed that, after 48h, cellulase from *Aspergillus sp.* retained more than 80% of its original activity in 30%(v/v) Choline Chloride-Glycerol, Choline Chloride- Ethylene glycol, while the enzyme lost its activity when incubated under the same conditions with Choline Chloride-Malonic acid. Malonic acid, in form of cation, probably interacts strongly with the enzyme resulting in its deactivation, on the contrary, according to the literature, glycerol is a protein stabilizer and often is used as biocatalyst dissolving media [15]. Moreover rice husk was pretreated with selected DES and with NaOH. Then the biomass suspensions in DES was diluted with the hydrolysis medium (enzyme in citrate buffer) and the NaOH treated biomass was filtered and washed prior to enzymatic hydrolysis. Performance of enzyme hydrolysis of the two pretreated biomass samples were compared. The DES pretreatment allowed a 2.8-3 fold increase in sugar release with respect to the untreated biomass hydrolysis while only 2.3 increase was observed after NaOH pretreatment.

Single step pretreatment

According to the reported literature, results on cellulase stability in the presence of DES pointed out the feasibility of the integration of DES-mediated lignin disruption step and the enzymatic hydrolysis step. As reported in Fig. 2, several advantages could be obtained through this approach with respect to the current approach described in Fig. 1: the process time would be reduced because the whole process would be carried out under low temperature and pressure avoiding intermediate cooling step, washing step could be avoided because cellulases retain their activity in presence of DES, detoxification step could be removed because not toxic concentration of inhibitors are produced during DES pretreatment.

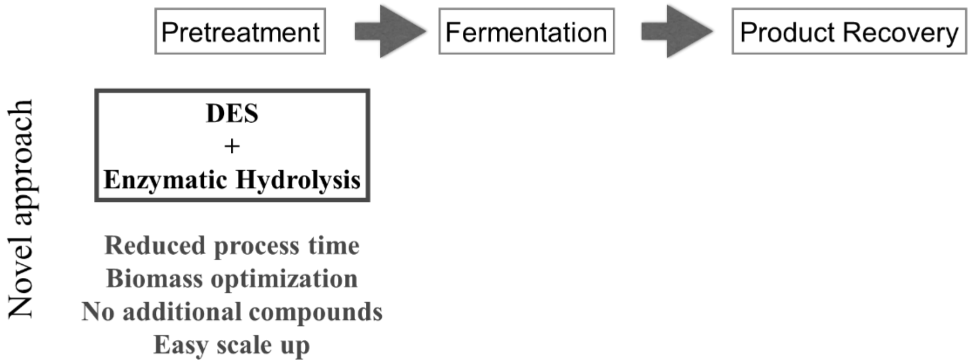

Figure 2. Advantages of process integration of DES mediated lignin disruption and enzymatic cellulose hydrolysis.

Conclusion and perspectives

The present work reviews main results available in the literature referring to DES pretreatment of lignocellulosic biomass. The conditions adopted during DES pretreatment and the tests reported in literature on cellulases stability in the presence of DES encourages further studies regarding this new class of solvents aimed at further reduce the operating temperature and avoid the use of intermediate steps. So, the novel pretreatment approach could reduce process time, hand work, and the use of additional compounds therefore making easier the scale-up of the process.

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