SCREENING OF DEEP EUTECTIC SOLVENTS FOR HIGH LIGNIN SOLUBILITY AND LACCASE ACTIVITY ASSISTED BY COSMOS-RS

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ABSTRACT

Lignin is a naturally occurring aromatic polymer and byproduct from the pulp and paper industry that can be used as raw material for conversion into high-value products and applications. However, currently most of the produced lignin is burned to generate energy. A promising and sustainable approach towards lignin valorization is the enzymatic depolymerization into new biochemicals. Because of lignin´s hydrophobicity and limited water solubility, and the lower compatibility of lignin dissolving organic solvents with laccase, alternative solvents, such as deep eutectic solvents (DES), capable of dissolving high amounts of lignin are potential candidates for this approach. In this work, DES aqueous solutions with potential to dissolve Kraft lignin without compromising laccase activity on lignin were screened and analyzed by COSMO-RS predictions. *In silico* predictions have been verified by experimental results measuring laccase activity with ABTS as substrate. The results indicated the existence of promising combinations that could enhance laccase enzymatic activity. The best systems involved halogenated and inorganic salts combined with alcohols, phenols, and carboxylic acids.

Keywords: Lignin. Deep eutectic solvent (DES). Laccase. Depolymerization. COSMO-RS.

1 INTRODUCTION

Lignin, along with cellulose and hemicellulose, is one of the main constituents of lignocellulosic biomass. It is the second most abundant naturally occurring polymer on the planet and is an important source of aromatic compounds.[1] However, currently, most of the produced lignin in industry is burned to generate energy.[2] One of the main challenges in increasing the value of lignin is to overcome its heterogeneity.[2] In this context, obtaining monomers by depolymerization process can be a way to transform lignin into high-value products.[2,3] This can be achieved through various methods, including thermochemical treatment, mechanical treatment, chemical catalysis, or biological treatment[4]. A more sustainable option could be one that falls within the biotechnological scope, such as enzymic processes.[5] Enzymes, whether from fungal or bacterial origin, have been evaluated for their potential to depolymerize both phenolic and non-phenolic components of lignin.[3] Water is the universal solvent for enzyme-based bioprocesses. However, since lignin has limited solubility in water, enzyme depolymerization is often inefficient. Therefore, searching for alternative solvents within the green chemistry sphere towards lignin depolymerization using enzymes is desired[2]. One of the major solvent players that have been studied in processes involving lignocellulosic biomass processing is deep eutectic solvents (DES).[6] DES are promising eco-friendly solvents to enhance lignin valorization, due to their ability to effectively solubilize lignin.[7,8]

Therefore, this study aimed at applying COSMO-RS as a predictive tool for the identification of DES aqueous solutions capable of simultaneously dissolving lignin and maintaining or enhancing the laccase activity towards improved enzymatic lignin depolymerization.

2 MATERIAL & METHODS

Owing to the numerous possible combinations of substances to form DESs, COSMO-RS is used as an initial screening tool to select the most promising solvents, avoiding the experimental trial and error methodology. Firstly, the activity coefficients of a lignin model compound (GG, guaiacylglycerol-beta-guaiacyl ether) at infinite dilution ($\ln(\gamma^{\infty}_{GG,DES})$) were calculated using a combination of 73 HBAs (hydrogen bond acceptors) and 146 HBDs (hydrogen bond donors) in an equimolar mixture (total number of 10658 combinations). The activity coefficients at infinite dilution in water ($\ln(\gamma^{\infty}_{water,DES})$) for the same HBA:HBD combinations of DES were also determined following the rational used by Pederson et al.[9] who verified that $\ln(\gamma^{\infty}_{water,DES})$ could be used as decisive parameter to predict enzymatic activity in green solvents. Subsequently, the results of $\ln(\gamma^{\infty}_{GG,DES})$ and $\ln(\gamma^{\infty}_{water,DES})$ were combined to select DES with potential of simultaneously dissolving lignin and maintaining or enhancing laccase activity. All calculations were performed using the software COSMOthermX21 (COSMOlogic GmbH & Co KG, Leverkusen, Germany) with the parametrization BP_TZVP_21.ctd, using COSMO-files. Turbomole program package was used to produce the COSMO-files.

The activity of laccase from *Trametes versicolor* in preselected DES mixtures with water have been analyzed based on the procedure described by Toledo et al.[10] with some modifications, using ABTS as the substrate. Chemicals for the preparation of

DES and other solutions were acquired from Honeywell Fluka, Sigma, PanReac AppliChem, TCI, Aldrich, Fischer Scientific and Alfa Aesar and used without further purification.

3 RESULTS & DISCUSSION

The trend of GG solubility in DES is displayed in Figure 1(A), where blue and yellow colors represent low and high $\ln(\gamma^{\infty}_{GG,DES})$ values, respectively. The higher the solubility of the solute in DES, the lower is the $\ln(\gamma^{\infty})$ value. From the analysis of Figure 1(A), it becomes apparent that the HBA has a higher impact on defining the best solvent for GG than the HBD. Halogenated salts (34-63) as HBAs showed the highest ability to solubilize lignin, because they showed the lowest $\ln(\gamma^{\infty}_{GG,DES})$ values. Among HBDs, the best ones were amides (1-7), alcohols (8-35), phenols (36-56), carboxylic acids (73-123), amino acids (91-115) and terpenes (139-141).

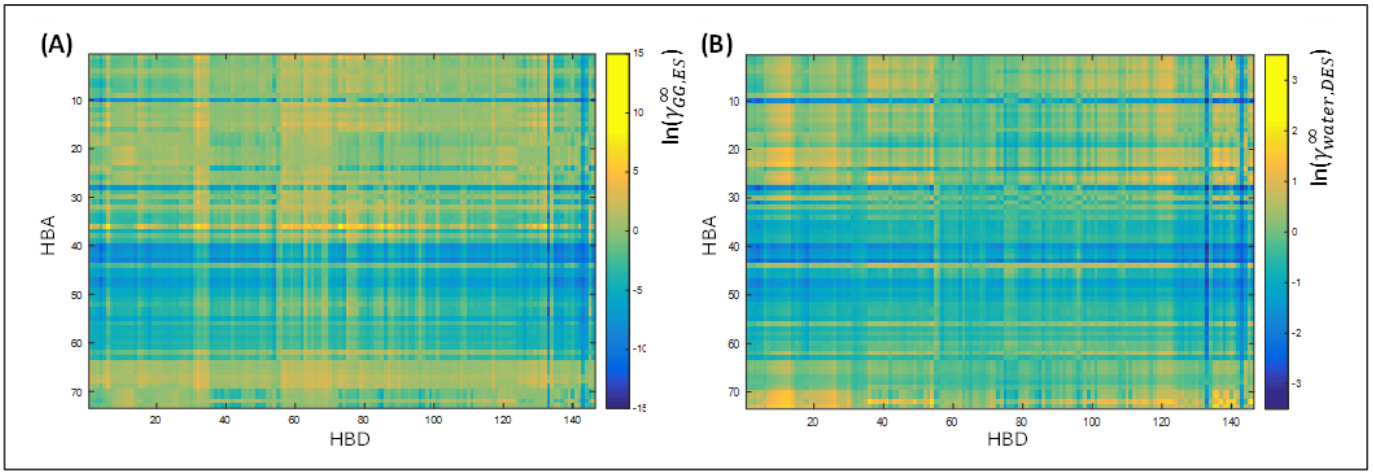

Figure 1 (A) Logarithmic activity coefficients at infinite dilution of GG ($\ln(\gamma^{\infty}_{GG})$) predicted by COSMO-RS in 10658 possible HBA and HBD combinations in equimolar mixture at 25 °C. **(B)** Logarithmic activity coefficients at infinite dilution ($\ln(\gamma^{\infty,water}_{DES})$) of water in 10658 possible HBA and HBD combinations in equimolar mixture predicted by COSMO-RS at 25 °C. The structural families for HBA are: Amides (1-3), amino acids (4-16), carboxylic acids (17-22), inorganic salts (28-33), halogenated salts (34-63), sugars (64-69), phenols (70-71) , terpenes (72-73); and for HBD are: Amides (1-7), alcohols, (8-35), phenols (36-56), sugars (57-72), carboxylic acids (73-123), amino acids (124-133), nitrogenated compounds (134-138), terpenes (139-141), and others (142-146).

However, DES with high potential to dissolve lignin might not be suited for enzyme reactions with laccase. Therefore, it is also necessary to evaluate how these possible HBA and HBD combinations affect laccase activity. DES were prepared with inorganic salts, halogenated salts and amino acids as HBAs, while alcohols were used as HBDs. As reported by Toledo et al.[10], who previously evaluated the impact of aqueous DES solutions on the enzymatic activity of laccase, a correlation between $\ln(\gamma^{\infty,water}_{DES})$ and the enzymatic activity of laccases in these solvents can be found. When water activity coefficients of DES are close to zero, higher laccase activities are achieved. In this sense, $\ln(\gamma^{\infty,water}_{DES})$ was calculated for all combinations proposed in this work and screened for $\ln(\gamma^{\infty,water}_{DES})$ values close to zero. As shown in Figure 1(B), HBA exhibited the strongest influence in defining the best solvent. The best HBA candidates were amides (1-3), amino acids (4-16), and sugars (64-69), while the most effective HBD were amides (1-7), alcohols (8-35), phenols (36-56), sugars (57-72), and carboxylic acids (73-123).

Through the analysis of the results and their trends obtained by COSMO-RS, it was possible to selected the best possible combinations of HBA:HBD (1:1) that might potentiate the enzymatic activity of laccase and simultaneously dissolve lignin. Therefore, from a range of 10658 possibilities, 229 possible combinations of DES were found based on halogenated and inorganic salts as HBAs, and alcohols, phenols, and carboxylic acids as HBDs. The next step in this study was to measure laccase activity in some of the HBA:HBD combinations predicted for DES preparation for validation.

From Figure 2, it can be seen that variations in HBA:HBD combinations with different structures impact enzymatic activity. Both the structure of the HBA and the HBD influence laccase activity, which is consistent with observations made by other studies[10,11] [Ch]DHC demonstrated the highest efficacy as an HBA for the enzymatic reaction, followed by urea, acetamide, $[N_{4444}]$Br, $[N_{4444}]$Cl, and ethanolamine. Comparing ionic HBAs, [Ch]DHC, with three hydroxyl groups, exhibited greater laccase activation compared to $[N_{4444}]$Br and $[N_{4444}]$Cl, lacking hydroxyl groups.

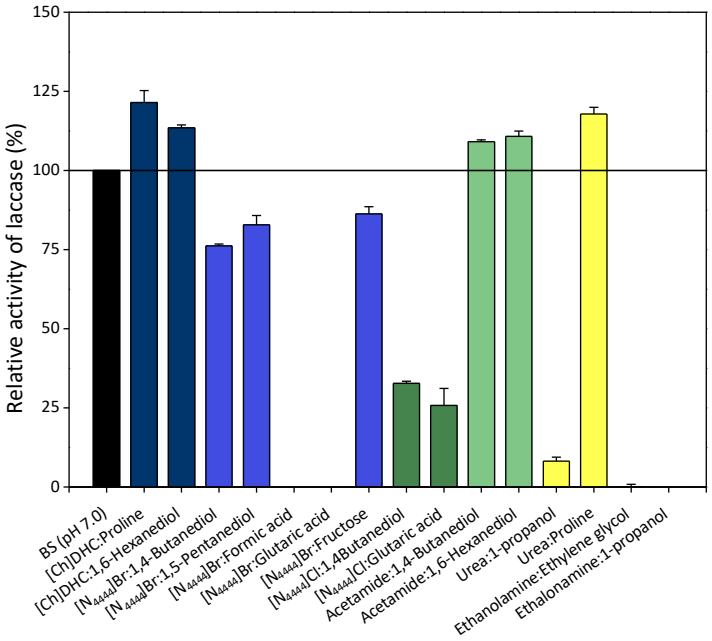

Figure 2 Relative laccase activity (%) at 25 °C in the presence of various HBA:HBD (1:1) combinations screened by COSMO-RS for the formation of DES aqueous solutions with a 25 % w/w water content. Different colors represent different HBAs used to prepare the DES.

4 CONCLUSION

The use of adjusted data from the literature combined with the prediction of activity coefficients by COSMO-RS to screen a wide range of DES towards improved enzymatic depolymerization of lignin shows great potential. The obtained results indicate that the most suitable DES combinations of HBA and HBD might comprise halogenated and inorganic salts with alcohols, phenols, and carboxylic acids. Experimental evaluation of laccase activity on ABTS in some of the most promising DES proved successful and revealed HBA:HBD combinations that lead to enhanced laccase activity in comparison to aqueous buffer.

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