Title: Lignocellulosic Biomass Valorisation using Deep Eutectic Solvents for Sustainable Biorefineries

Abstract: Concerns regarding the depletion of fossil fuel reserves and the environmental impacts of their usage have motivated the development of alternative fuel resources derived from non-food substrates such as lignocellulosic biomass (LBM). This research aimed to develop a sustainable bioprocess to utilise Sugarcane Bagasse (SB), an abundant LBM, from different sugarcane varieties. Deep eutectic solvents (DESs) were utilised to selectively extract lignin and increase SB digestibility for enzymatic saccharification and bioethanol production. FE-SEM, FTIR, TGA, and XRD analysis exhibited structural degradation of SB, following DES pre-treatment, with maximal delignification of 81.2% and enzymatic digestibility of 98.5% utilising choline chloride: lactic acid (ChCl:LA) DES. A one-pot pre-treatment and saccharification process in which DESs, and enzymes are used concurrently, eliminated several intermediate and washing steps to establish a cost-effective and energy-efficient bioconversion method. The biocompatibility of DESs was demonstrated by the retention of 90% of enzyme stability in ChCl:GLY (10% w/v) after 24 h and a maximum glucan conversion of 72.6%. Using response surface methodology, one-pot process parameters for optimal saccharification yield were optimised. A modified Michaelis-Menten kinetics model was applied to analyse the effect of enzyme concentration on reaction rate. Additionally, the DES pre-treatment process was integrated with microwave heating for efficient delignification of SB and the conventional DESs were incorporated with Lewis acids ($MgCl_2.6H_20$, $NiCl_2.6H_20$) in an effort to provide the acidity essential for fractionation while preserving the maximum biocompatibility of DESs. In this context, choline chloride: ethylene glycol: $NiCl_2.6H_2O$ (ChCl:EG:NI) at a molar ratio 1:2:0.016 with 20 w% water as a co-solvent produced the most promising results, with 84% delignification and 99% enzyme digestibility. Py-GC/MS and TGA investigations were used to examine the delignification process employing binary ChCl:EG DES and the novel ternary DES. Py-GC/MS analysis of lignin revealed that SB pre-treated with ChCl:EG:NI released the least phenolic compounds, indicating the maximum delignification efficiency. Furthermore, the one-pot integrated process produced 43.6 g/L of ethanol with 8.7 g ethanol per 100 g dry SB and 50.6 % theoretical yield. The integrated approach decreased the net selling price of ethanol by 30% to 9.75 $/gal, according to a preliminary cost study. The life cycle assessment revealed that the multi-unit process caused 10% more global warming and 4% more water depletion than the one-pot method. Thus, this research illustrates the potential of biocompatible DESs, and provides new opportunities for developing an effective and sustainable biomass conversion process.