# ABSTRACT

Various environmental concerns, including alarming climate change, have facilitated the need for sustainable energy alternatives in recent years. Lignocellulosic biomass (LCB), as the most abundant source of organic material on earth, can be a useful renewable feedstock for the production of biofuels and various value-added chemicals. In recent years, Ionic Liquid (IL), especially 1-ethyl-3-methylimidazolium acetate (EmimAc) is identified as a promising 'green solvent' for the pretreatment of LCB. Nevertheless, the IL pretreatment technology needs to be optimized for increasing process efficiency and minimizing its inhibitory effects on the Glycoside Hydrolases (GH) during the hydrolysis step. In this aspect, water can be utilized as a potential cosolvent with IL. Besides, IL-tolerant GH enzymes need to be developed along with glucose-tolerant β-glucosidases for efficient production of sugars from IL pretreated LCB. Thus, the present dissertation focuses on understanding the molecular insight into the deconstruction of lignocellulosic biomass to sugars in varying concentrations of EmimAc and water binary mixed solvent systems. Here, molecular-level behavior of cellulose microcrystal, macromolecular lignin (61 guaiacyl units), endoglucanase (RmCel12A), xylanase (TmXYN10B), and β-glucosidase (H0HC94) was investigated using atomistic molecular dynamics simulations. Dissolution of both cellulose and lignin took place in 50% and 80% EmimAc cosolvent systems. Loss of a significant number of intrachain and interchain hydrogen bonds (HBs) and formation of cellulose-Ac HBs governed the cellulose decrystallization process. Radial distribution function indicated the crucial role of Ac anions for the solvation of alkyl tail and hydroxyl groups in lignin. As the water content increased, water–anion network cluster formed, leading to the saturation of anion's HB ability, and the cosolvent property of water gradually decreased. Moreover, while studying the IL-tolerance of RmCel12A, the enzyme was stable up to 40% EmimAc. However, the essential enzyme motion and native hub residues at the active site were lost in 60% EmimAc, as evidenced by the Principal Component Analysis (PCA) and Protein structure network (PSN), respectively. Some non-catalytic residues (D13, R18, N55, Y119, and E203) were predicted as mutation targets for increasing IL tolerance. On the other hand, the reduced activity of TmXYN10B in high IL content (35% EmimAc) was caused by the alteration in both dominant modes and clique-community pattern of the active site. The Emim cations strongly interacted with the negatively charged active site of both the RmCel12A and TmXYN10B to inhibit their activity. Finally, the effects of low (0.1 M) and high (0.8 M) glucose concentrations were studied to understand the glucose-induced uncompetitive inhibition of H0HC94. PSN analysis indicated significant remodeling of the active site in terms of cliques and communities. In addition, six secondary binding sites (SBS) were identified, which could induce the uncompetitive inhibition of H0HC94. To improve the glucose tolerance, the role of conformational change and SBS were further predicted in HOHC94 mutants (C174V and H229S). Overall, this study unveils molecular insights into the deconstruction of lignocellulosic biomass in EmimAc-water mixed solvent systems for designing a 'One-pot' process of simultaneous pretreatment and hydrolysis at an industrial scale.



**Keywords:** lignin, xylanase, β-glucosidase, protein structure network, radial distribution function, principal component analysis, molecular dynamics simulation.