Designing tailored microbial and enzymatic response in ionic liquids for lignocellulosic biorefineries

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Out of myriad of challenges that still need to be overcome to realize lignocellulosic biorefineries, the capital and operational cost (CAPEX and OPEX, respectively) are second to the biomass cost itself. In addition, cost effective, feedstock flexible, and efficient pretreatment technologies are being researched to pretreat the recalcitrant biomass and enhance the accessibility of the cellulolytic enzymes to the cellulose polymer for the production of fermentable sugars. In this regard, ionic liquids (ILs) as a pretreatment solvent is gaining significant interest from the researchers worldwide due to its exceptional ability to solubilize lignocellulosic biomass (Remsing et al. 2006, Dadi et al. 2006; Singh et al. 2009). Strategies to develop cheap ILs, solvent recycling, and IL-based consolidated and integrated one-pot processes are being developed simultaneously with need to minimize IL and enzyme loading, water use, and processing steps (Cruz et al. 2013; Socha et al. 2014; Sun et al. 2017; Xu et al. 2015). To minimize CAPEX and OPEX and enable profitable biorefineries that implement unique benefits of the biomass solubilizing and lignin fractionation ability of some ILs, there is an increase interest in the design of simplified one-pot process (Fig. 1) with minimum separation and washing (Konda et al. 2014). Although simultaneous saccharification and fermentation (SSF) is easier to implement because of process compatibility, consolidated one-pot pretreatment-saccharification-fermentation (PSF) is not common for most pretreatment technologies that employ acids and bases for pretreatment to overcome biomass recalcitrance. At minimum, this needs pH adjustment prior to SSF and adds complexity due to salt formation and other issues for solvent

recovery and recycle and recovery of clean lignin stream. Towards the goal of cost effective and efficient processing of lignocellulose using ILs, there is a need for microbes and cellulolytic enzymes that function in the presence of ILs. This has led to an intense research into understanding the fundamental interaction of cellulolytic enzymes and microbial hosts with ionic liquids.

The early reports of ILs inhibiting enzymatic and microbial activities opened up the general area of study on how cellulose and microbes interact with non-aqueous solvent and the efforts on the development of IL tolerant cellulases and microbial hosts. Petkovic et al. reported on detailed metabolic profile of a filamentous fungi, *Penicillium* sp. in the presence of 16 ILs comprising imidazolium, cholinium or pyridinium cation and associated the high tolerance of this filamentous fungi to its ability to alter cell biochemistry in response to ILs (Petkovic et al. 2009). They used cluster study and Quantitative Structure-Activity Relationship (QSAR) in an attempt to link the toxicity of ILs to chemical structure for ease of IL toxicity evaluation. There have been numerous reports detailing the decrease of microbial IL tolerance with the increase of alkyl chain length (Petkovic et al. 2009, Nanchaiah and Francis 2011). Khudyakov et al. suggested that molecular mechanism of IL tolerance for *Enterobacter lignolyticus* (SCF1)—a soil bacterium from tropical rain forest included downregulation of membrane porin to reduce uptake of IL, upregulation of osmo-protectant transporters and drug efflux pumps to reduce intracellular IL concentration and cell membrane modeling via increase of cyclopropane fatty acid in the cell membrane (Khudyakov et al. 2012) and further used targeted functional screening of DNA to decipher mechanism of this bacterium to IL tolerance that involved a single gene *eilA* encoding a 52-kDa protein, which is homologous to the universal major facilitator superfamily membrane transporters (Rüegg et al. 2014). Although mechanistic details of IL tolerance are still unknown for *Firmicutes*, Wu et al. recently quantified gene transcriptions related to IL tolerance and biomass hydrolysis in *Firmicutes* and reported the

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Fig. 1 Consolidated ‘One-Pot Pretreatment-Saccharification-Fermentation’ scheme for fuels and chemicals from lignocellulosic biomass using ionic liquids. Panels show cellulosic plant cell wall (a), swollen cell wall in IL (b), and completely solubilized biomass in IL (c)

exciting finding of increase of transcription of xylanase, endoglucanase and beta glucosidase in the presence of IL (Wu et al. 2016).

Cellulase is a cellulolytic enzyme that was discovered serendipitously due to US Army's rotting tent and garments at Solomon Island during World War II and now plays a key role in the realization of lignocellulosic biorefineries, and bio-based fuels and chemicals (Ragauskas et al. 2006, Le Crom et al. 2009). Realization that certain ILs inhibit the activity of cellulase sparked interest among researchers on mining microbial communities for more IL tolerant cellulase, engineering cellulase to impart IL tolerance, evolving cellulases on ILs to enhance IL tolerance and other creative strategies (Datta et al. 2010; Wolski et al. 2016; Xu et al. 2016). Since application of non-aqueous solvent for bio-catalysis is a relatively emergent topic, there is still a lot of fundamental science questions that need to be investigated. For example, does the Hofmeister series for IL make sense? Can the IL anions and cations be ordered in a series according to their kosmotropic and chaotropic natures? Since ILs are designer solvents and the combinations of anion and cation pairs are limitless, a more attainable question for the biorefinery-centric application is to limit the query to the ILs that show promise for overcoming the biomass recalcitrance and are suitable for bioconversion of

lignocellulosic biomass. In this regard, imidazolium-based ILs and especially 1-ethyl-3-methylimidazolium acetate ($[C_2mim][OAc]$) has emerged as a gold standard to compare the efficiency of other ILs (Singh et al. 2009; Li et al. 2010). Enigmatically, $[C_2mim][OAc]$ IL has evaded the generalization based on Hofmeister series in terms of understanding the nature of cellulase inhibition.

Based on the findings that halotolerant cellulases are more IL tolerant and that halotolerant cellulase on average exhibit more negative surface charges, clever engineering of cellulases to modify the positive-to-negative charge ratios have gained interest (Nordwald and Kaar 2013). Recent publications, with well conceptualized design of experiments, are providing molecular level details on the mechanism of cellulase inhibition in ILs (Burney et al. 2015; Johnson et al. 2016). Briefly, more negative cellulase surface lessen the anion binding to active sites and prevent cellulase denaturation. The added advantage of this approach is the hypothesis that negative enzyme surface may also minimize impact of cellulase inhibition due to lignin (Nordwald et al. 2014). More recent combined experimental and computational investigation has provided detailed insight of molecular level mechanism of cellulase inhibition and denaturation in imidazolium IL and shedding light on biphasic manner of cellulase inactivation

(Summers et al. 2017). These detailed insights, growing number of draft genome sequences, clever engineering of cellulase, crosslinked, and modified cellulase that lead to more stable cellulase and enabling its recyclability all point to the brighter horizon for promise of lignocellulosic industry employing ILs for bioconversion. The potential of exploiting the designer nature of ILs begs the question: can the ILs be designed to illicit desired response from enzymes and microbes to turn the problem on its head and make the ILs to not just be synergistic with biocatalyst but boost the response for carbon efficient conversion at high biomass loadings?

Compliance with ethical standards

Conflict of interest Seema Singh declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

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