139. Optimization of Recombinant Cellulase Mixtures for Degradation of [C2C1Im][OAc] Pretreated Biomass

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Project Goals

The overall goal of this project is to optimize the ratios of a minimal set of cellulolytic enzymes that maximize glucose yields from 1-ethyl-3-methylimidazolium acetate ([C2C1Im][OAc]) pretreated biomass at 70 °C and 20% [C2C1Im][OAc]. Achieving this goal requires meeting two specific aims 1) develop a platform for characterizing large numbers of enzymes sampled from available glycosyl hydrolase and auxiliary enzyme families databases (e.g., CAZy) and from newly available studies of extremophilic microbial communities for their ability to remain stable and active at thermophilic temperatures and in the presence of [C2C1Im][OAc] and 2) develop an experimental design approach to optimizing both the set of enzymes in the mixture and their ratios.

Salts with Tm < 100 °C are called ionic liquids (ILs), and the IL 1-ethyl-3- methylimidazolium acetate ([C2C1Im][OAc]) has been shown to effectively pretreat lignocellulosic biomass such that enzymes can readily saccharify the cellulose into glucose at high yields (1, 2). Technoeconomic modeling at JBEI shows that an industrial- scale lignocellulosic biofuel process based on [C2C1Im][OAc] can be cost-competitive with fossil fuels (3). To sustain economic feasibility, the enzymes need to maintain robust activity at ≥70 °C in ≥20% ionic liquid, and the number and quantity of enzymes used must be minimized. Since biomass-degrading microorganisms have not evolved in [C2C1Im][OAc] environments, identifying C2C1Im][OAc] tolerant enzymes is challenging. Our strategy was to develop the JBEI Suite for Automated Lignocellulosic Saccharification (jSALSA), a high-throughput enzyme screening platform in which enzymes are characterized for their temperature, pH and [C2C1Im][OAc] concentration optima on a range of substrates, including [C2C1Im][OAc] pretreated biomass. Enzymes found to be stable and active in a minimum of 20% [C2C1Im][OAc] are then used in a design of experiments for mixtures to determine the ratios of a minimal set of recombinant enzymes that maximize glucose yields from [C2C1Im][OAc] pretreated biomass. Here we demonstrate this process to optimize the ratios of an endoglucanase, cellobiohydrolase and b-glucosidase mixture.

References

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