



# Rational approach to optimize cellulase mixtures for hydrolysis of regenerated cellulose containing residual ionic liquid

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## ARTICLE INFO

### Article history:

Received 2 September 2011

Received in revised form 20 October 2011

Accepted 22 October 2011

Available online 30 October 2011

### Keywords:

Ionic liquid

Cellulose pretreatment

Enzymatic cellulose hydrolysis

Kinetic modeling

## ABSTRACT

For the efficient production of glucose for platform chemicals or biofuels, cellulosic biomass is pretreated and subsequently hydrolyzed with cellulases. Although ionic liquids (IL) are known to effectively pretreat cellulosic biomass, the hydrolysis of IL pretreated biomass has not been optimized so far. Here, we present a semi-empirical model to rationally optimize the hydrolysis of pretreated  $\alpha$ -cellulose – regenerated from IL and containing residual IL from the pretreatment. First, the influence of the IL MMIM DMP on the individual cellulases endoglucanase I, cellobiohydrolase I and  $\beta$ -glucosidase was investigated. Second, an enzyme loading-dependent model was developed to describe kinetics for the individual cellulases and cellulase mixtures. Third, this model was used to optimize the cellulase mixture for the efficient hydrolysis of regenerated cellulose containing residual IL. Finally, we could significantly increase the initial hydrolysis rate in 10% (v/v) MMIM DMP by 49% and the sugar yield by 10% points.

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## 1. Introduction

One of today's key challenges is the development of new processes for the utilization of biomass. For the economical conversion from biomass to biofuels or platform chemicals, it is a prerequisite to efficiently hydrolyze cellulose to glucose at high yields, as cellulose represents 25–55% of the biomass (Kumar et al., 2009). The enzymatic cellulose hydrolysis has the benefits of highly selective conversion at energy saving low temperatures and pressures (Lynd et al., 2002). Though, the efficiency of the enzymatic cellulose hydrolysis is limited by long reaction times, due to declining reaction rates during the hydrolysis. Besides residual lignin and hemicellulose the main reason for the slow hydrolysis is the highly organized structure of the undissolved cellulose substrate, limiting the accessibility of the enzyme to the cellulose polymer chains (Alvira et al., 2010; Jeoh et al., 2007). Key parameters influencing the cellulose accessibility include available surface area, particle size, porosity and crystallinity (Arantes and Saddler, 2010). Biomass is therefore pretreated to achieve higher cellulose hydrolysis rates. Biomass pretreatment is an extensively studied research field, and various pretreatment methods are already available (Kumar et al., 2009; Sousa et al., 2009).

However, all pretreatment methods pose disadvantages that limit their industrial applicability up to now. Therefore, it is still necessary to explore alternative methods and gain in-depth understanding of the underlying influence factors to improve the bio-

mass conversion. An emerging biomass pretreatment method applies ionic liquids to effectively increase cellulose accessibility (Pinkert et al., 2009, 2010). A number of ionic liquids is known to dissolve cellulose and wood which can be regenerated by the addition of water, resulting in cellulose with low crystallinity and high porosity (Swatloski et al., 2002; Zavrel et al., 2009). In particular, imidazolium-based ionic liquids were found to effectively dissolve cellulose (Zakrzewska et al., 2010). The regenerated cellulose can be hydrolyzed up to 20-fold faster, thereby significantly reducing hydrolysis times (Engel et al., 2010; Zhao et al., 2009). However, residual ionic liquid from the pre-treatment negatively influences reaction rates and yields (Datta et al., 2010; Engel et al., 2010). To increase efficiency of regenerated cellulose hydrolysis containing residual ionic liquid from the pre-treatment, the influence of the ionic liquid on the individual cellulases and the optimization of the cellulase mixture for this particular pretreatment remains to be investigated (Engel et al., 2010). Of course, for economical reasons, the ionic liquid has to be recycled nearly quantitatively and therefore, the residual ionic liquid that was not removed prior to enzymatic hydrolysis, has to be recovered at a later process stage, e.g. by nanofiltration.

The adaptation of cellulase mixtures for optimized cellulose hydrolysis has to be performed for each particular cellulosic substrate and for each pretreatment method. Impurities originating from the pretreatment have to be considered, including residual solvents, lignin or hemicellulose (Banerjee et al., 2010a,b,c). Mixture optimizations have been performed for cellulose (Baker et al., 1998; Gusakov et al., 2007), lignocellulose substrates (Banerjee et al., 2010b; Berlin et al., 2007), and different pretreatment methods (Rosgaard et al., 2007). Until now, most efforts to identify

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