Substrate Dependency and Effect of Xylanase Supplementation on Enzymatic Hydrolysis of Ammonia-Treated Biomass

Rajesh Gupta · Tae Hyun Kim · Yoon Y. Lee

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Abstract Pretreatment based on aqueous ammonia was investigated under two different modes of operation: soaking in aqueous ammonia and ammonia recycle percolation. These processes were applied to three different feedstocks with varied composition: corn stover, high lignin (HL), and low lignin (LL) hybrid poplars. One of the important features of ammonia-based pretreatment is that most of the hemicellulose is retained after treatment, which simplifies the overall bioconversion process and enhances the conversion efficiency. The pretreatment processes were optimized for these feedstocks, taking carbohydrate retention as well as sugar yield in consideration. The data indicate that hybrid poplar is more difficult to treat than corn stover, thus, requires more severe conditions. On the other hand, hybrid poplar has a beneficial property that it retains most of the hemicellulose after pretreatment. To enhance the digestibility of ammonia-treated poplars, xylanase was supplemented during enzymatic hydrolysis. Because of high retention of hemicellulose in treated hybrid poplar, xylanase supplementation significantly improved xylan as well as glucan digestibility. Of the three feedstocks, best results and highest improvement by xylanase addition was observed with LL hybrid poplar, showing 90% of overall sugar yield.

Keywords Hybrid poplar · Corn stover · Xylanase · Aqueous ammonia · Pretreatment

Introduction

Various pretreatment technologies are being used to improve the digestibility of carbohydrates in biomass by making the cellulosic part more accessible by enzymes [1]. Among the major findings of recent pretreatment research is that a given pretreatment
reagent exhibits selectivity toward a certain type of reaction attacking specific chemical bonds in lignin–hemicellulose–cellulose matrix. Pretreatment methods operated at low pH including steam explosion [2, 3], hot water treatment [4–6], controlled pH treatment, and dilute acid treatment [7, 8] remove substantial amount of hemicellulose. On the other hand, pretreatment methods applying high pH such as ammonia fiber explosion [9–11], lime pretreatment [12, 13], and ammonia recycle percolation (ARP) [14, 15] show little effect on hemicellulose but high interaction with lignin. In low pH pretreatments, the liquid contains hemicellulose sugars and other degradation byproducts. These byproducts are known to be inhibitory in subsequent bioconversion process. The examples of the inhibitory compounds are phenolics derived from lignin degradation, furan derivatives (hydroxymethylfurfural and furfural) from sugar decomposition, and aliphatic acids [16, 17]. Sugar–lignin condensation reactions in pretreatment liquid further reduces the sugar yield [18–22]. Glucose/xylose co-fermenting microorganisms were found to be very sensitive to these inhibitory chemicals [23, 24]. Various methods have been used to detoxify these toxins [17], but it bears substantial additional cost. From this viewpoint, hemicellulose retention is a positive feature in pretreatment because it eliminates the need of converting sugars in pretreatment liquid [25].

In most of the pretreatment studies, process conditions of pretreatment are optimized in terms of glucan digestibility. One of the factors that limit the digestibility of pretreated biomass is insufficient xylanase activity in the “cellulase” used in the hydrolysis [45]. This is particularly true for substrates with high xylan content. According to Berlin et al., the hydrolysis rate of different organosolv-treated hardwood did not show any correlation with the filter paper activity of different enzyme preparations, yet showed significant correlation with the endogenous xylanase activity [26]. Covalent and non-covalent association of xylan with cellulose and lignin is an essential factor for holding the structural integrity of cell wall [27]. Removal of xylan either by pretreatment or by xylanase would enhance enzymatic hydrolysis of cellulose. Xylanase not only degrade xylan but also assists in delignification as observed in bio-bleaching of pulp [28].

In Fig. 1, lignin vs xylan content of various biomass feedstocks is shown. This data has been taken from the DOE website (http://www.eere.energy.gov/biomass/progs/search1.cgi). The data indicate that there is an inverse relationship between the lignin and xylan content of biomass. These feedstocks were divided into three different categories: Regions 1, 2, and 3 represent the feedstocks with high xylan and low lignin, low xylan and high lignin, and moderate xylan and moderate lignin, respectively. In this study, feedstocks representing the three regions were selected: corn stover, high-lignin (HL) hybrid poplar, and LL hybrid.

![Fig. 1 Relation between lignin and xylan content in different biomass](image-url)