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Proteins at heterogeneous (lignocellulose) interfaces

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Lignocellulosic biomass is a renewable resource capable of addressing the increasing worldwide demand for energy and the movement toward low carbon footprint, liquid transportation and aviation biofuels. Purposely grown energy crops (wood and grasses) and crop residues (corn stalks, sugarcane bagasse, and wheat straw) are available for conversion to biofuels if attractive process economics are achieved in hydrolyzing these lignocellulosic materials to sugars and converting the sugars to biofuels and bioproducts. Cellulase enzymes that hydrolyze cellulose to glucose currently contribute operating expenses of $0.15–$0.20 per liter of ethanol out of a total of $0.53. The goal is to decrease enzyme costs to 3–5 ¢/L. The high cost is in part due to the high loading of cellulases needed to make up activity losses when the enzymes bind to lignin rather than the cellulose substrate that is located in close proximity to the lignin. We address the concept of using liquid chromatography columns packed with biomass to efficiently probe partitioning of cellulases and other proteins on the surfaces of various forms of lignocellulose. The correlation of elution profiles to fundamental adsorption behavior provides a pathway to a deeper understanding of inhibition of cellulose hydrolysis due to interactions of proteins at heterogeneous lignocellulosic interfaces.

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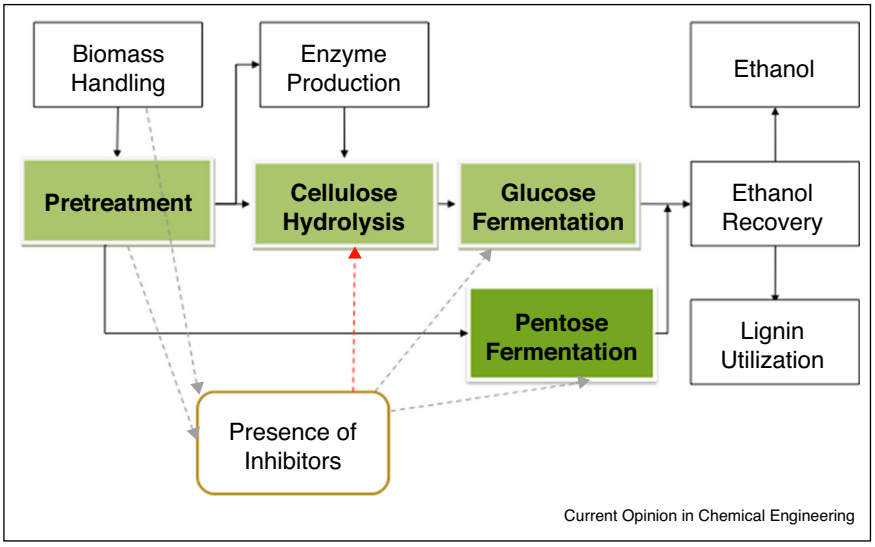
Introduction

Renewable energy resources have drawn people's attention because of concerns about depletion of fossil fuels and increasing worldwide demand for energy [1••]. Biofuels from renewable resources fit into two categories: 'first generation biofuels' derived from starch or sugar based feedstocks such as corn and sugarcane, and the 'second generation biofuels' produced from lignocellulosic biomass. Production processes of first generation biofuels are relatively mature and currently supply over 100 billion liters of fuel ethanol worldwide [2,3•,4•,5]. Second generation biofuels based on agricultural or forest residues would increase fuel supply while avoiding use of arable land to grow cellulosic crops instead of food crops. A comprehensive accounting and analysis of cellulosic biomass in the US shows that there are 343 million tons/yr available in 2017, and could grow to 1.2 billion tons by 2040 assuming a price of $60/ton biomass (dry basis) [6]. Of this total approximately 30% correspond to residues from agriculture for food production, municipal wastes, and forestry.

Extensive research has been carried out to improve the conversion efficiency of lignocellulosic feedstock to bioethanol. A recent report on design for a commercial wood to ethanol facility based on hydrothermal pretreatment illustrates how key improvements that have occurred since 2008 have played a role in reducing costs since no chemicals are added, and capital costs are lower than other types of pretreatments [7,8]. Research on hydrothermal pretreatment has expanded considerably as indicated by a 19× increase in papers published in this area over the last 10 years [7,8]. At this point, technical problems related to biomass inhibitors and enzyme cost still need to be solved [8–13].

The unit operations necessary for converting cellulose are shown in Figure 1 with inhibitors being generated at many points. Hydrolysis is preceded by cooking of biomass in liquid hot water, which generates lignin-derived inhibitors of cellulase enzymes while exposing both lignin and cellulose. While enzyme inhibitors may be removed by washing of the biomass solids after pretreatment, exposed lignin that is closely associated with cellulose displays a solid surface that adsorbs cellulase which hinders the ability of these enzymes to interact with the pretreated cellulose and catalyze hydrolysis [14,15]. Hence, inhibition and deactivation must be addressed if efficient use of enzymes is to occur and for enzyme costs are to be reduced. We propose that chromatography be employed to infer and characterize interactions of cellulase enzymes with the heterogeneous solid interfaces that define lignocellulosic biomass.

Figure 1



Conversion of cellulosic biomass to ethanol.

Enzymes and inhibitors

Enzymes make up one of the major costs of bio-ethanol production and may contribute up to $1.47/gallon ($0.40/L) to bioethanol production costs for corn stover [12] although costs are now approaching about half this amount. This reduced cost reflects the effects of a combination of more efficient production of enzymes, addition of helper enzymes to moderate inherent inhibitory effects of different biomass plant cell wall constituents, and better engineering of cellulose hydrolysis processes [7,11].

The enzymatic hydrolysis step is preceded by pretreatment of the lignocellulosic biomass to open up the structure of the lignocellulosic material, thus increasing enzyme accessibility to cellulose [13,14]. These processes are sometimes accompanied by removal of hemicellulose or reduction of lignin content. Phenolic compounds released by lignin play a major role in deactivating and inhibiting the enzymes [13,16,17] but may be removed by washing. However, the lignin that remains in the cellolusic material also impacts negatively enzyme activities [18]. The mechanism is not fully understood, due to the complex and non-uniform structure of lignin and the difficulties in characterizing binding sites of enzymes to lignin. However, it is known that cellulases, especially β-glucosidase, bind to lignin non-productively and become deactivated [17]. In order to compensate for this effect, a higher enzyme loading is used to achieve maximum extents of conversion of the cellulose in the pretreated material. These factors cause a pretreatment conundrum: pretreatment leads to higher (and ultimately complete) yields, but also causes a greater surface area of lignin to be generated resulting in the potential for non-productive interactions between enzyme and biomass [10] and the need to carry out hydrolysis at high enzyme loadings.

Currently many pretreatment methods exist, involving physical (chipping, milling, grinding), chemical (liquid hot water, stream explosion, AFEX, dilute acid, Organosolv, alkaline, etc.) or biological (brown or white fungi) processes [19,20,21•,22–25]. One solution would be to remove lignin resulting in a cellulose material that is readily hydrolyzed at low enzyme levels. However, the expense of such a pretreatment (which is essentially a pulping process) out-weighs savings in enzyme costs. While pretreatment conditions enhance accessibility, they may also expose lignin or other structures that are capable of adsorbing cellulases. This inevitably leads to increased operating costs due to biocatalyst consumption [25].

The capital cost of liquid hot water pretreatment is 1.5–5× less than other pretreatments [7]. Further cost reduction may be achieved by reducing the amount of enzyme added (i.e., enzyme loading). This review addresses how liquid chromatography may be used to probe lignocellulose obtained by liquid hot water pretreatments where added chemicals are not used with the goal of identifying conditions that reduce enzyme loadings.

Adsorption of proteins by lignin: protein properties that affect binding

The variability of commercially available cellulase mixtures in terms of different catalytic activities complicates observations and accurate measurements of adsorption of enzymes. Cellulose hydrolyzing enzymes consist of many