Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Mechanisms employed by cellulase systems to gain access through the complex architecture of lignocellulosic substrates

Bryon S Donohoe[1] and Michael G Resch[2]

To improve the deconstruction of biomass, the most abundant terrestrial source of carbon polymers, en route to renewable fuels, chemicals, and materials more knowledge is needed into the mechanistic interplay between thermochemical pretreatment and enzymatic hydrolysis. In this review we highlight recent progress in advanced imaging techniques that have been used to elucidate the effects of thermochemical pretreatment on plant cell walls across a range of spatial scales and the relationship between the substrate structure and the function of various glycoside hydrolase components. The details of substrate and enzyme interactions are not yet fully understood and the challenges of characterizing plant cell wall architecture, how it dictates recalcitrance, and how it relates to enzyme–substrate interactions is the focus for many research groups in the field. Better understanding of how to match pretreatments with improved enzyme mixtures will lead to lower costs for industrial biorefining.

Addresses
[1] National Renewable Energy Laboratory, Biosciences Center, 15013 Denver West Parkway, Golden, CO 80401, United States
[2] National Renewable Energy Laboratory, National Bioenergy Center, 15013 Denver West Parkway, Golden, CO 80401, United States

Corresponding author: Donohoe, Bryon S (bryon.donohoe@nrel.gov)

Current Opinion in Chemical Biology 2015, **29**:100–107

This review comes from a themed issue on **Energy**

Edited by **Timothy DH Bugg** and **Michael G Resch**

For a complete overview see the Issue and the Editorial

Available online 31st October 2015

http://dx.doi.org/10.1016/j.cbpa.2015.08.014

Introduction

The most abundant form of terrestrial carbon is in the form of plant biomass. Developing technologies to convert plant cell wall carbohydrates and lignin to fuels and chemicals will aid in reducing carbon dioxide in the atmosphere, dependence on oil importation, and improve energy independence. A deep understanding of the effects of thermochemical pretreatment and saccharification mechanisms will enable improvement of enzyme saccharification of biomass essential to the development of economically sustainable biorefineries.

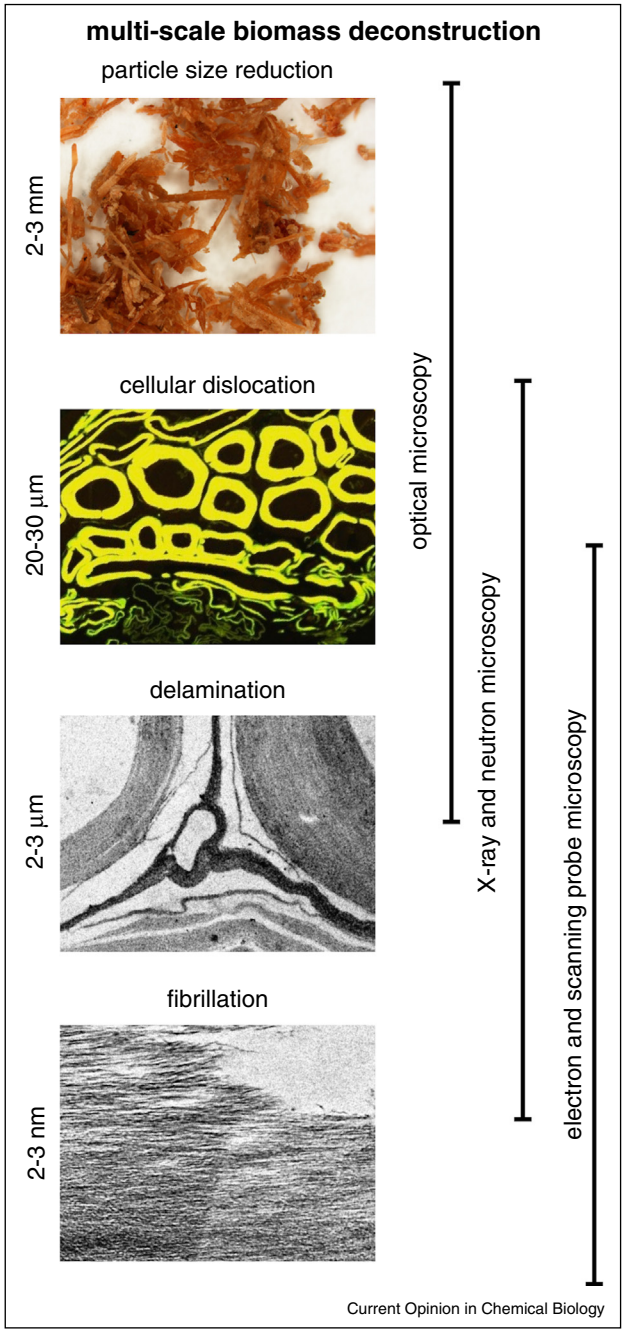
Enzymatic hydrolysis, in which lignocellulosic biomass is converted into fermentable sugars may be the most complex step in biomass conversion. At the molecular scale, the hydrolysis mechanisms of glycoside hydrolases have been extensively characterized and recently reviewed by Payne *et al.* [1••]. However, the complex nature of the plant cell wall presents many factors that impede enzymes such as substrate accessibility, lignin interference, cellulose crystallinity, and product inhibition. Nonetheless, enzymatic hydrolysis for biomass deconstruction offers the potential of high yields at mild operating conditions and higher selectivity than those of purely thermochemical processes. From the macromolecular scale through the cellular scale, we are only beginning to understand how a combination of enzymes, with a range of specificities, work together to gain and maintain access to complex lignocellulosic substrates. In this review we highlight recent progress to elucidate the enzymatic cell wall deconstruction mechanisms with emphasis on visualization techniques to understand the relationship between cell wall architecture and the function of carbohydrate active enzyme (CAZyme) components [2].

Direct substrate characterization

Determining what changes enzymes have made to lignocellulosic substrates relies on a foundation of knowledge about cell wall architecture that has benefited from new developments in imaging technologies along with careful consideration of sample preparation techniques (Figure 1). The direct observation of partially digested substrates has provided important new insights, across a range of scales, into biomass deconstruction and can be used elucidate cellulose mechanisms [3–7].

One of the most widely accessible techniques for substrate visualization is fluorescence microscopy. In comparison to other techniques, fluorescence imaging is fast, sensitive, and can be used for time-resolved imaging. Possibly the most informative use of fluorescence microscopy is immuno-fluorescence imaging using monoclonal antibodies to identify plant cell wall polysaccharides. This has been shown to be effective in characterizing plant cell walls before and after deconstruction [3,8]. Confocal microscopy was even used to guide the development of kinetic models that could suggest improvements in cellulase cocktails by revealing mechanisms and rate-limiting steps during cellulose degradation [9]. The

Figure 1



Multi-scale imaging of plant cell wall deconstruction by thermochemical pretreatment and enzymatic hydrolysis. The range of scales and typical microscopy modalities used are indicated.

results suggested that exposing new enzyme binding sites is a crucial rate-limiting step.

The only real disadvantage of fluorescence microscopy for addressing critical questions in biomass conversion is its lateral resolution is diffraction limited to roughly 250 nm. This is sufficient to visualize major cell wall layers and detect enzymes, but insufficient to monitor single enzymes or interrogate cell wall architecture. Super-resolution techniques however, have been in development over the past several years, achieve lateral resolution down to tens of nanometers, are now becoming commercially available and more widely used. These techniques use specialized illumination schemes, non-linear fluorophore responses, and image processing based localization to achieve stunning results. Super-resolution was used to measure the binding and movement of individual Cel7A CBH enzymes on cellulose surfaces and document their stop-and-go progression [7]. These new techniques are only beginning to be applied to plant samples, like the recent demonstration of super-resolution imaging to visualize cellulose bundles in onion epidermis cell walls [10]. Fluorescence techniques benefit from the continual development of fluorescent probes for investigating plant cell wall architecture and deconstruction and were reviewed recently by Paes [11]. Among the developments highlighted is photoactivatable fluorescent-labeled proteins fused to cellulase enzymes or CBMs to improve the resolution of localization and dynamics studies down to 10–50 nm [12].

In another recent review of biomass imaging, Bubner *et al.* point out the advantages that atomic force microscopy (AFM) has in terms of high resolution and being able to perform imaging on fully hydrated samples [13]. This is clearly true for imaging cellulose model substrates; however imaging real plant cell wall substrates still requires extensive and careful sample preparation that is sometimes discounted. Even for studies on model substrates, attention to minimal modification, and immobilization onto a surface are required for high-quality, interpretable data [4]. A recent study developed a simple and gentle protocol for cell wall preparation for AFM imaging of microfibrils and cell wall matrix materials at the inner surface of the epidermal cell walls in onion epidermis [14•]. With these techniques, microfibrils were made clearly visible in a near native state. A striking observation was that no obvious cross-links were seen among microfibrils. Instead, the microfibrils simply appear to come into close proximity with one another over distances of several tens of nanometers.

Another advanced imaging technique used to reveal the complex 3D architecture of plant cell walls is electron tomography [15•]. Sakar *et al.* compared tradition chemical fixation, high-pressure freezing followed by freeze substitution and resin embedding (HPF-FS), and vitreous sectioning methods to preserve plant tissues and detect structural differences in plants with genetically altered cell walls. Their study provides a novel view into the complexity of the cell wall, and they conclude that cryopreservation provides closest-to-native preservation. Electron tomography was also used as the primary tool to