Multiple Levers to Solve Recalcitrance for Lignocellulosic Solubilization

Evert K. Holwerda[1,2] (evert.k.holwerda.th@dartmouth.edu), Robert S. Worthen,[1,2] Natalie M. Ruppertsberger,[1,2] Michael L. Balch,[1,2] Brian H. Davison,[2] Zeng-Yu Wang,[3,2] Richard A. Dixon,[4,2] Debra Mohnen,[5,2] C. Neal Stewart,[6,2] Daniel G Olsen,[1,2] Gerald Tuskan,[2] Mark Davis,[7,2] Michael E. Himmel,[7,2] Jeffrey V. Zurawski,[8,2] Robert M. Kelley,[8,2] Charles E. Wyman,[9,2] Lee R. Lynd,[1,2] and **Paul Gilna**[2]

[1]Dartmouth College, Hanover, New Hampshire; [2]BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee; [3]The Samuel Roberts Noble Foundation, Ardmore, Oklahoma; [4]University of North Texas, Denton, Texas; [5]University of Georgia, Athens; [6]University of Tennessee, Knoxville; [7]National Renewable Energy Laboratory, Golden, Colorado; [8]North Carolina State University, Raleigh; [9]University of California, Riverside

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) designing plant cell walls for rapid deconstruction and (2) developing multi-talented microbes or converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. BESC biomass formation and modification research involves working directly with two potential bioenergy crops (switchgrass and *Populus*) to develop varieties that are easier to break down into fermentable sugars. We are testing large numbers of natural variants and generating specific and modified plant samples as well as developing genomics tools for detailed studies into poorly understood cell wall biosynthesis pathways. BESC research in biomass deconstruction and conversion targets CBP by studying thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction and manipulating these microorganisms for improved conversion, yields, and biofuel titer. BESC researchers provide enabling technologies in biomass characterization, 'omics, modeling and data management in order to (1) understand chemical and structural changes within biomass and (2) to provide insights into biomass formation and conversion mechanisms.

The primary barrier to economically competitive cellulosic biofuels is the resistance of plant cell walls to deconstruction – termed recalcitrance. Overcoming this barrier may be accomplished via multiple recalcitrance "levers" including:

1. starting with nature's best with respect to feedstocks and biocatalysts;
2. biotechnology to improve plants, enzymes, and microbes; and
3. non-biological processing prior to or during solubilization and fermentation.

Here we will report progress at applying and evaluating these levers. Studies aimed at individual levers include:

- comparison of the effectiveness of various biocatalysts at mediating plant cell wall solubilization;
- targeted modification of plants to decrease recalcitrance without sacrificing growth;
- targeted modification of thermophilic anaerobes to improve ethanol yield and titer without sacrificing fermentation capacity; and
- mechanical milling during fermentation, termed co-treatment.

In addition, we have evaluated solubilization of several second year field-trial lignocellulosic feedstocks, engineered for reduced recalcitrance with three different microbial solubilization systems; *Caldicellulosiruptor bescii*, *Clostridium thermocellum* and *Saccharomyces cerevisiae* with commercial fungal enzymes. This first-of-a-kind dataset will be used to gain new insights into the interplay between plant modification and choice of biocatalyst.

Based on our results, we conclude that there are powerful, emergent strategies to overcoming plant cell wall recalcitrance, and that systematically exploring combinations of these levers is a promising approach to enabling the cost-effective production of cellulosic biofuels.

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