In Vitro Cell.Dev.Biol.—Plant (2009) 45:193–198
DOI 10.1007/s11627-009-9213-y

INVITED REVIEW

The DOE BioEnergy Science Center—a U.S. Department of Energy Bioenergy Research Center

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Received: 6 February 2009 / Accepted: 20 April 2009 / Published online: 28 May 2009 / Editors: P. Lakshmanan; D. Songstad

Abstract The BioEnergy Science Center, a nationally and internationally peer-reviewed center of leading scientific institutions and scientists, is organized and in operation as a U.S. Department of Energy Bioenergy Research Center. This Oak Ridge National Laboratory-led Center has members from top-tier universities, leading national labs, and private companies organized as a single project team, with each member chosen for its significant contributions in the Center's research focus areas. The recalcitrance of cellulosic biomass is viewed as (1) the most significant obstacle to the establishment of a cellulosic biofuels industry, (2) essential to producing cost-competitive fuels, and (3) widely applicable, since nearly all biofuels and biofeedstocks would benefit from such advances. The mission of the BioEnergy Science Center is to make revolutionary advances in understanding and overcoming the recalcitrance of biomass to conversion into sugars, making it feasible to displace petroleum with ethanol and other fuels.

Keywords Cellulosic biomass · Biofuels · Recalcitrance · Ethanol · Consolidated bioprocessing · Poplar · Switchgrass

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Background and History

In December 2005, two U.S. Department of Energy (DOE) programs working together convened a Biomass to Biofuels Workshop (the Office of Biological and Environmental Research in the Office of Science, and the Office of Biomass Programs in the Office of Energy Efficiency and Renewable Energy) for the purpose of defining barriers and challenges to the rapid expansion of the production of ethanol from cellulose. A core barrier was identified: the resistance, or "recalcitrance," of cellulosic biomass to the conversion into sugars which can then be fermented into ethanol. The two programs articulated a joint research agenda involving the application of modern tools of biological research. The result of this workshop was the publication of a roadmap for speeding the research related to cellulosic ethanol production (DOE/SC-0095 2006).

In the past few years, biological research has seen a major transformation with the emergence of an approach known as "systems biology." Arising from human genome research, this approach involves the application of advanced techniques, tools and high-throughput technologies, computational modeling, and also the adoption of team science. Systems biology is at the core of the DOE Genomics:GTL (GTL) program (http://genomicsgtl.energy.gov/benefits/index/shtml). GTL (formerly known as "Genomes to Life") is a program of the Office of Biological and Environmental Research of the U.S. Department of Energy Office of Science.

The GTL research program is focused on developing technologies that can be used to understand plants and microbes and to enable biological solutions to challenges in energy, environment, and climate. DOE's "mission challenge" related to energy is described as understanding the principles of the structural and functional design of plants and microbes, and developing the capability of modeling, prediction, and engineering of optimized enzymes and microorganisms for the production of such biofuels as ethanol and hydrogen (http://genomicsgtl.energy.gov/benefits/index.shtml).

To focus the research resources of GTL on the biological challenges of biofuel production, DOE announced in June 2007 the establishment of three new Bioenergy Research Centers (BRCs). Each center is projected to receive $135 million over a 5-yr period to pursue the basic research related to high-risk, high-return biological solutions for bioenergy applications. The new scientific knowledge generated by these three BRCs is expected to lay a foundation of methods and tools for the emerging biofuel industry. The purpose of this paper is to provide insight into the BRC which is being lead by Oak Ridge National Laboratory (ORNL).

A competitive, peer-review process resulted in the selection of Oak Ridge National Laboratory in Tennessee to lead the DOE BioEnergy Science Center (BESC); the University of Wisconsin-Madison to lead the DOE Great Lakes Bioenergy Research Center (GLBRC); and DOE's Lawrence Berkeley National Laboratory to lead the DOE Joint BioEnergy Institute (JBEI). Each of these three centers represents a multidisciplinary partnership with research expertise in the physical and biological sciences, including genomics, microbial and plant biology, analytical chemistry, and computational biology.

The BESC team consists of ten institutional partners and individual investigators selected for their extensive experience in biomass research at seven other institutions. The new Joint Institute for Biological Sciences systems biology research facility at ORNL serves as the central hub for coordinating research activities among the various BESC partners. Through developing the scientific basis for understanding biomass recalcitrance, BESC's researchers hope to lay the foundation for the re-engineering of plants, microbes and enzymes at the system, cellular and molecular levels to improve the productivity of bioenergy crops, and to reduce the cost of conversion.

BESC Partners

DOE's Oak Ridge National Laboratory (ORNL), Oak Ridge, Tennessee. DOE's largest science and energy laboratory, ORNL is a world leader in poplar genome research, with strong programs in comparative and functional genomics, structural biology, and computational biology and bioinformatics. The ORNL Spallation Neutron Source and supercomputers at the ORNL National Leadership Computing Facility are used in BESC for investigating the activity of enzyme complexes.

Georgia Institute of Technology, Atlanta. Georgia Tech's Institute for Paper Science and Technology provides BESC with expertise in biochemical engineering and instrumentation for high-resolution analysis of plant cell walls.

National Renewable Energy Laboratory (NREL), Golden, Colorado. NREL has over 30 yr of experience in biomass analysis and biofuel research, and houses premiere facilities for analyzing biomass surfaces. NREL also has a long history of establishing biofuel pilot plants and working with industry for commercial development of technologies.

University of Georgia, Athens, Georgia (UGA). UGA's Complex Carbohydrate Research Center maintains state-of-the-art capabilities in mass spectrometry, NMR spectroscopy, chemical and enzymatic synthesis, computer modeling, cell and molecular biology, and immunocytochemistry for studying the structures of complex carbohydrates and the genes and pathways controlling plant cell-wall biosynthesis.

University of Tennessee, Knoxville, Tennessee (UT). UT conducts successful programs in bioenergy-crop genetic and field research (particularly switchgrass) and biotechnological applications of environmental microbiology.

Dartmouth College, Hanover, New Hampshire. Dartmouth's Thayer School of Engineering is a leader in the fundamental engineering of microbial cellulose utilization and consolidated bioprocessing approaches.

ArborGen, Summerville, South Carolina. ArborGen is a world leader in forest genetics research, tree development, and commercialization.

Verenium Corporation, Cambridge, Massachusetts. Verenium is a biofuels-focused biotechnology company and developer of specialty enzymes found in diverse natural environments and optimized for targeted applications.

Mascoma Corporation, Lebanon, New Hampshire. Mascoma is a leader in developing microbes and processes for economical conversion of cellulosic feedstocks into ethanol.

The Samuel Roberts Noble Foundation, Ardmore, Oklahoma. This non-profit research foundation is devoted to improving agricultural production and to advancing the development of switchgrass and other grasses through genomic research. Their activities are conducted by the Agricultural Division and the Foundation's Plant Biology and Forage Improvement programs.

Individual researchers from the University of California, Riverside; DOE's Brookhaven National Laboratory (Upton, New York); Cornell University (Ithaca, New York); Virginia Polytechnic Institute and State University (Blacksburg, Virginia); North Carolina State University (Raleigh, North Carolina); University of Minnesota (St. Paul, Minnesota); and Washington State University (Pullman, Washington) specialize in biomass pretreatment, characterization of plant-associated microbes, cellulose and enzyme modeling, consolidated bioprocessing, and lignin biochemistry.

BESC focuses on the fundamental understanding of the resistance of cellulosic biomass to enzymatic breakdown into sugars. This is thought to be the single-greatest obstacle to cost-effective production of biofuels (Lynd et al. 2008) (Fig. 1). BESC's approach to making biomass easier to degrade involves (1) designing plant cell walls for rapid deconstruction and (2) engineering a multi-talented microbe tailor-made for converting plants into biofuel in a single step, an approach known as consolidated bioprocessing. In this way, the problem is approached from two directions; both from the perspective of being able to design optimized biofuel feedstock crops and also from the "deconstruction" part of the problem. In BESC, these two research areas are not being pursued independently of each other, but are coupled in a single integrated plan.

The BESC Research Strategy

The unifying theme of BESC is to understand and overcome the recalcitrance of lignocellulosic biomass for conversion to fermentable sugars. This is a complex problem since the cell walls of plants are largely made up of cellulose and hemicellulose (polysaccharides that make up most of the mass of plant cell walls) and lignin that cross-links them together. This structure contributes mechanical strength and support to elements of the plant body. In addition, the lignin has properties which interfere with enzymatic conversion of the polysaccharides (DOE/SC-0095 2006). The researchers now working on these challenges in BESC were among the first to identify this biomass recalcitrance as a major obstacle to biomass conversion processes (Lynd et al. 1999) and are responsible for making fundamental advances in a wide range of related science (Lynd et al. 2005; Himmel et al. 2007).

BESC will pursue the following goals in three research focus areas (Fig. 2):

Focus Area 1. *Biomass formation and modification*—Goals: develop a thorough understanding

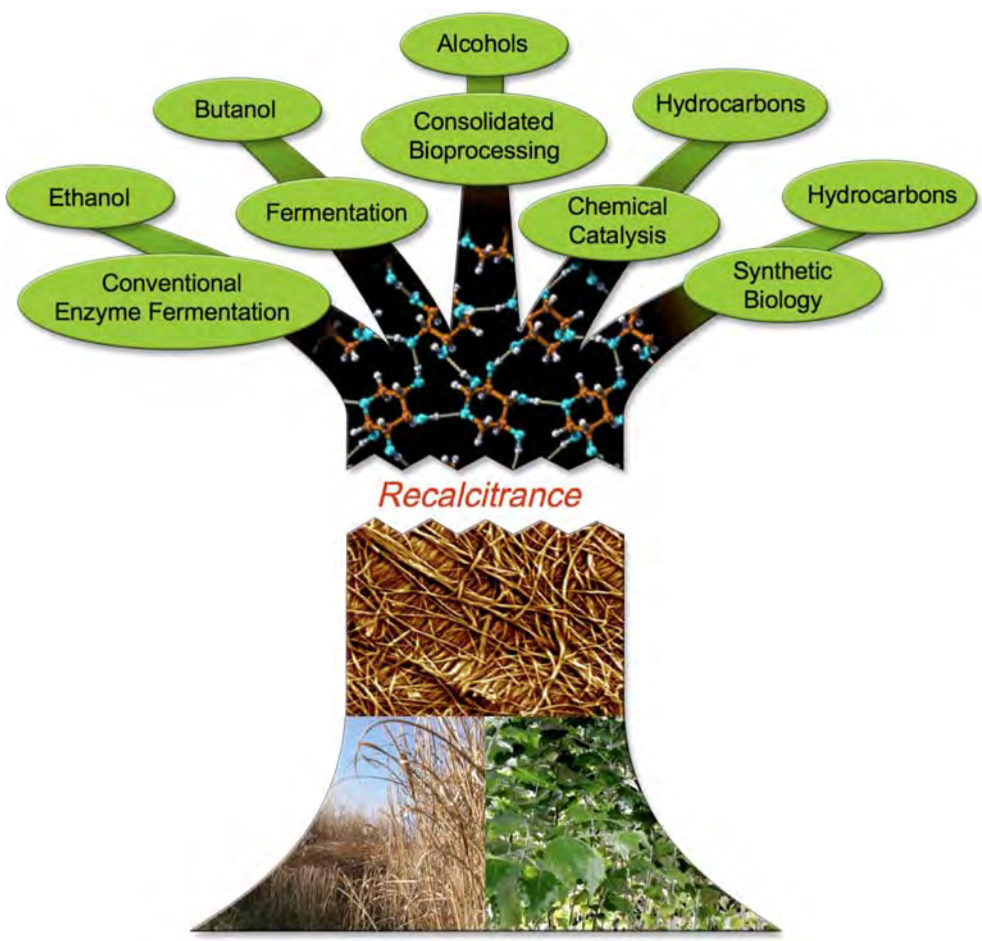

Figure 1. Access to the sugars in lignocellulosic biomass is the current critical barrier. Solving this problem will cut processing costs significantly for cellulosic biofuels.

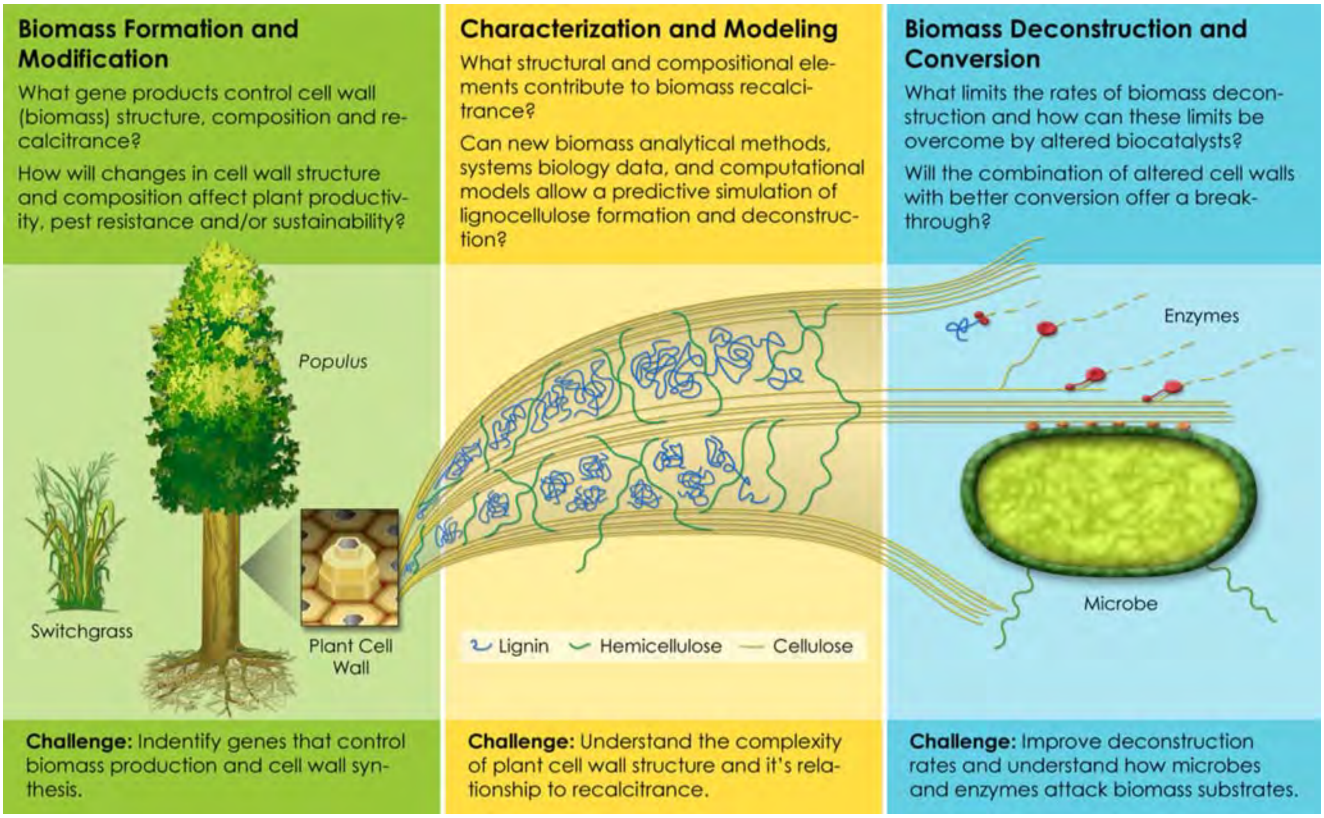

Figure 2. The BESC scientific approach integrates three science focus areas.

of the genetics and biochemistry of plant cell-wall biosynthesis so the process can be modified to reduce biomass recalcitrance.

Focus Area 2. *Biomass deconstruction and conversion*—Goals: develop an understanding of enzymatic and microbial biomass deconstruction; characterize and mine biodiversity; and use this knowledge to develop superior biocatalysts for consolidated bioprocessing (CBP).

Focus Area 3. *Characterization and modeling*—Goals: develop a high-throughput pretreatment and characterization pipeline for the systematic study of the structure, composition, and deconstruction of biomass. In a cross-cutting approach, integrate the scientific knowledge gained using chemical, spectroscopic, and imaging methods and computational modeling and simulation.

Focus Area 1. Biomass Formation and Modification. BESC's biomass formation and modification research involves working directly with two potential bioenergy crops—switchgrass and poplar—to develop varieties that are easier to break down into fermentable sugars yielding ultimately improved biofuel yields. Currently, little is known about how cellulose and hemicelluloses are synthesized; distributed within cell walls; and attached to each other, to lignin, or to cell-wall proteins (DOE/SC-0095 2006). Molecular, genetic, genomic, biochemical, chemical, and bioinformatics tools are being used by BESC researchers in this Focus Area to advance the understanding of cell-wall biosynthesis, cell-wall structure, and biological consequences of plant cell-wall modification. In addition, BESC is developing computational models of cell-wall biosynthesis that will help in poplar and switchgrass.

One major focus in plant cell-wall biosynthesis is the identification of genes that affect biomass production and to use this information to generate plants with reduced recalcitrance. Specifically:

– To identify and characterize gene products involved in cell-wall biosynthesis and structure, and to establish which genes alter biomass recalcitrance;
– To determine how changes in cell-wall composition and structure affect plant recalcitrance and to begin to develop a systems biology understanding of the genetic basis for cell wall structure and recalcitrance.

To accomplish the above, BESC is conducting fundamental research on the enzymes involved in plant cell-wall polysaccharide (cellulose, hemicellulose, and pectin) and lignin synthesis, a plant cell-wall biosynthesis gene database will be established and curated, and biosynthetic and regulatory genes encoding proteins involved in specific polysaccharide and lignin synthesis pathways will be investigated. Genes will be under- and over-expressed in *Populus* (*Populus trichocarpa*) and switchgrass (*Panicum virgatum*), via both stable and transient expression systems, and biomass from the resulting stable genetic variants will be characterized through various screening methods to evaluate any impact on cell-wall structure.

Focus Area 2. Biomass Deconstruction and Conversion. Two key hypotheses drive BESC's biomass deconstruction and conversion research: (1) microorganisms can be engineered to enable consolidated bioprocessing (CBP), a one-step, microbe-mediated strategy for directly converting plant biomass into ethanol; and (2) enzymes and microbial biocatalysts can be engineered to take advantage of recalcitrance-reducing plant modifications to achieve better biomass deconstruction. CBP will enable fundamental process cost savings through the establishment of a much simplified industrial process (Lynd et al. 1999). One model organism being studied in BESC for CBP development is *Clostridium thermocellum*, a bacterium that rapidly degrades pure cellulose and then ferments the resulting sugars into ethanol. This microbe's strategy for combined biomass deconstruction and conversion employs cellulosomes—multifunctional enzyme complexes that specialize in degrading cellulose (Brown et al. 2007). Understanding cellulosomes is important for rapid improvement in the deconstruction of more complex plant cell walls. BESC is studying the structures and activities of these multi-enzyme complexes to design new variants with better cell-wall-deconstruction capabilities. In addition to working with *C. thermocellum*, BESC researchers are investigating samples from hot springs at Yellowstone National Park to identify heat-tolerant enzymes and microbes with superior biomass-degrading functions that can be used to discover additional strategies for new CBP microorganisms.

In this Focus Area, we are exploring several fundamental frontiers: (1) mining the natural diversity of biomass-degrading enzymes and microbes; (2) studying how different biomass features affect the activities of enzymes and microbes; (3) examining the relationship between enzyme structure and function; (4) investigating how enzymes and microbes interact with pretreated cell walls; and (5) testing strategies for using microbial cultures for biomass deconstruction and conversion. An over-arching goal is to integrate information obtained from these various investigations into comprehensive conceptual and computational models of cellulose deconstruction in both natural and engineered environments.

Focus Area 3. Characterization and Modeling. Advancing BESC goals to develop improved plant materials and CBP methods that facilitate cost-effective conversion of biomass to fermentable sugars will require detailed knowledge of (1) the chemical and physical properties of biomass that influence recalcitrance, (2) how these properties can be altered by engineering plant biosynthetic pathways, and (3) how biomass properties change during pretreatment and how such changes affect biomass–biocatalyst interactions during deconstruction by enzymes and microorganisms.

BESC researchers are applying modern analytical technologies to examine chemical and structural changes that occur to the plant cell walls that have been genetically modified. Switchgrass and poplar samples generated in BESC will be catalogued, bar coded, and analyzed in detail for chemical composition. This biomass undergoes pretreatment and enzyme-digestibility studies followed as needed by detailed chemical, physical, and imaging analysis. The resulting data is being used to build relationship models between biomass structure and recalcitrance. Characterizing and modeling cell-wall synthesis pathways, biomass structure and composition, and microbe–enzyme interactions with biomass surfaces is expected to provide knowledge which will stimulate the coordinated development of (1) CBP microbes and (2) new generations of switchgrass and poplar optimized for deconstruction. The combination of characterization, modeling, and data sharing will help define the genomic and physical basis of plant cell-wall recalcitrance and deconstruction.

A strength of BESC is the crosscutting integration of diverse experimental, theoretical, and computational approaches. For example, high-throughput physical characterization of biomass will provide the basis for subsequent data mining that can reveal previously unknown correlations between recalcitrance and biomass structure.

Translation of BESC Science into Commercial Applications

BESC is supported by a governing board and a scientific advisory board populated with internationally known academic and bioenergy industry leaders. This structure is similar to what might be found in a biotech startup company, and is intended to provide the perspective that a corporate board might have, as well as the insight and objectivity that an external science advisory board brings. In addition, BESC has formed a "commercialization council" of technology-transfer and intellectual property management professionals from partner institutions to evaluate the commercial potential of new inventions arising from BESC research and to promote and facilitate the transfer of new discoveries to industry. New BESC inventions are posted on the program's website (http://www.bioenergycenter.org/licensing). The BESC inventions disclosed at this point in the program (in the middle of year #2) come from eight of our 17 partners, and arise from research in all three focus areas. The subject matter of these early inventions includes plant and microbial genetic transformation techniques, special methods of microscopy, and innovations in biomass sample handling.

BESC has among its goals the effective, coordinated commercialization of these technologies through licensing to companies pursuing biofuels development. The translation of BESC research results into applications testing and potential commercial deployment is an important step toward reaching DOE's bioenergy objectives. Further, toward our goal of forming external relationships, dissemination of results of the research, and generating information about commercial opportunities, BESC offers companies the opportunity to become BESC Industry Affiliates.

BESC's home base is less than 40 mi from a pre-commercial switchgrass-to-ethanol demonstration plant which represents a significant addition to our ability to move BESC scientific discoveries into commercial use. The $40-million facility funded by the Tennessee Biofuels Initiative will be an innovative pilot-scale biorefinery and state-of-the-art research and development facility for cellulosic ethanol or ethanol from non-food sources (http://www.utbioenergy.org/TNBiofuelsInitiative). The pilot-scale biorefinery is expected to be a catalyst for a new biofuel industry for Tennessee. The facility will produce cellulosic ethanol as a transportation fuel from two different non-food biomass feedstocks, corn stover (cobs and fiber) and switchgrass.

Education and Outreach

By leveraging successful educational and training programs already in place at partner academic institutions, BESC will offer students, postdoctoral staff, and scientists interdisciplinary research opportunities that cut across a broad range of biofuel-related fields. To extend the reach of BESC science to diverse locations and communities, collaborative workshops for training students and scientists and an open seminar series reporting scientific progress will be held at partner institutions. BESC also will provide opportunities each year for non-BESC scientists to participate in research at one or more partner sites.

In addition to our efforts to prepare graduate students and postdocs, our center has taken a novel approach in our education efforts. In collaboration with the Creative Discovery Museum in Chattanooga, we have developed lesson plans aimed at grades 4–6 to educate and inform students about the basics of energy production and utilization. These lessons include basic concepts such as the carbon cycle, lignocellulosic biomass as substrate for the production of biofuels, and technical and economic obstacles to a biobased fuel economy. The hands-on activities and guided questions are also designed to meet educational objectives for these grades. This program has been piloted in a hundred classrooms in North Georgia and Tennessee and will be made available to schools nationwide. We have also begun to pilot interactive "science night" programs offered to students and the general public through local schools, museums, and community centers.

Acknowledgement The BioEnergy Science Center (BESC) is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.

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