# Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda

## A Research Roadmap Resulting from the Biomass to Biofuels Workshop Sponsored by the U.S. Department of Energy

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# Introduction

In his 2006 State of the Union address (Bush 2006), the president outlined the new Advanced Energy Initiative (AEI) to help overcome America's dependence on foreign sources of energy (AEI 2006) and the American Competitiveness Initiative to increase R&D investments and strengthen education (ACI 2006). He seeks to reduce our national dependence on imported oil by accelerating the development of domestic, renewable alternatives to gasoline and diesel fuels.

"With America on the verge of breakthroughs in advanced energy technologies, the best way to break the addiction to foreign oil is through new technologies." —White House Press Release on the State of the Union Address and AEI (January 31, 2006)

Breakthrough technologies to realize the potential of cellulosic biofuels can be expedited by application of a new generation of biological research created by the genome revolution. Overcoming barriers to development of these fuels on an industrial scale will require high-performance energy feedstocks and microbial processes, both to break down feedstocks to sugars and to ferment sugars to ethanol. A focused set of investments linking revolutionary biofuel technologies with advances from the biological, physical, computational, and engineering sciences will quickly remove barriers to an efficient, economic, and sustainable biofuel industry.

# Joint Workshop Challenges Biofuel Science and Technology Communities

Two Department of Energy (DOE) offices are teaming to advance biofuel development and use: The Office of Biological and Environmental Research (OBER) within the Office of Science (SC) and the Office of the Biomass Program (OBP) within the Office of Energy Efficiency and Renewable Energy (EERE) (see descriptions of the two DOE programs, pp. 17 and 19). These offices are challenging their communities to identify critical science needs to support a substantial and sustainable expansion of biomass-derived fuels, specifically cellulosic ethanol. In the jointly sponsored Biomass to Biofuels Workshop held December 7–9, 2005, in Rockville, Maryland, more than 50 scientists representing a wide range of expertise convened to define barriers and challenges to this new biofuel industry. The workshop concentrated on improvement of biomass crops and their processing to transportation fuels. Although the focus was ethanol, the science applies to additional fuels that include biodiesel and to other bioproducts or coproducts having critical roles in any deployment scheme.

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The current approach to introducing biofuels relies on an "evolutionary" business and economic driver for a steady but moderate entry into the market. Technologies for implementing this new industry are being tested either by producing higher-value products from renewables (such as lactic acid) or as incremental additions to current corn-ethanol refineries (such as the conversion of residual corn-kernel fibers to ethanol).

This report is a workshop-produced roadmap for accelerating cellulosic ethanol research, helping make biofuels practical and cost-competitive by 2012 (\$1.07/gal ethanol) and offering the potential to displace up to 30% of the nation's current gasoline use by 2030. It argues that rapidly incorporating new systems biology approaches via significant R&D investment will spur use of these technologies for expanded processing of energy crops and residues. Furthermore, this strategy will decrease industrial risk from use of a first-of-a-kind technology, allowing faster deployment with improved methods. Ultimately, these approaches foster setting more aggressive goals for biofuels and enhance the strategy's sustainability.

# **America's Energy Challenges**

The triple energy-related challenges of the 21st Century are economic and energy growth, energy security, and climate protection. The United States imports about 60% of the petroleum it consumes, and that dependency is increasing.\* Since the U.S. economy is tied so closely to petroleum products and oil imports, disruptions in oil supplies can result in severe economic and social impacts. Conventional oil production will peak in the near future, and the resulting energy transition will require a portfolio of responses, including unconventional fossil resources and biofuels. Environmental quality and climate change due to energy emissions are additional concerns. Annual U.S. transportation emissions of the greenhouse gas (GHG) carbon dioxide ( $CO_2$ ) are projected to increase from about 1.9 billion metric tons in 2004 to about 2.7 billion metric tons in 2030 (EIA 2006).

### **The Promise of Biofuels**

Fuels derived from cellulosic biomass<sup>\*\*</sup>—the fibrous, woody, and generally inedible portions of plant matter—offer an alternative to conventional energy sources that supports national economic growth, national energy security, and environmental goals. Cellulosic biomass is an attractive energy feedstock because supplies are abundant domestically and globally. It is a renewable source of liquid transportation fuels that can be used readily by current-generation vehicles and distributed through the existing transportation-fuel infrastructure. Ethanol from corn grain is an increasingly important additive fuel source, but it has limited growth potential as a primary transportation fuel.\*\*\* The U.S. "starch-based" ethanol industry will jump start a greatly expanded ethanol industry that includes cellulosic ethanol as a major transportation fuel.

Cellulose and hemicelluloses, found in plant cell walls, are the primary component of biomass and the most plentiful form of biological material on earth. They are polysaccharides made up of energy-rich sugars that can be converted to ethanol (see sidebar, Understanding Biomass, p. 53). Current methods to break down biomass into simple sugars and convert them into ethanol are inefficient and constitute the core barrier to producing ethanol at quantities and costs competitive with gasoline.

Biological research is undergoing a major transformation. The systems biology paradigm-born of the genome revolution and based on highthroughput advanced technologies, computational modeling, and scientificteam approaches—can facilitate rapid progress and is a readily applicable model for biofuel technology. Systems biology is the core of the OBER Genomics: GTL program, whose goal is to achieve a predictive understanding of the complex network of interactions that underpin the biological processes related to biofuel production. Biological challenges to which GTL can apply systems biology approaches include enhancing the productivity of biomass crops optimized for industrial processing, improving enzyme systems that deconstruct plant cell walls, and increasing the yield of ethanol-producing microorganisms. Systems biology tools and knowledge will enable rational engineering of a new generation of bioenergy systems made up of sustainable energy crops for widely varying agroecosystems and tailored industrial processes. This research approach will encourage the critical fusion of the agriculture, industrial biotechnology, and energy sectors.

# A Growing Mandate for Biofuels: Policy, Legislative, and Other Drivers

A primary goal of the president's 2001 National Energy Policy (NEP) is to increase U.S. energy supplies, incorporating a more diverse mix of domestic resources to support growth in demand and to reduce national dependence on imported oil (NEPDG 2001). AEI accelerates and expands on several policy and legislative mandates (AEI 2006). It aims to reduce the nation's reliance on foreign oil in the near term and provides a 22% increase in clean-energy research at DOE for FY 2007, accelerating progress in renewable energy.

According to AEI, the United States must move beyond a petroleumbased economy and devise new ways to power automobiles. The country needs to facilitate domestic, renewable alternatives to gasoline and diesel fuels. The administration will accelerate research in cutting-edge methods of producing such "homegrown" renewable biobased transportation fuels as ethanol from agricultural and forestry feedstocks including wood chips,

\*\*Cellulosic biomass, also called lignocellulosic biomass, is a complex composite material consisting primarily of cellulose and hemicellulose (structural carbohydrates) bonded to lignin in plant cell walls. For simplification, we use the term cellulosic biomass.

\*\*\*In 2004, 11% of the U.S. corn harvest yielded 3.4 billion gallons of ethanol (NRDC 2006), roughly 1.7% of the 2004 fuel demand. Thus if all corn grain now grown in the United States were converted to ethanol, it would satisfy about 15% of current transportation needs.

<sup>\*</sup>Gasoline and diesel constituted 98% of domestic transportation motor fuels in 2004, with ethanol from corn grain supplying most of the remaining 2%. Annual gasoline consumption in 2004 was about 139 billion gallons, and 3.4 billion gallons of ethanol were used primarily as a fuel extender to boost gasoline octane levels and improve vehicle emissions.

stalks, and switchgrass. AEI would foster the early commercialization of advanced biofuel technologies, enabling U.S. industry to lead in deploying biofuels and chemicals internationally.

Achieving the goal of displacing 30% of the nation's current gasoline use by 2030 would require production levels equal to roughly 60 billion gallons a year (Bgal/year) of ethanol (see Table 1. Comparisons of 2004 Gasoline and Ethanol Equivalents, this page). An annual supply of roughly a billion dry tons of biomass will be needed to support this level of ethanol production. A recent report by the U.S. Department of Agriculture (USDA) and DOE finds potential to sustainably harvest more than 1.3 billion metric tons of biomass from U.S. forest and agricultural lands by mid-21st Century (Perlack et al. 2005). Investments in R&D and infrastructure are needed to realize this feedstock potential.

The U.S. Energy Policy Act of 2005 (EPAct; Appendix A, Provisions for Biofuels and Biobased Products in the Energy Policy Act of 2005, p. 186) has established aggressive near-term targets for ethanol production. A key provision requires mixing 4 Bgal of renewable fuel with gasoline in 2006. This requirement increases annually to 7.5 Bgal of renewable fuel by 2012. For 2013 and beyond, the required volume will include a minimum of 250 million gallons (Mgal) of *cellulosic* ethanol. Another section of the EPAct authorizes funds for an incentive program to ensure the annual production of 1 Bgal of cellulosic biomass-based fuels by 2015. Ethanol is the most common biofuel produced from cellulose, but other possible biofuel compounds can be produced as well.

Other important legislative drivers supporting biofuels are the Biomass R&D Act of 2000 and Title IX of the Farm Bill 2002 (U.S. Congress 2000; U.S. Congress 2002). The Biomass R&D Act directed the departments of Energy and Agriculture to integrate their biomass R&D and established the Biomass Research and Development Technical Advisory Committee (BTAC), which

2004	Gasoline (billion gallons)	Ethanol Equivalents (billion gallons)
U.S. consumption, 2004	139	200
About 60% from imports	83	120
Requirements to displace 30% of 2004 U.S. consumption	42	60
• Biomass requirements at 80 gal/ton		• 750 Mton
• Land requirements at 10 ton/acre and 80 gal/ton		• 75 Macre
• Numbers of refineries at 100 Mgal/ refinery		<ul> <li>600 (each requiring 160 miles<sup>2</sup> net or 125,000 acres)</li> </ul>

advises the Secretary of Energy and the Secretary of Agriculture on strategic planning for biomass R&D. As a precedent to the current presidential initiative, in 2002 BTAC set a goal requiring biofuels to meet 20% of U.S. transportation fuel consumption by 2030 as part of its vision for biomass technologies (BTAC 2002). Title IX supports increased use of biobased fuels and products and incentives and grants for biofuel and biorefinery R&D.

In addition to legislative mandates, several independent studies have acknowledged the great potential of biofuels in achieving a more diverse domestic energy supply (NCEP 2004; Greene et al. 2004; Lovins et al. 2005). Growing support for developing biomass as a key energy feedstock is coming from a variety of national and international organizations (GEC 2005; Ag Energy Working Group 2004; IEA 2004). Although these reports differ in the amounts of gasoline that could be replaced by ethanol from biomass, they all agree on three key issues: (1) Current trends in energy use are not sustainable and are a security risk; (2) No single solution will secure the energy future—a diverse portfolio of energy options will be required; and (3) Biofuels can be a significant part of the transportation sector's energy solution.

In its evaluation of options for domestic production of motor fuels, the National Commission on Energy Policy (NCEP) recommended cellulosic biomass as an important topic for near-term federal research, development, and demonstration and found that "cellulosic ethanol has the potential to make a meaningful contribution to the nation's transportation fuel supply in the next two to three decades" (NCEP 2004).

The Natural Resources Defense Council (NRDC) has projected that an aggressive plan to develop cellulosic biofuels in the United States could "produce the equivalent of nearly 7.9 million barrels of oil per day by 2050 ... more than 50 percent of our current total oil use in the transportation sector and more than three times as much as we import from the Persian Gulf alone" (Greene et al. 2004). This corresponds to roughly 100 Bgal/year ethanol. NRDC also recommends \$1.1 billion in funding between 2006 and 2012 for biomass research, development, and demonstration with 45% of this funding focused on overcoming biomass recalcitrance to ethanol processing. This level of funding is expected to stimulate a regular flow of advances needed to make ethanol cost-competitive with gasoline and diesel.

An independent analysis from the Rocky Mountain Institute found that significant gains in energy efficiency and the large-scale displacement of oil with biofuels, mainly cellulosic ethanol, would be key components of its strategy to reduce American oil dependence over the next few decades (Lovins et al. 2005).

To illustrate the widespread support for fuel ethanol, the Governors' Ethanol Coalition, an organization devoted to the promotion and increased use of ethanol, now includes 32 member states as well as international representatives from Brazil, Canada, Mexico, Sweden, and Thailand. In a recent report, the coalition called for rapid expansion of ethanol to meet at least 10% of transportation fuel needs "as soon as practicable" and for development of "lignocellulosic-based" fuels for expansion beyond those levels (GEC 2005). "The use of ethanol, particularly biomass-derived ethanol, can produce significant savings in carbon dioxide emissions. This approach offers a no-regrets policy that reduces the potential future risks associated with climate change and has the added benefit of economic development."

# **Benefits of Biofuels**

Biofuels, especially corn-derived and cellulosic ethanol, constitute the only renewable liquid transportation fuel option that can be integrated readily with petroleum-based fuels, fleets, and infrastructure. Production and use of biofuels can provide substantial benefits to national energy security, economic growth, and environmental quality.

### **National Energy Security Benefits**

"National security is linked to energy through the dependence of this country and many others on imported oil—much of it located in politically troubled parts of the globe. As such, the potential for largescale failures in the global production and distribution system presents a real threat." — Governors' Ethanol Coalition (GEC 2005)

Today the United States is dependent on oil for transportation. Alternative, domestically based, and sustainable fuel-development strategies, therefore, are essential to ensuring national security. America accounts for 25% of global oil consumption yet holds only 3% of the world's known oil reserves. About 60% of known oil reserves are found in sensitive and volatile regions of the globe. Increasing strain on world oil supply is expected as developing countries become more industrialized and use more energy. Any strategy to reduce U.S. reliance on imported oil will involve a mix of energy technologies including conservation. Biofuels are an attractive option to be part of that mix because biomass is a domestic, secure, and abundant feedstock. Global availability of biomass feedstocks also would provide an international alternative to dependence on an increasingly strained oil-distribution system as well as a ready market for biofuel-production technologies.

### **Economic Benefits**

A biofuel industry would create jobs and ensure growing energy supplies to support national and global prosperity. In 2004, the ethanol industry created 147,000 jobs in all sectors of the economy and provided more than \$2 billion of additional tax revenue to federal, state, and local governments (RFA 2005). Conservative projections of future growth estimate the addition of 10,000 to 20,000 jobs for every billion gallons of ethanol production (Petrulis 1993).

In 2005 the United States spent more than \$250 billion on oil imports, and the total trade deficit has grown to more than \$725 billion (U.S. Commerce Dept. 2006). Oil imports, which make up 35% of the total, could rise to 70% over the next 20 years (Ethanol Across America 2005).

Among national economic benefits, a biofuel industry could revitalize struggling rural economies. Bioenergy crops and agricultural residues can provide farmers with an important new source of revenue and reduce reliance on government funds for agricultural support. An economic analysis jointly sponsored by USDA and DOE found that the conversion of some cropland to bioenergy crops could raise depressed traditional crop prices by up to 14%. Higher prices for traditional crops and new revenue from bioenergy crops could increase net farm income by \$6 billion annually (De La Torre Ugarte 2003).



Fig. 1. Reduced Carbon **Dioxide Emissions of Ethanol** from Biomass. When compared with gasoline, ethanol from cellulosic biomass could dramatically reduce emissions of the greenhouse gas, carbon dioxide (CO<sub>2</sub>). Although burning gasoline and other fossil fuels increases atmospheric CO<sub>2</sub> concentrations, the photosynthetic production of new biomass takes up most of the carbon dioxide released when bioethanol is burned. [Source: Adapted from ORNL Review (www.ornl.gov/info/ornlreview/ v33\_2\_00/bioenergy.htm)]

### **Environmental Benefits**

#### **Climate Change**

When fossil fuels are consumed, carbon sequestered from the global carbon cycle for millions of years is released into the atmosphere, where it accumulates. Biofuel consumption can release considerably less  $CO_2$ , depending on how it is produced. The photosynthetic production of new generations of biomass takes up the  $CO_2$  released from biofuel production and use (see Fig. 1. Reduced Carbon Dioxide Emissions of Ethanol from Biomass, this page). A life-cycle analysis shows fossil  $CO_2$  emissions from cellulosic ethanol to be 85% lower than those from gasoline (Wang 2005). These emissions arise from the use of fossil energy in producing cellulosic ethanol. Nonbiological sequestration of  $CO_2$  produced by the fermentation process can make the biofuel enterprise net carbon negative.

A recent report (Farrell et al. 2006) finds that ethanol from cellulosic biomass reduces substantially both GHG emissions and nonrenewable energy inputs when compared with gasoline. The low quantity of fossil fuel required to produce cellulosic ethanol (and thus reduce fossil GHG emissions) is due largely to three key factors. First is the yield of cellulosic biomass per acre. Current corn-grain yields are about 4.5 tons/acre. Starch is 66% by weight, yielding 3 tons to produce 416 gal of ethanol, compared to an experimental yield of 10 dry tons of biomass/acre for switchgrass hybrids in research environments (10 dry tons at a future yield of 80 gal/ton = 800 gal ethanol). Use of corn grain, the remaining solids (distillers' dried grains), and stover could yield ethanol at roughly 700 gal/acre. Current yield for nonenergy-crop biomass resources is about 5 dry tons/acre and roughly 65 gal/ton. The goal for energy crops is 10 tons/acre at 80 to 100 gal/ton during implementation. Second, perennial biomass crops will take far less energy to plant and cultivate and will require less nutrient, herbicide, and fertilizer. Third, biomass contains lignin and other recalcitrant residues that can be burned to produce heat or electricity consumed by the ethanol-production process.

Energy crops require energy inputs for production, transportation, and processing—a viable bioenergy industry will require a substantial positive energy balance. Figure 2. Comparison of Energy Yields with Energy Expenditures, this page, compares results for cellulosic and corn ethanol, gasoline, and electricity, demonstrating a substantially higher yield for cellulosic ethanol. Over time a mature bioenergy economy will substitute biomass-derived energy sources for fossil fuel, further reducing net emissions.



Fig. 2. Comparison of Energy Yields with Energy Expenditures. The fossil energy-replacement ratio (FER) compares energy yield from four energy sources with the amount of fossil fuel used to produce each source. Note that the cellulosic ethanol biorefinery's projected yield assumes future technological improvements in conversion efficiencies and advances that make extensive use of a biomass crop's noncellulosic portions for cogeneration of electricity. Similar assumptions would raise corn ethanol's FER if, for example, corn stover were to replace current natural gas usage. The corn ethanol industry, already producing ethanol as an important additive and fuel extender, is providing a foundation for expansion to cellulosic ethanol. [Source: Figure, based on the Argonne National Laboratory GREET model, is derived from Brinkman et al. 2005. Other papers that support this study include Farrell et al. 2006 and Hammerschlag 2006.]

#### **Other Environmental Benefits**

Perennial grasses and other bioenergy crops have many significant environmental benefits over traditional row crops (see Fig. 3. *Miscanthus* Growth over a Single Growing Season in Illinois, p. 9). Perennial energy crops provide a better environment for more-diverse wildlife habitation. Their extensive root systems increase nutrient capture, improve soil quality, sequester carbon, and reduce erosion. Ethanol, when used as a transportation fuel, emits less sulfur, carbon monoxide, particulates, and GHGs (Greene et al. 2004).

# **Feasibility of Biofuels**

The United States could benefit substantially by increasing its use of domestic, renewable fuels in the transportation sector, but can biofuels be produced at the scale needed to make a real difference in transportation consumption of fossil fuels? More specifically, is there enough land to provide the needed large-scale supply of biomass, is the use of biofuels sustainable agriculturally, can biofuels become cost-competitive with gasoline, and is cellulosic-biofuel production technically feasible for energy? The short answer to all these questions is yes, and this section summarizes recent reports that support this view.

### **Land Availability**

A major factor influencing the extent to which biofuels will contribute to America's energy future is the amount of land available for biomass harvesting. Are biomass resources sufficient to meet a significant portion of transportation-fuel consumption, and how would harvesting biomass for energy affect current agricultural and forestry practices?

In 2005, a study jointly supported by DOE and USDA examined whether land resources in the United States are sufficient to sustain production of over 1 billion dry tons of biomass annually, enough to displace 30% or more of the nation's current consumption of liquid transportation fuels. By assuming relatively modest changes in agricultural and forestry practices, this study projects that 1.366 billion dry tons of biomass could be available for large-scale bioenergy and biorefinery industries by mid-21st Century while still meeting demand for forestry products, food, and fiber (Perlack et al.



2005) (see sidebar, A Billion-Ton Annual Supply of Biomass, p. 10). This supply of biomass would be a sevenfold increase over the 190 million dry tons of biomass per year currently used for bioenergy and bioproducts. Most of this biomass is burned for energy, with only 18 million dry tons used for biofuels (primarily corn-grain ethanol) and 6 million dry tons used for bioproducts.

The biomass potential determined by the "billion-ton" study is one scenario based on a set of conservative assumptions derived from current practices and should not be considered an upper limit. Crop-yield increases assumed in this study follow business-as-usual expectations. With more aggressive commitments to research on improving energy crops and productivity, the biomass potential could be much greater. Energy-crop yield is a critical factor in estimating how much land will be needed for large-scale biofuel production, and this factor can be influenced significantly by biotechnology and systems biology strategies used in modern plant breeding and biomass processing.

Many potential energy crops (e.g., switchgrass, poplar, and willow) are essentially unimproved or have been bred only recently for biomass, compared to corn and other commercial food crops that have undergone substantial improvements in yield, disease resistance, and other agronomic traits. A more complete understanding of biological systems and Fig. 3. *Miscanthus* Growth over a Single Growing Season in Illinois. *Miscanthus* has been explored extensively as a potential energy crop in Europe and now is being tested in the United States. The scale is in feet. These experiments demonstrate results that are feasible in development of energy crops. [Image source: S. Long, University of Illinois]

# A Billion-Ton Annual Supply of Biomass: Summary of Potential Forest and Agricultural Resources

In 2005, a study jointly supported by DOE and USDA examined whether land resources in the United States are sufficient to sustain production of over 1 billion dry tons of biomass annually, enough to displace 30% or more of the nation's current consumption of liquid transportation fuels (Perlack et al. 2005). Assuming relatively modest changes in agricultural and forestry practices, this study projects that 1.366 billion dry tons of biomass (368 million dry tons from forest and 998 million dry tons from agriculture) could be available for large-scale bioenergy and biorefinery industries by mid-21st Century while still meeting demand for forestry products, food, and fiber (see Fig. A. Potential Biomass Resources, below). This supply of biomass would be a sevenfold increase over the 190 million dry tons of biomass per year currently used for bioenergy and bioproducts. Most of this biomass is burned for energy, with only 18 million dry tons used for biofuels (primarily corn-grain ethanol) and 6 million dry tons used for bioproducts.

Land area in the United States is about 2 billion acres, with 33% forestlands and 46% agricultural lands consisting of grasslands or pasture (26%) and croplands (20%). Of the estimated 368 million dry tons of forest biomass, 142 million dry tons already are used by the forest products industry for bioenergy and bioproducts. Several different types of biomass were considered in this study. Residues from the forest products industry include tree bark, woodchips, shavings, sawdust, miscellaneous scrap wood, and black liquor, a by-product of pulp and paper processing. Logging and site-clearing residues consist mainly of unmerchantable tree tops and small branches that currently are left onsite or burned. Forest thinning involves removing excess woody materials to reduce fire hazards and improve forest health. Fuelwood includes roundwood or logs burned for space heating or other energy uses. Urban wood residues consist primarily of municipal solid waste (MSW, e.g., organic food scraps, yard trimmings, discarded furniture, containers, and packing materials) and construction and demolition debris (see Table A. Potential Biomass Resources, this page, and Fig. B. Biomass Analysis for the Billion-Ton Study, p. 11).



Fig. A. Potential Biomass Resources: A Total of More than 1.3 Billion Dry Tons a Year from Agricultural and Forest Resources.

### **Table A. Potential Biomass Resources**

Biomass Resources	Million Dry Tons per Year
Forest Biomass	
Forest products industry residues	145
Logging and site-clearing residues	64
Forest thinning	60
Fuelwood	52
Urban wood residues	47
Subtotal for Forest Resources	368
Agricultural Biomass	
Annual crop residues	428
Perennial crops	377
Miscellaneous process residues, manure	106
Grains	87
Subtotal for Agricultural Resources	998
Total Biomass Resource Potential	1366





Several assumptions were made to estimate potential forest biomass availability. Environmentally sensitive areas, lands without road access, and regions reserved for nontimber uses (e.g., parks and wilderness) were excluded, and equipment-recovery limitations were considered. As annual forest growth is projected to continue to exceed annual harvests, continued expansion of standing forest inventory is assumed.

Among agricultural biomass resources, annual crop residues are mostly stems and leaves (e.g., corn stover and wheat straw) from corn, wheat, soybeans, and other crops grown for food and fiber. Perennial crops considered in the study include grasses or fast-growing trees grown specifically for bioenergy. Grain primarily is corn used for ethanol production, and miscellaneous process residues include MSW and other by-products of agricul-tural resource processing.

A total of 448 million acres of agricultural lands, largely active and idle croplands, were included in this study; lands used permanently for pasture were not considered. Other assumptions for agricultural biomass resources include a 50% increase in corn, wheat, and small-grain yield; doubling the residue-to-grain ratio for soybeans; recovery of 75% of annual crop residues with more efficient harvesting technologies; management of all cropland with no-till methods; 55 million acres dedicated to production of perennial bioenergy crops; average biomass yield for perennial grasses and woody plants estimated at 8 dry tons per acre; conversion of all manure not used for on-farm soil improvement to biofuel; and use of all other available residues.

application of the latest biotechnological advances will accelerate the development of new biomass crops having desirable attributes. These attributes include increased yields and processability, optimal growth in specific microclimates, better pest resistance, efficient nutrient use, and greater tolerance to moisture deficits and other sources of stress. Furthermore, many biotechnological advances for growing better biomass crops will be used to improve food crops, easing the pressure on land area needed to grow food. Joint development of these biotechnological advances with other countries will help moderate the global demand for crude oil. In an idealized future scenario with greater per-acre productivity in energy, food, and fiber crops and decreased demand for transportation fuels resulting from more efficient vehicles, the United States could have sufficient land resources to produce enough biomass to meet all its transportation-fuel needs.

### **Agricultural Sustainability of Biomass Production**

Sustainable practices for growing and harvesting biomass from dedicated crops will be essential to the success of large-scale biofuel production. Capital costs of refineries and associated facilities to convert biomass to fuels will be amortized over several decades. These capital assets will require a steady annual supply of biomass from a large proportion of surrounding land. Therefore, a thorough understanding of the conversion pathway and of biomass harvesting's long-term impacts on soil fertility is needed to ensure sustainability. Vital nutrients contained in process residues must be returned to the soil. Perennial crops expected to be used for biofuels improve soil carbon content and make highly efficient use of mineral nutrients (see sidebar, The Argument for Perennial Biomass Crops, p. 59). Additional information about the composition and population dynamics of soil microbial communities is needed, however, to determine how microbes contribute to sustaining soil productivity (see section, Ensuring Sustainability and Environmental Quality, p. 68). Mixed cultivars of genetically diverse perennial energy crops may be needed to increase productivity and preserve soil quality. Because conventional annual food and fiber crops are grown as monocultures, relatively little research has been carried out on issues associated with growing mixed stands.

### Today – Fuel Ethanol Production from Corn Grain (Starch Ethanol)

In 2004, 3.41 Bgal of starch ethanol fuel were produced from 1.26 billion bushels of corn—11% of all corn grain harvested in the United States. This record level of production was made possible by 81 ethanol plants located in 20 states. Completion of 16 additional plants and other expansions increased ethanol-production capacity to 4.4 Bgal by the end of 2005; additional planned capacity is on record for another 1 Bgal from 2006 to 2007 (RFA 2005). Although demand for fuel ethanol more than doubled between 2000 and 2004, ethanol satisfied less than 2% of U.S. transportation-energy demand in 2004. In the United States, ethanol is produced in corn wet or dry mills. Corn *wet* mills fractionate the corn grain for products like germ and oil before converting the clean starch to sugars for fermentation or for such valuable food products as high-fructose corn syrup and maltodextrins. The corn fiber by-product usually is sold as animal feed. In corn *dry* mills, the grain is ground, broken into sugar monomers (saccharified), and fermented. Since the grain is not fractionated, the only by-product is the remaining solids, called distillers' dried grains with solubles, a highly nutritious protein source used in livestock feed. A bushel of corn yields about 2.5 gal ethanol from wet-mill processing and about 2.8 gal from dry grind (Bothast and Schlicher 2005). Some 75% of corn ethanol production is from dry-mill facilities and 25% from wet mills.

# Tomorrow – Biorefinery Concept to Produce Fuel Ethanol from Cellulosic Biomass

Cellulosic ethanol has the potential to meet most, if not all, transportationfuel needs. However, due to the complex structure of plant cell walls, cellulosic biomass is more difficult than starch to break down into sugars. Three key biomass polymers found in plant cell walls are cellulose, hemicellulose, and lignin (see Lignocellulosic Biomass Characteristics chapter, p. 39). These polymers are assembled into a complex nanoscale composite, not unlike reinforced concrete but with the capability to flex and grow much like a liquid crystal. The composite provides plant cell walls with strength and resistance to degradation and carries out many plant functions. Their robustness, however, makes these materials a challenge to use as substrates for biofuel production.

Traditional cellulosic biorefineries have numerous complex, costly, and energy-intensive steps that may be incompatible or reduce overall process efficiency. The current strategy for biochemical conversion of biomass to ethanol has its roots in the early days of wood chemistry. Developed in the 1930s for wartime use in Germany, it is used in Russia today. This process involves three basic steps, each element of which can be impacted by cellulosic biomass research (see Fig. 4. Traditional Cellulosic Biomass Conversion to Ethanol Based on Concentrated Acid Pretreatment Followed by Hydrolysis and Fermentation, p. 14). After acquisition of suitable cellulosic biomass, biorefining begins with size reduction and thermochemical pretreatment of raw cellulosic biomass to make cellulose polymers more accessible to enzymatic breakdown and to free up hemicellulosic sugars, followed by production and application of special enzyme preparations (cellulases) for hydrolysis of plant cell-wall polysaccharides to produce simple sugars. Final steps in the process include fermentation, mediated by bacteria or yeast, to convert these sugars to ethanol and other coproducts that must be recovered from the resulting aqueous mixture. Recent research and development has reduced dramatically the cost of enzymes and has improved fermentation strains to enable simultaneous saccharification and fermentation (SSF), in which hydrolysis of cellulose and fermentation of glucose are combined in one step.



to enable simultaneous saccharification and fermentation (SSF, green boxes surrounded by orange), in which hydrolysis of cellulose and fermentation of glucose are combined in one step. Cellulosic biomass research is targeting these steps to simplify and increase the yield of biomass production and processing (see Fig. 5. p. 15). [Source: Adapted from M. Himmel and J. Sheehan, National Renewable Energy Laboratory]

> Figure 5. A Biorefinery Concept Incorporating Advanced Pretreatment and Consolidated Processing of Cellulose to Ethanol, p. 15, depicts key targets for simplifying and improving the biorefinery concept. Feedstock research seeks first to increase biomass yields and enhance biomass characteristics to enable more efficient processing. Advanced biocatalysts will augment or replace thermochemical methods to reduce the severity and increase the yield of pretreatment. More robust processes and reduction of inhibitors would allow elimination of the detoxification and separation steps. Developing modified enzymes and fermentation organisms ultimately will allow incorporation of hydrolysis enzyme production, hydrolysis, and fermentation into a single organism or a functionally versatile but stable mixed culture with multiple enzymatic capabilities. Termed consolidated bioprocessing (CBP), this could enable four components comprising steps 2 and 3 (green boxes) in Fig. 4 to be combined into one, which in Fig. 5 is called direct conversion of cellulose and hemicellulosic sugars. Further refinement would introduce pretreatment enzymes (ligninases and hemicellulases) into the CBP microbial systems as well,



discussed in this roadmap are based on first developing technologies to allow more energy-efficient and chemically benign enzymatic pretreatment. Saccharification and fermentation would be consolidated into a simple step and ultimately into a single organism or stable mixed culture (consolidated bioprocessing), thus removing multiple whole steps in converting biomass to ethanol. Also see Fig. 6. p. 16. [Source: Adapted from M. Himmel and J. Sheehan, National Renewable Energy Laboratory]

reducing to one step the entire biocatalytic processing system (pretreatment, hydrolysis, and fermentation). These process simplifications and improvements will lessen the complexity, cost, and energy intensity of the cellulosic biorefinery.

In addition to polysaccharides that can be converted to ethanol, the lignin in plant cell walls is a complex polymer of phenylpropanoid subunits that must be separated from carbohydrates during biomass conversion. Energy-rich lignin can be burned for heat, converted to electricity consumable by other steps in the ethanol-production pathway, or gasified and converted to Fischer-Tropsch (FT) fuels (see Fig. 6. Mature Biomass Refining Energy Flows: Example Scenario, p. 16, and Table A. Summary of Energy Flows in Mature Biorefinery Concept, p. 16). For more information, see Deconstructing Feedstocks to Sugars, p. 85, and Sugar Fermentation to Ethanol, p. 119. For an overview of how genomics can be applied to developing new energy resources, see megasidebar, From Biomass to Cellulosic Ethanol, p. 26.



Fig. 6. Mature Biomass Refining Energy Flows: Example Scenario. A mature integrated cellulosic biomass biorefin-

ery encompasses biological and thermochemical processes, demonstrating the efficiencies possible with a fully integrated design. This scenario incorporates the consolidated bioprocessing (CBP) concept, in which all biological processes are incorporated into a single microbe or microbial community. Energy derived from feedstocks is chemically and physically partitioned to ethanol and other products. Dotted arrows from above indicate energy inputs needed to run machinery. The thermochemical portion releases energy that can be used, for example, to sustain necessary temperatures, both heating and cooling, and to power pumps and other ancillary equipment. Table A is a summary of energy flows in this biorefinery concept. [Source: Adapted from L. Lynd et al., "Envisioning Mature Biomass Refineries," presented at First International Biorefinery Symposium, Washington, D.C. (July 20, 2005).]

### Table A. Summary of Energy Flows in Mature Biorefinery Concept

#### Products

- 54% ethanol
- 5% power (electricity)
- 10% diesel
- 6% gasoline

#### **Production Inputs**

- 21% captured for process energy or lost
- <5% agricultural inputs (e.g., farming costs, feedstock transport)

### Ethical, Legal, and Social Issues (ELSI)

Using biomass to produce biofuels holds much promise for providing a renewable, domestically produced liquid energy source that can be a viable alternative to petroleumbased fuels. Biofuel R&D, therefore, aims to achieve more than just scientific and technological advances per se. It is conducted to accomplish important societal needs, with the broader goals of bolstering national energy security, economic growth, and the environment. Analyzing and assessing the societal implications of, and responses to, this research likewise should continue to be framed within the context of social systems and not simply in terms of technological advances and their efficacy (see sidebar, Ethical, Legal, and Social Issues for Widespread Development of Cellulosic Biofuels, this page).

# EERE OBP Platform for Integrated Biorefineries

The Department of Energy's strategic plan identifies its energy goal: "To protect our national and economic security by promoting a diverse supply and delivery of reliable, affordable, and environmentally sound energy." One of several strategies identified to achieve this goal is to "research renewable energy technologies—wind, hydropower, biomass, solar, and geothermal—and work with the private sector in developing these domestic resources."

The department's Office of Energy Efficiency and Renewable Energy (EERE) Office of the Biomass Program (OBP) elaborates on that goal: "Improve energy security by developing technologies that foster a diverse supply of reliable, affordable, and environmentally sound energy by providing for reliable delivery of energy, guarding against energy emergencies, exploring advanced technologies that make a fundamental improvement in our mix of energy options, and improving energy efficiency."

Major outcomes sought include the following.

- By 2012, complete technology development necessary to enable startup demonstration of a biorefinery producing fuels, chemicals, and power, possibly at an existing or new corn dry mill modified to process corn stover through a side stream.
- By 2012 (based on AEI), complete technology integration to demonstrate a minimum sugar selling price of \$.064/lb, resulting in a minimum ethanol selling price of \$1.07/gal. Ethanol would be produced from agricultural residues or dedicated perennial energy crops.

# Ethical, Legal, and Social Issues for Widespread Development of Cellulosic Biofuels

S ocietal questions, concerns, and implications clearly may vary according to the evolutionary stage of biofuel development. Acceptance and support from diverse communities will be needed. Further, societal and technological interactions at earlier phases of research, development, demonstration, deployment, and decommissioning (RDDD&D) will affect interactions at later phases. Within the context of social systems, three overarching questions emerge.

- What are the possible long-term implications of biofuel development and deployment for social institutions and systems if the strategy "works" as anticipated and if it does not?
- How are individuals, organizations, and institutions likely to respond over time to this development and the changes integral to its deployment?
- What actions or interventions (e.g., regulations) associated with biofuel development and its use and deployment will probably or should be taken at local, regional, and national levels to promote socially determined benefits and to avoid, minimize, or mitigate any adverse impacts?

Broad topics raised at the workshop included the following:

- Sustainability of the total integrated cycle.
- Competing interests for land use.
- Creation and use of genetically modified plants. Who creates and uses them, who decides based on what criteria, and how might or should they be regulated?
- Creation and use of genetically modified microbial organisms in a controlled industrial setting.
- Individuals and groups that have the authority to promote or inhibit R&D, demonstration, and use.
- Groups most likely to be affected (positively or negatively) by biofuels at all evolutionary stages of RDDD&D on the local, national, and global levels.



Fig. 7. DOE Energy Efficiency and Renewable Energy Strategic Goals as They Relate to Development of Biofuels. [Source: *Multi Year Program Plan 2007–2012*, OBP, EERE, U.S. DOE (2005)] The program's overarching strategic goal is to develop biorefinery-related technologies to the point that they are cost- and performance-competitive and are used by the nation's transportation, energy, chemical, and power industries to meet their market objectives. The nation will benefit by expanding clean, sustainable energy supplies while also improving its energy infrastructure and reducing GHGs and dependence on foreign oil. This goal is in alignment with DOE and EERE strategic goals as shown in Fig. 7. DOE Energy Efficiency and Renewable Energy Strategic Goals as They Relate to Development of Biofuels, p. 18.

Planning documents of EERE's OBP describe advances the program seeks for four critical objectives: (1) Alter feedstocks for greater yield and for converting larger portions of raw biomass feedstocks to fuel ethanol and other chemicals; (2) decrease costs and improve enzyme activities that convert complex biomass polymers into fermentable sugars; (3) develop microbes that can efficiently convert all 5- and 6-carbon sugars released from the breakdown of complex biomass polymers; and (4) consolidate all saccharification and fermentation capabilities into a single microbe or mixed, stable culture.

A commercial industry based on cellulosic biomass bioconversion to ethanol does not yet exist in the United States, but several precommercial facilities are in development. The Canadian company, Iogen Corporation, a leading producer of cellulase enzymes, operates the largest demonstration biomass-to-ethanol facility, with a capacity of 1 Mgal/year; production of cellulosic ethanol from wheat straw began at Iogen in April 2004. OBP has issued a solicitation for demonstration of cellulosic biorefineries (U.S. Congress 2005, Section 932) as part of the presidential Biofuels Initiative.

# **DOE Office of Science Programs**

The DOE Office of Science (SC) plays key roles in U.S. research, including the contribution of essential scientific foundations to DOE's national energy, environment, and economic security missions (see Fig. 8. DOE Office of Science Programs and Goals as They Relate to Development of Biofuels, p. 20). Other roles are to build and operate major research facilities with open access by the scientific community and to support core capabilities, theories, experiments, and simulations at the extreme limits of science. An SC goal for the Office of Biological and Environmental Research (OBER) is to "harness the power of our living world and provide the biological and environmental discoveries necessary to clean and protect our environment and offer new energy alternatives." SC's goal for its Office of Advanced Scientific Computing Research (OASCR) is "to deliver computing for the frontiers of science" (U.S. DOE 2004).

To address these priorities, OBER and OASCR are sponsoring the Genomics:Genomes to Life (GTL) program. Established in 2002, GTL uses genome data as the underpinnings for investigations of biological systems with capabilities relevant to DOE energy and environmental missions. The GTL scientific program was developed with input from hundreds of scientists from universities, private industry, other federal



Fig. 8. DOE Office of Science Programs and Goals as They Relate to Development of Biofuels. [Derived from Office of Science Strategic Plan and Genomics:GTL Roadmap]

agencies, and DOE national laboratories. Providing solutions to major national problems, biology and industrial biotechnology will serve as an engine for economic competitiveness in the 21st Century. DOE missions in energy security are grand challenges for a new generation of biological research. SC will work with EERE to bring together biology, computing, physical sciences, bioprocess engineering, and technology development for the focused and large-scale research effort needed—from scientific investigations to commercialization in the marketplace. Research conducted by the biofuel R&D community using SC programs and research facilities will play a critical role in developing future biorefineries and ensuring the success of EERE OBP's plans.

The nation's investment in genomics over the past 20 years now enables rapid determination and subsequent interpretation of the complete DNA sequence of any organism. Because it reveals the blueprint for life, genomics is the launching point for an integrated and mechanistic systems understanding of biological function. It is a new link between biological research and biotechnology. Fig. 9. Understanding Biological Capabilities at All Scales Needed to Support Systems Biology Investigations of Cellulosic Biomass. Capabilities are needed to bring together the biological, physical, computational, and engineering sciences to create a new infrastructure for biology and the industrial biotechnology in the 21<sup>st</sup> Century. This figure depicts the focus



GTL's goal is simple in concept but challenging in practice—to reveal how the static information in genome sequences drives the intricate and dynamic processes of life. Through predictive models of these life processes and supporting research infrastructure, GTL seeks to harness the capabilities of living systems. GTL will study critical properties and processes on four systems levels—molecular, cellular, organismal, and community—each requiring advances in fundamental capabilities and concepts. These same concepts and capabilities can be employed by bioprocess engineers to bring new technologies rapidly to the marketplace.

Achieving GTL goals requires major advances in the ability to measure the phenomenology of living systems and to incorporate their operating principles into computational models and simulations that accurately represent biological systems. To make GTL science and biological research more broadly tractable, timely, and affordable, GTL will develop comprehensive suites of capabilities delivering economies of scale and enhanced performance (see Fig. 9. Understanding Biological Capabilities at All Scales Needed to Support Systems Biology Investigations of Cellulosic Biomass, this page). In vertically integrated bioenergy research centers, these capabilities will include the advanced technologies and state-of-the-art computing needed to better

Fig. 10. Creating a **Common Research** Agenda. The EERE Office of the Biomass Program's Multi Year Program Plan 2007-2012 contains a roadmap for biofuel development that identifies technological barriers to achieving goals defined in Fig. 7, p. 18. These challenges include the need for new feedstocks, their deconstruction to fermentable sugars, and fermentation of all sugars to ethanol. Within the DOE Office of Science, OBER and OASCR's roadmap for the Genomics:GTL



program outlines scientific goals, technologies, computing needs, and a resource strategy to achieve the GTL goal of a predictive understanding of biological systems. This document is a roadmap that links the two plans.

understand genomic potential, cellular responses, regulation, and behaviors of biological systems. Computing and information technologies are central to the GTL program's success because they will allow scientists to surmount the barrier of complexity now preventing them from deducing biological function directly from genome sequence. GTL will create an integrated computational environment that will link experimental data of unprecedented quantity and dimensionality with theory, modeling, and simulation to uncover fundamental biological principles and to develop and test systems theory for biology.

# Biomass to Biofuels Workshop: Creating a Common Research Agenda to Overcome Technology Barriers

A product of the Biomass to Biofuels Workshop, this roadmap analyzes barriers to achieving OBP goals (as described herein) and determines fundamental research and capabilities (as described in the GTL Roadmap) that could both accelerate progress in removing barriers and allow a more robust set of endpoints (see Fig. 10. Creating a Common Research Agenda, this page). Relating high-level topical areas and their goals to key scientific milestones identified by workshop participants could help achieve progress toward OBP goals in collaboration with SC (see Table 2. Overcoming Barriers to Cellulosic Ethanol: OBP Biological and Technological Research Milestones, p. 23).

## Table 2. Overcoming Barriers to Cellulosic Ethanol: OBP Biological and Technological Research Milestones

Office of the Biomass Program (OBP) Barrier Topic	Technology Goals	Science Research Milestones
Feedstocks Develop sustainable technologies to supply biomass to biorefineries	Better compositions and structures for sugars production Domestication: Yield, tolerance Better agronomics Sustainability	Cell-wall architecture and makeup relative to processability Genome sequence for energy crops Domestication traits: Yield, tolerance Cell-wall genes, principles, factors New model systems to apply modern biology tools Soil microbial community dynamics for determining sustainability
Feedstock Deconstruction to Sugars Develop biochemical conversion technologies to produce low-cost sugars from lignocellulosic biomass	Pretreatment EnzymesReduced severityReduced wasteHigher sugar yieldsReduced inhibitorsReduction in nonfermentable sugarsEnzyme Hydrolysis to SugarsHigher specific activityHigher thermal toleranceReduced product inhibitionBroader substrate rangeCellulases and cellulosomes	<ul> <li>Cell-wall structure with respect to degradation Modification of the chemical backbone of hemicellulose materials to reduce the number of nonfermentable and derivatized enzymes</li> <li>Cell-wall component response to pretreatments</li> <li>Principles for improved cellulases, ligninases, hemicellulases</li> <li>Understanding of cellulosome regulation and activity</li> <li>Action of enzymes on insoluble substrates (fundamental limits)</li> <li>Fungal enzyme-production factors</li> <li>Nonspecific adsorption of enzymes</li> <li>Origin of inhibitors</li> </ul>
Sugar Fermentation to Ethanol Develop technologies to produce fuels, chemicals, and power from biobased sugars and chemical building blocks	<b>Cofermentation of Sugars</b> C-5 and C-6 sugar microbes Robust process tolerance Resistance to inhibitors Marketable by-products	Full microbial system regulation and control Rapid tools for manipulation of novel microbes Utilization of all sugars Sugar transporters Response of microorganisms to stress New microbial platforms Microbial community dynamics and control
<b>Consolidated Processing</b> Reduce process steps and complexity by integrating multiple processes in single reactors	Enzyme Production, Hydrolysis, and Cofermentation Combined in One Reactor Production of hydrolytic enzymes Fermentation of needed products (ethanol) Process tolerance Stable integrated traits All processes combined in a single microbe or stable culture	Fundamentals of microbial cellulose utilization Understanding and control of regulatory processes Engineering of multigenic traits Process tolerance Improved gene-transfer systems for microbial engineering Understanding of transgenic hydrolysis and fermentation enzymes and pathways

The workshop was organized under the following topical areas: Feedstocks for Biofuels (p. 57); Deconstructing Feedstocks to Sugars (p. 85); Sugar Fermentation to Ethanol (p. 119); and Crosscutting 21st Century Science, Technology, and Infrastructure for a New Generation of Biofuel Research (p. 155). A critical topic discussed in several workshop groups was Lignocellulosic Biomass Characteristics (p. 39). These five topics and plans would

tie the two offices' roadmaps together and also serve as a key driver for implementing the combined roadmaps in pursuit of a high-level national goal: Create a viable cellulosic-biofuel industry as an alternative to oil for transportation. These topics and their relationships are discussed in subsequent chapters outlining technical strategy and detailed research plans developed in the workshop.

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References continued on p. 28.

# From BIOMASS to CELLULOSIC ETHANOL

# **Biomass Feedstock**

Plant Residues and Energy Crops

#### Biotechnology offers the

promise of dramatically increasing

ethanol production using cellulose, the most abundant biological material on earth, and other polysaccharides (hemicellulose). Residue including postharvest corn plants (stover) and timber residues could be used, as well as such specialized high-biomass "energy" crops as domesticated poplar trees and switchgrass.

Biochemical conversion of cellulosic biomass to ethanol for transportation fuel currently involves three basic steps:

- Pretreatments to increase the accessibility of cellulose to enzymes and solubilize hemicellulose sugars
- Hydrolysis with special enzyme preparations to break down cellulose to sugars
- Fermentation to ethanol

Making cellulosic biomass conversion to ethanol more economical and practical will require a science base for molecular redesign of numerous enzymes, biochemical pathways, and full cellular systems.

### DOE GTL program contributions needed to

- · Control cell-wall composition for energy production
- Develop appropriate model systems for energy crops
- Improve quantity and quality of perennial herbaceous and woody biomass crops
- Domesticate energy crops for stress tolerance
- Develop sustainable management practices

### Pretreatment

Goal: Make cellulose more accessible to enzymatic breakdown (hydrolysis) and solubilize hemicellulose sugars



Hemicellulose

Cellulose exists within a matrix of other polymers, primarily hemicellulose and lignin. Pretreatment of biomass with heat, enzymes, or acids removes these polymers from the cellulose core before hydrolysis.

Pretreatment, one of the most expensive processing steps, has great potential for improvement through R&D.

[Figure adapted from N. Mosier et al. 2005. "Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass," Bioresource Technology **96**(6), 673–86. Reprinted with permission from Elsevier.]



### DOE GTL program contributions needed to

- Optimize and exploit biological catalysts
- · Reduce thermochemical treatments and waste
- Increase simple sugar yields and concentration

All recommendations for DOE Genomics:GTL program contributions stem from the 2005 workshop sponsored by the DOE Office of Science and Office of Energy Efficiency and Renewable Energy. The report and this image are available at www.doegenomestolife.org/biofuels/.

# **Applying Genomics for New Energy Resources**

## **Hydrolysis**

Goal: Break down cellulose into its component sugars using enzyme preparations



(1) A cellulase enzyme breaks down pretreated cellulose fragments into double glucose molecules (cellobiose), which are then split by another cellulase type (2) into single glucose residues.



Cellulose is made up of double glucose molecules (cellobiose).

Enzymes such as cellulases synthesized by fungi and bacteria work together to degrade cellulose and other structural polysaccharides in biomass. Optimizing these complex systems will require a more detailed understanding of their regulation and activity.

## Fermentation to Ethanol

Goal: Convert sugars to ethanol using microbes

Glucose and other sugers

Biorefiner

crobial Fermentan

Microbes ferment sugars to ethanol, which is then separated from the mix of ethanol, water, microbes, and residue and purified through distillation.



### DOE GTL program contributions needed to

- Increase specific activities
- Increase thermal tolerance
- Reduce product inhibition
- Broaden substrate range

### Consolidate Processing Steps

Integrate hydrolysis and fermentation steps into a single microbe or mixed stable culture that

- Produces hydrolytic enzymes
- · Ferments sugars to ethanol
- Is process tolerant
- · Has stable integrated traits

### DOE GTL program contributions needed to

- Eliminate solid-liquid separation step
- Coferment 5- and 6-carbon sugars from biomass feedstocks
- Increase process tolerance and resistance to inhibitors
- Return minerals to soils

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# **Background Reading**

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