

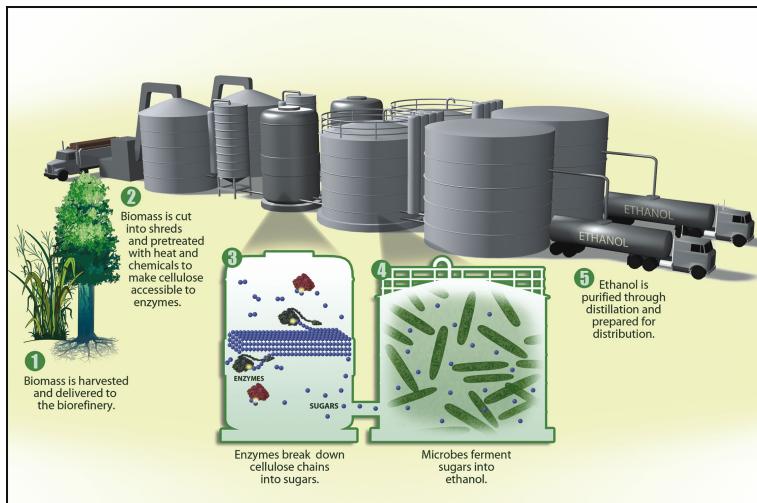
DOE 
Bioenergy
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genome programs of the U. S. Department of Energy Office of Science
founder of the Human Genome Project and leader in systems biology research

Credit or Source :

Genomic Management Information System, Oak Ridge National Laboratory

How Cellulosic Ethanol Is Made



From Biomass to Cellulosic Ethanol

Ethanol from cellulosic biomass—the most abundant biological material on the planet—has the potential to revolutionize the fuel ethanol industry and decrease U.S. dependence on imported oil. Despite its abundance, cellulosic biomass is a more complex feedstock that requires more extensive processing than corn grain, the primary feedstock for conventional fuel ethanol production in the U.S. Several scientific breakthroughs are needed to make cellulosic ethanol production cost efficient enough to operate at a commercial scale.

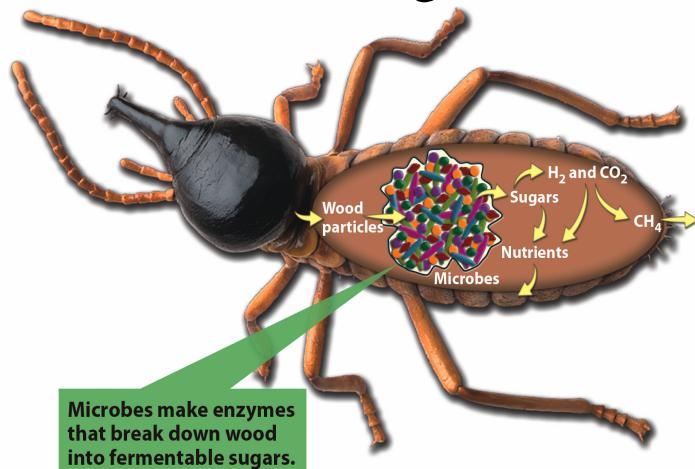
This figure highlights some key processing steps in an artist's conception of a future, large-scale, cellulosic ethanol production facility. (1) Cellulosic biomass from trees, grasses, or agricultural wastes is harvested and delivered to the biorefinery. (2) Biomass is ground into small, uniform particles. Thermal or chemical pretreatment separates cellulose, a tough polymer of tightly bound sugar chains, from other biomass materials and opens up the cellulose surface to enzymatic attack. (3) A mix of enzymes is added to break down cellulose into simple sugars. (4) Microbes produce ethanol by fermenting sugars from cellulose and other biomass carbohydrates. (5) Ethanol is separated from water and other components of the fermentation broth and purified through distillation.

To bring down costs, continued progress is needed in the development of energy crops dedicated to biofuel production, biomass-collection technologies, pretreatment methods that minimize the release of inhibitory by-products, and more efficient enzymes and microbes robust enough to withstand the stresses of industrial processing.

Credit or Source :

Genome Management Information System, Oak Ridge National Laboratory

The Termite Gut: Nature's Microbial Bioreactor For Digesting Wood & Making Biofuels



The microbial community within a termite's gut is one of nature's most efficient bioreactors—typically converting 95% of cellulose into simple sugars within 24 hours. More than 200 species of microbes make up this community, and they produce a bounty of wood-busting enzymes that could be put to work in biorefineries making ethanol from several forms of cellulosic biomass.

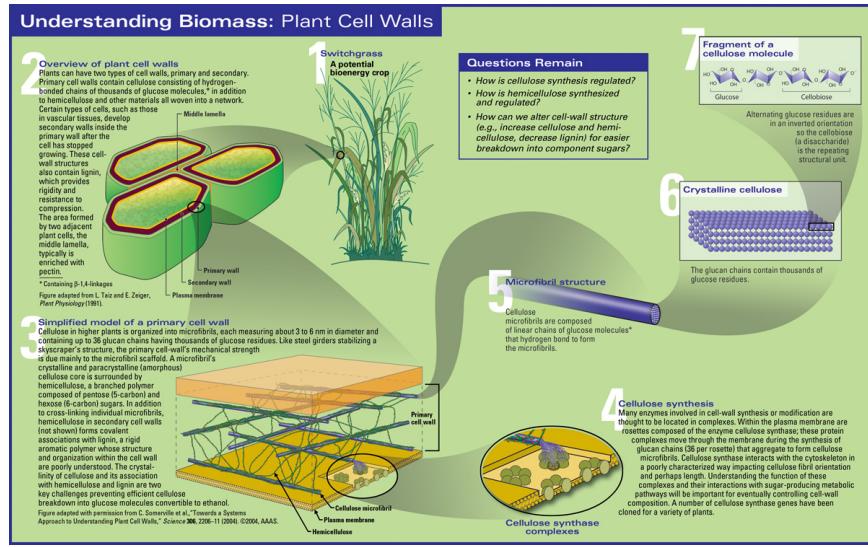
This diverse array of microbial capabilities that could jumpstart a new biofuel industry is the result of a codependent strategy for survival. Without wood-eating microbes, a termite would not be able to extract nutrients and energy from wood, and without the termite to grind wood into tiny pieces and provide an oxygen-free habitat within its gut, the microbes would not be able to survive. In addition to efficiently degrading cellulose into sugars, some termite-gut microbes are biochemically capable of generating other potential fuels such as hydrogen or methane. Hydrogen produced by one group of microbes is consumed by other gut microbes that create energy-producing by-products the termite can use. Investigating the termite-gut community reveals a vast collection of biological pathways that may one day be put to use for multiple energy applications.

A collaboration of researchers from the Department of Energy's Joint Genome Institute (DOE JGI), the California Institute of Technology, Diversa, and INBIO (National Biodiversity Institute of Costa Rica) has sequenced and analyzed microbial DNA extracted from the guts of hundreds of termites harvested from a nest in a Costa Rican rainforest. Preliminary results already have identified several novel enzymes capable of degrading cellulose into sugars, and the San Diego-based biotechnology company Diversa has used insights from this discovery to create a high-performance enzyme cocktail for processing plant biomass into biofuels. DOE JGI researchers continue to investigate other microbial communities in the guts of insects that consume different plant materials. The goal is to understand and reconstruct a diverse range of metabolic processes that could be scaled up for industrial biofuel production.

Credit or Source :

Genome Management Information System, Oak Ridge National Laboratory

Understanding Biomass: Plant Cell Walls



Description :

Explains plant cell wall structure and some of the issues preventing their efficient conversion to ethanol.

Citation :

"Genomics:GTL Transforming Cellulosic Biomass," U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy, June 2006, <http://genomicsgtl.energy.gov/biofuels/> and U.S. DOE. 2006. "Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda," DOE/SC/EE-0095, U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy, <http://genomicsgtl.energy.gov/biofuels/>.

Credit or Source :

Genome Management Information System, Oak Ridge National Laboratory

Understanding Biomass: Plant Cell Walls (Expanded)

Understanding Biomass: Plant Cell Walls

2 Overview of plant cell walls

Plants can have two types of cell walls, primary and secondary. Primary cell walls contain cellulose consisting of hydrogen-bonded chains of thousands of glucose molecules,* in addition to hemicellulose and other materials all woven into a network. Certain types of cells, such as those in vascular tissues, develop secondary walls inside the primary wall after the cell has stopped growing. These cell-wall structures also contain lignin, which provides rigidity and resistance to compression. The area formed by two adjacent plant cells, the middle lamella, typically is enriched with pectin.

* Containing β -1,4-linkages

Figure adapted from L'Taiz and E. Zeiger,

Plant Physiology (1991).



1 Switchgrass A potential bioenergy crop

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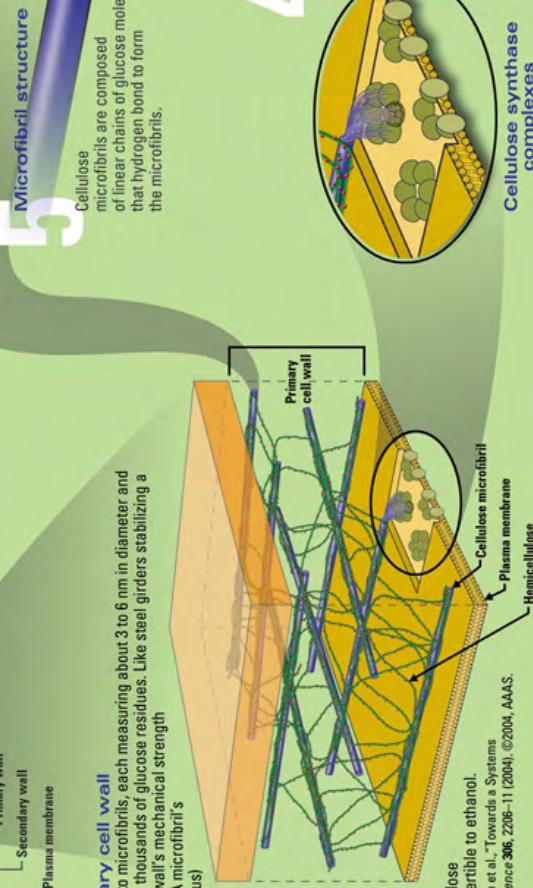
Plant Physiology (1991).

3 Simplified model of a primary cell wall

Cellulose in higher plants is organized into microfibrils, each measuring about 3 to 6 nm in diameter and containing up to 36 glucan chains having thousands of glucose bonds. Like steel girders stabilizing a skyscraper's structure, the primary cell-wall's mechanical strength is due mainly to the microfibril scaffold. A microfibril's

crystalline and paracrystalline (amorphous) cellulose core is surrounded by hemicellulose, a branched polymer composed of pentose (5-carbon) and hexose (6-carbon) sugars. In addition to cross-linking individual microfibrils, hemicellulose in secondary cell walls (not shown) forms covalent associations with lignin, a rigid aromatic polymer whose structure and organization within the cell wall are poorly understood. The crystallinity of cellulose and its association with hemicellulose and lignin are two key challenges preventing efficient cellulose breakdown into glucose molecules convertible to ethanol.

Figure adapted with permission from C. Somerville et al., "Towards a Systems Approach to Understanding Plant Cell Walls," *Science* **306**, 2206–11 (2004). ©2004, AAAS.



Questions Remain

- How is cellulose synthesis regulated?
- How is hemicellulose synthesized and regulated?
- How can we alter cell-wall structure (e.g., increase cellulose and hemicellulose, decrease lignin) for easier breakdown into component sugars?

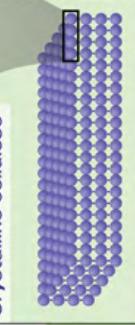
7

Fragment of a cellulose molecule



Alternating glucose residues are in an inverted orientation so the cellulose (a disaccharide) is the repeating structural unit.

Crystalline cellulose



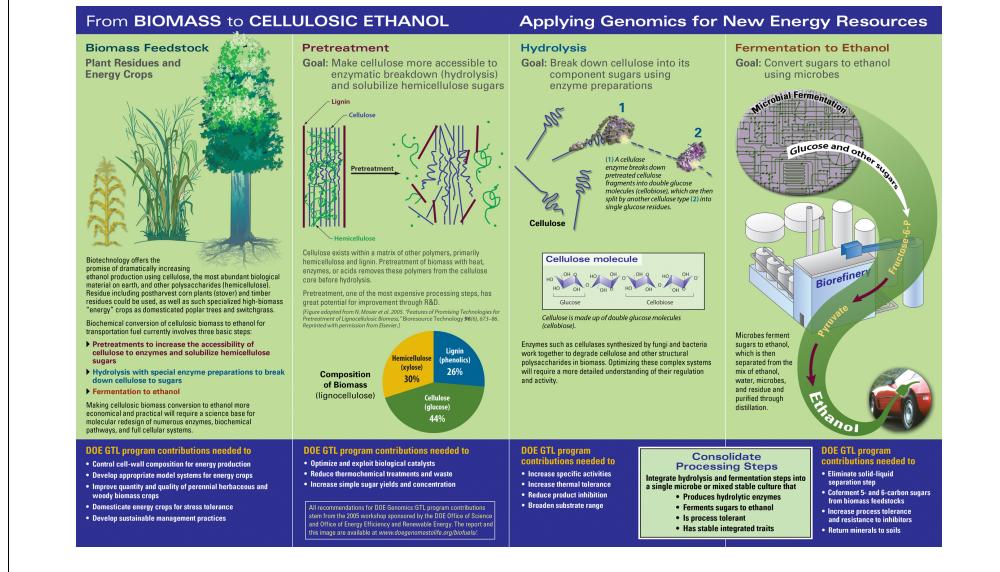
The glucan chains contain thousands of glucose residues.

4 Cellulose synthesis

Many enzymes involved in cell-wall synthesis or modification are thought to be located in complexes. Within the plasma membrane are rosettes composed of the enzyme cellulose synthase; these protein complexes move through the membrane during the synthesis of glucan chains (36 per rosette) that aggregate to form cellulose microfibrils. Cellulose synthase interacts with the cytoskeleton in a poorly characterized way impacting cellulose fibril orientation and perhaps length. Understanding the function of those complexes and their interactions with sugar-producing metabolic pathways will be important for eventually controlling cell-wall composition. A number of cellulose synthase genes have been cloned for a variety of plants.

Genome Management Information System, Oak Ridge National Laboratory

From Biomass To Cellulosic Ethanol



Description :

Depicts the process used to convert biomass (plant matter) into cellulosic ethanol and the improvements needed to optimize these processes.

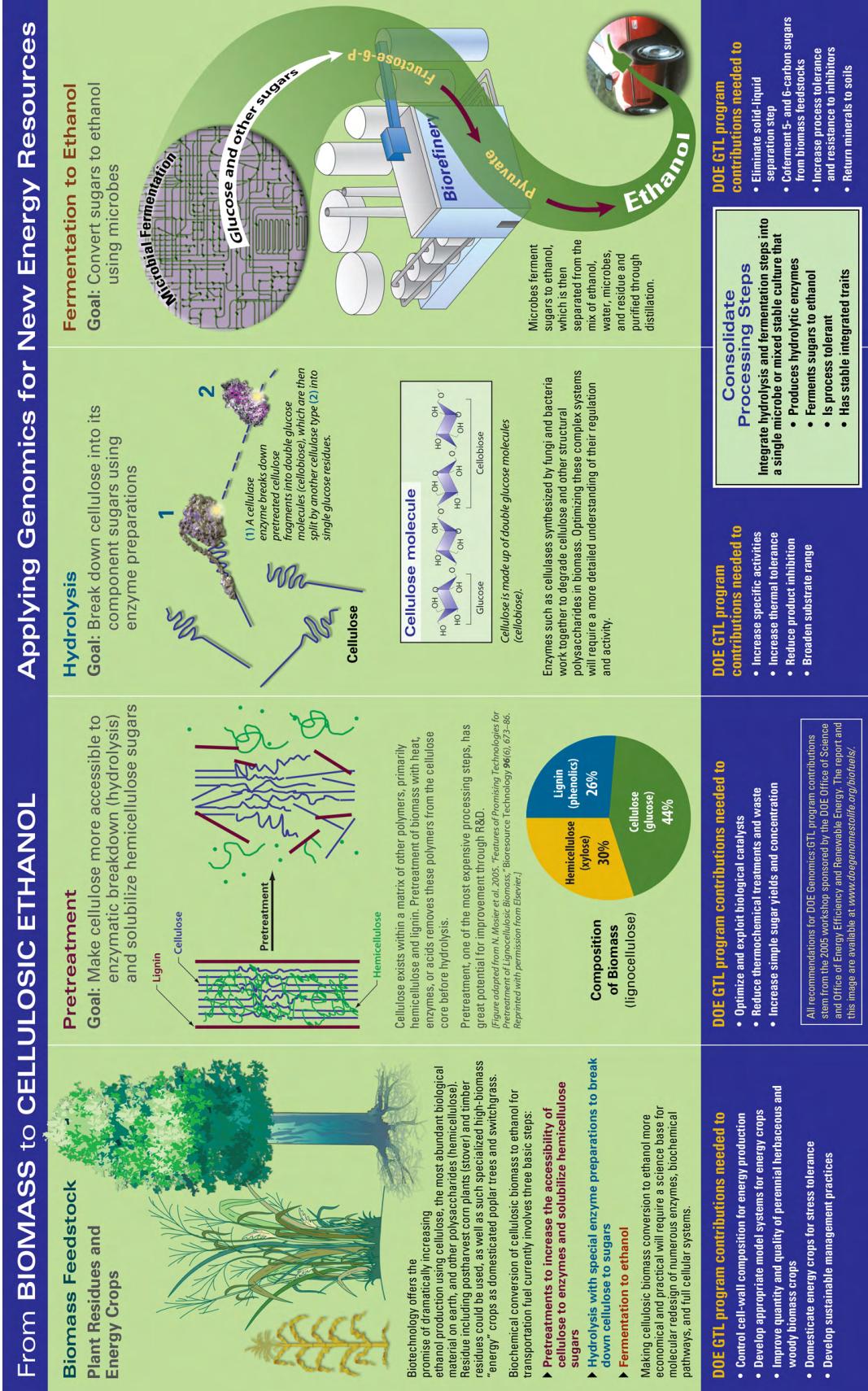
Citation :

"Genomics:GTL Transforming Cellulosic Biomass," U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy, June 2006, <http://genomicsgtl.energy.gov/biofuels/> and U.S. DOE. 2006. "Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda," DOE/SC/EE-0095, U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy, <http://genomicsgtl.energy.gov/biofuels/>.

Credit or Source :

Genome Management Information System, Oak Ridge National Laboratory

From Biomass To Cellulosic Ethanol (Expanded)



Genome Management Information System, Oak Ridge National Laboratory

Cellulosic Biomass Feedstock: Poplar/Trees



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: (1) Pretreatments to increase the accessibility of cellulose to enzymes and solubilize hemicellulose sugars; (2) Hydrolysis with special enzyme preparations to break down cellulose to sugars; and (3) 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; and Develop sustainable management practices.

Citation : "Genomics:GTL Transforming Cellulosic Biomass," U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy, June 2006, <http://genomicsgtl.energy.gov/biofuels/> and U.S. DOE. 2006. "Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda," DOE/SC/EE-0095, U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy, <http://genomicsgtl.energy.gov/biofuels/>.

Credit or Source : Genome Mgt. Info. Sys., Oak Ridge National Laboratory

Cellulosic Biomass Feedstock: Corn Stover



Description :

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: (1) Pretreatments to increase the accessibility of cellulose to enzymes and solubilize hemicellulose sugars; (2) Hydrolysis with special enzyme preparations to break down cellulose to sugars; and (3) 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.

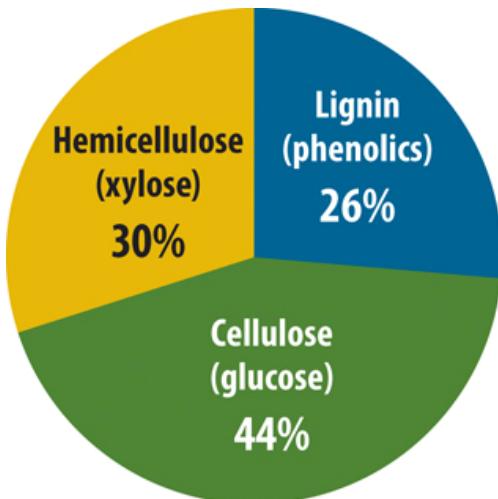
Citation :

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Credit or Source :

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Composition of Biomass (Lignocellulose)



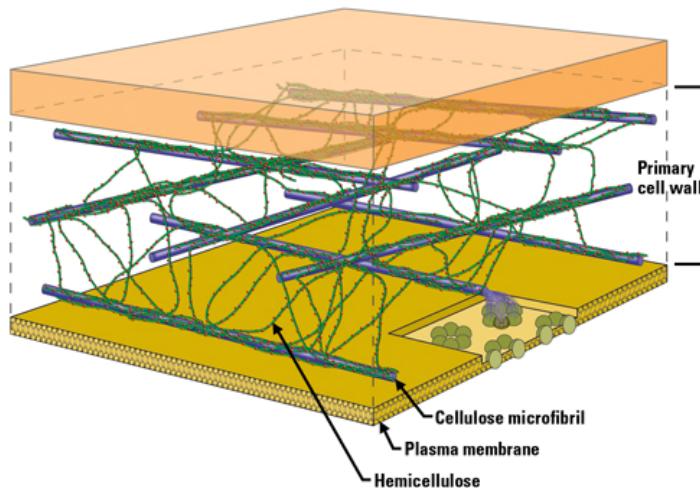
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Simplified Model of a Primary Cell Wall



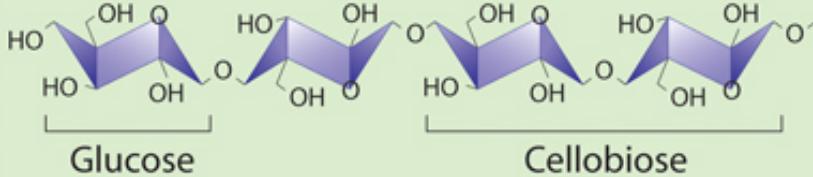
Cellulose in higher plants is organized into microfibrils, each measuring about 3 to 6 nm in diameter and containing up to 36 glucan chains having thousands of glucose residues. Like steel girders stabilizing a skyscraper's structure, the primary cell-wall's mechanical strength is due mainly to the microfibril scaffold. A microfibril's crystalline and paracrystalline (amorphous) cellulose core is surrounded by hemicellulose, a branched polymer composed of pentose (5-carbon) and hexose (6-carbon) sugars. In addition to cross-linking individual microfibrils, hemicellulose in secondary cell walls (not shown) forms covalent associations with lignin, a rigid aromatic polymer whose structure and organization within the cell wall are poorly understood. The crystallinity of cellulose and its association with hemicellulose and lignin are two key challenges preventing efficient cellulose breakdown into glucose molecules convertible to ethanol.

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Credit or Source : Figure adapted with permission from C. Somerville, Stanford University, 2004. Source: Genome Management Information System, Oak Ridge National Laboratory

Fragment of a Cellulose Molecule

Fragment of a cellulose molecule



Alternating glucose residues are in an inverted orientation so the cellobiose (a disaccharide) is the repeating structural unit.

Description :

Alternating glucose residues are in an inverted orientation so the cellobiose (a disaccharide) is the repeating structural unit. 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.

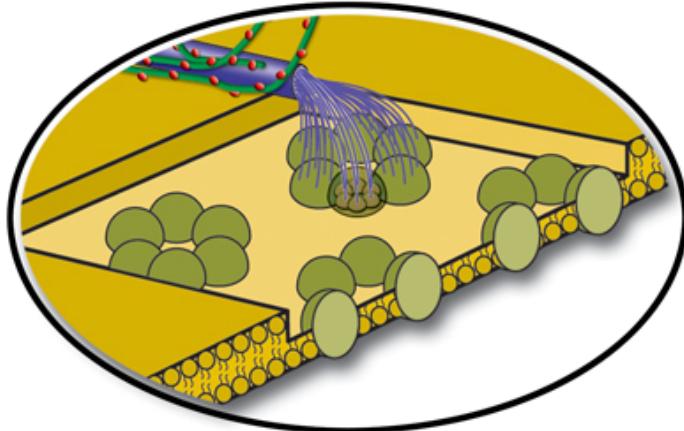
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Credit or Source :

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Cellulose Synthase Complexes



Cellulose synthase complexes

Description :

Cellulose synthesis: Many enzymes involved in cell-wall synthesis or modification are thought to be located in complexes. Within the plasma membrane are rosettes composed of the enzyme cellulose synthase; these protein complexes move through the membrane during the synthesis of glucan chains (36 per rosette) that aggregate to form cellulose microfibrils. Cellulose synthase interact with the cytoskeleton in a poorly characterized way impacting cellulose fibril orientation and perhaps length. Understanding the function of these complexes and their interactions with sugar-producing metabolic pathways will be important for eventually controlling cell-wall composition. A number of cellulose synthase genes have been cloned for a variety of plants.

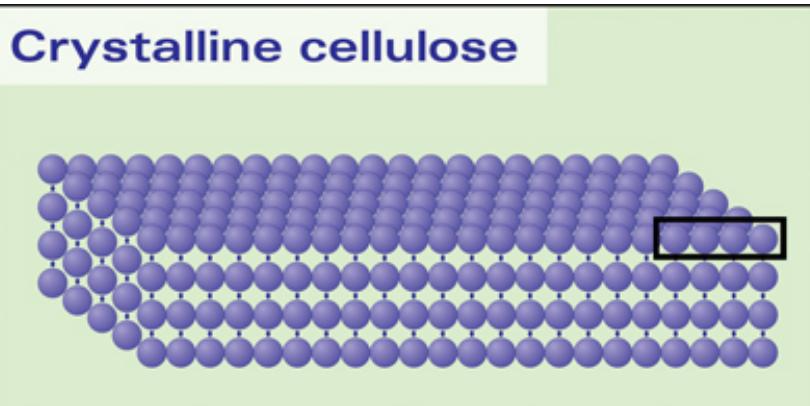
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Crystalline Cellulose



The glucan chains contain thousands of glucose residues.

Description :

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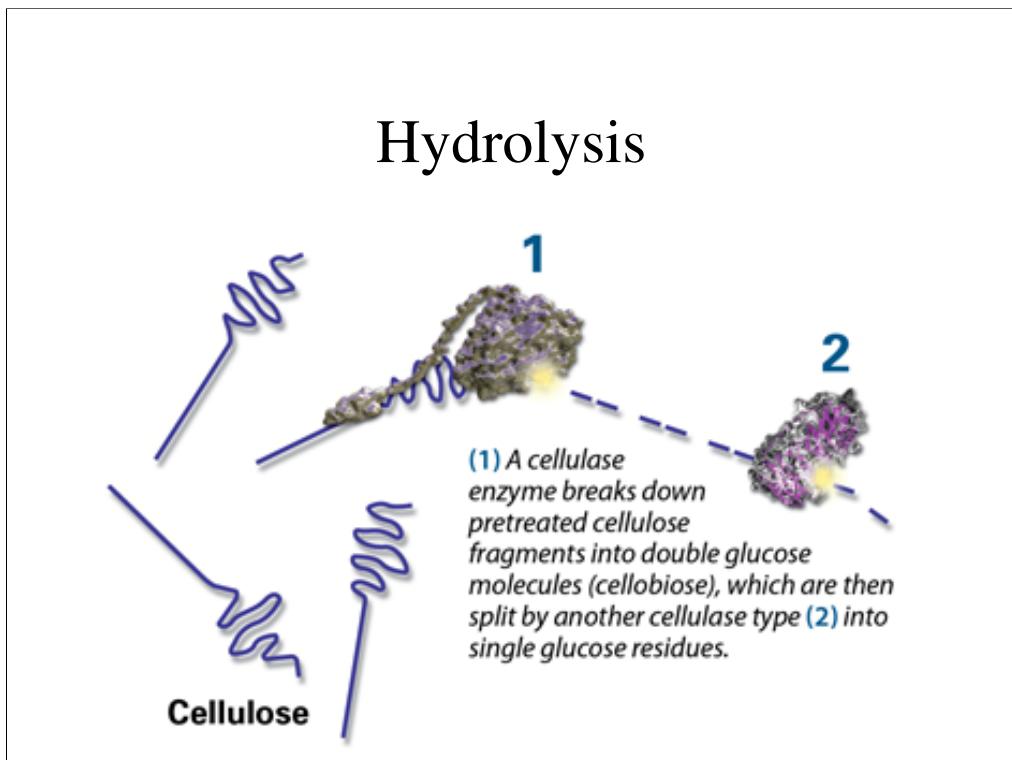
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Hydrolysis



Description :

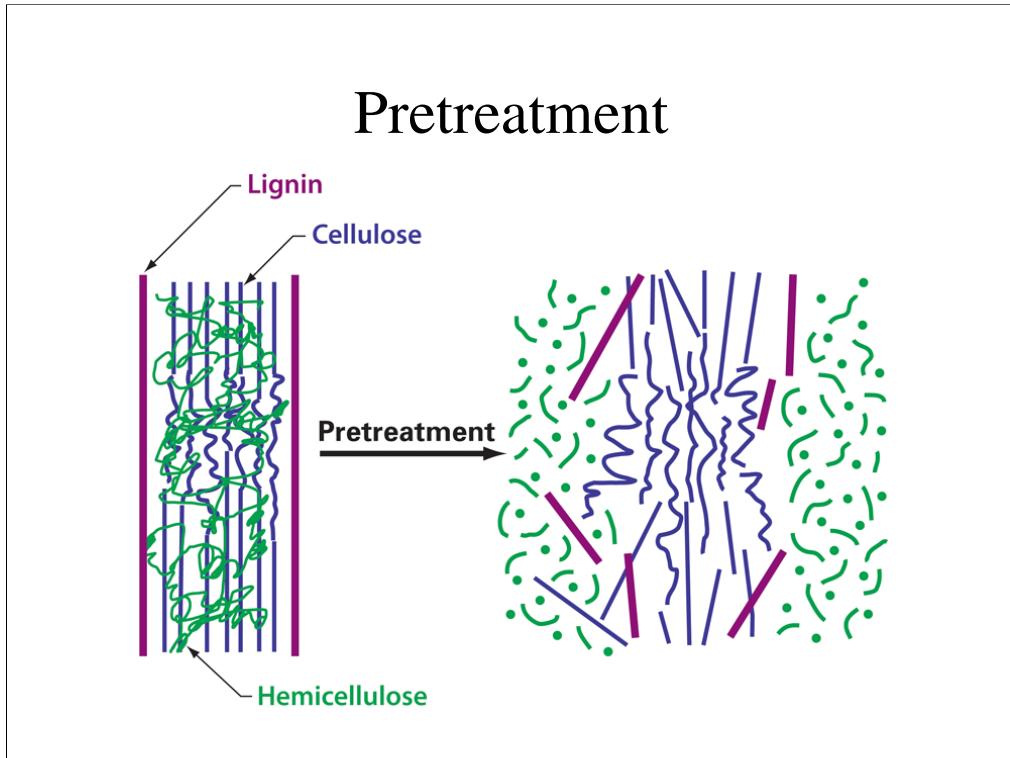
Goal: Break down cellulose into its component sugars using enzyme preparations. Enzymes such as cellulases synthesized by fungi and bacteria work together to degrade cellulose and other structural polysaccharides in biomass. Optimizing these complex systems will require a more detailed understanding of their regulation and activity.

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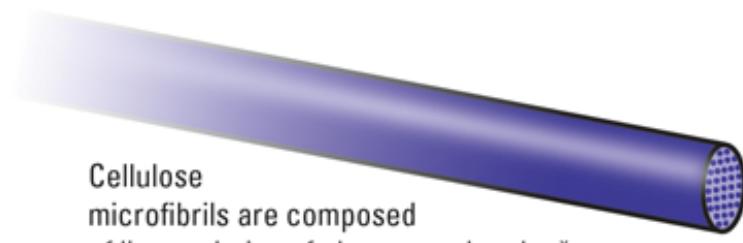
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Credit or Source :

Figure adapted from N. Mosier et al. 2005. "Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass," Bioresource Technology 96(3), 673–86. Source: Genome Management Information System, Oak Ridge National Laboratory

Microfibril Structure



Cellulose microfibrils are composed of linear chains of glucose molecules* that hydrogen bond to form the microfibrils.

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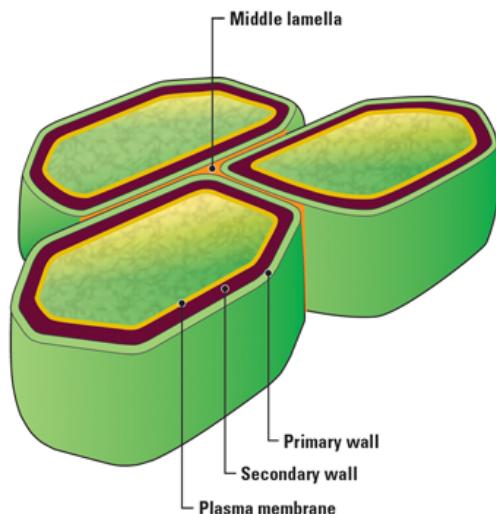
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Overview of Plant Cell Walls



Description :

Plants can have two types of cell walls, primary and secondary. Primary cell walls contain cellulose consisting of hydrogen-bonded chains of thousands of glucose molecules, (Containing b-1,4-linkages) in addition to hemicellulose and other materials all woven into a network. Certain types of cells, such as those in vascular tissues, develop secondary walls inside the primary wall after the cell has stopped growing. These cell-wall structures also contain lignin, which provides rigidity and resistance to compression. The area formed by two adjacent plant cells, the middle lamella, typically is enriched with pectin.

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Credit or Source :

Figure adapted from L. Taiz and E. Zeiger, Plant Physiology (1991). Source: Genome Management Information System, Oak Ridge National Laboratory

Cellulosic Biomass Feedstock: Switchgrass



Description :

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: (1) Pretreatments to increase the accessibility of cellulose to enzymes and solubilize hemicellulose sugars; (2) Hydrolysis with special enzyme preparations to break down cellulose to sugars; and (3) 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.

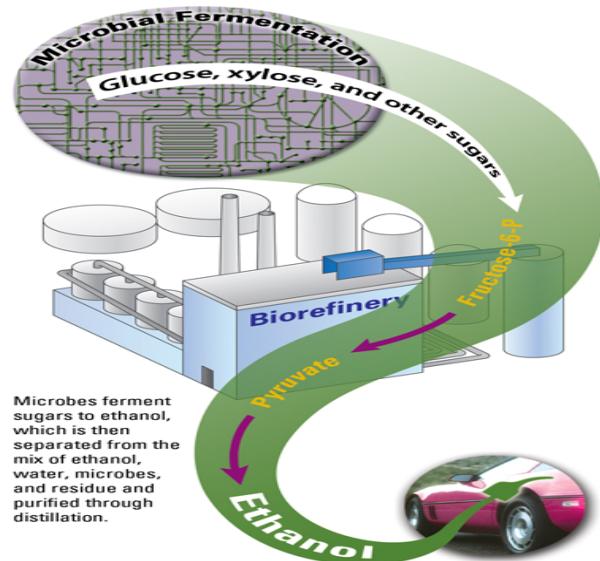
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Credit or Source :

Genome Management Information System, Oak Ridge National Laboratory

Fermentation to Ethanol



Description :

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

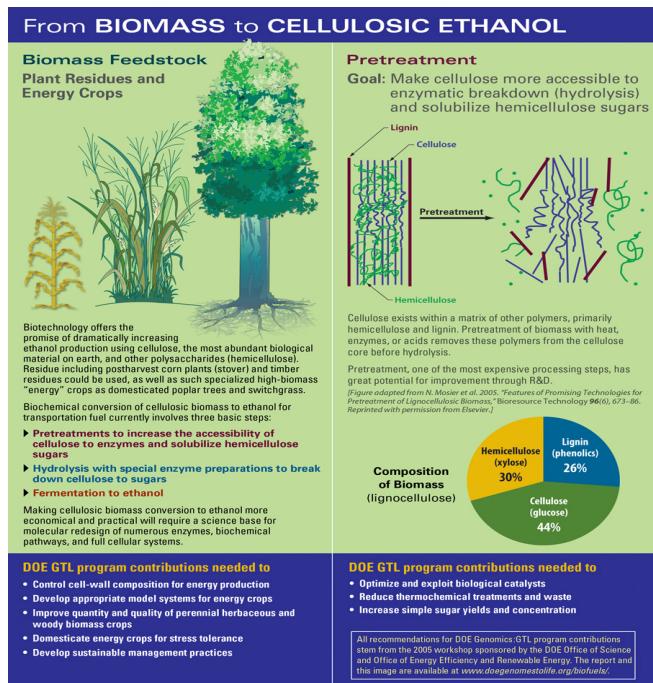
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Credit or Source :

Genome Management Information System, Oak Ridge National Laboratory

From Biomass to Cellulosic Ethanol (Part a)



Citation :

"Genomics:GTL Transforming Cellulosic Biomass," U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy, June 2006, <http://genomicsgtl.energy.gov/biofuels/> and U.S. DOE. 2006. "Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda," DOE/SC/EE-0095, U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy, <http://genomicsgtl.energy.gov/biofuels/>.

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From BIOMASS to CELLULOUSIC ETHANOL

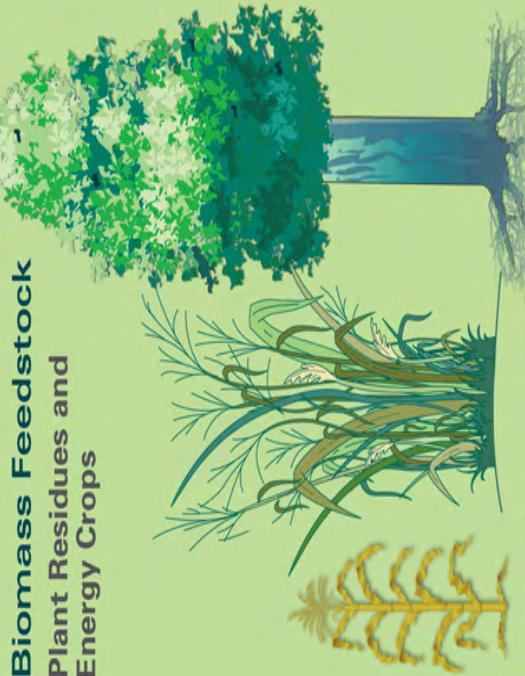
Biomass Feedstock

Plant Residues and Energy Crops

Pretreatment

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

From Biomass to Cellulosic Ethanol (Part a Expanded)



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**

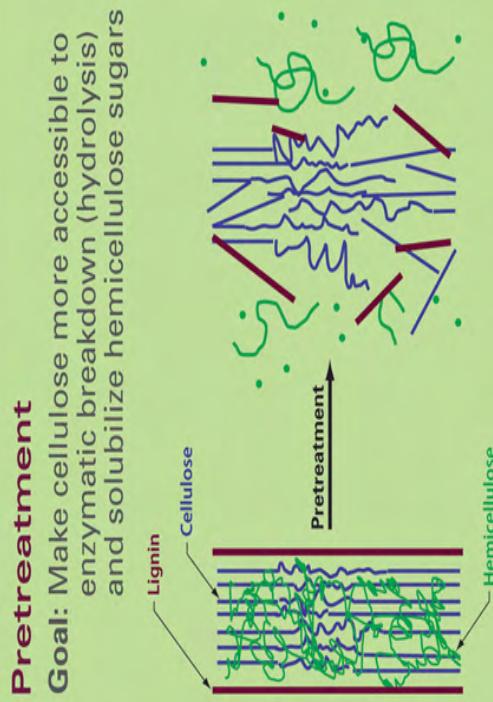
► **Hydrolysis with special enzyme preparations to break down cellulose to sugars**

► **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

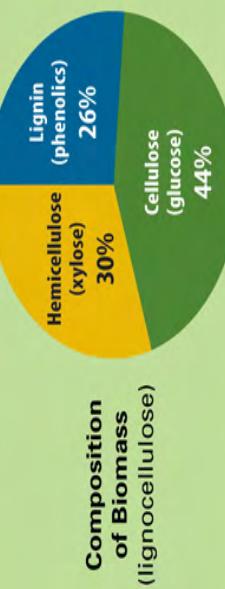
- Control cell-wall composition for energy production
- Develop appropriate model systems for energy crops
- Improve quantity and quality of perennial herbaceous and woody biomass crops
- Domesticate energy crops for stress tolerance
- Develop sustainable management practices



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), 6773-86. Reprinted with permission from Elsevier.]



Credit/Source:
Genome
Management

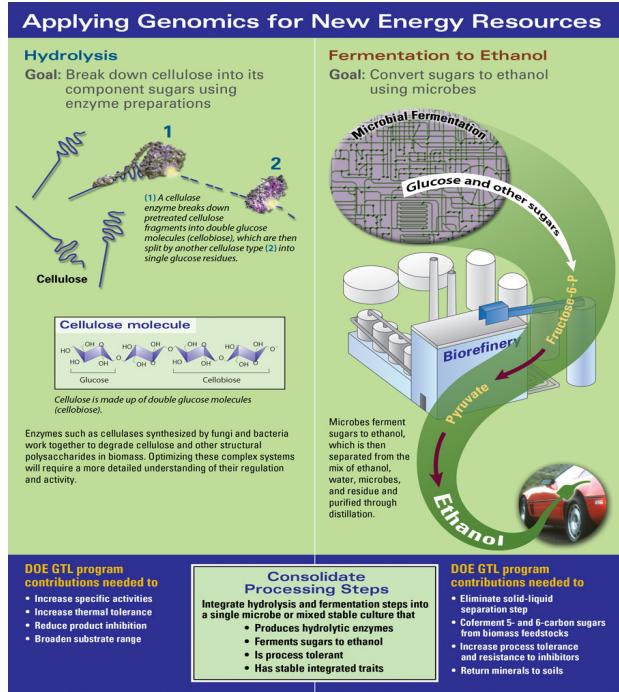
DOE GTL program contributions needed to

- 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.doe genomeinfo.org/biofuels/](http://www doe genomeinfo org/biofuels/).

System, Oak
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Laboratory

From Biomass to Cellulosic Ethanol (Part b)



Citation :

"Genomics:GTL Transforming Cellulosic Biomass," U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy, June 2006, <http://genomicsgtl.energy.gov/biofuels/> and U.S. DOE. 2006. "Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda," DOE/SC/EE-0095, U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy, <http://genomicsgtl.energy.gov/biofuels/>.

Credit or Source :

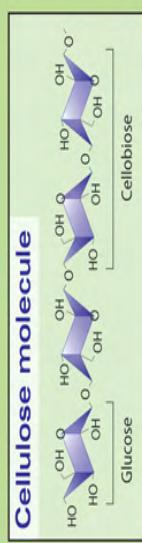
Genome Management Information System, Oak Ridge National Laboratory

Applying Genomics for New Energy Resources

Biomass to Cellulosic Ethanol (Part b Expanded)

Fermentation to Ethanol

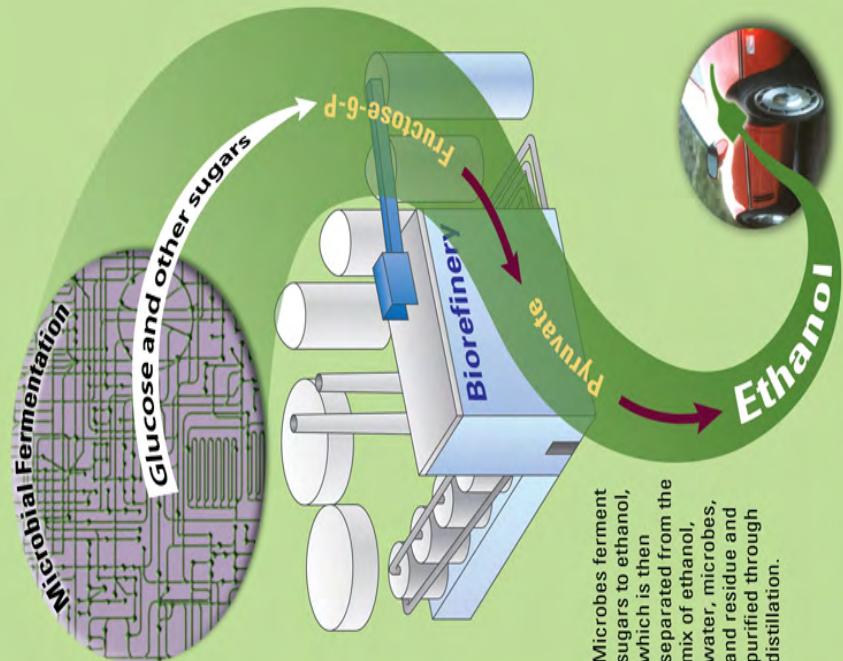
Goal: Convert sugars to ethanol using microbes



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.

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



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Information

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

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

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

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