BioEnergy Science Center and Beyond:

An integrated Biofuel Strategy

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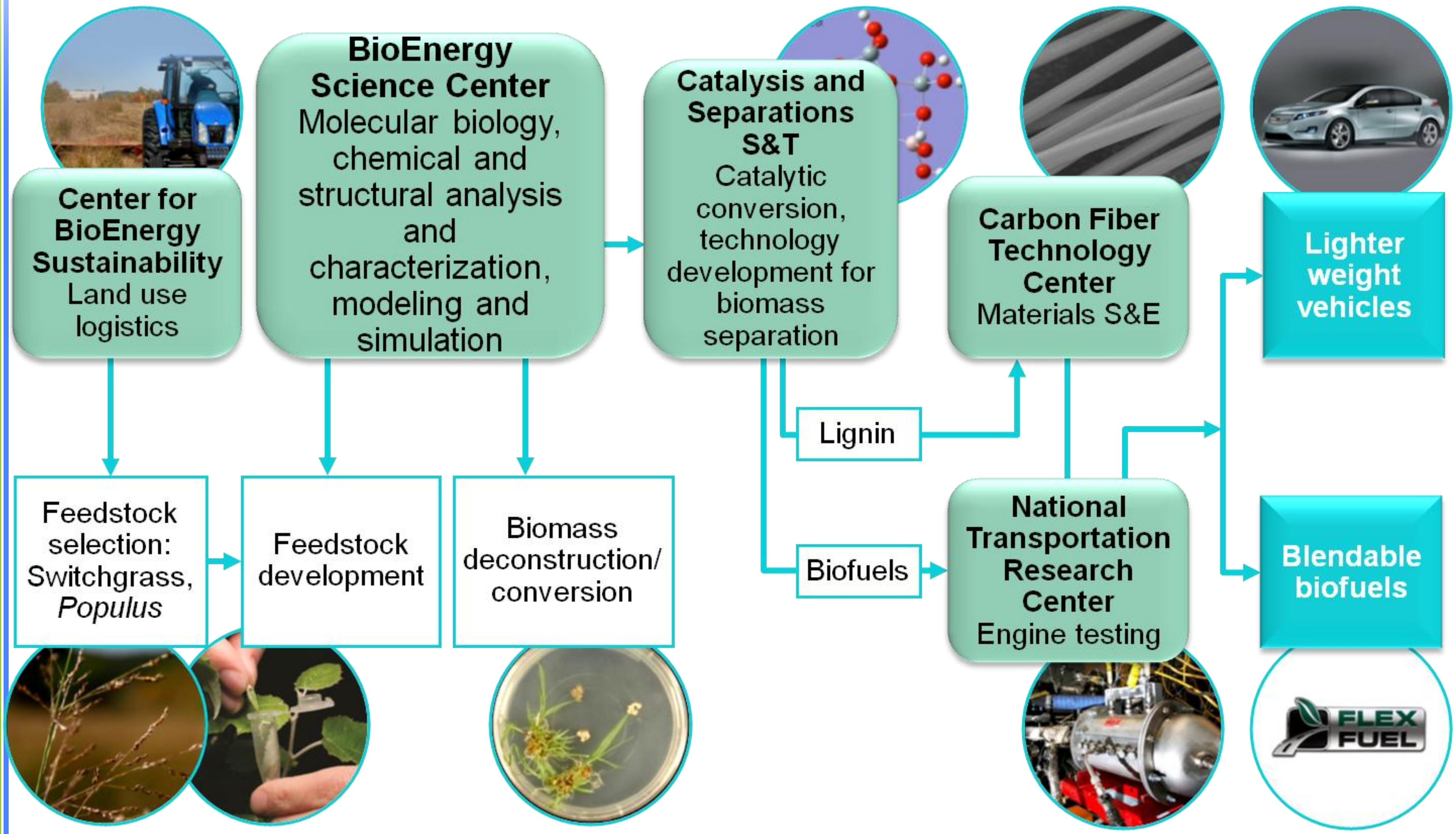
Bioscience and biotechnology for sustainable mobility



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The BioEnergy Science Center

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A multi-institutional DOE funded center performing basic and applied science dedicated to improving yields of biofuels from cellulosic biomass


BESC Headquarters at ORNL

- Samuel Roberts Noble Foundation
- National Renewable Energy Laboratory
- Brookhaven National Laboratory
- Cornell University
- University of Minnesota
- Washington State University
- University of California–Riverside
- North Carolina State University
- Virginia Polytechnic Institute
- University of California–Los Angeles

- Oak Ridge National Laboratory (ORNL)
- University of Georgia
- University of Tennessee
- Dartmouth College
- West Virginia University
- Georgia Institute of Technology
- ArborGen, LLC
- Ceres, Incorporated
- Mascoma Corporation

322 personnel across 19 U.S. Institutions

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Technical Focus: Enhanced Access to Sugars in Lignocellulosic Biomass

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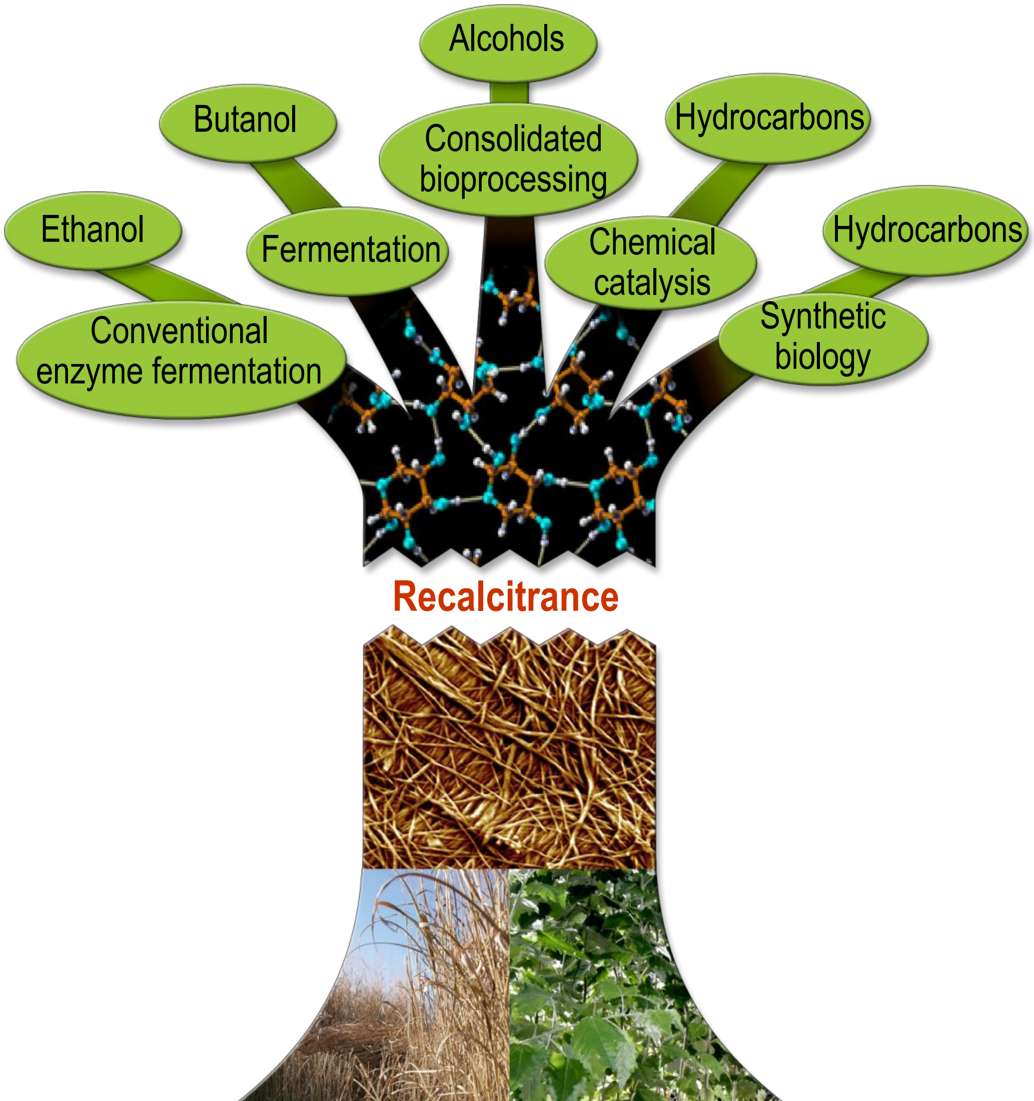

- Key Mission: Deliver science breakthroughs that impact the biofuels industry.
- Our focus is on understanding and overcoming recalcitrance in plants.
- This will lead to not only fuels but also new co-products.

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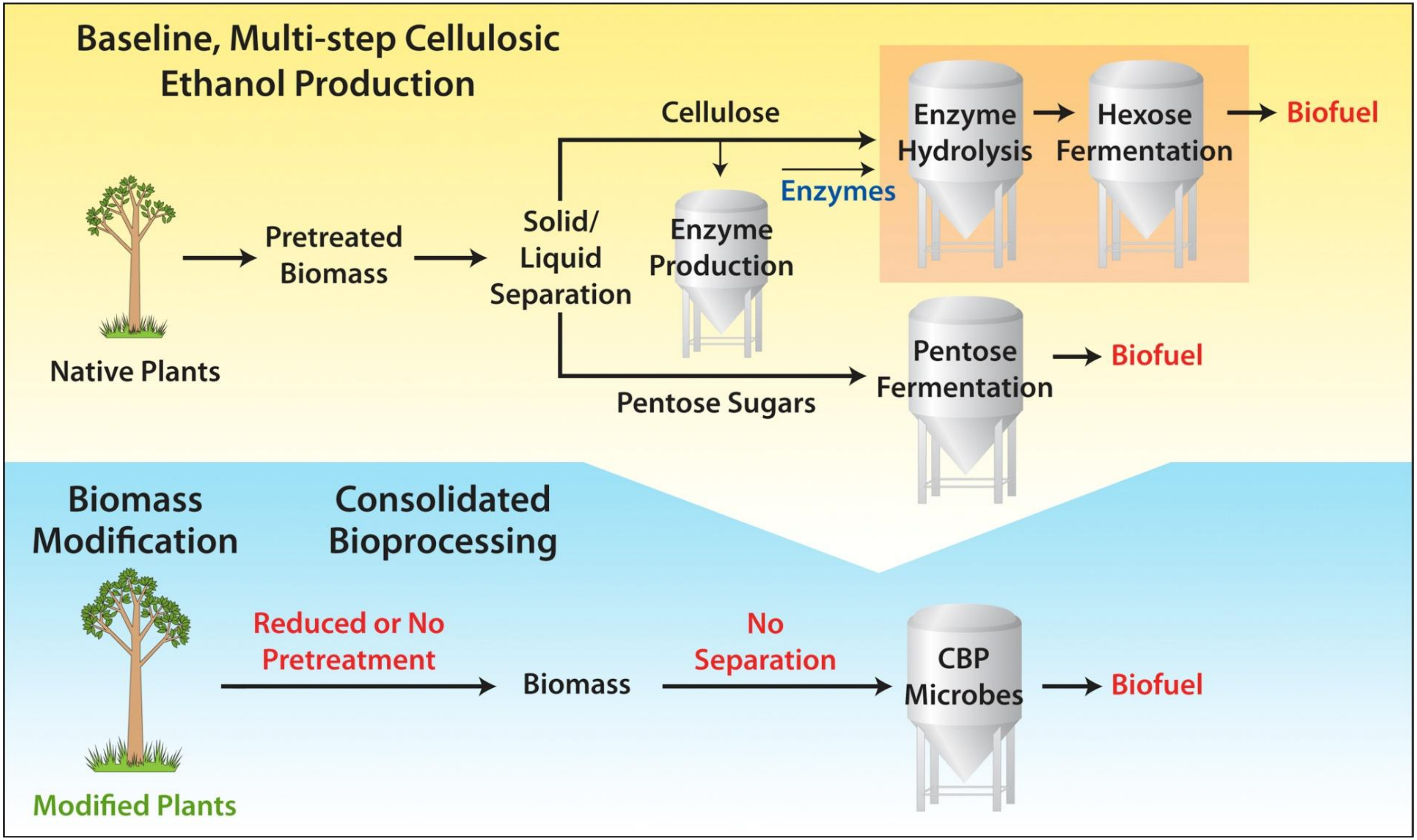
BESC Seeks to Revolutionize How Biomass is Processed



Source: Fu C and Wang Z-Y (Noble)

Dramatic Improvement achieved in Switchgrass Transformation Efficiency

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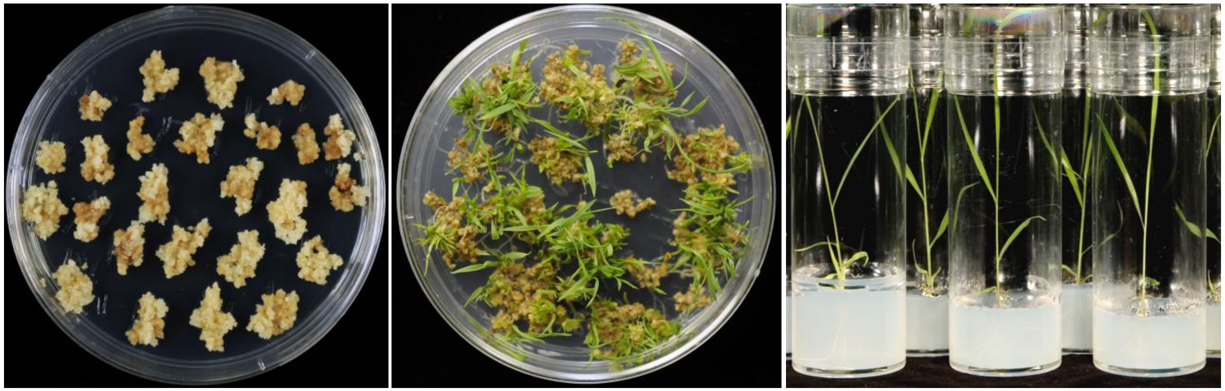

- Achieved a transformation efficiency of more than 90% (previously less than 5 %) with high reproducibility.

- On the basis of a transformability screen on ten thousand Alamo seeds plated.

- Timeline to produce in vitro rooted plants reduced to 4 months from 5 months.

- A large number of plants (>400) have been successfully produced in the last 4 months.

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Genetic block in lignin biosynthesis in switchgrass increases biofuel yields

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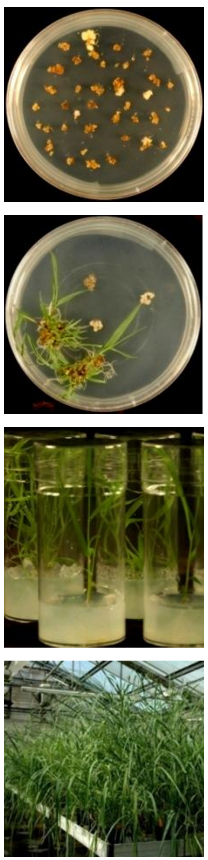
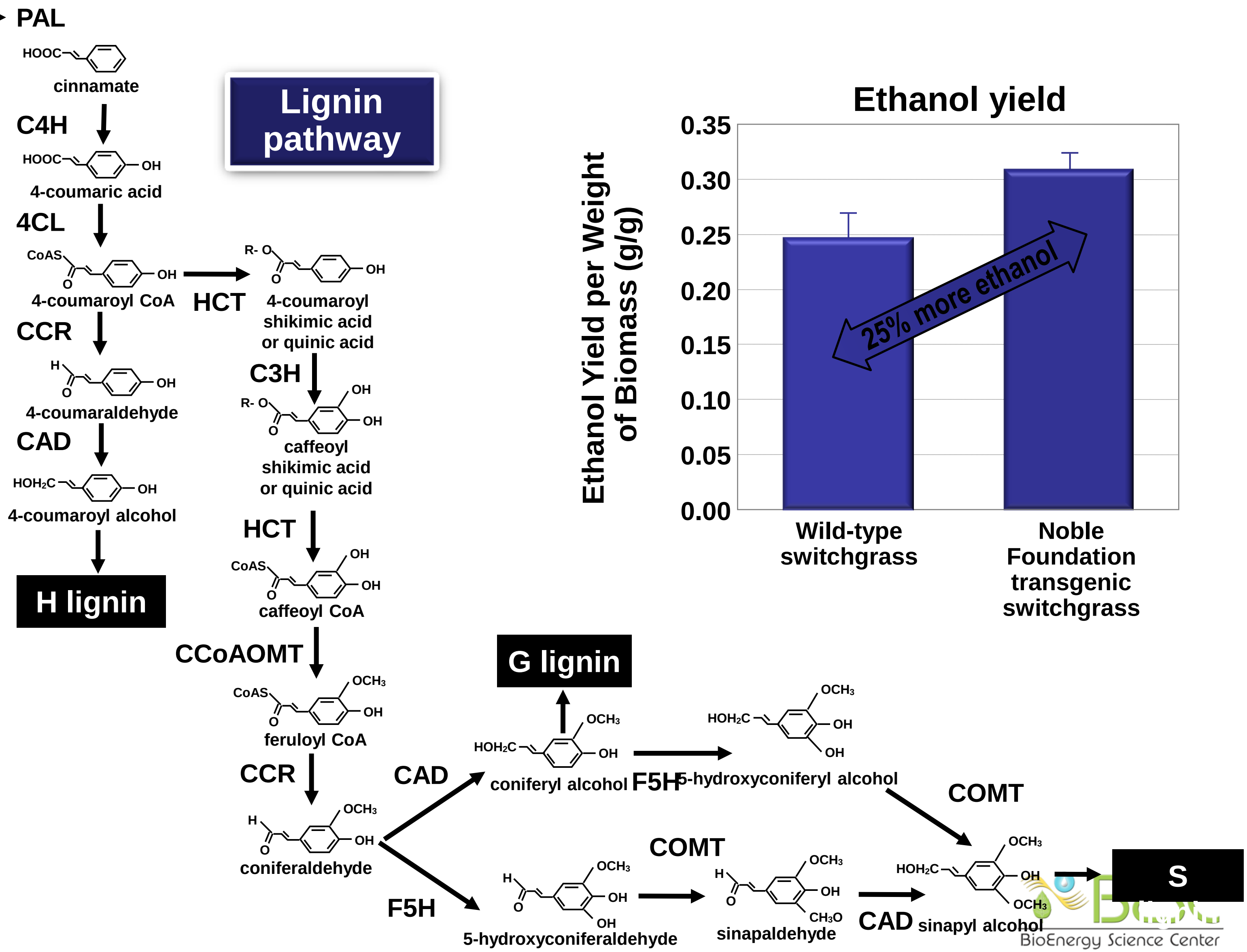
Agrobacterium-mediated transformation of switchgrass





THE SAMUEL ROBERTS NOBLE FOUNDATION

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Citation: Fu et al. Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass. February 14, 2011, doi: 10.1073/pnas.1100310108

Mutation of Key TF Increases Pith Cell Wall Thickness

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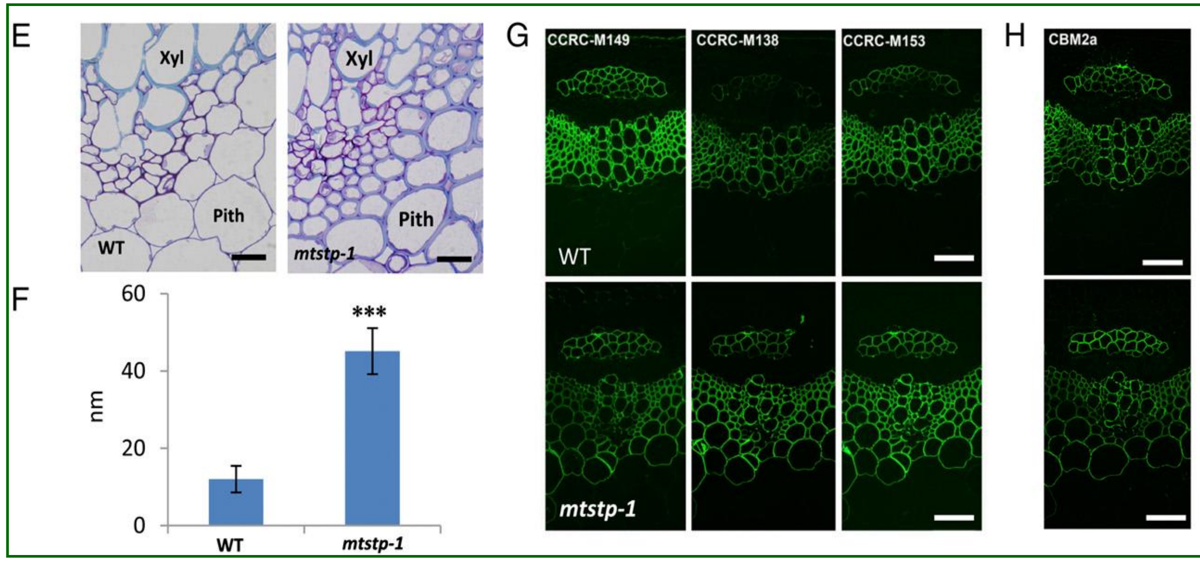
- Mutants with secondary cell wall thickening in pith cells leading to an ~50% increase in biomass density in stem tissue of the *Arabidopsis* mutants.
- Repression of TFs that activate secondary wall synthesis were confirmed by in vitro assays and in plant transgenic experiments.



Phenotypic analysis of the Mtstp-1 mutant in *Medicago*.
(E) Light microscopy of pith cell walls in WT and mutant.
(F) Quantification of cell wall thickness of the WTand mutant sections.
(G and H) Detection of xylan and cellulose by immunohistochemistry using monoclonal antibodies against distinct xylan epitopes (G) and a carbohydrate-binding module that binds crystalline cellulose (H) in stem sections. Antibody and CBM names are indicated. (Scale bar: E, 20µm;G and H, 10µm.)

- **The discovery of negative regulators of secondary wall formation in pith opens up the possibility of significantly increasing the mass of fermentable cell wall components in bioenergy crops.**

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The University of Georgia

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Mutation of WRKY transcription factors initiates pith secondary wall formation and increases stem biomass in dicotyledonous plants
H Wang, U Avcib, J Nakashimaa, MG Hahn, F Chena, and RA Dixon (Noble, CCRC-UGa); PNAS (2010)

Mining variation to identify key genes in biomass composition and sugar release

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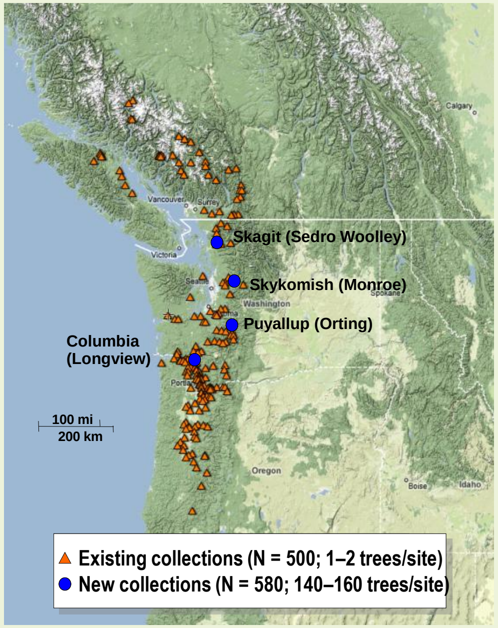
Collected ~1300 samples for *Populus* association and activation-tag study

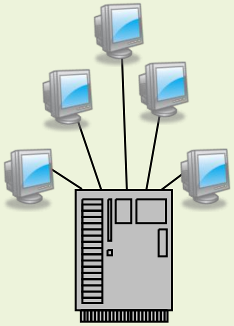


High-throughput screening pipeline

- Create genetic marker map to identify allelic variation
- Identify marker trait association

Sugar release assay

Cell wall biosynthesis database



Establish common gardens for association and activation-tag populations with thousands of plants

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High-Throughput Characterization Pipeline for the Recalcitrance Phenotype

Screening thousands of samples

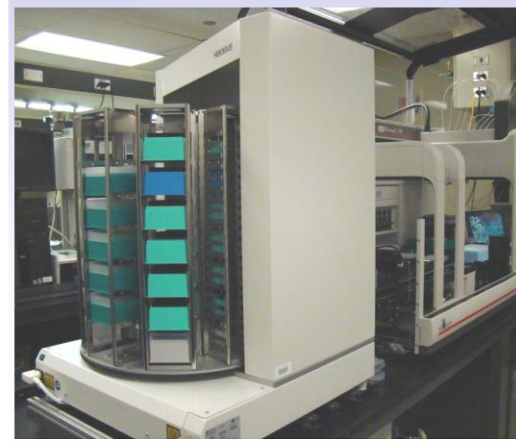
Composition analytical pyrolysis, IR, confirmed by wet chemistry

→

Pre-treatment new method with dilute acid and steam

→

Enzyme digestibility sugar release with enzyme cocktail



Detailed chemical and structural analyses of specific samples

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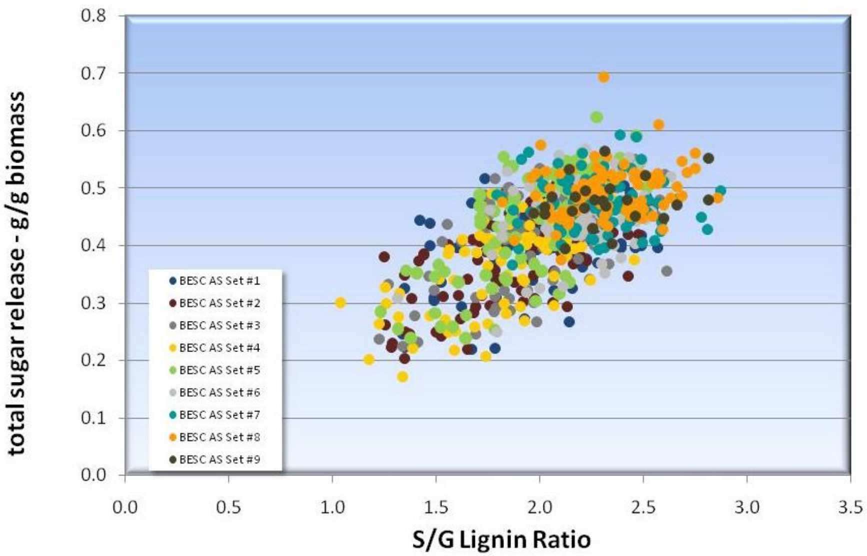
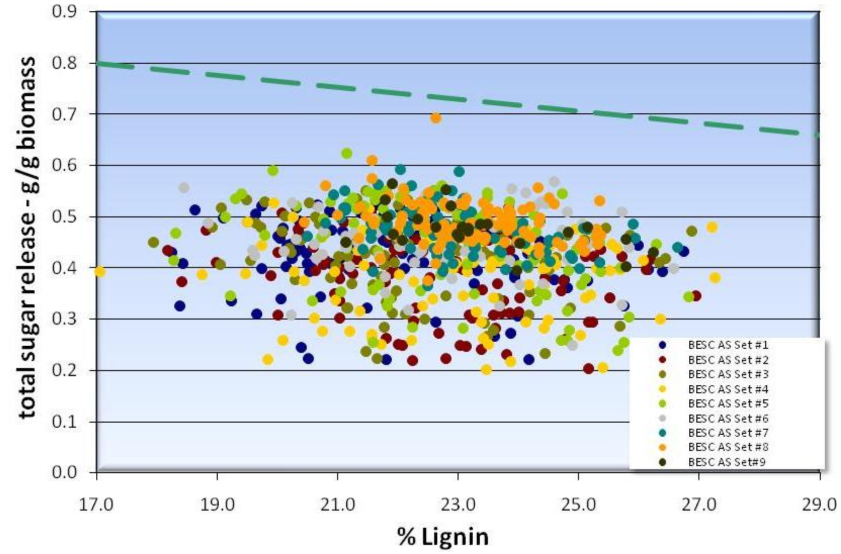
Source: NREL

High-throughput screening to analyze natural *Populus* trees

- Screening of 1200 natural *Populus* trees
- Hot water as pretreatment only
- Sugar release varies from 25% to >90% of theoretical value





Environmental vs genetic?

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Screening *Populus* Natural Variation

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BESC Reduced Recalcitrancs Bioenergy Feedstock

Confirmed in Common Gardens

Identified 19 genes that control reduced recalcitrance in *Populus*

Identified 6 individual genotypes that yield 85% of their sugar with no pretreatment

August 12, 2011 last update

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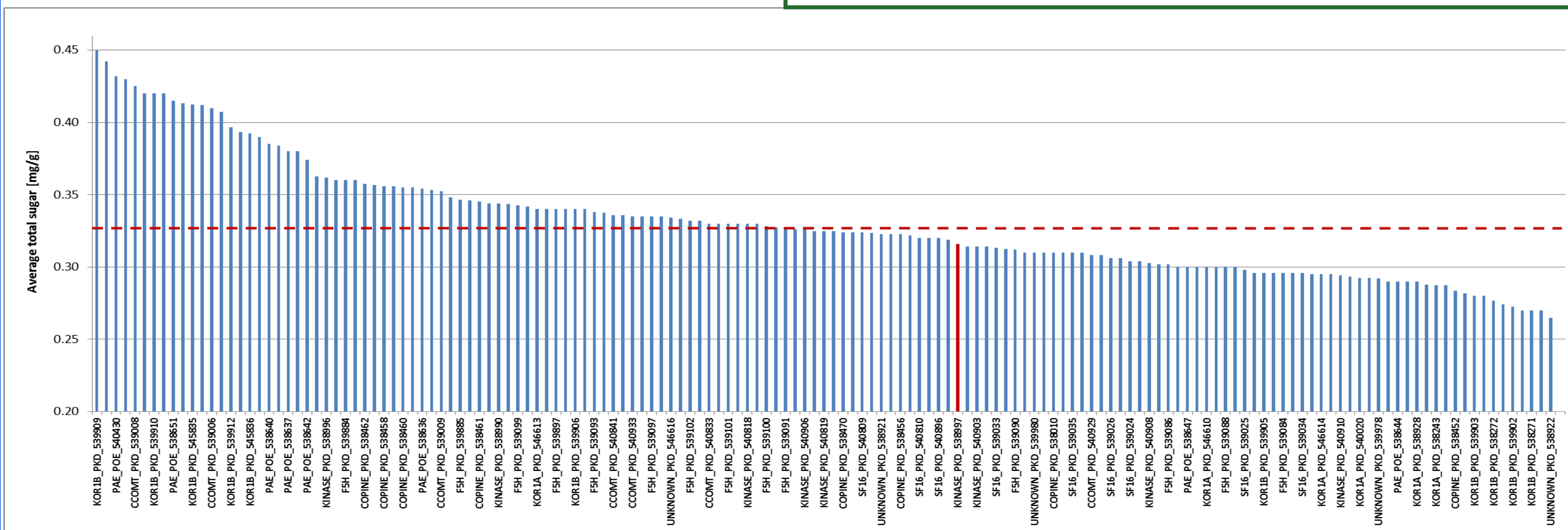
Genetically improved *Populus* feedstocks show increased sugar release

- Over 300 different *Populus* cell wall transgenics created.
- Several show a high sugar release, low recalcitrance, normal growth phenotype.

Identified 16 genetically modified, reduced recalcitrance lines in *Populus* / switchgrass which yield improved amounts of fermentable sugars



Source: ORNL Populus group

Characterization tools for feedstock and microbial samples

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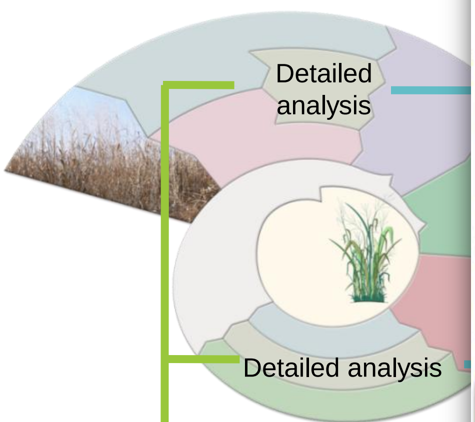
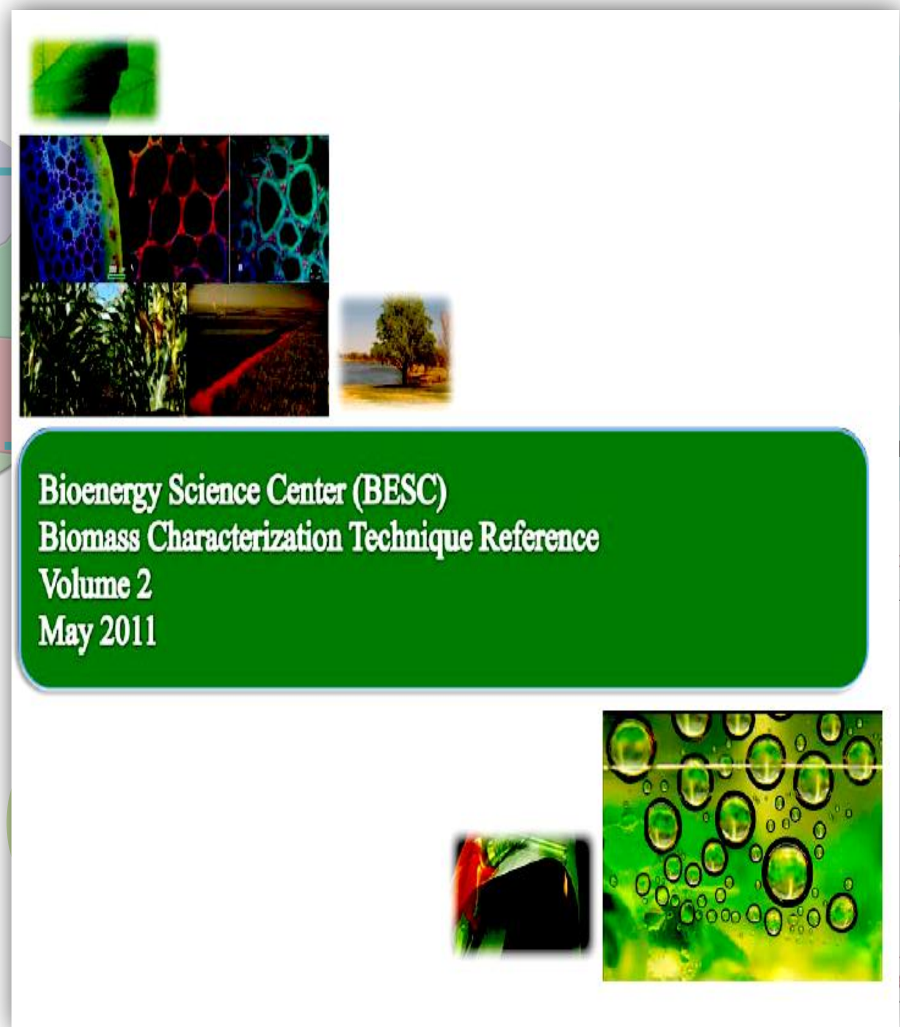


Chemistry


Bioenergy Science Center (BESC)
Biomass Characterization Technique Reference
Volume 2
May 2011

AFM of switchgrass showing cellulose microfibrils

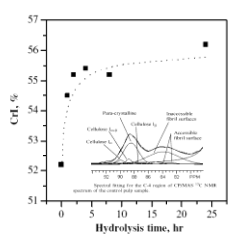
NREL National Renewable Energy Laboratory

Immunolocalization using wall antibodies on switchgrass

The University of Georgia

OAK RIDGE National Laboratory

The University of Georgia



NMR for cellulose crystallinity

Georgia Tech | Institute of Paper Science and Technology

lignin in biomass

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Field testing of improved feedstocks

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Over 40 *Populus* constructs in stoolbeds in South Carolina. (Arborgen)

Over 1000 different *Populus* genotypes growing in 4 common gardens, Pacific Northwest (ORNL)

Field assessment of genetically improved switchgrass in Texas (Ceres)

Field assessment of genetically improved switchgrass in Tennessee (UT)

Genetic tools for *C. thermocellum*

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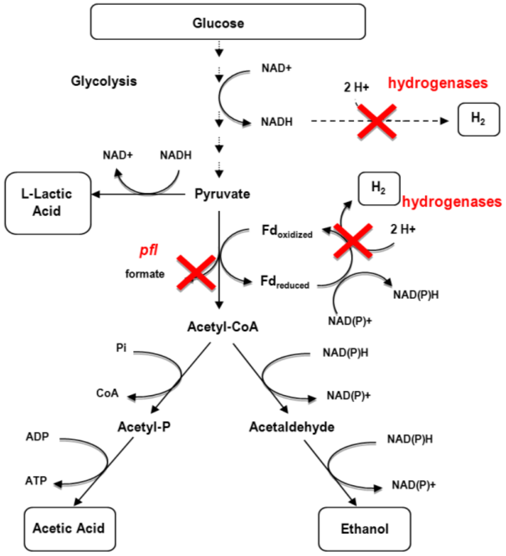
- BESC has developed and applied genetic tools for *Clostridium thermocellum* (a key CBP microbe) for economical production of biofuels from cellulosic feedstocks.
- BESC has discovered ways to overcome key inhibitors of microbial fermentation efficiency, e.g. microbial strain improvements that lead to enhanced ethanol or acetate tolerance.
- These discoveries are significant as end-product titer and inhibitory byproducts are important contributors to capital and downstream processing costs.

Gene	Locus	Description
celS	Cthe2089	Cellulosomal GH48
celY	Cthe0071	Non-Cellulosomal GH48
cipA	Cthe3077	Cellulosomal scaffoldin
cipADdocII	Cthe3077	Domain that attaches CipA to cell surface
ech	Cthe3019-3024	Ech hydrogenase
hfs	Cthe0425-0428	Hfs hydrogenase
ldh	Cthe1053	Lactate dehydrogenase
Gene D01	CtheD01	Central metabolism gene
pta	Cthe1029	Phosphotransacetylase
rnf	Cthe2430-2435	Ferredoxin oxidoreductase
spo0A	Cthe0812	Sporulation initiation factor
Gene D02	CtheD02	Central metabolism gene
Gene D03	CtheD03	Central metabolism gene
adhE	Cthe0423	Bi-functional aldehyde/alcohol dehydrogenase
pyrF	Cthe0951	orotidine 5'-phosphate decarboxylase
hpt	Cthe2254	hypoxanthine phosphoribosyltransferase
cat	From pNW33N	Chloramphenicol acetyltransferase
kan	From pIKM1	Kanamycin resistance gene
neo	From pUB110	Kanamycin resistance gene
tdk	From *T. saccharolyticum*	Thymidine kinase
Gene M01	Thermophilic anaerobe	Central metabolism gene
Gene M02	Thermophilic anaerobe	Central metabolism gene
Gene M03	Thermophilic anaerobe	Central metabolism gene



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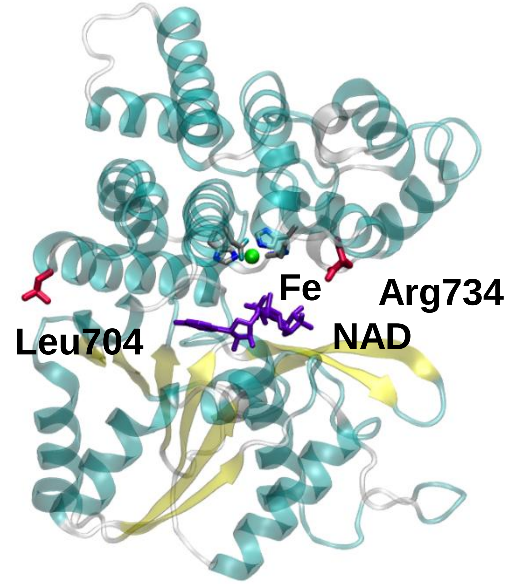
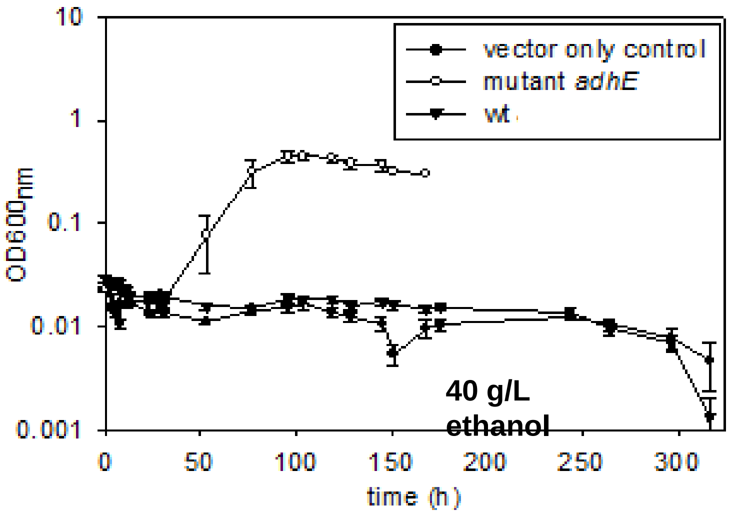
Source: Guss et al. , Dartmouth, ORNL.

Single microbial gene is linked to increased ethanol tolerance

- Ethanol intolerance is an important metric in terms of lignocellulosic biofuels process economics.
- Tolerance has often been described as a complex and likely multigenic trait for which complex gene interactions come into play.
- A mutated alcohol dehydrogenase (AdhE) with altered co-factor specificity was shown to enhance ethanol tolerance in *Clostridium thermocellum*, a candidate consolidated bioprocessing microbe.
- The simplicity of the genetic basis for this ethanol-tolerant phenotype informs rational engineering of mutant microbial strains for cellulosic ethanol production.

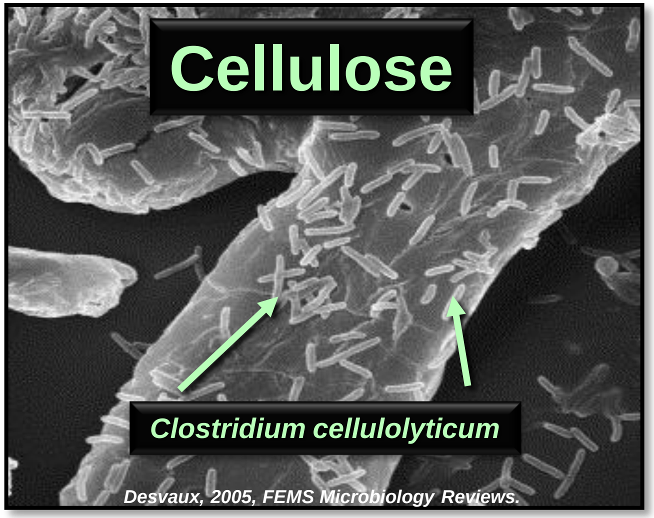




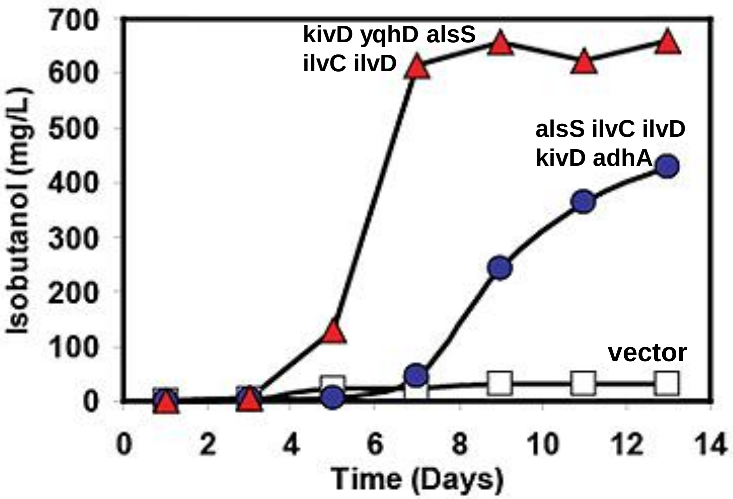
Citation: Brown S. D. et al. Mutant alcohol dehydrogenase leads to improved ethanol tolerance in *Clostridium thermocellum.* PNAS. 2011, *Accepted.* The invention is available for licensing: U.S. 61/346,660

Microbe engineered to produce isobutanol directly from cellulose

- BESC researchers engineered a native cellulose-degrading microbe, *Clostridium cellulolyticum,* to produce isobutanol.
- Demonstrating the ability to combine CBP (consolidated bioprocessing) with production of next generation biofuels.



C. Cellulolyticum **growing on cellulose substrate**



Isobutanol production on cellulose:
Isobutanol pathway mutants vs. Vector control

Citation: Higashide (UCLA), Li (ORNL), Yang (ORNL), & Liao (UCLA) Applied Environmental Microbiology (2011) Apr;77(8):2727-33.

BESC will revolutionize how biomass is processed and converted

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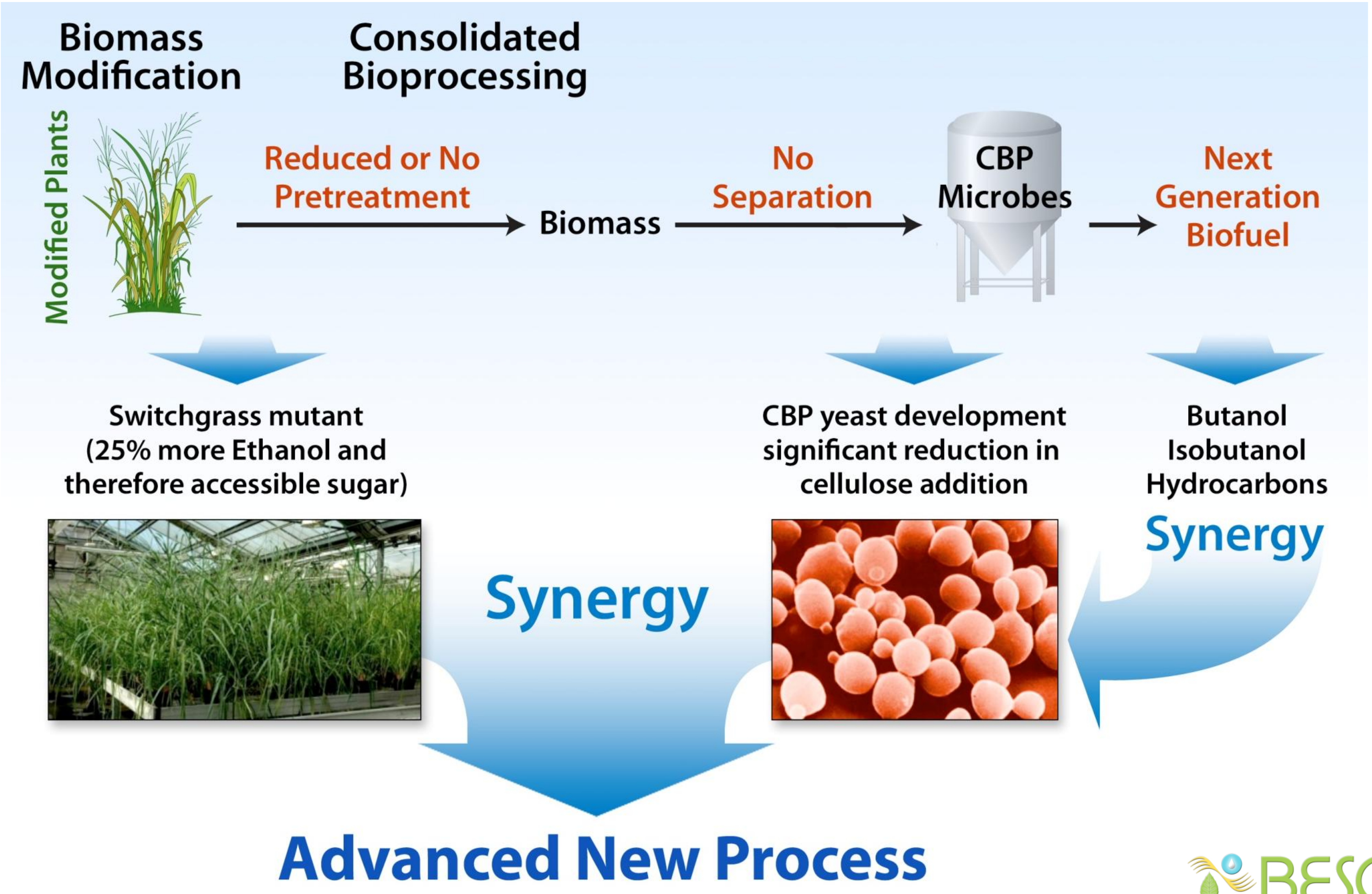

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BESC Impact: Fundamental breakthroughs critical to making biofuels a viable energy option

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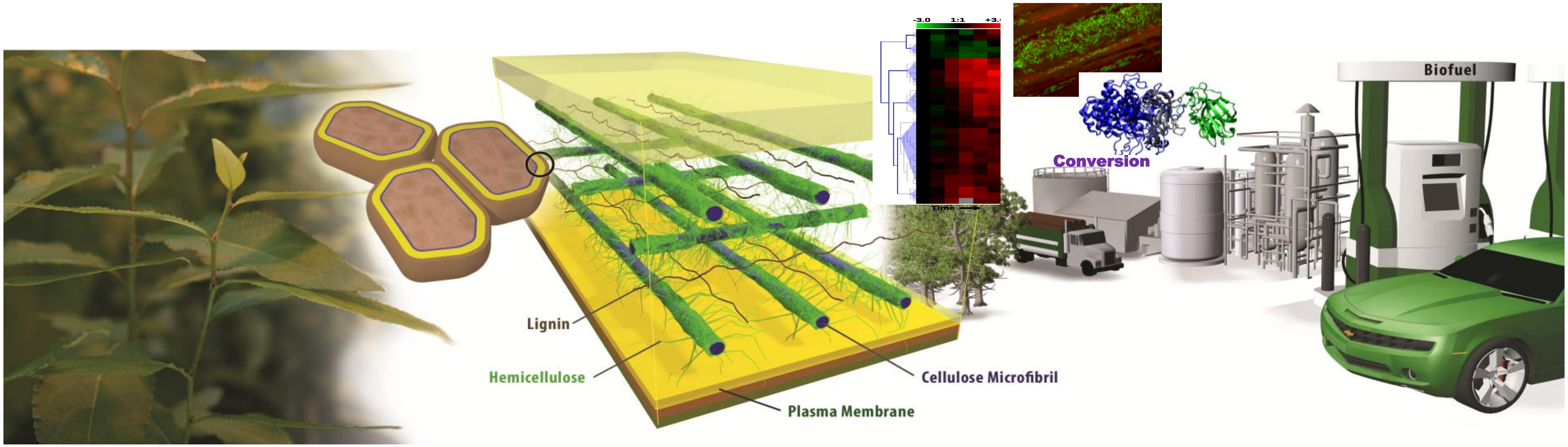

Source: Yunqiao Pu Y, Kosa M, Kalluri U. C., Tuskan G. A. and Ragauskas A. J. "Challenges of the utilization of wood polymers: how can they be overcome?", Appl. Microbiol and Biotech. Online 28 July 2011.

- Generated a genetically improved switchgrass that yields 30% increased biofuel and requires 3- to 4-fold less enzyme for processing.
- Identified a panel of natural variants of *Populus* that release 85% of sugar with minimal or no pretreatment.
- Developed genetic tools and discovered ways to overcome key inhibitors of microbial fermentation efficiency, e.g. ethanol or acetate tolerance.
- Demonstrated the production of isobutanol directly from cellulose.
- Deployed a first-of–a-kind high-throughput platform for determining recalcitrance properties of tens of thousands of feedstocks samples.

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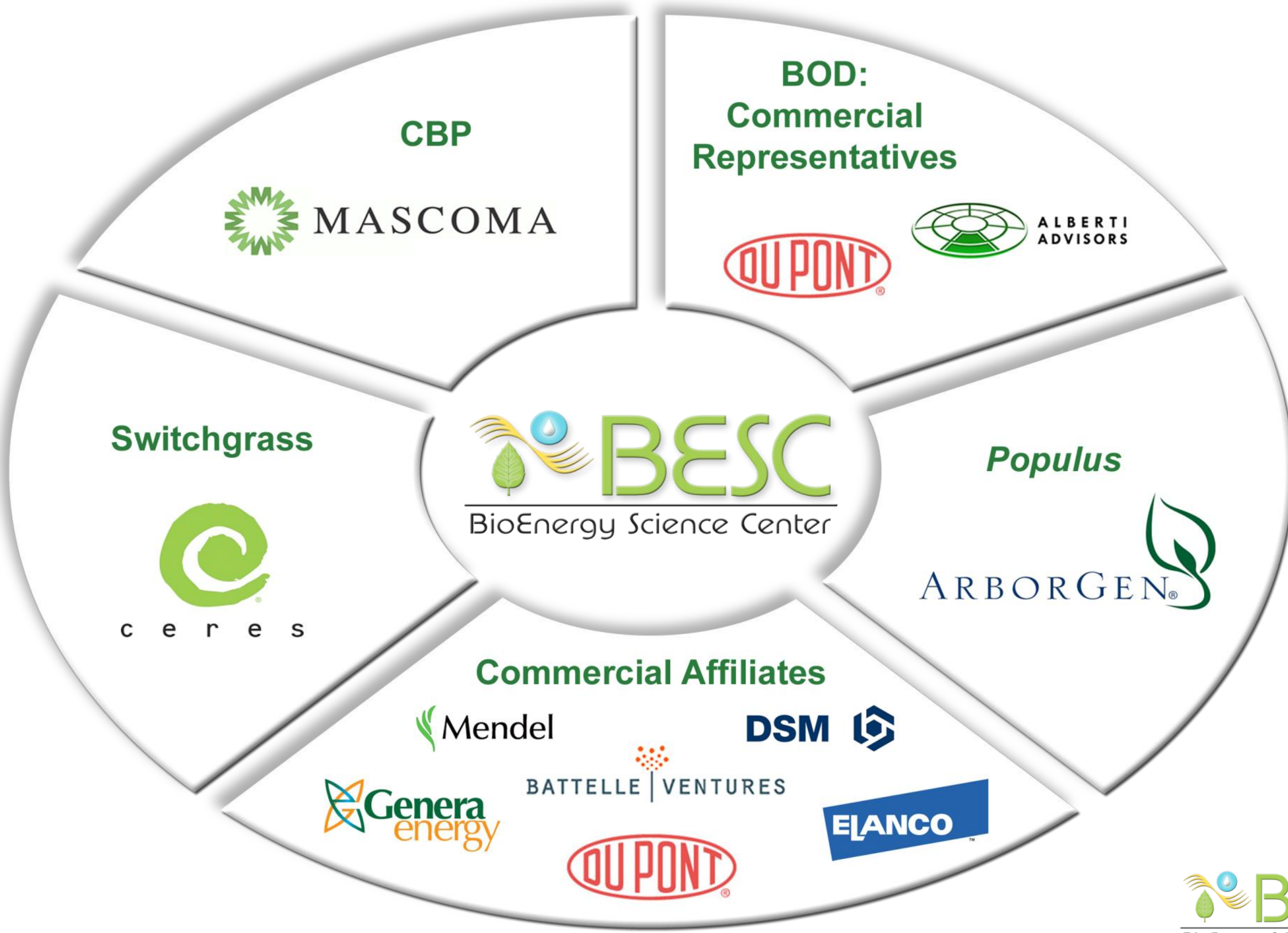
Industrial partners facilitate strategic commercialization



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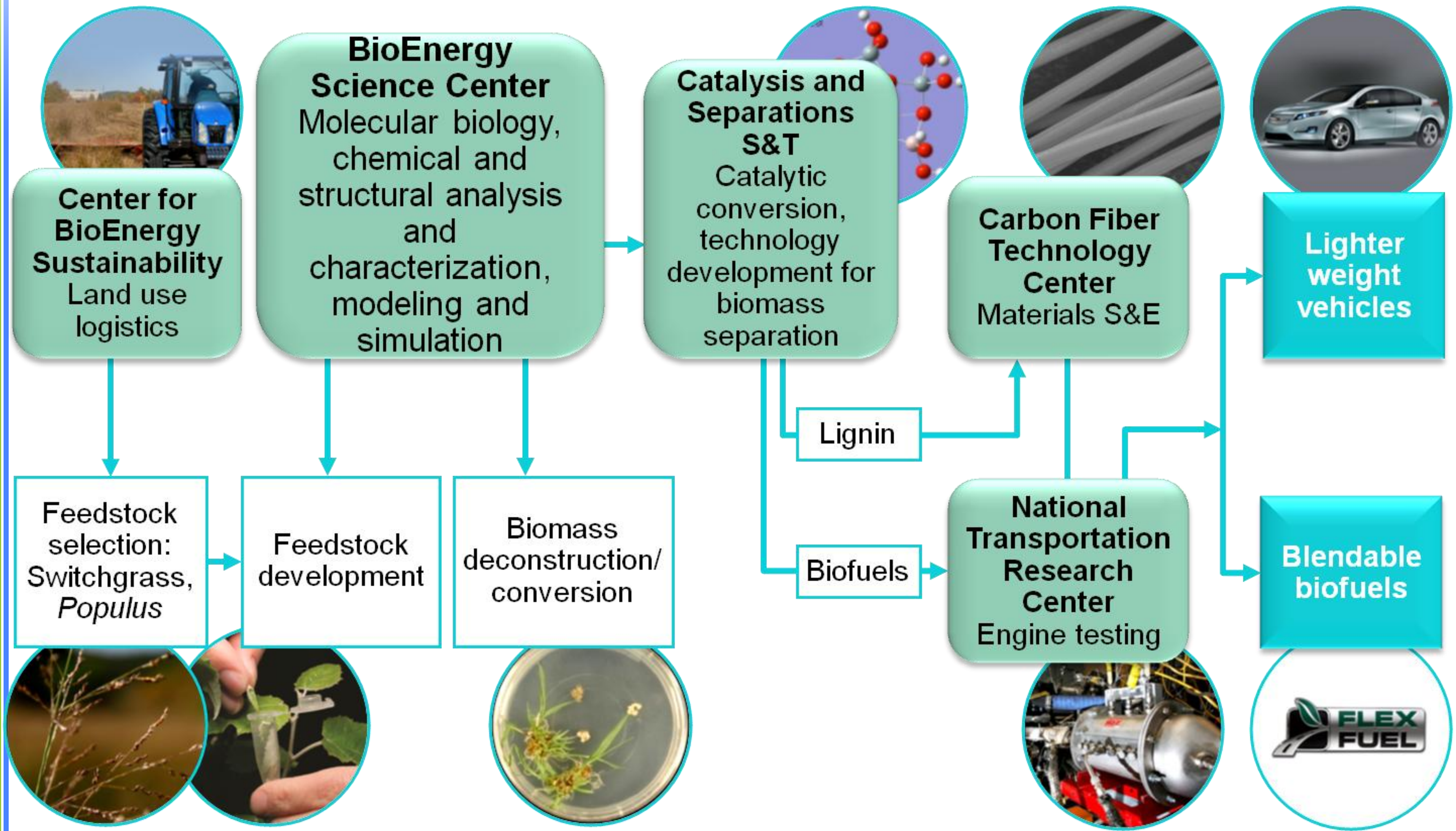
Bioscience and biotechnology for sustainable mobility



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Converting ethanol to hydrocarbons

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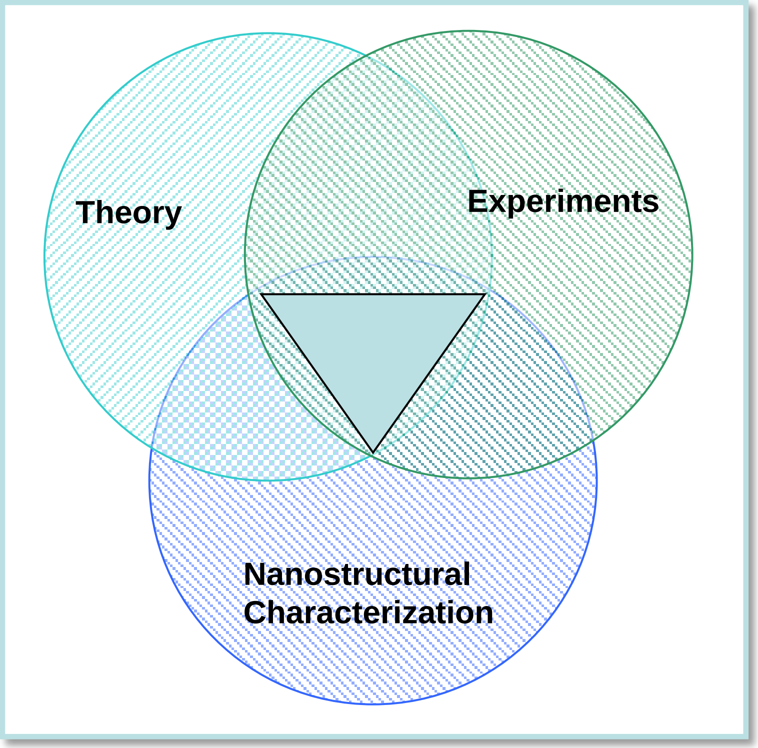
- Methanol Gasification plants operated in New Zealand in 1979
- Dow is planning Ethanol to Ethylene plant in Brazil
- Catalytic ethanol gasification



Approach combines theory and experimental studies to speed up the discovery process and overcome deficiencies of "trial and error" approach

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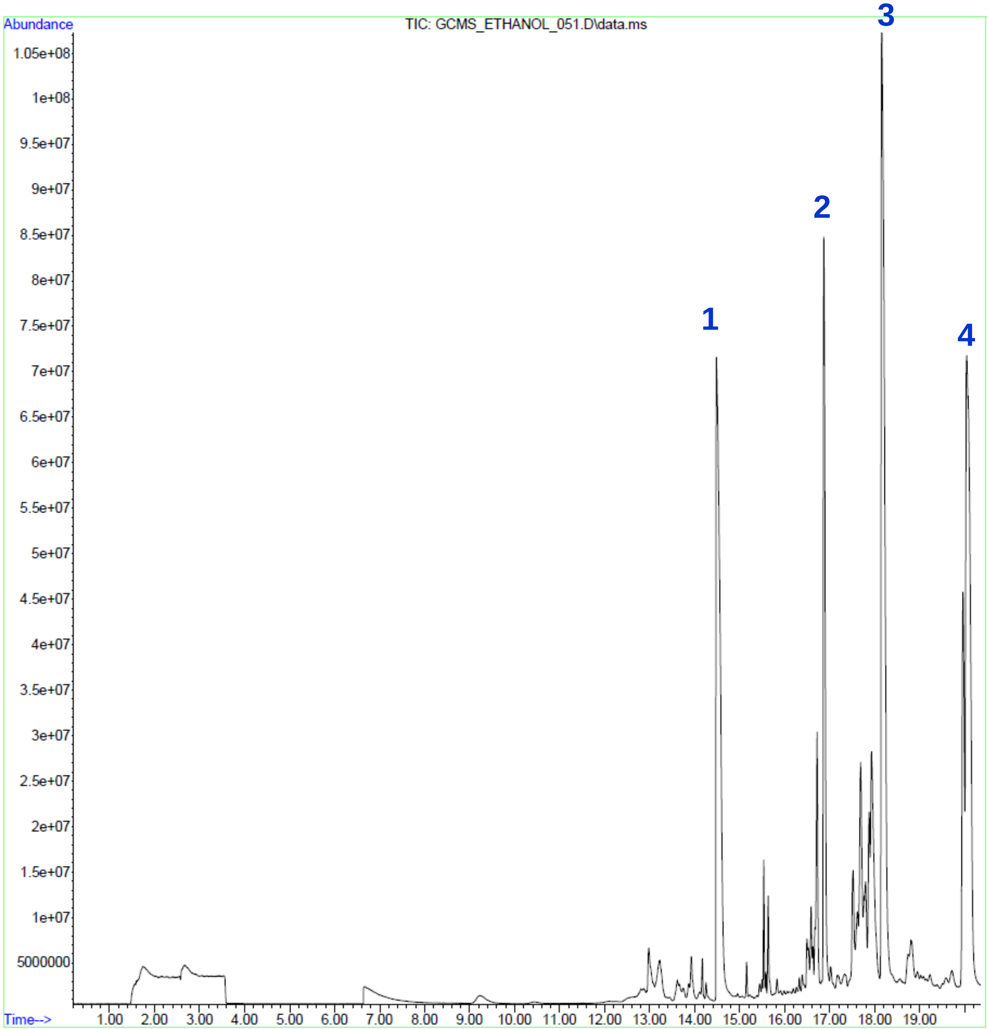
Ethanol over Catalyst



- Temperature 400ºC
- Products
  - Ethylene
  - Water
  - Propene
  - Propane
  - Cis-Bicyclo[4.2.0]octa-3,7-diene
  - 1-Propene-2-methyl
  - 2-Butene
  - Ethanol [1]
  - Ethyl ether
  - Cyclobutane
  - Benzene [2]
  - Benzene -1-ethyl-3-methyl
  - Benzene-1-ethyl-4-methyl
  - Toluene [3]
  - Ethylbenzene
  - m-Xylene [4]

Ethanol (5%+95% water v/v) over Catalyst

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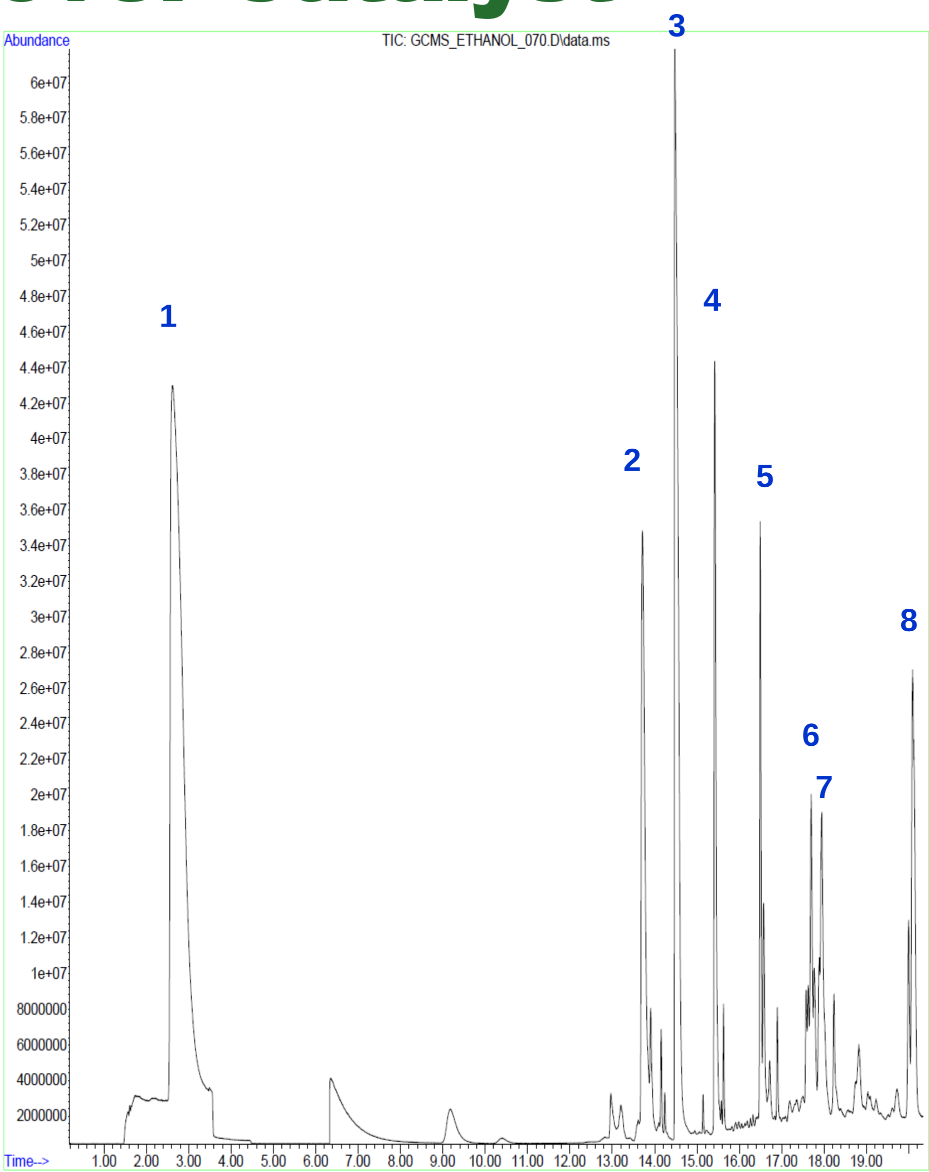

- Temperature 400C
- Products
  - Ethylene [1]
  - Water
  - Propane
  - Propene
  - Acetaldehyde
  - Isobutane [2]
  - 1-Propane-2-methyl
  - 2-Pentene
  - Ethanol [3]
  - Butane-2-methyl [4]
  - Pentane-2-methyl [5]
  - Pentane-3-methyl
  - Cyclopentane, methyl
  - Benzene
  - Hexane-2methyl
  - Hexane-3-methyl
  - Benzene-1-ethyl-3methyl [6]
  - Benzene-1-ethyl-4methyl [7]
  - Toluene
  - Ethylbenzene and m-xylene [8]

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ORNL Research Directed Toward Production of Multiple Value-added Streams from Biomass Feedstock

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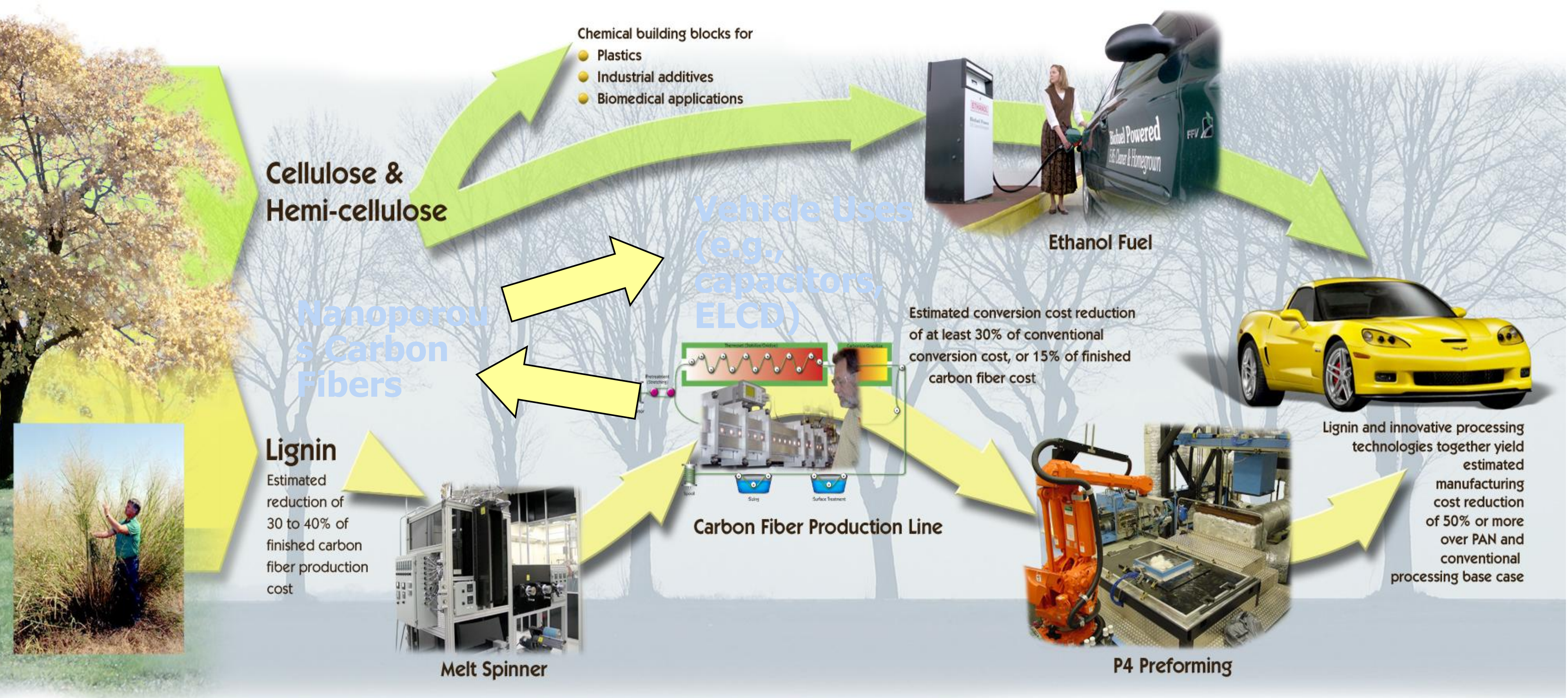

DOE Office of Vehicle Technologies Lightweight Materials Program

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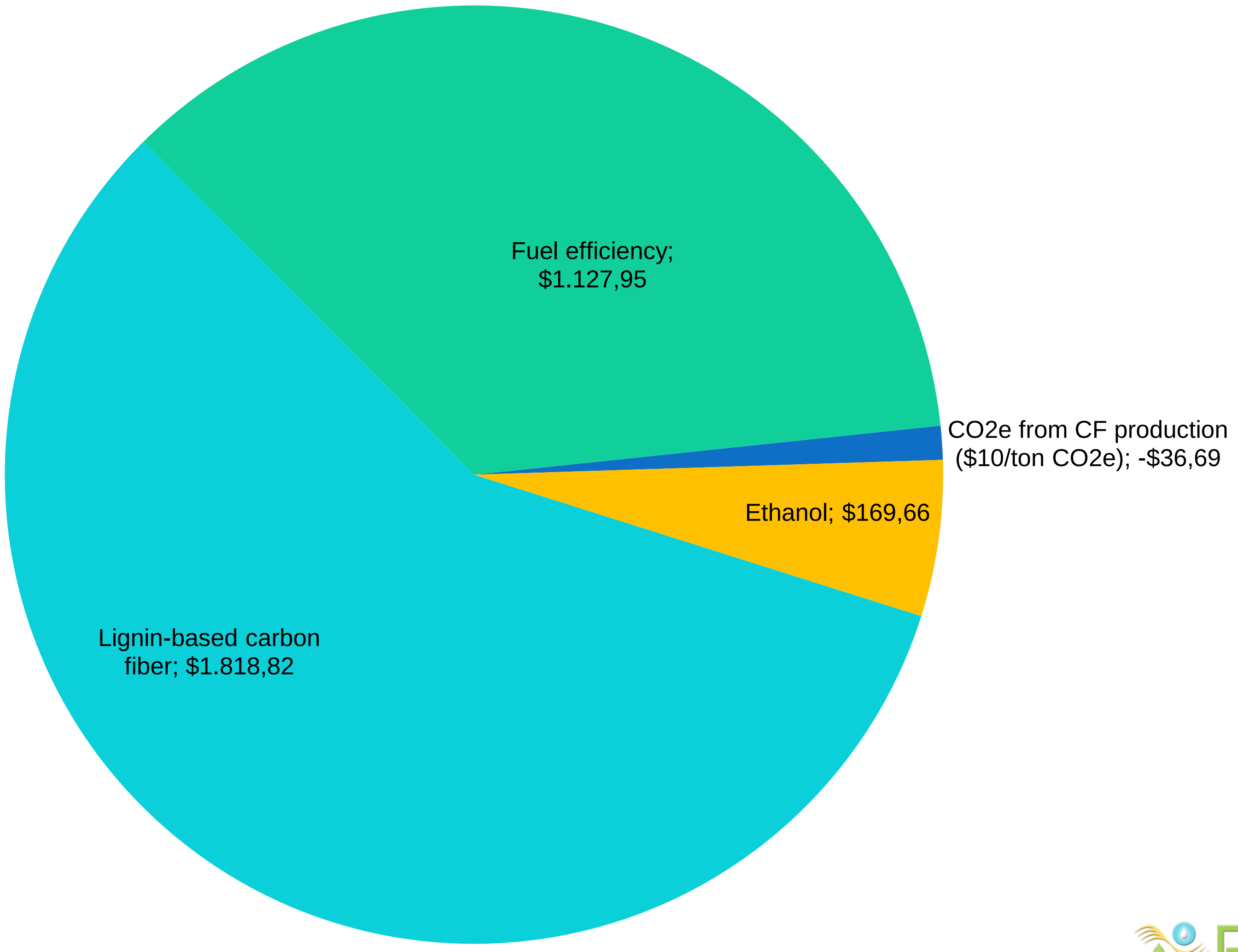
Integrated Biomass Strategy



Estimated production cost of lignin-based carbon fiber (Kline economic model – $ per lb: study conducted by DOE)

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Lignin → **Precursor Spinning** → **Oxidation 2-4 Stages $0.99**

$0.50

Note: The Kline economic model currently does not take into account:
- Purification of lignin has been eliminated (potential for lignin cost < 50¢/lb)
- Each 1¢ reduction in lignin price reduces carbon fiber cost by 2¢/lb
- Precursor fiber spinning speed currently almost 3-times Kline baseline

Note: The Kline economic model currently assumes stabilization of lignin fiber will be comparable to conventional stabilization of PAN fiber:
- Stabilization time reduced to minutes
- May be possible to eliminate conventional stabilization step entirely

Carbonization 1-2 Stages $0.71 → **Graphitization 1-2 Stages $0.77** → **Surface Treatment $0.33** → **Spooling $0.41**

Total $3.71/lb → **Updated for latest findings, cost = < $3/lb**

Note: The Kline economic model currently assumes a carbon fiber yield of 35%. In fact, carbon fiber yield has been increased to almost 55% using more appropriate lignin chemistry and processing conditions.

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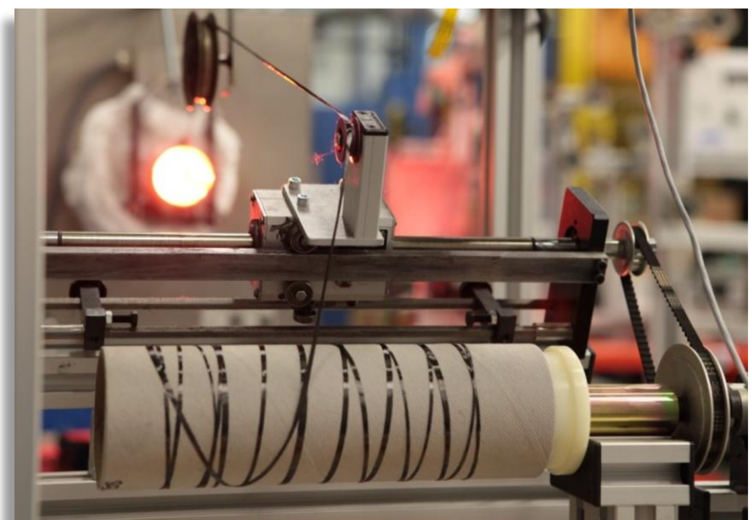
ORNL Carbon Fiber Processing Equipment)

Multi-pass oxidation oven

Oxidized fiber entering carbonization furnace



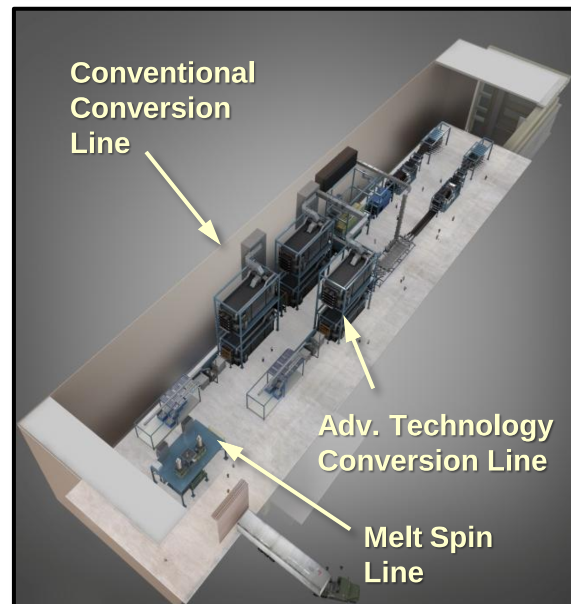
Finished carbon fiber being spooled

Pilot-scale, multiple-tow carbon fiber conversion line (≈ 20 lb/day)

Carbon Fiber Technology Center (≈ $50 million)

- North America's most comprehensive carbon fiber material and process development capabilities
- Development and demonstration of carbon fiber technology for energy and national security applications
- Low-cost and high-performance fibers
- Fast, energy efficient processing
- Capability to evaluate micrograms of candidate materials and produce up to 25 tonnes/year of carbon fibers
- Produce fibers for large-scale material and process evaluations by composite manufacturers
- Train and educate workers
- Grow partnerships with US industry

Facility and equipment



Thank you

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