

Biocombustibles de segunda generación a partir de nuevas materias primas / Second generation biofuels from new feedstocks

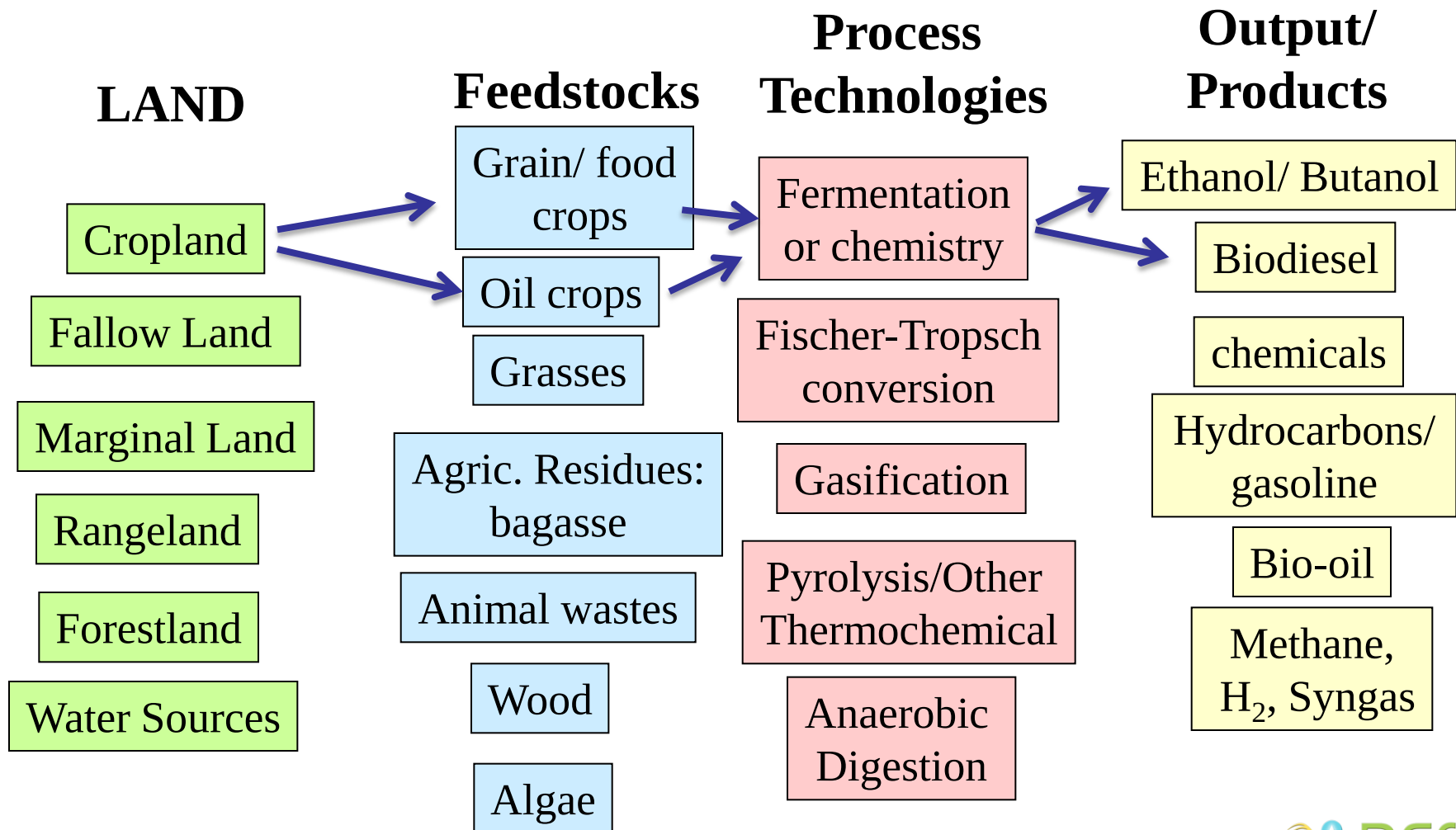
**Simposio de Bioeconomía en América Tropical
9 y 10 de octubre del 2013
San Jose, Costa Rica**

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White Cliff Biosystems, and
BioEnergy Science Center (BESC)
Oak Ridge National Laboratory
Tennessee, USA

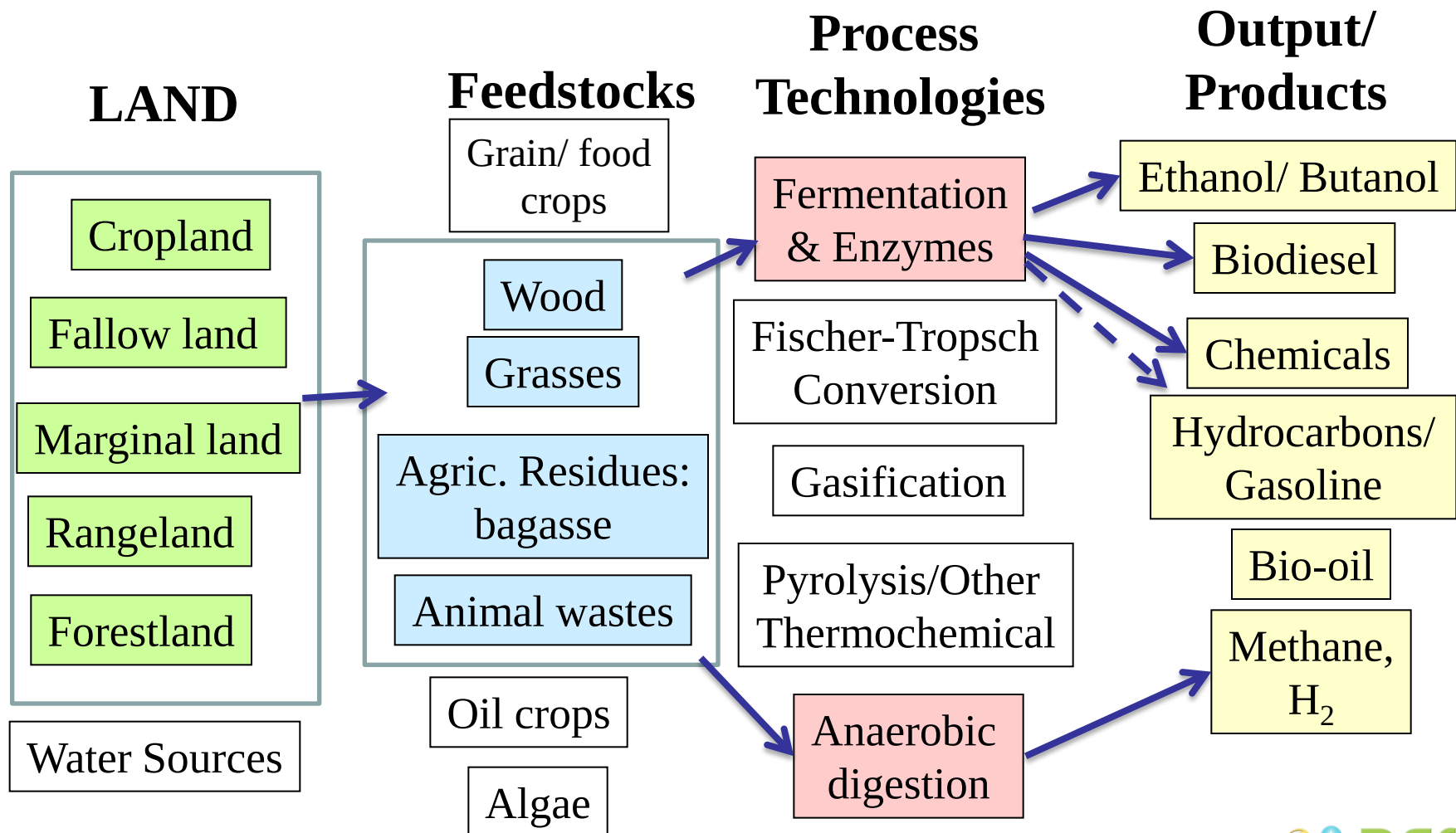
“Definitions” in biofuel deployment

- 1st generation (full commercial exists)
 - Sucrose and starch to ethanol
 - Oil-seeds into biodiesel
- 2nd generation (in deployment)
 - Cellulosics into ethanol (& butanol)
 - Cellulosics to thermochemical biocrude
- 3rd generation and beyond (technology development)
 - Whole biomass to advanced biocrude/ and bio-oil
 - Sucrose and starch fermentation into advanced biofuels (petroleum compatible ie: (iso)butanol)
 - Cellulosic fermentation to advanced biofuels
 - Algal oils into biodiesel

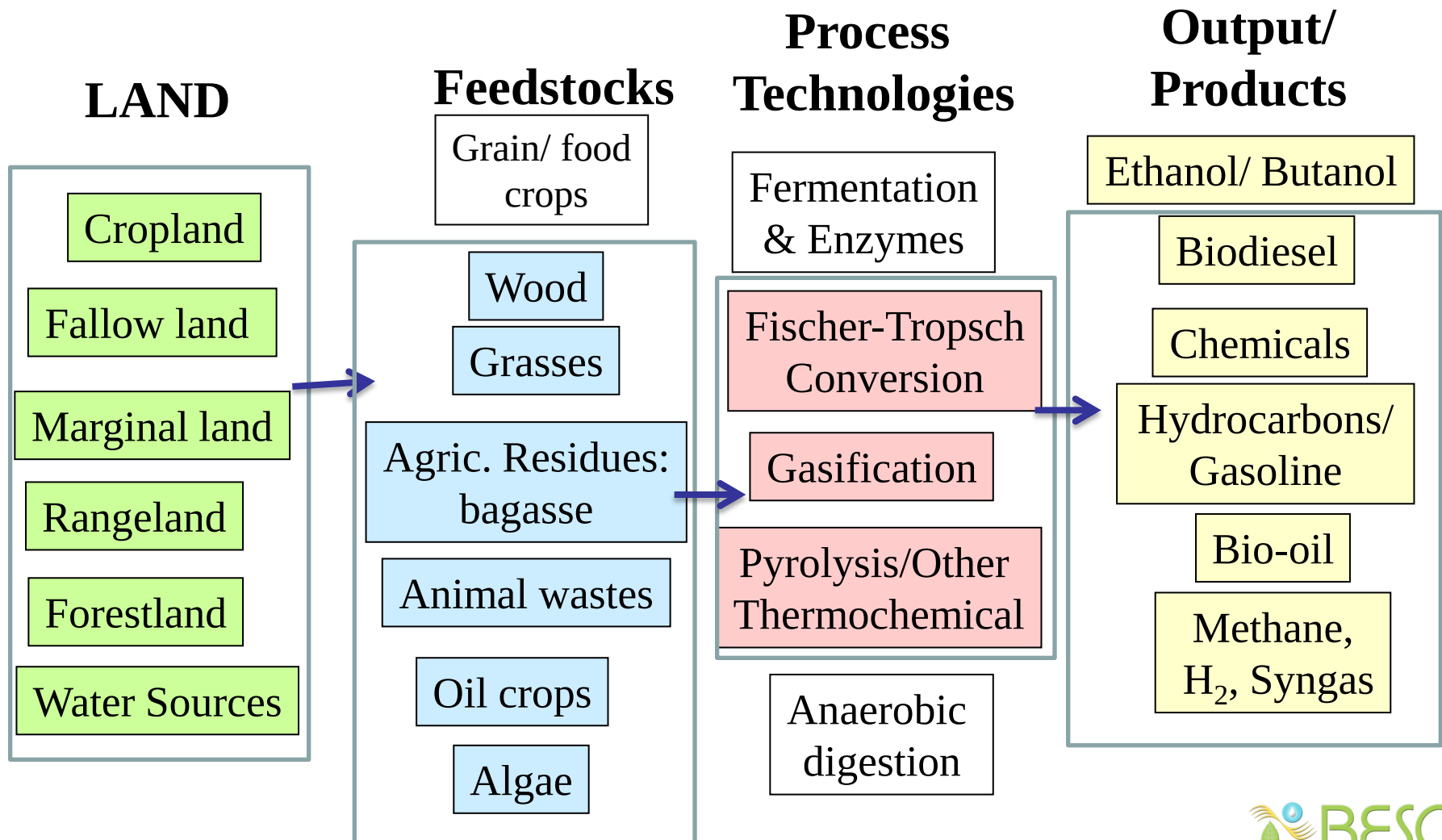
First Generation Biofuels



2nd (&3rd) Generation Biofuels: Biochemical



2nd (&3rd) Generation Biofuels: Thermochemical

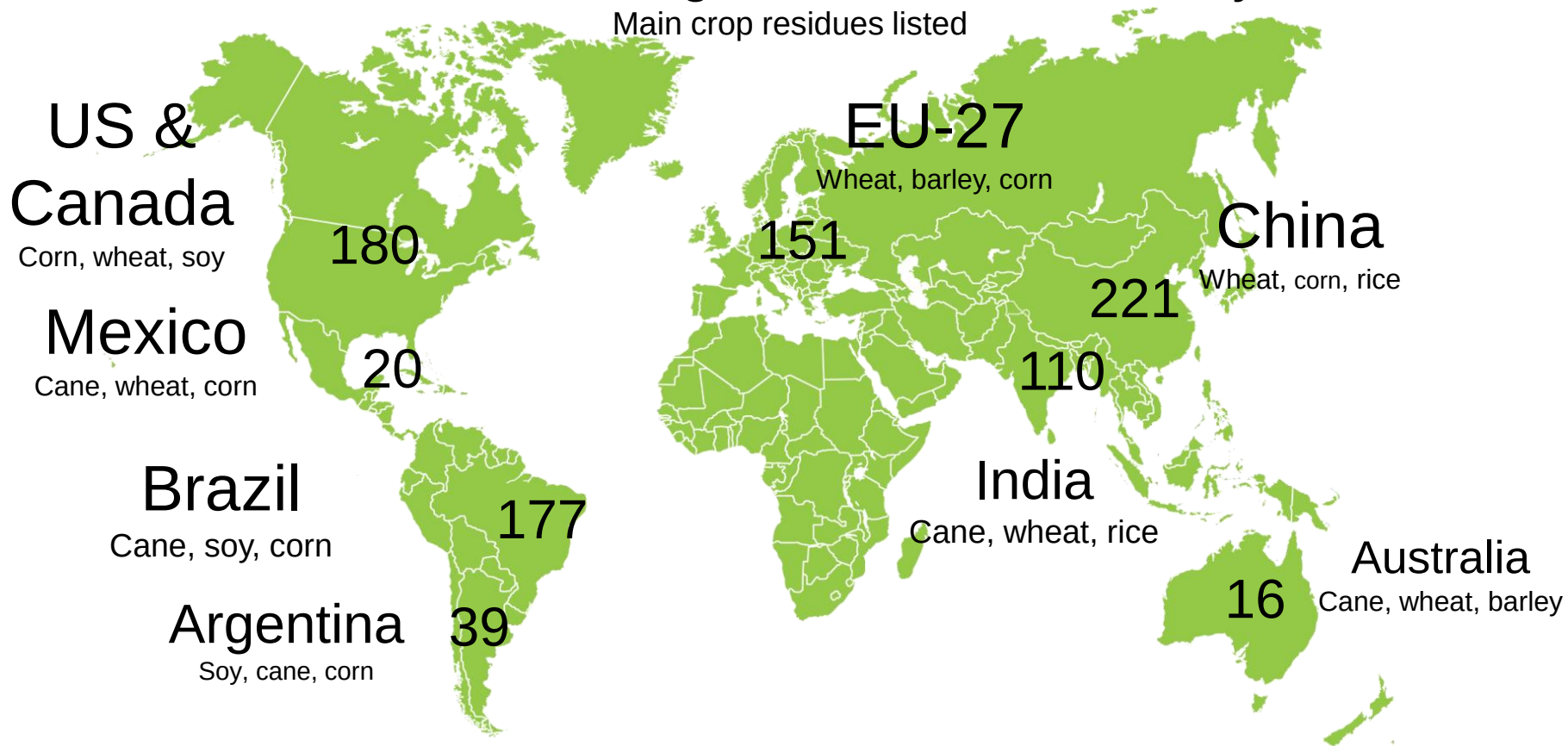


Global Biofeedstocks (2030):

914 Million MT of residues* can be available in eight regions and can replace half of the gasoline needs

Million MT of Agricultural residues only

Main crop residues listed



*This assumes 75% of residues are left on field

Based on presentation from Novozymes
Brian Davison ORNL

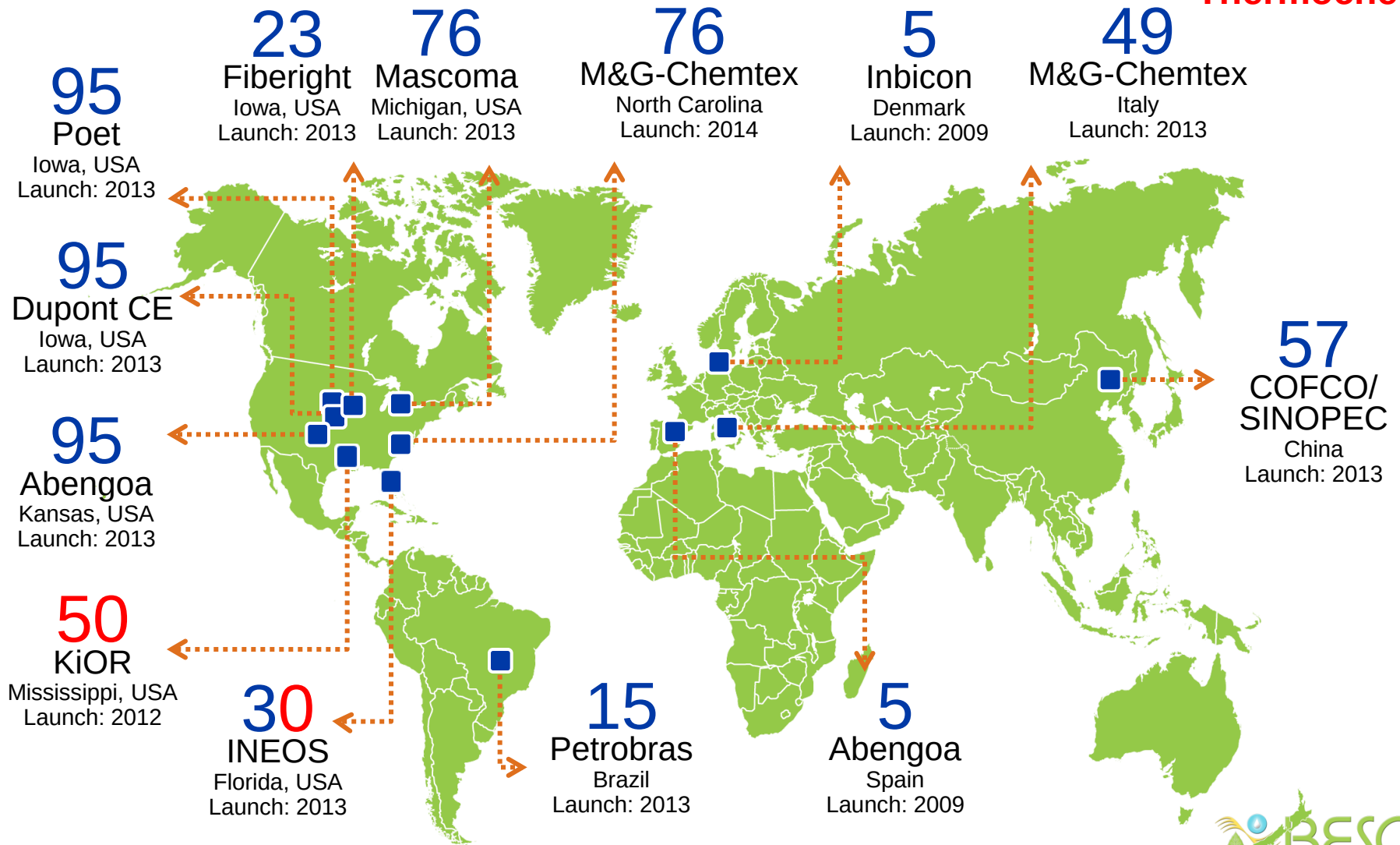
Cellulosic Biofuels Industry is Emerging (2013)



U.S. DEPARTMENT OF
ENERGY

Millions of liters
per year by
2017

Biochemical
Thermochem



Volumes and launch dates as announced by the respective companies
Brian Davison ORNL



KiOR Biocrude Production in the US

- Technology uses Fluid Catalytic Cracking (FCC) technology
- KiOR converts wood chips from Southeastern US
- Product is bio-crude oil and process gas for cogen of electricity
- Biocrude must be refined to fuels and KiOR works with Chevron
- First plant converts ~500 MT wood/day to ~50 million L/ year
- In September *KiOR, Inc. announced shipment of 1.35 million liters cellulosic diesel* fuel as of the end of August 2013 from its first commercial-scale facility in Columbus, Mississippi.
- KiOR plans a second facility in Natchez, MS
@ ~150 million L/ y
- They are interested in conversion of bagasse to crude oil

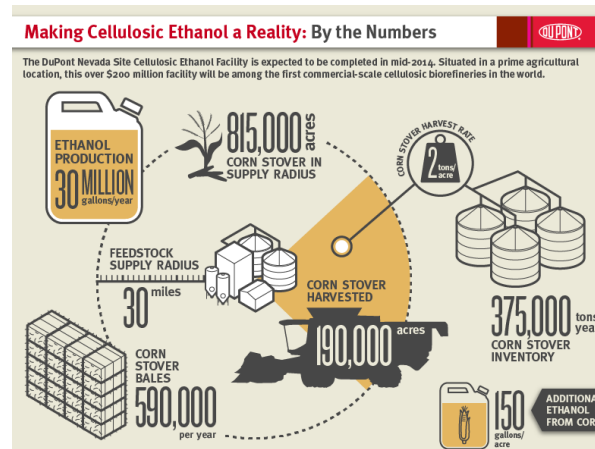
www.kior.com



DuPont Cellulosic Ethanol in the US



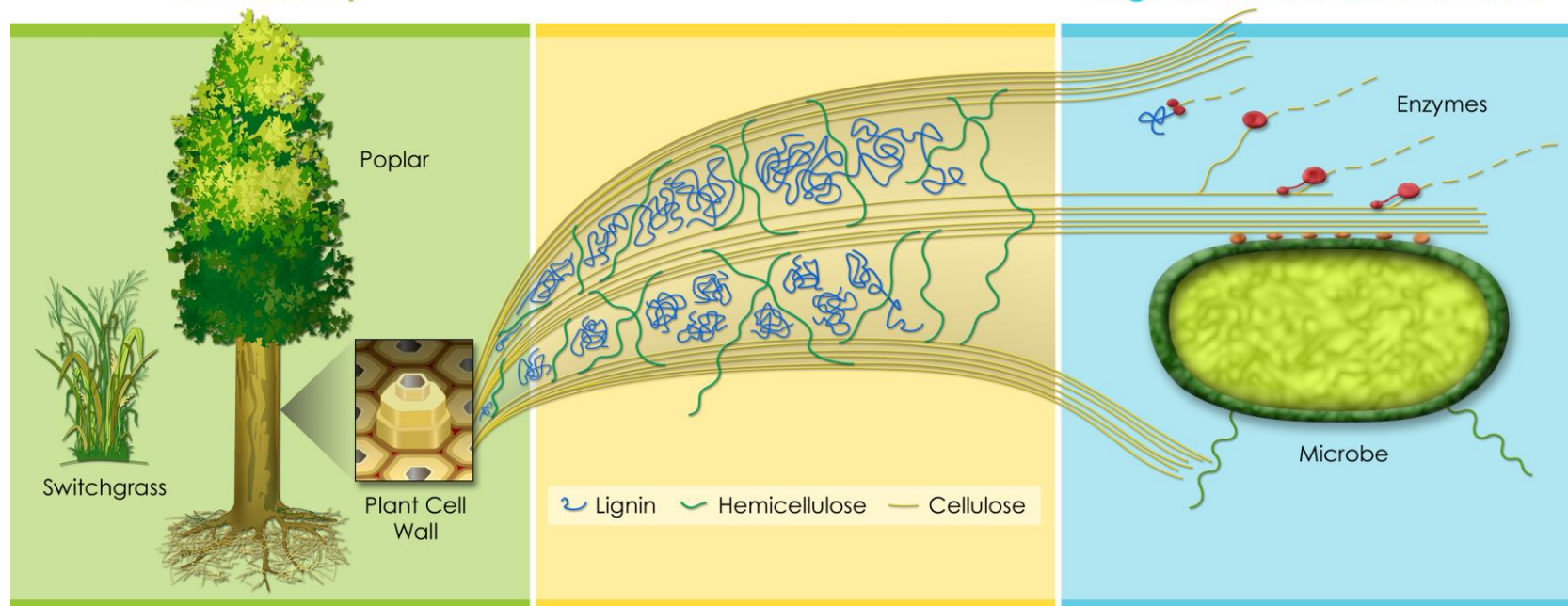
- DuPont technology uses enzyme breakdown & fermentation
 - DuPont bought the enzyme producer Genencor
- DCE operated a ~950,000 L/yr demo plant in Tennessee since 2009
- Plant being build in Iowa corn belt in the US: Nevada, Iowa
 - Produce ~95 million liters of cellulosic ethanol per year, using corn residues and expected complete mid-2014
- \$9 million grant from the Iowa Power Fund, combined with over \$226 million in matching funds by DuPont Cellulosic Ethanol
- Facility will use 375,000 dry tons of corn residues (stover) per year
- DuPont is interested in collaboration



ORNL BioEnergy Center: A Two-pronged Approach to Increase Biomass Sugars Availability

Modify the plant cell wall structure to increase accessibility

Improve combined microbial approaches that release sugars and ferment into fuels



Both utilize rapid screening for relevant traits followed by detailed analysis of selected samples

Switchgrass example
(USDA-BESC)

Yeast example
(Mascoma-BESC)

Potential of non-transgenic plant feedstock for bioenergy applications

Natural variation

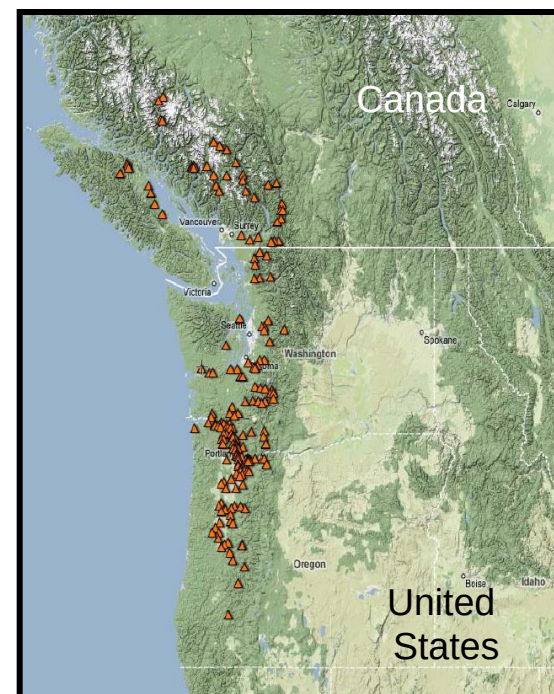
- Wide range of compositional characteristics
- Potential to mine natural sources of plant material
- Non-GMO accelerates development of feedstock resources

Switchgrass variation

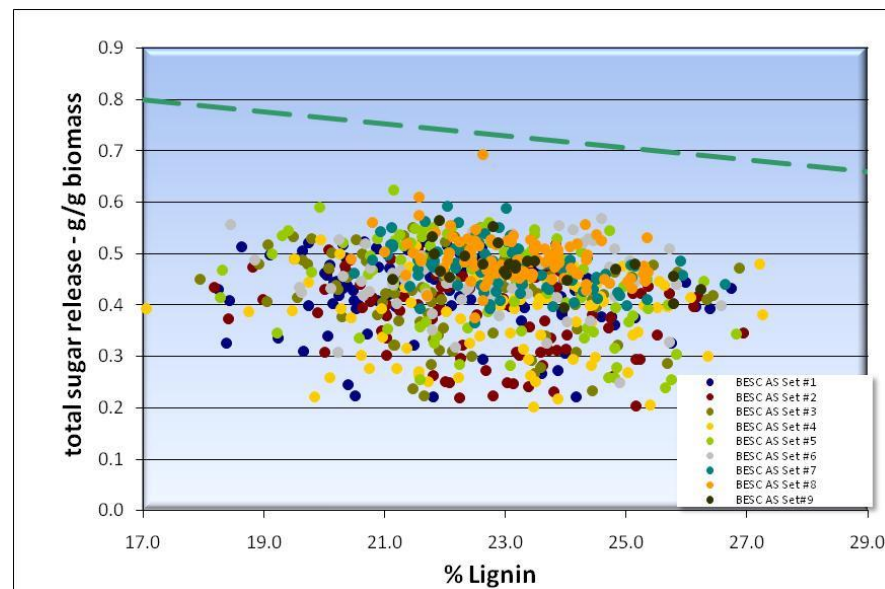
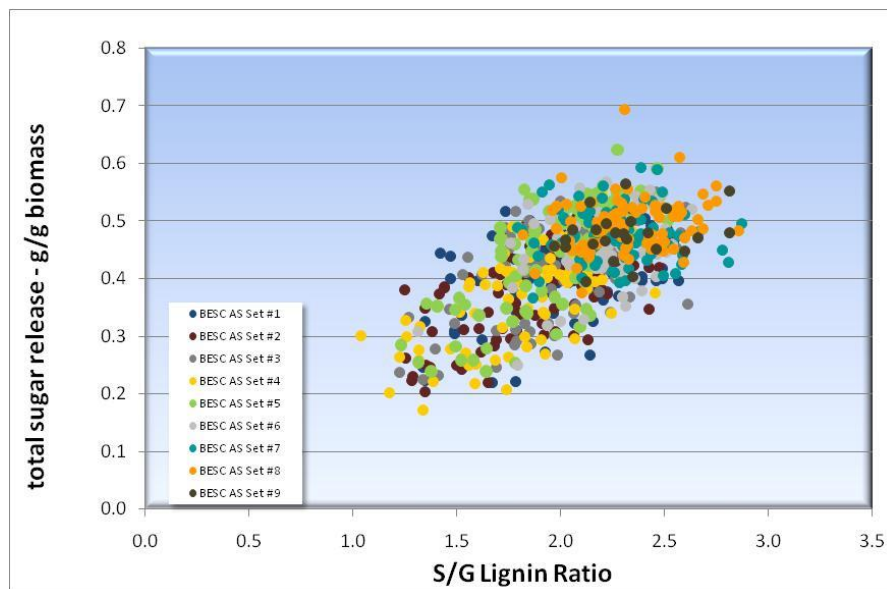
- Upland and lowland varieties
- Distributed across the Eastern US
- and Canada

Populus

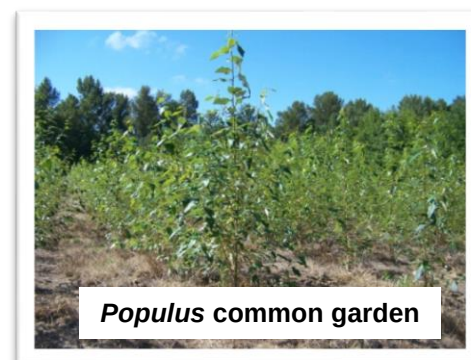
- Distributed in northwestern US and Canada
- Found in diverse climates and terrain
- Genome sequence is known (*P. trichocarpa*)



High-throughput Screening to Analyze Natural *Populus* Trees

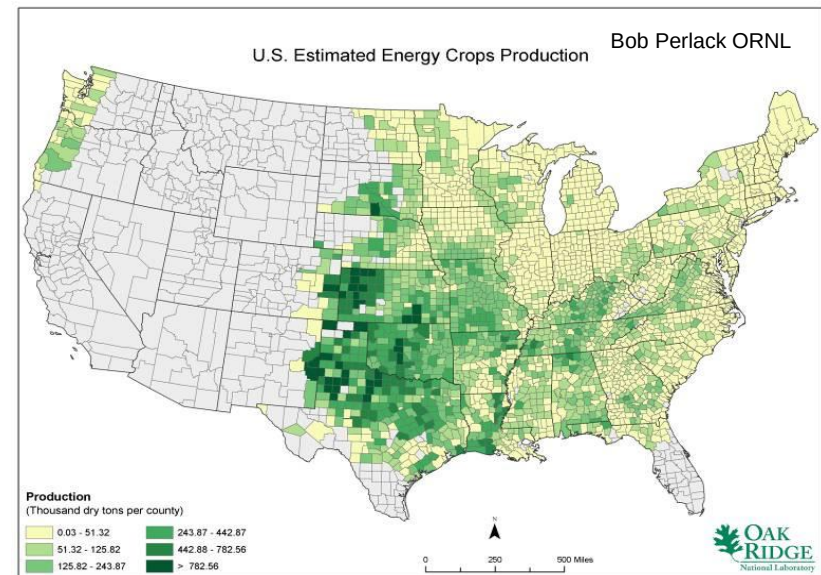


- Screening of ~1200 natural *Populus* trees, grow in three common gardens in California
- Sugar release varies from 25% to over 90% – huge!
- Lignin varied from 17% to 27% of dry weight – huge!
- Environmental vs genetics? Fermentation testing of common garden trees underway – appears to be genetics



Benefits of switchgrass *Panicum virgatum* L.

- Native U.S. prairie grass, non-invasive
- Perennial crop good for ten+ years
- Production starts by the second year
- High production possible (>25 MT/hectare)
- Requires low nitrogen, water inputs
- Harvested with convention equipment
- Numerous varieties available for most of the Eastern U.S.

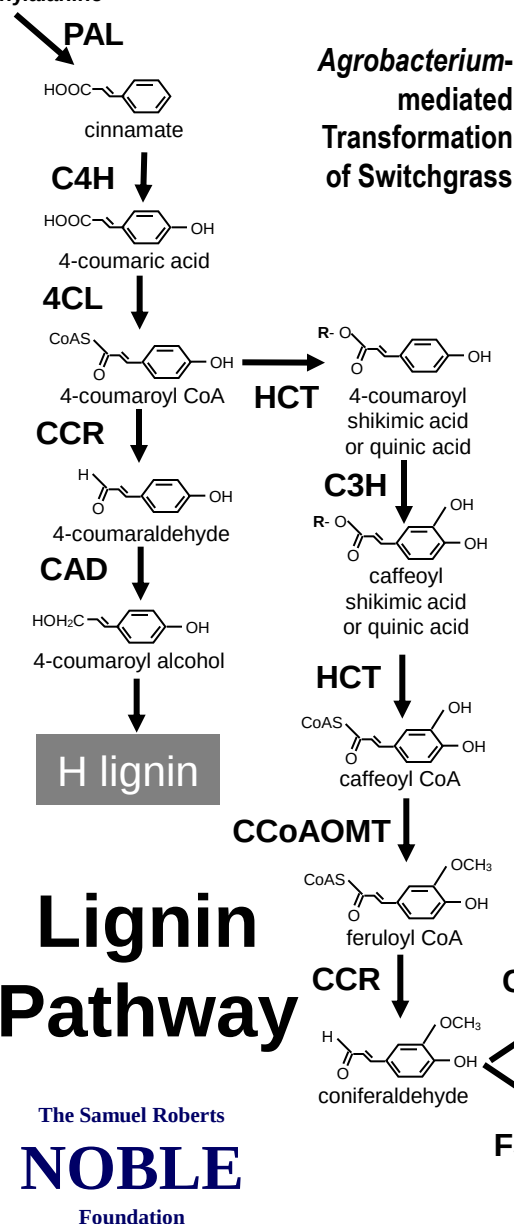


Genetic Block in Lignin Biosynthesis in Switchgrass Increases Ethanol Yields

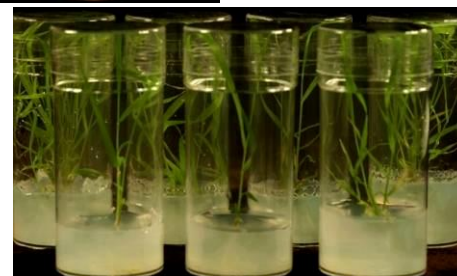
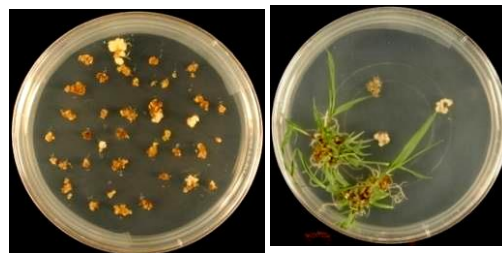
X. Fu, Z. Wang, Noble
J Mielenz, ORNL

Proc Natl Acad Sci USA 108 (9), 3803-3808

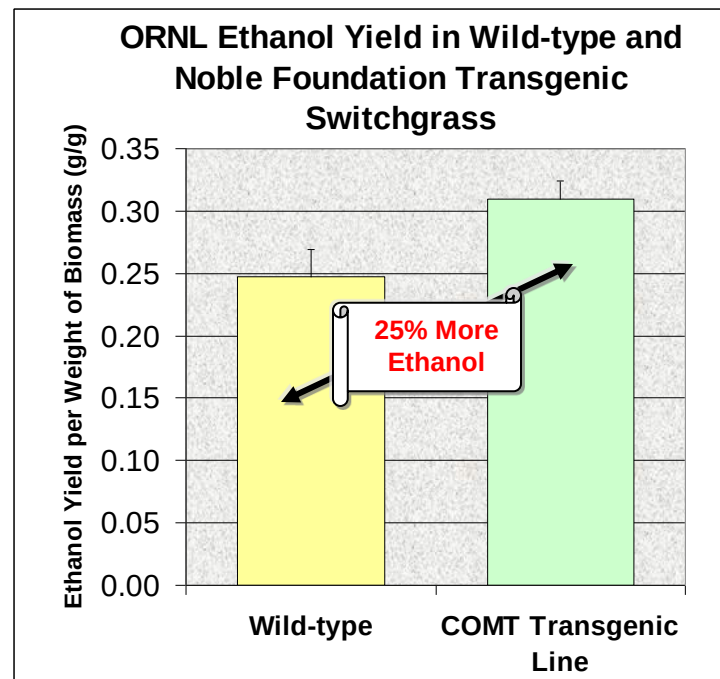
Phenylalanine



Agrobacterium-
mediated
Transformation
of Switchgrass



G lignin



COMT Mutant Switchgrass is similar to Wild-type

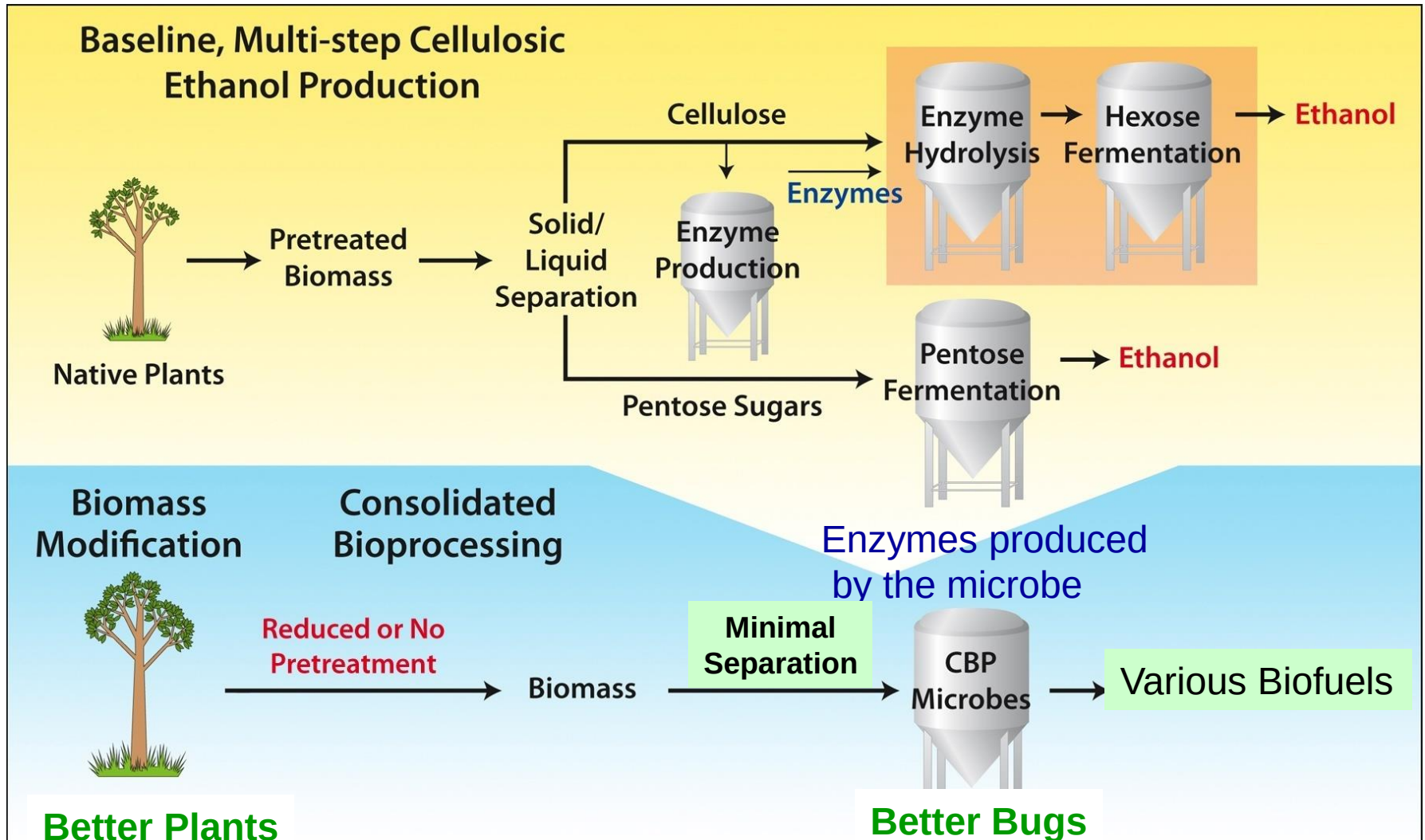
- Lignin decreased about 16% in COMT2 & 3
- Plant grew normally compared to wild-type (WT)
 - Height, stem diameter, growth rate
- Decreased lignin content did not impact cellulose levels
- Small increase in hemicellulose was detected



Wild-type (L) and 3
Transgenic switchgrass plants (R)

Fu, Mielenz* et al., Proc Natl Acad Sci USA. 108 (9), 3803-3808.*

Reduction of bioprocess steps: Consolidated Bioprocessing (CBP)



Fermentation by modified switchgrass and genetically engineered Mascoma yeast

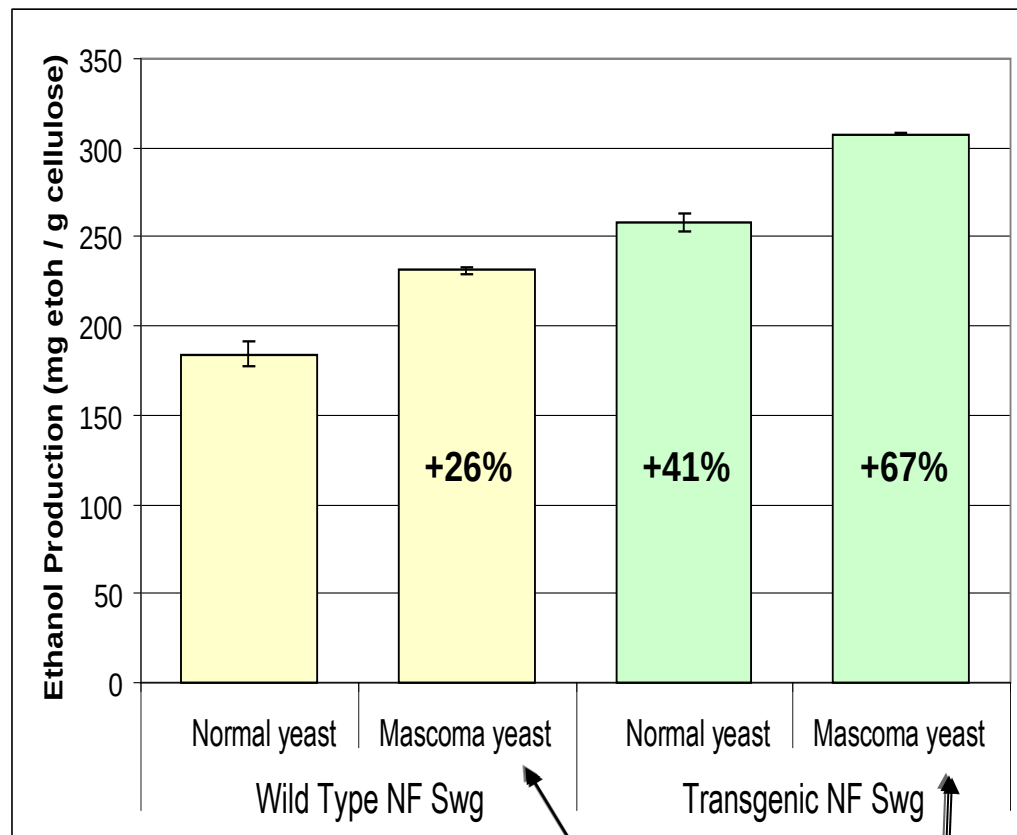
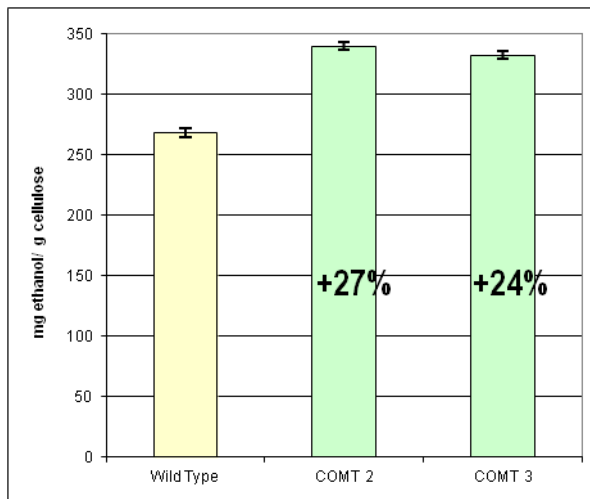
- Variables: native and modified yeast and switchgrass
- Biomass used was wild type and transgenic COMT switchgrass after dilute acid pretreatment (180°C, 0.5% H₂SO₄ 7.5 min.)
- Mascoma yeast expressing cellulase enzyme (cellobiohydrolase II)
- Determined rate of fermentation and net ethanol yield on substrate

Improved switchgrass and new fermentation yeast demonstrate synergy

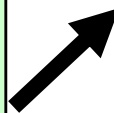
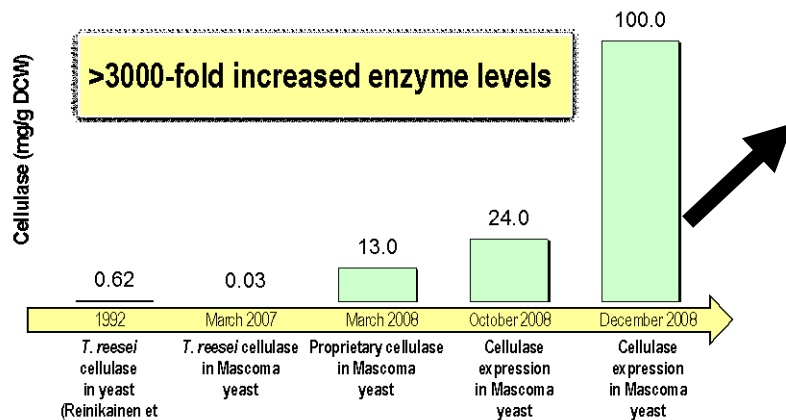


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COMT Transgenic Switchgrass Produced More Ethanol

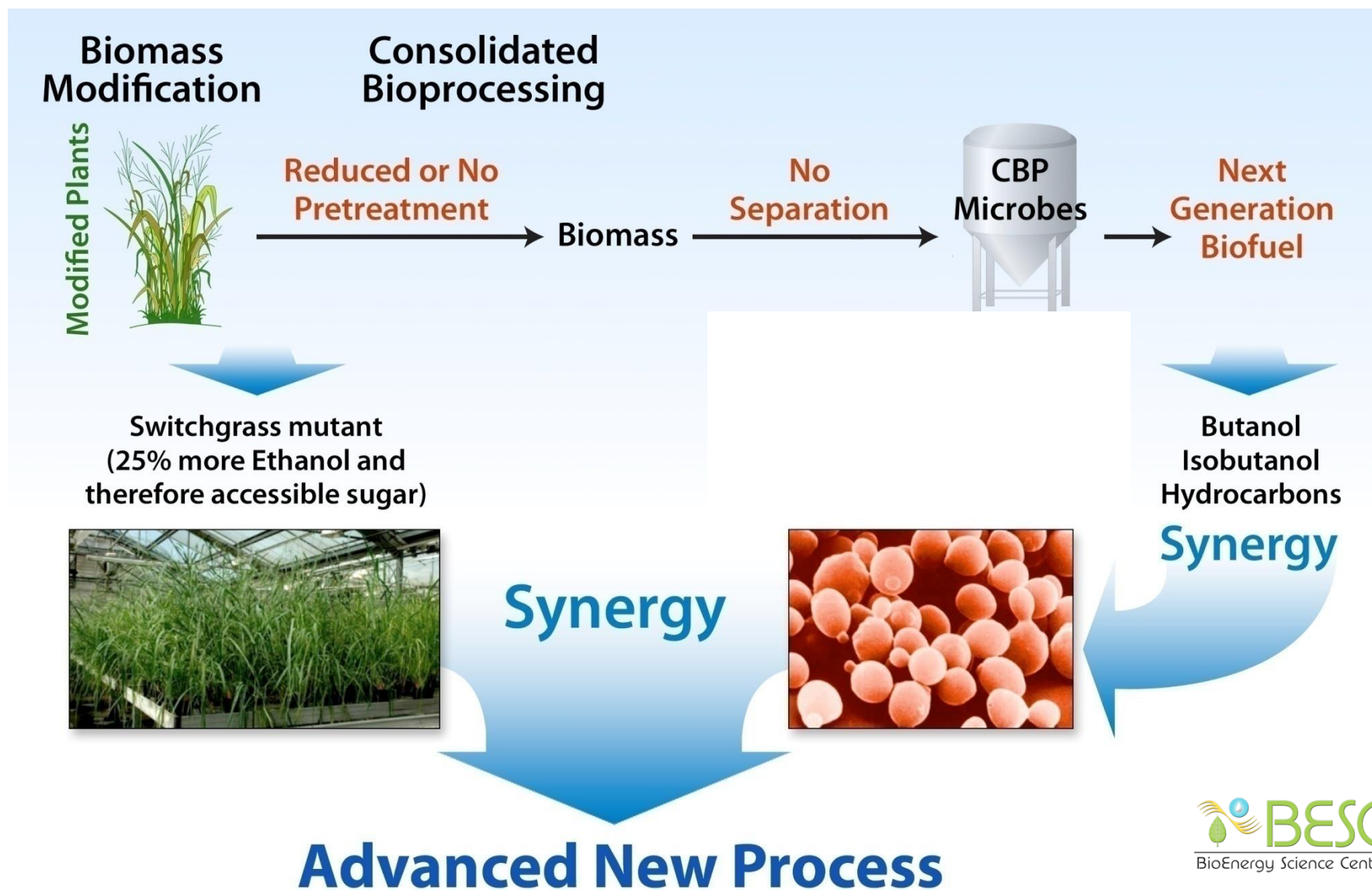


Mascoma development of a CBP yeast



Mascoma Corp yeast expressing a cellobiohydrolase

Ultimate process of improved feedstock and improved bioconversion



Potential tropical semi-arid feedstock with growing interest: *Agave* species

- Agaves are among the most water-use efficient plants in the world due to its crassulacean acid metabolism (CAM)
- Daytime: plant stomata remain closed saving water;
Nighttime: CO₂ is taken up and held for daytime conversion to carbohydrates with sunlight
- Current uses are largely for alcoholic beverages such as tequila from *A tequilana* or mescal from related *Agave* species as well as for fiber production
- Can other species grown for shorter time be used for biofuel production in warm semi-arid regions?



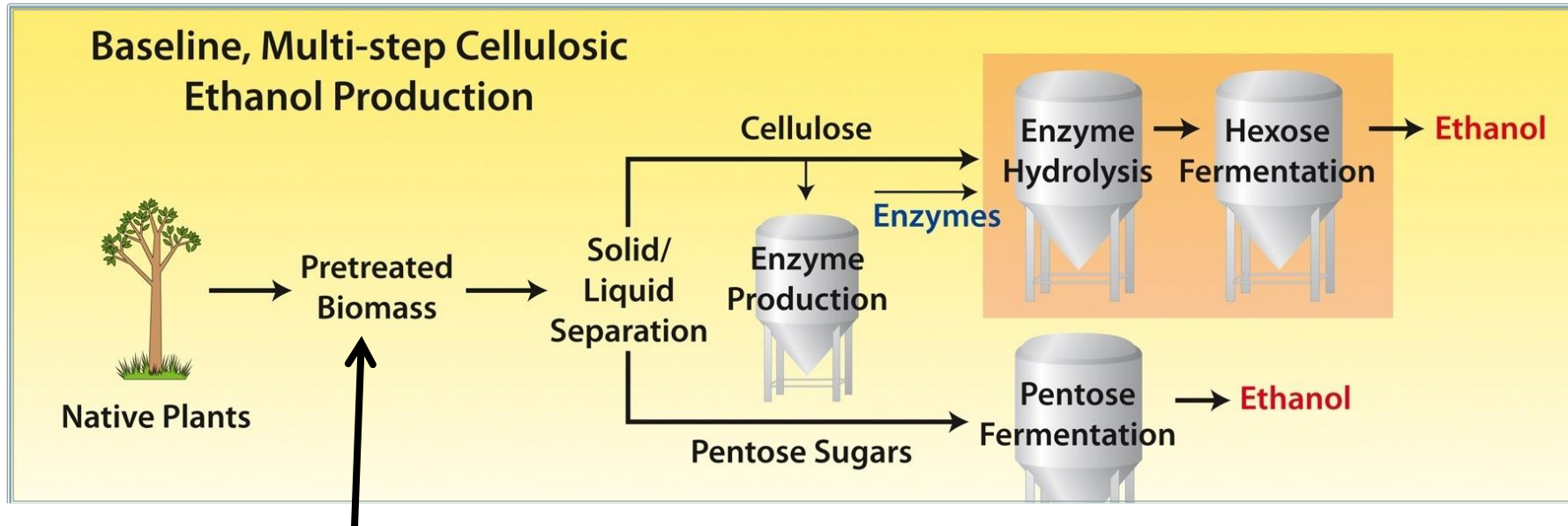
Evaluation of Agave for biofuels at ORNL with interest in U.S. grown species

Agave under investigation	Cold Hardiness
<i>Agave americana</i>	-9 deg C*
<i>Agave americana var. marginata</i>	-7 deg C**
<i>Agave americana var. gainsville</i>	-9 deg C**
<i>Agave decipiens</i>	-1 deg C**
<i>Agave ghiesbreghtii</i>	-4 deg C*
<i>Agave havardiana</i>	-23 deg C*
<i>Agave lechuguilla</i>	-18 deg C*
<i>Agave neomexicana</i>	-29 deg C*
<i>Agave parryi var. truncate</i>	-9 deg C*
<i>Agave salmiana ferox</i>	-4 deg C*
<u><i>Agave tequilana</i></u>	-4 deg C*
<i>Agave univitatta var. compacta</i>	-7 deg C*

*Agaves Yuccas and related species, M & G Irish, Timber Press

**J Notestein

Commercial Cellulosic Ethanol Production



Many pretreatment processes can destroy sugars

Question: How can current biofuel production processes be applied to *Agave* other than beverage species produce biofuels

Summary

- 2nd generation biofuels are moving to market via multiple commercial operations
- Both thermochemical and biochemical processes are being commercialized with some feedstock preferences
- Improvement of the bioconversion economics for biorefineries will occur via synergy of improved feedstock AND better fermentation microorganisms
- Sugarcane bagasse and other agricultural residues plus *Agave* provide opportunities for tropical America to produce 2nd generation biofuels
- International partnering will occur as feedstock is local but the processing technology can be transferred.

Thank-you

Notice:

36th annual Symposium on Biotechnology for Fuels and Chemicals

April 28 – May 1, 2014

Clearwater Beach, Florida, USA

19 **technical** sessions of 7 speakers plus 400 posters

700+ attendees ~25% international, ~30% industry

Brazil and Korea lead the international attendance

Abstracts are due in November: <http://www.simbhq.org/meetings/>

Chairs: Jim McMillan, Steve Decker, NREL, USA

Steve Brown, ORNL, Thomas Klasson, USDA, USA