



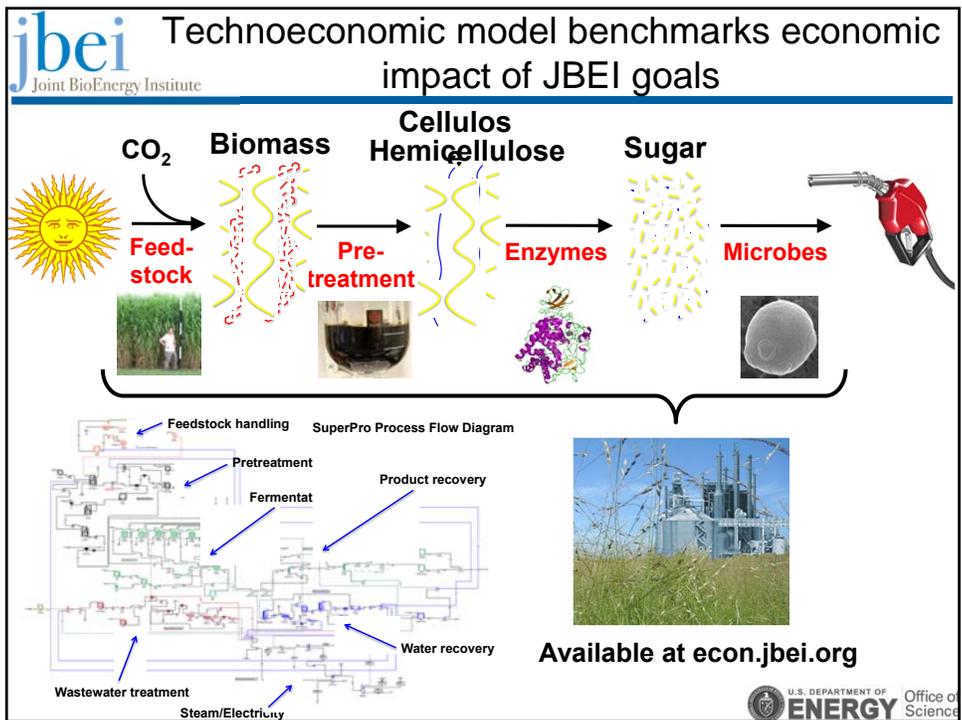
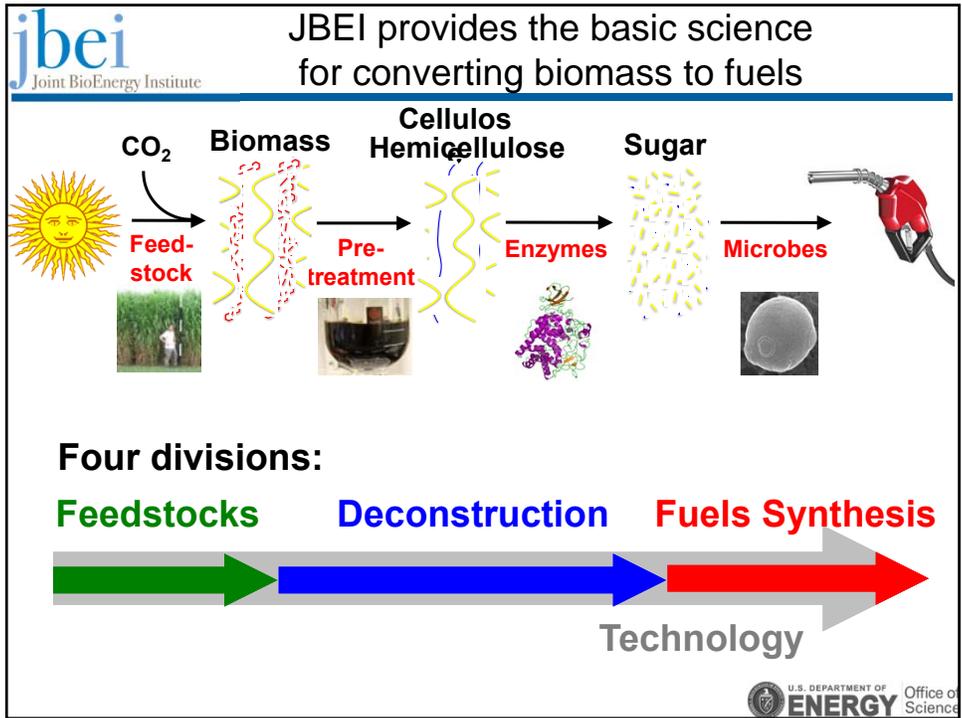
## Use-inspired, team-based basic science

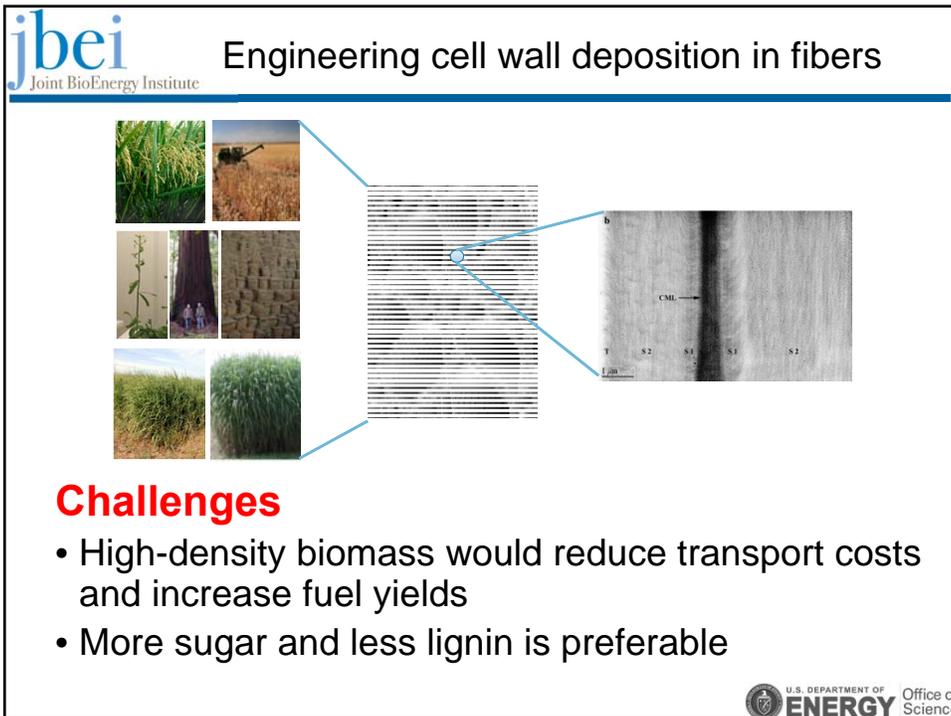
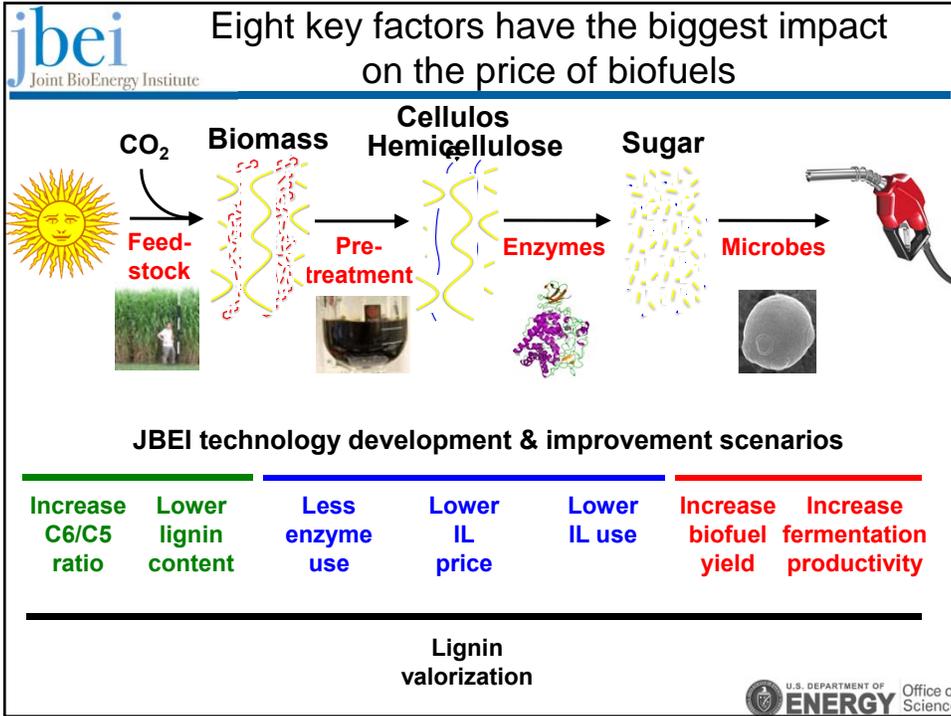
Jay Keasling, CEO

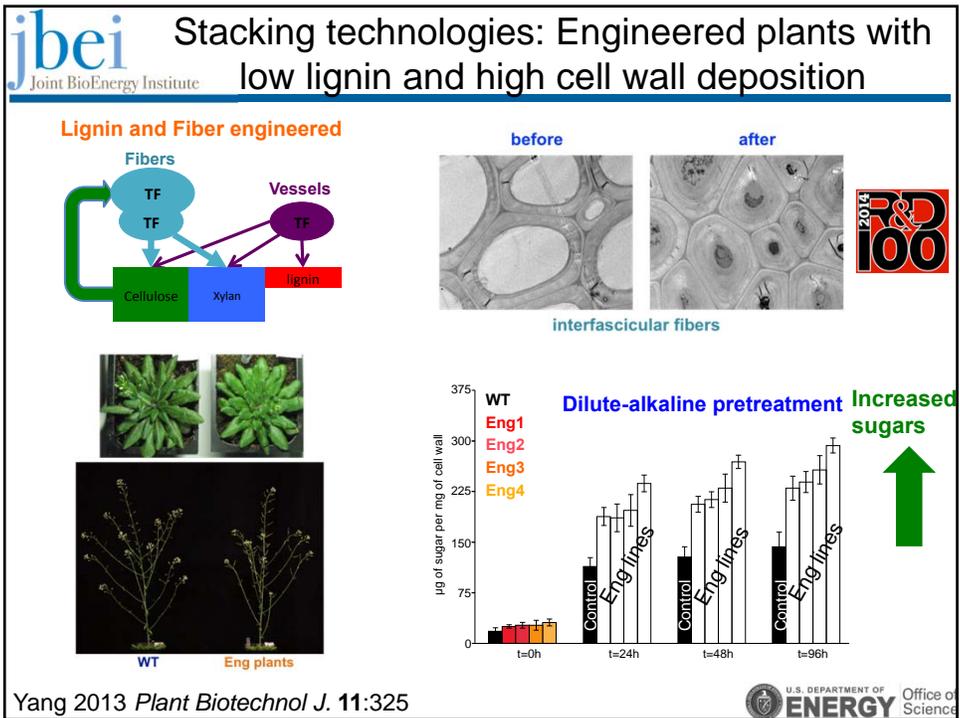
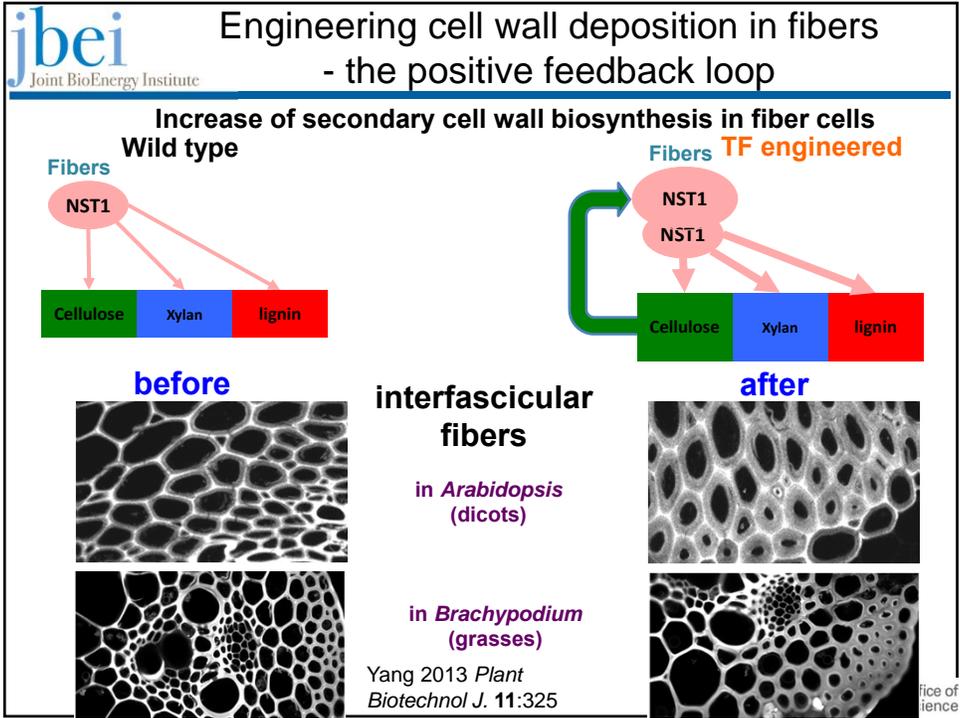


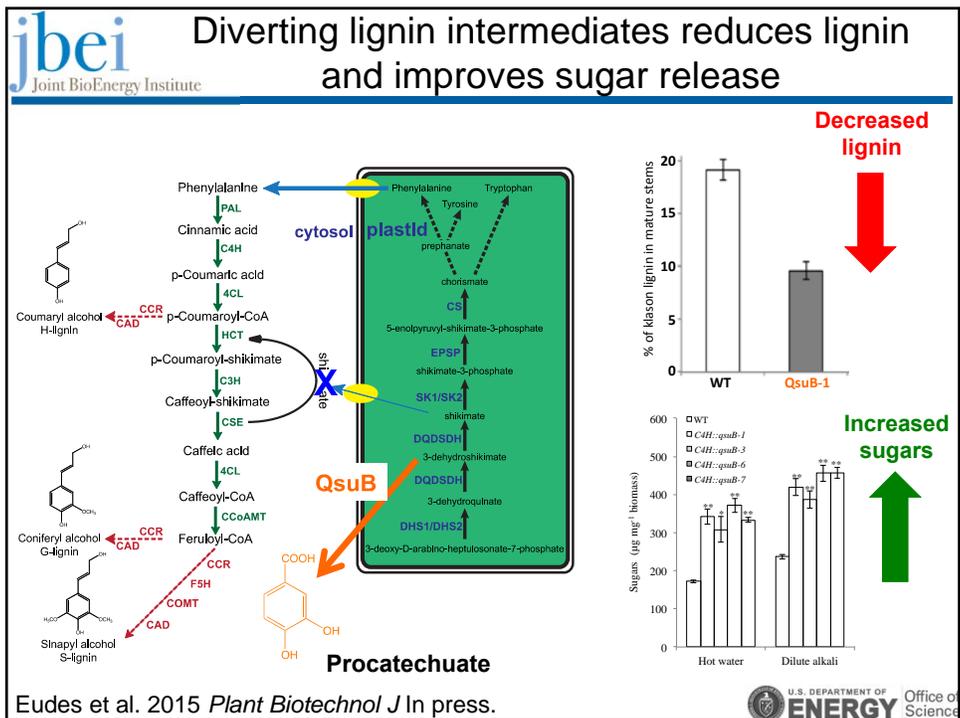
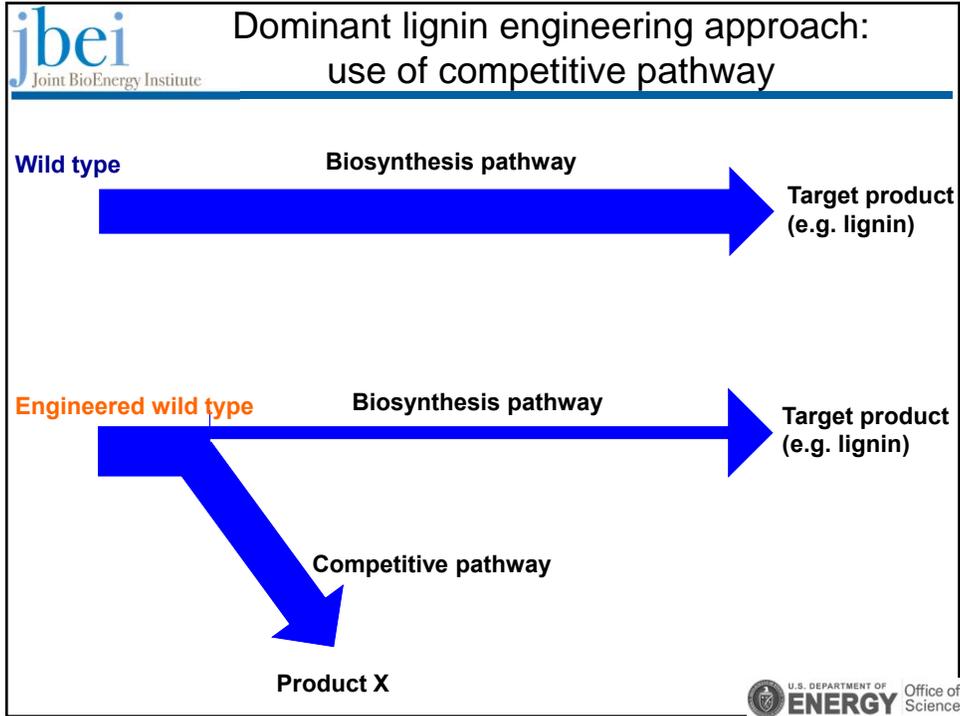
Joint BioEnergy Institute (JBEI)  
Seven partners – One location



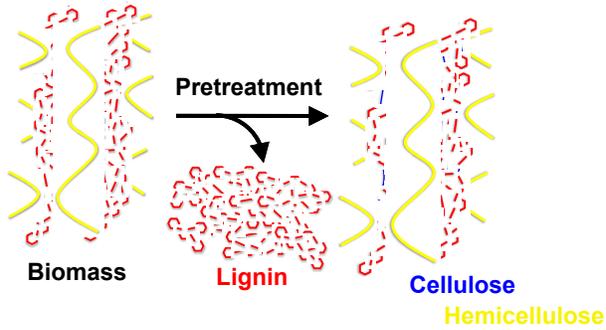








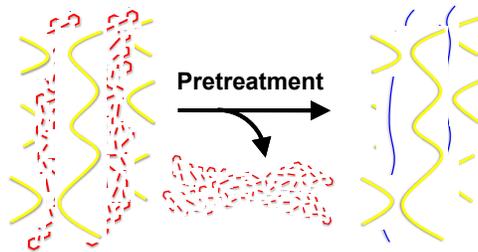
## Some key challenges in converting lignocellulosic biomass to fuels



### Challenges

- We need better pretreatment processes that yield cleaner cellulose/hemicellulose

## Ionic liquids pretreatment produces clean cellulose/hemicellulose



Ionic liquids



Mix  
Heat  
Stir

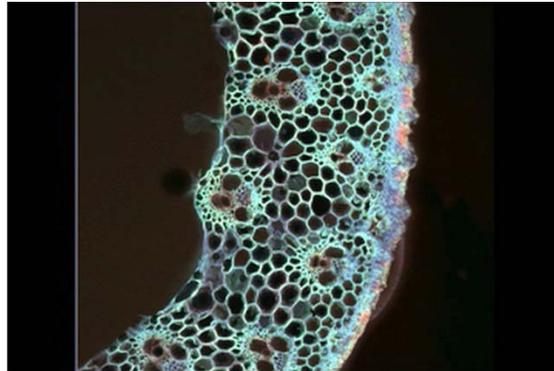


Add  
Anti-solvent



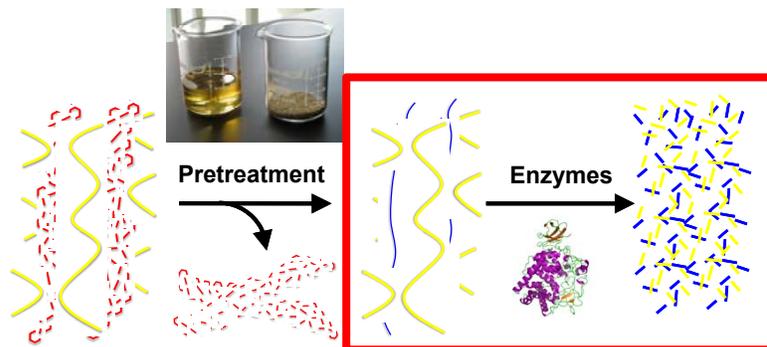
Biomass

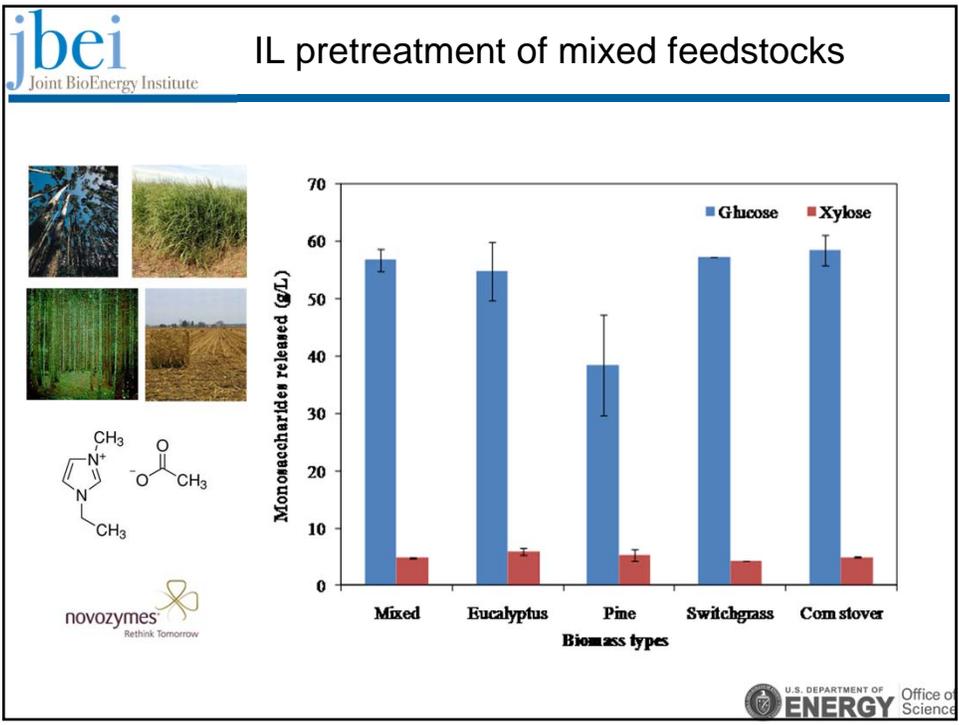
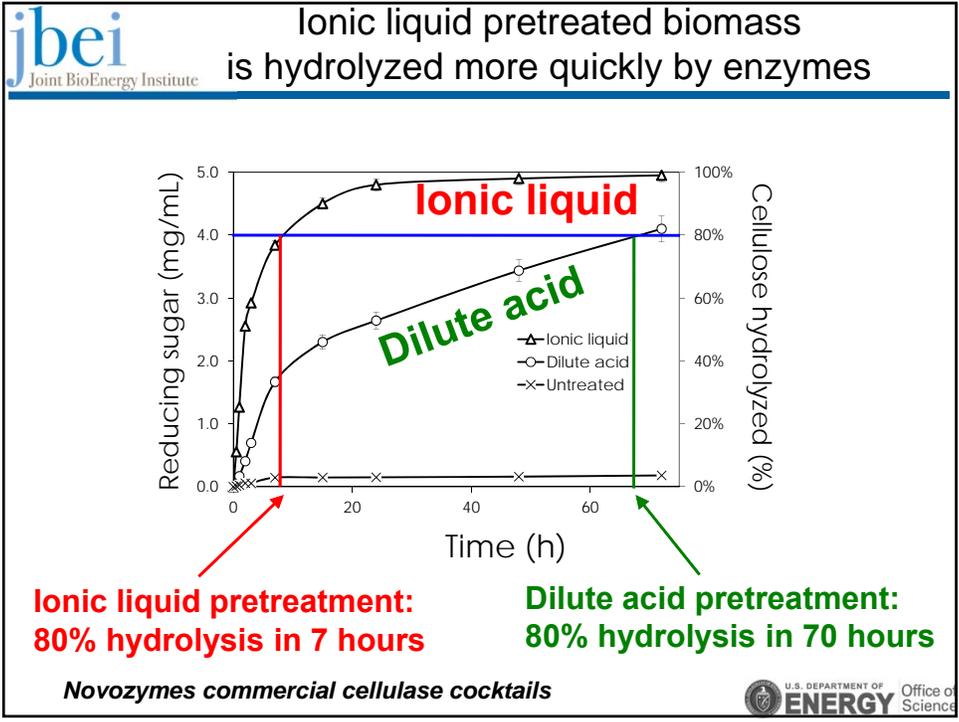
## Switchgrass undergoing IL pretreatment

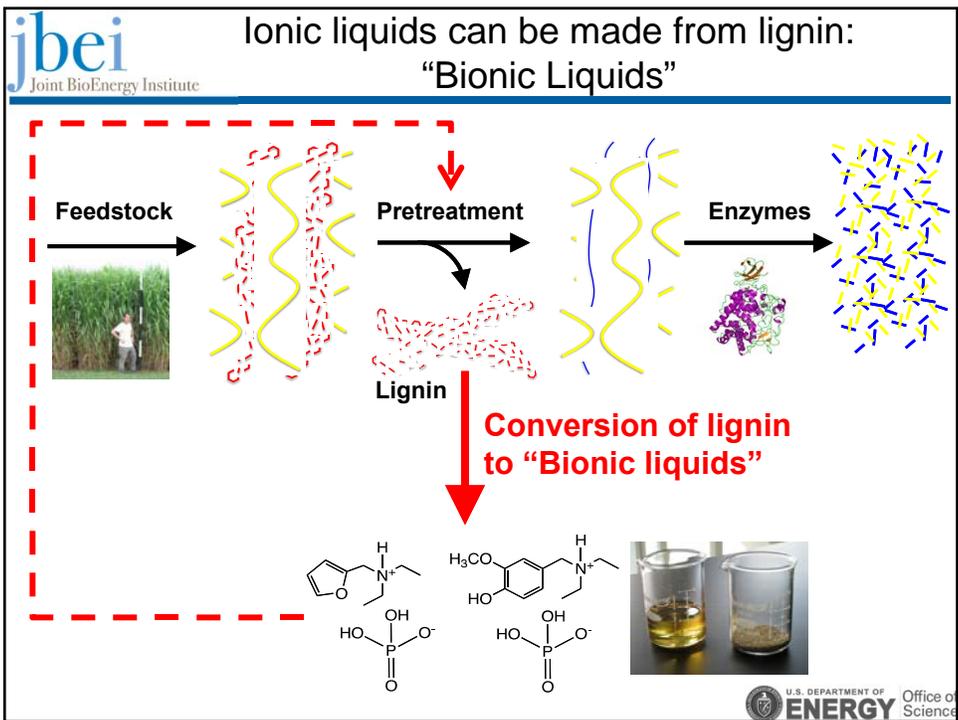
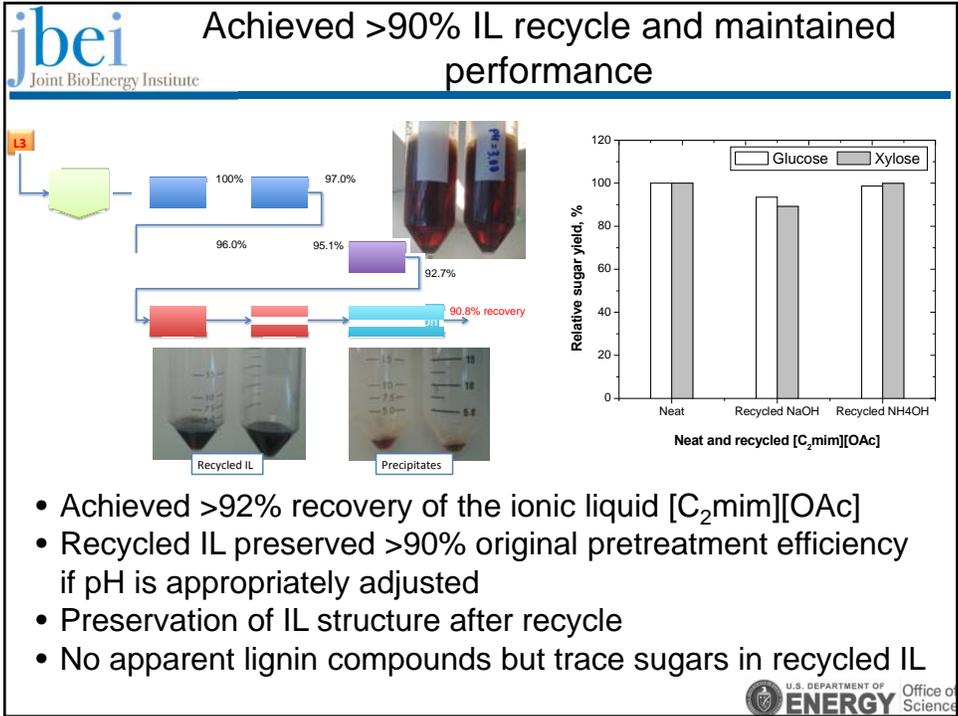


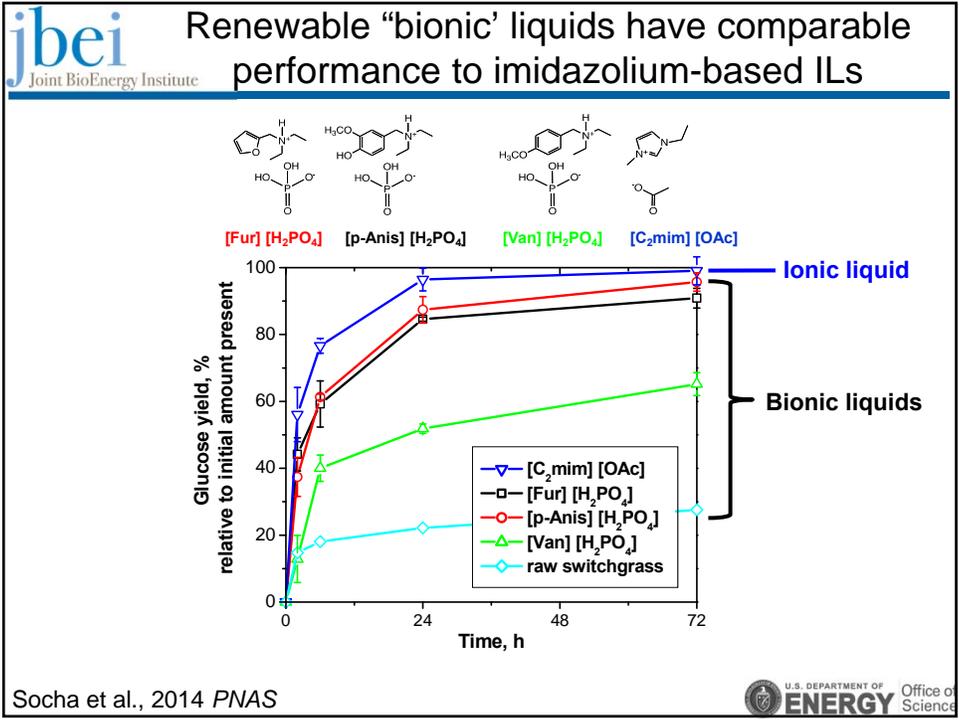
- [C<sub>2</sub>mim][OAc], 120°C
- In situ studies using bright field microscopy
- Complementary Raman and fluorescence studies indicate that lignin is solvated first, then cellulose

## Does pretreatment with ionic liquids improve cellulose hydrolysis?









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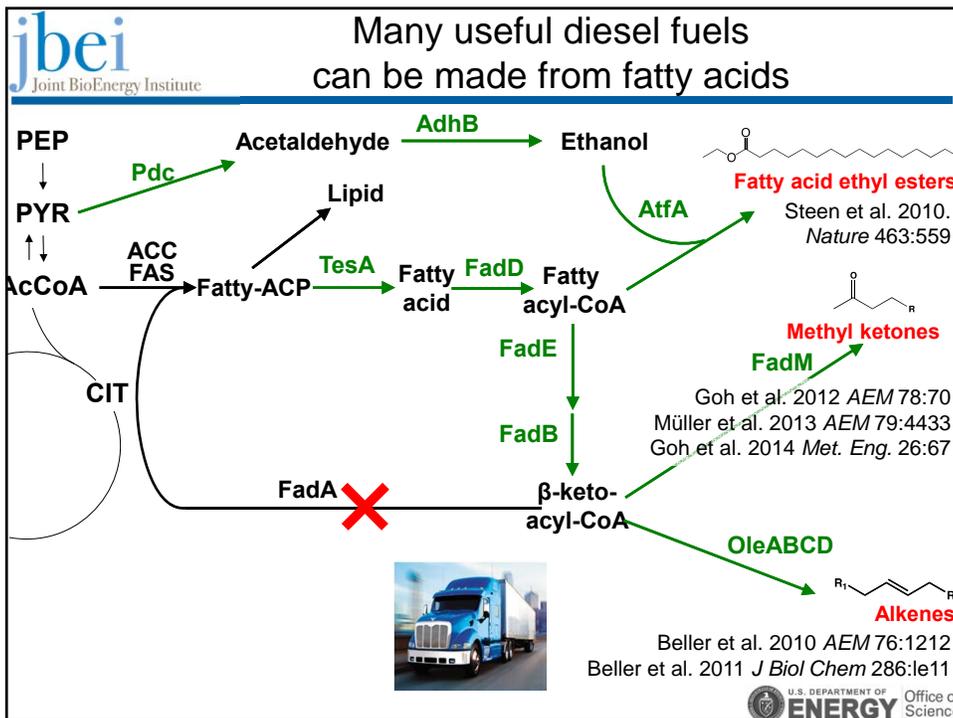
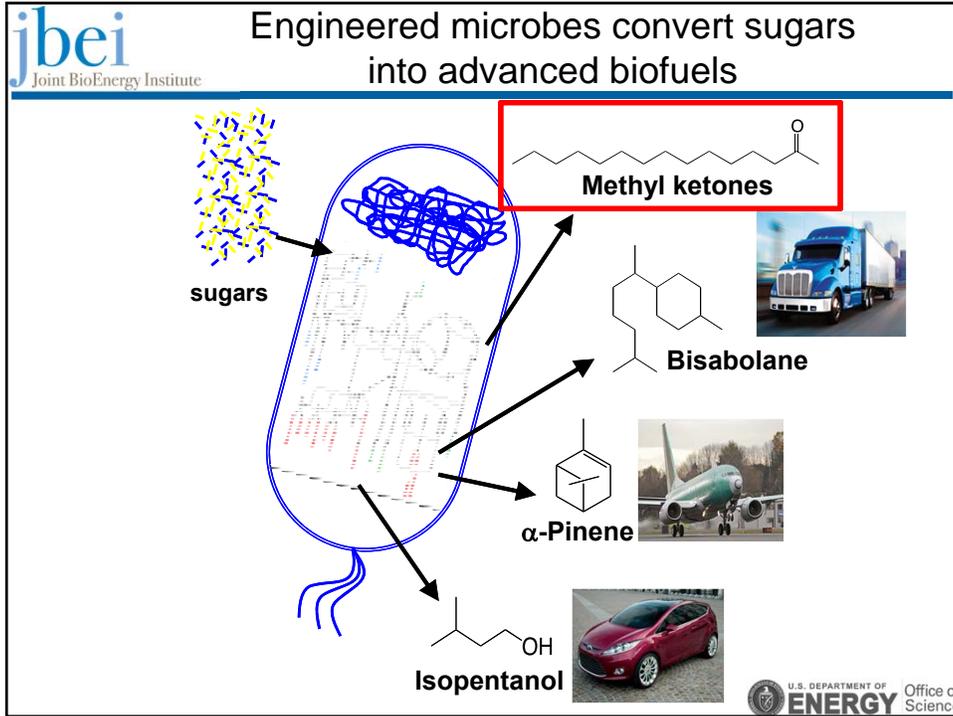
### Some key challenges in converting lignocellulosic biomass to fuels

**Challenges**

- Biofuels are needed for all kinds of engines, particularly diesel and jet engines
- Many fuel-producing organisms can only utilize a fraction of the sugars from biomass

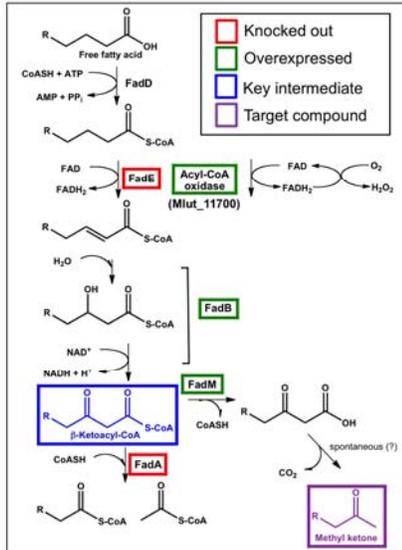
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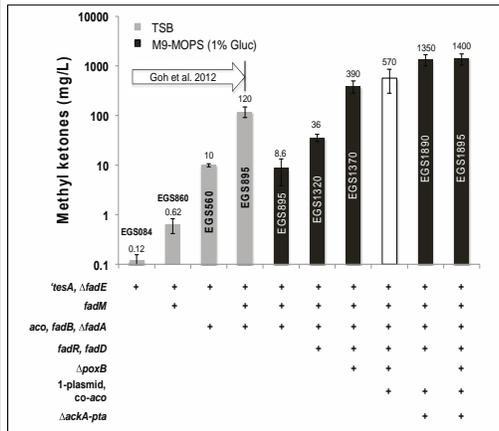


## Diesel-range methyl ketones in *E. coli*

### Re-engineering $\beta$ -oxidation

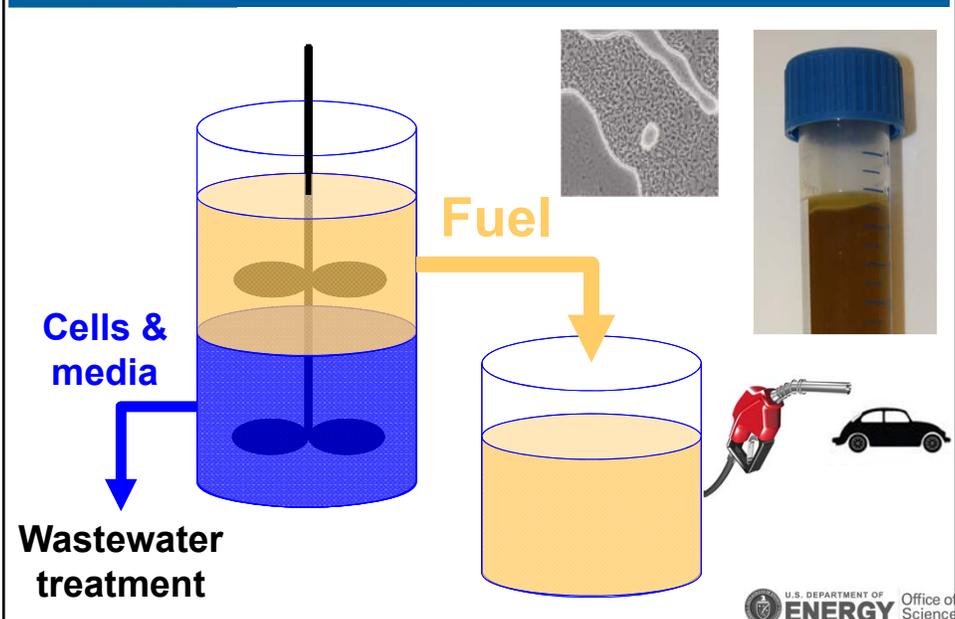


### Titers exceeding 1.4 g/L (1% Glu, M9) 40% of maximum theoretical yield



Goh et al. 2014 *Metab. Eng.* 26:67

## Phase separation allows simple purification of fuel



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## Engineered microbes convert sugars into advanced biofuels

**sugars**

**Methyl ketones**

**Bisabolane**

**$\alpha$ -Pinene**

**Isopentanol**

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## Improving isopentanol production using proteomics and metabolomics

Construct pathway  
Quantify proteins

1

2

"top"

g isopentanol / g glucose

**46% theoretical yield and 1.5 g/L tite**

**Highest reported yield (12%)**

Chou and Keasling (2012)  
Zheng et al. (2013)  
1A  
2A  
2A-mk  
3A  
3A-mk  
3A-mk-NudB  
3A-NudB

Concentration (mg/L)

Isopentanol

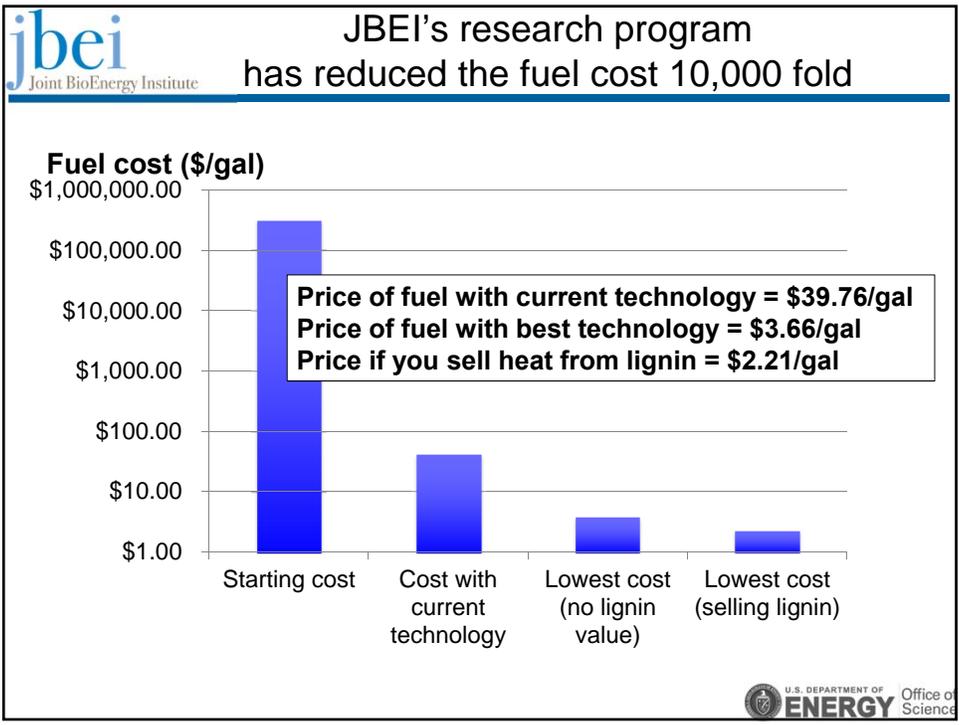
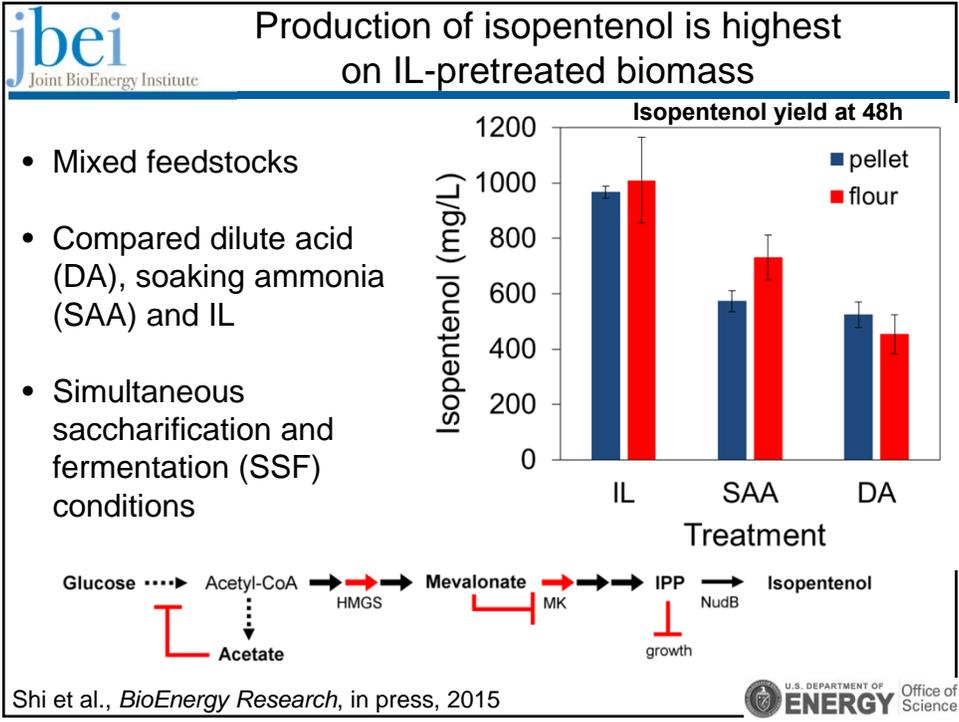
Acetate

Glucose

Strain

Chou & Keasling, 2012. *Appl. Env. Micro.* 78:7829  
George et al. 2014 *Biotech Bioeng* 111:1648

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## JBEI products are shared with the scientific community

- Data made available through JBEI-created databases
  - disseminated to external databases, including Kbase
- JBEI computational tools made available to the DOE community
- JBEI collaborates with Kbase to develop new tools for rapid synthetic biology
- JBEI research tools help advance other Genomic Science Programs:
  - Nano-initiator Mass Spectrometry (Northen) enables ENIGMA research

**Tools**

- Genomes
- Metagenomes
- Omics
- Structure

ELN EDD LIMS

**Grass Expression & Phylogenomics**

MASCP SUBA RiceNet

Plant Proteomes Grass systems biology

**Other GSP-related Resources**

img img/m phytozome MIG-RAST

**KBase**  
PREDICTIVE BIOLOGY  
DOE Systems Biology Knowledgebase

BESC  
BioEnergy Science Center

GREAT LAKES BIOENERGY

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## Thanks to ...

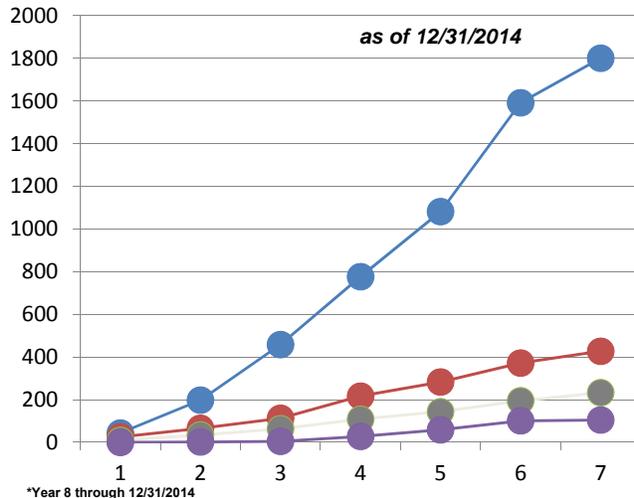
### The JBEI Team

Thank you  
Working Genomes Association

### BER for funding

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## DOE Bioenergy Research Centers: Publications, Patents, Licenses



Total  
**Publications<sup>1</sup>**  
1,799

Total  
**Invention Disclosures**  
427

Total  
**Patent Applications**  
233

Total  
**Licenses/Options<sup>2</sup>**  
105

**Total Patents: 24**

<sup>1</sup> Represents publications "in print" and "accepted/in press"  
<sup>2</sup> Represents patent applications licensed/options



## Moving BRC advances to the marketplace

The grid includes logos for the following companies and organizations:

- Pacific Ethanol, Inc., Dow, Ceres, Weyerhaeuser, POET, Monsanto, Scion, Roesein, Novozymes, Shell, Evolva
- Cargill, Borregaard LignoTech, firstgreen PARTNERS, Lignol, GlucanBio, Boeing, Amyris
- FuturaGene, DSM, LS9, INC., GSF, ARBORGEN, CNH Industrial, Xylome
- FDC Enterprises, aligna technologies, AGRICEN SCIENCES, Renmatix, A&C, Canada, Greenwood, IOGEN
- tesetagen, MillerCoors, allopartis, GINKGOBIOWORKS, PlantProbes, Nextval, TECHNOLOGY HOLDING, GE
- genomatica, ZeaChem, LYGO, syngenta, opxbio, Soil Net, bp
- Mendel BIOTECHNOLOGY, opxbio, ExxonMobil, DECAGON DEVICES, T.B.R., verdezyne, NIDUS, ADM
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- GM, Hyrax ENERGY, Caliper, a PerkinElmer company, DUPONT, SuGenit, BASF, MASCOMA, storenso
- Afingen, viridia, Show Me Energy Cooperative, GreenEarth Institute, DYADIC, Edeniq, MBG, ABENGOA
- Semba, BESC, jbei, GREAT LAKES BIOENERGY

