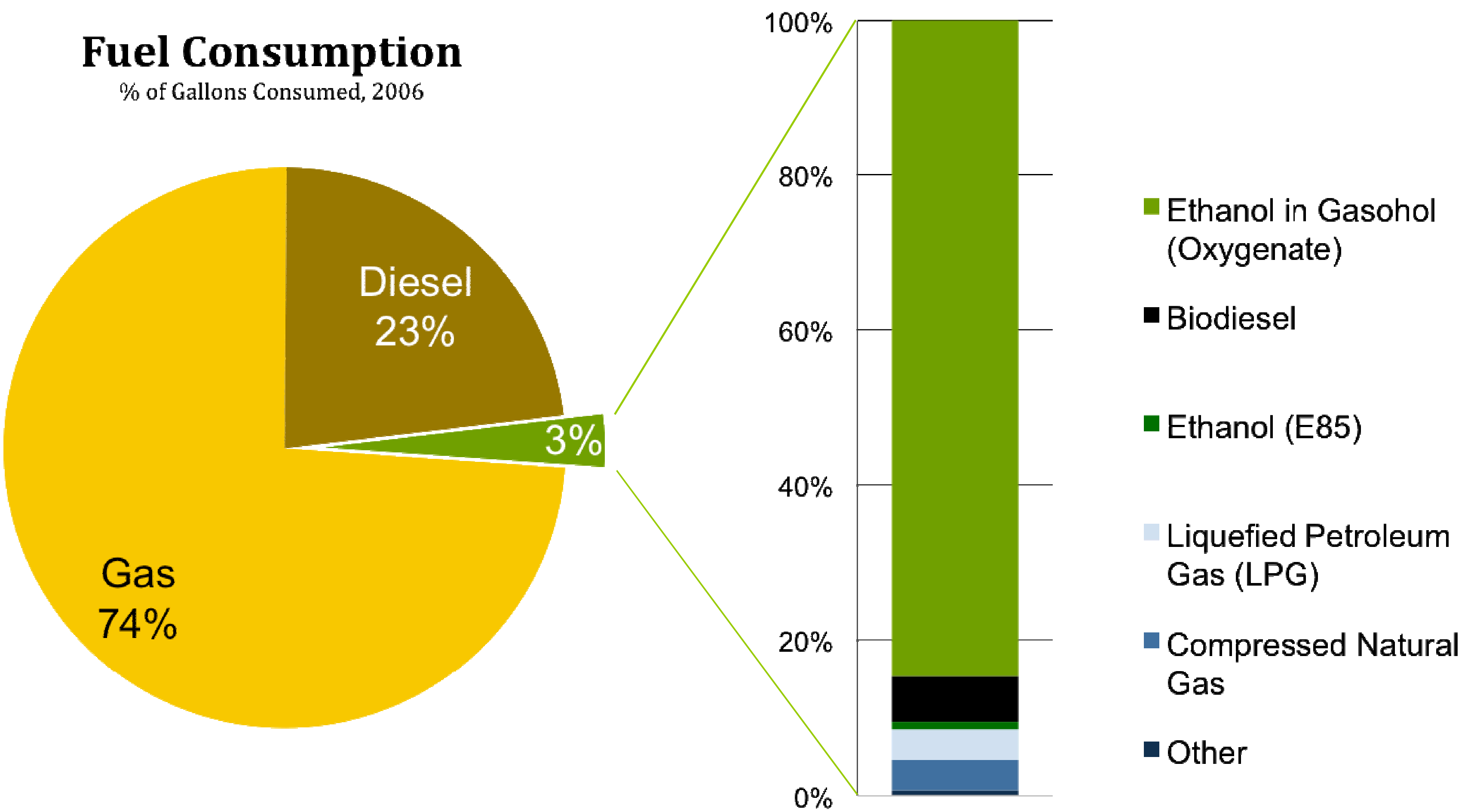
Sandia Bioscience

SAND2011-2199P

Sandia BioFuels Overview

PETROLEUM DOMINATES TRANSPORT FUELS

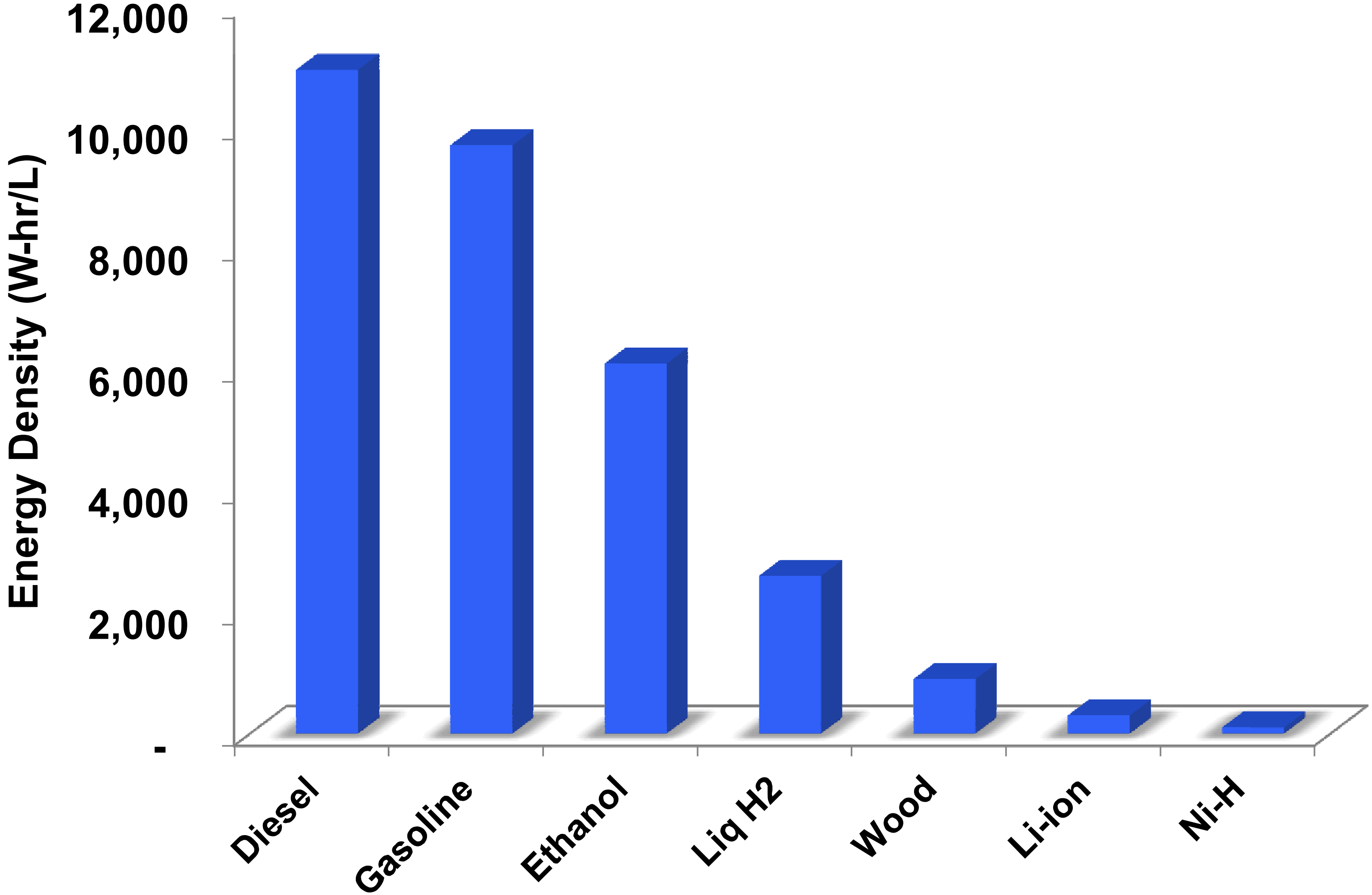


Source: Alternatives to Traditional Transportation Fuels, 2007, Release Date: April 2009
Statistics: Estimated Consumption of Vehicle Fuels in the United States, by Fuel Type

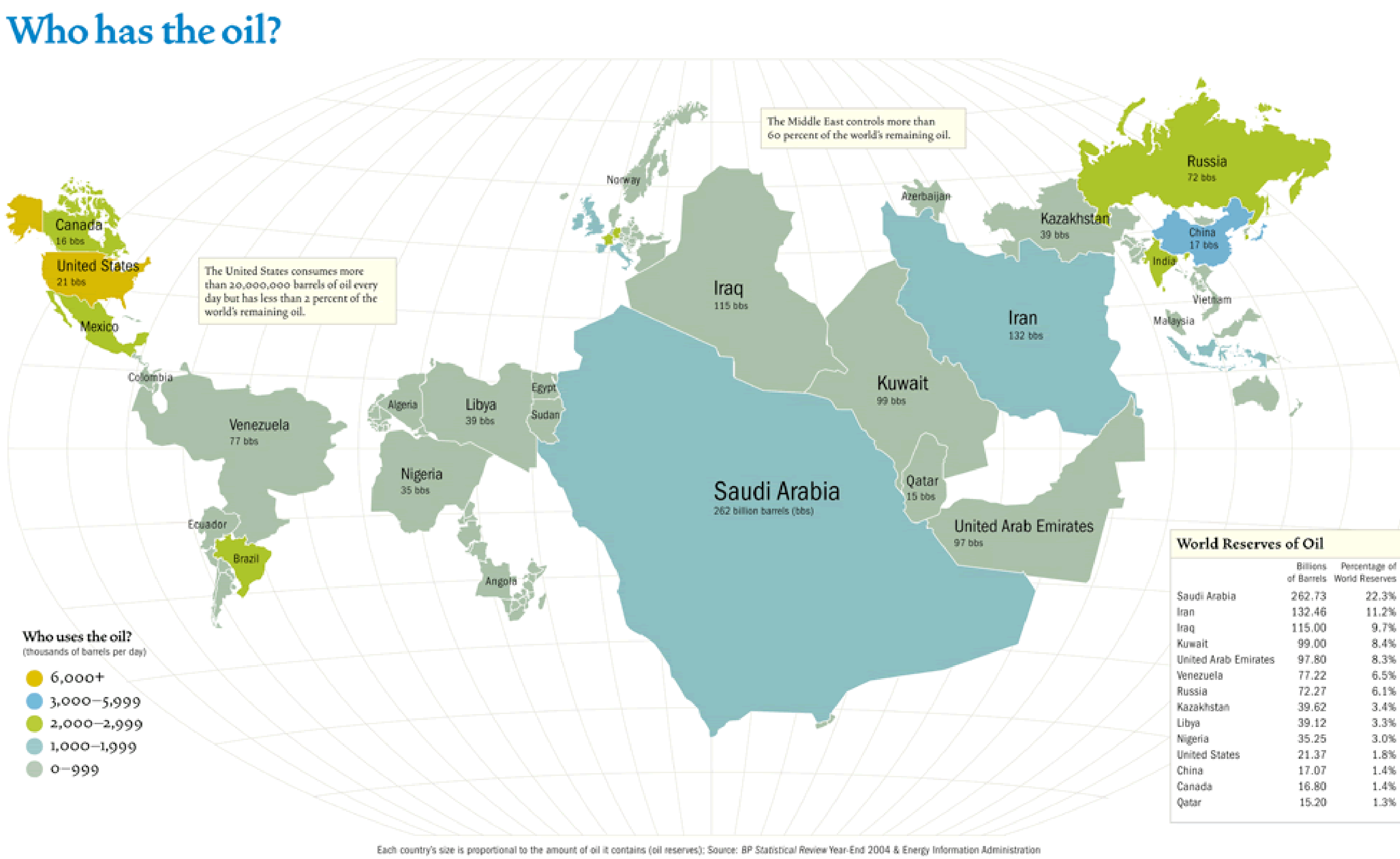
Petroleum Is a Wonder Resource

- Liquid
- Cheap
- Loaded with Energy

Petroleum Is Packed with Energy



PETROLEUM RESERVES LOCATED MOSTLY OUTSIDE US

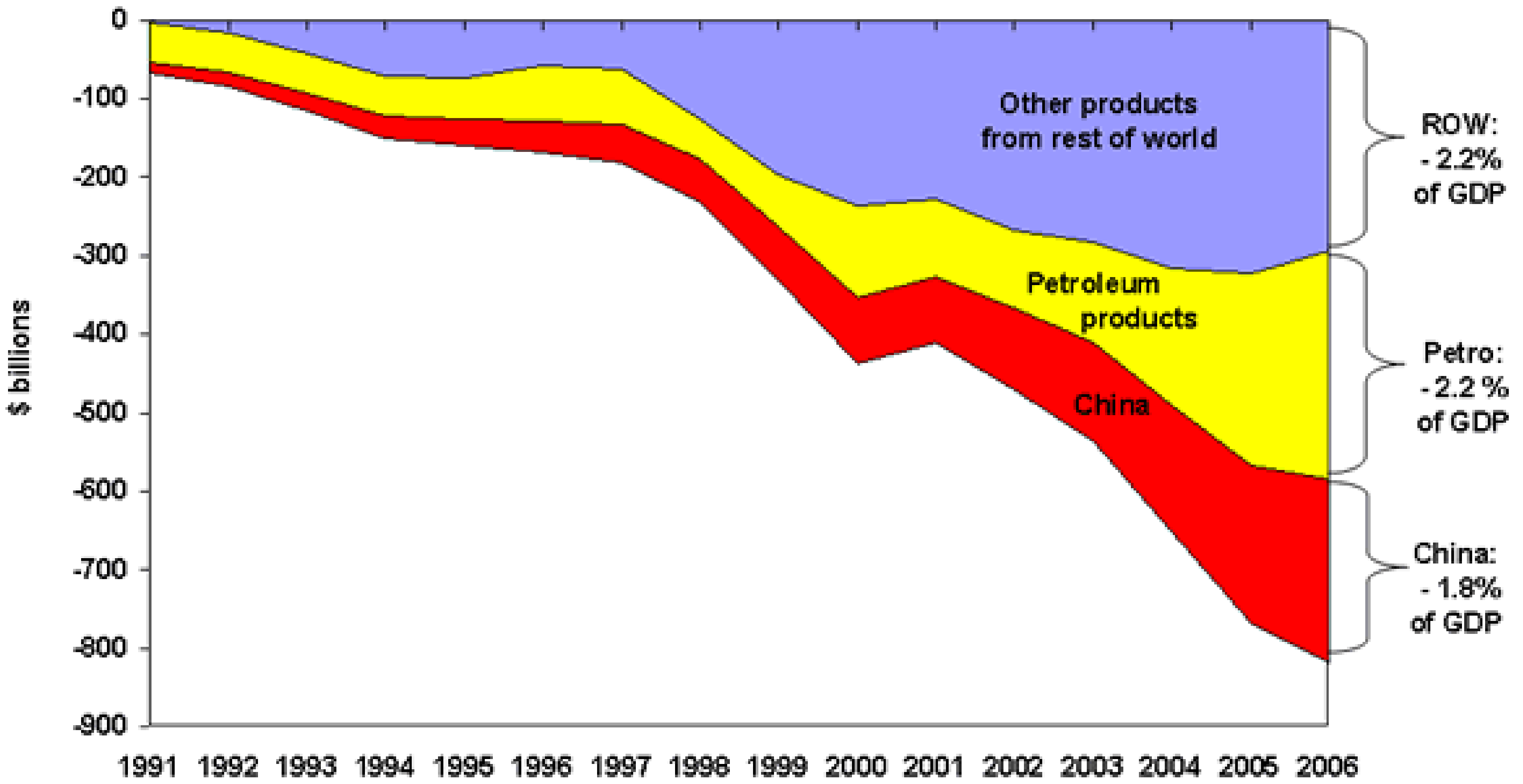


World Reserves of Oil

	Billions of Barrels	Percentage of World Reserves
Saudi Arabia	262.73	22.3%
Iran	132.46	11.2%
Iraq	115.00	9.7%
Kuwait	99.00	8.4%
United Arab Emirates	97.80	8.3%
Venezuela	77.22	6.5%
Russia	72.27	6.1%
Kazakhstan	39.62	3.4%
Libya	39.12	3.3%
Nigeria	35.25	3.0%
United States	21.37	1.8%
China	17.07	1.4%
Canada	16.80	1.4%
Qatar	15.20	1.3%

Source: EIA and BP (2004)

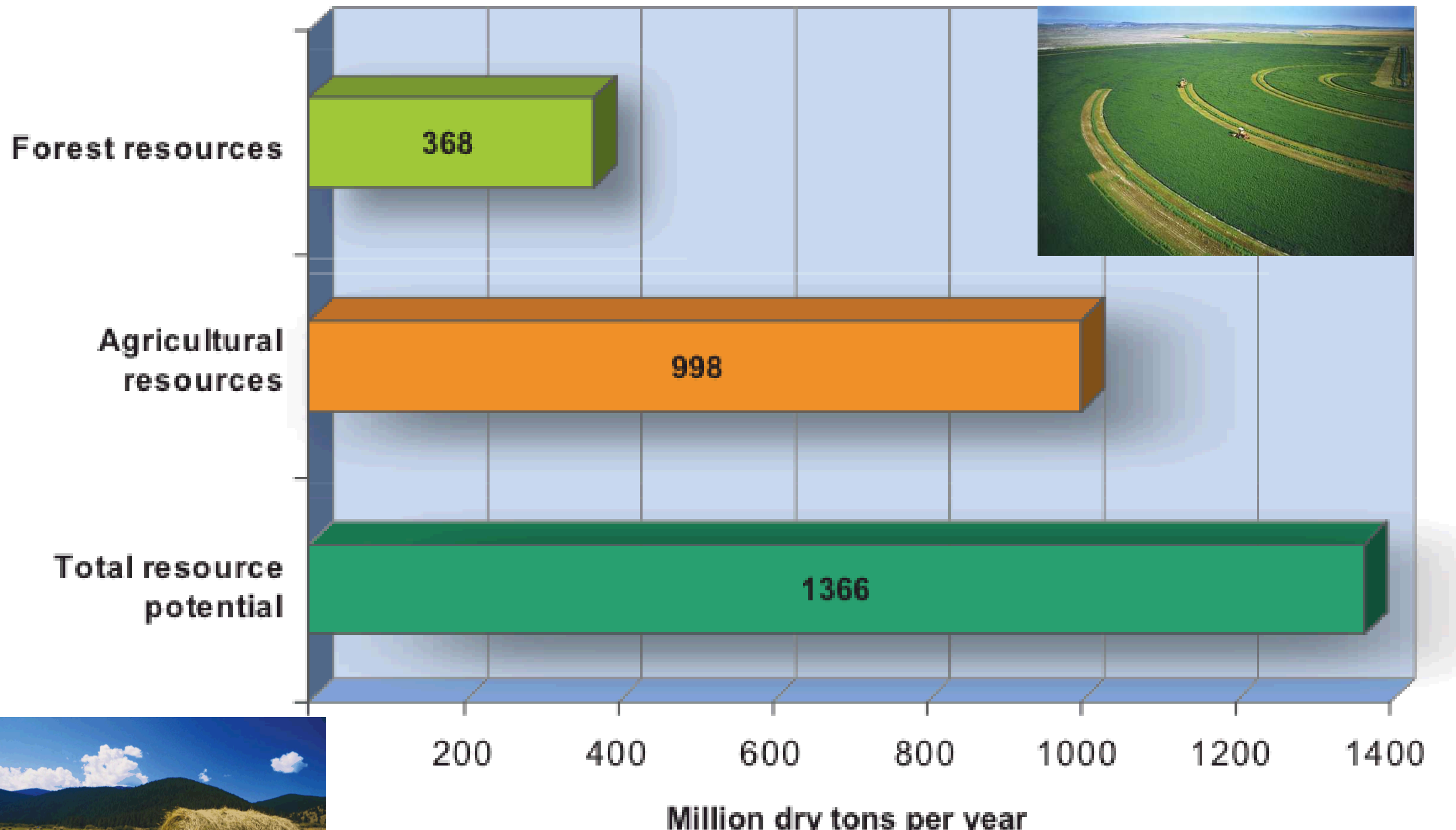
SIGNIFICANT WEALTH TRANSFERRED FROM U.S.



US Census Bureau

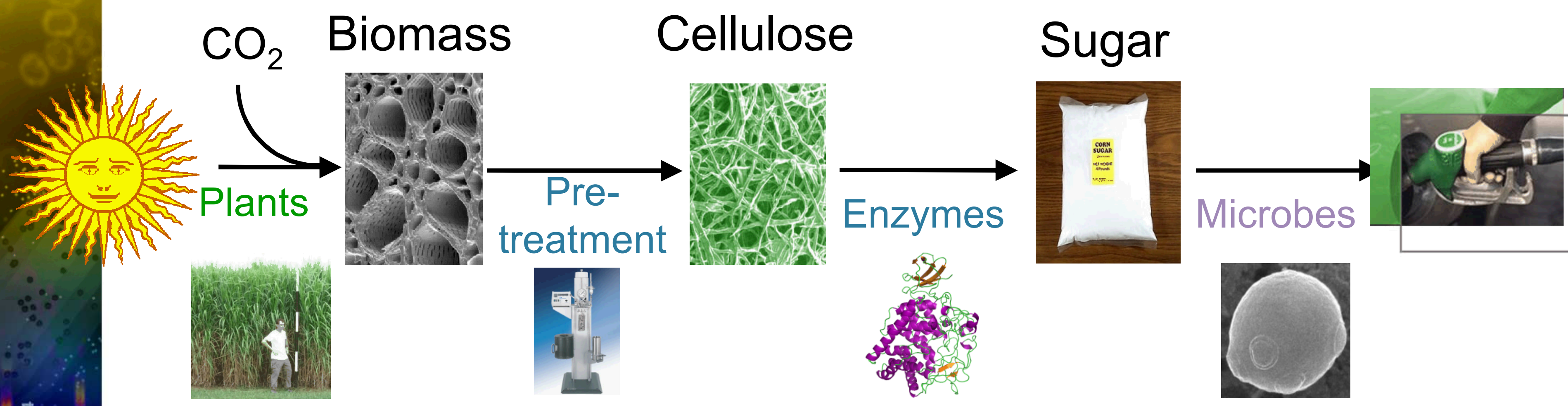
Displacement of Petroleum is Critically Needed

1.3 Billion Tons/Yr of Biomass in U.S.



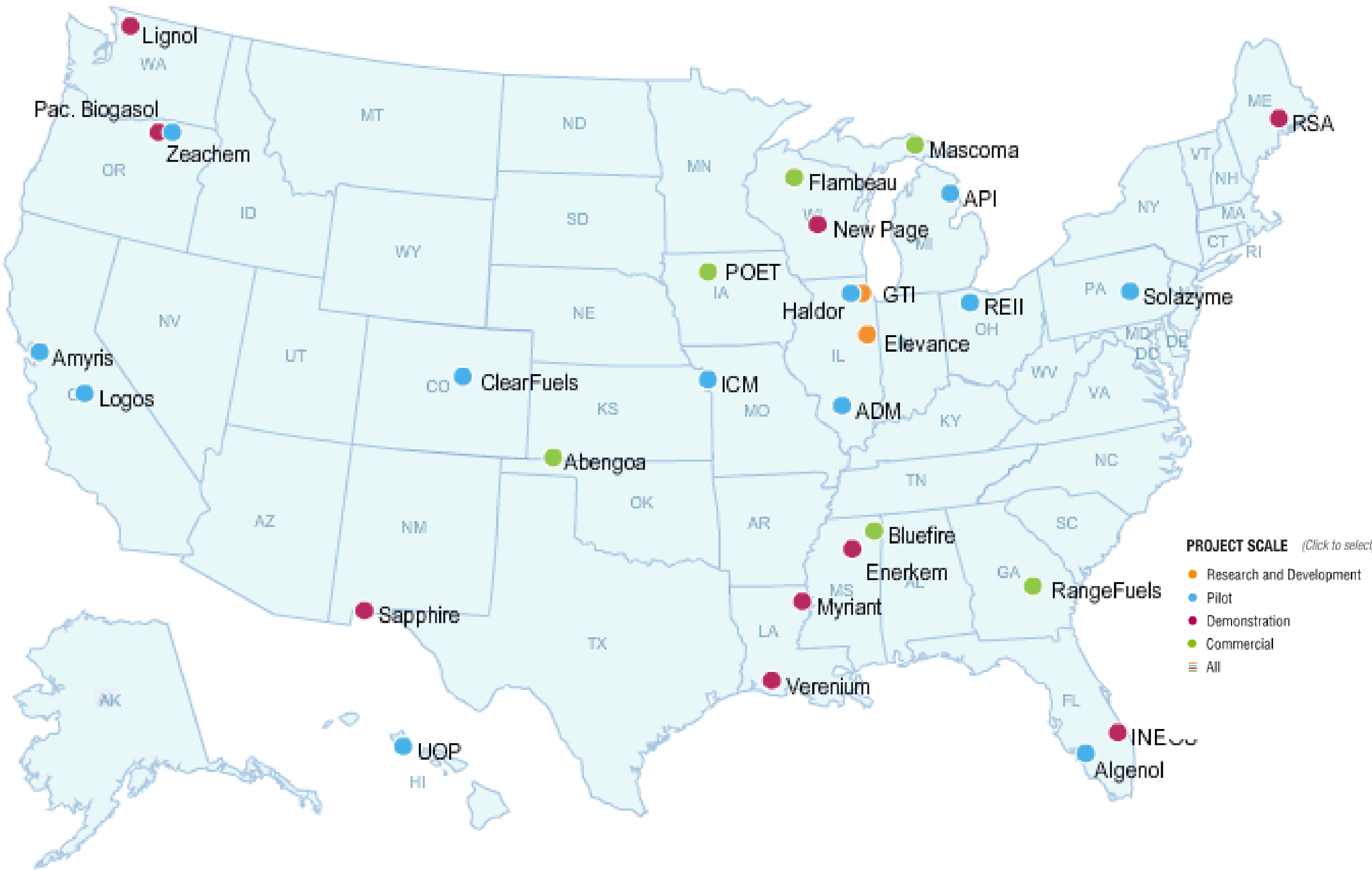
DOE Billion Ton Vision (2005)
- Perlack et al.

BIOFUELS FROM LIGNOCELLULOSIC BIOMASS



POTENTIALLY DISPLACING 30-40% OF U.S. TRANSPORTATION FUEL

DEPT OF ENERGY INVESTING IN 27 LC BIOFUELS PROJECTS



Developing Next Generation BioFuels: Joint BioEnergy Institute (JBEI)

Unified Research & Operations

- $134M, 5-year DOE OBER program
- Highly focused research agenda
- Single operation and facility

Six Partners

- Three DOE National Laboratories
- Two Universities
- One Foundation

Berkeley Lab | Sandia National Laboratories | Lawrence Livermore National Laboratory | UC Davis University of California | Carnegie Institution for Science

Four Science Divisions

- Feedstocks
- Deconstruction
- Fuels Synthesis
- Cross-cutting Technologies

Industry Partnership Program

- Underpin growth of biofuels industry
- Ensure technology transfer to the developing biofuels industry

FEEDSTOCKS DIVISION

Goal

- Establish scientific basis for breeding plants that can be economically and efficiently processed to produce fermentable sugars

Approach

- Understand biosynthesis and function of matrix polysaccharides and lignin
- Use this knowledge to make targeted modifications of the plant cell wall

Model Plant Systems

- *Arabidopsis*, rice, switchgrass

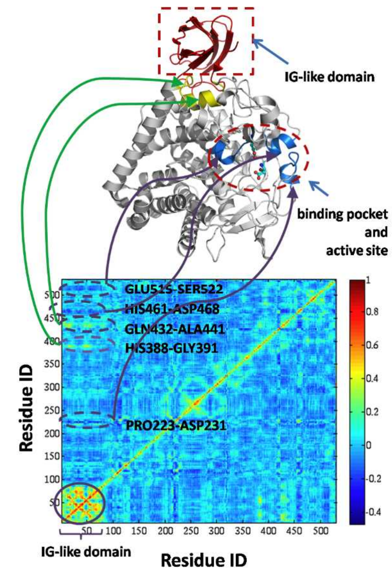
DECONSTRUCTION DIVISION

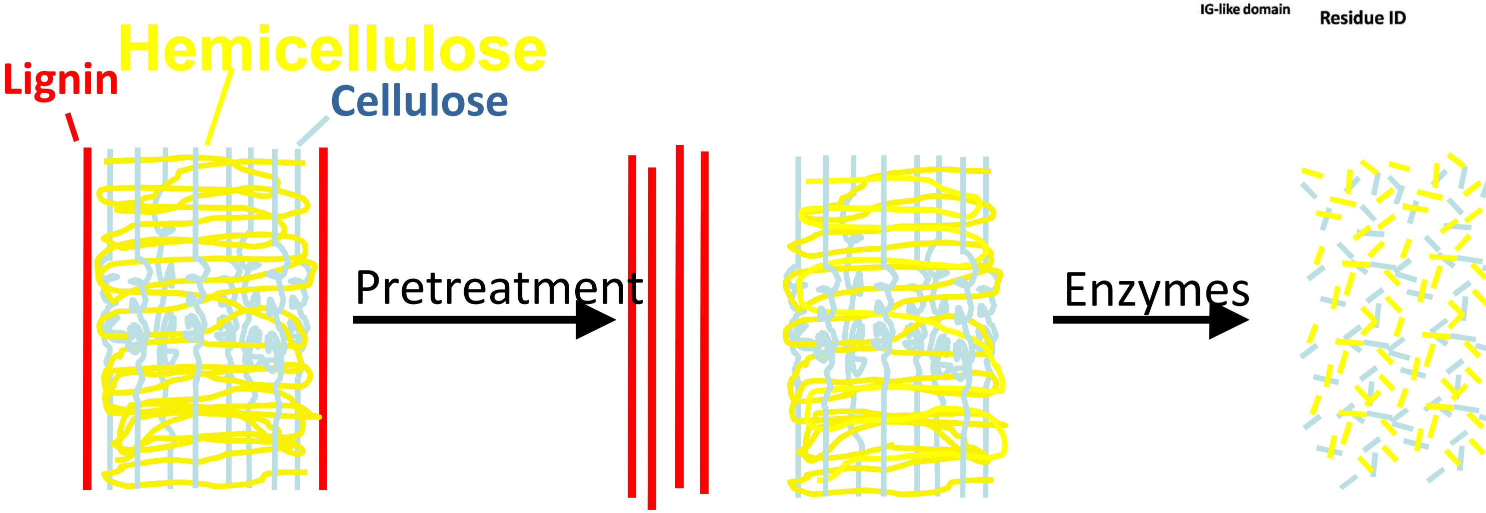
Goal

- Develop effective methods for biomass deconstruction

Approach

- Understand chemical and structural changes to biomass during pretreatment
- Develop new, highly-effective solvent-based deconstruction





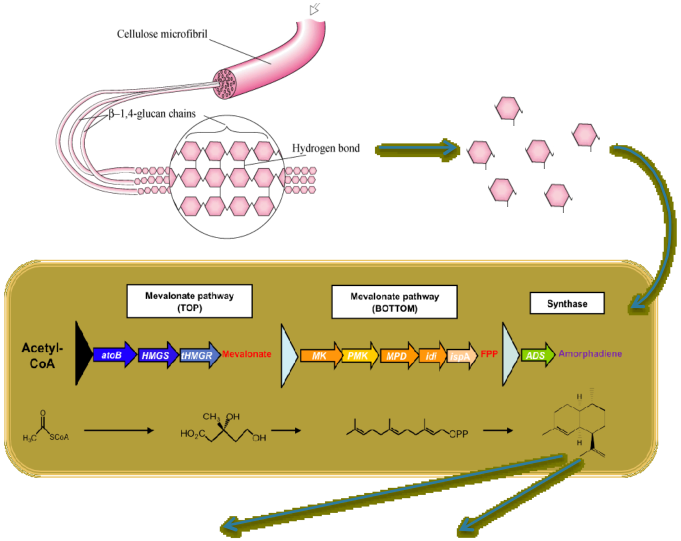
Fuels Synthesis Division

Goal

- Engineer microbes to produce advanced biofuels from biomass monomers

Approach

- Develop tools for synthetic biology for biofuels production
- Engineer microorganisms to consume both C_5 & C_6 sugars
- Develop metabolic pathways for advanced biofuels
- Engineer organisms to produce and withstand high concentrations of biofuels

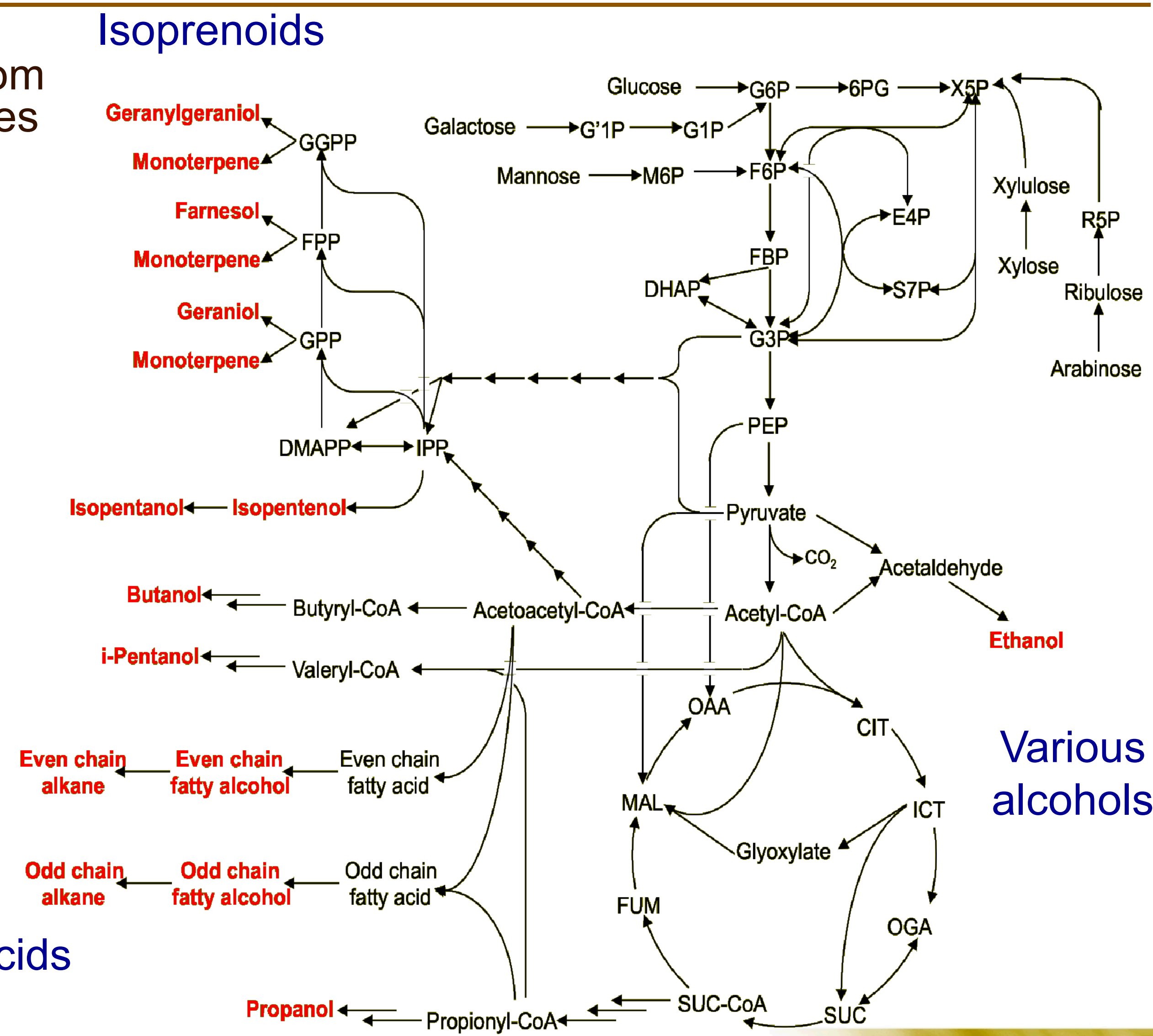


Model organisms

- *Escherichia coli*
- *Saccharomyces cerevisiae*
- *Sulfolobus sulfataricus*

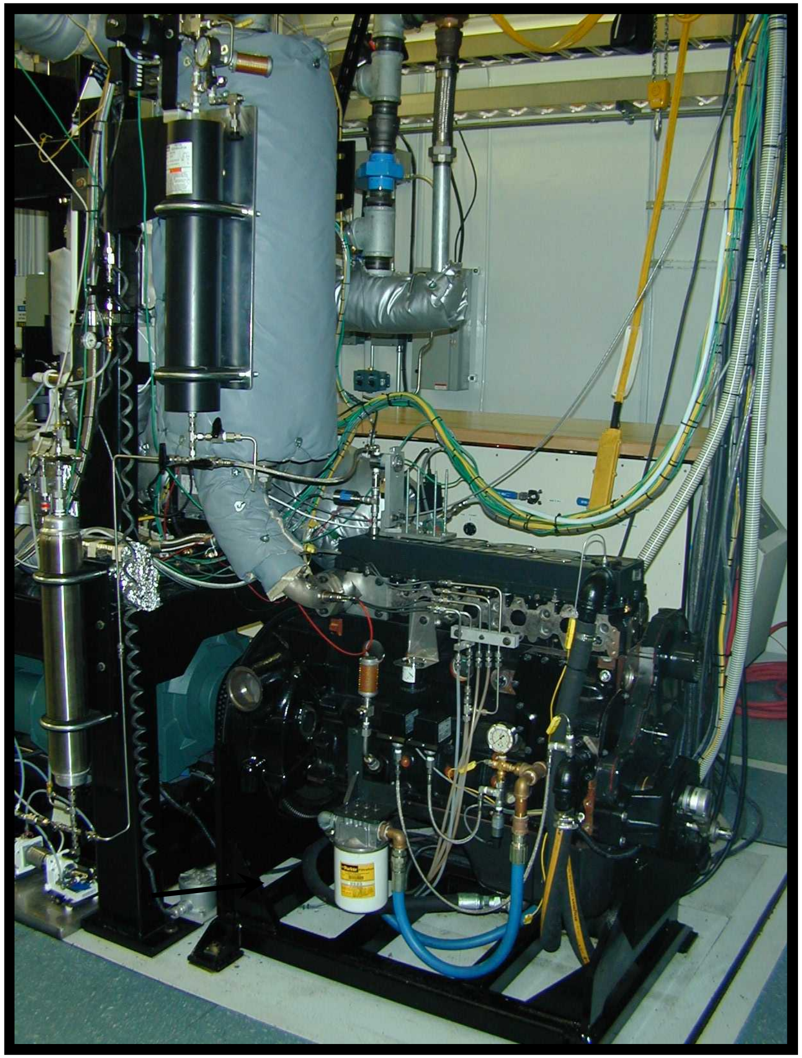
Some alternatives to ethanol

- Potential fuels from central metabolites
  - Alkanes
  - Alcohols
  - Esters
- Bio-gasoline
- Bio-jet fuel
- Bio-diesel



Advanced Biofuel Testing in Advanced Engines

- Biofuel tests conducted in a Homogeneous Charge Compression Ignition (HCCI) engine.
  - HCCI is an advanced low-temperature combustion (LTC) process that can provide <u>high efficiency and ultra-low NOx and particulate emissions</u>.
  - HCCI is being widely studied by R&D groups worldwide as a candidate for future high-efficiency engines.
  - Results are also indicative of performance in spark-ignition and diesel engines.



Combustion Research Facility, SNL

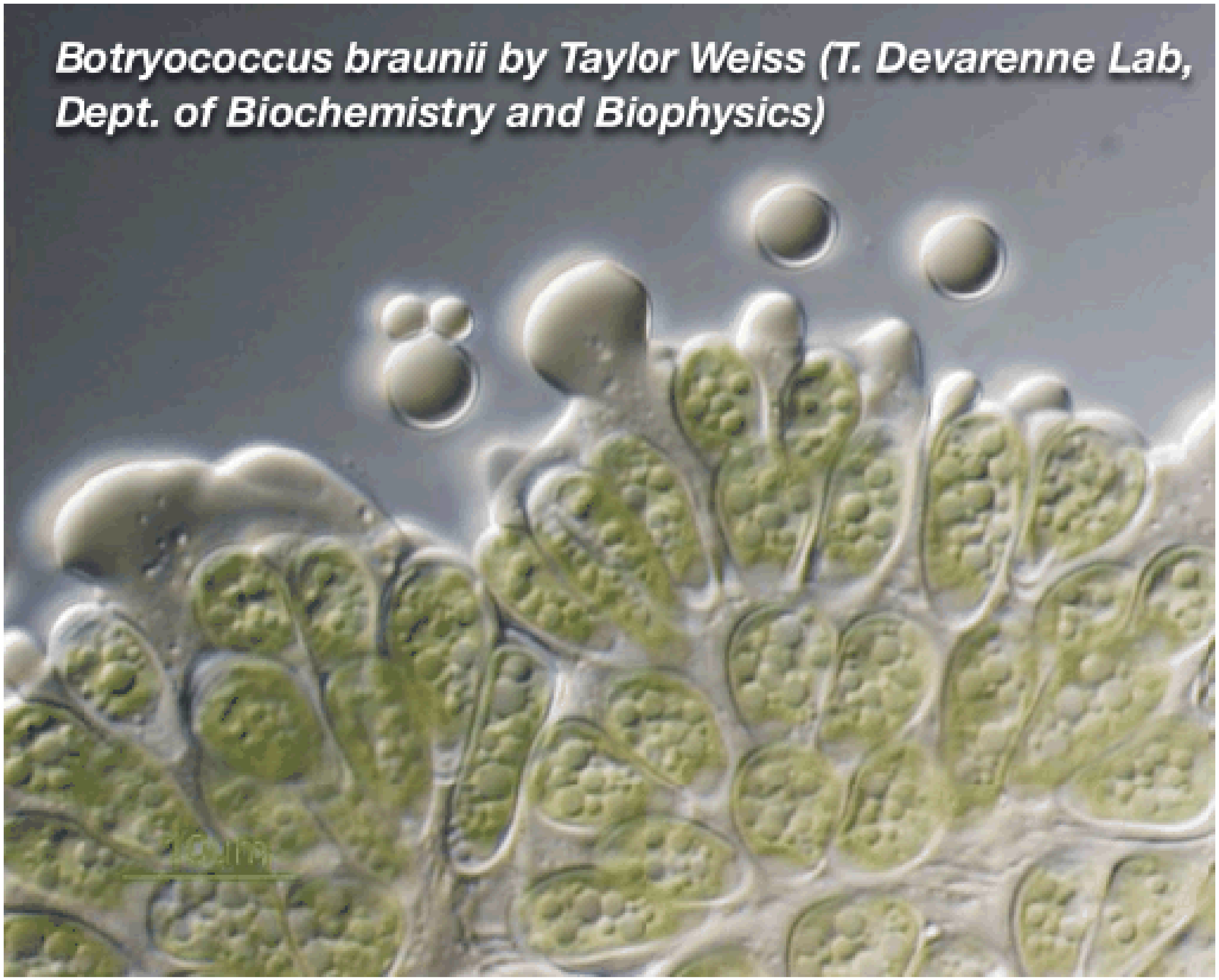
JBEI ACCOMPLISHMENTS

- 115 Publications
- 270 Presentations
- 43 Technical Disclosures
- 18 Patent Applications; 2 Software Copyrights Registered
- Hosted 8 Visiting International Researchers (Chile, Brazil, Sweden, Australia, New Zealand)
- 34 Student Interns and 13 Graduate Students
- Hosted two high school student summer outreach programs
- Participated in Cleantech to Market Program at Haas School of Business 2008-2010

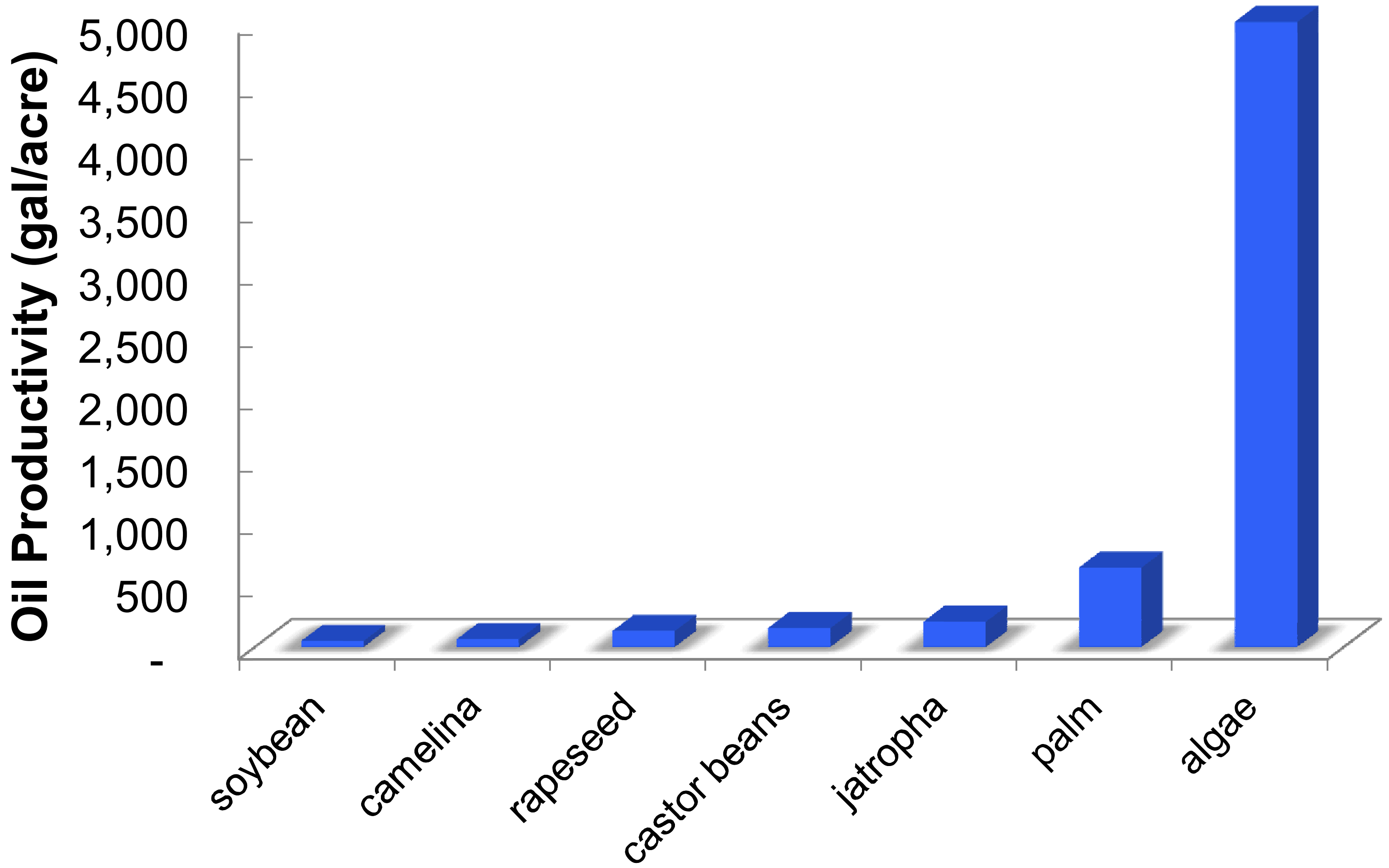
Lignocellulosic Biofuels Potentially Displaces 30-40% of US Transportation Fuel

What Next?

Renewable Oil from Algae



Spectacular Productivities



Algae productivity from Kristina Weyer, "Theoretical Maximum Algal Oil Production", 2008 Algae Biomass Summit. Other data taken from Joshua Tickell, "From the Fryer to the Fuel Tank" (2003)

ALGAE CONVERSION TO DROP-IN FUELS

	Petroleum ULSD	*Biodiesel (FAME)*	*Green Diesel*
Oxygen Content, %	0	11	0
Specific Gravity	0.84	0.88	0.78
Sulfur content, ppm	<10	<1	<1
Heating Value MJ/kg	43	38	44
Cloud Point, °C	-5	-5 to +15	-30 to -10
Distillation, °C	200 to 350	340 to 355	265 to 320
Cetane	40	50-65	70-90
Lubricity	Baseline	Good	Baseline
Stability	Baseline	Poor	Baseline

Jennifer Holmgren - UOP LLC, "Refining Biorenewables with the UOP/ENI Ecofining™ Process" (2007)

Algae Do Not Need Fresh Water

Brackish water for algae

Fresh water for crops

ALGAE DO NOT NEED ARABLE LAND

Non-arable lands for algae

Arable land for crops

ALGAE STATE OF TECHNOLOGY

NBT (Israel) – founded in 1975

Earthrise (CA) – founded in 1976

- 10,000 tons/year of algae produced globally for nutraceuticals and food
- Production costs $5-30/kg algae

Top Challenges for Algae

- Produce cheap oily algae (~$0.50/kg)
  - Mitigate pond crashes
    - Forensics to identify etiological agents
    - Diagnostics for early detection
  - Maximize biomass production
    - CFD modeling guides reactor design and operations
- Harvest algae inexpensively
  - Effect bioflocculation through mechanistic understanding of triggers
- Oil extraction from wet algae
- Maximize value of co-products
  - Algae residue conversion to fuels and nutrients

SANDIA CORE CAPABILITIES IN ALGAE

- ***Spectroscopy*** for broad area interrogation of algae pond productivity and health.
- ***Applied Biology*** for GMOs, nutrient and CO_2 use, and laboratory to pond scale-up.
- ***Metabolic Engineering*** for enhanced biofuel production.
- ***Harvesting, De-watering, and Extraction*** technologies.
- ***Techno-Economic Modeling*** to inform technical and policy decisions.

Scale Matters in Algae Production

Ideal Conditions

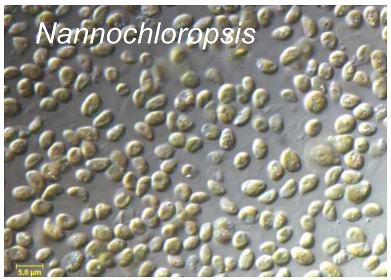
Reality

Reality introduces:

- Predators
- Light intensity variations
- Temperature cycles (daily and seasonal)
- Mixing

Challenges being bottom of the food chain

Algae



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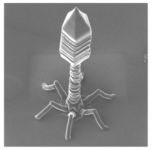
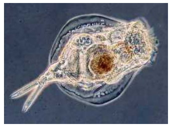
Etiological Agents

Virus

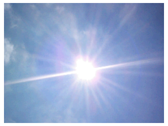


Bacteria

Zoo-plankton



Environment (Temp, salinity, pH, CO2, nutrients)



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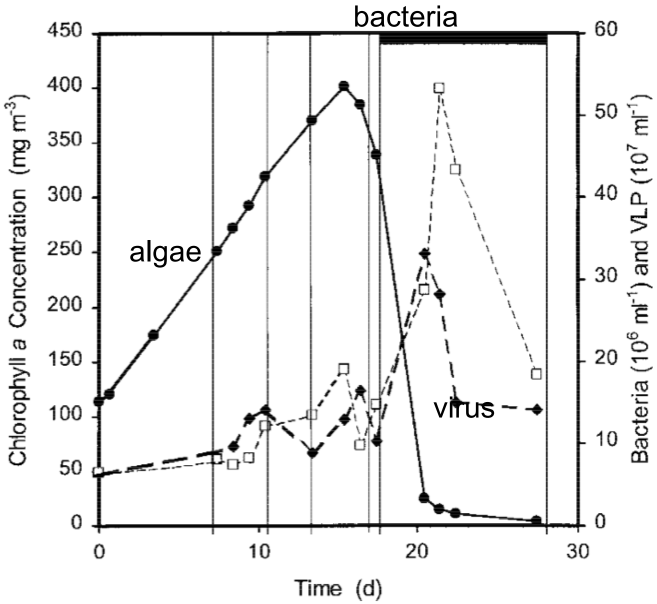
Collapse



Herman Gons et al., Antonie van Leeuwenhoek, 81: 319-326, 2002.

"Perhaps the most worrisome component of the large-scale algal cultivation enterprise is the fact that algal predators and pathogens are both pervasive and little understood."
- DOE Draft Algal Biofuels Technology Roadmap (2009)

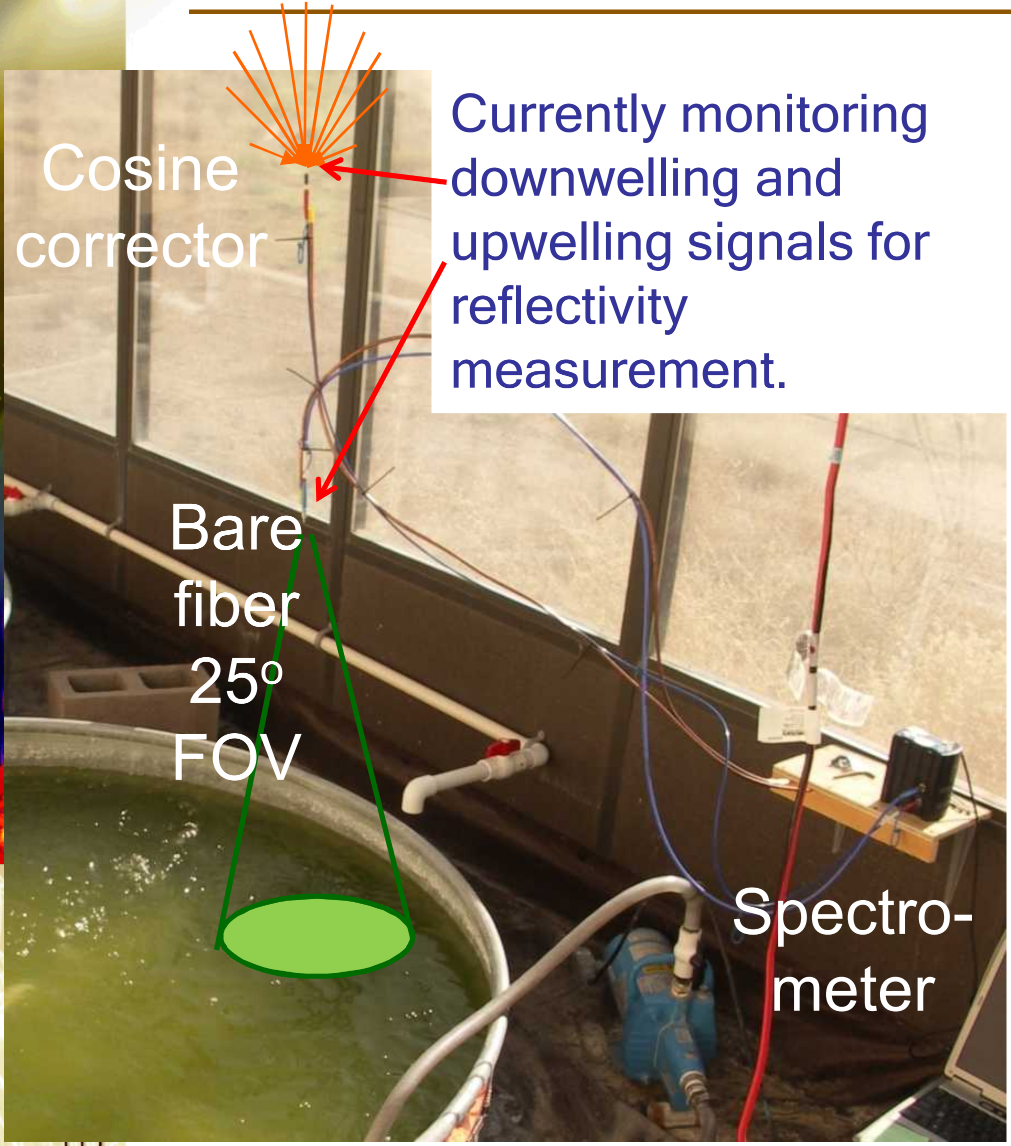
Lane et al. (2010)

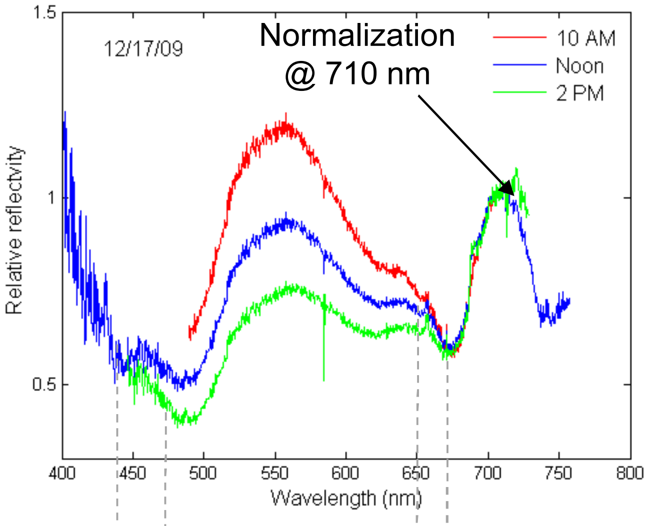
Sandia has Lab-scale Cultivation Systems that Enable Algae Biology and Scale-up Research

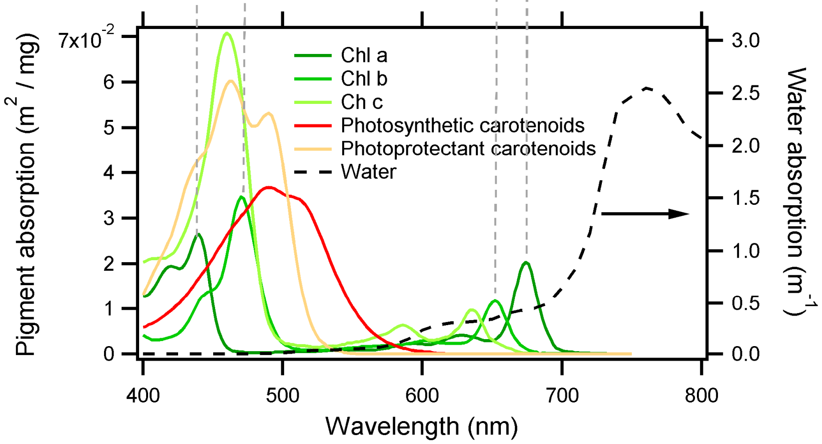
Species being grown:
- ***Botryococcus braunii***
- ***Neochloris oleoabundans***
- ***Chlamydomonas reinhardtii***
- ***Thalassiosira pseudonana***
- ***Nannochloropsis***

LABORATORY AND REMOTE SENSING SPECTRA ARE BEING USED TO DEVELOP EMPIRICAL CORRELATIONS FOR SCALE-UP

Currently monitoring downwelling and upwelling signals for reflectivity measurement.

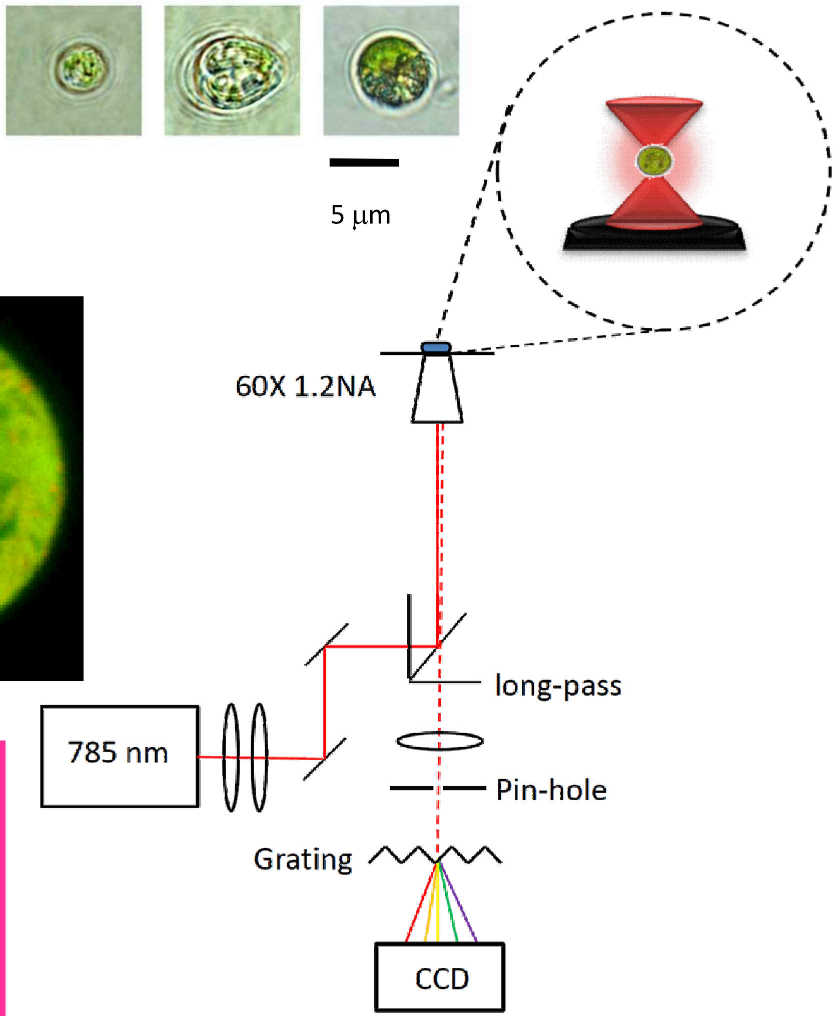


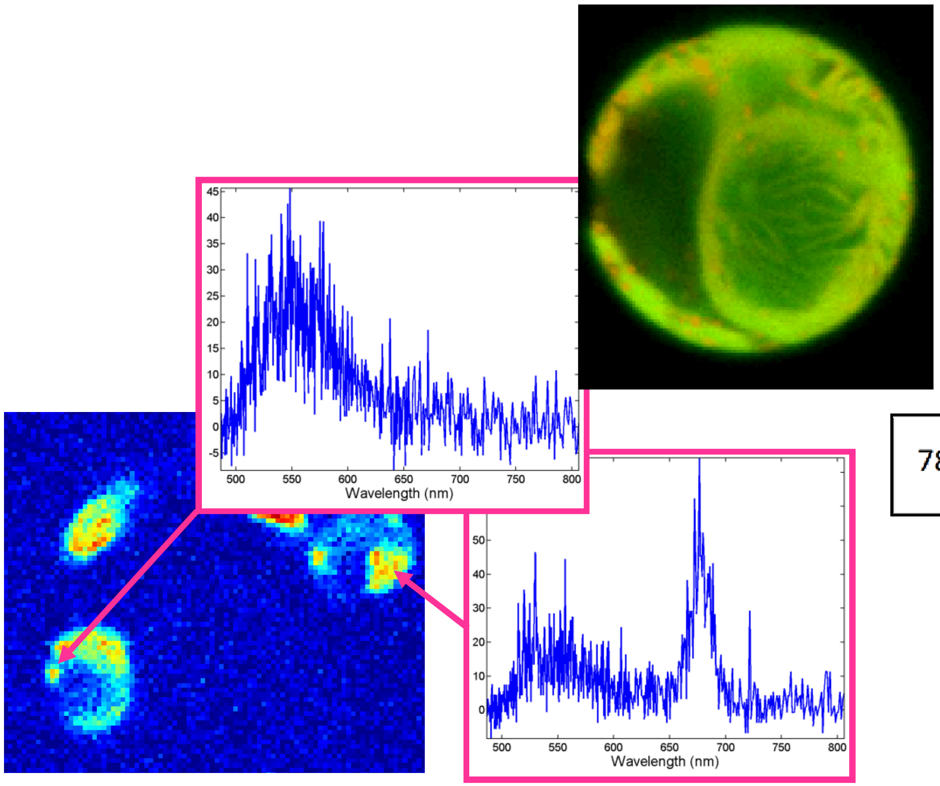




WE HAVE DEVELOPED NOVEL LIPIDOMICS CAPABILITIES

We have developed laser trapping Raman spectroscopy unit for single cell observations of lipid accumulation



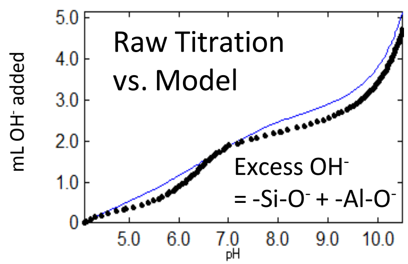


LDRD PI: Seema Singh

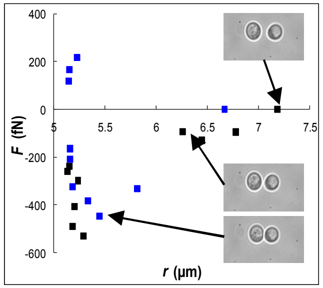
NSF SBIR w/BaySpec

We are Developing Novel Algal Dewatering Technologies

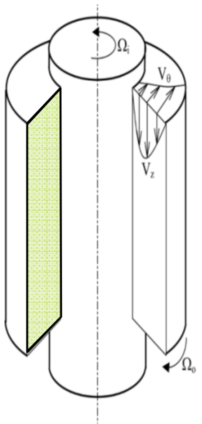
Surface potential measurements



Surface interaction force measurements

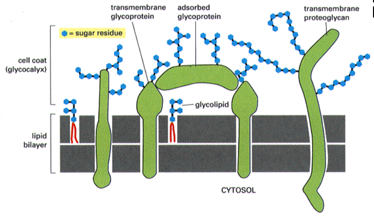


Controlled flow population dynamics measurements

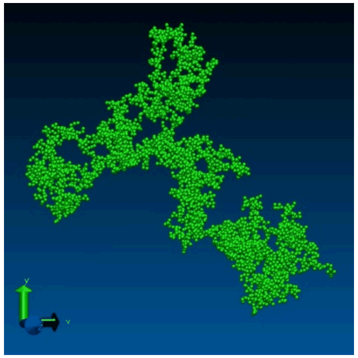


Predicting and optimizing floc dynamics at scale

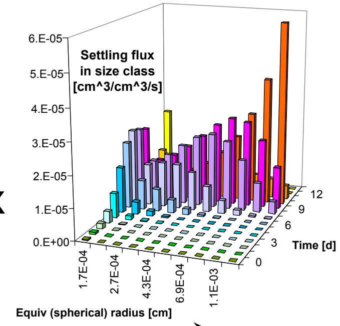
Surface complexation models



Floc aggregation simulations



Predict size and flux distributions



nanometer | micrometer | millimeter | meter

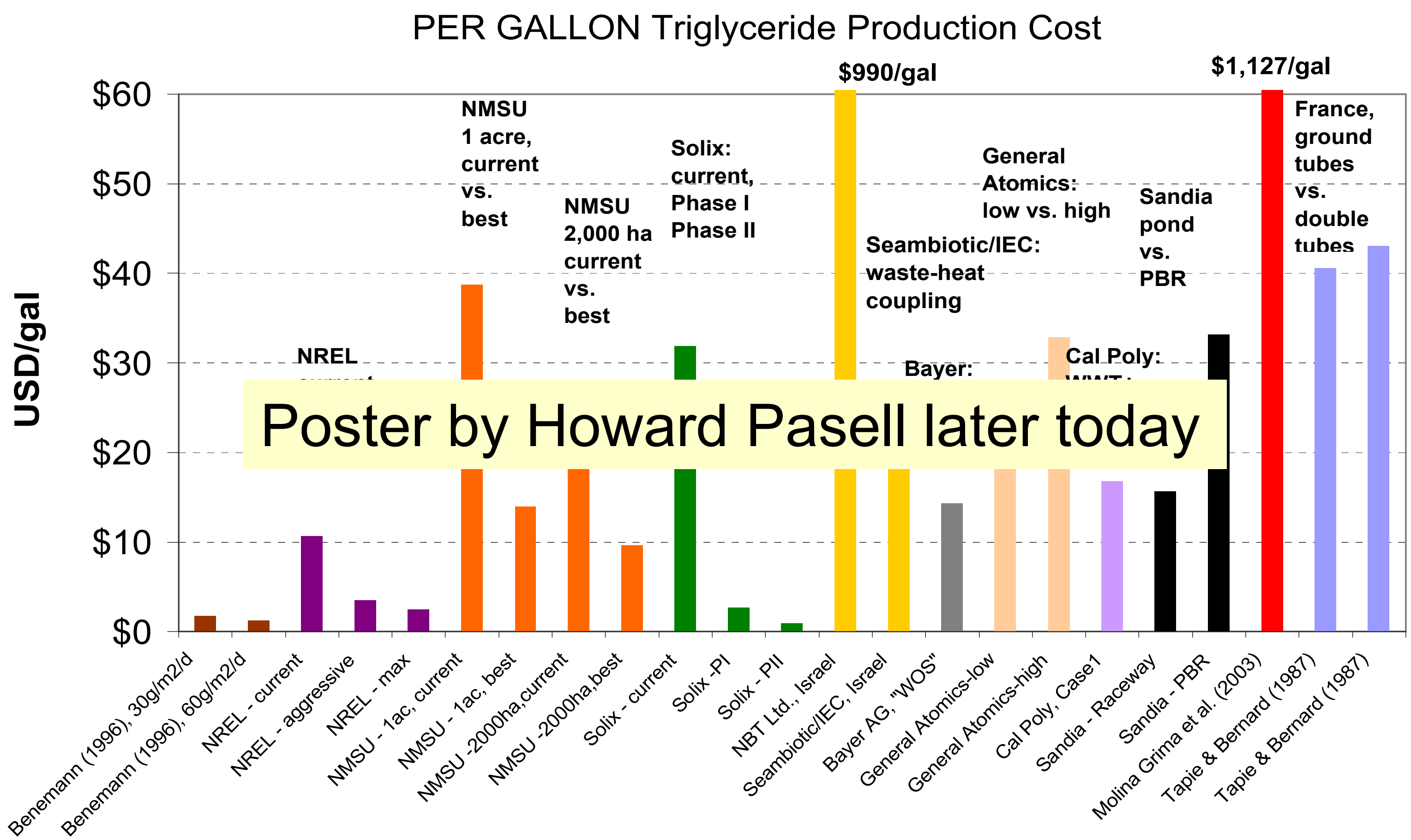
LDRD PI: John Hewson

Summary

- Biofuels represent an enormous potential for displacing petroleum
- Technical and economic challenges remain for both lignocellulosics and algae
- Sandia plays an integral role in overcoming these barriers

Back-up Slides

No Consensus Exists on the Feasibility of Algal Biofuels

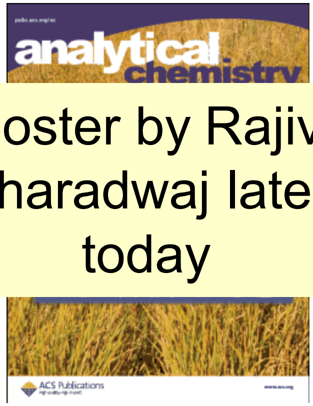


Average = $ 109 USD/gal

Variability is wide, Std. Dev. = $ 301 USD/gal

Biofuels Program Accomplishments – FY10

- Continuing scientific success at JBEI; related program growth and recognition
- JBEI received "Top Government Funded Research Institution in Biofuels" Award – Biofuels Digest, December, 2010
- Sustainable Algal Biofuels Consortium funded at $6M (ASU-led), $1.2M to SNL
- Algal pond crash forensics project funded by DOE OBP at $800k over two years
- Participation in algal biofuels EFRC
- Awarded NSF SBIR on algal lipidomics with BaySpec
- New start LDRD ($1M/yr for 3 years) on establishing a link between biofuels and combustion science



Poster by Rajiv Bharadwaj later today

Biofuels Program: Strategic Relationships and Programs

jbei Joint BioEnergy Institute

SABC Sustainable Algal Biofuels Consortium *cultivating energy solutions*

Berkeley Lab

ASU

NREL

Sapphire Energy

DSM

MIT

Montana State University

Monsanto

Pacific Northwest National Laboratory

Community Power Corporation The Modular Biopower Company

GM

POET Energy inspired.

GE imagination at work

novozymes Rethink Tomorrow

CU

Lockheed Martin

Identify etiological agents and abiotic factors to limit crashes

- Limited success using
  - Extremophiles
  - Photobioreactors
  - GMO
- Our approach
  - Early detection
  - ID genetic signatures
  - Isolate agent(s)
  - Confirm crash in lab
  - Develop mitigation strategy

Solix Enclosed PBRs

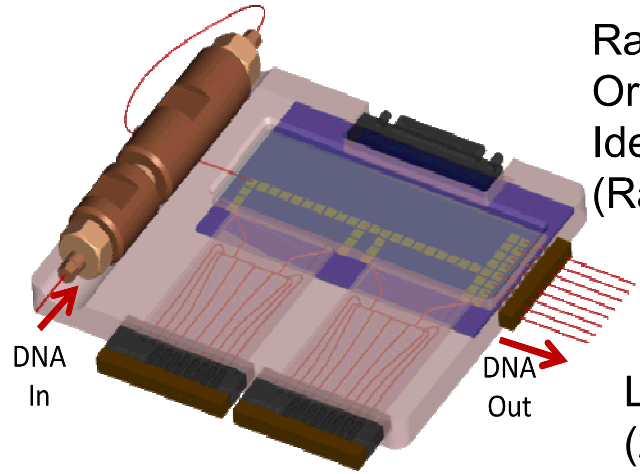
Lane et al. (2010)

SANDIA BIOSCIENCE

Sandia National Laboratories

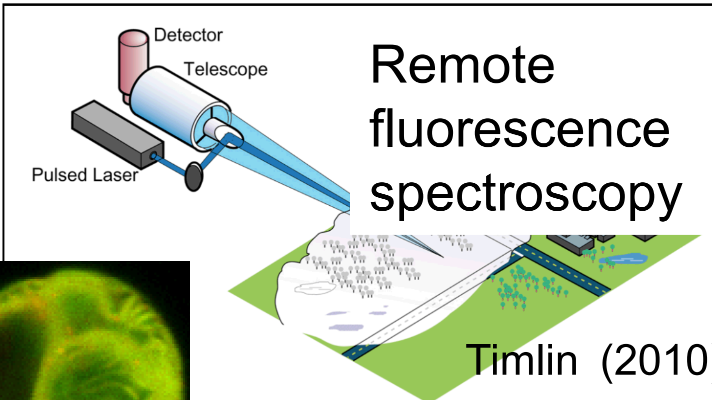
Adv. diagnostics for early agent detection and identification

- Microfluidics for ID of agents in complex samples
- Spectroscopy for stand-off culture diagnostics
- <u>Early detection and ID of agents is critical</u>



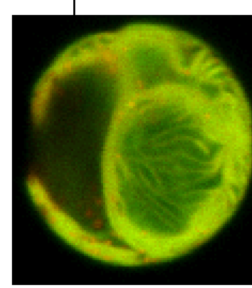
Rapid Threat Organism Identification (RapTOR)

Lane et al. (2010)



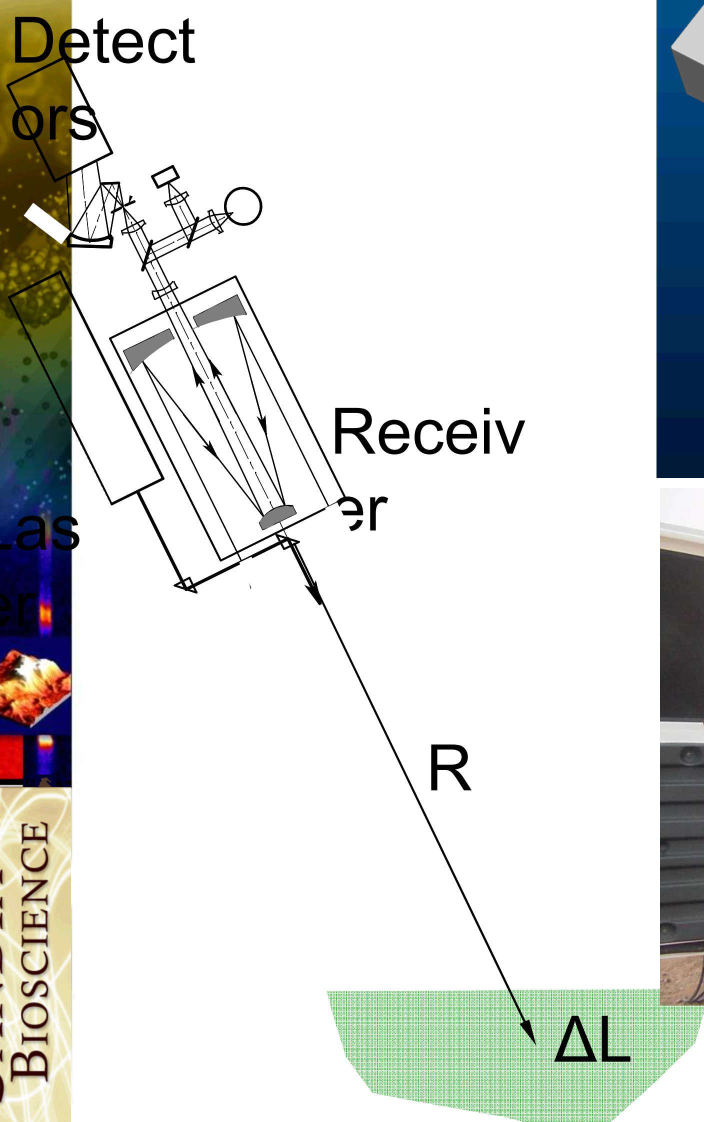
Remote fluorescence spectroscopy

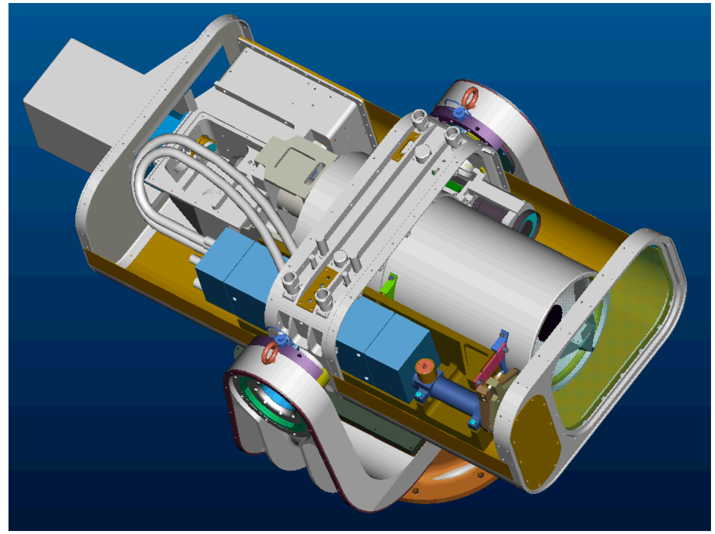
Timlin (2010)



Hyperspectral image of *Dunaliella*

Evaluating Utility of Deploying Fluorescence Lidar Instrument





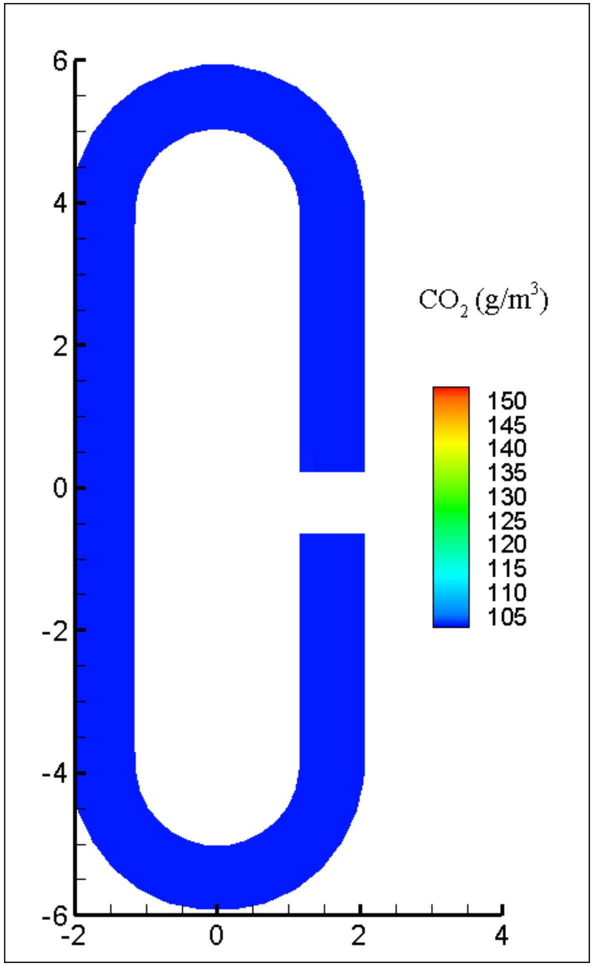
SANDIA BIOSCIENCE

CFD modeling to improve algae production

- Environmental Fluid Dynamics Code (EFDC)
- CE-QUAL couples kinetics with 22 variables

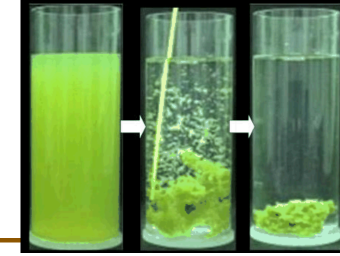
$$\frac{\partial}{\partial t} B(\mathbf{x},t) = \left(P - B_M - P_R - W_S \frac{\partial}{\partial z} \right) B(\mathbf{x},t)$$

- Inform engineering design, farm operations, and strain selection to maximize productivity at each site



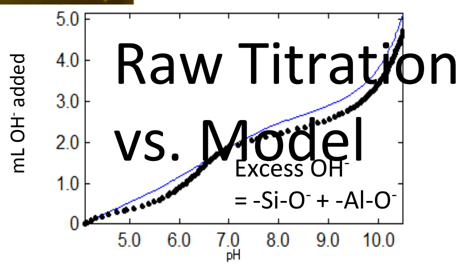
James and August (2010)

Flocculation mechanics



Wageningen University

Surface potential measurements



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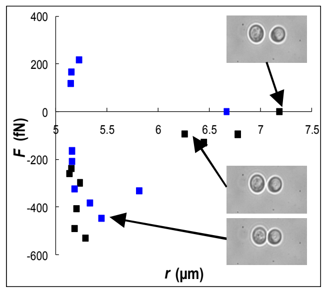

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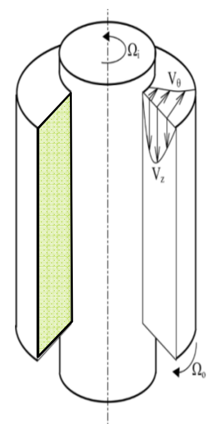

Predicting and optimizing floc dynamics at scale

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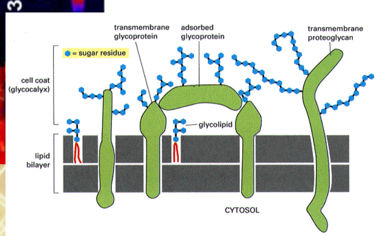

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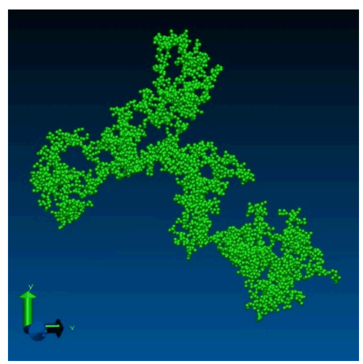

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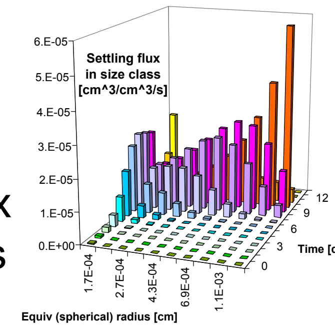


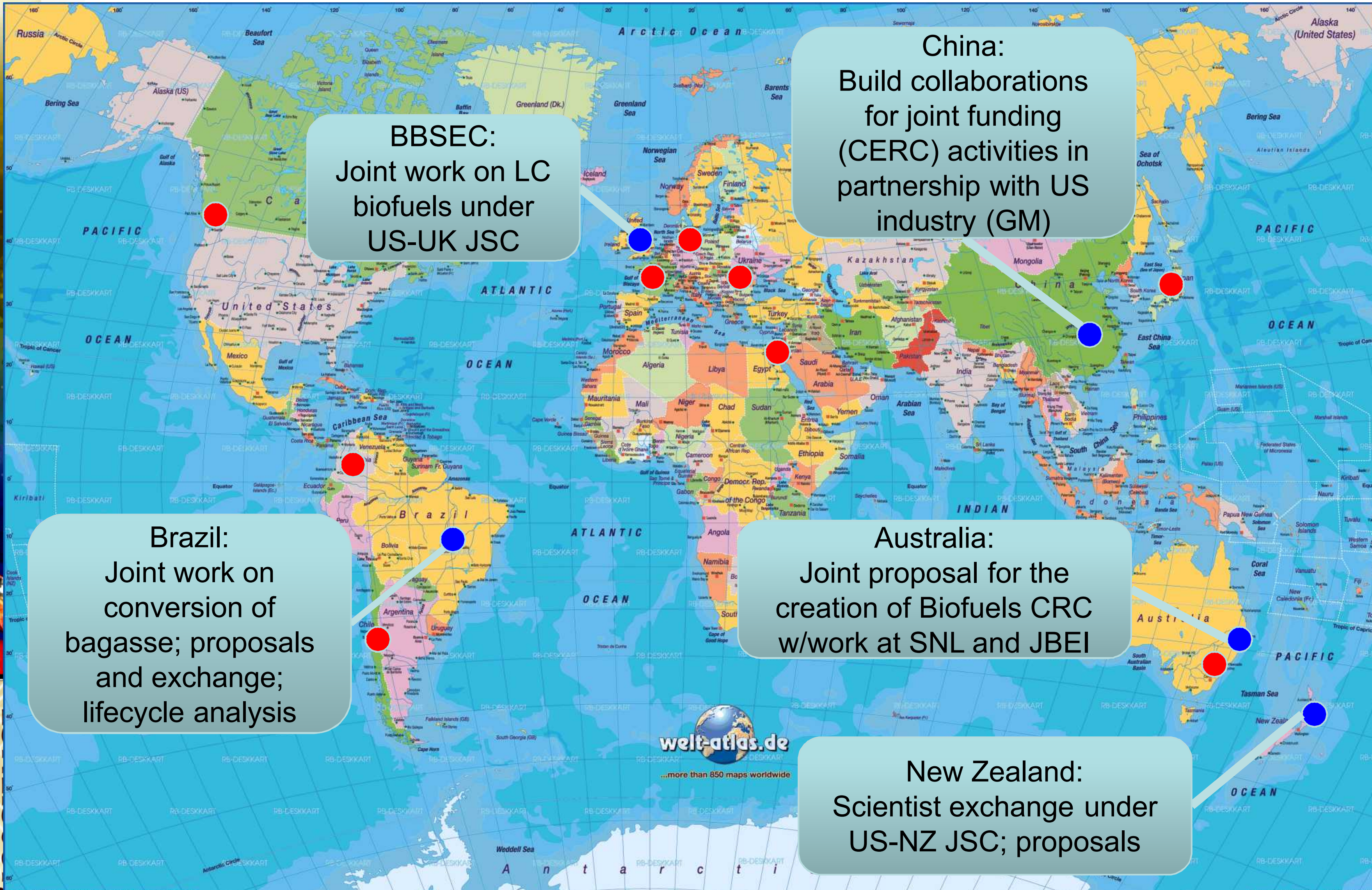
nanometer micrometer millimeter meter

Sandia Biosciences

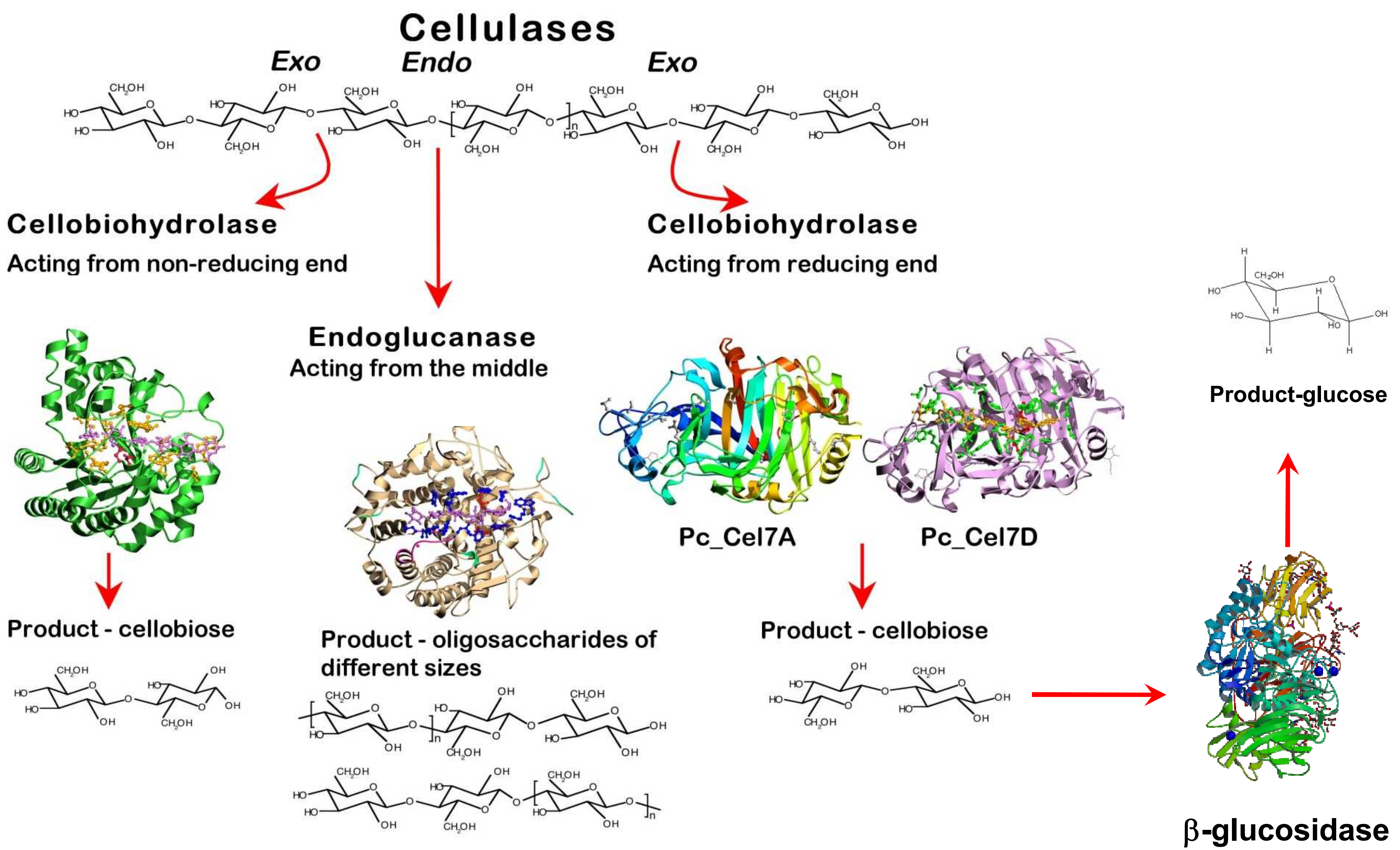
SNL Algae Projects

- Pond crash forensics (DOE)
- Integrated Biorefineries (Sapphire)
  - CFD Modeling, pond diagnostics, and CO2 reduction strategies
- Sustainable Algae Biofuels Consortium (SABC)
  - Biochemical conversion if algal residue and fuel testing
- Lab Directed Research and Development
  - Bench to raceway
  - Algae flocculation mechanism
  - Algae lipidomics
  - Algae growth kinetics modeling
  - Metabolic engineering of cyanobacteria and diatoms

KEY SNL GLOBAL BIOFUEL PARTNERSHIPS



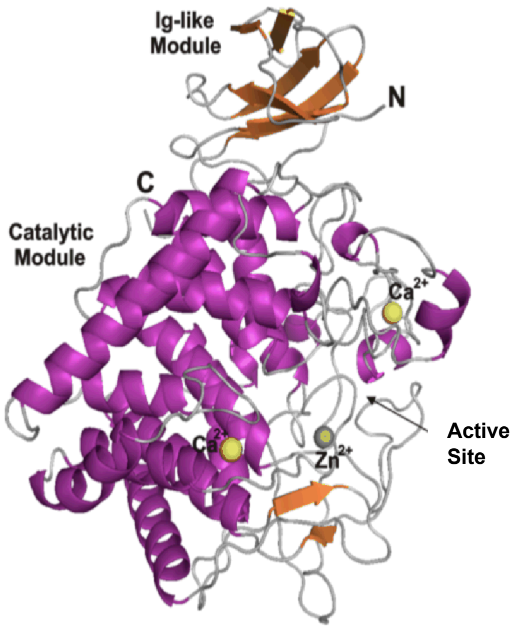
Cellulases Act Synergistically to Hydrolyze Cellulose to Glucose



Cellulase Research at JBEI

Objectives:

- Identify, discover and characterize cellulases that function in relevant biorefinery environments (e.g., high T, low pH).
- Optimize performance of individual cellulases.
- Optimize performance of recombinant cellulase cocktails.

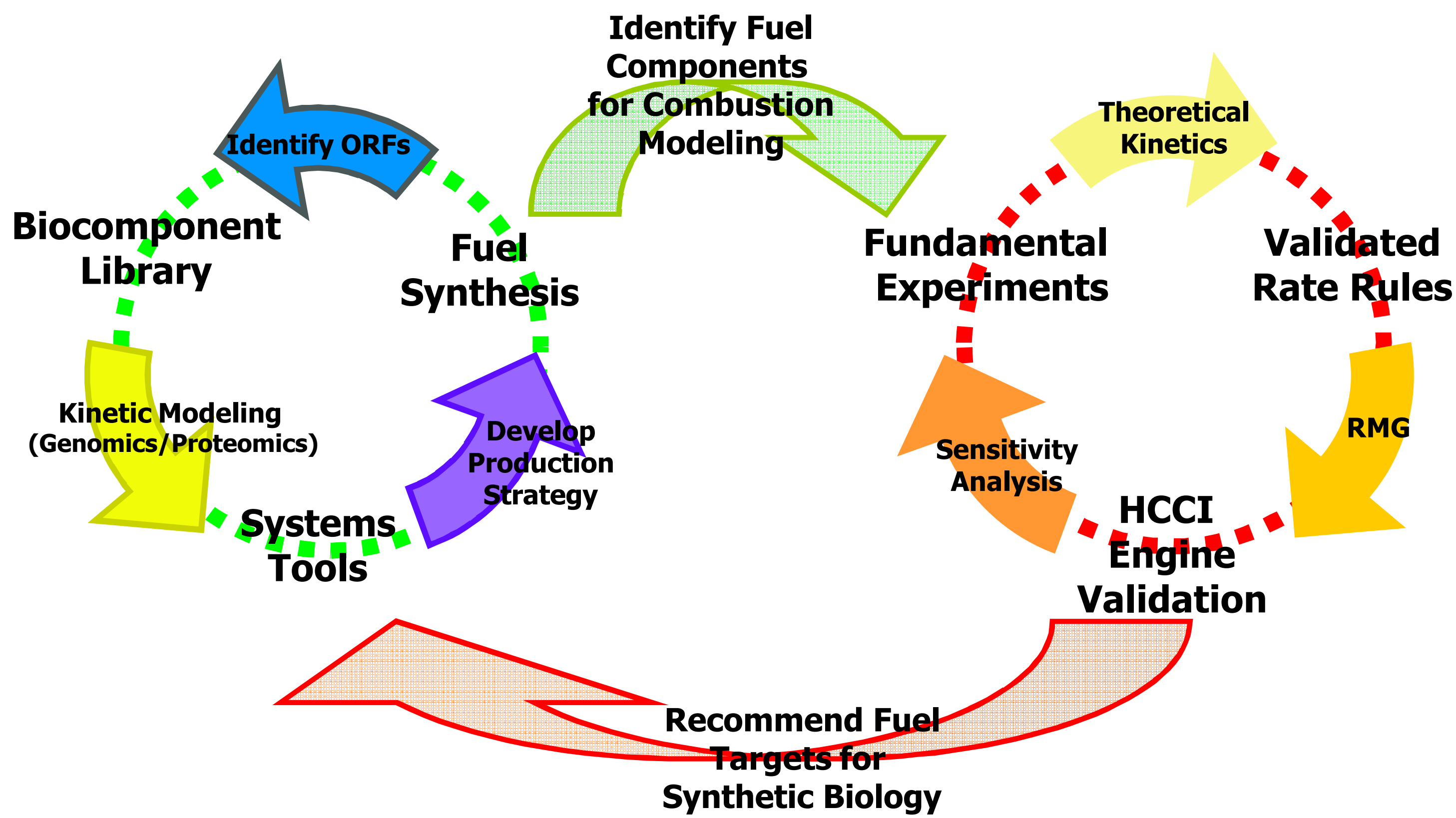


Example:

Cel9A from *Alicyclobacillus Acidocaldarius*

- Structure Determination
- Structural Characterization
- Structure Based Design
- Dynamics

Technical Goals of LDRD are Interconnected but Modular

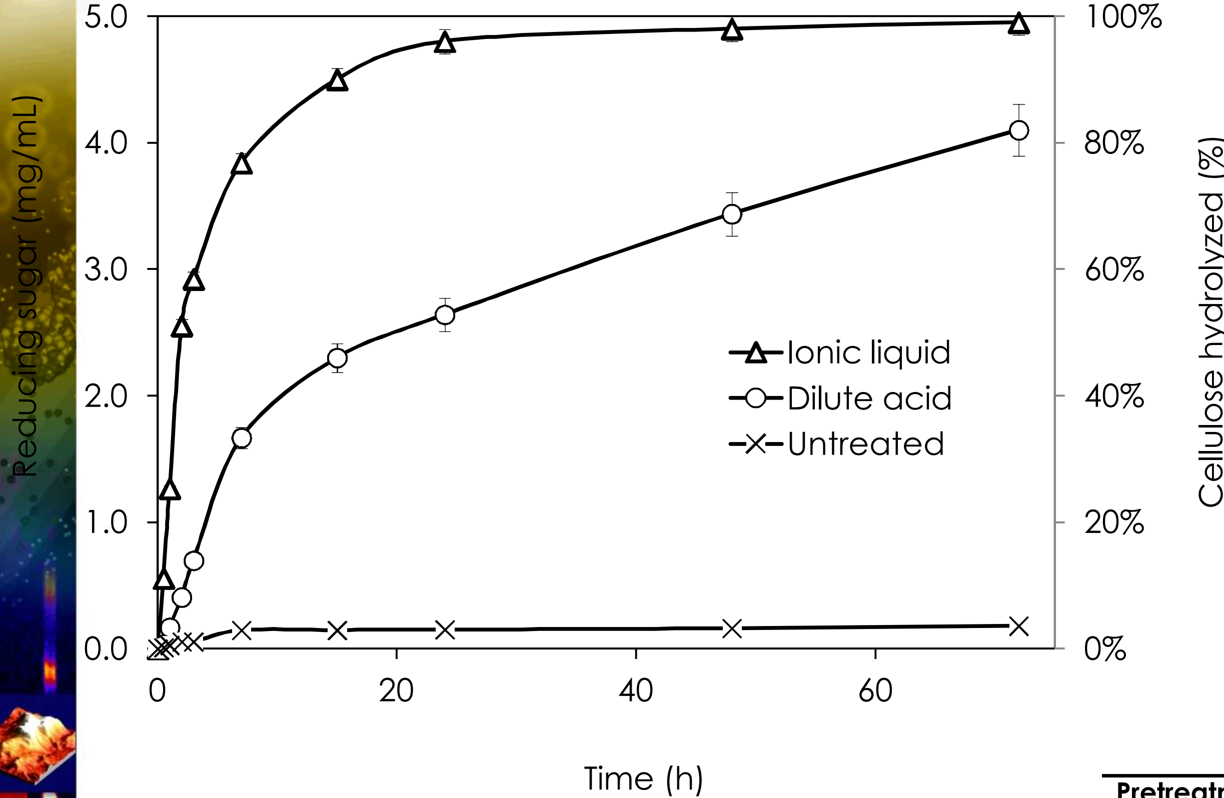


Starting points already set for both sides

Specific strategy in any area can be modified if necessary – "links" are transferable

Craig Taatjes *Masood Hadi* *John Dec*

Switchgrass Hydrolysis After IL Pretreatment has Improved Kinetics



Hydrolysis yield of 90% in ~16 hours after IL pretreatment

>90 hours to reach same level after dilute acid pretreatment

Novozymes commercial cellulase cocktails:

- ***5g glucan/L biomass loading***
- ***50mg protein/g glucan of cellulase (NS50013)***
- ***5mg protein/g glucan of β-glucosidase (NS50010)***

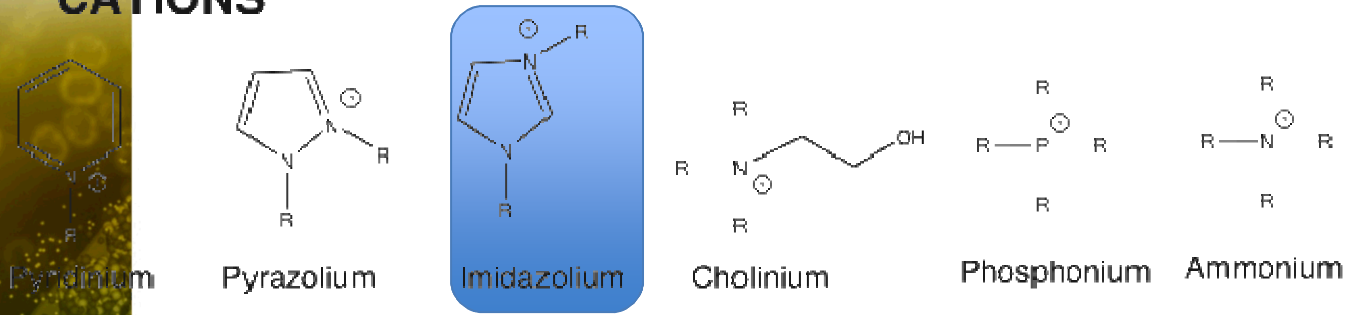
Pretreatment method	Initial enzymatic rate (mg/L/min)	Rate enhancement
Untreated	0.34 ± 0.03	-
Dilute acid	1.11 ± 0.12	3.3
Ionic liquid	18.50 ± 1.56	54.4

Li C. et al. *Bioresource Technology*, 2010, 101 (13) 4900-4906

IONIC LIQUIDS AS LIGNOCELLULOSE SOLVENTS

Room Temperature, Molten Salts

CATIONS



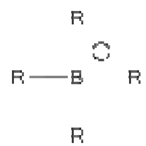
Cation determines:
- stability
- properties

Water immiscible ⟹ Water miscible

ANIONS

PF_6^- $NH(SO_2CF_3)_2^-$ RSO_4^- SCN^- $CH_3SO_3^-$ Cl^-

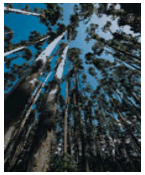
$CH_3CO_2^-$



Anion determines:
- chemistry
- functionality

1-ethyl-3-methyl imidazolium acetate, [C2mim][OAc], dissolves > 20 wt% cellulose

Understanding Biomass Deconstruction


Eucalyptus


Corn stover


Pine


Rice straw


Arabidopsis


Switchgrass

Feedstocks Division and USDA

↓ IL pretreatment

Solubilized Biomass

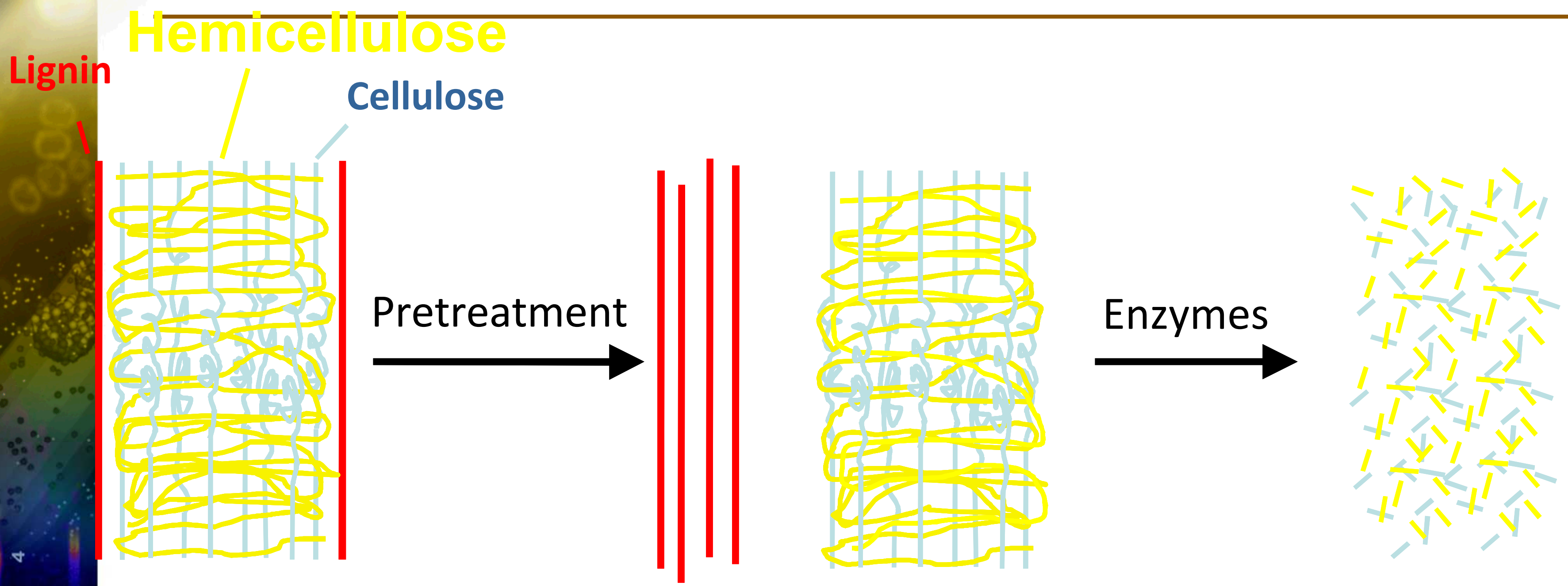
↓ Anti-solvent addition

Recovered Biomass

↓ Enzymatic hydrolysis

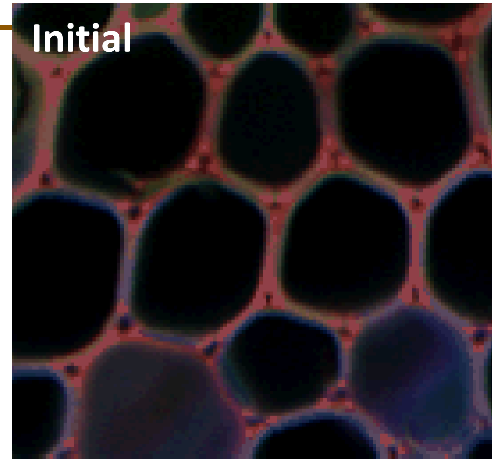
Hydrolysate

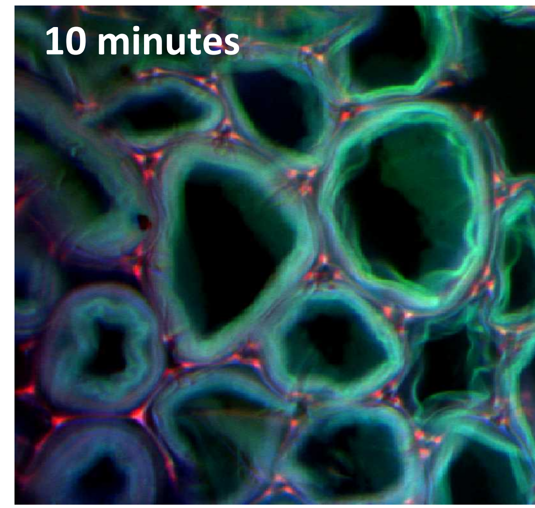
Deconstruction Goals



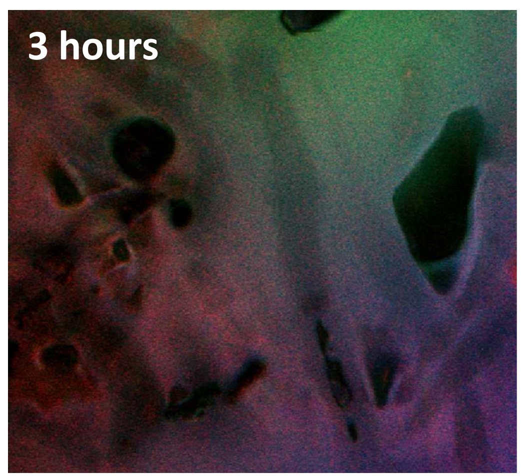
- Effective pretreatment that:
  - Delignifies biomass
  - Produces amorphous cellulose
- Improved lignocellulolytic enzymes:
  - Specific industrial environments
  - Targeted discovery of enzymes from microbial communities

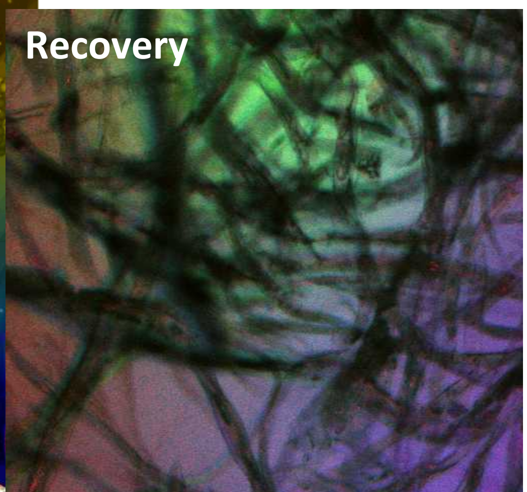
Confocal Fluorescence Provides Insight into IL Pretreatment

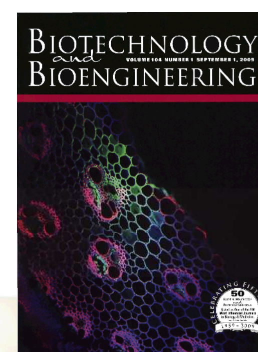




Switchgrass + [C2mim][OAc]



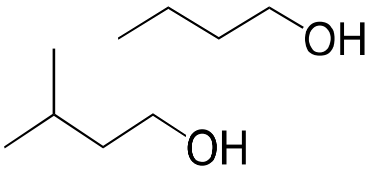


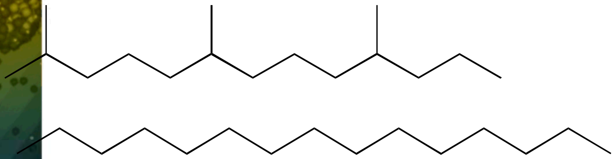


Singh, S.; Simmons, B.A.; Vogel, K.P *Biotechnology and Bioengineering,* 2009, 104(1), 68-75 – Featured Cover Article

Initial JBEI Biofuel Targets

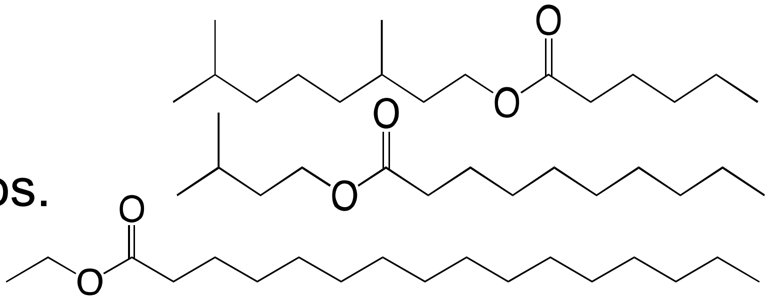
Alcohols
- Low miscibility with water
- High octane
- Appropriate vapor pressure

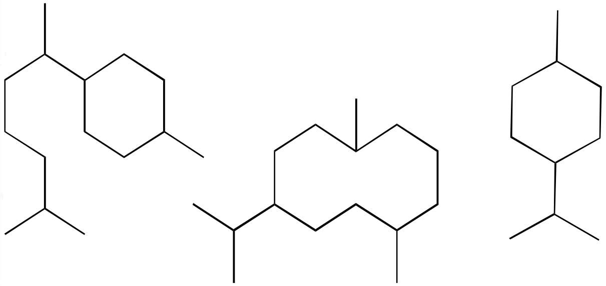




Alkanes
- Diesel (or gasoline) replacements
- Appropriate cetane (or octane) numbers

Esters
- Diesel replacements
- Appropriate cetane nos.

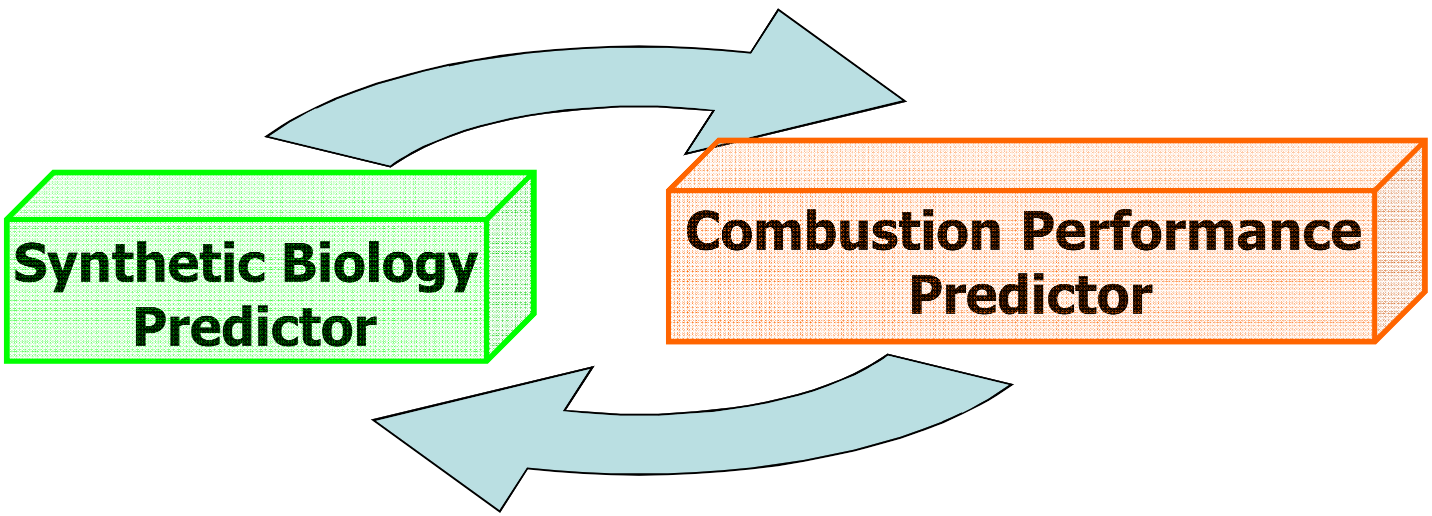




Cyclic alkanes/alkenes
- Jet fuel replacements
- Low freezing point

Tailoring Next-Generation Biofuels and their Performance in Advanced Engines LDRD

- No trick to get multiple researchers to take money from the same pot – key is to structurally enforce co-development
- "Ideal Program":

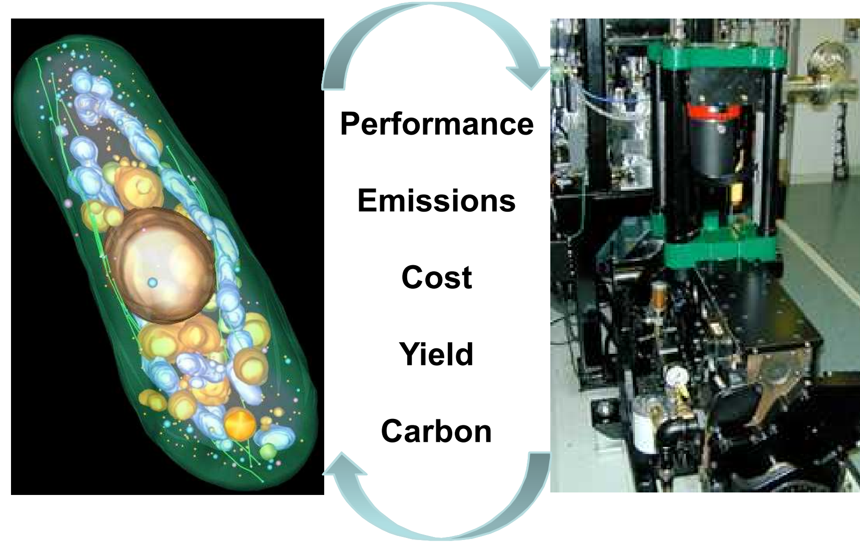


- *Predicting combustion is a big problem!*
  - *We'll concentrate on one crucial part – ignition chemistry*
- *Predicting synthetic biology is a big problem!!*
  - *We'll concentrate on novel and flexible biosynthetic platform(s)*

Craig Taatjes ***Masood Hadi*** ***John Dec***

US-China CERC Project: Characterization, Optimization, and Combustion of Biofuels

Task PIs: Blake Simmons (SNL/JBEI), Jay Keasling (JBEI), Angela Violi (UM)



Objectives

Evaluate advanced biofuels produced by JBEI in engines and provide performance feedback

Critical path

Identify most promising biofuel candidates at JBEI

Evaluate biofuels in a wide range of combustion platforms

Deliverables

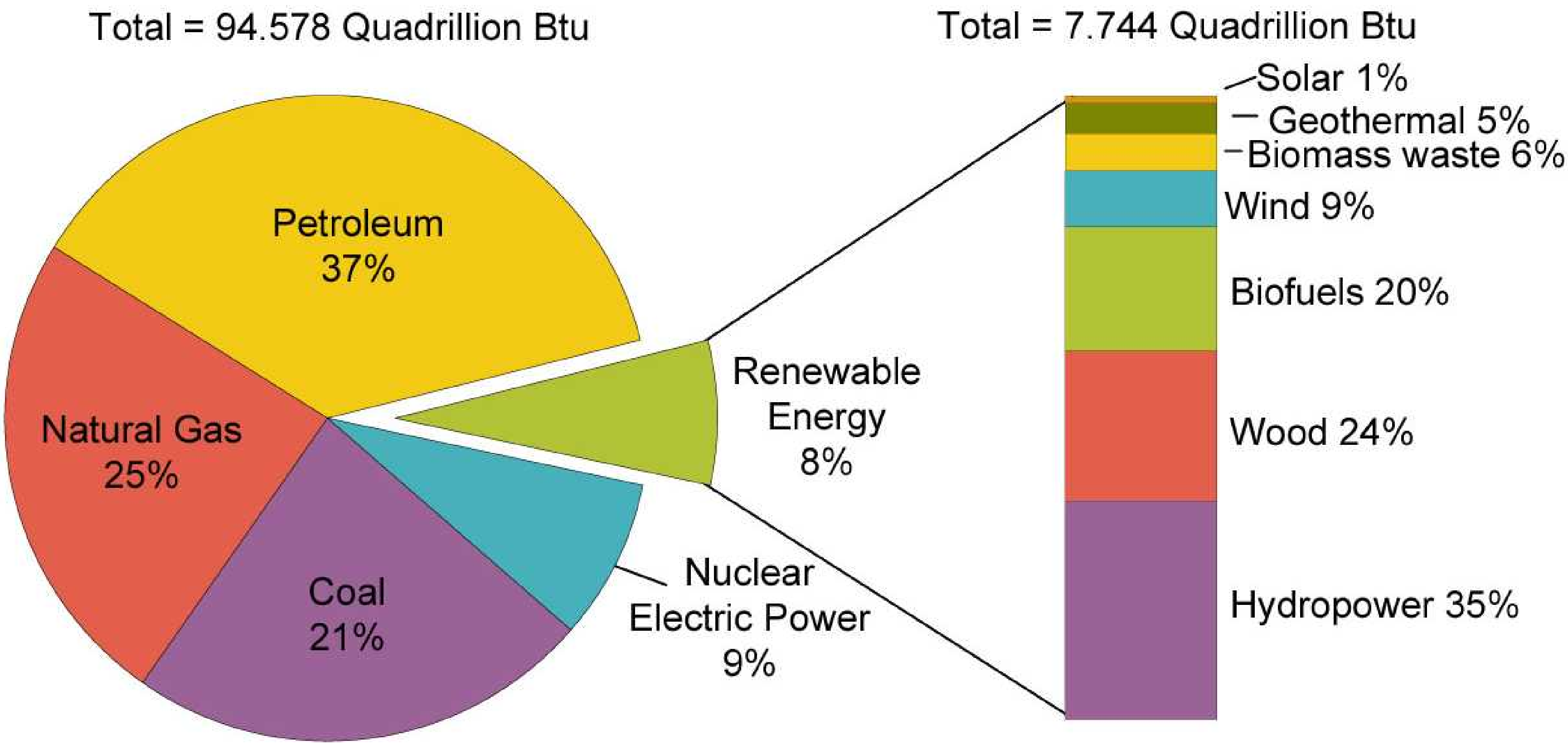
Isoprenoid and sesquiterpene biofuel evaluation

Down selection to optimized biofuels for advanced engines based on performance

Other Lignocellulose Biofuels Projects

- Monsanto CRADA
- DSM CRADA
- Trojan Horse LDRD – ended FY10 – business development mode
- Mobile Biorefinery CRADA w/CPC (DoD funded)
- Sustainability and LCA efforts

US ENERGY CONSUMPTION (2009)



(Keasling, 2011)

Sandia is a Key Partner in National and International Algae Programs

Sustainable Algal Biofuels Consortium
cultivating energy solutions

DOE OBP: Biochemical Conversion of Algal Residuals in Fuel Production and Testing

Sapphire Energy

DOE OBP Integrated Biorefinery: Scaling algal biofuels

International Programs *DOE OBP: Systems Analysis and Techno-Economic Modeling for Canada and Israel*

Internal Investments:

- *Metabolic Engineering in Biodiesel Feedstocks: Cyanobacteria and Diatoms (PI: Anne Ruffing)*
- *From Algae to Oilgae (PI: Seema Singh)*
- *Cultivation Scale-up: From Benchtop to Raceways (PI: Jeri Timlin)*
- *First Principles in Flocculation of Algae (PI: John Hewson)*
- *Modeling Algae Growth in Raceways (PI: Patricia Gharagozloo)*