

Development of Microalgal Biofuels: A National Laboratory Perspective



NREL is a national laboratory of the U.S. Department of Energy Office of Energy Efficiency and Renewable Energy operated by the Alliance for Sustainable Energy, LLC

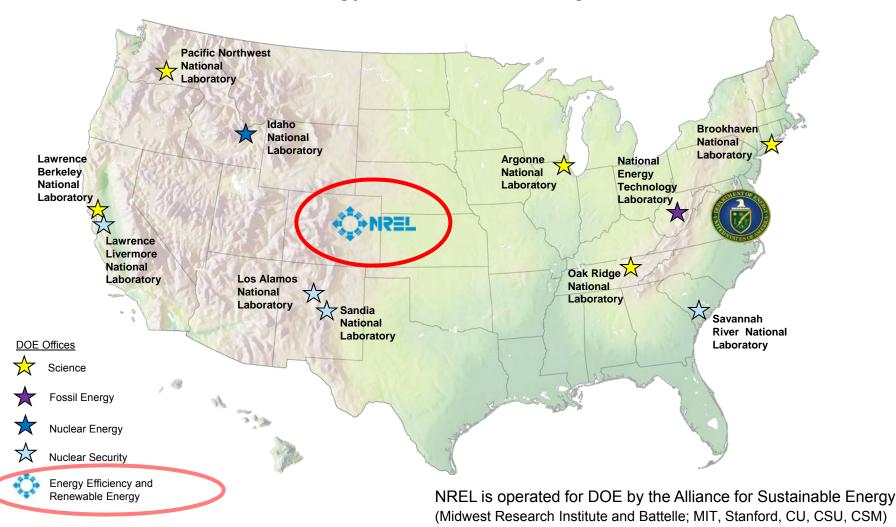
Outline

Biofuel Challenges: Renewable Fuel Standard and Energy Density Potential of Algal Biofuels: Myth vs reality DOE's Aquatic Species Program: What's changed since 1996? Role of the US Government in Developing Algal Biofuels NREL's Microalgal Biofuels Program Conclusions

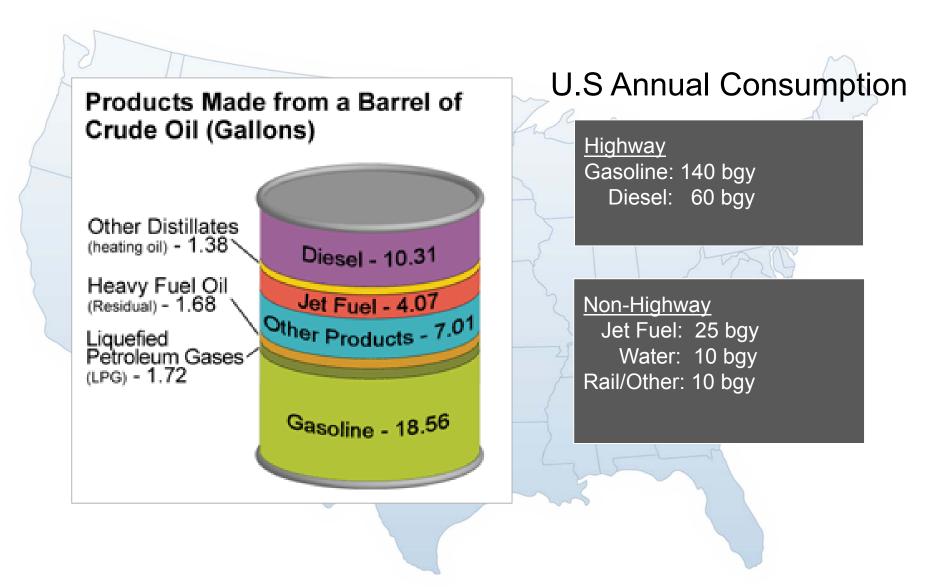


U.S. Dept of Energy National Labs

NREL is the only DOE National Laboratory dedicated to renewable and energy efficient technologies



U.S. Petroleum Transportation Fuels



U.S. Biofuels Current Status

U.S. Consumption Gasoline: 140 bgy Diesel: 60 bgy



Biodiesel¹

- ~175 commercial plants
- 2.7 bgy capacity (2009)
- 0.5 bg produced (2009)





Corn Ethanol²

- ~200 commercial plants
- 13.0 bgy capacity (+ 1.4 bgy planned) (2009)
- 10.5 bg produced (2009)



Cellulosic Ethanol³

- 30 demo plants DOE-funded
- ~.250 bgy capacity projected
- Additional industry-only funded plants

bg = billion gallons; bgy = billion gallons per year

Sources: 1- National Biodiesel Board, 2- Renewable Fuels Association, 3- DOE Biomass Program



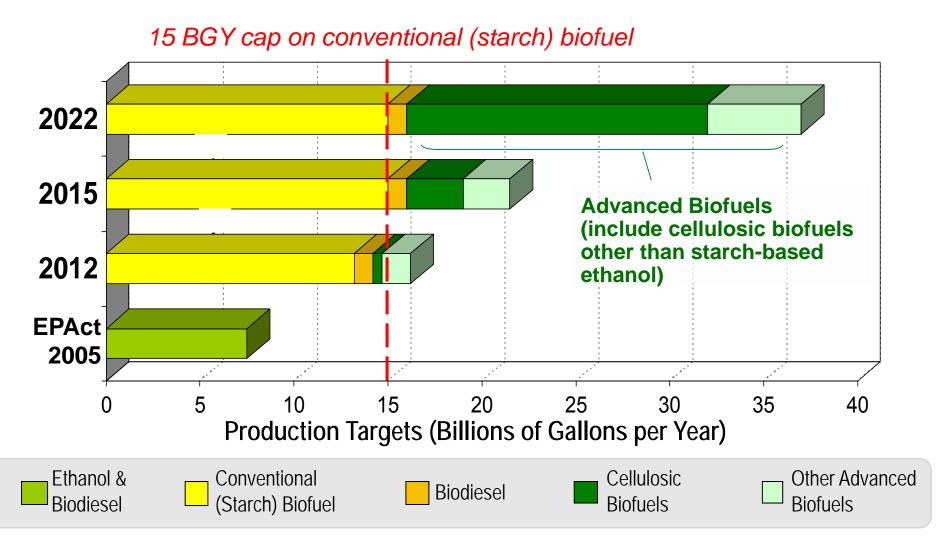






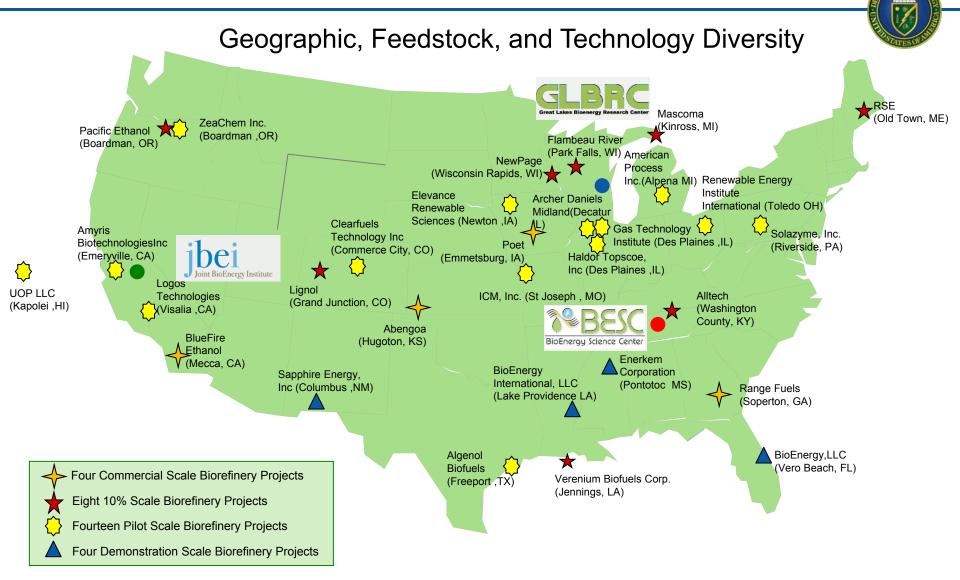
~2,000 E85 stations

U.S. Renewable Fuel Standard (RFS)



RFS in the Energy Independence and Security Act (EISA) of 2007

Major DOE Biofuels Project Locations



Biofuel Challenges: Energy Density

Cellulosic ethanol addresses the gasoline market

• U.S. gasoline usage: 140 billion gallons/year (bgy)

Does not address need for higher-energy density fuels

- U.S. diesel usage: 60 bgy
- U.S. jet fuel usage: 25 bgy

Energy Densities

Ethanol	Gasoline	Biodiesel	Diesel/Jet Fuel
76,330 Btu/gal	116,090 Btu/gal	118,170 Btu/gal	128,545/135,000 Btu/gal

Dilemma: Biodiesel from current oilseed crops cannot come close to meeting U.S. diesel demand (60 billion gal/year)

- 0.5 bgy biodiesel (2009) uncertainty exists
- Soy oil (2.75bg; 2007); replaces <5% of demand</p>



Alternative sources of oils are needed!

Routes to Algal Biofuels

Defining a Biofuels Portfolio From Microalgae

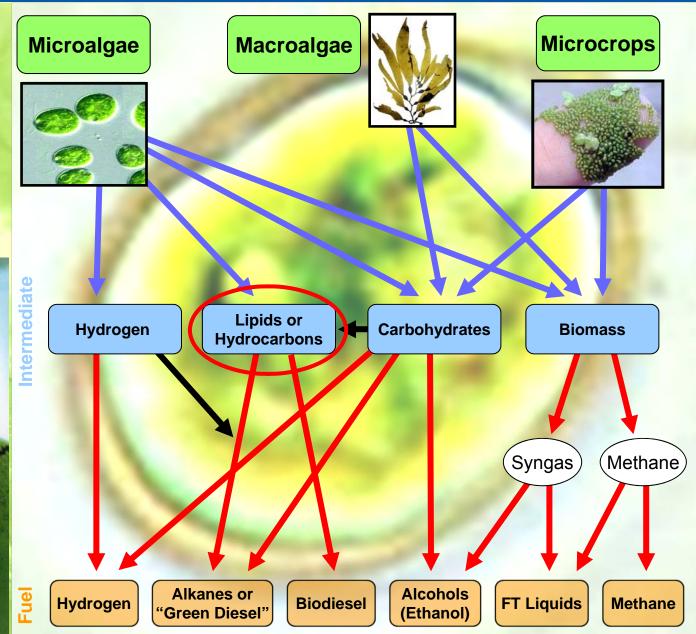
trends



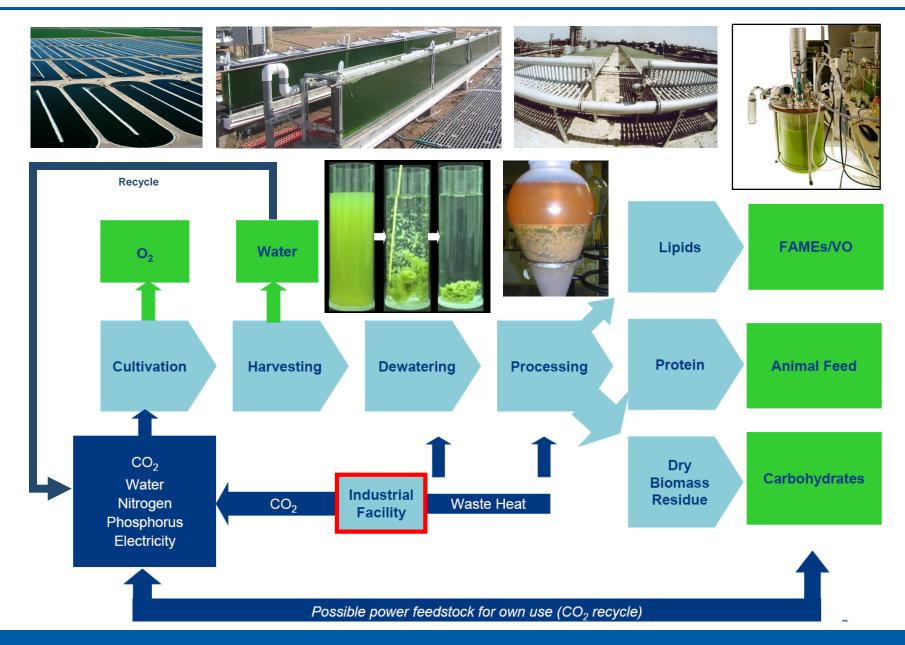
TRENDS

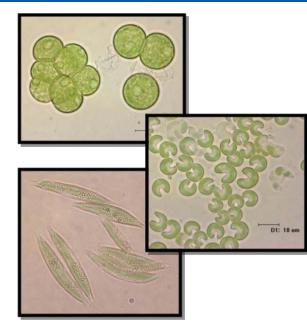
Wastewater bioreactors
 Information from cell culture aroma
 Exploring relationships between biological objects

Create your own virtual journal online with BioModNet Reviews http://wwices.html.our Recommend access to your librarian today.



General Cultivation Processing Flow Sheet



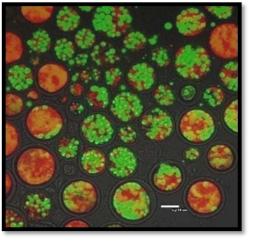


• High-lipid content (up to 50%); rapid growth; more lipids than terrestrial plants -- *10x - 100x*

- Can use non-arable land; growth possible in fresh, brackish, saline and wastewater
 - No competition with food, feed or fiber
 - Utilize large waste CO₂ resources (i.e., flue gas)

Fluorescence micrograph showing stained algal oil droplets (green)

• Potential to displace significant U.S. petroleum fuel usage – requires existing infrastructure



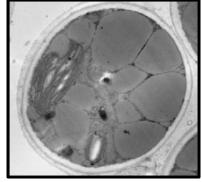
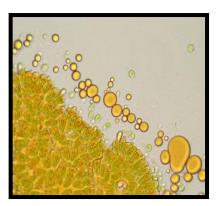
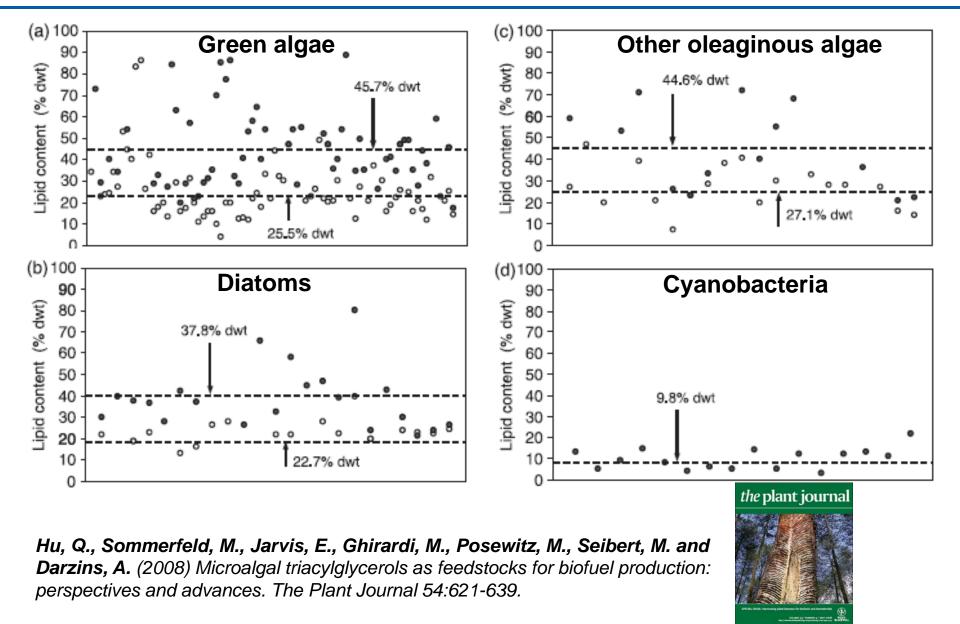


Image courtesy: Q. Hu, ASU



Cellular Lipid Content of Algae



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Superior Oil Yields

Crop	Oil Yield Gallons/acre
Corn	18
Cotton	35
Soybean	48
Mustard seed	61
Sunflower	102
Rapeseed	127
Jatropha	202
Oil palm	635
Algae (20g/m²/day-15%)	1267



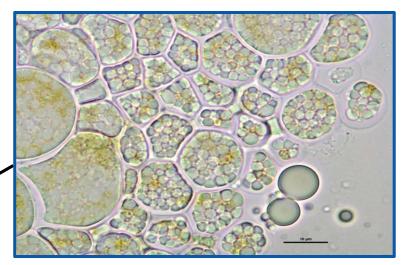
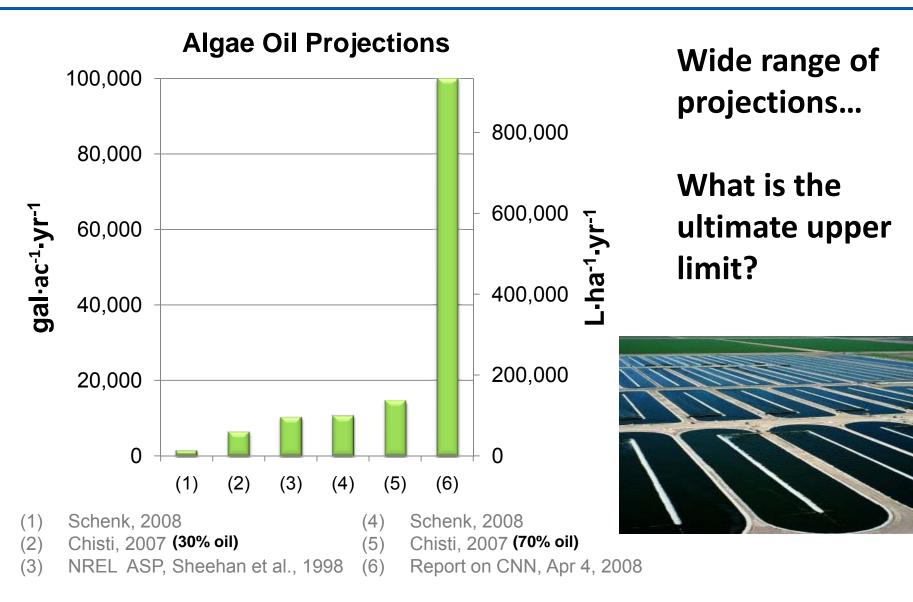
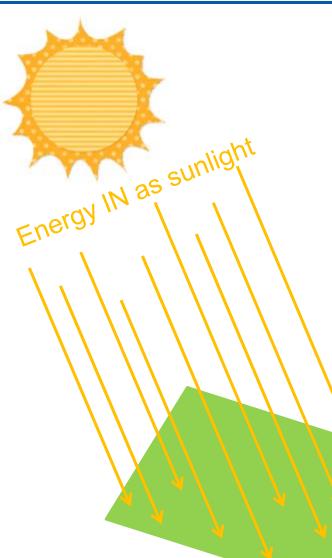


Image courtesy of Lee Elliott, CSM

Myth vs Reality



Need to Obey Laws of Thermodynamics



1st law: conservation of energy

$$E_{in} - E_{out} = E_{stored}$$

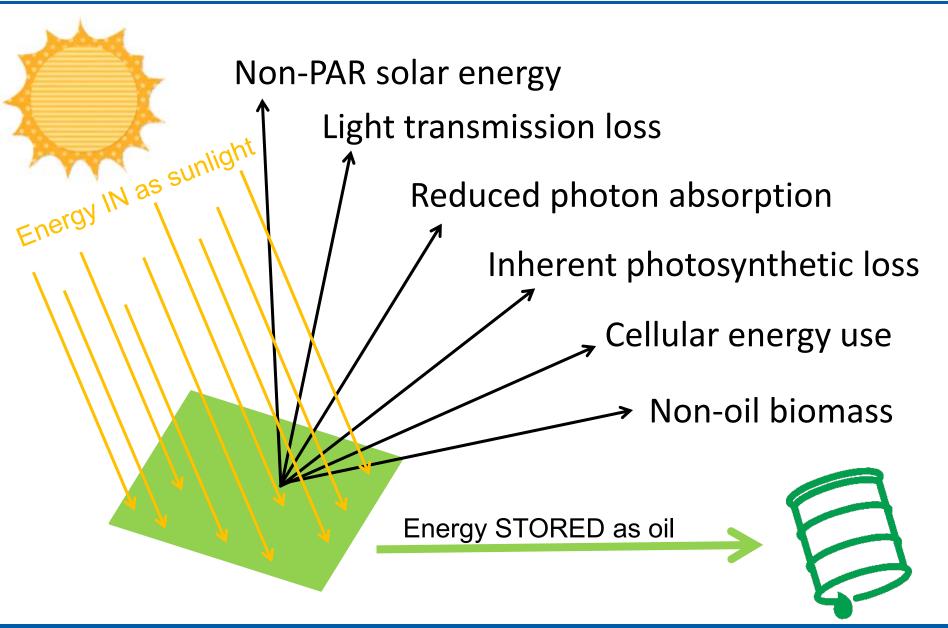
2nd law: 100% efficiency is not possible

 $E_{in} > E_{stored}$

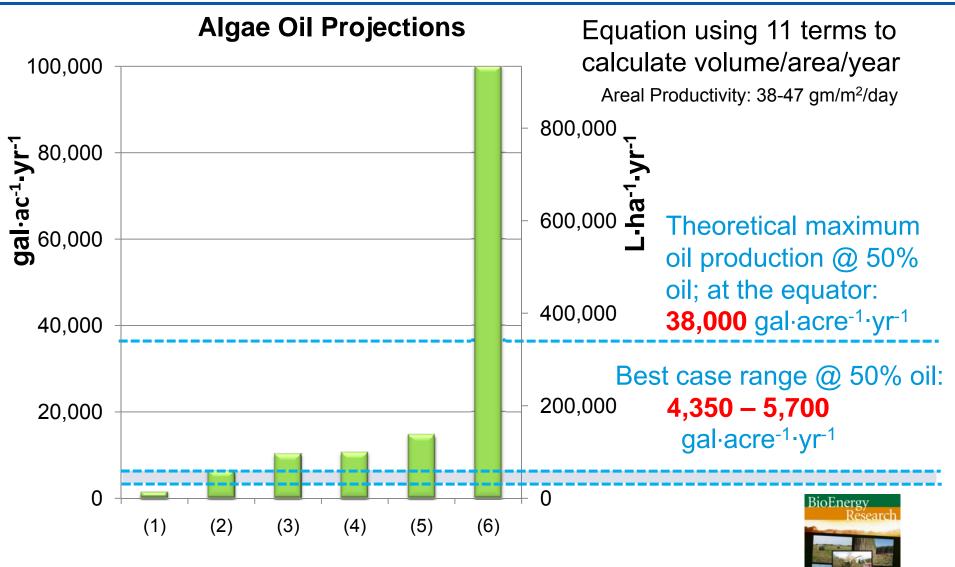
Energy STORED as oil



Inefficiences galore.....

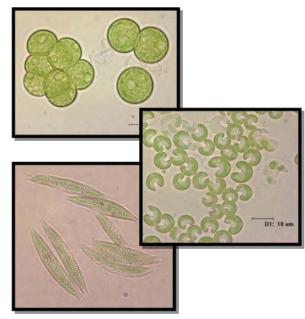


Industry needs to well grounded....

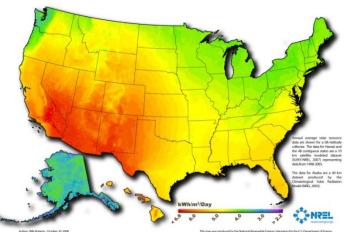


Weyer, K., Bush, D., Darzins, A. and Willson, B. (2009). Theoretical Maximum Algal Oil Production. (BioEnergy Research, Online First [Open Source])

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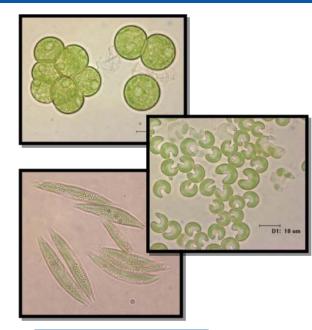


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- Potential to displace significant U.S. petroleum fuel usage requires existing infrastructure









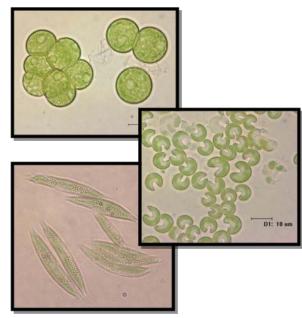
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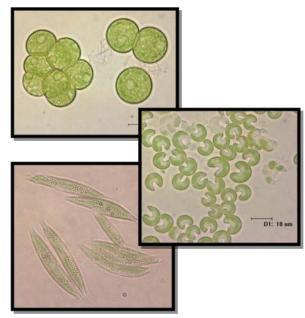
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Seambiotic: Ashkelon, Israel



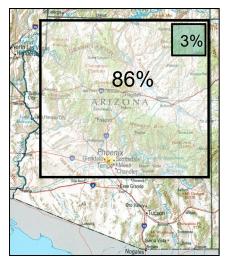




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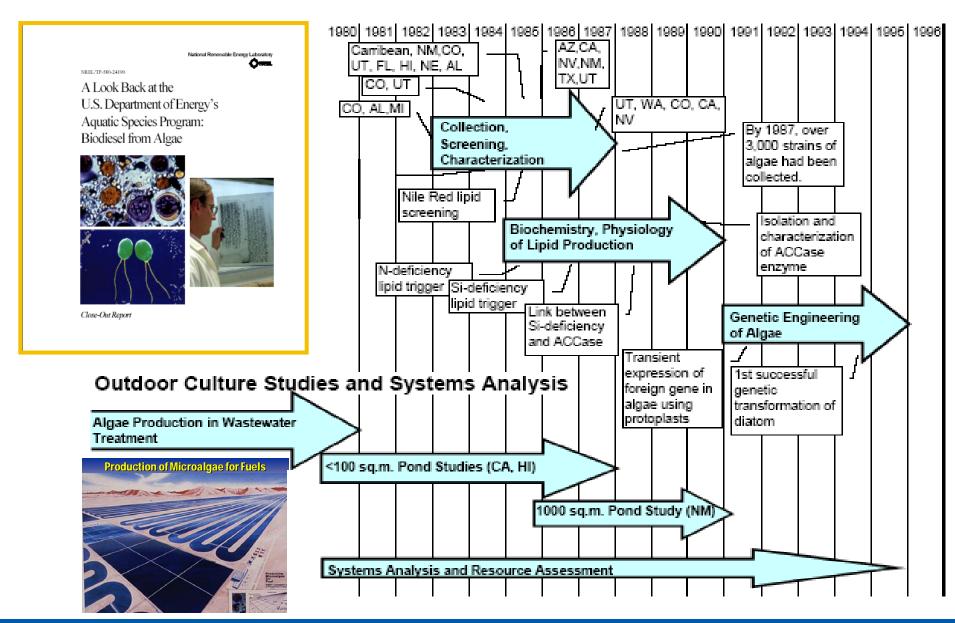
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	Soybean	Algae
gal/year	3 billion	3 billion
gal/acre	48	1267
Total acres	62.5 million	2.4 million





DOE's Aquatic Species Program



What's Changed Since 1996?

- Record oil prices; increasing worldwide demand for energy
- CO₂ capture, GHG reduction; energy security
- Industrial interest (>150 algal companies)
- Interest by oil industry, venture capital, end users, utilities and governments
- Explosion in biotechnology
 ExonMobil

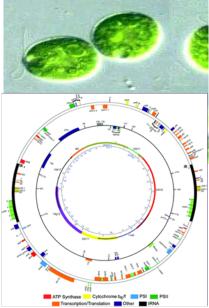


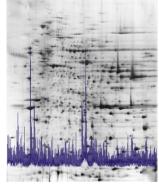


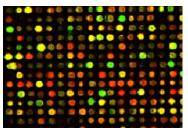




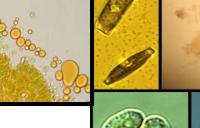
ConocoPhillips

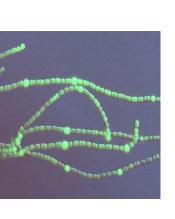






Role of the US Government in developing algal biofuels





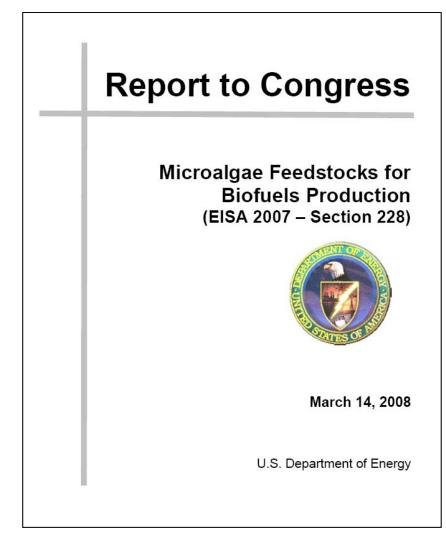
Sun Liah

CO CO Pyruval (Sugar

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Congressional Algae Report

Microalgae Feedstocks for Biofuels Production



Report Outline

- Executive Summary
- Introduction
- Historical Review of Technical Progress
- Microalgae Oil Production: Biology and Physiology
- Microalgae Oil to Biofuels
- Current Activities/Funding Support for Algae Biofuels
- Resource and Technoeconomic
 Assessment
- Conclusions and Recommendations

National Renewable Energy Laboratory and Air Force Office of Scientific Research Joint Workshop On Algal Oil for Jet Fuel Production February 19-21, 2008 Arlington, VA





http://www.nrel.gov/biomass/algal_oil_workshop.html

Algal Biofuels Technology Roadmap Workshop

Sponsored by the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy (EERE), Office of the Biomass Program



- Venue: Univ. of Maryland Dec 9-10, 2008
- **Participants**: ~200 scientists, engineers and other experts and stockholders
- **Goal**: Define activities needed to resolve barriers associated with commercial scale algal biofuel production
- Workshop: plenary talks and breakout sessions covering technical, industrial, resource, and regulatory aspects
- Information: <u>http://www.orau.gov/algae2008/</u>

http://www.orau.gov/algae2008pro

 Progress: First draft of Roadmap complete; Request for Information (RFI)



the world. They may represent a sustainable pathway for helping to meet the U.S. biofuel production targets set by the Energy Independence and Security Act of 2007

Microalgae are single-cell, photosynthetic organisms known for their rapid growth and high energy content. They are capable of doubling their mass several times per day, and more than half of that mass consists of lipids or triacylglycerides— the same material found in vegetable oils. These bio-oils can be used to produce such advanced biofuels as biodiesell, green diesel, green gasoline, and green jet fuel.



Higher oil prices and increased interest in energy security have stimulated new public and private investment in algal biofuels research. The Biomass Program is reviving its Aquatic Species Program at the National Renewable Energy Laboratory Non-Competitive with Agriculture: (NREL) to build on past successes and drive down the cost of largescale algal biofuel production. Private investors as well as programs within the Defense Advanced Research Projects Agency (DARPA) and Air Force Office of Scientific Research

(AFOSR) are also sponsoring

research at NREL, Sandia, and

other laboratories. Substantial

research and development

challenges remain.

Undernanding of Fresh Water:

of climates (including deserts)

mpressive Productivity

Mitigation of CO2

http://www1.eere.energy.gov/biomass/pdfs/algalbiofuels.pdf

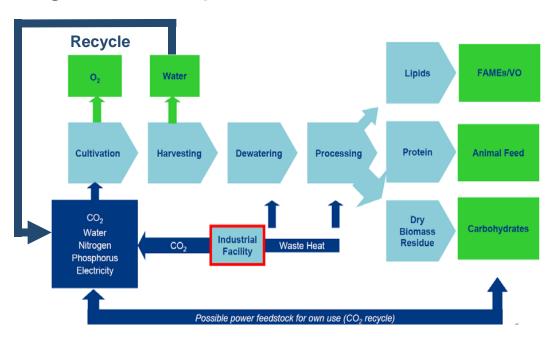
*Funding opportunity DE-PS36-09GO39010-RFI

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December 9-10, 2008 University of Maryland, Inn and Conference Center

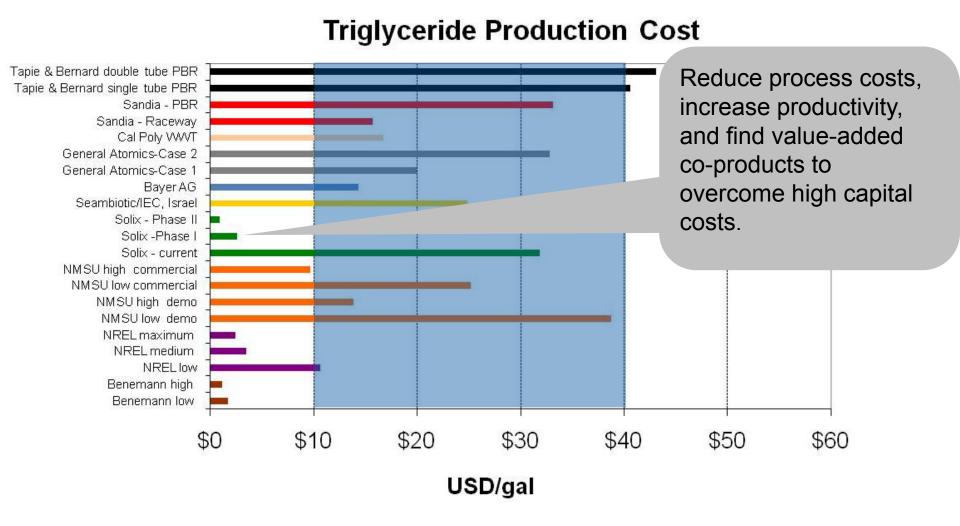
Fundamental and applied research needed to resolve uncertainties associated with commercial-scale algal biofuel production:



- Algal Biology
- Cultivation
- Harvest/dewatering
- Extraction/fractionation
- Conversion to fuels
- Co–products
- Systems integration
- Siting & Resources
- Regulation & Policy



Technoeconomic Analysis



Photobioreactors Haven't Been Ruled Out

Algal Biofuels Award

Awarded to:

National Alliance for Advanced **Biofuels and Bioproducts** (NAABB)

Leader: **Donald Danforth Plant Science** Center

With Partners: 2 national laboratories, 10 university and 13 industry partners

Objectives and Approach: Develop a systems approach for sustainable commercialization el (such as

appropriation

renewable ga bioproducts. Integrate res

universities,

+ \$35M in FY10

jet fuel) and

nies, ories to

overcome the critical barriers

- Develop and demonstrate the science and technology necessary to significantly increase production of algal biomass and lipids
- Explore co-products, including animal feed, industrial feedstocks, and additional energy generation.
- Multiple test sites will cover diverse environmental regions to facilitate broad deployment.

\$44M DOE + \$11M cost share

NREL's Algal Biofuels Program

Rebuilding NREL Algae Program

In 2006, recognizing the limitations on biofuels based on terrestrial biomass, NREL began a strategic initiative to revive its program in algal biofuels.

Leveraging research funding from a number of sources to revive a program dormant for more than a decade

- Partnerships with industry and academia
- International partnerships
- Internal funding
- DOE
- DOD



Chevron Algae CRADA

2nd Collaborative Research and Development Agreement (CRADA) under Chevron/NREL Alliance

Goal: Identify and develop algae strains that can be economically harvested and processed into finished transportation fuels







DOE US - Israel Collaboration

Development of Novel Microalgal Production and Downstream Processing Technologies for Alternative Biofuels Applications

Joint NREL/SNL/Israel-US Private Industry Collaboration

Tasks:

- Develop extraction methods
- Thermochemical conversion of algal feedstocks
- Physics-based modeling/analysis
- Life Cycle Analysis (LCA)

Seambiotic



Image courtesy: A. Ben-Amotz, Seambiotic

DOE US - Canada Collaboration

Isolation and preliminary characterization / assessment of scale-up potential of photosynthetic microalgae for the production of both biofuels and bio-active molecules in the US and Canada

Joint NREL/SNL/NRC Partnership

Tasks:

- Bioprospecting for strains with suitable characteristics for cultivation in northern latitudes – marine environments
- Evaluation of strains for lipid productivity and bio-active molecule production
- Siting analysis for selected regions of Canada and northern US
- Techno-economic modeling



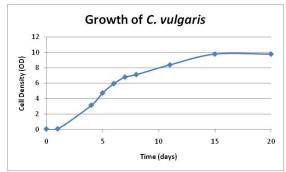
Chlorella vulgaris: NREL Model System

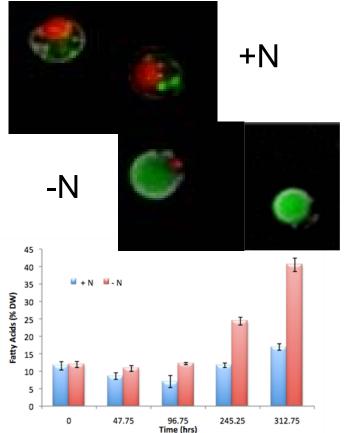
Chorella vulgaris UTEX395

- · Rapid growth to high cell density
- · Visible accumulation of oil after N depletion
- High fatty acid content after N starvation
- Capable of heterotrophic growth
- Reports of successful genetic manipulation

Projects currently underway

- Digital gene expression (Transcriptomics)
- Proteomics (AFOSR)
- Development of novel harvest methodologies
- Development of transformation methodologies
- Regulated enzymatic disruption of algal cell walls as an oil extraction technology
- Identification of novel promoters

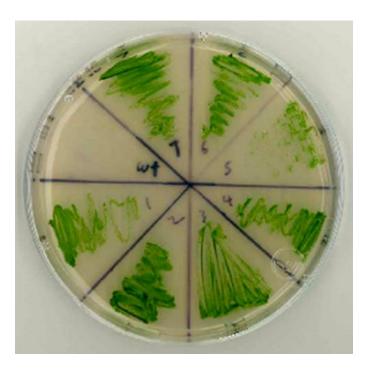


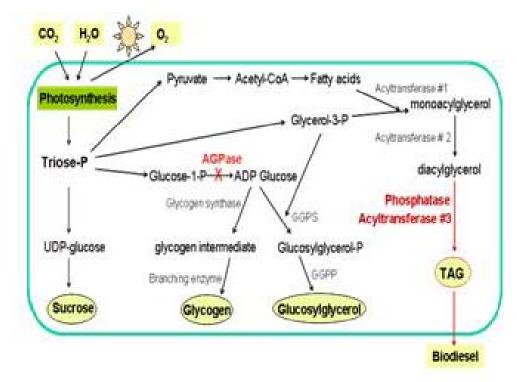


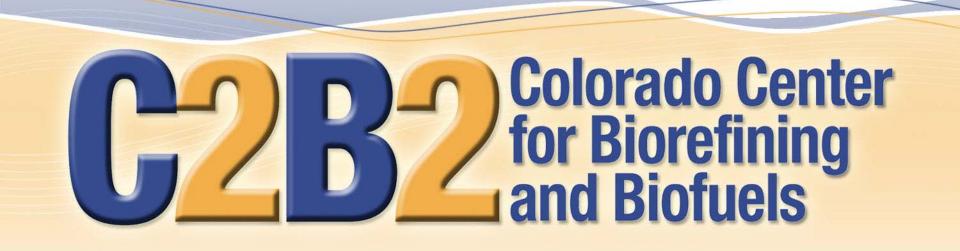
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Pathway Engineering in Cyanobacteria

- Biodiesel from Cyanobacteria (Synechocystis PCC 6803)
- Development of Novel Cyanobacterial Biofuels







Establishment of a Bioenergy-Focused Microalgal Strain Collection Using High-Throughput Methodologies











Current Corporate Sponsors (21)

Catchlight Energy Ceres Chevron Cobalt Biofuels ConocoPhillips Ecopetrol Flad Architects

> Colorado Center for Biorefining

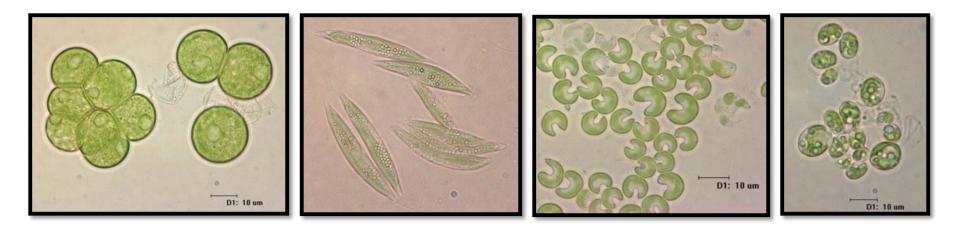
General Motors Genesis Biofuel Gevo GICON Kimberly-Clark Korth O'Neil Engineering LiveFuels

Mascoma OpX Bioproducts Shell Global Solutions Solix Biofuels Sundrop Fuels Valero ZeaChem



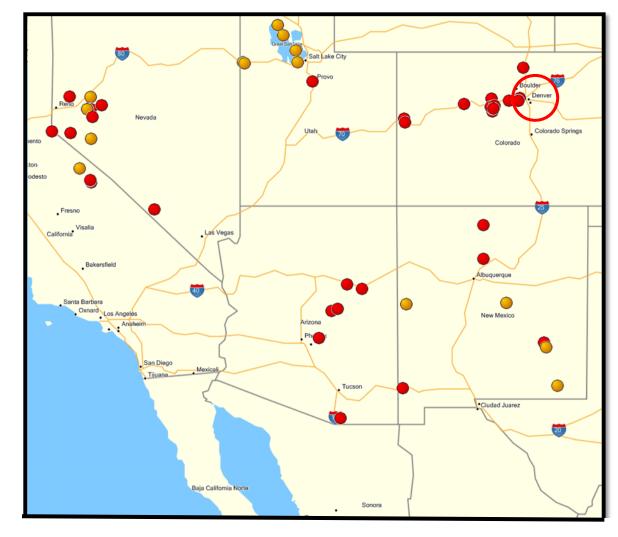
Project Description

- **Goal:** Isolate and characterize 500 microalgal strains utilizing rapid, high-throughput methodologies
 - Sample unique aquatic environments in the US Southwest.
 - Sort microalgal cells using Fluorescent Activated Cell Sorting (FACS).
 - Characterize algae based on morphology and lipid accumulations
 - Cryopreserve microalgae isolates for long-term storage.



Locations of Sample Collection Sites

- Targeted sites from diverse locations in the Southwest
- Site determination
 - Locally: Based on visible algal growth
 - Regionally: Documentation of productivity and visible growth
- 69 samples collected:
 - ➢ 31 from Colorado
 - ➢ 9 from Utah
 - ➢ 8 from New Mexico
 - 6 from Arizona
 - 5 from California
 - 10 from Nevada



Red: 2008 sampling trip

Orange: 2009 sampling trip

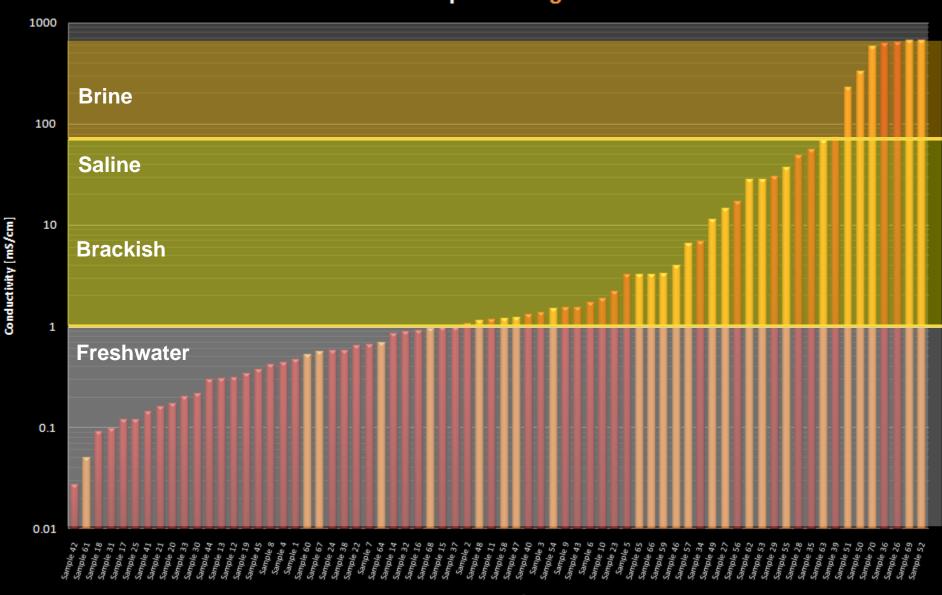
Specific Conductivity and Water Quality

- Typical specific conductivity ranges for water from fresh to brine and the corresponding salinity ranges.
 - ➢ Conductivity measures the ionic activity of water.
 - Conductivity is a very good measure of TDS and salinity.
 - Most influential ions contributing to conductivity: Na+, Cl-, Ca2+, K+, Mg2

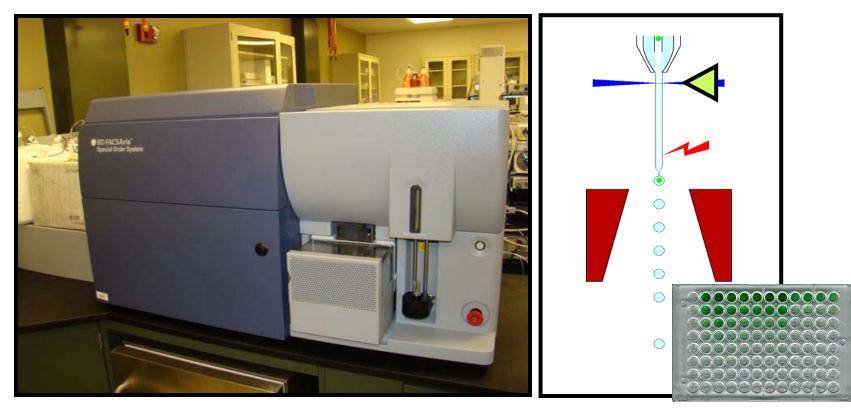
Water Type	*Specific Conductivity (mS/cm)	Salinity (ppt)
Fresh Water	<1.0	<0.5
Brackish Water	1.0-47.0	0.5-30
Saline Water	47.0-73.0	30-50
Brine	>73.0	>50
Sea Water (Boothbay Harbor, Maine)	65.4	42.78

Specific conductivity is normalized at 25 degrees Celsius. Source: <u>http://www.ourlake.org/html/specific_conductivity.html</u>

Water Sample Conductivities of all Sample Sites 2008 Samples: Red 2009 Samples: Orange

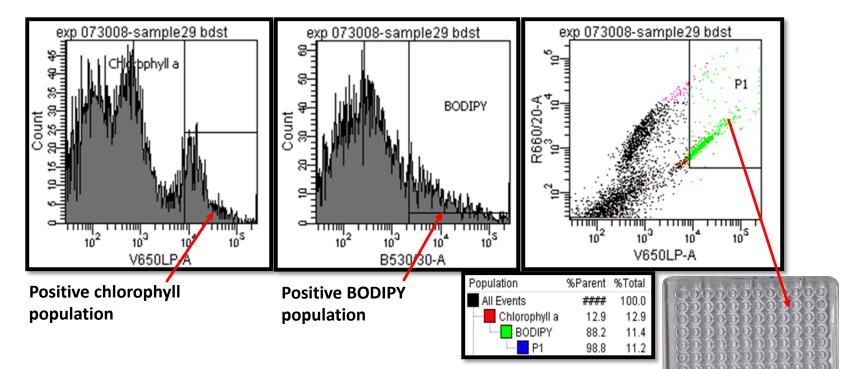


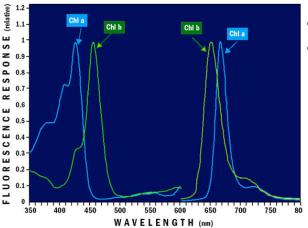
Fluorescence Activated Cell Sorter (FACS)



- NREL's Custom BD FACSAria[™]
 - analyzes particle fluorescence and sorts microalgae based on pre-set parameters.

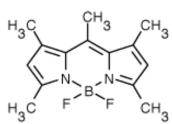
High-Speed FACS Sorting





• Chlorophyll *a* excitation, 405nm, emission at >650nm.

• Non-polar lipid probe Bodipy excitation, 488nm, emission at 530/30nm.

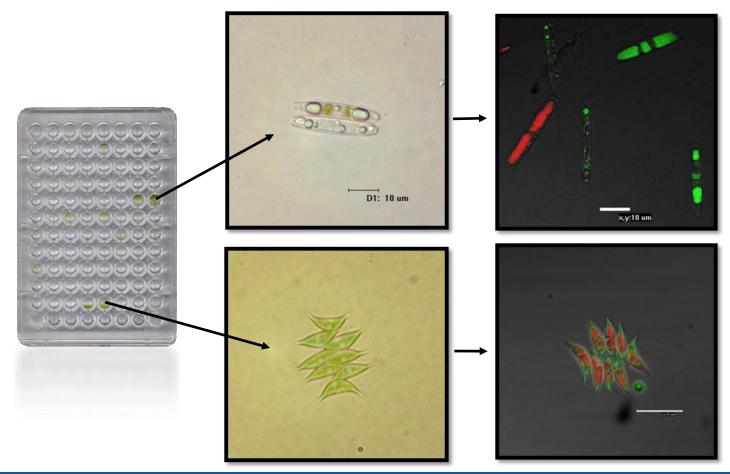


96 well plate

Sort single events in population P1 which are positive for **both** chlorophyll **and** BODIPY

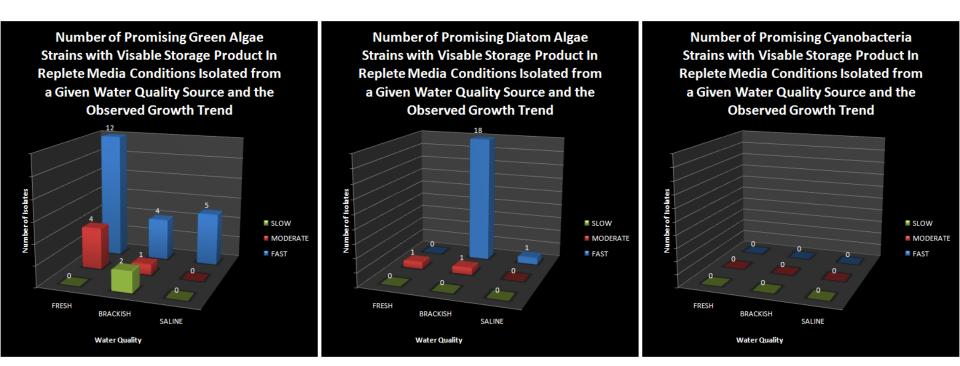
Clonal Isolate Characterization

- **Step 1:** Image positive wells with brightfield microscopy; compare morphologies and transfer unique isolates into fresh media.
- **Step 2:** Stain cells with fluorescent lipophilic probe (Bodipy) and image intracellular non-polar lipid distribution by confocal microscopy.



Population Analysis

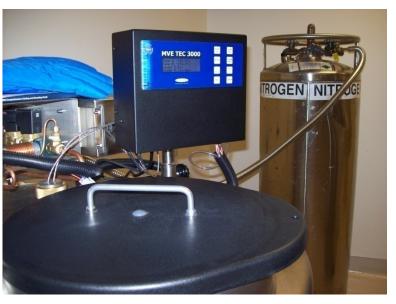
Green algae and diatoms from fresh, brackish and saline environments identified as potential bioenergy-feedstock strains



Algal Cryopreservation System



Chart[®] MVE 800 Series-190 vapor-phase LN₂ cryopreservation tank





Cryopreservation of Isolated Strains

- 91% of strains successfully cryopreserved in 5% MeOH in growth media
 - Protocol adapted from UTEX

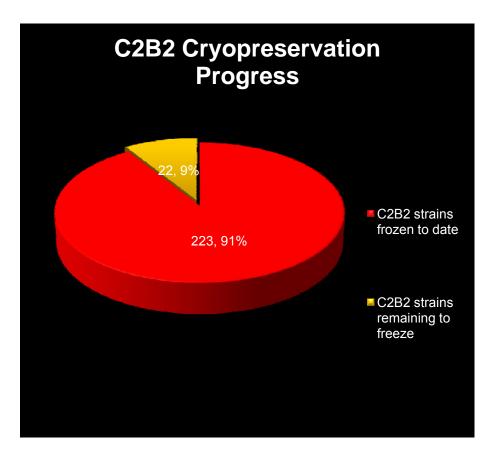


Plate A OD(λ =750) initial measurement

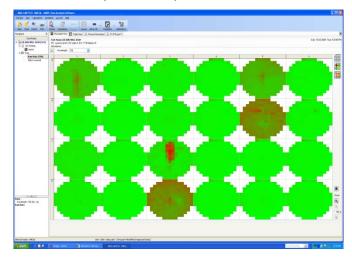
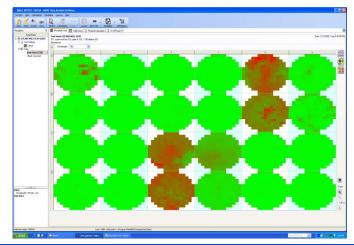


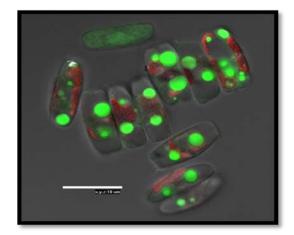
Plate A OD(λ =750) final measurement

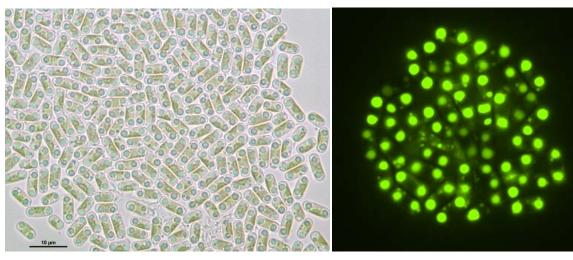


Future work

Phase II: Detailed Lipid Production Analysis of Most Promising Strains

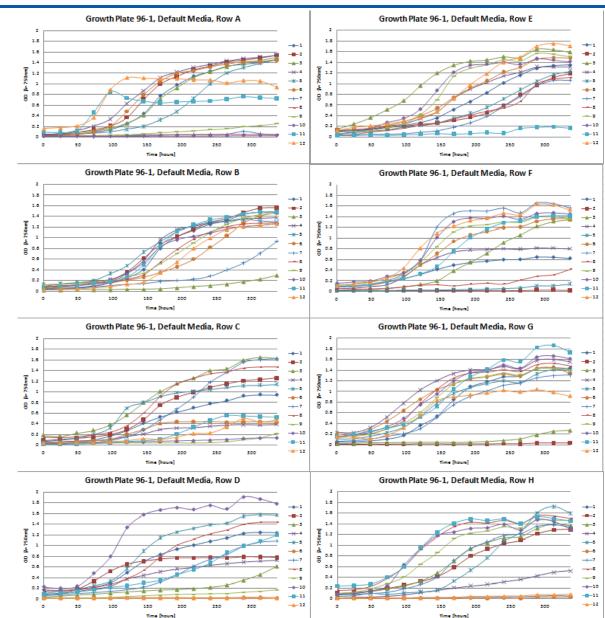
- Screen/select most promising strains
- Optimize lipid productivity
- Obtain fatty acid methyl ester profiles
- Determine taxonomic classifications
- Rapidly quantitate lipid content and profile.





High Throughput Growth Screening

- Tracked growth of the entire culture collection in a 96 well plate format to get a quantitative picture of the growth of all strains over a 14 day period
- Goal: Identify strains that should be examined more closely



NREL Funded Algal Biofuels Projects

Laboratory Directed Research & Development (LDRD) Awards

"Development of a Comprehensive High-Throughput Technique for Assessing Lipid Production in Algae"

"Use of Digital Gene Expression (DGE): Tag Profiling for High Throughput Transcriptomics in Microbial Strains Involved in Advanced Biofuel Production"

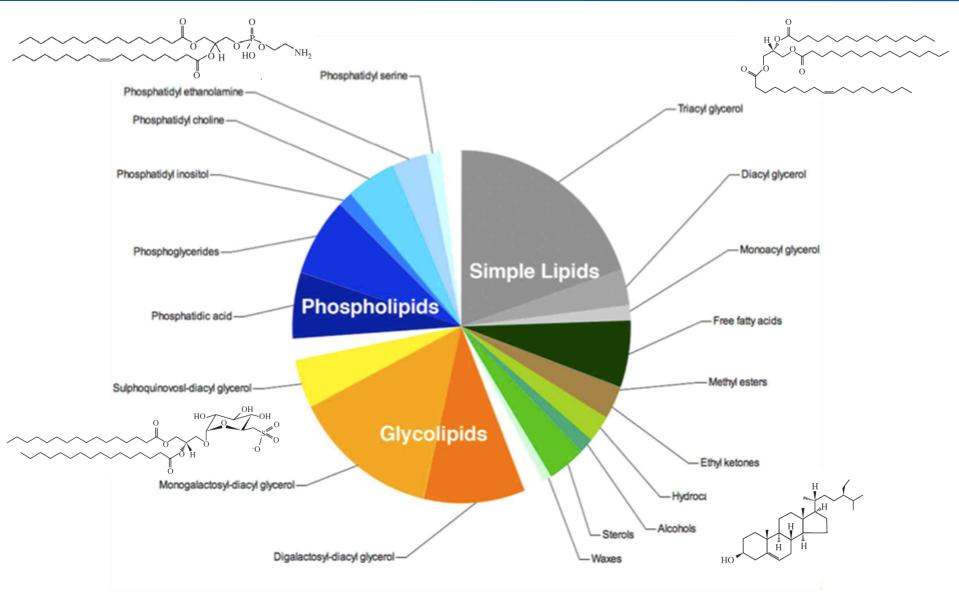
"Biodiesel from Cyanobacteria"

"Regulated Enzymatic Disruption of Algal Cell Walls as an Oil Extraction Technology"

"Identification of Novel Promoters in Green Algal Species"

"Development of Novel Cyanobacterial Biofuels"

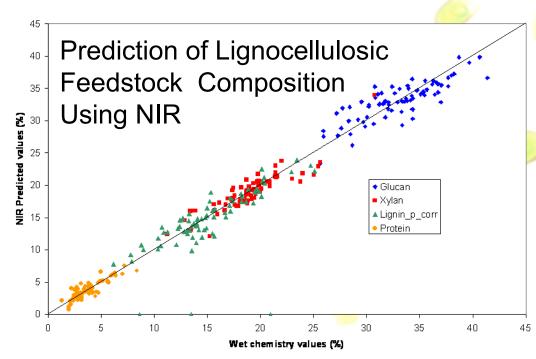
Algal Lipids



Courtesy of Peter Williams, Bangor University, UK

Characterization of microalgal biomass

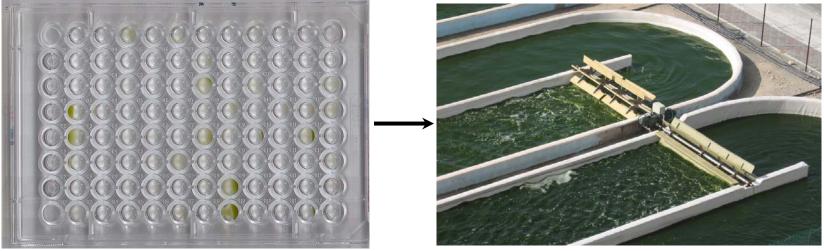
- 1. Development of a high-throughput microalgae analytical platform
- 2. Chemometric correlation of IR spectra with microalgal lipids



Error varies with constituent, but for NREL models, well-predicted samples have typical uncertainties in glucan and xylan of ~2%

1. High-throughput analytical platform

- Lipid analysis is needed to assess the suitability of algal biomass as feedstock for biodiesel production
- Not all lipids are equal; e.g. phospholipids are less desirable due to a lower conversion efficiency of lipid to fuel
- High-throughput techniques would allow the screening of a large number of potential candidates for large-scale culturing



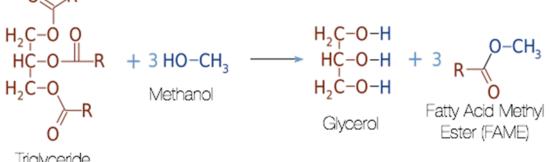
Courtesy of Ami Ben-Amotz, Seambiotic, Israel

Lipid extraction and *trans*-esterification





Lipids are traditionally extracted using Hexane-Isopropanol and Chloroform-Methanol as solvent systems



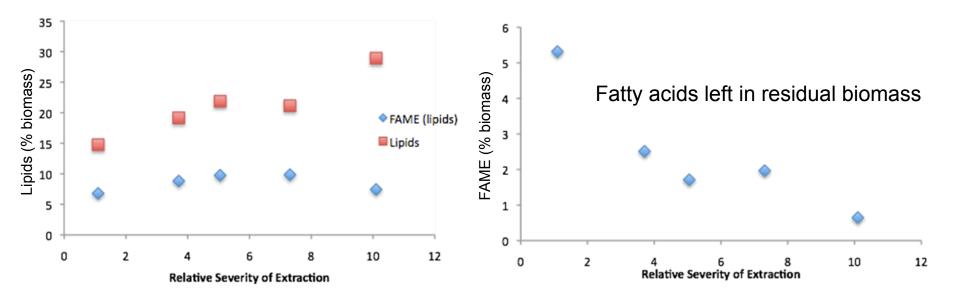


Automated lipid extraction the Accelerated using Solvent Extractor (ASE 200, Dionex) can analyze 24 samples per run, using only 200 mg biomass per sample

Triglyceride

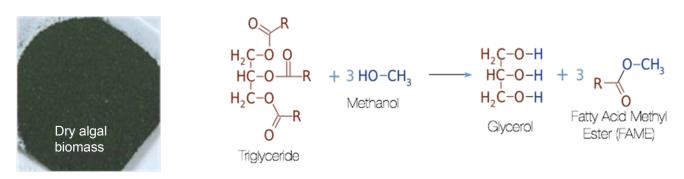
Fatty acid content in the lipid fraction are determined by acid catalyzed transesterification

Solvent-based extraction inaccuracies

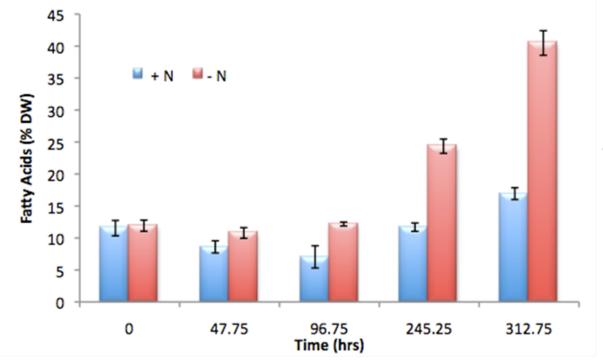


Variability in total extracted lipids and completeness of the extraction (based on severity of extraction conditions) using the solvent-based ASE system prompts the question for an alternative lipid quantification protocol for microalgae

Direct Biomass Trans-esterification

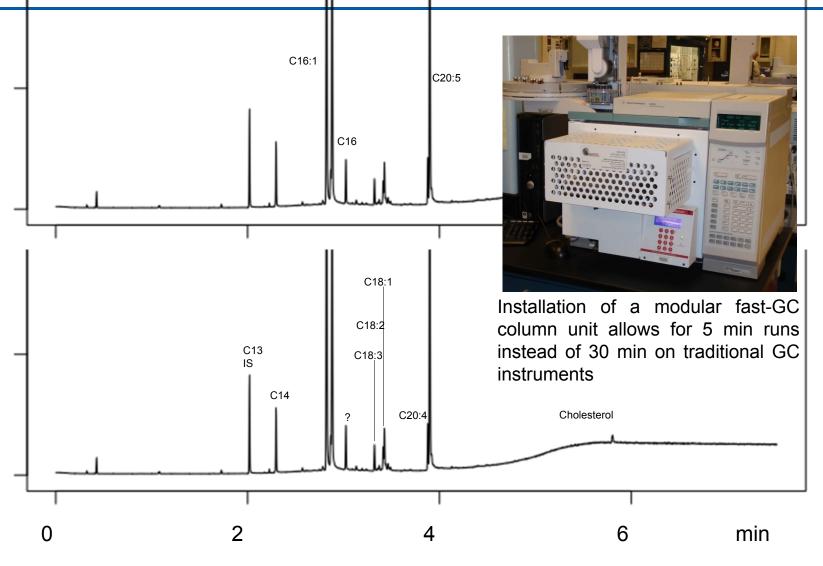


Fatty acid content in the biomass fraction are determined by acid catalyzed *trans*-esterification



Increase in total fatty acid content of *Chlorella vulgaris* strain under nitrogen starvation

Fatty acid profiling by fast-GC



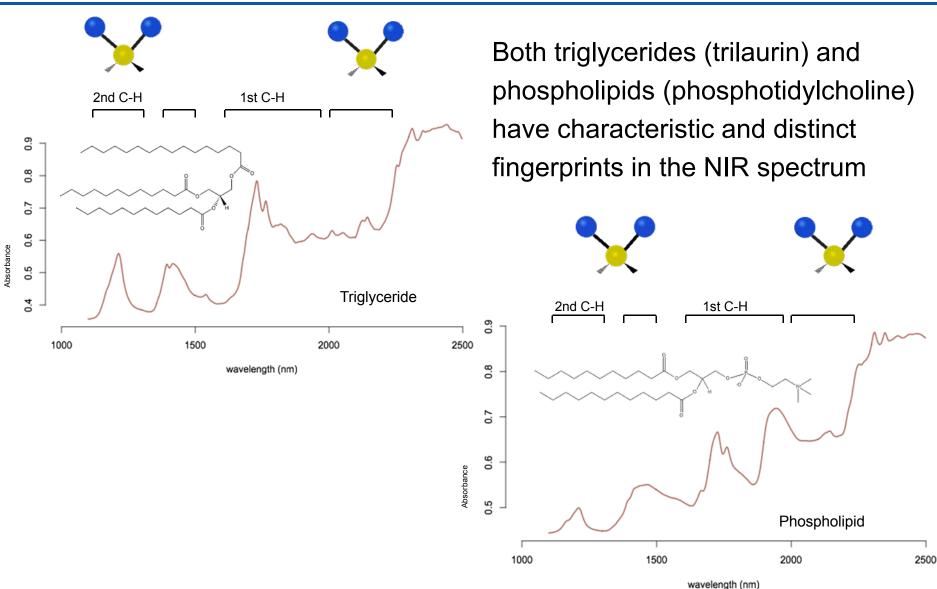
GC-FID FAME fingerprint of *Nannochloropsis* sp. lipids (HCI:MeOH)

2. IR spectroscopy for lipid quantification

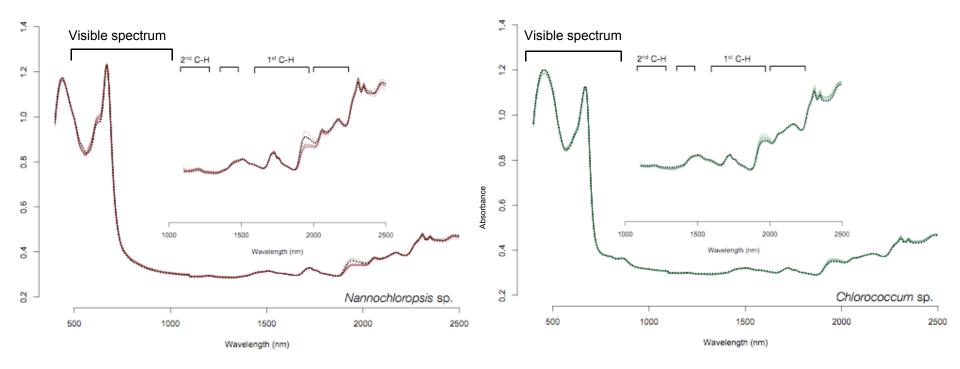
- Spectroscopic techniques are being used routinely as an alternative for long laborious analytical chemical methods
- Chemical bonds of a molecule absorb energy in the IR region of the spectrum
- Lipids have characteristic NIR spectra. Spectra contains overtones and combinations from stretching and bending of bonds in molecules.
- IR spectroscopy can distinguish between polar and neutral lipids

Time per sample:	
Wet Chemical methods (ASE/HPLC/GC) IR fingerprinting	> 1 day < 1 min

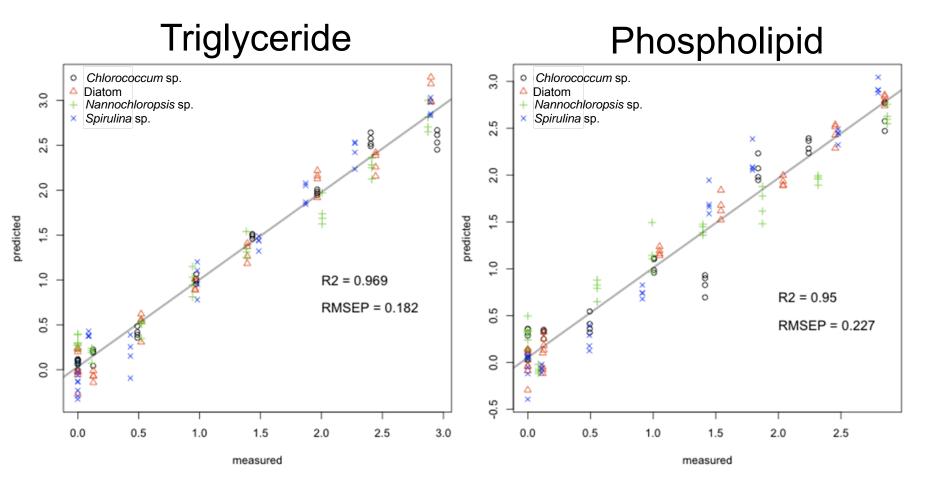
NIR fingerprinting of lipids



NIR fingerprinting of algal biomass



NIR Multivariate PLS Regression



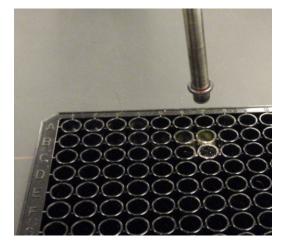
Exogenous lipid spike content (measured) versus the predicted values by NIR spectra show a highly significant correlation (R2 > 0.94)

High-Throughput Algal Strain Screening

- ✓ Triglycerides and phospholipids have characteristic IR fingerprints
- ✓ Successful linear multivariate calibration models for single species and for multiple species of algae, based on the measured spike concentrations
- ✓ A combined multiple species model (NIR and FTIR) is robust in predicting the lipid content across species
- ✓ NIR models appear to perform better compared with FTIR models





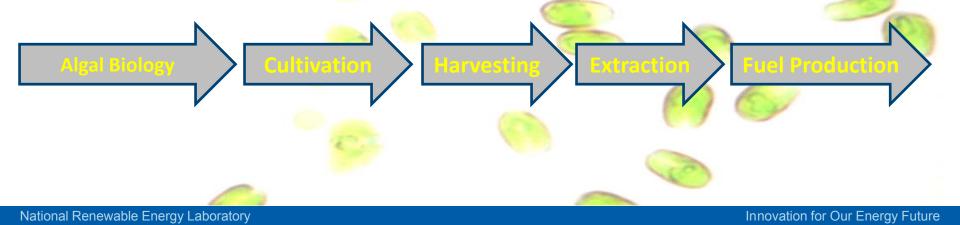


Conclusions

- The potential of algal biofuels is significant
- Production of fuels from algae have been demonstrated; microalgae can be grown and harvested; lipids extracted and converted to transportation fuels
- Algal biofuels are not, however, currently economical
- Infrastructure does not exist for an algal biofuels industry
- The unanswered questions remaining at the end of the ASP remain the focus of intense R&D efforts.
- Many of the conclusions of the ASP have been reconsidered

Conclusions

- This time, the combined drivers of peak petroleum, energy security, sustainability, and climate change provide significant incentive to move beyond R&D into commercialization.
- A greater understanding of the underlying <u>biological</u> and <u>engineering</u> principles is necessary before a commercial scale-up is feasible.
- NREL is focusing its research efforts on all parts of the algal biofuels process value chain.



Acknowledgments

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NREL Algal biofuels research:

http://www.nrel.gov/biomass/proj_microalgae.html

A national laboratory of the U.S. Department of Energy Office of Energy Efficiency & Renewable Energy







Wind

