NAABB Final Report

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Presentation Overview

- **Biofuels: one solution to the energy crisis**
- **Algae as a Bioenergy Crop**
- **Challenges for Algae production**
- **Overview of the National Alliance for Advanced Biofuels and Bioproducts (NAABB)**
  - Algal Biology and Strain Development
  - Algal Cultivation
  - Algal Harvesting and Extraction
  - Conversion to Biofuels
  - Coproduct Development
  - Sustainability of Algae as Biofuel Crop
- **Summary and Future Work**

US Renewable Fuel Standard

Biofuels Policy Mandate:

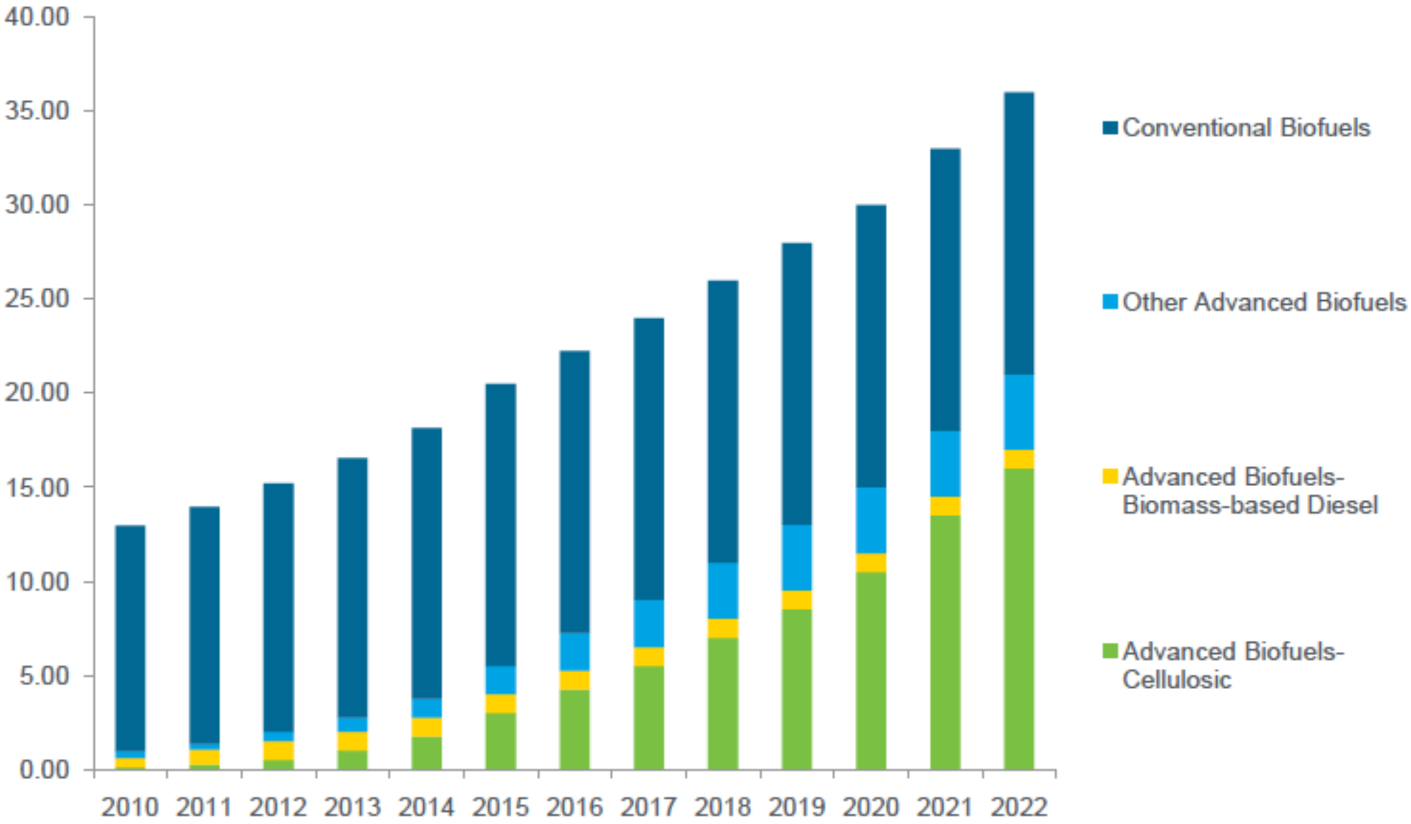
EISA (2007). "Energy Independence and Security Act of 2007", H.R.6

110th Congress

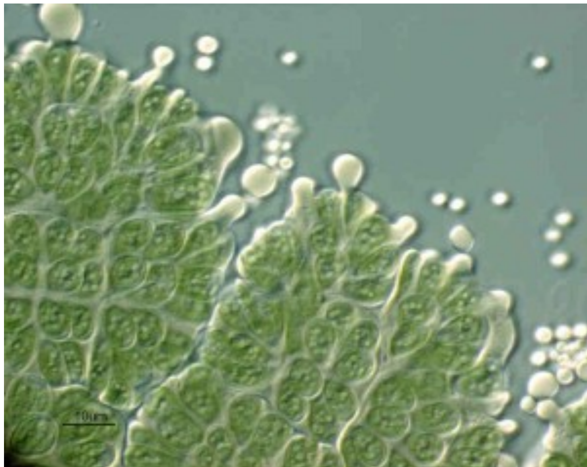
Public Law No: 110-140

December 19, 2007.

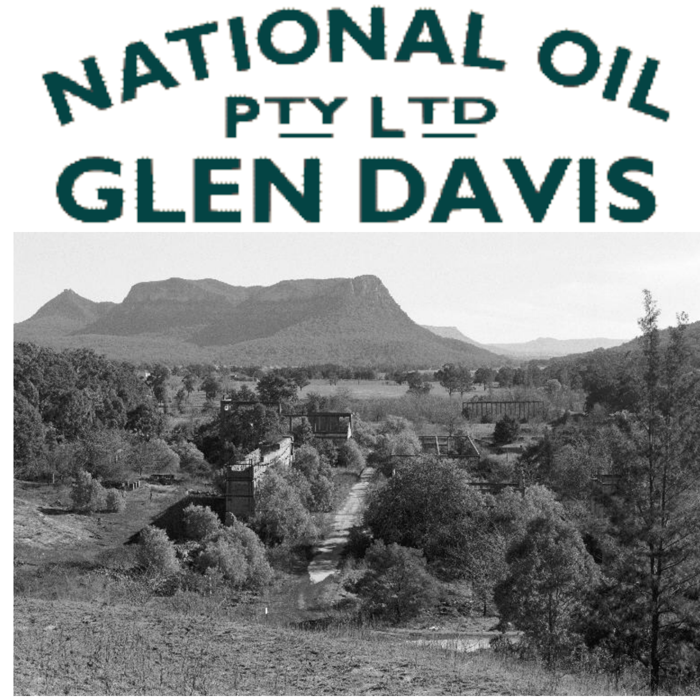
EISA RFS2 Renewable Biofuels Production Targets
In Billions of Gallons per Year (BGY)



Algae is the original source of today's fossil fuels

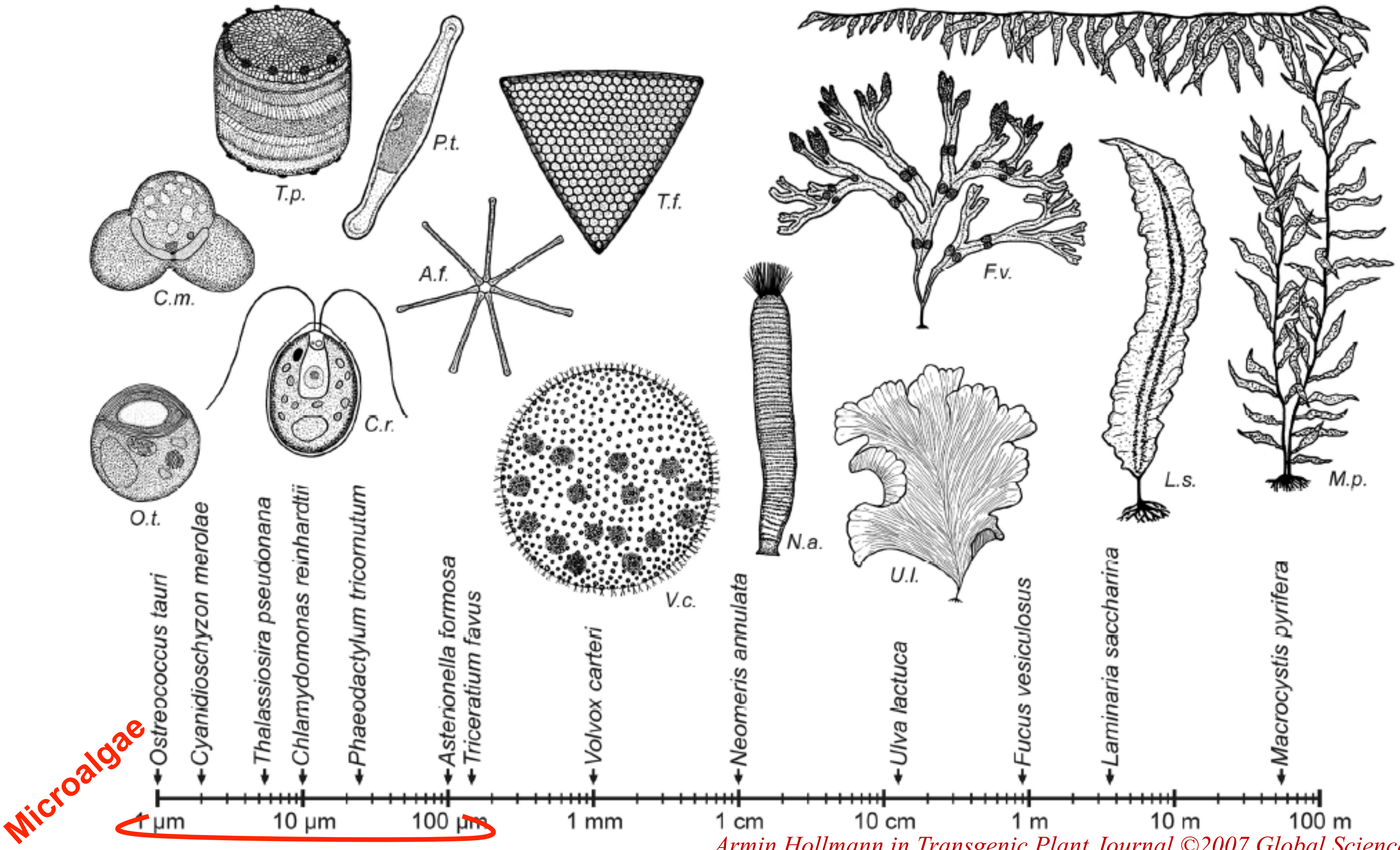


Boghead



http://www.glendavisworks.com.au/gallery.html

Algal Species Size Span 8 Orders of Magnitude



Armin Hollmann in Transgenic Plant Journal ©2007 Global Science Books

The Algae Advantage

Rapid growth rate

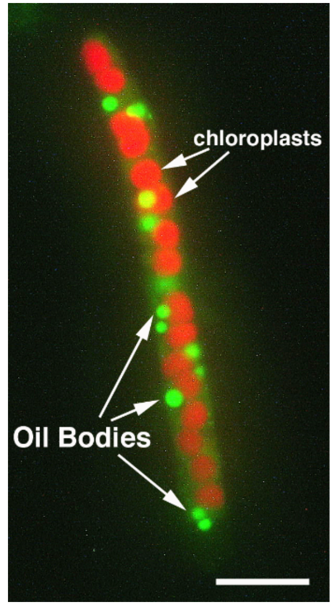
Double in 6-12 hours

High oil content

4-50% lipids (fats)

All biomass harvested

100%



Continuous harvesting

24/7, not seasonally

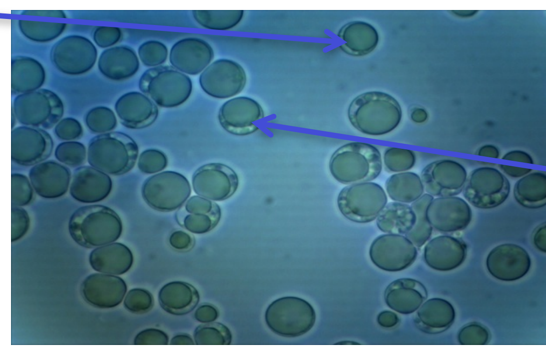
Sustainable

Capture up to 90% of injected CO_2

Utilize waste water

Non-food

4-50%
Lipid (fat) biomass



50-90%
Other biomass

Over-productivity of Algae

Olympic nightmare: A red tide in Yellow Sea

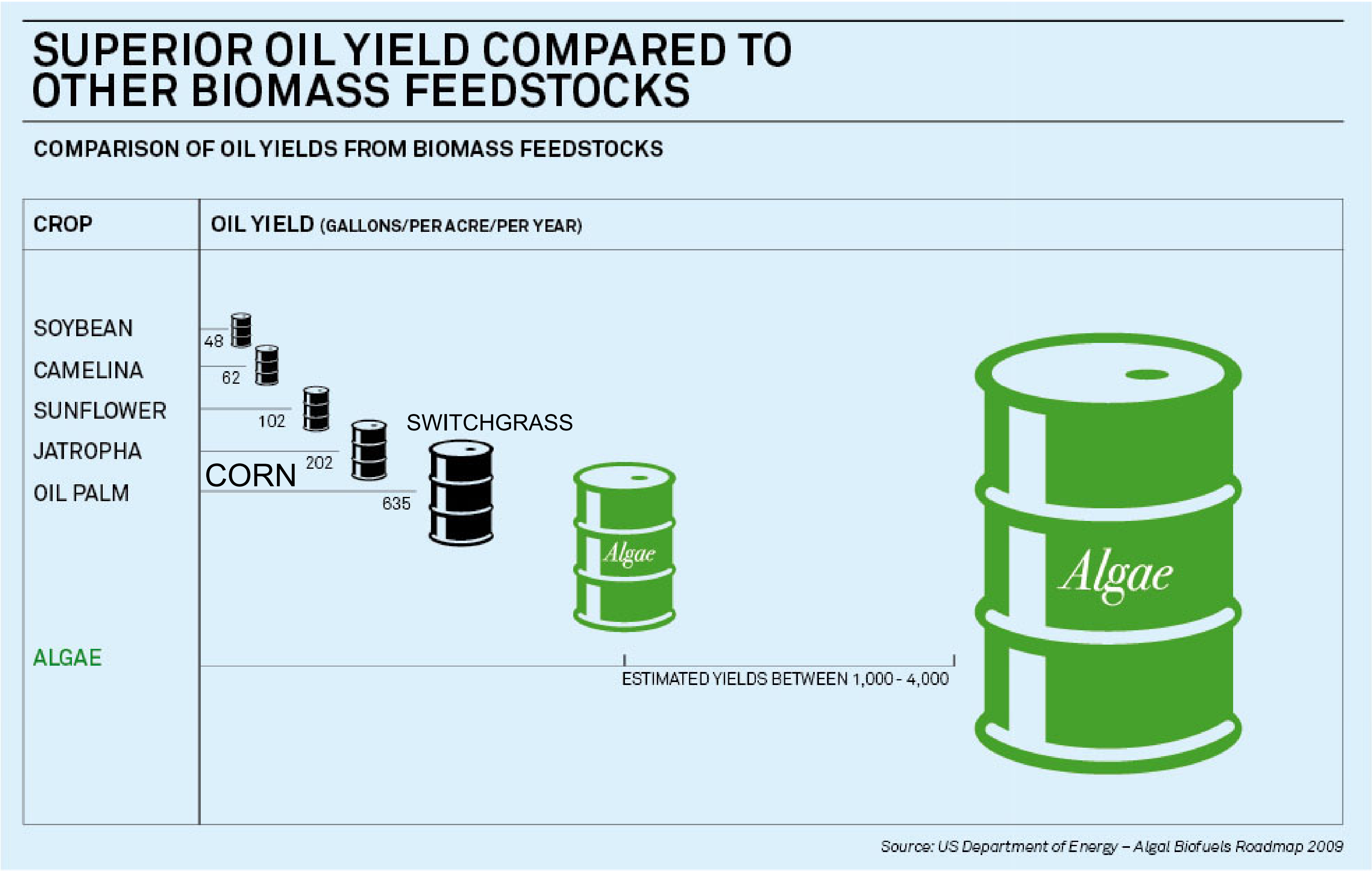
By Jim Yardley
Published: Monday, June 30, 2008

Photo Source: NBC Olympics Website

- Qingdao, China
- Green alga (*Ulva prolifera*)
- late May - early July 2008
- > 200,000 tons biomass
- < 17 km^2 coastal area (~ 4,200 acres)

> 47 tons/acre

The Promise of Algae-Based Biofuels

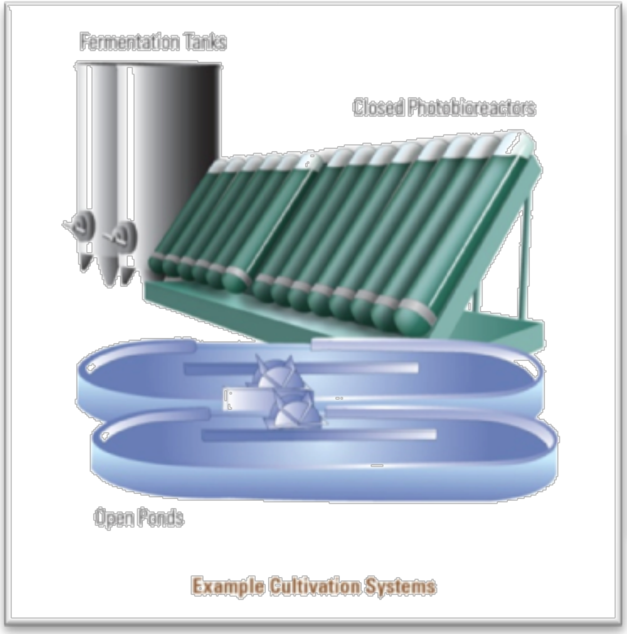


(Source: U.S. DOE, "National Algal Biofuels Technology Roadmap," 2009)

Technical Challenges for Algae Biofuels

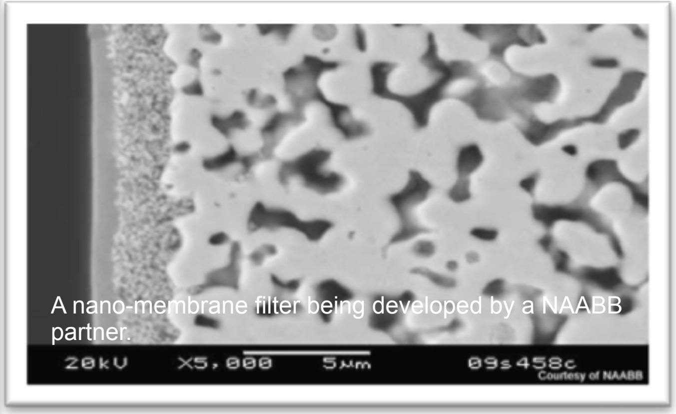
National Alliance For Advanced Biofuels and Bio-products

Biology and Cultivation



What conditions are needed for maximum growth and lipid content?

Biomass Harvesting and Recovery



A nano-membrane filter being developed by a NAABB partner.

Current methods for harvesting algae and lipid oil are too expensive and hazardous.

Conversion and End-use

What improvements can be made to make algae biofuel more competitive and sustainable?

Purpose of NAABB

NAABB Members

National Alliance For Advanced Biofuels and Bio-products

Lead Institution ★

The Donald Danforth Plant Science Center, St. Louis, MO

National Laboratories ●

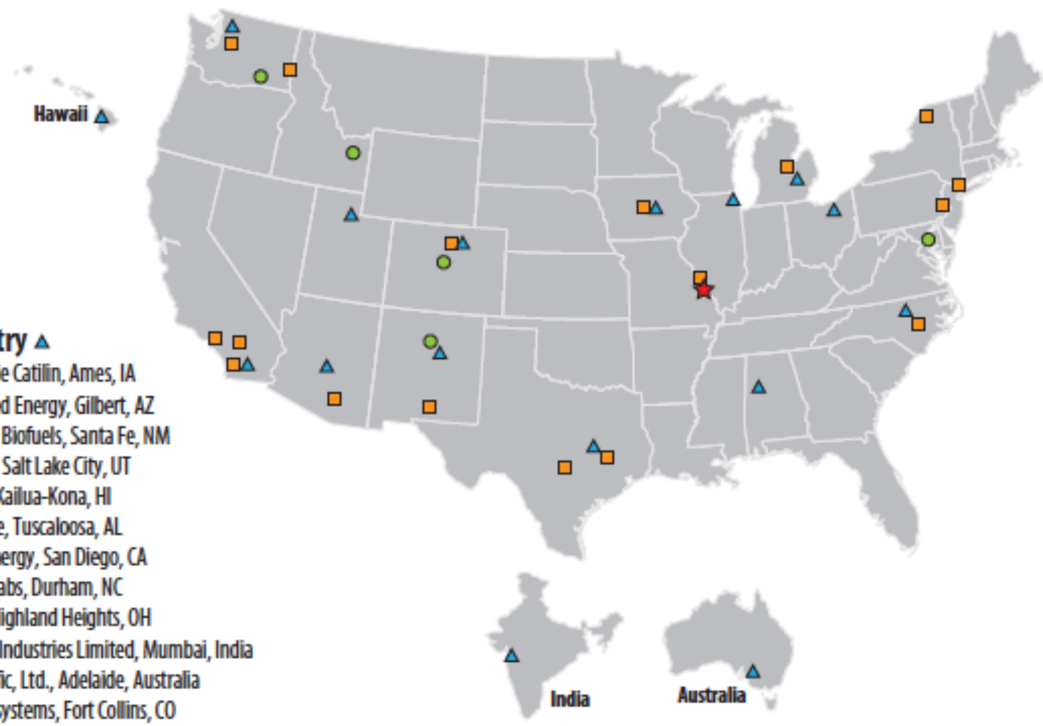
Los Alamos National Laboratory/New Mexico Consortium, Los Alamos, NM
Pacific Northwest National Laboratory, Richland, WA
Idaho National Laboratory, Idaho Falls, ID
National Renewable Energy Laboratory, CO
United States Department of Agriculture – Agricultural Research Service, Washington, DC

Universities ■

Brooklyn College, Brooklyn, NY
Clarkson University, Potsdam, NY
Colorado State University, Fort Collins, CO
Iowa State University, Ames, IA
Michigan State University, East Lansing, MI
New Mexico State University, Las Cruces, NM
North Carolina State University, Raleigh, NC
Texas AgriLife Research / Texas A&M University System, College Station, TX
University of Arizona, Tuscon, AZ
University of California Los Angeles, Los Angeles, CA
University of California Riverside, Riverside, CA
University of California San Diego, San Diego, CA
University of Pennsylvania, Philadelphia, PA
University of Texas, Austin, TX
University of Washington, Seattle, WA
Washington State University, Pullman, WA
Washington University St. Louis, St. Louis, MO

Industry ▲

Albemarle Catilin, Ames, IA
Diversified Energy, Gilbert, AZ
Eldorado Biofuels, Santa Fe, NM
Genifuel, Salt Lake City, UT
Cellana, Kailua-Kona, HI
Inventure, Tuscaloosa, AL
Kai BioEnergy, San Diego, CA
Palmer Labs, Durham, NC
Phycal, Highland Heights, OH
Reliance Industries Limited, Mumbai, India
Pan Pacific, Ltd., Adelaide, Australia
Solix Biosystems, Fort Collins, CO
Targeted Growth, Seattle, WA
Terrabon, Bryan, TX
UOP a Honeywell Company, Des Plaines, IL
Valicor, Dexter, MI



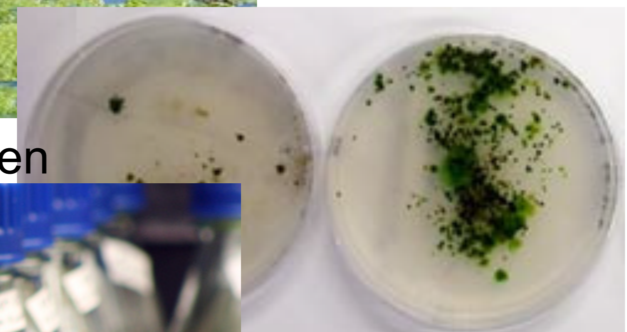
Algal Biology Accomplishments – isolation

Screened 2,200 independent algal isolates to find the best candidates for biofuels

- 30 of the best strains deposited into the UTEX (algae culture collection at the University of Texas)
- New micro-GC/MS techniques were developed for measuring lipid quantity and fatty acid profiles
- A genetic bar-coding system was developed for tracking algal strains

1. Sample

2. Isolate



3. Screen

4. Characterize

5. Validate

Algal Biology Accomplishments - engineering

NAABB National Alliance For Advanced Biofuels and Bio-products

Sequenced 8 new alga genomes

- 2 adapted mutants were re-sequenced
- Genomic annotations were completed for 3 genomes
- Two new web-based algal functional genome and metabolic mapping annotation tools were developed
- 250 transcriptomes were completed from 10 investigators across 8 institutions

Fifty gene targets identified for improving biomass and oil yield

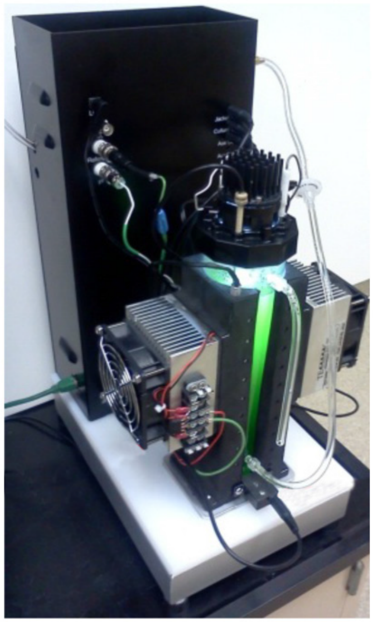
- Transcriptome analysis revealed genes that could increase oil accumulation

Table 2. NAABB Genome Projects

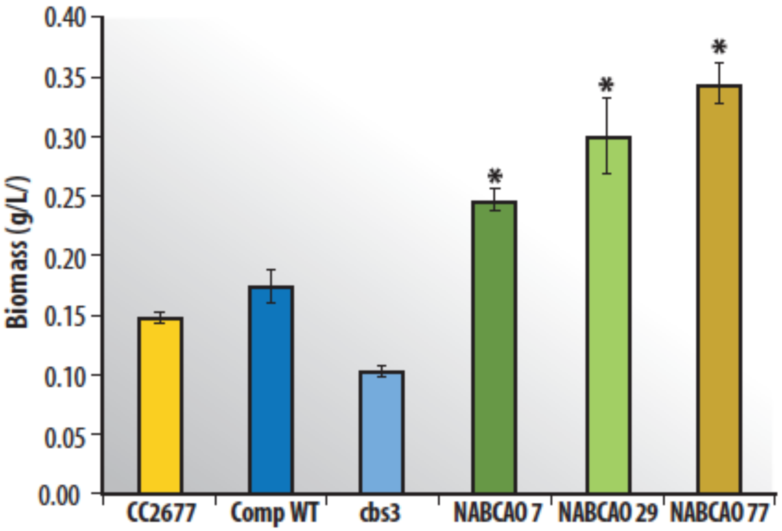
Genome	Code	Assembly Quality	Size, Mbp
Picochlorum sp.	NSC	Improved high quality draft	15.2
Auxenochlorella protothecoides UTEX25	CPI	Improved high quality draft	21.4
Chrysochromulina tobin	CAF	High quality draft	75.9
Nannochloropsis salina CCMP1776	NSK	Improved high quality Ddraft	27.7
Tetraselmis sp. LANL 1001	TSG	Standard draft	220
Chlorococcum sp. DOE 0101	CPT	Standard draft	120
Chlorella sp. DOE1412	CSJ	Standard draft	55
Chlorella sorokiniana Phycal 1228	CSJ	Standard draft	55

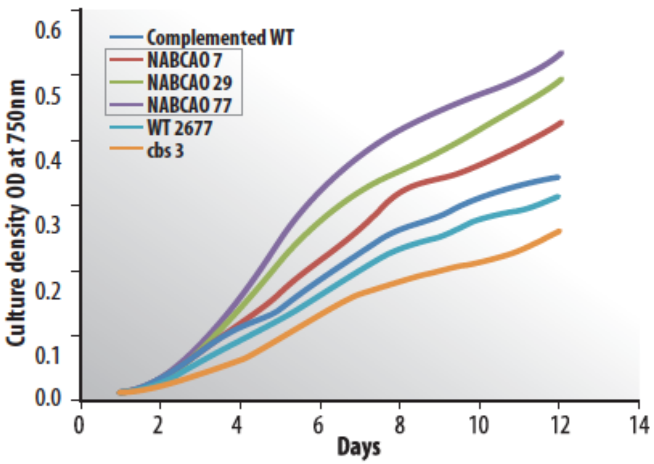
Algal Biology Accomplishments - engineering

Developed an algal transformation pipeline to increase biomass yield and lipid production



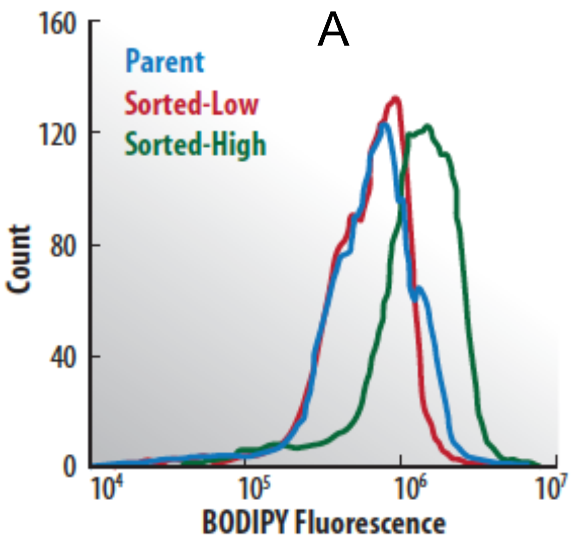
- Transgenic algae w/ self-adjusting light-harvesting antenna (NABCAO lines)
- Response to changing light levels or culture densities
- Adjust their chlorophyll ratios and antenna size
- Transgenics show productivity 2x greater than the wildtype



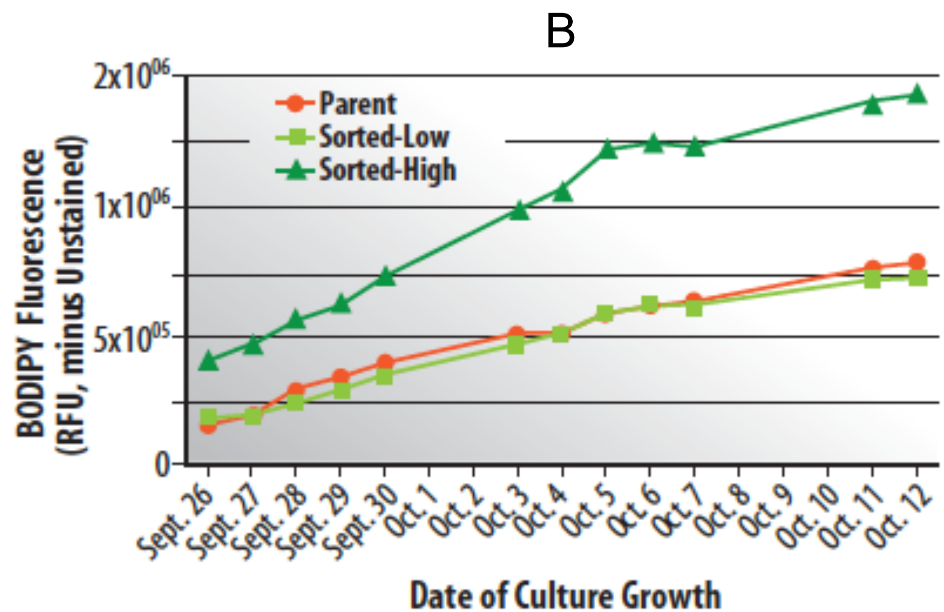


Developed new molecular tools for improving production strains

Algal Biology Accomplishments - evolution



Directed evolution resulted in new strains with 50% improved oil yields

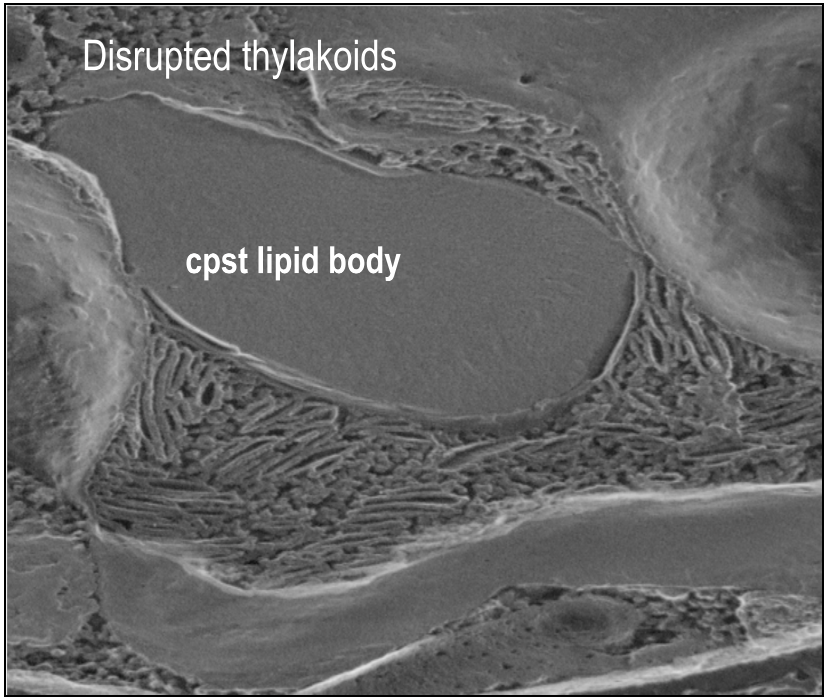


(A) Histograms of BODIPY fluorescence of parent and sorted populations

(B) During nitrogen starvation, all cultures accumulated lipids, with the sorted-high population outperforming the parent on all days (avg 2x improvement).

Algal Biology Accomplishments - lipids

Demonstrated the impact of lipid remodeling on cellular structure



- Nitrogen stress induces the breakdown of cellular components (autophagy):
  - Ribosomes
  - Peroxisomes
  - Chloroplasts
- It also causes the release of substrates for metabolism and biosynthesis
- Thylakoid rearrangement allows large lipid bodies to form
- NAABB studies enabled a better understanding of cellular response to N-starvation

Cultivation Accomplishments – pond systems

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Small Scale Test Beds Cultivation Studies (PNNL/NMSU/Texas A&M)

Large Scale Test Bed/Cultivation Studies & Biomass Production

Solix Biofuels PBR System

Cellana Inc., Kona Site

Closed System + **Open System**

Photobioreactors (PBRs) | Open Raceway Ponds

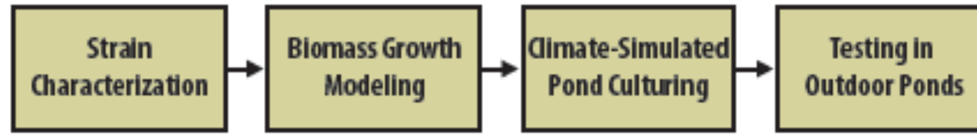
Contamination-minimized Monocultures (continuous production; inoculates open ponds)

Consistent Batch Production (harvested 3-7 days after inoculation; re-inoculate at end of last day)

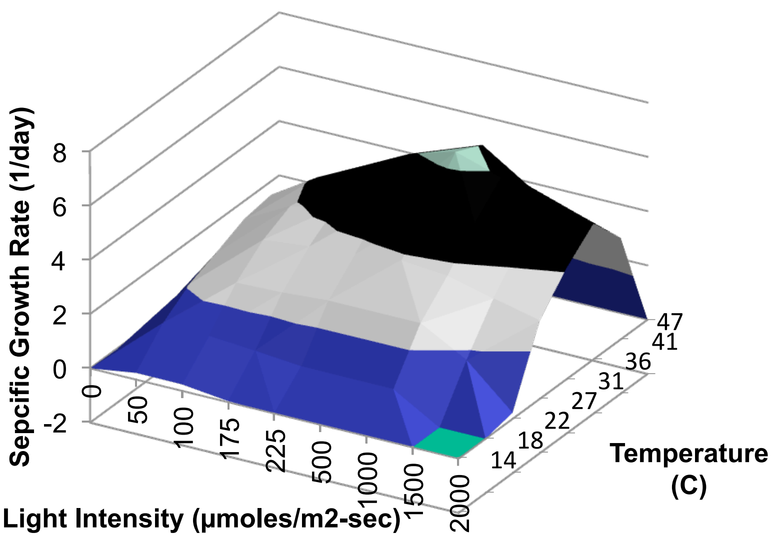
Covered by US Patents 7,770,322 & 5,541,056, Similar Patents/Patents pending in Europe, Australia, South Africa, Brazil, Japan, Mexico

Cultivation Accomplishments – tools, methods

Developed a microalgae growth model to evaluate the best strain and climate pairings



- A biomass growth model was developed which in conjunction with the Biomass Assessment Tool (BAT) to evaluate:
    - Productivity in various environmental conditions
    - Monoculture vs. mixed species



Cultivation Accomplishments - ARID

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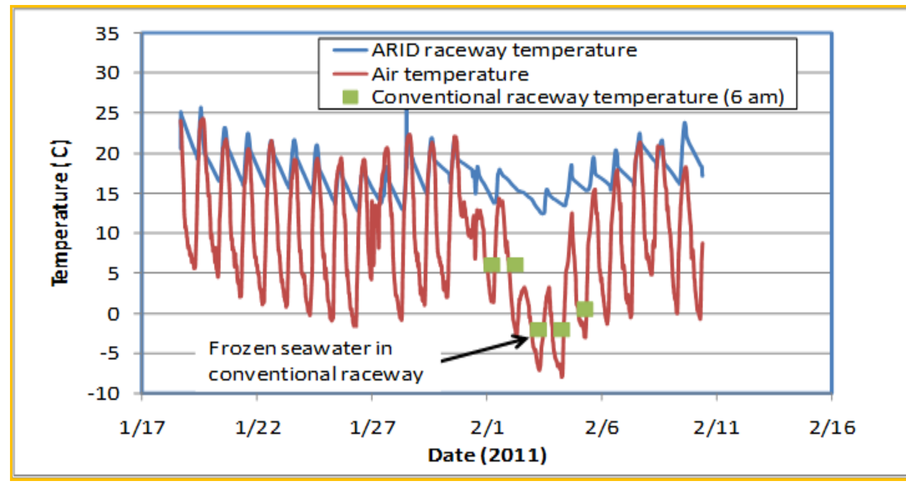
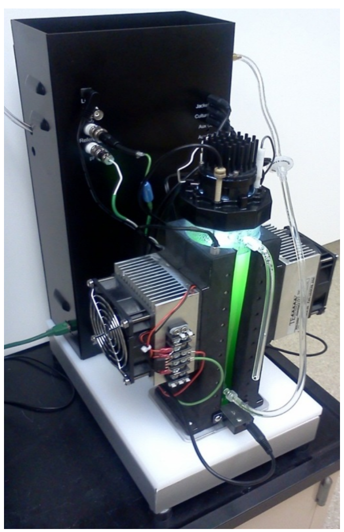
Developed ARID, a unique, low-energy pond system that maintains optimum conditions

- **The ARID raceway system has been shown to be cost – CAPEX > 8% OPEX < 45%**



Conducted & analyzed large-scale cultivation trials on 8 algae strains



A commercial photobioreactor system that mimics a pond environment has been developed and a start-up company, *Phenometrics*, initiated

Accelerating Algal Discoveries
Phenometrics

Harvesting Accomplishments - dewatering

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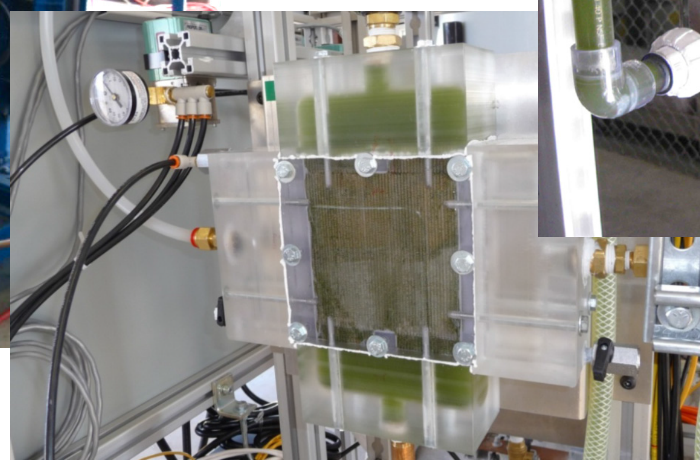
Evaluated 9 innovative harvesting and extraction technologies at lab scale

- **Current harvesting technologies (i.e. centrifuge) are major bottlenecks to cost effective production of biofuels from algae**
- **Need to concentrate from 1 g/L to 40 or 100 g/L prior to extraction**

Electrocoagulation



Filtration

Ultrasonic

Harvesting Accomplishments – scale up

Scaled-up three innovative harvesting technologies

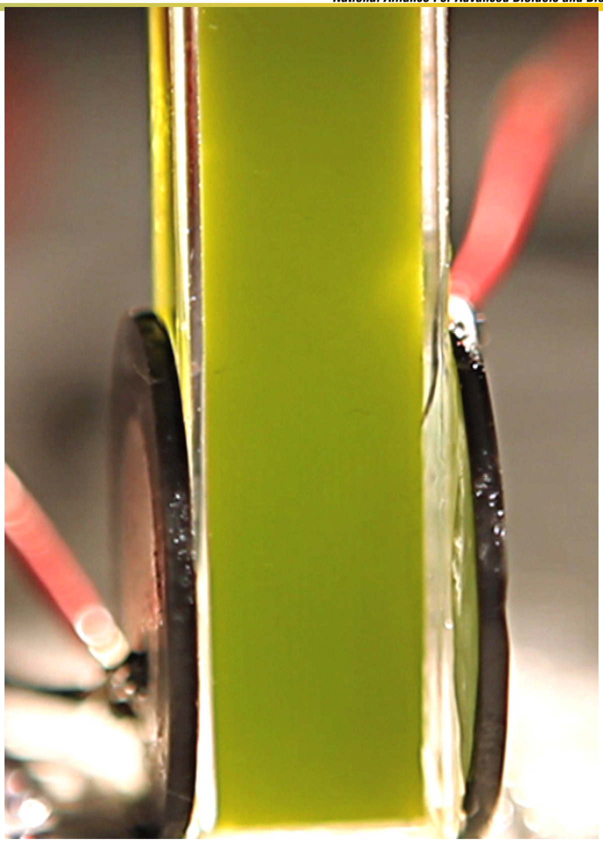
Technology	Energy Input (kWh/kg)	Chemical Cost (USD/Kg)	Electricity Cost (USD/kg)	OPEX (USD/kg)	OPEX (USD/Gal)	PEL
Baseline Harvesting Technologies						
Centrifuge Baseline	3.300	0.000	0.264	0.264	1.799	56.98
Dissolved Air Floatation	0.250	0.008	0.020	0.028	0.191	4.317
Spiral Plate Separation	1.418	0.000	0.113	0.113	0.773	24.47
NAABB Harvesting Technologies						
Chitosan Flocculation	0.005	0.055	0.000	0.055	0.377	0.093
$AlCl_3$ Flocculation	0.120	0.046	0.010	0.056	0.380	2.072
Electrolytic Harvesting*	0.039	0.004	0.003	0.007	0.049	0.673
Membrane Filtration*	0.046	0.000	0.004	0.004	0.025	0.789
Ultrasonic Harvesting*	0.078	0.000	0.006	0.006	0.043	1.347
Baseline Extraction Technologies						
Pulsed Electric Field	11.52	0.000	0.922	0.922	6.280	198.9
Wet Hexane Extraction	0.110	0.001	0.009	0.010	0.068	1.904
NAABB Extraction Technologies						
Solvent Phase Algal Migration	1.648	0.947	0.132	1.079	7.352	28.45
Ultrasonic Extraction	0.384	0.000	0.031	0.031	0.209	6.630
Nanoparticle Mesoporous	0.008	54.35	0.001	54.36	370.5	0.137
Supercritical	1.174	0.000	0.094	0.094	0.640	20.27

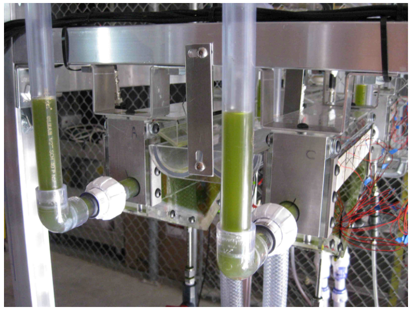
Table 3. Baseline feasibility assessment of harvesting and extraction technologies.

**The highlighted harvesting technologies were selected for scale-up.*

Harvesting Accomplishments - ultrasonic

- **Sound waves aggregate algae**
  - No moving parts; no chemical additives
  - Harvested cells are still viable
  - 100X concentration effect in a single pass
  - Low energy input: 0.01-0.04 kWhr/m^3
  - Low cost: 1-4 cents per gal lipid
- ***Pilot Test* September 2012*:***
  - *N. oculata* from Solix Biosystems
- ***Scaled-up Harvester:***
  - 45-225L/hr using 9 modules
  - Scaled harvester module delivered energy to the liquid layer 100-fold more effectively than laboratory-scale unit





Large scale concentration

Harvesting Accomplishments - filtration

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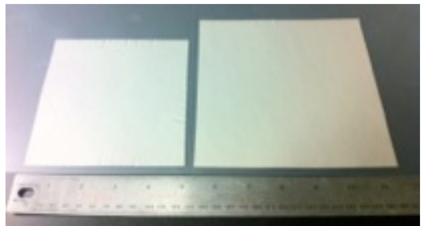
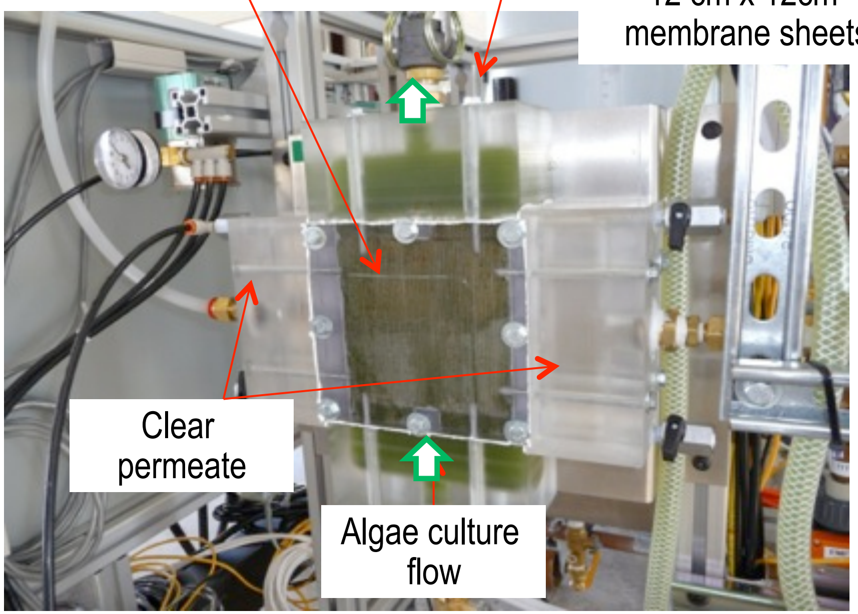
- Developed thin porous Ni alloy metal sheet membranes
- Field tests using mobile unit performed in Pecos, TX

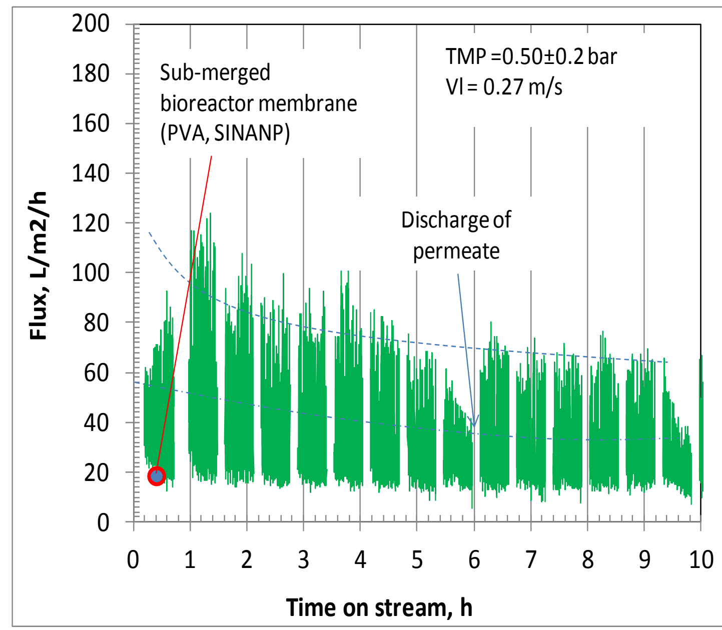
Online observation of the membrane surface



Membrane module assembled from 18 of 12 cm x 12cm membrane sheets



Filtration was conducted at relatively low liquid flow velocity with liquid/bubble slug flow



Harvesting Accomplishments – electrocoagulation

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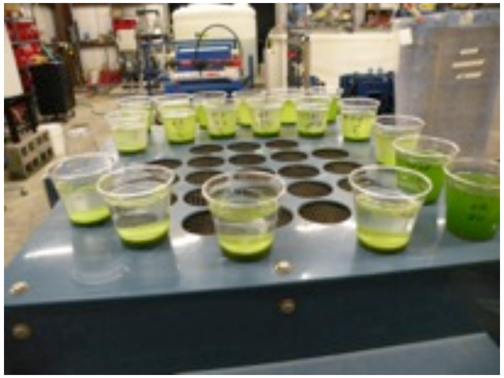
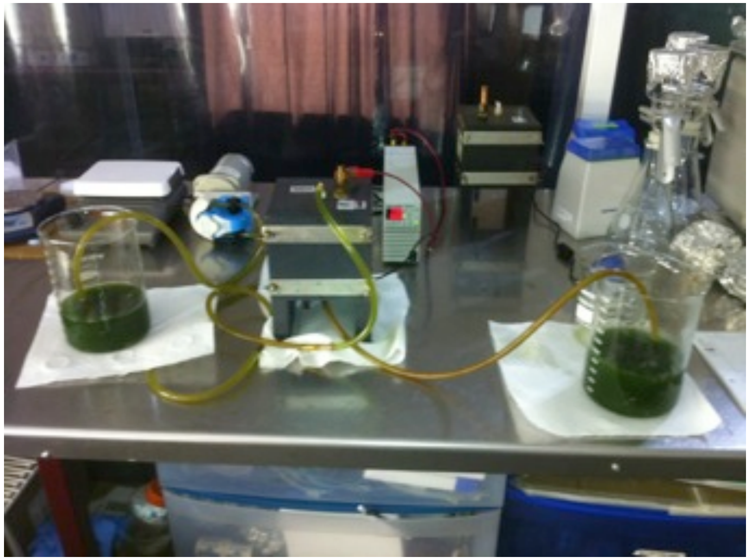
- Reactive metallic electrodes to produce positively charged ions that induce coagulation of the negatively charged microalgae
- Pilot Test July 2012, Pecos Texas
- Final Solids – 8%
- Energy - 0.04 kW/m^3
- Loading 270 m^3/kg hr





Conversion Accomplishments

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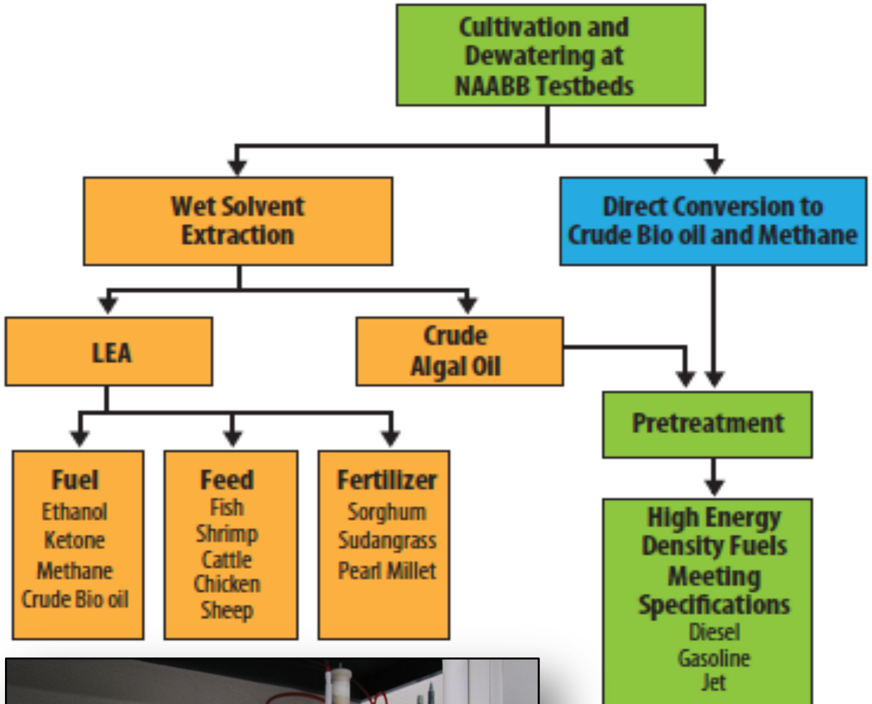
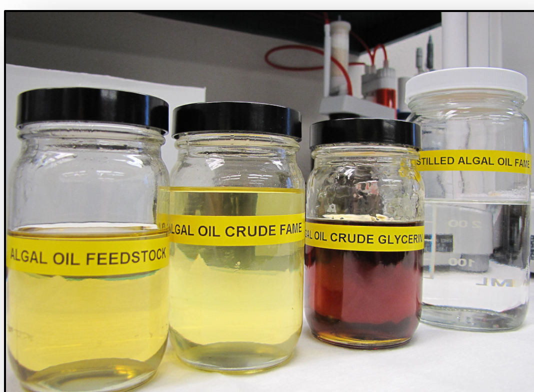
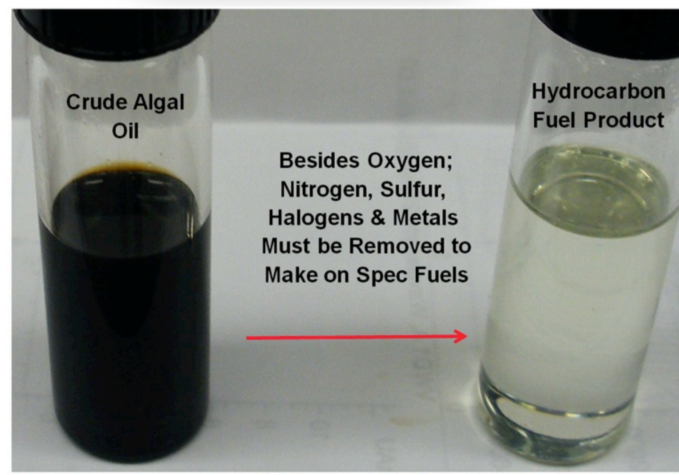
Developed detailed algal biomass and biofuel characterization methods







"Contaminants" for Conversion are "Nutrients" for Cultivation

Characterization of Jet Fuel from HTL Conversion

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Developed processes and economic models for 8 fuel production pathways

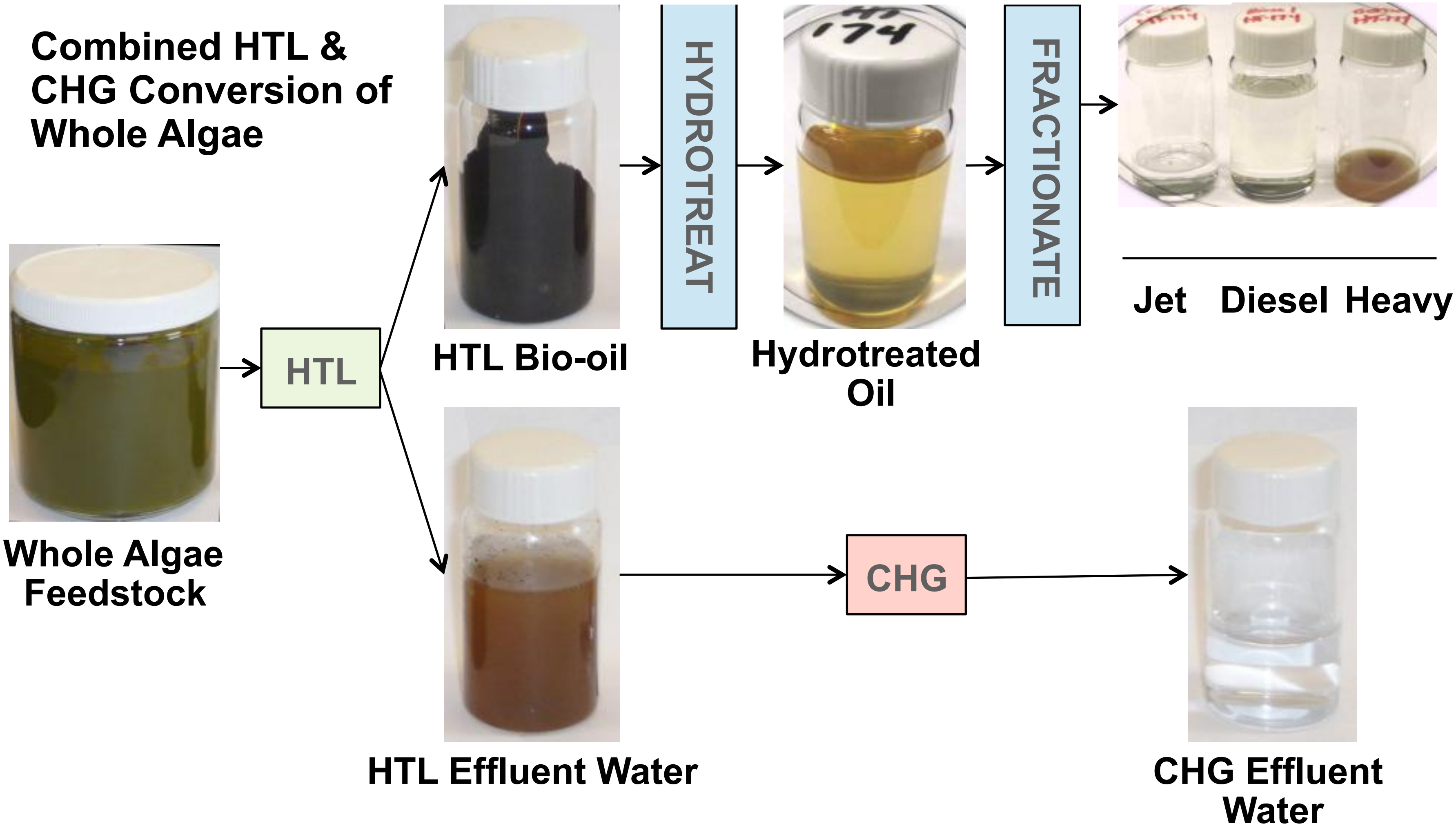
Table 4. NAABB algal biofuels met the jet fuel specification.

Algal Biomass Source				Cellana *N. oceanica* (High Lipid)	Solix *N. salina* (High Lipid)	TAMU Pecos *N. salina* (Low Lipid)	Cellana *N. oceanica* (High Lipid)	Cellana *N. oceanica* (Low Lipid)
Extraction Process				Inventure FAME	Valicor Wet Solvent	Valicor Wet Solvent	Valicor Wet Solvent	PNNL HTL
Crude Oil Type				Distilled FAME	Crude Lipid Extract	Crude Lipid Extract	Crude Lipid Extract	HTL Bio-Oil
Parameter	**D7566 HEFA Specification**	**Jet A**	**Jet A1**					
Density (g/L)	730 - 770	775 - 840	775 - 840	755	753	756	749	780
Freeze Point (°C) max	-47	-40	-47	-49	-63	-62	<-80	-57
Flash Point (°C) min	38	38	38	43	40	45	40	59
Distillation								
10% Recovered Temp (T10) °C max	205	205	205	156	160	150	152	167
Final Boiling Point (°C) max	300	300	300	279	271	284	264	272
T50-T10 min	15	–	–	36	34	39	28	37
T90-T10 min	40	–	–	92	85	84	70	75

Conversion Accomplishments – HTL & CHG

Produced jet/diesel fuel that met ASTM specifications using NAABB strains and production pathways

Combined HTL & CHG Conversion of Whole Algae

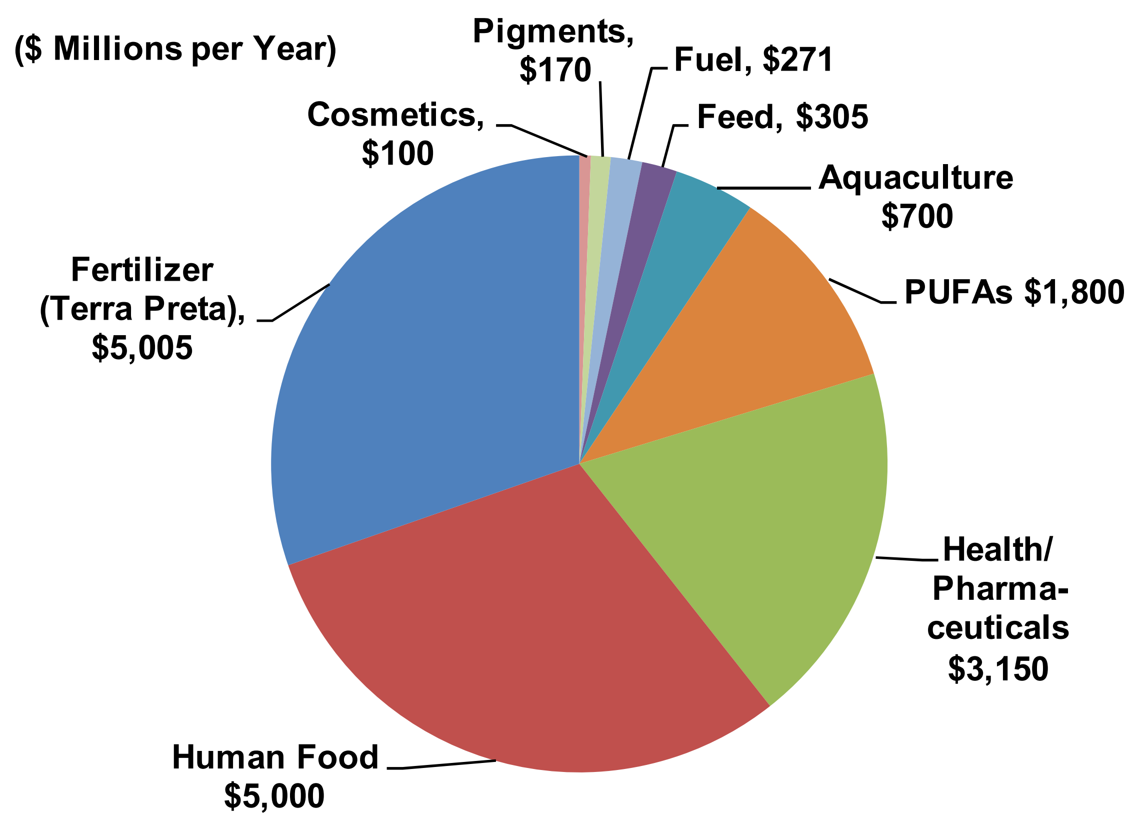


Algae to Oil – The Hydrothermal Process

Algal Bioproducts World Markets

National Alliance For Advanced Biofuels and Bio-products

Total Value of Algae Products, World Markets: 2010



(Source: Pike Research)

Agricultural Coproducts Accomplishments

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Demonstrated palatability of LEA through *in vivo* tests

Table 5. Summary of the feeding studies conducted on animals and mariculture.

Type of Animal Tested	Performance	Digestibility/Palatability
Fish (red drum and hybrid striped bass)	Replaced up to 10% of crude protein from fishmeal and soy protein concentrate with LEA without causing substantial reductions in fish performance.	Excellent
Shrimp	At least a 20% inclusion level of LEA could replace the expensive soybean and/or fish meals in shrimp feed.	Excellent
Cattle	Supplementation of LEA stimulated forage utilization to a similar extent as cottonseed meal in cattle (100 mg N/kg body weight).	Blends of LEA and conventional protein supplements will minimize concerns of palatability. Does not impair fiber digestion.
Sheep	LEA may be a viable protein and mineral supplement for sheep; however, caution is advised for diets containing greater than 20% LEA due to slight reductions in performance.	Good
Pigs	Use of LEA is not recommended at this time. Supplementation with 5–20% LEA was tested and reductions in growth and weight gain were noted.	Not palatable
Chicken	Inclusion of 5% LEA in young broiler chicken and laying hens diets may be viable.	Good

Evaluated the use of LEA as a fertilizer

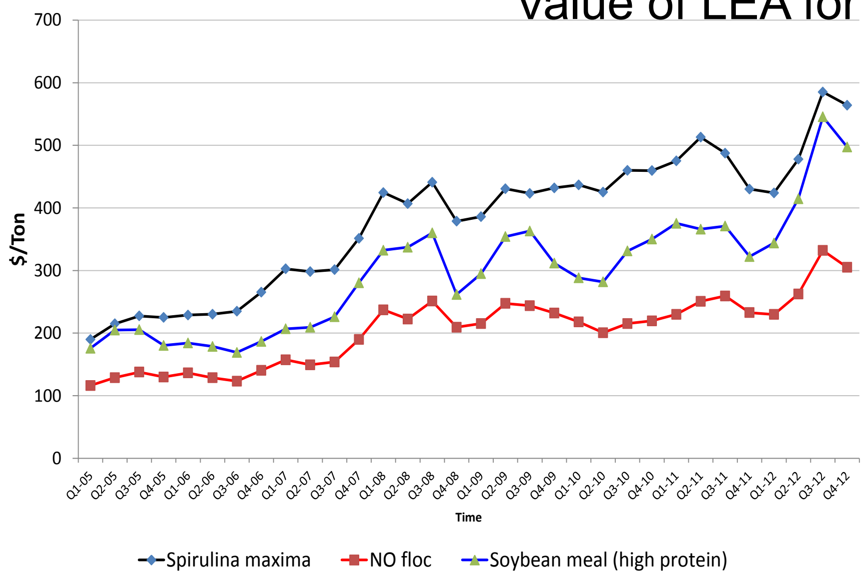
Agricultural Coproducts Accomplishments

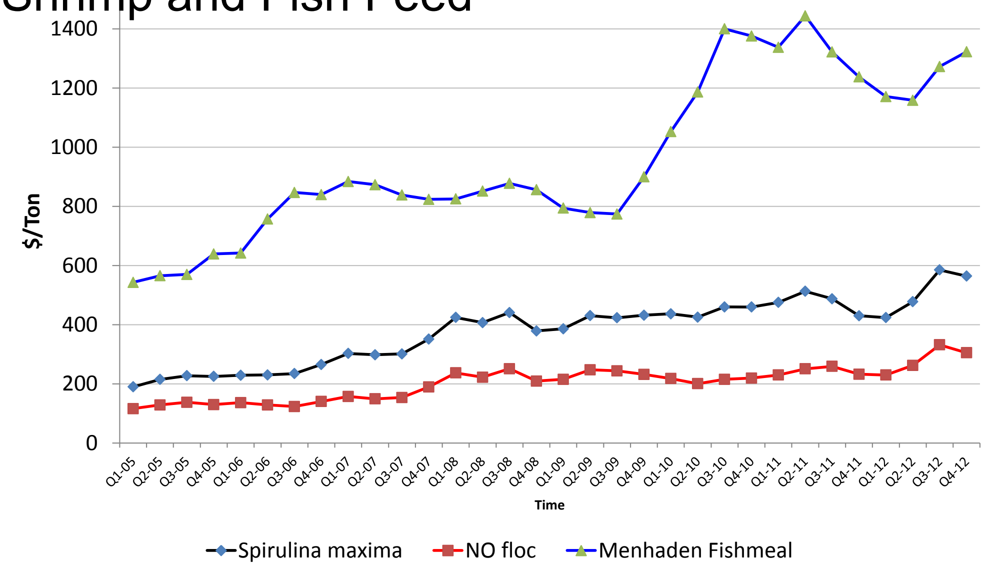
NAABB National Alliance For Advanced Biofuels and Bio-products

Extensively characterized LEA for feed value and contamination concentration

- **Based on fractions of energy, protein, fat, etc. in LEA and whole algae; the value of these ingredient in mariculture rations are:**
  - Whole algae averages $82/ton more than soybean meal – about $373/ton in 2013
  - LEA averages $94/ton less than soybean meal – about $200/ton in 2013
  - A non-market advantage of feeding LEA to mariculture is it replaces a portion of fishmeal in the ration thus protecting the ocean's fish population

Value of LEA for Shrimp and Fish Feed



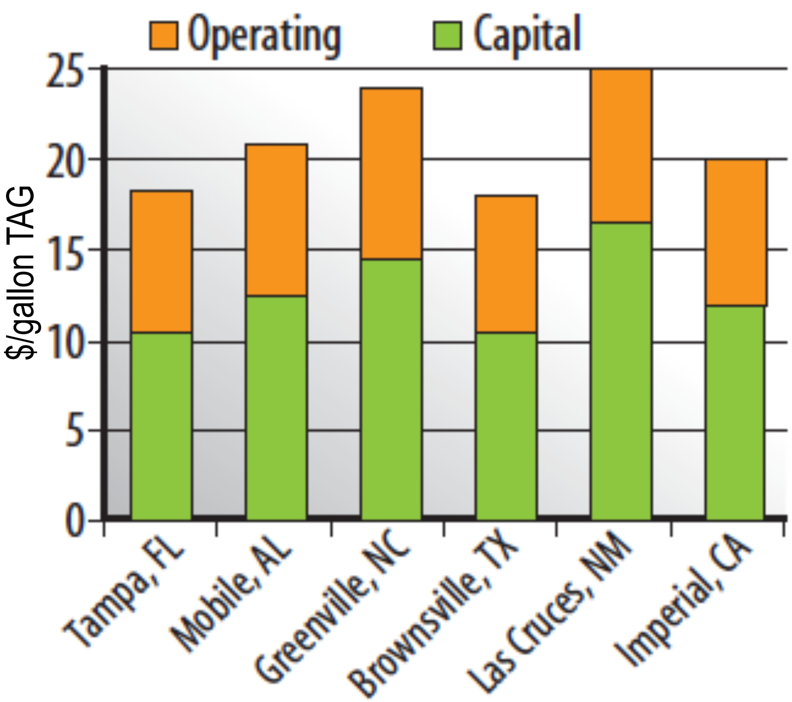


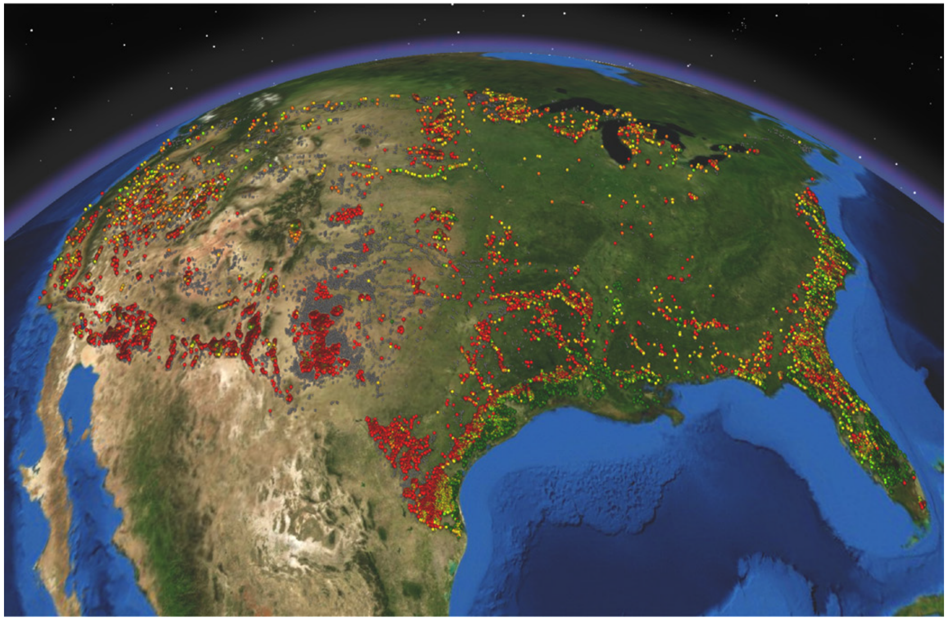
Sustainability Accomplishments

NAABB National Alliance For Advanced Biofuels and Bio-products

Analyzed production data from outdoor algal cultivation facilities

- **Mean annual biofuel production capability**





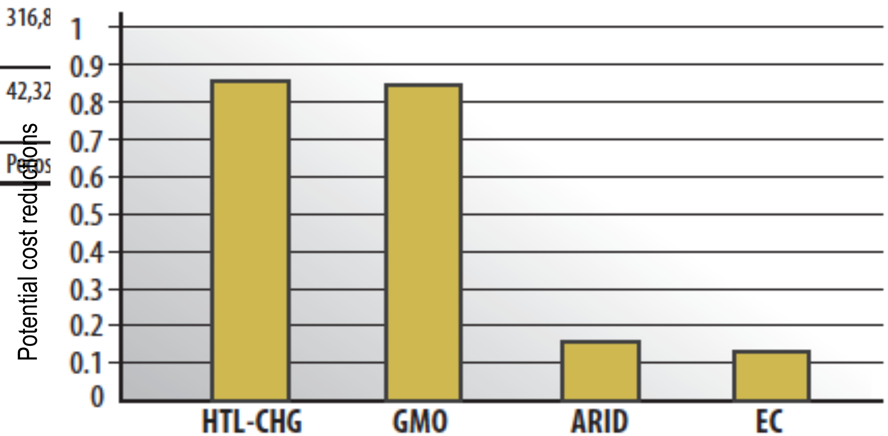
Resource-feasible algae production sites in the United States were selected from the BAT analysis

Determined that a robust growth regime over the entire year is more important in lowering GHG emissions than selecting for peak productivity

NAABB Scenarios for Overall Economic Feasibility

Table 10. Summary of the technologies analyzed for the seven alternative scenarios.

	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6	Scenario 7
Products	Crude TAG & LEA	Crude TAG & LEA	Crude TAG & LEA	Crude HTL oil & methane	Crude HTL oil & methane	Crude HTL oil & methane	Crude HTL oil & methane
Cultivation	Open pond w/ liners	Open pond w/ liners	ARID w/liners	Open pond w/ liners	Open pond w/ liners	ARID w/liners	ARID w/liners
Feedstock strain g/m2/d	Generic 7.4	Generic 7.4	Generic 9.3	Generic 7.4	Generic 19.4	Generic 9.3	GMO 23.2
Harvesting	Centrifuge	EC	EC	Centrifuge	EC	EC	EC
Extraction	Wet solvent extraction	Wet solvent extraction	Wet solvent extraction	HTL-CHG	HTL-CHG	HTL-CHG	HTL-CHG
Nutrient recycling	No	No	No	Yes	Yes	Yes	Yes
Biomass Production (tons/yr)	119,900	119,900	152,200	119,900	316,8		
Crude Oil Production (gallons/yr)	4,679,000	5,096,000	6,470,000	13,506,000	42,32		
Location	Pecos, TX	Pecos, TX	Tucson, AZ	Pecos, TX	Pecos		



Developed a rigorous approach for assessing technologies to fully evaluate 7 scenarios

NAABB innovations:
Electrocoagulation harvesting (EC)
Open pond cultivation system (ARID)
Hydrothermal liquefaction (HTL)
Genetically modified algae strain (GMO)

Sustainability Accomplishments – cost reductions

NAABB National Alliance For Advanced Biofuels and Bio-products

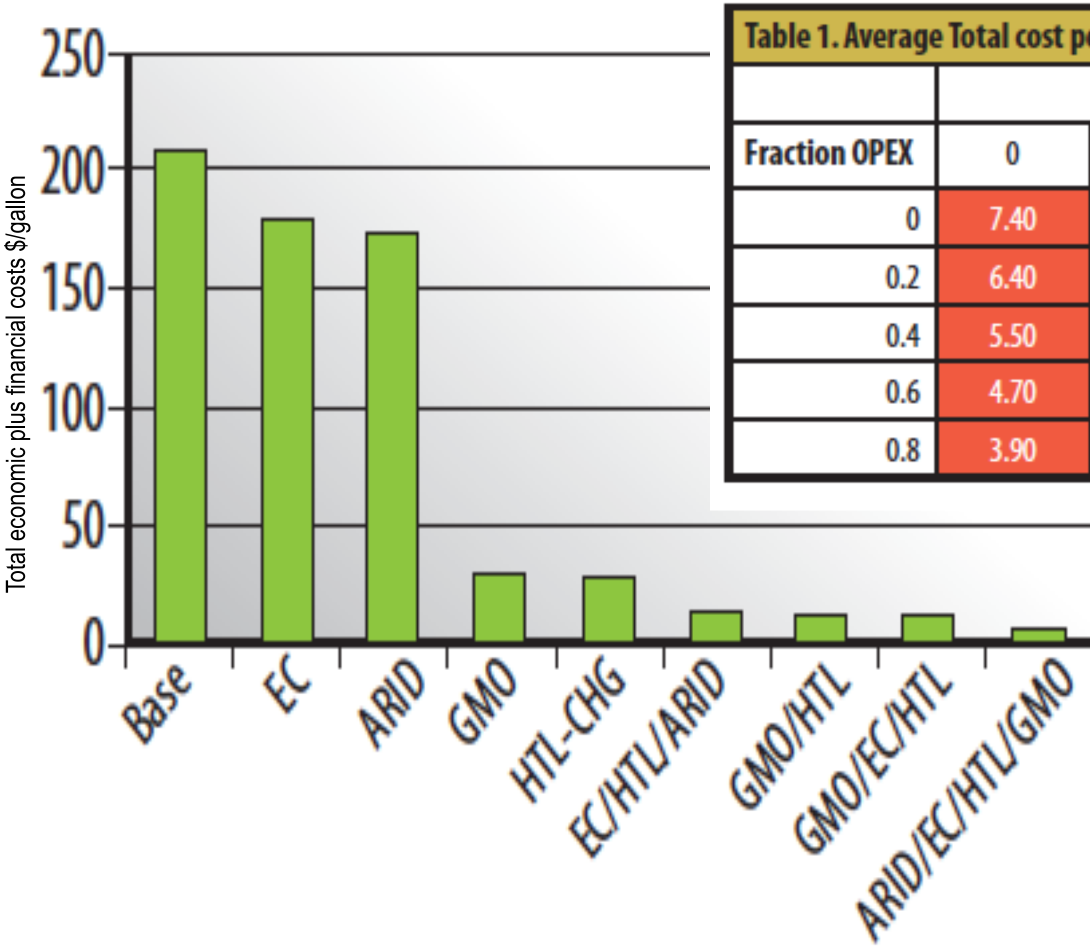


Table 1. Average Total cost per Gallon for Biocrude Oil ($/Gallon)

	Fraction Reductions in CAPEX				
Fraction OPEX	0	0.2	0.4	0.6	0.8
0	7.40	6.40	5.40	4.50	3.50
0.2	6.40	5.50	4.50	3.60	2.60
0.4	5.50	4.60	3.70	2.80	1.90
0.6	4.70	3.80	2.90	2.10	1.40
0.8	3.90	3.10	2.30	1.60	0.80

$7.50 /gal biocrude can be achieved under these scenarios

$2 /gal biocrude will require significant cost reductions in CAPEX and OPEX

Outlook for the future

NAABB – National Alliance For Advanced Biofuels and Bio-products

NAABB improved each step of the production process;
but to create a sustainable biofuels industry, improvements are needed

A new concept for algae farms is needed.

Capital expenditure & resource utilization must be minimized while maximizing productivity

Improve Biomass Productivity
Target: 2-4 X Increase (g/m^2)

- Transfer genes to production organisms
- Validate GMO strain outdoors
- Reduce pond crashes

Improve Cultivation-Harvesting Efficiency
Target: 1.5X

- Reduce energy & extend season
- H_2O and CO_2 management
- Demonstrate harvesting scale-up

Improve Extraction-Conversion Yield
Target: 2 X Increase in Yield

- Optimize HTL processing/ upgrading
- Integrate with CHG Processing
- Enable Nutrient Recycle

Integration Tools

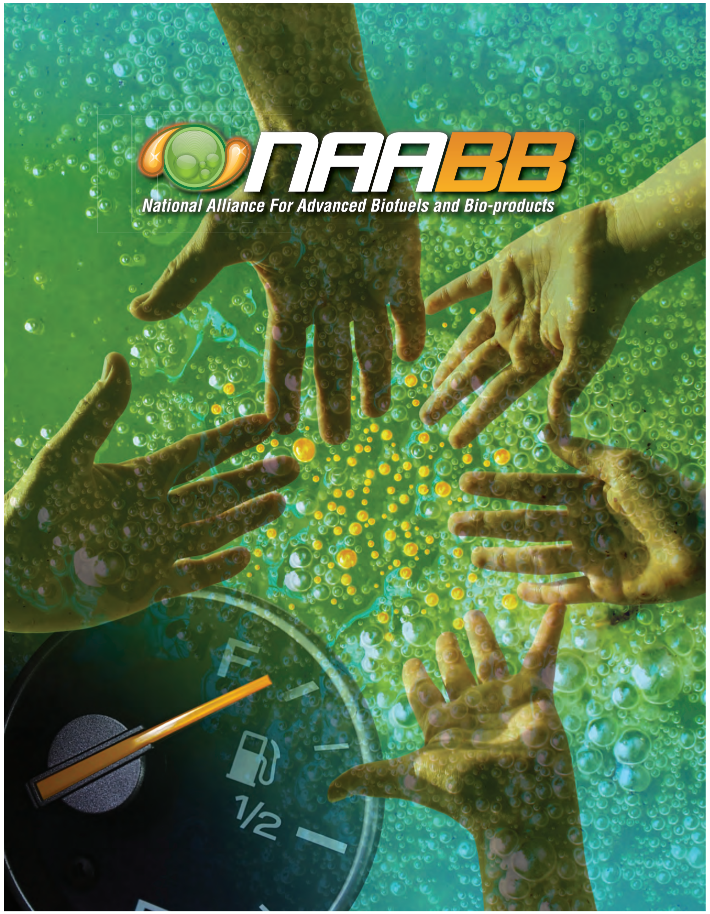
- ✓ Growth models
- ✓ Resource Assessment Models
- ✓ Sensitivity analysis to optimize conversion systems
- ✓ Tools to optimize algae to climate conditions
- ✓ LCA for recycle of water nutrients and energy balances

NAABB by the numbers

- >100 scientific publications and 5 theses
- New Journal: ALGAL RESEARCH (Elsevier)
- New Conference Series: *International Conference on Algal Biomass, Biofuels and Bioproducts*
- Contributed 30 top algae strains culture collection at the University of Texas

- 33 Intellectual Property Disclosures
  - Molecular biology tools – 10
  - Cultivation – 5
  - Harvesting and Extraction – 7
  - Fuel conversion – 8
  - Co-products and other – 3
- New company: *Phenometrics*

NAABB Reports



For more detail about NAABB Accomplishments:

Final Technical Report (500 page pdf)

1. NAABB consortium background and organization
2. Main R&D accomplishments
3. Short summaries of individual NAABB project
4. URL: TBD

Synopsis (27 page pdf):
URL: http://www.energy.gov/eere/bioenergy/related-links-0

To all the NAABB members and the DOE-EERE:

Thank You!!