The Promise and Challenge of Algae as Renewable Sources of Biofuels

Biomass Program Webinar
September 8, 2010

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DOE-EERE-Office of Biomass Program

Webinar Outline

1. Introduction to DOE Biomass Program and our emerging algal biofuels initiative (25 minutes)

2. Overview of DOE's *National Algal Biofuels Technology Roadmap:* defining the algal biofuels supply chain and the remaining R&D challenges (30 minutes)

   - Q&A Period (10 minutes)

3. Presentations from Biomass Program algae R&D consortia: NAABB, Cellana LLC, CAB-Comm, and SABC (60 minutes)

   - Q&A Period (10 minutes)

Introduction to DOE Biomass Program and our Emerging Algal Biofuels Initiative

Biofuels Interest & Motivation

- **Energy Security & Desire for Reduced Dependence on Imports**
  - Oil imports of ~10-M bbl/day (~150-Bgal/yr) … over half of U.S. oil use … two thirds for transportation fuels
  - Subject to supply disruption from volatile regions
  - Represents ~$300-B/yr burden on the U.S. economy (at $80/bbl) … supports interests hostile to the U.S.
  - Increasing competition (China, India, etc.) & price volatility with limited global petroleum production capacity and supplies
- **Desire for Reduced Greenhouse Gas Emissions**
  - Concern that fossil fuel emissions have adverse environmental impacts
- **Attractive Characteristics of Biomass-Based Biofuel**
  - Can displace fossil carbon fuels with more carbon-neutral fuels
  - Can be produced domestically, contributing to U.S. economy
- **Issues**
  - Must be *affordable & sustainable* at commercial scale
  - Energy balance & GHG footprint depend on approach & processes

Snap-Shot of U.S. Transportation Fuel Use

Fuel Use & CO_2 Emissions Give Context for Biofuels Production Targets

- **Current U.S. Consumption/Demand for Transportation Fuels***:

Gasoline blends:	~140-B gal/yr
Diesel:	~ 45-B gal/yr
Aviation:	~ 25-B gal/yr
Total:	~ 210-B gal/yr

*DOE/EIA Annual Energy Outlook 2008

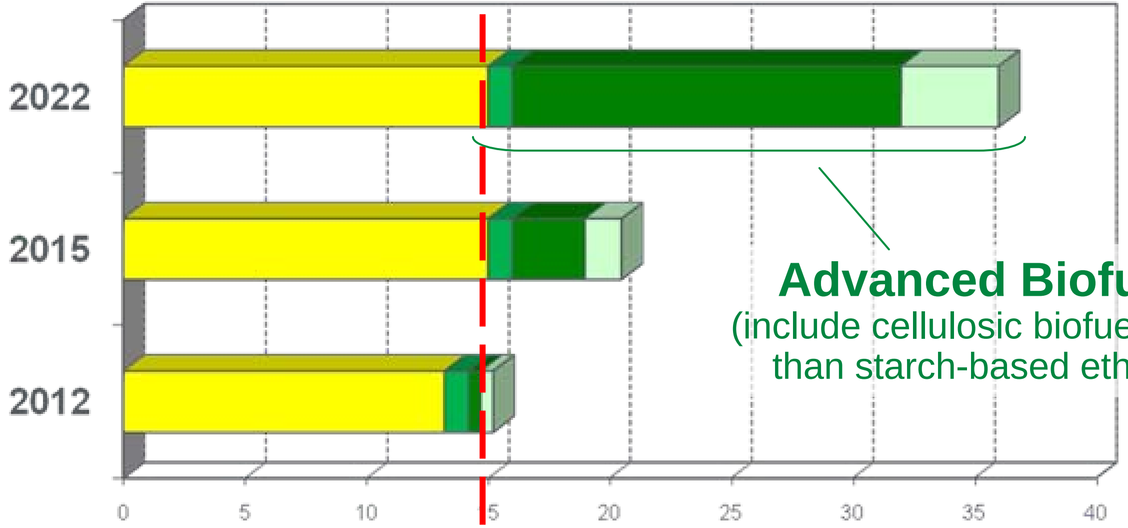
- **Burning each of these fuels produces ~ 20 pounds of CO_2 / gallon**
  - 210-B gal/yr x 20-lbs CO_2/gal ~ 2.1 Billion tons of CO_2 / year
  - Compared with ~ 4-Billion tons CO_2 from stationary sources (power plants, cements plants, ethanol plants, etc.)
  - Capture and sequestration of CO_2 and/or re-use of emitted carbon from transportation vehicles is impractical
- **Improved end-use efficiency can complement biofuels in helping:**
  - **Reduce reliance on imported petroleum**
  - **Reduce GHG emissions**

Biofuel Policy Drivers for DOE and others
EPACT-2005 and EISA-2007

Renewable Fuel Standard (RFS2)

- 🟨 Conventional (Starch) Biofuel
- 🟩 Biomass-based diesel
- 🟩 Cellulosic Biofuels
- ⬜ Other Advanced Biofuels

15 BGY cap on conventional (starch) biofuel



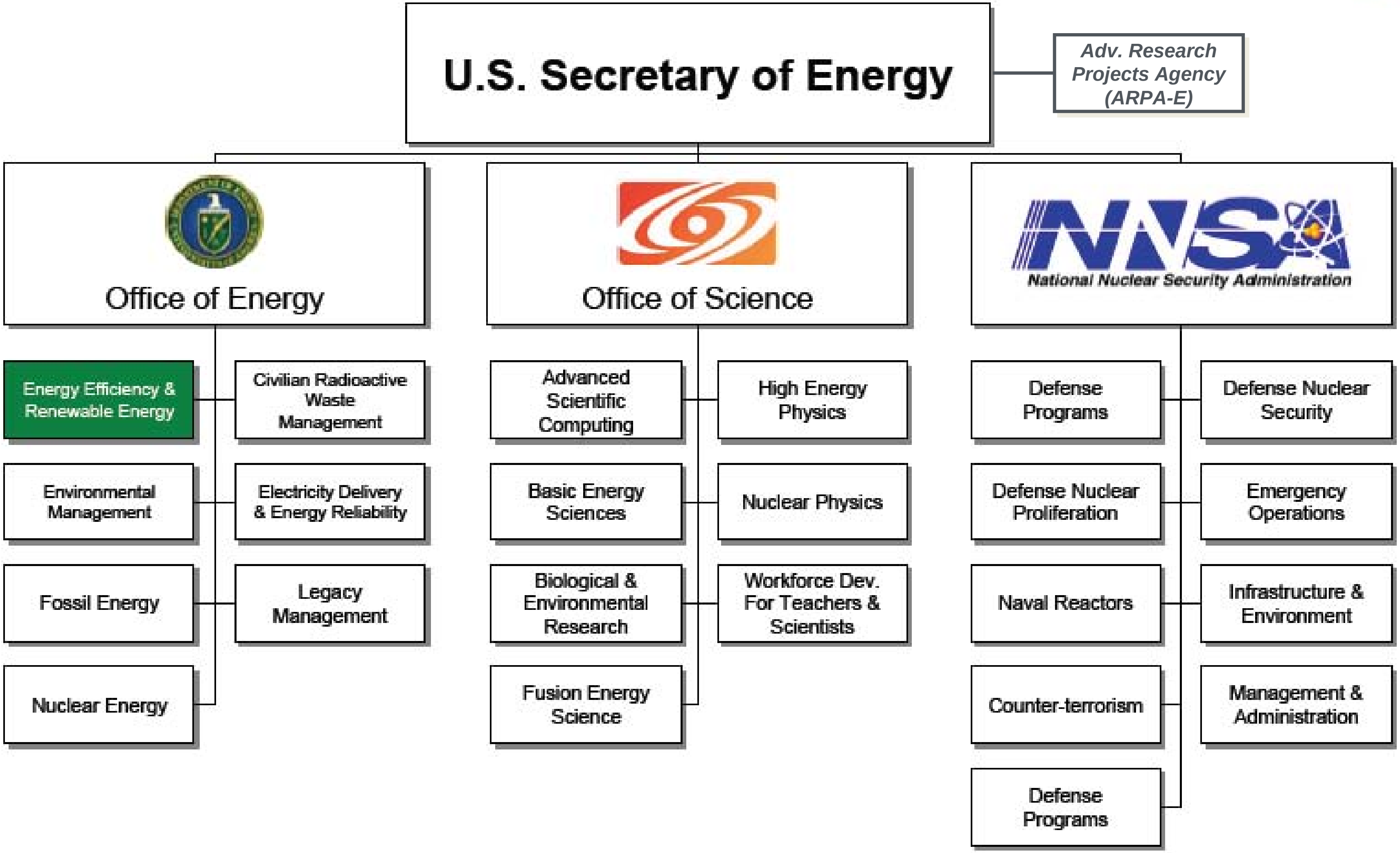
Advanced Biofuels
(include cellulosic biofuels other than starch-based ethanol)

Mandated Biofuel Production Targets (Billions of Gallons)

EISA defines ***Cellulosic Biofuel*** as “renewable fuel derived from any cellulose, hemicellulose, or lignin that is derived from renewable biomass and that has lifecycle greenhouse gas emissions…that are *at least 60 percent less* than baseline lifecycle greenhouse gas emissions.” The EPA interprets this to include cellulosic-based diesel fuel.

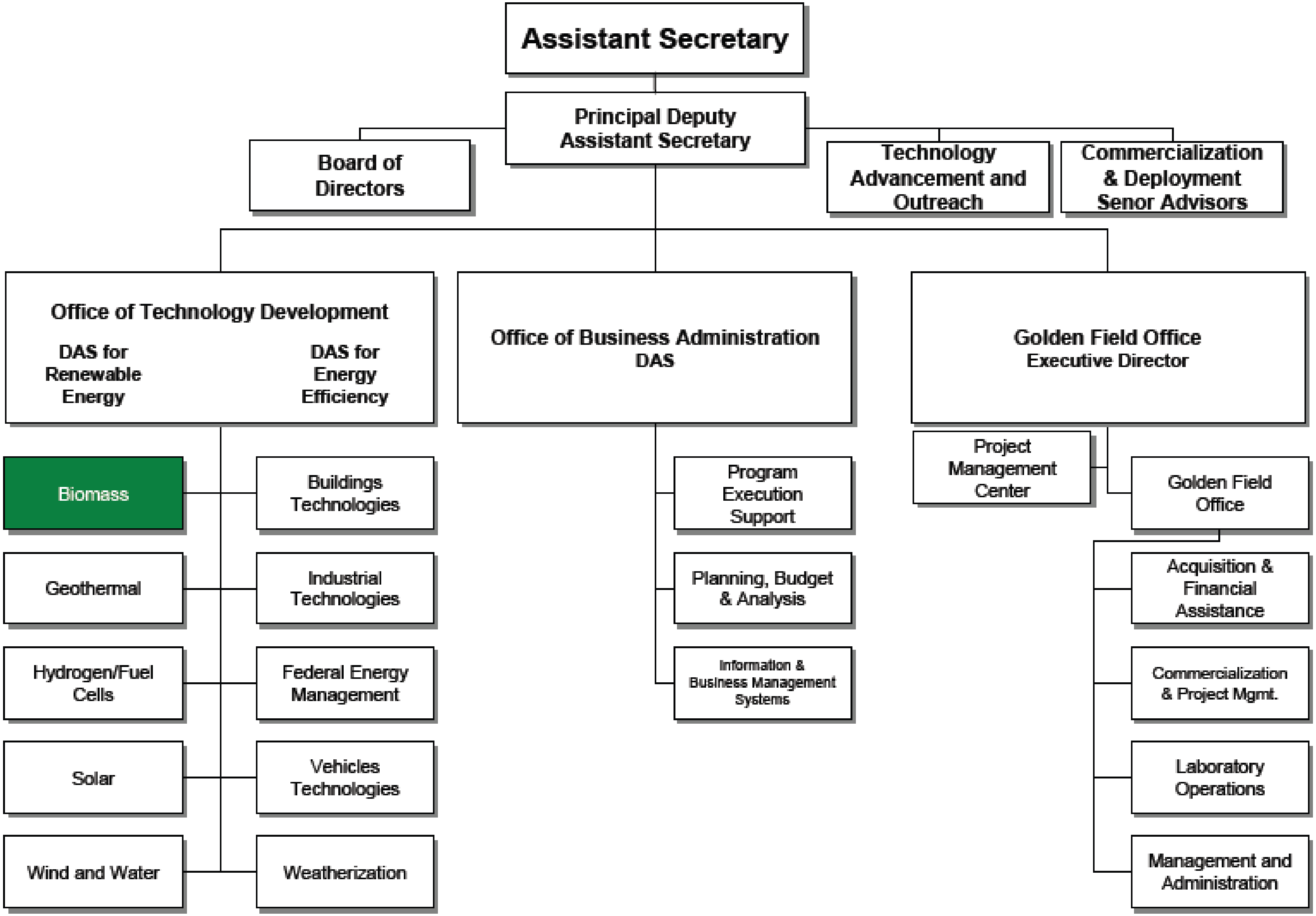
EISA defines Advanced ***Biofuel*** as “renewable fuel, other than ethanol derived from corn starch, that has lifecycle greenhouse gas emissions…that are *at least 50 percent less* than baseline lifecycle greenhouse gas emissions.” This includes biomass-based diesel, cellulosic biofuels, and other advanced fuels such as sugarcane-based ethanol and algae-based biofuels.

EERE within DOE Organization Chart



- U.S. Secretary of Energy
  - *Adv. Research Projects Agency (ARPA-E)*
  - Office of Energy
    - Energy Efficiency & Renewable Energy
    - Civilian Radioactive Waste Management
    - Environmental Management
    - Electricity Delivery & Energy Reliability
    - Fossil Energy
    - Legacy Management
    - Nuclear Energy
  - Office of Science
    - Advanced Scientific Computing
    - High Energy Physics
    - Basic Energy Sciences
    - Nuclear Physics
    - Biological & Environmental Research
    - Workforce Dev. For Teachers & Scientists
    - Fusion Energy Science
  - NNSA National Nuclear Security Administration
    - Defense Programs
    - Defense Nuclear Security
    - Defense Nuclear Proliferation
    - Emergency Operations
    - Naval Reactors
    - Infrastructure & Environment
    - Counter-terrorism
    - Management & Administration
    - Defense Programs

OBP within EERE Organization Chart



Assistant Secretary

Principal Deputy Assistant Secretary

Board of Directors

Technology Advancement and Outreach

Commercialization & Deployment Senor Advisors

Office of Technology Development
DAS for Renewable Energy
DAS for Energy Efficiency

Biomass

Geothermal

Hydrogen/Fuel Cells

Solar

Wind and Water

Buildings Technologies

Industrial Technologies

Federal Energy Management

Vehicles Technologies

Weatherization

Office of Business Administration
DAS

Program Execution Support

Planning, Budget & Analysis

Information & Business Management Systems

Golden Field Office
Executive Director

Project Management Center

Golden Field Office

Acquisition & Financial Assistance

Commercialization & Project Mgmt.

Laboratory Operations

Management and Administration

Successive Generations of Biofuels

Corn Ethanol

- Commercially available (no DOE research ongoing)
- Reduced GHG emissions
- Capped by RFS

Mature Commercial Technology

Cellulosic Ethanol

- Focus of current DOE research
- Potential to lower GHG emissions 86%
- Uses biomass from waste and non-agricultural land

Emerging Technologies

Advanced Biofuels

- Emerging efforts on new advanced biofuels and pathways, including algae
- Exploit opportunities to reduce environmental footprint
- Energy content and fuel economy similar to petroleum-based fuels

Increasing Energy Densities and Fuel Infrastructure Compatibility

DOE/EERE OBP Mission and Strategic Focus
Advanced *Biofuels and Biopower*

Mission: *Develop and transform the abundant and renewable biomass resources of the U.S. into cost-competitive, high-performance biofuels, bioproducts, and biopower through targeted research, development, and demonstration.*

Focus:

- Cellulosic Ethanol
- Advanced Renewable Light-Duty, Diesel, and Aviation Replacement Fuels
- Biopower

oals:

Achieve Affordable, Sustainable Production at Commercial Scales
Reduce GHG Emissions
Increase Resource Use Efficiency
Increase Systems Performance and Reliability
Increase National Energy Security
Reduce U.S. Reliance on Imported Petroleum
Contribute to Domestic U.S. Economy

Advanced Biofuels Initiative

Renewed Interest in Algae as Biofuel Feedstock

Biomass Program

Algal Biofuels

Biofuels made from microalgae hold the potential to solve many of the sustainability challenges facing other biofuels today.

Algal biofuels are generating considerable interest around the world. They may represent a sustainable pathway for helping to meet the U.S. biofuel production

Renewed Interest and Funding

Higher oil prices and increased interest in energy security have stimulated new public and private

Benefits of Algal Biofuels

Impressive Productivity: Microalgae, as distinct from seaweed or macroalgae, can potentially

Growing America's Energy Future

U.S. DEPARTMENT OF ENERGY BIOMASS PROGRAM

National Algal Biofuels Technology Roadmap

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

- The DOE Office of Energy Efficiency and Renewable Energy Biomass Program established an "Advanced Biofuels Initiative" in 2008

- An element is the "Algae Pathway"
  - Stakeholder workshop held December 10, 2008

- National Algal Biofuels Technology Roadmap released June 28, 2010 http://www1.eere.energy.gov/biomass/

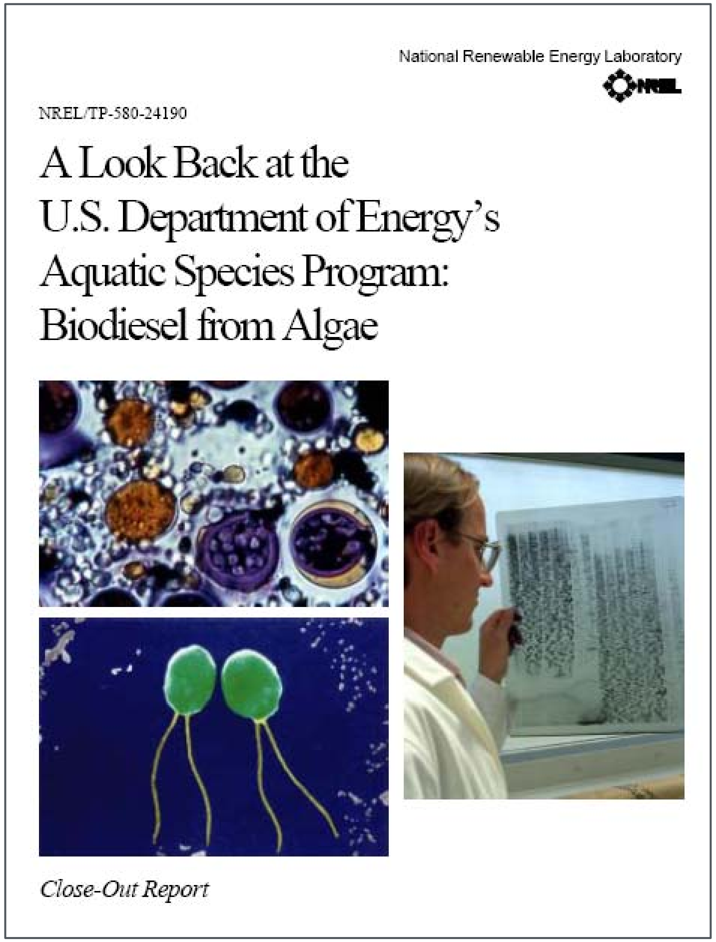
Building on Past DOE Investments in Algae Aquatic Species Program … *A Look Back*

Initial algae biofuels R&D during the period 1978-1996

DOE investment ~ $25M

Excerpt from ASP Close-Out Report (1998) …

*In 1995, DOE made the difficult decision to eliminate funding for algae research within the Biofuels Program … **[T]his report should be seen not as an ending, but as a beginning. When the time is right, we fully expect to see renewed interest in algae as a source of fuels and other chemicals. The highlights presented here should serve as a foundation for these future efforts.***



National Renewable Energy Laboratory

NREL

NREL/TP-580-24190

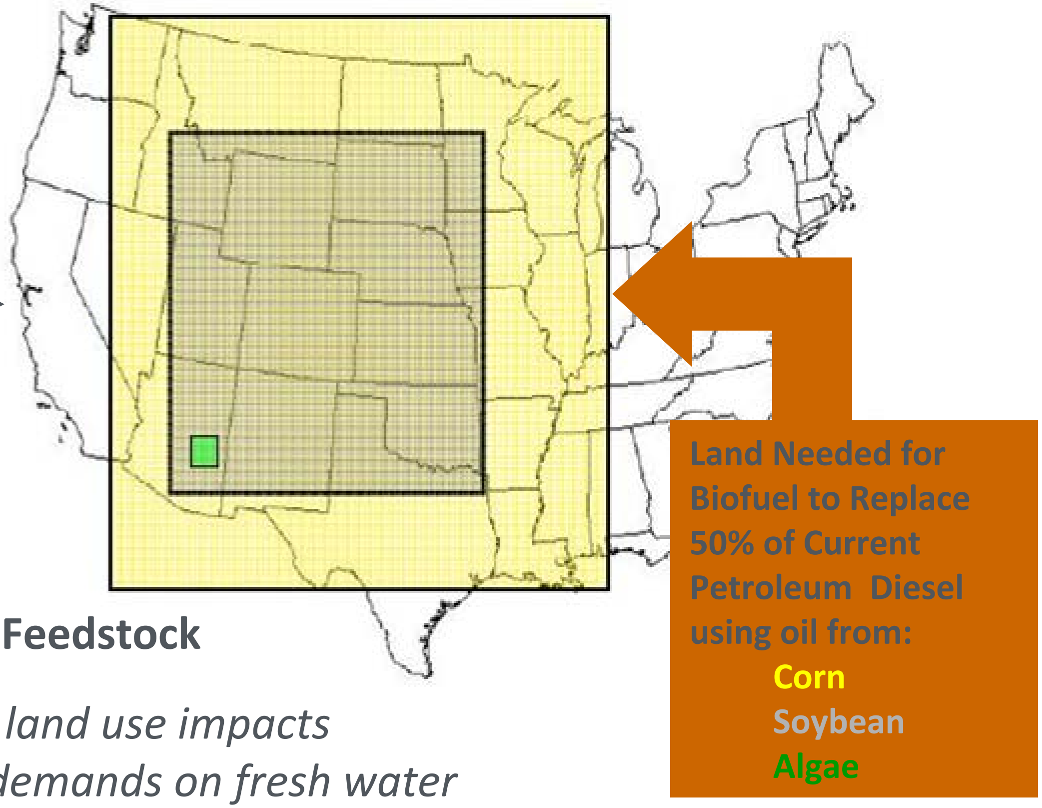
A Look Back at the
U.S. Department of Energy's
Aquatic Species Program:
Biodiesel from Algae

Close-Out Report

The Potential Advantages of Algae

Gallons of Oil per Acre per Year (approximate)	
Corn	**18**
Soybeans	**48**
Safflower	83
Sunflower	102
Rapeseed	127
Oil Palm	635
Micro Algae	**700 - 7000**

Notional example for photosynthetic microalgae oil production



Land Needed for Biofuel to Replace 50% of Current Petroleum Diesel using oil from:

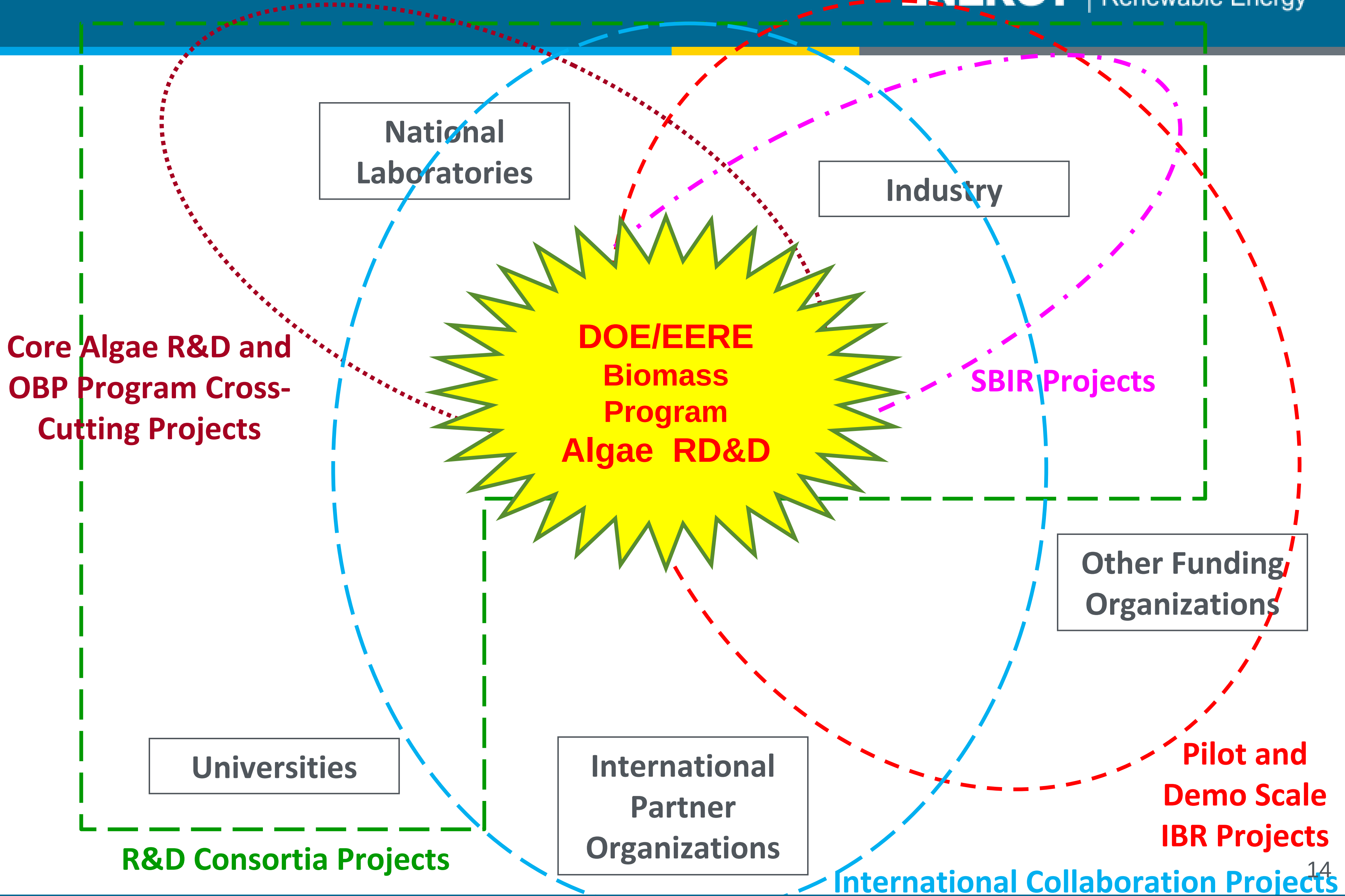
Corn
Soybean
Algae

Key Attributes of MicroAlgae as Biofuel Feedstock

- *Reduced land footprint and indirect land use impacts*
- *Can use non-fresh water to reduce demands on fresh water*
- *High production potential for both whole biomass and neutral lipids*
- *Potential source of high quality feedstock for advanced biofuels production*
- *Need not compete with agricultural lands and water for food/feed production*
- *Can potentially recycle CO_2, organic carbon, & nutrients from waste streams*

However, affordable and productive commercial scale operations not yet demonstrated

OBP Algae Program Investment Areas and Participants



National Laboratories

Industry

DOE/EERE Biomass Program Algae RD&D

Core Algae R&D and OBP Program Cross-Cutting Projects

SBIR Projects

Other Funding Organizations

Universities

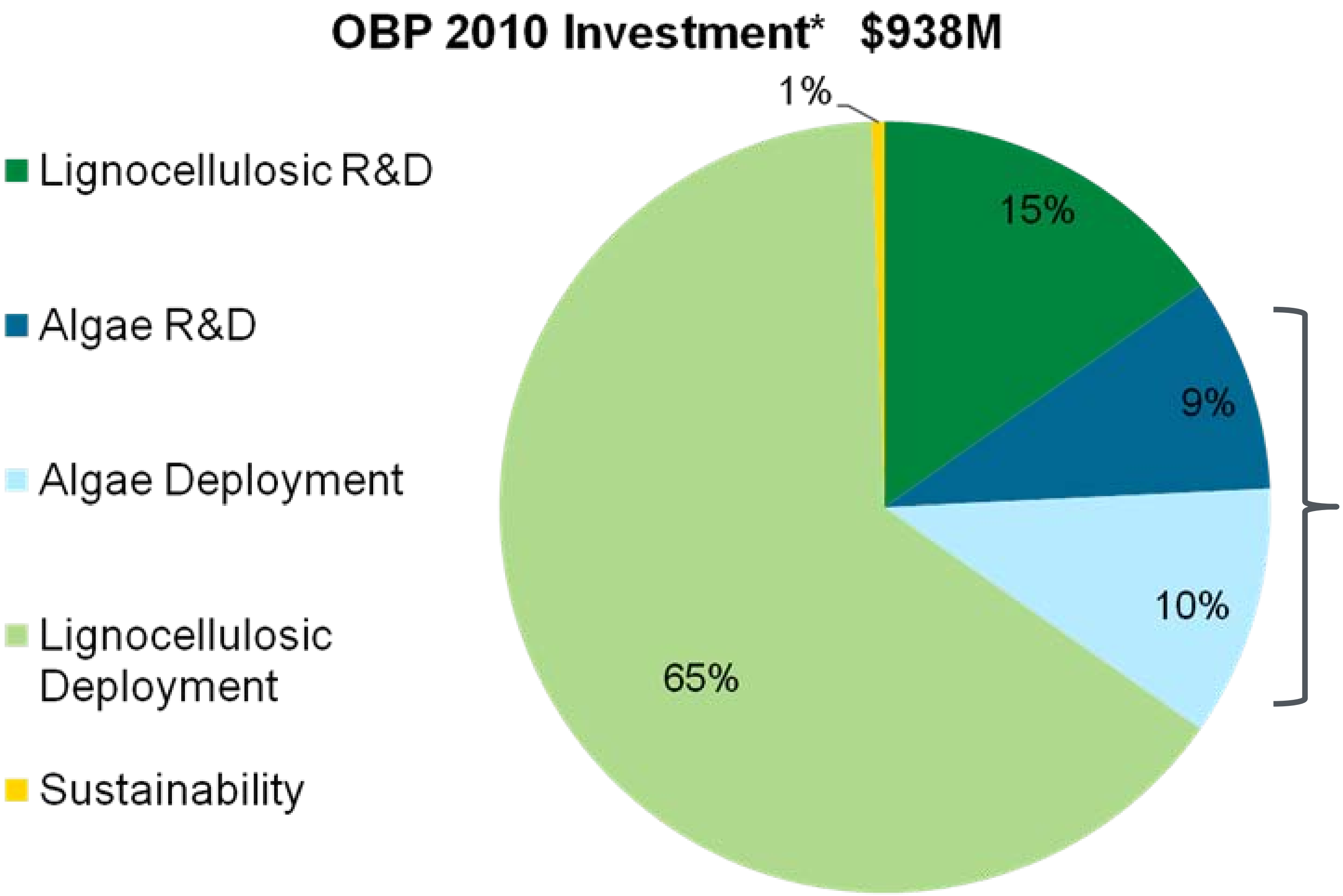
International Partner Organizations

Pilot and Demo Scale IBR Projects

R&D Consortia Projects

International Collaboration Projects

FY2010 Biomass Program Budget Breakdown

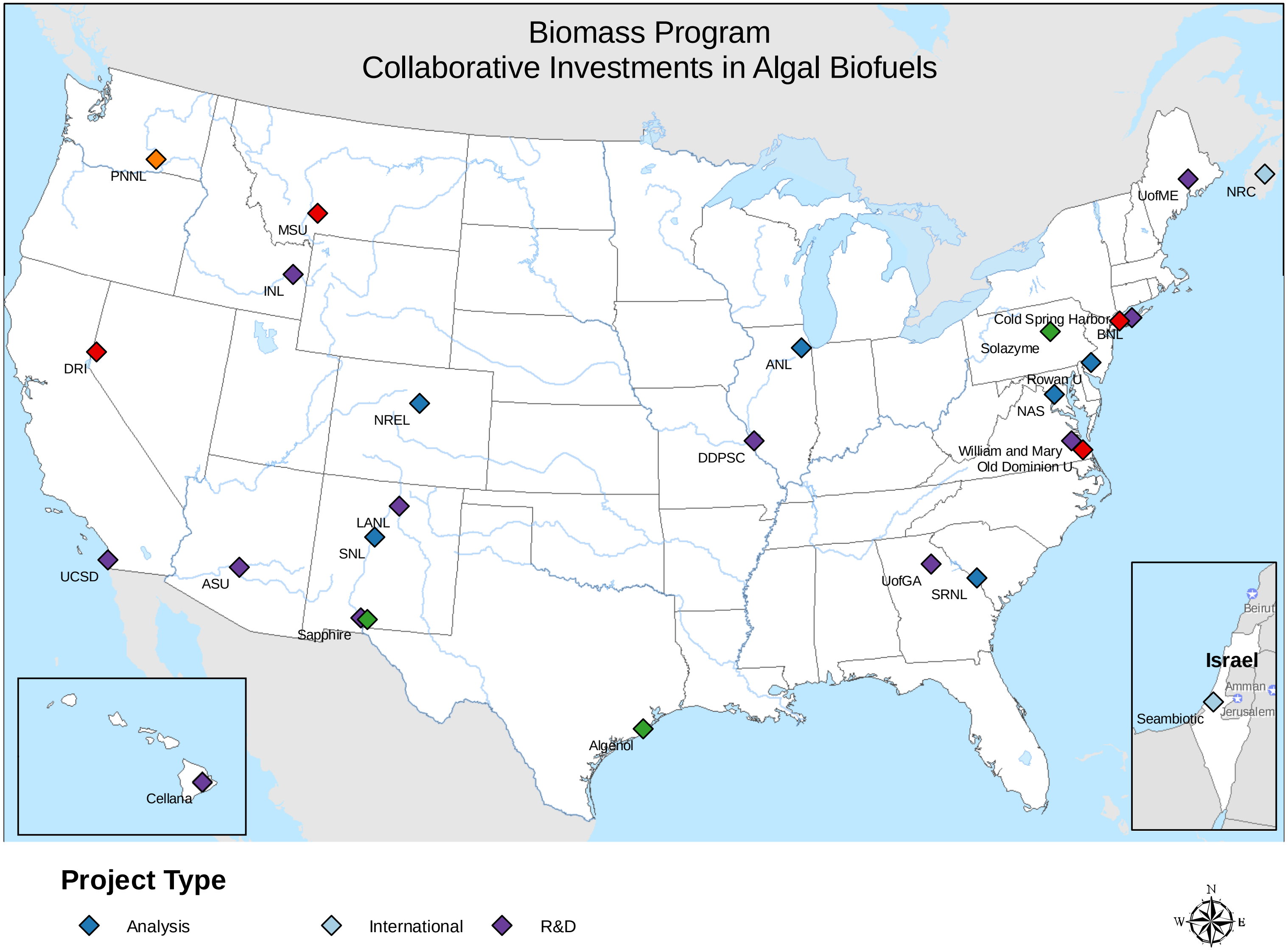


*Includes regular FY2010 appropriations and 2009 ARRA funds

The 2010 investment in algae totals ~ $180M, and includes:

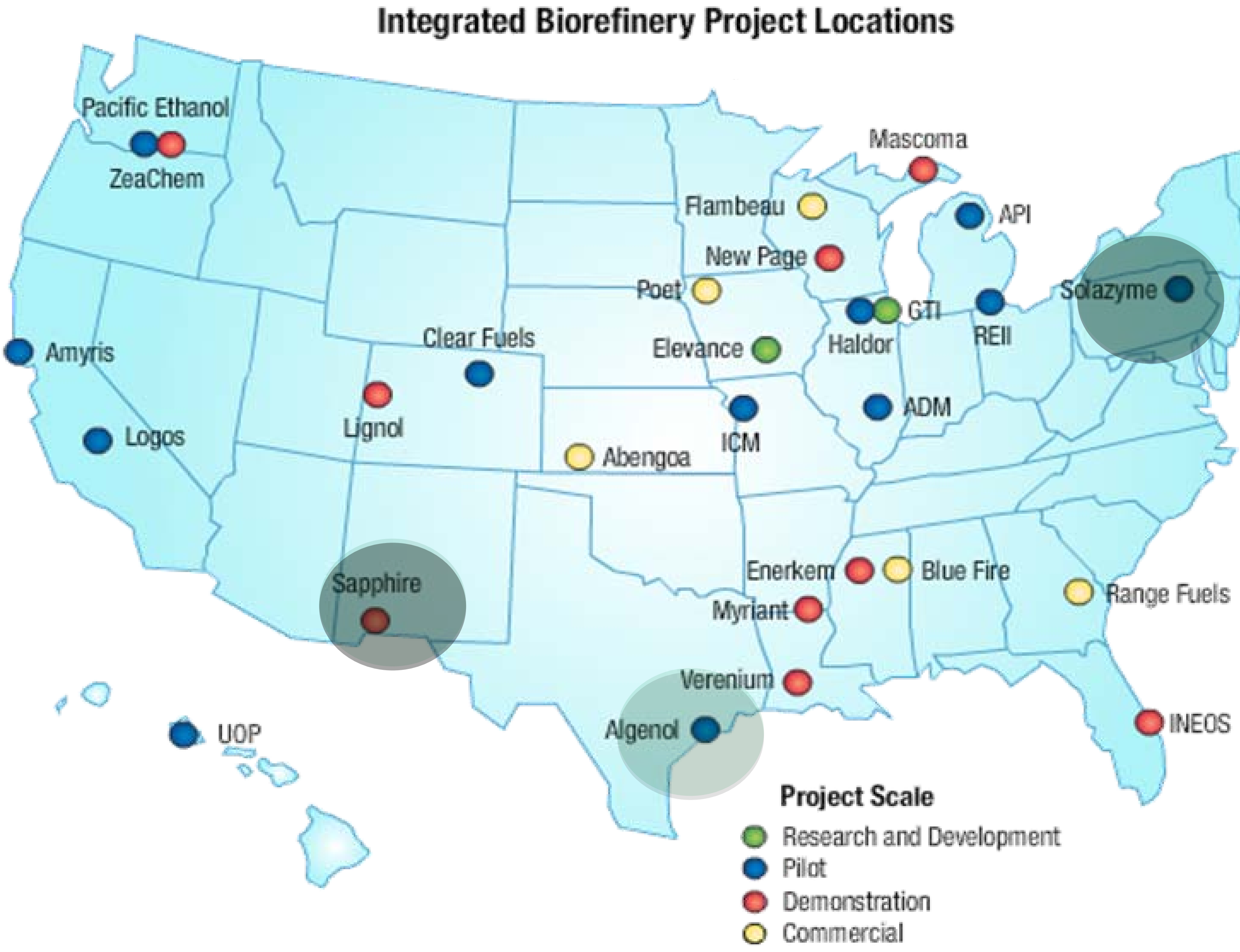
- **$49M for the NAABB consortium**
- **$35M for algae R&D, as directed by Congress**
- **$50M for the Sapphire to deploy open pond algal biofuel system**
- **$25M for the Algenol to pilot an photobioreactor algal biofuel system**
- **$22M for Solazymes to pilot a heterotrophic algal biofuel system**

DOE/EERE OBP Algae Biofuels Project Map



Locations of IBR Projects

Algae projects are circled:
Algenol, Sapphire, and Solazyme



Integrated Biorefinery Project Locations

For more information, visit: http://www.eere.energy.gov/biomass/integrated_biorefineries.html

Algae R&D Consortia Projects

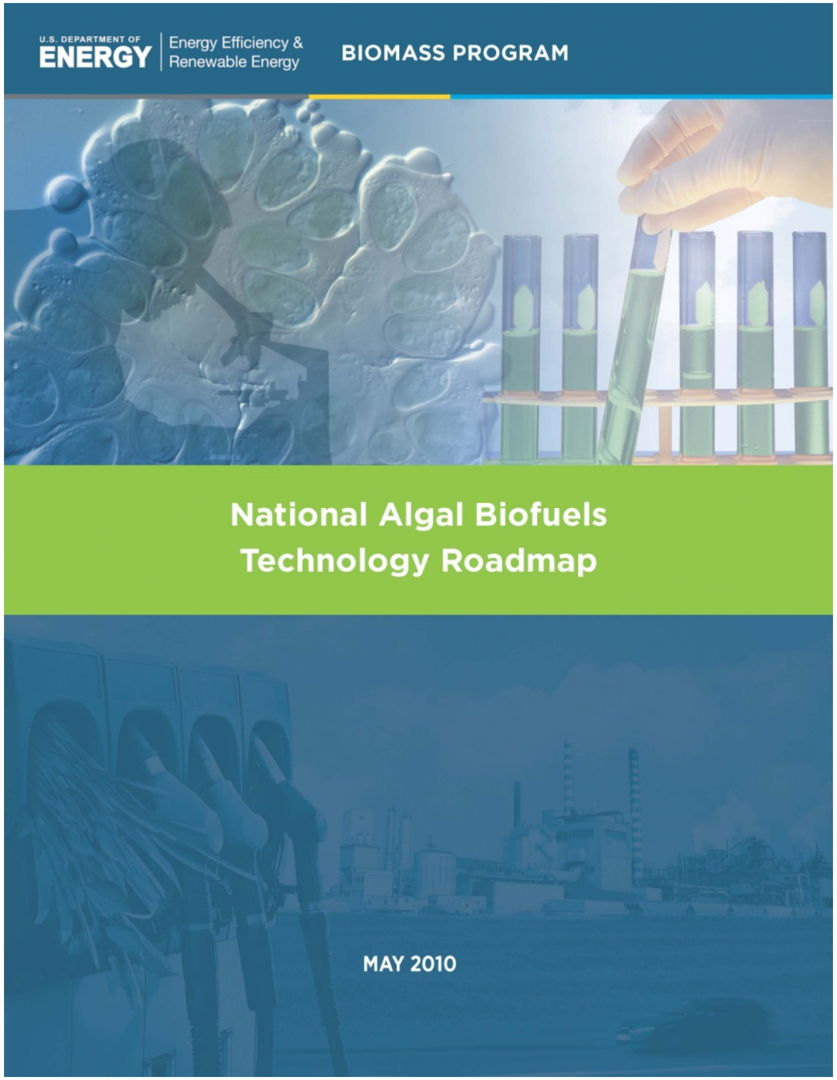
- **National Alliance for Advanced Biofuels and Bioproducts (NAABB)**
  - $49M in Recovery Act funds
  - Led by the Donald Danforth Plant Sciences Center
  - Director: Dr. Jose Olivares (Los Alamos National Laboratory)
  - Biology, Cultivation, Harvest/Dewater, Extraction, Thermochemical Conversion, Sustainability, Co-products
- **Cellana Consortium (Cellana)**
  - up to $9M in appropriated funds
  - Led by Cellana, Inc.
  - Director: Dr. Mark Huntley (U. Hawaii)
  - Cultivation (marine hybrid system), systems integration, co-products
- **Consortium for Algal Biofuels Commercialization (CAB-Comm)**
  - up to $9M in appropriated funds
  - Led by UC San Diego
  - Director: Dr. Steve Mayfield (UCSD)
  - Crop protection, Lifecycle Analysis
- **Sustainable Algal Biofuels Consortium (SABC)**
  - **up to** $6M in appropriated funds
  - Led by Arizona State University
  - Director: Dr. Gary Dirks
  - Biochemical conversion, Fuel Testing

DOE's *National Algal Biofuels Technology Roadmap*

Defining the algal biofuels supply chain and the remaining R&D challenges

National Algal Biofuels Technology Roadmap: Shaping the Biomass Program's Algae RD&D portfolio

- DOE held a workshop in 2008 that brought together over 200 stakeholders from different algae interest groups (academia, DOE national labs, industry, etc.)
- The discussions at the workshop helped define the state of the field and the existing R&D challenges- these were captured in a "Roadmap" document released by DOE last July
- The challenges laid out in the Roadmap help guide Biomass Program RD&D in Algae



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BIOMASS PROGRAM

National Algal Biofuels Technology Roadmap

MAY 2010

Available for Download: http://www1.eere.energy.gov/biomass/pdfs/algal_biofuels_roadmap.pdf

The Algal Supply Chain: How we get from Algae to Fuel

Algal Cultivation

Algal Harvesting and Processing

Conversion of algae components into biofuels and other products

The Algal Supply Chain: How we get from Algae to Fuel

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Algal Cultivation

- Strain Biology/Selection
- Cultivation Strategy
- Resources and Siting

Algal Harvesting and Processing

- Harvesting/Dewatering Technology
- Fractionation/Extraction Technology

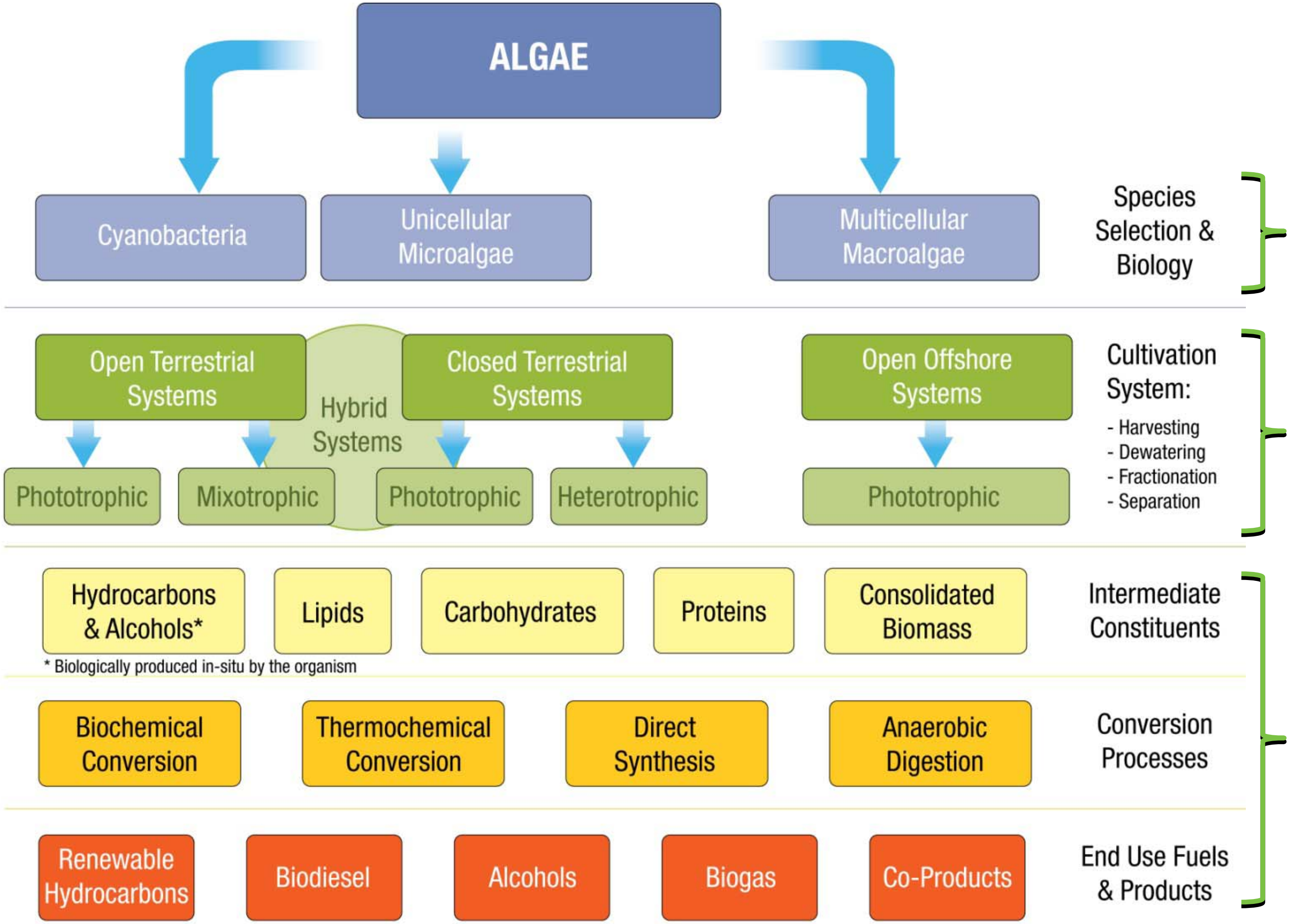
Conversion of algae components into biofuels and other products

- Fuel Synthesis, Conversion or Upgrading Technology
- Infrastructure and Market of Fuel or Product

Is this economically and environmentally sustainable?

Can the system be scaled up to the necessary scales for fuels?

The Algal Supply Chain: Multiple Pathways from Algae to Fuel



ALGAE

Cyanobacteria

Unicellular Microalgae

Multicellular Macroalgae

Species Selection & Biology

Open Terrestrial Systems

Hybrid Systems

Closed Terrestrial Systems

Open Offshore Systems

Phototrophic

Mixotrophic

Phototrophic

Heterotrophic

Phototrophic

Cultivation System:
- Harvesting
- Dewatering
- Fractionation
- Separation

Hydrocarbons & Alcohols*

Lipids

Carbohydrates

Proteins

Consolidated Biomass

Intermediate Constituents

* Biologically produced in-situ by the organism

Biochemical Conversion

Thermochemical Conversion

Direct Synthesis

Anaerobic Digestion

Conversion Processes

Renewable Hydrocarbons

Biodiesel

Alcohols

Biogas

Co-Products

End Use Fuels & Products

Select Algae

- **Select Cultivation Strategy**
- **Harvest Algae**
- **Process algae into components**

Convert or Upgrade Components Into Biofuels and Other Products

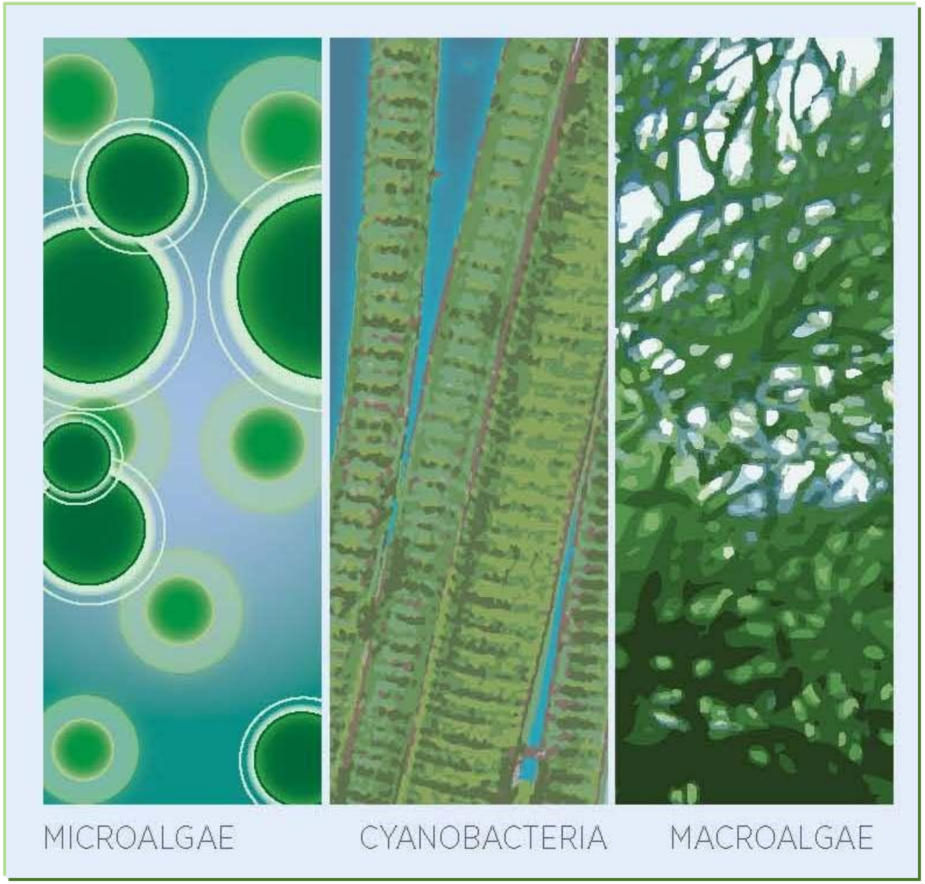
Algal Strain Selection

The **"Ideal Algae"** would be:

- **Productive**
- **Stable in culture**: robust in response to environmental changes and predators/pathogens

Algae producers have an important decision to make…

- Species of algae chosen will effect all downstream processing including the type of biofuel produced
- Could also consider cultivating mixed algae communities



Different "types" of algae exist

Algal Strain Selection

Important Challenges for R&D:

We need to understand more about **algal biology-** what controls the production of important molecules needed for biofuel production, and what makes them stable in culture

With this knowledge can we select, breed or engineer **more ideal algal strains**?

Cyclotella

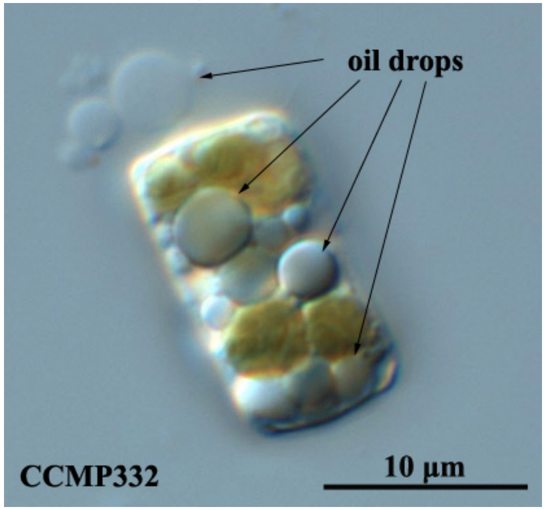
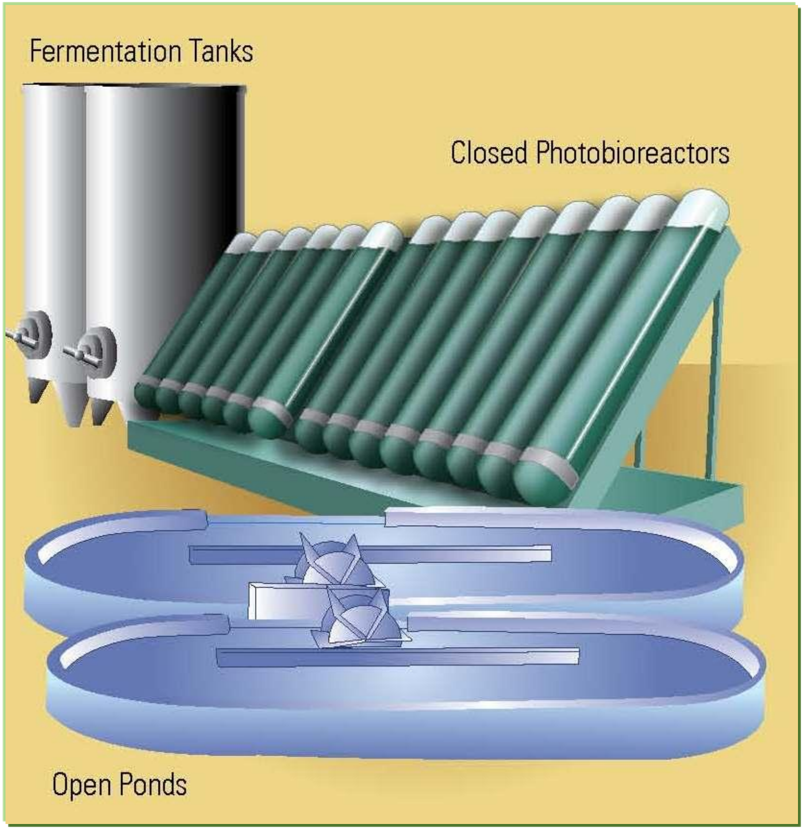


Photo Source: R. Andersen/CCMP

Algal Cultivation Strategies

Several Types of Algae Cultivation Vessels Exist



Open systems- typically outdoor open ponds

Closed systems- enclosed clear plastic vessels (bags or tubes) known as Photobioreactors, or dark tanks (such as fermentation tanks)

Offshore systems- growing algae in the open ocean- usually contained in some way (bags or ropes)

Algal Cultivation Strategies

Important Challenges for R&D:

What is the best strategy for cultivating algae when you **balance productivity with economics**?

- Unlikely to be one-size-fits-all approach for every region
- Sunlight or sugars?
- System must also be optimized for production of desired product (ex. lipid or whole biomass)

Raceway Pond- Cellana LLC, HI

Closed Photobioreactor System- Arizona State University

Fermentation Tank- NREL

Resources & Siting for Cultivation

- Resources required to grow algae:
  - Land
    - Algal productivity eases land requirement
    - Can use non-arable land
  - Water
    - Many algae can grow in non-fresh water
  - CO_2 or Sugars
  - Nutrients
  - Electricity

- Siting options (location)- many propose to cultivate algae coupled with:
  - Wastewater Treatment (provides nutrients and non-fresh water source)
  - Aquaculture (provides infrastructure)
  - Point CO_2 sources (CO_2 re-use)
  - Marine Environments (ample non-fresh water)
  - Sugar Waste Streams (ex. pulp and paper)

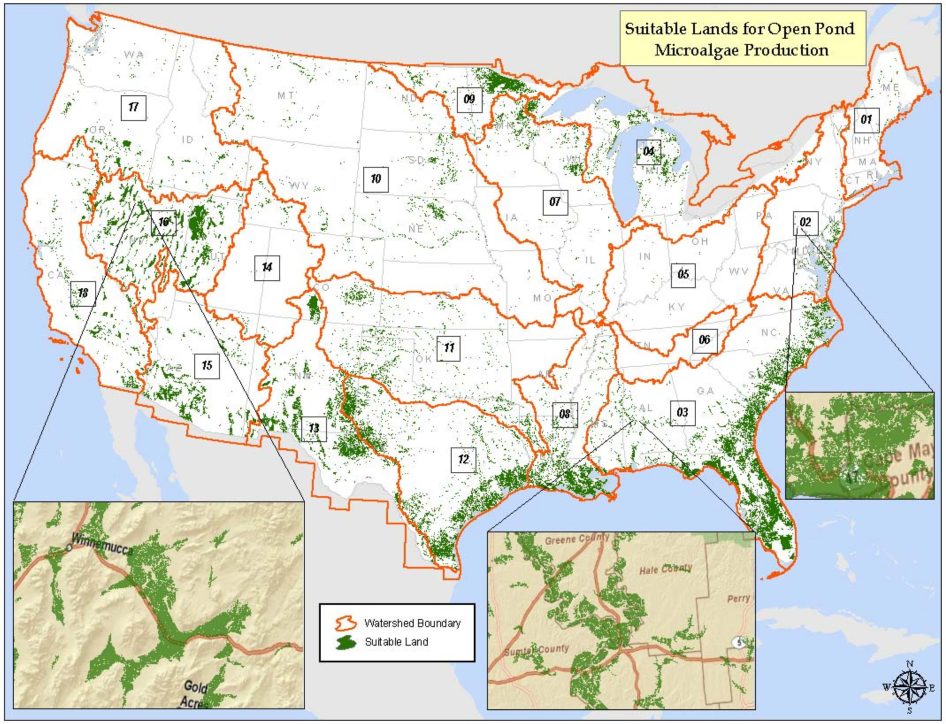
Proximity, sustainable availability, and cost of all resources will effect price of biofuel

Resources & Siting for Cultivation

Challenges for R&D:

Can one access all necessary inputs to cultivate algae and still maintain a cost-effective and sustainable process?

Is system recycling of water, nutrients, and energy feasible and necessary?



Pacific Northwest National Laboratory Report to DOE.
Wigmosta, MS et al., *manuscript in preparation.*

Harvesting & Dewatering

Algae in culture are relatively dilute- most of the water must be removed before algae can be processed into fuel. This is a very energy-intensive step in making algae biofuels. Current technologies are either expensive, unscalable, and/or adversely affect down-stream processing.

Challenges for R&D:

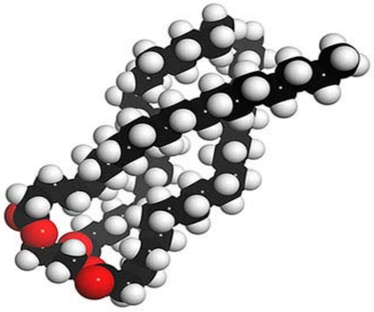
- Many technology options currently exist that must be evaluated. Develop improved harvesting and dewatering technologies
- New or improved technologies must reduce energy intensity, capital and operating costs, and have scalability!
- Downstream processes that can handle wet algae are advantageous

Fractionation & Extraction

Once harvested, algal biomass must be separated into its different useful components

Carbohydrates **Proteins** **Lipids**

Triacylglyceride (TAG)



Fatty Acid (FA)

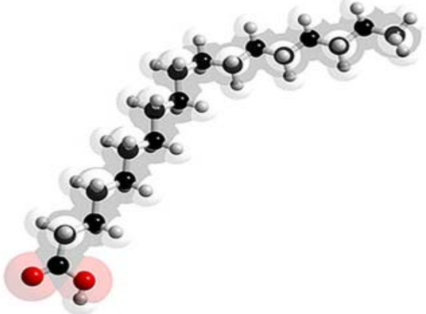


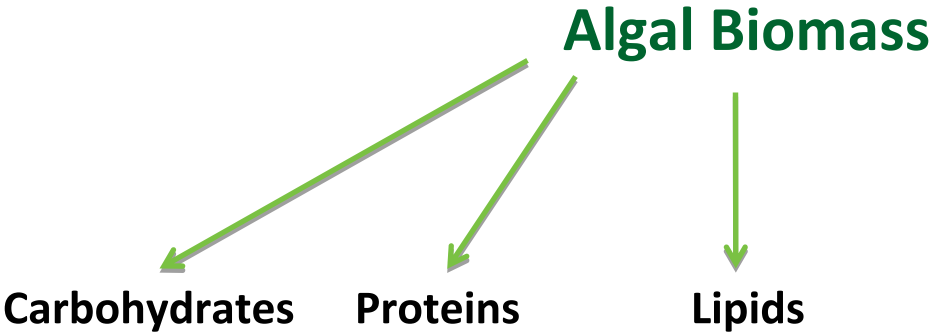
Photo Source: 3dChem Website

Challenges for R&D:

Similar to harvesting, fractionation and extraction technologies need to be improved in terms of cost, energy-use, and scalability. Yield of desired product is also important.

Also being explored are ways to convert whole algae into fuels, or get the algae to directly produce the desired fuel through selection or engineering

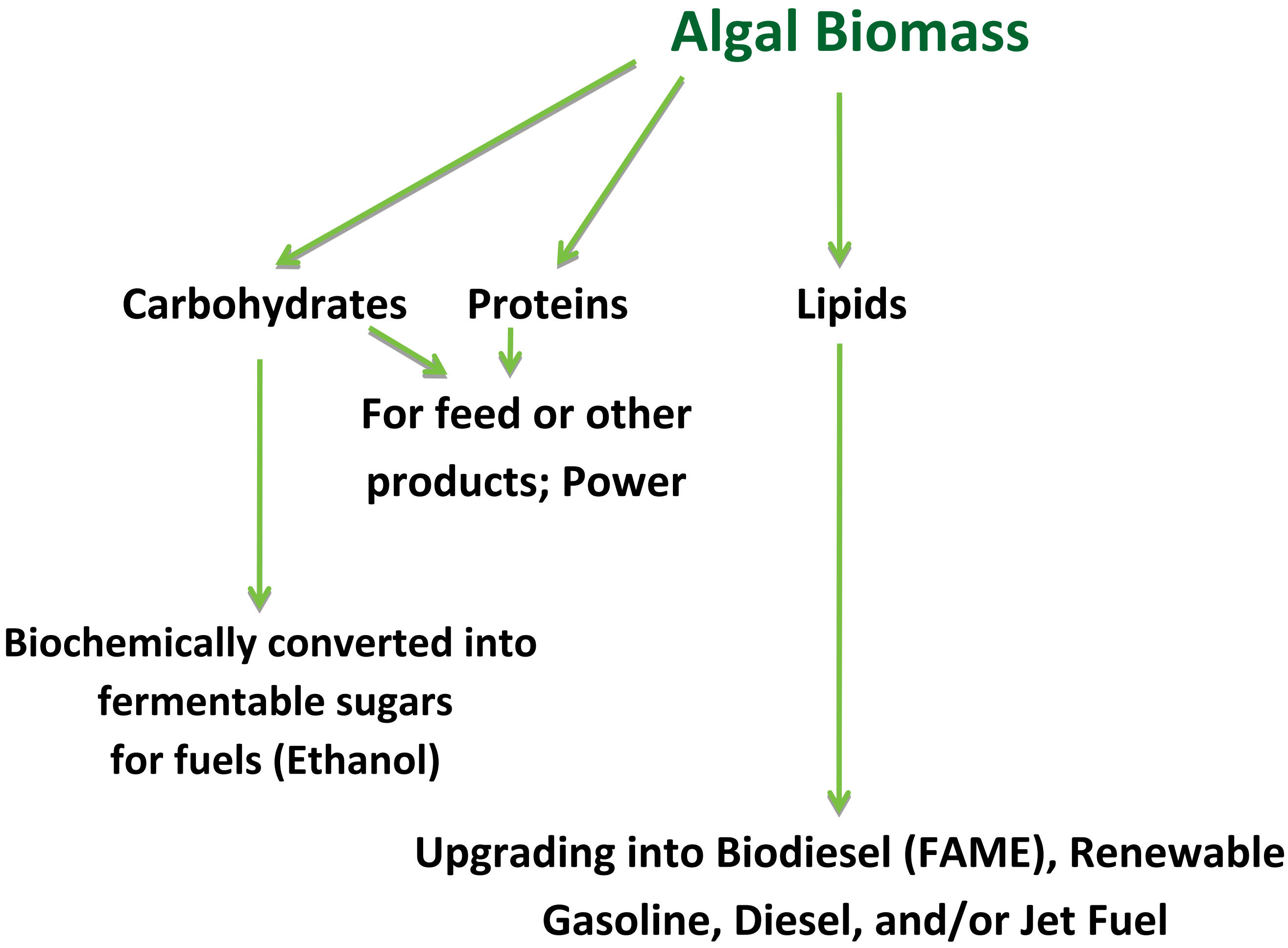
Conversion to Fuel and Products



Algal Biomass

Carbohydrates **Proteins** **Lipids**

Conversion to Fuel and Products



Algal Biomass

Carbohydrates

Proteins

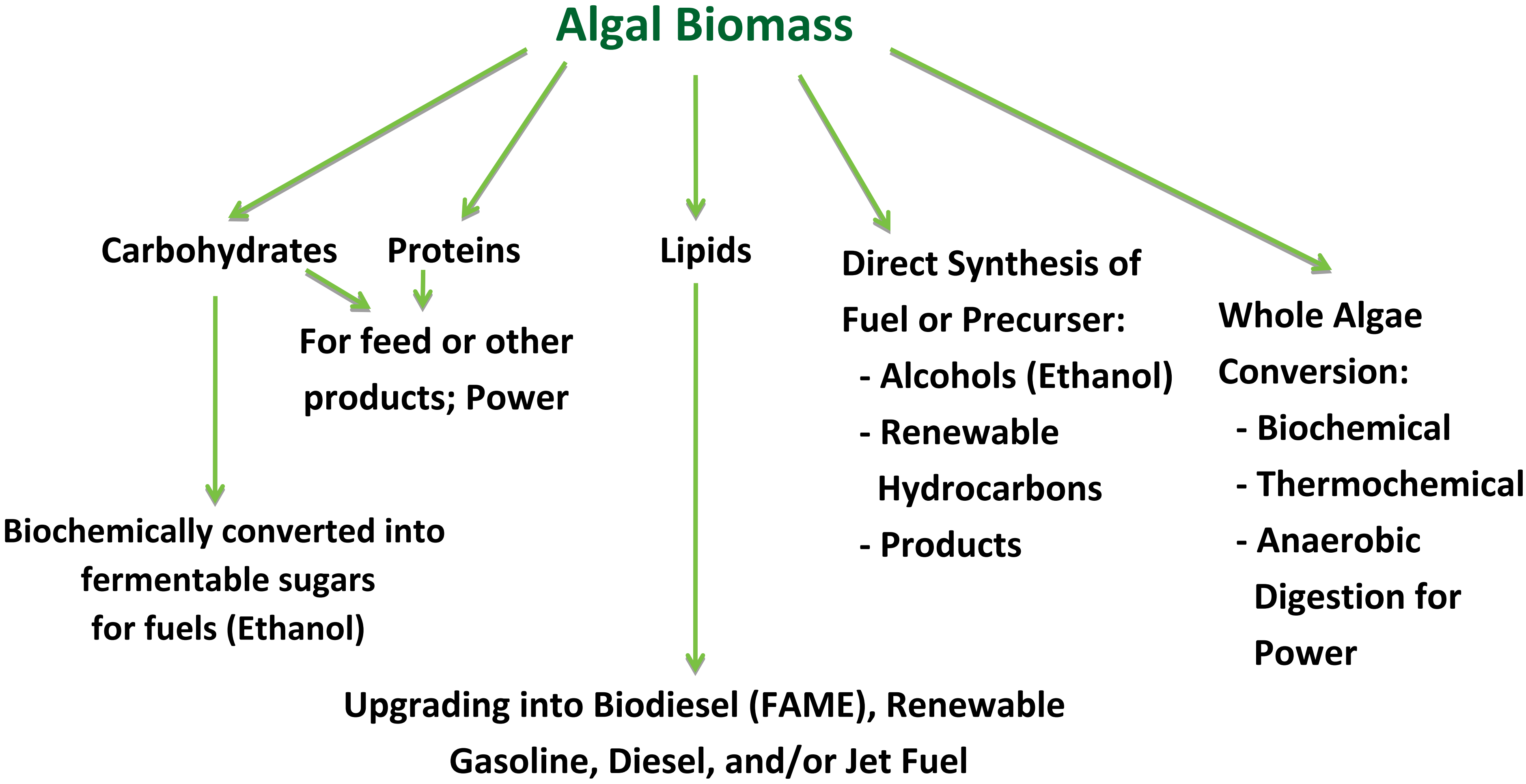
Lipids

For feed or other products; Power

Biochemically converted into fermentable sugars for fuels (Ethanol)

Upgrading into Biodiesel (FAME), Renewable Gasoline, Diesel, and/or Jet Fuel

Conversion to Fuel and Products



Algal Biomass

Carbohydrates

Biochemically converted into fermentable sugars for fuels (Ethanol)

Proteins

For feed or other products; Power

Lipids

Upgrading into Biodiesel (FAME), Renewable Gasoline, Diesel, and/or Jet Fuel

Direct Synthesis of Fuel or Precurser:

- Alcohols (Ethanol)
- Renewable Hydrocarbons
- Products

Whole Algae Conversion:

- Biochemical
- Thermochemical
- Anaerobic Digestion for Power

Conversion to Fuel and Products

Challenges for R&D:

- Investigate many technology options for converting algal biomass into different biofuels
  - Also consider production of co-products that will aid in cost-effectiveness of entire system
  - Issues include catalysts, energy intensity, GHG emissions, conversion rates

A gasifier being used by a NAABB partner to convert whole algal biomass to fuels

Extracted Algal Oil: Solazyme, CA

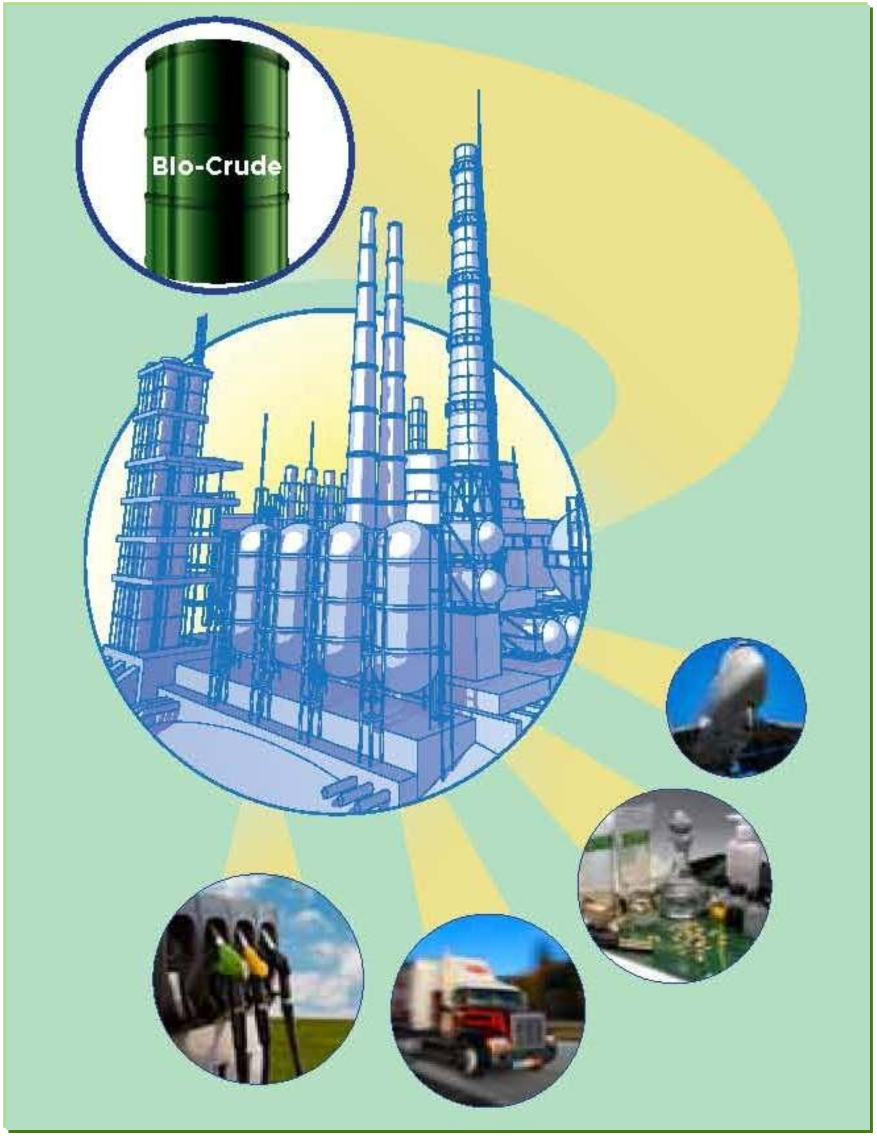
Fuel Products & End Uses

Products:

- Renewable Gasoline, Diesel, and Jet Fuel
- Biodiesel
- Ethanol (and other alcohols)
- Higher-value Products (animal/fish feed, fertilizer, food supplements)
- Biogas - Power

Challenges:

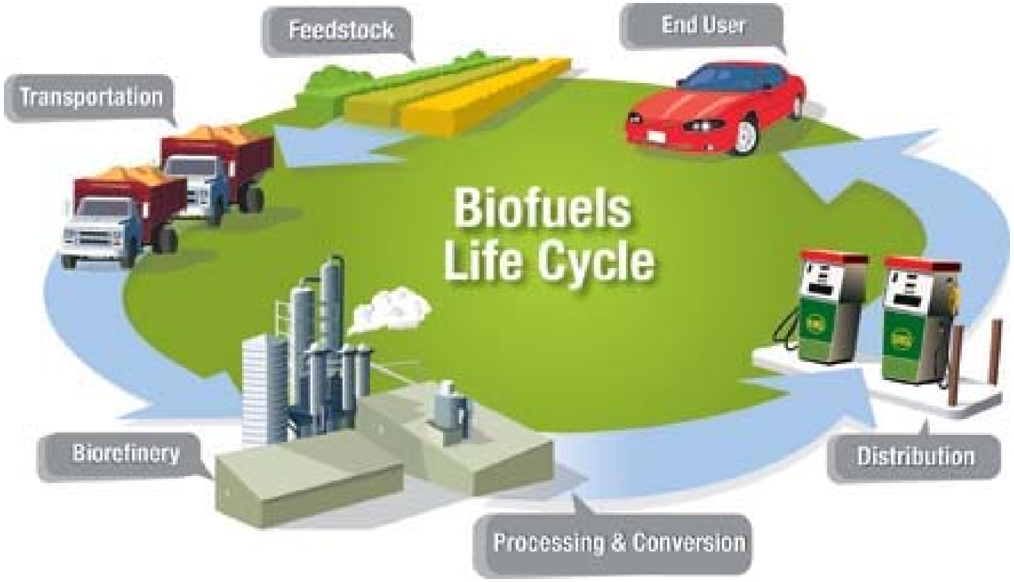
- Fuel infrastructure compatibility and market entry
- Co-product markets
- Storage and handling of biomass, fuels, and products
- Government and industry regulations and standards



Analysis & Sustainability of Algae Biofuels Systems

Technology R&D must be coupled with analysis of the economics and sustainability of the entire system, to ID technology options that improve the cost and GHG emission profile of the final product

- Techno-Economic Analysis (cost)
- Life-Cycle Analysis (GHG emissions, water footprint, etc.)



Algal Biofuels Technology Challenge Areas Re-cap

1. Feedstock supply: Strain selection/development, cultivation strategy, siting & resources

2. Feedstock processing: Harvesting/dewatering, fractionation and extraction

3. Conversion into Fuel: synthesis, conversion, or upgrading into fuels and co-products

4. Infrastructure, Fuel and Product Markets, and Regulations/Standards

5. Systems Integration and Scale-up of all Technologies***

6. Sustainable Practices: Life Cycle and Techno-Economic analyses, siting and resources management

The Faces of Team Algae

DOE- HQ

DOE- Golden Field Office

Valerie Reed , PhD
Conversion R&D Team Lead, Algae Team Lead

Joyce Yang, PhD
Microbiology/Genetics

Joanne Morello, PhD-AAAS Fellow
Plant Pathology/ Plant Biology
Microbiology

Ron Pate- on loan from Sandia
Applied Physics/Electrical and
Systems Engineering & Analysis

Roxanne Dempsey
(not pictured)
Project Management and
Infrastructure Development

Daniel Fishman-BCS
Algae Ecology

Christine English, PhD- Navarro
Biochemistry/Molecular Biology

Information Resources

- Office of Biomass Program - http://www1.eere.energy.gov/biomass/
- EERE Info Center - www1.eere.energy.gov/informationcenter
- *National Algal Biofuels Technology Roadmap* - http://www1.eere.energy.gov/biomass/pdfs/algal_biofuels_roadmap.pdf
- Biomass R&D Initiative – www.biomass.govtools.us
- Grant Solicitations - www.grants.gov

- DOE Office of Science - http://www.er.doe.gov/
- DOE Loan Guarantee Program Office - http://www.lgprogram.energy.gov

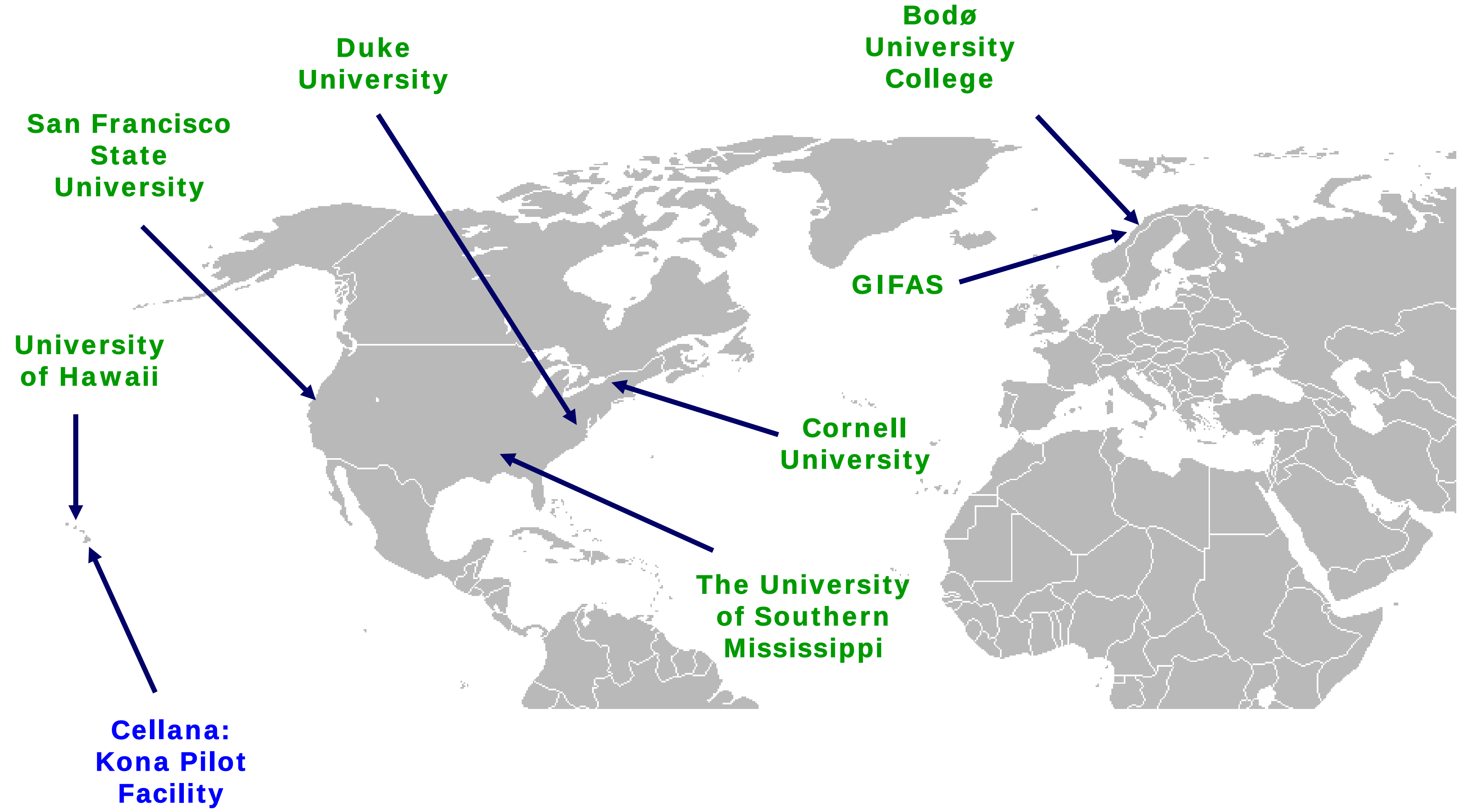
CELLANA
ALGAE TECHNOLOGY. NATURALLY.

Large-scale Production of Fuel and Feeds from Marine Microalgae

Mark Huntley
Chief Science Officer, Cellana

Department of Energy Webinar
8 September 2010

Our Consortium



Bodø University College

Duke University

San Francisco State University

GIFAS

University of Hawaii

Cornell University

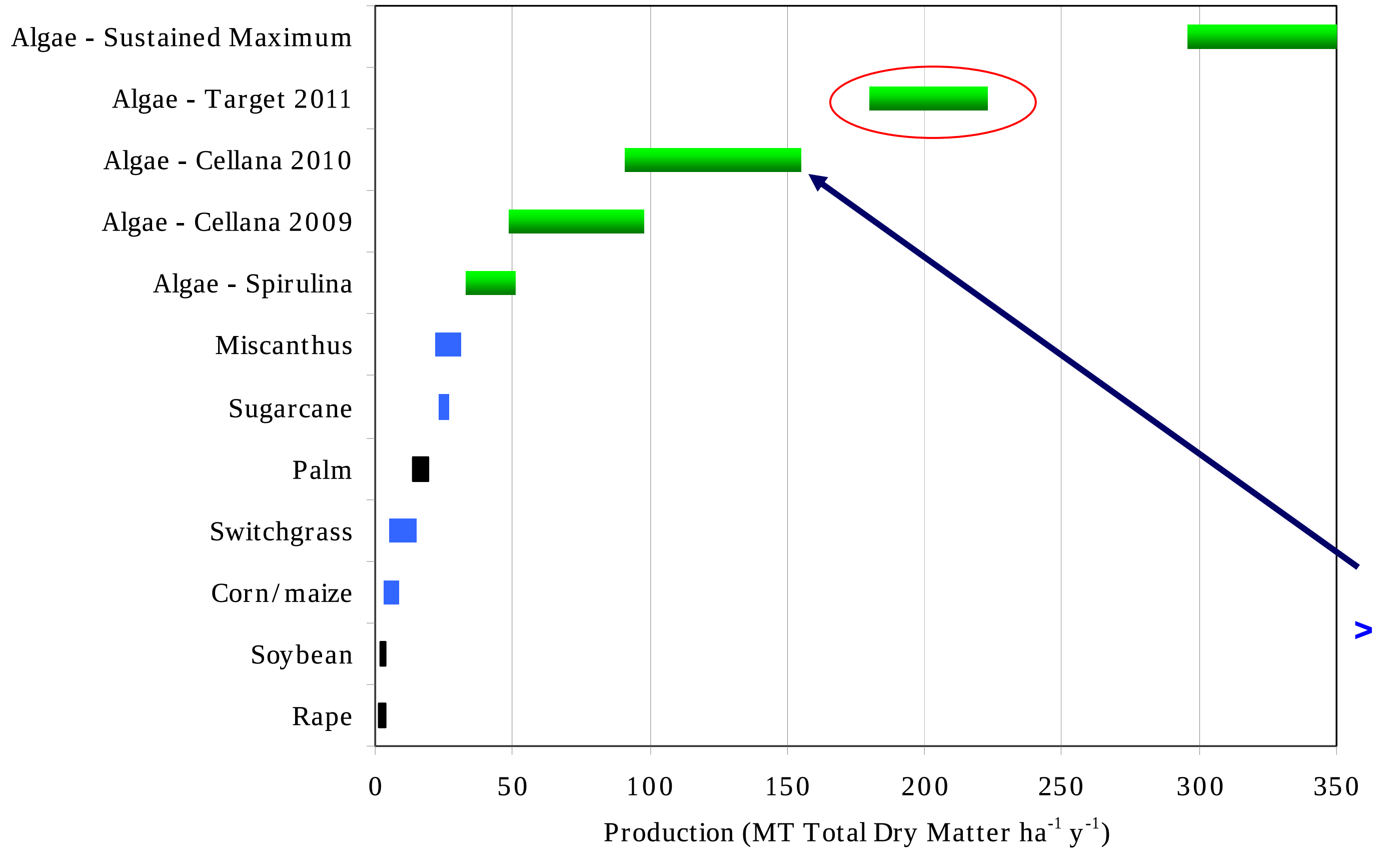
The University of Southern Mississippi

Cellana: Kona Pilot Facility

- University Members
- Consortium Lead

What we like about marine algae

Algae are more productive than terrestrial energy crops



PRODUCTION at KPF:

>3,800 gal/acre/yr

What we like about marine algae

Algae use less land

Bioenergy Crop	) Total Land in Cultivation (km^2	Algae Land Required (km^2)
Sugarcane	227,250	22,700
Oil palm	138,547	9,896
Maize	1,580,300	26,338
Rapeseed	308,053	2,282
Total	2,254,150	61,216
Total (%)	100%	2.7%

Land now in production could be reduced >95%

Source: FAO – 2007 data

What we like about marine algae

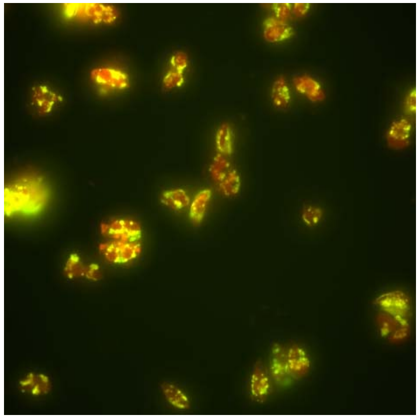
Algae make the right kinds of oils

Fatty Acids	Weight %				
	Palm Oil	Soy Oil	Algae Oil 1	Algae Oil 2	Algae Oil 3
C12:0	0.2				
C14:0	1.1		19.7	14.3	14.5
C16:0	44.0	6.5	29.9	36.2	22.0
C16:1	0.0	0.0	41.2	31.9	42.8
C18:0	4.5	4.2	2.0	2.4	3.7
C18:1	39.2	28.0	0.0	6.9	4.6
C18:2	10.1	52.6	0.0	1.5	2.5
Other	1.1	8.7	7.2	6.8	9.9

Shorter chain lengths and high saturation are generally favorable

Source: Cellana

What we like about marine algae



Agricultural land is not needed, and

for Cellana’s process…

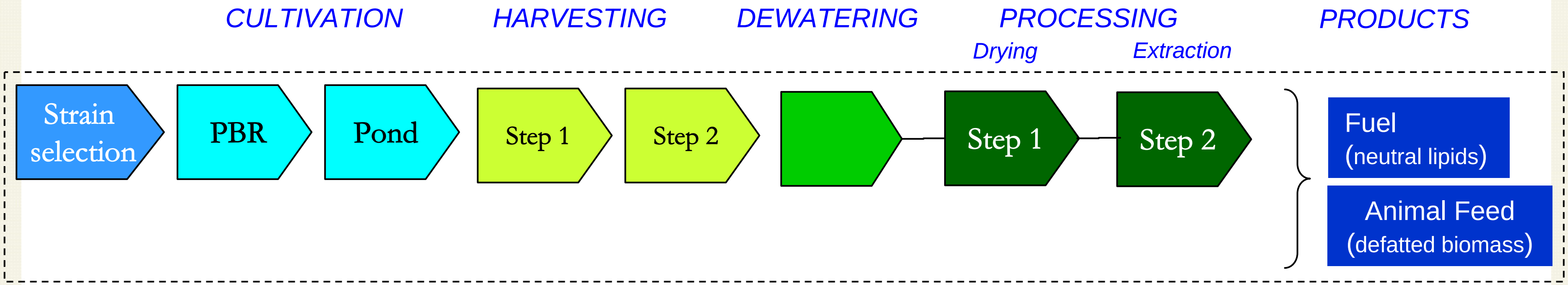
No herbicides needed

No pesticides needed

No GMOs needed

No freshwater needed - we use seawater

The Cellana algae-to-product pathway



CULTIVATION

HARVESTING

DEWATERING

PROCESSING

Drying

Extraction

PRODUCTS

Strain selection

PBR

Pond

Step 1

Step 2

Step 1

Step 2

Fuel (neutral lipids)

Animal Feed (defatted biomass)

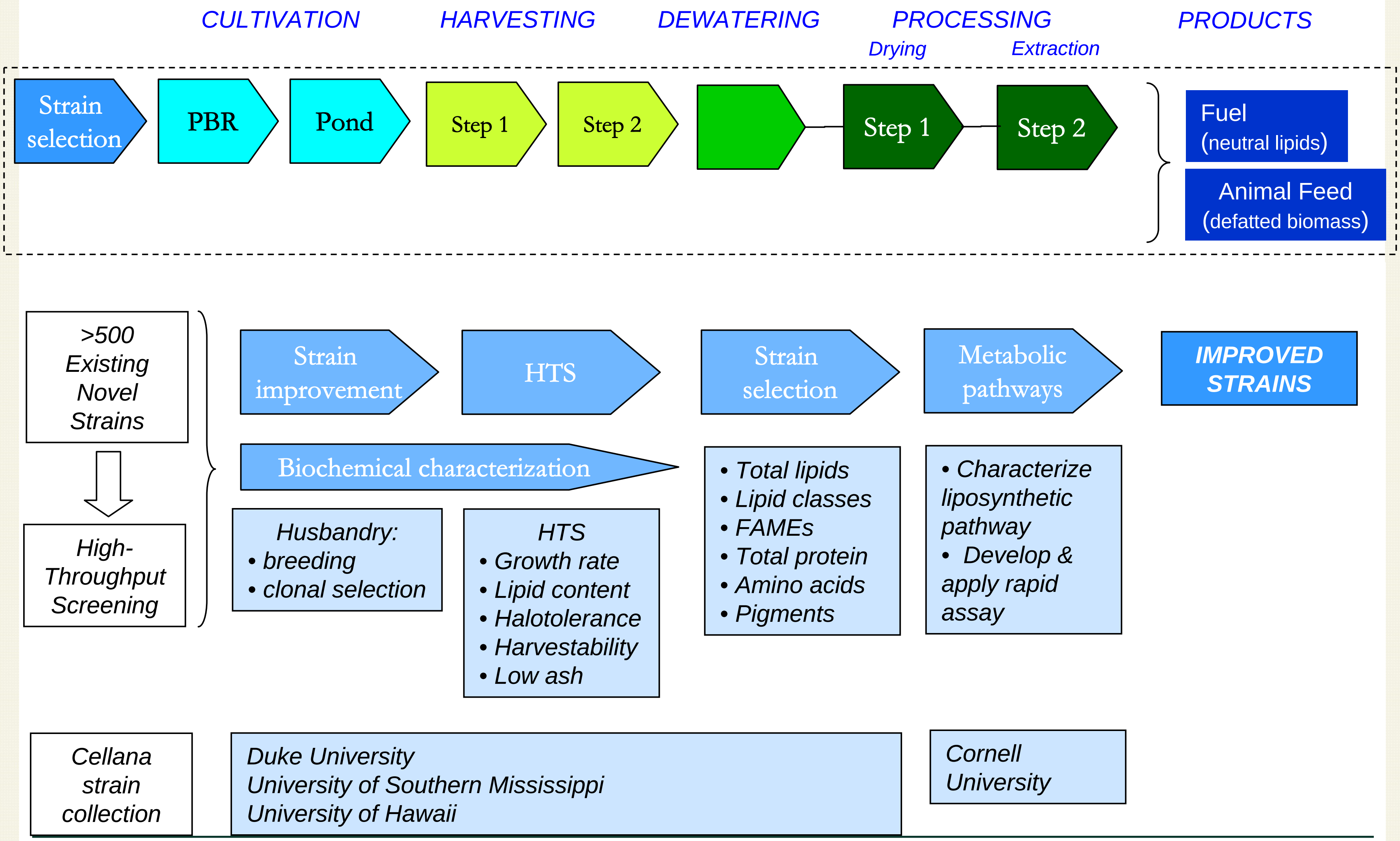
Kona Pilot Facility

Producing 1 MT dry weight per month

Status

- Consortium operating 3 yrs
- Integrated production system operating 1 yr
- World-class pilot facility (KPF)
- Fuels demonstrated
- Feeds demonstrated
- Unique strain collection
    - >500 strains newly isolated from nature
    - comprehensively screened

Strain Development



CULTIVATION *HARVESTING* *DEWATERING* *PROCESSING* *PRODUCTS*

Drying *Extraction*

Strain selection → PBR → Pond → Step 1 → Step 2 → Step 1 → Step 2

Fuel (neutral lipids)

Animal Feed (defatted biomass)

>500 Existing Novel Strains

High-Throughput Screening

Strain improvement → HTS → Strain selection → Metabolic pathways → ***IMPROVED STRAINS***

Biochemical characterization

Husbandry:
- *breeding*
- *clonal selection*

HTS
- *Growth rate*
- *Lipid content*
- *Halotolerance*
- *Harvestability*
- *Low ash*

- *Total lipids*
- *Lipid classes*
- *FAMEs*
- *Total protein*
- *Amino acids*
- *Pigments*

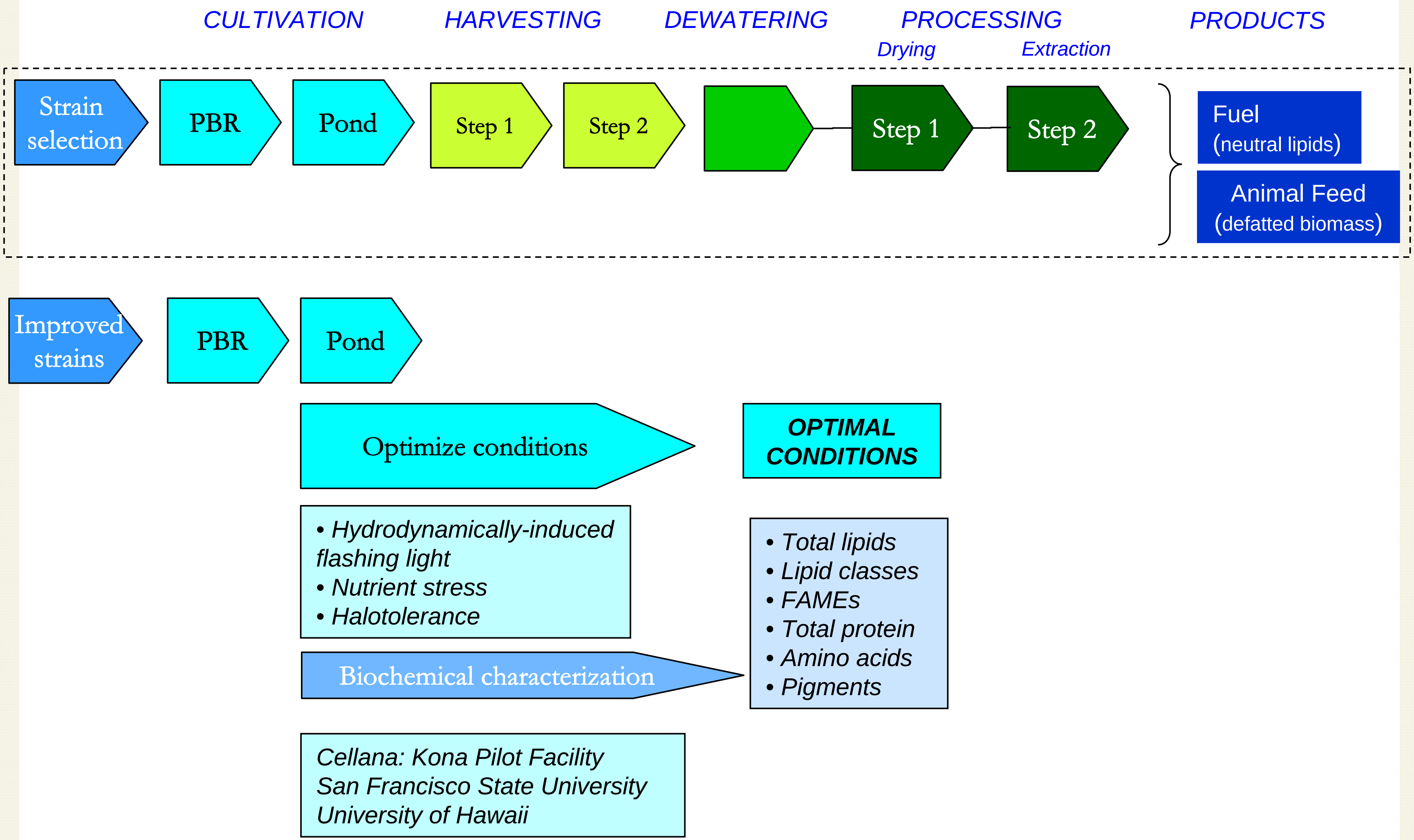
- *Characterize liposynthetic pathway*
- *Develop & apply rapid assay*

Cellana strain collection

Duke University
University of Southern Mississippi
University of Hawaii

Cornell University

Feedstock supply - *Cultivation*



CULTIVATION *HARVESTING* *DEWATERING* *PROCESSING* *PRODUCTS*

Drying *Extraction*

Strain selection → PBR → Pond → Step 1 → Step 2 → → Step 1 → Step 2

Fuel (neutral lipids)

Animal Feed (defatted biomass)

Improved strains → PBR → Pond

Optimize conditions → **OPTIMAL CONDITIONS**

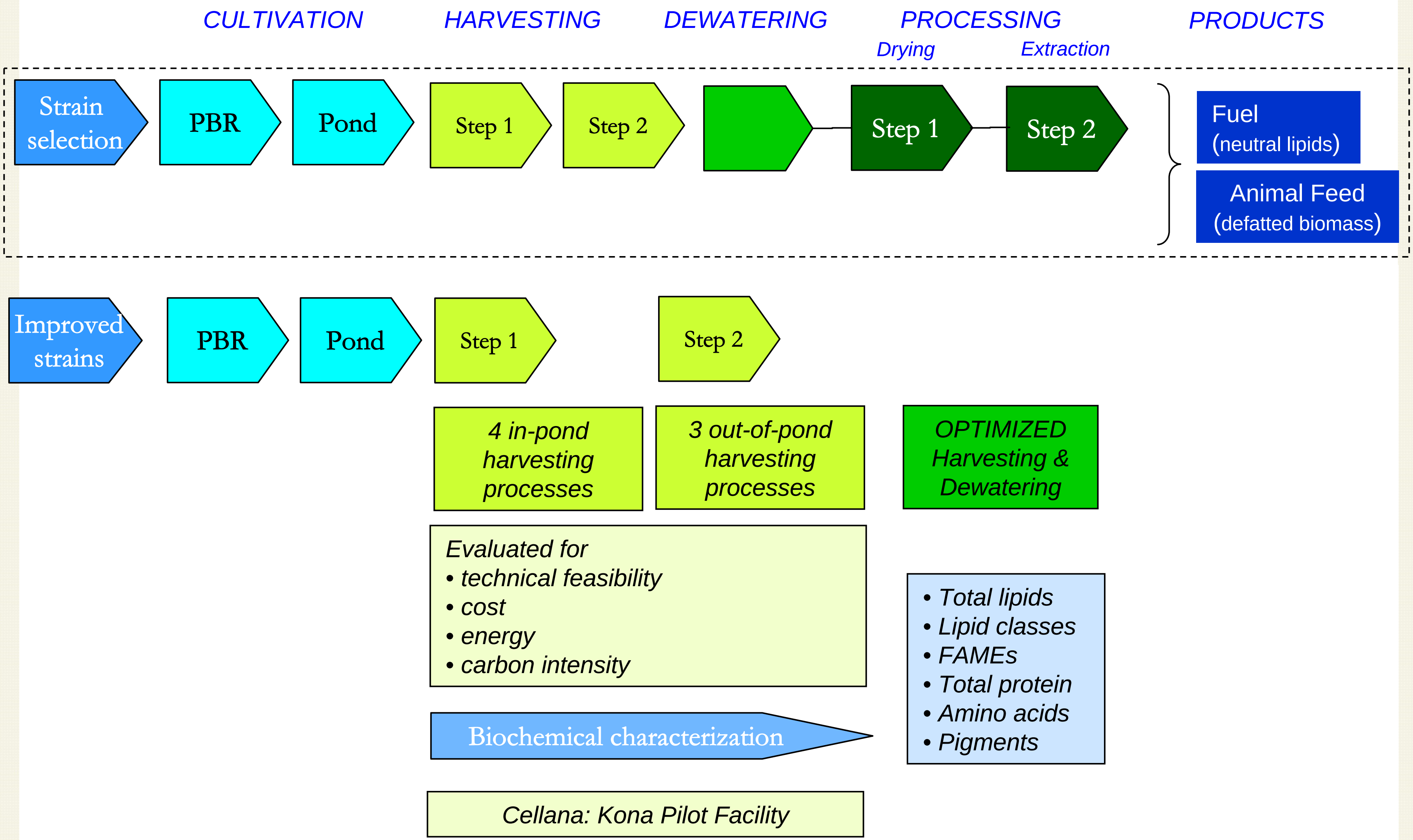
- *Hydrodynamically-induced flashing light*
- *Nutrient stress*
- *Halotolerance*

Biochemical characterization →

- *Total lipids*
- *Lipid classes*
- *FAMEs*
- *Total protein*
- *Amino acids*
- *Pigments*

Cellana: Kona Pilot Facility
San Francisco State University
University of Hawaii

Feedstock supply – *Harvesting and Dewatering*



CULTIVATION *HARVESTING* *DEWATERING* *PROCESSING* *PRODUCTS*

Drying *Extraction*

Strain selection → PBR → Pond → Step 1 → Step 2 → → Step 1 → Step 2

Fuel (neutral lipids)

Animal Feed (defatted biomass)

Improved strains → PBR → Pond → Step 1 → Step 2

4 in-pond harvesting processes

3 out-of-pond harvesting processes

OPTIMIZED Harvesting & Dewatering

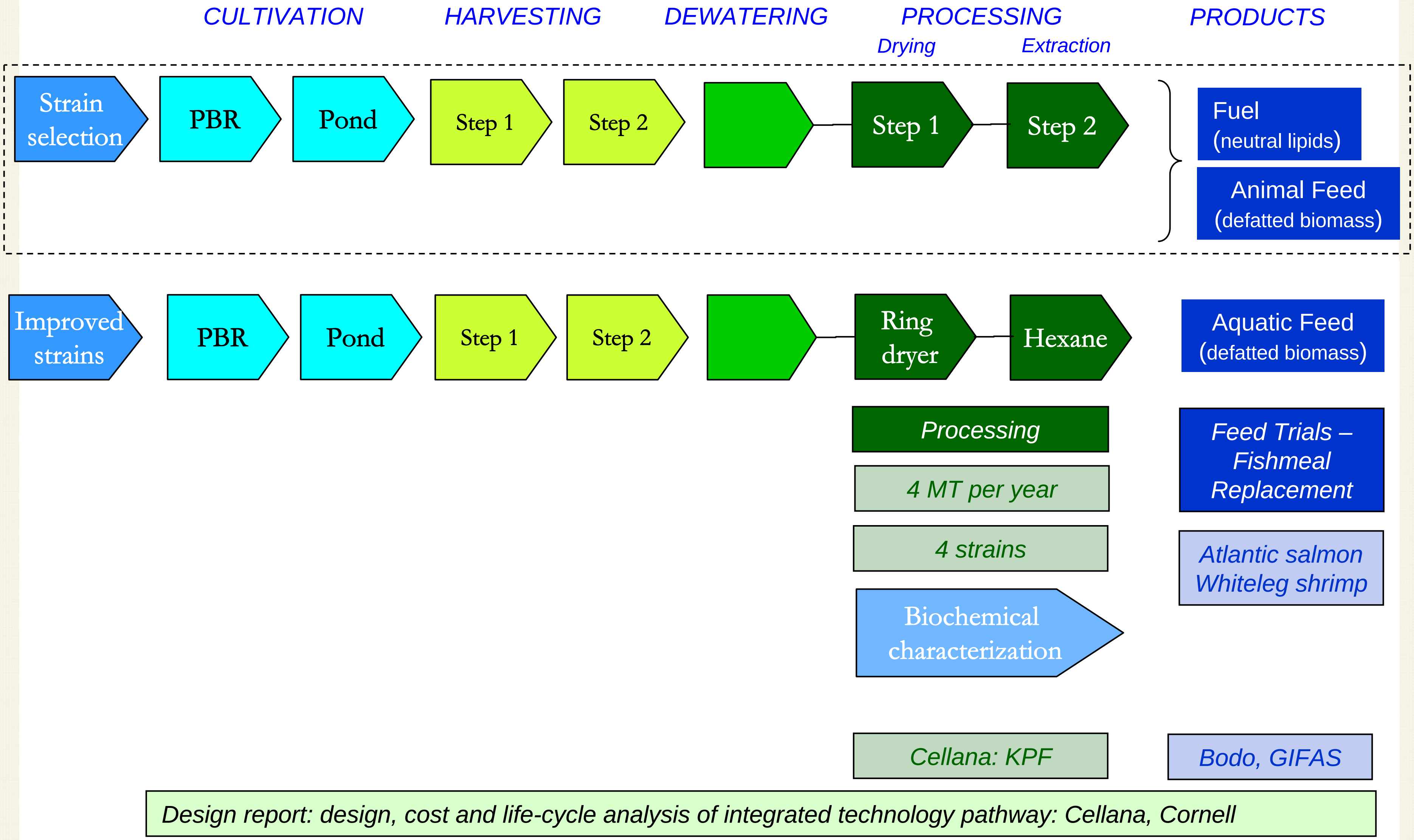
Evaluated for
- *technical feasibility*
- *cost*
- *energy*
- *carbon intensity*

Biochemical characterization

- *Total lipids*
- *Lipid classes*
- *FAMEs*
- *Total protein*
- *Amino acids*
- *Pigments*

Cellana: Kona Pilot Facility

Feedstock logistics – *Extraction & Co-Products*



CULTIVATION *HARVESTING* *DEWATERING* *PROCESSING* *PRODUCTS*

Drying *Extraction*

Strain selection → PBR → Pond → Step 1 → Step 2 → → Step 1 → Step 2

Fuel (neutral lipids)

Animal Feed (defatted biomass)

Improved strains → PBR → Pond → Step 1 → Step 2 → → Ring dryer → Hexane

Aquatic Feed (defatted biomass)

Processing

4 MT per year

4 strains

Biochemical characterization

Feed Trials – Fishmeal Replacement

Atlantic salmon
Whiteleg shrimp

Cellana: KPF

Bodo, GIFAS

Design report: design, cost and life-cycle analysis of integrated technology pathway: Cellana, Cornell

Key Features of the Cellana algae-to-product pathway

Low energy consumption

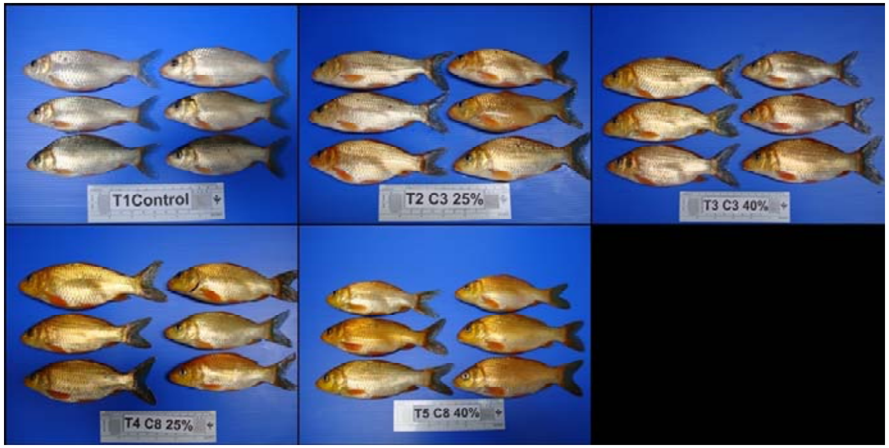
Net energy production is highly positive

Low carbon intensity

2x lower than sugarcane ethanol, under California Low-Carbon Fuel Standard

Sustainable co-products

Feed production is more
sustainable than fishmeal



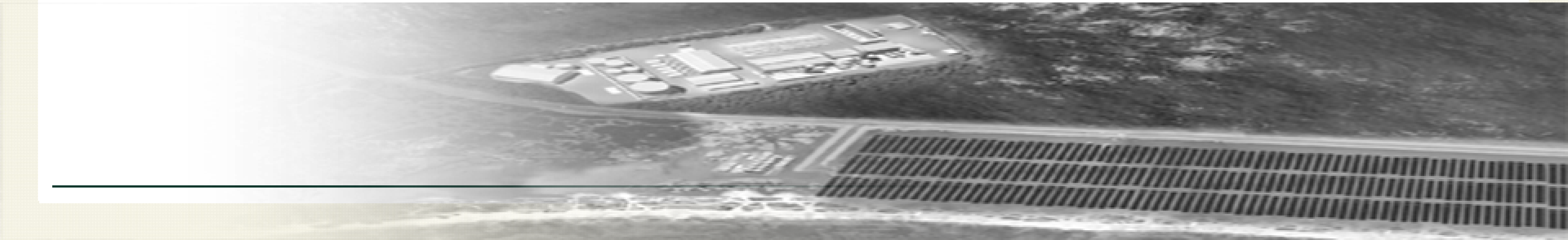
Carp grown on algae meal instead of fishmeal

Source: Bodo University

Summary

- Integrated Cellana pathway now demonstrated at pilot scale
  - Producing 1 MT per month

- Develop new strains
- Optimize cultivation conditions
- Evaluate novel harvesting, dewatering lineups
- Demonstrate co-product value as fishmeal replacement in aquatic feeds
- Develop updated design, cost & LCA for 1000-ha facility



SABC

Sustainable Algal Biofuels Consortium

Cultivating Energy Solutions

SABC Project: Biochemical Conversion of Algal Biomass and Fuel Testing

Lead Institution: Arizona State University
Leader: Gary Dirks (ASU)
Funds: Federal - $6M
Industry Cost Share - $1.5M
Project duration: Two years

U.S. DEPARTMENT OF ENERGY
ASU
NREL NATIONAL RENEWABLE ENERGY LABORATORY
Sandia National Laboratories
GT
SCHOOL OF MINES COLORADO 1874
SRS ENERGY
novozymes Rethink Tomorrow

Biochemical Conversion of Algal Biomass and Fuel Testing

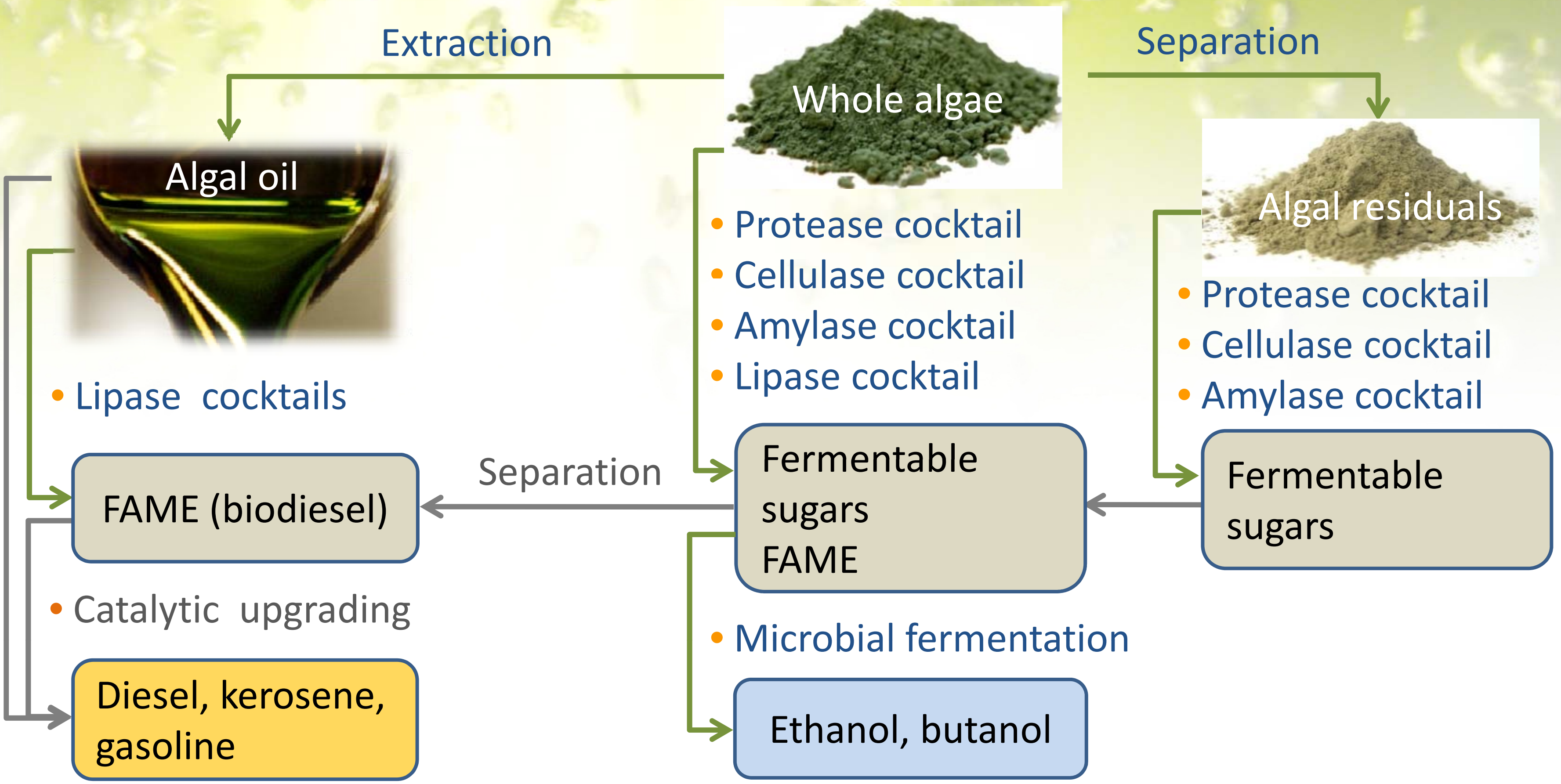
Objective:

The primary objective is to evaluate biochemical (enzymatic) conversion as a potentially viable strategy for converting algal biomass into lipid-based and carbohydrate-based biofuels. Secondary objective is to test the acceptability of algal biofuels as replacements for petroleum-based fuels.

Approach:

- Develop a feedstock matrix of algal biomass based on species and growth/process conditions
- Determine and characterize biochemical composition of selected strains
- Explore multiple enzymatic routes to hydrolyze and convert untreated or pretreated whole algal biomass, oil extracts, and algal residuals
- Determine *fit-for-use* properties of algal derived fuels, fuel intermediates

Multiple Biochemical Conversion Strategies and Routes of Algal Feedstocks into Biofuels



Extraction

Whole algae

Separation

Algal oil

- Lipase cocktails

FAME (biodiesel)

- Catalytic upgrading

Diesel, kerosene, gasoline

- Protease cocktail
- Cellulase cocktail
- Amylase cocktail
- Lipase cocktail

Fermentable sugars FAME

Separation

- Microbial fermentation

Ethanol, butanol

Algal residuals

- Protease cocktail
- Cellulase cocktail
- Amylase cocktail

Fermentable sugars

Potential Process Improvements from Biochemical Conversion

- Biochemical processing of whole algae has the potential to eliminate costly drying and extraction steps
- Application of multiple enzyme cocktails to whole algae enables simultaneous or sequential production of lipid-based and fermentable sugar-based fuel intermediates
- Simultaneous enzymatic hydrolysis, esterification and transesterification of whole algae or algal oil extracts to produce FAMEs reduce process steps and yield potentially cleaner fuel intermediates for final processing to biodiesel or further upgrading to other fuels
- Biochemical processing under mild reaction conditions may minimize the formation of side products and preserves other potentially valuable co-products (e.g., proteins, carotenoids, vitamins)
- Biochemical processing of algal biomass may be easier than that of lignocellulosic feedstocks due to simpler biochemical composition and structure

SABC Team and Organization

- The project led by Dr. Gary Dirks and administered by **ASU**
- The R&D will be carried out primarily by **ASU**, **NREL** and **SNL**
- Additional contributions from Georgia Institute of Technology, Colorado Renewable Energy Collaboratory, Colorado School of Mines, SRS Energy, Lyondell Chemical Company, and Novozymes.
- 24 month scope of work primarily focused on biochemical conversion of algal residuals and whole algal cells

Two Main Technical Tasks:

Task 1 Investigate several promising biochemical options for converting both whole algae and algal residues into transportation fuels

Task 2 Produce samples of those fuels (both lipid and carbohydrate based) and perform fuel testing to determine if those fuels are *fit-for-purpose*

Task 1: Biochemical Conversion of Whole Algae/Algal Residuals into Fuels

Subtask 1.1. Produce selected algae for biochemical conversion

Subtask 1.2. Develop a fundamental understanding of algal chemical composition and structure

Subtask 1.3. Identify and test a variety of pretreatment options and hydrolytic enzyme preparations to facilitate release of fermentable sugars and conversion of algae residues/whole algae into fuel intermediates/products

Task 2: Product Performance of Algal-Derived Hydrocarbon Fuels and Blend Components

Subtask 2.1: Chemical analysis and basic characterization – FAME, diesel

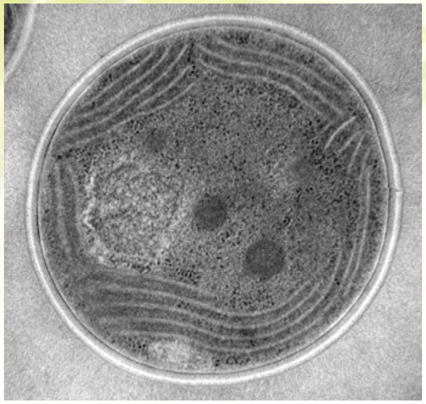
Subtask 2.2: Chemical analysis and basic characterization – alcohols from biochemical conversion.

Subtask 2.3: ASTM specification performance and property assessment

Subtask 2.4: Fuel stability assessment (i.e., storage and thermal stability)

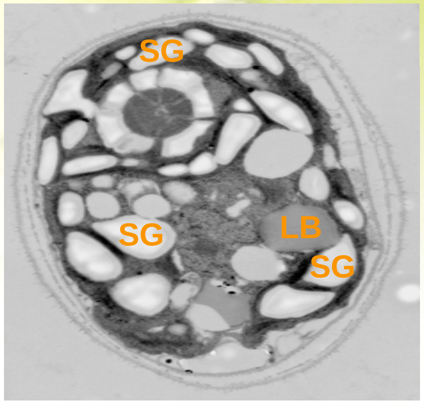
Biochemical Composition of Algae is Species-Specific

SG = starch granule   LB = lipid body

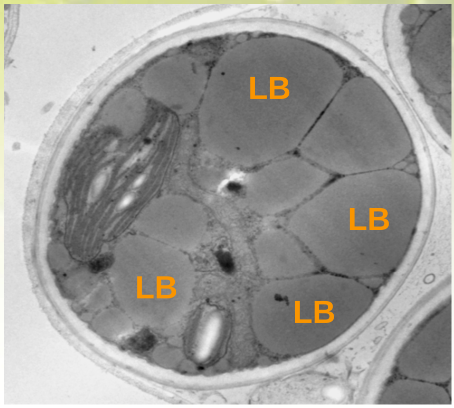


Synechosystis

Palmellococcus



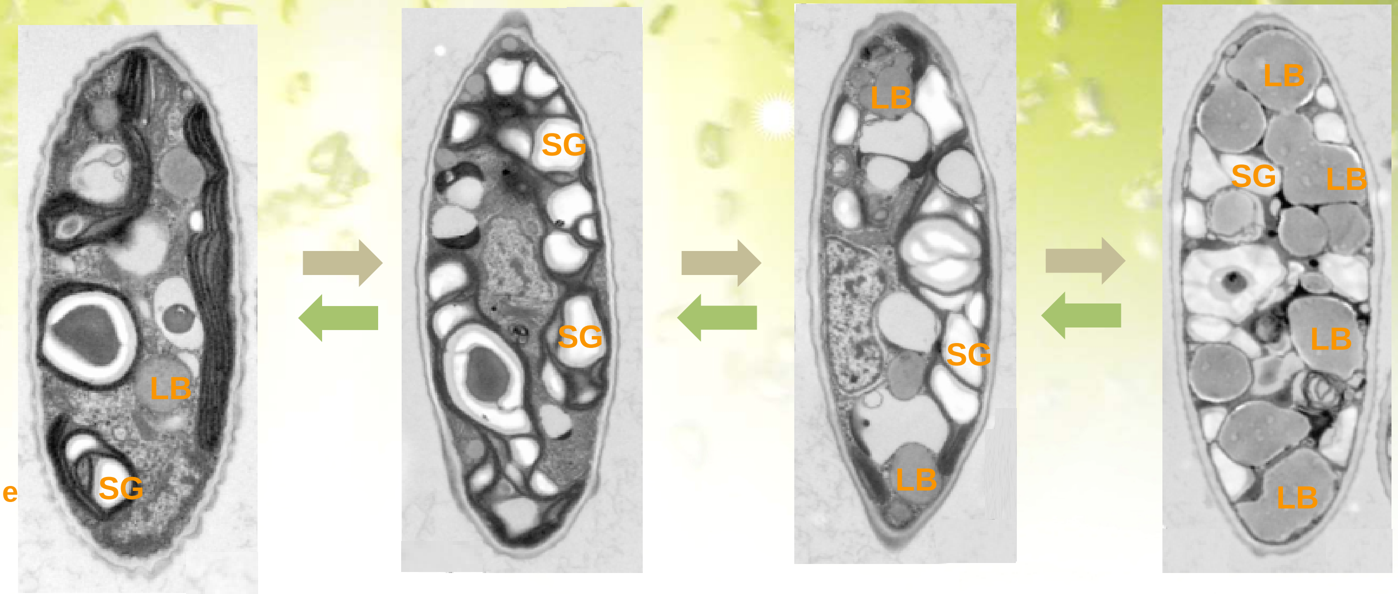
Chlamydomonas



Pesudochlorococcum

Lipid (% dwt)	8	8	15	60
Starch (% dwt)	0	5	45	6
Protein (% dwt)	60	50	20	15

Biochemical Composition of Algae is Condition-Dependent



LB = lipid bodies
SG = starch granule

Reversible transformation of a *Scenedesmus* cell under various culture conditions

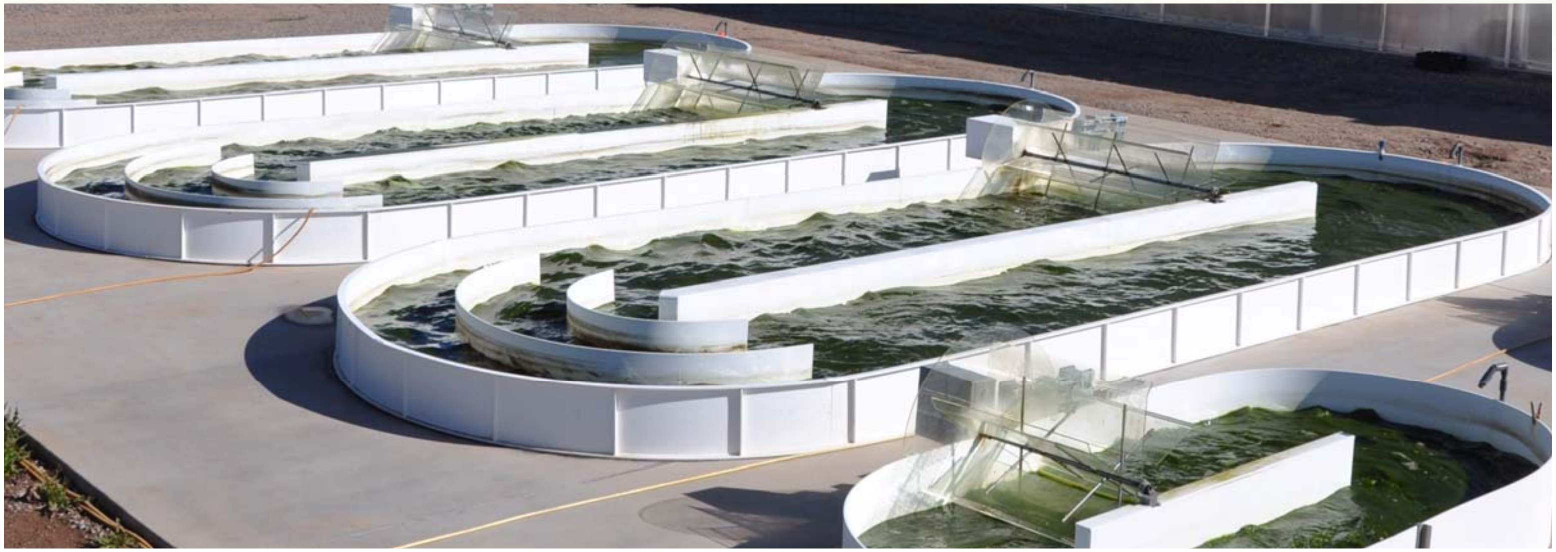
Lipid (% dwt)	10	15	20	45
Starch (% dwt)	8	35	25	15
Protein (% dwt)	55	30	20	10
Cell wall (% dwt)	10	12	14	16

SABC

Sustainable Algal Biofuels Consortium

Cultivating Energy Solutions

Open Raceways Available for Algal Feedstock Production



Photobioreactors Available for Algal Feedstock Production

Major Milestones and Timetable

Phase I:

Small-scale screening for fuel feedstock production and biochemical processing features

Phase II:

Integration of process operations down-selected in Phase I

Phase III:

Scale-up of integrated process for production and testing of fuels

Milestones:

Down-select strains and culture optimization
(Target date: Month 9)

Produce sufficient lipid-rich and carbohydrate-rich algal biomass
(The entire project)

Multiple routes for pretreatment/enzymatic hydrolysis evaluated
(Month 10)

Milestones:

Down-select best strains and processes for maximum lipid and ethanol yields
(Month 15)

Milestones:

Report on chemical analysis and ASTM standards testing for algal biofuels (Month 23)

Final report on fuel production using a biochemical or a combined chemical-biochemical approach and identification of critical elements for future work/cost reduction (Month 24)

Summary of Expected Outcomes

Subtask 1.1 and Subtask 1.2 (NREL, Sandia, CSU, ASU)

- Support biomass growth of g to kg quantities (per selected species/growth condition)
- Complete compositional analysis of algal biomass with the generation of a compositional library as a function of species, growth conditions

Subtask 1.3 (NREL, Sandia, ASU, Novozymes)

- Identify a number of pretreatment options and test existing commercial enzymes to develop baseline
- Explore the development and testing of new pretreatment steps and algae specific enzyme formulations
- While initial focus on algal residuals, also test biochemical conversions on whole cell algae and test whether conversion of whole cell algae will facilitate lipid extraction while at the same time producing fermentable sugars in order to produce a new paradigm in algal biomass processing

Subtask 2 (NREL, ASU, SRS, Lyondell)

- Detailed chemical analysis and basic characterization of the impurities present in the fuels produced from algal biomass generated in Task 1
- Assessment of compliance with ASTM specifications for chemical composition, performance, and stability requirements algal derived biofuels

SABC

Sustainable Algal Biofuels Consortium

Cultivating Energy Solutions

Thank for your time and attention!

U.S. DEPARTMENT OF ENERGY ASU NREL NATIONAL RENEWABLE ENERGY LABORATORY Sandia National Laboratories GT SCHOOL OF MINES COLORADO 1874 SRS ENERGY novozymes Rethink Tomorrow

NAABB

National Alliance For Advanced Biofuels and Bio-products

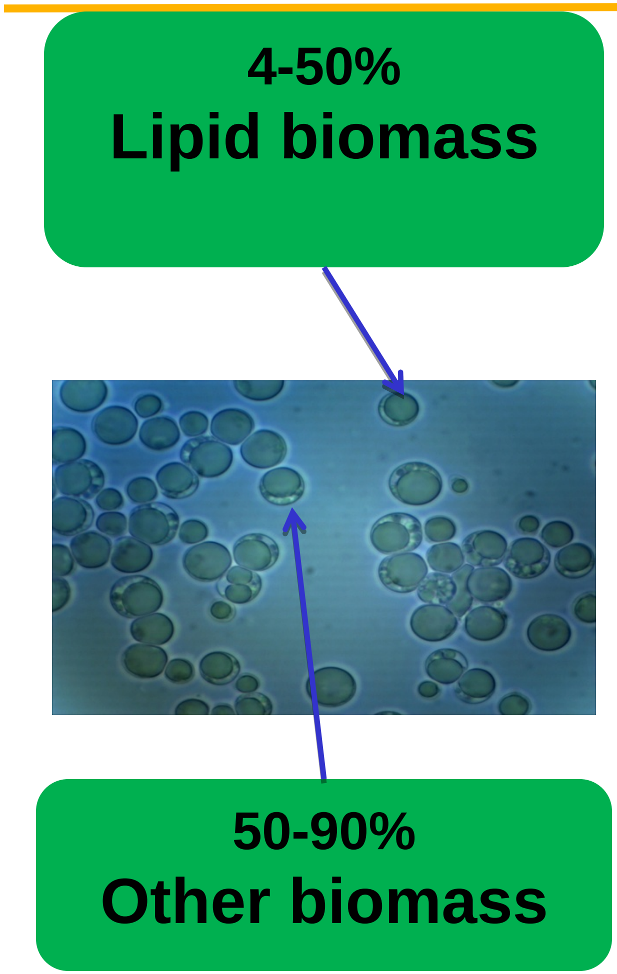
The Promise and Challenge of Algae as a Renewable Source of Biofuels

José A. Olivares
NAABB Executive Director
Los Alamos National Laboratory and
The Donald Danforth Plant Science Center

DOE-Office Of Biomass Program Webinar, Sept. 8, 2010

LANL LA-UR 10-01077

Biofuels from Algae



4-50%
Lipid biomass

50-90%
Other biomass

Rapid growth rate

Double in 6-12 hours

High oil content

4-50% non-polar lipids

All biomass harvested

100%

Continuous harvesting

24/7, not seasonally

Sustainable

Capture up to 90% of injected CO2

Utilize waste water

Non-food

Headliner Productivity

The New York Times

Olympic nightmare: A red tide in Yellow Sea

By Jim Yardley

Published: Monday, June 30, 2008

BEIJING — With less than six weeks before it plays host to the Olympic sailing regatta, the city of Qingdao has mobilized thousands of people and an armada of small boats to clean up an algae bloom that is choking large stretches of the coastline and threatening to impede the Olympic competition.

Photo Source: AP News Eye Press

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

  (~ 4,200 acres)

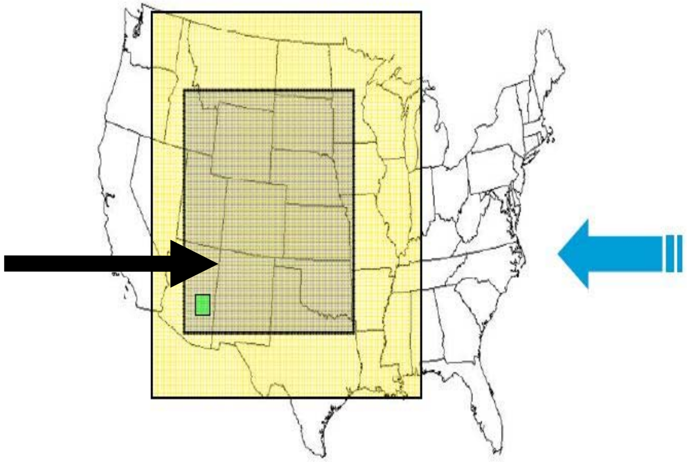
> 47 tons/acre

The Promise of Algae-Based Biofuels

Algae has potential advantages over corn, cellulosic materials, and other crops as an alternative to petroleum-based fuels

	Gallons of Oil per Acre per Year
Corn	**18**
Soybeans	**48**
Safflower	**83**
Sunflower	**102**
Rapeseed	**127**
Oil Palm	**635**
Micro Algae	**1000 - 7000**

Figure courtesy of Sandia National Laboratory



The amount of land required to replace 50% of the current petroleum diesel usage using corn, soybean, and algae.

Land Needed for Biofuel to Replace 50% of Current Petroleum Diesel using oil from:
Corn
Soybean
Algae

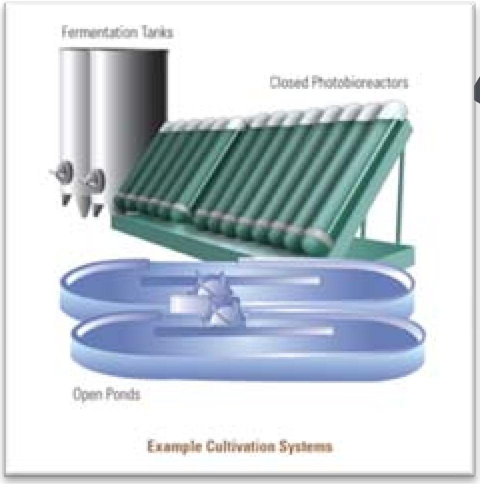
- *High biomass productivity potential*
- *Oil feedstock for higher energy-content fuels*
- *Can avoid competition with agricultural lands and water for food & feed production*
- *Can use non-fresh water, resulting in reduced pressure on limited fresh water resources*
- *Captures CO2 and recycles carbon for fuels and co-products*

Goal –> *Algae to Jetfuel*

www.spiegel.de

Technical Challenges

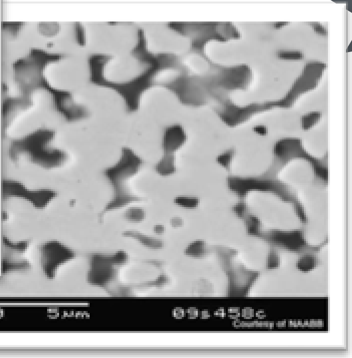
Biology and Cultivation



- Cultivation system design
  - Temperature control
  - Invasion and fouling
- Cultures
  - Growth, stability, and resilience
- Input requirements
  - CO_2, H_2O sources, energy
  - Nitrogen and phosphorous
- Siting and resources

Biomass Harvesting and Recovery

- Energy efficient harvesting and dewatering systems
- Biomass extraction and fractionation
- Product purification

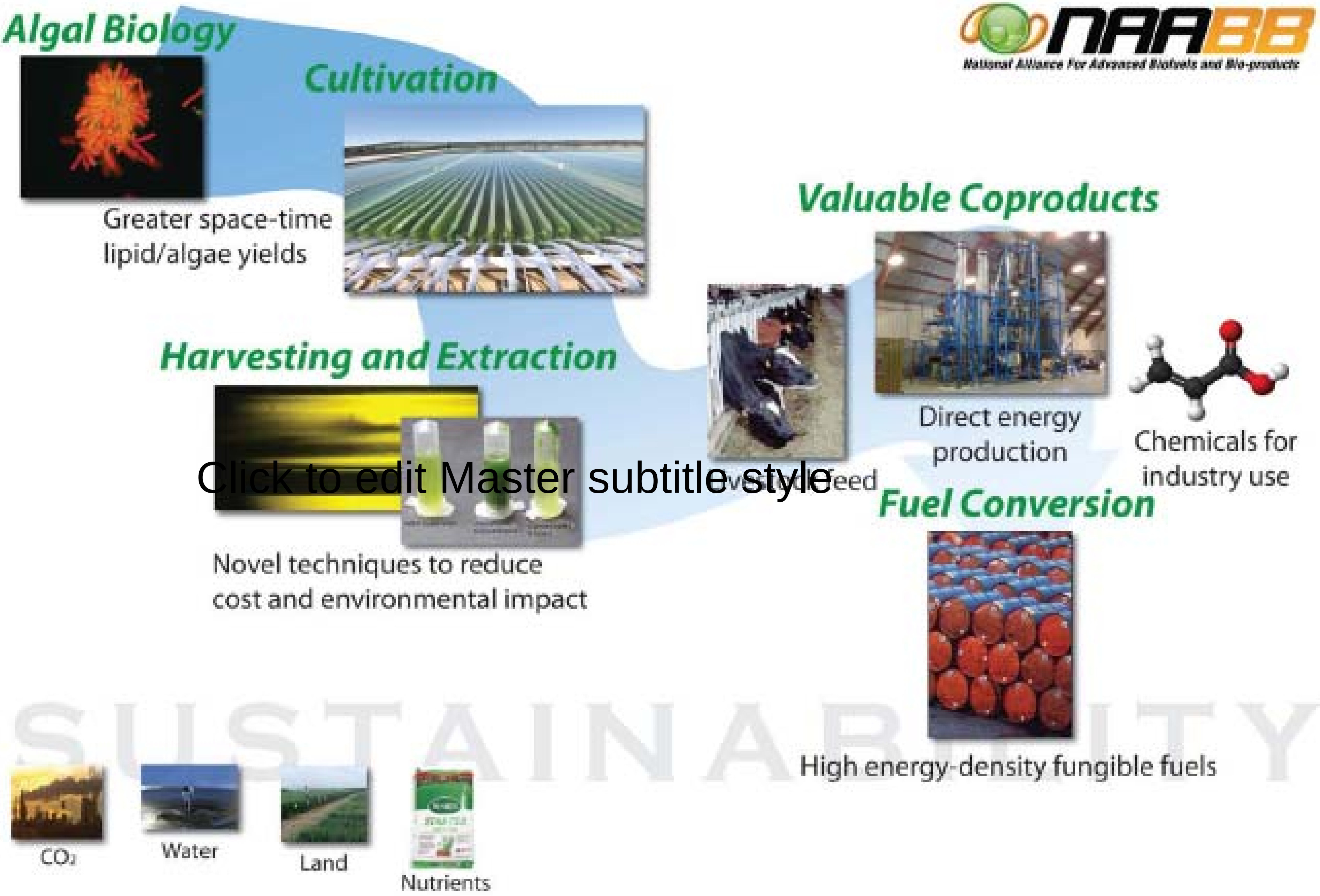


A nano-membrane filter being developed by a NAABB partner.

Conversion and End-use

A gasifier being used by a NAABB partner to convert algal biomass to fuels

- Process optimization
  - Thermochemical
  - Biochemical
- Fuels characteristics
- Co-Products



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Development and Commercialization Value Chain

NAABB Intellectual Property

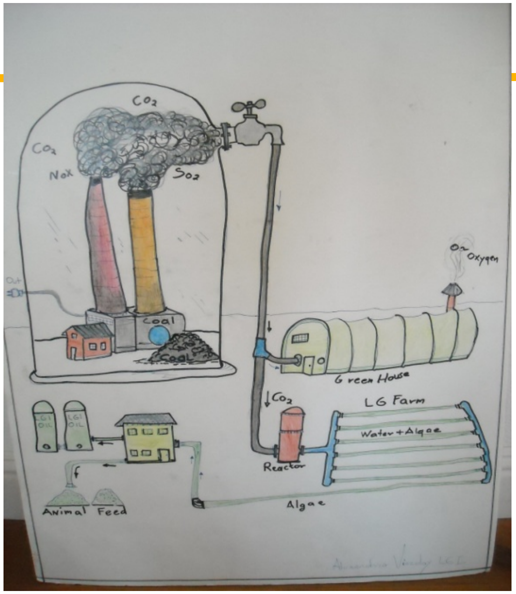
1. ***All inventions belong to the originating/inventing organization(s)***

2. ***Invention Disclosure to 'parent organization' and NAABB leadership***
   - Inventorship is verified
   - NAABB disseminates info to members under the non-disclosure agreement (NDA)
   - 30 Day Disclosure Information Period

3. ***Commercial License***
   - 60 Day faith effort to negotiate licenses
   - If one NAABB member is interested, exclusive OR non-exclusive rights will be granted
   - If multiple NAABB members are interested, non-exclusive rights will be negotiated for each

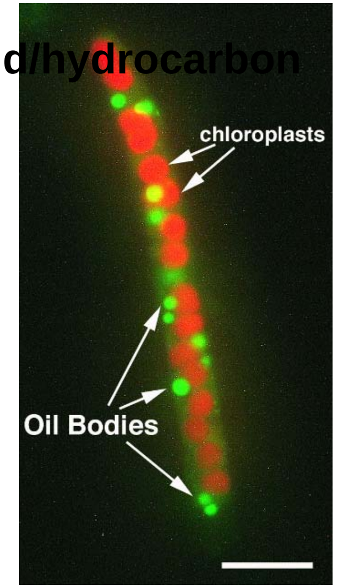
4. ***Copyrights***
   - Parent organization can seek copyright OR NAABB members may seek to elect title

NAABB Specific Objectives



- **Developing technologies for cost-effective production of algal biomass and lipids**
  - **Algal Biology -** Increase overall productivity of algal biomass accumulation and lipid/hydrocarbon content
  - **Cultivation -** Increase overall productivity by optimizing sustainable cultivation and production systems
  - **Harvesting/Extraction -** Develop cost-effective and energy efficient harvesting and lipid extraction technologies
- **Developing economically viable fuels and coproducts**
  - **Fuel Conversion –** Develop technologies to convert lipids/hydrocarbons and biomass residues into useful fuels
  - **Valuable Coproducts -** Develop a set of valuable coproducts to add profitability and provide flexibility to allow responsiveness to changing demands/opportunities in the market.
  - **Providing a framework for a sustainable algal biofuels industry**
  - **Sustainability Analysis –** Quantitatively assess the energy, environment, economic viability and sustainability of the NAABB approaches to guide our strategy

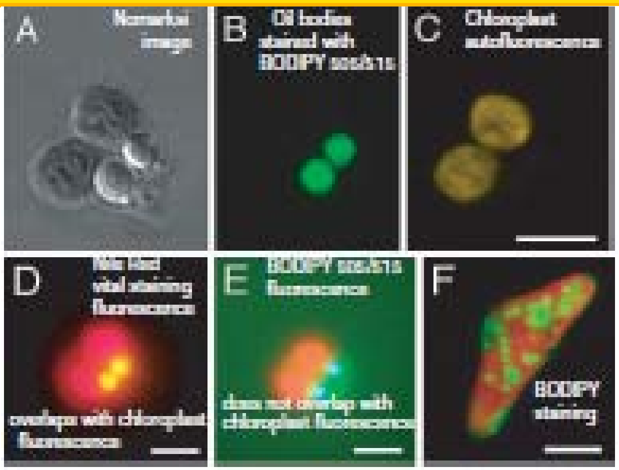
NAABB Algal Biology Objectives

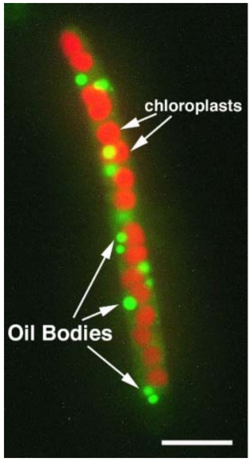


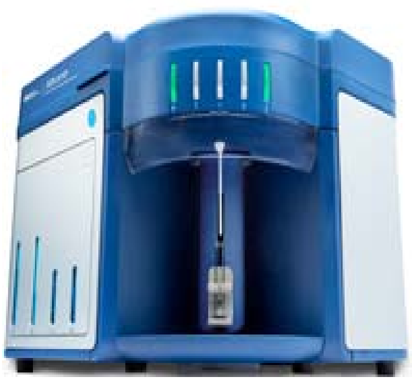
Increase overall productivity of algal biomass accumulation and lipid/hydrocarbon content

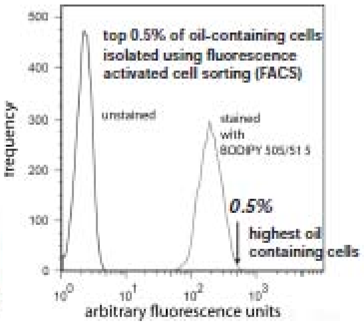
- **Mining natural diversity** (Brooklyn, UW)
- **Mutagenesis for increased lipid production** (WUSL)
- **Systems biology for lipid production**
  - Genomics, proteomics, transcriptomics (LANL/PNNL, UCLA)
- **Crop protection**
  - Adaptive evolution (U of Az)
  - Genetic modification for environmental traits (Danforth)
- **Maximizing yield**
  - Screening tools (WSU), Metabolic regulation (LANL), Nutrient and ionomics (ARS/Danforth)
- **Maximizing lipid production**
  - Gene ID (WUSL, Danforth), Transcriptomics (UCLA, TG), Lipid secretory system and lipid packaging (Danforth, UW, AXI)
- **Maximizing production of hydrocarbons**
  - Isoprenoids (LANL, TAMU, UAz)

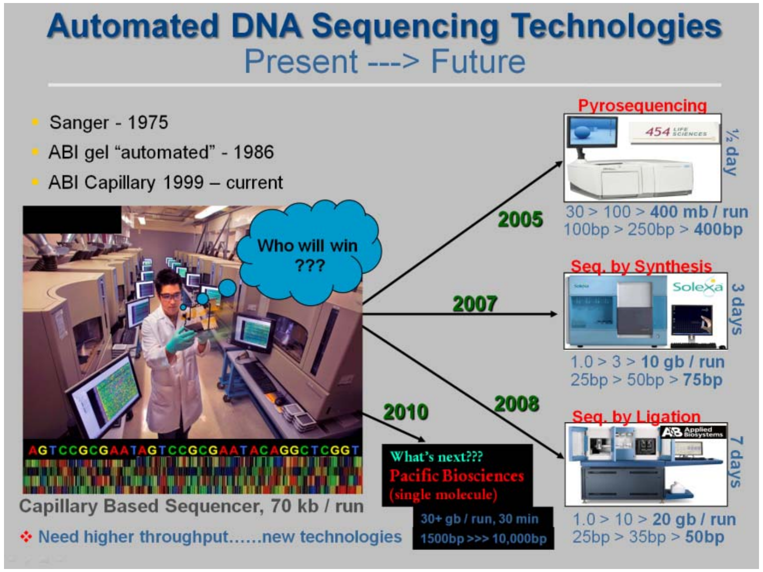
Phenotypic and Genotypic Analysis











Automated DNA Sequencing Technologies

Present ---> Future

- Sanger - 1975
- ABI gel "automated" - 1986
- ABI Capillary 1999 – current

Who will win ???

2005

Pyrosequencing

½ day

30 > 100 > **400 mb / run**
100bp > 250bp > **400bp**

2007

Seq. by Synthesis

3 days

1.0 > 3 > **10 gb / run**
25bp > 50bp > **75bp**

2008

Seq. by Ligation

7 days

1.0 > 10 > **20 gb / run**
25bp > 35bp > **50bp**

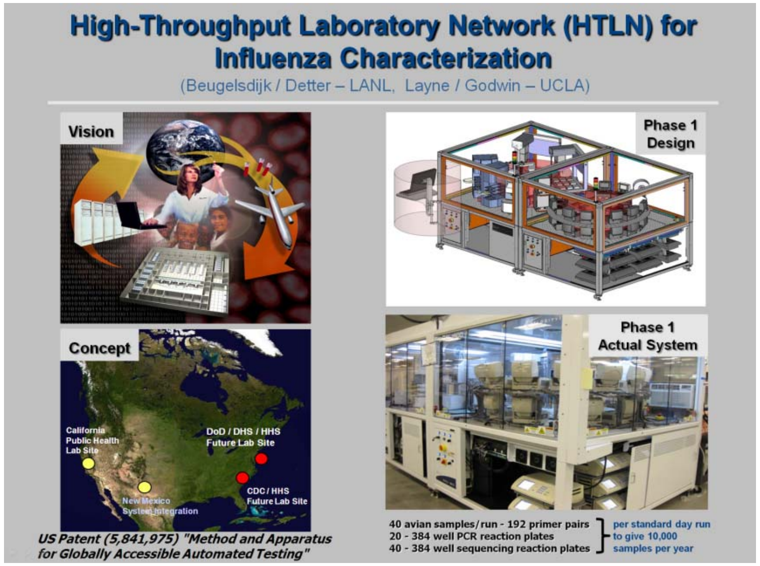
2010

What's next???
Pacific Biosciences
(single molecule)

30+ gb / run, 30 min
1500bp >>> 10,000bp

Capillary Based Sequencer, 70 kb / run

❖ Need higher throughput……new technologies



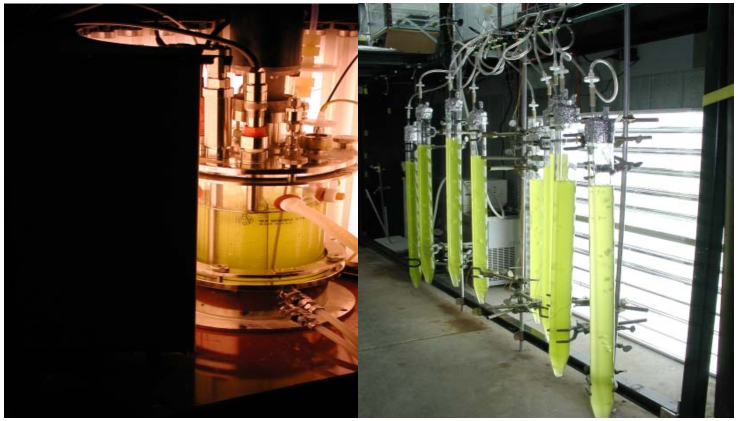
High-Throughput Laboratory Network (HTLN) for Influenza Characterization

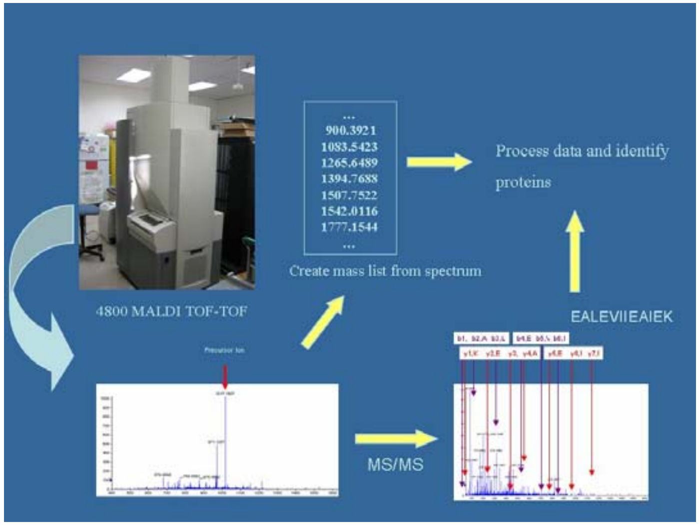
(Beugelsdijk / Detter – LANL, Layne / Godwin – UCLA)

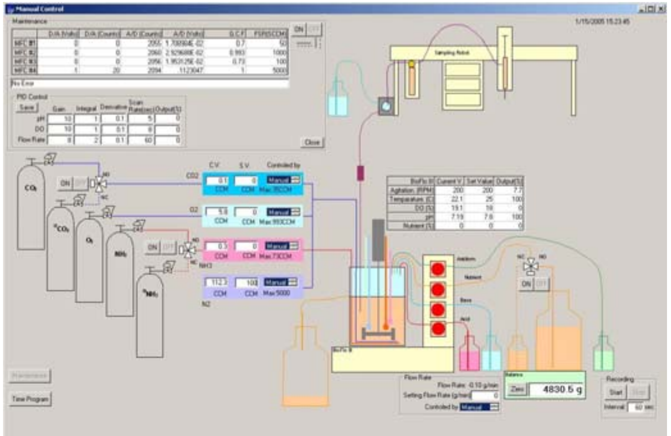
US Patent (5,841,975) "Method and Apparatus for Globally Accessible Automated Testing"

40 avian samples/run - 192 primer pairs 20 - 384 well PCR reaction plates 40 - 384 well sequencing reaction plates	per standard day run to give 10,000 samples per year

Proteomic and Metabolic Analysis







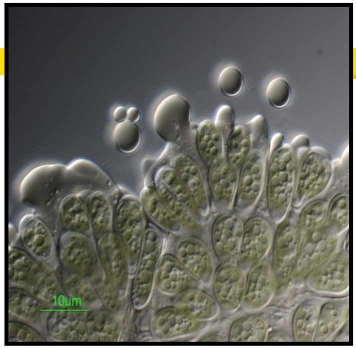
	D/A (Volts)	D/A (Counts)	A/D (Counts)	A/D (Volts)	G.C.F	FS(SCCM)
MFC #1	0	0	2055	1.708984E-02	0.7	50
MFC #2	0	0	2060	2.929688E-02	0.993	1000
MFC #3	0	0	2056	1.953125E-02	0.73	100
MFC #4	1	20	2094	1.123047	1	5000

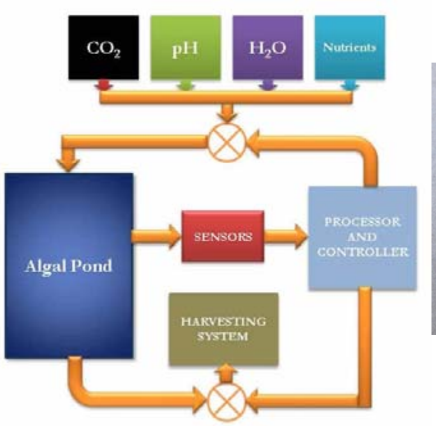
Save	Gain	Integral	Derivative	Scan Rate(sec)	Output(%)
pH	10	1	0.1	5	0
DO	10	1	0.1	8	0
Flow Rate	8	2	0.1	60	0

BioFlo III	Current V	Set Value	Output(%)
Agitation (RPM)	200	200	7.7
Temperature (C)	22.1	25	100
DO (%)	19.1	18	0
pH	7.19	7.8	100
Nutrient (%)	0	0	0

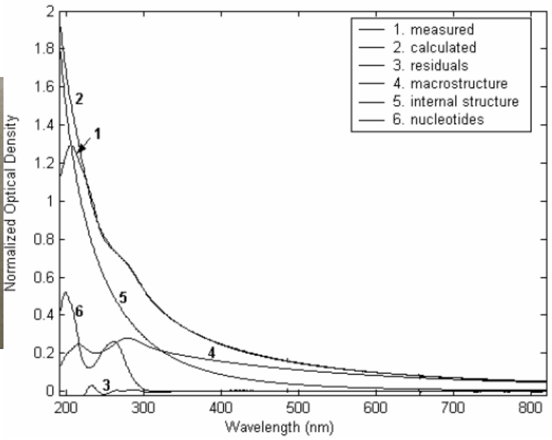
Flow Rate
Flow Rate: 0.10 g/min
Setting Flow Rate (g/min): 0
Controlled by: Manual

Cultivation – Productivity, Environment, Nutrients, Water




CO_2
pH
H_2O
Nutrients
Algal Pond
SENSORS
PROCESSOR AND CONTROLLER
HARVESTING SYSTEM

Real-time In-situ Monitoring (JA Thomasson, TAMU)



Alupoaei, **Biosensors & Bioelectronics,2003**

NAABB Algal Harvesting and Extraction Strategies

- Sedimentation, filtration, dried air flocculation
- Centrifugation alone 15% solids
- Centrifugation and drying >90%
- Belt filter press - 30%
- Attached growth systems - surfaces
- Bioharvesting

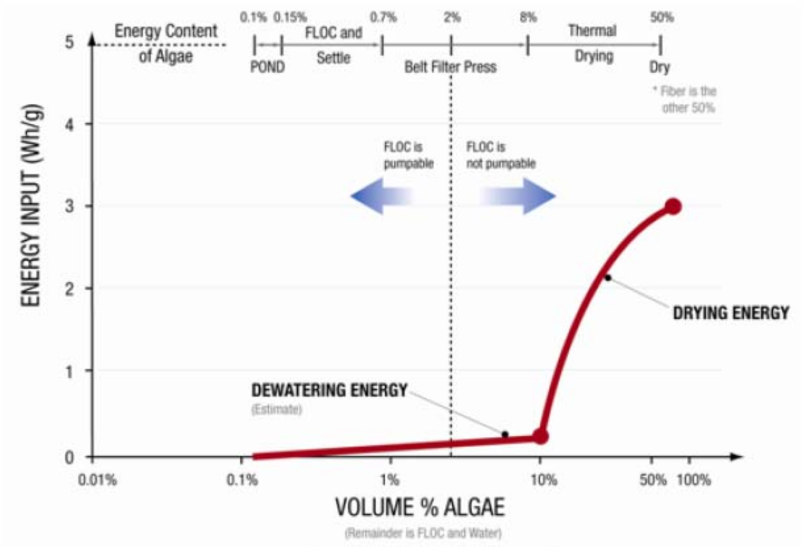


Figure 4: Approximate energy curve for harvesting, dewatering, and drying considering a process of flocculation, sedimentation, belt filter pressing, and drum oven heating.

NAABB will develop cost-effective and energy efficient harvesting and lipid extraction technologies

Harvesting technologies

Acoustic focusing (LANL)

Hybrid capacitive deionization/electro deionization (CDI/EDI) (TAMU)

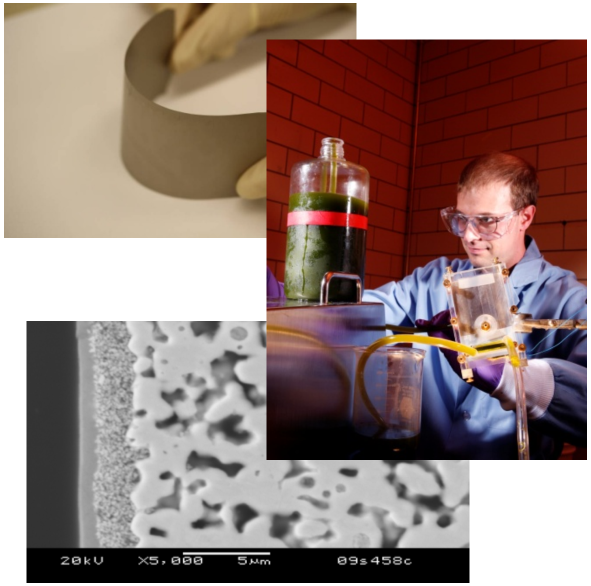
Membranes and flocculants (PNNL)

Extraction Technologies

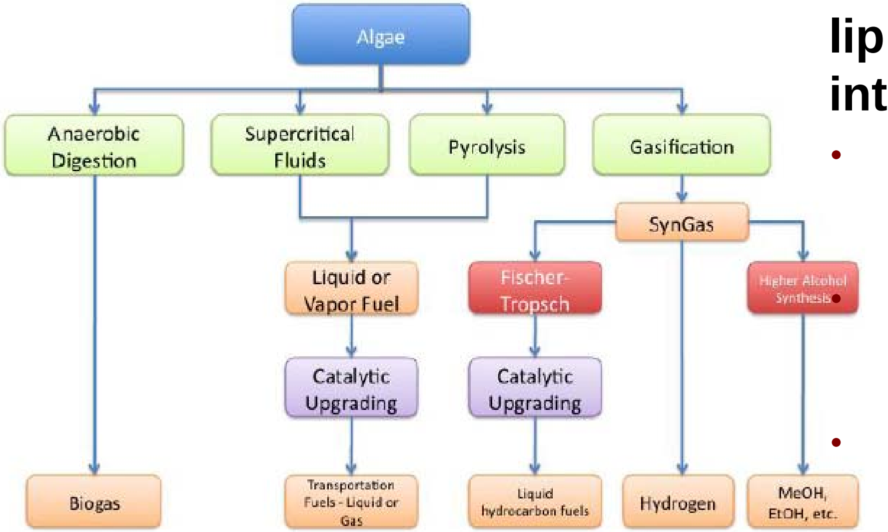
Acoustic technologies (LANL)

Mesoporous nanomaterials (MNM) (Catilin)

Amphiphilic solvents (TAMU)



NAABB Conversion Strategies



- **Develop technologies to convert lipids/hydrocarbons and biomass residues into useful fuels**
  - **Fuel characterization** • *Physical and chemical properties of algal esters and biofuels* • *Thermophysical and transport properties of biofuels* (CSU, UOP, NMSU, UA)
  - **Lipid conversion to fuels** • *Catalytic decarboxylation and deoxygenation* • *Catalytic and supercritical transesterification* (UOP, DE, LANL,CAT, PNNL, NMSU)
  - **Biomass conversion to fuels** • *Catalytic gasification* • *Thermochemical gasification and power* • *Fast pyrolysis and hydroprocessing* • *Anaerobic fermentation to EtOH and gasoline* (CSU, UCSD, TER, GEN)

Pyrox-type dual fluid-bed gasification plant with 4 dry-tons per day capacity

Fuel properties characterization CSU Engine Lab, UOP,

Animal and Mari-culture Industry

peptides, carbohydrates,
lipids, vitamins,
pigments, minerals and
other valuable trace
elements

Amino acid content
Digestibility coefficient
Biological value
Net protein utilization
Protein efficiency ratio

Consortium for Algal Biofuels Commercialization (CAB-Comm)

The San Diego Center for Algae Biotechnology - UCSD
University of Nebraska - Lincoln
Rutgers University
UC Davis

CAB-Comm
Academic Partners

UC San Diego SIO	U Nebraska Lincoln	Rutgers University	UC Davis Life Cycle Associates
S. Mayfield	D. Weeks	P. Falkowski	A. Kendall
S. Burkart	J. Van Etten	C. Dismukes	S. Unnesch
S. Golden	G. Oyler	D. Bhattacharya	
J. Golden	J. Nickerson		
S. Briggs	H. Cerutti		
S. Kay			
B Palenik			
G. Mitchell			
M. Hildebrand			
J. Shurin			
B. Brahamsha			

CAB-Comm
Commercial Partners

Sapphire Energy

General Atomics

Life Technologies

Sempra Energy

Chevron

Praxair

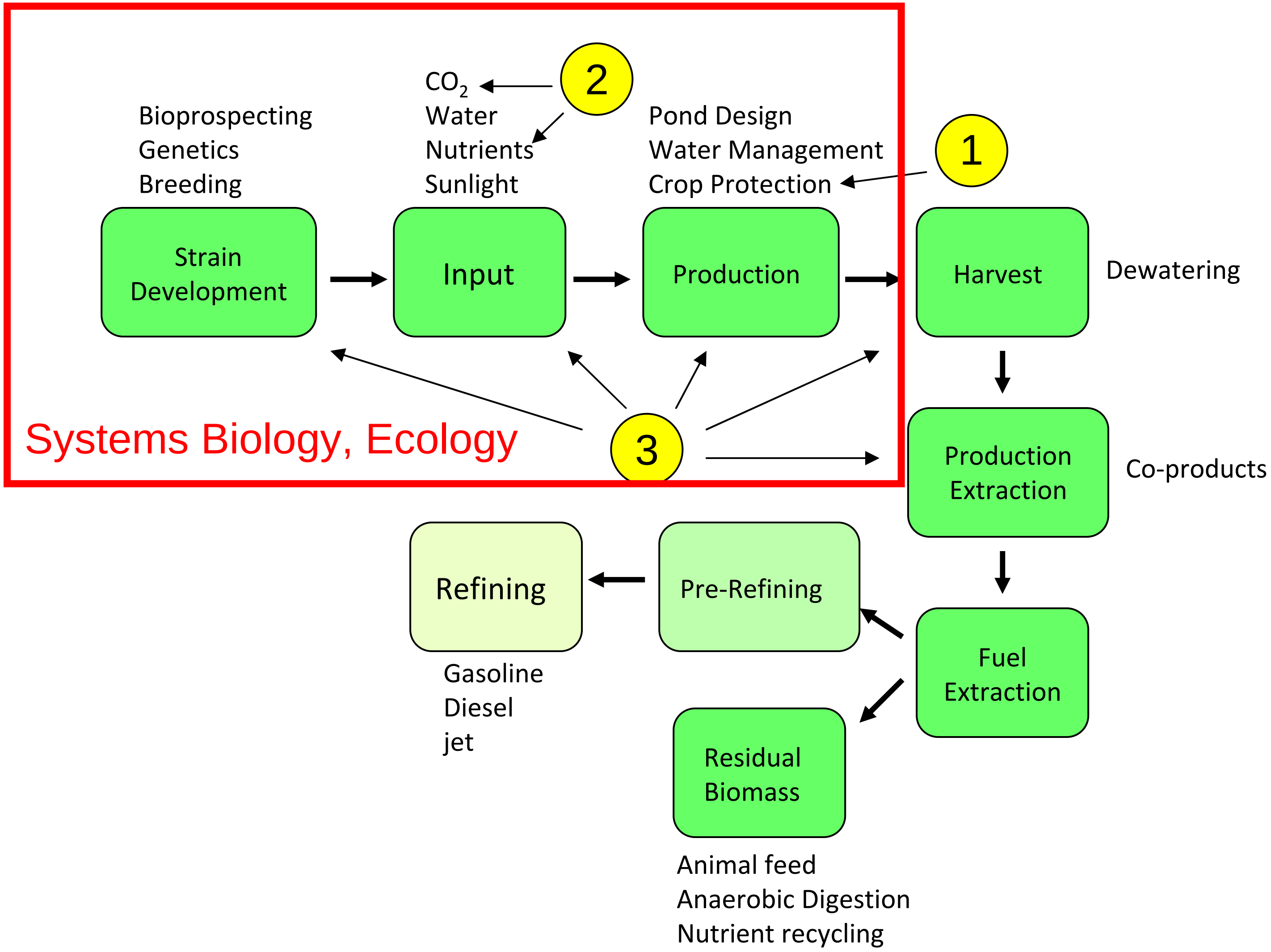
W.R. Grace

Research Areas

Systems Biology and Ecology

1. Crop Protection / Yield Optimization
2. Nutrient Utilization and Recycling
3. Genetic Tools

Algal Biofuels Production Chain
DOE Roadmap



Bioprospecting
Genetics
Breeding

Strain Development

CO_2
Water
Nutrients
Sunlight

Input

Pond Design
Water Management
Crop Protection

Production

Harvest

Dewatering

Production Extraction

Co-products

Fuel Extraction

Pre-Refining

Refining

Gasoline
Diesel
jet

Residual Biomass

Animal feed
Anaerobic Digestion
Nutrient recycling

Systems Biology, Ecology

1

2

3

Specific Projects and Associated Research Groups

I. Crop Protection

1. Characterization of algal genetic resistance to chytrid fungi. (Briggs, Mayfield)
2. Crop protection by secretion of extracellular products and their potential roles in suppressing growth of competing species. (Falkowski, Dismukes, Bhattacharya).
3. Develop anti-viral technologies (Van Etten)
4. Identify and characterize quorum sensing molecules (QSMs) from algae that act as high-density growth inhibitors. (Nickerson)
5. Production of Antimicrobials for Crop Protection in Eukaryotic Algae (Burkart, Mayfield)
6. Develop strategies for finding or constructing grazer/competitor resistant strains (Brahamsha, Goldens, Palenik, Shurin)

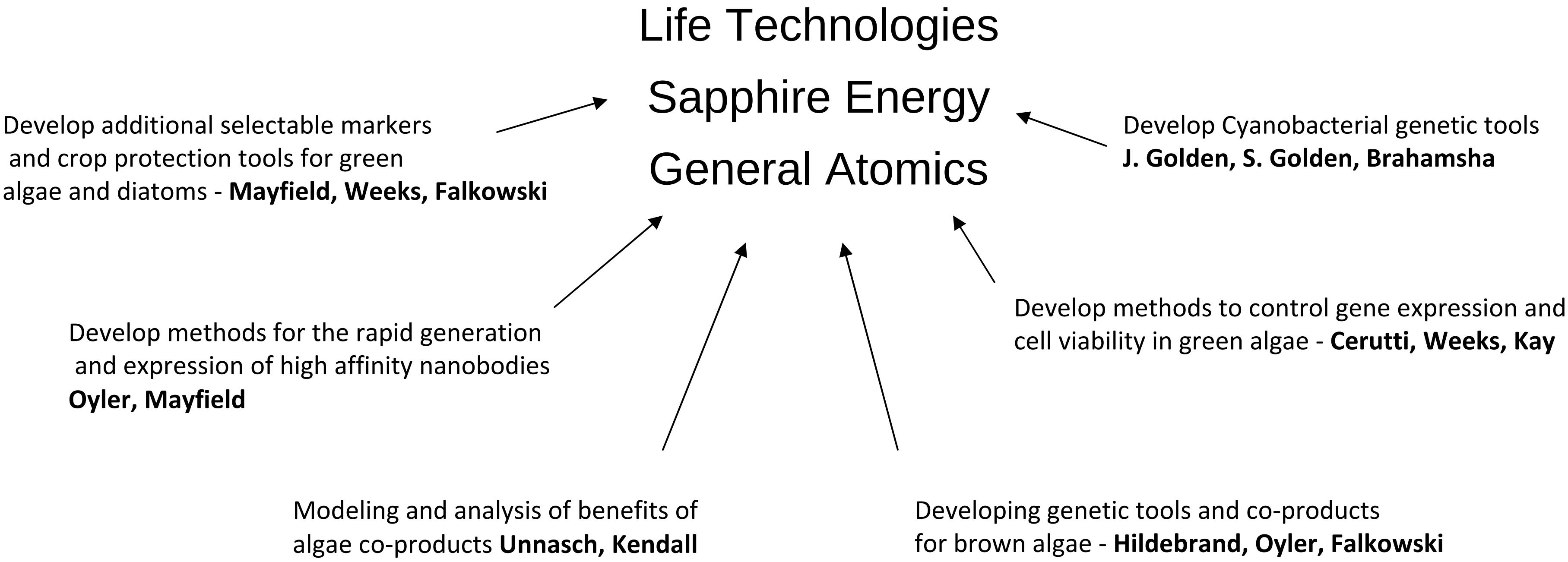
II. Nutrient Utilization and Recycling

1. Physiological characterization of elite algae strains within the abiotic matrix that regulates growth and carbon partitioning. (Mitchell, Shurin)
2. Characterization of carbon dioxide utilization in cyanobacteria at the molecular and cellular levels. (Dismukes, Falkowski)
3. Biological nutrient supply & protection. (J. Golden, S. Golden, Brahamsha)
4. Develop and Characterize a Model Pond. (Palenik, Shurin, Brahamsha, Briggs, S. Golden and J. Golden, Mitchell)
5. Modeling and analysis of nutrient recycling loops (Unnasch, Kendall, Mitchell)

III. Genetic Tools to Enablement Crop Protection and Co-products Production

1. Develop additional selectable markers and crop protection tools for green algae and diatoms (Mayfield, Weeks, Falkowski).
2. Develop new methods to control gene expression and cell viability in green algae. (Cerutti, Weeks)
3. Develop Cyanobacterial genetic tools (J. Golden, S. Golden, Brahamsha)
4. Developing genetic tools and co-products for brown algae (Hildebrand, Oyler).
5. Develop methods for the rapid generation and expression of high affinity nanobodies to promote crop protection, facilitate harvesting, and express high-value co-products. (Oyler)
6. Modeling and analysis of benefits of algae co-products (Unnasch, Kendall)

Developing Genetic Tools
for Green Algae, Brown Algae and Cyanobacteria



Life Technologies
Sapphire Energy
General Atomics

Develop additional selectable markers and crop protection tools for green algae and diatoms - **Mayfield, Weeks, Falkowski**

Develop Cyanobacterial genetic tools
J. Golden, S. Golden, Brahamsha

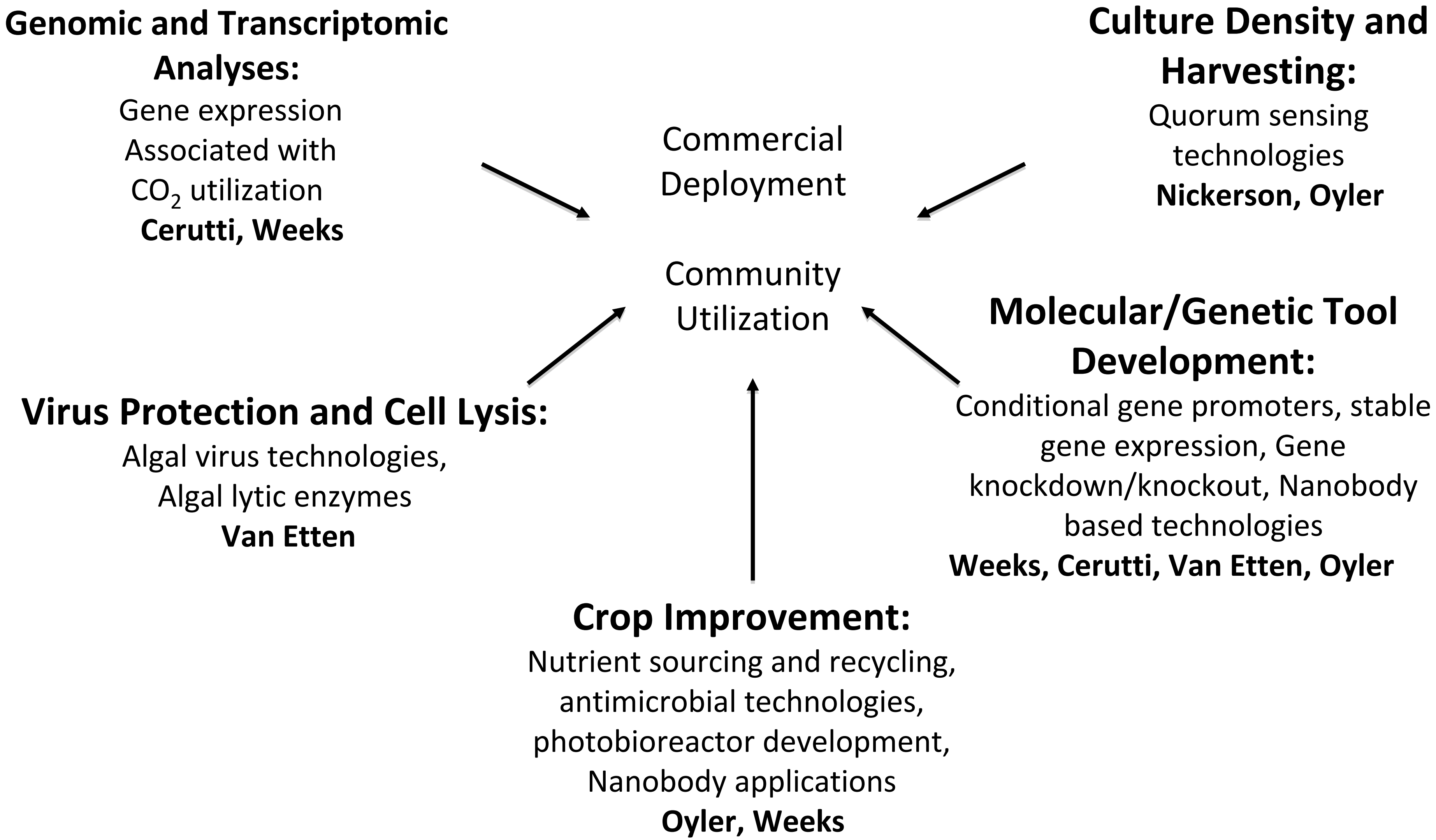
Develop methods for the rapid generation and expression of high affinity nanobodies
Oyler, Mayfield

Develop methods to control gene expression and cell viability in green algae - **Cerutti, Weeks, Kay**

Modeling and analysis of benefits of algae co-products **Unnasch, Kendall**

Developing genetic tools and co-products for brown algae - **Hildebrand, Oyler, Falkowski**

Nebraska Center for Algal Biology and Biotechnology (UNL-NCABB)



Commercial Deployment

Community Utilization

Genomic and Transcriptomic Analyses:
Gene expression
Associated with
CO_2 utilization
Cerutti, Weeks

Culture Density and Harvesting:
Quorum sensing
technologies
Nickerson, Oyler

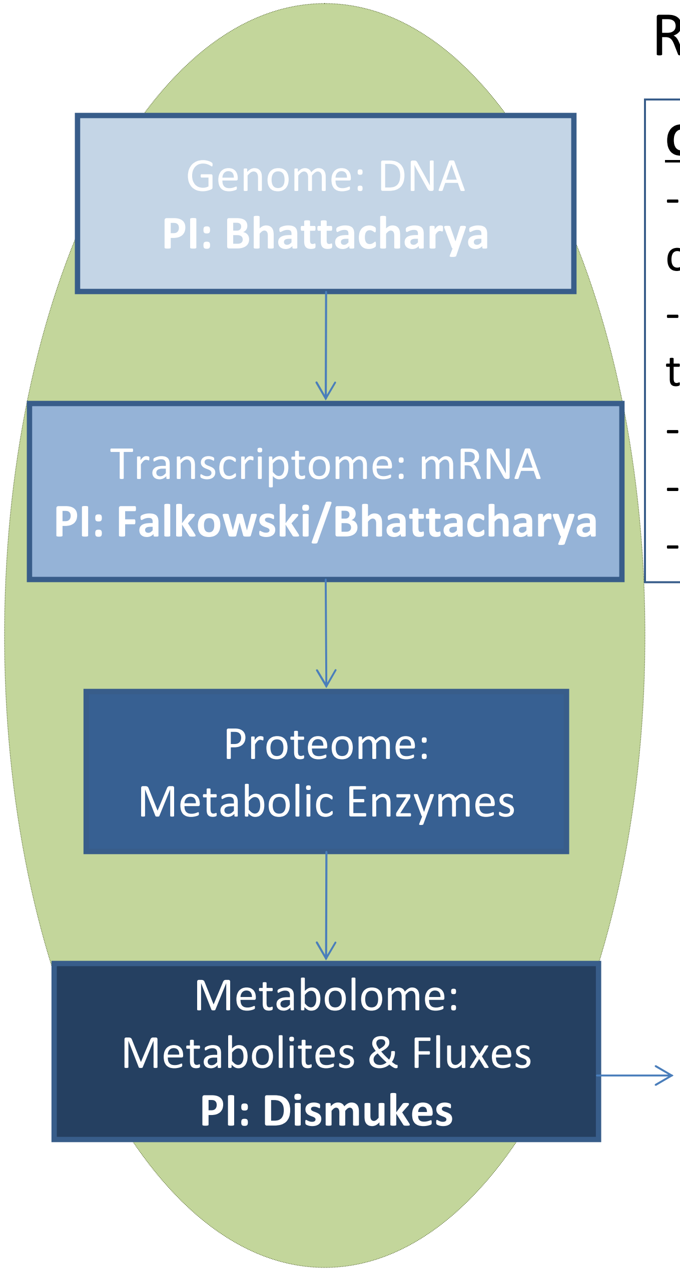
Virus Protection and Cell Lysis:
Algal virus technologies,
Algal lytic enzymes
Van Etten

Molecular/Genetic Tool Development:
Conditional gene promoters, stable
gene expression, Gene
knockdown/knockout, Nanobody
based technologies
Weeks, Cerutti, Van Etten, Oyler

Crop Improvement:
Nutrient sourcing and recycling,
antimicrobial technologies,
photobioreactor development,
Nanobody applications
Oyler, Weeks

Crop protection via allelopathy in algae &cyanobacteria

Rutgers University



Genome: DNA
PI: Bhattacharya

Transcriptome: mRNA
PI: Falkowski/Bhattacharya

Proteome:
Metabolic Enzymes

Metabolome:
Metabolites & Fluxes
PI: Dismukes

<u>Conditions to analyze</u>

- Co-culture w/ competitors, pathogens
-Allelopathic additives to ↓ competitors
-Genetic manipulations
-Culture extremes
-Nutrient perturbations

Methods

-Measure photosynthetic parameters

-Identify excreted 2^o metabolites & signaling

-knock-down/in enzymes & signaling with RNAi

-Compare genomes & transcriptomes

-Generate map of genome methylation

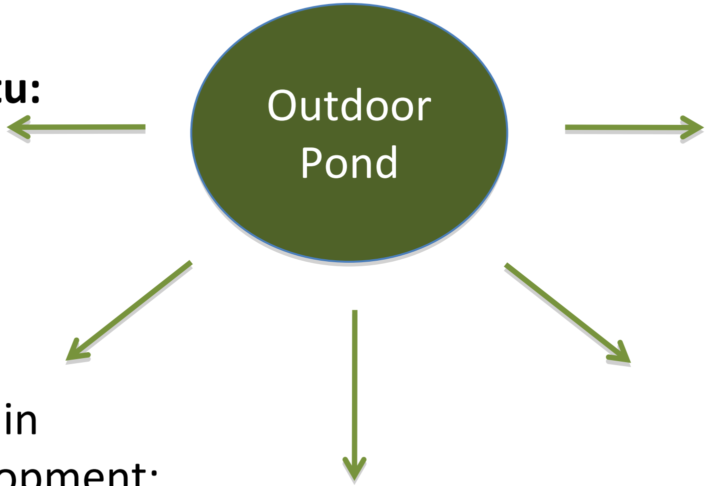
Create RNA libraries

Optimize conditions for crop protection

<u>Allelopathic molecules</u>

- Microcystins
- Saxitotoxins
- Anatoxins
- Cylindorspermopsin

Crop protection studies using model ponds



Outdoor Pond

Algal protein expression in situ:
Briggs, Palenik

Contaminant and grazer diversity: 18S, 16S rRNA libraries, imaging flow cytometry
Brahamsha, Palenik

Algal physiology and genetics:
Characterization of model cyanobacterial production strain BL0902 and genetic tool development:
S Golden, J Golden, Brahamsha

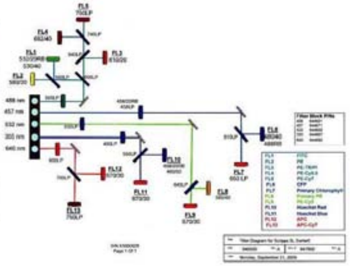
Pond Ecology:
Role of algal and grazer population diversity in pond stability
Shurin, Palenik, Brahamsha, Mitchell

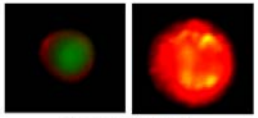
Crop improvement:
Development of grazer resistant cyanobacterial strains
J Golden, S Golden, Brahamsha

Developing Genetic Tools and Crop Protection Strategies for Brown Algae

(Hildebrand, Oyler)

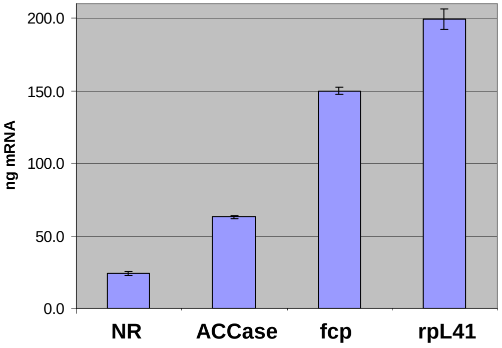
1. Mutagenesis and flow cytometry for strain improvement

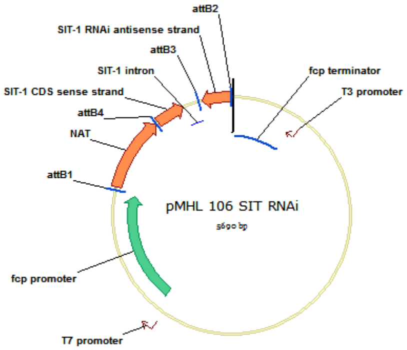




Nile Red Staining of Lipids

2. Diatom expression control elements that regulate mRNA levels and Gateway™-based expression vectors

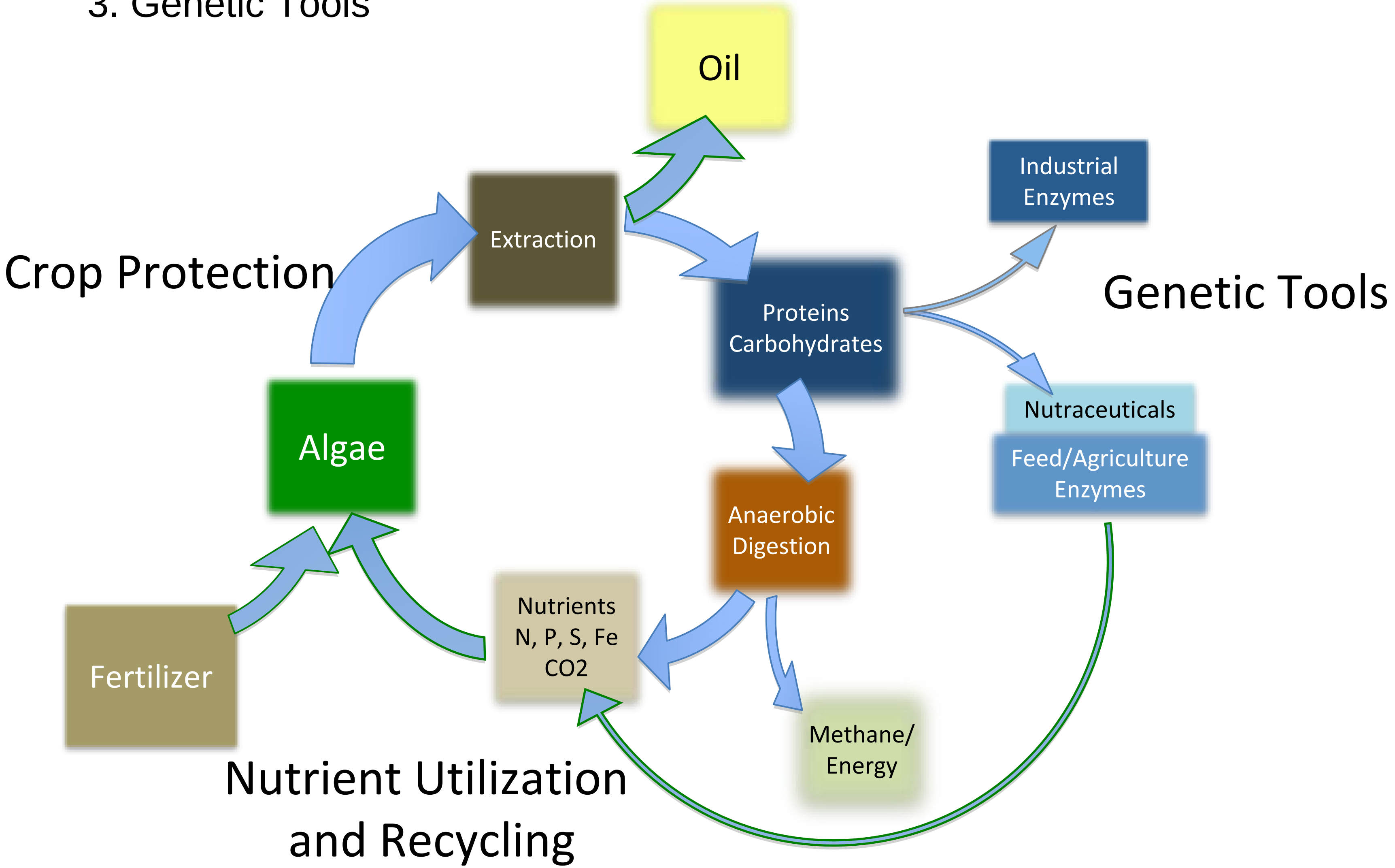




3. Expression of GFP-tagged protein in diatoms (red = chlorophyll, green = GFP)

Algae Biofuel Production Cycle Systems Biology and Ecology

1. Crop Protection
2. Nutrient Utilization and Recycling
3. Genetic Tools



Oil

Extraction

Crop Protection

Industrial Enzymes

Genetic Tools

Proteins Carbohydrates

Nutraceuticals

Algae

Feed/Agriculture Enzymes

Anaerobic Digestion

Nutrients
N, P, S, Fe
CO2

Fertilizer

Methane/ Energy

Nutrient Utilization and Recycling

The San Diego Center for Algae Biotechnology
SD-CAB

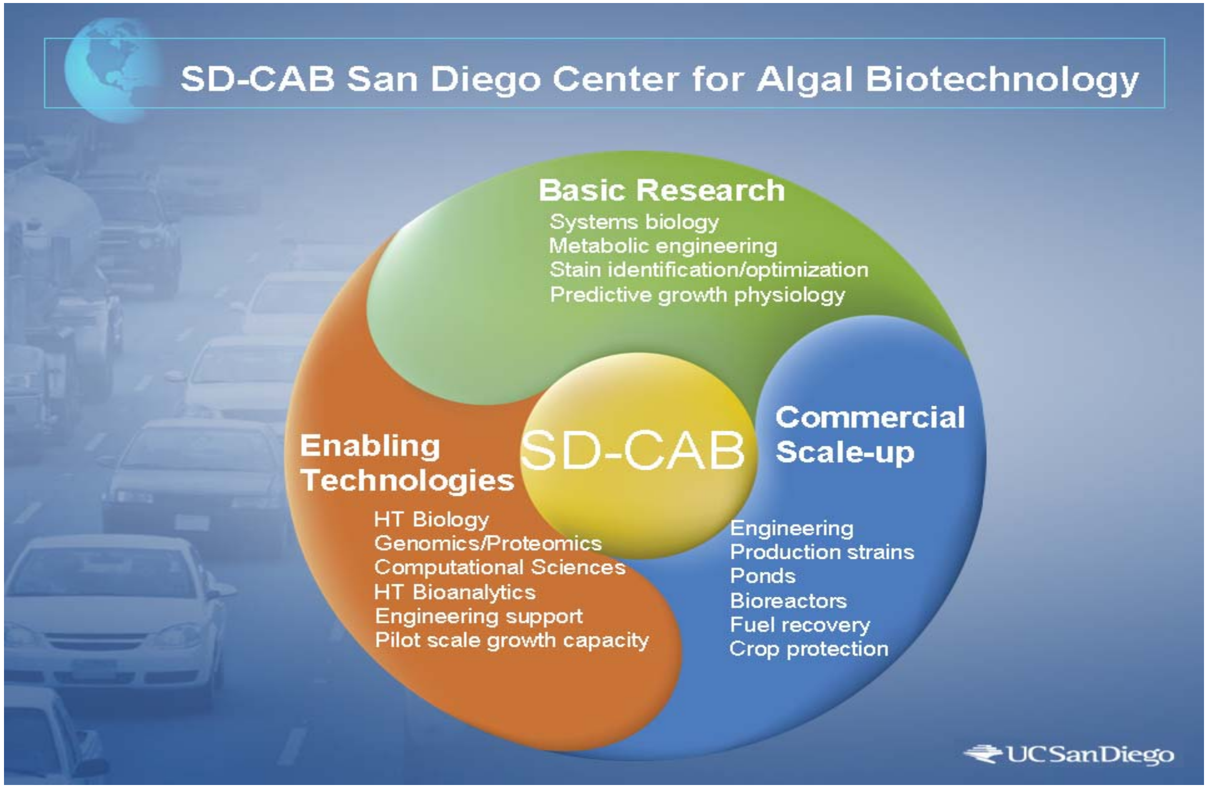
http://algae.ucsd.edu/

THE
SCRIPPS
RESEARCH
INSTITUTE

Biological Sciences
where discovery comes to life UC San Diego

SCRIPPS INSTITUTION OF OCEANOGRAPHY
UCSD

Mission: The San Diego Center for Algae Biotechnology (SD-CAB) was established to support the development of innovative, sustainable and commercially viable algae-based biotechnology solutions for renewable energy, green chemistry, bio-products, water conservation and CO2 abatement.



SD-CAB San Diego Center for Algal Biotechnology

SD-CAB

Basic Research
- Systems biology
- Metabolic engineering
- Stain identification/optimization
- Predictive growth physiology

Enabling Technologies
- HT Biology
- Genomics/Proteomics
- Computational Sciences
- HT Bioanalytics
- Engineering support
- Pilot scale growth capacity

Commercial Scale-up
- Engineering
- Production strains
- Ponds
- Bioreactors
- Fuel recovery
- Crop protection

UC San Diego