“Algae Testbed Public Private Partnership (ATP³): Multi-Region, Long-Term Algae Biomass Cultivation Trials”

John A. McGowen, PhD, PMP
Director of Operations and Program Management
Arizona Center for Algae Technology and Innovation (AzCATI)
Arizona State University

BIO World Congress on Industrial Biotechnology
May 13, 2014
Agenda

• AzCATI Introduction
• ATP³ Objectives
  – Open, Collaborative Testbed Network
  – High Impact Data from Long Term Cultivation Trials
• Cultivation Trials
  – Site Alignment “Year 1 Harmonization” and validation of UFS Experimental Framework
  – Preliminary Data from Phase 2 – Spring ’14
  – Data management and availability
• Summary and Acknowledgements
The Arizona Center for Algae Technology and Innovation (AzCATI), part of ASU LightWorks, formed in 2010 through the support of Science Foundation of Arizona to serve as a hub for research, testing, and commercialization of algae-based products.

**Connect** – Serve as an intellectual and physical hub to foster local, state, national and international research and education on algae-based solutions for a sustainable environment

**Advance** – Enable development of innovative and sustainable technologies for production of microalgae feedstocks for biofuels and bioproducts

**Collaborate** – Facilitate collaborations among universities, national laboratories, and industry to accelerate technology development and commercialization

**Educate** – Provide innovative learning and educational environments and opportunities for training next generation scientists and engineers for the biotechnology workforce

**Launch** – Serve as a national test bed to accelerate the advancement of algae technology development and commercialization
AzCATI Research and Development Priorities

- Strain development for multiple applications
- Carbon capture and bioremediation from industrial/municipal/Ag sources
- Development of next generation algal mass culture systems and processes
- System scale-up and systems/processes integration
- Evaluation of algae products/co-products
- LCA and techno-economical assessment of algae-based biotechnologies
- Development of State/National test bed facilities
A key priority for AzCATI was the development of test bed facilities that can be State and National resources for universities, industry and the National Laboratories.
ASU and the DOE Funded RAFT: ATP³’s Two Main Objectives

Collaborative Open Testbeds
• Establish **network** of facilities for the algal research community and **increase stakeholder access** to real-world conditions for algal biomass production.
• **Accelerate** applied algae research, development, investment, and commercial applications for biofuel feedstock production.

High Impact Data from Long Term Algal Cultivation Trials
• Design and implement a unified experimental program across different **regional, seasonal, environmental and operational conditions** comparing promising production strains at meaningful scales.
• **Data made widely available** to the TEA/LCA and overall research community allowing for a robust analysis of the state of technology.
## ATP³ Quad Chart Overview

### Timeline

- **Project start date:** 2/1/2013
  - Pre-Award (at risk) 11/12-1/13
- **Project end date:** 1/30/2018
- **Percent complete:** <30%

### Budget

Total project cost: $17.05M  
DOE Commitment (up to $15M)

- Collaborative Testbed: $7.3M  
  - DOE share: $5.25M  
  - Contractor share: $2.05M
- High Impact Data: $9.75  
  - DOE share: $9.75M  
  - Contractor share: N/A

### Barriers

- **Ft-B** Sustainable Production: Existing data on the productivity and environmental effects of biomass feedstock production systems...are not adequate
- **St-E** Best Practices for Sustainable Bioenergy Production
- **At-A/At-C** Lack of comparable, transparent and reproducible analysis and inaccessibility and unavailability of data

### Partners

- ASU (AzCATI)
- National Renewable Energy Laboratory
- Sandia National Laboratories
- Cellana
- Cal-Poly
- Touchstone Research Laboratory
- Georgia Tech
- Florida Algae LLC
- UTEX
- Commercial Algae Management
- Valicor Renewables
ATP³ Project Timeline

ATP³ Phases:

1. **Months 1-12**: Coordinate mobilization of partnership and initiate work to perform both functions – Go/No Go for Phase 2 (January 2014).
   **Major Milestones:**
   - ATP organization, systems and processes established
   - Methodologies harmonized across all partner sites
   - Initial cultivation trial and detailed experimental planning completed
   - Biomass stocks available

2. **Months 13-36**: Long term cultivation trials implementation and building customer base as a user facility – Go/No Go for Phase 3
   **Major Milestones:**
   - Cultivation trials executed, data distribution implemented and state of algal biofuels technology design report completed
   - Capability of testbed network to serve stakeholder community demonstrated

3. **Months 37-60**: Sustainable Testbed Operations.
   **Major Milestones:**
   - State of algal biofuels technology design report updated with customer data
   - Value network created and funding secured to sustain network in out years
Collaborative Open Testbeds

ATP³ offers access to a wide array of services, capabilities and facilities:

- Strain Identification & Isolation
- Analytical Services
- Education & Training
- Biomass Production & Supply
- Equipment Testing
- Stakeholder Access to Facilities

Regional testbed facilities for the partnership are physically located in Arizona, Hawaii, California, Ohio, Georgia, and Florida.
Variety of Cultivation Systems, Types, and Scale

<table>
<thead>
<tr>
<th>ATP³ Partner Site</th>
<th>Cultivation Capacity Total (Liters) (unit scale range)</th>
<th>Annual Production Capacity (AFDW)</th>
<th>Yr. Outdoor Operations Began</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASU (AzCATI)</td>
<td>235K (200 - 125K)</td>
<td>1.5 - 2.0 MT</td>
<td>2006</td>
</tr>
<tr>
<td>Cal Poly (CP)</td>
<td>100,000 (1K - 10K)</td>
<td>1.0 - 1.5 MT</td>
<td>2007</td>
</tr>
<tr>
<td>Cellana (KDF)</td>
<td>750,000 (200 - 120K)</td>
<td>12 - 15 MT</td>
<td>2008</td>
</tr>
<tr>
<td>Florida Algae (FA)</td>
<td>30,000 (500-1K)</td>
<td>&lt;0.5 MT</td>
<td>2011</td>
</tr>
<tr>
<td>Touchstone (TRL)</td>
<td>525,000 (500 - 125K)</td>
<td>3 - 4 MT</td>
<td>2012</td>
</tr>
<tr>
<td>Georgia Tech. (GT)</td>
<td>6000 (1K-1.5K)</td>
<td>&lt; 0.1 MT</td>
<td>2013</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,600,000 L</strong></td>
<td><strong>330,000L</strong></td>
<td><strong>17 - 25 MT</strong></td>
</tr>
</tbody>
</table>
Variety of independent and vertically integrated downstream harvesting unit ops

Provide service to ATP³ customers
- Produce algal biomass in the form of slurry, paste and dry powers
- Serve as baseline technologies for the improvement of future harvesting/dewatering and oil extraction processes (Valicor and OpenAlgae platforms)

Support DOE’s TEA, sustainability, and resource modeling
- Generate harvesting data for the current harmonized model
- Provide more options to generate data on the selection of harvesting methods
- Provide feedstock for lipid extraction and other downstream product applications
ATP³ sets standards and conducts harmonized, rigorous, and objective long term cultivation trials to provide a realistic assessment of the state of technology for algal based biofuels and bioproducts.

- Our Unified Field Studies (UFS) at the 6 testbed sites along with our Advanced Field Studies (AFS) enable comparison of promising production strains at meaningful scale across variable conditions.
- Our Scientific Data Management System and validated, harmonized SOP’s for analytical and production processes ensures data integrity across all sites.
- Our data from the UFS and AFS will be made publicly available and provide a critical resource to TEA and LCA analysis yielding high impact, validated data.
Unified Field Studies – Harmonized Data

**Goal**
Eliminate Measurement Variability from Unified Field Study experimental effectors

**Objectives:**
- Need to lock down uncertainty around measurements
- Statistical analysis of UFS data should not be influenced by analytical variability
- Unified biochemical data ingest to data management system

**Productivity metrics:**
- Volumetric Dry Weight
- Ash Free Dry Weight
- Lipids (total FAME)
- Carbohydrates
- Protein
• System and scale variation has the potential to induce unwanted, non-geographical related variability between testbeds as a function of:
  – system design
  – scale of operation
  – source water/nutrients
  – sampling protocols
  – productivity measurement protocols
  – operator skill/training/experience/consistency
  – other...

Need to harmonize procedures and systems across sites
Mitigation of Site-to-Site Variance (Systems and Processes)

Harmonized Systems via

– Uniform design of indoor seed cultivation (800 ml columns and 2’x2’ flat panels)
– Uniform design of mini-pond system
– Uniform (and automated) water quality monitoring on outdoor production units (YSI)
– Uniform light intensity measurements through adoption of same - LiCor LI190 PAR Quantum Sensor (integrated into YSI units)

Harmonized Processes via

– Rigorous verification and validation of production methodologies for measuring biomass productivity (AFDW, OD, Nutrients) through a round-robin framework parallel to analytical harmonization
– Indoor and outdoor cultivation SOP’s (pond cleaning, inoculation, sampling protocols, nutrient adds, transfers/splits)
– Standardized data reporting

Harmonized experimental framework - Unified Field Studies (UFS)
Mini-pond System Harmonization
Pond Install and Initial Operation
Production Harmonization Progress: Seed and Mini-Pond System Alignment

Next step was to perform a verification run exercising the whole system: Experiment UFSBASELINE1 - seed to ponds, sampling, reset/re-seed of ponds, sensor management and data collection and data management.

GOAL - demonstrate sites can execute on an experimental plan in a coordinated fashion!
Production Harmonization Progress: Initial Baseline Verification of Mini-Ponds

• Purpose of UFS Baseline run: Exercise the whole system
  – Seed production in columns and panels
  – Seed transfer and inoculation of all 6 ponds
  – Calibration and maintenance of sensors
  – Daily grab sample measurements
  – Sample preservation (for analytical and molecular testing (i.e., pond crash forensics)
  – Pond splits/re-seeding
  – Spreadsheet management, data entry and QA/QC
  – Run ponds for an extended period of time
    • target was to get at least 4 continuous weeks of cultivation with one major reset during the experiment
    • “real world” conditions (rain, temp swings, things blowing into ponds, contamination, equipment issues etc., etc., etc.,)
Production Harmonization Progress:
Initial Baseline Verification of Mini-Ponds – Original Schedule

- Two Stage experiment, minimum two week run each stage, early October start.
- 1 factor, 2 level experiments run in triplicate during each stage – goal to reset with culture from one pond as seed source for second stage – indoor seed for reset if crash/other issue
  - First stage: pH (all other factors fixed – strain, starting conc., paddlewheel speed, depth, TN, etc.)
  - Second Stage: TN two level, high/low to allow for one set to shift to lipid after hitting target concentration
Production Harmonization Progress:
Initial Baseline Verification of Mini-Ponds – Productivity

### Average AFDW (g/L) vs. DATETIME

<table>
<thead>
<tr>
<th>SiteID</th>
<th>ASU</th>
<th>Cellana</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Operational Condition
- High N
- Low N
- pH 7.8
- pH 8.5

### Mean (Average Areal Biomass Productivity (g/m2-day)) vs. Operational Condition

- High N
- Low N
- pH 7.8
- pH 8.5

*Nannochloropsis Oceanica (KA32 – Cellana)*
Lipid content between ponds and sites – ash free basis

Data curves courtesy of Lieve Laurens
2014 Seasons: Unified Field Studies

- Season = 13 weeks with a total of 8 weeks dedicated to experimentation

Spring 2014 = March – May

*Nannochloropsis* Batch vs Semi-continuous

Summer 2014 = June – August

*Nannochloropsis* and *Chlorella* Batch vs Semi-continuous

Fall 2014 = September – November

*Nannochloropsis* and *Chlorella* Batch vs Semi-continuous

Winter 2014 = December – February

*Nannochloropsis* and *Chlorella* Batch vs Semi-continuous
Spring 2014 UFS: Batch vs Semi-continuous Cultivation

• Pond experimentation will run for 8 weeks of a 13 week season with one week in between two 4 week runs if needed for instrument calibration and pond re-starts.

• Alternatively, ponds can run for a continuous 8 weeks if they are performing well.

<table>
<thead>
<tr>
<th>2014/2015 Unified Field Study</th>
<th>Spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental Outline</td>
<td>M A M</td>
</tr>
<tr>
<td>Seeds production/pond prep</td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13</td>
</tr>
<tr>
<td>Nannochloropsis Only</td>
<td></td>
</tr>
<tr>
<td>Strain switch</td>
<td></td>
</tr>
<tr>
<td>Chlorella Only</td>
<td></td>
</tr>
<tr>
<td>Data sharing/reports</td>
<td></td>
</tr>
</tbody>
</table>
## Standard Experimental Conditions and Sampling

### Factors Set Point

<table>
<thead>
<tr>
<th>Factors</th>
<th>Set Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous N (mg/L)</td>
<td>105 mg/l</td>
</tr>
<tr>
<td>pH (Nanno)</td>
<td>7.9</td>
</tr>
<tr>
<td>Depth (cm)</td>
<td>25</td>
</tr>
<tr>
<td>PW speed (Hz)</td>
<td>20</td>
</tr>
<tr>
<td>Inoculum (g L⁻¹)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

### Samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD@ 750nm</td>
<td>8 am, M – F</td>
</tr>
<tr>
<td>DW</td>
<td>8 am M, W, F, T0, TF</td>
</tr>
<tr>
<td>AFDW</td>
<td>8 am M, W, F, T0, TF</td>
</tr>
<tr>
<td>Mass balance (lipid/FAME, carbs, starch, protein). Parameter must have an AFDW associated with all samples</td>
<td>T0 (inoculation or directly post-dilution), TF (prior to a harvest or dilution occurring) – samples MUST be taken within 1 hour of AFDW/OD (8:00 am ± 1 hour) or a new AFDW sample is required</td>
</tr>
<tr>
<td>Nutrients</td>
<td>8 am M, W, F, TF</td>
</tr>
<tr>
<td>Weather data</td>
<td>Real time (hourly)</td>
</tr>
<tr>
<td>In-situ sensors</td>
<td>Real time (15 minute intervals)</td>
</tr>
<tr>
<td>Microscopic exam</td>
<td>8 am M, W, F</td>
</tr>
<tr>
<td>Genetic Analysis, qPCR</td>
<td>TF (once per experiment), upon pond health decrease,</td>
</tr>
<tr>
<td>Manual checks (pH, temp, salinity, depth)</td>
<td>Daily; AM and PM</td>
</tr>
<tr>
<td>% Shading</td>
<td>Monthly; AM, Mid, PM</td>
</tr>
<tr>
<td>Water chemistry</td>
<td>Monthly ICPMS testing</td>
</tr>
</tbody>
</table>
Spring 2014 UFS: Batch vs Semi-continuous Cultivation
ATP³ uses a web-based scientific data management system (SDMS) to store & retrieve data about samples – Existence & Location (what & where they are) – Composition (what they look like) – History (how were they produced)

Standardizing how we collect and store these data across all ATP³ sites is absolutely necessary for success – Each site use standardized spreadsheets to collect defined primary data about samples & experiments – Local data champions at each site are responsible for getting data into the system – NREL hosts & maintains the web-based system

The ATP³ SDMS is derived from existing systems that NREL researchers developed with BETO support!!
Using the existing infrastructure and expertise of OpenEI.org will provide a rapid, robust, and low-cost solution for making the ATP³ datasets public.
High Impact Data – Long Term Cultivation Trials:

- All sites operational with identical seed and mini-pond systems
- Analytical and Production Harmonization completed Q4’13
- Phase 2 Experimental Plan Execution underway
  - Spring 2014 Run on track to wrap end of May
  - To be presented in detail at ABBB in Santa Fe in June
- Phase 2 UFS continue with seasonal runs through 2015.
- Advanced Field Studies (AFS) begin late this year and run thru 2015 as well – leverages other assets, including larger cultivation scales/systems available at sites
- Data management system implemented with multi-site data flowing into NRE:L managed SDMS system
- First data release to the public from cultivation trials will occur by mid to late Summer 2014 with quarterly updates thereafter
Acknowledgements
Acknowledgements

ASU
Gary Dirks
John McGowen
Thomas Dempster
Milt Sommerfeld
William Brandt
Jessica Cheng
Jordan McAllister
Sarah Arrowsmith
David Cardello
Theresa Rosov
Mary Cuevas
Jeffrey Prairie
Richard Malloy
Xuezhi Zhang
Henri Gerken
Pierre Wensel
Linda Boedeker
Sarah Mason
Travis Johnson
Sydney Lines

NREL
Phil Pienkos
Lieve Laurens
Ed Wolfrum
David Crocker
Ryan Davis
Stefanie Van Wychen
Eric Knoshaug

Sandia National Labs
Ron Pate
Todd Lane
Patricia Gharagozloo
Thomas Reichardt
Jessica Drewry

Cellana
Valerie Harmon
Egan Rowe
Emily Knurek
Kate Evans
Peter Prentiss
Reyna Javar
Kari Wolff
Keao Bishop-Yuan
Lynn Griswold
Christina Boyko
Charlie O’Kelley

Florida Algae
Steven Schlosser
Chris Withstandley
Mary Riddle
Nancy Pham Ho (FIT)

Touchstone Research Laboratory
Xueyan Liu
Phil Lane
Doug Amie
Brian Gordon

ASU Undergrads
Wyatt Western
Sichoon Park
Priya Pradeep
Terr Snell
Catherine Achukwu
Christine Yi

Cal Poly
Tryg Lundquist
Braden Crowe
Eric Nicolai

Commercial Algae Management
Albert Vitale
Robert Vitale

G.Tech Undergrads
Fariha Hassan
Jerry Duncan
Frazier Woodruff
Shusuke Doi
Hao Fu
Patricia Penalver-Argueso
Allison Dunbar

Gerard Nguyen
Deven Diliberto
Jack Sunderland
Dan Averbuj
Ann Marie Sequeira
Lauren Miller
Michele Hendrickson
Emily Wang

Cal Poly Undergrads
Aydee Melgar
Gulce Ozturk
Kaitlyn Jones
Michael Antoine
Trung K Tran
Jake Bender
Heather Freed
Daniel McBroom
Michele Hendrickson

UTEX
Schonna Manning
Jerry Brand

G.Tech Undergrads
Samantha Lui
Michele Hendrickson

Soroush Aboutalebi