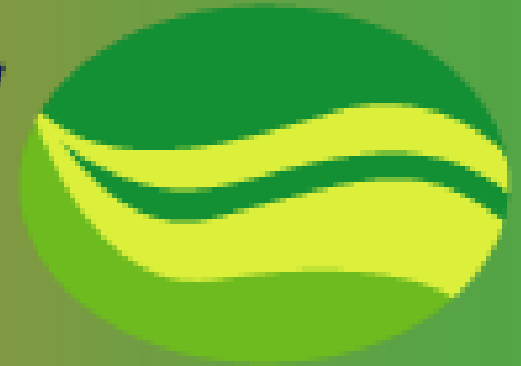# RAFT
Regional Algal Feedstock Testbed

DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

WBS: 1.3.5.111

March 7, 2019

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ARIZONA   NM STATE UNIVERSITY   Pacific Northwest NATIONAL LABORATORY   TEXAS A&M AGRILIFE RESEARCH

Goal Statement

- **Create long-term cultivation data necessary to understand and promote algae biomass production**.
  - Support the development of innovative technologies to capture and recycle water and nutrients – impaired water
- M.8.1 Algal feedstock production
  - M.8.1.1 Development of technically viable, sustainable and cost effective algae production
- Support the Biomass Program's goals to model pathways for significant (>1 billion gallons per year) volumes of cost-competitive algal biofuels by 2022.
  - Energy Independence and Security Act of 2007 (EISA)
  - Energy Policy Act of 2005 (EPAct 2005)

Quad Chart Overview

Timeline

Project start date:10/1/2013
Project end date: 9/30/2018
Percent complete: 100%

Barriers

Barriers addressed

- Ft-A Feedstock Availability and Cost:
- Ft-B Sustainable Production

Budget

	Total Costs Pre FY17	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	$ 4,129,374	$ 1,350,761	$ 319,865	$0
DOE Funded FFRC (PNNL)	$ 1,434,453	$ 365,968	$ 287,747	$0
Project Cost Share (Comp.)*	$ 262,645	$ 140,846	$ 47,495	$0

Partners

- New Mexico State University (17.5%)
- Pacific Northwest Laboratory (27.5%)
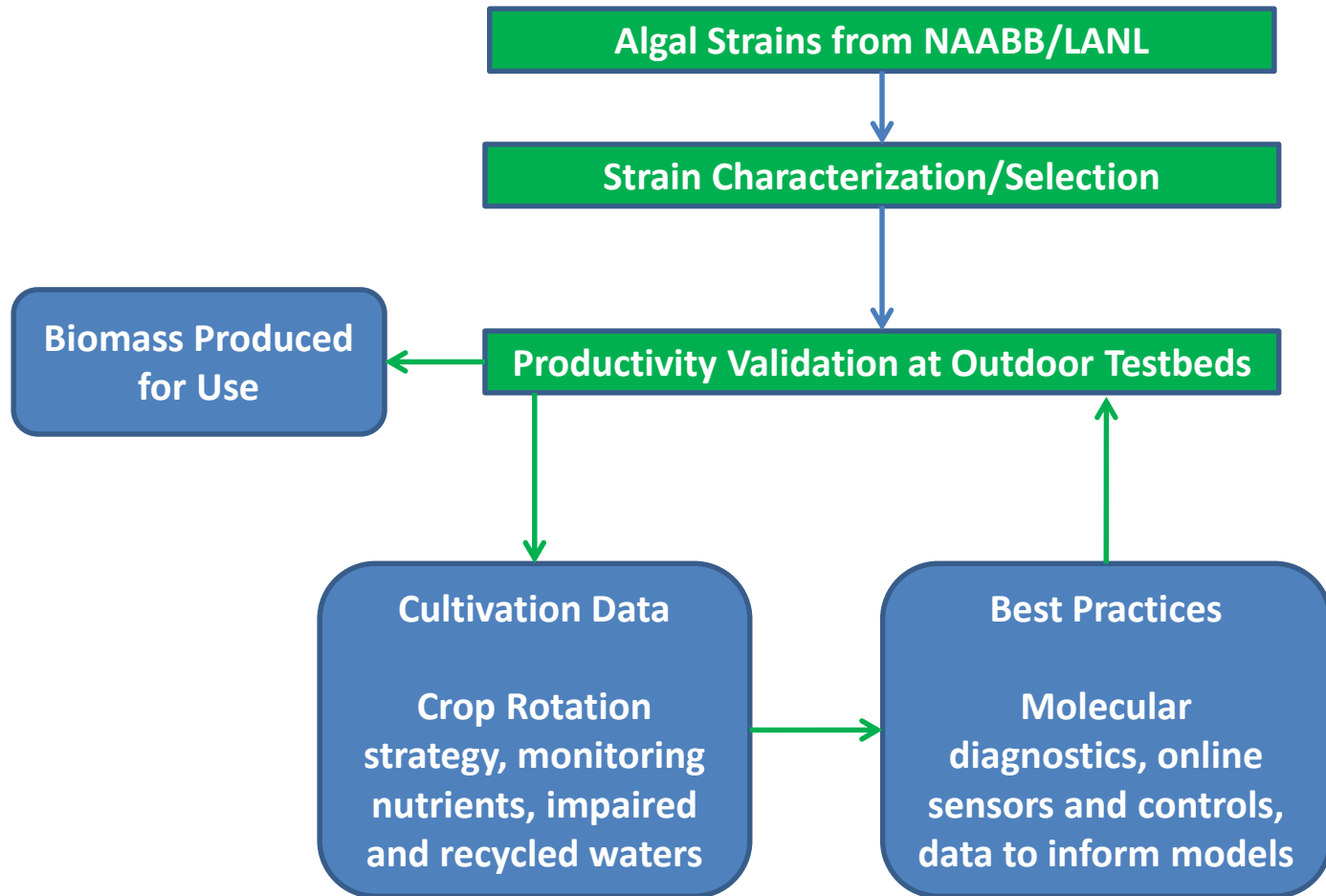- Texas A&M Agrilife Research (20%)

1 - Project Overview

- Long term algal cultivation data in outdoor pond testbeds.
  - Develop Best Management Practices
    - Real-time culture health and productivity monitoring
    - Molecular diagnostics
    - "Open-access" data management

  - Improve and refine cultivation and operational models
    - Pond and model feedback-enhanced systems
    - Biomass Assessment Tool (BAT) model

  - Increase sustainability of algae biomass production
    - Optimize biomass productivity using impaired waters
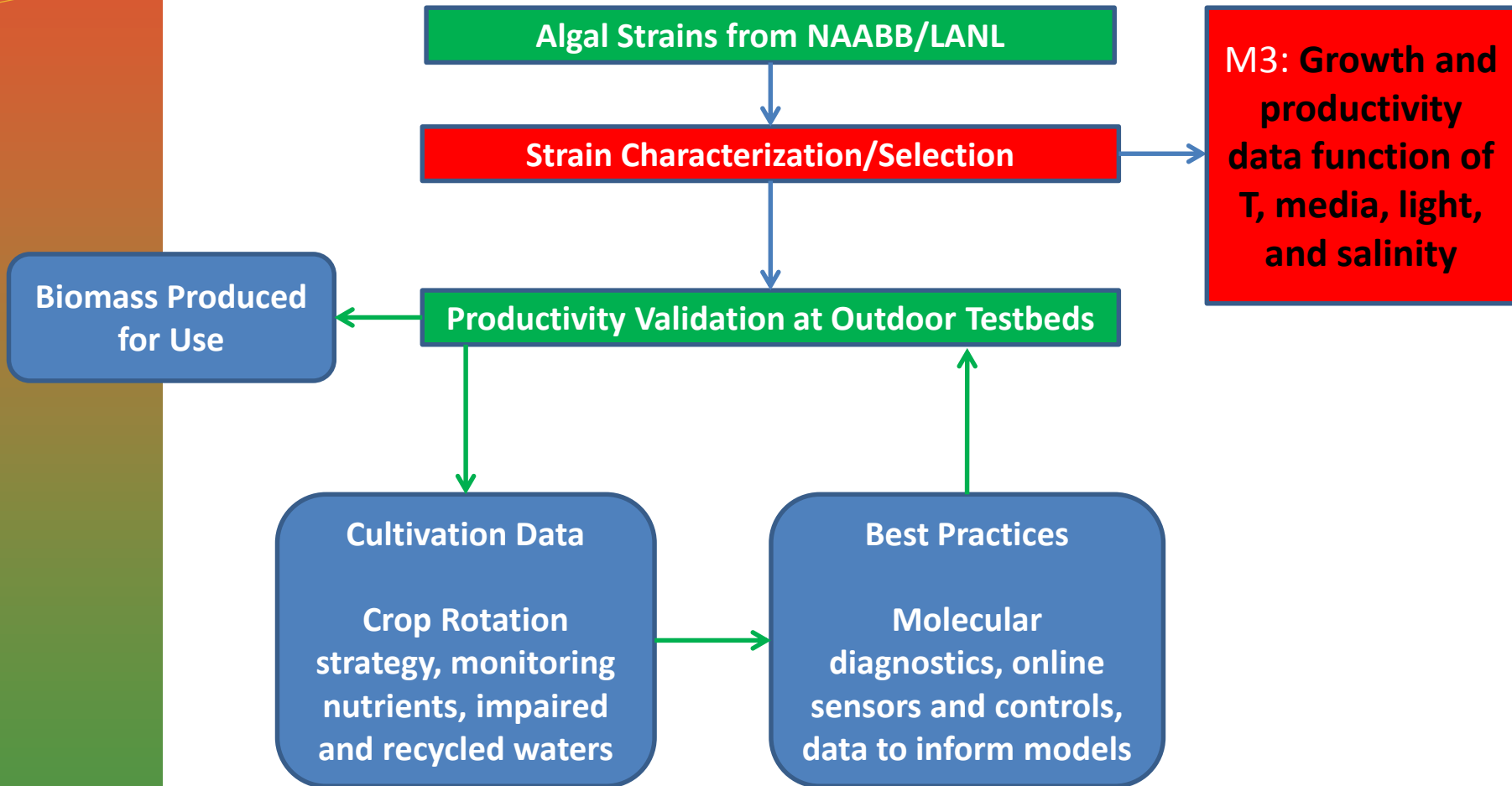   - Strain selection and crop rotation for year-round cultivation

2 – Approach (Management)

- Critical Success Factors
  - **DL1 & DL2 Data Management; QA/AC ; R&D Plan – 6   - complete**
  - **M1 Management Information System in Place – 12 - complete**
  - **M2 Long term cultivation data for 2 strains over 2 seasons – 22**
  - **DL3 : Long term R&D Report and Phase 3 Plan Go/No Go**
  - **M3 Growth and productivity data for 3 strains function of T, media, light, salinity – 30**
  - **M4 Growth and cultivation data in 2 impaired waters and/or nutrient recycle systems – 42**
  - **M5 Long term cultivation data 2 strains in 3 different regions – 46**
  - **Final Report**
- Challenges
  - Contamination/Culture Stability – included part in final report
  - Timeliness of putting together final report
- Management Approach
  - Biweekly teleconferences & Quarterly reports
  - Biannual meetings & Annual site visits by PI and PM to Sites

2 – Approach (Technical)

```
Algal Strains from NAABB/LANL
            │
            ▼
Strain Characterization/Selection
            │
            ▼
```

Biomass Produced for Use ◄── Productivity Validation at Outdoor Testbeds
                                              │
                                              ▼

Cultivation Data

Crop Rotation strategy, monitoring nutrients, impaired and recycled waters ──► **Best Practices**

Molecular diagnostics, online sensors and controls, data to inform models

3 – Technical Accomplishments

Algal Strains from NAABB/LANL

Strain Characterization/Selection

M3: **Growth and productivity data function of T, media, light, and salinity**

Productivity Validation at Outdoor Testbeds

Biomass Produced for Use

Cultivation Data

Crop Rotation strategy, monitoring nutrients, impaired and recycled waters

Best Practices

Molecular diagnostics, online sensors and controls, data to inform models

Strains

Chlorella sorokiniana DOE 1412

- NAABB strain isolated by Dr. Juergen Polle's group (CUNY)
- Single spherical cells that typically divide into four daughter cells
- **Cold sensitive**
  - Will not grow below 14 °C
- **Heat tolerant** to 42 °C
  - Optimum range is 27-42 °C
- **Brackish tolerant** <20ppt
- **Growth in Secondary Waste Water**
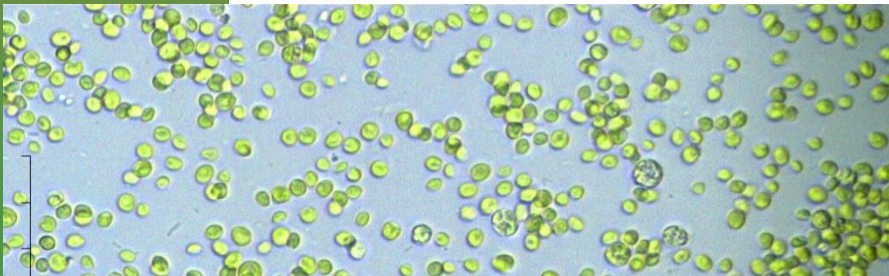
Monoraphidium minutum

- Dominated *Chlorella* culture during winter cultivation at Texas A&M Highly variable cell size and morphology
- Impressive cold growth in cold
  - Optimum range is 17-27 °C
- **Heat sensitive**
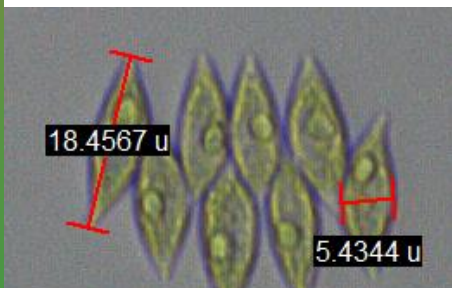- **Brackish tolerant** <20ppt
- **Growth in Secondary Waste Water**
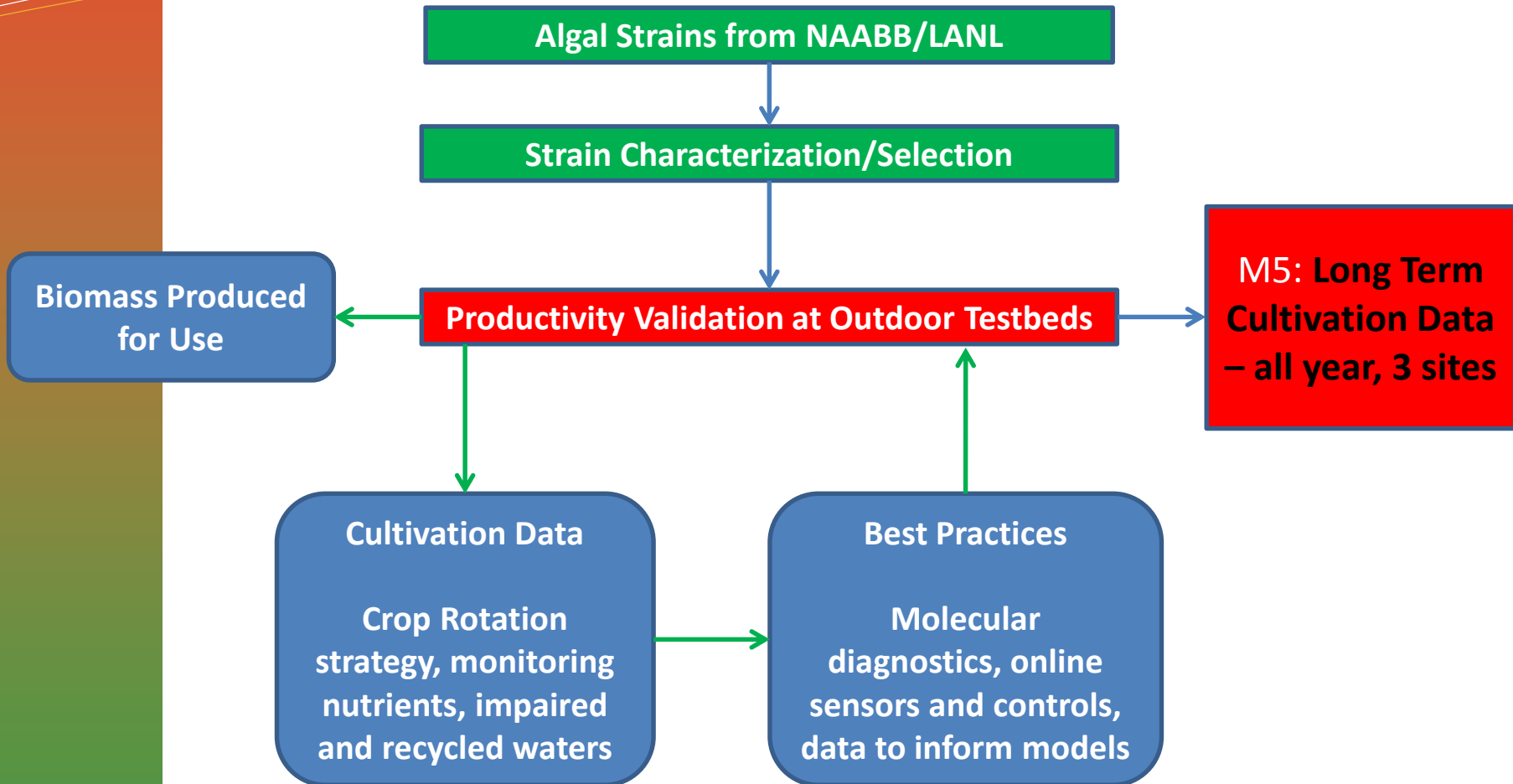
Scenedesmus obiquus and others

- NAABB strain isolated by Dr. Juergen Polle's group (CUNY)
- Large four to eight-celled colonies, occasionally single cells
- Winter hardy
- **Heat tolerant** to 38 °C
  - Growth rates <1 day^{-1} at 10 °C
  - Growth rates >3 day^{-1} at >22 °C
  - Optimum range is 22-38 °C
- **Salinity sensitive** <5ppt
- Settles rapidly

12 Total Strains

- Source
- Optimum Temperature
- Min/Max Temp Tolerance
- Season
- Field Trials
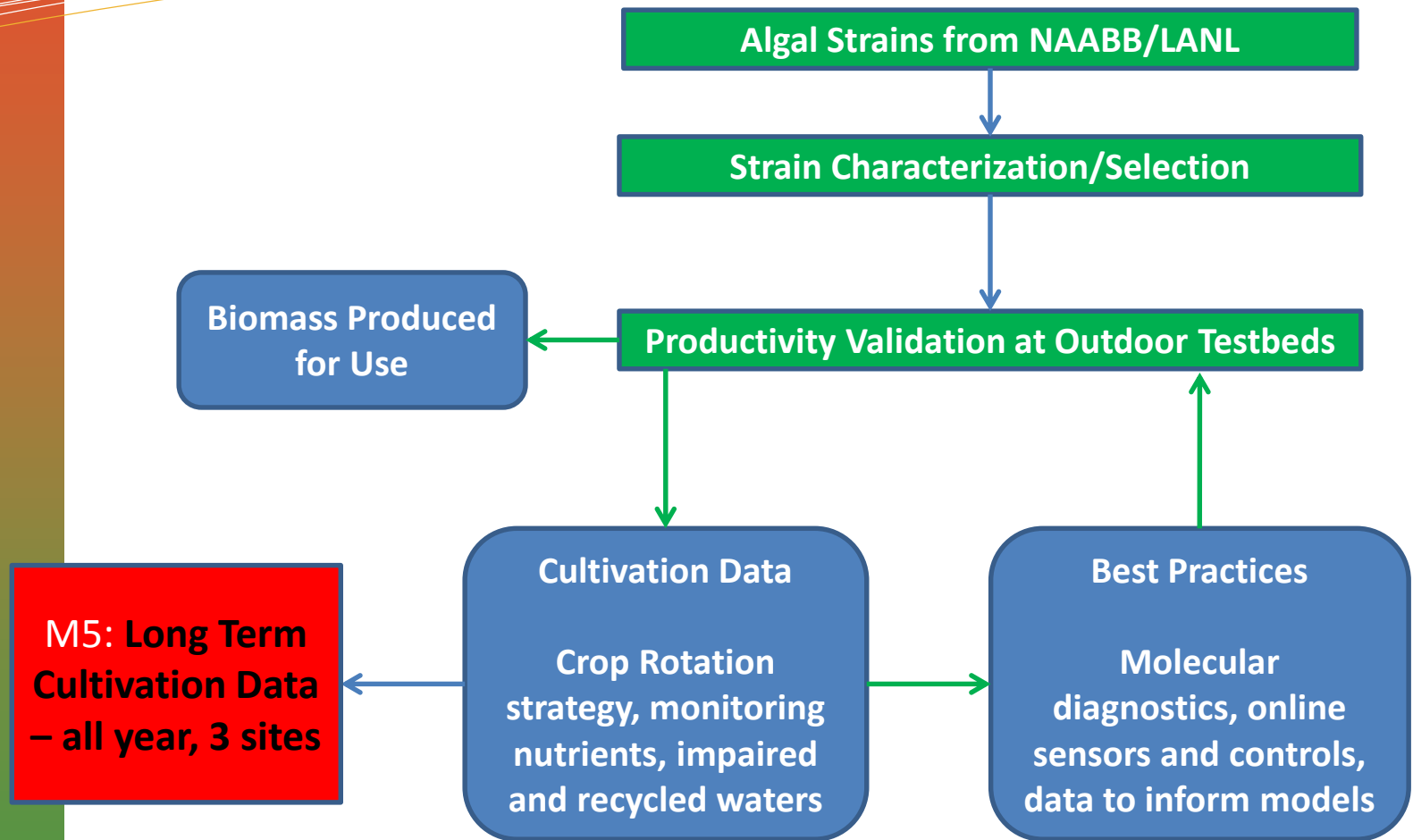- Respiratory biomass loss
- Salinity tolerance

```
                    ┌─────────────────────────────────┐
                    │   Algal Strains from NAABB/LANL  │
                    └─────────────────────────────────┘
                                     │
                                     ▼
                    ┌─────────────────────────────────┐
                    │  Strain Characterization/Selection │
                    └─────────────────────────────────┘
                                     │
                                     ▼
┌──────────────┐    ┌─────────────────────────────────┐    ┌──────────────────┐
│   Biomass    │◄───│  Productivity Validation at      │───►│  M5: Long Term   │
│ Produced     │    │  Outdoor Testbeds                │    │  Cultivation Data│
│ for Use      │    └─────────────────────────────────┘    │  – all year,     │
└──────────────┘              │              ▲             │  3 sites         │
                              ▼              │             └──────────────────┘
                    ┌──────────────┐    ┌──────────────┐
                    │ Cultivation  │───►│ Best         │
                    │ Data         │    │ Practices    │
                    └──────────────┘    └──────────────┘
```

Biomass Produced for Use

Algal Strains from NAABB/LANL

Strain Characterization/Selection

Productivity Validation at Outdoor Testbeds

M5: Long Term Cultivation Data – all year, 3 sites

Cultivation Data

Crop Rotation strategy, monitoring nutrients, impaired and recycled waters

Best Practices

Molecular diagnostics, online sensors and controls, data to inform models

Productivity

Site	Strain	# of Runs	Longest (days)	Range of linear productivity (g/m²/day)
Tucson, AZ	*C. sorokiniana*	61	66	5-23
	M. minutum	8	74	2-13
	S. obliquus	29	106	2-24
Pecos, TX	*C. sorokiniana*	20	34	2-20
	M. minutum	28	65	4-13
	S. obliquus	25	71	2-14
Las Cruces, NM	*C. sorokiniana*	43	41	3-16
	M. minutum	19	58	1-10
	S. obliquus	15	58	4-28

3 – Technical Accomplishments

Algal Strains from NAABB/LANL

Strain Characterization/Selection

Productivity Validation at Outdoor Testbeds

Biomass Produced for Use

Cultivation Data

Crop Rotation strategy, monitoring nutrients, impaired and recycled waters

Best Practices

Molecular diagnostics, online sensors and controls, data to inform models

M5: **Long Term Cultivation Data – all year, 3 sites**

Cultivation Data

https://raft.Arizona.edu/cultivation-data

Sortable table

- Experiment label
- Strain
- Reactor
- Location
- Depth
- Max Productivity
- Max Concentration

Other Information

- Start Date
- Duration (days)
- Average Productivity
- Harvest Strategy
- Objective
- Weather station
- Comments

Continuous Data

Continuous data. *Monoraphidium Minutum*

PW1 and PW2 (6 inch culture depth)
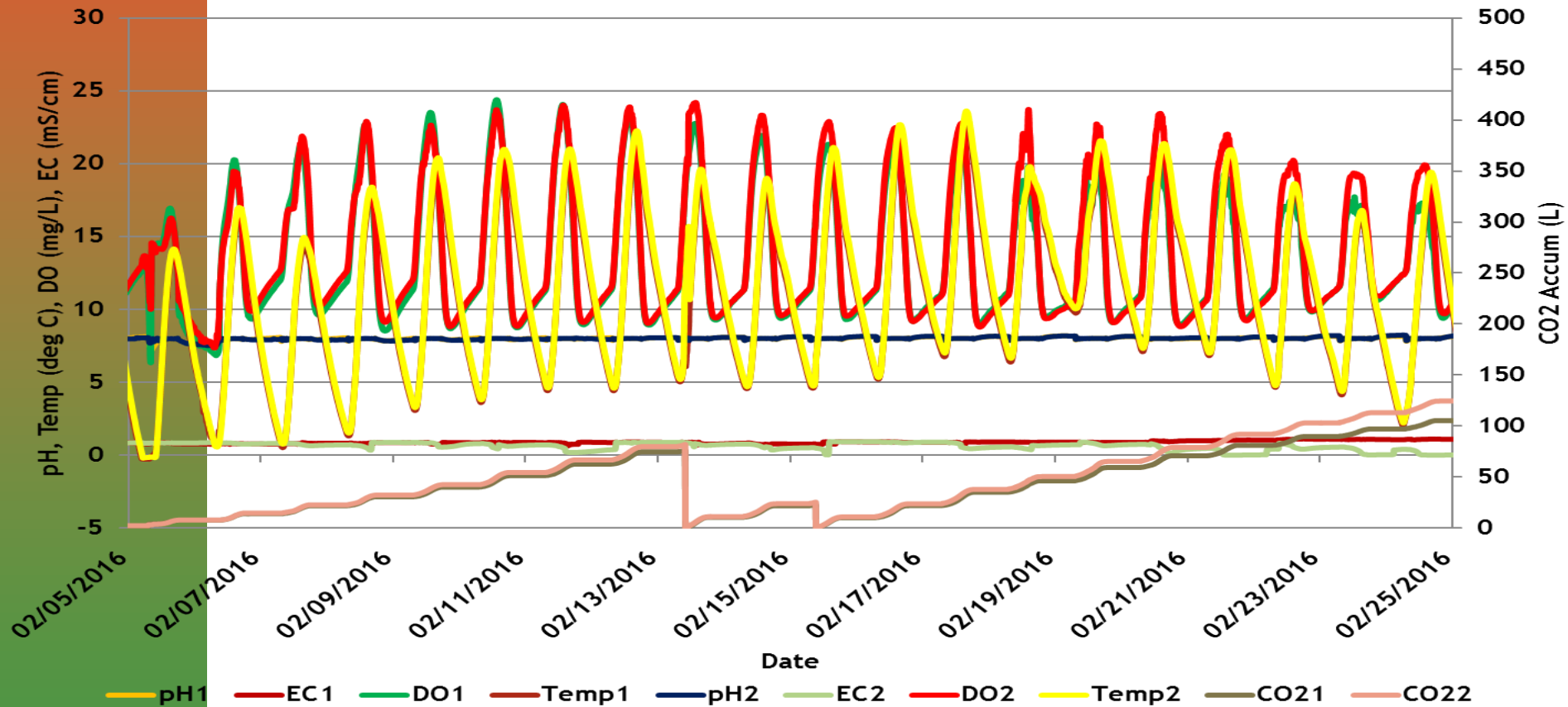
Cultivation Data

- Crop Protection
- Native Polyculture – NMSU
- *Galdieria sulphuraria – NMSU*
- Municipal Wastewater - NMSU
- Saline well water – TAMU
- Nutrient monitoring (N, CO_2) – UA
- Continuous cell mass monitoring – UA
- Flue gas - UA
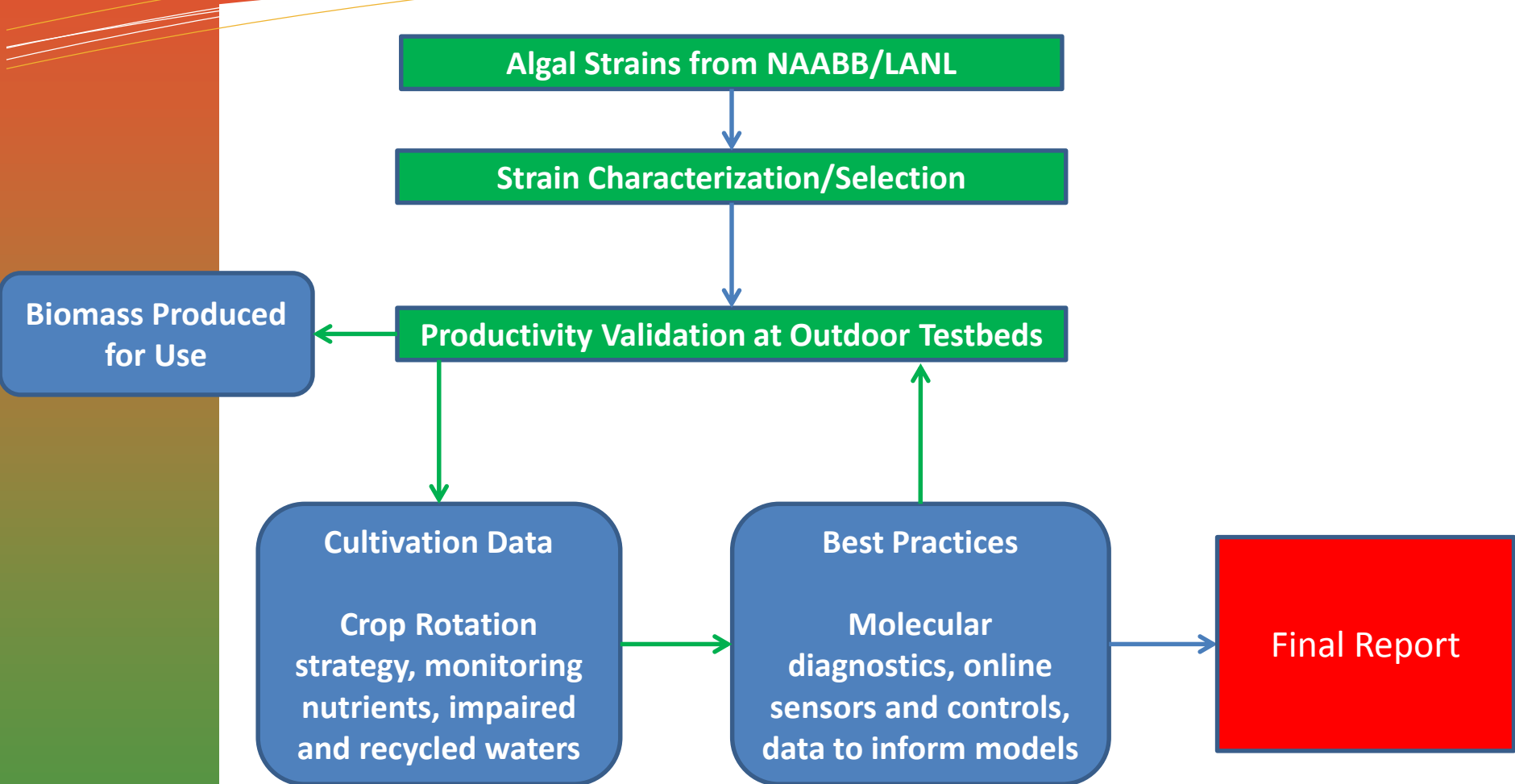
Best Practices

- Characterization
  - Laboratory analysis
  - Link to growth and production models
- Strain maintenance
- Scale up – microscopic and molecular monitoring
- Outdoor Cultivation
  - Discrete and continuous monitoring
  - Robust data sheets
  - Secondary (N, P, cell composition)
  - 75% harvest end of linear growth
- Control Strategies
- Molecular diagnostics – culture health

On-line Sensors and Control

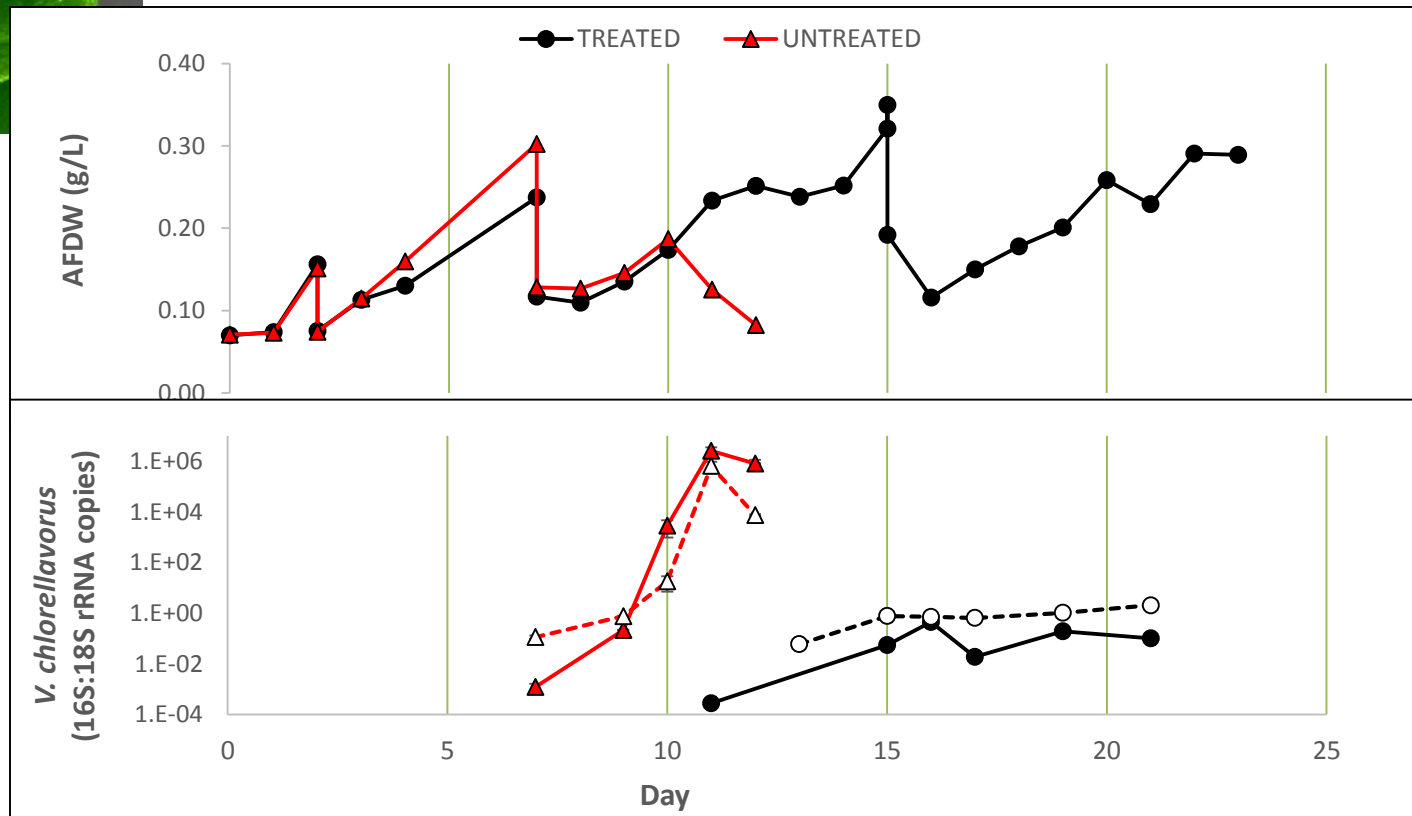Optical density measurement compartment

Sampling pump

Sensor controller electronics compartment
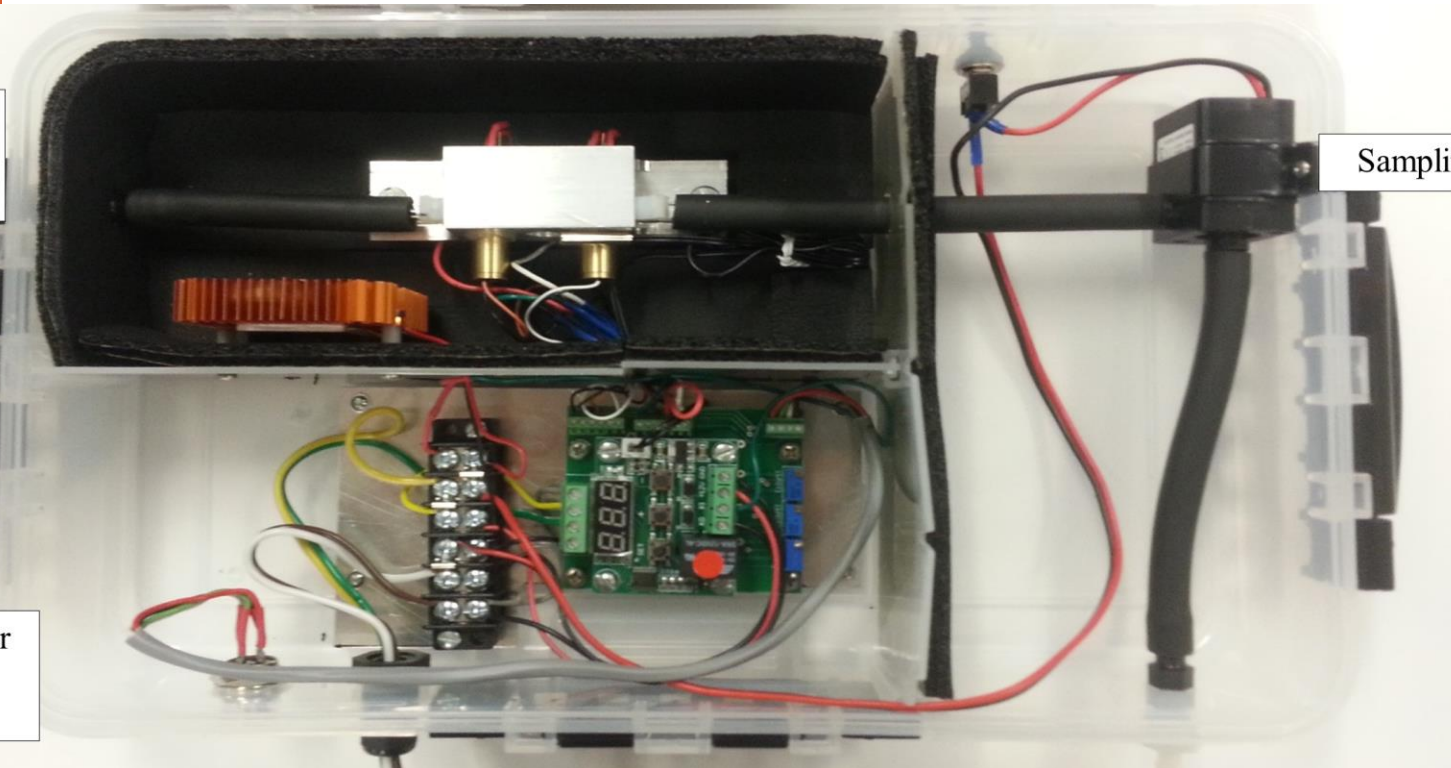
Continuous Growth Data
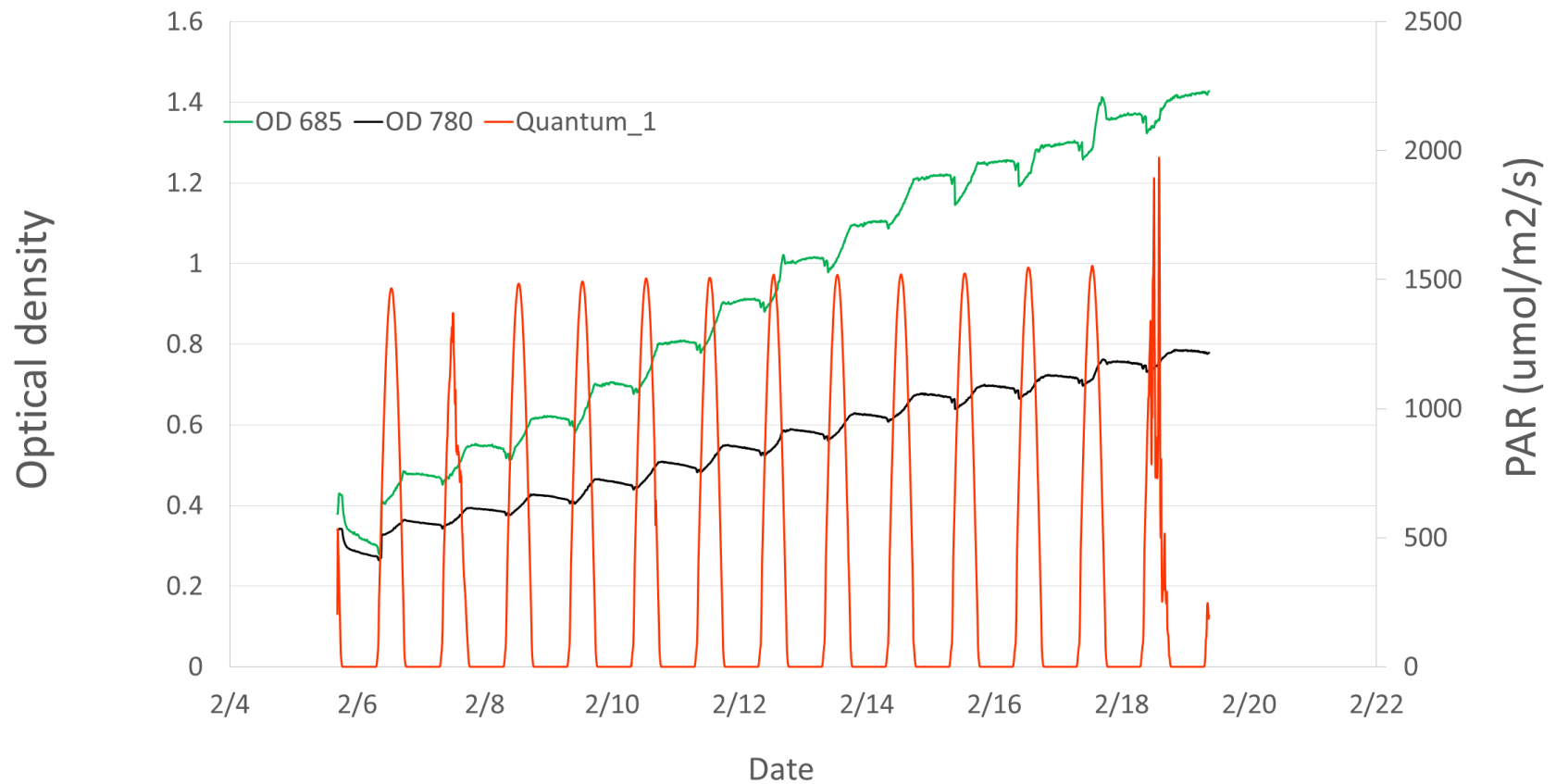
Molecular Diagnostics
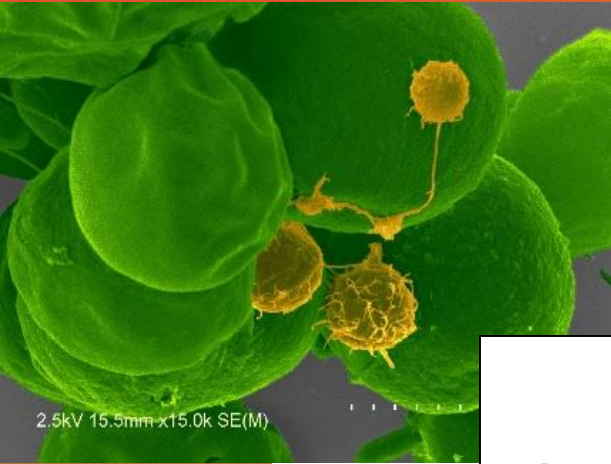
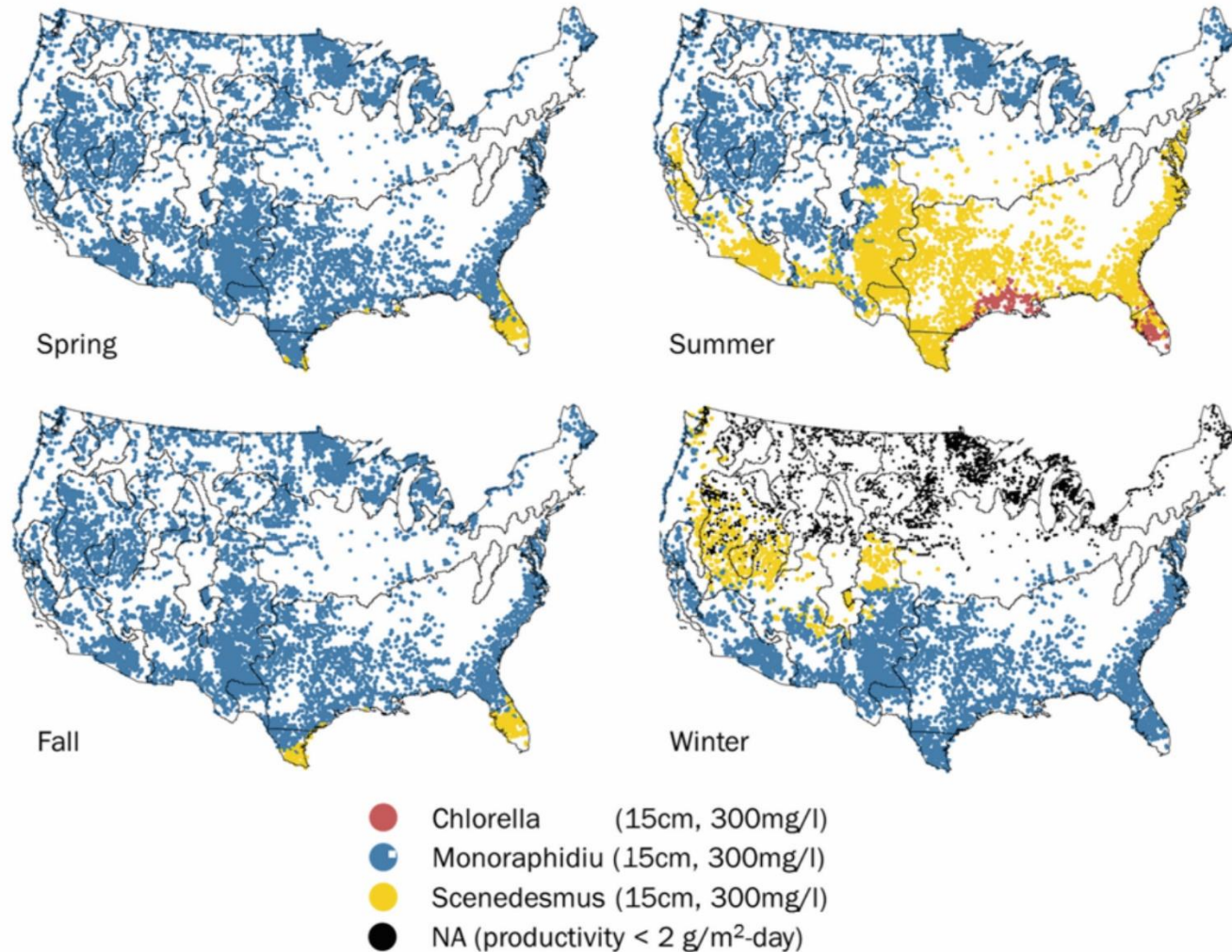**Benzalkonium Chloride (Biocide) Treatment 2 ppm**

Models

- Biomass growth model
  - LEAPS reactors
  - Respiration
- Biomass Assessment Tool (BAT)
  - Strain rotation
  - Culture depth
  - Harvest Strategy
  - Strain
- Publication list (Huesemann, Waller, Skaggs)

Model
(Seasonal Optimal Cultivation Strategy)

Spring

Summer

Fall

Winter

🔴	Chlorella	(15cm, 300mg/l)
🔵	Monoraphidiu (15cm, 300mg/l)	
🟡	Scenedesmus (15cm, 300mg/l)	
⚫	NA (productivity < 2 g/m²-day)	

4 – Relevance

- Created a strategy to obtain the cultivation data necessary to understand and promote algae biomass production
- Public data storage
  - 272 experiments
- Publications (34), Presentations (37), Patents (3)
- Personnel
  - 14 Principal investigators
  - 27 Technical Staff
  - 3 Post doctoral fellows
  - 12 graduate students
  - 13 undergraduate students
  - 2 high school interns

4 – Relevance

- Lessons Learned
  - Strain Selection
  - Molecular Diagnostics
  - Cultivation and Modeling
- Pond Crashes
  - Table in report
  - UA Experience – ciliates, microburst, vampire, etc.

- Finishing up publications
  - 3 accepted with revisions
  - 1 PhD dissertation
- Web site work containing more explanation
  - Data
  - Strains
  - Reactors

Summary

1. Completed all milestones and deliverables
2. Finished final report
3. **https://raft.Arizona.edu/cultivation-data**

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Additional Slides

Publications, Patents, Presentations, Awards, and Commercialization – Full List in final report

- **Papers**
  - Huesemann, M.H., M. Wigmosta, N. Sun, Eric Myers, Braden Crowe, R. Skaggs, and P. Waller, "Successful validation of the PNNL Microalgae Biomass Growth Model for *Chlorella sorokiniana* (DOE 1412) using outdoor pond culture data from Arizona, Florida, Texas, and California," *Algal Research*, in preparation.
  - Edmundson SJ and M Huesemann, Characterization of algal strains with cold season production potential. *Journal of Applied Phycology*, in preparation.
  - Huesemann, M.H., P. Williams, S. Edmundson, P. Chen, R. Kruk, V. Cullinan, B. Crowe, and T. Lundquist, "Laboratory Environmental Algae Pond Simulator (LEAPS) Photobioreactors: Validation Using Outdoor Pond Cultures of *Chlorella sorokiniana* and *Nannochloropsis salina*", *Algal Research,* submitted.
  - Huesemann M, B Crowe, P Waller, A Chavis, S Hobbs, S Edmundson, and M Wigmosta (2016) A validated model to predict microalgae growth in outdoor pond cultures subjected to fluctuating light intensities and water temperatures. *Algal Research* 13, 195-206. http://dx.doi.org/10.1016/j.algal.2015.11.008
  - Huesemann, M.H., M. Wigmosta, B. Crowe, P. Waller, A. Chavis, S. Hobbs, S. Edmundson, B. Chubukov, V.J. Tocco, and A. Coleman, "Estimating the maximum achievable productivity in outdoor ponds: Microalgae biomass growth modeling and climate-simulated culturing", In: *Micro-Algal Production for Biomass and High-Value Products*, Dr. Stephen P. Slocombe and Dr. John R. Benemann (Eds.), CRC Press, Taylor and Francis, LLC, 2016.
  - Edmundson SJ and M Huesemann (2015). The dark side of algae cultivation: characterizing night biomass loss in three species of photosynthetic algae, *Chlorella sorokiniana*, *Nannochloropsis salina*, and *Picochlorum* sp. *Algal Research* 12, 470-476. doi:10.1016/j.algal.2015.10.012
  - Attalah, S., P. Waller, G. Khawam, R. Ryan. 2015. Energy productivity of High Velocity Algae Raceway Integrated Design (ARID-HV). Transactions of the ASABE. In press.
  - Jia, F., Kacira, M., Ogden, K. L., Multi-Wavelength Based Optical Density Sensor for Autonomous Monitoring of Microalgae. Sensors 15(9): 22234-22248 (2015). doi:10.3390/s150922234
  - Khawam, G., P. Waller, S. Attallah, R. Ryan. 2014. ARID raceway temperature model evaluation. Transactions of the ASABE. 57(1): 333-340 DOI 10.13031/trans.57.10198

Publications, Patents, Presentations, Awards, and Commercialization

- **Papers**
  - Selvaratnam, T. Pegallapari, A, Montelya, F., Rodriquez, G, Nirmalakhandan, N, Lammers, PJ, Van Voorhies, W. 2014. Feasibility of algal systems for sustainable wastewater treatment. Renewable Energy
  - Xu, B., P. Li, and P. Waller. 2014. Study of the flow mixing in a novel open-channel raceway for algae production. Renewable Energy. 62:249-57. doi:10.1016/j.renene.2013.06.049
  - Angelova, A., Park, S.H., Kyndt, J., Fitzsimmons, K., and Brown, J.K. 2014. Sonication based isolation and enrichment of *Chlorella protothecoides* chloroplasts for Illumina genome sequencing. J. Appl. Phycol. 26: 209-218. DOI: 10.1007/s10811-013-0125-1.
  - Jia, F., M. Kacira, L. An, C. C. Brown, K. L. Ogden, and Brown, J.K. 2017. Autonomous detection of abiotic and biotic disturbance in a microalgal culture system using a multi-wavelength optical density sensor. J. appl. Phycol. (in revision).
  - Park, S.H., Steichen, S., Ogden, K., and Brown, J.K. Molecular detection of microalgal species using single nucleotide mismatch design. J. Phycol.
  - Park, S.H., Steichen, S.A., Li, X., Ogden, K., and Brown, J.K., 2016. Association of *Vampirovibrio chlorellavorus* with rapid decline of *Chlorella sorokoriana* in an outdoor cultivation system. J. Appl. Phycol.
  - Steichen, S., and Brown, J.K. 2017. Real-time, quantitative detection of *Vampirovibrio chlorellavorus*, a bacterial pathogen of *Chlorella* biofuel species. J. Phycol.
  - Li, M., Shadman, F., Ogden, K. (2016). Removal of Gallium from Wastewater by Adsorption on Algae-Based Biosorbents (Under review). Chemosphere.
  - Ogden, K. L. "Algae as a Bio-Feedstock" Chemical Engineering Progress November: 63-66 (2014)
  - Lynn Wendt, Chenlin Li, Georgiy Kachurin, Bradley Wahlen, Kimberly Ogden, J Austin Murphy Evaluation of a high-moisture stabilization strategy for harvested microalgae blended with herbaceous biomass: Part I – storage performance. Algal Research (Under review).
  - *Song Gao, Peter Waller, Kimberly Ogden, Renhe Qiu (2017) Scenedesmus obliquus*, nutrient demand, nutrient storage and growth limitation. Bioresource Technology (under review)

- **Patent Application**
  - Jia, F., M. Kacira, K. Ogden, G. Ogden. 2015. Optical device for in-line and real-time monitoring of microalgae. University of Arizona.
- **Thesis**
  - Steichen, S. 2016. Tracking an algal predator: monitoring the dynamics of *Vampirovibrio chlorellavorus* in an outdoor culture. M.S. Thesis. University of Arizona, Tucson, AZ.
  - Li, M. (2019).Removal of Heavy Metals from semiconductor wastewater (Unpublished doctoral dissertation). University of Arizona, Tucson, Arizona.
  - Acedo, Margarita."Microalgae cultivation using holographic optical elements reactors and flue gas as a CO2 carbon source". PhD dissertation., University of Arizona, Tucson, May 2018.
  - Renhe Qiu. May 2017, Effects of Chemical Environmental Conditions on Cell Growth and Lipid Production of Microalgae Chlorella Sorokiniana, PhD Thesis, University of Arizona.
  - Khawam, George. Effects of shading on evaporation and algae growth in experimental open pond raceways. PhD Thesis, Univeristy of Arizona, 2017.
  - Jude Udeozor, "Effect of Ga(III) and As(V) on the growth rate, fatty acid and lipid content of Chlorella and Scenedesmus." MS Thesis, University of Arizona, May 2017.
  - Song Gao, Modeling shading, salinity and nutrients in an algae growth model. PhD Thesis, University of Arizona, May 2017.

- **Tours/Presentations to companies**
  - Evonik 10/13
  - IHI NeoG Algae 9/14
  - Veolia 10/14
  - Hala Ghandour is the Deputy Managing Director of Expo 2020 Dubai 11/14
  - Dr. Juan Manuel Verdugo Rosas - Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food - a unit from the Federal Executive Branch of the Government of Mexico). 1/15
  - Dr. Petya Stoykova from Bulgaria's Agrobioinstitute. A USDA Borlaug Fellow in the Fall 2014.
  - Private industry group working in algae for biofuels (NDA in place with TAMU) – multiple meetings – 12/13-1/14
  - Global Algae Innovations (November/December 2014)
  - Dr. Haubruge and Laurence Leblanc of IDELUX, the economic development arm of the Luxembourg region of Belgium, and Dr. Eric Haubruge, Vice Recteur in charge of International Relations of Liege University as well as Aurore Richel; plus Yves Dubus from Belgian Trade Commission – February 2015

Publications, Patents, Presentations, Awards, and Commercialization

- **Presentations**

  - Huesemann M, SJ Edmundson, M Wigmosta, and L Brown (2015) Increasing annual microalgae biomass productivity through crop rotation: Characterization and modeling of winter and summer strains. Algal Biomass Summit. Washington, D.C.

  - Edmundson, S.J, Huesemann, M. H.The Dark Side of Algae Cultivation: a comparison of biomass loss at night in three photosynthetic algae, *Chlorella sorokiniana*, *Nannochloropsis salina* and *Picochlorum* sp." 2014 ABO conference in San Diego.

  - Park, S-H., Kyndt, J., Acedo, M., Best, O., and Brown, J.K. 2014. RNA-Seq transcript expression profiling of low phosphate adaptation of *Auxenochlorella protothecoides* and for efficient biofuel production. 4th International Conference on Algal Biomass, Biofuels, and Bioproducts, Santa Fe Convention Center, Santa Fe, NM. June 15-18, 2014.

  - Y. Mehdipour, S. Gao, P. Waller, K. Ogden (2016) Biomass concentration, settling rate and chemical flocculants for Scenedesmus obliquus and Chlorella sorokiniana The 6th International Conference on Algal Biomass, Biofuels & BioproductsSan Diego, USA

  - K.L. Ogden (Keynote Speaker), J. Brown, P. Waller, M. Kacira, S. Attalah, F. Jia (2016) Crop rotation and monitoring strategy for year round production of microalgae The 6th International Conference on Algal Biomass, Biofuels & Bioproducts, San Diego, USA

  - Gao, P. Waller, K. Ogden, S. Attalah, M. Husesmann (2016) Addition of salinity and shading to an algae growth model and evaluation in the ARID raceway The 6th International Conference on Algal Biomass, Biofuels & Bioproducts, San Diego, USA

  - George Khawam, Peter Waller, Kimberly Ogden, Said Attalah, Song Gao.   Shading in algae biofuel experimental paddlewheel bioreactors. Poster award. AZSEC. September 15, Flagstaff. Arizona.

  - Song Gao, Renhe Qiu, Peter Waller, Kimberly Ogden, A study on improving nitrogen and phosphorus inputs for growing Scenesdesmus oliquus 7th International Conference on Algal Biomass, Biofuels, and Bioproducts. Miami, Florida.

  - Lammers, P, Seger, M, Holguin, O, Csakan, N, Chavez, G, Nui, R, Boeing, W, Schaub, T. Quality control systems reveal the yin and yang of outdoor algae cultivation. Algal Biomass, Biofuels and Bioproducts meeting in Sante Fe on June 15-18th 2014

Publications, Patents, Presentations, Awards, and Commercialization

- **Presentations**
  - Ogden, K., Lacombe, L. and Nakasko, P. Production of food, energy, and fuel from microalgae in holographic diffractive optic-solar glass reactors. AIChE Meeting, San Francisco, CA 11/16.
  - Ogden, K. (Invited), Brown, J., Waller, P., Attalah, S., Kacira, M., Fei, J., Gaos, S., Steichen, S., Qiu, R. Algal Cultivation Strategies for Advanced Biomass Yield. 5th Pan American Conference on Plants and Bioenergy, Santa Fe, NM 8/16.
  - Ogden, K. Li, X. Effect of temperature and salt on laboratory growth and pathogenicity of Vampirovibrio chlorellavorus in a cultivated Chlorella sp. host. AIChE Meeting, Salt Lake City, Utah, 11/15.
  - Ogden, K. RAFT Overview, Harmonization Meeting, Golden, CO 10/15.
  - Ogden, K. A perspective on how microalgae can address the water, energy, food nexus. ICOSSE 2015, Hungary, 6/15.
  - Ogden, K. Benefits and challenges of the algal biofuels industry. Arizona Board of Regents, 3/14.
  - Lammers, P. Algal-based system for energy-positive wastewater treatment: Algae in Hot Water. AOAIS Conference, Korea 2014
  - Ogden, K. L. Status and challenges to making biofuels from algal biomass cost competitive. International Algal Conference, Taiwan 10/13.
  - Ogden, K. L. Ryan, R, Waller, P. A novel patented open cultivation system for microalgae using recycled water. AIChE Annual Meeting 11/13.
  - Ogden, K. L. What is the Future of Making Biofuel from Algae? UA Women's Group. Tucson, AZ 3/14.
  - Ogden, K. L. Benefits and challenges of the algal biofuels industry. US/China Conference on Sustainable Manufacturing Wuhan China 3/14.
  - Ogden, K. L. RAFT Project. Biomass 2014, Washington DC 8/14.
  - Ogden, K. L. Status and Update of RAFT Project. BIO PacRim conference. San Diego CA 12/14.
  - Ogden, K.L., Barnhart, A, Snyder S, Nuclear Renewable Energy. Arizona Governor's Energy Board 1/15.
  - Qiu, R. Ogden, K. L. Using wastewater for algal cultivation. AIChE Annual Meeting, Atlanta, GA 11/14.
  - Van Voorhies, W. and Lammers, P. Characterization of an extremophile algae, *Galdieria sulphuraria*, for biomass production. Algal Biomass, Biofuels and Bioproducts meeting in Sante Fe on June 15-18th 2014

Publications, Patents, Presentations, Awards, and Commercialization

- **Presentations**
  - Acedo, Margarita; Qui, Renhe; Vazquez, Manuel; Lopez, Paola; Ogden, Kimberly. April, 2015. "Overview of the regional algae feedstock testbed (RAFT) project. Poster presentation at Arizona Student Energy Conference (AZSEC), University of Arizona, Tucson, AZ.
  - Renhe Qiu, Maxime Fournier. Solar Fuels 101, Third Arizona Student Energy Conference (AzSEC). Tempe, AZ. April 2014.
  - Renhe Qiu. Centrate as a Water Source for Algae Culture, Arizona Board of Regents (ABOR) Meeting on "Using Wastewater for Mass Culture of Algae for Food, Feed and Fuel". Mesa, AZ. May 2014.

- **Supply Biomass**
  - Testbeds have supplied biomass to the PNNL HTL project
  - UA Testbed supplied biomass multiple times to INEL for preservation study
  - Algae to Utah State and UNLV for PhD Research