



DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

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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
- By 2017, model the sustainable supply of 1 million metric tons ash free dry weight (AFDW) cultivated algal biomass.
- 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: 9/30/2017 Percent complete: 80% Barriers addressed – Ft-A Feedstock Availability and Cost:

Barriers

- Ft-B Sustainable Production

	Total Costs FY 12 –FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17-Project End Date)
DOE Funded	\$2,723,193	\$1,533,180	\$1,403,328	\$1,045,432
DOE Funded FFRC (PNNL)	\$556,752	\$371,426	\$506,275	\$765,547
Project Cost Share (Comp.)*	\$176,807	\$144,746	\$43,374	\$115,064

Budget

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.
- Alternative Non-Potable Water Sources and Nutrient Evaluations (N,P, CO₂)
 - Brackish and recycled water
 - Wastewater
- **3**. Population Dynamics and Control Strategies
 - Control of invasive species
 - Mixed cultures
 - Neural network control system
- 4. Biomass Production and Characterization
 - Improving data mining tools for in-depth analysis of environmental, yield, crash data sets
 - Preliminary biomass analysis (primarily ATP3 methods)



2 – Approach (Management)

Critical Success Factors

- BM2 Complete strain characterization for cold weather strains complete 10/14
- BM3 Complete long term cultivation studies of cold weather strains complete 3/15
- **BM4** Complete strain characterization for SW summer strains **complete 5/15**
- **BM5** Complete long term cultivation studies of SW summer strains complete 9/15
- CM1 Obtain strain characterization data for 2 additional RAFT production strains for warm and or cold season applications - complete 12/15
- CM2 All Phase 2 RAFT testbed data available in data management platform for and assessable for data analysis using relational data tools – complete 3/16
- CM3 Complete installation of continuous OD sensor at testbeds and begin continuous data collection – complete 6/16
- D1 Complete experimental plan for RAFT testbeds for FY17 year using BAT model to select seasonal strain rotations strategies and specific operating parameters (culture depth, harvest strategies) to maximize biomass productivity. complete 9/16
- CM4 Crop protection strategies selected in laboratory studies at NMSU validated in UA and TAMU testbeds for summer/fall strains – complete 12/16



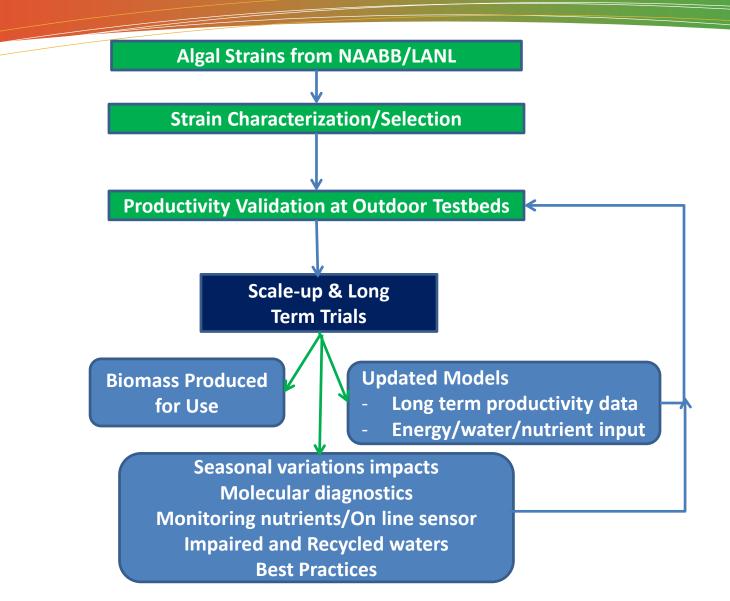
2 – Approach (Management)

Critical Success Factors – upcoming

- CM5 Workshop completed to analyze Phase 3 cultivation data from testbeds and benchmark experimental plan. – April 2017
- CM6 Utilize the BAT modeling framework to conduct a comprehensive trade off analysis to identify priority sites for algal biomass production incorporating the RAFT experimental results related to strain-specific performance, strain rotation and harvest strategies, and operational best practices. – June 2017
- CM7 Phase 3 RAFT testbed data available in data management platform for and assessable for data analysis using relational data tools. All RAFT data available to public – September 2017
- **D D1.** RAFT Final Report with Best Practices Summary September 2017
- Challenges
 - Contamination/Culture Stability
 - Complexity of "Sharing data" or "public data" via relational database.
- Management Approach
 - Biweekly teleconferences & Quarterly reports
 - Biannual meetings & Annual site visits by PI and PM to Sites

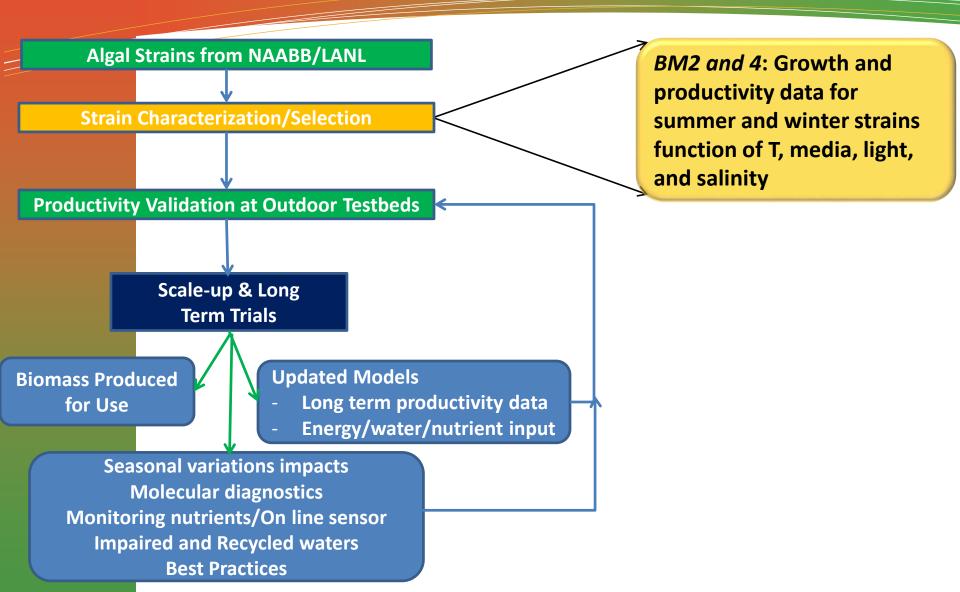


2 – Approach (Technical)





3 – Technical Accomplishments

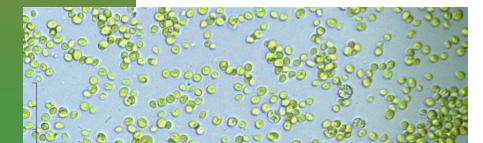




Strains

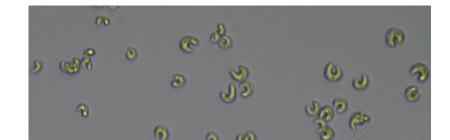
Chlorella sorokiniana DOE 1412

- 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

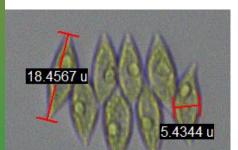
- Dominated Chlorella culture during winter cultivation at Texas A&M Highly variable cell size and morphology
- Impressive cold growth in cold
 - Optimum range is 17-27 °C
- Heat sensitive
- Brackish tolerant <20ppt
- Growth in Secondary Waste Water





Scenedesmus obliquus and others

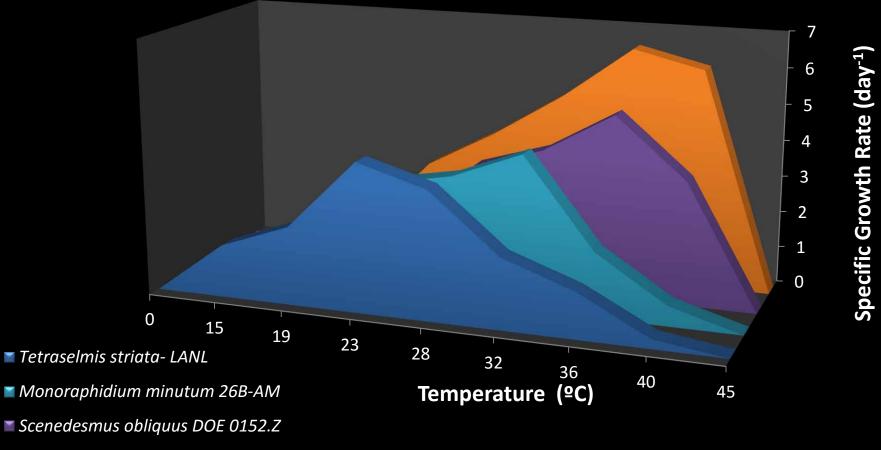
- NAABB strain isolated by Dr. Juergen Polle's group (CUNY)
- Large four to eight-celled colonies, occasionally single cells
- Winter hardy
- Heat tolerant to 38 °C
 - Growth rates <1 day⁻¹ at 10 °C
 - Growth rates >3 day⁻¹ at >22 °C
 - Optimum range is 22-38 °C
- Salinity sensitive <5ppt
- Settles rapidly



- Other Strains Investigated
 - Nannocloropsis salina
 - Picochlorim sp.
 - Tetraselmis striata
 - Stichococcus minor
 - Tisochrysis lutea
 - Synechococcus elongates
 - Chlorella antartica
 - Tetraselmis striata Cellana
 - Tetraselmis striata UTEX



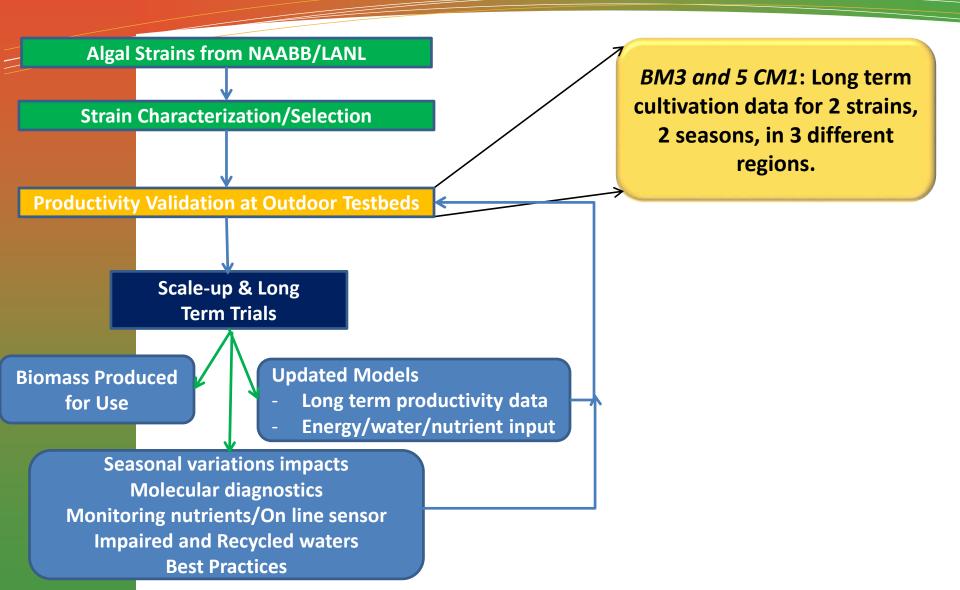
Growth rate under ideal conditions



Chlorella sorokiniana DOE1412

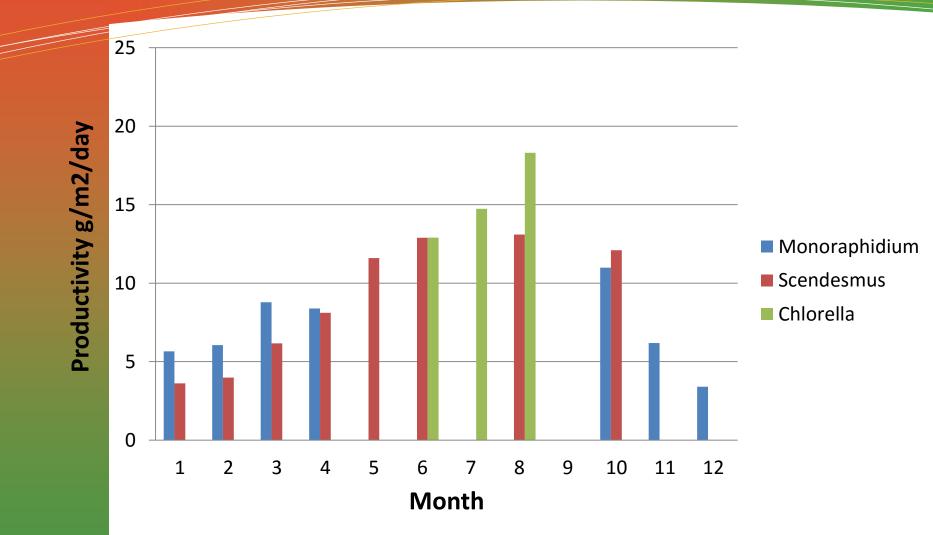


3 – Technical Accomplishments



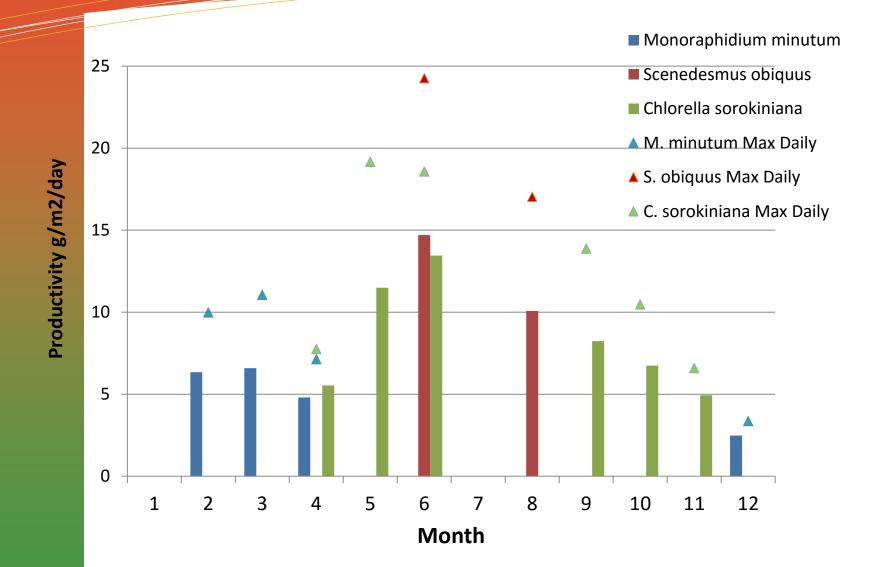


Seasonal Productivity in Pecos - 2016



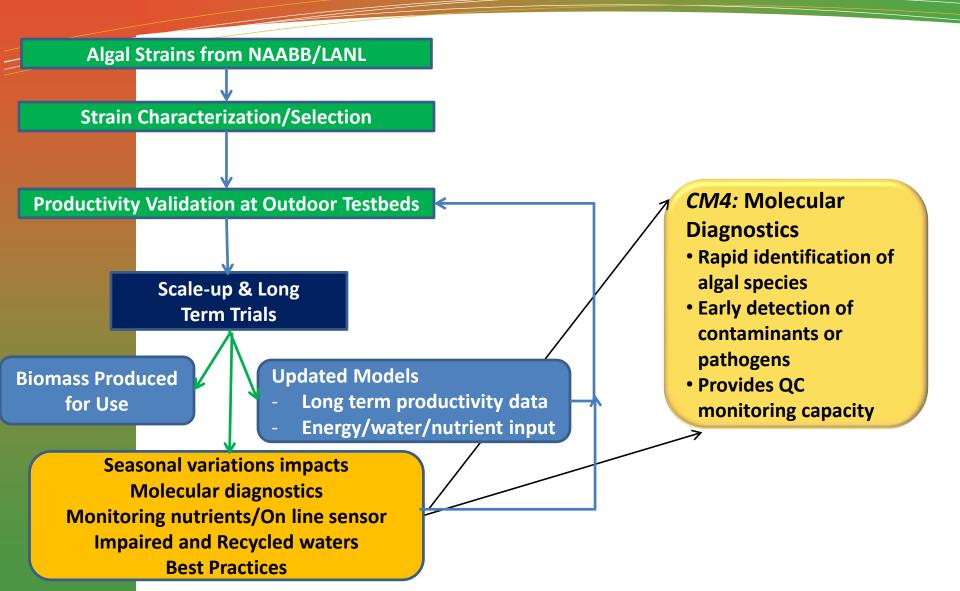


Seasonal Productivity in Tucson





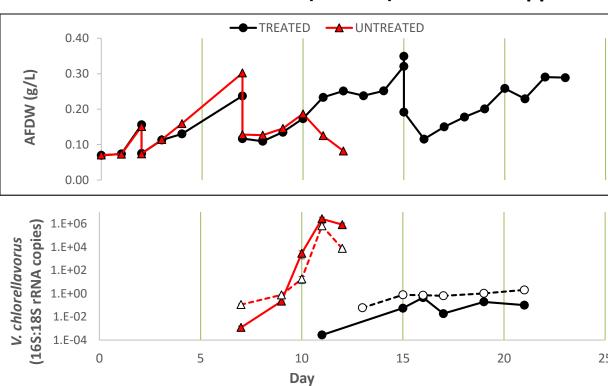
3 – Technical Accomplishments



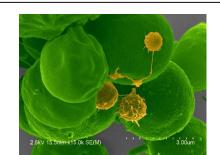


Pest Management Strategies: Vampirovibrio chlorellavorus

- Pathogen detection by quantitative PCR
 - Limit of detection ~ 19 V. chlorellavorus DNA copies
- Untreated culture crashed early and accumulated a high pathogen concentration
- Treated culture sustained
- V. chlorellavorus whole genome sequence released (LANL and UA)



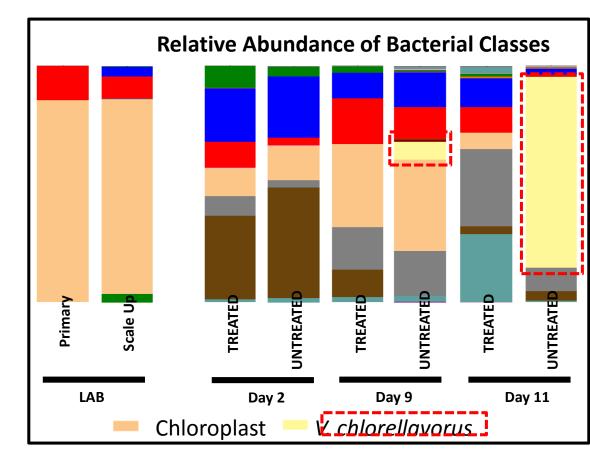






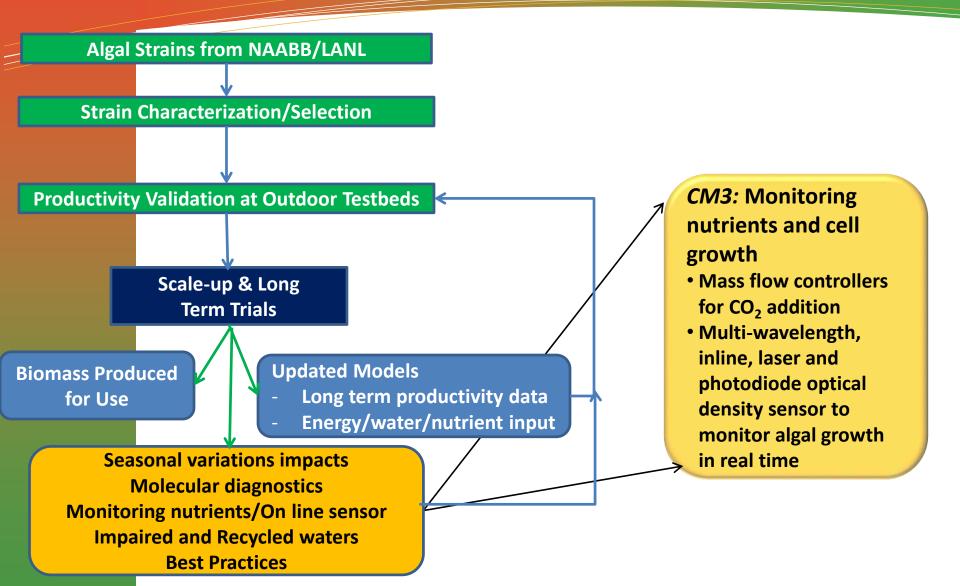
Bacterial community DNA analysis after biocide treatment

- Deep sequencing of total bacterial communities
- Outdoor culture community rapidly became complex
- V. chlorellavorus displaces variety of beneficial bacteria
 - TREATED D11 = 615 bacteria types
 - UNTREATED D11 =
 191 bacteria types
- Treatment induced recovery of bacterial community





3 – Technical Accomplishments

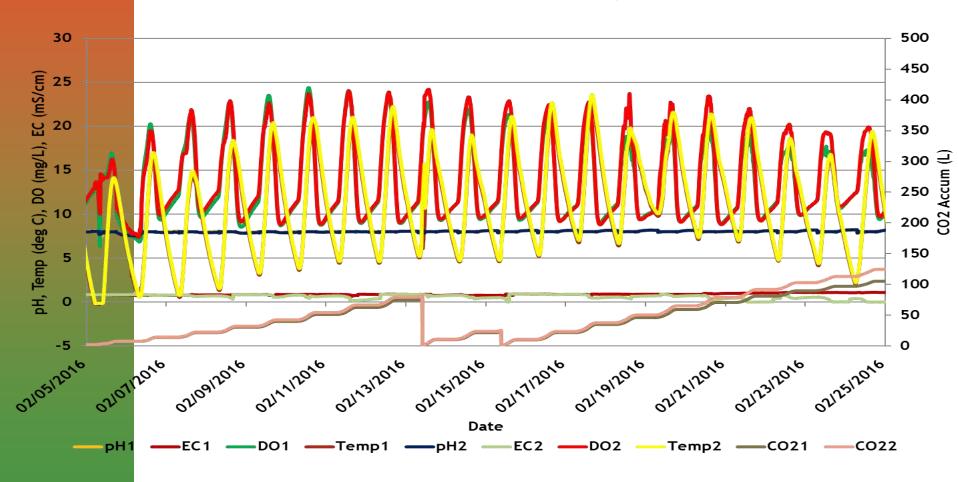




Continuous Data

Continuous data. Monoraphidium Minutum

PW1 and PW2 (6 inch culture depth)

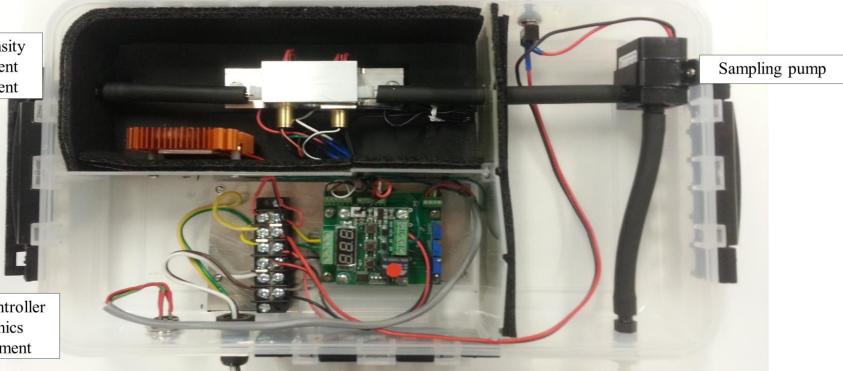




On-line Optical Density Data

Optical density measurement compartment

Sensor controller electronics compartment





Continuous Growth Data

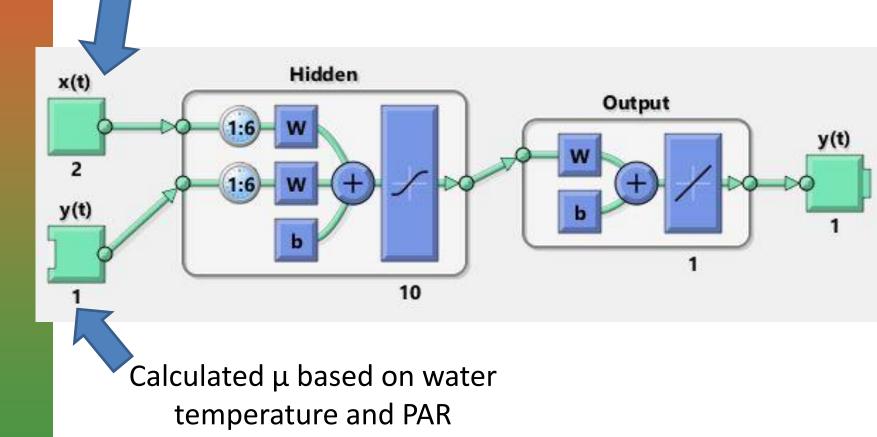






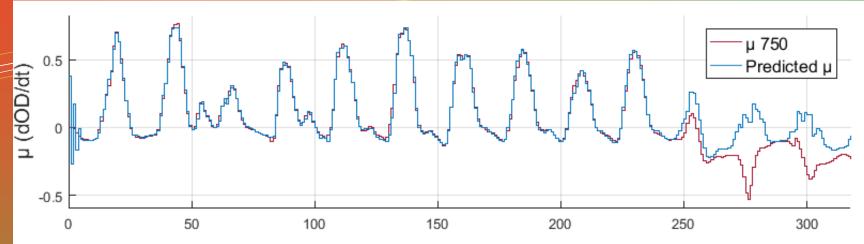
Continuous Growth Data

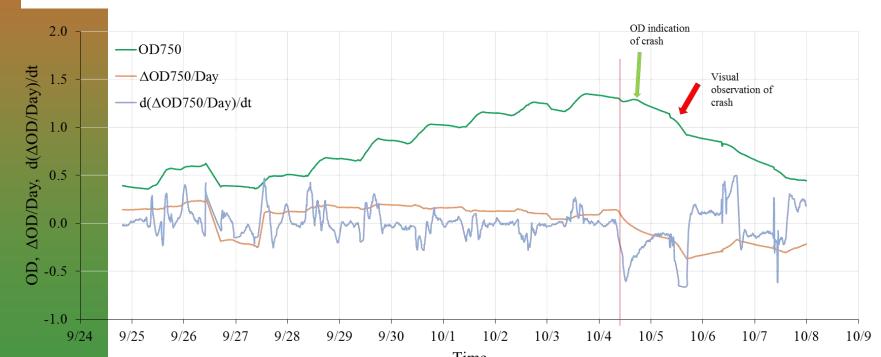
Calculated μ from OD data continuous





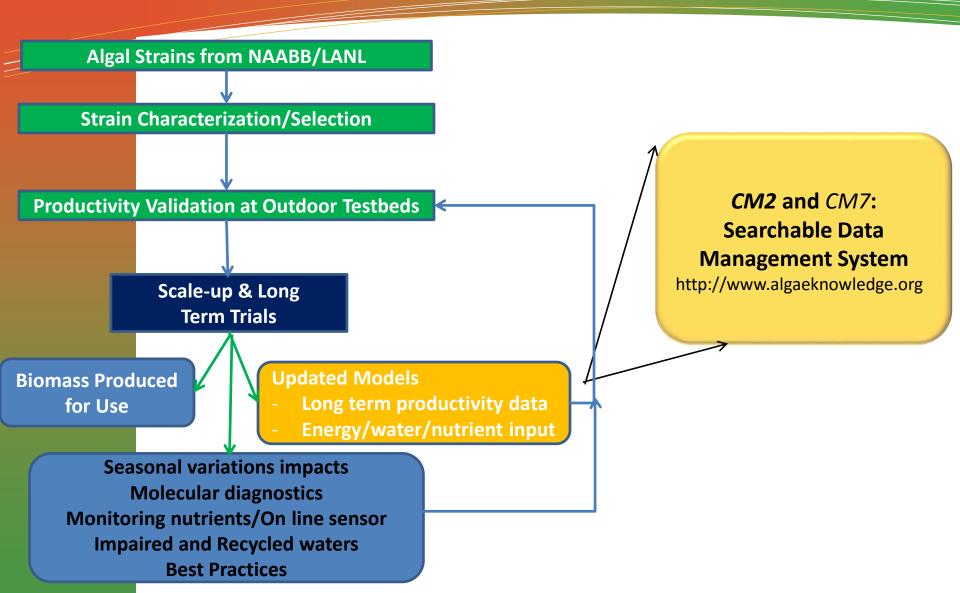
Continuous Growth Data



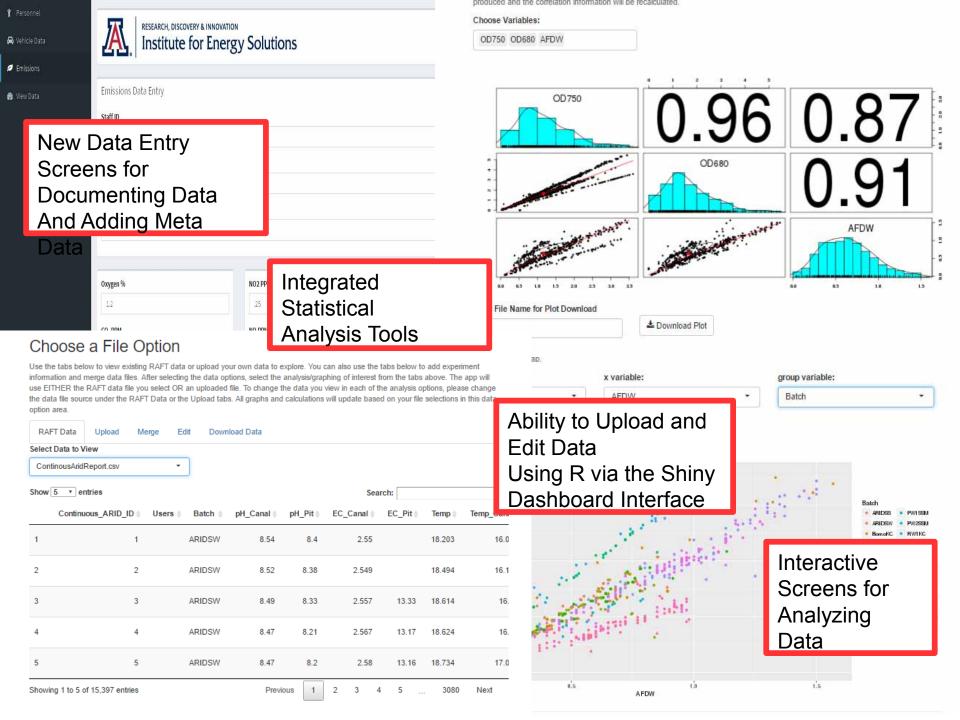




3 – Technical Accomplishments



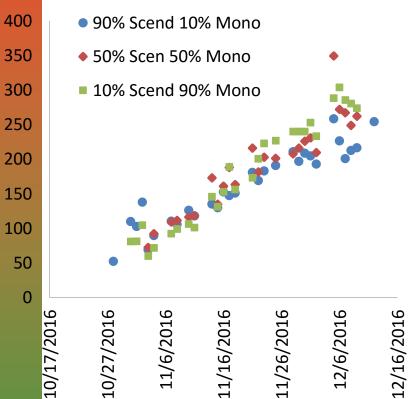
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Types of data available





Mixed cultures – similar performance

Scenedesmus obliquus – October (Pecos				
Water type	Productivity g/m²/day	Average g/m²/day		
Freshwater	9.8	9.4		
	9.1			
Well Water	12.3	11.4		
	10.6			
Wastewater	8.1	8.4		
	8.7			



Types of Data

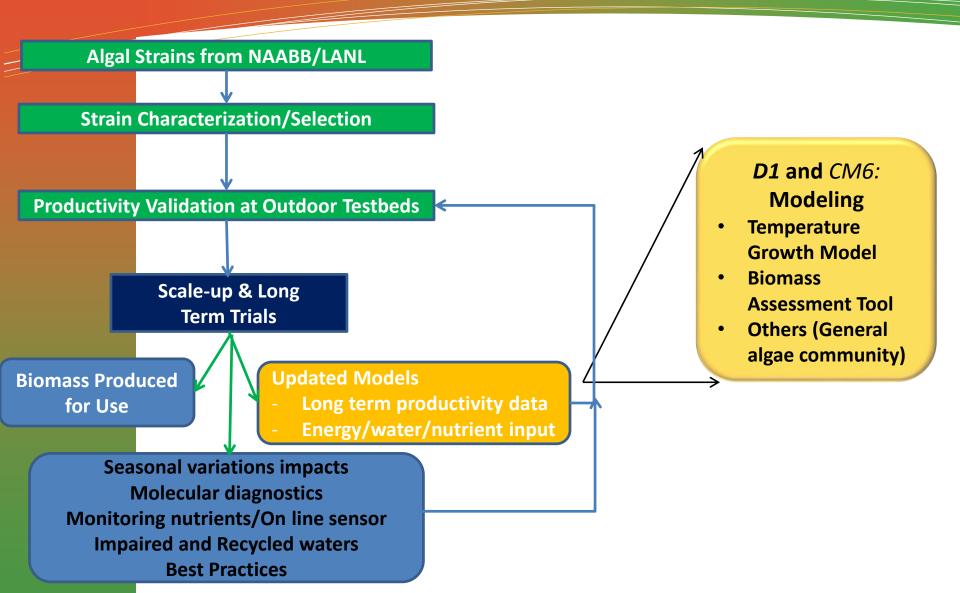
- Depth 6 to 10 inches
- Reactor type (size and ARID)
- Recycled water
- Wastewater
- Mixed cultures, pure culture
- Nutrients High and low nitrogen/phosphate
- Continuous OD and CO₂ data

Algae Strain	Lipid	Protein	Carbohydrate	Ash
Monorophidium minutum	25%	49%	21%	5%
Chlorella sorokiniana	18%	60%	14%	8%
Scenedesmus obliquus	21%	49%	19%	12%

Data will be available demonstrating that all Project Objectives are met



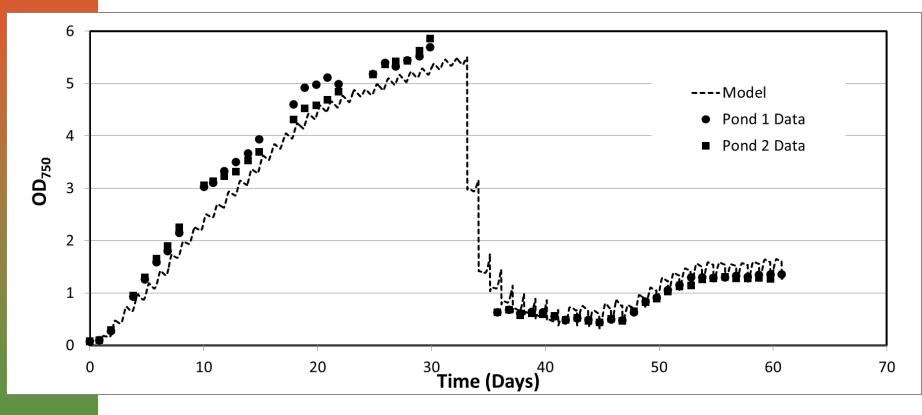
3 – Technical Accomplishments





Validation for Chlorella sorokiniana

models



Arizona outdoor ponds

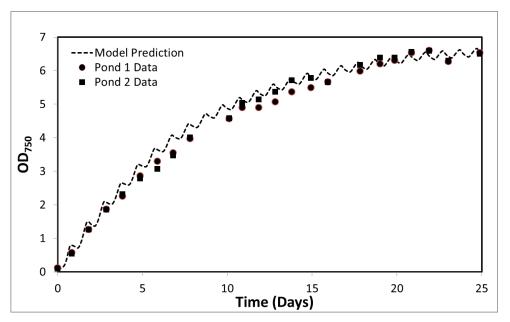
- Batch
- Semi-continuous with different dilution rates



BAT/Growth Modeling in Support of Experimental Design

Demonstrate growth model performance for:

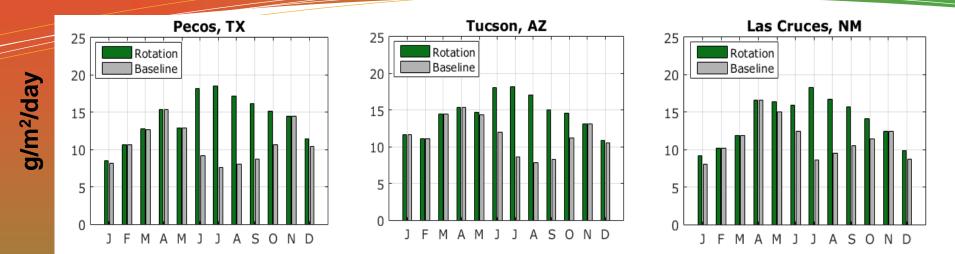
- Multiple species
 - Chlorella (warm)
 - Scenedesmus (all)
 - Monoraphidium (cool)
- Multiple locations
 - AZ, TX, FL
- Multiple seasons
 - Summer, spring, winter



Model Validation for Florida simulation for July in PNNL indoor LED Ponds



BAT/Growth Modeling in Support of Experimental Design



Provide feedback and guidance for design, monitoring and interpretation of test bed experiments

- Assess most effective operating depths (10cm, 15 cm, 20 cm) for given strains and seasonal climates
- Evaluate alternative harvest strategies and production
 - volumes: 25%, 50%, 75%, 90%
 - Frequency f(growth curve slope)
- Compare relative effectiveness of alternative strain rotation patterns for alternative locations



4 – Relevance

- Created a strategy to obtain the cultivation data necessary to understand and promote algae biomass production
- Developed a strategy for selecting strains for outdoor culturing via a priori characterization and modeling
- Developed cultivation protocols for strain specific media design for outdoor ponds.
- Refined systematic strain characterization and selection methodology to enable careful expansion of DOE's strain portfolio for alternative climatic and water conditions.
- Successfully cultivated 3 strains over multiple seasons in multiple locations in multiple cultivation systems
- Assembled preliminary, accessible comprehensive data base to enable testing and validation of algal pond and biophysical growth models.
- Provided biomass to other researchers (e.g. HTL studies)
- Determined root cause and management strategy for *Chlorella sorokiniana* pond crashes



4 – Relevance

- Provided QC monitoring of algal cultures at all stages of growth and scaleup using molecular identification tools
- Developed methodologies to identify algal species, and detect contaminants and/or pathogens in algal cultures
- Demonstrated new sensor technology to support advanced, more efficient cultivation control strategies.
- Achieved real time OD monitoring of algal biomass in PBR and open raceways.
- Tech transfer and innovative approach/technologies for algal growth and health monitoring
 - Ability to monitor bacterial and algal communities
 - Patent filed for online OD sensor for continuous OD monitoring



5- Future Work

- Continue to upgrade data management system
- Validate models with testbed data
- Continue nutrient & water monitoring
- Couple bacterial & algal community monitoring to further gauge status of algal health
- Integrate real time OD and algal biomass monitoring with other variables (i.e. DO, pH) to enable autonomous control of algae production
- Finish experimental crop-rotation plan publish results



Summary

- Overview Long term cultivation at 3 testbeds using multiple strains
- 2. Approach –

Screen strains for optimal temperature and light;

Semi continuous growth over all seasons in outdoor systems;

Provide data to modelers – receive feedback

- **3.** Technical Accomplishments/Progress/Results Milestones and deliverables on track for:
 - Characterization
 - Outdoor cultivation,
 - Monitoring (bacteria, continuous growth),
 - Modeling
- **4. Relevance** Model/productivity driven comprehensive research plan to meet DOE objectives
- 5. Future work Continue to obtain year round crop rotation data, complete data base, update models, and publish results



Additional Slides



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. <u>http://dx.doi.org/10.1016/j.algal.2015.11.008</u>
- 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
 38



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, University 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



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.
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- 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