DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

WBS: 1.3.5.111

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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
  – Integration and Scale up
- 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: 33%

Barriers

Barriers addressed

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

Budget

	FY 13 Cost	FY 14 Costs	Total Planned Funding (FY 15-9/30/17)
DOE Funded Universities		$1,033,237.41	$4,766,762.59
DOE Funded (PNNL)		$550,000	$1,650,000
Project Cost Share (Comp.)*		$73,332	$131,771

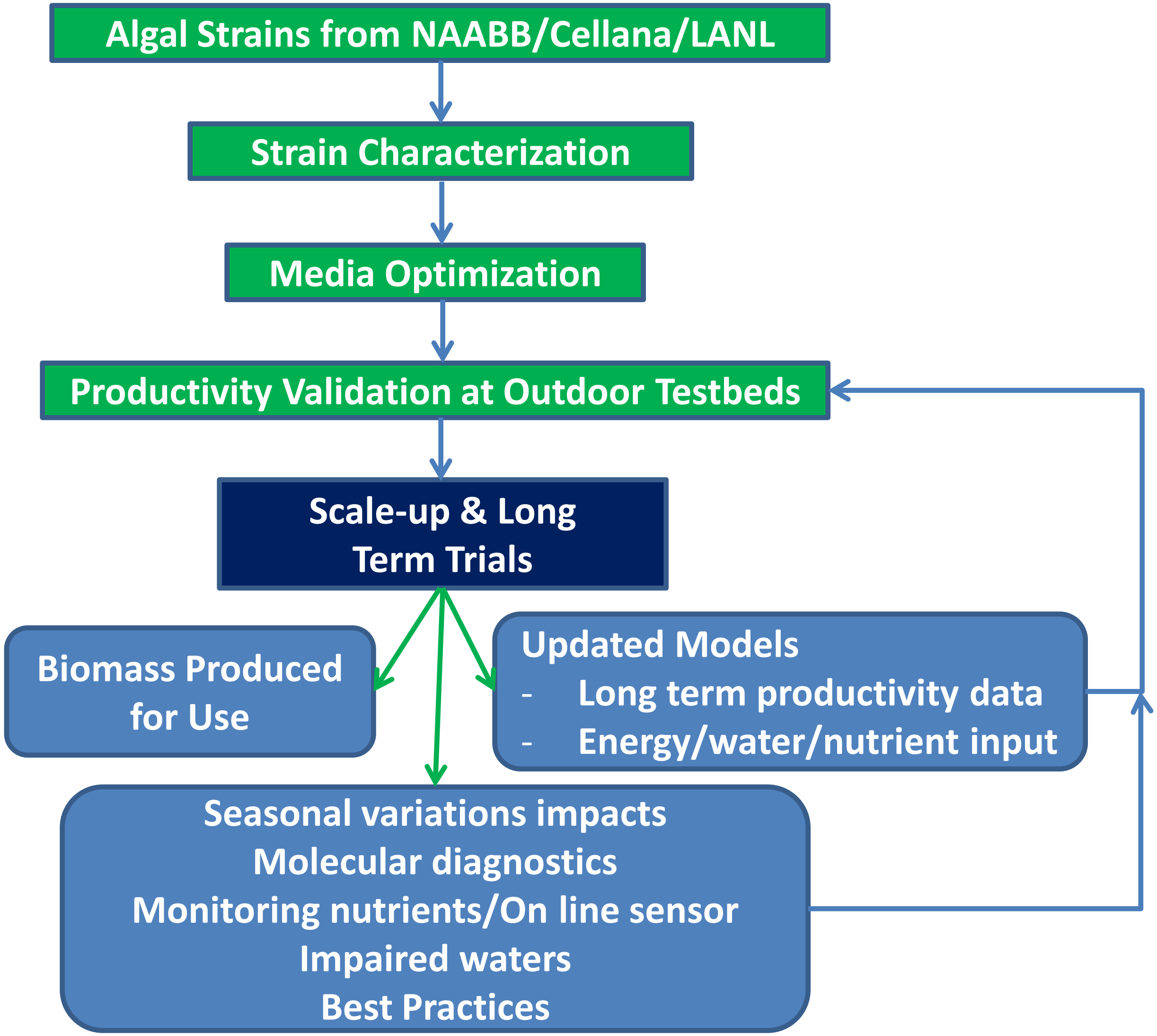
Partners

- 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 techno-economic models
    - Pond and model feedback-enhanced systems
  - Increase sustainability of algae biomass production
    - Optimize biomass productivity using impaired waters
    - Strain selection and crop rotation for year-round cultivation

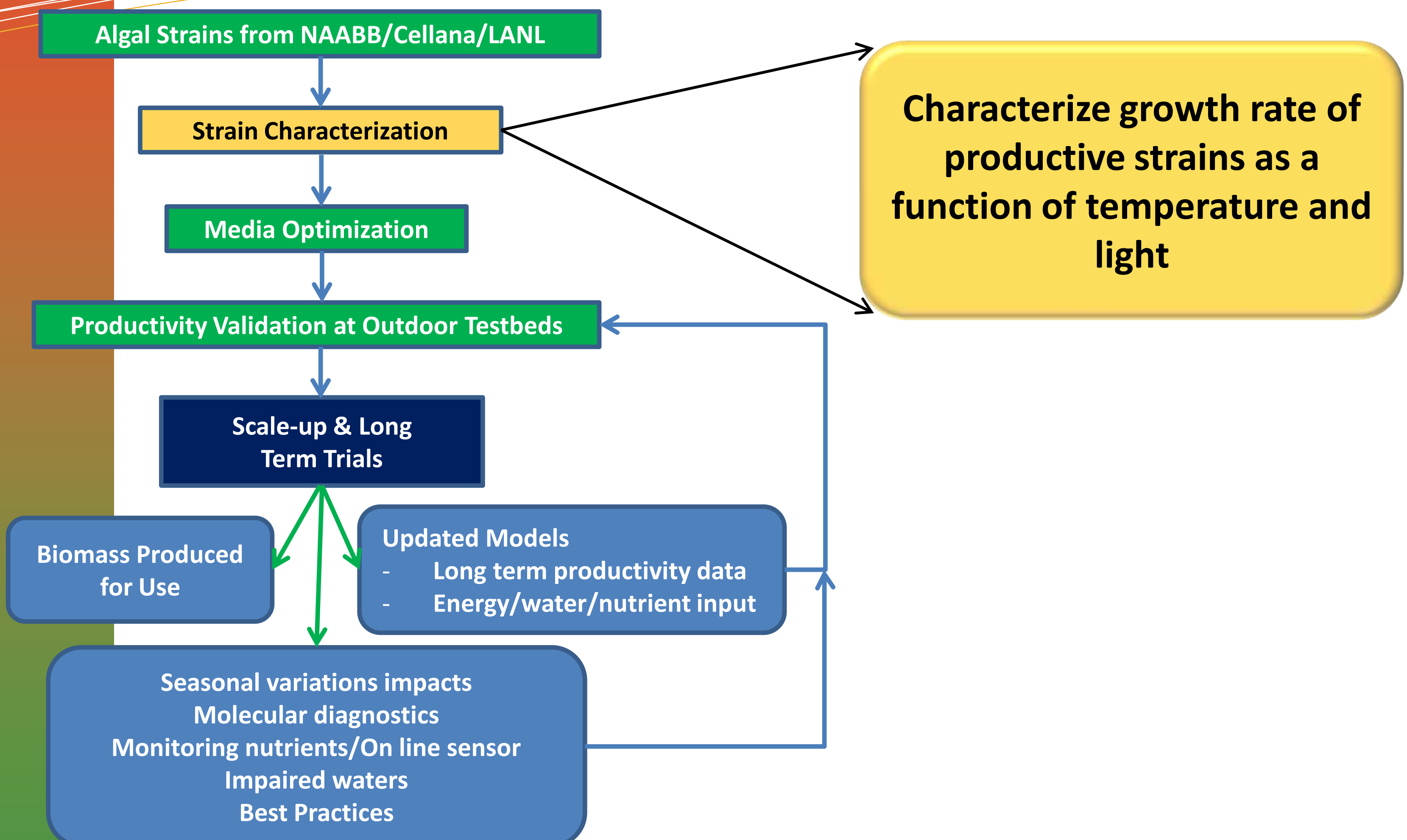
2 – Approach (Technical)



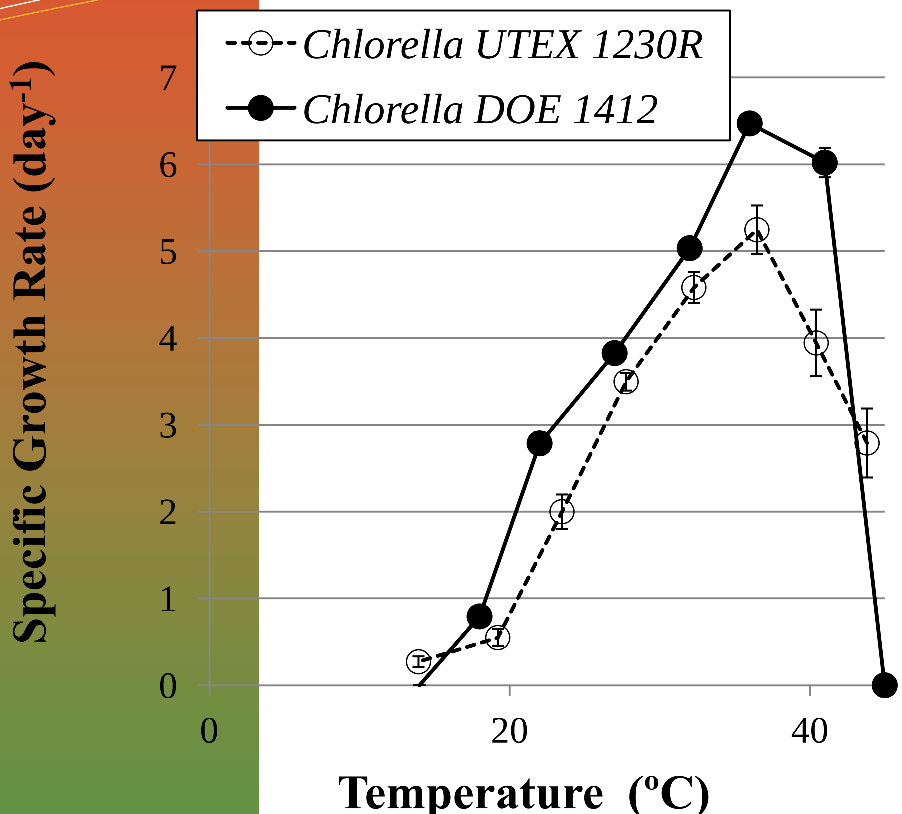
2 – Approach (Management)

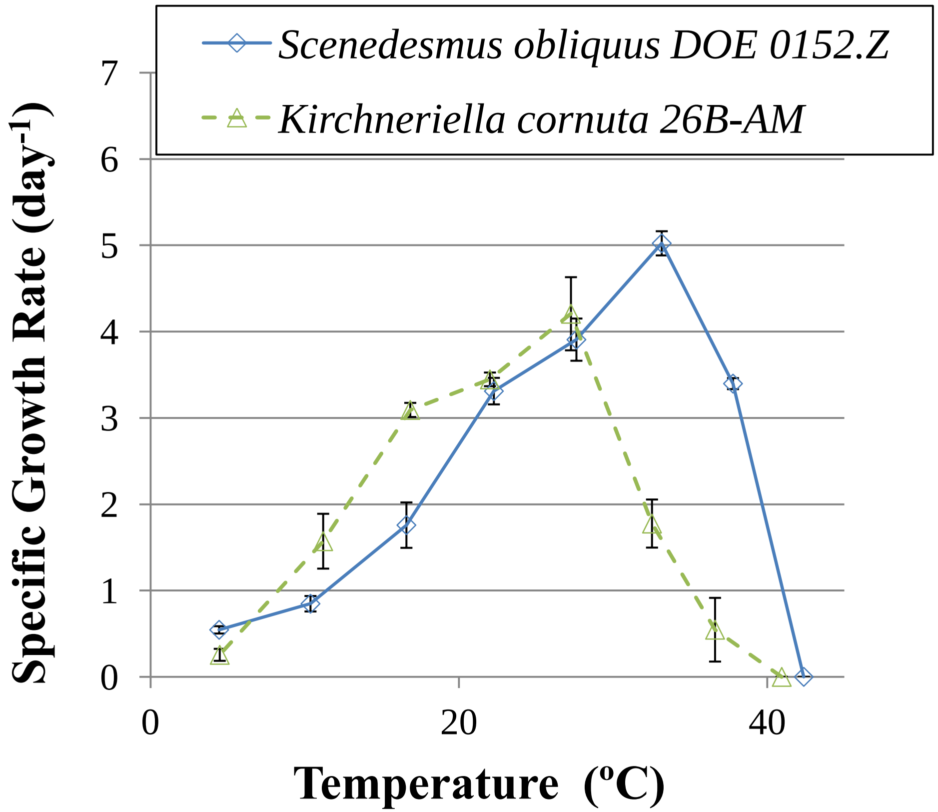
- Critical Success Factors
  - **DL1 & DL2 Data Management; QA/AC ; R&D Plan – 6 - complete**
  - **M1 Management Information System in Place – 12 - complete**
  - *M2 Long term cultivation data for 2 strains over 2 seasons – 22 – in progress*
  - DL3 : Long term R&D Report and Phase 3 Plan Go/No Go
  - *M3 Growth and productivity data for 3 strains function of T, media, light – 30 - in progress*
  - M4 Long term cultivation data 2 strains in 3 different regions – 36
  - *M5 Growth and cultivation data in 2 impaired waters and/or nutrient recycle systems – 42 – laboratory data and preliminary outside*
- Challenges
  - Contamination/Culture Stability
  - Complexity of “Sharing data” or “public data”
- Management Approach
  - Biweekly teleconferences & Quarterly reports
  - Biannual meetings & Annual site visits by PI and PM to Sites

3 – Technical Accomplishments



Characterization

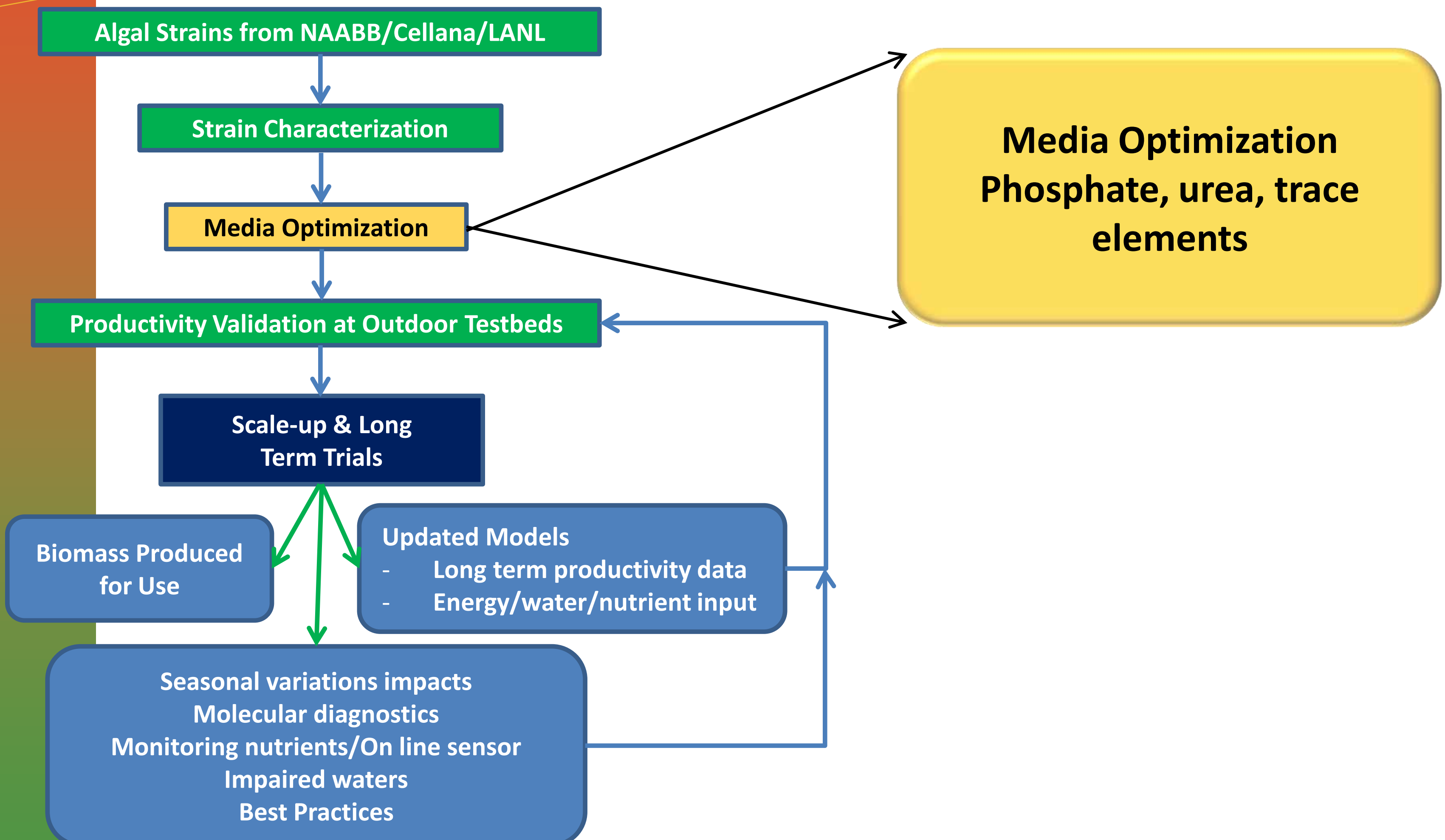




High growth rate even up to low 40s Summer strain

Growth even at 10 C Two winter strains

3 – Technical Accomplishments

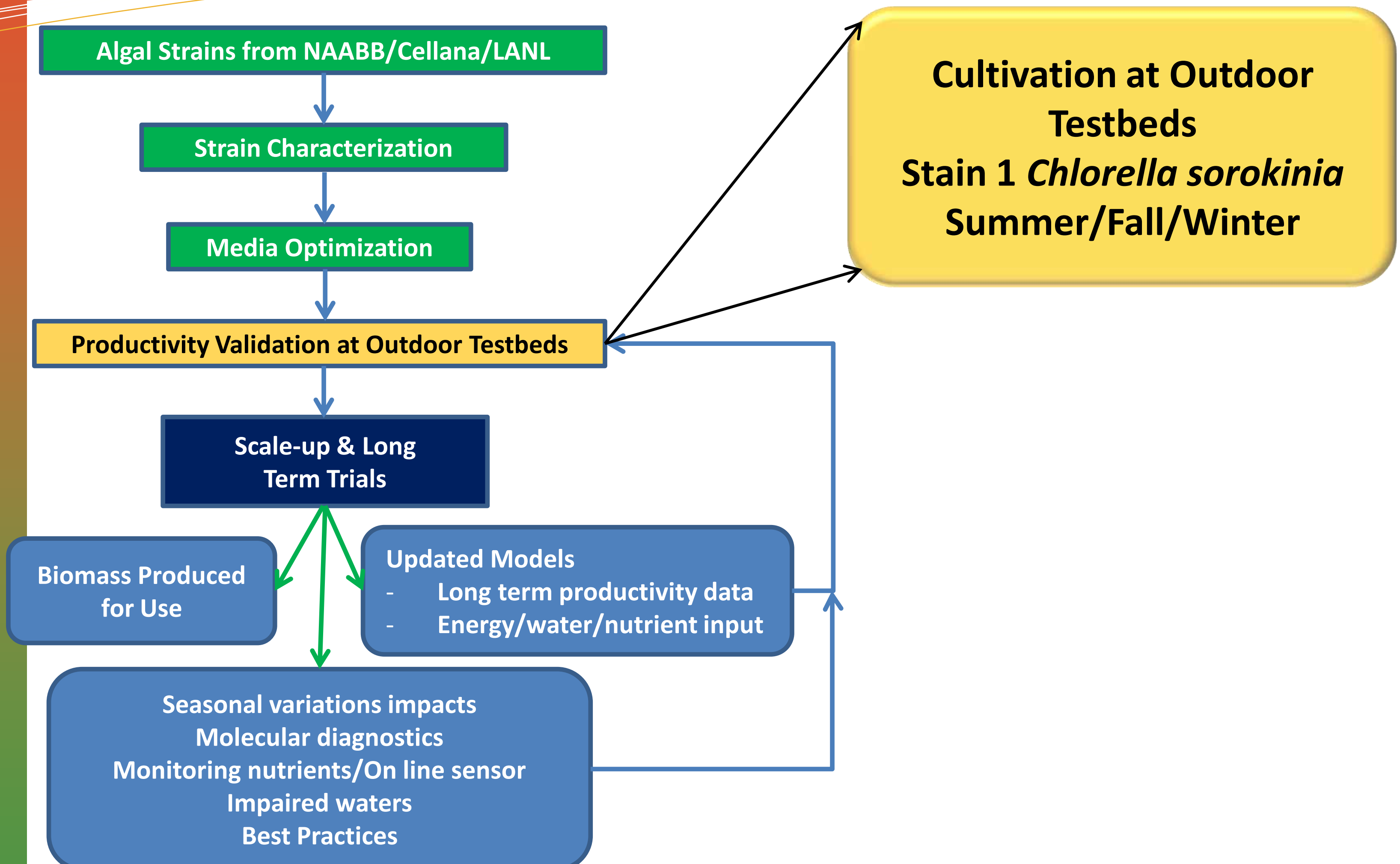


Media Optimization

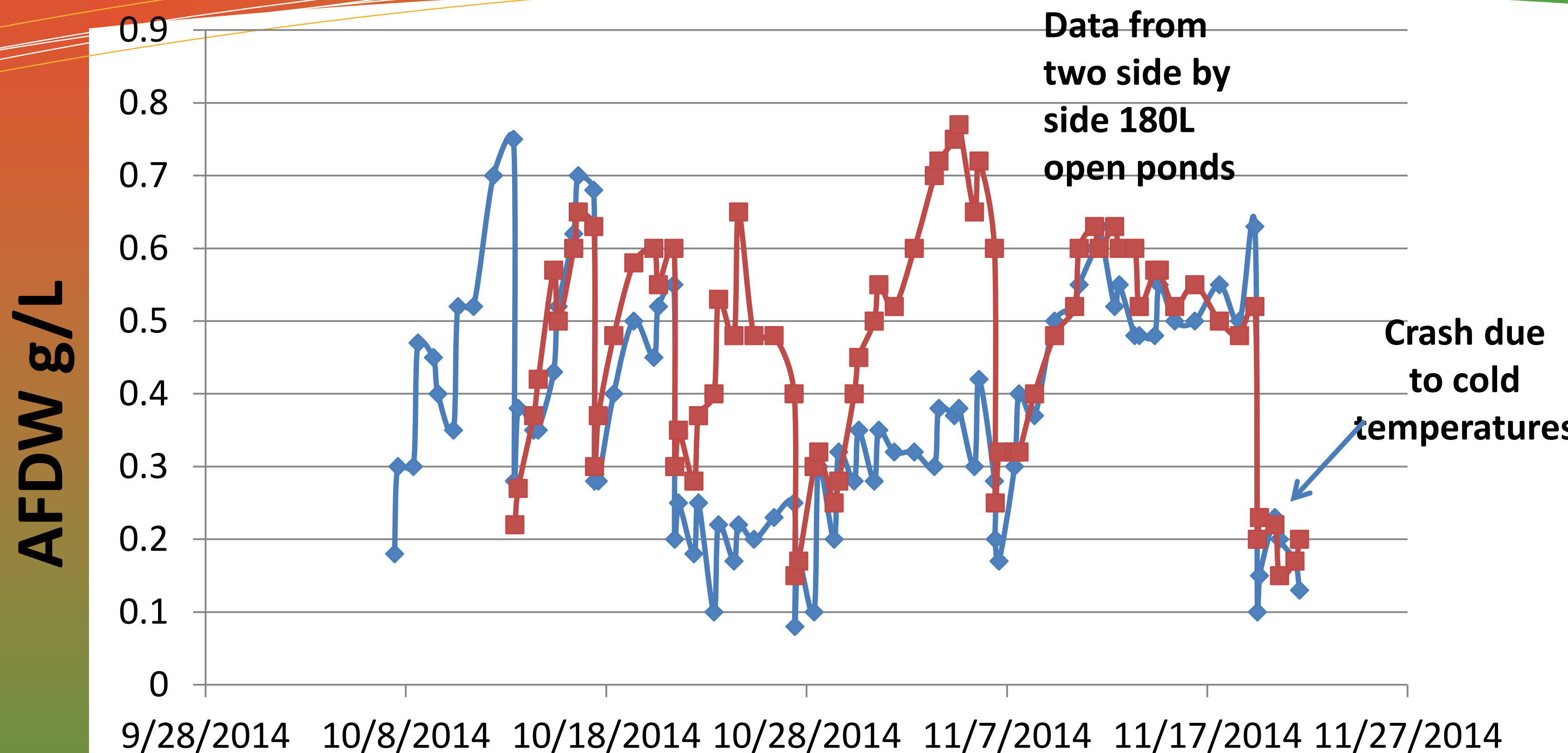
Chemical	Common name	g/L
Na_2CO_3	Soda Ash	0.02
NaCl	TruSoft	5
(NH2)2CO	Urea	0.1
$MgSO_4 * 7H_2O$	Magnesium sulphate	0.012
$NH_4H_2PO_4$	MAP	0.035
KCl	Potash	0.175
FeCl	Iron Chloride	0.0035
Di Sodium EDTA		0.00436
Trace metals solution		1
Vitamin solution (100 X)	Vitamin solution	0.005

- Complete Media Optimization
  - *Chlorella sorokinia (DOE 1412)*
  - *Scenedesmus obliquus*
  - *Kirchneriella* sp.
- **Cost is about 1/10 that of BG11 with same growth rate**

3 – Technical Accomplishments



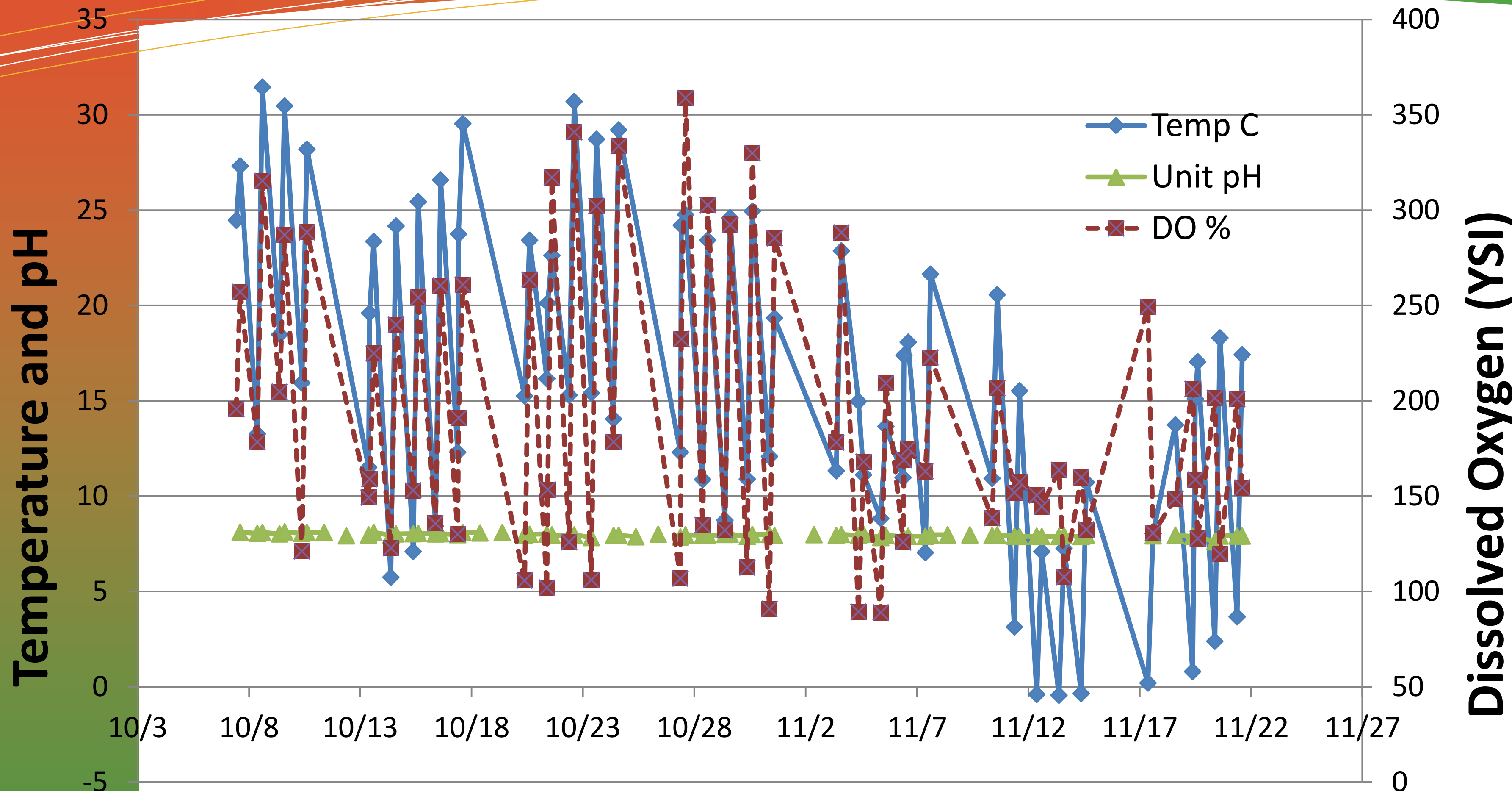
Chlorella sorokiniana



Chlorella is a productive strain but it is difficult to have consistency in production even in side by side reactors

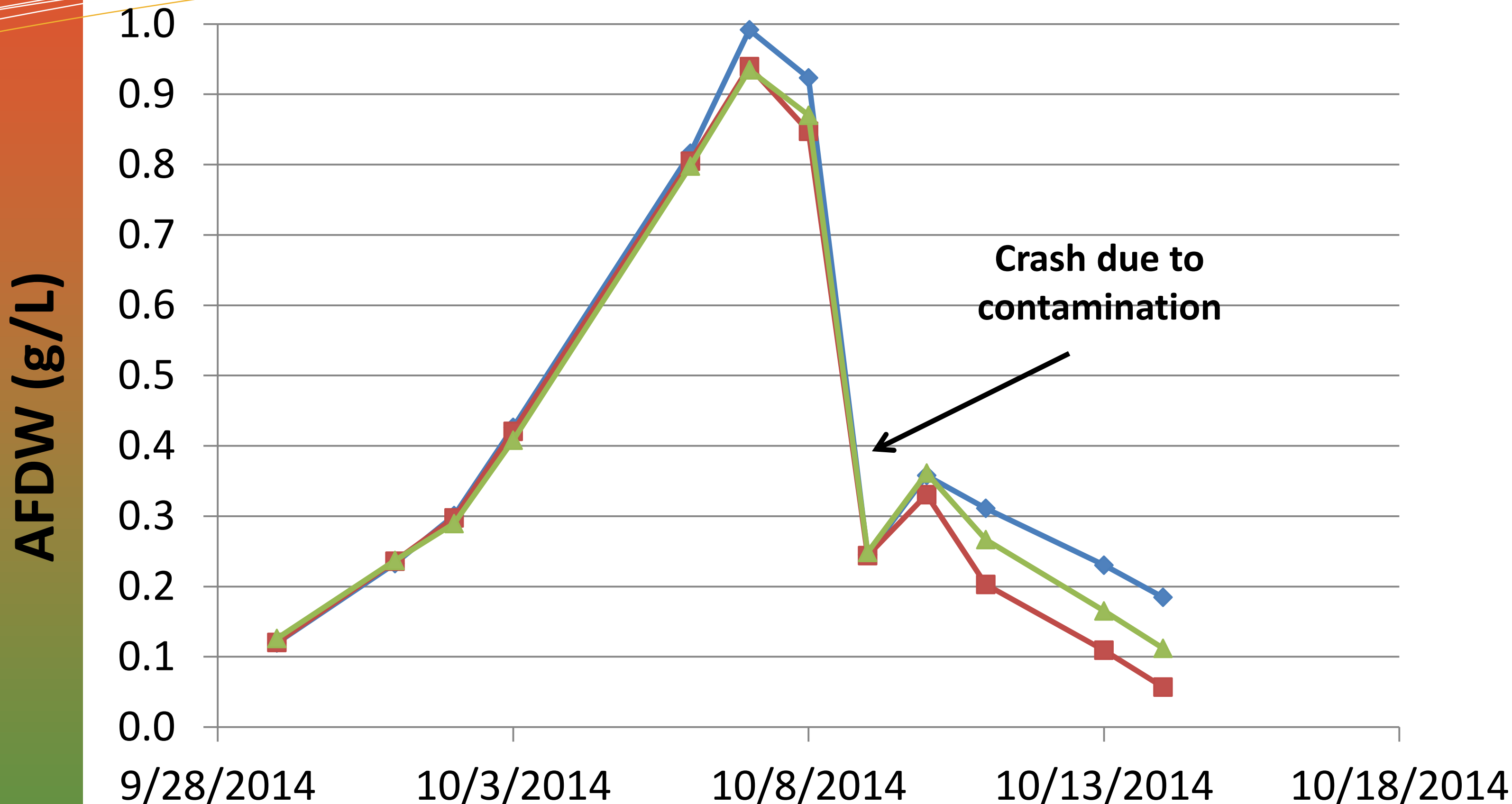
10 to 20 $g/m^2/day$

Chlorella sorokiniana



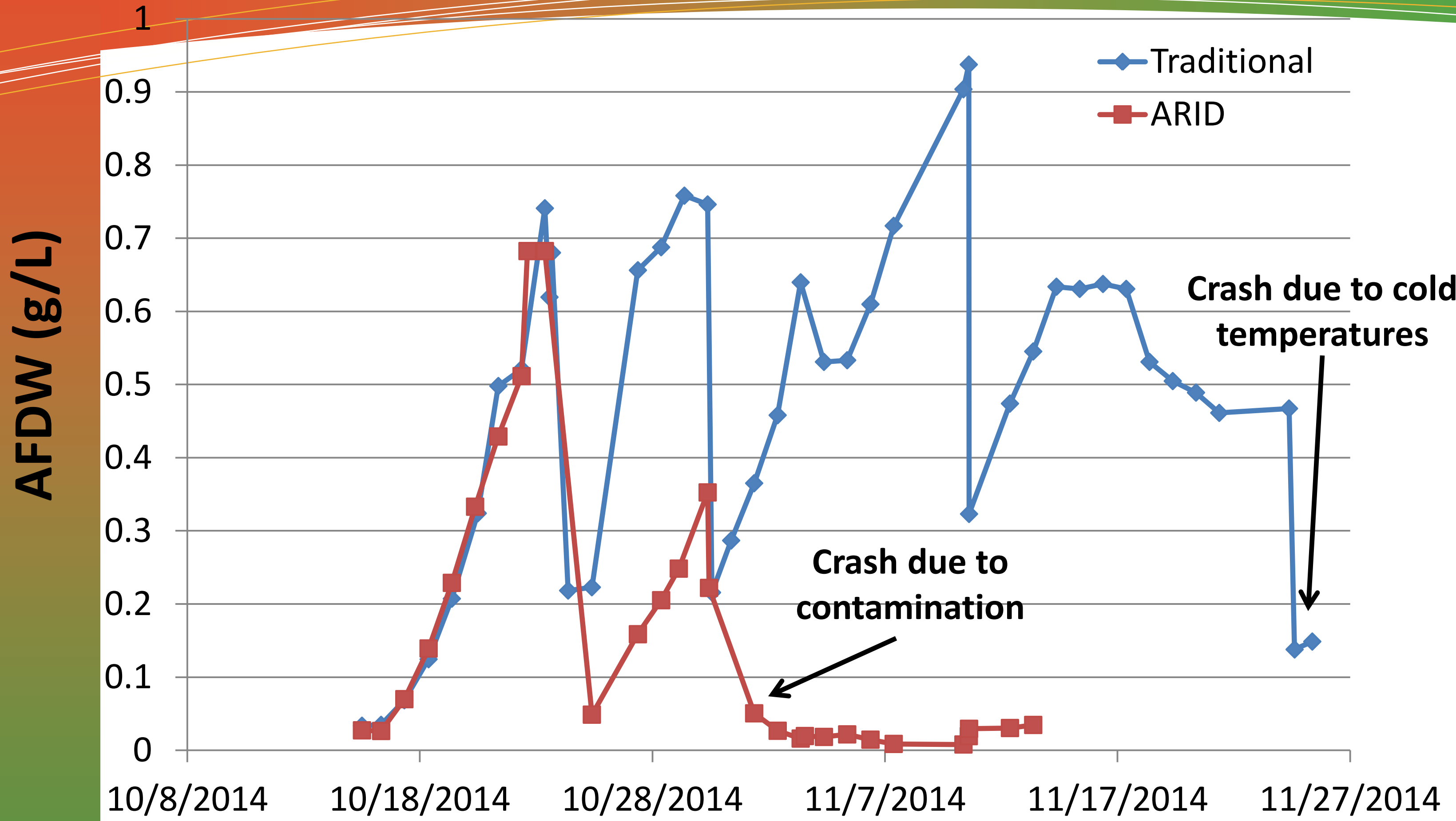
At all sites monitor T, DO, salinity, weather, water and nutrient addition, harvest volume, OD, AFDW, and control pH using CO_2

Chlorella sorokiniana



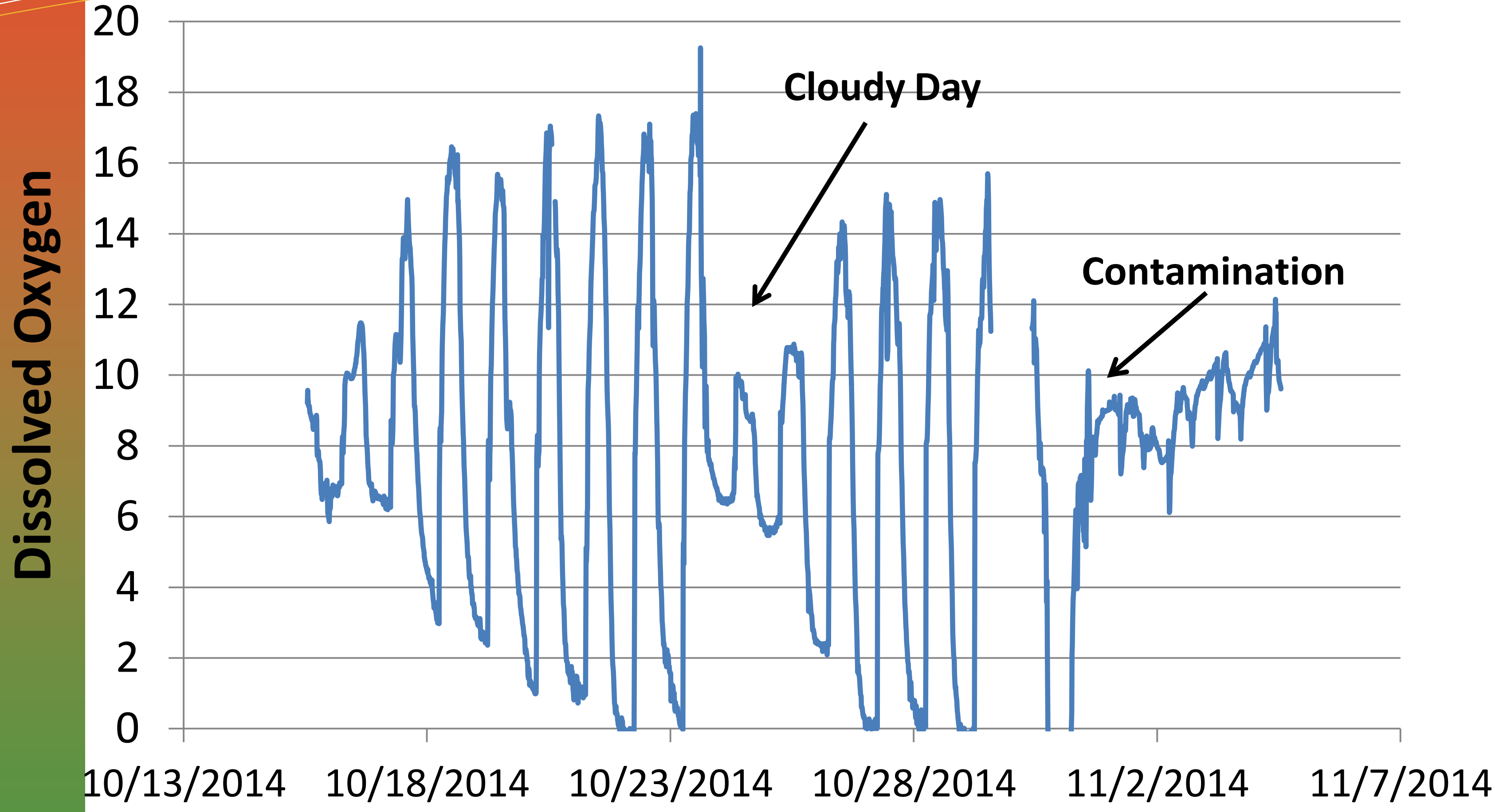
Productivity 7 to 13 g/m²/day (open systems)
30 g/m²/day (closed system)

Chlorella sorokiniana

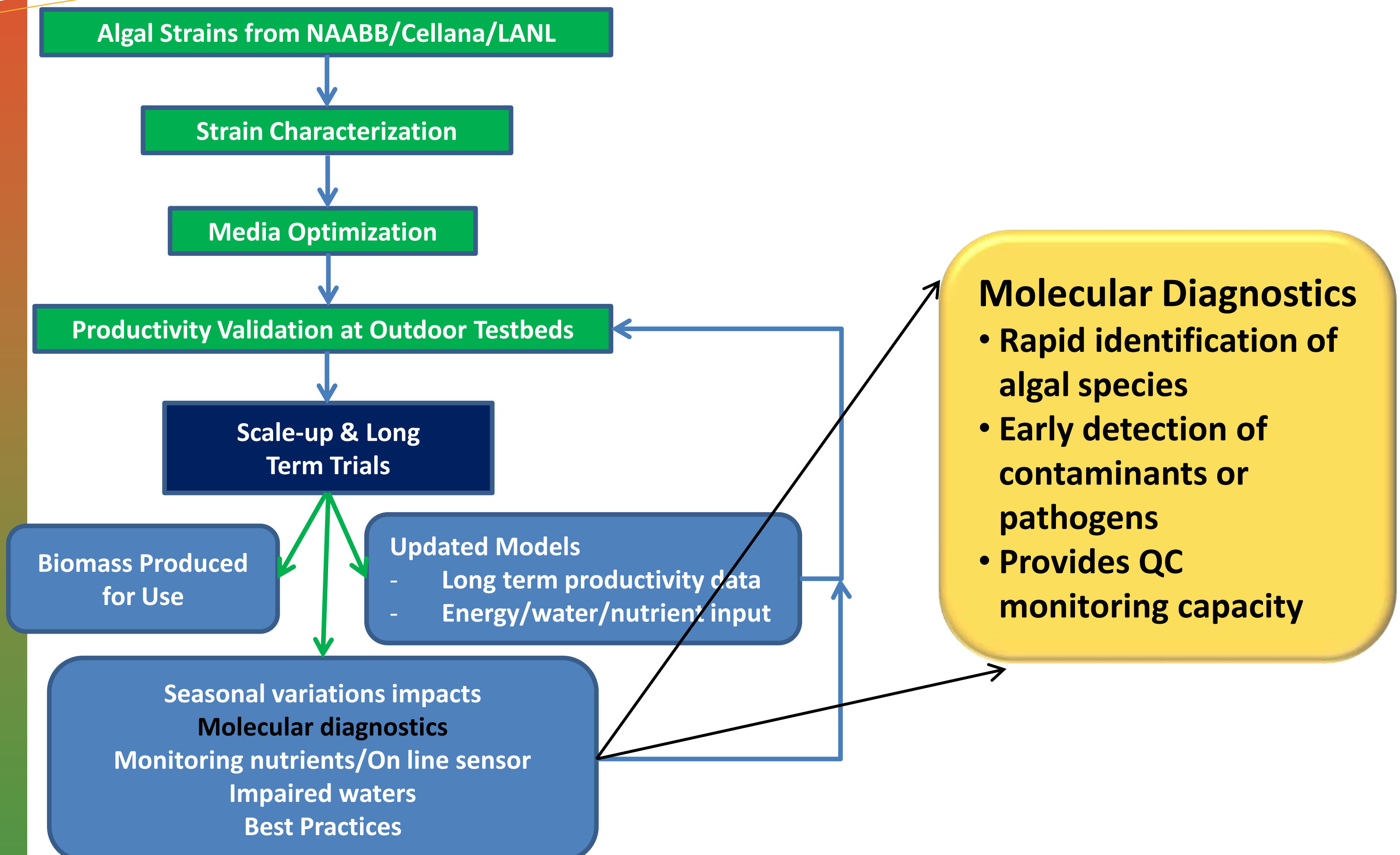


Productivity (9 to 10 g/m²/day fall 21 g/m²/day summer) – but culture crashes requiring monitoring of culture health

Early Indication – Dissolved Oxygen



3 – Technical Accomplishments



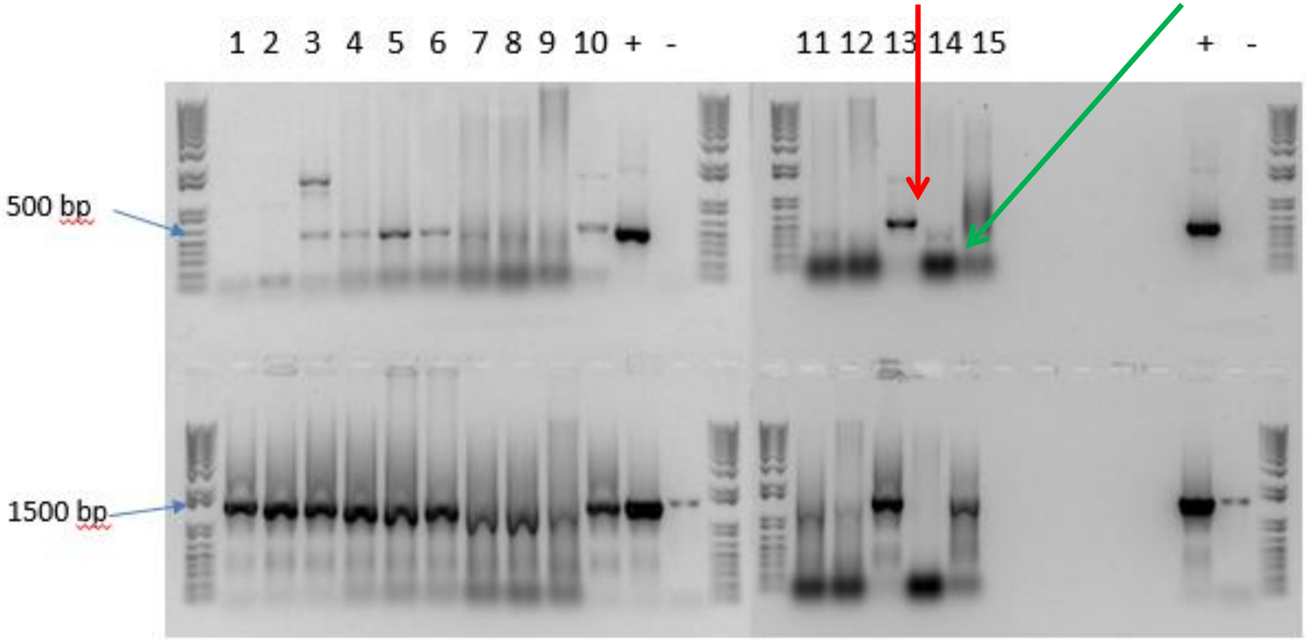
Vampirovibrio chlorellavorus

Top Panel: *V. chlorellavorus* specific primers (40 cycles)
-Expected size: 528

Bottom Panel: 16S degenerate primers (40 cycles)
-Expected size: ~1500 bp

Vampiro present Pond Crash

Vampiro not present



Samples From RAFT05

1. ARID 10/15/14
2. BT1 10/15/14
3. BT2 10/15/14
4. ARID 10/19/14
5. BT1 10/19/14
6. BT210/19/14
7. ARID 10/31/14
8. BT1 10/31/14
9. BT210/31/14
10. ARID 11/3/14
11. BT1 11/3/14
12. BT211/3/14
13. ARID 11/10/14
14. BT1 11/10/14
15. BT211/10/14

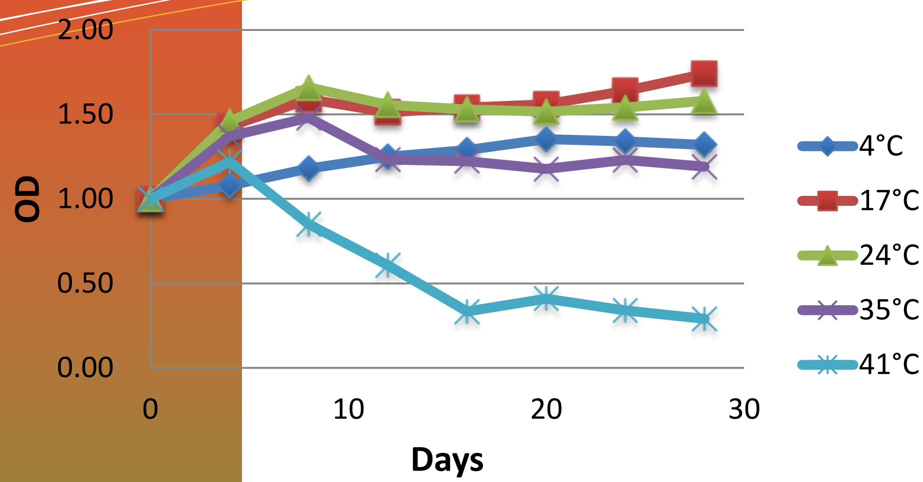
+. *V. chlorellavorous* 16S

-. ddH2O

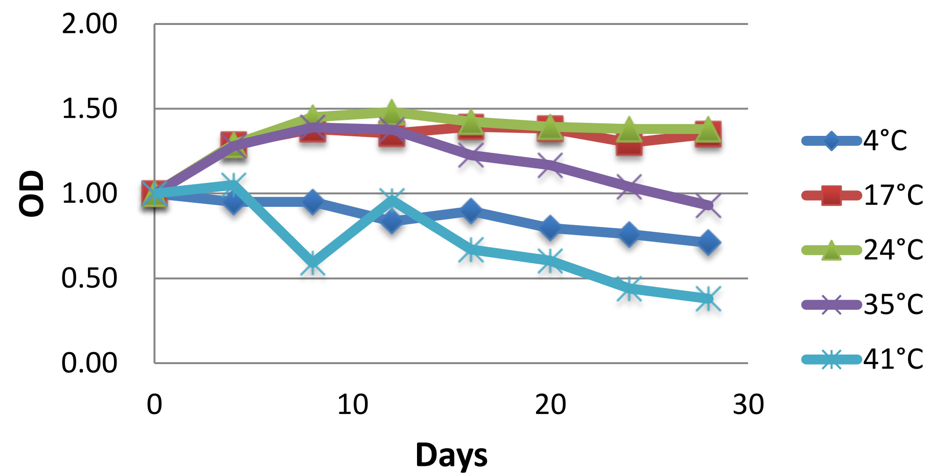
Molecular monitoring enabled the 'association' of *Vampirovibrio* with production 'crashes' of *Chlorella* spp. in AZ and NM

Relationship between T and *Vampiro*

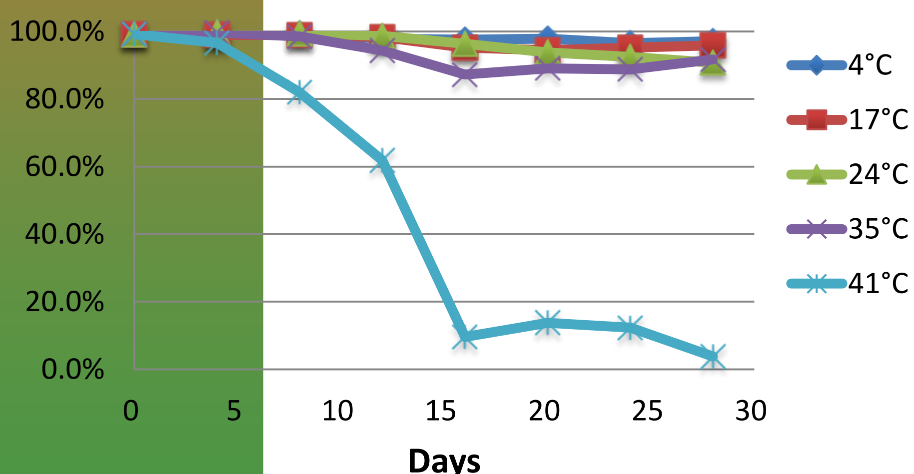
OD(DOE 1412 Control) at 750nm



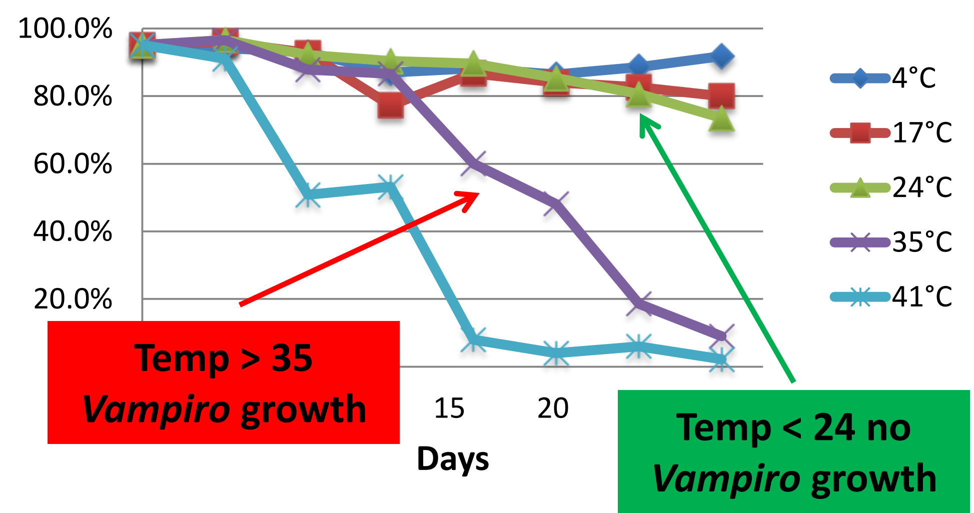
OD(DOE 1412 w/VV) at 750nm



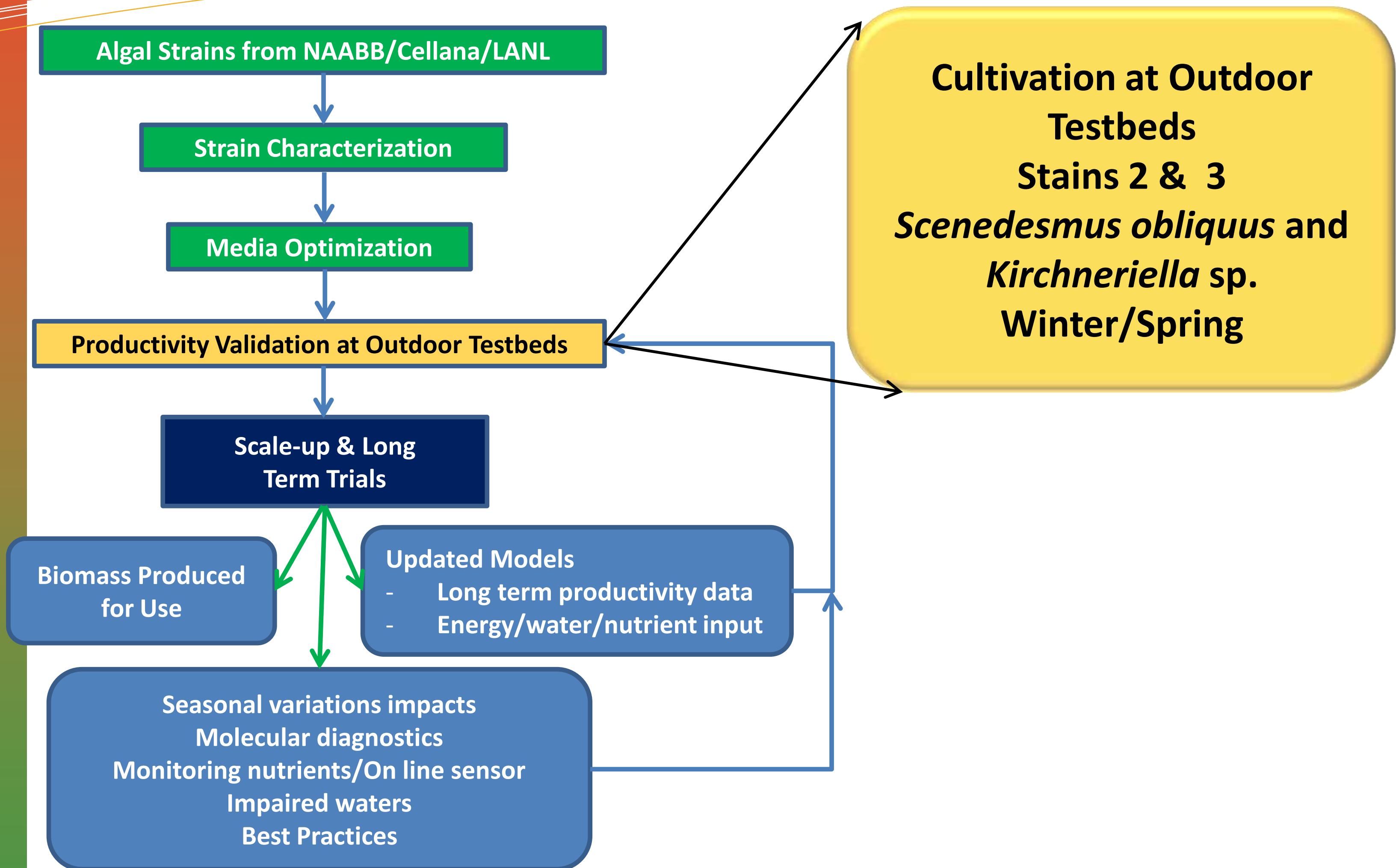
% of Living Cells(DOE 1412 Control)



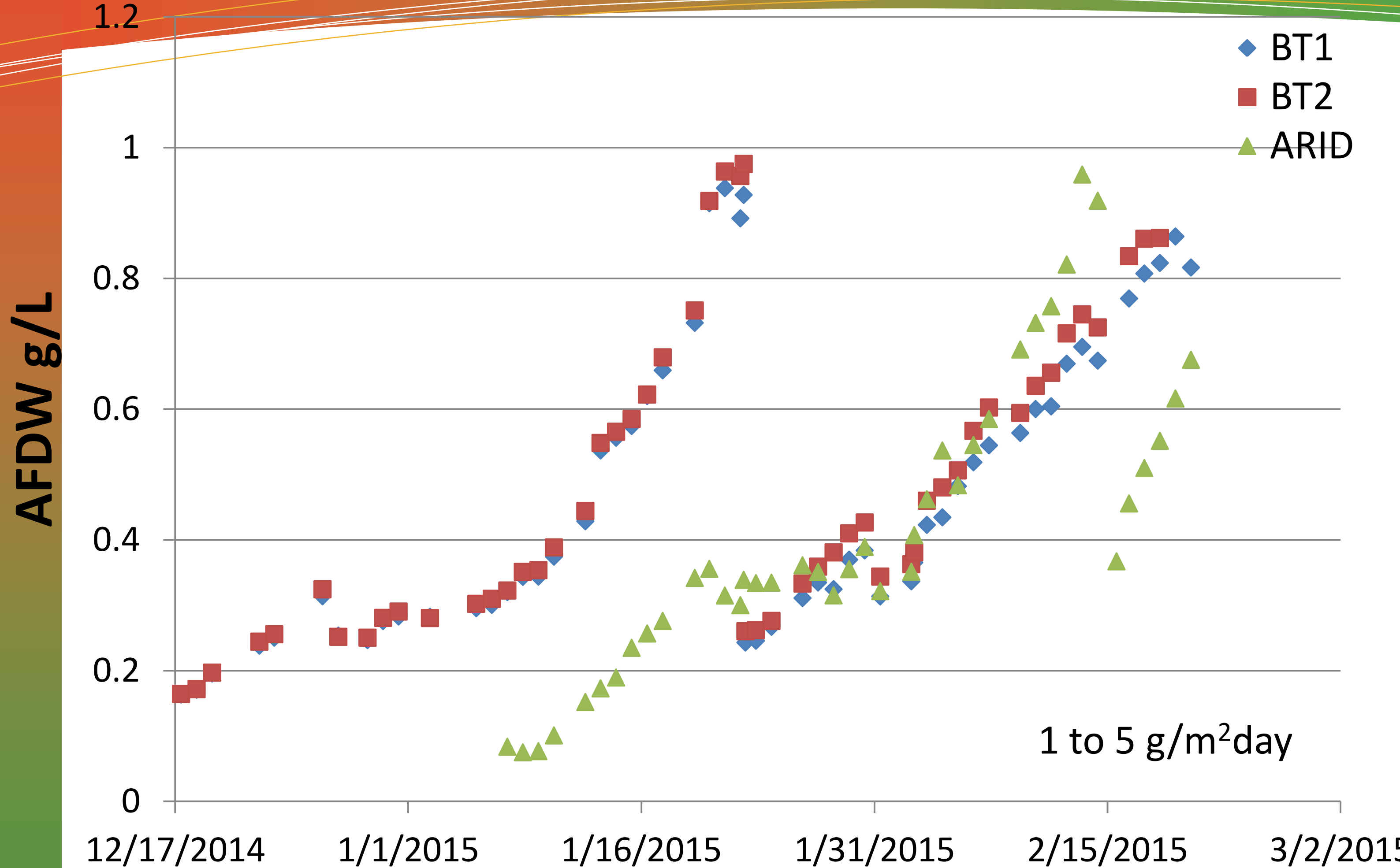
% of Living Cells(DOE 1412 w/VV)



3 – Technical Accomplishments

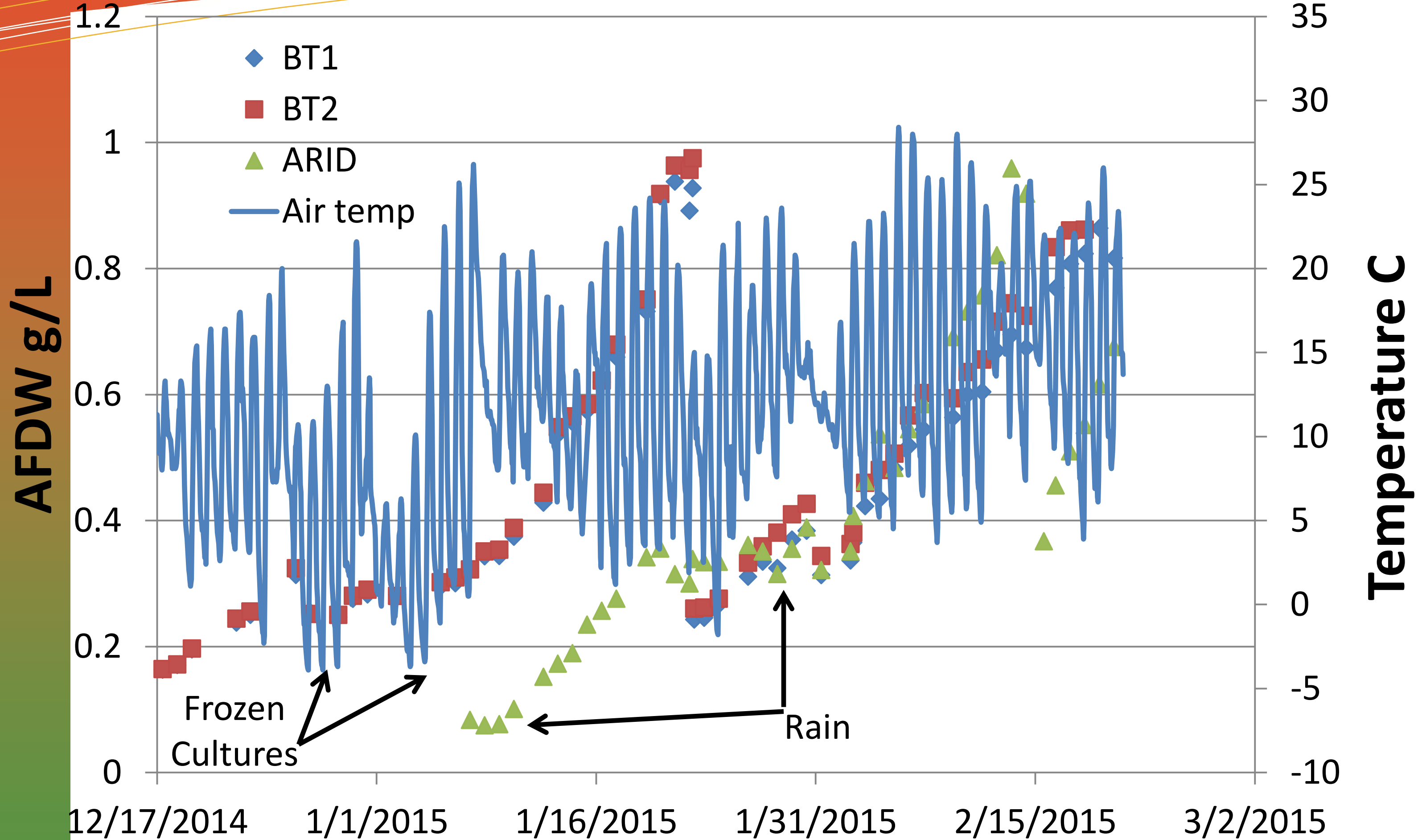


Scenedesmus



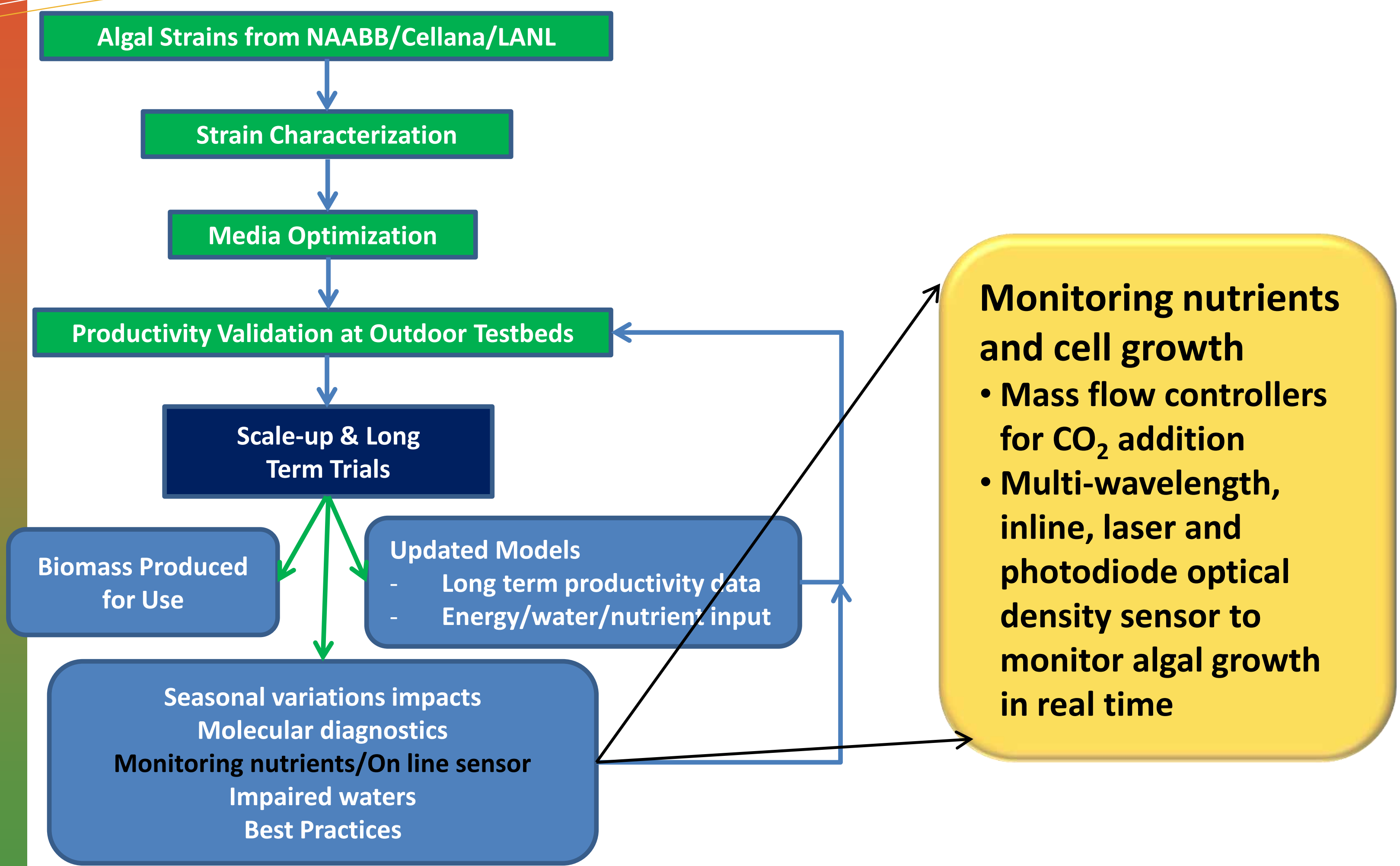
Winter cultivation studies have begun – able to grow semicontinuous culture for 2-3 months at 2 sites

Scenedesmus



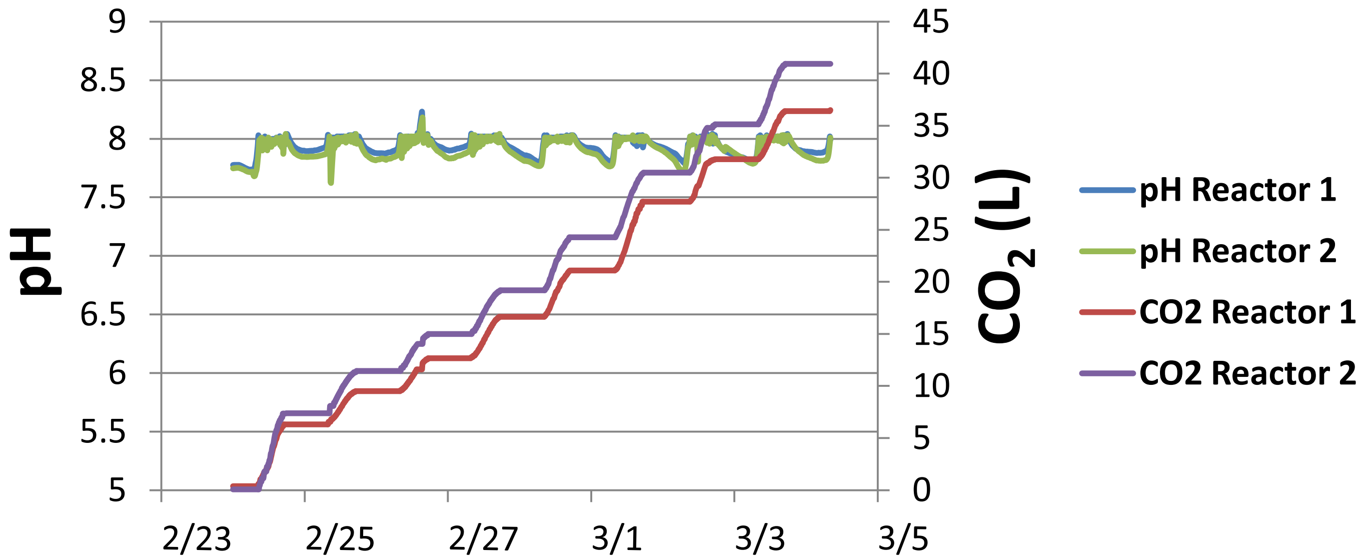
Temperature (frozen ponds) and rainy days limiting growth but strains recover w/o restart

3 – Technical Accomplishments

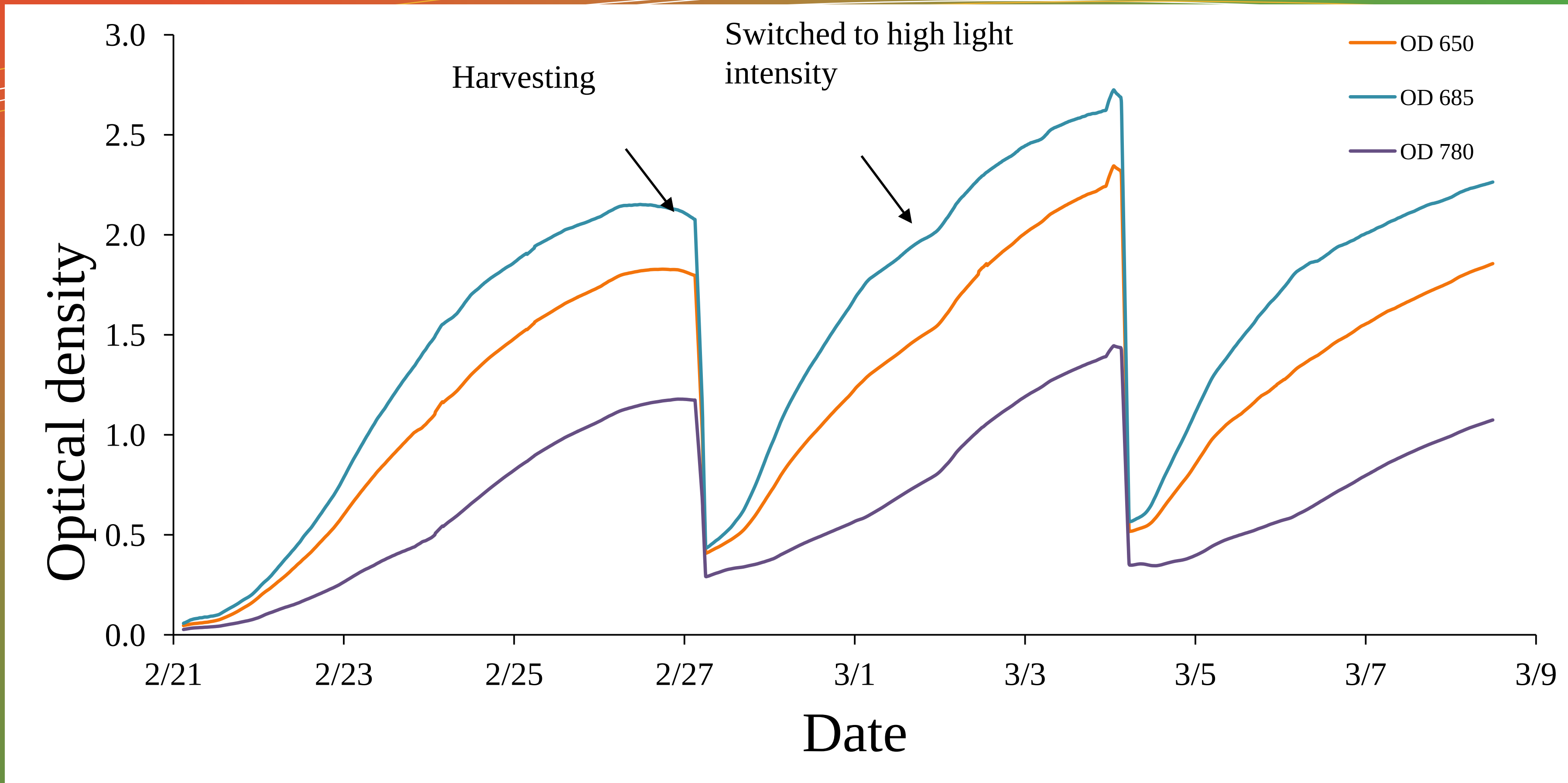


Established Techniques

- Water chemistry – ICP/MS
- Phosphate – colorimetric kit and/or IC
- Urea – urease kit/ Berthelot assay
- CO_2
  - Need to optimize set point
  - Combination of on/off and mass flow controllers



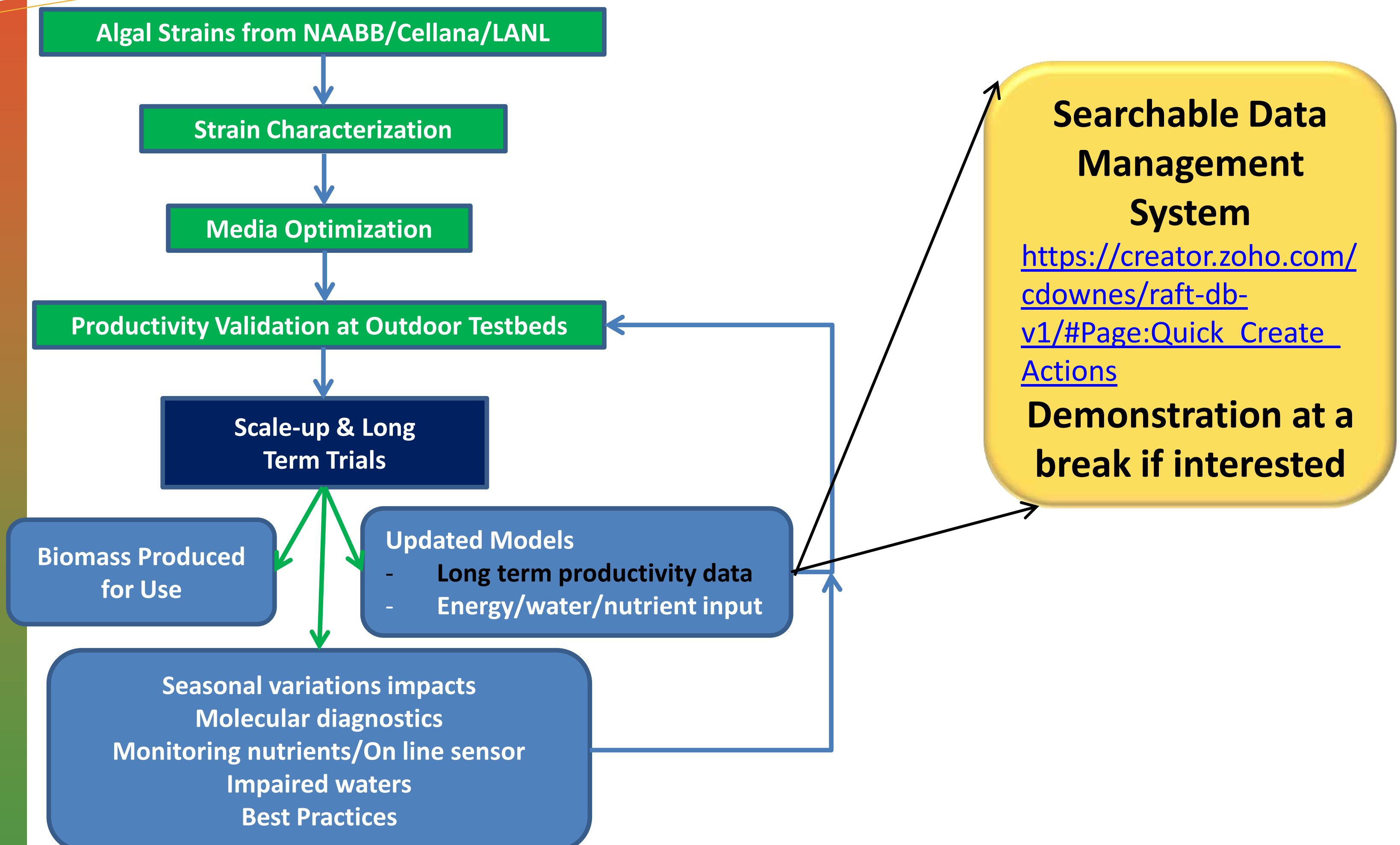
Continuous Real time OD Measurement

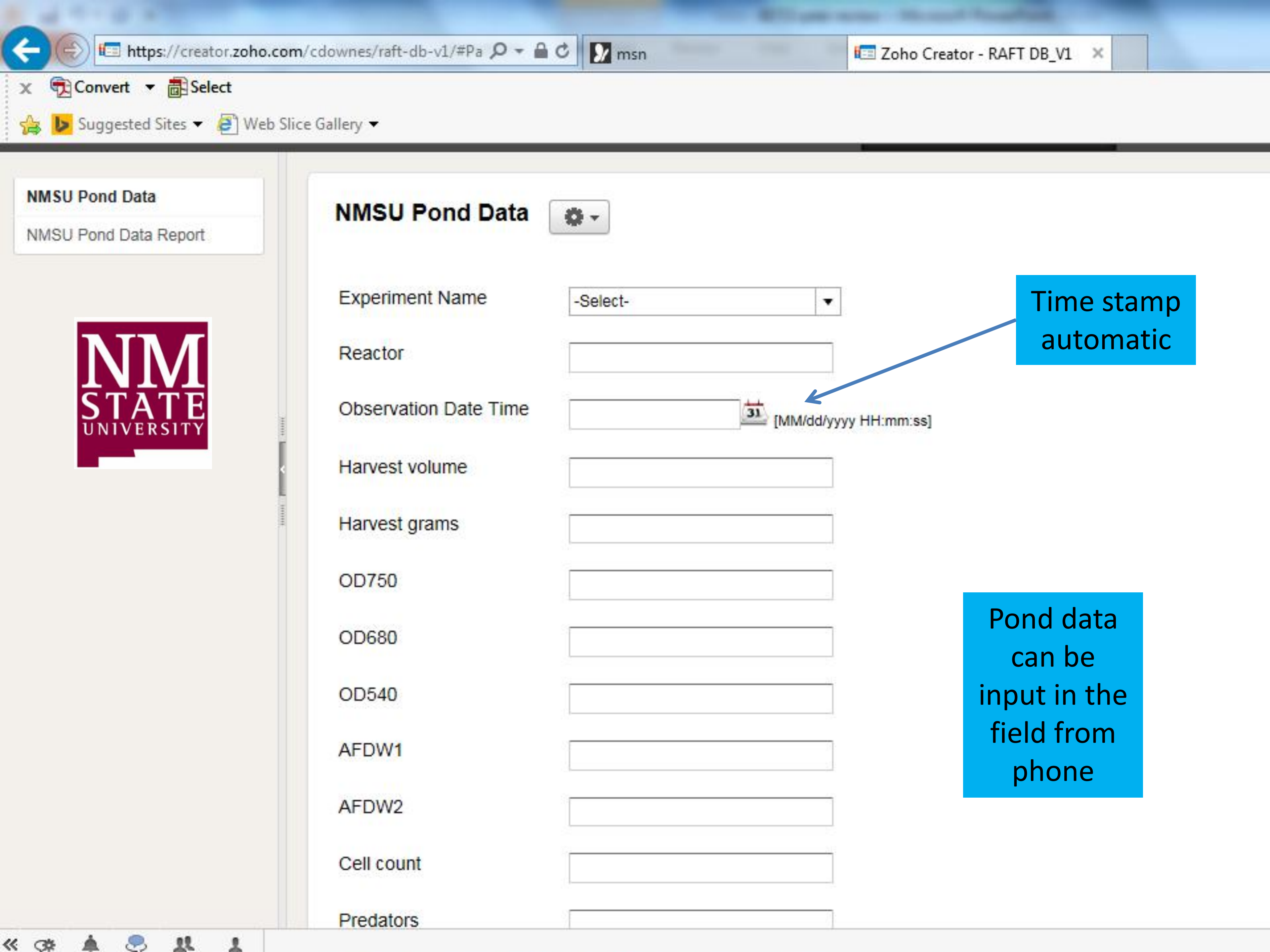


Continuous cultivation of *Chlorella sp*. in PBR: continuous light, pH control, T, and OD readings in new multi-wavelength laser diode/photodiode based sensor (IP Disclosure Filed)

Correlates well with AFDW (g/L) AFDW= $0.41 * OD_{780} + 0.055$ g L^{-1} ($R^2 = 0.95$)

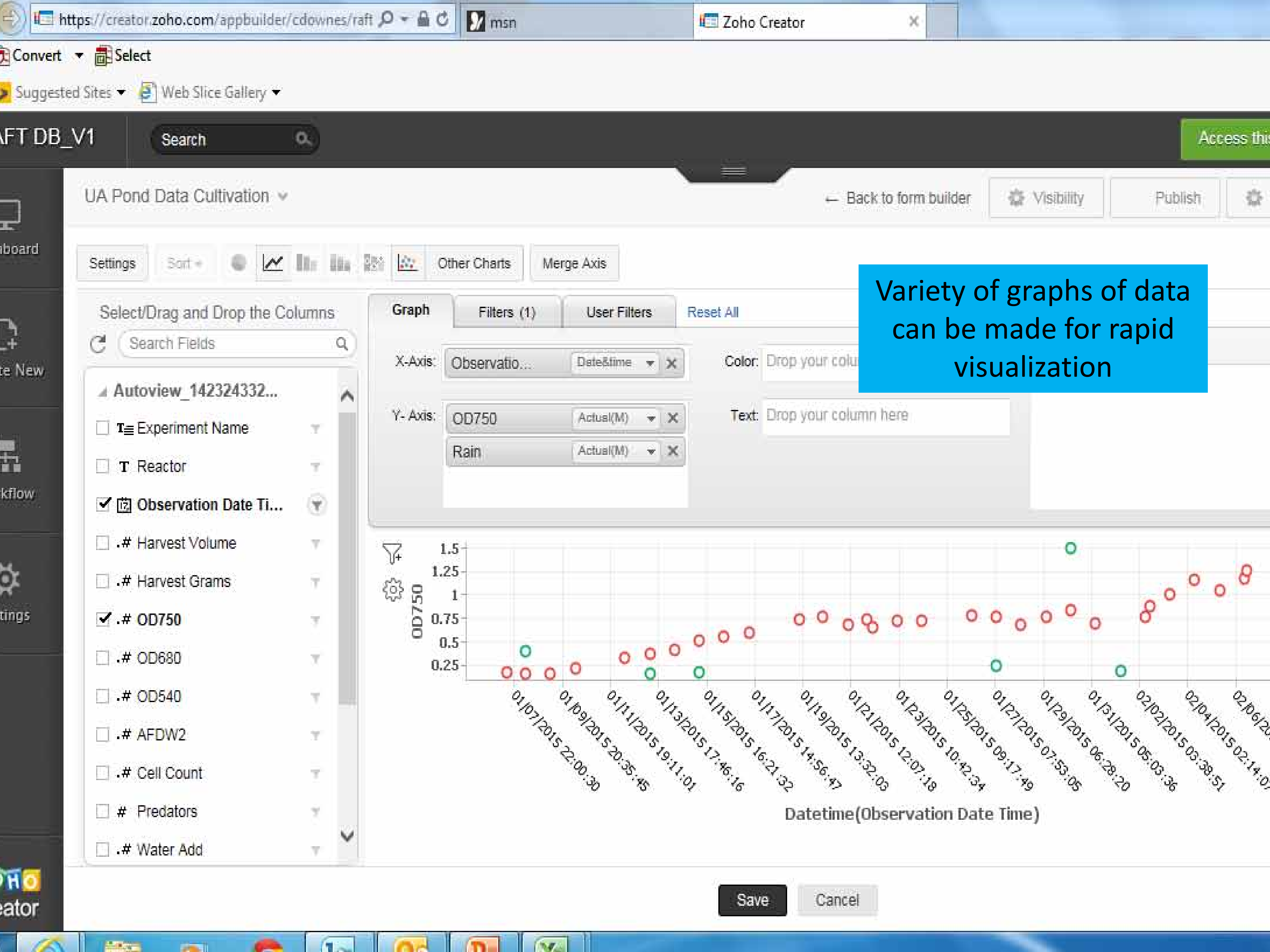
3 – Technical Accomplishments

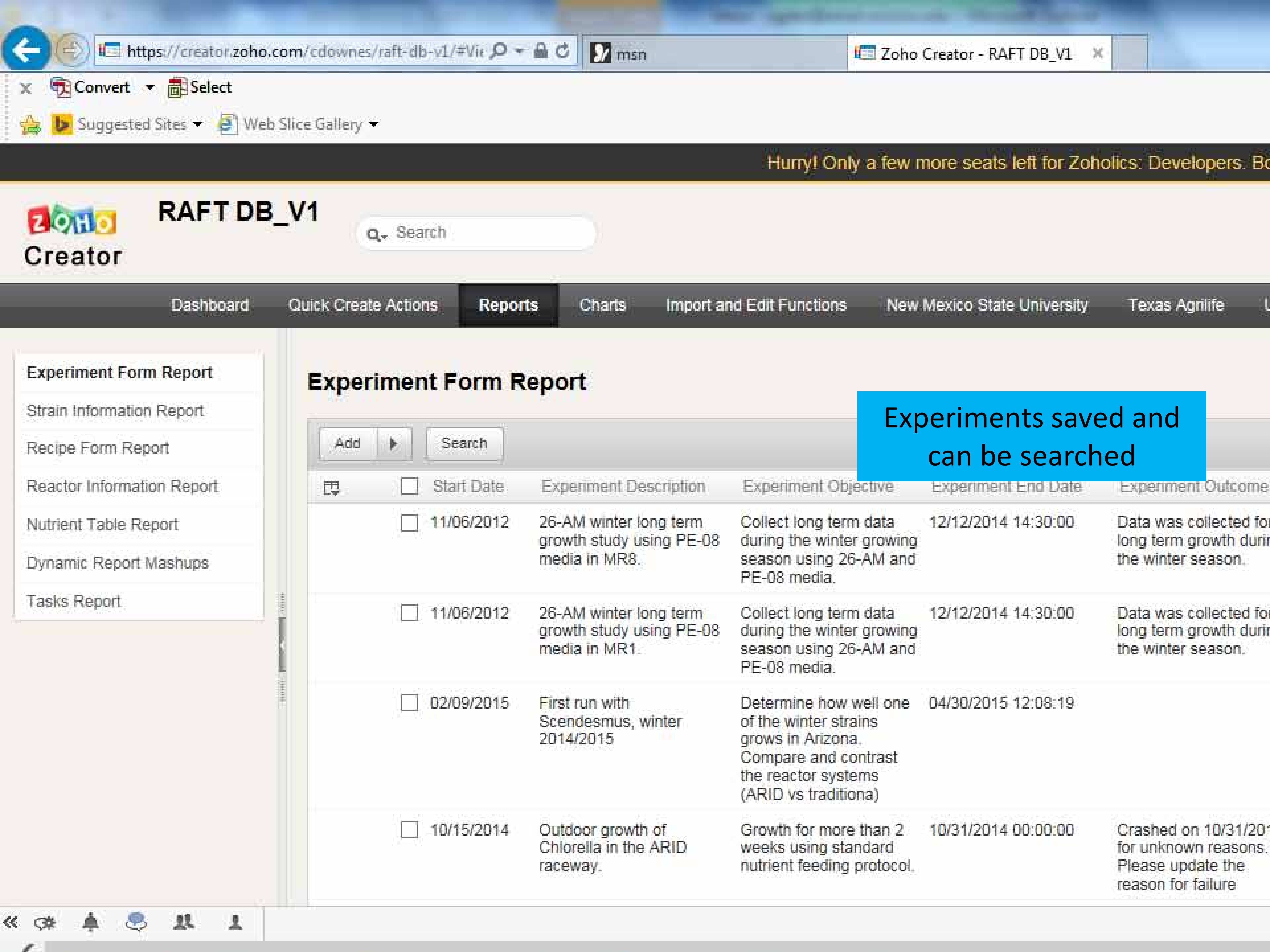




Time stamp automatic

Pond data can be input in the field from phone

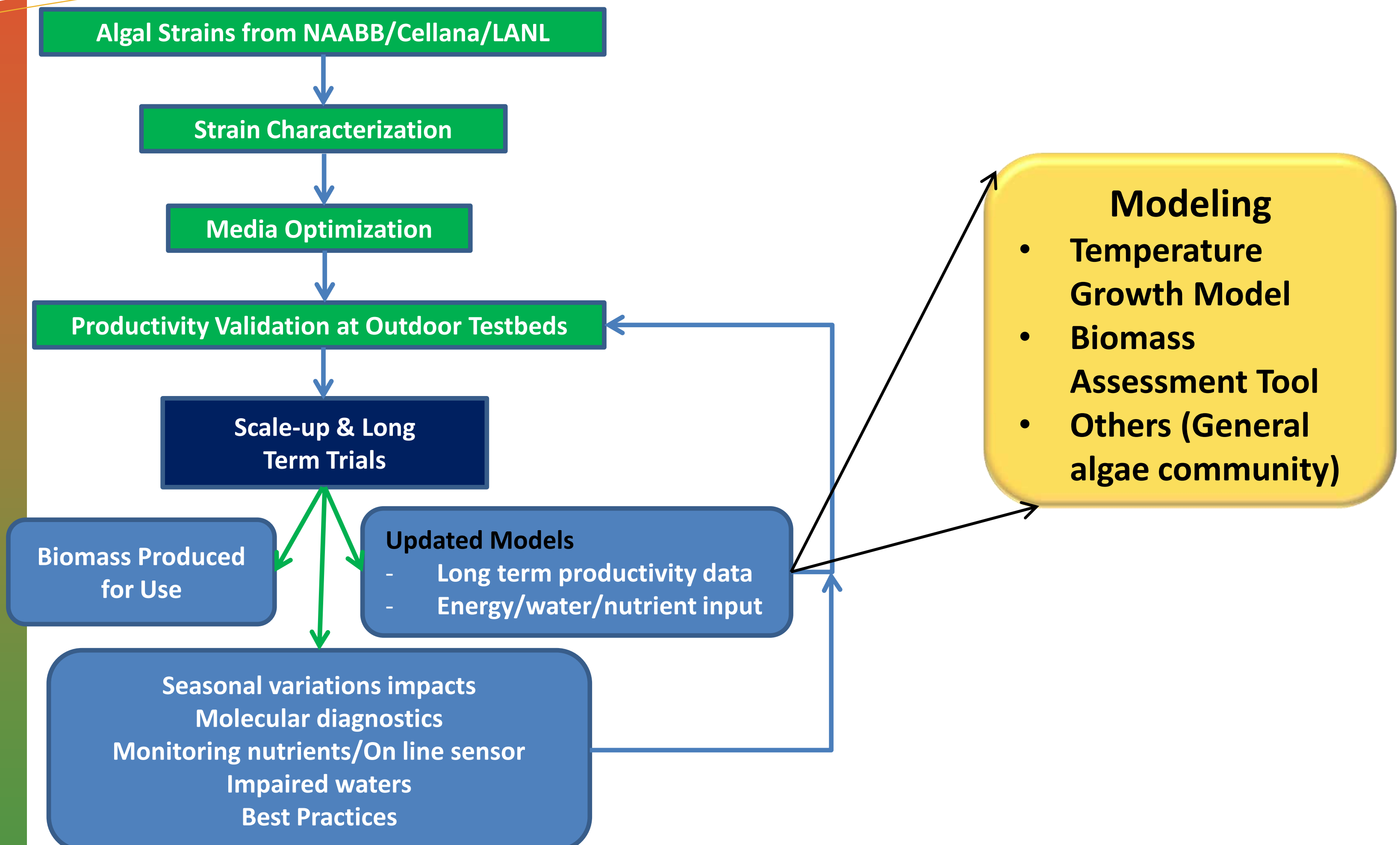

Variety of graphs of data can be made for rapid visualization
Zoho Creator
UA Pond Data Cultivation
Back to form builder
Visibility
Publish
Settings
Sort
Other Charts
Merge Axis
Select/Drag and Drop the Columns
Search Fields
Graph
Filters (1)
User Filters
Reset All
X-Axis: Observatio... Date&time
Y- Axis: OD750 Actual(M)
Rain Actual(M)
Color: Drop your colu
Text: Drop your column here
Autoview_142324332...
Experiment Name
Reactor
Observation Date Ti...
Harvest Volume
Harvest Grams
OD750
OD680
OD540
AFDW2
Cell Count
Predators
Water Add
1.5
1.25
1
0.75
0.5
0.25
OD750
01/07/2015 22:00:30
01/09/2015 20:35:45
01/11/2015 19:11:01
01/13/2015 17:46:16
01/15/2015 16:21:32
01/17/2015 14:56:47
01/19/2015 13:32:03
01/21/2015 12:07:18
01/23/2015 10:42:34
01/25/2015 09:17:49
01/27/2015 07:53:05
01/29/2015 06:28:20
01/31/2015 05:03:36
02/02/2015 03:38:51
02/04/2015 02:14:07
Datetime(Observation Date Time)
Save
Cancel



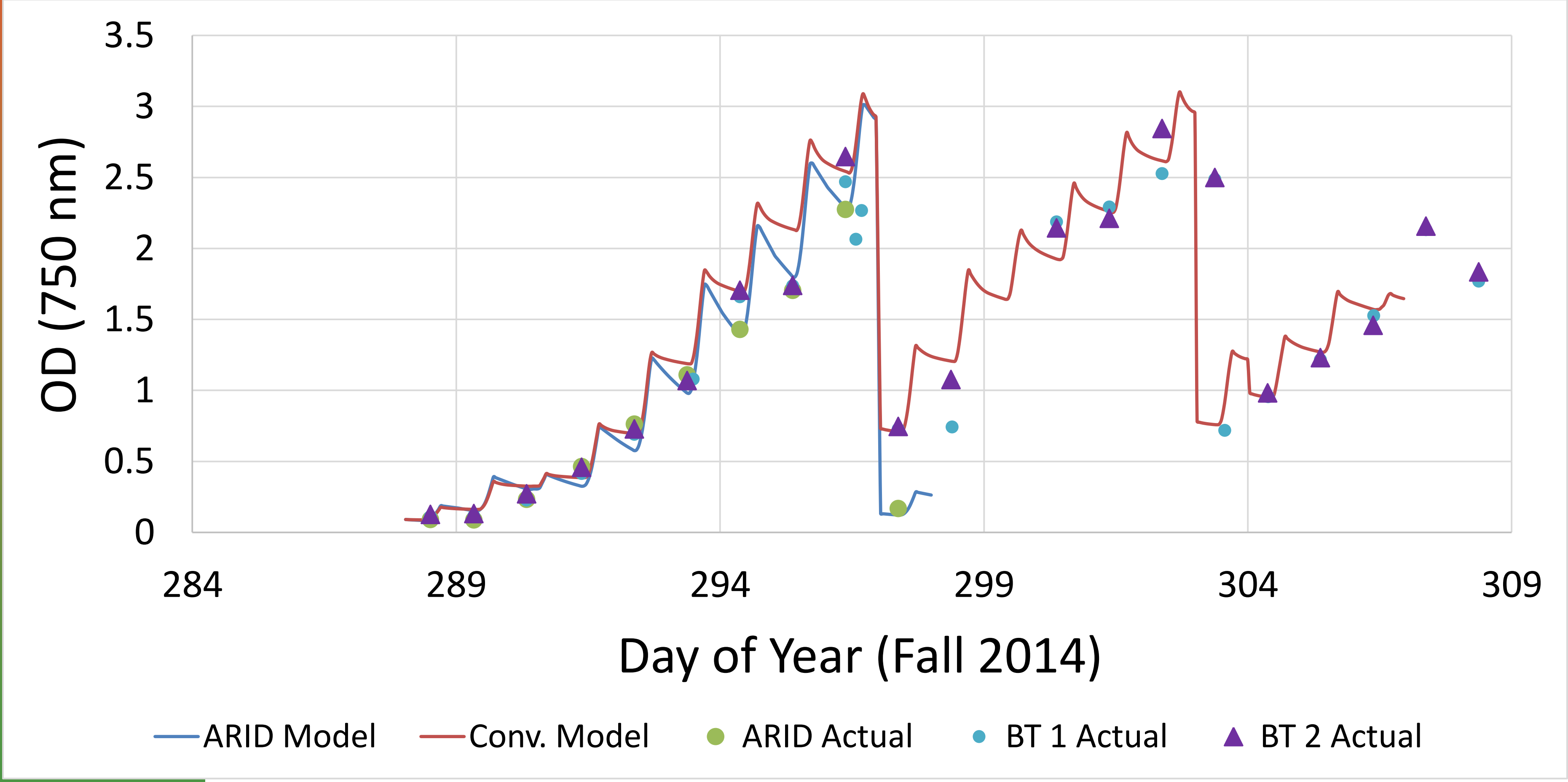
Experiment Form Report

Start Date	Experiment Description	Experiment Objective	Experiment End Date	Experiment Outcome
11/06/2012	26-AM winter long term growth study using PE-08 media in MR8.	Collect long term data during the winter growing season using 26-AM and PE-08 media.	12/12/2014 14:30:00	Data was collected fo long term growth duri the winter season.
11/06/2012	26-AM winter long term growth study using PE-08 media in MR1.	Collect long term data during the winter growing season using 26-AM and PE-08 media.	12/12/2014 14:30:00	Data was collected fo long term growth duri the winter season.
02/09/2015	First run with Scendesmus, winter 2014/2015	Determine how well one of the winter strains grows in Arizona. Compare and contrast the reactor systems (ARID vs traditiona)	04/30/2015 12:08:19	
10/15/2014	Outdoor growth of Chlorella in the ARID raceway.	Growth for more than 2 weeks using standard nutrient feeding protocol.	10/31/2014 00:00:00	Crashed on 10/31/201 for unknown reasons. Please update the reason for failure

3 – Technical Accomplishments



Comparison between outdoor pond growth and model in ARID and Conventional raceways



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, managed and 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 of *Chlorella sorokiniana* pond crashes

4 – Relevance

- Provided QC monitoring of algal cultures at all stages of growth and scale-up 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
  - Invention disclosure filed for inline OD sensor for continuous OD monitoring
  - Continuous OD monitoring with inline OD sensor currently being tested outdoors

RAFT
Regional Algal Feedstock Testbed

5- Future Work

Algal Strains from NAABB/Cellana/LANL

Strain Characterization

Additional summer strains - *Tetraselmus*

Media Optimization

Develop media for additional summer strains

Productivity Validation at Outdoor Testbeds

Continue winter strain productivity testing throughout spring and switch to summer strains

Scale-up & Long Term Trials

Scale up at Pecos from 180L to 3,800 and 7,600 L

Biomass Produced for Use

Updated Models
- Long term productivity data
- Energy/water/nutrient input

Continue to upgrade data management system
Validate models with testbed data
Provide feedback to testbeds
Continue nutrient, energy & water monitoring

Seasonal variations impacts
Molecular diagnostics
Monitoring nutrients/On line sensor
Impaired waters
Best Practices

Impaired water studies

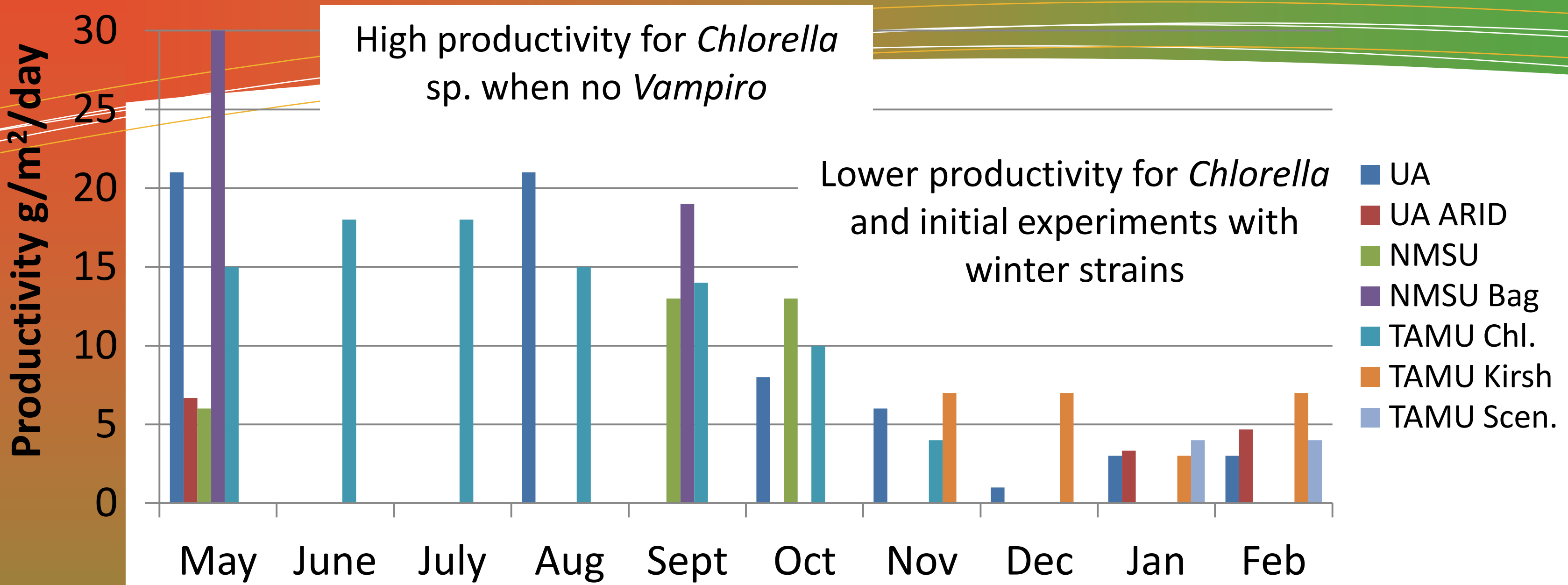
- NMSU – primary effluent
- TAMU – Galveston waste water, Pecos agricultural wells
- UA – secondary effluent, recycled media

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

5 – Future Work

- Go/No Go Decision in Summer 2015
  - Establish outside reviewer strategy with DOE
  - Submit written summary report
    1. Demonstrated significant technical progress against R&D Plan
    2. Two seasons of cultivation data obtained and provided to DOE harmonization modeling group
    3. Productivity data for two freshwater and two impaired water strains obtained
    4. Data from seasonal variances captured
  - Present future strategy/research plan
  - Address questions

Summary



1. **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 -** Established data management system; Defined system for monitoring growth, productivity, nutrients and culture health
4. **Relevance** - Model/productivity driven comprehensive research plan to meet DOE objectives
5. **Future work -** Impaired waters; Larger scale; Process control

Additional Slides

Publications, Patents, Presentations, Awards, and Commercialization

- **Papers**
  - 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., M. Kacira, K. Ogden. 2015. Multi-wavelength laser diodes based real time optical sensor for microalgae production application. *Bioresource Technology*
  - 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
  - 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
- **Invention Disclosures**
  - Jia, F., M. Kacira, K. Ogden, G. Ogden. 2014. Optical device for in-line and real-time monitoring of microalgae. (Invention Disclosure) University of Arizona.
- **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**
  - 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.
  - 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
  - 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
- Supply Biomass
  - Testbeds have supplied biomass to the PNNL HTL project
  - Biomass available to other researchers upon request