DOE Bioenergy Technologies Office (BETO)

2023 Project Peer Review

Innovations in Algae Cultivation

April 4, 2023

Renewable Carbon Resources – Algae

David Hazlebeck

Global Algae Innovations

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Global Algae Innovations
Algae Solutions to Global Dilemmas

Vision

Harness the unparalleled productivity of algae to provide food and fuel for the world, dramatically improving the environment, economy, and quality of life for all people

- Founded Dec 2013
- Algae for commodities
- Technology development in 8-acre Kauai Algae Farm
- Radical advances throughout the entire process
- Selected as XPRIZE Carbon Removal milestone award winner in 2022
- Scaling–up suite of novel technologies in new San Luis Obispo County Farm

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Project Overview – Goals

- **Develop tools for innovations in cultivation**
  - Test systems from laboratory through mass cultivation
  - Spectroscopic measurement of algae composition
- **Use tools and innovative methods to achieve improved productivity**
  - Increase productivity by 50%
  - Achieve 31% lipid content with 2 to 3-day lipid formation
  - Cultivation methods consistent with overall process that achieves
    - $3.00/gallon of gasoline equivalent (GGE) for biofuel intermediate at 5,000-acres
    - 50% GHG reduction for biofuel relative to petroleum fuels

Project Overview – Accelerating Cultivation R&D

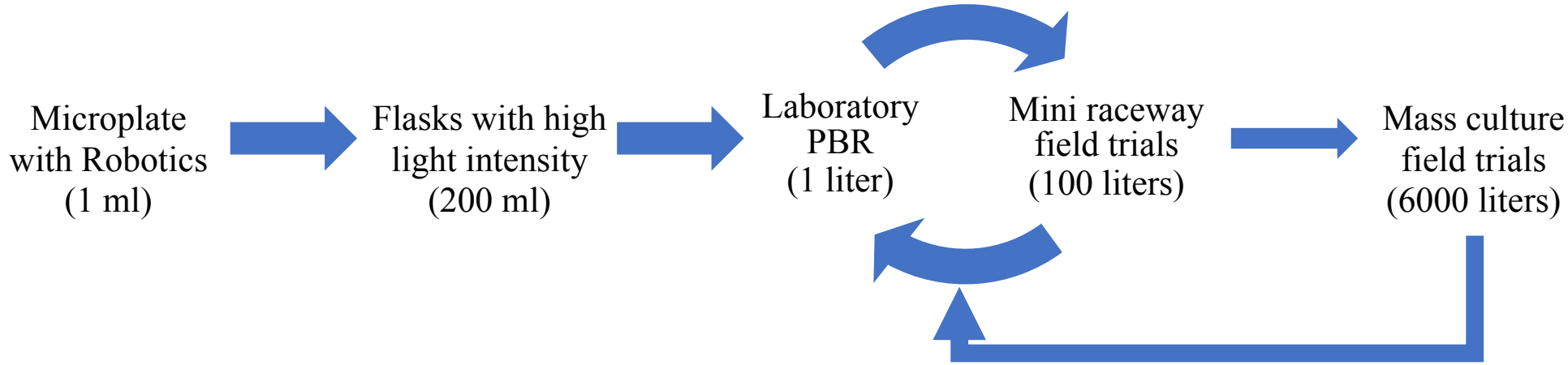
- **Difficulty in scaling down advanced cultivation systems**
  - Many of our cultivation advances have not been deployed below 0.2-acre
  - Actual methods cannot be duplicated in very small systems like mini-raceways
  - Need higher fidelity systems for mimicking the culture conditions achieved in large-scale raceways in smaller-scale test systems
- **Realtime measurement of algae composition**
  - Tracking impact of cultivation condition changes on algae composition
- **Over 100 ways to increase productivity through cultivation methods**
  - Many are too expensive or to energy intensive, such as most closed photobioreactors
  - All advances must be constrained through detailed techno-economic analysis (TEA)
  - Planning 20 patent applications this year of which 4 are from this project

Approach

1. **Budget Period 2 (complete)**
   - Prepare samples and down select sample preparation for spectroscopy
   - Build out photobioreactors, mini-raceways, and intermediate raceways
2. **Budget Period 3 (complete)**
   - Spectroscopic method to predict protein and lipid content of algae
   - Correlate productivity between different experimental system scales
   - Use systems to develop cultivation methods to improve productivity and lipid content
   - Down-select to two methods or tools for more detailed research
3. **Budget Period 4 (expected to start in May or June)**
   - Implement spectroscopic method at the farm and use to accelerate research
   - Obtain consistent lipid content of 45-50% with protein of 30-35%
   - Optimize process lipid formation method
     - Minimize area
     - Higher productivity and consitency
     - Higher protein content in final product

Approach – Test Systems

- **New cultivation test systems**
  - Microplate tests with pH, temperature, light control
  - Shaker Flasks with air bubbling and high intensity light
  - New photobioreactor with programable pH, temperature, light, dissolved oxygen, depth, mixing rate, and air/liquid contacting
  - New outdoor sloped mini-raceways with propeller pumps and added air/liquid contacting
  - New 60m^2 raceways with same advanced methods as larger-scale raceways



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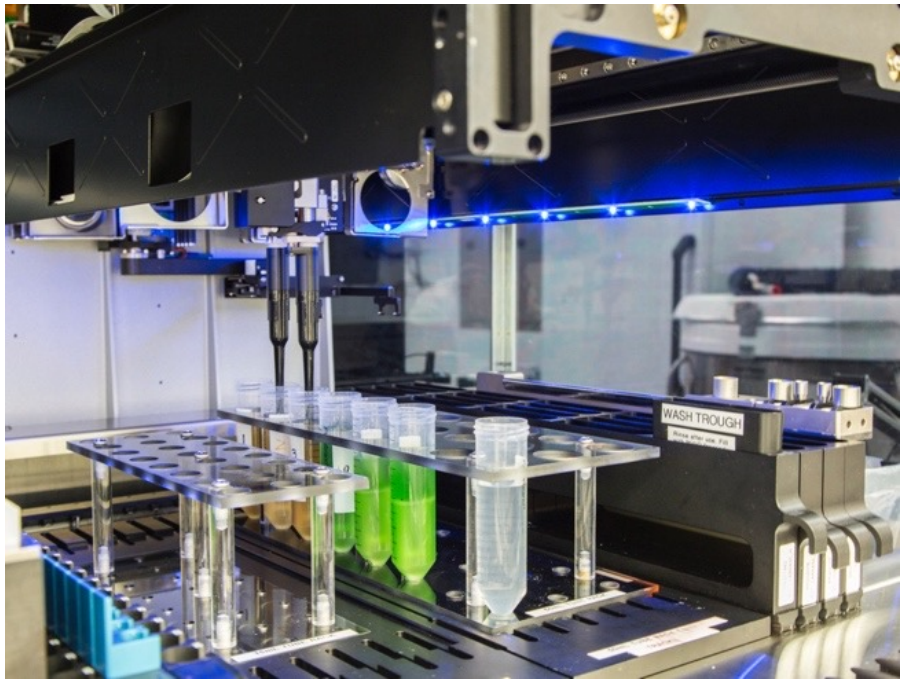
Approach - Rapid compositional analysis by spectroscopic prediction

- Robotic processing and data collection
- Multivariate spectroscopy,, sample presentation, configuration
- Novel machine learning algorithms on complex spectra
  - Partial Least Squares linear regression analysis
  - Artificial Neural Networks (ANN)
- Initial samples to establish sample preparation methods
- Approximately 200 samples with wide variety of compositions
  - 80% of samples used for model development
  - 20% to of samples test the models



Approach - Cultivation Improvements

- **TEA constrained**
  - Update TEA throughout for decision making and analysis of cultivation options

- **Cultivation Improvements**
  - Nutrient mix and timing of additions
  - Abiotic conditions
  - Biotic conditions
  - New strains or co-cultures
  - Lipid formation method
  - Respiration control

Approach – Testing in Outdoor, Open Raceways

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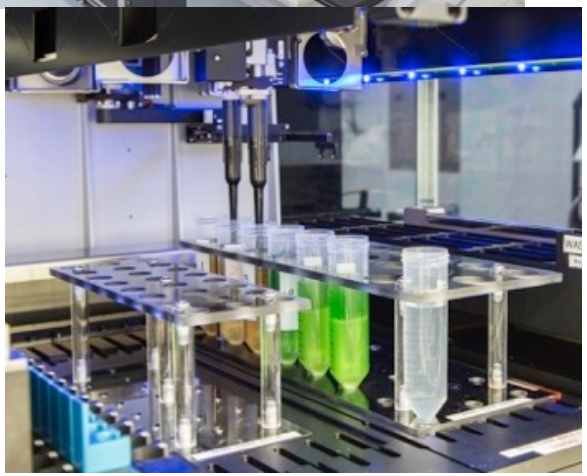
Approach – Key Technical Challenges

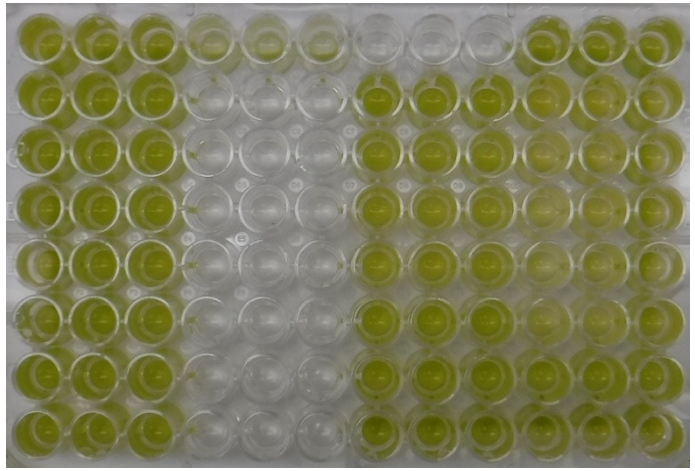
- **Tool development**
  - Bringing three new test systems on-line with multiple bioreactors in each system
  - Developing correlations for cultivation between difference test scales
  - Developing correlations for cultivation between laboratory and outdoors
  - Narrowing sample preparation methods to optimum for spectroscopic analysis
  - Obtaining wide range of samples to develop spectroscopic compositional analysis

- **Cultivation improvements**
  - Balancing resources between tool development and cultivation improvements
  - Improving productivity and lipid content from prior high levels

Microplate & flask testing



- 96-well microplate format for growth experiments
- Screen algae for OD based specific growth rate and final OD as indicator of biomass produced



Operational issue
Need microplate pH measurement
– *being added on a new project*

- Grow in 1-liter flasks in incubator on stir plates
- High light (>1000 umol/m^2s) during part of the day
- Daily samples for analytical testing
- Transfer every 48 hours

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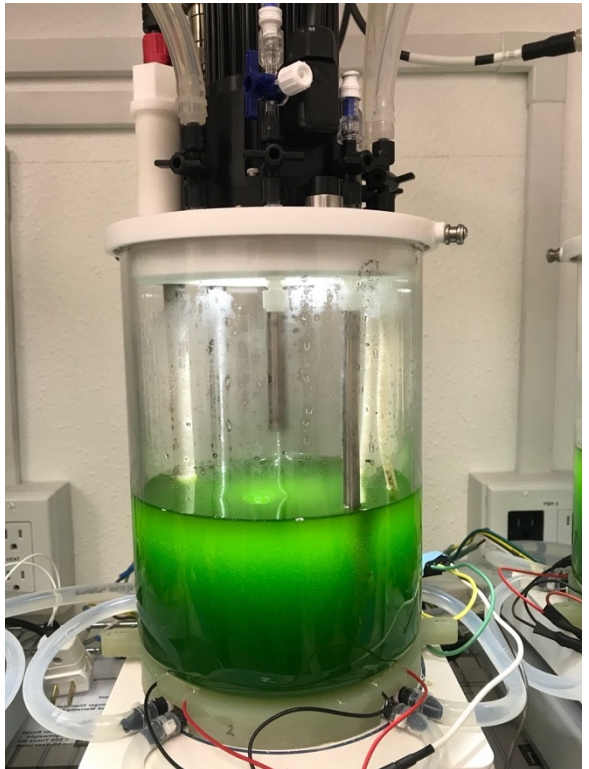
Laboratory Photobioreactors

PLC controlled growing system

- 6 reactors dedicated to this project enable triplicate experiments
- LED illuminator to simulate natural sunlight including seasonal and daily scripts
- Top-down light
- Heater and thermoelectric coolers
- Peristaltic pumps for media and harvesting
- Variable mixing rate
- Filtered N_2/O_2 and CO_2 additions to control pH and dissolved oxygen
- Monitors temperature, pH, DO, OD, and conductivity

Operational issues

1. Light distribution and intensity – *adjusted scripts based on detailed intensity measurements at 25 locations; worked with vendor to improve output*
2. Control & data acquisition – *longer than anticipated to shakedown all reactors; variability between reactors.*



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2m² sloped raceways

- **Depth control from 2.5 – 16.5 cm**
- **Automated concentrated media feed**
- **Automated recycled or fresh media feed**
- **Automated controls for pH, dissolved oxygen, and temperature**
- **Continuous instrumentation monitoring via sondes**
  - **Temperature**
  - **Dissolved Oxygen**
  - **Conductivity**
  - **pH**
- **Volume monitoring via pressure sensor**

Operational issues

1. Time between propeller pump failures – *alternate supplier work with us to improve reliability*
2. Too large of a depth range – *built two sets of shells*
3. Complicated by fertilizer issue – *procured new fertilizer*
4. Inability to adequately simulate air-liquid contacting/dissolved oxygen level –*new equipment added*

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Intermediate Scale Raceways - 60 m^2

- Automated concentrated media feed
- Automated recycled or fresh media feed
- Automated controls for pH, dissolved oxygen, and temperature
- Continuous instrumentation monitoring via sondes
  - Temperature
  - Dissolved Oxygen
  - Conductivity
  - pH
- Volume monitoring via pressure sensor
- Goal: Good correlation with full-scale raceways

Operational issue

Appears to simulate full-scale raceways well but a fatal design flaw in sump seals and pumps precludes getting accurate productivity data - *Need to shift to different sump design, use same sealing as full raceways, and better pumps; not enough funding available to make the change*

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Larger-scale raceways available to perform testing at mass culture scale

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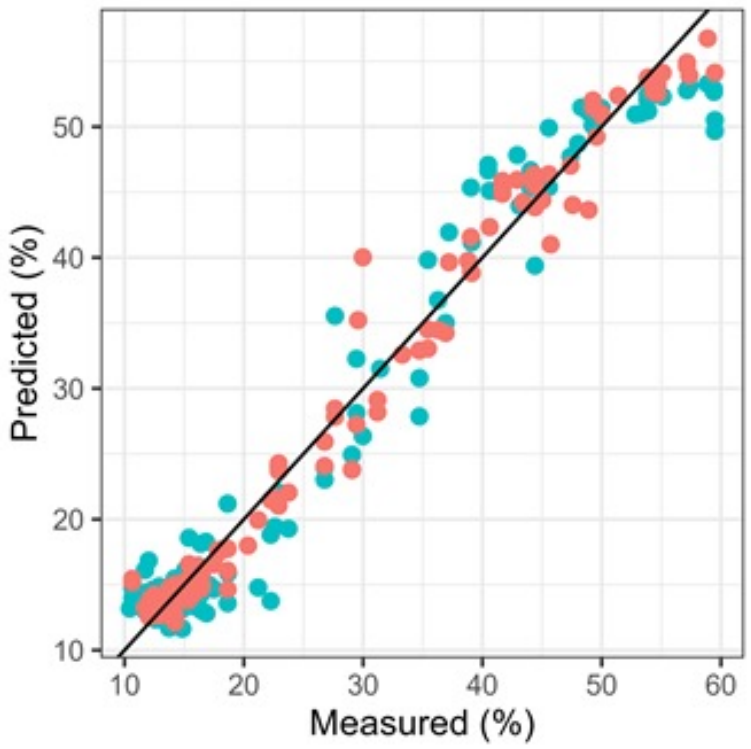
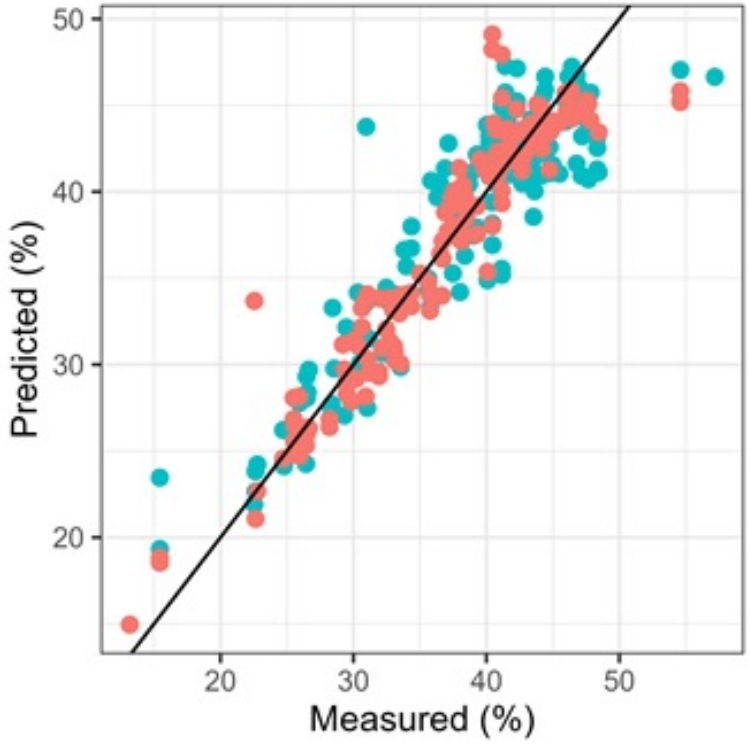
Spectroscopic Measurement of Algae composition

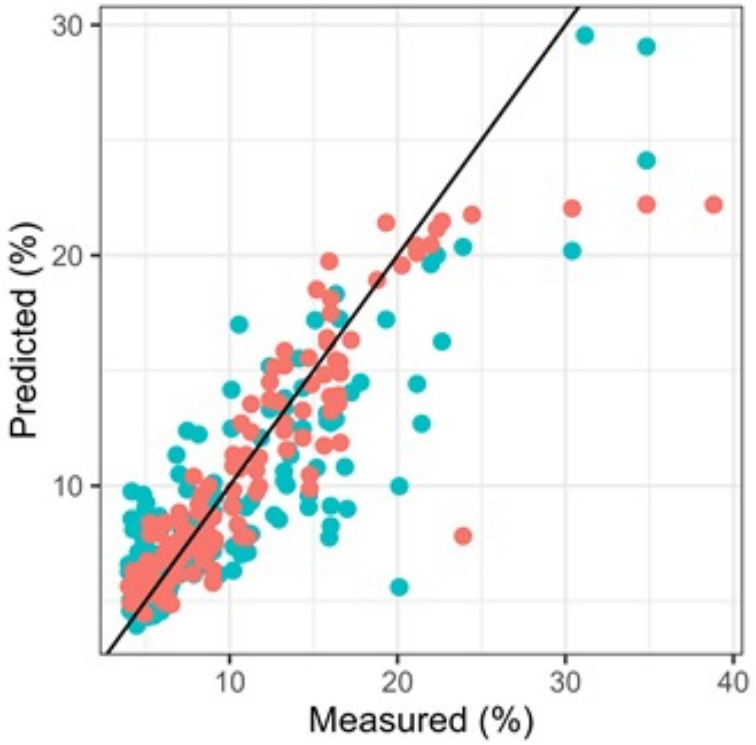
Lipid



Protein



Carbohydrate



Lessons learned:

1. Many more samples than anticipated to define sample preparation method
2. Parametric testing required for most sample preparation variables

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Only two of the biotic approaches tested had a significant impact on productivity

Method	Productivity relative to control
Bleach	88%
Peroxide	102%
Biofilm prevention	100%
Proprietary 1	101%
Proprietary 2	103%
Proprietary 3	123%

Plan to apply for patents this year

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Abiotic methods

Proprietary 5	Fall	117%
Proprietary 4	Fall	143%
Proprietary 5	Fall	145%
Proprietary 6	Fall	115%
Proprietary 7	Winter	105%
Proprietary 4,6	Winter	158%
Proprietary 4,6,7	Winter	165%

Plan to apply for patents this year

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Progress and Outcomes – lipid formation and new strains

- **Lipid content**
  - Goal: 31% lipid content with 2 to3 day lipid formation in outdoor open raceways
  - Achieved: 49% lipid content with 2-day lipid formation in outdoor open raceways
    56% lipid content with 3-day lipid formation in outdoor open raceways
  - Results replicated in laboratory reactors simulating outdoor conditions at Global Algae and at Colorado School of Mines
- **New strains**
  - 26 new green algae strains that grow well at higher pH
  - Best to-date in the direct air capture pH range is a *Chlorella sorokiniana*
  - Several other strains are promising, but have not been fully vetted

Impact of Successful Completion

- **New tools available for cultivation research and development**
  - Photobioreactors
    - Global Algae on multiple DOE projects;
    - University of California at San Diego on multiple DOE projects
  - Mini-raceways
    - Global Algae on multiple DOE projects;
    - Colorado School of Mines and Pacific Northwest National Laboratory
  - Instrument for rapid algae composition measurement (through Global Algae Equipment)
- **Improved economics and life cycle of algal biofuels**
  - 50% lipid content in outdoor raceways from ~26% before project
  - 50% higher productivity

Summary

- **New tools for cultivation research and development**
  - Test systems from microplate through mass-culture
  - Spectroscopic method for rapid analysis of algae composition
- **Cultivation improvements to date**
  - Achieved increase in lipid content from 26% to 40-50% in all seasons
  - Achieved increase in productivity of 15-50% depending on season
- **Status**
  - Completed tool development and scoping cultivation research and development
  - Moving to detailed development of two down-selected approaches
    1. Further development and use of the rapid algae composition measurement
    2. Further development of the lipid formation method

QUAD Chart Overview

Timeline

- *BP2 start date: June 2020*
- *Project end date: Dec 2024*

	FY22 Costed	Total Award
DOE Funding	$1,150,000	$4,500,000
Project Cost Share	$288,000	$1,125,000

TRL at Project Start: 3
TRL at Project End: 4

Project Goal

Develop tools - new test systems correlated from laboratory though mass-culture and a spectroscopic algae composition measurement. Use tools to conduct R&D on cultivation improvements to increase productivity, lipid content, and process robustness.

End of Project Milestones

- Increase productivity by 50%
- Increase lipid content to 31%

Funding Mechanism

FY19 Multi-topic FOA - CIPA

Project Partners:

- Hamilton Robotics

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Additional Slides

Responses to Previous Reviewers' Comments

- No recommendations from previous reviewer comments

Publications, Patents, Presentations, Awards, and Commercialization

- None to date
- Planning 4 patents from scoping research and development
- After detailed development plan to commercialize analytical instrumentation and method

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