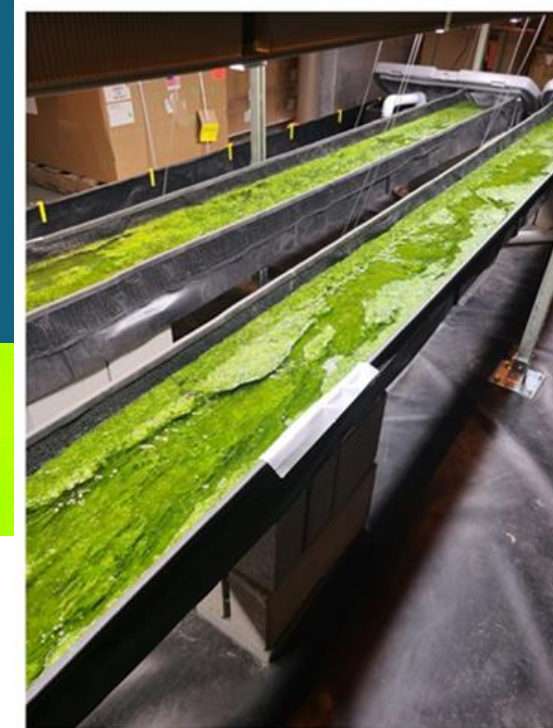
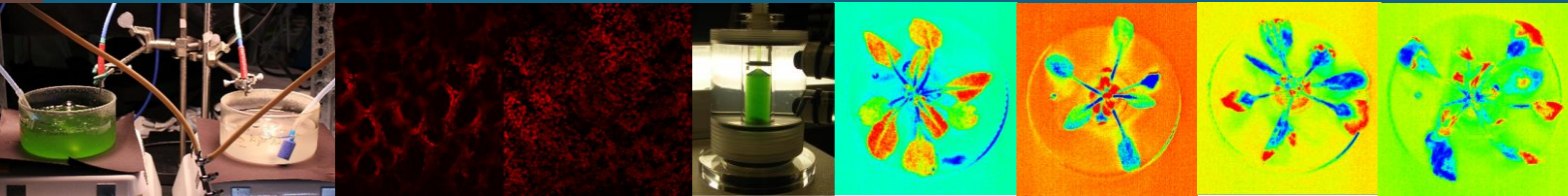


# Attached Periphytic Algae Production & Analysis



March 23, 2021  
Advanced Algae Systems

*PRESENTED BY*

**Ryan W Davis**

**Sandia National Laboratories**

This presentation does not contain any proprietary, confidential,  
or otherwise restricted information



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## Project Overview



- Attached algae cultivation provides means to surmount current cost drivers for algae biomass production, including nutrients and CO<sub>2</sub>, harvesting, and maintaining culture stability.
- We are testing attached algae production strategies based on Walter Adey's Algae Turf Scrubber concept and utilizing contaminated/nutrient laden sources waters for CO<sub>2</sub> and nutrients
- We are generating data to support TEA, LCA, GIS-RA, and CONOPS for scale-up, and identifying opportunities for ecosystem services (e.g. nutrient interception / HAB prevention) for further value-addition
- Project Objective: The current year goal is to demonstrate the ability for attached algae cultivation to simultaneously achieve 24 g/m<sup>2</sup>/day and <25% ash using compromised surface waters. The three-year goal of the project is to demonstrate attached algae cultivation coupled to water treatment with a net biomass cost of <\$450 /ton AFDW.

# I – Management



Task Name	FY21 Budget Authority
Task 1: Validation of flow-way optimization for biomass production	\$225,000
Task 2: Lab-scale flow-way harvesting optimization	\$225,000
Task 3: Systems engineering and Computational Fluid Dynamics modeling for flow-way design and operational optimization	\$225,000
Task 4: TEA, LCA, GIS-RA, CONOPS	\$75,000
Project Total	\$750,000

## Go/No-Go Decision

Name	Description	Criteria	Date
<b>Environmentally simulated, channeled flow-way biomass compositional quality</b>	Demonstrate ability of channeled flow-way to simultaneously provide productivities of at least 24 g m <sup>-2</sup> day <sup>-1</sup> with ash content <25%	~50% ash reduction is required for both bio- and thermochemical processing route viability	12/31/2020

# I - Management



Sandia National Laboratories [Ryan Davis, Eric Monroe] – Systems design, construction, operation, and analysis at Sandia Livermore Algae Testbed Facility, field deployment coordination and evaluation

Pacific Missile Range Facility, Barking Sands (U.S. Navy) [Brooke McFarland, Angela Merritt] – Stake-holder, deployment partner for reducing nutrient loading in discharge from base WWTP

Texas Agrilife, Georgia Southern University [Anthony Siccardi] – deployment operations for marine/estuarine source waters, Osso Bay, Laguna Madre, TX; Savannah River, Priest's Landing, GA

Colorado State University [Jason Quinn] – TEA/LCA/GIS-RA, and ecosystems services assessment (HAB prevention)

## **Industry Partners:**

Imperial Irrigation District [Steve Charlton] – Stake-holder, cost-share partner for metals remediation at Salton Sea

Imperial Valley Research Center [Wally Hale, Candace Nelson] – O/M support, biomass logistics for Salton Sea deployment, Brawley, CA

Hydromentia, Inc. [Mark Zivojnovich] - Consultation, flow-way hardware, and scale-up support

Global Algae Innovations [Rodney Corpuz, Leila Kamakele] – Installation, O/M, biomass logistics for deployment at Pacific Missile Range Facility – Barking Sands

Algix, Inc. [Ryan Hunt] – Evaluation of filamentous algae biomass for BloomFoam<sup>®</sup> application

Bimonthly meetings with SNL, CSU, GSU project teams. Frequent communication with PMRF, IID/IVCRC, Hydromentia, GAI, and Algix for deployment updates, timely delivery of samples

# Key partnerships & Industry Engagement



BLOOM Foam™ Algae Shoe



# 2 - Approach

6

- **Deployment and operation of field and testbed pilots:**

- Livermore Algae Testbed trials, and marine/estuarine (Corpus Christi, TX, riverine (Alamo River, CA), & WWTP-integrated (Navy Barking Sands, HI) deployments at partners sites. Challenges: austere/remote sites, limited physical security, harsh environments, source waters pose ES&H risks in some locales.

- **Determine productivity and composition:**

- AFDW, ash content, proximate analysis at Sandia. Challenges: dewatering in the field, reliable feedstock logistics

- **Identify periphytic cohorts:**

- 16S/18S amplicon sequencing, microscopy. Challenges: high biological diversity, heterogeneity on flow-ways

- **Quantify source water impacts:**

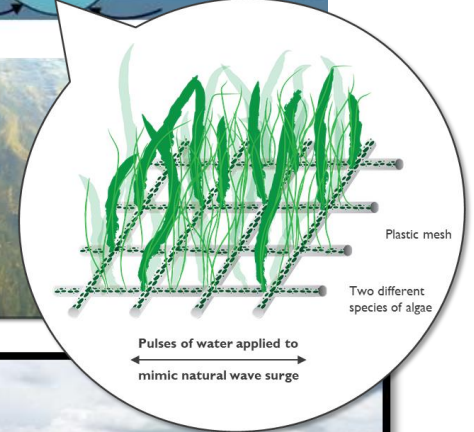
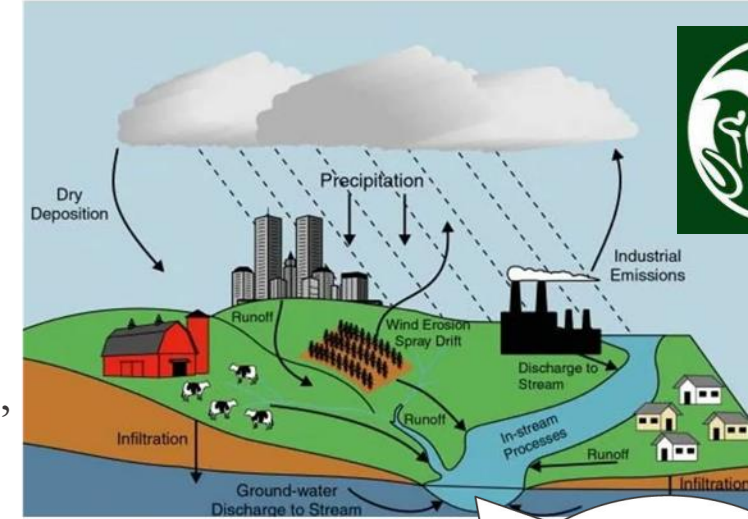
- Mass balances for NO<sub>3</sub>, NH<sub>4</sub>, PO<sub>4</sub>, BOD, TSS, RCRA-8 metals. Challenges: reliable sampling and sample logistics, high cost, flow-totalization

- **Provide biomass for applications testing:**

- partnering SNL, INL, and PNNL AAS projects, Algix – BloomFoam. Challenges: meeting biomass quality requirements (high ash), determining impact of shipping & storage

- **Conduct sustainability and economic modeling:**

- TEA, LCA, GIS-RA, ecosystem services - Colorado State U (Quinn). Challenges: identifying realizable deployment scenarios, competing technologies



## 2 – Approach

Comparing technoeconomic feasibility of algae biomass production methods



### Periphytic Algae 'Turf'

e.g., *Hydromentia* – Vero Beach, Florida



- Polyculture – resilient and resistant to crashes
- Growth: 5-20+ g/m<sup>2</sup>/day (AFDW)
- No N/P nutrients or external CO<sub>2</sub> added
- Harvest & dewatering simple, but ash reduction needed
- Requires energy for water pumping to maintain flow
- Polyculture biomass focus - low neutral lipids & much higher ash
- Similarities with open field agriculture

VS

### Algae Raceway Pond

e.g., NBT – Eilat, Israel



- Monoculture – vulnerable to crashes
- Growth: 5-20+ g/m<sup>2</sup>/day (AFDW)
- Needs fertilizer & CO<sub>2</sub>
- Harvest & dewatering more difficult & energy-intensive
- Requires energy for water supply and paddle wheel flow/mixing
- Lipid focus (historical)



Attached algae cultivation shown to reduce cost of biomass production compared to Open Raceway Ponds \$460/ton, *without* ecosystem services credits based on zero nutrient and CO<sub>2</sub> costs, low-cost harvesting and system upkeep, culture resilience. Baseline outdoor productivities of 4-11 g/m<sup>2</sup>/day established in multi-season trials

Profitable utilization of biomass is lacking in the established Algae Turf Scrubber CONOPS – this project provides fundamental data supporting scale-up models, including biomass yield and ash content, proximate composition, water quality improvement data, and supporting energy and bioproduct applications testing from various source water types to enable systems optimization and siting for nutrient interception and biomass yield, for scale-up scenario planning.

Providing biomass for pilot-scale applications testing - Project provided 4x 25Gal (15-20% solids) harvested biomass samples to BETO Feedstocks logistics (deashing, storage, INL), CAP/Fractionation and Bioprocessing (NREL/SNL), and thermochemical conversion (HTL, PNNL) projects, and industry partner, Algix, Inc., for testing.

Enabling efficient production of desirable strains - dominant genus in marine/estuarine surface waters is *Ulva*, an industrially produced (and wild-harvested) strain in many countries including N. and W. Europe, S. Africa, and Asia. Multi-month stable cultivation of seed cultivars, *Tribonema minus*, provided by MicroBio Engineering, and *Cladophora* sp. for improved value and reduced ash, with pilot-scale productivity 24 g/m<sup>2</sup>/day AFDW, 22% ash, providing a path towards achieving the end of project goal of

Establishing water resource & contaminant interception potential – Demonstrated 6-month continuous interception of 90% of non-point source N/P nutrients, >60% B.O.D (organics), and various trace metals (Ni>Zn>Cu>Cd>Pb>Hg>As>Se) at bioaccumulating levels (600-2000x that of the source water)

Demonstrating ecosystem services value - Great Lakes deployment scenario suggests Attached Algae system outperforms incumbent technologies for harmful algae bloom mitigation (incl. septic system repair cost and regulatory scenarios for WWTP discharge).

See referenced manuscripts in supplementary slides

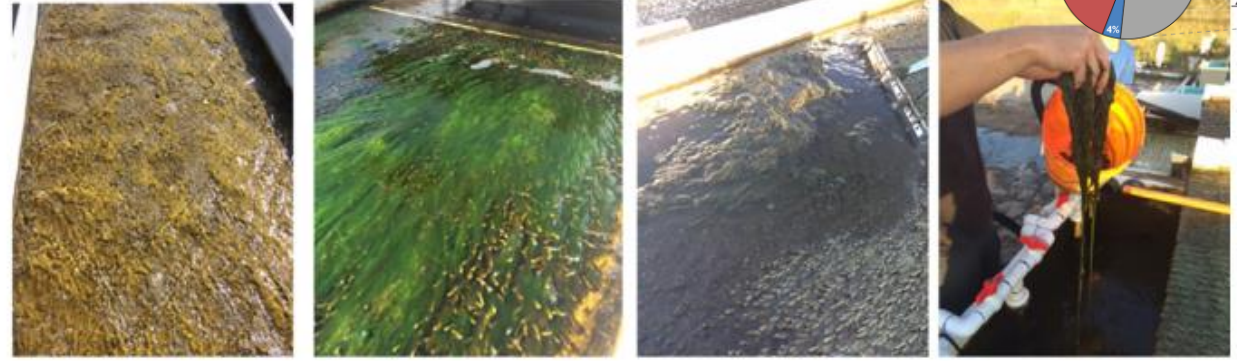


# 4 – Progress and Outcomes

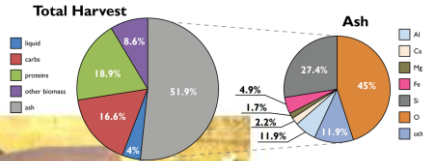
Successful completion of multi-year deployments for establishing biomass productivities in marine/estuarine and riverine source waters



40x micrograph



Pioneer algal turf (benthic diatoms) → Established algal turf (*Ulva lactuca*) → Weekly harvest (low cost) → Biomass!



Seasonal average productivity Corpus Christi, TX  
Marine/estuarine source water: 4 - 10 g/m<sup>2</sup>/day\*



Seasonal average productivity Salton Sea, CA  
Riverine source water: 5 - 10 g/m<sup>2</sup>/day\*

\*Low: Oct - April, High: March - Sept



## 4 – Progress and Outcomes

### Savannah River – Georgia Southern

**Team lead:** Anthony Siccardi

**Deployment site:** Priest's Landing, Savannah GA, Estuarine, compromised by surface runoff

- 12x 20' flow-ways for triplicate comparison of flow-way substrates



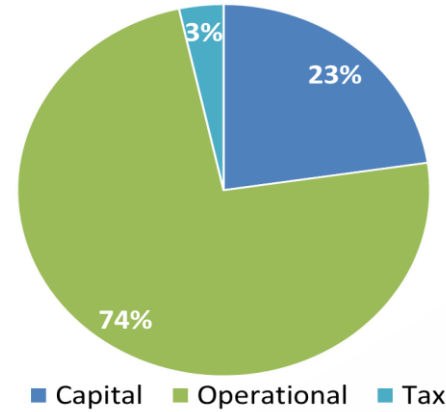
- 400' flow-way long system for evaluation of nutrient draw-down
  - NH<sub>3</sub>/NO<sub>3</sub>: 2.3±0.9 ppm
  - PO<sub>4</sub>: 0.4±0.3 ppm
- Achieved >15 g/m<sup>2</sup>/day summertime, 7±2 g/m<sup>2</sup>/day wintertime (6 months continuous), 32% protein, 55% carbohydrate, 5% TAG.



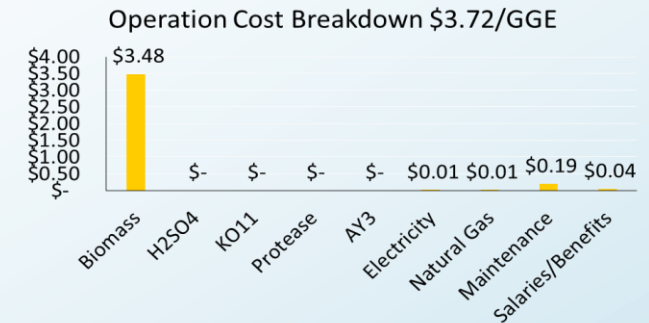
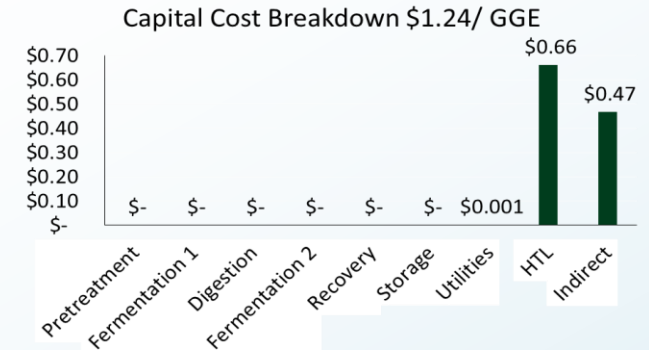
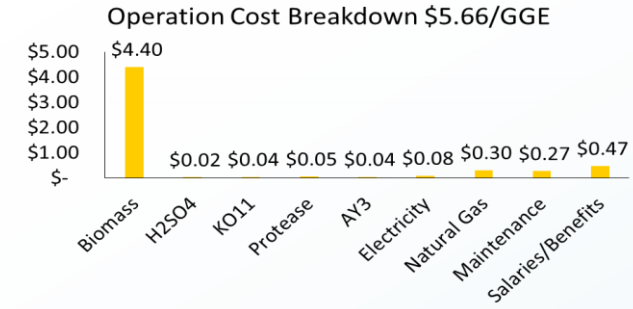
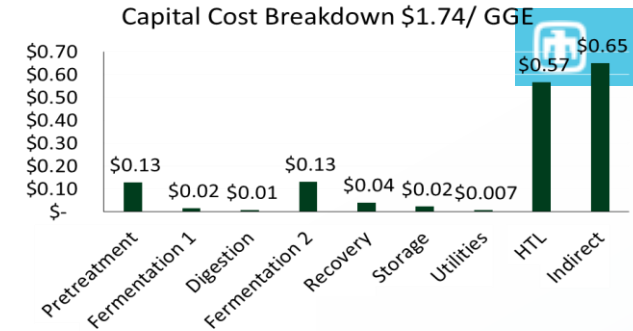
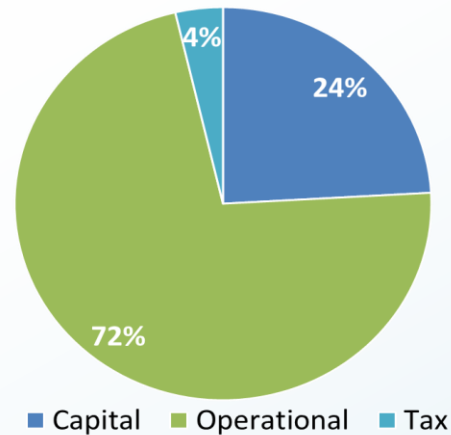
# 4 – Progress and Outcomes

- Integrated TEA/LCA for Attached Algae processing for fuels: fractionation versus HTL
- Minimum Fuel Selling Price for fractionation processing is \$7.67, HTL only is \$5.16
- Cost does not include potential premiums for co-products, incl.
  1. Water clean-up credits
  2. \$8 - \$14 /gal for isobutanol
  3. Co-products
- Biomass production cost is major cost driver, based on modeled cost of \$460/ton, attached algae polyculture production should compare favorably against conventional raceway (suspended) cultivation

Fractionation + HTL Cost Breakdown \$7.67/GGE



HTL Only Cost Breakdown \$5.16/GGE

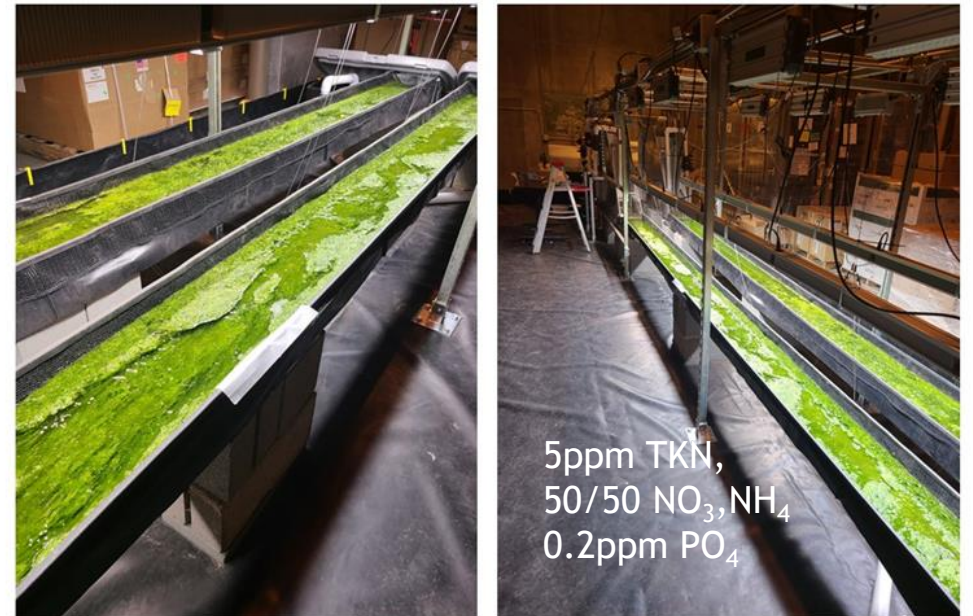
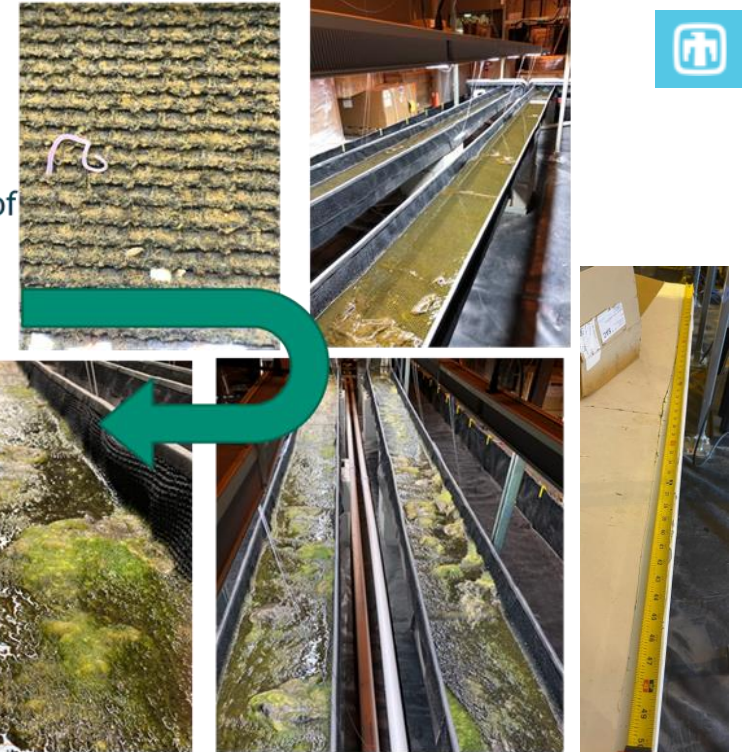


## 4 – Progress and Outcomes

Continuous Attached Algae Flow-Way operation in environmentally-controlled, pilot-scale cultivation (2x 1' x 30' flow-ways) at Sandia Algae Testbed, for detailed assessment of,

- 1) Inoculation strategies focusing on designed polycultures that are amenable with contact with local periphyton, and conveying improved productivity and biomass quality
  - 2) Reduce new harvesting strategies to practice, including slough-induction & collection
  - 3) Test not-currently proven/non-fieldable analyses for biomass production and species succession, e.g. various N/P loading, spectroradiometric monitoring, controlled contaminant experiments
- 22% ash content from attached algae achieved in Oct 2020, lowest report to date among reports that differentiate ash & biomass in “Turf Algae”-relevant literature; pooling biomass for conversion tasks with PNNL and SNL
  - >24 g/m<sup>2</sup>/day, 20-25% ash achieved 12/2020 - 2/2021 fulfills FY21 annual milestone

Transition of  
pioneer to  
mature  
algae turf



## 4 – Progress and Outcomes

### PMRF-Kauai WWTP-integrated deployment

- FY21 Deployment - home of Sandia's Modular Attached Algae System
- Navy base with independent waste- water treatment facility
- Facultative pond discharges to the Pacific Ocean
  - NH<sub>3</sub>/NO<sub>3</sub>: 10±2 ppm, PO<sub>4</sub>: 1 ppm, 8kg/day VTSS
  - *These waters are associated with local coliform & avian botulism of local concern*
  - *Kauai-local stake-holders and project partners*



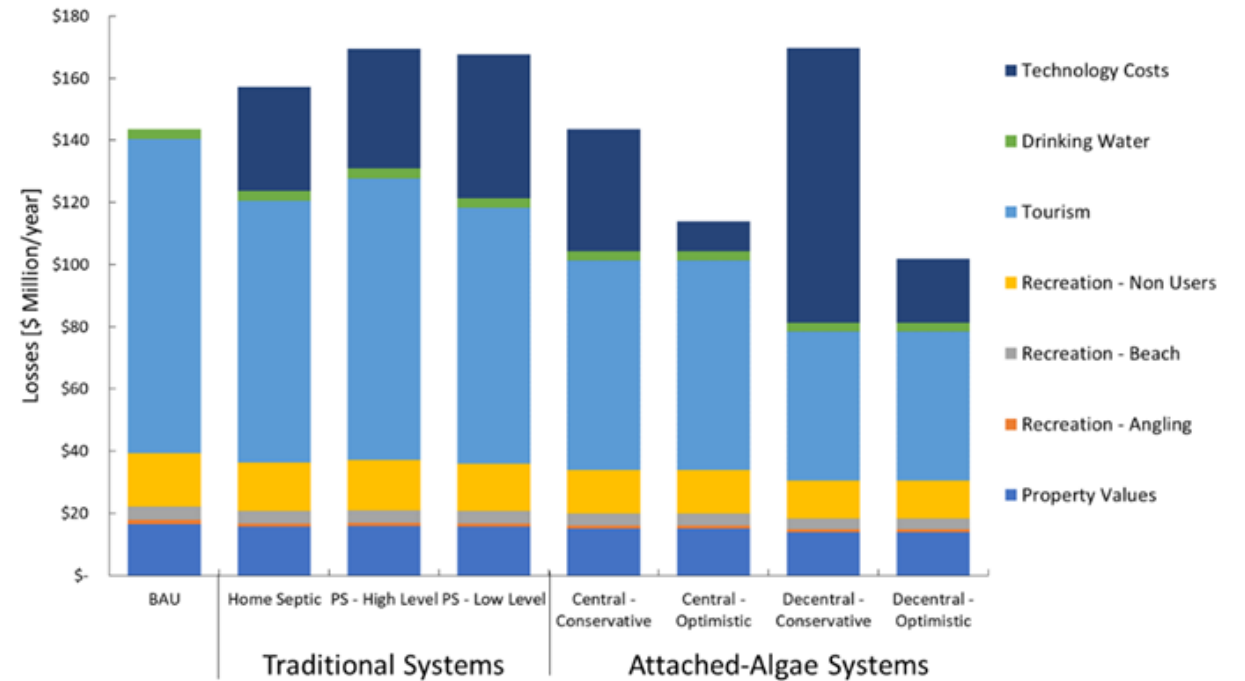
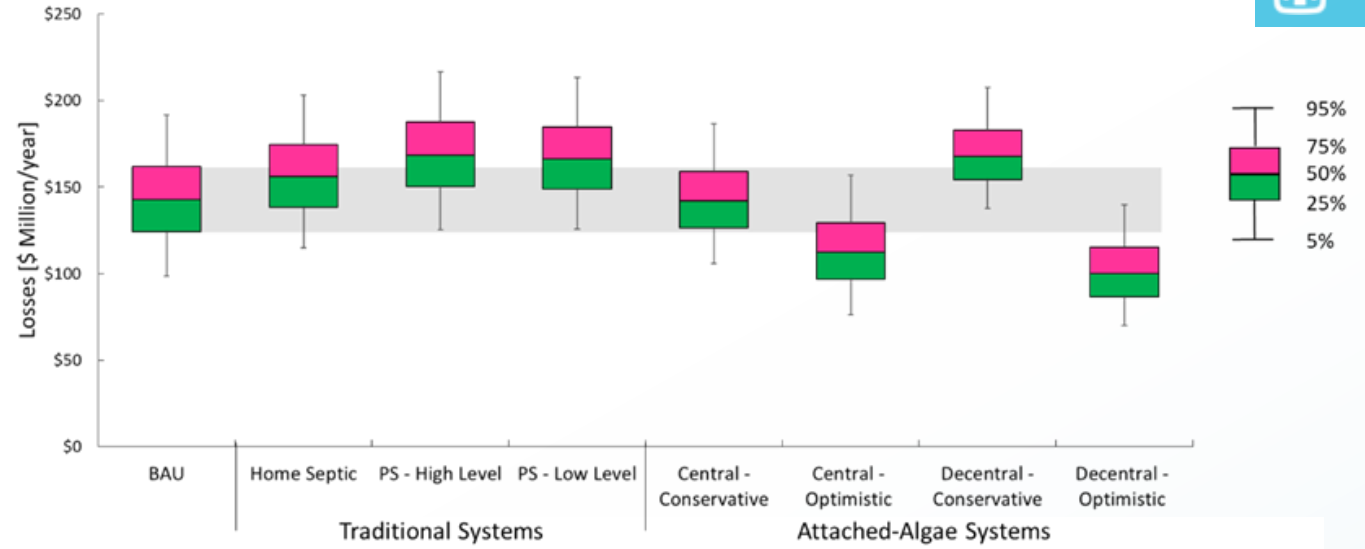
*Power station, flow-way hardware delivered to PMRF, site preparations complete 6/2020; Subcontracting & detailed installation and O/M manuals completed 12/2020*



Eric Monroe

# 4 – Progress and Outcomes

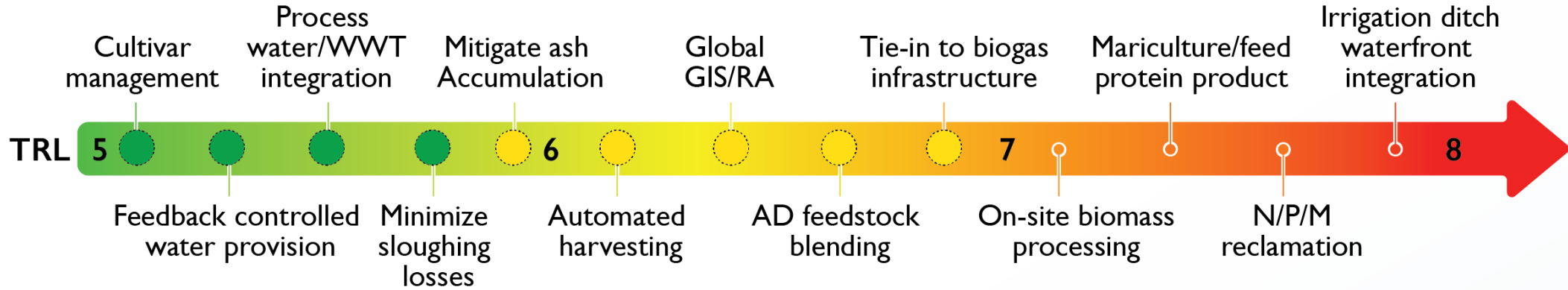
- Ecosystem service evaluation based on harmful algae bloom mitigation in Lake Erie, considering Attached Algae deployment scenarios, various waste-water treatment discharge limit scenarios, and septic repair.
- For all scenarios, including with decreasing future dry reactive phosphorus release rates, both centralized and decentralized Attached Algae deployment scenario show improved economic potential compared to costs associated with conventional nutrient minimization approaches.
- The largest HAB-associated cost are tourism, property value, and recreation - these exceed the technology mitigation costs in almost all scenarios
- Opportunities for engaging nutrient credit trading markets and as a HAB prevention technology are being pursued through GIS-RA, watershed modeling, and industry engagement efforts at Sandia, CSU, and the Electric Power Research Institute.



# 4 – Progress and Outcomes



## Sandia's Turf Algae Technology Maturation Plan (FY21)



- Currently funded tech. dev. tasks (BETO, SNL)
- Proposed tech dev

## Potential Customer Segments and Associated Value Prop(s)



## 5 – Summary



- Attached algae cultivation provides means to surmount current cost drivers for algae biomass production, including nutrients and CO<sub>2</sub>, harvesting, and maintaining culture stability.
- Successfully achieved >24 g/m<sup>2</sup>/day, <25% ash in pilot-scale environmentally simulated conditions, and 4-10 g/m<sup>2</sup>/day, >50% ash in multi-year pilot-scale outdoor marine/estuarine and riverine source water; marine/estuarine biomass dominated by *Ulva*, riverine biomass dominated by *Cladophora*.
- New WWTP-coupled system installation underway through collaboration with U.S. Navy, with support from Global Algae Innovations.
- Providing quantities of biomass (10Kg DW equivalent ea.) for conversion testing at SNL, INL, PNNL, and for use by Algix, Inc for BloomFoam<sup>®</sup>
- Data that has been generated is being used to support TEA, LCA, GIS-RA, and CONOPS for scale-up, and identifying opportunities for ecosystem services (e.g. nutrient interception / HAB prevention) for further value-addition with EPRI, Colorado State



# Quad Chart Overview



## Timeline

- Project Start Date: 10/1/2019
- Project End Date: 9/30/2021

	FY20	Active Project
DOE Funding	(10/01/2019 - 9/30/2021)	\$2.25M (\$750,000 /yr)

## Project Partners

- Colo State University
- Imperial Irrigation District / IVCRC
- Global Algae Innovations

## Barriers addressed

Biomass Availability and Cost (Aft-A)  
Sustainable Algae Production (Aft-B)  
Sustainable Harvesting (Aft-D)

## Project Goal

The current year goal is to demonstrate the ability for attached algae cultivation to simultaneously achieve 24 g/m<sup>2</sup>/day and <25% ash using compromised surface waters.

## End of Project Milestone

The end goal of the project is to demonstrate attached algae cultivation coupled to water treatment with a net biomass cost of <\$450 /ton AFDW.

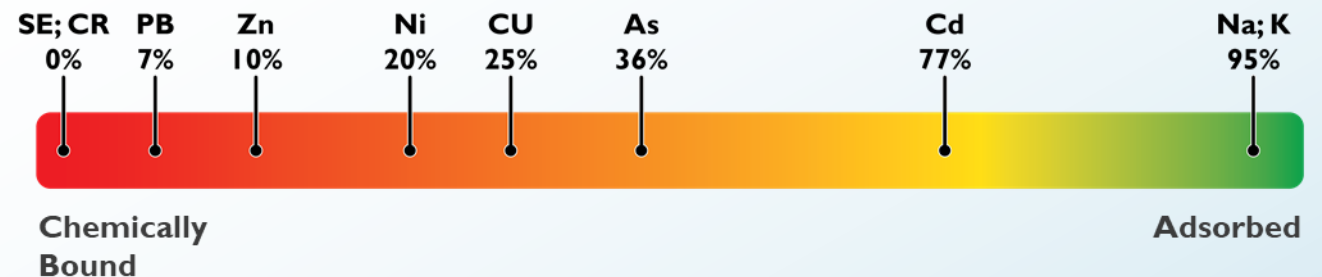
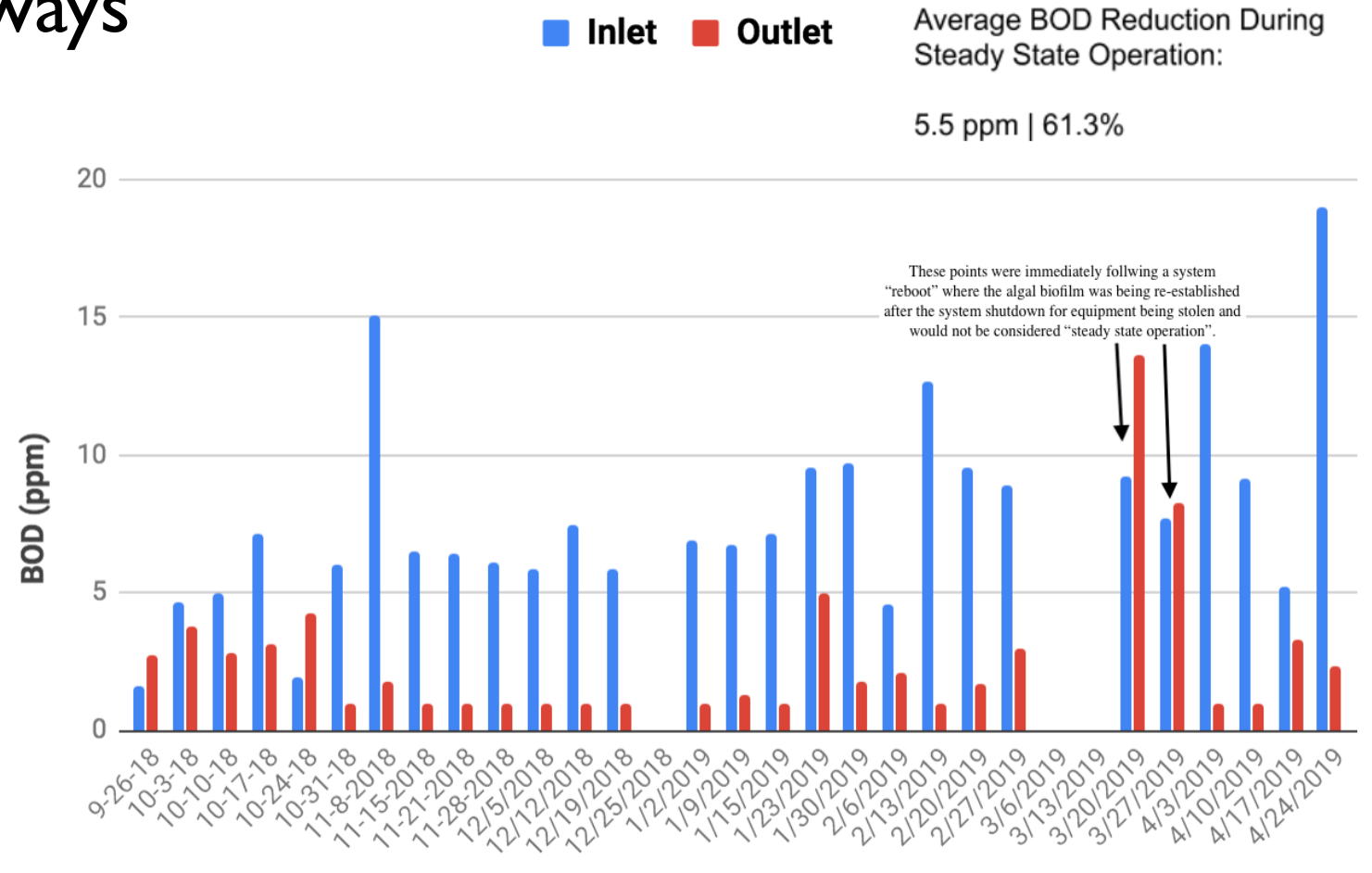
## Funding Mechanism

BETO-AAS-AOP

# Remediation of nutrients, metals and biological oxygen demand in attached algae flow-ways

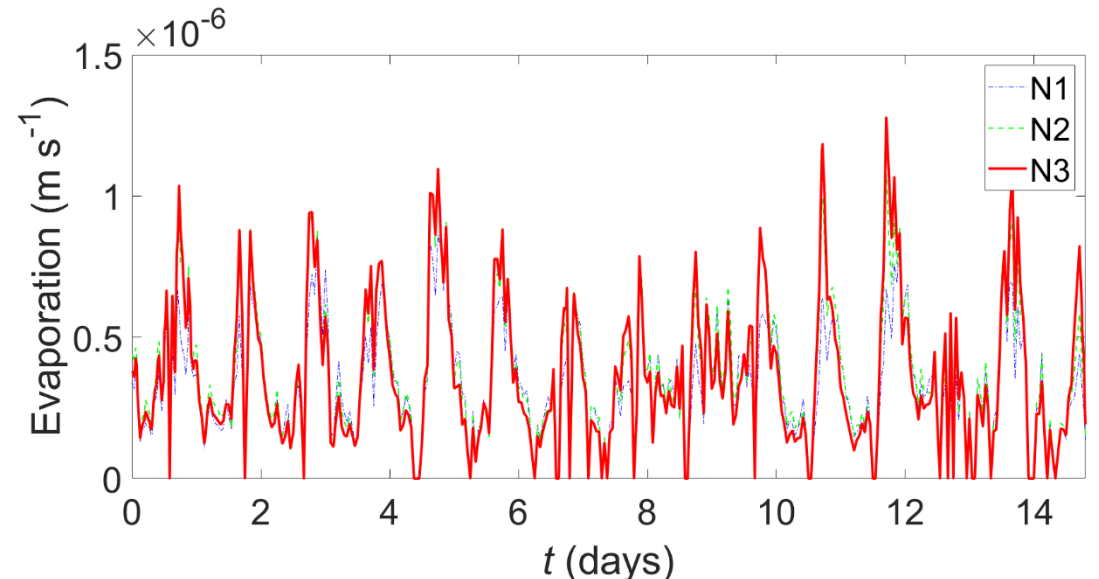
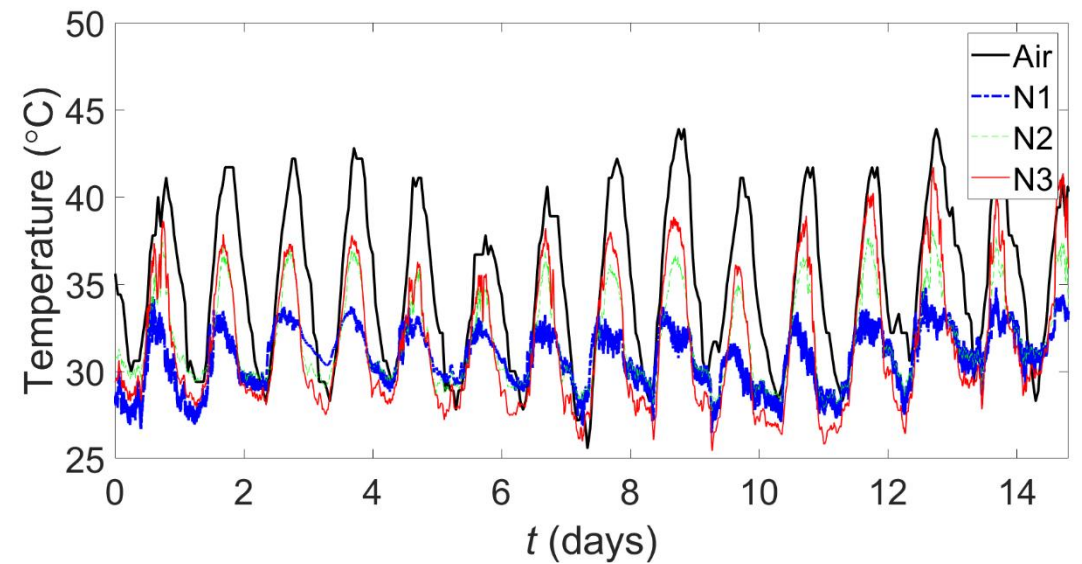


- TN and TP recovery 300~500 mg/m<sup>2</sup>/day and 15~30 mg/m<sup>2</sup>/day in multi-year outdoor Salton Sea and Laguna Madre pilots
- BOD reduction > 60% from 6-month trial at Salton Sea
- Biomass compositional analysis reveals significant (15-60%) interception of RCRA-identified metals of concern at Salton Sea, and bound versus adsorbed state of metals in the harvested biomass.
- Applications for nutrient credits and as a HAB prevention technology are being pursued through sustainability modeling efforts at CSU.



# Predictive evaporation framework for attached-algae flow-ways

- **Mean water temperatures** were observed at 30.9, 31.8, and 31.6 °C at N1, N2, and N3
- **Mean evaporation** rate at this flow-way is estimated at  $3.3 \times 10^{-7}$ ,  $3.8 \times 10^{-7}$ , and  $3.7 \times 10^{-7}$  m s<sup>-1</sup> at N1, N2, and N3, approximately double reported pan evaporation rate for Salton Sea/Imperial Co, CA
- **The results** obtained from the attached-algae flow-ways are **consistent with results published by Quiroz-Arita et al. (2020)** that demonstrated that algae biomass influences the water temperature, and water temperature affects the evaporation rate in the system
- **The next steps** will incorporate the development of the **water temperature and algae biomass models that will include the evaporation sub-system**
- **Future directions** of the prediction framework for evaporative losses in algae flow-ways **will include model calibration and validation approach using experimental work** at the attached-algae flow-way testbed at Sandia National Laboratories



# Metagenomics analysis for characterizing variation in microbial ecology

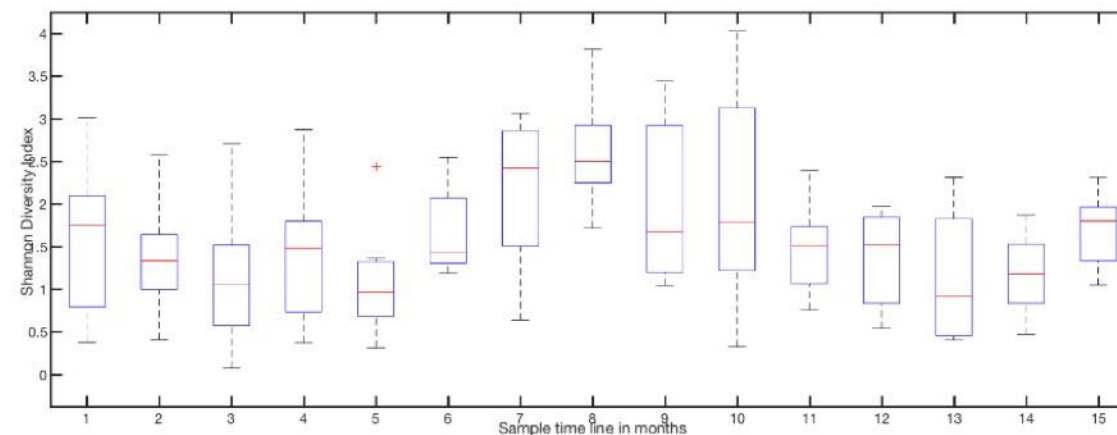
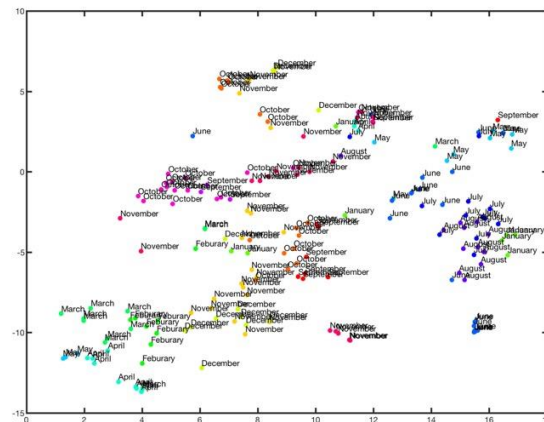
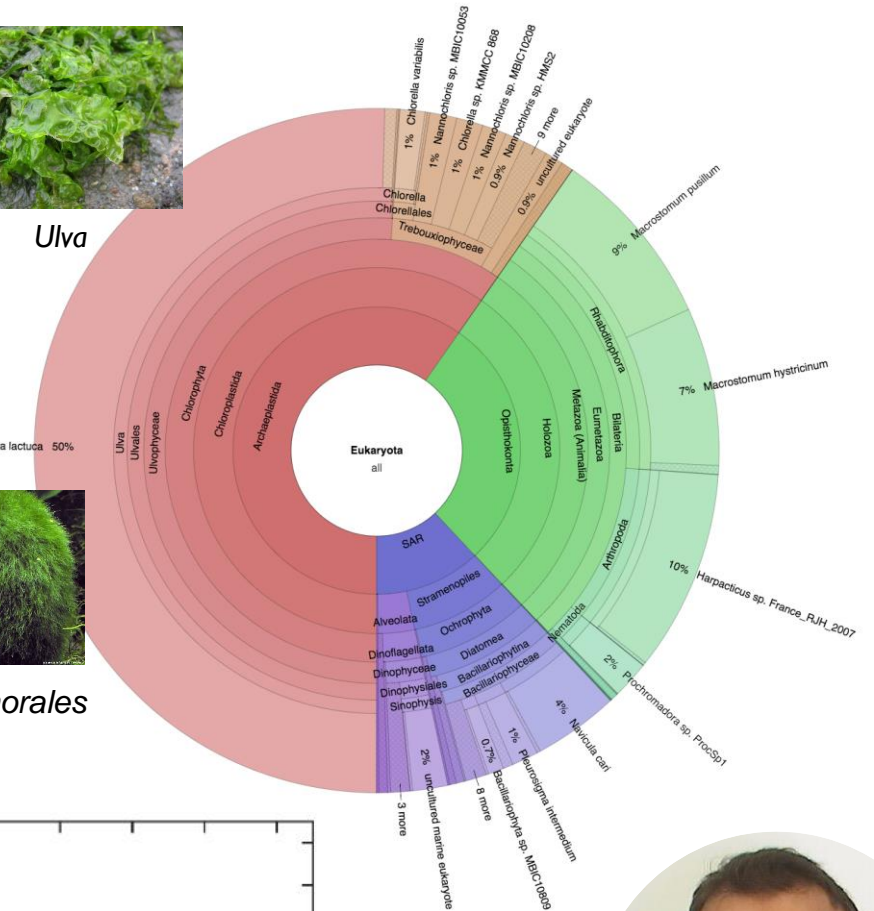
- 300,000 OTUs, 8 – 10x more than raceway algae polycultures
- Diversity in samples show repeatable seasonal variation
- Data being used to identify strains that are associated with high productivity for development of seed cultures for rapid onset of high yield biomass production.
- **No indication of system harboring toxigenic algae species**



*Ulva*



*Cladophorales*

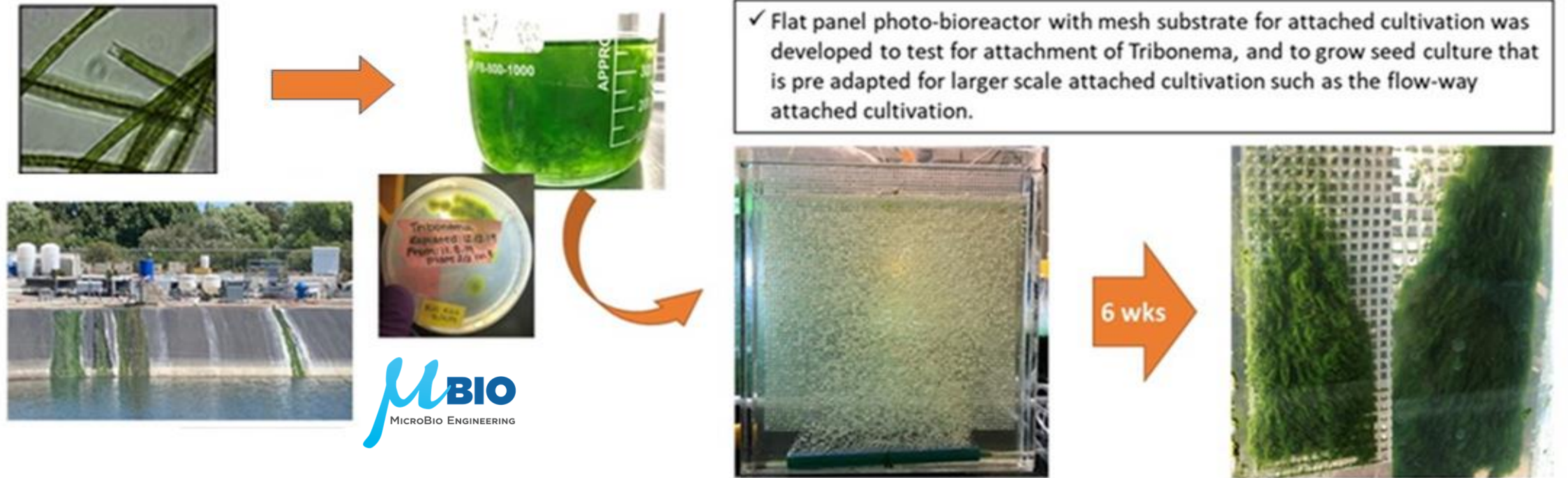


**MAGPie**



Kunal Poorey

# Development of filamentous culture seeding capabilities

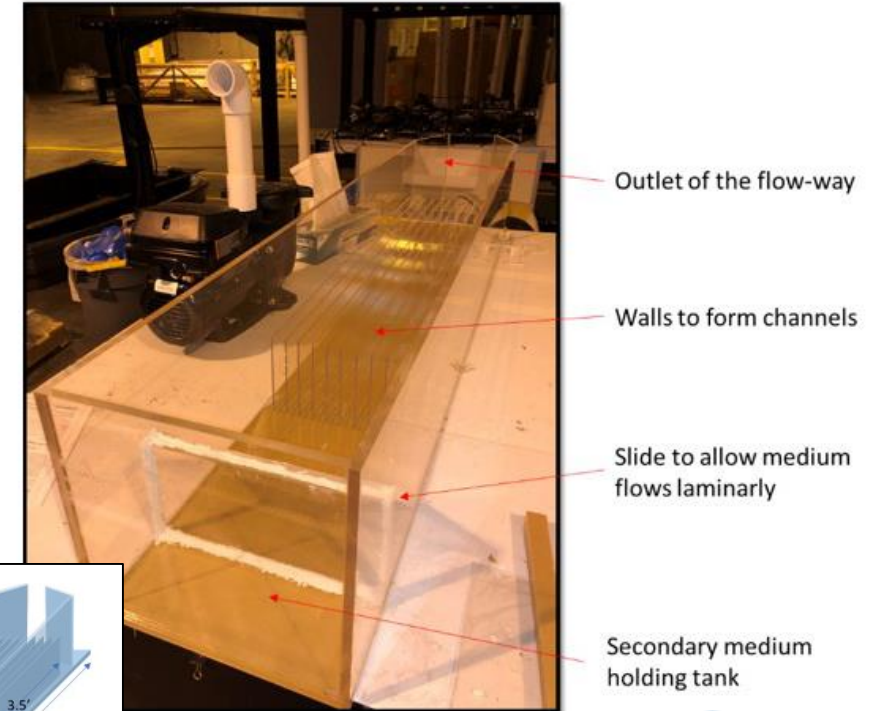
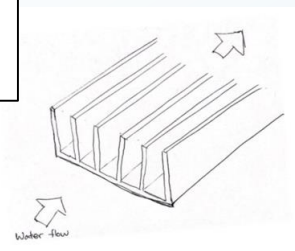
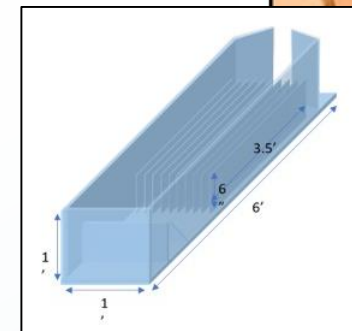
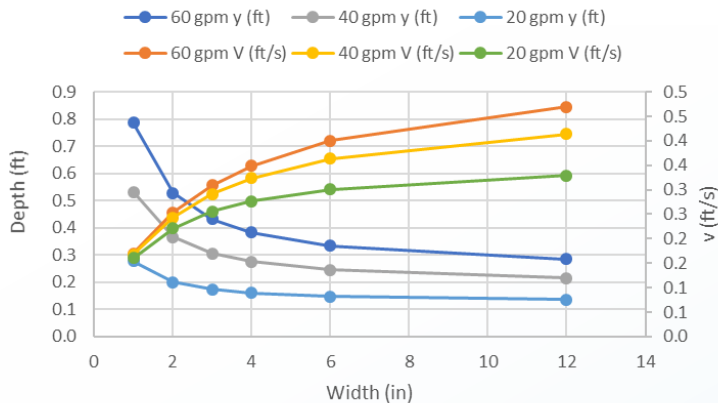
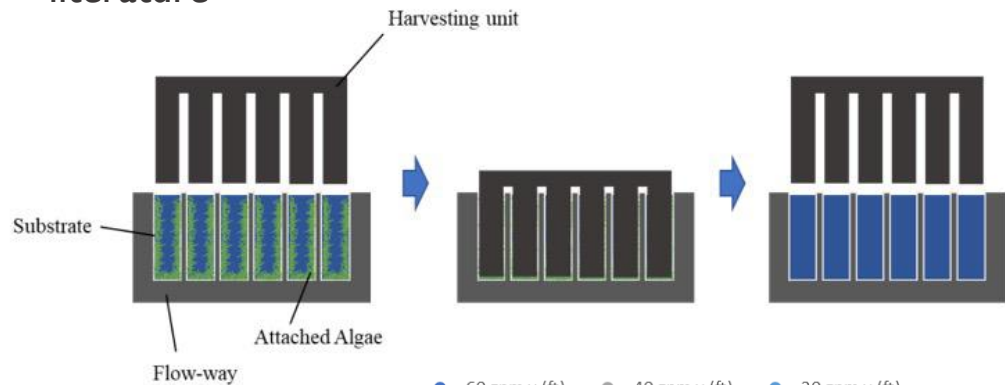


*Tribonema* and *Cladophora* biomass from 4 sets of week-long cultivation and harvest trials was subjected to ash-free dry weight (AFDW) assessment using standard muffle-furnace based gravimetry, with rinsing of the growth mesh prior to harvest with DI water. The results of these studies indicated ash-free dry weight biomass productivities of  $37 \pm 3$  g m<sup>-2</sup> day<sup>-1</sup> for *Tribonema* and  $33 \pm 4$  g m<sup>-2</sup> day<sup>-1</sup> for *Cladophora* in fresh to brackish growth medium in fulfillment of the Go/No-Go criterion for FY20

# Demonstrate attached algae substrate modification to reduce ash and improve effective flow-rate and nutrient loading regimes



- Higher tunability to variable nutrient loading and flow-rates
- **Lowering ash content** in a biomass since they are attached on the vertical walls (ash might accumulate on the bottom of the channels)
- 22% ash content from attached algae achieved in Jan 2020, lowest report to date among reports differentiating ash & biomass in “Turf Algae”-relevant literature



Sungwhan Kim



Reduce algae biomass production costs and improve cultivation stability to facilitate algae production scale-up with nutrient interception and prevention of harmful and nuisance algae blooms (low-cost biomass + nutrient credits/ecosystem services)

## Peer-reviewed Publications:

1) DeRose, Davis, Monroe, Quinn “Economic viability of proactive algae bloom mitigation technologies” (2021) *Great Lakes Research* (R1 received)

“Results show that on average, Lake Erie communities lose \$142 M ( $\pm$  \$29M) year-1 from HAB’s without mitigation technology. Use of attached-algae systems show an average savings of \$12-42M per year from HAB mitigation. Attached algae systems are the only nutrient reduction technology to show net-positive cash flow when compared with traditional nutrient removal systems. Additional considerations included stochastic uncertainty and different future eutrophication scenarios, which increased the effectiveness of attached-algae systems.”

2) Kim, Quiroz-Arita, Monroe, Huysman, Mitchell, Siccardi, Davis “Application of attached algae flow-ways for coupling biomass production with the utilization of dilute non-point source nutrients in the Upper Laguna Madre, TX” (2021) *Water Research* 191, 116816

“The predicted ash-free biomass productivity using ML models resulted in root-squared-mean errors (RSME) from 1.78 to 1.86 g/m<sup>2</sup>/day, and R<sup>2</sup> values from 0.67 to 0.75 using different methods. The greatest contributor to net productivity was total solar irradiation, followed by air temperature, salinity, and pH”

3) DeRose, DeMill, Davis, Quinn “Integrated techno economic and life cycle assessment of the conversion of high productivity, low lipid algae to renewable fuels” *Algal Research* (2019) 38, 101412

“Ash content is cost-driver for utilization of algae turf biomass for fuels, based on bioconversion, HTL, and combined scenarios”

## Patents:

1) Davis, Monroe, Kim “Algal harvesting and water filtration” US Patent Appl 16/904,202

2) Davis, Monroe “Harvesting of cyanobacterial biomass and bioproducts” US Patent Appl 16/903,035