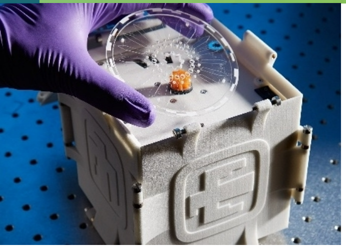
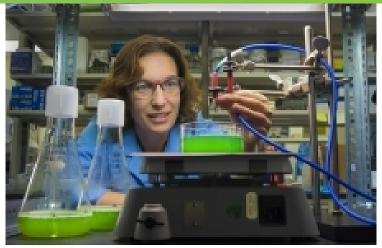
SAND2022-13696C

Sandia National Laboratories

Biological dynamics of periphytic algae and microbes in long term Turf Algae cultivation

PRESENTED BY

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Principal Member of Tech Staff, 08622 Bioresource & Environmental Security

Oct 5, 2022

ALGAE BIOMASS SUMMIT

Oct 3-28, 2022 | www.algaebiomasssummit.org

U.S. DEPARTMENT OF ENERGY

Understanding algae cultivation techno-economics for biomass and ecosystem services

Periphytic Algae ‘Turf ‘

e.g., Hydromentia – Vero Beach, Florida

VS

Algae Raceway Pond

e.g., NBT – Eilat, Israel

Polyculture – resilient and resistant to crashes

Growth: 5-20+ g/m^2/day (AFDW)*

No N/P nutrients or external CO_2 added

Harvest & dewatering simple, but ash reduction needed

Requires energy for water pumping to maintain flow

Polyculture biomass focus - low neutral lipids & higher ash

Similarities with open field agriculture

- Monoculture – vulnerable to crashes
- Growth: 5-20+ g/m^2/day (AFDW)*
- Needs fertilizer & CO_2
- Harvest & dewatering more difficult & energy-intensive
- Requires energy for water supply and paddle wheel flow/mixing
- Lipid focus (historical)

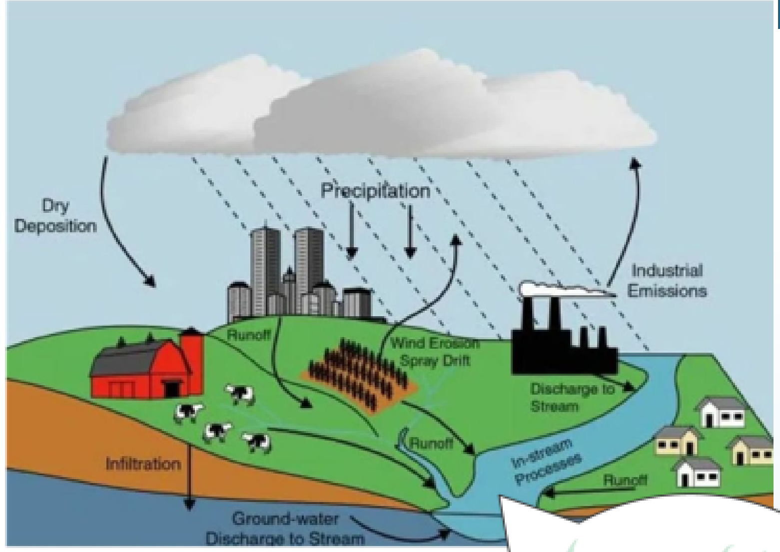
Biomass production using waste nutrients

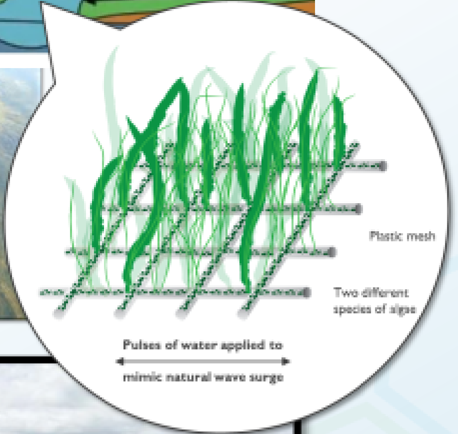
N/P equivalents for algae cultivation:

100 MGGE/year max. from municipal wastewaters

>1 BGGE/year from agricultural runoff

(30% fertilizer runoff, 70% livestock effluent)





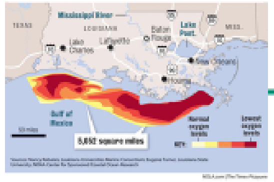
Symptoms of a waste nutrient problem

Algae-induced aquatic hypoxia: "Dead Zones"

>600 confirmed algal-bloom induced dead zones world-wide, **up >800% since 70's**

>$4B annual loss in US alone as a result of harmful algae blooms (NOAA)

Why?



Fertilizer runoff

Algae Bloom

Eutrophication (algae decomposition)

Hypoxia

Turf Algae systems were found to compare favorably on a cost basis compared to legacy nutrient abatement technologies for HAB prevention (DeRose et al *Great Lakes Res*, 2021; DeRose et al *Algal Res*, 2019)

Attached periphytic algae cultivation concept

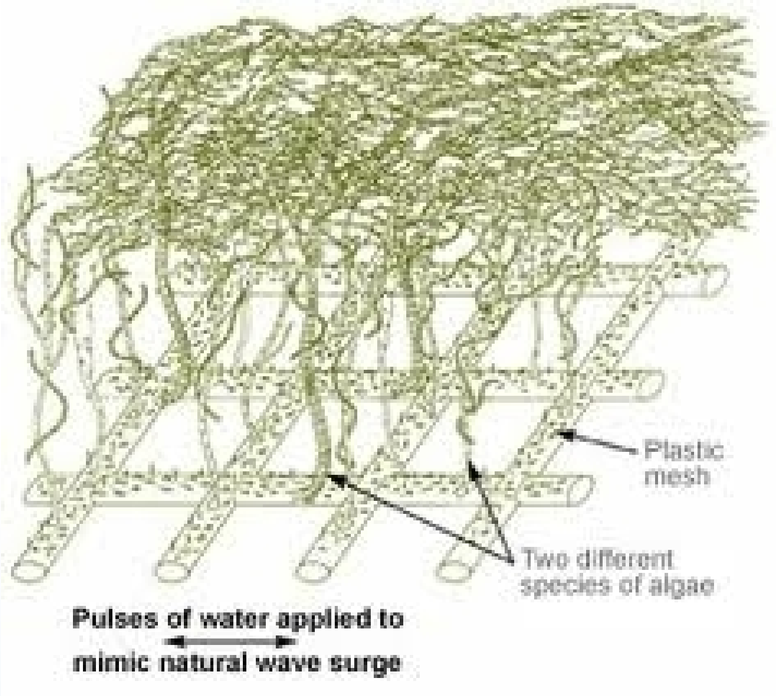
Provide habitat for natural filamentous algae assemblages to proliferate among baseline biofilm

Attached growth allows utilization of dilute nutrients, ie. flow rate can be adjusted based on nutrient concentration variability

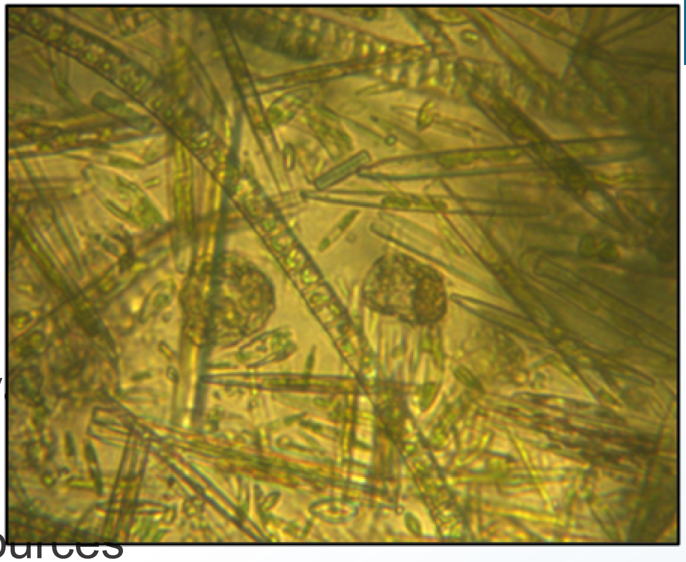
Mixotrophy benefits from carbon sources (BOD) in runoff, symbiosis with microbial biofilm

Potential for dramatic decrease in hydrodynamic residence time for water treatment: 35x improvement in L/m^2 versus suspended algae in Open Raceways Ponds

Regular harvesting to maintain log-phase growth



Turf Algae System Features:



40x established culture micrograph: Agricultural/storm runoff attached microalgae consortium

Water Chemistry

*The system **Removes**:*

Ammonium, nitrates, phosphate, & trace metals (at ppb levels)

Mineral and atmospheric carbon, common organics (60 - 90% BOD remov

Suspended solids (>70% TSS removal)

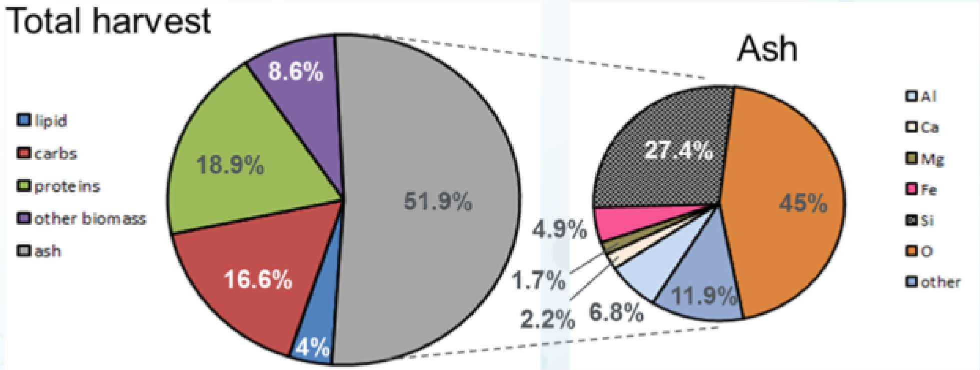
Select dissolved contaminants, including SH_2 and gross alpha emission sources

*The system **Adds**:*

Oxygen to depleted surface waters

raises pH via carbonate buffering

Evaporative cooling

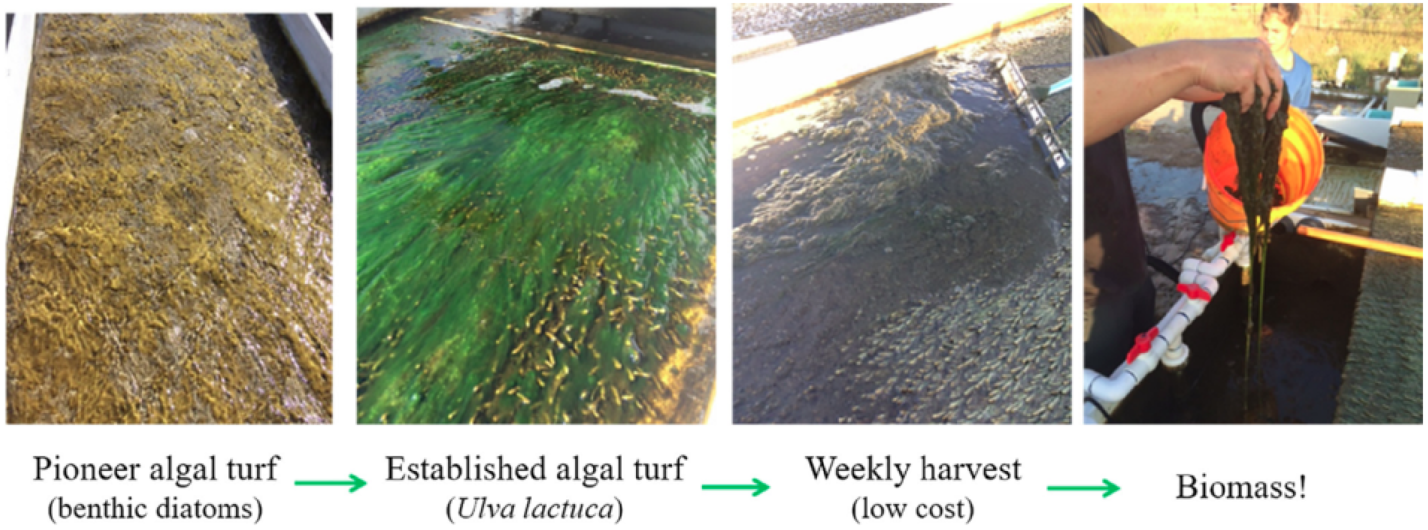


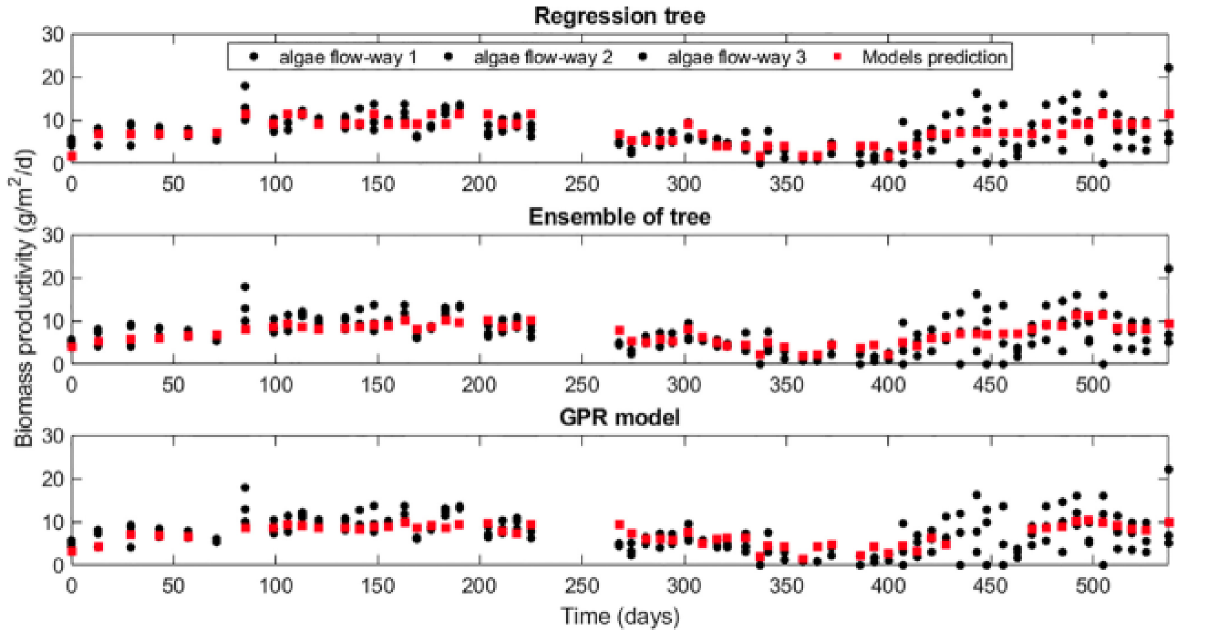
Biomass Product

Demonstrated for energy (biomethane, HTL biocrude, fractionation/fermentation), materials (BloomFoam®), and soil application (high carbon content, slow-release fertilizer) for agricultural decarbonization

Corpus Christi Flow-Way Deployment: Utilization N/P from runoff for biomass cultivation

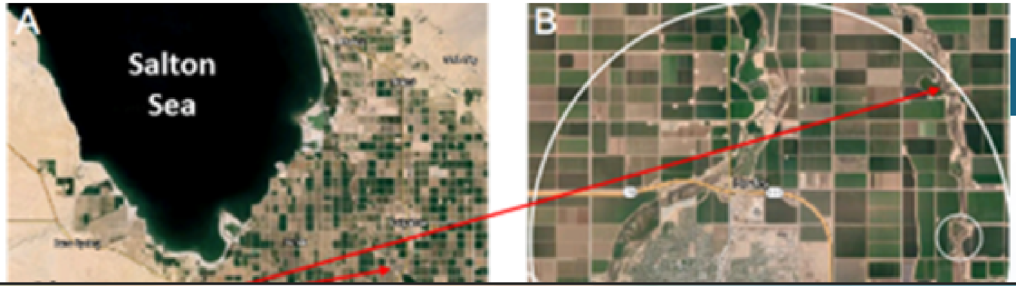
- Two-year trial for determining biomass yield potential from dilute nutrient interception
- Cultivated biomass used for experimental trails of biomass de-ashing and ensiling for improved biomass logistics
- System resilience demonstrated through long-term continuous operations, including weathering Hurricane Harvey
- Machine learning for prediction of system performance as a function of weather variables





Texas A&M AgriLife

Salton Sea Deployment: Toxic metals remediation



- Fresh/agricultural runoff source water, partnering with Imperial Irrigation District, Imperial Valley Research Center

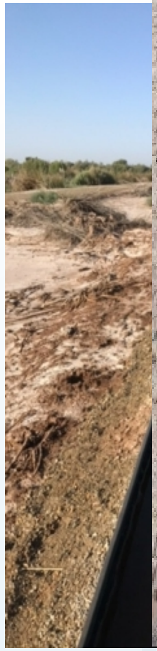
- Austere site, sourcing water from the Alamo River tributary

- Waters heavily laden with N/P, organics, metals (Hg, Pb), & metalloids (Se, As)

- Side-by-side raceway & floway operation for comparative assessment

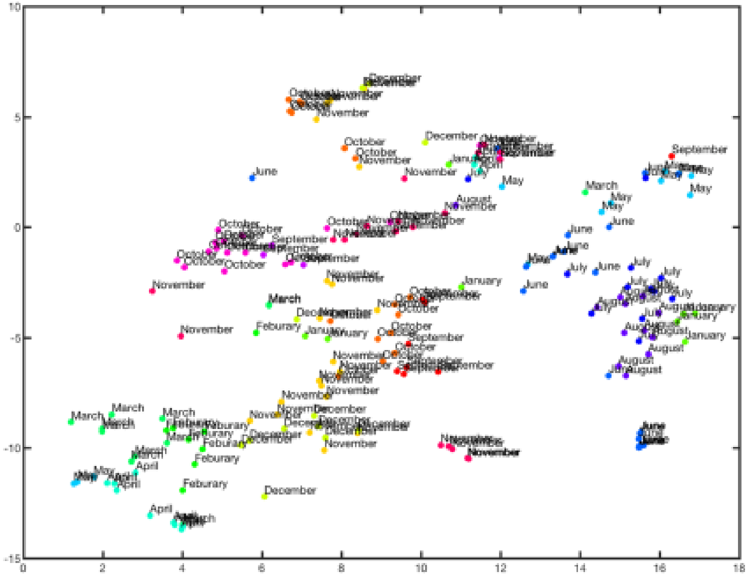
Salton Sea Algae Flow-Way Deployment

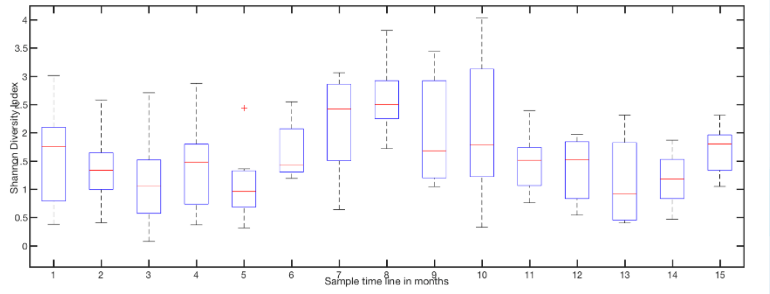
- 900-ft flow-way (80m^2) in Brawley, CA on Alamo River tributary to Salton Sea, operational Oct 2016 – Sept 2020
- State of California and Imperial Irrigation District tasked with seeking means to remediate Se and As accumulation in wetlands fauna
- Austere site: no physical security or facilities, pumping provided by renewable power pumping station
- Source water: untreated agriculture runoff



Microbiome analysis for characterizing variations 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, and other desirable trains for development of seed cultures.
- **No indication of system harboring toxigenic algae species**





Ulva

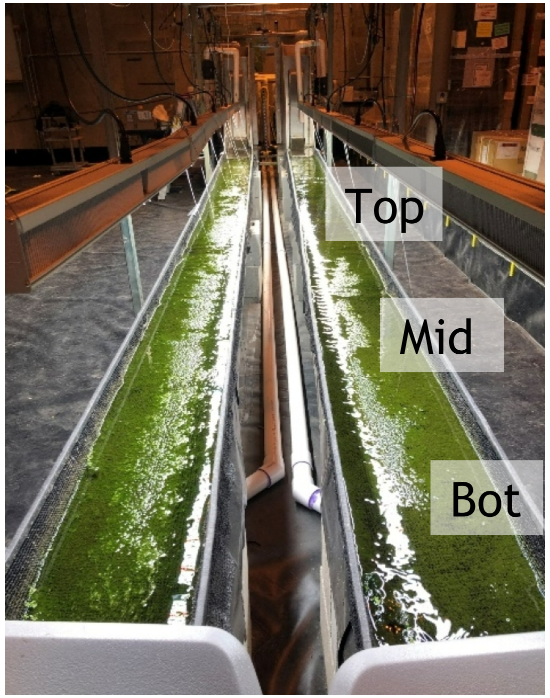
Cladophorales

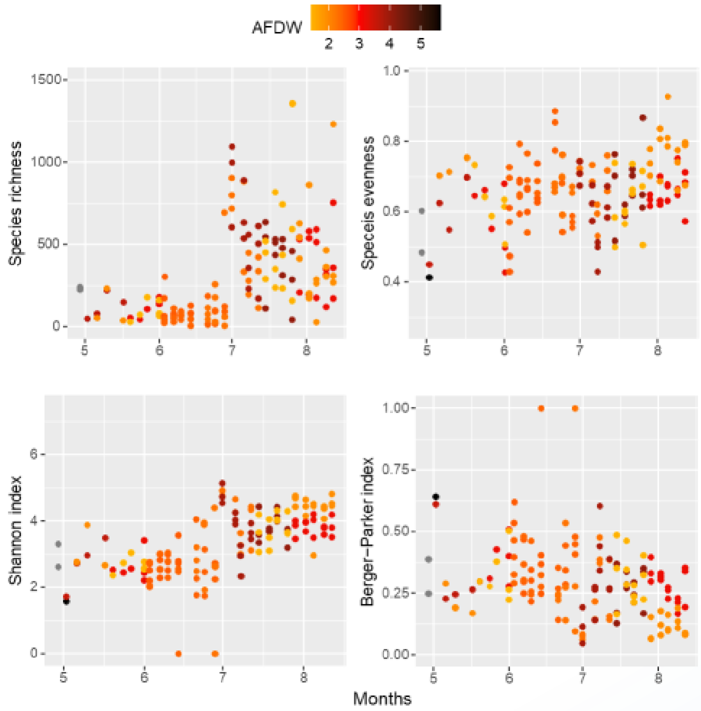
Pilot-scale flow-way operation at the testbeds

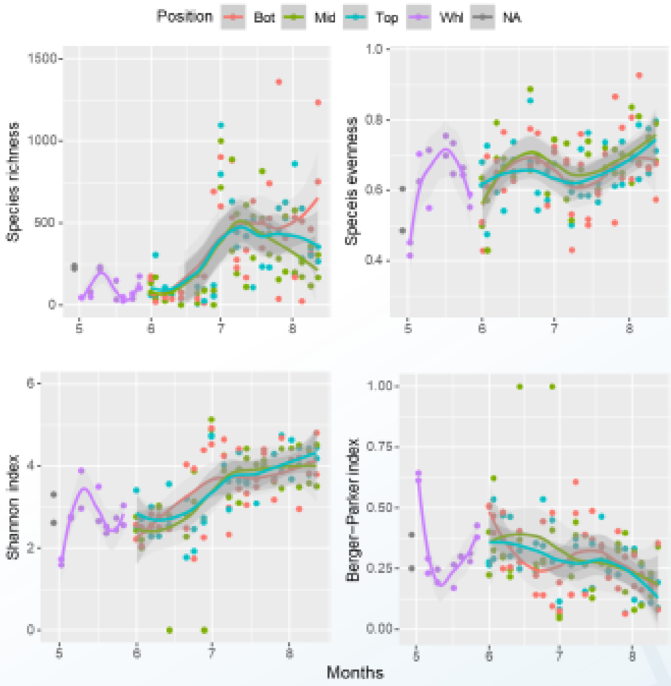
Operating Conditions

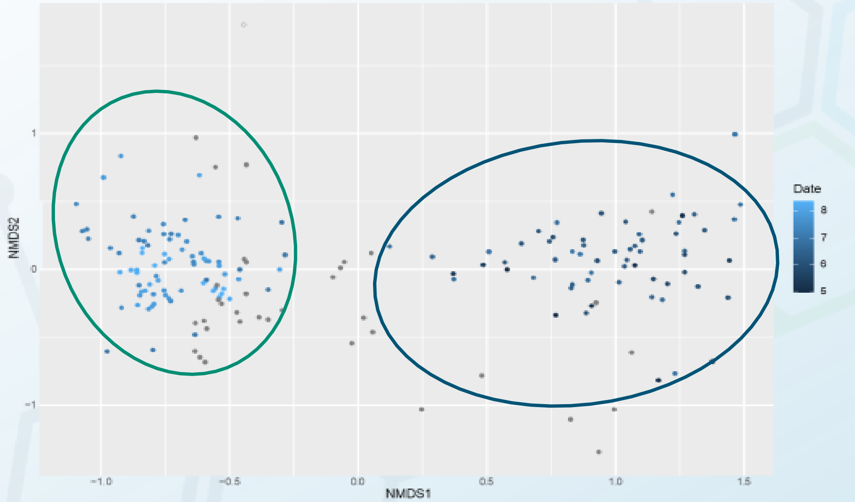
- Two of 20ft-long 1ft-wide gently sloped (~1°) flow-way
- Medium circulated with periodic addition of nutrients (fed-batch)
- Nutrients: 5 ppm N_{total} (2.5 ppm NO_3^- + 2.5 ppm NH_4^-), 0.5 ppm P (KH_2PO_4), 40 ppm Ca^{+2}, 20 ppm Mg^{+2} , 60 ppm HCO_3^- , trace element, and vitamins.
- Total working volume: 750L, pumping rate: ~23 LPM (6 GPM)
- LED lights were environmentally simulated (0~1000 μE)
- No control on water temperature, but it was kept between 15-20 °C
- Biomass was harvested every 2 weeks, sloughed biomass collected at the end of the flow-way were also separately collected.
- Water chemistry (pH, ORP, temperature, conductivity, dissolved oxygen, saturation level, and salinity) was measured through the probe installed in the head tank.

Microbiome analysis: Diversity analysis of bacterial community



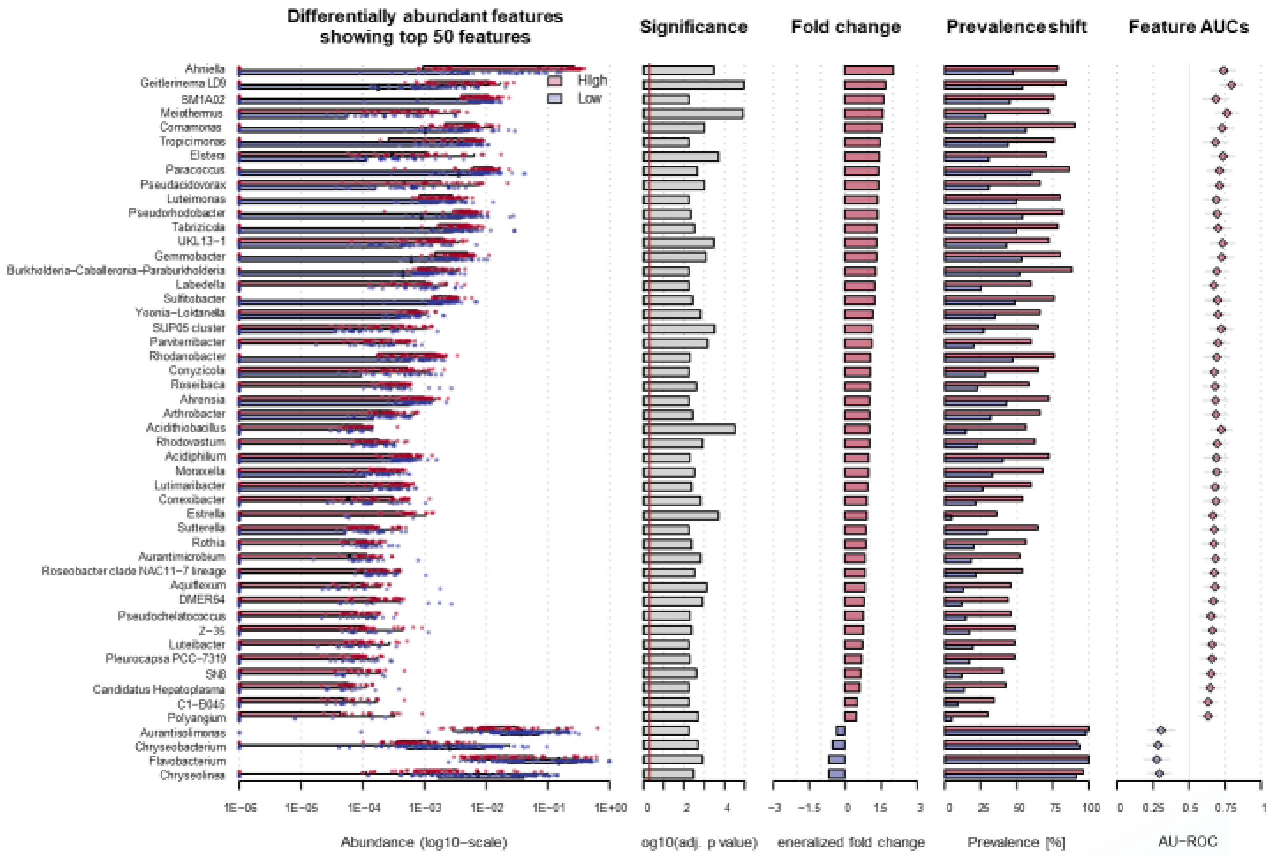


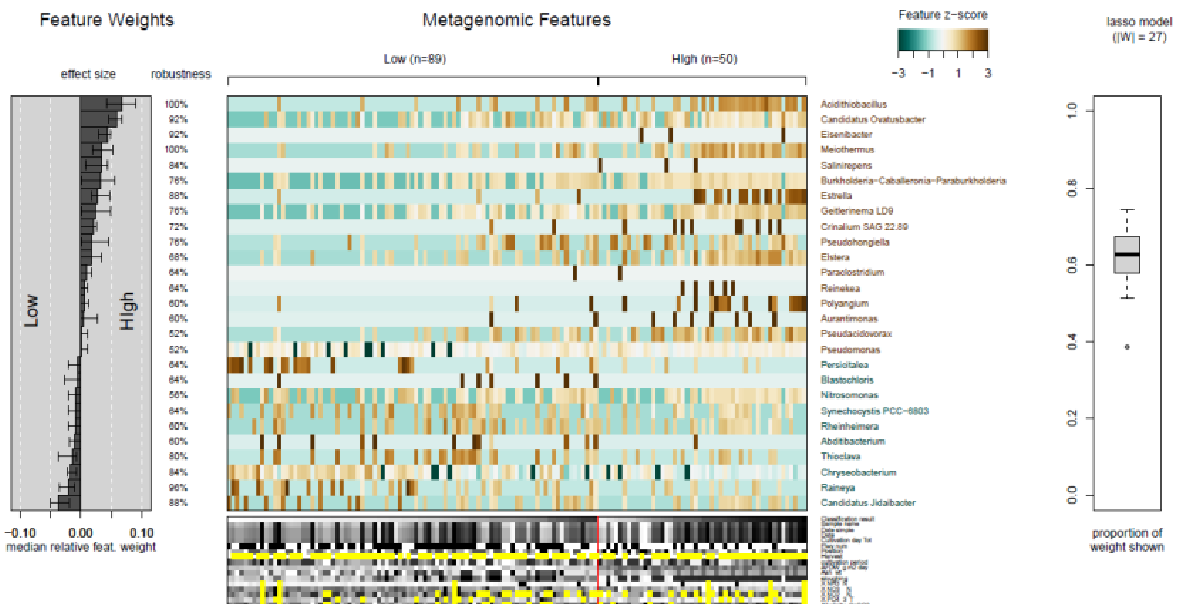


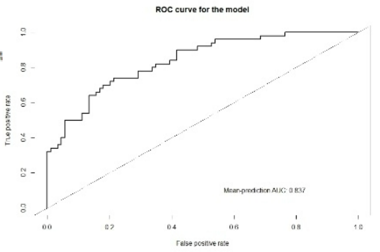


- Bacterial community at genus level were first analyzed in terms of their diversity: alpha diversity over time and beta diversity
- Diversity of bacterial community increased over time along with their richness and evenness. Beta diversity shows there are two clusters formed based on date indicating big change in diversity between earlier and later cultivation period.
- No significant effect of biomass productivity and position of flow-way on diversity changes.

Association with algal productivity

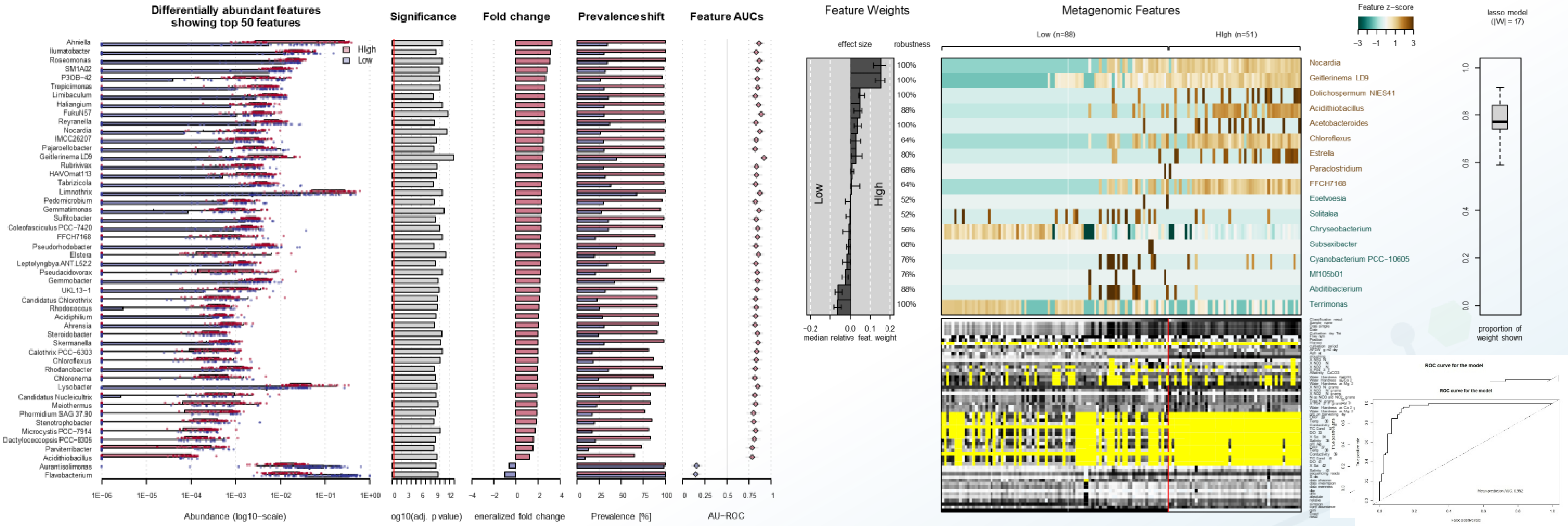






- Relative abundance of microbiome between two group (high (>3 $g/m^2/day$) and low ash-free biomass productivity) was assessed.
- Abundances of top 50 features across two different groups (high vs. low) have shown significantly different.
- Cross-validation accuracy is high with AUC = 0.837
- Relative abundances of 27 bacterial genera collectively associated with ash-free biomass productivity of the flow-way with 17 of them positively associated and 10 of them negatively associated.

Association with sloughing activity



- Relative abundance of microbiome between two group (high (>20%) and low sloughing activities) was assessed.
- Abundances of top 50 features across two different groups (high vs. low sloughing activities) have shown significantly different.
- Cross-validation accuracy is very high with AUC = 0.952
- Interested in those associated with low sloughing activities, *Chryseobacterium*, *Flavobacterium*, and *Terrimonas*. gen.

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- John Benemann (MicroBio Engineering)

Sandia's Legacy in Attached Algae RD&D

Support from EERE-BioEnergy Technologies Office (BETO), the Sandia BioScience Center, and cost-matching from municipal and industry partners has resulted in 4 field deployments for investigating sensitivities to water chemistry, environmental factors, and engineering system stability; and establishment of a new environmentally simulated Algae Testbed Facility to support specific application domains

2013 - 2016: Preliminary bench-scale testing

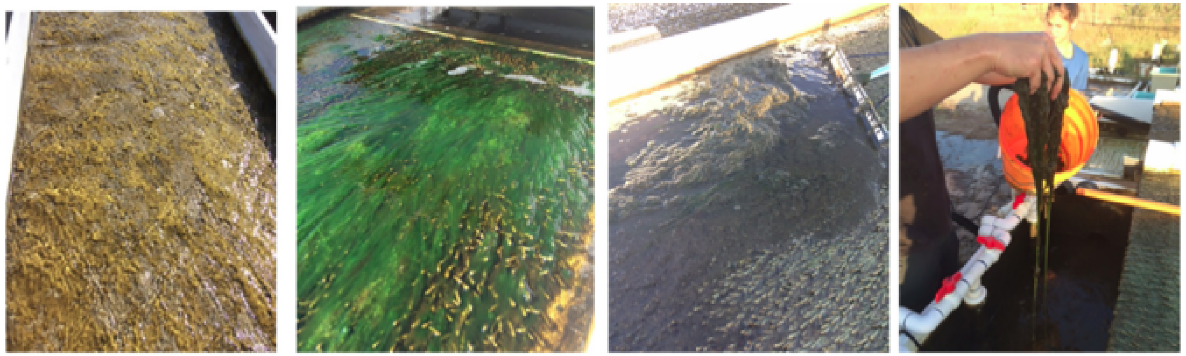
2015 - 2018: Osso Bay, Corpus Christi, TX (marine / estuarine)

2016 - 2020: Salton Sea, Brawley, CA (riverine, metals remediation)

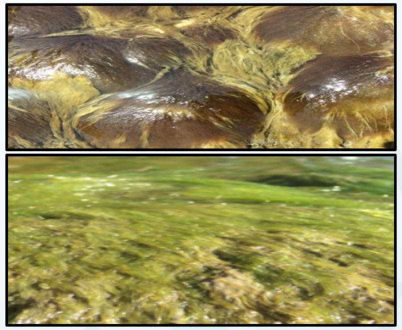
2017 - present: Sandia Testbed Facility – Livermore, CA (recirculating, environmentally simulated pilot)

2018 - 2019: Savannah River, Priest's Landing, GA (riverine / estuarine, substrate optimization)

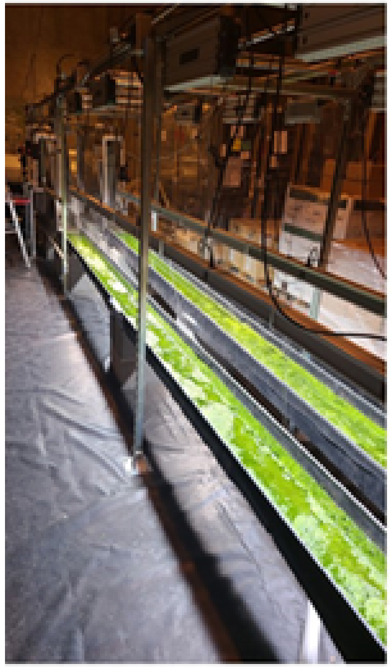
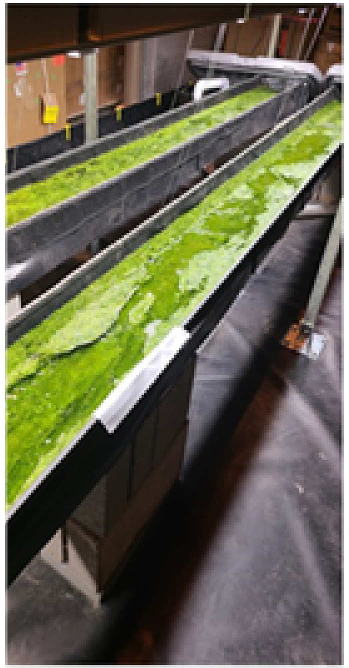
2021 -present: Barking Sands, Kekeha, HI (WWTP outfall)



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



Sandia's Algae Testbed Facility for evaluation of algae systems

Algae Testbed Facility:

Environmental simulated algae cultivation:

3x 1000L Open Algae Raceways (paddle-wheel driven, CO_2 injected)

6x 100L Open Algae Raceways (paddle-wheel driven, CO_2 injected)

2x 30' Attached Algae Floways (single pass / recirculating)

3x 80L Bubble-column Photobioreactors

incl. associated media pumping, biomass harvesting, and process monitoring

Applied Biosciences Laboratory:

10x 1L ePBR Photobioreactors

2x MC1000 OD Multi-Cultivator Apparatus

4x Diel Cycle Incubators

incl. full analytical suite, including nutrients, bio-intermediates, and bioproducts

Bioinformatics Hub:

Biota Computational Cluster / Server

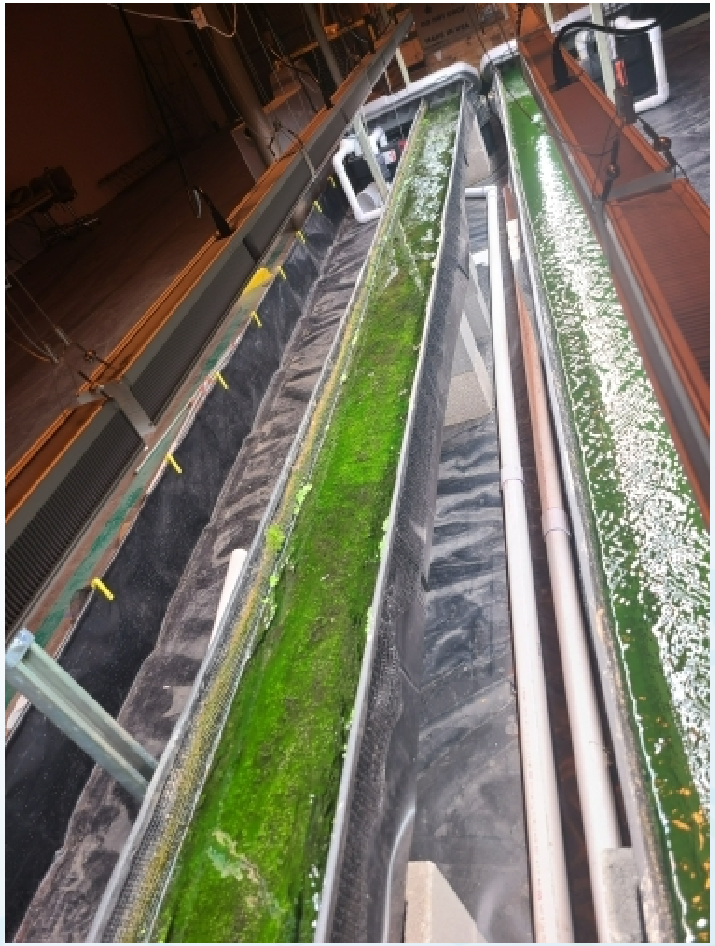
Computational Fluid Dynamics

Amplicon Sequencing Bioinformatics Pipeline

MAGPie

https://www.sandia.gov/research/research_foundations/bioscience/index.html

Sandia-Livermore Turf Algae Flow-Ways



- On-going operation of Sandia's environmentally simulated flow-ways
- 'Slough-based' harvesting
- Sample collection for microbiome sequencing
- Biomass pooling for conversion testing

  - approx. 7 Gal of 10-15% solids AFDW pooled with **average ash content of 15-20% w/w** for pilot HTL testing with PNNL (for comparison to high ash sample, provided April 2021).

- FY22 New patents issued: Davis, Monroe, Kim "Algal harvesting and water filtration" US Pat No 11186507; Davis, Liu "Enrichment of amino acids from biomass residuum" US Pat No 11326193; Davis, Tran-Gyamfi, Wu, Simmons "Biochemical upgrading of high protein biomass and grain

Commercial deployment for water treatment

Two multi-acre algae turf (aka 'marsh treatment') facilities operating in FL since 2004

Used specifically for treatment of reverse osmosis brine by-product prior to discharg to remove hydrogen sulfide and other contaminants, including *gross alpha particles* and ammonia

(Indian River County 2030 Comprehensive Plan, Chapter 3B: "Potable Water Sub-Element", Suppl #11, Oct 12, 2010)

Sources of gross alpha particles could be any actinide (Z>84), but Uranium, Radon, Radium, and Thorium are the most likely sources in ground water.

("Natural Radioactivity in Groundwater – A Review", USGS, Reston VA, 1988)

Sandia's Turf Algae Technology Maturation Plan and Commercialization Efforts (FY21-22)

Feedback controlled water provision

Mitigate ash accumulation

US Domestic GIS/RA

N/P/M reclamation

Minimize sloughing losses

Automated harvesting

Tie-in to CA-biogas infrastructure

Agricultural CO2 credits

Cultivar management

Process water/WWT integration

AD feedstock blending

On-site biomass logistics

Irrigation ditch, waterfront integration

TRL	5	6	7	8	9

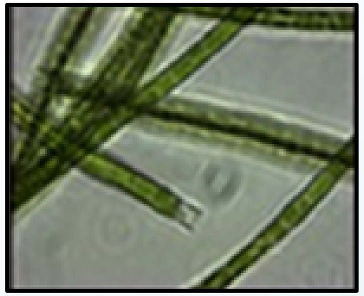
ENERGY I-CORPS U.S. Department Of Energy

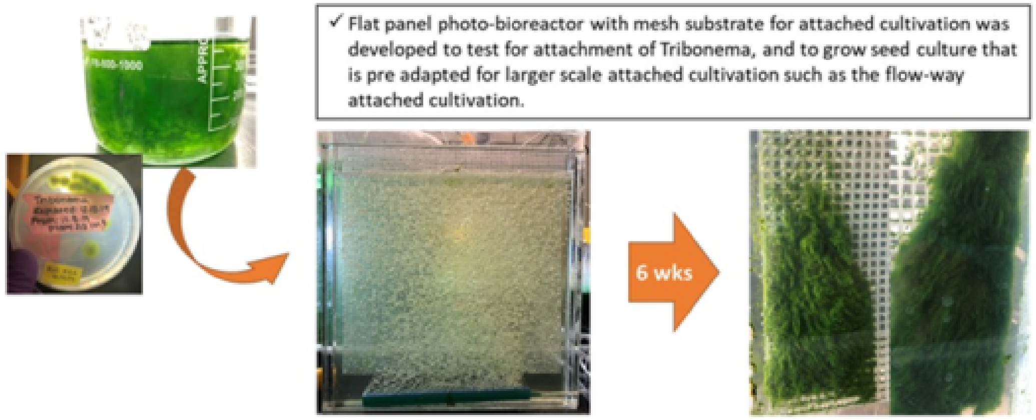
μBIO MicroBio Engineering

Demonstrated technology improvements (dashed green box)

On-going tech. dev (yellow box)

Cultivar dev. at Sandia Testbed





Industry Partnerships

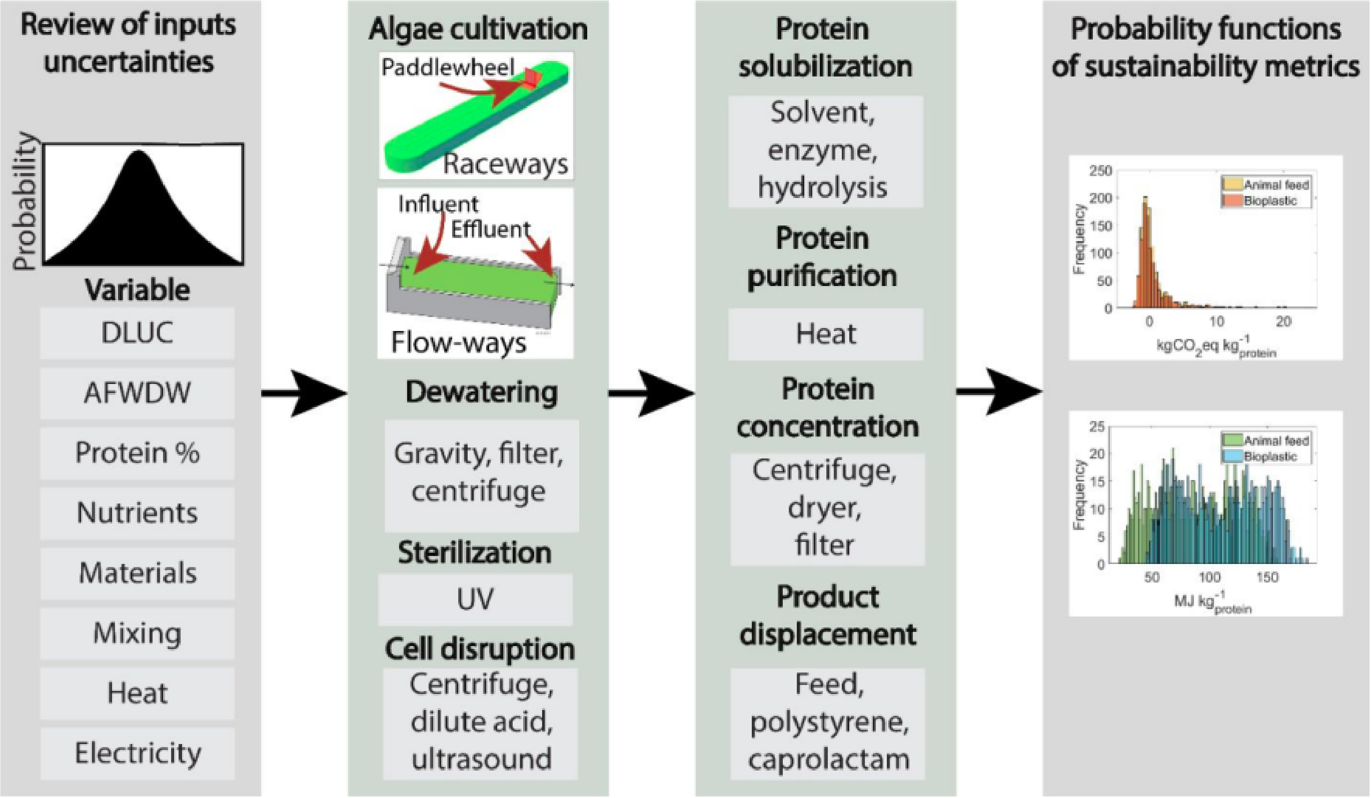
Imperial Irrigation District www.iid.com
MicroBio Engineering www.microbioengineering.com
HydroMentia Inc www.hydromentia.com
Global Algae Innovations www.globalgae.com
Algix Inc www.algix.com
HelioBioSys Inc www.heliobiosys.com
Zivo BioScience Inc www.zivobioscience.com

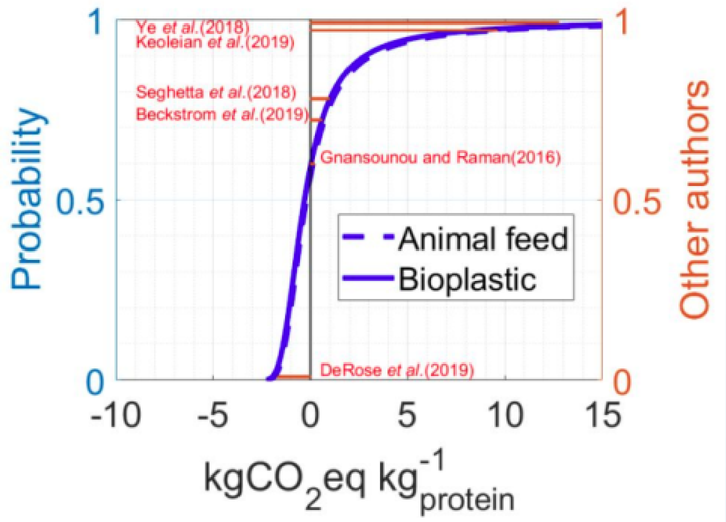
U.S. DEPARTMENT OF ENERGY

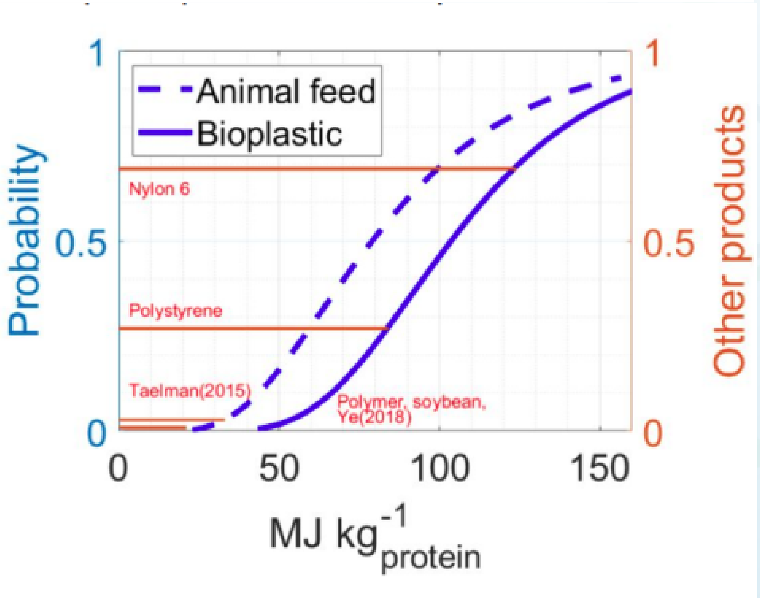
Sustainability & risk assessment for utilization of Turf Algae biomass for bioproducts

Stable probabilistic distributions of energy consumption and CI for algae biomass based on currently available (high protein) algae and processing technologies show:

1) >50% probability for negative carbon emissions for both feed and biopolymer scenarios
2) >67% probability for energy cost reduction for monomers production versus nylon-6







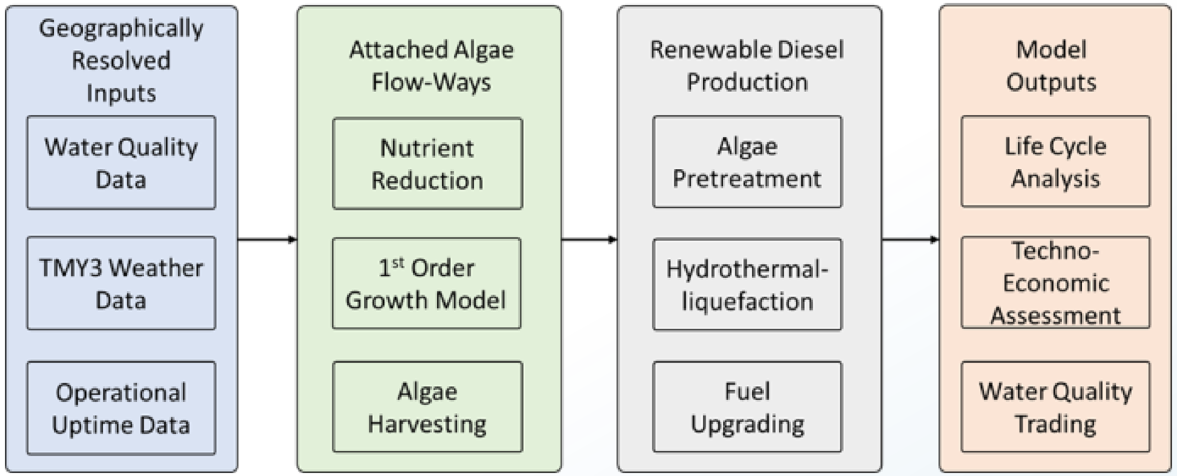
Quiroz-Arita et al *Sus Energy & Fuels* 2021

GIS analysis provides siting logistics for positive ROI from Turf Algae deployments for nutrient credits + biofuel production

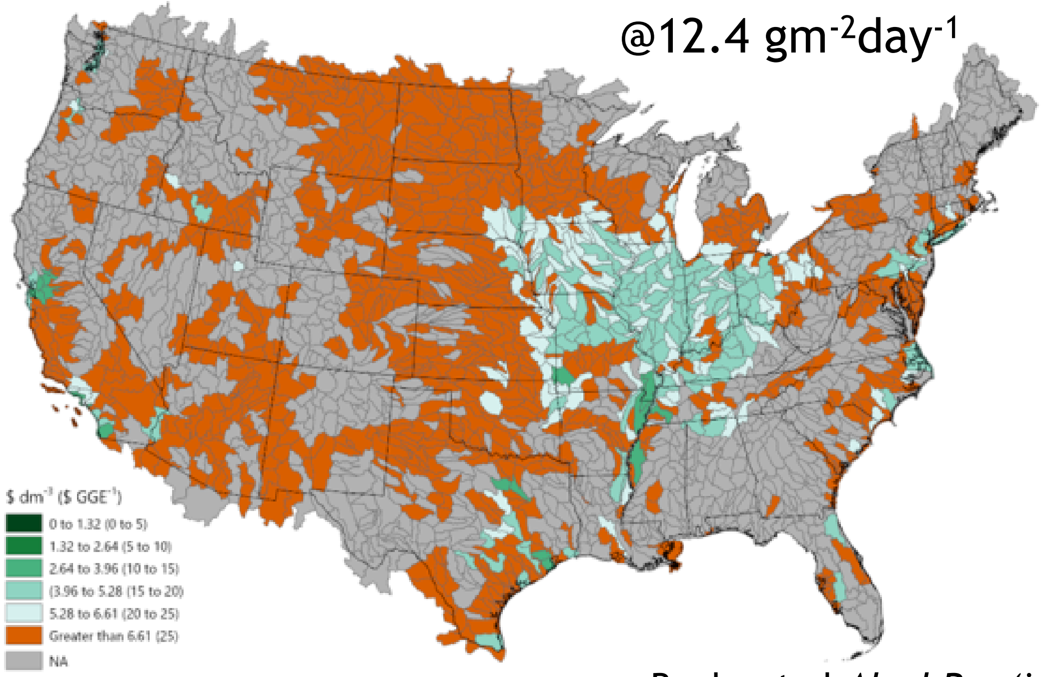
TEA/LCA-GIS scenarios based on nutrient remediation + HTL conversion using Turf Algae

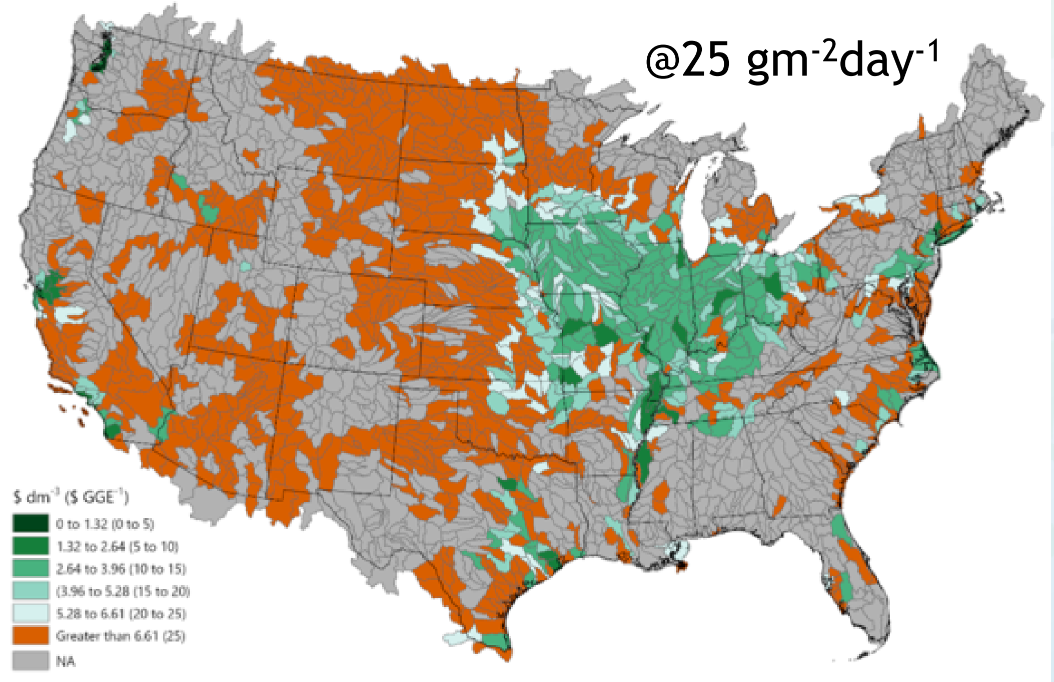
At least 1Mt/yr of biomass would be available in the US from net ROI+ ATS deployments at a nutrient credit of $19.0 /lbs nutrient, assuming $3.50 /GGE, and $15.2 /lbs nutrients (+ carbon), assuming $5.00/GGE.

Currently offered nutrient credit values are $10-$15/lbs nutrient (EPRI), whereas cost of waste water treatment is $100 /lbs nutrient. No Carbon Credit was assumed in this analysis



Map: Net fuel costs offset by nutrient credits





Banks et al *Algal Res* (in review)

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