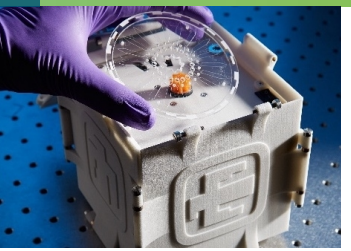
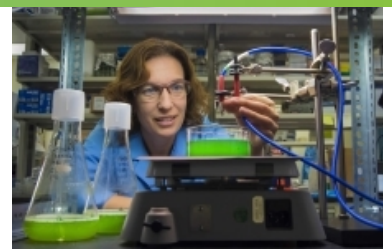
SAND2024-14074C

way System for Enhanced Biomass Production

PRESENTED BY

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Algae Biomass Summit 2024

Oct 22, 2024

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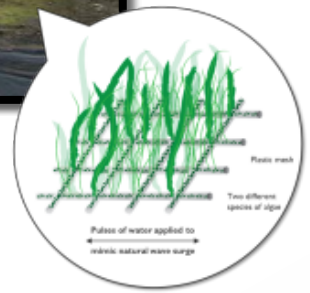
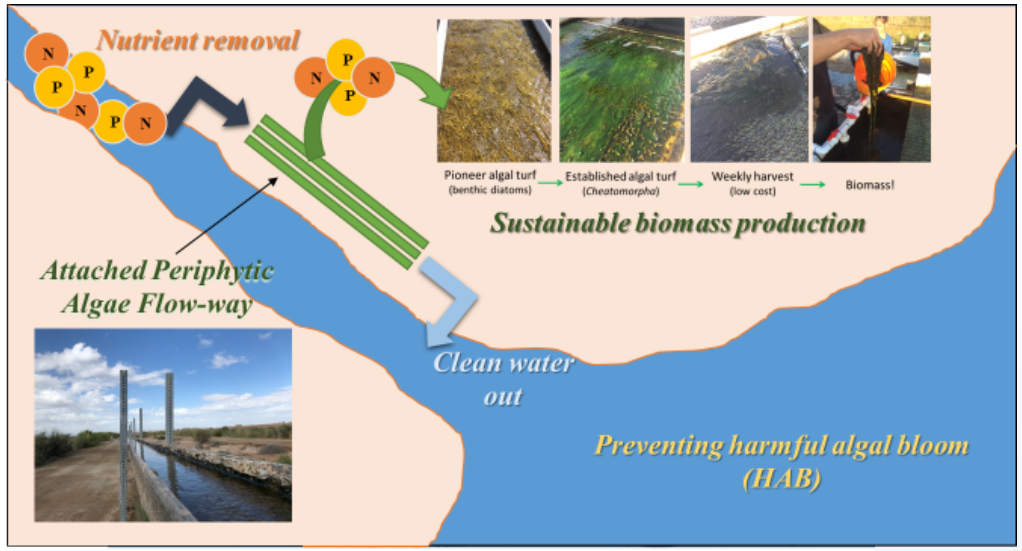
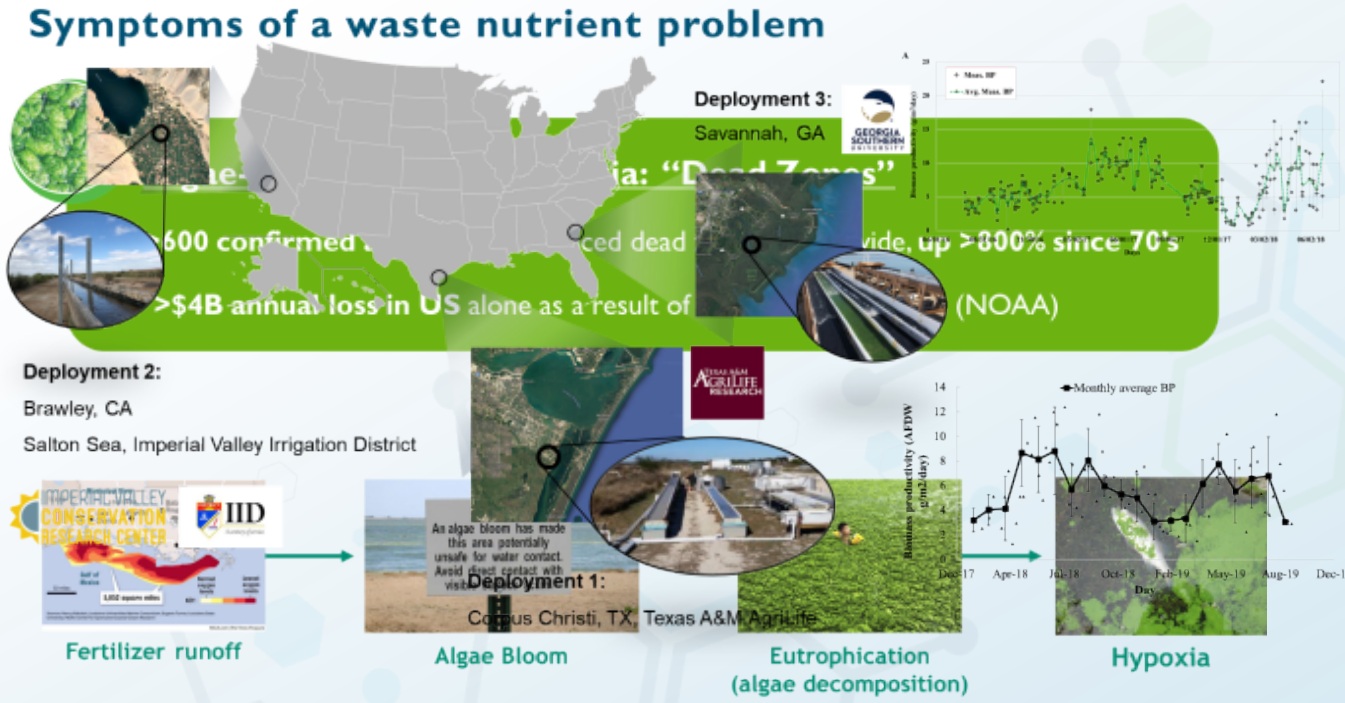
Attached Algae Flow-way

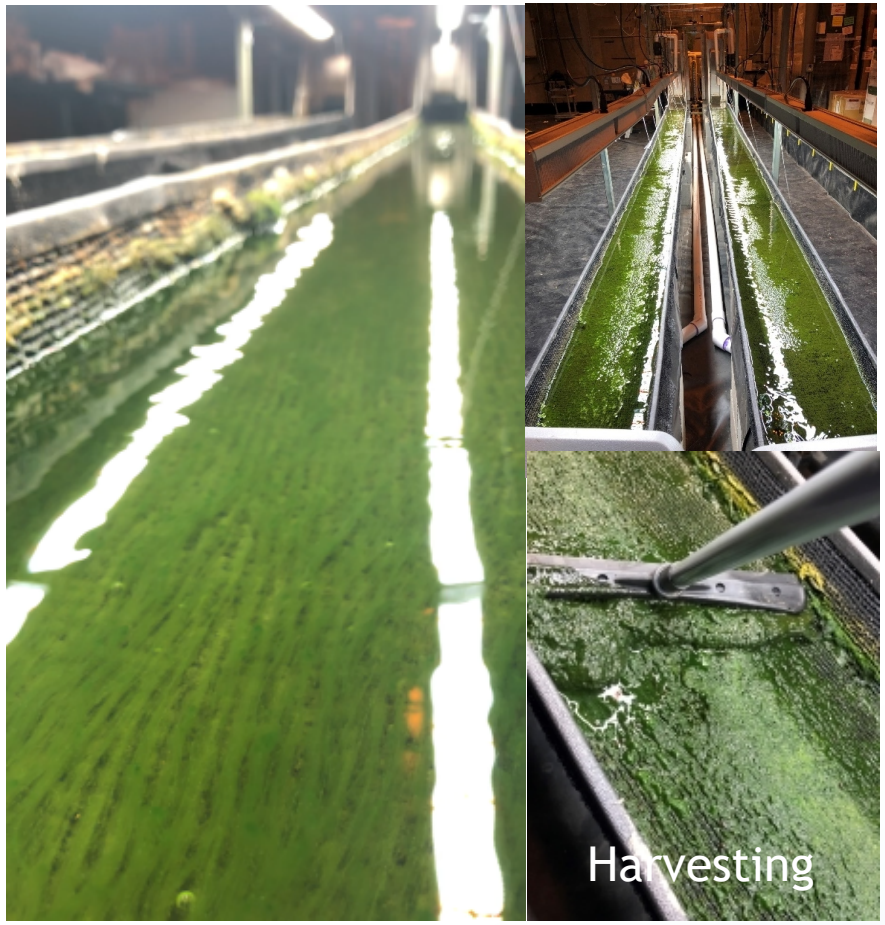




- Provide habitat for natural filamentous algae assemblages to proliferate
- Attached growth allows utilization of dilute nutrients, ie. flow rate can be adjusted based on nutrient concentration variability
- Potential symbiotic mixotrophy benefits from carbon sources in agricultural runoff
- Potential for dramatic decrease in hydrodynamic residence time for water treatment: 35x improvement in L/m^2 versus conventional raceways
- Regular harvesting to maintain log-phase growth



Attached Algae Flow-way in Pilot-scale



Operating Conditions

- Two of 20ft-long 1ft-wide gently sloped (~1°) flow-way
- Medium circulated with periodic addition of nutrients (fed-batch)
- Nutrients: Under high nutrient condition [15 ppm N_{total} ($NaNO_3$), 1.5 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 maintained low and with diurnal cycle (0 - 800 µE/m^2/s)
- 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.

Strategies for Improvement

Problem Statement

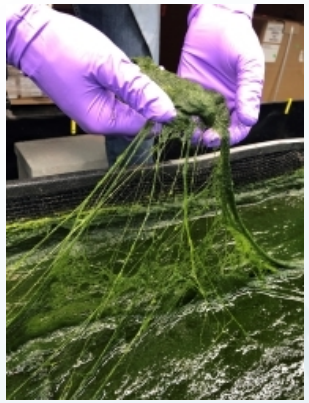
- Sustainable biomass production was achieved, but ash-free biomass productivity is not yet competitive.
- Biomass quality needs to be improved -> lower the ash content and higher the lipid content

Strategies to tackle problems

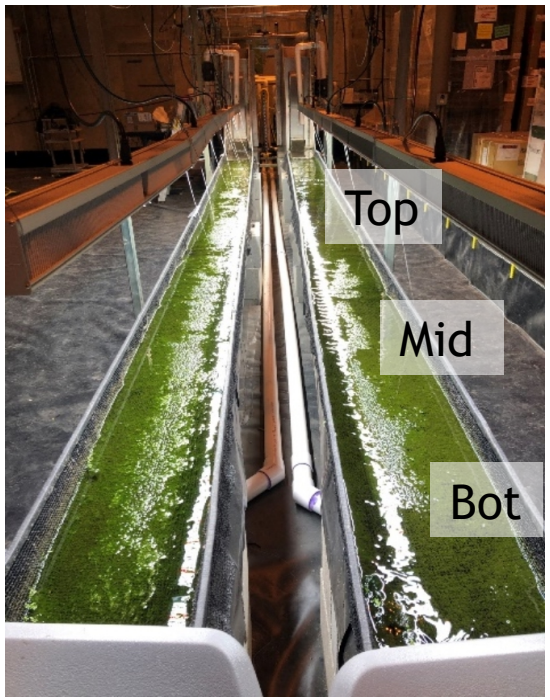
- Biological approach: Understanding dynamics of microbial community over time and their potential impact on biomass productivity and quality
- Mechanical approach:
  - Design of the flow-way – change the geometry of the system to increase surface to volume ratio
  - Operating parameters – optimize the operating parameters such as length of HRT, pulse flow frequency, flow-rate, harvesting method and frequency.

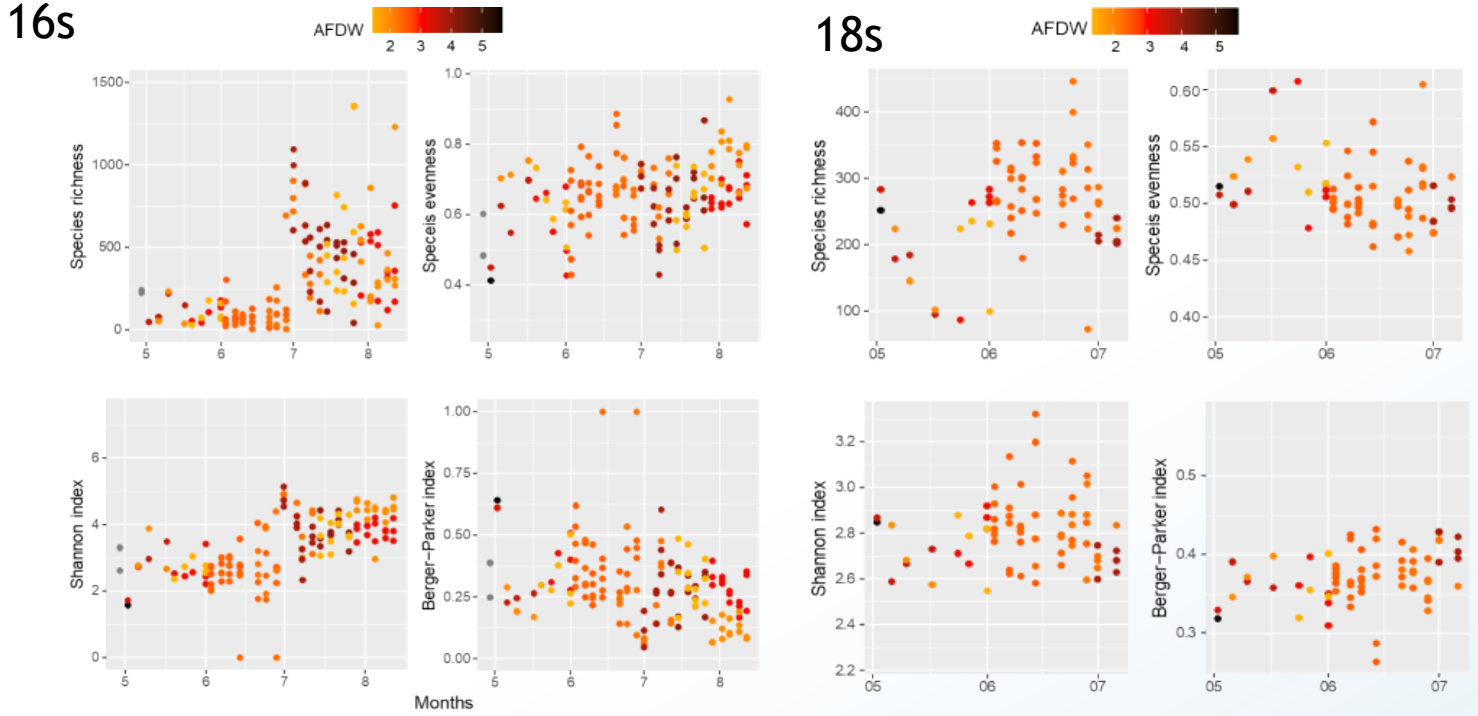
High productivity

High quality

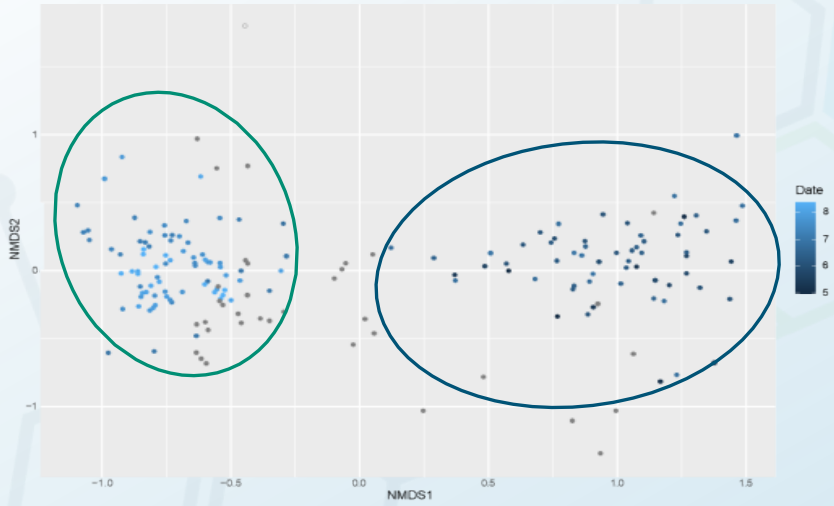


Long-term microbial community dynamics

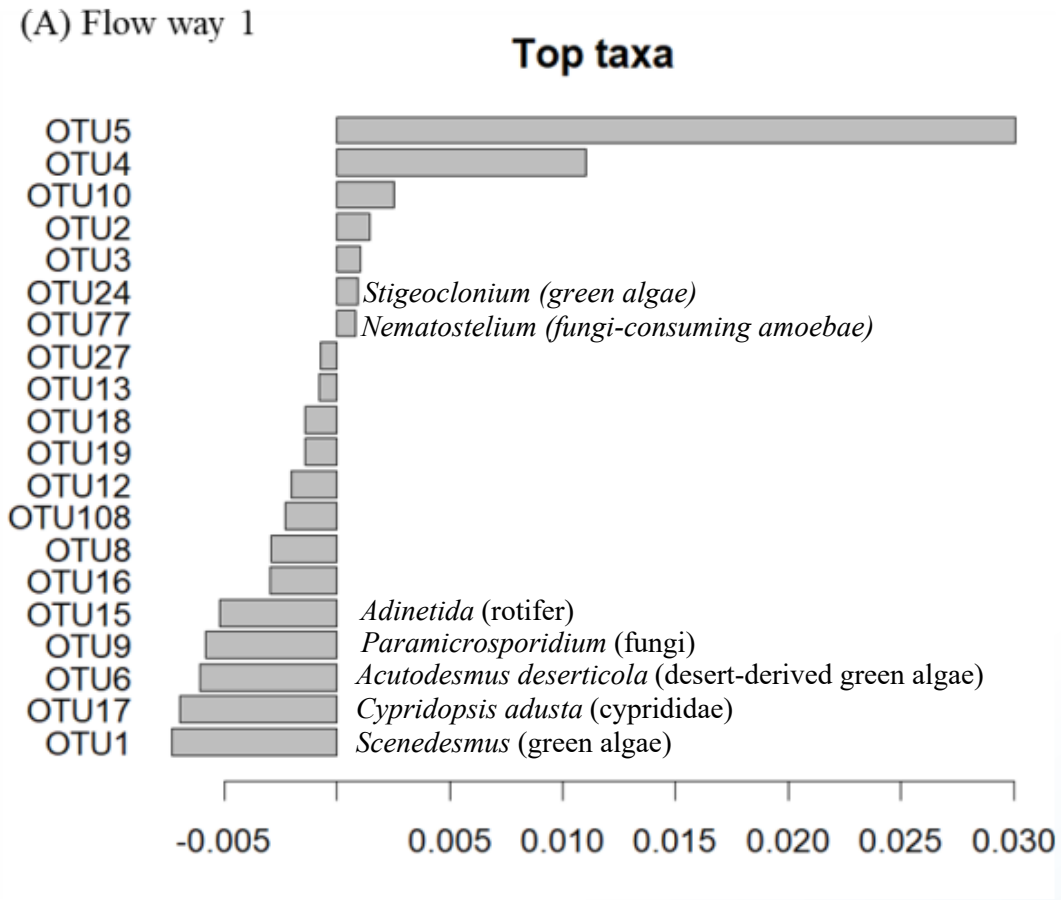


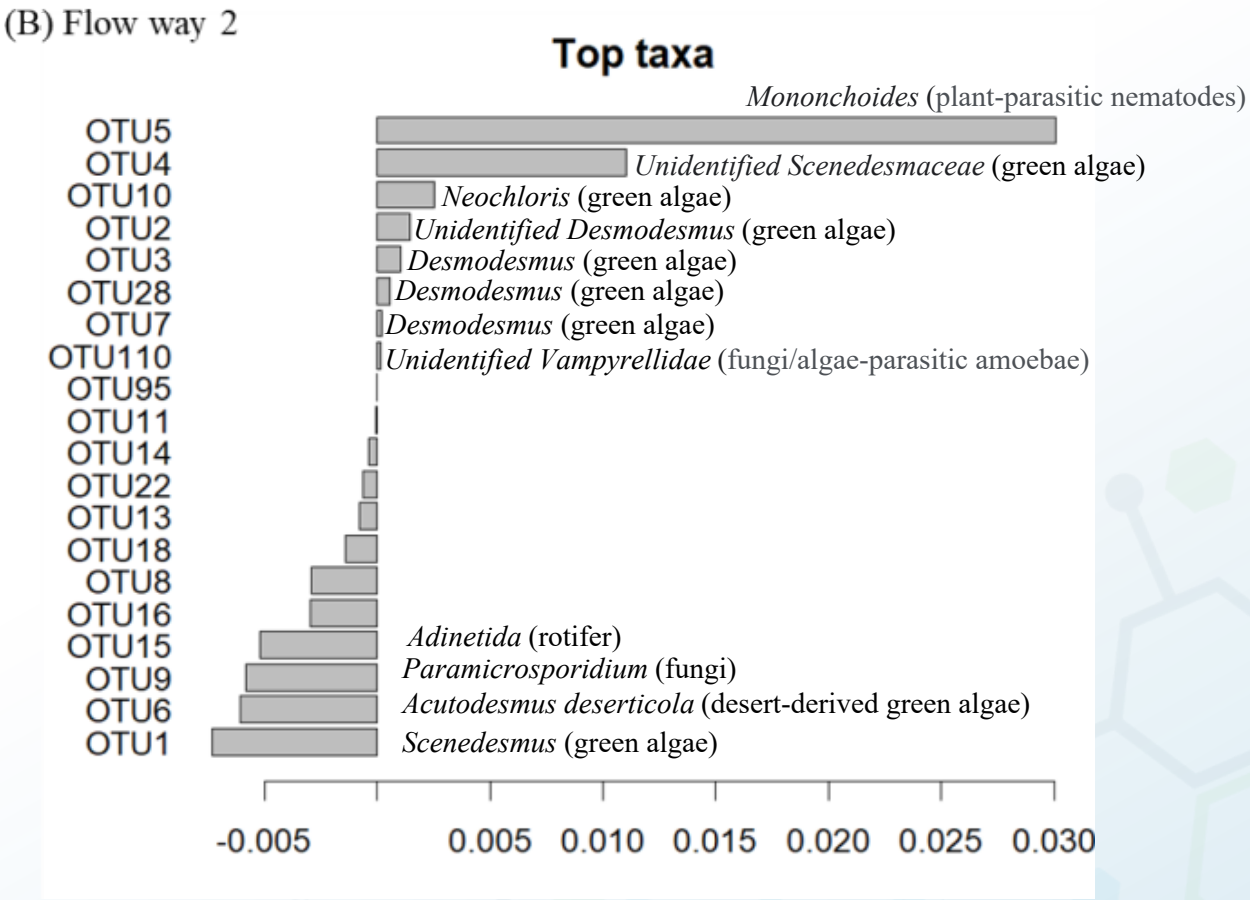


- Bacterial community at genus level were first analyzed in terms of their diversity: alpha diversity over time and beta diversity
- Diversity of bacterial community increased over time along with their richness and evenness. Beta diversity shows there are two clusters formed based on date indicating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



Short-term microbial community dynamics

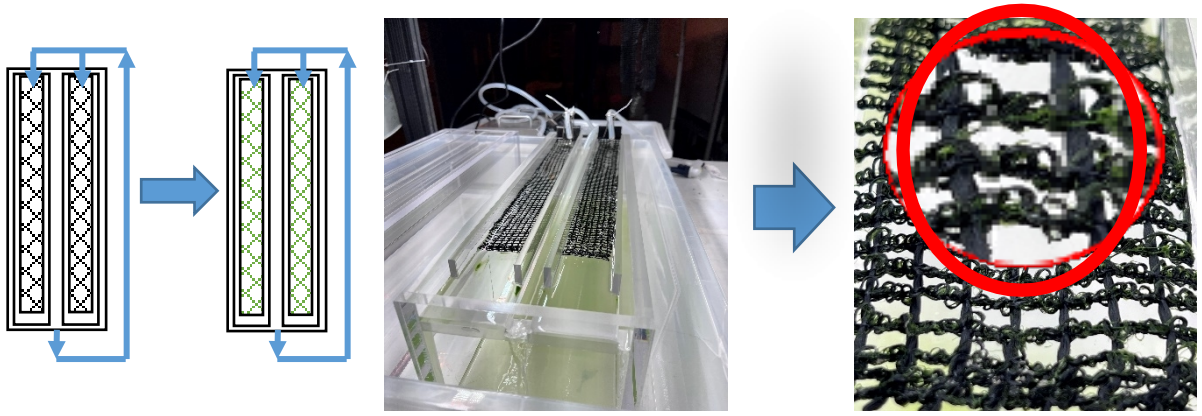




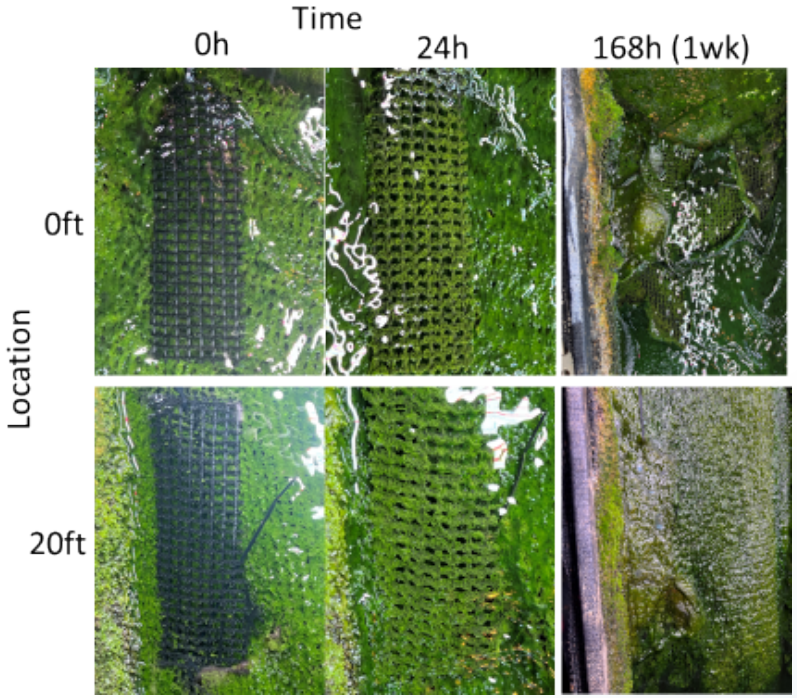
- Ten taxa affected to microbial community differences. Top five taxa are common in both flow ways
- Except the worm and amoebae, green algae taxa led to the microbial community differences

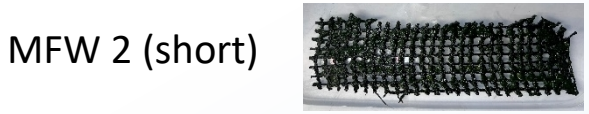
Seeding Strategy

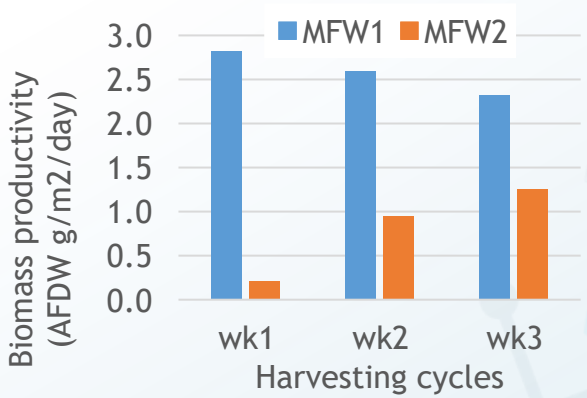
- With new substrates (no bio-activation)

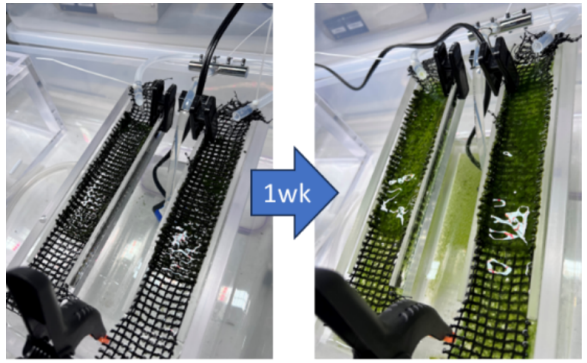


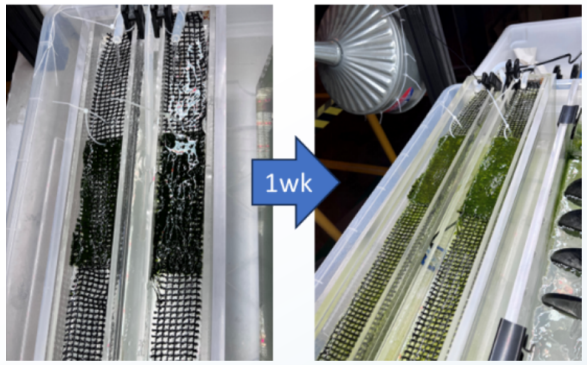
- Bio-activation durations: long vs. short periods (*S. obliquus UTEX393*)

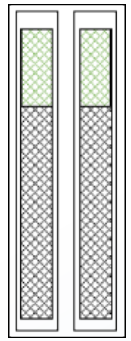


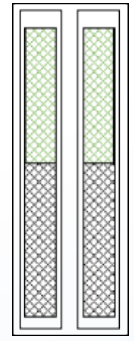


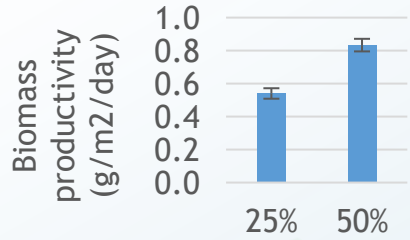








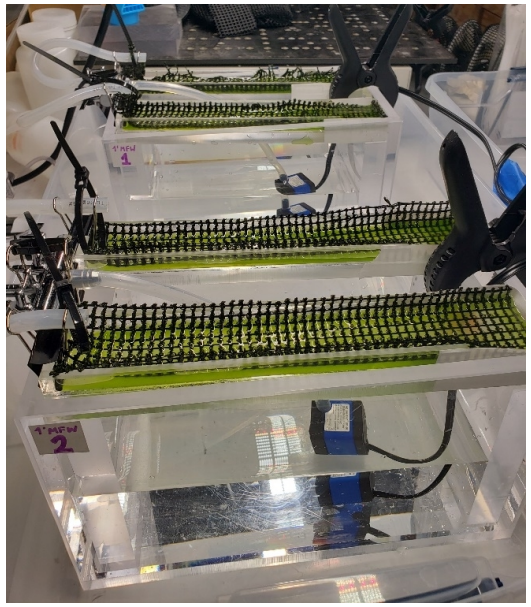




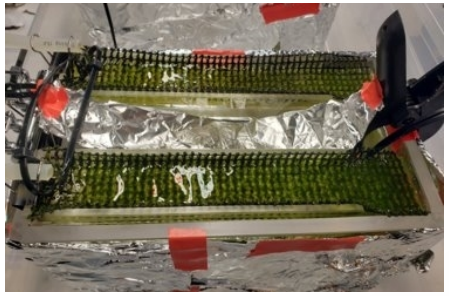
- Different substrate conditions were compared.
  - New substrates vs. bio-active substrates (substrates pre-covered with biofilm)
  - Different bio-activation duration: short vs long
- Different seed substrate portions to empty substrate were compared:
  - 25% vs 50% of surface area were compared.
- As a results, **long-term bio-activation** provided much better initial cell attachment and cell growth on the empty substrate. Also, **50% surface area** showed much stable cell growth.

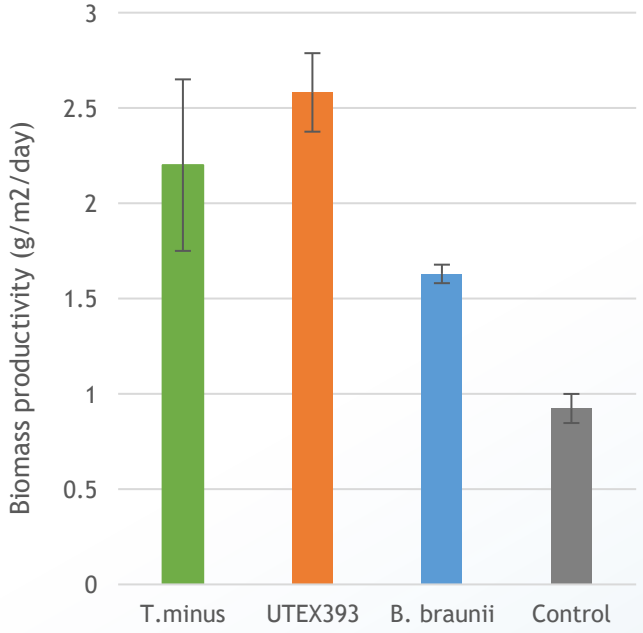
Seeding Strategy

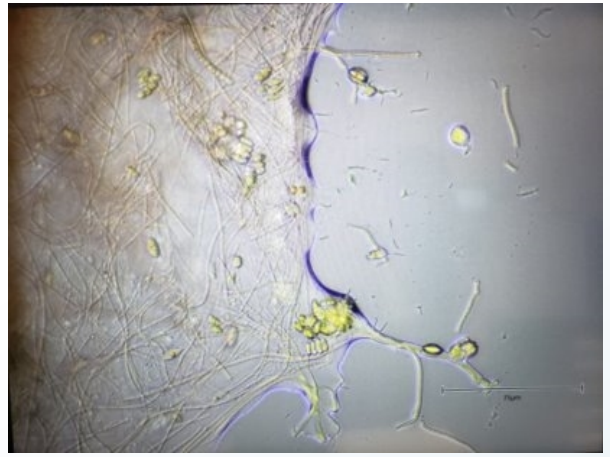
- *Biomass productivity with target strains seeded*

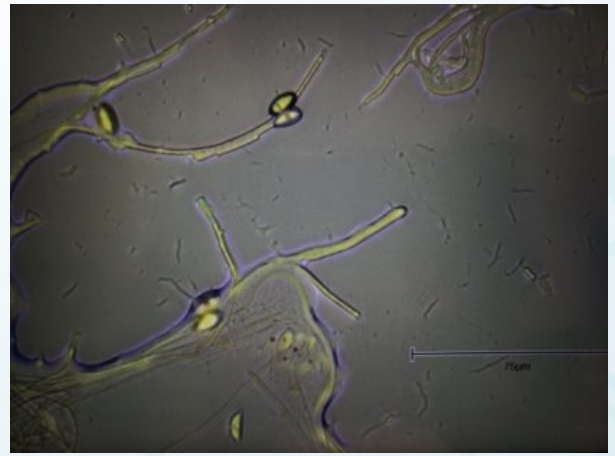


7 Days









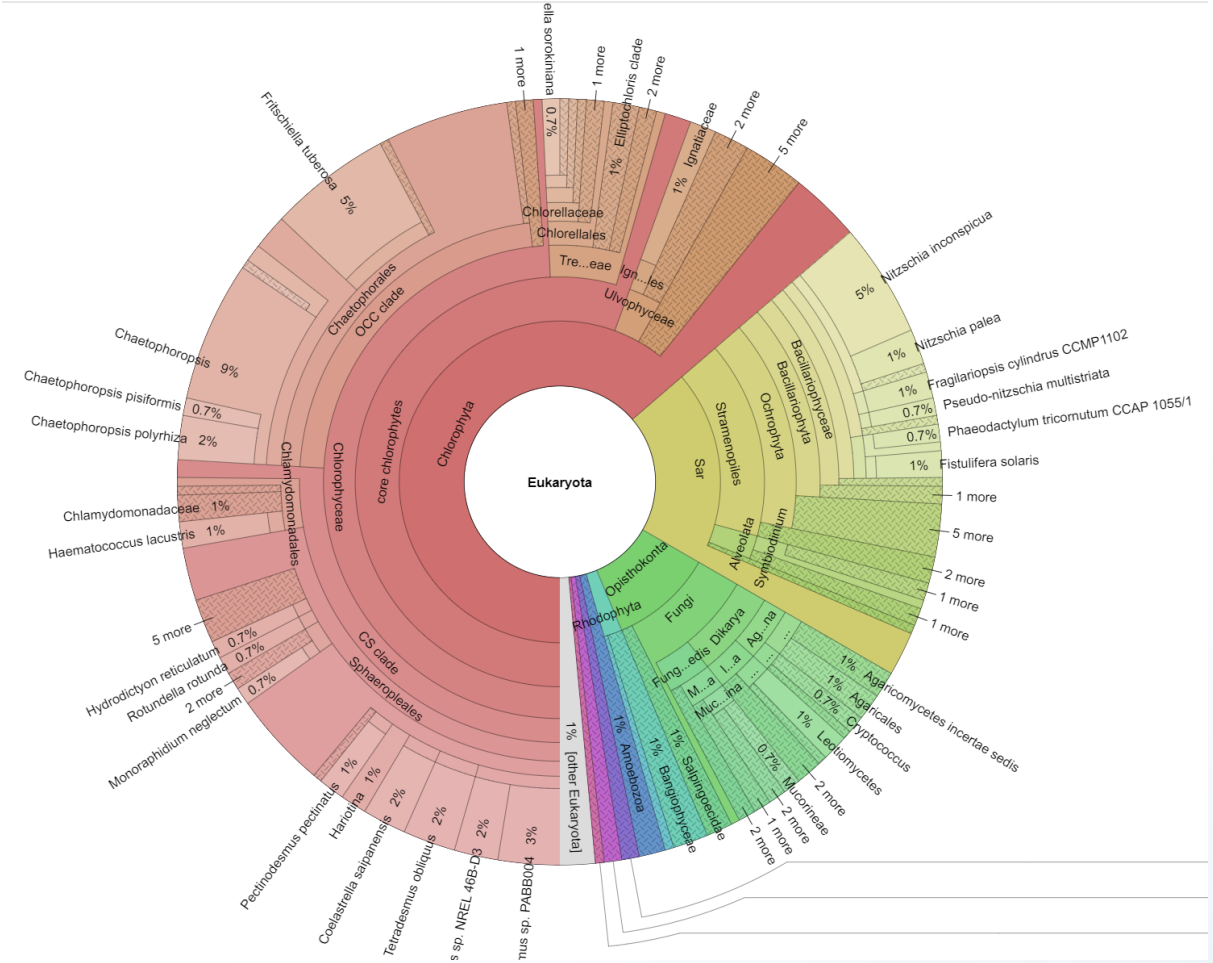
Microscopic images *Scenedesmus obliquus UTEX393* attached on filamentous algae confirming their epiphytic growth.

- Concentrated *T. minus, S. Obliquus UTEX393 and B. braunii* were seeded on top of the substrates, stayed on the substrates for 12 hours before the start of recirculation.
- Biomass was harvested and media was completely changed every 1 week for 3 weeks.
- 3-week average biomass productivities with *T.minus*, *S. obliquus UTX393* and *B. braunii* **improved by ~140%, ~180%, and ~75%, respectively** (2.2±0.5, 2.6±0.2, and 1.6±0.0 g/m^2/day vs. 0.9±0.1 g/m^2/day).
- Different harvesting strategies were employed for the culture of each strains.

Seeding Strategy

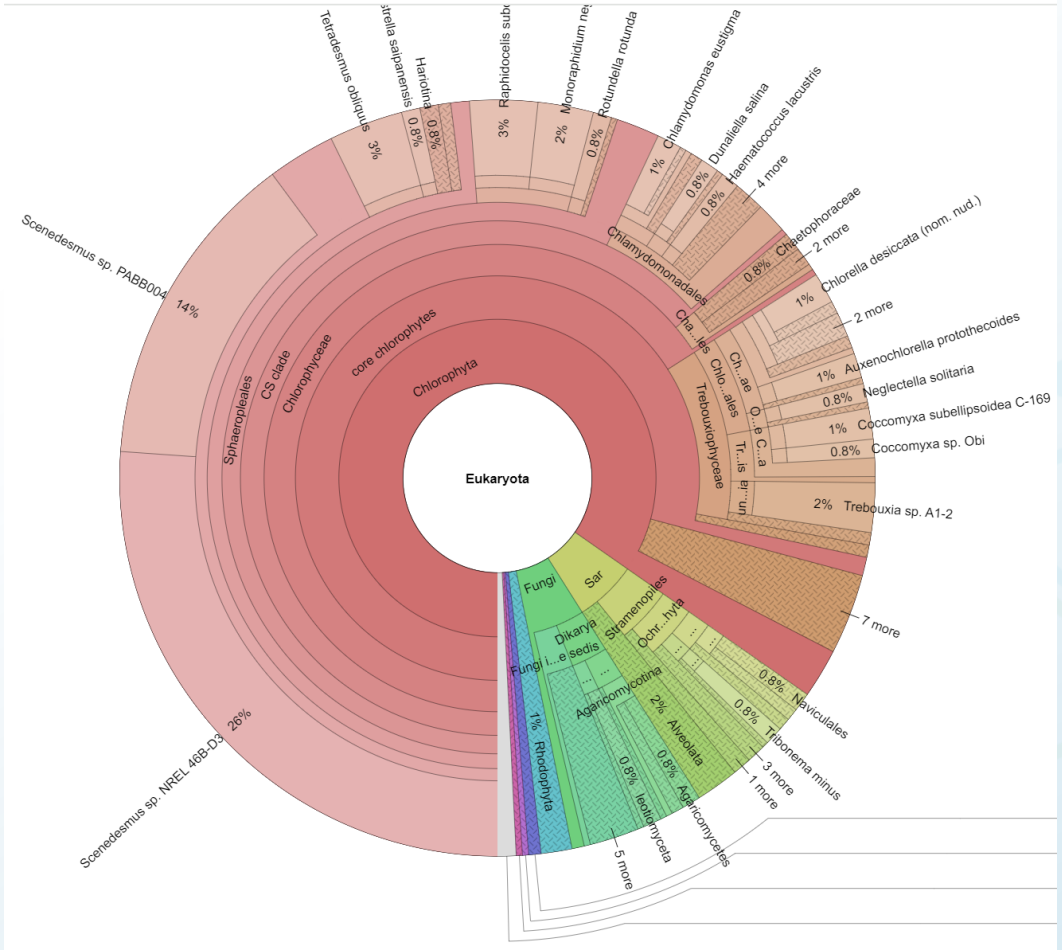
Initial condition

- Scenedesmus sp. NREL 46B-D3 (2%)
- Tetradesmus obliquus (2%)
- Scenedesmus sp. PABB004 (3%)



1st wk

- Scenedesmus sp. NREL 46B-D3 (26%)
- Tetradesmus obliquus (3%)
- Scenedesmus sp. PABB004 (14%)
- 7% → 43%



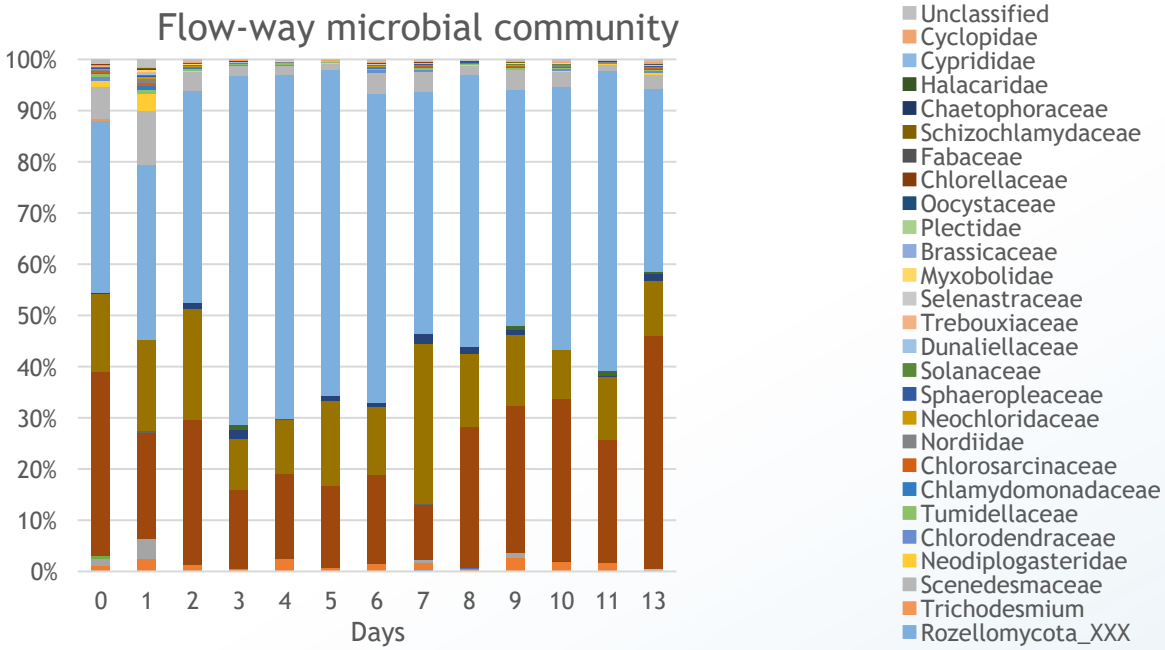
Seeding Strategy

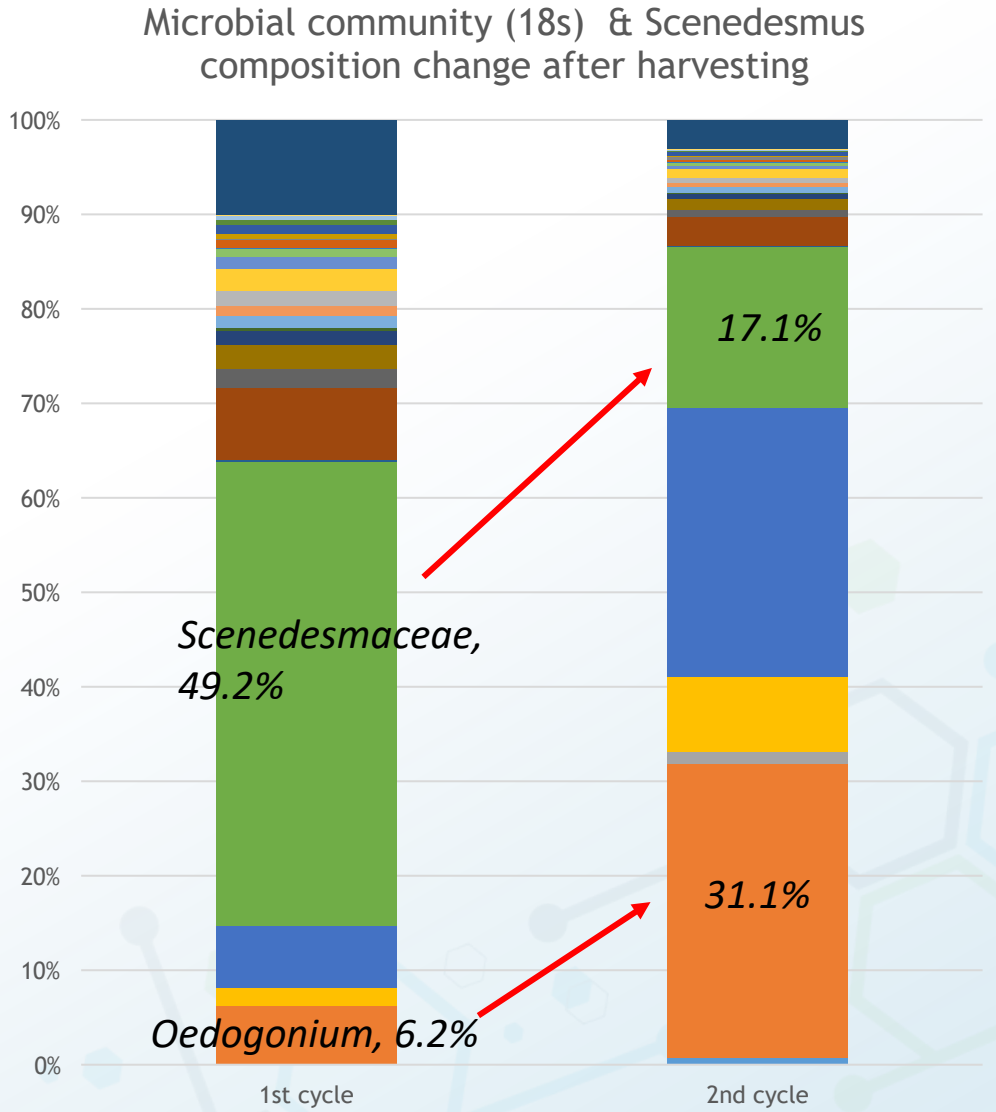
Microbial community analyses:

- Effort to improve accuracy of 18s sequencing result: Sample preparation, DNA extraction methods, and different database being tested.

Sample description	Sample mass (g)	DNA concentration (ng/uL)	A260/A280	A260/A230
Original wet biomass	0.2680	10-300	1.87	2.10
Liquid nitrogen	0.2504	40-150	1.83	2.12
Freeze-drying	0.2213	100-500	1.84	2.11

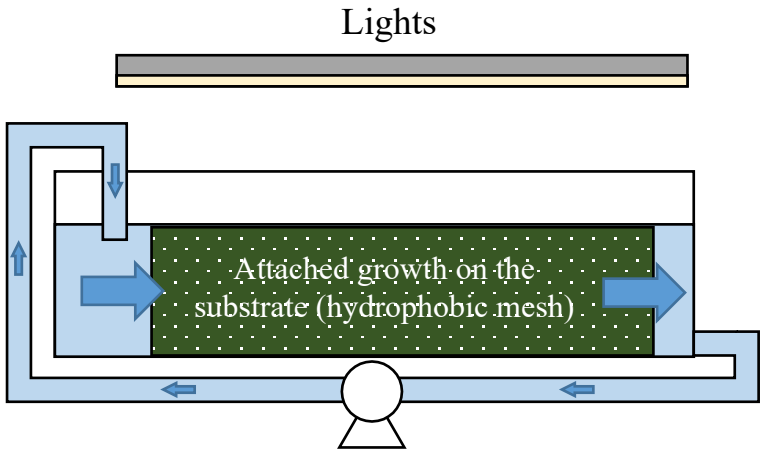
- Annotation using specific database for filamentous algae extracted from PR2 followed by SILVA database could be most reliable outcome.

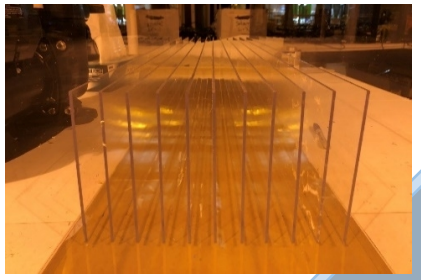


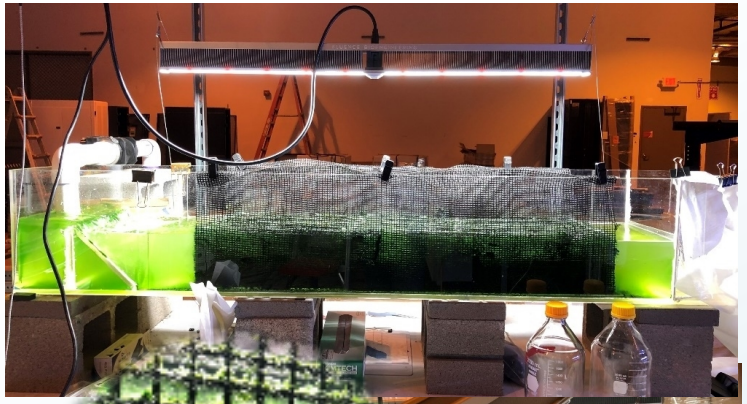


- Fatty acids based: 1 - 2% → 4.4 – 6.5% → 3.0 – 3.7%
- Crude lipids based: 1.9 – 3.8% → **10.3 – 25.1%** → **6.3 – 11.5%**

Multi-channel Attached Algae Flow-way (MCF)







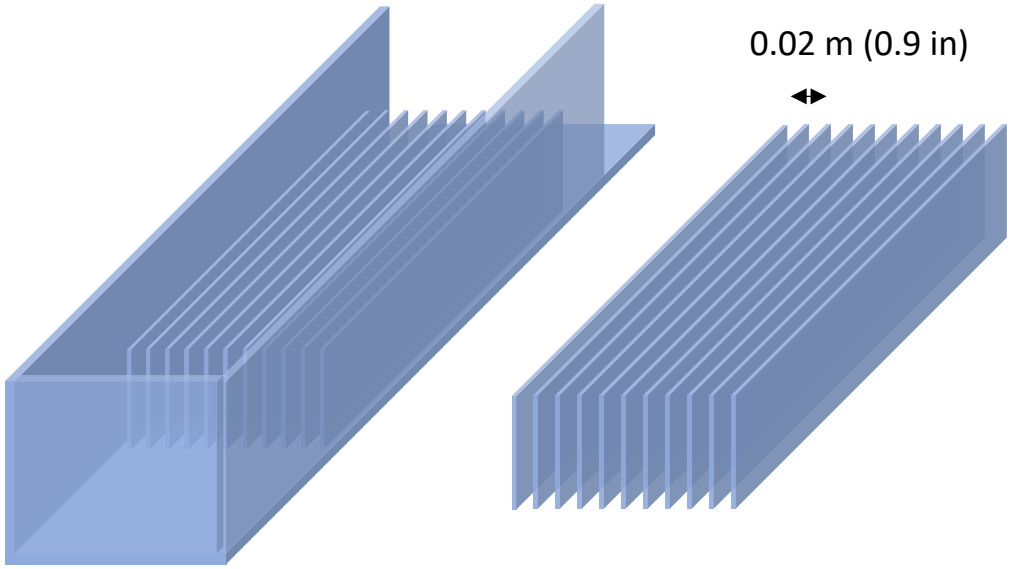
Higher ash at the bottom

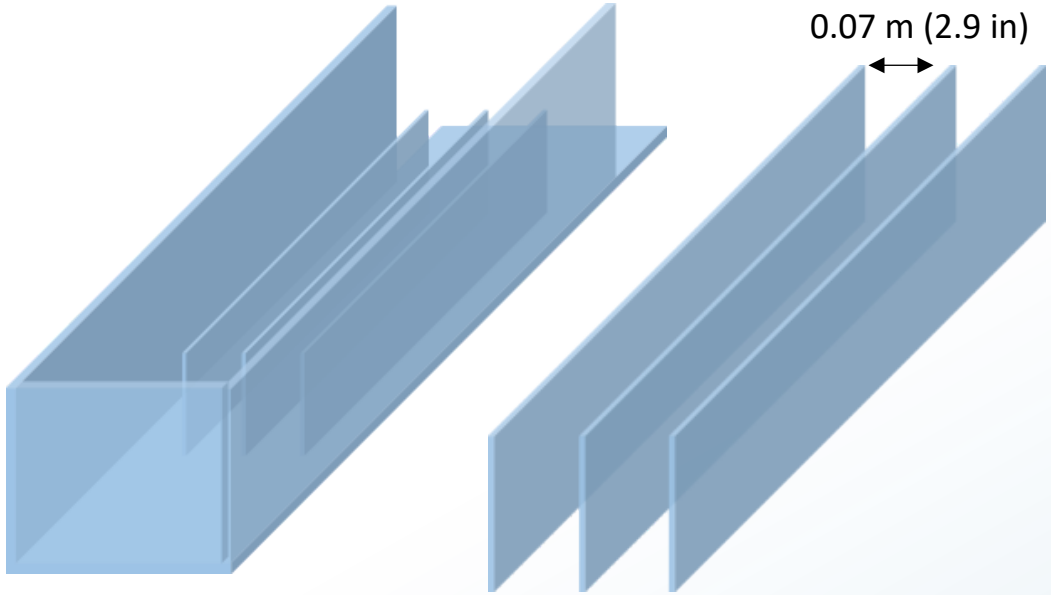
Footprint biomass productivity increased by ~300%

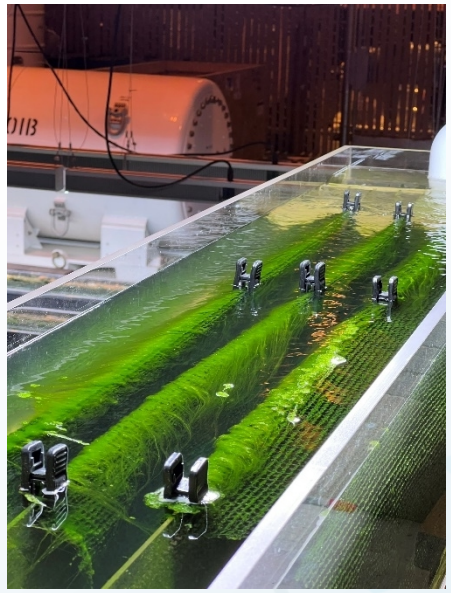
Operating condition:

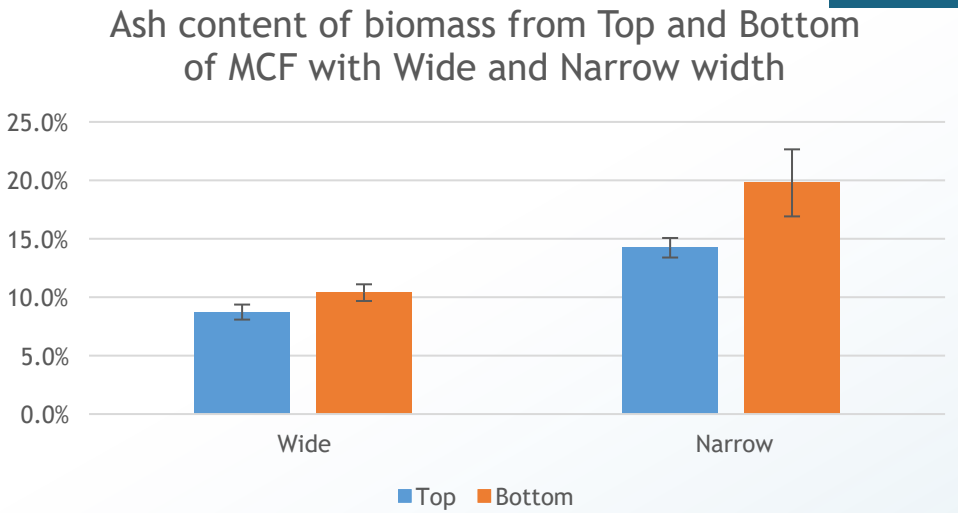
- Essentially maintained same as our flow-way pilot system
- Media is supplied at ~20GPM into the head tank and let flow over to the channeled flow-way
- A pass-through media is recirculated into the system.
- The light simulates the diurnal cycle.
- The depth of water is maintained to completely submerge the channels.

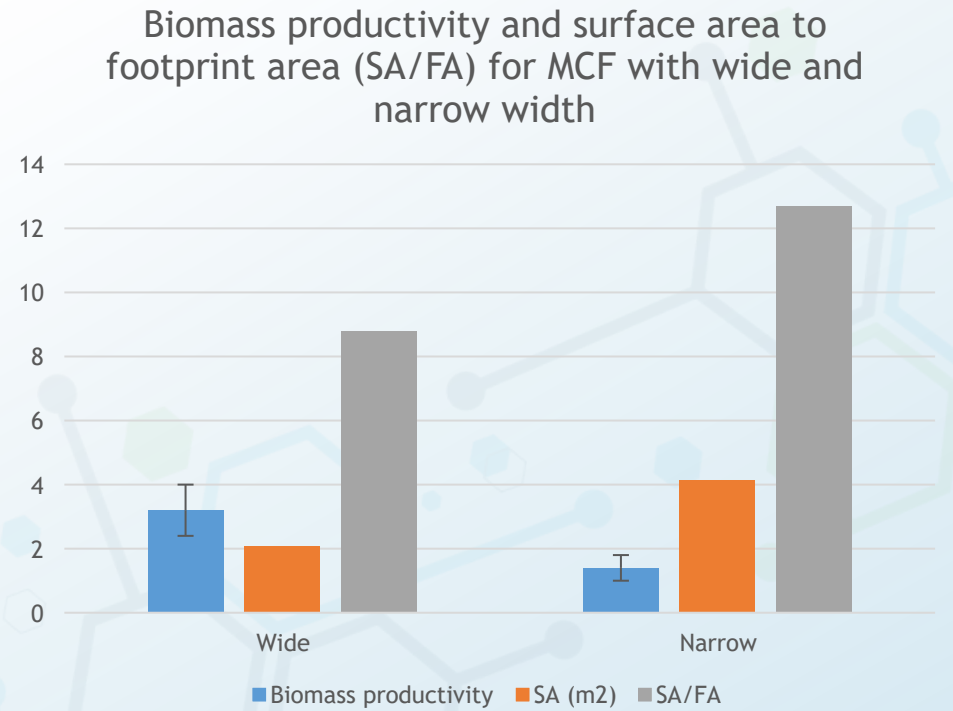
MCF Design and Process Optimization



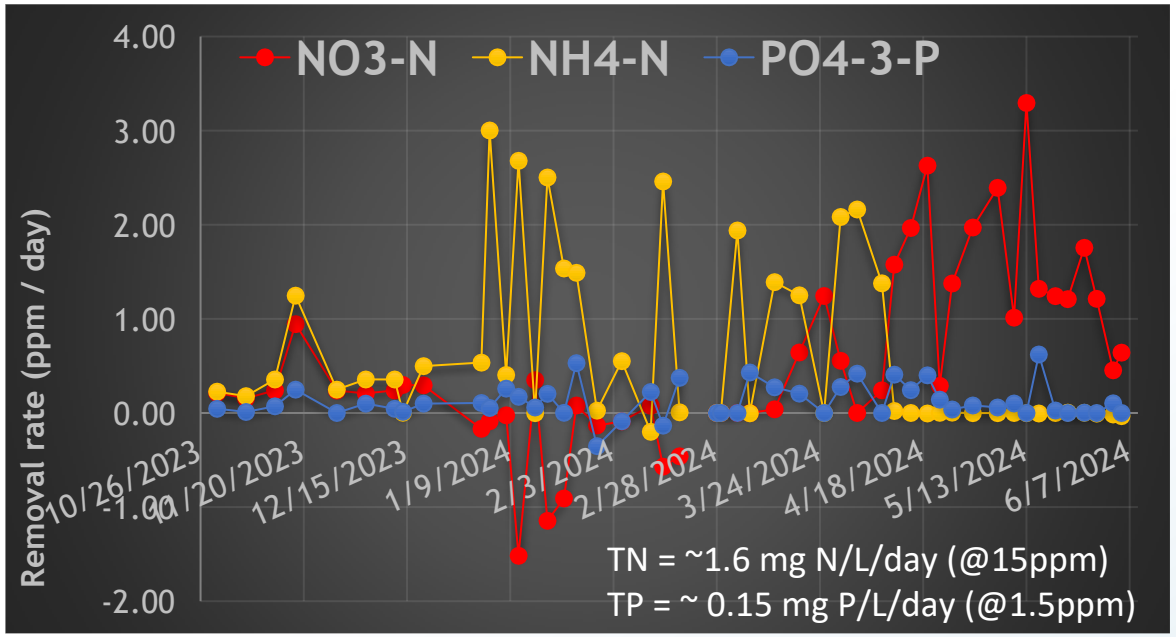


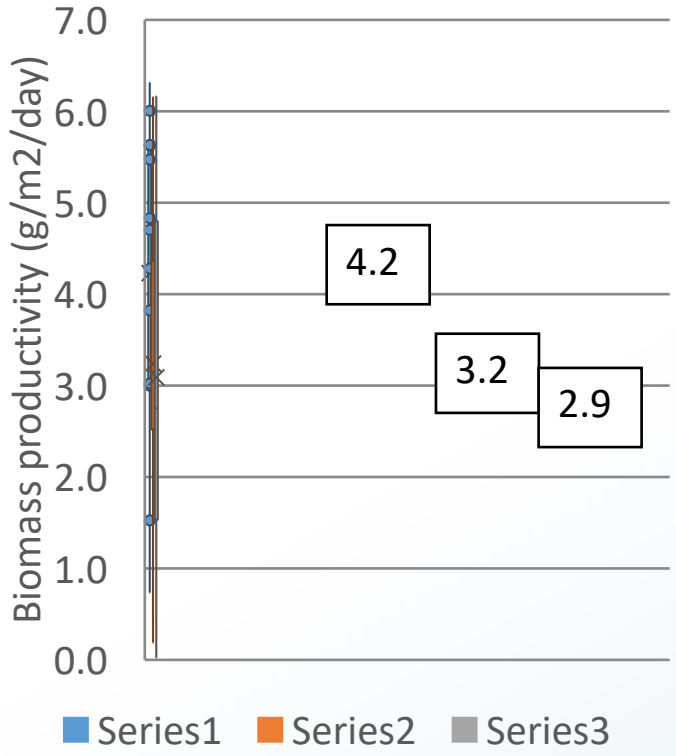


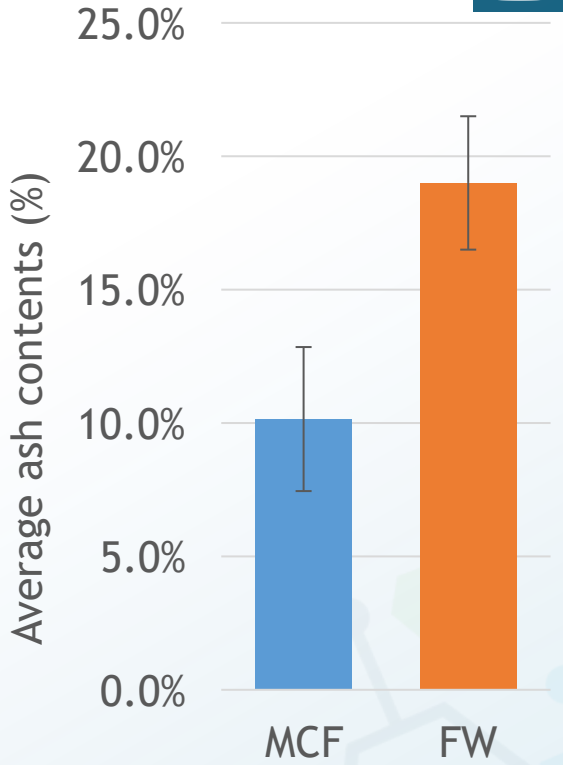




MCF Design and Process Optimization

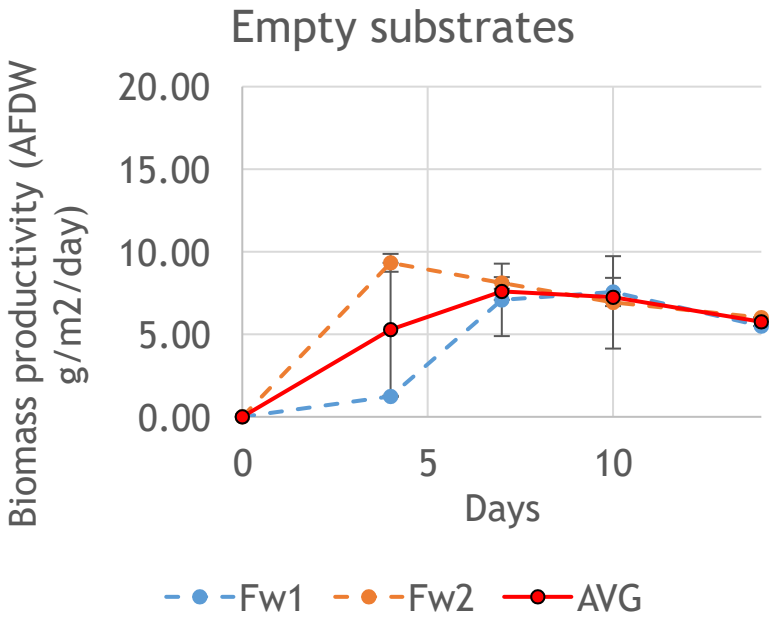


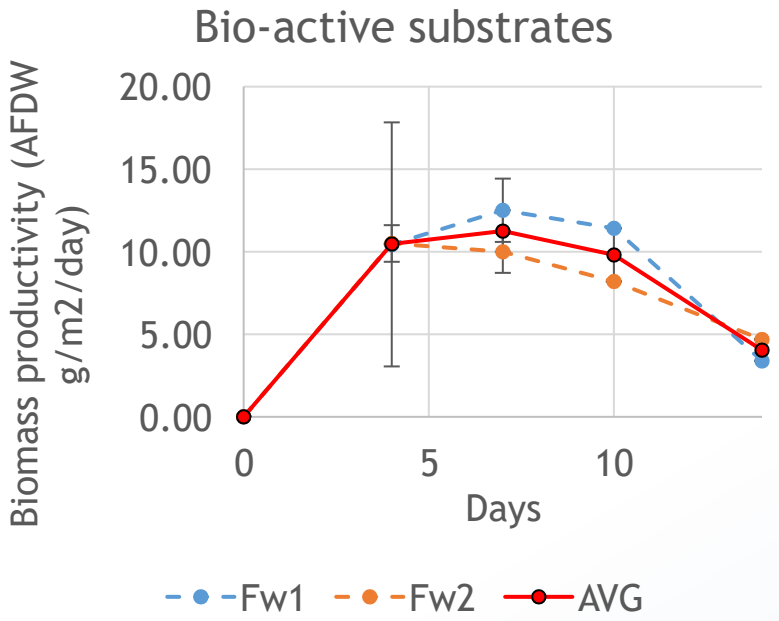


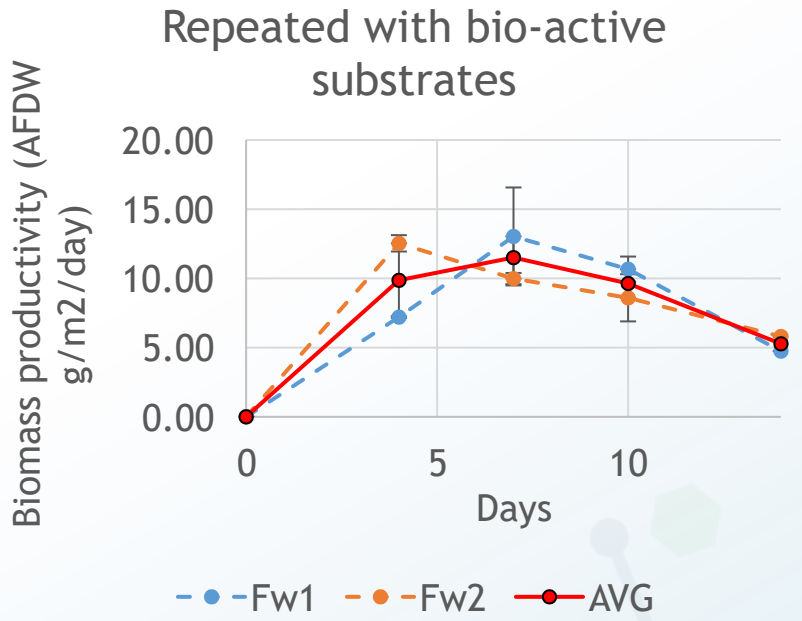


- MCF had average **of 4.2±1.7 g/m2/day**, which is **30-45% higher** than average productivities of both flowway 1 and 2 during the same period. Average ash content of the biomass harvested from MCF was **10.1%**, **46.6% lower** than the average ash content of biomass from flowways.

Harvesting Strategy







1. Initial biomass productivity change: Biomass productivities for different harvesting day (D-4, D-7. D-10, D-14) from new un-seeded substrates
2. Biomass productivity from bio-active substrates change
3. Biomass productivity changes after harvesting substrates from #2.

- Overall productivity experiments indicated that biomass productivity could be at its **maximum around their first week (Day 7),** which were **31%, 180%, and 117% higher** than productivity in D-14.

	D-7	D-14	Improvement
#1	7.6 ± 0.5	5.8 ± 0.3	31%
#2	11.3 ± 1.3	4.0 ± 0.7	180%
#3	11.5 ± 1.5	5.3 ± 0.5	117%

Acknowledgments

Colorado State University

HydroMentia

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