Removal of pathogenic bacteria in algal turf scrubbers

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What is an algal turf scrubber (ATS)?

- Ecologically engineered system in which benthic algae remove pollutants from water
  - Benthic algae – algae attached to a submerged surface, typically filamentous
  - Wastewater, stormwater, polluted surface waters
  - Benthic algae is harvested every 5-14 days depending on season and nutrient loading: continuous growth cycle similar to a lawn
- Algae is typically cultivated in a long narrow channel lined with a substratum
  - Pulsing mechanism (tipping bucket) used to provide wave pulse to increase productivity (mimics turbulence of natural systems)
  - Process water can be recirculated or flowthrough
- Mixed community of algae and bacteria grow on substrate
  - Community structure is seasonally variable (temperature, light, nutrient loading)
  - Material exchanges between algal and bacteria community
  - Algae is indigenous to water being treated

What is an algal turf scrubber (ATS)?

What can ATS do?

- Studied and observed to remove pollutants from a variety of waste, storm and natural waters.
  - Nutrients: assimilation, precipitation (N, P)
  - Sediment
  - Metals: adsorption, assimilation and precipitation (Fe, Ca, Mg, Pb, Cu…)
  - Organic compounds: high DO enhances bacterial decomposition via UV breakdown
- Treated water has higher pH, higher DO
- Pollutants are stored in or on the algal biomass which is periodically harvested allowing for regeneration of the algal turf
  - Biomass can be used as a fertilizer and has the potential for the production of biofuels through anaerobic digestion, fermentation or oil extraction
- Designated as urban best management practice in Chesapeake Bay watershed for nutrient and sediment

Previous research: algal/bacteria association

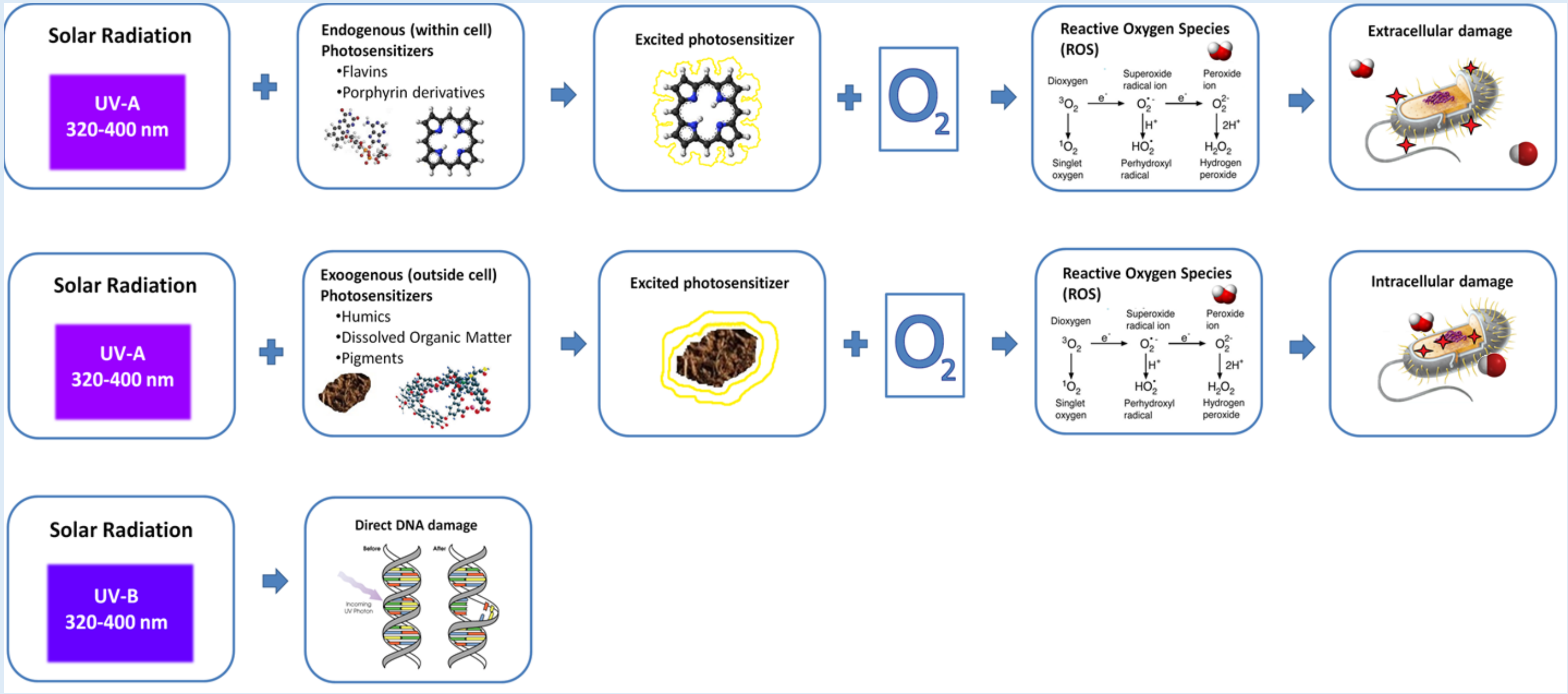
- No current studies significantly demonstrate the ability of ATS to remove pathogenic bacteria from water column.
  - One published study on an algal biofilm does provide a preliminary observation on removal
  - Has been suggested that flowthrough ATS would provide inconsistent removal of pathogenic bacteria
- Filamentous algae have been shown to harbor pathogenic bacteria (E. coli).
  - Algal provide protection from UV radiation and provide a carbon source to heterotrophic bacteria through algal exudates
- Marine algal turfs have been observed to capture fine sediments from land erosion.
  - May also allow for the capture of microorganisms?

Previous research: treatment ponds

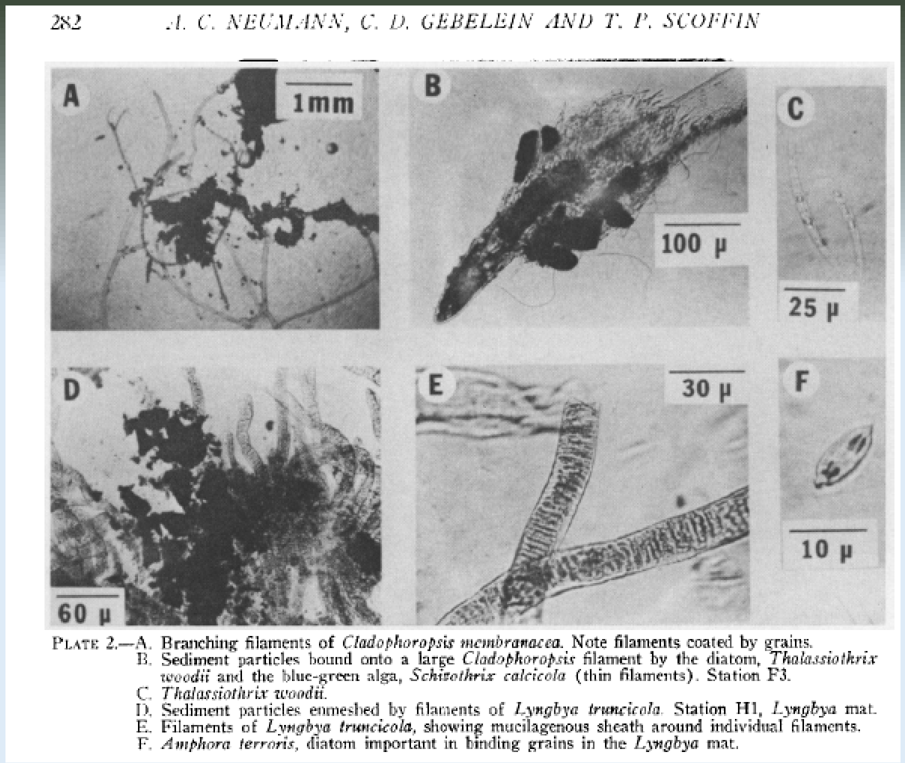
- Treatment ponds have been observed to achieve a high removal efficiency of pathogenic microorganisms
  - High Rate Algal Ponds (HRAPs), waste stabilization ponds (WSPs)
  - The algal community provides oxygen to the water column through photosynthesis
    - Oxygen feeds into production of Reactive Oxygen Species (ROS) via photosensitizing reactions in presence of solar ultraviolet radiation (UV)
  - Removal also occurs through predation and sedimentation
- WSPs (HRAP in particular) can be considered an analog to possible removal mechanisms in ATS

Pathogen removal mechanisms in ATS

- Algal turf is cultivated under a thin film of water; minimal solar energy loss through water column
  1. Direct UV disinfection – disinfection from UV-B radiation
  2. Indirect UV disinfetion – disinfection from reactive oxygen species
     - ROS formed when UV light excites photosensitizer compounds in water reacting with DO
     - Can occur outside the cell (exogenous) or inside of the cell (endogenous) depending on photosensitizer compounds



Pathogen removal mechanisms in ATS



- Interwoven filaments of algal turf act as a filter trapping sediment particles as well as organic material
  - Bacteria also attached to sediment and organic particles
  - May also trap bacteria?
  - Filaments also exude ‘sticky’ carbohydrates which facilitate adsorption on to surface of algae

Research questions

- If the ATS can facilitate the removal of nutrients, metals and sediment is it also able to remove microbial pollutants from the water column?
- What are the effects of a periodic wave pulse on pathogen removal from the water column? Does it re-suspend bacteria from algae?
- How does the growth stage of the algal turf influence pathogen removal?
    - Removal of a mature algal turf versus a harvested algal turf
- Does removal vary among pathogens?
    - Aquatic pathogens vs enteric pathogens

Research objectives

- Evaluate the ATS for the potential to remove pathogens
  1. Observe pathogen remove of ATS with surge mechanism and without surge mechanism
  2. Observe pathogen removal of ATS using a mature algal turf and a harvested algal turf
  3. Observe difference in removal using an aquatic pathogen (Flavobacterium columnare) and enteric bacteria (non pathogenic Escherichia coli)

Experimental plan

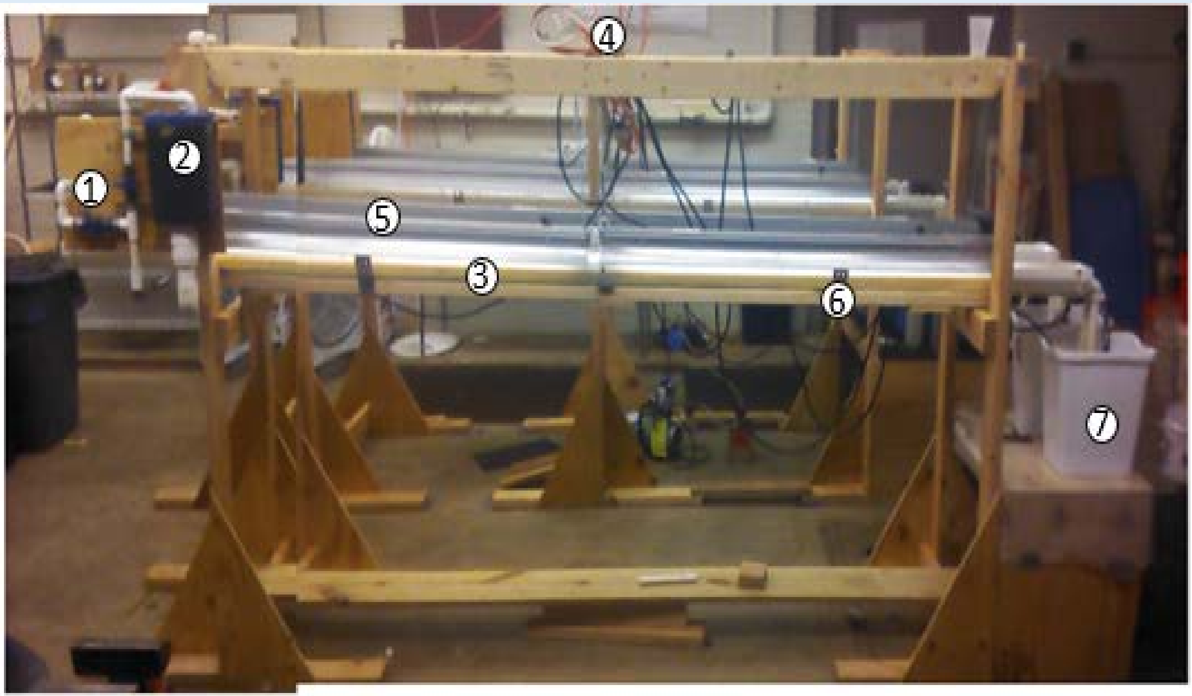
- Design lab scale ATS to use for answering the research questions
  - Should have same characteristics of ATS: long narrow channel and periodic wave pulse.
- Experiment is multifactor design
  - Two factors tested: the presence of algae and UV in the reactor
  - Allows observation of how the various combinations of factors affect the removal of bacteria
- Grow bacteria, spike reactors and observe removal of bacteria from water column over time (CFU/mL) using plated samples
  - Disinfect reactors after experiment
- Observe the change in bacterial concentration in algal turf (CFU/mg-wet wt. algae) using samples collected at beginning and end of experiment
  - Determine how much of bacteria stays in turf and effect of surge on concentration

1. Algae with UV
2. Algae with no UV
3. No algae with UV
4. No algae, no UV

Methods: lab scale ATS design

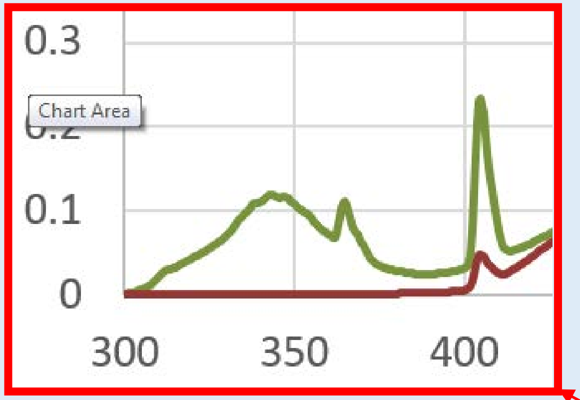
- Recirculating system with float activated surge device
- Constructed using off the shelf materials available at local hardware stores

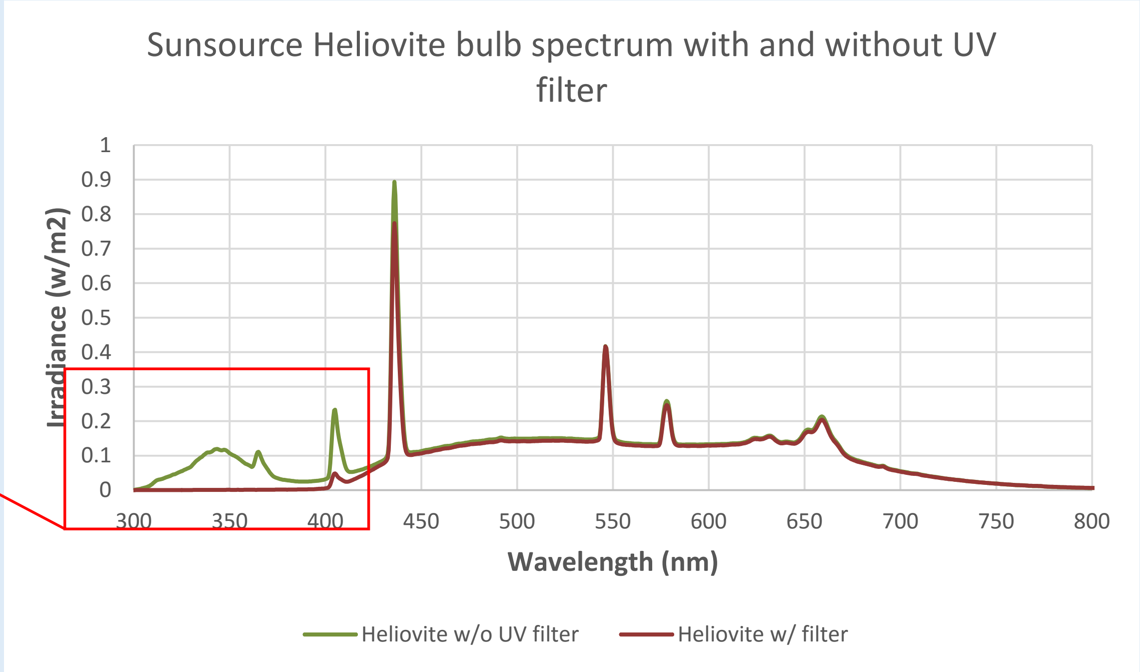
1. Valve and surge mounting board
2. Surge device
3. 305cm vinyl rain gutter
4. Mounted power strips
5. 122cm T8 fluorescent fixture
6. Gutter mounting bracket
7. Reservoir/pump



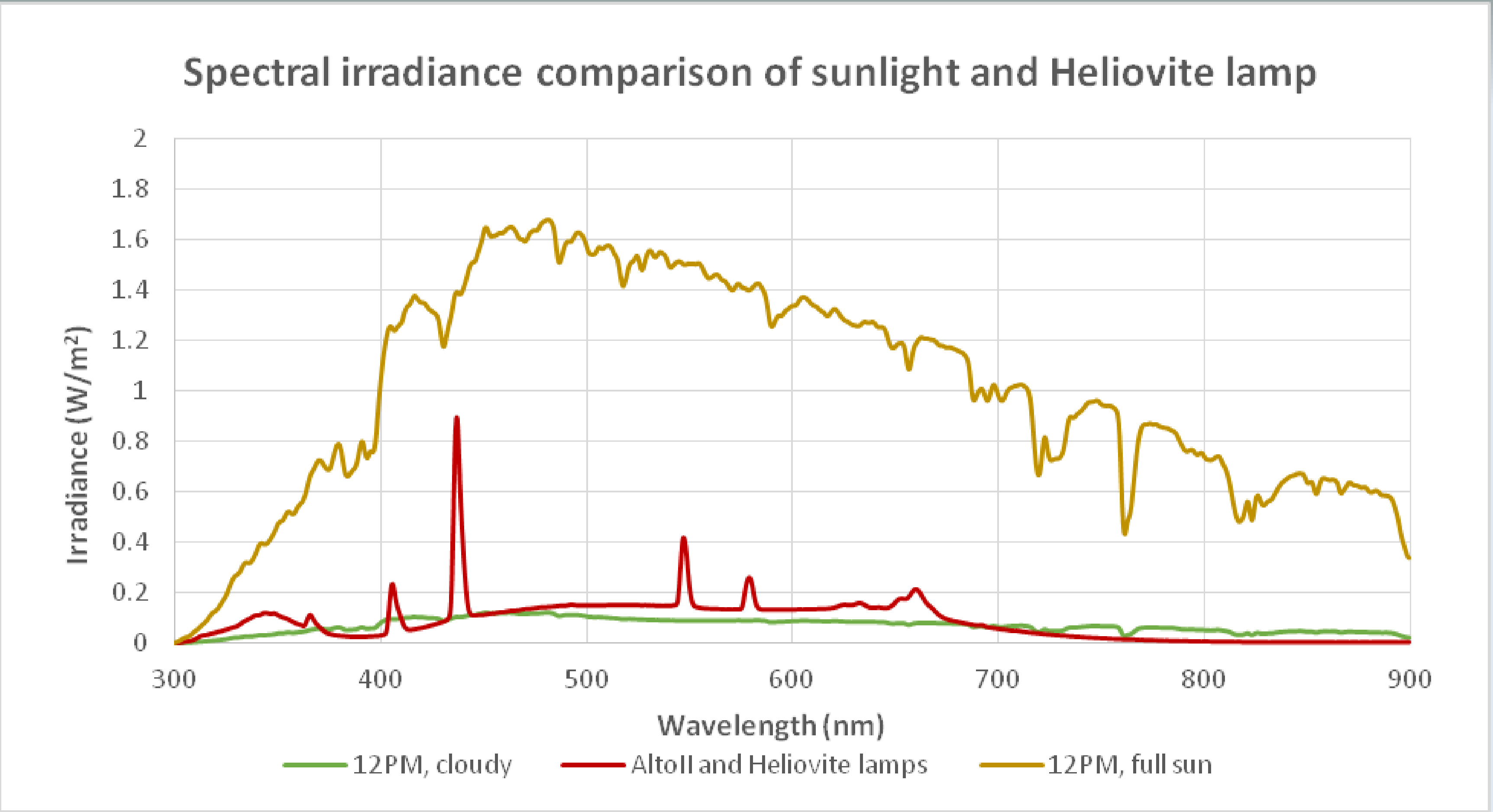
Methods: lab scale ATS lighting

- Algae grown using 48” 32W Phillips Alto II fluorescent lamps
- UV A&B supplemented using Sunsource Heliovite UV lamps.
- During experiment all reactors utilize 2 Phillips lamps and 2 Heliovite lamps
  - No UV treatments will utilize lamp filters to remove UV wavelength
  - Non filtered UV light fixtures will need to be ¼” higher than filtered light fixtures to match intensity of filtered UV lamps.

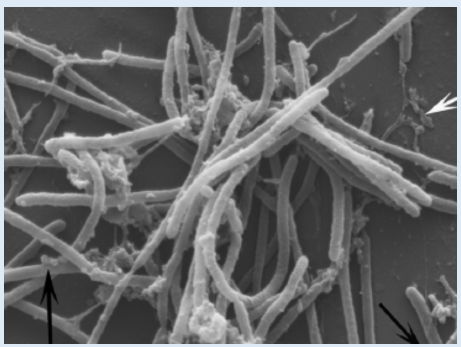


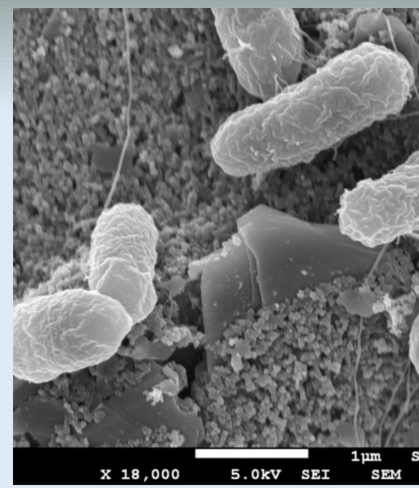


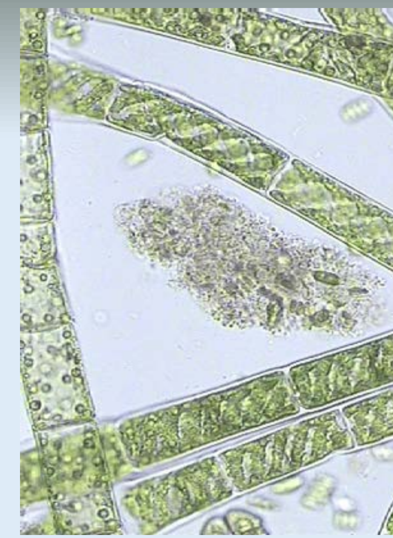
Methods: lab scale ATS lighting



Methods: bacterial and algal cultures





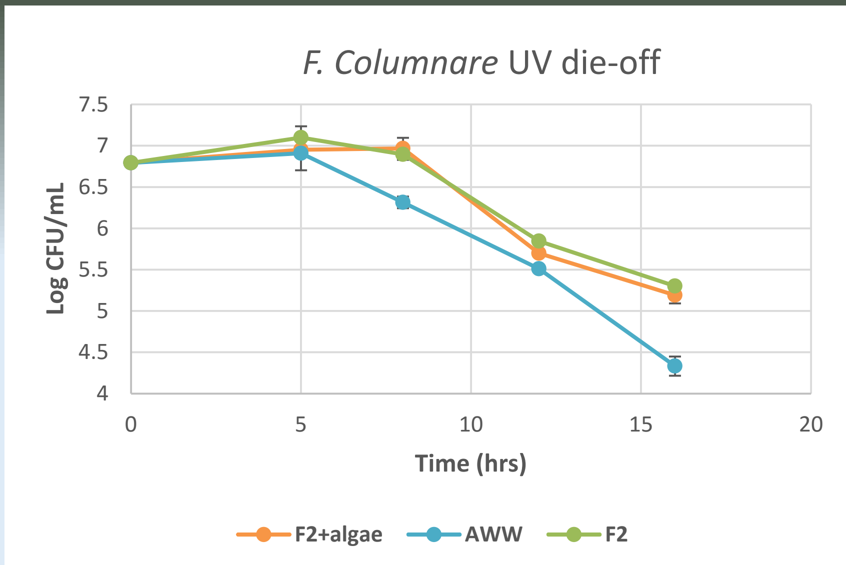


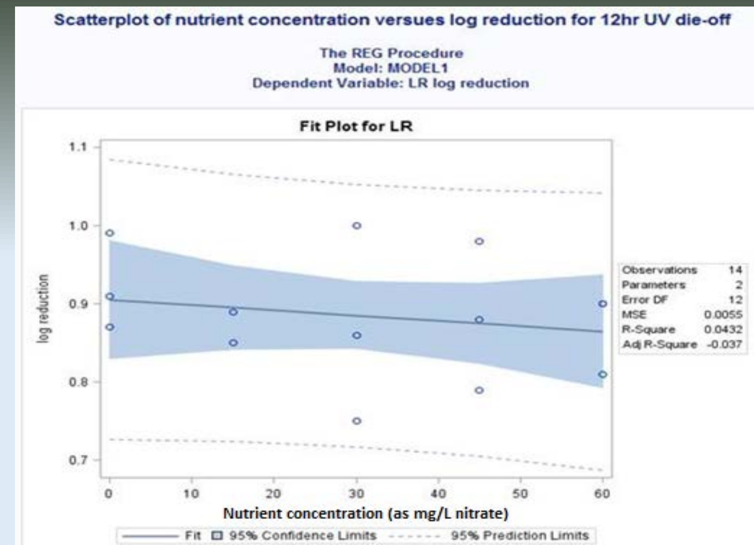
- *F. columnare* BG27
  - Aquaculture pathogen affecting catfish, tilapia
  - 4-12 µm in length
  - UV resistant cell pigments
  - Readily forms biofilm

- *E. coli* DH10B
  - Strain does not ferment lactose and Resistant to Streptomycin
  - Non-pathogenic, enteric (37°C)
  - ~2 µm in length

- Algae
  - Mixed algae culture isolated from pilot scale ATS receiving aquaculture wastewater
  - Filamentous: *Oedogonium, Rhizoclonium* and *Sirogonium*
  - Suspended: *Scenedesmus, Closterium* and *Oocystis*

Preliminary experiments





- F. columnare die off tested on aquaculture wastewater, F/2 media and F/2 media with algal exudates under UV/no UV supplemented light.
  - AWW had faster die off compared to other treatments – using F/2 would not artificially increase die off
- Variable concentration of F/2 under UV/no UV light tested for difference in die off after 24 hours
  - Determine if F/2 would need to be held constant using dosing pump
  - No significant difference in die off observed over the range of F/2 concentration
- UV lamps did not significantly affect algal productivity (not shown)

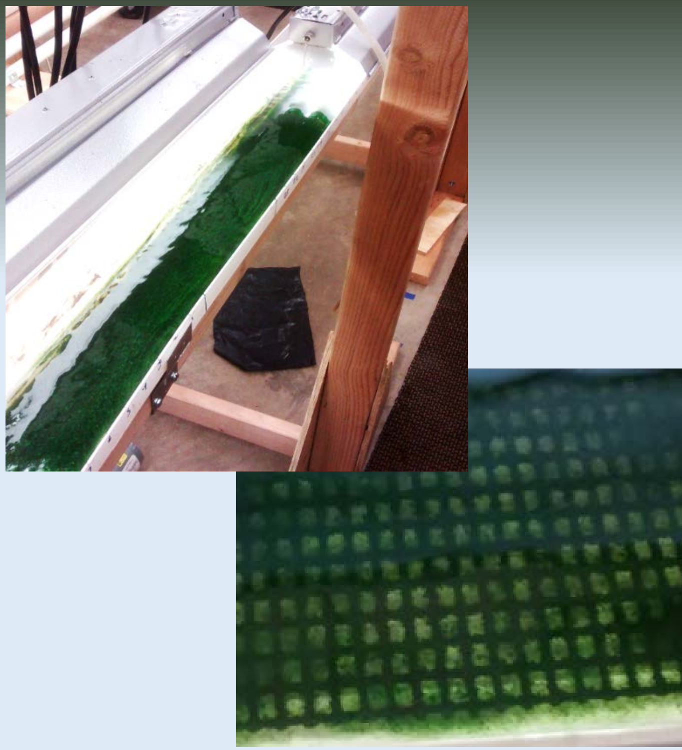
Experiment parameters

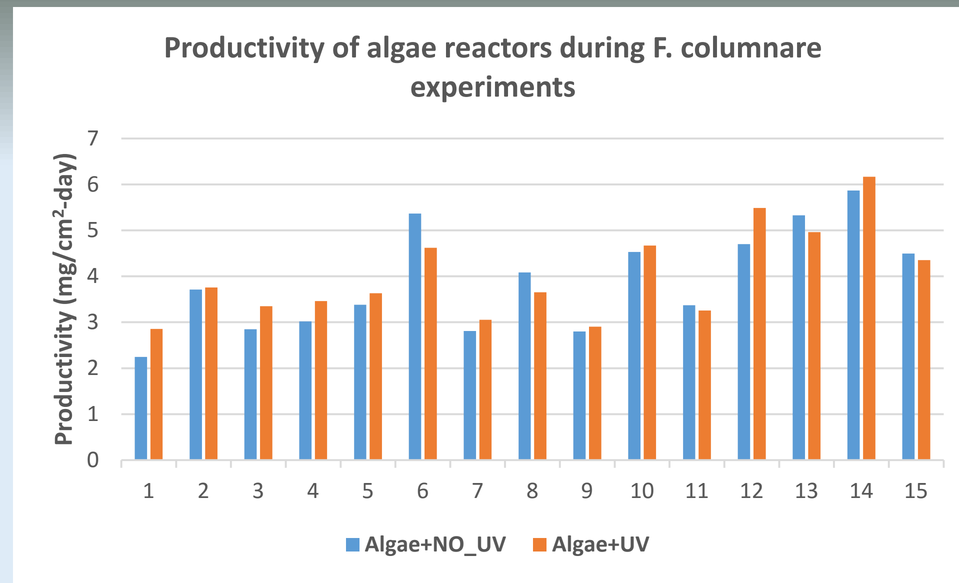
- Algae grown over 9 day period under 16/8 diurnal lighting at ~250 µmol m^{-2} s^{-1} PAR without UV lamps
  - Algae harvested on day 1 with experiments on day 2 and 9
    - Lighting on for 24 hours during experiments
  - System volume kept at 5.7L dechlorinated tap water with daily addition of F/2 algae media
- All reservoirs kept at 1.5L with a HRT of ~15 sec
  - Total system volume of mature algae treatments greater than no algae and harvested algae
  - Total light exposure of column of water for 24 hr period is avg. 10 hrs
- Flowrate set at 6 LPM
- Surge set at avg. 0.7L @ 15 seconds
- Reservoir amended with buffers for pH control
- E. coli experiments utilized pH control with HCl
- Reservoirs spiked with 175-200mL of overnight bacterial culture
- All reactors disinfected to remove biofilm after each experiment with 500ppm chlorine
  - Algae substratum removed from algae reactors during disinfection

Data collection

- To determine concentration of bacteria in water column, ~1mL of water was sampled from each reservoir. Sampled water was serially diluted with PBS and plated in triplicate on respective agar.
  - F. columnare: Modified Sheih with Tobramycin
  - E. coli: MacConkey agar with Streptomycin
- To determine concentration of bacteria in algae, 1mg wet weight of algae was sampled from treatments with algal turf. Algae was sampled within 1' of the upstream side of the channel and 1 of the downstream outlet. Algae was processed in a food grade blender with 9mL of PBS before being serially diluted and plated.
- Water quality data was collected at each sampling event using hand held pH, EC, Temp probe and separate DO probe.
- Agar plates were incubated 24-48 hours depending on bacteria and enumerated for CFU/mL and CFU/mg-wet wt. algae.

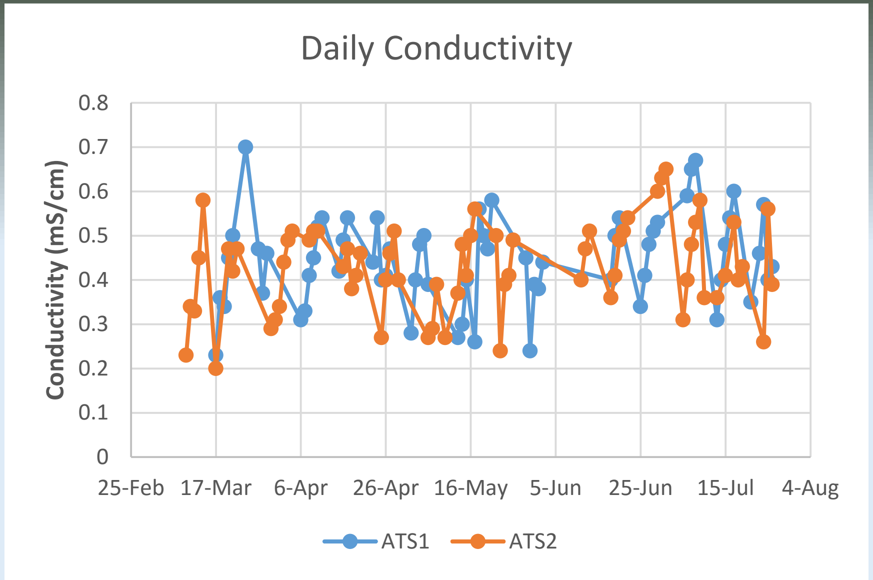
Results: Algal productivity

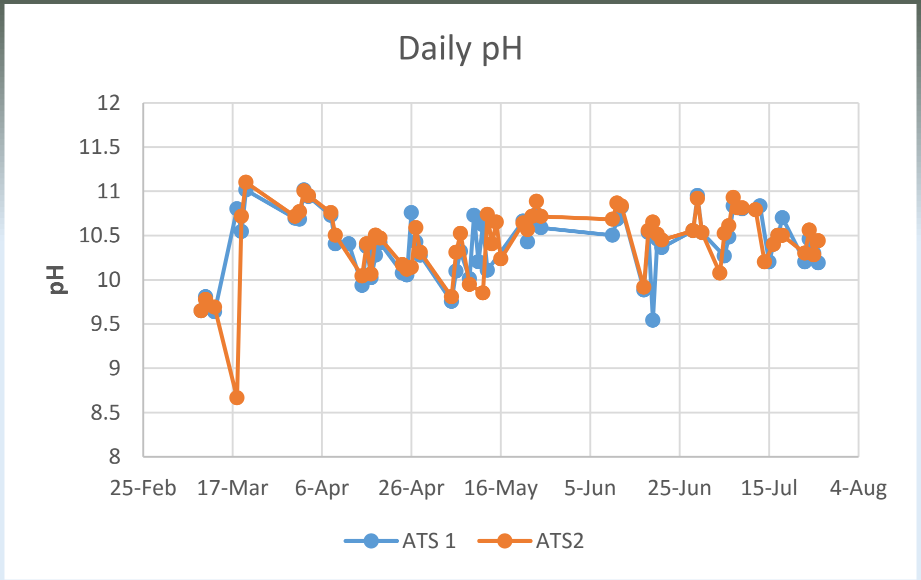




- Overall daily productivity during the 9 day harvest cycle
- Productivity inhibited by disinfection after each experiment
  - Disinfection removes any colonized algae attached to gutter underneath void space in substratum
  - Impact on physical removal?

Results: Algal productivity water quality





- Constant conductivity not maintained during growth of algal turf.
  - No water exchange of reservoirs in between harvests/experiment.
- No significant variance of water quality parameters between ATS1 and ATS2
  - Avg pH=10.4, Avg Temp=25.3 C, Avg EC=.44 mS/cm

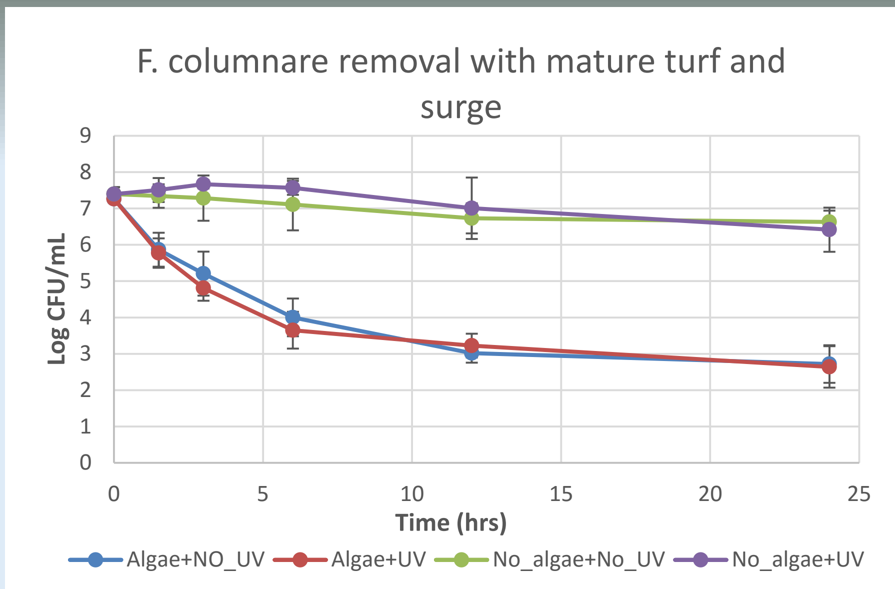
Results: experiment water quality

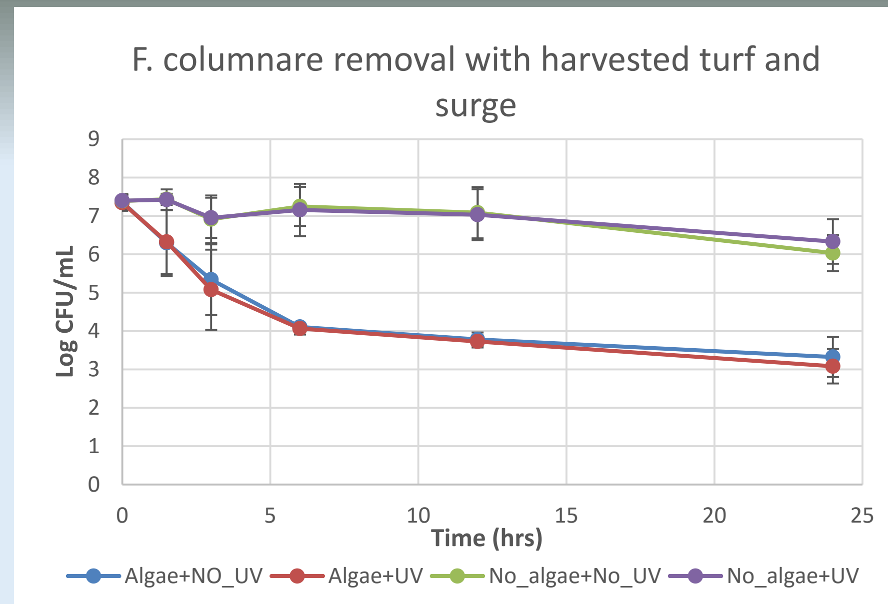
F. columnare: mature turf with surge								
	pH		EC		Temp.		DO	
Treatment	Avg.	Std.	Avg.	Std.	Avg.	Std.	Avg.	Std.
Algae+NO_	8.49	0.57	0.41	0.03	26.09	0.73	No_data	No_data
Algae+UV	8.56	0.55	0.41	0.03	26.08	0.54	No_data	No_data
No_algae+I	8.02	0.49	0.47	0.06	26.22	0.74	No_data	No_data
No_algae+I	7.96	0.43	0.45	0.06	25.78	0.63	No_data	No_data

F. columnare: harvested turf with surge								
	pH		EC		Temp.		DO	
Treatment	Avg.	Std.	Avg.	Std.	Avg.	Std.	Avg.	Std.
Algae+NO_	8.15	0.60	0.45	0.04	26.30	0.58	No_data	No_data
Algae+UV	8.21	0.61	0.45	0.04	26.12	0.47	No_data	No_data
No_algae+I	7.61	0.40	0.47	0.08	26.38	0.52	No_data	No_data
No_algae+I	7.64	0.40	0.50	0.09	25.96	0.48	No_data	No_data

- pH significantly varied between algae and no algae treatments
    - Buffers did not prevent pH change due to algal productivity
- DO measurements were not taken for *F. columnare*

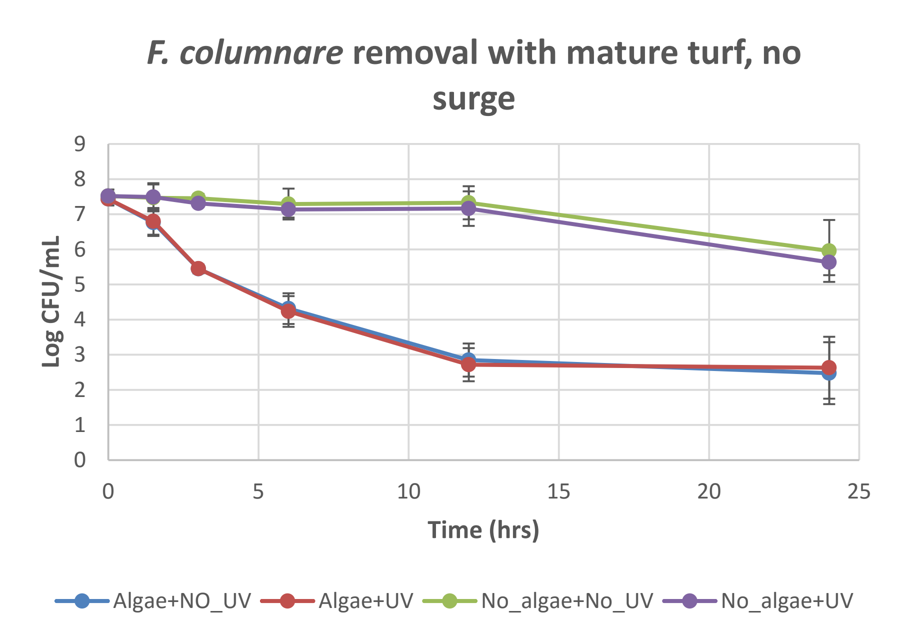
Results: *F. columnare* removal w/ surge

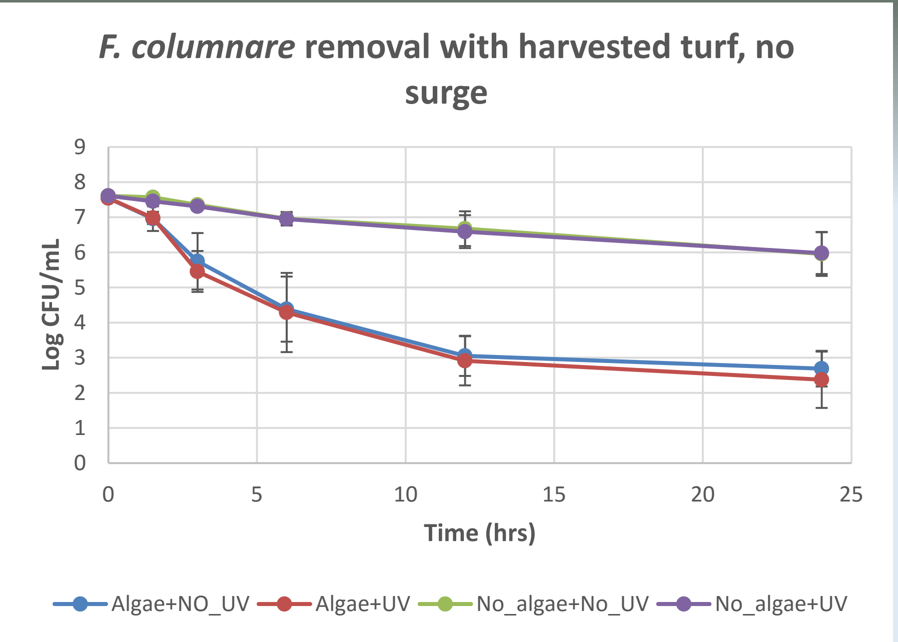




- For both mature and harvested turf, the log removal of algae treatments was significantly different from no algae treatments but not each other.
- Estimated baseline of log 2.15 (±0.57) background F. columnare
- Data points at 12 and 24 hours are estimates since background bacteria created noise at plated low sample dilutions impairing accurate enumeration of F. columnare

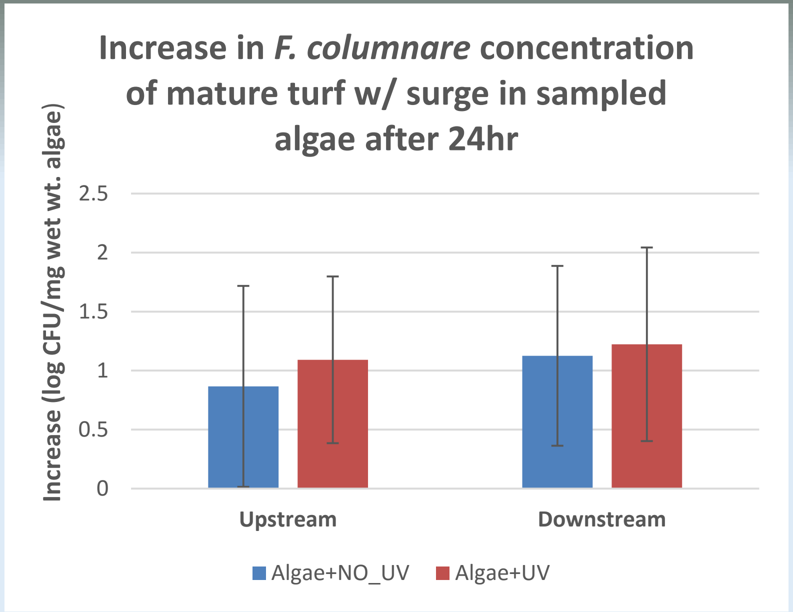
Results: *F. columnare* removal w/o surge

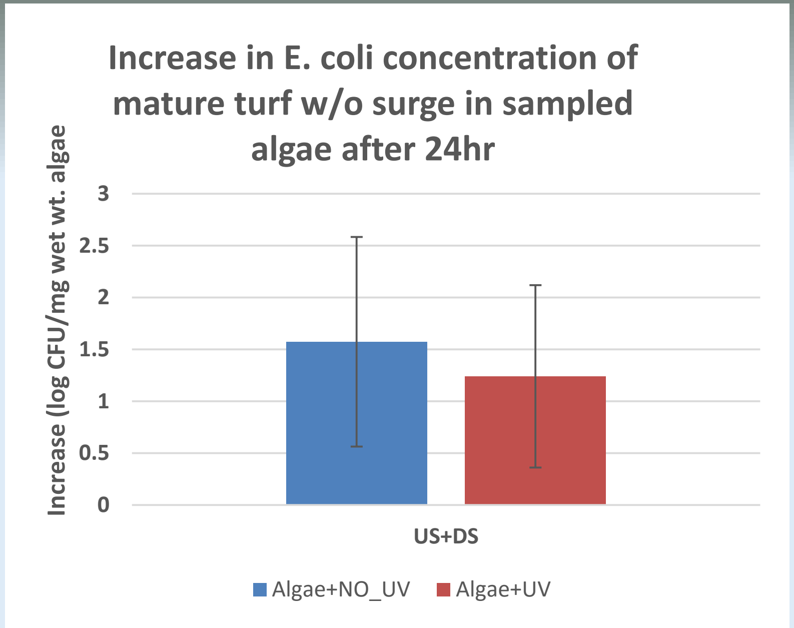




- Data set incomplete (n=4) requires 1 more sample: no statistical analysis yet
- Slightly greater overall removal compared to surge but probably not significant
- Removal curve similar to surge
- Baseline of log 2.5 (±0.75) *F. columnare*

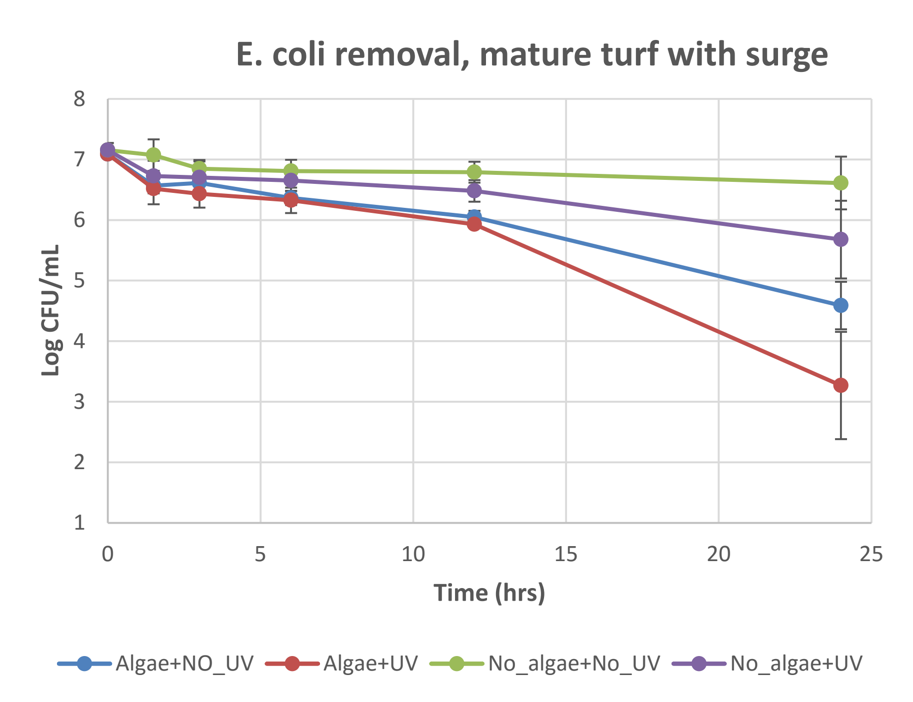
Results: *F. columnare* in sampled turf

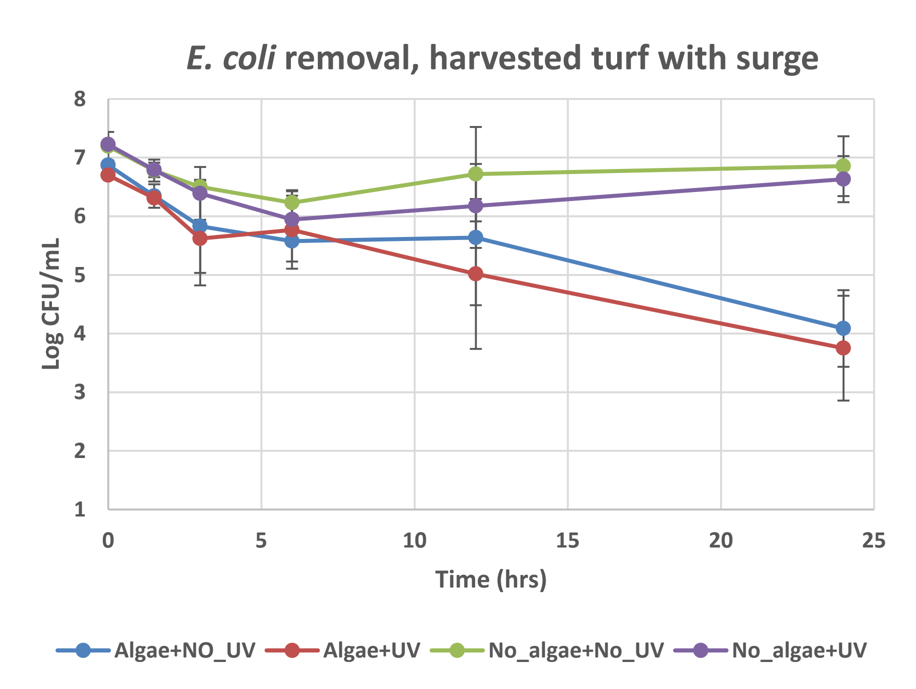




- Enumeration of *F. columnare* in sampled mature algal turf difficult due to amount of background bacteria on plate after incubation: data is a rough estimate of concentration
- No surge used a composite sample between upstream and downstream ends since flow was assumed uniform at both ends of channel
- No significant difference between upstream and downstream ends of ATS

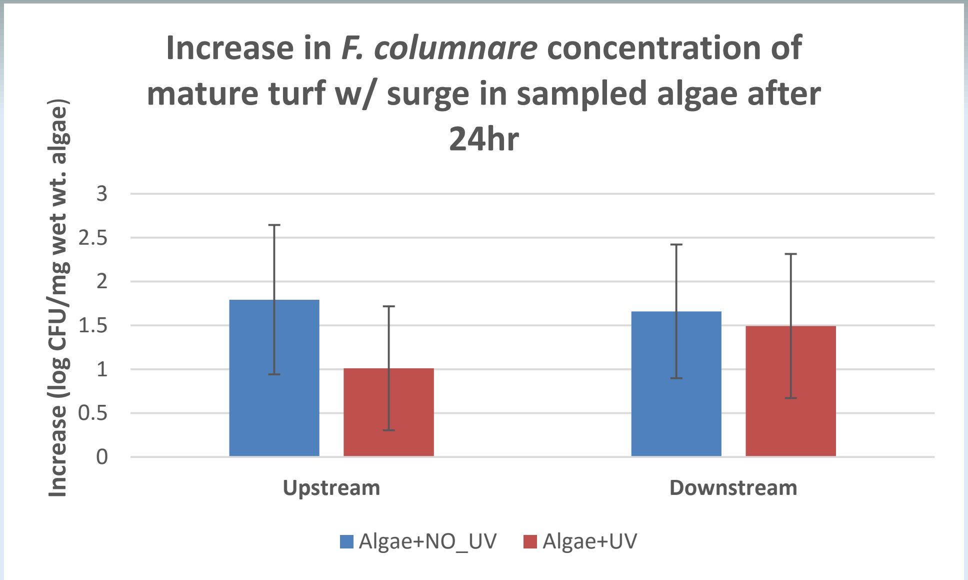
Results: *E. coli* removal w/ surge + pH ctrl





- Effect of physical removal by algal turf and UV disinfection can be seen with E. coli
  - Algae+UV treatment greatest removal
- N=2, more samples needed
- Removal difference after 12 hrs
  - Stress from lack of nutrients?
  - Buildup of ROS?

Results: *E. coli* in sampled turf



- MacConkey agar with Streptomycin allowed for a reduction of background bacteria and more accurate enumeration than *F. columnare*
- Slightly more bacteria found in algae from no UV treatment but doesn't appear significant
- No significant difference between upstream and downstream ends of ATS

Discussion

- The operating parameters of the reactor may lend itself to representing a conservative removal possible in ATS.
  - How well can this translate to larger ATS systems?
- For the most part, water quality parameters did not vary significantly except for pH during *F. columnare* experiments.
  - Although pH wasn't controlled for *F. columnare* experiments the higher pH of algal treatments probably did not greatly affect removal since *F. columnare* is more tolerant to high pH
- The pH control of algae treatments used during experiments with E. coli probably inhibited the rate of removal but allowed for the separation of treatment affects.
- Concentration of bacteria in algae did not significantly differ between upstream and downstream ends of the ATS. This might not be the case for larger ATS which have much longer channel lengths.
- Physical removal of bacteria appears to be a function of turf surface area rather than volume.
  - Lower flowrate = greater removal?
  - An effect of algal species and turf formation?

Conclusions

- Overall, the data suggest that a recirculating ATS can remove bacteria from the water column and removal is both a physical and photochemical process.
    - ATS systems removal probably inconsistent due to environmental variables (sunlight, temperature, water quality) and would probably require recirculating water instead of flow through for significant removal
    - Treated water might meet standards for recreation or agricultural reuse?
- The use of surge does not significantly impact pathogen removal
- The growth stage of the algal turf does not significantly impact removal
- Algal turf is more effective at removing larger bacteria (F. columnare) and less effective with smaller bacteria (E. coli).
- Although the level of UV radiation in the reactors is a fraction of full sunlight, the effects of UV radiation do contribute to removal as seen with the algae+UV treatment achieving highest log removal with E. coli.

Future work

- Complete remaining E. coli experiments with pH control
- Observe removal of E. coli in lab scale ATS without pH control
- Develop a mathematical model for removal in ATS
    - Batch reactor model for lab scale ATS
    - Plug flow for landscape scale ATS
    - Adsoprtion isotherm needed to explain physical removal by algal turf?
- Observe E. coli removal in landscape scale ATS