The Shape of Things to Come:

Reverse-engineering periphyton colonization processes using 3D Printing

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Overview

- Motivation
- Background
- Methodology and Experimentation
- Preliminary Observations
- Conclusions & Future research

Algal turf scrubbing (ATS) has been used for years for pollutant recovery from water.

Peach Bottom ATS, Delta, PA

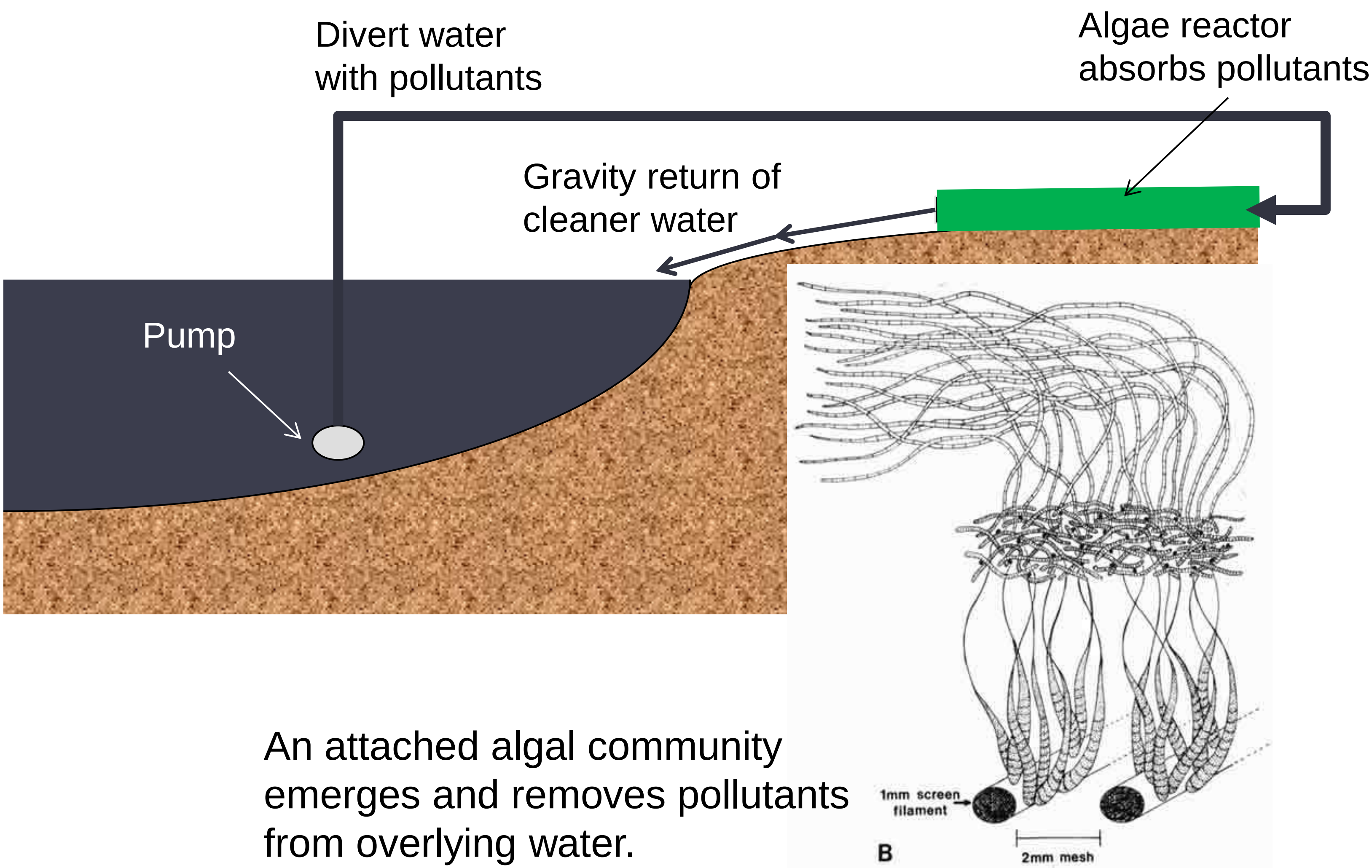
USDA ARS
Beltsville, MD

Baltimore Harbor
Baltimore, MD

Great Wicomico
River ATS, VA

Taylor Creek ATS
Lake Okeechobee, FL

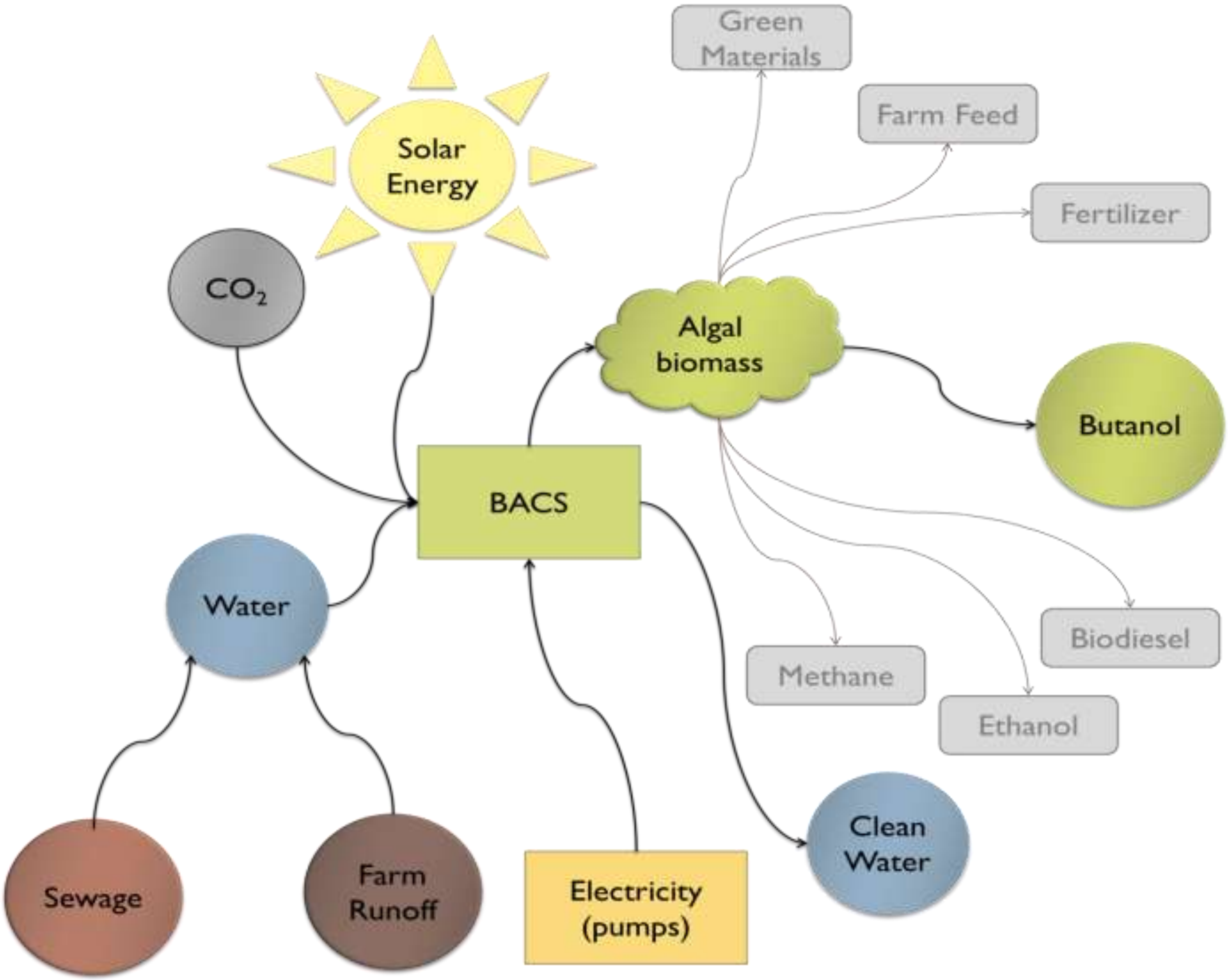
The ATS process entails flowing a thin film of water over a growth substratum.



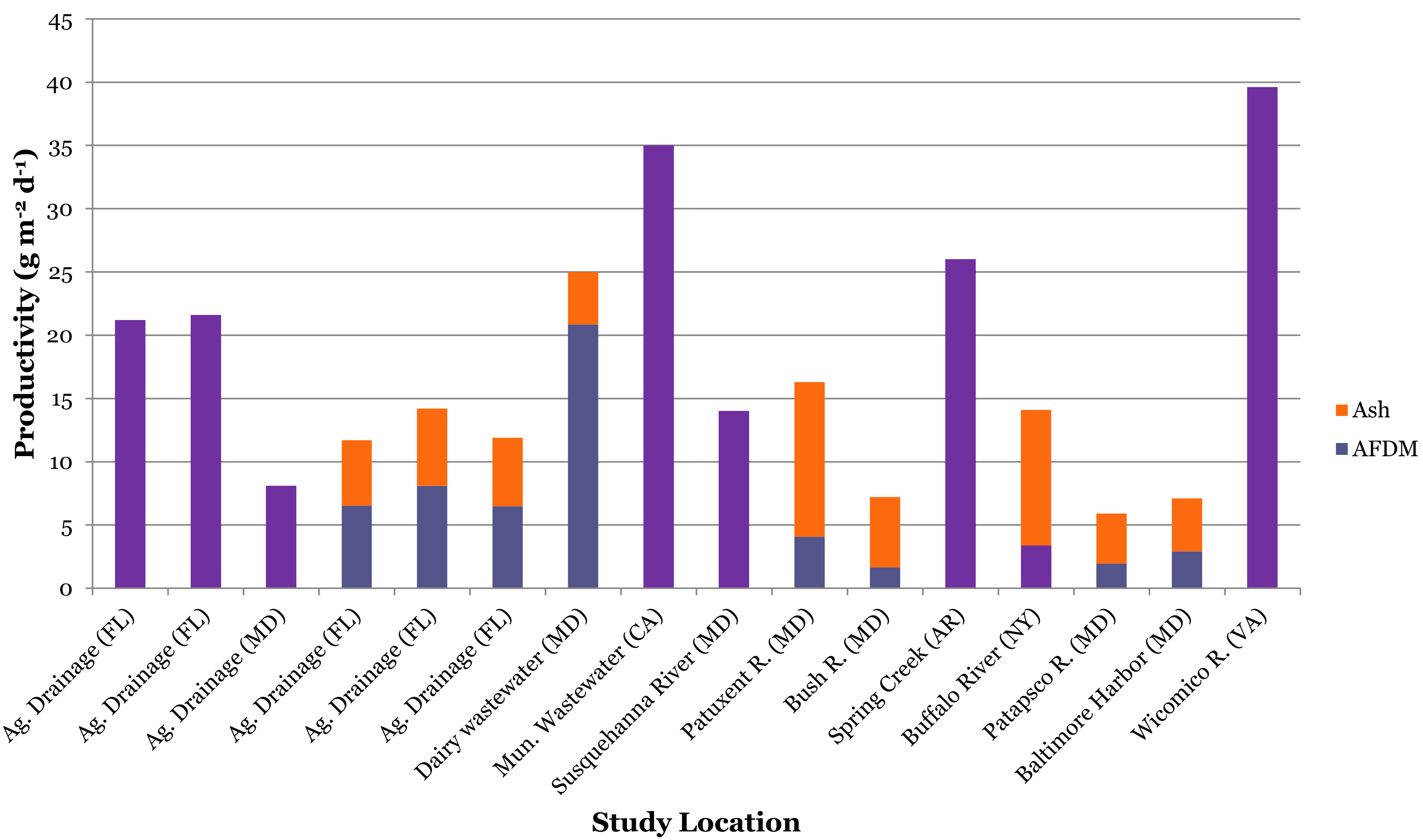
Adey and Loveland (2006)

An attached algal community emerges and removes pollutants from overlying water.

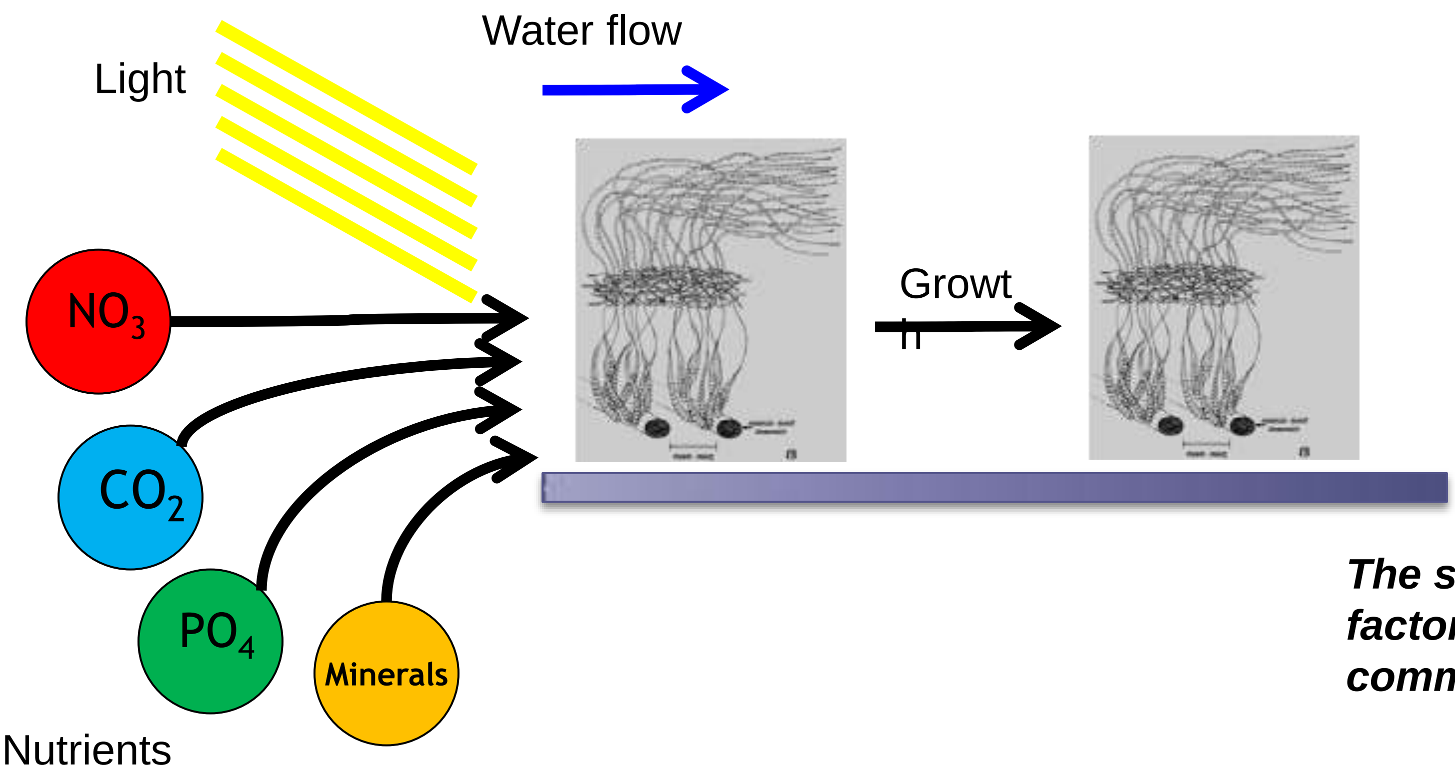
The application of ATS has been limited because of economics.



Reported annual mean productivities and ash content of various ATS units are varying with typically low quality.



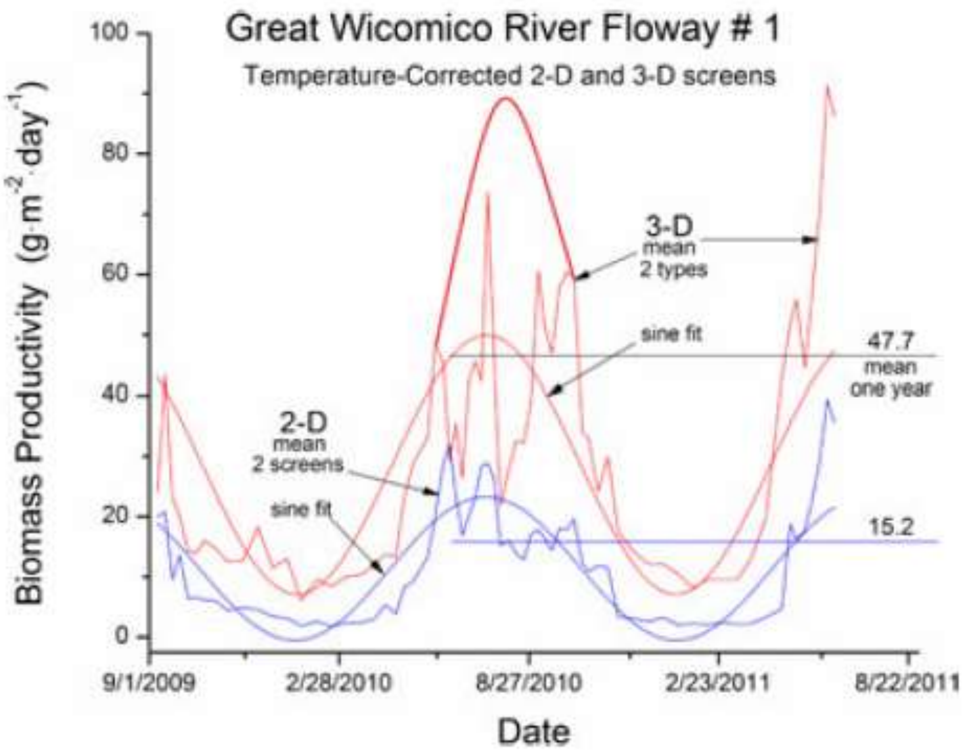
What approaches are available for controlling the algal community?



The substratum is a factor in periphyton community growth.

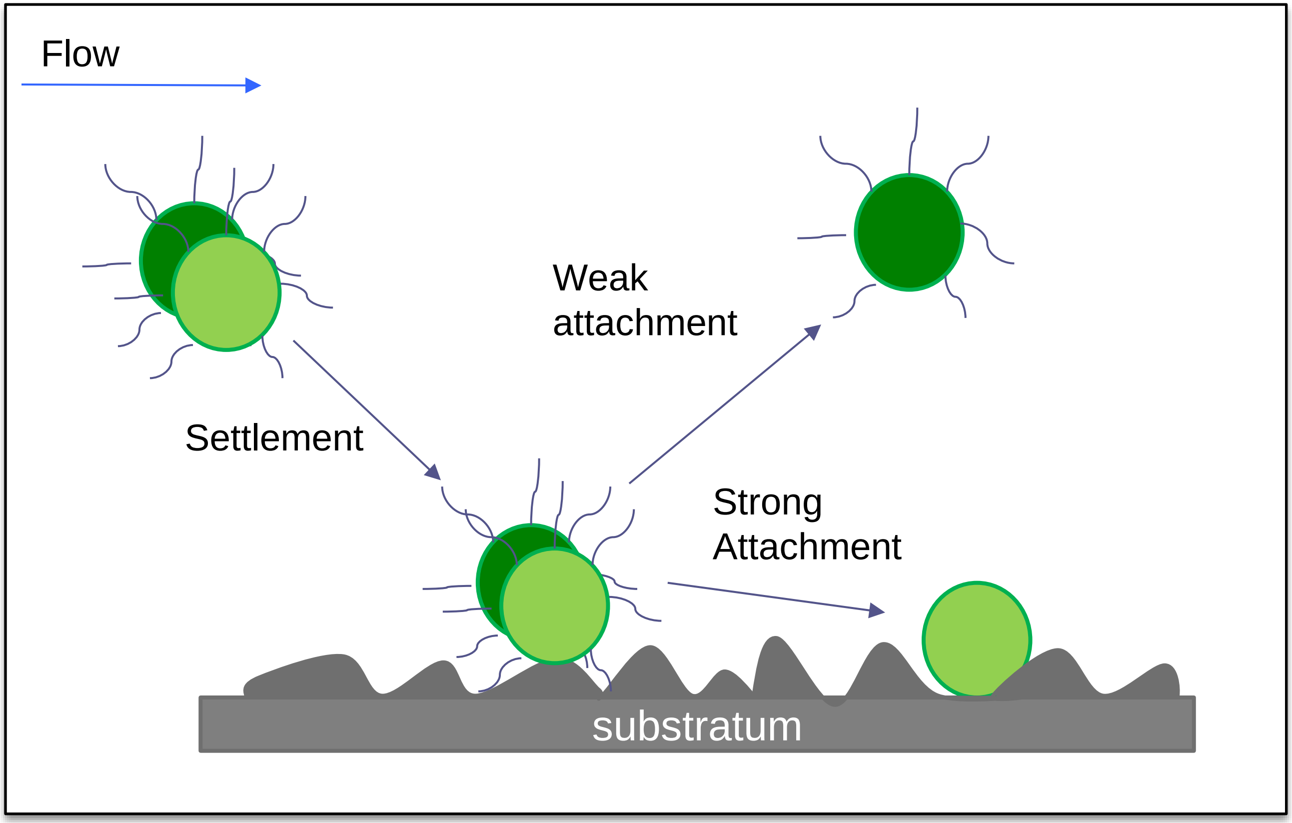
New designs for 3-dimensional substrata are being investigated for their effect on net productivity.

3D screen (Adey et al 2013)

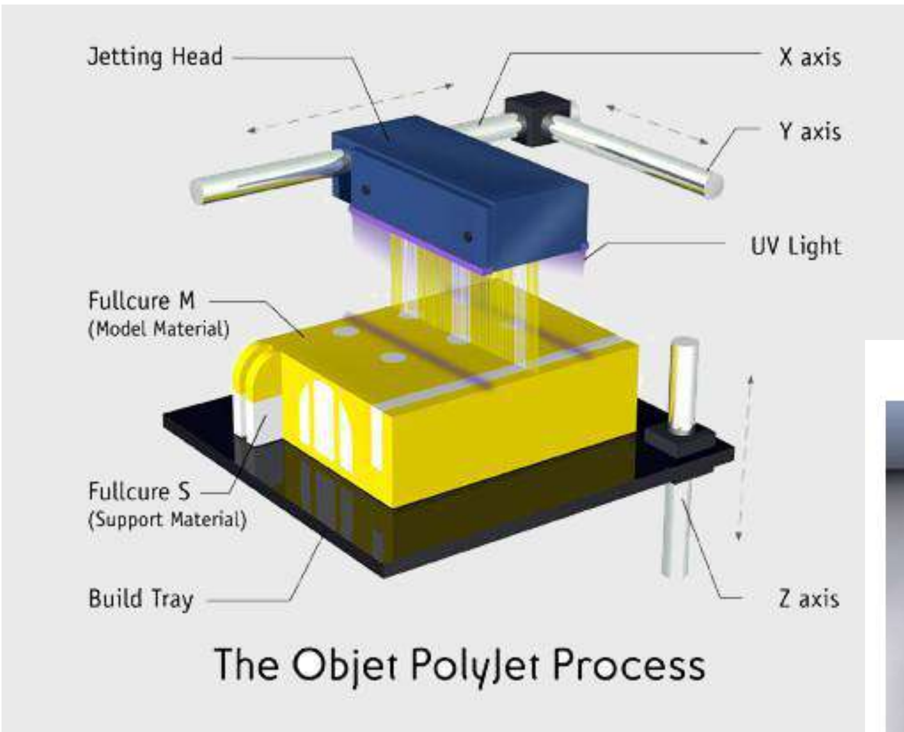


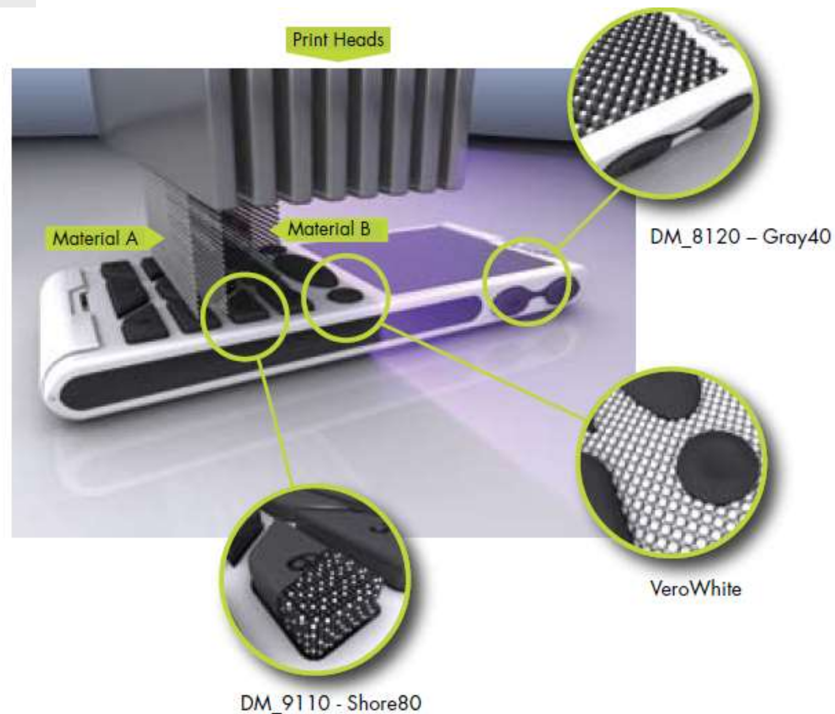
From Adey et al. (2013). Algal turf scrubber (ATS) floways on the Great Wicomico River, Chesapeake Bay: Productivity, Algal Community Structure, Substrate and Chemistry. J. Phycol. 49, 489-501.

We submit that substratum characteristics act as a filter on the colonizing periphyton community.



3D printing affords a tool for rapid surface prototyping and replication.





Auburn 3D Printed Biofilm Surfaces (3D-PBS) Laboratory

Capabilities:

Stratasys Objet 30 printer

- Printing at µm scale
- Solids modeling

Nanovea Profilometer

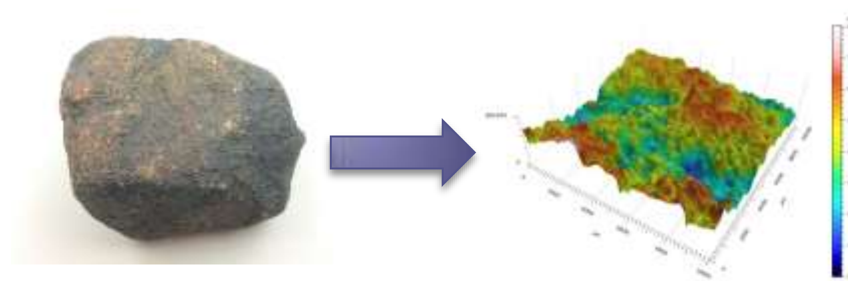
- Surface measurement at the nm scale
- Surface modeling

Benthic algae bioreactors

- Algal culturing and cultivation
- Microscopic and biochemical analysis

Objectives of the lab:

- Reverse engineer and replicate roughness of natural surfaces
- Experiment on dynamics of colonization, growth, and species competition
- Investigate surface roughness as a factor in periphyton community development



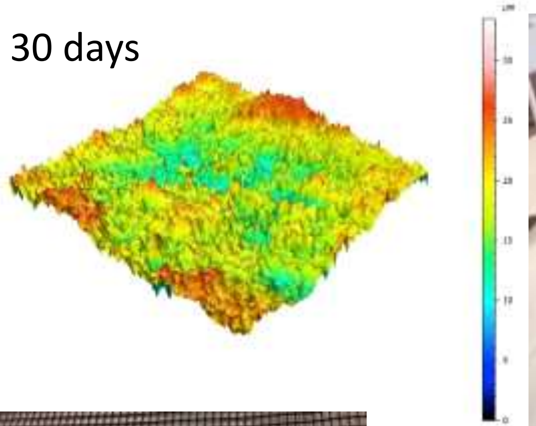
Three preliminary experiments:

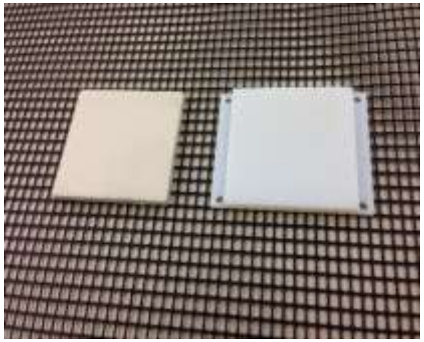
- Recruitment of species from natural waters on 3D-printed surfaces
- Investigation of roughness effects on algal growth in lab ATS bioreactor
- Exploration of colonization on 3D printed complex geometries

Experiment #1: Recruitment of species from natural waters

Purpose of Experiment: What will colonize these things?

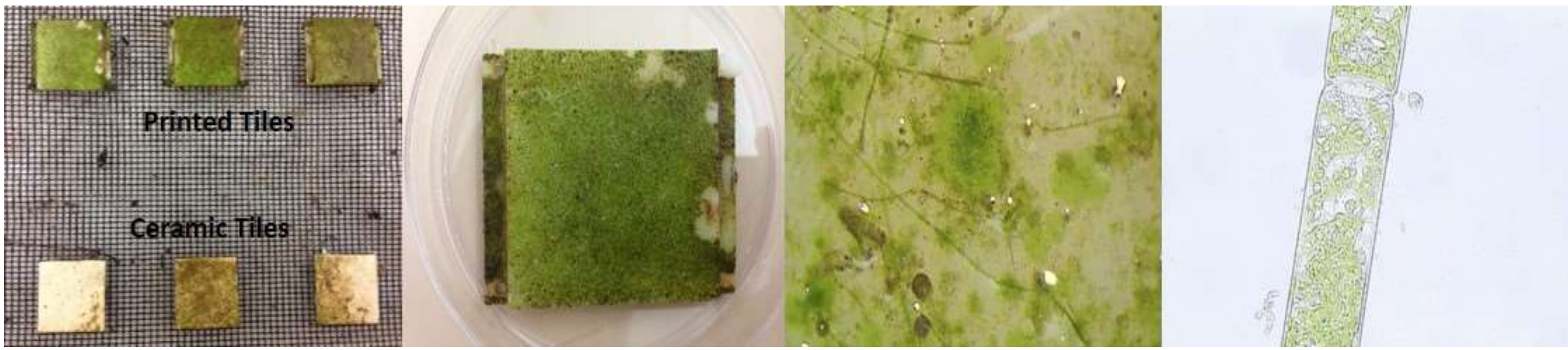
- Investigating possibility of colonization of algal species on printed tiles in natural streams
- Printed tiles with average roughness Ra: 0.198 - 0.932 µm
- Unglazed ceramic tiles for experimental control
- Placed in various Alabama streams for 30 days





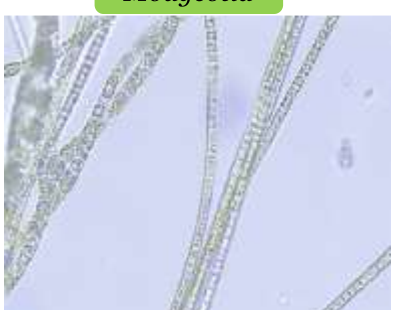
Experiment #1 Preliminary Results

- Printed tiles showed the ability of attachment and colonization of algal species

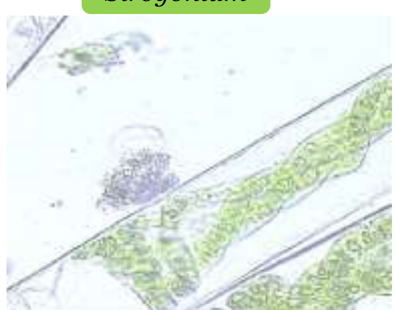


- A variety of algal species were identified by micrograph analysis (10 identified overall)

Mougeotia

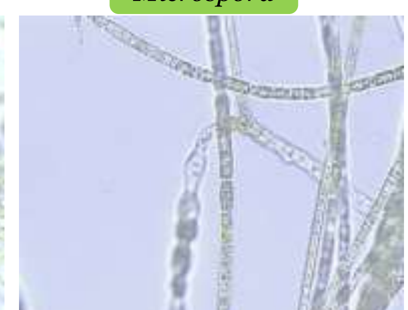


Sirogonium

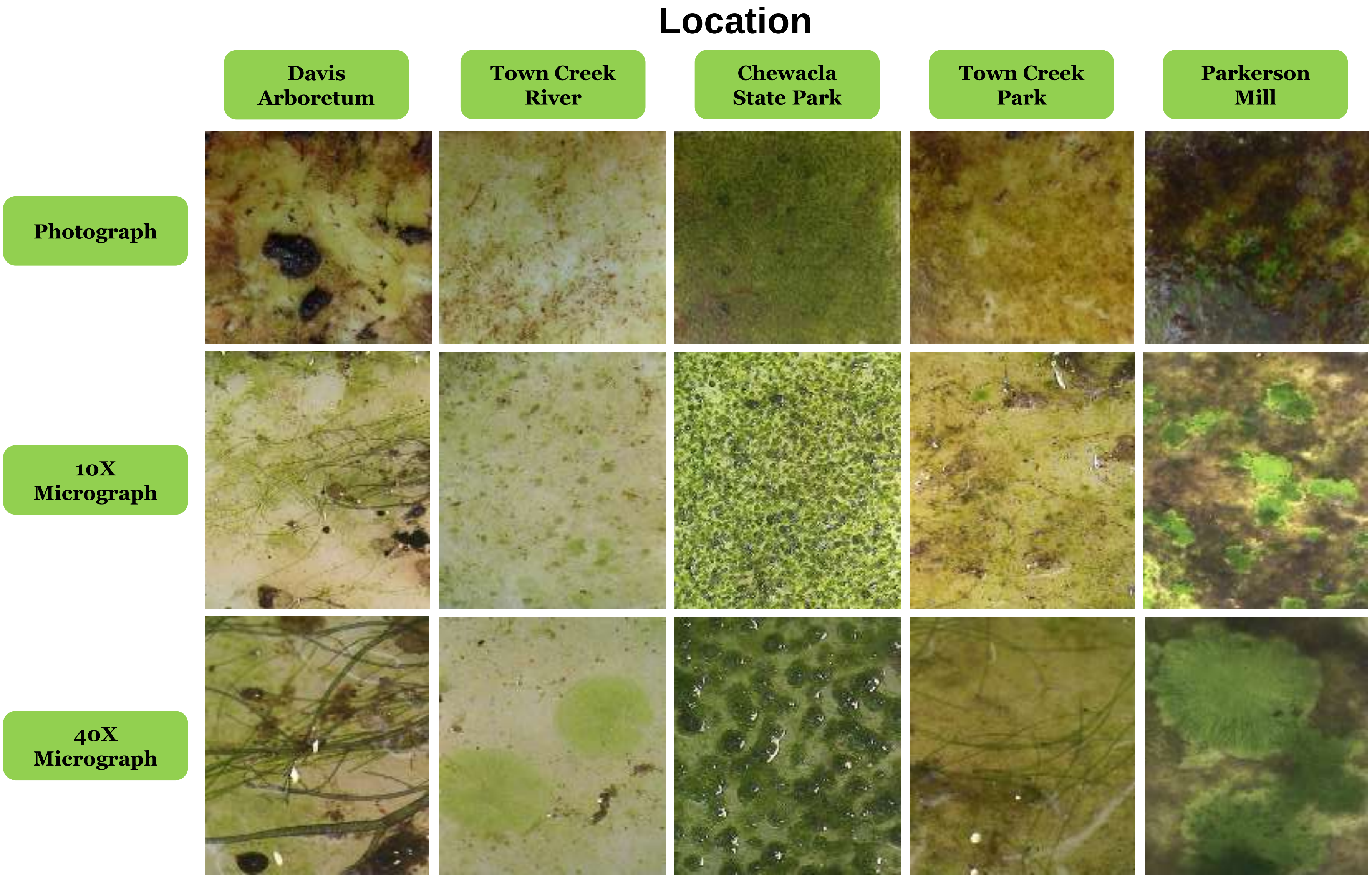


Spirogyra

Microspora



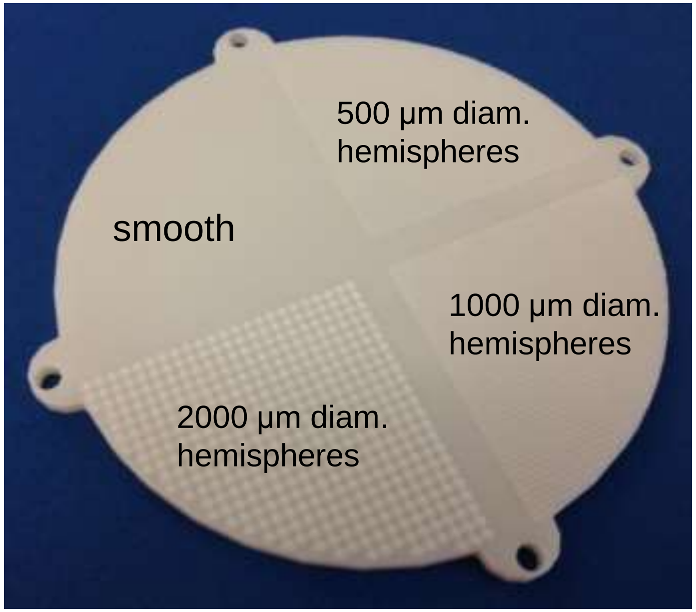
Experiment #1: Preliminary Observations

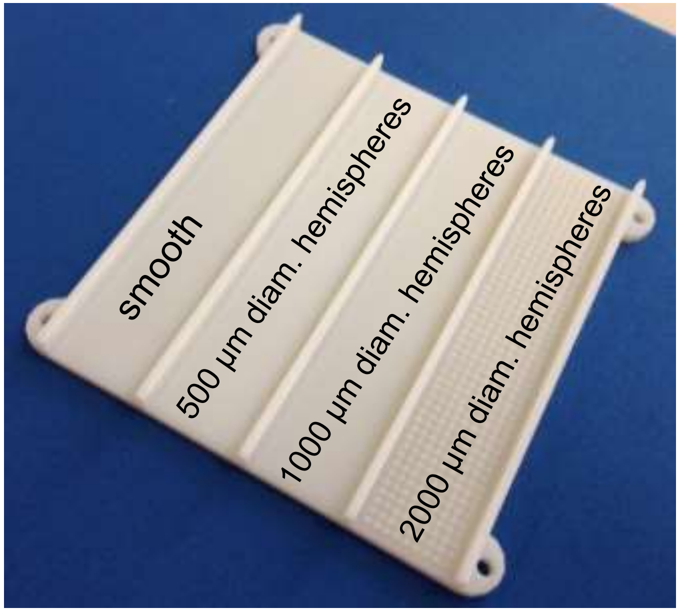


Experiment #2: 3D-printed growth plates in controlled bioreactor cultivator

Purpose of the experiment:

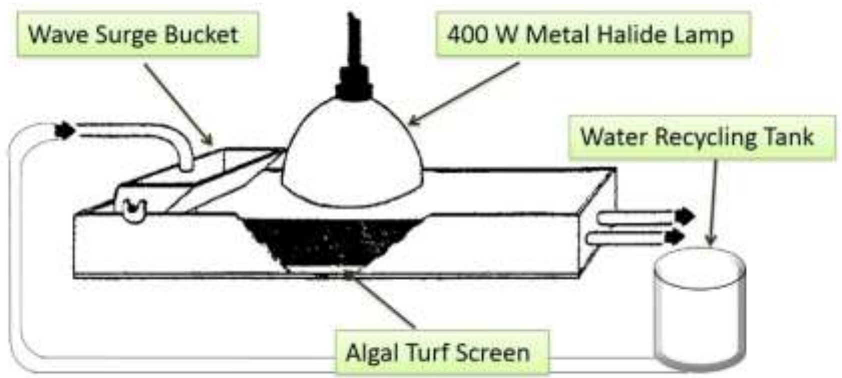
- To demonstrate algal biofilms can attach and colonize 3D printed polymer surfaces
- To investigate the effect of surface feature sizes on algal attachment and growth density





Experiment #2 Description

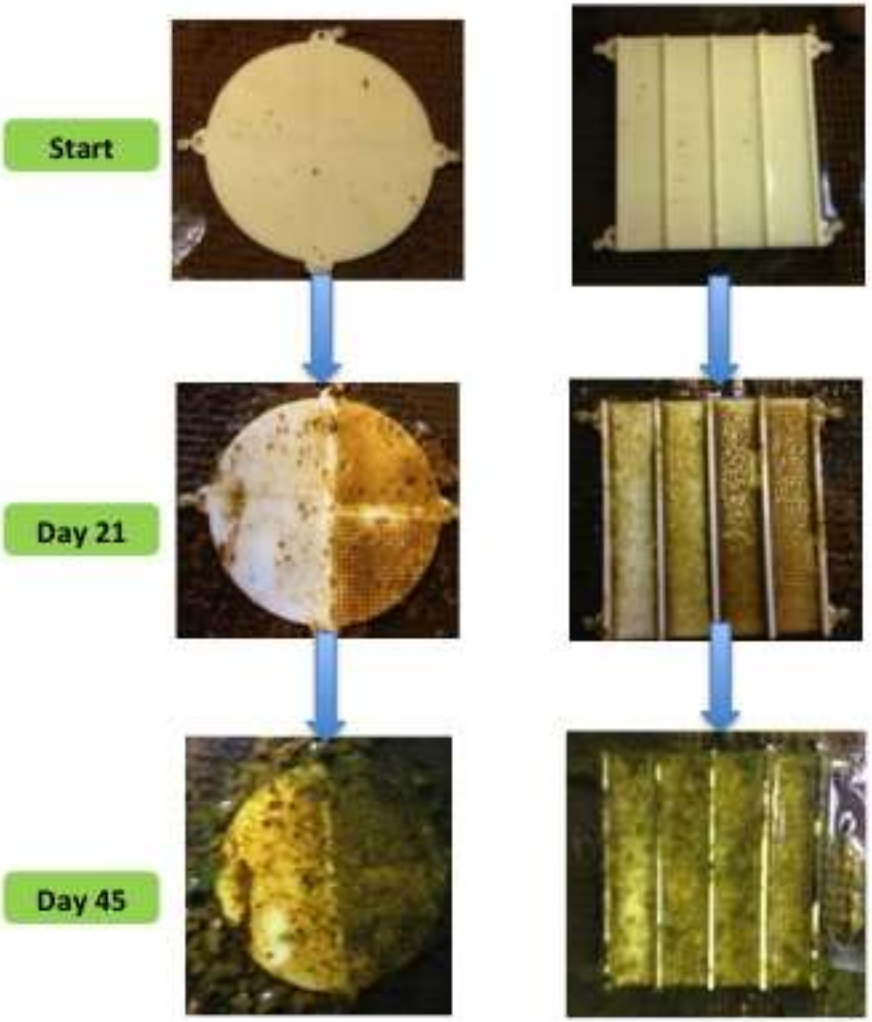
- Indoor recirculating ATS with flow rate of 45 L min^{-1} with a tipping frequency of 4 min^{-1}
- Two 400 W metal halide grow lamps
- Experiment duration was 45 days
- Inoculated with mixed algal community dominated by *Spirogyra communis*
- Dosed daily with commercial F/2 media



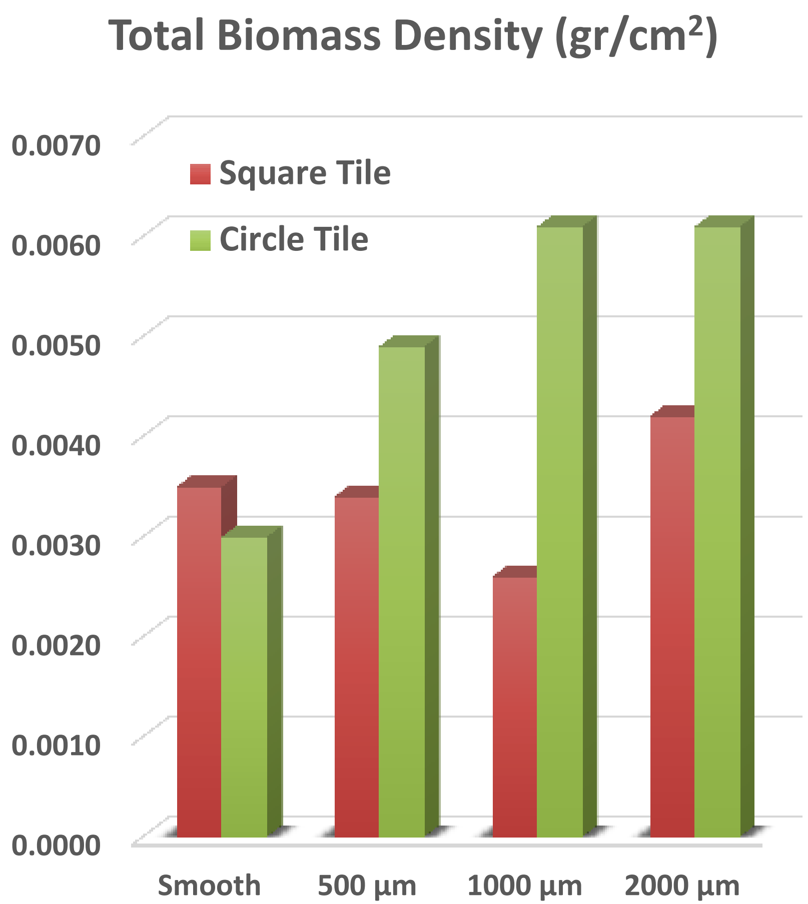
Reference:

- Mulbry, W. W. & Wilkie, A. C. Growth of benthic freshwater algae on dairy manures. *J. Appl. Phycol.* **13,** 301–306 (2001).

Experiment #2 Preliminary Results: Algal colonization density directly correlates with surface roughness.



Images by K. Kardel, AU-ISEN

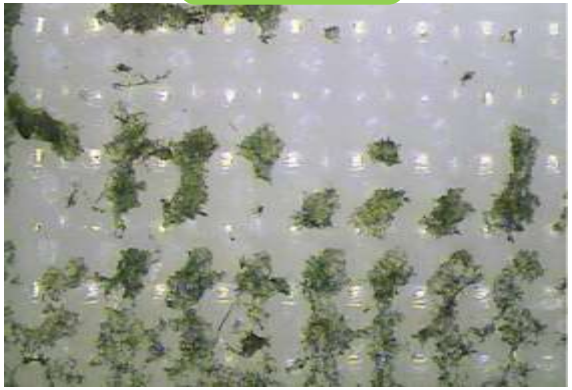


Experiment #2: Preliminary Observations

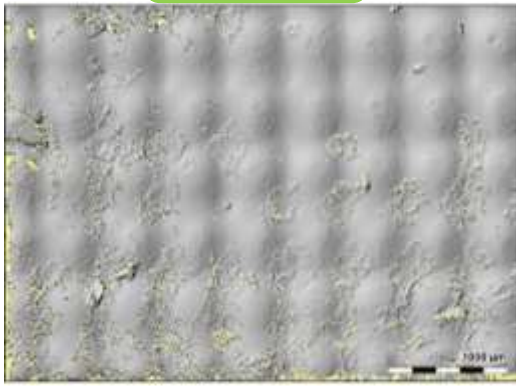
- Areas with larger diameter hemispheres (1000 & 2000 µm) presented larger amount of residual biomass
- Zones of refugia were greater for larger diameter hemispheres
- Possible design approach: Optimize surface roughness to maximize regeneration rate following harvest.

Post-harvest

30x Micrograph



Pseudo-photo



Pre-harvest

Post-harvest

Experiment #3: Colonization on complex geometries

Purpose of Experiment:

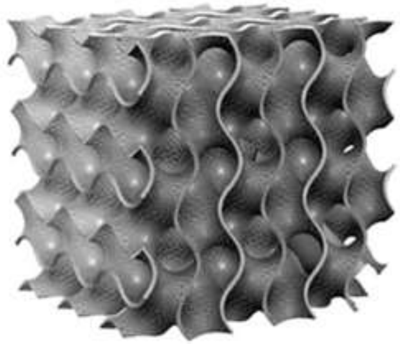
- Design and manufacturing of 3D printed cube structures with high surface area
- Based on a mathematical model for a gyroid:

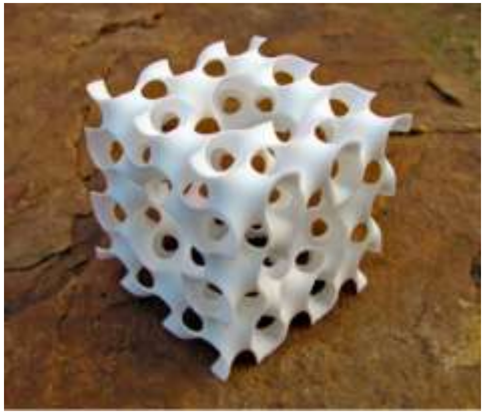
  $$\sin x \times \cos y + \sin y \times \cos z + \sin z \times \cos x = 0$$

- Inoculated with *Spirogyra communis*
- Dosed daily with commercial F/2 media
- Experiment duration: 30 days
- Frequency of Rocker: 70 rpm

Dimensions: 20×20 mm

Surface Area: 8127 mm^2

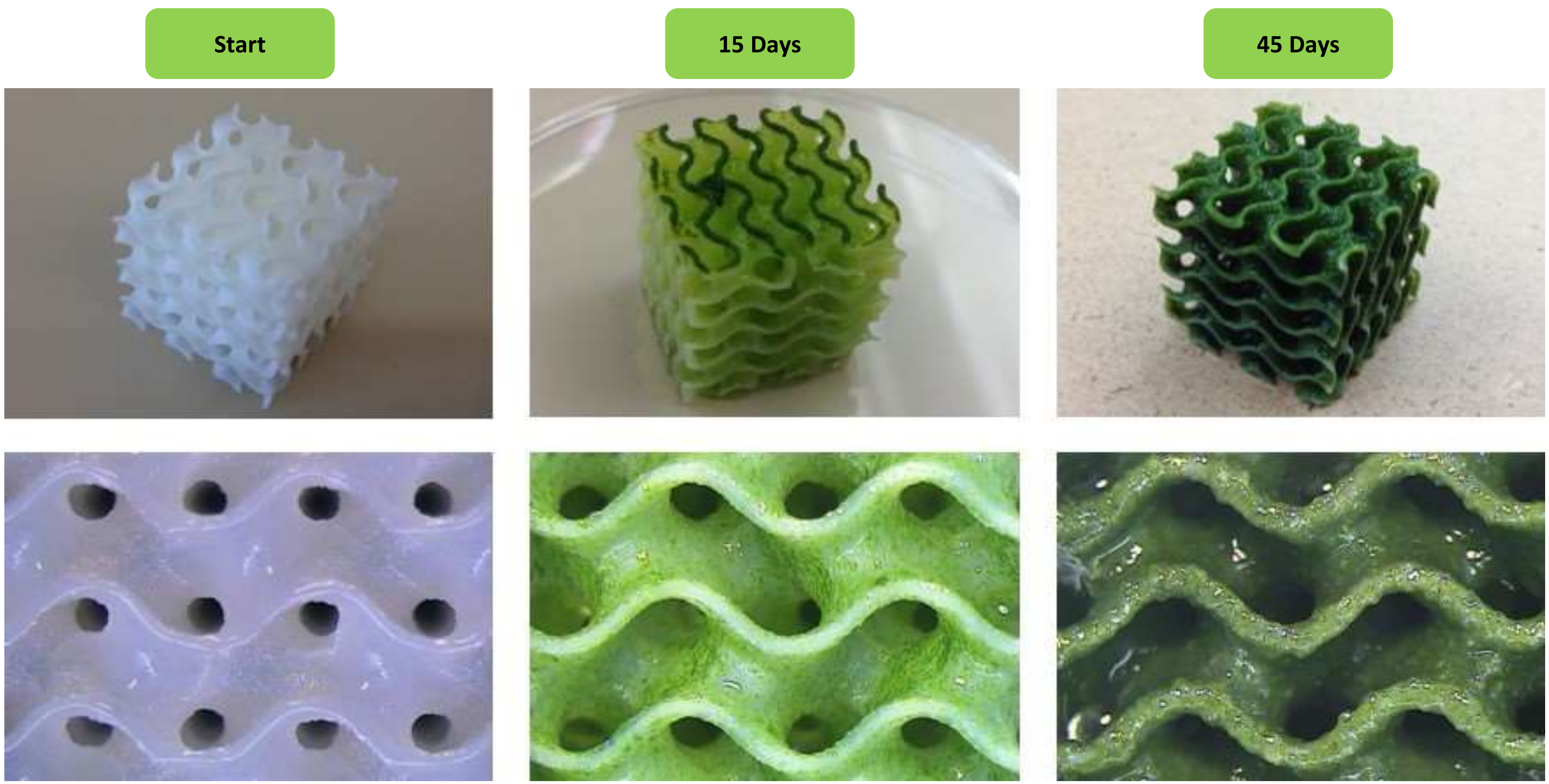




Preliminary Observations

Experiment #3:

- Attachment of algal spores on high surface area
- Observed colonization deep inside the gyroid
- Novel biofilter media for novel ecologies



Conclusions & Future Research

- Additive manufactured substrata can support algal biofilms
- Surface roughness affects rate of colonization.
  - *An optimal topography may exist for each species*
- Interstitial spaces provide refugia for recolonization and regrowth
  - *An optimal spacing may exist for harvest optimization and biomass yield*
- Potential to design complex geometries for novel microecological patterning
  - *Structures may be designed for synthetic microbial ecologies*

Acknowledgements

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Reference

Portions of this presentation are published in:

Kardel, K., Carrano, A.L., Blersch, D., and Kaur, M. 2015. Preliminary development of 3D printed custom substrata for benthic algal biofilms. *3D and Printing and Additive Manufacturing Journal* 2(1): 12-19.