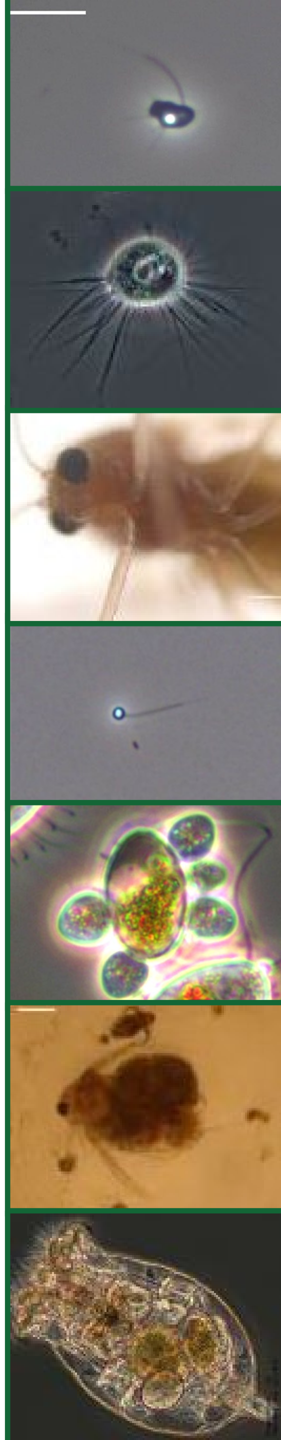
Barriers to Scale: Algae Crop Protection Workshop

Session 2 Report Out: Alternative Crop Protection Approaches to Chemicals and Pesticides

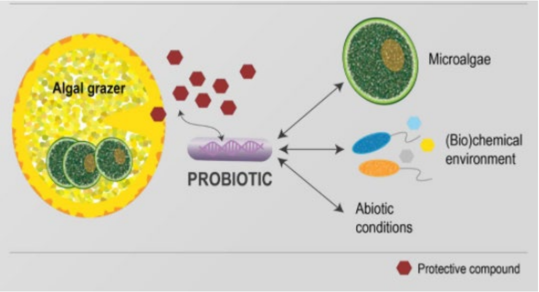
Moderator: Philip Lee

Rapporteur: Amanda Barry

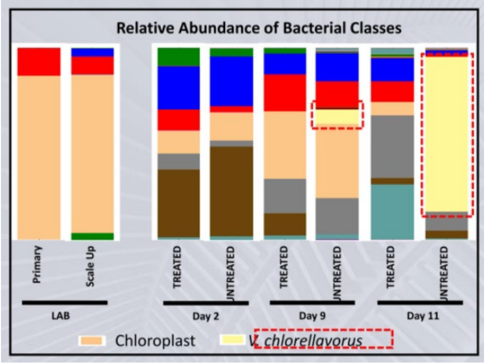


Panel Discussion Highlights

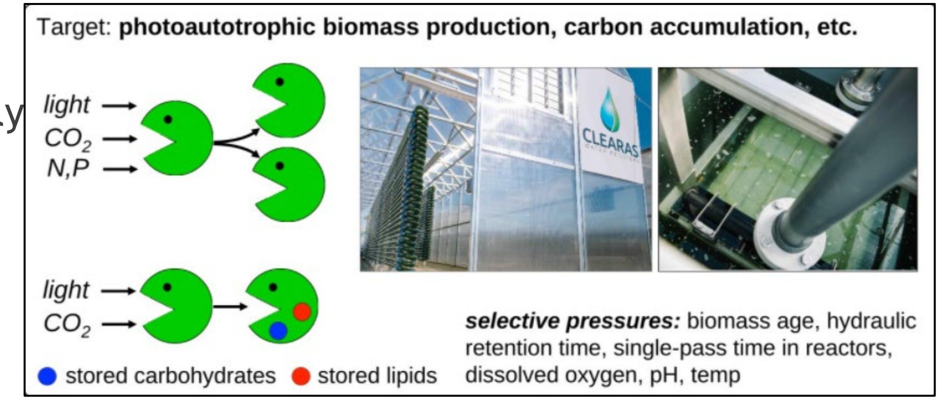
- Session overview:
  - Expense of crop protection can be prohibitive even if successful
  - Sapphire: Combination of pesticides and other controls (i.e. nutrients, alkalinity, fertilizer) were shown to be needed for continuous multi-year cultivation of *Desmodesmus*
  - Industry wants to move away from pesticides
- Rhona Stuart:
  - Biological control agents (BCAs) underexplored in algae cultivation, unlike plant agriculture
  - Leveraging the microbiome
    - Deterring grazing by rotifers with protective bacterium does not displace native microbiome; niche/role in bacterial community is important
  - Understanding basic biology of pests important
    - Parasitic chytrid fungi can proliferate without lysing algae; providing parasite nutrients killing can be deterred



- Kim Ogden:
  - Biggest pest at University of Arizona ponds *Vampirovibrio chlorellavorus*
  - Chemical treatment: Benzalkonium chloride (2 ppm) effective treatment
  - Pest displaces beneficial bacteria in the culture; recovers after treatment
  - Engineering treatment: Used reactor design to control dissolved oxygen levels at night to aerate for ciliate or other pest control



- Jeremy Guest:
  - Selective pressure with designed reactors and processes for strain resilience under certain conditions
  - Competitive advantage for natural organism with desired function
  - Strategy from wastewater: Wastewater treatment must function effectively 365 days a year continuously
  - When starting with very different mixed communities with same selective pressure, all can achieve stable performance with fluctuating community population
  - Can influence diversity with this selection: Older biomass/sludge has less diversity than younger population



Group Participants

1. Lauren Illing, BCS / BETO, strategic workshop planning, facilitation and report developme
2. Philip Lee, AST / BETO, Project monitor
3. Frederick (Eric) Mayer, CEO of LightWater Corporation, an algae startup in Orlando Florida
4. Amanda Barry, R&D Manager, Sandia National Labs - microalgae biochemistry and molecular biology
5. Zackary Johnson, Duke University, Marine Algae Industrialization Consortium (MAGIC), marine algae cultivation/products/ecology
6. Pedro Cardoso, Deputy Manager of Lisbon Experimental Unit at A4F-Algae for Future.
7. Fritz Vorisek, Staff Scientist for UK CAER
8. Brian Harriman, Synthetic Genomics, R&D Coordinator
9. Ken Reardon, Professor, Colorado State University - bioprocess engineering, microbial ecology, systems biology
10. Ainnatul Adawiyah binti Ahmad Termizi, PhD student at The University of Queensland Australia
11. Ghazala Butt, Associate Professor in Department of Botany, GC University Lahore, Pakistan, working with algal culturing and taxonomy
12. Jeremy Guest, Associate Professor; University of Illinois at Urbana-Champaign; mixed community cultivation, wastewater, biorefinery design, TEA/LCA
13. Dorinde Kleinegris, NORCE Norwegian Research Centre AS, PI on microalgae & associate prof. @University of Bergen
14. Todd Lane, Distinguished member of Technical Staff, Sandia National Laboratories
15. Kunal Poorey, Staff Scientist, Sandia National Labs, Computational Biology, Microbiome Analysis, Data Science
16. Diana Gomes, A4F-Algae for future, Researcher
17. Xavier Mayali, Lawrence Livermore, algal-microbe interactions
18. Valerie Harmon, Microalgae Production Consultant, Harmon Consulting Inc.
19. Adity Biswas, Associate Research Scientist @ The tru Shrimp Company
20. Patrick Thomas, PhD student @ University of Oldenburg, community ecology
21. Ryan Simkovsky, University of California San Diego, Project Scientist
22. Joshua Podlevsky / Sandia National Labs -- researcher / molecular and microbiology employing CRISPR
23. Juliana Abraham, Research Scientist at Center for Environmental Systems, Stevens Institute of Technology
24. Ty Samo, Lawrence Livermore National Lab, microbial ecology, microscopy
25. Sanjaya Lama, Ph.D. student, Hasselt University, Microalgae cultivation and Harvesting
26. Lieve Laurens, NREL, Scientist and group lead, algae biochemistry and biotechnology
27. Shawn Starkenburg, LANL, Microbial Genome Scientist
28. Dave Hazlebeck, Global Algae Innovations, algae cultivation and processing
29. Brad Wahlen, Idaho National Laboratory, Biochemistry, algae feedstock logistics and bioprocessing
30. Scott Edmundson, Research Botanist/Ecologist, Pacific Northwest National Laboratory

Group Discussion Highlights

- **Question 1: Barriers** What is preventing pest resistant strain (or populations) R&D?

1.7. Lack of understanding of the native microbiome roles/function	17
1.5. Tool development required e.g. transformation, breeding, evolution	11
1.1. Deciding on a host Which algae to use? The "favorite' strain keeps changing	10
1.4. Pest-specificity, a strain that is resistant to one pest might be susceptible to another	9
1.14. natural communities assemblages are different at different scales, making predictions difficult.	8
1.6. currently time and money are focused on lipid production R&D	7
1.13. lack of long term community dynamics data	7
1.2. Deciding on a pest target	6
1.8. reliable pest "models" to accurately (and predictably) measure resistance	6
1.10. focus on monocrops	6
1.11. Strong selective pressures causing pests to adapt and overcome any resistance traits in the algae	4
1.12. Lack of concerted efforts on commercial strains	3

Group Discussion Highlights

- **Question 2: Strategies** What non-chemical strategies have been or should be considered or may be feasible/economically viable for crop protection?

1.1. Changes to media composition	14
1.2. Biological control	13
1.6. Consortia - Algae and others e.g. microbiome	8
1.14. Harvest and operating reactor strategies	8
1.4. Mechanical methods e.g. filter to remove big pests, physical disruption to destroy weaker pests	7
1.8. Probiotics	7
1.5. Pest resistant lines e.g. by breeding, GM, or evolution	6
1.3. Integrated pest management	5
1.13. Community engineering based on ecological theory Same as above responses about consortia/polycultures, but focus on using ideas from niche theory/coexistence theory to design communities that we know a priori are likely to coexist over time, have complementary traits that induce overyielding, and confer resistance to grazers, pathogens, and invasion by algal competitors	5
1.7. Algal polyculture	4
1.9. Crop rotation	3
1.18. Process engineering to create selective pressures for resilient communities	3

Group Discussion Highlights

- **Question 3: Overcoming Barriers / Path Forward** What's needed to overcome barriers related to the top (1-3) priority non-chemical strategies

1.1. Changes to media composition

1.2. Biological mechanisms / consortia

1.3. mechanical / operational

How can we overcome barriers?

What do we need to understand in order to develop these strategies?

General discussion:
Missing opportunities if we focus on comparisons to terrestrial systems.

Following model of wastewater treatment systems with selection of mixed communities may be better for biomass production (for specific products, would need to better understand selection pressures).

Group Discussion Highlights

- **Question 3: Overcoming Barriers / Path Forward** What's needed to overcome barriers related to the top (1-3) priority non-chemical strategies

1.1. Changes to media composition

1.1.6. How can we overcome barriers?

1.1.6.1. always test viability of different media compositions under different conditions

1.1.6.2. Agree. This is what I am currently doing in the lab

1.1.6.3. Sourcing crashed cultures for isolating pests remains difficult

1.1.6.4. Development of test systems, including isolated agents and realistic culture systems

1.1.6.5. Need more reproducible pest models for testing different media compositions

  1.1.6.5.1. Yes, supporting pest model/pest cultivation methods development for experimentation is critical for this to work

1.1.6.6. need more isolation techniques/strategies

1.1.6.7. shared and open database of which common pests are sensitive to certain changes in pH, salinity, etc

1.1.6.8. Automation and control

1.1.6.9. systematic study and rapid determination of media water chemistry over time needs be part of this

1.1.6.10. Need to understand/predict the chemical composition of growth media/water quality for large-scale production with integrated water and nutrient recycling, i.e., what is the steady state water quality of an algae farm?

  1.1.6.10.1. Yes water quality is very important especially during recycle and need to look at seasonal variations

1.1.6.11. Medium should support highest specific growth rate of alga while suppresses the proliferation of the pest

1.1.7. What do we need to understand in order to develop these strategies?

1.1.7.1. trade off between media constituents and cost, as well as how those changes affect productivity, etc.

Group Discussion Highlights

- **Question 3: Overcoming Barriers / Path Forward** What's needed to overcome barriers related to the top (1-3) priority non-chemical strategies

1.2. Biological mechanisms / consortia

1.2.7. How can we overcome barriers?

1.2.7.1. Research- Better understandings of roles and mechanisms

1.2.7.2. Developing tools to specifically explore/target the microbiome

1.2.7.3. Research into community interactions and the role of different resources on community interactions

1.2.7.4. Basic ecological research on members of phycosphere both beneficial and harmful

1.2.7.5. Sourcing crashed cultures for isolating pests remains difficult

1.2.7.6. I think we're missing a lot of impactful opportunities if we let comparisons to terrestrial crops drive decision-making related to algae. The turnover in algal cultivation systems is many orders of magnitude faster, and establishment, etc., are fundamentally different. I would consider stopping trying to perfectly engineer a consortia and instead focus on creating the right conditions which create a competitive advantage for target functions among the population. If it's a pure culture, this is certainly more challenging.

1.2.7.7. Research into predicting culture stability

1.2.7.8. Find selective pressures in the growth environment that will yield pest-resistant and productive communities

1.2.7.9. Better understanding of what the community members require in order to manipulate who is present

1.2.7.10. If products are important as well as predictable performance in a conversion process that takes advantage of respective products, then the crop analogy holds and long term pest dynamics at relevant scales are needed, to understand what triggers 'virulence' in response to the other parameters of the culture/crop environmet

1.2.8. What do we need to understand in order to develop these strategies?

1.2.8.1. understanding how pests recognize crops to enable better interference or binding systems

1.2.8.2. how the biology affects the system as a whole...downstream processes and potential products

Group Discussion Highlights

- **Question 3: Overcoming Barriers / Path Forward** What's needed to overcome barriers related to the top (1-3) priority non-chemical strategies

1.3. mechanical / operational

1.3.4. How can we overcome barriers?

1.3.4.1. Limit costs associated with operational strategies

1.3.4.2. A better cream separator

1.3.4.3. Filtering out larger pests

1.3.4.4. Sourcing crashed cultures for isolating pests remains difficult

1.3.4.5. We need better harvest systems

  1.3.4.5.1. needs to remove everything

1.3.4.6. We need to know what pests to target

1.3.4.7. Continuous monitoring of OD culture health

1.3.4.8. a pond immune system

1.3.4.9. Advanced sensors and control strategies for key nutrients (other than DO, IC, pH)

  1.3.4.9.1. Conductivity

1.3.5. What do we need to understand in order to develop these strategies?

1.3.5.1. costs and how these mechanisms affect the system and productivity