SAND2019-2294C

The Good, the Bad, and the Algae: Chemical and Biological Analyses of Microalgal Cultures

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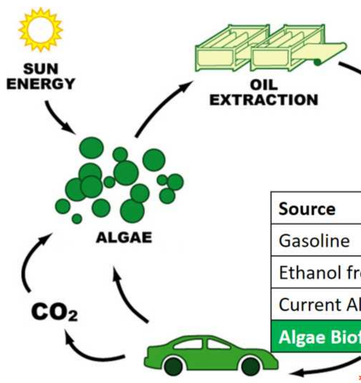
Biofuel is the future, but there are serious economic barriers to overcome before it becomes our reality.



Target selling prices for algae biomass in 2022 is $494/ton AFDW (ash-free dry weigh; 2014$) **to achieve $5/GGE** (gasoline gallon equivalent). **BETO MYPP, 2014**

Source	Price /lb	Price /gal
Gasoline	$0.29	$2.49*
Ethanol from Corn	$0.35	$2.92*
Current Algae Biofuel	$0.85- $3.67*	$7.06 - $30.46
Algae Biofuel Target	**$0.10 – 0.25***	**$1.50 - $2.10**

*Approximate national average for 2017
*2017 DOE Alternative Fuel Price Report
*Sun et al, *Energy,* 36 (2011) 5169-51795.
*BETO Multi-Year Program Plan, March 2016

Nannochloropsis salina

- Microalgae contain 25-60% lipids, do not require arable land, are not a source of food, and do not cause environmental harm.
- Adaptable/found in many systems: freshwater, marine, high pH, high salinity, and high nutrient municipal wastewater systems.
- Microalgae utilize atmospheric CO_2 and are responsible for up to 40% carbon fixation worldwide, reducing harmful greenhouse gas.

Hannon et al, 2010; Douskova et al, 2009

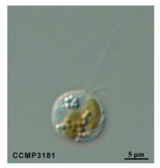
Crashed ponds are caused by various algal predators:


Brachionus plicatilis, marine rotifer


Marine planktonic copepod, *Calanus*

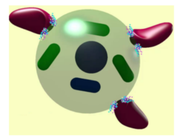

Oxyrrhis marina, dinoflagellate


alga infected with chlorovirus



Poterioochromonas, a golden algae or chrysophyte


Numerous parasitic chytrids attack the filament of a green alga

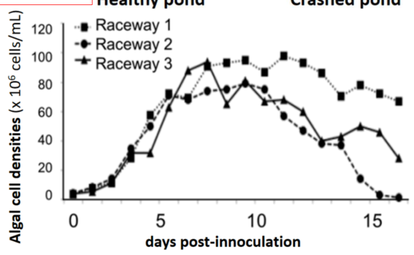

Vampirovibrio chlorellavoras bacterial predation on green alga, *Chlorella*.

Algal population crashes cause **losses of up to 30% of annual crop production** from the typical open raceway system.
Richardson et al, 2014.


Healthy pond

Crashed pond

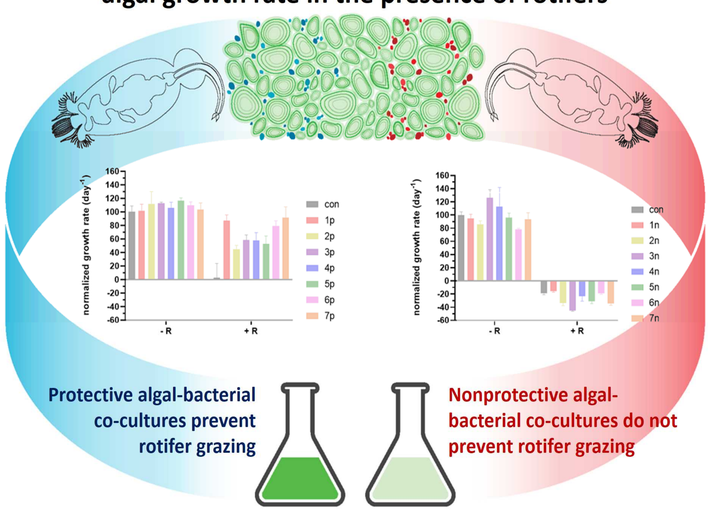
Pond crashes: *N. salina* growth in biological replicate raceways at Texas Agrilife. Raceways show moderate to severe **biomass loss** as a result of algal predation.



Carney et al. 2016

We are investigating the chemical ecology of algal culture systems in order to protect algal ponds from grazing, allow for early detection of pond crashes, and fight antimicrobial resistance by identifying novel compounds.

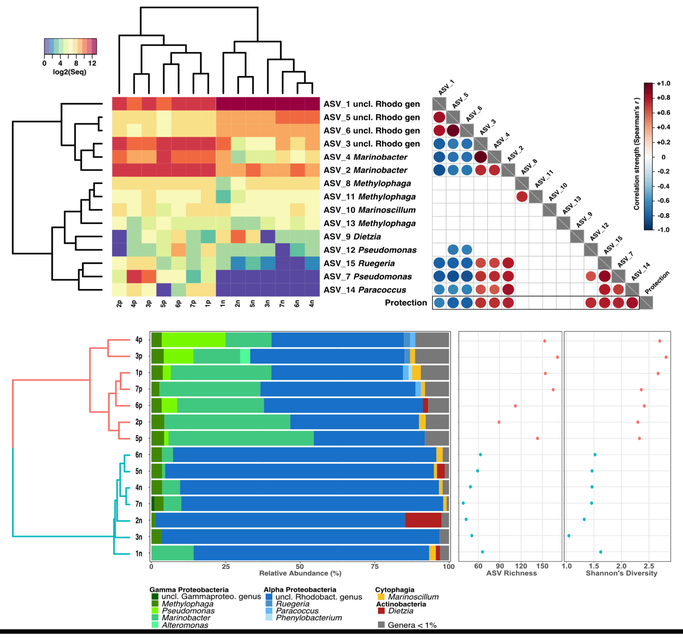
Algal-bacterial co-cultures differentially impact the algal growth rate in the presence of rotifers



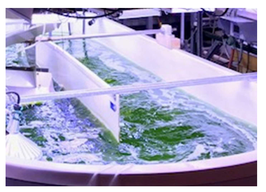
We obtained seven algal-bacterial co-cultures from crashed rotifer cultures, maintained them in co-culture with *Microchloropsis salina*, and used a microalgal survival assay to determine that algae present in each co-culture were protected from rotifer grazing and culture crash. After months of routinely diluting and maintaining these seven algal-bacterial co-cultures, we repeated the assay and found the opposite result: none of the seven bacterial communities protected the microalgae from rotifer grazing.

We performed 16S rRNA gene amplicon sequencing on the protective and nonprotective co-culture samples and identified substantial differences in the makeup of the bacterial communities. Protective bacterial communities consisted primarily of Alphaproteobacteria (Rhodobacteraceae) and Gammaproteobacteria (*Marinobacter*, *Pseudomonas*, *Methylophaga*) while nonprotective bacterial communities were less diverse and missing many putatively crucial members. We are currently performing experiments for metagenomics and metabolomics analyses to further understand the microbial and chemical ecology of the protective bacterial communities.

Our data support inoculating production ponds with a diverse, protective microbiome may be a novel, low-cost method to reduce the frequency of pond crashes **(Fisher *et al*, 2019, in submission).**

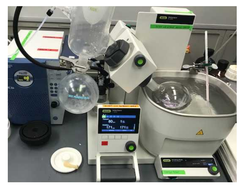


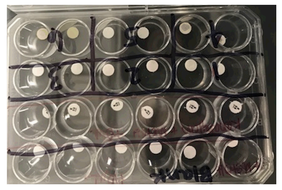
Natural Product Discovery in Algal Cultures



1) Collect high-biomass algae pond samples

2) Extraction using ethyl acetate

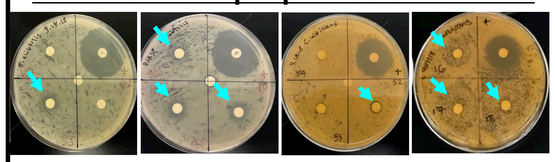




3) Dry fraction using rotovap

4) Add 300 ug to filter disks

Antimicrobial properties confirmed



Bacillus subtilis (left) and *Candida albicans* (right) were sensitive to several chemical mixtures from algal-bacterial cultures, as shown by the 'halos' here (arrows).

The primary goal of this three month EE LDRD was to leverage current DOE projects and analyze the "chemical waste" from algal-bacterial ponds to discover new antimicrobials.

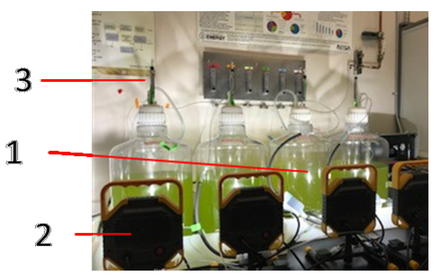
We hypothesized that algal-bacterial co-cultures contain antimicrobials generated by bacteria in tough competition to thrive/survive in high-nutrient production pond ecosystem. We isolated 75 chemical fractions and screened against bacterial and fungal targets. We identified 25 chemical mixtures that elicited an anti-microbial growth inhibition response.

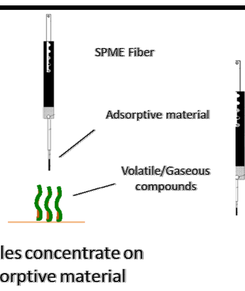
We are currently exploring options for follow-on funding to fund analysis of these extracts for chemical identification. We are looking for ways to apply this new natural products isolation and testing pipeline to other scientific problems for future impact at Sandia, future collaborations, and external sponsors.

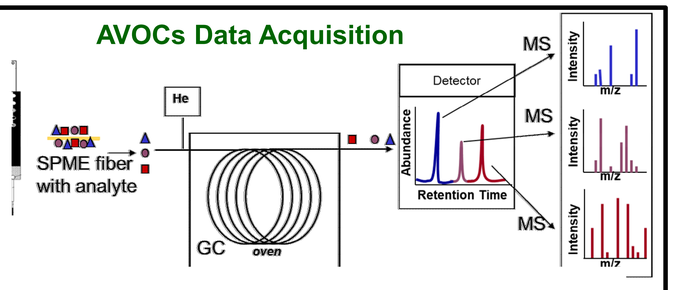
Microchloropsis salina was grown at 5M cells/mL in four 15-L cultures of ESAW with air and 1%-CO_2. After 48-hours of growth, *Brachionus plicatilis* were added to algae cultures and volatile organic compounds (VOCs) were sampled for 24-48 hrs using PDS/DVB SPME fibers for 60 min exposures.

An untargeted GC-MS approach characterized VOCs using 70 eV electron ionization on a quadrupole mass analyzer. Deconvoluted experimental spectra were determined via comparison to the NIST14 spectrum database (Match>70%) and retention index matching for *M. salina*-only cultures, *B. plicatilis*-infected *M. salina* cultures, ESAW control, and SPME fiber blanks.

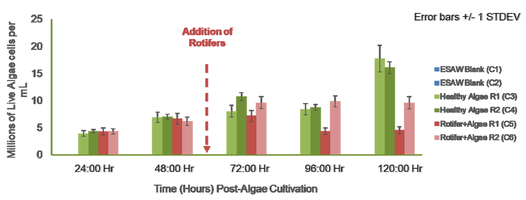
Volatiles were attributed to each condition if: 1) present in at least 2 or 3 replicates per timepoint, 2) greater than 10x abundance of corresponding abundance in media-only control. The addition of *B. plicatilis* to healthy cultures of *M. salina* produced an abundance of putatively identified carotenoid-derived products such as trans β-ionone and 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanone. Concurrent to VOC analysis, daily measurements of live algal density confirmed that rotifer-inoculated cultures displayed lower live algal counts relative to the uninoculated controls **(Reese & Fisher, *et al*, 2019, in preparation).**



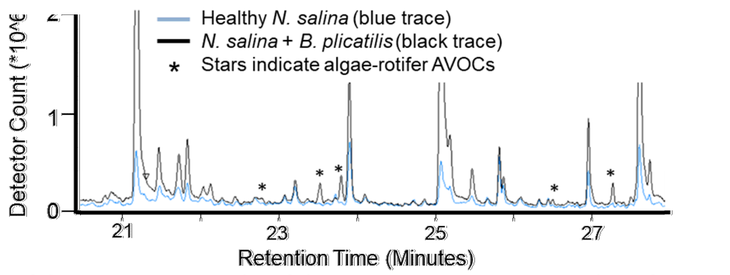




Experimental Setup: Growing *N. salina* and *B. plicatilis* cultures (1) with simulated sunlight sources (2) were sampled with SPME fibers (3); AVOCs characterized using GC-MS

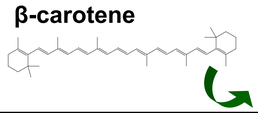


ced pond crashes successfully performed for cultures
. salina infected with *B. plicatilis*. AVOCs were
pled at multiple timepoints after rotifer addition.

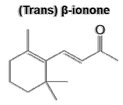


GC chromatogram of AVOCs signals unique to the rotifer-infected cultures, identified through the use of carefully chosen controls (SPME fiber blanks, media blanks, uninfected algae).

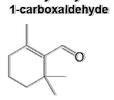
β-carotene



(Trans) β-ionone



2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde



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