An Extremophile Approach to Stable Mixotrophic Algae Cultivation

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Acknowledgements

Realization of Algae Potential  
U.S. Dept of Energy - EE0006316  
(2014-2016)

A Novel Platform for Algal Biomass Production Using Cellulosic Mixotrophy  
U.S. Dept of Energy - EE0007562  
(2016-1018)

Arizona State University - Lightworks

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Platform Design Concepts
Arid Region Focus

1. Dryland and non-arable land use to:
   a) minimize food/fuel tradeoffs
   b) minimize land-use change related CO2 emissions

2. Design for maximum possible water efficiency
   a) Utilize plastic enclosures and condensate collection
   b) Rotate strains for seasonal PBR thermal management
   c) Recycle water, nutrients and minimize blowdown volumes

3. Energy Recovery Pathway: HTL-CHG

4. Application Agnostic: high-value -> WWT -> bioproducts via metabolic engineering -> fuel
Temperature and pH limits of life

Rothschild & Mancinelli Nature 409, 1092 - 1101 (2001)
Galdieria sulphuraria

Thermo-acidophilic algae (Cyanidiales)

**Galdieria sulphuraria**

- Unicellular red alga
- Extremely acidophilic (pH 0-4) – self limiting
- Moderately thermophilic, maximum sustained growth temperature is 56°C
- High Rubisco CO$_2$/O$_2$ discrimination
  - Uemura et al. 1997
- Photoautotrophic, mixotrophic and Heterotrophic growth (up to 100 g/L)
  - Graverholt et al. 2007
Cyanidiales (Red microalgae) **Key Phenotypes**

- Evolved geographically isolated hot springs
- Small genomes (~15 Mbp) with high genetic diversity
- Thermotolerance: 20 – 56°C; >63°C for several peak afternoon hours
- Autotrophic, mixotrophic and heterotrophic growth modes
- Optimum pH range 1 to 4; no growth at pH 7 (self-limiting)
- Metabolic pH drop via NH$_4^+$ assimilation and H$^+$ transport
- **Easy and stable outdoor cultivation phenotypes in closed systems with passive solar heat gain**

18S phylogeny of *Galdieria* and *Cyanidium* strains obtained from Culture Collection of Microorganisms from Extreme Environments (CCMEE)

Yellowstone National Park (YNP)

Mixotrophs

Strict Autotroph
Inorganic Carbon Provision At Low pH

The equilibrium concentration of CO₂ at pH 2 is 99% lower than at pH 7 due to the absence of bicarbonate.

Counter-balanced by a 500-fold higher exchange rate between dissolved CO₂ and atmospheric CO₂ at pH 2 relative to pH 7.


\[ G. \text{ sulphuraria} \] growth is independent of CO₂ concentration above 1% v/v in air.
Phenotypic Variation for pH and Temp in Cyanidiales

G.s. = Galdieria sulphuraria, C.c. = Cyanidium caldarium,

Collaboration with Andreas Weber (HHU)
Control of the biochemical composition of *Galdieria sulphuraria* cells. Material from NMSU algae cultivation testbed; Freeze/etch electron micrograph, courtesy of U. Goodenough, Washington University; both supported by DOE-NAABB.
Cyanidioschyzon merolae
YNP-1A

Chloroplast
Mitochondrion
Nucleus
Eisosomes
Outdoor Cultivation Systems

Vertical PBR array, 1m x 1.2m
4 cm light path, 0.5 meter spacing
0.3 VVM,
1% CO₂ in air

Vertical PBR array, 0.8 m x 14.6 m
10 cm light path, 1.5 meter spacing
0.3 VVM, 1% CO₂ in air
Autotrophic Outdoor Growth

![Graph showing AFDW (g/L) over time with various data points indicating average growth, water addition, restarts, rain, and average temperature.](image-url)
Carbon Sources for *G. sulphuraria*

- Photosynthesis
- Hexoses (glucose, mannose, galactose, fructose, sorbose, rhamnose, fucose)
- Pentoses (arabinose, lyxose, ribose, xylose)
- Hexiols (mannitol, sorbitol, dulcitol, fucitol)
- Pentiols (adonitol, xylitol, arabitol)
- Tetriols (meso-erythritol)
- Triols (glycerol)
- Disaccharides (sucrose)
- Acetate
- Amino Acids (glutamate, aspartate)

Outdoor Mixotrophic Productivity in Vertical Flat-Panel PBR, 4 cm light path, 50-L volume

Nitrogen added on day 16 was taken up with only minor effect on growth

Sucrose degraded outside cells followed by preferential glucose utilization over fructose
G. sulphuraria (5587.1) Heterotrophic Growth on Sugars
Mixotrophic Productivity Boost in 48’ vPBR
10 cm light path

*Galdieria sulphuraria*, pH 2.5, 50 L/m²
sucrose added at 25 mM on 8/12
10 cm light path, 12.2 m², 1,220 L
1.5 m spacing between reactors

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Outdoor sucrose-grown culture micrograph
bar = 10 \mu m

Photoautotrophic Culture

Mixotrophic w/ sucrose
Waste Carbon and Nutrients
Undiluted Anaerobic Digester “Centrate”

- Centrate_CM_ASU medium (green symbols) has additional ammonium relative to pure Centrate (red)
- Achieves the same N:P ratio as in optimized growth medium (CM_ASU).
- Extremely high NH$_4^+$ tolerance
Two Algae-Related, Tenure/Tenure-Track Faculty Searches at ASU

Senior-Level Search
Open Rank

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Thank You for Your Attention!

ATP³
Algae Testbed
Public-Private Partnership

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