Cultivation and Lipid Profiles of Thermo-Tolerant, Mixotrophic Red Algae (*Galdiera sulphuraria*) in Inexpensive Closed Photobioreactors

*P. J. Lammers*1, W. Van Voorhies 1, T. Selvaratnam 1, N. Nirmalakhandan 1, M. Seger 1, A. Unc 1, H.K. Reddy 1, S. Deng 1, R. Roth 2, U. Goodenough 2, and R. Castenholz 3

1 New Mexico State University, USA; 2 Washington University, USA; 3 University of Oregon, USA
Water Availability is the Major Resource Limitation for Large-Scale Algae Cultivation in the Arid Southwest

• 8-9 feet pan evaporation per year
• Fresh water supplies are both highly limited and legally entangled

**Alternatives**

1. **Municipal and Agricultural Waste Water**
   *Energy positive WWT with nutrient extraction*

2. **Produced water from Oil and Gas Extraction**
   – 10 to 20 barrels of water per barrel of oil
   – Producers pay premium for re-injection
   – Brackish and requires clean up but useable (El Dorado Biofuels – Jal, NM)

3. **Brackish and Saline Aquifers**
Evaporation Control in Closed Cultivation Systems

- Plastic bioreactors with various mixing systems
- Greenhouse effect – hence thermo-tolerant algae species.
- Enable efficient CO₂ enrichment (1% in gas phase) with ultra-low gas-flow rates
  - 2 L-gas/min/400-L-liquid = 0.005 VVM
  - We observe no O₂ inhibition <30% peak O₂
  - 0.02% gas-phase evaporative water loss/day = ~200 mL of water loss/400-L of culture volume
Temperature data for 8 inch depth, “lay flat” PBR. The area between the red lines indicates acceptable temperature range for *Cyanidium/Galdieria* acid thermophiles.

The area between the green lines shows temperature range in which more temperate algae like *Chlorella* would grow.
Galdieria sulphuraria

• Unicellular red alga
• Extremely acidophilic (pH 0-4) – self limiting
• Moderately thermophilic, maximum sustained temperature is 56°C
• Photoautotrophic, mixotrophic and
• Heterotrophic growth (up to 100 g/L)

• High Rubisco CO2/O2 discrimination
Versatile Mixotrophic Metabolism

Sweet life at pH 1

- 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)

\~1\%\ Dissolved\ Inorganic\ Carbon\ Levels\ in\ Below\ pH\ 5\ Relative\ to\ pH\ 8\ in\ *Galdieria*\ Cultivation\ Systems

Can\ this\ really\ work?
**Galdieria** and *Cyanidium* strains obtained from Culture Collection of Microorganisms from Extreme Environments

18S phylogeny of 4 strains under study received from R. Castenholz

**Galdieria sulphuraria** CCMEE 5587.1

Volumetric Growth Rate in 6 mL tube cultures, 20-L Carboys is 0.11-0.12 grams/Liter/day (40 °C, pH 2.5)

200-L raceways: 10-16 g/m²/day (0.1-0.16 g/L/day) in raceway @ Variable Temps; pH 2.5; 10 cm depth; 5% CO2; ultra-low gas flow rate @ <1 PSI to inflate bag
*Galdieria sulphuraria* growth is independent of CO2 concentration between 1%, 5% and 10% in air.

Growth of 5587.1 in 20 l carboys. Carboys grown at 40°C with 24h light in incubator and 1, 5 and 10% CO2 enrichment. 2/1-3/22/13. N=2 for each line. SD plotted.
Outdoor Growth of *Galdieria sulphuraria* in an Enclosed Raceway
10 cm Depth, 5% CO₂ in Air, @ 2 L/min, O₂ in gas phase is ~30% during daytime

Bag Growth and Temperature

\[
y = 0.165x + 0.159 \\
R^2 = 0.978
\]

0.165 grams/L/day
16.5 grams/m²/day
Panel A: cell organization during photosynthetic growth stage, Panel B: cell morphology after nitrogen-limitation showing abundant lipid and starch bodies.

Bacterial survivability test at different pH and temperature conditions: *E. coli* 13-B6 (wastewater isolate highly resistant to multiple antibiotics)

(Adrian Unc and Mark Seger; NMSU)

**Viable *E. coli* quantification**

(M.L. agar)

- **40°C**
- **48°C**
Hydrothermal processing uses water, temperature and pressure to convert organic feedstock into fuel

- Wet process with no additional solvents
- Process conditions are wet slurry at 10-20% dry solids, 160-350°C
- Sequential HTL allows substantial pre-extraction of saccharides, amino acids and organic phosphate prior to liquefaction step

Hydrothermal Liquefaction Results from Wet Red Algal Biomass

*Galdieria sulphuraria* (CCMEE 5572) Ave FAME 8.9% (SD 1.2) N=3

<table>
<thead>
<tr>
<th>Galdieria (5572)</th>
<th>T=275 °C, 60 bar</th>
<th>T=300 °C, 91 bar</th>
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<tbody>
<tr>
<td>Oil yield (%)</td>
<td>15.43</td>
<td>25.32</td>
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Authors and Collaborators:

• Wayne Van Voorhies, Nick Csakan, Adrian Unc, Mark Seger, N. Nirmalakhandan, Thinesh Selvaratnam, Shuguang Deng, Harvind Reddy (NMSU)
• Tanner Schaub, Omar Holguin, Barry Dungan (NMSU)
• R. Roth and Ursula Goodenough (Washington University)

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