Pond Reliability: ATP3 Unified Field Studies
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Introduction to Unified Field Studies
Establish Collaborative Open Testbeds at five geographically distinct production facilities in order to quantify seasonal and environmental variability in algae open pond reliability

- Southeast Coastal: FL
- Western Coastal: CA
- Pacific Tropical Coastal: HI
- Southeast Inland: GA
- Southwest Desert Inland: AZ
Generate High-Impact Data from Long Term Cultivation Trials

- **Unified Field Studies (UFS)**
  - All 5 sites executing same production methods, systems, including seed production systems, and strains
- **Scientific Data Management System and Harmonized SOPs for analytical and UFS production methods**
  - Data integrity
  - Strong QC capability of data
- **Data publicly available via National Renewable Energy Lab website**
Unified Field Study Experimental Design

- **Five Sites:** Southwest desert inland, Southwest coastal, Southeast coastal, Southeast inland, and Pacific tropical coastal.
- **Four Seasons:** Spring (March – May), Summer (June – August), Fall (September – November), Winter (December – February)
- **Two Years:** 2014 and 2015
- **Two Strains:** *Nannochloropsis oceanica* (Cellana strain KA32), *Chlorella vulgaris* (ASU strain LRB_AZ_1201)

**Experimental Treatments:** Harvest strategies: frequency *(Once per week (1x) vs Three times per week (3x)) and dilution rates (0.11, 0.214 and 0.3 per day)*
### Standard Experimental Methods, Conditions and Sampling

#### Treatments in triplicate:

**Harvest Strategy**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Set Point</th>
</tr>
</thead>
</table>
| Aqueous N (mg L⁻¹)  | 136.4 mg L⁻¹  
|                     | 70 mg L⁻¹  
|                     | 36.8 mg L⁻¹  |
| pH                  | 7.9                              |
| Depth (cm)          | 25                               |
| PW speed (Hz)       | 20                               |
| Inoculum (g L⁻¹)    | 0.05                             |

#### Harvest Frequency =

- 1x per week: Monday
- 3x per week: Mon, Wed, Fri

#### Dilution Rate =

- 0.11:
  - 75% harvest and media replacement once per week or 25% harvest and media replacement three times per week.
- 0.214:
  - 50% harvest and media exchange three times per week
- 0.3:
  - 70% harvest and media exchange three times per week

#### Samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD@ 750nm</td>
<td>Dawn +60 min, M – F</td>
</tr>
<tr>
<td>DW</td>
<td>Dawn +60 min M, W, F, T0, TF</td>
</tr>
<tr>
<td>AFDW</td>
<td>dawn +60 min M, W, F, T0, TF</td>
</tr>
<tr>
<td>Mass balance</td>
<td>T0 (inoculation or directly post-dilution), TF (prior to a harvest or dilution occurring) – samples MUST be taken within 1 hour of AFDW/OD or a new AFDW sample is required</td>
</tr>
<tr>
<td>Nutrients</td>
<td>Dawn +60 min M, W, F</td>
</tr>
<tr>
<td>Weather data</td>
<td>Real time (hourly)</td>
</tr>
<tr>
<td>In-situ sensors</td>
<td>Real time (15 minute intervals)</td>
</tr>
<tr>
<td>Microscopic exam</td>
<td>dawn +60 min M, W, F</td>
</tr>
<tr>
<td>Genetic Analysis, qPCR</td>
<td>Weekly, upon pond health decrease,</td>
</tr>
<tr>
<td>Manual checks (pH, temp, salinity, depth)</td>
<td>Daily; AM and PM</td>
</tr>
<tr>
<td>% Shading</td>
<td>Monthly; AM, Mid, PM</td>
</tr>
<tr>
<td>Water chemistry</td>
<td>Monthly ICPMS testing</td>
</tr>
</tbody>
</table>

**Factors**

- **Set Point**
  - Aqueous N (mg L⁻¹)
    - 136.4 mg L⁻¹
    - 70 mg L⁻¹
    - 36.8 mg L⁻¹
  - pH
    - 7.9
  - Depth (cm)
    - 25
  - PW speed (Hz)
    - 20
  - Inoculum (g L⁻¹)
    - 0.05
Pond Reliability Metrics

• **Reliability**: The ability of an asset (in this case a pond) to perform its intended function without failure for a specified period of time under specified conditions.

• **Primary Metric**
  – Mean Time Between Failure (MTBF) in Days
    • A harmonized metric as per the *Society for Maintenance and Reliability Professionals*
    • Average length of operating time between failures; error-free performance time
    • This metric can only be calculated if a pond fails
Other Pond Reliability Metrics

- **# of Pond Failures**
  - Pond Failure can be system/human failure or biological contamination or stress causing the pond productivity to drop below acceptable levels
  - When ponds failed, they were typically re-inoculated from a healthy pond in the same treatment, or from seed produced indoors when available

- **Mean Time to Reset (MTTR) in Days**
  - Total amount of time spent performing a reset (in this case draining, cleaning/calibrating and re-inoculating a pond)/number of resets (in this case one pond)

- **Using MTBF and MTTR other valuable metrics can be calculated**
  - **Reliability Coefficient**
    - Error-free performance time (MTBF)/error-free performance time (MTBF) + time to reset (MTTR)
  - **Failure Rate (Probability of Failure)**
    - 1/MTBF
Reliability Metrics to Calculate Effort

• Primary Metrics
  – Annual # of Pond Resets
    • 365 / (MTBF + MTTR)
  – Annual Reset Effort
    • Annual # of resets x MTTR
Results: Location and Season
The Southeast Coastal site in Florida had the highest total number of failures as well as a significant difference in time between failures, while the Western Coastal site in California had the fewest number of failures. MTBF between all sites but Florida were not significantly different.
The Spring and Winter seasons had the highest MTBF, while Fall season had the lowest amount of time between failures.
All Sites: Seasons and Strains

# of Failures and Yield

ASU Seasons and Strains: # of Failures

CP Seasons and Strains: # of Failures

Cellana Seasons and Strains: # of Failures

FA Seasons and Strains: # of Failures (n)

GT Seasons and Strains: # of Failures (n)

- Reliability Coefficient = \( \frac{MBTF}{MTBF+MTTR} \)
- Is there a correlation between rainfall and failures?
- Is there a correlation between temperature and failures?
- Since location seems to be a significant driver of failure, what weather conditions lead to failure?
Can we use reliability metrics to determine optimal production methods?
Results: Strain
Algal Strain:
Mean Time Between Failures, Reset Effort

- Production strain will impact effort, which will affect production costs
- *Nannochloropsis* did not experience failures in 11 out of 20 experiments: Failure Rate = 0.08
- *Chlorella* only had one run that did not fail out of 14 experiments: Failure Rate = 0.1
Results: Harvest Strategy
Harvest Strategy: MTBF and Reset Effort

As dilution rates and harvest frequencies increased a trend of improved reliability and reduced reset effort of ponds occurred. Best fit for both was to a polynomial equation. Data is comprehensive for all strains, all seasons and all sites.
Results: Inoculum Source
UFS Inoculum Production Methods for System Initiation

Initial culture density ~0.1-0.2 g/L
Average growth rate ~0.3 g/L/day
Growth period is 9-10 days
Final culture density ~3.5 g/L

12-2’x2’ panels yield provide biomass for a minimum target of 0.05 g/L (AFDW) initial mini-pond concentration (sites have 14 available)

Initial culture density ~0.2 g/L
Average growth rate ~0.15-0.2 g/L/day
Growth period is 9-10 days
Final culture density ~2.0 g/L
Drain, clean, calibrate

Advantage of no grow out, right back to harvesting immediately! But is this quality inoculum?
Inoculum Source: MTBF and Reset Effort

Inoculum Source: Mean Time Between Failures

P-value = 0.0000868951

Inoculum Source: Annual Reset Effort

- Annual # of Repairs Average
- Annual Repair Effort (# Repairs * MTTR) Average

P-value = 0.000058
• It is possible to utilize reliability metrics presented here to evaluate production methodologies to reduce pond failures.
• Only the Southeast Coastal area had a low MTBF, other locations tested did not show any difference in pond reliability with MTBFs of approximately three weeks
  – An in depth analysis of what drives the lower MTBF in this region is warranted
• Fall season was the season with the shortest MTBF, however differences between seasons were not significantly different in the overall data set
• MTBF was significantly different between strains indicating that strain selection will have a significant effect on effort and cost
• A strong positive trend exists for both MTBF and reset effort as harvest frequency and dilution rate increase
• Inoculum source had a significant effect on MTBF of ponds
Future work

- Currently working on a publication to define reliability metrics
- Data processing to attempt to identify what is driving the trends observed in pond reliability
- An attempt to identify if any correlations can be found with the pond ecosystem composition as measured with both 16S and 18S sequencing data collected over the course of the UFS studies
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Thank you for your time and attention!