Simulation of Outdoor Pond Cultures Using Indoor LED-lighted and Temperature-Controlled Raceway Ponds

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The Challenge

- How to determine which laboratory strains should be selected for outdoor pond cultivation, i.e., exhibit high biomass and lipid productivity?

The Solution

- Integrated approach to predict real-world performance of novel strains

Strain Characterization ➔ Biomass Growth Modeling ➔ Productivity Mapping ➔ Climate-Simulated Pond Culturing
Overview

- Climate-Simulation Pond Capability
- Simulation of *Chlorella* 1412 Outdoor Arizona Pond Cultures
- Identification of Maximum Achievable Annual Biomass Productivity for LANL *Picochlorum* Strain
PNNL Climate Simulation Ponds
LED Light Spectrum Approximates Sunlight
Potential Uses of LED-lighted Ponds

▸ Study effects of different levels of constant temperatures and light
▸ Simulate actual variations in temperature and light observed in outdoor ponds to validate climate-simulated culturing concept
▸ Predict performance of outdoor ponds at any geographic location and season of choice
Batch and Semi-continuous *Chlorella* 1412 Pond Cultures in Arizona
Observed Fluctuations in Sunlight Intensity and Water Temperature
Simulating *Chlorella* 1412 Outdoor AZ Pond Cultures in PNNL’s Indoor LED-Lighted Ponds: Light Intensity Scripts
Simulating *Chlorella* 1412 Outdoor AZ Pond Cultures in PNNL’s Indoor LED-Lighted Ponds: Water Temperature Scripts
Simulating Chlorella 1412 Outdoor AZ Pond Cultures in PNNL’s Indoor LED-Lighted Ponds: Biomass Growth (Batch Culture)
Simulating *Chlorella* 1412 Outdoor AZ Pond Cultures in PNNL’s Indoor LED-Lighted Ponds: Invasion by Predators (Rotifers)
Simulating *Chlorella* 1412 Outdoor AZ Pond Cultures in PNNL’s Indoor LED-Lighted Ponds: Biomass Growth (Semi-Continuous Culture)

Dilution Rate = 0.45/day  \[\rightarrow\]  Dilution Rate = 0.21/day
Maximum Achievable Annual Biomass Productivity of LANL *Picochlorum* sp.

Questions:

- Where is the best outdoor pond location for this strain?
- What is the maximum achievable annual biomass productivity?
- Can predicted productivity be validated in climate-simulation ponds?
Integrated Strategy

\[ \mu = f(I, T) \]

Light absorption coefficient \((k_a)\)

Strain Characterization

\[
\text{Biomass growth} = f(I, T, k_a, d, t)
\]

Location specific:
- Light scripts
- Temperature scripts
- Productivity

Biomass Growth Model

Productivity Mapping

Climate-Simulated Culturing

BAT Inputs

Location specific:
(2600 stations)
- Land availability
- Resource availability
- Weather data

Biomass Assessment Tool (BAT)
Strain Characterization: Specific Growth Rate as a Function of Temperature
Strain Characterization: Photosynthetic Oxygen Evolution as a Function of Light Intensity at Different Temperatures
Strain Characterization: Scatter-corrected Light Attenuation Coefficient

\[ I = I_0 \cdot e^{-k_a \cdot OD \cdot L} \]

\[ k_{sa} = k_a \cdot \frac{K_{OD}}{K_{OD} + OD} \cdot \frac{K_L}{K_L + L} \]
Identification of Geographic Locations of Optimal Annual Biomass Productivity of WT in Outdoor Ponds
Generation of Light Intensity & Water Temperature “Scripts” (Time Series) for Ponds at Optimal Location

Light Intensity

Water Temperature
Predict Monthly Biomass Productivity at Best Pond Location Operated at Optimal Dilution Rate (0.25/day)
Validation of *Picochlorum* sp. Performance in Climate-Simulation LED-lighted Raceway Ponds
Biomass Concentration as a Function of Time
Conclusions

► Integrated Screening and Testing Approach
  - Integrated low-risk approach to predict real-world performance of novel promising strains at optimal pond location

► Biomass Growth Modeling and Integration with BAT
  - Predictive screening of strains for high biomass productivity
  - Identification of best pond culture locations
  - Model-guided optimization of pond design and operation

► Climate-Simulated Culturing
  - Measure seasonal and annual biomass productivities under conditions simulating hypothetical ponds at best geographic locations (per model)
EXTRA VIEWGRAPHS
LED-Lighted Pond Capabilities

- 800 L culture volume and depth up to 26 cm.

- Access to clean seawater and groundwater enables culturing of freshwater and marine strains.

- Light intensity can be increased up to 2700 µmoles/m²-sec.

- The LED spectrum (4500 LEDs) is very similar to that of sunlight.

- Pond water temperature controllable from ca. 6 to 48 °C.

- Simulate daily light and water temperature fluctuations of hypothetical outdoor ponds at any geographic location and season of choice.

- pH control via intermittent CO₂ sparging.

- Biomass can be harvested with a continuous centrifuge.
Light intensity \((l)\) decreases exponentially with light penetration distance \((z)\) according to Beer-Lambert’s Law

\[-k_aBz\]

- \(k_a = \) biomass light absorption coefficient
- \(B = \) biomass concentration

Light intensity can be predicted in each discretized culture volume layer.

The specific growth rate \((\mu)\) in each volume layer can be calculated if the relationship between \(\mu\) and light intensity \((l)\) and temperature \((T)\) is known.

Biomass concentration \((B)\) increases exponentially with time \((t)\).
Model Assumptions & Input Parameters

► Model Assumptions
- Light and temperature are the main determinants of biomass growth and productivity
- There are no other growth-limiting factors (N, P, CO₂, mixing)

► Physical Input Parameters
- Incident light intensity \( (I_o) \) as a function of time (1 min)
- Water temperature \( (T) \) as a function of time (5 min)
- Culture depth \( (d) \)
- Dilution rate \( (D) \) for continuous or semi-continuous cultures

► Biological Species-Specific Input Parameters
- Maximum specific growth rate \( (\mu) \) as a function of temperature
- Maximum specific growth rate \( (\mu) \) as a function of light
- Rate of biomass loss in the dark \( (\mu_{dark}) \) as a function of \( T \) & \( I_{avg} \)
- Scatter-corrected biomass light absorption coefficient \( (k_a) \)
Alignment with Goals of DOE BETO

- Achieve annual productivity of 20 g/m² -day by 2014 (equivalent to 1500 gallons per acre per year)*
- Achieve annual productivity of 25 g/m² -day by 2018 (equivalent to 2500 gallons per acre per year)*