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Growth and Lipid Measurements Towards Modeling of *Dunaliella salina*

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Problem

Why biofuels?

- Current sources of energy are in decline, harmful to the environment, and often obtained from foreign suppliers.
- To reduce green house gasses, climate change and our dependence on foreign oil we need alternative clean and domestic energy sources.
- Algae-based biofuels are a promising component to a long-term renewable energy solution.

Why Algae?

- Algae can be engineered or stressed to produce large quantities of oil with favorable characteristics for biodiesel.
- Algae can be grown in waste/brackish/sea water, reducing the impact on fresh water supplies.
- Algae mitigate atmospheric CO_2.
- Algae can be grown on non-arable land, decreasing the impact on the food supply.
- Algae growth and harvesting still require much optimization to reduce the cost of oil production and improve efficiency.

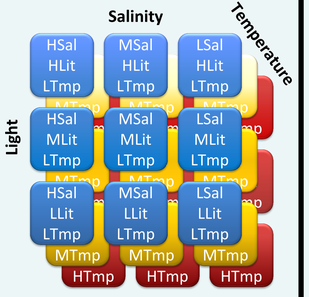
- How can we easily optimize algae growth and lipid production for different environmental conditions?
- What bioreactor designs yield the best growth efficiencies?
- What types of algae works best a different times of years or different locations?

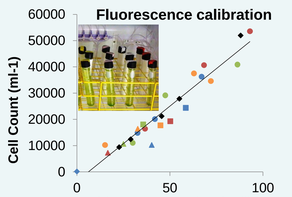
Need a realistic model

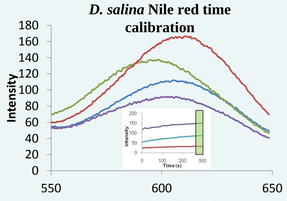
- We need to be able to optimize algae growth and lipid production in large commercial scale systems.
- It is too time consuming and expensive to test various solutions on a commercial scale.
- A computational model facilitates faster and cheaper optimization.
- However, the necessary data are lacking to create the needed constitutive relations for algae growth and lipid production.

Approach

- **Multi-factorial Measurements**
  - Measure effect of light intensity, temperature and salinity on growth multiple key marine algal species
  - Use in-situ measurement methods and parallel growth to reduce time needed
- **Constitutive Relations**
  - Determine relationships between environmental variables and growth
  - Apply to algae growth model
- **Photobioreactor Models**
  - Develop model for closed photobioreactor systems
  - Expand model for marine algal species with salinity dependence
  - Add lipid production to model







Measurement Techniques

- **Algae Selection:**
  - Marine, triacylglycerol (TAG) producing, readily available
  - *Dunaliella salina, Chlorella sorokiniana, Nannochloropsis oculata, Nitzschia frustulum*
- **Factors:**
  - Sample 4 salinities, 3 light intensities, 4 temperatures
- **Growth:**
  - Measure chlorophyll a fluorescence
  - Excite at ~440 nm, emit at ~670 nm
  - Calibrate chlorophyll fluorescence with known standards of chlorophyll concentration
  - Calibrate chlorophyll concentration with cell counter for each algae species
- **Lipids:**
  - Use Nile-red-stained lipid fluorescence
  - Excite at ~530 nm, emit at ~600 nm
  - Calibrate for cell penetration time period for each algae species

EFDC and Water Quality Model

- Modified version of the Environmental Protection Agency's Environmental Fluid Dynamics code and U. S. Army Corp of Engineer's water-quality model.
- Models algae growth based on constitutive relations

B – biomass concentration (gC/m³)
P – production rate (1/d)
B_M – metabolism rate (1/d)
P_R – predation rate (1/d)
f – growth limiting constitutive relations (non-negative, less than or equal to 1)

$$\frac{\partial}{\partial t} B(\mathbf{x},t) = (P - B_M - P_R) B(\mathbf{x},t) + \frac{B_L}{V}$$

$$P = \mu_{opt} \cdot \left[f_1(N) f_2(I) f_3(T) f_5(S) \right]$$

- Tracks nutrients, salinity, temperature, light, CO_2 and O_2 concentrations
- Allows for sources and sinks of parameters
- Currently assuming no settling (well mixed)

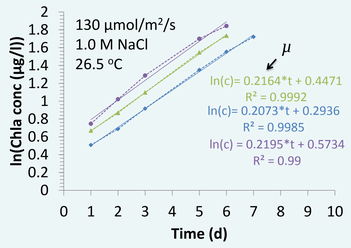
Results

Growth Measurements

- Measured growth at 23.5 26.5, 29.5, and 31.5 °C for 3 light intensities (50, 130, 280 µmol/m²/s) and 4 salinities (1, 1.5, 2, 2.5 M NaCl) in parallel
- Bubble air into samples
- Light/dark cycle = 16:8
- Measured in triplicate and averaged
- Calculate specific growth rate, μ, by fitting data to exponential growth curve:

$$C_{Chla} = C_0 \, e^{\mu t}$$

$$\ln(C_{Chla}) = \mu t + \ln(C_0)$$



Plot of measured chlorophyll concentration

Constitutive Relation Determination

Salinity:

- Bell curve with different above and below optimum decay coefficients and non-zero asymptote

$ksal_1$ = 0.0001 (ppt NaCl)$^{-2}$
$ksal_2$ = 0.0002 (ppt NaCl)$^{-2}$
S_{opt} = 90 ppt NaCl (1.01 M)
f_{sal} = 0.6

$$f(S) = \frac{\mu(S)}{\mu_{opt}} = \begin{cases} \left(f_{sal} \exp\left(-ksal_1 \left(S - S_{opt} \right)^2 \right) + \left(1 - f_{sal} \right) \right) & when \;\; S \le S_{opt} \\ \left(f_{sal} \exp\left(-ksal_2 \left(S - S_{opt} \right)^2 \right) + \left(1 - f_{sal} \right) \right) & when \;\; S > S_{opt} \end{cases}$$

Light Intensity:

- Steele's equation for light intensity constitutive relation including light extinction

I_{opt} = 150 µmol/m²/s
FD = ⅔ (from 16:8 light/dark cycle)

$$f(I_0) = \frac{\mu(I_0)}{\mu_{opt}} = \frac{2.718 \cdot FD}{Kess \cdot \Delta z} \left(e^{-\alpha_B} - e^{-\alpha_T} \right)$$

$$\alpha_B = \frac{I_0}{FD \cdot I_{opt}} e^{-Kess(H_T + \Delta z)}, \quad \alpha_T = \frac{I_0}{FD \cdot I_{opt}} e^{-Kess \cdot H_T}$$

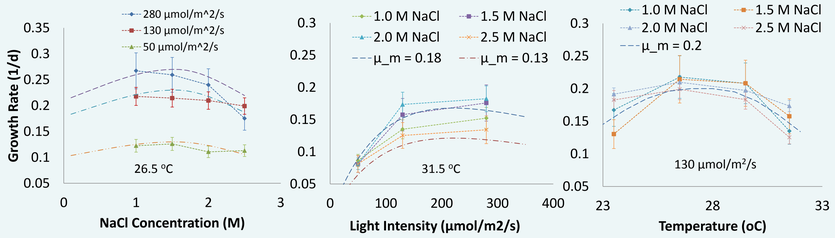
$$Kess = Ke_b + Ke_{Chl} \frac{B}{CChl}$$

Temperature:

- Gaussian curve with differing above and below optimum decay coefficients and a range of optimal temperature.

k_{T1} = 0.02 (°C)$^{-2}$
k_{T2} = 0.025 (°C)$^{-2}$
$T_{opt,1}$ = 27 °C
$T_{opt,2}$ = 28 °C

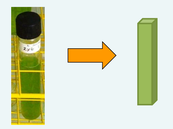
$$f(T) = \frac{\mu(T)}{\mu_{opt}} = \begin{cases} \exp\left(-k_{T1} \left(T - T_{opt,1} \right)^2 \right) & T \le T_{opt,1} \\ 1 & T_{opt,1} < T \le T_{opt,2} \\ \exp\left(-k_{T2} \left(T - T_{opt,2} \right)^2 \right) & T > T_{opt,2} \end{cases}$$

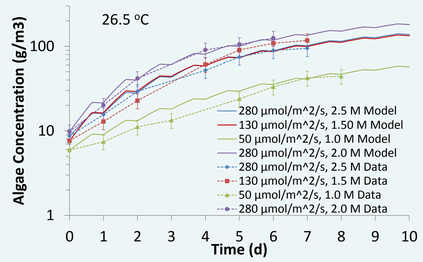


Comparisons between measured data and constitutive relations.

Lab-scale Model and Data Comparison

- Predicted *D. salina* growth in test tube based on constitutive relations (measurement (*S,I,T*) and literature (*N*))
- Relations chosen work relatively well, more improvements could be made

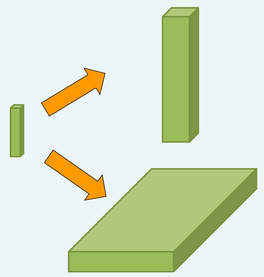


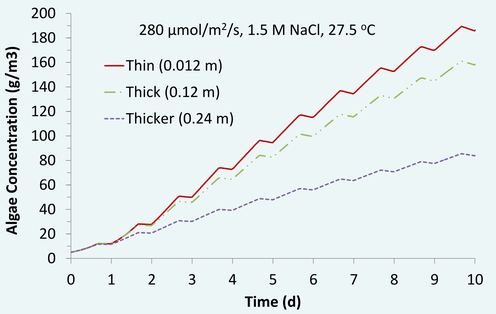


Plot of measured and modeled algae concentration over time in test tube photobioreactor

Model Expansion

- Model can be expanded to larger scale bioreactors like open channel raceways, column and flat plate airlift bubble mixing photobioreactors, and closed channel tubular photobioreactors.
- Able to compare various bioreactor geometries
- Model shows dependencies on photobioreactor thickness due to reduced light penetration.

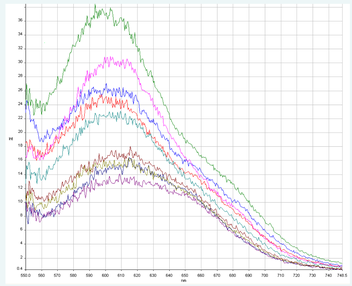




Plot of modeled algae concentration over time for different photo bioreactor thicknesses

Lipid Measurements

- Lipids were measured later in the exponential growth phase and after.
- No appreciable variations were seen during the growth
- When CO_2 bubbling was stopped, no appreciable difference in lipid quantity was measurable.
- Beta carotene build-up in *Dunaliella salina* caused interference and error with the Nile Red measurements especially at high light conditions.



Nile Red fluorescence measurements for *Dunaliella salina*

Conclusions

- Completed multi-factorial measurements of *D. salina* growth
- Added salinity growth dependence to existing EFDC model
- Created airlift bubble type photobioreactor model (e.g.. test tube, column, plate)
- Developed salinity, temperature, and light intensity constitutive relations for *D. salina*
- Modeled *D. salina* in airlift photobioreactor and compared to measurement data
- Modeled effect of photobioreactor thickness
- Knowledge gained will enable computational models to optimize algae growth in real-world conditions with varying temperature, light, and salinity over the course of a day or year.
- Through a validated constitutive growth model, algae performance and production efficiency can be predicted for various growth conditions, including different weather climates and reactor designs.
- The model will enable improved design and algae strain selections.

National Nuclear Security Administration

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