The Dark Side of Algae Cultivation:

A comparison of biomass loss at night in three photosynthetic algae, *Chlorella sorokiniana*, *Nannochloropsis salina* and *Picochlorum* sp.

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PNNL, Marine Sciences Laboratory
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What is the **Dark Side**?

- Solar energy comes in circadian periods
  - Photosynthetic organisms must deal with long periods of no illumination
  - There is an energetic cost in dealing with the dark
- Daily productivity lost to cellular metabolism over the dark period = *Dark Biomass Loss*

**Dark biomass loss acts as a daily ‘tax’ on solar gains**
If you are being taxed on a daily cycle you would probably like to know the rate!

Many studies use an assumed 10% estimate.

In Microalgae, ratio of dark respiration : photosynthesis ranges from 0.004 to 0.59 (Geider and Osborne 1989).

Would you invest in a factory that might lose 60% of its product every night?
Why Study **Dark Biomass Loss in Algae**?

- **Screen** high productivity strains
  - High biomass loss = waste of solar gains

- **Predictive modeling** of outdoor production
  - Dark losses are currently unavoidable in large ponds

- **Understand** underlying mechanisms
  - May lead to practical methods to mitigate losses
Most dark biomass loss studies use $O_2$ exchange in short, low volume incubations as a proxy for biomass loss

- Difficult to extrapolate to real world biomass changes

Current knowledge gaps:

- Dark biomass loss
- Species specificity
- Effect of temperature
- Effect of growth phase

$\Delta O_2$ exchange $\Delta$ Biomass
Methodology: hunting for “dark matter”

Three commercial biomass candidates tested:

1) *Nannochloropsis salina* CCMP 1776
2) *Chlorella sorokiniana* DOE 1412
3) *Picochlorum* sp. LANL

![Typical growth and loss curve as a function of temperature](image)
Methodology: *the hunt for “dark matter”*

- Algae cultivated at three optical densities (OD$_{750}$)
- Simulated growth phases
- 500 µmol photons/m$^2$/sec surface light intensity

**Thermal Gradient Incubator**
- Algae incubated in the dark at a range of temperatures ~10-30 °C
- Cultures continually aerated

- Light incubation
- Dark incubation

![Flasks wrapped in foil](image)

![Chiller](image)

![PID-controlled heater](image)

![Aluminum block](image)
Methodology: *the hunt for “dark matter”*

- Ash-free dry weight (AFDW): glass fiber filters (GF/F), dried to constant weight at 105 °C and ignited at 540 °C.

- Biomass losses as % were calculated from the difference in biomass over the dark incubation.

- Biomass loss rates ($\mu_{\text{dark}}$) calculated following:

  $$\mu_{\text{dark}} = \frac{\ln B_f - \ln B_i}{\Delta t}$$

  Where initial and final biomass were measured by either AFDW or optical density at 750nm (OD$_{750}$).
Dark Biomass Loss as a Function of Temperature

*Nannochloropsis salina CCMP 1776*
Dark Biomass Loss as a Function of Temperature

*Picochlorum* sp. WT-LANL

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>% Biomass Loss (AFDW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>2.4%</td>
</tr>
<tr>
<td>19</td>
<td>4.1%</td>
</tr>
<tr>
<td>25</td>
<td>3.6%</td>
</tr>
<tr>
<td>29</td>
<td>6.0%</td>
</tr>
</tbody>
</table>
Dark Biomass Loss as a Function of Temperature

**Chlorella sorokiniana DOE1412**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>% Biomass Loss (AFDW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>4.6%</td>
</tr>
<tr>
<td>17</td>
<td>7.2%</td>
</tr>
<tr>
<td>24</td>
<td>6.7%</td>
</tr>
<tr>
<td>30</td>
<td>6.8%</td>
</tr>
</tbody>
</table>
Quick Summary:
- Temperature has a moderate Arrhenius-type effect on dark loss
- 10% appears to be a relatively good estimate!

What about the knowledge gaps?
- Dark biomass loss (AFDW)
- Effect of temperature
- Species specificity
- Effect of growth phase
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- Effect of temperature
- Species specificity (3 out of ~10 million)
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What about the knowledge gaps?
- Dark biomass loss (AFDW)
- Effect of temperature
- Species specificity
- Effect of growth phase
Dark Biomass Loss as a Function of Growth Phase

% Biomass Loss (AFDW)

- Nannochloropsis salina (CCMP 1776)
- Chlorella sorokiniana (DOE 1412)
- Picochlorum sp (LANL-WT)

Growth phase prior to dark incubation

Exponential
Linear
Stationary

All cultures at 24 °C

Growth phase prior to dark incubation
\( \mu_{\text{dark}} \) as a Function of Growth Phase

Growth Phase Prior to Dark Incubation

- Exponential
- Linear
- Stationary

\( \mu_{\text{dark}}(\text{day}^{-1}) \)

-0.55
-0.45
-0.35
-0.25
-0.15
-0.05

- Nannochloropsis salina (CCMP 1776)
- Chlorella sorokiniana (DOE 1412)
- Picochlorum sp

October 31, 2014
Conclusions

1. A general Arrhenius-type trend of increasing biomass loss corresponded with increasing dark incubation temperature

2. $\mu_{\text{dark}}$ rates were highly variable between species tested ranging from -0.55 to -0.04 1/day.

3. $\mu_{\text{dark}}$ rates were highly dependent on the growth phase prior to the dark incubation.
Rate and extent of biomass loss in the dark is a species specific physiological characteristic.

\( \mu_{\text{dark}} \) is influenced by environmental conditions:

- Light intensity (prior to dark phase)
- Night culture temperature
Acknowledgements

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Implications of Findings

- Algae are a diverse polyphyletic group, loss rates are expected to be highly variable among algae
  - Individual screening required for accurate predictive modeling
  - Highly productive strains likely to have low dark biomass loss rates

- Future research should address influence of:
  - Photoperiod
  - Daytime temperature
  - Heterotrophic interactions
  - Aeration/dissolved oxygen
Dark Biomass Loss appears to be non-linear

Non-linear loss (Grobbelaar and Soeder 1985)

Fig. 1. Respiration rates of Coelastrum sphaericum in the dark from an outdoor pond at different temperatures.
PNNL Outdoor Ponds