Cyanobacteria and Biosequestration: The Effects of High CO₂ on Calcifying *Synechococcus*

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**Abstract**

Increased carbon dioxide levels in the atmosphere and its influence on climate change is a growing concern in the scientific, political, and social communities. Methods of mitigation are being tested to explore carbon capture and storage through the biosynthesis of stable carbon-containing compounds using different strains of calcifying cyanobacteria. By utilizing marine genera, the cyanobacteria could potentially be grown in brine waste waters, conserving valuable fresh water resources. In this experiment, two strains of *Synechococcus* were cultured in flasks with varying levels of CO₂ (air, 5% CO₂, and 15% CO₂). Growth of each culture was monitored by measuring optical density and pH levels. Morphological characteristics of each culture were analyzed through light microscopy and scanning electron microscopy. Preliminary results have shown inconsistent morphology and growth. Cultures started from previous experiments lacked duplication of observed filamentous morphology, but exhibited better growth in high CO₂ levels. The incubation of cultures in media with varying levels of calcium chloride will be used to analyze and compare the sequestration of carbon through calcium carbonate production. Analysis of the chemical composition of precipitates in the media and the exopolysaccharide sheath (ESP) and surface layer (S-layer) of cells will verify the presence of calcium carbonate. Methods include the use of Scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) and polarized light microscopy. If experimental outcomes verify efficient production of calcium carbonate from sources of high CO₂, these cyanobacteria may be viable systems for capturing carbon from coal fired power plants.

**Results**

**Growth of Synechococcus PCC 7002 and PCC 8806 under 3 CO₂ levels**

<table>
<thead>
<tr>
<th>Experiment 1: S7002 15% CO₂</th>
<th>Experiment 1: S8806 5% &amp; 15% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>O.D. (730 nm)</strong></td>
<td><strong>Mean pH</strong></td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>1.2</td>
<td>1.4</td>
</tr>
</tbody>
</table>

**Conclusions**

- S7002 grows well under high CO₂ concentrations
- Potential adaptations to CO₂ levels after inoculating Exp. 2 from Exp. 1
- Morphology of both strains is variable between replicates and CO₂ concentration
  - Filamentous characteristics were not replicated
  - Possible CaCO₃ deposit on the surface of cells seem with SEM, but need to confirm with EDX
- Additional experiments and analyses must be conducted to confirm CaCO₃ presence

**Challenges and Future Studies**

- Calcing conditions
  - Need high pH: stronger buffers (CHES, CAPSO, AMP, CAPS) or from careful control of sparging
- Practical application
- Method for detecting CaCO₃ precipitation
- FTIR (Fourier transform infrared spectroscopy)
- Polarized light microscopy looks for presence of calcite
- Track disappearance of soluble Ca in media
- Variation in growth and morphology
- Compare cultures in media with variable nitrate levels

**Methods**

- Species of interest: *Synechococcus* (PCC 7002 and PCC 8806)
- Series of experiments: compare S7002 and S8806 with each other then in variable media
  - A+ media (buffered with Trizma)
  - ASN III media (unbuffered resembling sea water)
  - Incubated in a shaker with sparging at 3 CO₂ levels
- Study growth, morphology, and production of recalcitrant carbon; CaCO₃
- Track growth measuring optical density (730 nm) and monitor pH
- Examine morphology through light microscopy
- Cell surface characteristics SEM
- Investigate CaCO₃ production

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