Cyanobacteria and Biosequestration: The Effects of High CO_2 on Calcifying *Synechococcus*

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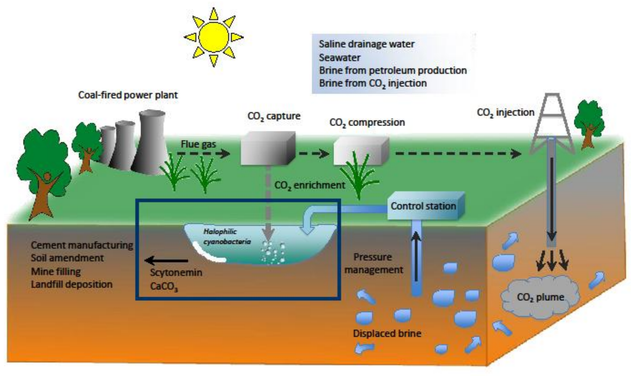
Abstract

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 CO_2: air, 5% CO_2, and 15% CO_2. Growth of each culture was monitored by measuring optical density and pH level. 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 CO_2 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 CO_2, these cyanobacteria may be viable systems for capturing carbon from coal fired power plants.

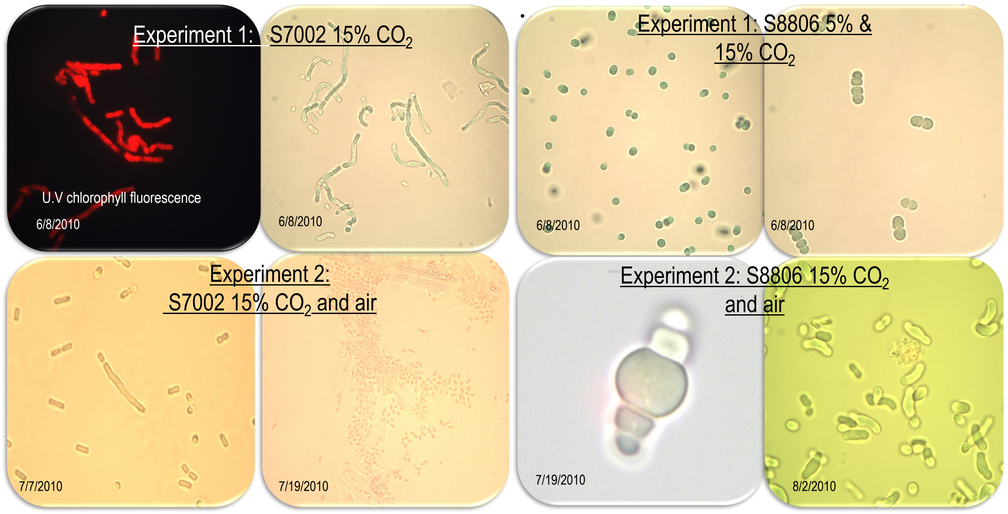


Methods

- 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 CO_2 levels
- Study growth, morphology, and production of recalcitrant carbon; $CaCO_3$
- Track growth measuring optical density (730 nm) and monitor pH
- Examine morphology through light microscopy
- Cell surface characteristics SEM
- Investigate $CaCO_3$ production

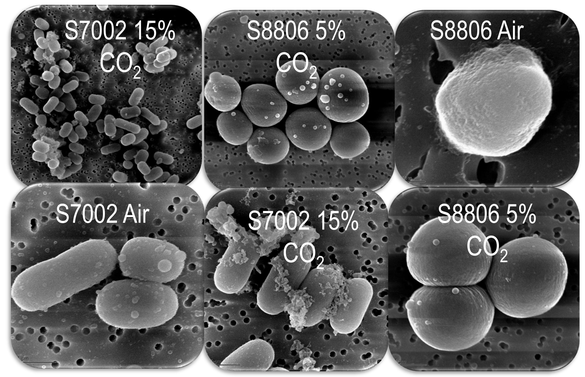
Results

Light Microscopy 100 x magnification



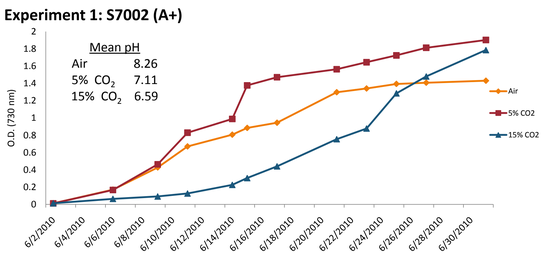
*10 µL of each culture was deposited on slide for analysis. Cell measurements were taken using ImageJ to compare size between CO_2 variation by measuring x & y coordinates in pixels and converting to microns; S8806 was more variable in cell size

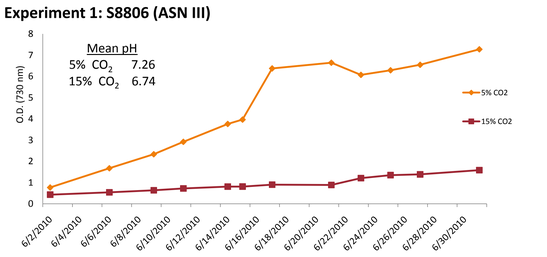
SEM Pictures

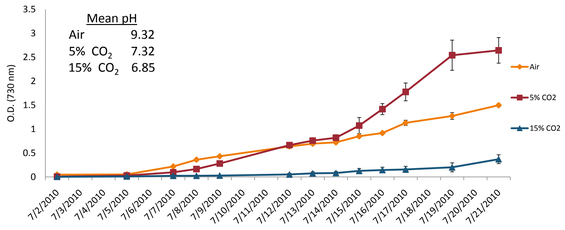


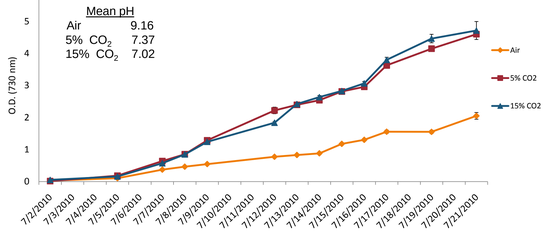
*180 µL of sample was combined with 20 µL of glyceraldehyde and taken to SEM lab. Variable shapes, associations and surface were observed . Possible that washing took healthy cells in some samples (S8806 Air)

Growth of Synechococcus PCC 7002 and PCC 8806 under 3 CO_2 levels









Conclusions

- S7002 grows well under high CO_2 concentrations
- Potential adaptations to CO_2 levels after inoculating Exp. 2 from Exp. 1
- Morphology of both strains is variable between replicates and CO_2 concentration
  - Filamentous characteristics were not replicated
- Possible $CaCO_3$ deposit on the surface of cells seem with SEM, but need to confirm with EDX
- Additional experiments and analyses must be conducted to confirm $CaCO_3$ presence

Challenges and Future Studies

- Calcifying conditions
  - Need high pH: stronger buffers (CHES, CAPSO, AMP, CAPS) or from careful control of sparging
  - Practical application
- Method for detecting $CaCO_3$ precipitation
  - SEM-EDX ((Scanning electron microscopy-energy-dispersive X-ray spectroscopy)
  - 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

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