Exploring cyanobacterial cell culture systems to investigate the protein complex that initiates photosynthesis.

Catherine Escobar[1], David A Flesher[2], Jieun Shin[1], Christopher J. Gisriel[1], Gary W. Brudvig[1]
[1]Department of Chemistry, Yale University
[2]Department of Molecular Biophysics and Biochemistry, Yale University

Introduction

- Most plants, algae, and cyanobacteria use photosynthesis to convert solar energy into chemical energy. **Cyanobacteria** are prokaryotic organisms and the model organism used is *Synechocystis* sp. PCC 6803. The initiation of cyanobacterial photosynthesis begins by water splitting in the **Photosystem II (PSII) protein complex** (figure 1).
- To study this process, PSII must be isolated from the cyanobacterial cells. To achieve this, large volumes of cyanobacterial cultures must be grown. It is vital to avoid contamination and to optimize cell growth procedures to maximize cell viability

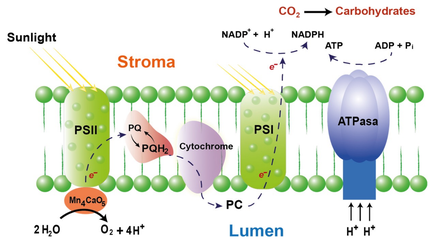


Figure 1. Figure 1. Cartoon depiction of the proteins involved in photosynthetic electron transport. PSII (left) is the protein complex initially responsible for deriving reducing equivalents from water.

- PSII is found in the thylakoid membranes. It harnesses sunlight and, the energy of which drives the catalytic mechanism of its metallocofactor, the oxygen-evolving complex (OEC).
- The OEC is a Mn_4CaO_5 cluster situated on the lumenal side of PSII. The tetramanganese cluster of the OEC is the catalytic center where water oxidation occurs in PSII.

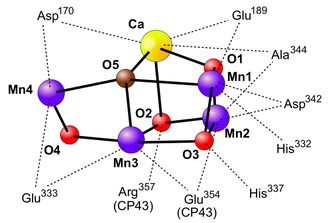


Figure 2. Structures of the OEC and its ligating amino acids.

Objectives

- This research project explores cell growth procedures with different media and instruments to develop an efficient and reliable protocol for successful cyanobacterial growth.
- Use UV/Vis spectrometry to establish a time frame for optimal harvesting of cyanobacterial cultures.
- Measure the rate oxygen evolution in PSII.

Methods

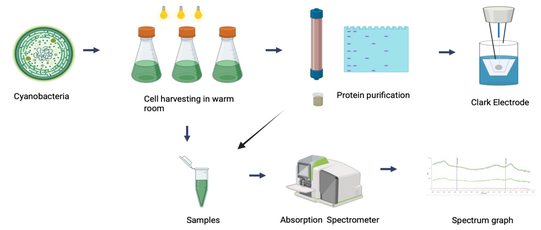
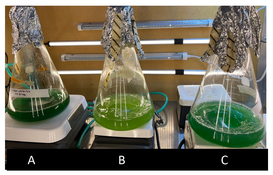


Figure 7. Experimental workflow. Cyanobacterial wild-type cultures were grown with media of collaborator's protocol under white light at 32 degrees C. An oxygen assay was obtained using a Clark electrode from purified PSII protein complexes. A UV/Vis spectrometer was used to obtain the absorbance spectra of whole cyanobacterial cells and purified PSII.

Observations

Growth Curve of Cyanobacteria Cells



Flask **A** grown in shaker. Flask **B (contaminated)** and **C** grown with stirring bar.

Figure 2. Three different cultures of cyanobacteria.

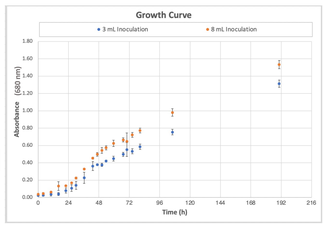


Figure 3. Growth curve for two different inoculation volumes.

Characterization and Concentration Measurement

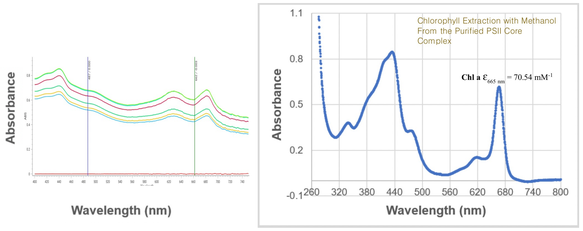


Figure 4 and 5. UV/Vis spectra of whole cells (left) and of the purified PSII (right). The spectrua are consistent with the known published results.

Oxygen Assay

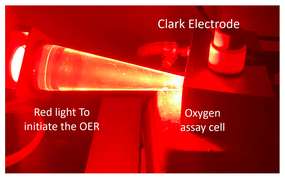


Figure 6. Clark electrode set up and red light to initiate the OER.

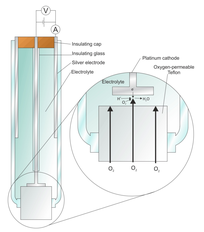


Figure 7. Principles of the Clark electrode.

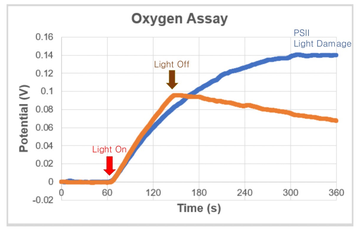


Figure 8. O_2 assay profile of the wildtype. Increase in the potential from the Clark electrode reading over time (pH 6.5, 25 ℃)

Oxygen Evolution Rate (OER)

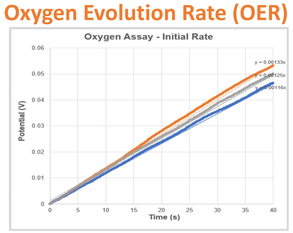


Figure 9. O2 assay profile for the initial 40 seconds after the light was turned on. The potential is observed to increase linearly

	SLOPE (V/s)	OER ($\mu mol\ O_2\ (mg\ Chl)^{-1}\ hr^{-1}$)
1	0.00133	3110
2	0.00125	2930
3	0.00116	2720
Average	0.00125	2920
SD		160

OER from WT:
$2920 \pm 160\ \mu mol\ O_2\ (mg\ Chl)^{-1}\ hr^{-1}$

Future Direction

The direction of the laboratory is to draw inspiration from a collaborative group in University of California Riverside that is successful at cyanobacterial cultures at desired volumes.

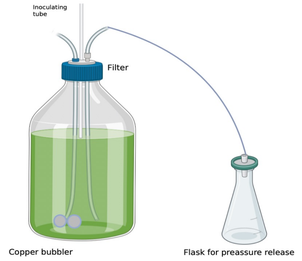


Figure 10. Carboy with cyanobacterial culture. The tentative set up uses tubbing for CO_2 delivery from $CuSO_4$ bubbling.

Conclusions

- Optimal time for cell harvesting was investigated from growth curve experiment
- Cell growth conditions were optimized and sterilization techniques for growing cyanobacterial cultures need further improvement
- The OER of the wild-type purified PSII protein complex studied with the Clark electrode

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