Optimization of Photobiological Hydrogen Production in *Synechocystis* sp. PCC 6803 Encapsulated in Silica Sol-Gel

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Subsurface Biosphere Initiative
Research and education focused on life below Earth's surface.

Introduction

As fossil fuel supplies continue to diminish and their use generates negative effects on the environment, the need for alternative fuel sources grows. Hydrogen gas is an increasingly viable energy source for the future, but will require further research before large scale production is feasible. Photobiological hydrogen production via cyanobacteria utilizes just water and sunlight, making it a particularly attractive option for the future.

The Cyanobacteria:

Cyanobacteria are phototrophic organisms capable of producing hydrogen in anaerobic conditions. This research was done with the cyanobacterium *Synechocystis* sp. PCC 6803. This organism accumulates glycogen in the light, which it breaks down during dark, anaerobic conditions, releasing hydrogen in the process. Figure 1 shows the process:

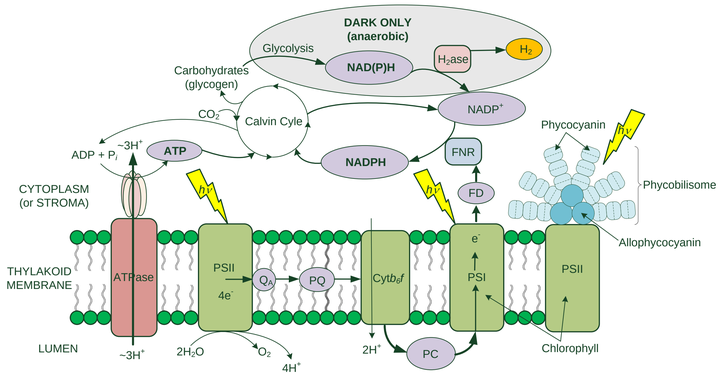

Figure 1. Oxygenic photosynthesis in the thylakoid membrane of *Synechocystis* PCC 6803. Light-harvesting pigments (chlorophyll, phycocyanin and allophycocyanin) absorb light, which is transferred to the reaction centers (PSII and PSI). Photosystem II splits water into protons and electrons and releases O_2. The protons create a potential gradient across the thylakoid membrane, which is used to produce ATP. The electrons are passed through the electron transport chain by carrier proteins to PSI, where they are used to create reduced ferredoxin and NADPH. During dark incubation, the hydrogenase enzyme creates hydrogen gas by utilizing the NAD(P)H that is synthesized from the glycogen stored during oxygenic photosynthesis.[1]

Methods

The goal of this research was to improve the amount of hydrogen *Synechocystis* sp. PCC 6803 can produce in multiple light/dark cycles. The following approaches were used:

Encapsulating Cells

The hydrogenase enzyme competes with other metabolic pathways for NAD(P)H. Encapsulating the cells in silica sol-gel reduces the competition by preventing growth and forcing cells into a steady-state metabolism. Previous studies show that encapsulated cells produce more hydrogen.[1]

Light Cycling

The hydrogenase enzyme does not function in the presence of O_2, therefore light cycling was used to allow the organism to enter anaerobic conditions while in the dark.

Mutants

Wild Type (WT) *Synechocystis sp.* PCC 6803 contains large light absorbing antennae called phycobilisomes. Antennae mutants (ΔcpcAB, ΔapcE, and ΔapcF) have modified light harvesting antennae that absorb less light and allow the cells to grow in brighter light conditions, increasing their efficiency compared to WT cells, which saturate at low light intensity.

BG-11 media: Nutrient rich media that gives cells what they need to thrive
EHB-1 media: Nitrogen and sulfur limited media that forces cells to store glycogen[2]

Optical density and fluorescence measurements were taken to assess cell growth and photodamage. Gas chromatography was used to assess the amount of hydrogen produced by the encapsulated cells.

Questions Addressed

-In which gel compositions do *Synechocystis* sp. PCC 6803 produce more hydrogen?
-Do the cells accumulate glycogen when encapsulated?
-When in liquid EHB-1, the cells break down their pigments. Does this happen when the cells are encapsulated? How long does it take the cells to recover?
-How much time do the cells need to spend in dark, anaerobic conditions to produce hydrogen?

Results

Gel Optimization

Bicarbonate (HCO_3^-) Concentration: Gels made with media that contained lower concentrations of HCO_3^- were more stable. The HCO_3^- concentration also had an effect on H_2 production (Fig. 2b). EHB-1 media is typically made with 46mM HCO_3^-.

Betaine Additive: As an osmoprotectant, betaine had no effect on gel stability, but did increase cell life and H_2 production (Fig. 2a).

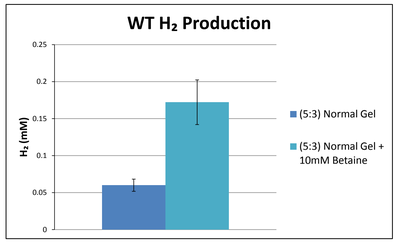

Figure 2a. Effect of betaine on H_2 production

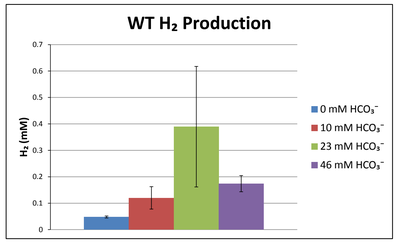

Figure 2b. Effect of HCO_3^- concentration in EHB-1 on H_2 production

Glycogen Accumulation

In liquid cultures, all four examined strains of *Synechocystis* store more glycogen when in EHB-1, deprived of nitrogen and sulfur. However, when encapsulated, EHB-1 media did not significantly affect glycogen storage (data not shown).

Maintenance and Repair

Cells in liquid culture break down phycocyanin and allophycocyanin when they are in the nutrient-deficient EHB-1, but switching them back to BG-11 media provides nutrients needed to repair and/or resynthesize these pigments (data not shown). Encapsulated cells react differently when moved from BG-11 to EHB-1 (Fig. 3).

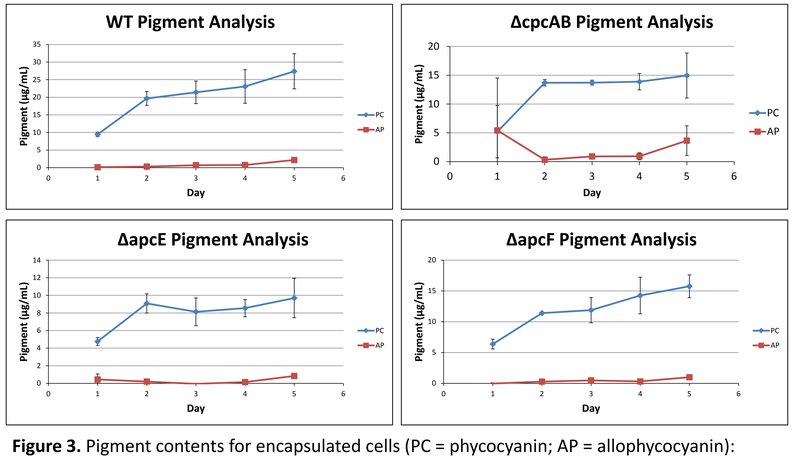

Figure 3. Pigment contents for encapsulated cells (PC = phycocyanin; AP = allophycocyanin):
Day 1: After 3 days encapsulation in BG-11, (12 hrs ON/12 hrs OFF light cycling)
Day 2: After 24 hours in EHB-1
Day 3: After 24 hour Hydrogen production cycle
Day 4: After 1 day of repair in BG-11 (12 hrs ON/12 hrs OFF light cycling)
Day 5: After 2 days repair, in BG-11 (12 hrs ON/12 hrs OFF light cycling)

Acknowledgements

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2. Burrows E, Chaplin F, Ely R. Optimization of media nutrient composition for increased photofermentative hydrogen production by Synechocystis sp. PCC 6803. International Journal of Hydrogen Energy 2008; 33: 6092–6099.
3. Campbell D, Hurry V, Clarke A, Gustaffson P, Oquist G. Chlorophyll Fluorescence Analysis of Cyanobacterial Photosynthesis and Acclimation. Journal of Microbiology and Molecular Biology 1998; 62: 667–683.

ϕPSII indicates how efficiently cells utilize absorbed light. The value decreases when the cells are moved from BG-11 to EHB-1, particularly in the ΔapcE.

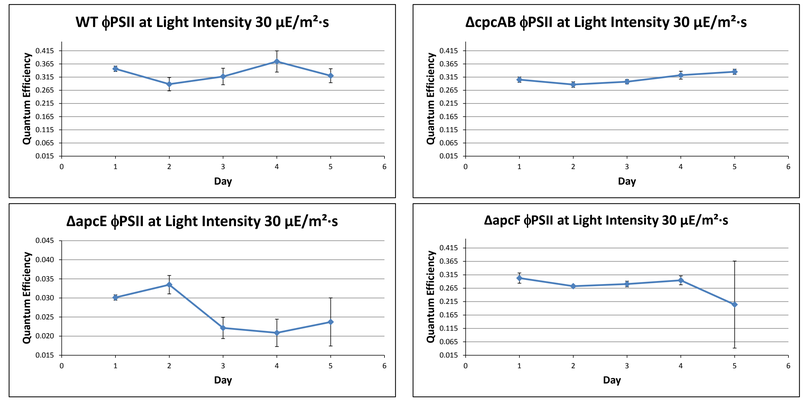

Figure 4. Quantum efficiencies of PS II for strains under the same media and light cycling conditions used for pigment analyses shown in Figure 3.

Hydrogen Production

Putting the cells in the dark in sealed containers forces them to function anaerobically, which allows the hydrogenase enzyme to produce hydrogen.

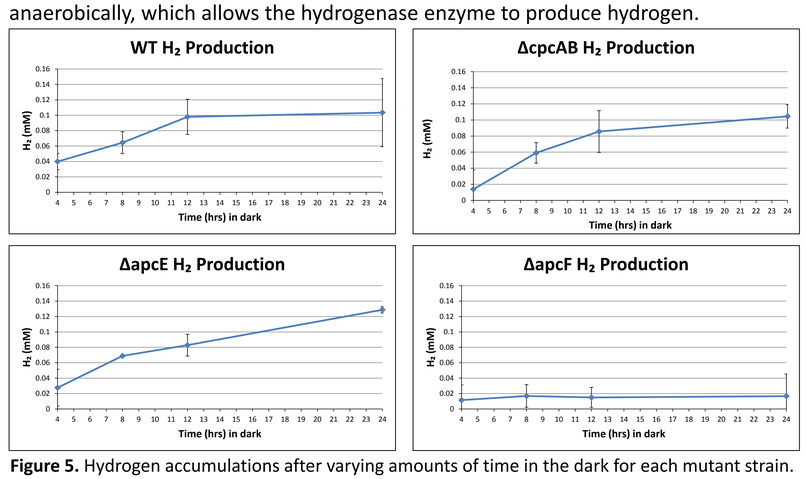

Figure 5. Hydrogen accumulations after varying amounts of time in the dark for each mutant strain.

Conclusions and Future Work

Optimization of hydrogen production in *Synechocystis* sp. PCC 6803 depends on a broad range of variables.

The composition of the gel greatly affects both hydrogen production and the long term stability of the gel. Because the high HCO_3^- concentration in EHB-1 media causes an increase in pH and thus a less stable gel, it is favorable that the cells produce more hydrogen in EHB-1 with a lower concentration of bicarbonate (Fig. 2b).

The data suggest that cells can maintain or increase their pigments during encapsulation (Fig. 3). However, the apparent rise in pigment content could result from greater or easier release of the pigments caused by decreased gel stability.

The activity of PS II decreases when the cells dark acclimate to make hydrogen and increases with repair. Just 1 day of repair was sufficient for most of the strains to recover pre-encapsulation PSII activity (Fig. 4). The ΔapcE strain seems to be more affected by the dark acclimation stage than the other strains, which may be due to the fact that ΔapcE is the most light-limited and, as a result, the slowest growing strain.

Generally, most hydrogen accumulation occurred in the first 12 hours of dark incubation (Fig. 5). Thus, keeping the cells in the dark for more than approximately 12 hours does not greatly increase the hydrogen yield.

Additional work is required before the process is truly optimized, including,
-Optimizing EHB-1 media for encapsulated cells rather than for liquid cultures.
-Improving glycogen accumulation in encapsulated cells
-Pigment and fluorescence analysis in encapsulated cells over longer durations