Optimal Chemical Environment for Algal Biohydrogen Production

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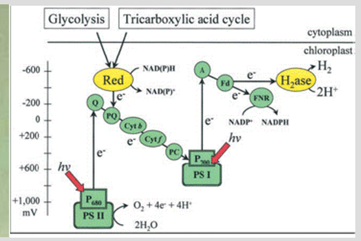
INTRODUCTION

Under certain conditions, green algae (such as Chlamydomonas reinhardtii) can produce hydrogen in place of oxygen gas under the simple reduction reaction $2H^+ + 2e^- \rightarrow H_2$, which is catalyzed by the [FeFe] hydrogenase complex. The production of hydrogenase by algae is transient, however, as oxygen is a strong inhibitor of hydrogenase. It was noted in the late 1990s that when deprived of a sulfur compound in its growth medium, algae switched from oxygen production to hydrogen production. Sulfur is limited to a small amount, allowing the algae to consume the remaining sulfur in order to grow to a high density. Upon complete consumption of the sulfur, the cells are unable to photosynthesize, shutting down oxygen production. However, the algae is still able to respire, which consumes the remaining oxygen in the chamber and creates anaerobic conditions. The lack of oxygen activates the enzyme hydrogenase, normally inhibited by oxygen, which catalyzes the reduction of protons into molecular hydrogen. This method is of interest to the bioenergy industry due to its potential to mass produce hydrogen for use as fuel.



MATERIALS & METHODS

Four liquid cultures of CC-125 Chlamydomonas Reinhardtii algae were obtained from the Chlamydomonas Resource Center at the University of Minnesota, St. Paul, along with the necessary reagents needed. In addition, 2 cultures of Chlamydomonas Reinhardtii on agar were provided. The reagents included a complete salts solution (ingredients below), from which the sulfur deprived medium is used, an acetate solution ($CH_3COONa \cdot 3H_2O$ -- 100.0g per 1L), a phosphate solution (HPO_4^{2-} and $H_2PO_4^-$ -- 288.0g and 144.0g per 1L, respectively), and a solution of Hutner's trace elements.

The concentration of the acetate solution were varied in order to test algae growth and hydrogen production under sulfur deprivation and acetate limitation.

Perform following tasks to prepare algae for growth

1. Control Algae
   a. Remove 16 mL water from 1L bottle of spring water
   b. Add in 5 mL each of complete salts solution, phosphate solution, and acetate solution
   c. Add in 1 mL of Hutner's trace elements
   d. Add 10 mL liquid culture of algae
2. Sulfur Deprived Algae
   a. Remove 16 mL water from 1L bottle of spring water
   b. Add in 5 mL each of sulfur-free salts solution, phosphate solution, and acetate solution
   c. Add in 1 mL of Hutner's trace elements (sulfur-free)
   d. Add 10 mL liquid culture of algae
3. Sulfur Deprived, Half-Acetate Algae
   a. Remove 16 mL water from 1L bottle of spring water
   b. Add in 5 mL each of sulfur-free salts solution and phosphate solution
   c. Add in 2.5 mL of acetate and 2.5 mL distilled water (dilute to half molarity)
   d. Add in 1 mL of Hutner's trace elements (sulfur-free)
   e. Add 10 mL liquid culture of algae
4. Sulfur Deprived, Acetate Limited
   a. Remove 16 mL water from 1L bottle of spring water
   b. Add in 5 mL each of sulfur-free salts solution and phosphate solution
   c. Add in 1 mL of acetate and 4 mL distilled water (dilute to a fifth of molarity)
   d. Add in 1 mL of Hutner's trace elements (sulfur-free)
   e. Add 10 mL liquid culture of algae

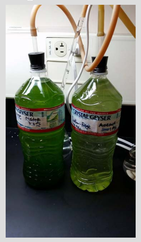
Connected each culture above to a 5 mL pipette filled with water using latex tubing. Left the pipette in an erlenmeyer flask filled with water (gas collection method).

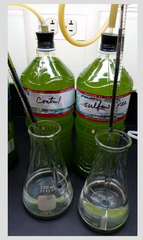
Allowed algae to grow under fluorescent plant light. Checked periodically for growth and gas production. Qualitative observations of algal growth, as well as quantitative measurements of gas collection and pH of solution, were measured.

RESULTS

Growth Observations

- The control bottle (with the normal, sulfur-containing growth medium) showed a deep green
- Small bubbles were observed in some of the sulfur-deficient cultures
- Sulfur-deprived algae were a lighter green than the control
- Cultures with limited acetate show fewer bubbles, overall lighter color
- Control 1: dark green
- Sulfur deficient, full acetate 1: pale, translucent green
- Sulfur deficient, half acetate: pronounced, dark green
- Sulfur deficient, ⅛ acetate: transparent green





pH Data

Culture	*Control 1*	*Control 2*	*Sulfur-free, full acetate 1*	*Sulfur-free, full acetate 2*	*Sulfur-free, half acetate*	*Sulfur-free, fifth acetate*
pH	*8.0*	*7.8*	*8.0*	*8.0*	*7*	*6.5*

Gas Evolution

Acetate, Sulfur deprived 2.5 mL	
Day	H_2 Level (mL)
0	1.42
6	2.62

Acetate, Sulfur deprived 1 mL	
Day	H_2 Level (mL)
0	2.93
6	2.92

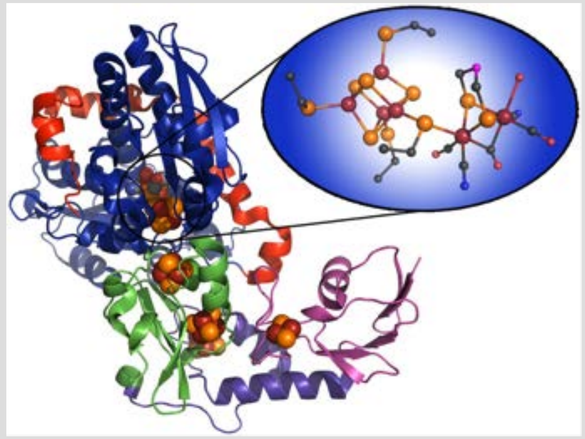
Control 1	
Day	O_2 Level (mL)
0	2.6
1	2.8
2	2.95
13	1.4
14	1.35
15	1.39
22	1.41

Control 2	
Day	O_2 Level (mL)
0	5.3
1	4.6
12	1.6
14	1.32
21	1.32

Full Acetate, Sulfur Deprived	
Day	H_2 Level (mL)
0	
1	4.5
12	4.1
14	2.23
21	2.15

Full Acetate, Sulfur deprived	
Day	H_2 Level (mL)
0	3.72
1	3.85
2	4
13	4
14	4
15	4.04
22	4.1

Hydrogenase Enzyme



SUMMARY / CONCLUSIONS

By manipulating the acetate concentration of the growth medium in addition to depriving the algae of sulfur in the growth medium, the growth rate of algae was observed and the amount of gas released was measured.

In the cultures in which the acetate was limited to 1mL per liter of solution, the culture was a lighter green, indicating less growth of the algae. Compared to the culture with a fifth of the acetate, the culture with half the acetate showed exceptional growth--the culture showed a dark, pronounced green. The data show that acetate limitation restricts the growth of the algae. The pH data show that the addition of acetate raises the pH. Since reducing the acetate concentration restricted algal growth, the optimal pH for algal growth is demonstrated by this experiment to be approximately 8.

However, the data did not show a significant or valid difference in gas production elsewhere in the experiment. All cultures yielded erroneous measurements, which fluctuated and occasionally decreased. This is likely due to the presence of different phases in the gas production/consumption of algae. There is likely the presence of an oxygen-consumption phase, a hydrogen-producing phase, and another consumption or termination phase. The gas measurement varied over the days, and a six-day window did not show the entire gas level. Thus, the measurements of the gas produced were invalid.

The data revealed that the algae grow best in pH 8, and the limitation of acetate stunts the growth and proliferation of algae. The gas collection yielded erroneous results that may be due to the presence of different gas production/consumption phases over time.

ACKNOWLEDGEMENTS / REFERENCES

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