*AlgEYE: Automation of Algal Hydrogen Production*

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## Background

A strain of green algae (Chlamydomonas reinhardtii) is known to produce hydrogen gas in an anaerobic state using an enzyme called hydrogenase. However, the oxygen sensitivity of the hydrogenase makes it difficult to keep hydrogen production ongoing. A way to circumvent the sensitivity to oxygen is to deprive the algae of sulfur[1]; however nutrient starvation has long term effects on the efficiency of photosynthesis[2]. Therefore, by creating a system that would automatically regulate the level of sulfur, hydrogen production will not be sacrificed to algal nutrient starvation.

Under normal conditions, Chlamydomonas fixate carbon dioxide from the atmosphere to produce starches and oxygen gas is the by product. However, when there is not enough oxygen in the environment, the algae cannot respire or continue with normal life processes (such as production of starches through photosynthesis), so the algae end up living off the starches they store. However, if light is still present, then water is still being split into oxygen gas and hydrogen ions in Photosystem II (PSII). The oxygen is used by the algae in its attempt to respire as much as it can and the hydrogen ions accumulate in the inner thylakoid. If there is no oxygen present, then an enzyme called hydrogenase takes effect in Photosystem I (PSI) and creates hydrogen gas from the hydrogen ions, instead of letting PSI reduce NADP+ into NADPH. It is believed that hydrogen production by microorganisms is in fact a safety valve to avoid over reduction of photosynthetic electron carriers[3] and therefore slow the rate with which starches are being consumed.

Melis et al found that sulfur deprivation induces "two important phenomena in the algae: accelerated starch accumulation and a gradual PSII degradation"[4]. Sulfur is an essential nutrient

that algae need to reproduce, so limiting it in return limits the number of cells, therefore letting starches accumulate. The gradual PSII degradation happens because NADPH production is gradually being switched to hydrogen gas production as the oxygen levels in the environment lower due to respiration. Ultimately, when the rate with which oxygen is being produced is less than the rate at which oxygen is being consumed, then anaerobic states are achieved and hydrogen gas is produced.

Given the various problems associated with bioreactors, researchers are currently trying to isolate the hydrogenase enzyme to avoid bioreactors altogether, however this is proving to be a challenging task. Therefore, an alternate approach would be to improve current bioreactors, and one way to do that would be through an automatic control system. An automatic control system creates a balance between the number of healthy algae and hydrogen production.

**Purpose**

The purpose of this project would be to maximize cost-efficiency of hydrogen production in algae through the use of an automated system that controls environmental factors. Hydrogen can be used to power hydrogen fuel cells, but the cost of hydrogen is too high to be economical. By creating an automatic control system that can effectively control the environment to better suite hydrogen production in algae, the price of hydrogen could be lowered, bringing wide spread hydrogen fuel cells closer to reality.

**Design Criteria**

If the automatic control system is to be successful, more hydrogen gas has to be produced over a prolonged period of time through the automated system than in a manually controlled bioreactor. Alternatively, if the system is able to increase the number of algae in the bioreactor

(i.e. make it greener), then the system is effectively managing the environment to ensure optimal algae growth and by extension, hydrogen production.

**Procedure**

Algal hydrogen production can be broken down into 3 stages:

1. Inoculation of Chlamydomonas reinhardtii
2. Growth and reproduction of Chlamydomonas, until it reaches the critical density needed to produce hydrogen
3. Automation of hydrogen production in Chlamydomonas reinhardtii

For each experiment, two reactors are created. Each one consists of a water bottle, media consisting of water with various salts and ions and a small sample of Chlamydomonas reinhardtii, strain CC-125. The information regarding the ratios for the various salts in the media was determined by other scientists. For this set up, one bioreactor has complete media and one bioreactor has sulfur deprived media. Each bioreactor is hermetically sealed to ensure no oxygen can get in so that an anaerobic state may be achieved.

For the Chlamydomonas to grow and reproduce, they need the right environmental conditions. A growth chamber was made from a box lined with white paper and several cool 23W fluorescent light bulbs dangling on the inside. Each bulb is placed 10 cm away from each bioreactor to ensure adequate illumination. The automatic control system uses a webcam to measure the average colour of the bioreactor to determine its health; the algae's health is directly proportional to the amount of chlorophyll, or the amount of green in the bioreactor. The system takes periodic measurements of the algae health as it reproduces, to give quantitative results as to the progress of the algae growth. The system may also send out an alarm for human intervention if the algae exit their expected growth cycle.

After the algae have grown to a certain density indicated by reaching a certain shade of green, the system will begin to take measurements that will control sulfur deprivation. The system periodically measures the colour of the algae in the bioreactor. If the algae bleach past a certain threshold in colour, the system sends a signal to the dosator to add sulfur solution to the bioreactor. As the sulfur is added, the algae stop producing hydrogen and return to oxygenic photosynthesis which allows them to regenerate in numbers and colour. The system waits for the algae to regenerate in colour, after which the cycle repeats The control system connects the webcam, laptop and the microcontroller that is hooked up to a servo motor that provides output to the dosator. For the control system, the input variable is the colour of the algae, and the output variable is ultimately the colour of the algae, as the addition of sulfur to the bioreactor lets the algae regain density be resuming normal photosynthesis.

**Results and Observations**

Since the algae were held up at customs for two months, the timeline for the project was delayed significantly. As a result, only one full month of testing was achieved however, even this is not enough considering the fact that algae takes time to reproduce to the level needed in a bioreactor and by extension, mistakes take even longer to fix.

Before the system could be tested, preliminary experimentation had to be conducted to ensure that the algae do indeed produce hydrogen in an anaerobic environment. The sample experiment that the algae came with was conducted however hydrogen was not produced. The experiment was then modified to limit possible sources of error in various ways including bioreactor set up, light intensity and heat in the environment. It was observed that algae grew faster at a temperature of $27^{o}$C, compared to the $20^{o}$C-$25^{o}$C suggested by the paper that the algae came with. Eventually, the automatic control system was modified to monitor algae growth, in

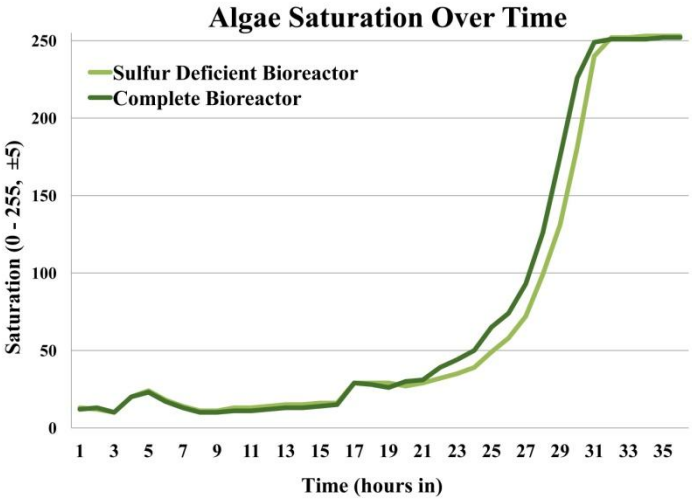


**Figure 1: Algae Saturation Over Time**

addition to controlling sulfur depletion. For a 710 mL bioreactor at 27$^{o}$C, the recorded growth model for algae is shown in Figure 1.

The uncertainty for the saturation comes from an uncertainty estimate as it was observed that slight aberrations in the pixels under bright light would cause slight fluctuation in the saturation values. As a result, pixel values were averaged throughout the various stages of measurement. It was also observed that the algae saturation follows an "S" curve as the algae reproduce exponentially for a period of time and then level off as they deplete the nutrients in the media.

**Conclusions**

It can be concluded that Chlamydomonas reinhardtii growth speeds are affected by temperature and light intensity fluctuations. Additionally, the automatic control system created was successful in converting qualitative measurements of colour into quantitative measurements. This means that algae can be automatically monitored and controlled, making processes that take a long time easier to maintain.

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