

Biohydrogen

By: Ghufran Siddiqui

Abstract

This project explores the hydrogen producing abilities of *Chlamydomonas reinhardtii* using copper addition and sulphur depletion. The different concentrations of copper the algae were put into were 0.4ppm, 0.8ppm, 1ppm, 1.6ppm and 2ppm. The second part is to deprive them of sulphur then compare the two methods. It was found that the most hydrogen is produced from the sulphur deficient method.

Background Information

As the world looks for alternative and environmentally friendly fuels it has gone in many directions, one of them being algae for bio-fuels. Algae have mostly been looked at as a way to produce bio-diesel, however in a better direction is using the algae for the production of hydrogen. Generating hydrogen via photosynthetic organisms is environmentally friendly and economical. Most other methods of producing hydrogen cause a lot of pollution and use a lot of energy; the use of algae is a feasible solution.

Sulphur Depletion

There are two methods that are known to be able to make *Chlamydomonas reinhardtii* switch into producing hydrogen. One is depriving them of sulphur and the other is by adding copper.

Depriving the algae of sulphur leads to photosynthesis shutting down in the cells. Sulphur is a valuable nutrient and is used in producing amino acids required to make enzymes that allow photosynthesis to occur. The photosynthetic enzymes which can no longer be produced properly due to the disruption of certain amino acids result in photosynthesis no being carried out. Once the plants stop to photosynthesize they use up the oxygen within the closed environment through

cellular respiration. Once the environment is anaerobic, hydrogenase functions to produce hydrogen gas.

Addition of Copper

The addition of copper works on the same basis as depriving of sulphur. By adding copper to the algae's environment their ability to produce oxygen is stopped. Copper hinders the ability of the algae to do photosynthesis properly by preventing photosystem II synthesis (which generates oxygen) to function. This happens because the copper ions disrupt the function of a gene (the Nac2 gene) required for photosystem II synthesis. Then the same effect occurs as in the sulphur-deficient media with the hydrogenase enzyme producing hydrogen.

Chlamydomonas reinhardtii

Chlamydomonas Reinhardtii algae are unicellular flagellates (with two flagellums) and are part of the Chlorophyta genus. These algae are normally found in soil and freshwater. They have a large chloroplast, a cell wall, two flagella and a large pyrenoid ("eyespot") which senses light.

Purpose

This project suggests the use of an alternative cleaner fuel. It suggests, more-so, the production of a well known fuel – hydrogen. The purpose of this experiment is to identify which method of producing hydrogen using Chlamydomonas Reinhardtii is best; depriving the algae of sulphur or by using the addition of copper.

Hypothesis

If the copper concentrations of the algae environment are tested at 100% of the regular copper concentration (0.4 ppm, control), 200% (0.8 ppm), 250% (1.0 ppm), 400% (1.6 ppm) and 500% (2.0 ppm) then it will be possible to determine which concentration of copper is the best to

produce the largest amount of hydrogen gas. The concentration of 1.0 ppm is expected to produce the most gas because this is the lowest level of concentration that is expected to cause damage to the culture because copper is an algacide. In a system that is not regulated for copper during the experiment such as the one that will be made, it is expected that the sulphur deficient method will work the best because the algae cannot repair themselves where they can in the copper method.

Procedure & Experiment

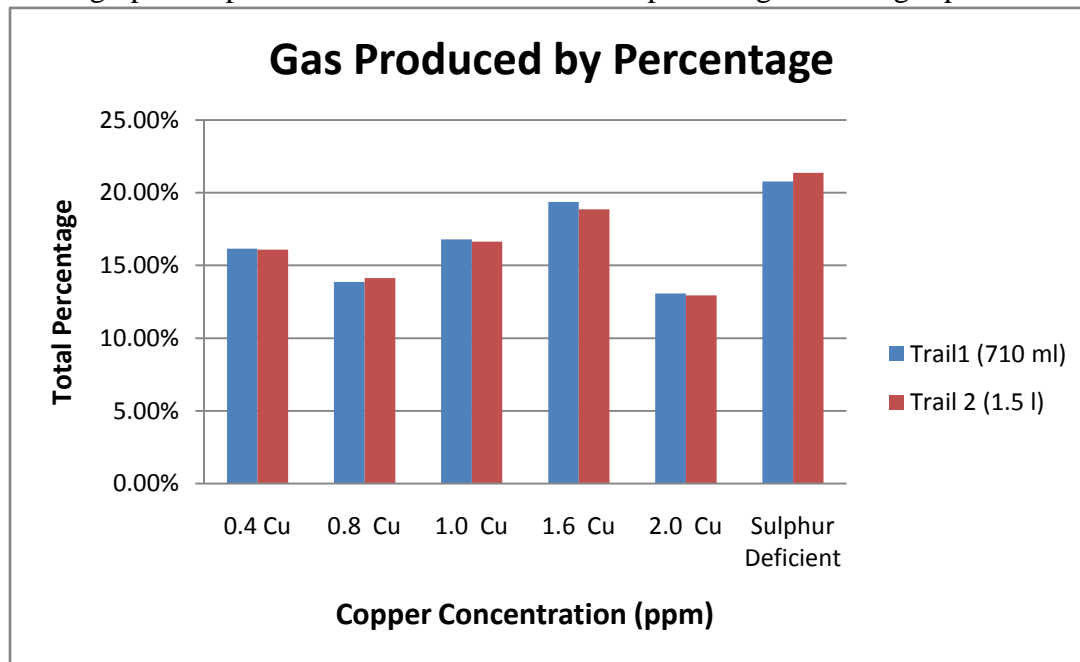
The experiment was set up with six water bottles. One control (0.4 ppm Cu), one with 0.8 ppm Cu, 1.0 ppm Cu, 1.6 ppm Cu, 2.0 ppm Cu and a sulphur deficient media water bottle. The water solutions made for the algae consisted of (ordered from Chlamydomonas Center) a complete salts solution, phosphate solution, acetate solution and trace elements solution. For the sulphur deficient media sulphur-free salts solution and sulphur free– trace elements solution was used.

The copper concentrations were changed using Copper (II) Sulphate Pentahydrate. This required making a solution where 0.786 g of Copper (II) Sulphate Pentahydrate was added to a litre of water. Each millilitre of this solution increased the copper concentration by 0.2 ppm in 1 litre of water. There were two trails of this experiment done. One with 710 ml bottles and one with 1.5 litre water bottles. The experiment lasted 10 days where the algae were grown under anaerobic conditions (and the appropriate copper or sulphur deficient environment) with 12 hour light/dark cycle. During this time the gas was captured using a balloon. After the ten days the balloons of each bottle were tested for how much gas was produced using the water displacement method. Each gas was also tested to see if the gas was hydrogen using the burning splint test.

Results & Observations

Copper Concentration (ppm)	Amount of gas produced (ml)		Type of gas	
	Trail 1 (710 ml)	Trail 2 (1.5 L)	Trail 1	Trail 2
Control (regular sulphur & copper) – 0.4 Cu	47	87	No hydrogen identified	No hydrogen identified
0.8 Cu	40.3	76.5	Hydrogen	Hydrogen
1.0 Cu	48.8	90	Hydrogen	Hydrogen
1.6 Cu	56.3	102	Hydrogen	Hydrogen
2.0 Cu	38	70	Hydrogen	Hydrogen
Sulphur Deficient	60.4	115.6	Hydrogen	Hydrogen
Total gas:	290.8	541.1	X	X

*This graph compares the two different sizes in a percentage of total gas produced.



Results and Discussion

In the graph and table, it can be seen how every media generated hydrogen –even if it was little- except the control bottle which did not indicate hydrogen through the burning splint test.

Knowing that algae can only produce hydrogen in an anaerobic environment it is possible to say that the environment within the control bottle never turned into anaerobic because there was no

limiting condition on the algae to prevent them from photosynthesizing and producing oxygen. Out of the different copper concentrations it can be seen that each increasing amount of copper produced more hydrogen than the last amount. The greatest amount of hydrogen generated was from the 1.6 ppm of copper, which produced 102 ml of hydrogen. However the next amount of 2.0 ppm copper produced less and a possible reason for this would be it damaged the algae more. The sulphur deficient method still produced more than the 1.6 ppm of Cu. The sulphur deficient method produced 115.6 ml of hydrogen which is only 13.6 ml (2.51%) more than the most effective copper method. The downside which is clearly observed from looking at the bottle is the yellow tinge which indicates the algae are dieing compared to the greener color of the copper and control (most healthy).

Application

Today, hydrogen generation is mostly being developed as a way to provide hydrogen for hydrogen fuel cells and this is the most effective use. The hydrogen generated from the algae would be used like any other hydrogen. It could be compressed and used in a hydrogen fuel cell to produce electricity.

Conclusions

Although the hypothesized amount of 1.0 ppm Cu was not the most effective it was found that 1.6 ppm Cu was the most effective in producing hydrogen. Overall, the sulphur-deficient method of producing hydrogen was more effective than the copper. The sulphur deficient algae produced 115.6 ml of hydrogen, 2.51% more than the copper. However, the algae in the sulphur-deficient media die much faster than the copper algae and for this reason copper could be better used. Knowing which method is best is important because in order for such renewable energies to become possible the most efficient methods must be utilized.

