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The Effect of Urea Concentration in Water on the Growth of *Chlorella* Algae

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Chlorella is a type of algae that is found in nature and has many uses. The specific organic compound being tested to influence *Chlorella* growth is urea. This compound is being tested to see what makes it grow more or less, and it is important because it can be used in biofuel production and causes hazards in bodies of water during eutrophication. In this experiment, we set up two sets of four bioreactors with four different concentrations of urea and measured the growth of the algae in each. We found that there is a generally positive relationship between urea added and cell growth, but there is a limit before the urea starts to decrease the growth. This means urea can be used to either boost growth for biofuel production or to reduce it to prevent eutrophication.

Introduction

Across the planet, eutrophication has become a growing problem in water sources. This process is the overgrowth of algae, causing other plants in the body of water to not be able to obtain the light that they need (French 2017). When these plants die, it can cause complications for all the other plants and animals in that body of water. These complications can include lack of oxygen in the water as well as a reduced pH level (Smith et al. 1999, Smith 2003). Researchers are looking for a way to reduce algae growth in bodies of water to prevent excessive eutrophication. One area of focus when it comes to preventing algae growth is reducing nitrogen sources in the water. Nitrogen sources have been shown to increase the amount of lipid production and cell growth in algae (Li et al. 2008). One such nitrogen source that is commonly found in bodies of water is urea. In our experiment, we will be testing the effect of urea concentration on the growth of algae, specifically *Chlorella*. Algae and its products can be converted into certain biofuels that can be used to power vehicles of all kinds (Demirbas 2011). Algae can produce fuels such as biodiesel, bioethanol, and multiple others (Demirbas 2011). Increasing the amount of algae growth can produce more algae that can be converted into biofuels. We hypothesize that the more urea there is in the growth media, the faster the rate of growth of algae, because urea is a nitrogen source and algae needs nitrogen to grow.

Methods

*=Mentor

In this experiment, we were looking for how urea concentration affected the growth of *Chlorella* in water. We followed the procedure for making bioreactors that's in the lab journal *Investigating Biology* French (2017). A modification we made to the experiment was choosing the independent variable urea to add to our water. We used one water bottle as our control group by adding no urea to it at all, and then we added 2mL, 4mL, and 6mL of 1M urea solution to the other three respectively. We ran two separate trials of the same experiment. For the first trial we set up four bioreactors and let them run for one week; for the second trail and we set up a new set of four bioreactors and ran the same experiment for one more week. To measure the initial amount of algae, we used a micropipette to take a 10µL sample of each bottle and placed them on a hemocytometer. We examined these samples under a microscope to evaluate the cell density of the algae. After one week had gone by, we took another 10µL sample of each bottle. To examine the growth of the algae, we observed the cell density on a hemocytometer yet again and calculated the change in density. This was calculated by multiplying the average cells per square by the dilution factor and then dividing the product by the volume of a square in mL.

Results

The data showed a significantly high amount of growth in the bioreactors with 2mL and 4mL of urea added as seen in Figure 1. The bioreactors with 6mL of urea added had a lower growth than the control group. All the bioreactors started at different cell densities, so there is an amount of fluctuation between trials.

Table 1: This table shows the amount of cell growth in each bioreactor for the two trials that were conducted. The cell growth for each week was found by subtracting the initial cell density from the final cell density. The average of both weeks is found in the rightmost column.

Amount of Urea Added (mL)	**Week 1 Cell Growth**	**Week 2 Cell Growth**	**Average Cell Growth**
0 (Control)	1.25×10^5	2.5×10^5	1.88×10^5
2	5.63×10^5	6.25×10^5	5.94×10^5
4	1.06×10^6	5.63×10^5	8.12×10^5
6	1.88×10^5	1.25×10^5	1.57×10^5

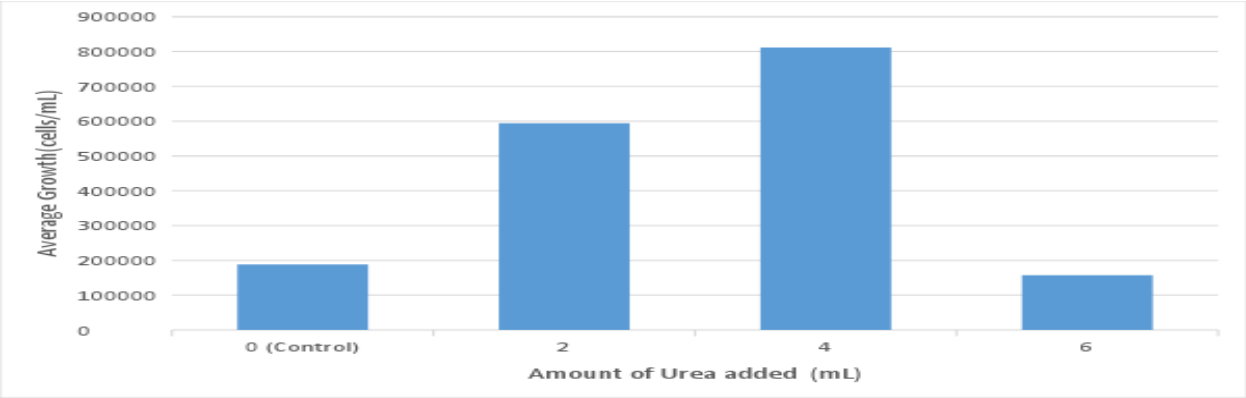

Figure 1: This graph shows the average change in cell density for the different amounts of urea that we added to the bioreactors. Reference Table 1 for the individual growth in each bioreactor.

Discussion

Our hypothesis was rejected by our results. Although there was a positive relationship between the amount of urea added and the average growth for the first 4mL, once the amount of urea hit 6mL, the relationship stopped. Urea is a nitrogen source, and algae needs nitrogen to grow. However, the large amounts of urea that we added into our fourth bioreactor could have oversaturated the water with nitrogen. This could have caused the algae to have too much nitrogen and lead to a decrease in cell growth. A revised hypothesis would be that the amount of urea added will lead to more algae growth until the urea concentration becomes too high and inhibits the algae's growth. A similar experiment was conducted to observe the relationship between algae growth and amounts of urea (Carder et al. 2017). In that experiment, they found that the relationship between urea added and algae growth was constantly positive (Carder et al. 2017). However, that experiment only tested the range of 40 µL to 120 µL, which is a significantly smaller range than what this experiment used (Carder et al. 2017). Their urea concentration was never high enough to have the same effects that our highest concentration had, but they did show increases in growth at the lower concentrations like our experiment did (Carder et al. 2017). Through this experiment, it can be seen that algae growth can be increased by certain amounts of urea, but there is a limit. Too much of a nitrogen source, such as urea, can limit the growth of algae in nature. This can be used to combat eutrophication and in the creation of biofuels.

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