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Article

Effects of various alkaline carbonic solutions on the growth of the freshwater algae Chlorophyceae

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SUMMARY

Modern day fossil fuels are prone to polluting our environment, which can provide major habitat loss to many animals in our ecosystems. Algae-based biofuels have become an increasingly popular alternative to fossil fuels because of their sustainability, effectiveness, and environmentally-friendly nature. To encourage algae growth and solidify its role as an emerging biofuel, we tested basic (in terms of pH) solutions on pond water to determine which solution is most efficient in inducing the growth of algae. We hypothesized that a calcium carbonate solution would induce the most algae bloom, because calcium is a catalyst in photosynthesis. Alkaline carbonate solutions were used to create an optimal environment for the growth of algae; three carbonic alkaline solutions were tested in samples of pond water with algae measured through visible light absorbance using a spectrophotometer. As hypothesized, calcium carbonate induced the most algae growth compared to the control sample, potassium carbonate, and sodium bicarbonate. In conclusion, calcium-containing solutions stimulated algae growth the most, likely due to this solution having properties to stimulate the photosynthetic process by enhancing the transcription and translation process for genes related to chloroplast proteins and enzymes. Our results suggest that calcium is important in enhancing algae growth, which may be important as alternatives for fossil fuels are developed.

INTRODUCTION

Algae has recently been seen as an ecological alternative to fossil fuels because of its renewable and sustainable properties (1). The growth of algae requires the consumption of excess CO2 in the atmosphere, which could in turn lower greenhouse emissions, which would better the environment. Algae can grow and thrive in all types and bodies of water (i.e., saltwater, freshwater, rivers, stream, lakes, and ponds) (2). In addition, Although its ideal growing conditions are in the summer with high humidity and temperatures ranging from 18ºC to 29ºC, many species of green marine algae can grow in almost all types of climate and weather. Algae can replicate 30 to 100 times faster than other biofuel sources such as corn when experiencing these conditions (1). Unlike other biofuels sources such as corn or soybean, algae is not a primary food source for humans or livestock (3). For these reasons, algae-based fuels are considered by many scientists to be the only renewable energy resource that can meet the global demand for fuel in the long term (3).

Algae is an aquatic plant that is often considered to be an indication of a struggling ecosystem (4). Excessive algae growth can starve or suppress other forms of aquatic life and block the sunlight necessary for their growth. Algae has been shown to remove oxygen from freshwater ecosystems, causing the organisms living in these waters to perish (5). Despite these shortcomings, the benefits of algae, especially as an emerging pillar in the biofuel industry, are often overlooked.

Aquatic unicellular green algae, or Chlorophyceae, a species of marine algae, have found their way into the biofuel industry due to their ability to reproduce quickly under ideal conditions (1). Algae is often implemented as a biofuel by fermenting its carbohydrates to make alcohols, including ethanol and butanol, as well as other products such as plastics and biochemicals. The current methods that have produced successful algal blooms, for the use of biofuels, are the implementation of nitrogen and phosphorus in the pond ecosystems, but often these chemicals are overused and have detrimental effects on the environment and aquatic organisms (4). One idea that was proposed to implement an ecological method to induce algal blooms using alkaline carbonate solutions (2). These solutions could affect the growth of algae because when their carbonate anion interacts with heated water, it decomposes into a metal oxide and carbon dioxide (6). The increased levels of carbon dioxide in pond ecosystems could then allow for algal blooms. In addition, the increased carbon dioxide in the system causes the pH to increase in aquatic environments, and the algal growth can significantly rise when the pH climbs to a range of 8.2 to 8.7(2).

The main goal of our study is to develop an alternative, eco-friendly solution to grow algae for the use of growing demand of biofuels, We analyzed which metal oxide from three different carbonate solutions stimulated the most algal growth; more specifically, we measured chlorophyll as a measure of algal growth. Of the three alkaline carbonate solutions: calcium carbonate, potassium carbonate, and sodium bicarbonate, we hypothesized that calcium carbonate would induce the most algal growth due to its ability to catalyze the photosynthetic process in plants. Our results did show that calcium carbonate induced the most algae, which demonstrates that the use of calcium carbonate solutions could provide an alternative, ecofriendly way to supply algae for biofuel use.

RESULTS

The rationale for the experiment was to see whether alkaline carbonate solutions influenced algae growth, which we measured by quantifying light absorbance, by using a spectrophotometer in the wavelength range of chlorophyll. Chlorophyll level is an accurate measure algae growth, as if chlorophyll level increases in a certain solution, this means that the algae levels are also increasing.

The wavelength range analyzed was from 430 nm to 650 nm, which is the wavelength corresponding to chlorophyll (2). Generally, we found that the addition of calcium carbonate induced algae growth over that observed in the control, whereas the addition of potassium carbonate and sodium bicarbonate did not. **Figure 1A** represents the control subject with no chemical treatment. As shown, in the wavelength corresponding to chlorophyll, minimal growth was observed (maximum: 0.03 OD). **Figure 1B** represents the results obtained with the addition of calcium carbonate (CaCO3). As displayed, there was much higher absorbance (maximum: 0.22 OD). **Figure 1C** represents the results obtained with the addition of potassium carbonate (K2CO3). As shown, similar to the control, minimal growth was observed (maximum: 0.03 OD). **Figure 1D** represents the results obtained with the addition of sodium bicarbonate (NaHCO3). As shown, there was minimal absorbance (maximum: 0.02 OD).

DISCUSSION

In this investigation, we compared algae growth using different alkaline carbonate solutions: sodium bicarbonate, potassium carbonate, and calcium carbonate. The calcium carbonate solution resulted in the greatest amount of absorbance in the wavelength 430nm - 650nm (the wavelength of chlorophyll), indicating the highest content of algae. These results could be due to calcium's role in photosynthesis, as it can regulate the transcription and translation of genes that encode the chloroplast proteins and enzymes (7). One possible reason why the sodium solutions did not produce large amounts of growth is that algae do not need sodium for photosynthesis. Though sodium is critical to cell metabolism, algae only use trace amounts to regulate metabolism (8). Similarly, the potassium carbonate solution also exhibited no growth, likely because potassium is associated with the movement of water molecules, nutrients, and carbohydrates in the plant tissue rather than directly with photosynthesis (9).

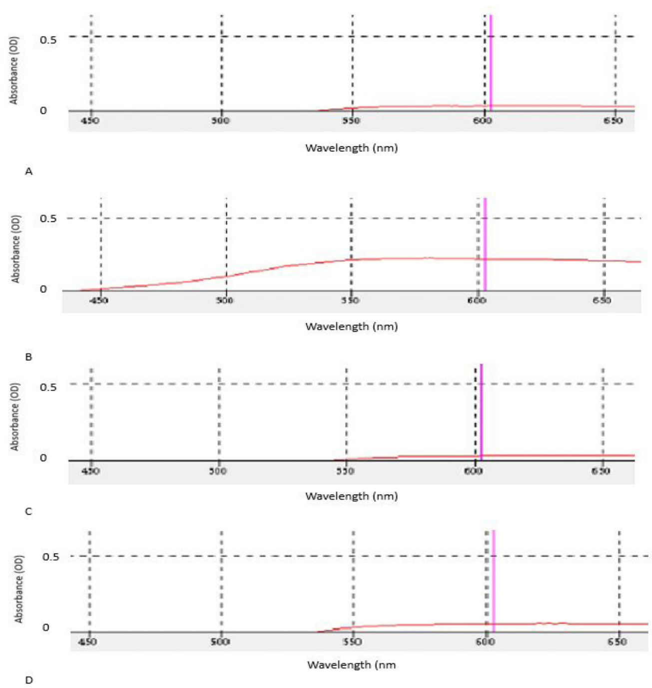

Figure 1: Visible Light Absorbance Graphs. A: Control; B: CaCO3; C: K2CO3; D: NaHCO3. Wavelength range of 450-650 nm is shown, which corresponds to chlorophyll. Vertical purple line indicates the maximum absorbance for each curve: Control (0.03 OD); CaCO3 (0.22 OD), C: K2CO3; (0.03 OD), NaHCO3 (0.02 OD).

Our study does have several limitations. One is the reliance of the results on one trial of the experiment, which was due to logistical and time constraints. The results must be validated by running the experiment again, with three replicates for each condition. Furthermore, ideally we would include a condition in which the pH is increased by adding K2CO3 and a non-basic source of calcium, such as calcium chloride. This experiment would strengthen our hypothesis that it is calcium that is important for algal growth and not just the pH increase. Furthermore, we did not control for contamination; for instance, a more rapidly growing bacterium in the calcium sample or the presence of common microorganisms that feed on algae (such as herbivorous zooplankton like rotifers, open water larvae, and aquatic mites which graze on algae and are commonly found in Georgia ponds) could have influenced our results. Additionally, the presence of microorganisms that penetrate algae's cell walls and extract their contents, like Vampyrella lateritia trophozoite, may have also skewed the results. The small sample volume used is also a limitation as it does not realistically account for the large-scale implications of this research, as the ultimate goal is to induce algal growth rate in urban algae farms for use as biofuel. Finally, the trial was only conducted over 24 hours, which is not the optimal time for algae to grow; in future experiments we should drive for 48-72 hour trials.

Algae-based biofuels are sustainable, effective and environmentally friendly, so it is important to study conditions that may enhance algae growth. Within the stated limitations, we view our work as an important preliminary step in understanding the crucial role calcium might have for this process. Our results may have significant implications for the eco-friendly production of algae, a biofuel that may have a critical role to play in meeting global long-term renewable energy demand.

MATERIALS AND METHODS

Pond water from Mountain Campus, Berry College (Rome, GA) was collected in a 1L container. The four samples of pond water, all identical samples, taken from the same pond were divided into 100mL solutions. We created 0.1M solutions of sodium bicarbonate (NaHCO3) (note that bicarbonate was used because sodium carbonate was unavailable), potassium carbonate (K2CO3), and calcium carbonate (CaCO3). The source for all three solutions was a standard laboratory supplier (Carolina Biological Supply, Burlington, NC). Using a pipette, 4.5 mL of a sodium bicarbonate solution was added to the beaker (#1). 1 mL of potassium carbonate solution was added to the beaker (#2) and 2 mL of the calcium carbonate solution was added to the beaker (#3), to dilute the solutions to 0.1M. The solutions were then tested with pH paper to ensure they were in the 8.2–8.7 range. One beaker was left as the control sample, with no chemical alterations. The three chemically treated samples of pond water were left on a west-facing windowsill for 24 hours to receive maximal sunlight. A spectrophotometer was used to measure algae growth by recording each sample's absorbance of visible light in the 430-650 nm range, corresponding to chlorophyll. Absorbance graphs from the spectrophotometer (which was calibrated at 600 nm) as well as the absorbance rate of each sample were recorded.

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