Nitrogen Starvation of Algae – a Stress for Lipids

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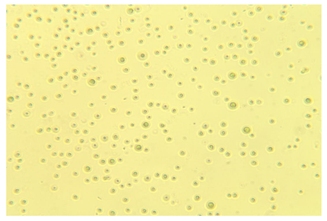
Abstract

Microalgae strains, such as *Chlorella vulgaris*, *Chlorella minutissima,* and *Scenedesmus obliquus* have been researched as potential feedstocks for biofuels because of their fast growth rate and ability to produce lipids. In order for commercial-scale algae biofuel plants to become a reality, however, the conversion of algal lipids into biofuels needs to become more efficient. One strategy to improve biofuel yields from algae is to induce lipid production using a two-stage nitrogen starvation method. In this method, microalgae are grown in nutrient-replete conditions such as wastewater to achieve high biomass and are then transferred to nitrogen-depleted conditions to stimulate lipid production and increase overall lipid content. The objective of this research is to test the viability of different aqueous conditions including tap water, distilled water, and deionized water, to determine which nitrogen-depleted medium results in the maximum lipid induction. The results will indicate the optimal media characteristics including pH, conductivity, and trace organics for maximum lipid induction. *Chlorella cf. vulgaris* was used for preliminary trials because of its availability and metabolic ability to produce lipids. The reason for using water instead of a modified growth medium (nitrogen lacking) is cost, availability, and ease of access for commercial purposes. Microscopic monitoring showed large amounts of lipids in both deionized and distilled water and very small amounts of lipids in tap water and the control. The experiment is ongoing and lipid content measurements by nuclear magnetic resonance will be used to confirm and quantify results obtained by microscopic monitoring.

Introduction

- Algae farming companies such as Algenol, Cellena, and Heliae produce algae at a commercial scale for the pharmaceutical industry.
- Successful practices for inducing lipid induction on a large scale are necessary for algae farming companies to capture the potential of algal biofuels.
- Lipid content is typically between 12-20% in *Chlorella* (Mujtaba et al., 2012, Tang et al., 2011).
- *Chlorella cf. vulgaris* was chosen because it is readily available and is known to have lipids.



Chlorella cf. vulgaris. 25x

Chlorella cf. vulgaris. 100x

Objective

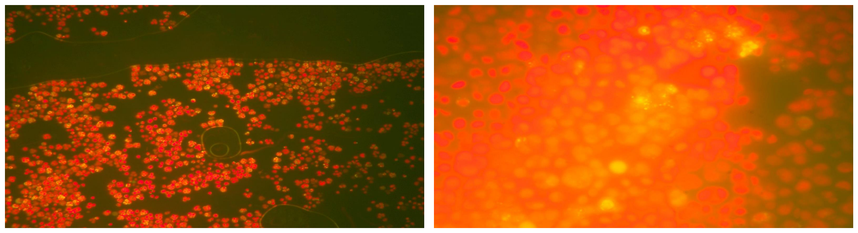
The objective of this experiment is to determine which of the three treatments (tap, deionized, and distilled water) are the most effective for inducing lipid production in *Chlorella cf. vulgaris.*

Materials & Methods

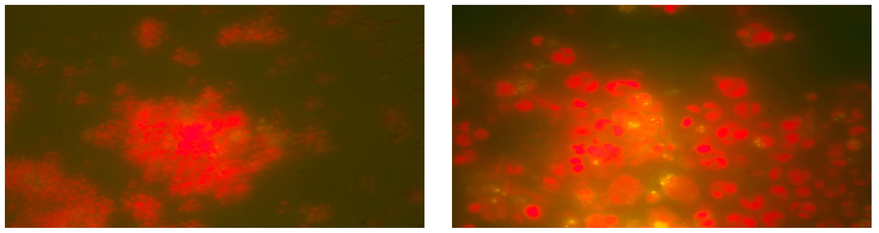
- Cells were cultivated in standard BG-11 growth medium.
- Two liters of algae were centrifuged into four equal pellets.
- Cells were then rinsed with the treatment liquid (BG-11, tap, deioinized, and distilled water) to clean the cells of the medium.
- Cells were then transferred to 500 L Erlenmeyer flasks and re-suspended in BG-11 (control), tap, deionized, and distilled water.
- 12 hour and 24 hour lipid content readings of each treatment were taken by Nuclear Magnetic Resonance (NMR).
- A sample from each of the treatments was taken for fluorescent microscopic analysis. Slides of each treatment were prepared and fluoresced under 40x and 100x.

Nile red stained photomicrographs of *Chlorella cf. vulgaris* after treatment at 40x and 100x

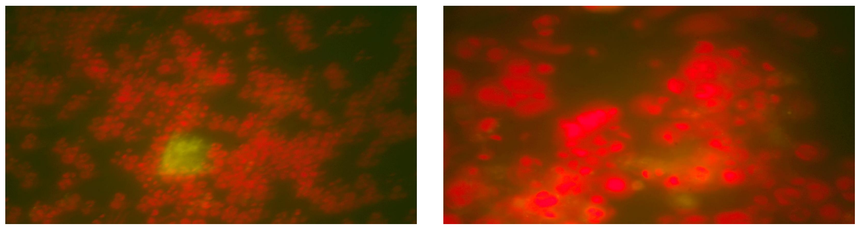
* Yellow fluorescence indicates lipids. Red fluorescence indicates chlorophyll.



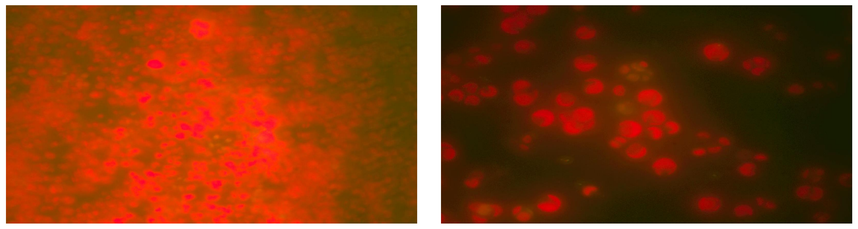
Deionized Water Treatment



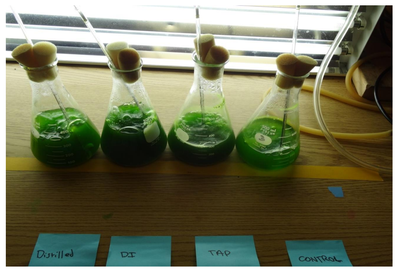
Distilled Water Treatment



Tap Water Treatment



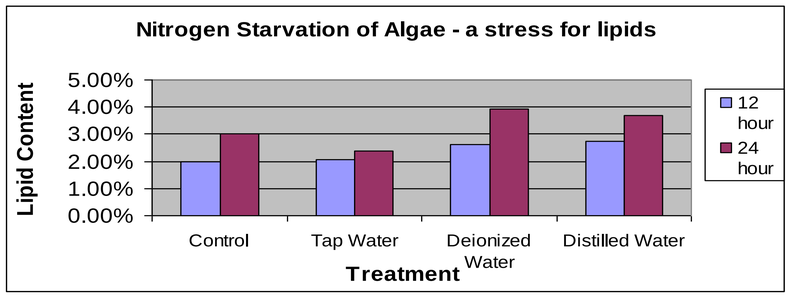
Control Treatment



- (Above) Experimental setup.
- (Left) NMR in use.

Results

- Microscopic, qualitative results were as follows: deionized water and distilled water induced the highest lipid production, as evidenced in the photomicrographs, while tap water and the control induced little to no lipid production.
- NMR quantification displayed the same trend as the microscopic observations.



Conclusion

- Qualitative evidence indicates that low conductivity and low pH (characteristics of deionized and distilled water) are the preferred characteristics of a nutrient-depleted medium for maximum lipid production in the strain *Chlorella cf. vulgaris.*
- Quantitative methods require further refinement in the process of determining lipid content.
- Continued analysis beyond 24 hours is ongoing to monitor trends.

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