



# The Effect of Exogenous Phytohormones on Algal Growth

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## Abstract

Microalgae are potential feedstocks for biofuels because of their fast growth rate and ability to produce lipids. One challenge for commercial-scale algal biofuels is the manipulation of algae to create high lipid content without adversely affecting biomass growth. The objective of this research is to investigate the effect of exogenous plant hormones on algal biomass growth and lipid content. Plant hormones are signal molecules that regulate plant growth and development. *Chlorella vulgaris*, a locally isolated strain with demonstrated ability to produce lipids, was selected as the test organism. *Chlorella vulgaris* was grown in BG-11 standard growth medium. Plant hormones of the auxin, cytokinin, and abscisic acid plant hormone classes were added, individually and in combinations, to determine their influence on cell biomass and lipid content. All experimental trials were conducted in triplicate. Preliminary trials have shown that all hormone treatments outperformed the control, and algae grew faster in all hormone combinations than in individual hormone treatments. The combination of trans-zeatin riboside (tZ) at 1 part per million (ppm), 1-naphthalene-acetic acid (NAA) at 5 ppm, and abscisic acid (ABA) at 5 ppm, exhibited the fastest growth rate. Among individual hormone treatments, the fastest growth rate was achieved by ABA at a concentration of 50 ppm.

## Introduction

- The algae farming industry is a growing industry, however, at this time algae companies are not able to grow algae for fuel purposes and turn a profit. Because of this many 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.

## Objectives

The objective of this experiment is to determine the effect of different phytohormone treatments on *C. cf. vulgaris*. This experiment aims to determine an optimal phytohormone application to enhance the biomass grown and lipid content of the algal cell.

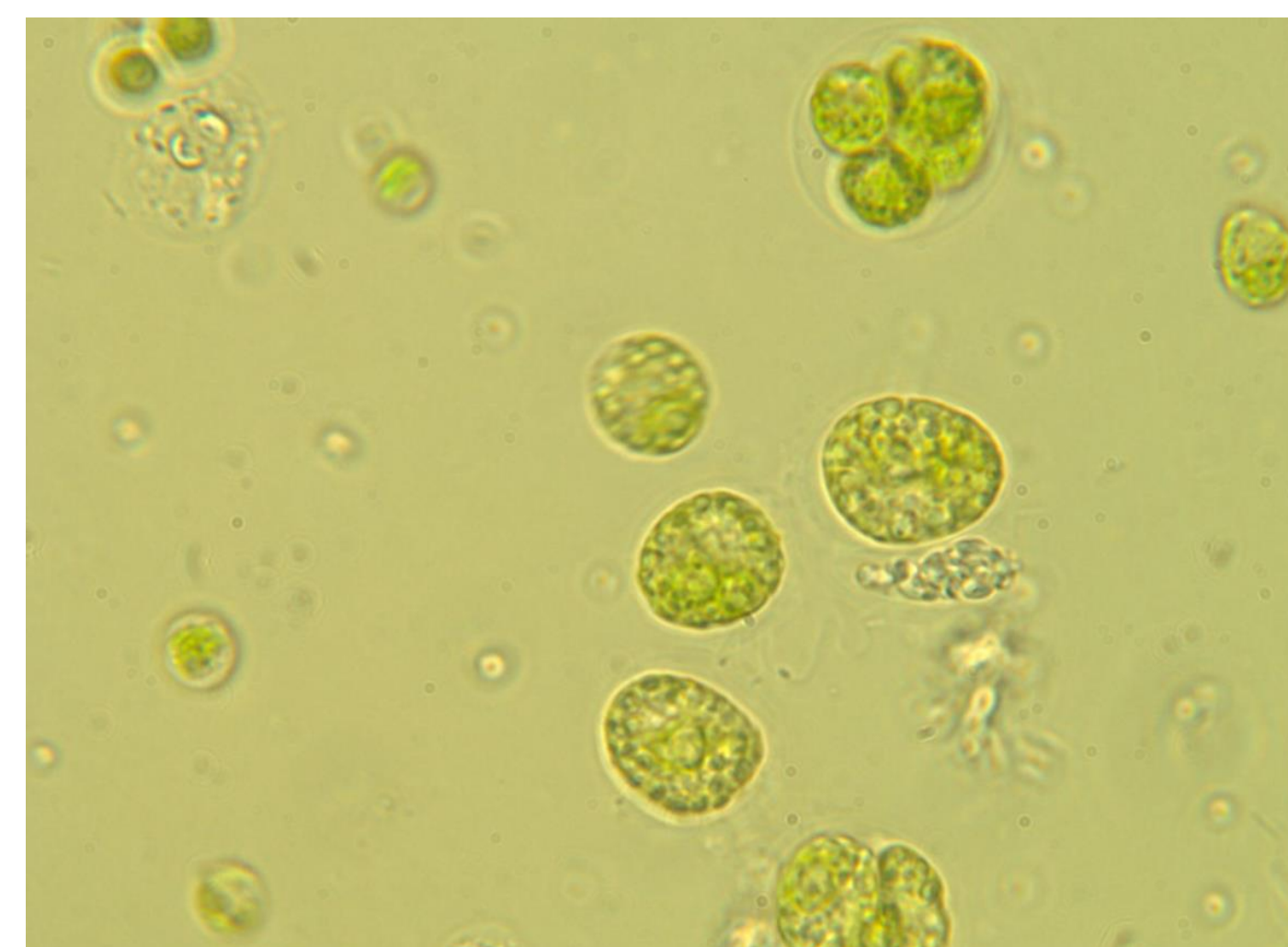


Figure 1. *C. cf. Vulgaris* at 100x magnification.



Figure 2. The set up of the singular hormone treatments.

## Methods

- Initial Algae Cultivation
  - *C. cf. vulgaris* was cultivated in standard BG-11 growth medium
  - Algae was cultivated for 7 days
- Experimental Setup
  - Each set was performed in triplicate
  - Total of 8 sets – 1 control, 4 singular hormone additions and three hormone combinations<sup>1</sup>
  - Erlenmeyer flasks (250-ml) with an active volume of 175mL (50 mL inoculum added to 125 mL of BG-11) were used for algae cultivation
  - Hormones were dissolved in 50% ethanol and appropriate concentrations were applied in 10µL aliquots<sup>2</sup>
  - Cultures were grown with light intensity of 300 µMol photons/m<sup>2</sup>/s
- Analytical Measurements
  - Algal growth was monitored using a spectrophotometer (680nm)<sup>3</sup>
  - Optical density was converted to biomass yield using standard curves generated by *C. cf. vulgaris*
  - Lipid content was measured by Nuclear Magnetic Resonance (NMR)

Table 1 – Biomass and Lipid % as a function of treatment

	Biomass Yield (g) <sup>a</sup>	Growth Rate (g/day)	Lipids (%)
Control	0.117 ± 0.007	0.017	1.20
ABA 50 PPM	0.138 ± 0.026	0.020	3.50
ABA 5 PPM	0.126 ± 0.030	0.018	3.14
NAA 5 PPM	0.128 ± 0.018	0.018	2.98
tZ 1 PPM	0.122 ± 0.002	0.017	2.34
tZ (1PPM) NAA (5PPM)	0.165 ± 0.033	0.027	3.86
tZ (1PPM) NAA (5PPM) ABA (50 PPM)	0.156 ± 0.042	0.026	7.10
tZ (1PPM) NAA (5PPM) ABA (5 PPM)	0.172 ± 0.020	0.029	6.71

<sup>a</sup> Biomass Yield= Final Weight – Initial Weight

## Results (continued)

All hormone treatments in singular and combination improved algal growth rates compared with the control. The more hormones added (the most being three) to the algae the more *C. cf. Vulgaris* biomass produced. Results also show that all hormone treatments increased lipid content. Similar to the hormone to biomass relationship, all hormone combination treatments outperformed all singular hormone treatments in lipid production. Hormone combination treatment tZ (1 ppm), NAA (5 ppm), and ABA (50 ppm) resulted in the highest lipid content (7.1%). Among the singular hormone treatments ABA at 50 ppm increased lipid content the most (3.5%).



Figure 3. Using nuclear magnetic resonance to determine algal lipid content.

## Conclusions

- Quantitative evidence indicates a positive relationship between exogenous phytohormone application and increased biomass as well as increased lipid content.
- Using several hormones in combination rather than on their own proved to be the most impactful.
- Among the hormone combination treatments, the treatment with the highest concentrations resulted in the greatest lipid content.
- Applying phytohormones to algae during exponential growth can induce artificially steeper growth rates for *C. cf. Vulgaris*.

## Future Work

Future work should investigate the relationship between these hormones and other strains of algae. Additionally, all hormones used should be researched at different concentrations and in all singular/multiple hormone treatment combinations. Statistical analysis of this research will create a clearer picture of how each individual hormone impacts algae cells.

## References

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