

Comparative Analysis of Single-Stage and Multi-Stage Lipid Extraction from *Chlorella vulgaris* for Biodiesel Applications

Nathan I. Martinez & Dr. Matthew Alexander
Wayne H. King Department of Chemical and Natural Gas Engineering

ABSTRACT

The continued reliance on fossil fuels contributes significantly to climate change, yet current biofuel production methods remain inefficient and unsustainable. This urgency underscores the need for viable eco-friendly alternatives to support a sustainable future. Microalgae have emerged as a promising source for biofuel production due to their high lipid content and rapid growth in diverse environments which can allow for minimal growth competition. This study investigates the potential of *Chlorella vulgaris* as both a biofuel source and a potential agent for wastewater treatment, contributing to water purification while generating lipid-rich biomass. A key focus of this work is optimizing the lipid extraction process. The central goal is to compare the efficiency of single-stage versus multi-stage extraction methods, with lipid yields measured through gravimetric analysis. Several experimental trials were conducted under varied culture conditions and extraction parameters to determine the most effective strategy for lipid production and extraction. The results are expected to show that the multi-stage extraction method yields a higher lipid content than the single-stage process because of its improved solvent saturation control, and it allows for a more complete extraction process. Anticipated obstacles such as contamination, cultivation inefficiencies, and cost barriers are addressed. The findings aim to provide crucial insight into scalable biofuel production systems and contribute foundational data for the future of algae-based technologies involving renewable energy. These outcomes will enable a quantitative comparison of extraction methods and highlight the practical implications for sustainable fuel development.

PURPOSE

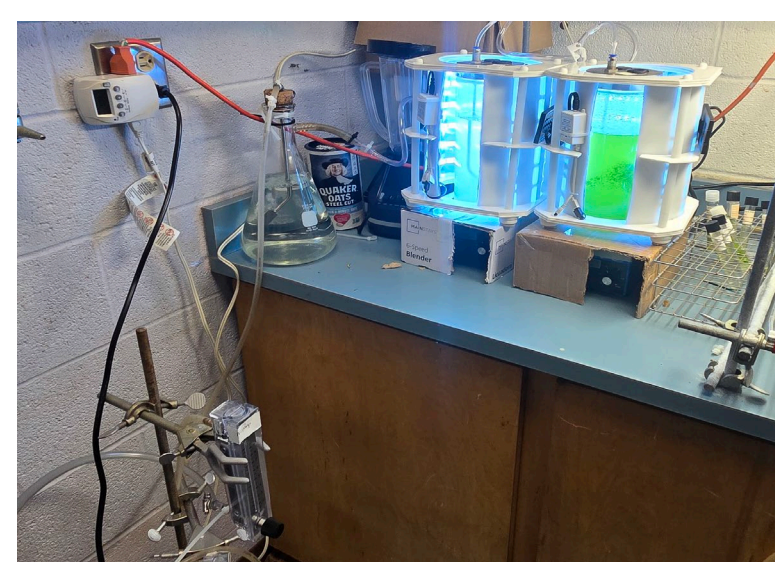
- Are the resources necessary for multiple extractions of lipids from microalgae worth expending when in comparison to what is needed for a single extraction?
- Rising sustainability concerns usher the need for a new fuel source or a better understanding of current methods in practice.

INTRODUCTION

- Biofuel** – Made from domestic oils and fats, and is a promising alternative fuel, but methods like algal biomass liquefaction require further research to enhance sustainability and efficiency (Singh & Gu, 2010).
- Lipid Extraction** – Essential for converting microalgae into biofuels, with newer eco-friendly methods like supercritical CO₂ and microwave-assisted extraction being developed to replace traditional solvents and improve commercial viability (Chisti, 2007).
- Microalgae** – Fast-growing, carbon-converting biofuel source with high lipid content and low competition for resources, but high cultivation costs and technical limitations still require further research to ensure long-term viability (Chisti, 2007).
- Wastewater Treatment** – Microalgae can grow in wastewater, improving water quality through oxygenation while serving as a low-cost cultivation method for biofuel production; ongoing research explores mixed cultures and advanced harvesting to improve consistency and efficiency (Pittman et al., 2011).

METHODS

- Culturation of Microalgae (*Chlorella vulgaris*)
 - Inoculation into Proteose Medium
- Drying of Microalgae
 - Approx. 130 deg. Celsius for 2 hours
- Addition of Separation Mixture
 - 5mL Chloroform
 - 10mL Methanol
 - 4 mL Water
- Vortex with Further Addition of Mixture
 - 5mL Chloroform
 - 5mL Water
- Gravimetric Centrifugation
 - 2500rpm for 30 minutes
- Separation of Layers
 - Top: methanol and water
 - Middle: residual biomass
 - Bottom: chloroform and lipids
- Purification by Evaporation
 - Top layer is discarded, middle is reused or discarded, bottom is left within a pre-weighed dish for chloroform to evaporate as lipids will be left to calculate.



*Apparatus for culturing of algae and inoculation



*Drying oven used to dry algae



*Centrifuge used to separate mixture into layers



*Fume hood used for safe evaporation of chloroform

RESULTS

Multi-Stage Extraction					
Container	Trial Number	Mass (mg)	Container	Trial Number	Mass (mg)
Ceramic (20 mL)	1	125.9 mg	Metal (40 mL)	1	176.7 mg
Ceramic (20 mL)	2	88.1 mg	Metal (40 mL)	2	117.6 mg
Ceramic (20 mL)	3	62.2 mg	Metal (40 mL)	3	103.5 mg

Single-Stage Extraction	
Container	Mass (mg)
Metal (40 mL)	163.3 mg
Ceramic (20 mL)	109.2 mg

RESULTS, CONT.

- Reuse of algae diminished lipid product.
- Lipids had green hue.



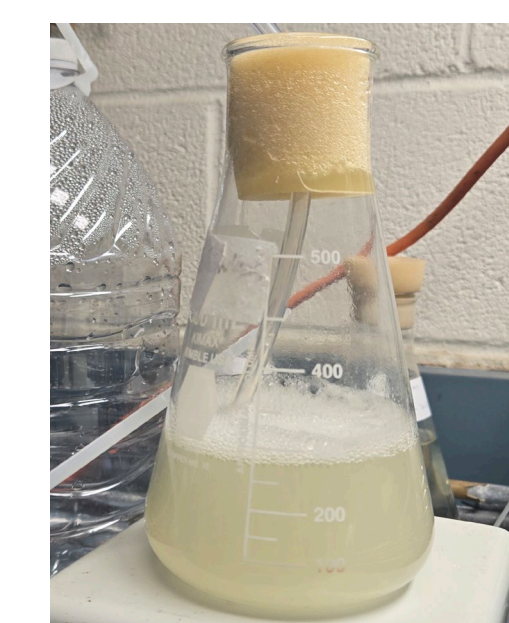
*Multi-Stage Extraction products 1 through 3 from left to right with metal on inside and ceramic on outside.



*Single-Stage Extraction products with metal on left and ceramic on right.

DISCUSSION

- The initial flask used to culture the algae became cloudy early on possibly because of the crystallization of salts.
- Multi-stage extraction is beneficial in terms of product but may not be worthwhile when it comes to resources consumed for ending product.
- The container used for heating seemed to influence the algae recovery and attributed to lower values in the ceramic section.



*Cloudy flask after inoculation



*Dwindling returns over the multiple extractions



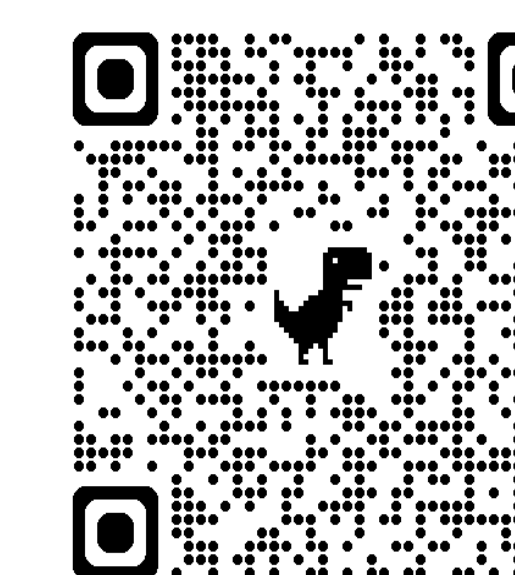
*More in metal compared to ceramic

CONCLUSIONS

- Multi-stage extraction is efficient but with the downside of gradual decline of lipid product.
- The container containing the dry algae can affect the overall outcome because of potential staining and sticking.
- Even in small samples, *Chlorella vulgaris* is a solid contender in the biodiesel field for lipid production.
- Concepts applied here can be utilized in more fields than chemical engineering.

REFERENCES

For the complete list of references, please scan this QR code.
 Thank you!



Acknowledgments

Heartfelt thank you to:

- Dr. Matthew Alexander
- TAMUK McNair Program & Coordinators
- Mr. Lee Ortegon