Developing Methods of Producing and Processing of Marine Microalgae to Biocrude

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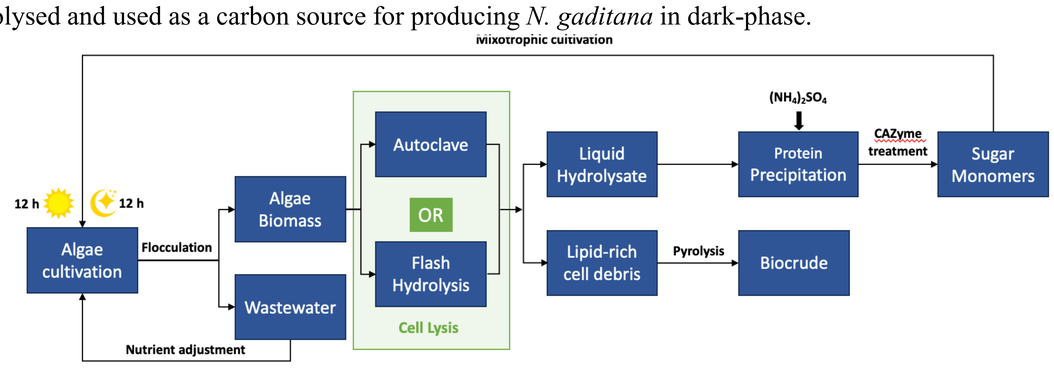
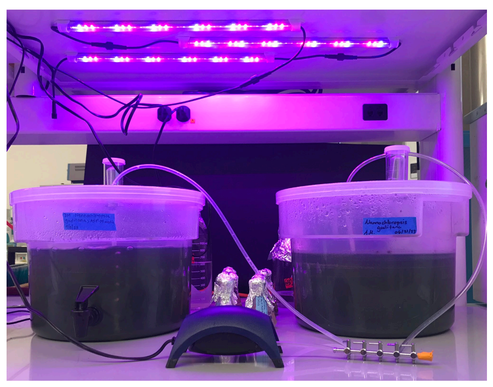
Project Overview

- Developing a closed-loop system of producing and processing of *Nannochloropsis gaditana* to biocrude
- Algal biomass will be separated and lysed using autoclave in a batch process, and flash hydrolysis in a continuous process.
- The algal components such as lipids and proteins will be fractionated, and the carbohydrates will be hydrolysed and used as a carbon source for producing *N. gaditana* in dark-phase.



Cultivation of *Nannochloropsis gaditana*



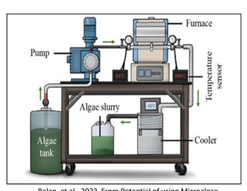
- *Nannochloropsis* is a marine, phototrophic organism.
- They can be cultivated in nutrient-rich media under the presence of red and blue lights (daytime) in the presence of CO_2 and organic carbon source (night-time).
- *N. gaditana* has high lipid and protein content as compared to other microalgal species

Biomass component	*Nannochloropsis gaditana*[3]	*Chlorella vulgaris*[1]	*Spirulina platensis*[2]
Lipids	54	25.1	10.7
Carbohydrates	12	35.0	12.8
Proteins	32	20.0	63.9
Ash	2	2.0	6.1

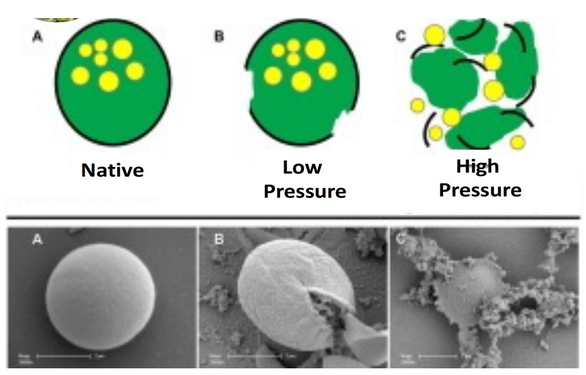
Algae Lysing

Autoclave (AC) 120°C, 15 psi, 15min. — **3%, 5%, 8%, 10% SL**

Flash Hydrolysis (FH) 230°C, 1500 psi, 10 sec. — **3% SL**



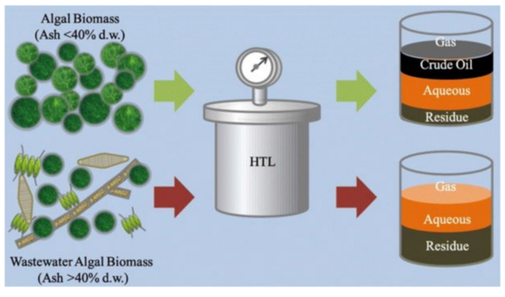
Balan, et al., 2023, From Potential of using Microalgae to Sequester Carbon dioxide and processing to bioproducts, accessed 7 September 2023



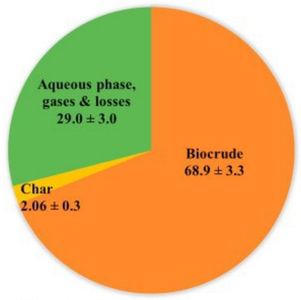
Yap, et al., 2014, From A mechanistic study of algal cell disruption and its effect on lipid recovery by solvent extraction, accessed 7 September 2023, https://doi.org/10.1016/j.algal.2014.07.001

- Two different methods were used to lyse *N. gaditana*
- Autoclave (AC) is a batch process and uses low temperature and long residence time (30-60 minutes)
- Flash hydrolysis (FH) a continuous process where the algal slurry (3-10%) is pumped into a hot tube maintained at high temperature for a short residence time (30 seconds).
- AC process results in partial cell lysis, while FH process results in complete cell lysis.

Biocrude Analysis



Chen, et al., 2017, From Effect of ash on hydrothermal liquefaction of high-ash content algal biomass, accessed 5 October 2023, https://doi.org/10.1016/j.algal.2017.05.010



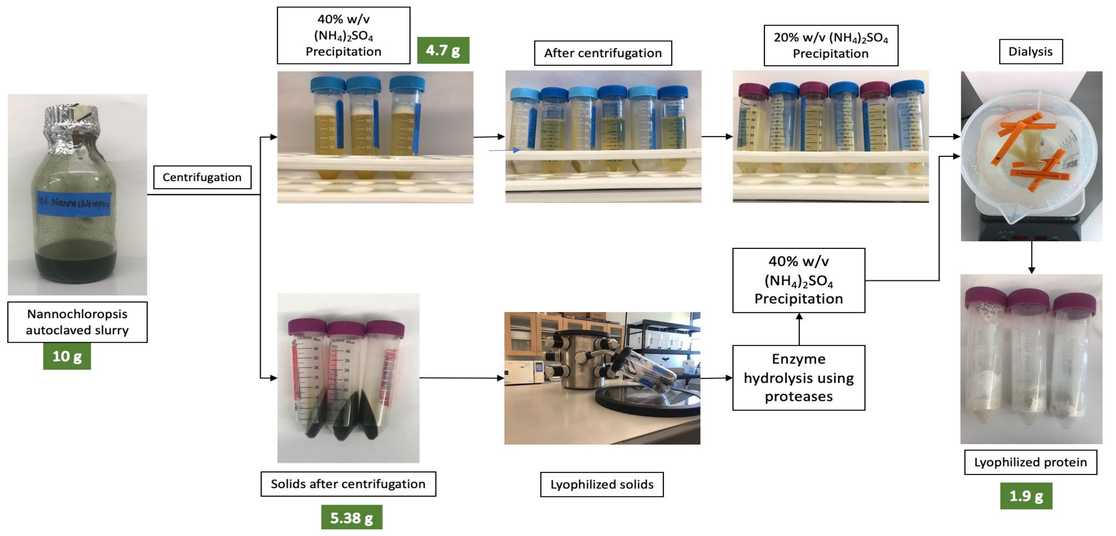
Teymouri, et al., 2018, From Evaluation of lipid extractability after flash hydrolysis of algae, accessed 5 October 2023, https://doi.org/10.1016/j.fuel.2018.03.044

- Lipid-rich cell debris, also known as biofuel intermediates (BI) obtained after cell lysing was used as the substrate for biocrude production
- Hydrothermal liquefaction (HTL) was performed at high temperature for 1 h after which the aqueous phase was separated and analysed for its composition.
- The figure on the right is a representative figure in which the *Chlorella vulgaris* BI was subjected to HTL at a temperature of 350 °C for 1 h yielding 68.9% biocrude.

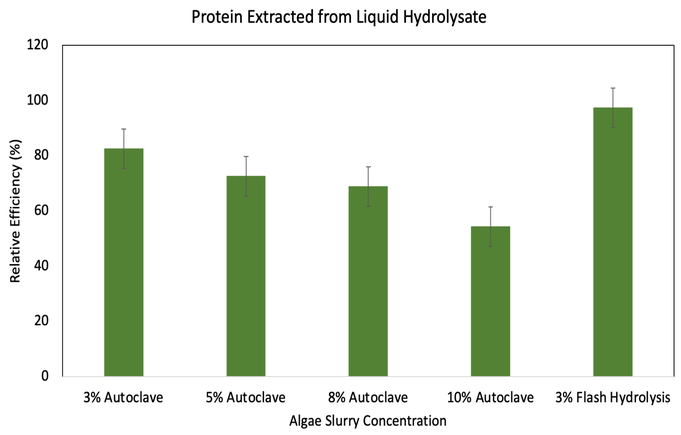
Acknowledgements

Financial support of this work was through the National Science Foundation CAREER Award # 1351413, PFI: AIR TT Grant# 1640593 awarded to Dr. S. Kumar; DOE Grant# FE0032203, UH Center for Carbon Management and Energy and UH Subsea Institute awarded to Dr. V. Balan.

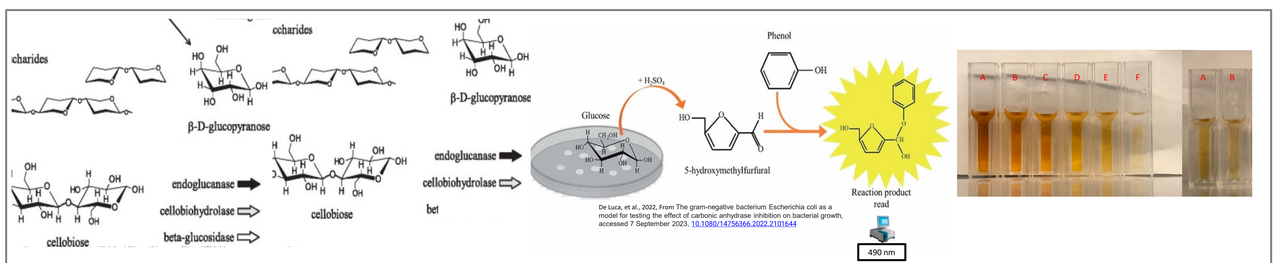
Protein Precipitation and Separation



- CHNS analysis was performed to calculate protein content of *N. gaditana.*
- N factor of 4.78 was used.[4]
- Total protein was calculated to be approximately 35% DW of biomass.
- After extraction, proteins were dialyzed to remove ammonium sulfate and lyophilized
- Proteins were measured gravimetrically.
- FH process resulted in 97.4% protein recovery from soluble hydrolysate when compared to AC process that recovery 85% at 3% solids loading.



Carbohydrate Recycling



De Luca, et al., 2022, From The gram-negative bacterium Escherichia coli as a model for testing the effect of carbonic anhydrase inhibition on bacterial growth, accessed 7 September 2023, 10.1080/14756366.2022.2101644

- *N. gaditana* carbohydrates were hydrolysed using dilute sulfuric acid (4%) or carbohydrate active enzymes (CAZymes).
- Phenol-sulfuric acid assay that measure the reducing sugar content was used to determine carbohydrate content
- *N. gaditana* was inoculated in the untreated carbohydrate-rich hydrolysate and growth was measured over 1 week.
- Reducing sugars concentration decreased from 9.4 mg/mL to 5.7 mg/mL which demonstrate the growth of *N. gaditana*

Future Work

- Enzymatic hydrolysis of soluble carbohydrates using CAZymes and measure sugar concentration
- Compare rate of algae growth in media containing acid-hydrolyzed or enzyme hydrolyzed carbohydrates sugar streams
- Perform FH at varying algal slurry concentrations and compared with AC process
- Produce and analyze biocrude using Gas chromatography followed by mass spectrometry method
- Analysis of FH and AC hydrolyzed algal proteins using MALDI-TOF.

Conclusions

- *N. gaditana* is being cultivated in 12 h light/dark cycle
- Carbohydrates extracted in the soluble stream were hydrolyzed and recirculated for mixotrophic growth mode.
- Flash hydrolyzing algae resulted in recovery of 97.4% of proteins from soluble stream
- Initial tests show microalgae capable of up taking recycled microalgal carbohydrates.

References

1. Alavijeh, R. S., Karimi, K., Wijffels, R. H., van den Berg, C., & Eppink, M. (2020). Combined bead milling and enzymatic hydrolysis for efficient fractionation of lipids, proteins, and carbohydrates of Chlorella vulgaris microalgae. *Bioresource technology*, *309*, 123321.
2. H. Sati, M. Mitra, S. Mishra, P. Baredar, Microalgal lipid extraction strategies for biodiesel production: A review, Algal Research. 38 (2019) 101413. https://doi.org/10.1016/j.algal.2019.101413.
3. Sung, MG., Han, JI., Lee, B. *et al.* Wavelength shift strategy to enhance lipid productivity of *Nannochloropsis gaditana*. *Biotechnol Biofuels* **11**, 70 (2018). https://doi.org/10.1186/s13068-018-1067-2
4. Laurens, L. M. (2016). *Summative Mass Analysis of Algal Biomass-Integration of Analytical Procedures: Laboratory Analytical Procedure (LAP)* (No. NREL/TP-5100-60943). National Renewable Energy Lab.(NREL), Golden, CO (United States).