The Future of Biodiesel & Wastewater Treatment

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ABSTRACT

Microalgae are an excellent vehicle for significant triglyceride production, which can be transformed chemically into biodiesel fuel. However, large volumes of water are necessary for successful microalgae cultivation. With ever-increasing demands for clean potable water, it makes sense to investigate using non-potable waters, such as wastewater and brackish waters, for microalgae culturing. The current research aim is to investigate whether the microalgae strains *Dunaliella salina*, and *Neochloris oleoabundans* are good candidates for industrial triglyceride production and treatment of wastewater by comparing their growth in the basic media versus growth in wastewater media that has additional sodium nitrate ($NaNO_3$) and sodium sodium phosphate dibasic (Na_2HPO_4) or dipotassium phosphate (K_2HPO_4) (depending on the corresponding media). The expected results are that the wastewater media will have a higher microalgae production and thus oil production than that of the basic media. With this in mind, microalgae might be a great candidate for industrial feedstock production and treatment of wastewater.

INTRODUCTION

Algae:

- **photosynthetic organisms that grow in a range of aquatic habitats, such as lakes, ponds, rivers, oceans, and even wastewater**
- Qualities:
  - Capacity to survive harsh environmental conditions (Ajala & Alexander, 2020a)
  - Produce Lipids
    - Triglycerides (TAG)
  - Great candidate to potentially replace fossil fuels (Hibel et al., 2015; Mondal et al., 2017; Morteo & Alexander, 2022; Patil et al., 2008)
  - Resource to potable water (Ajala & Alexander, 2020; Mondal et al., 2017)

PURPOSE OF THE STUDY

The current research's aim is to investigate if the microalgae strains, *D. salina* and *N. oleoabundans* are good candidates for industrial feedstock production and treatment of wastewater by comparing them to the medium that does not alter the concentrations of sodium nitrate ($NaNO_3$) and sodium phosphate dibasic (Na_2HPO_4) or dipotassium phosphate (K_2HPO_4) (depending on the corresponding media).

RESEARCH QUESTIONS

- Does a medium in higher sodium nitrate ($NaNO_3$) and sodium phosphate dibasic (Na_2HPO_4) or dipotassium phosphate (K_2HPO_4) (depending on the corresponding media) concentrations produce more triglycerides than that of the algae harvested in the non-altered medium?
- Is there a correlation between algae growth and triglycerides (oil production)?
- How does the growth of algae affect the treatment of wastewater and the overall release of triglycerides?

METHODS

- The following procedures will be performed to investigate if the microalgae strains, *Dunaliella salina*, and *Neochloris oleoabundans* are good candidates for industrial feedstock production and treatment of wastewater by comparing it to the medium that does not alter the concentrations of sodium nitrate ($NaNO_3$) and sodium phosphate dibasic (Na_2HPO_4) or dipotassium phosphate (K_2HPO_4) (depending on the corresponding media).

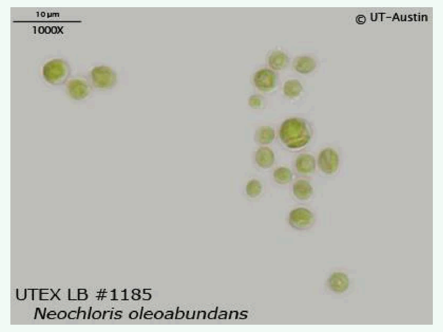
Figure 1

Dunaliella salina

Note. Purchased from UT-Austin.

Figure 2

Neochloris oleoabundans



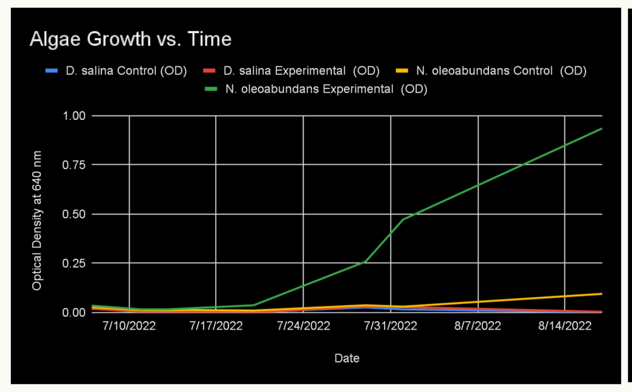
Note. Purchased from UT-Austin.

RESULTS

- As seen in Figure 5, it is evident that there was a successful seed culturing of *D. salina* and *N. oleoabundans*. To continue, as seen in Figures 6-8, the *N. oleoabundans* experimental group showed significant growth, decreased the levels of nitrate and phosphate (Table 3), and had a lipid percent of 21.45 (Table 4). At the same time, for both the control and experimental group of *D. salina* it appears that this set did not culture at all (Figures 6 and 8).

Figure 12

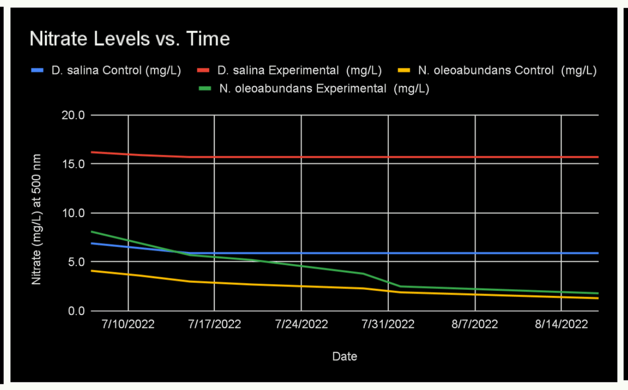
Algae Growth: OD readings at 640 nm



Note. OD reading over 3 weeks of Bioreactors 1-4.

Figure 13

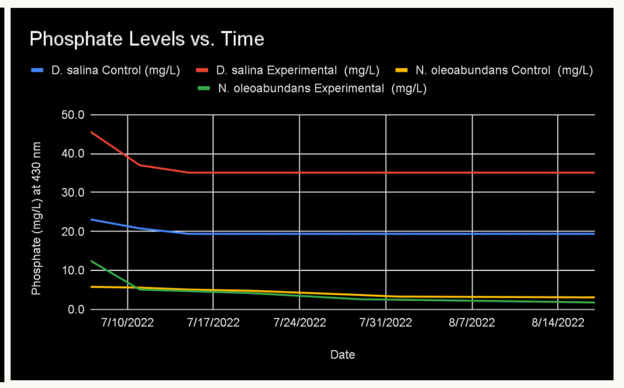
Nitrate readings at 500 nm



Note. Nitrate level readings over 3 weeks of Bioreactors 1-4 using the Hach DR/2000 Visible Spectrophotometer.

Figure 14

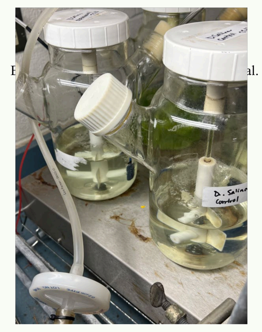
Phosphate readings at 430 nm



Note. Phosphate level readings over 3 weeks of Bioreactors 1-4 using the Hach DR/2000 Visible Spectrophotometer.

Figure 6

Bioreactors 1 and 2: D. salina



Note. Bioreactor 1 (front): *D. salina* control;

Figure 7

Bioreactors 3 and 4: N. oleoabundans

Bioreactor 3 (front): *N. oleoabundans* control; Bioreactor 4 (back): *N. oleoabundans* experimental.

Table 3

Biomass Per Dry Weight

	Biomass (gwd)
N. oleoabundans Control	0.04574285714
N. oleoabundans Experimental	0.1724285714
D. salina Control (seed culture)	0.5928
N. oleoabundans Control (seed culture)	0.14

Note. Biomass measured in grams.

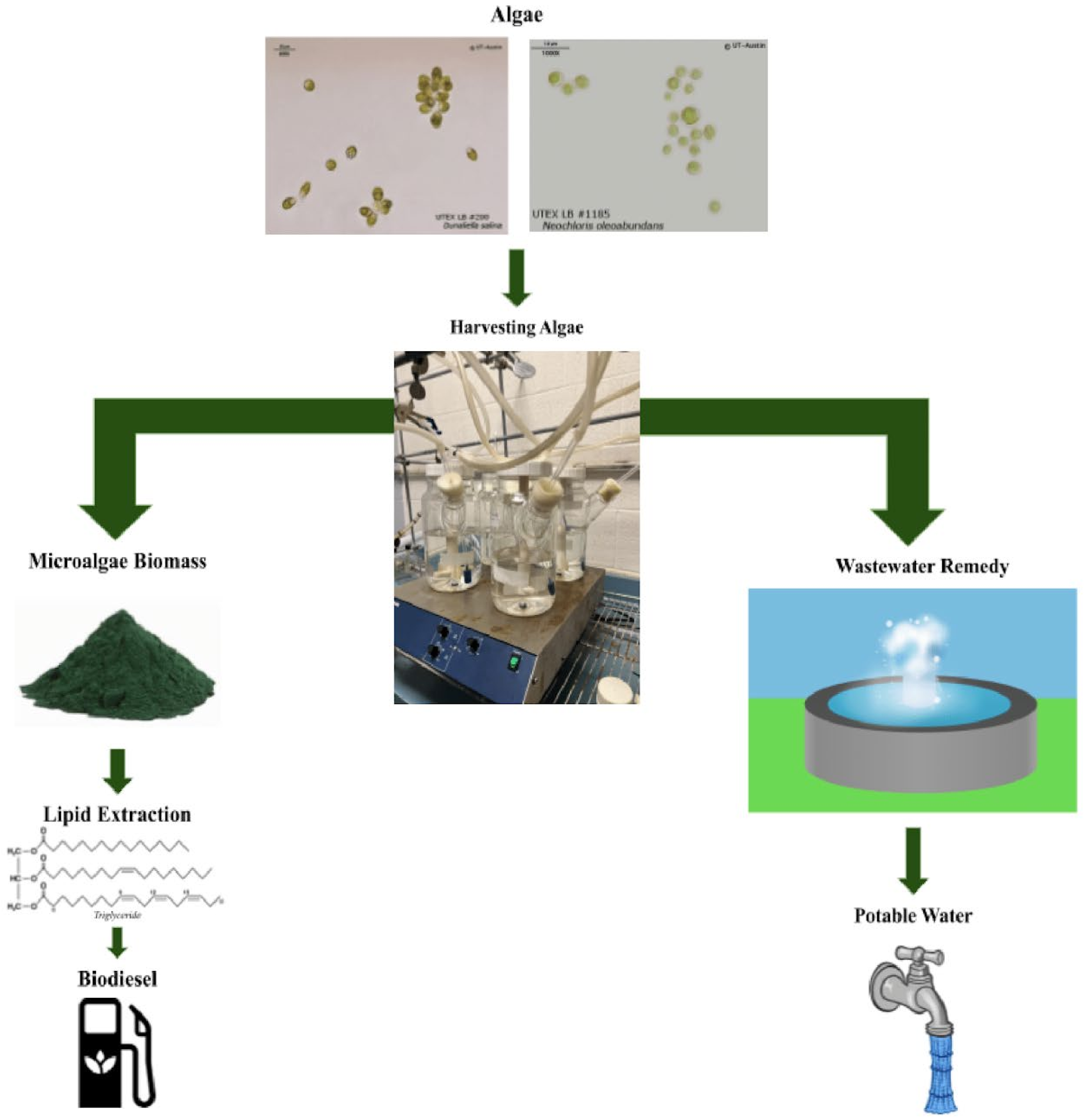
Table 4

Lipid Percent

	Lipid %
N. oleoabundans Control	54.966
N. oleoabundans Experimental	21.458
D. salina Control (seed culture)	5.735
N. oleoabundans Control (seed culture)	38.000

Note. The lipid percent from the biomass.

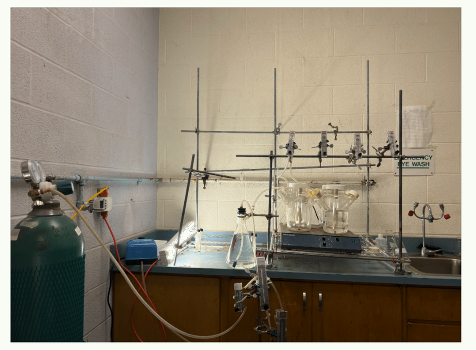
CONCLUSION



METHODS CONTINUED

Figure 3

Bioreactors & Experimental Set-Up



Note. The control groups are in the front while the experimental are in the back.

Figure 4

Hach DR/2000 Visible Spectrophotometer

Note. Used to measure optical density and Nitrate and Phosphate levels present in each case.

Figure 5

Seed Culturing of D. salina and N. oleoabundans

Note. 2 Erlenmeyer flask seed cultures (on stirrer plates).

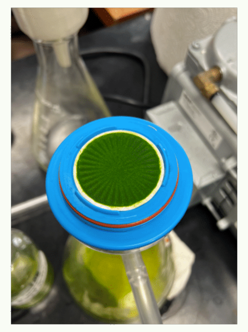
Figure 11

Lipid Trays

Note. 17: *N. oleoabundans* experimental; 18: *N. oleoabundans* control; 20: *N. oleoabundans* control seed culture; 21: *D. salina* control seed culture.

Figure 8

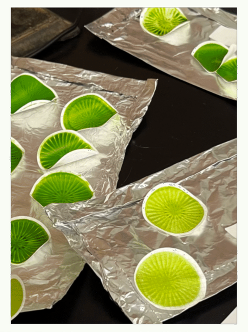
Vaccum Filtration



Note. Filtrating the *N. oleoabundans* to collect the biomass.

Figure 9

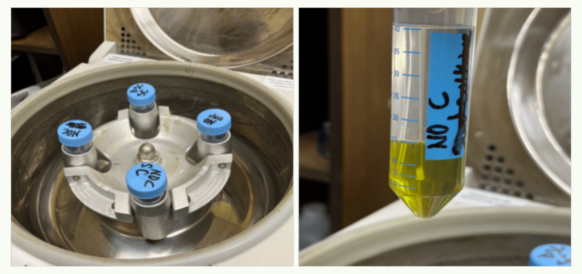
Dry Biomass



Note. Dry biomass used in lipid extraction.

Figure 10

Lipid Extraction & Separation



Note. Bottom layer: Lipids; Top layer: Water and methanol.

Table 1

Dunaliella salina's wastewater media

	Addition (mL)
$NaNo_3$	29 mL
Na_2HPO_4	50 mL
2x ERD media	921 mL
Total Volume (mL)	1,000 mL

Note. Derived from Morteo's Case 3 centrate.

Table 2

Neochloris oleoabundans' wastewater media

	Addition (mL)
$NaNo_3$	29 mL
K_2HPO_4	23 mL
Soil Extract Medium	948 mL
Total Volume (mL)	1,000 mL

Note. Derived from Morteo's Case 3 centrate.

DISCUSSION

- The reason behind the species, *Dunaliella salina*, not harvesting is still unknown. However, based on the seed culture results, it is predicted that it would not be a good candidate for industrial triglyceride production. At the same time, *Neochloris oleoabundans* would be a good candidate for industrial triglyceride production and wastewater treatment.

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