Micro'fat'ories: Increasing Microalgal Biomass and Lipids for Biofuel Production

Problem Space

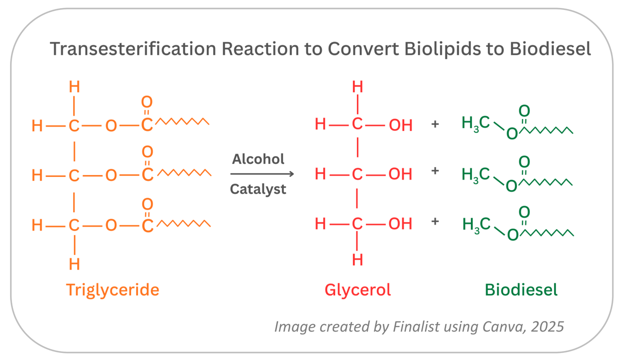
Fossil fuels

- **Dominant** energy source today. They accounted for 86% of world's energy mix in 2024.
- **High greenhouse gas** emissions drive climate change. In 2024, atmospheric CO_2 from burning fossil fuels reached 423 ppm, with emissions of 37.4 billion tons.
- **Non-renewable**, cause environmental pollution, and threats to biodiversity.

The Future of Energy: Green Power, Clean Future

Microalgae as a source of biofuel

- ✓ **Unicellular organisms:** Can be freshwater or marine and are fast growing.
- ✓ **Produce oil or biolipids** that can be converted to Biofuel.
- ✓ **Renewable and carbon neutral** – absorbs CO_2 as they grow
- ✓ **Sustainable**: Rapid growth, 20x more oil per acre than crops
- ✓ **Water efficient**: Grows on non-arable land and wastewater – no food competition



Current Challenges

Operational difficulties:

- ➢ **Low** and inconsistent **biomass** yield
- ➢ **Low lipid** content: difficult to maintain growth for high lipid production
- ➢ **Harvesting** and **extraction difficulties**
- ➢ **Competition** with cheaper fossil fuels

Engineering Goals

- **Improve Biomass Yield:** Vitamin B1 and B Complex may help increase algal metabolism by acting as cofactors in the metabolic pathways. Vitamin C may increase photosynthesis and protects the cells from being damaged. Indole 3-acetic acid (IAA) is a plant hormone that could help cell division.
- **Improve Oil Production:** Salt stress may increase the amount of lipids produced per cell, by changing the metabolic pathway from converting CO_2 from air to lipids instead of sugars and proteins.

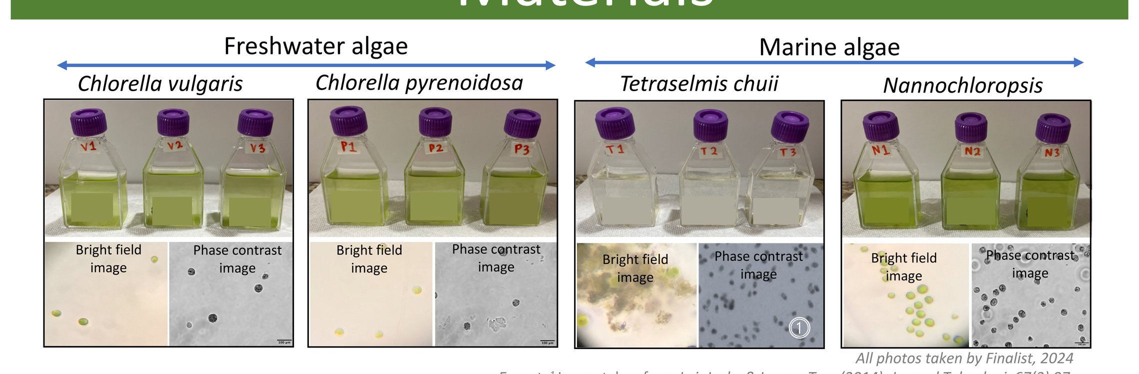
Questions Asked

A. Which freshwater and marine microalgae produce the most biomass in their standard growth conditions?

B. Will the addition of Vitamins B, C and plant hormone to standard growth media increase biomass?

C. Will saline stress given to freshwater microalgae increase their lipid production?

Materials



1. Microalgae:
- *Chlorella vulgaris*- culture and as sodium alginate algal beads
- *Chlorella pyrenoidosa*
- *Nannochloropsis*
- *Tetraselmis chuii*

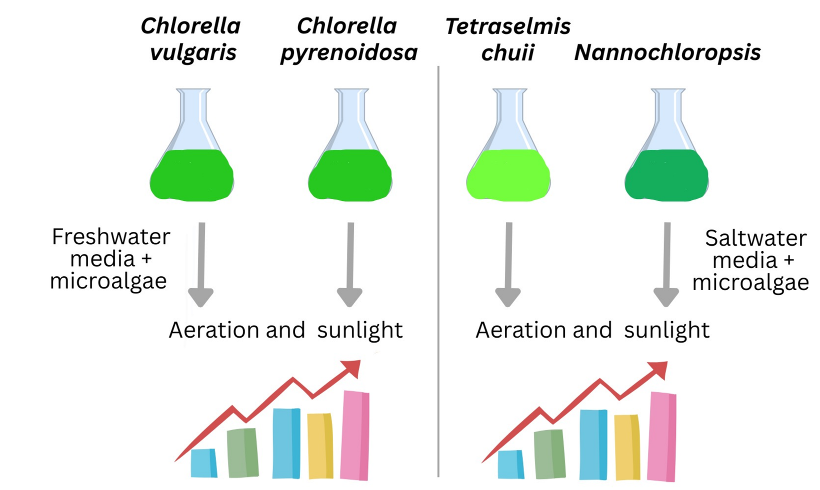
2. Additives:
- Vitamin B1 (6 mg/mL)
- Vitamin B Complex
  - Contains five key B-vitamins including B12 (1.2 mg/mL)
- Vitamin C (22.5 mg/mL)
- Indole 3-acetic acid (IAA, a plant growth hormone) (0.1mg/100mg solid)
- Sea Salt

3. Chemicals:
- Acetone
- Nile red dye

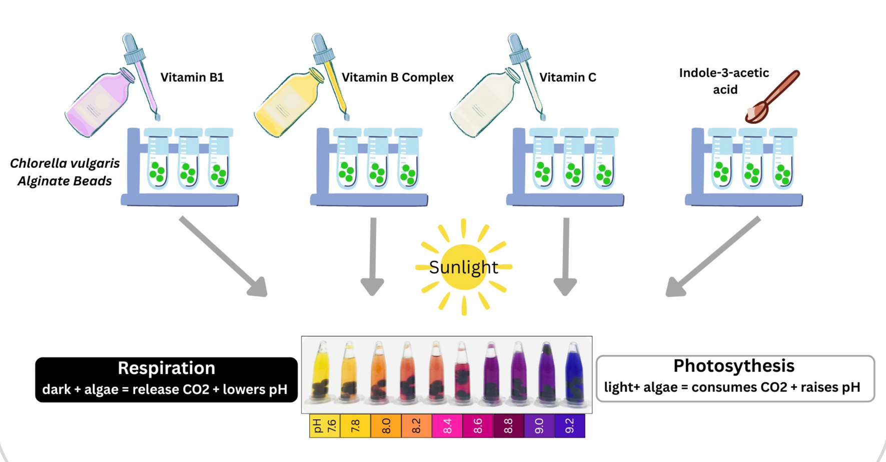
4. Other materials:
- Algal growth media – Fresh water (Bold's basal media),and saltwater media (Guillard and Ryther's f/2 media)
- 25 ml T-Flasks
- 500 ml wide mouth bottles
- Secchi Sticks
- DPBS (buffer for rinsing cells)
- 15 mL test tubes
- 10 ml Syringe
- 3 ml dropper
- 100-1000 µl pipette
- 20-200 µl pipette
- 2-20 µl pipette
- Disposable pipette tips
- Microcentrifuge tubes
- Fluorescence microscope
- Fluorescence plate reader
- Countess cell counter

Experimental Methods

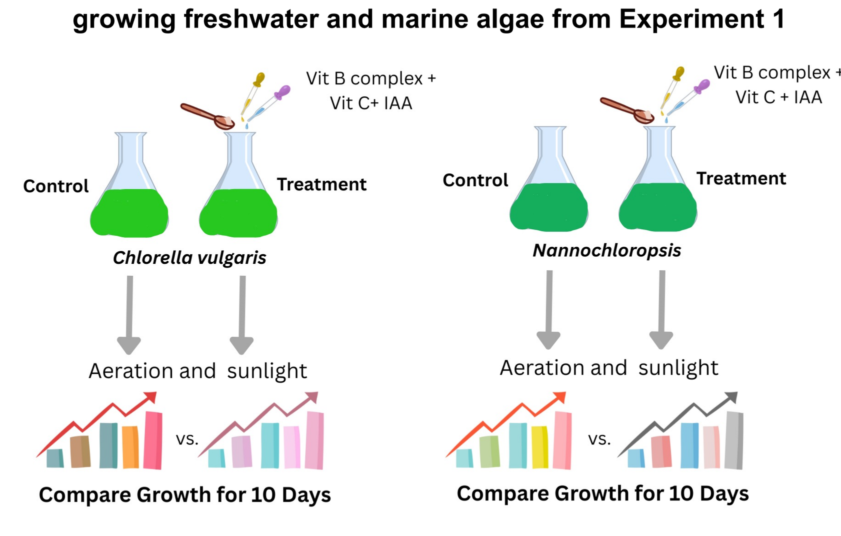
Experiment 1
Comparing growth kinetics of different microalgae



Experiment 2
Effect of individual additives on photosynthesis by *Chlorella vulgaris*

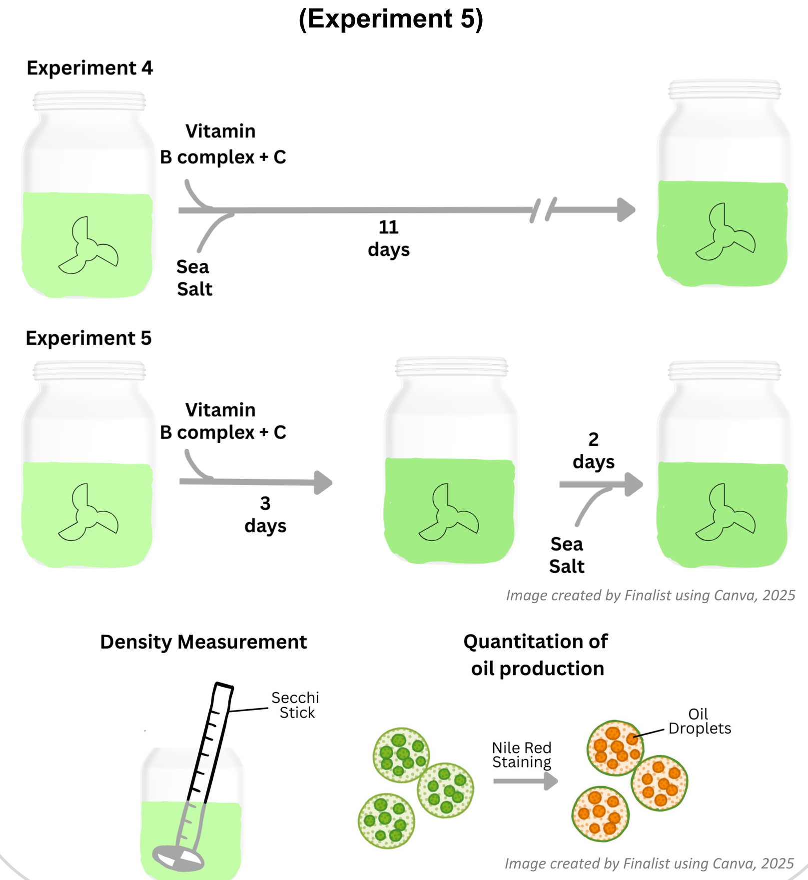


Experiment 3
Effect of vitamin B complex, C and IAA on the fastest growing freshwater and marine algae from Experiment 1

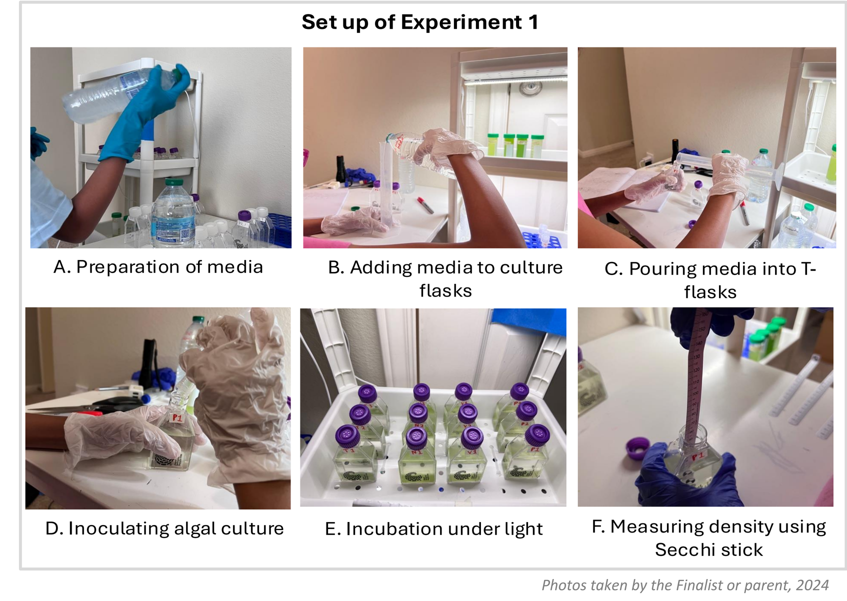


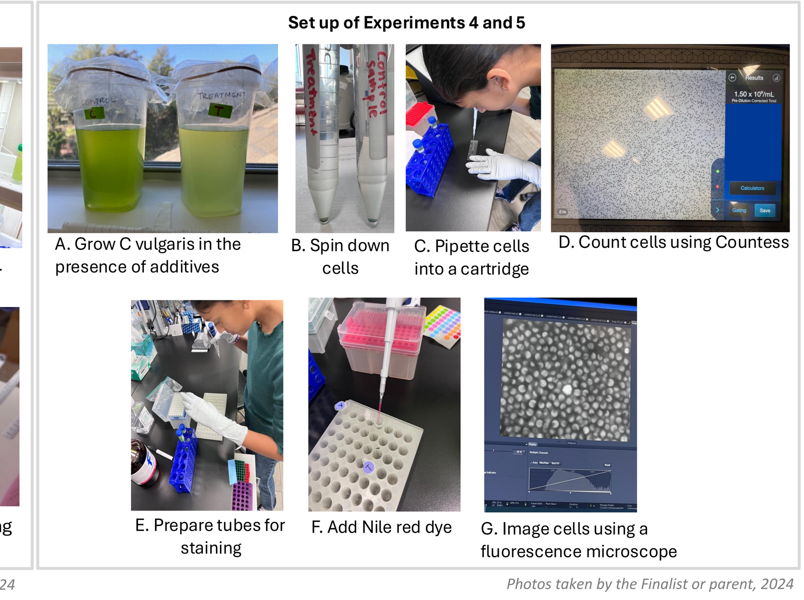
Experiments 4 and 5

Effect of vitamin B complex, C and sea salt on growth and lipid production by microalgae when added either simultaneously (Experiment 4) or sequentially (Experiment 5)



Experimental Setup





Data

Figure 1: Comparing growth kinetics of the microalgae: *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Nannochloropsis*, and *Tetraselmis*

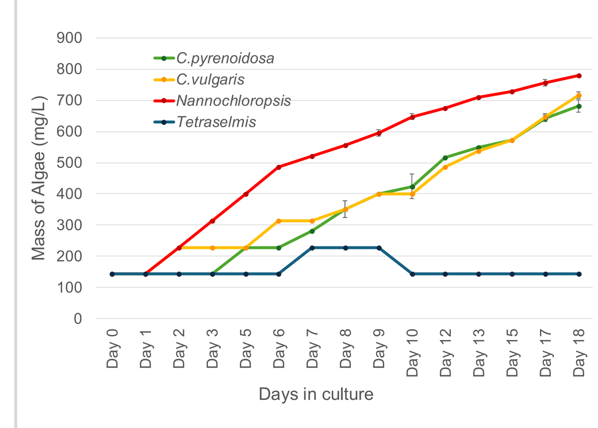


Figure 2: Effect of various additives on the photosynthesis rate of *Chlorella vulgaris*

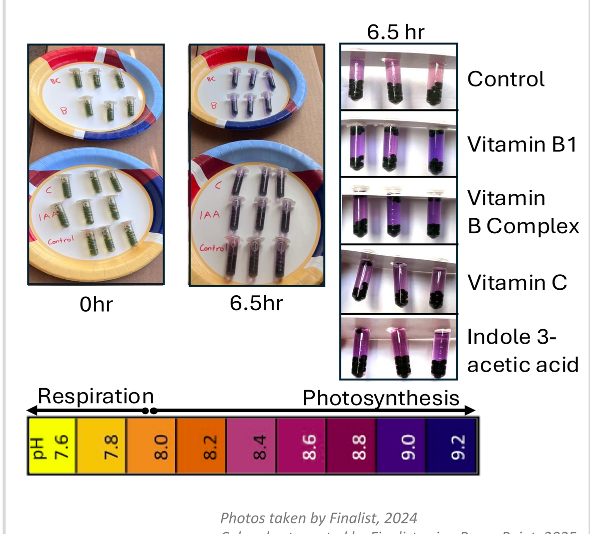
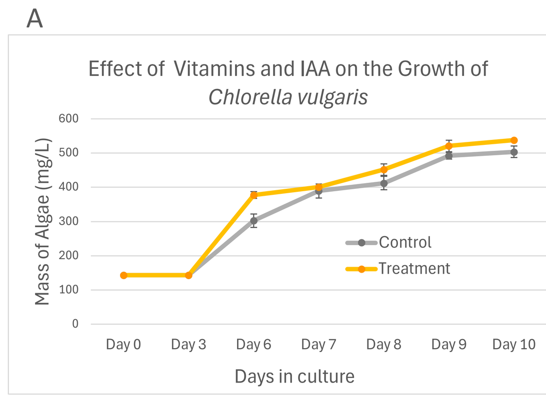


Figure 3: Effect of vitamin B complex, C and IAA on *C. vulgaris* and *Nannochloropsis*.

A



B

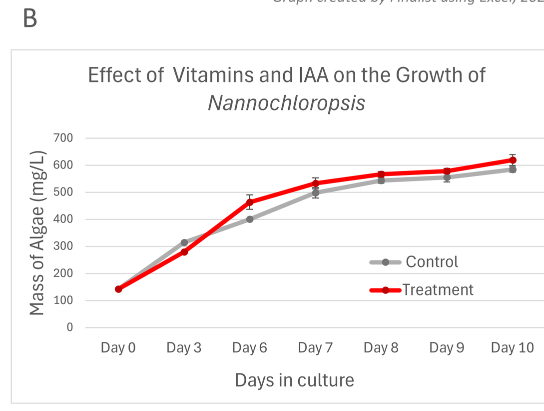


Figure 4a: Effect of Vitamin B complex, C, and the addition of salt simultaneously on the growth of *Chlorella vulgaris*

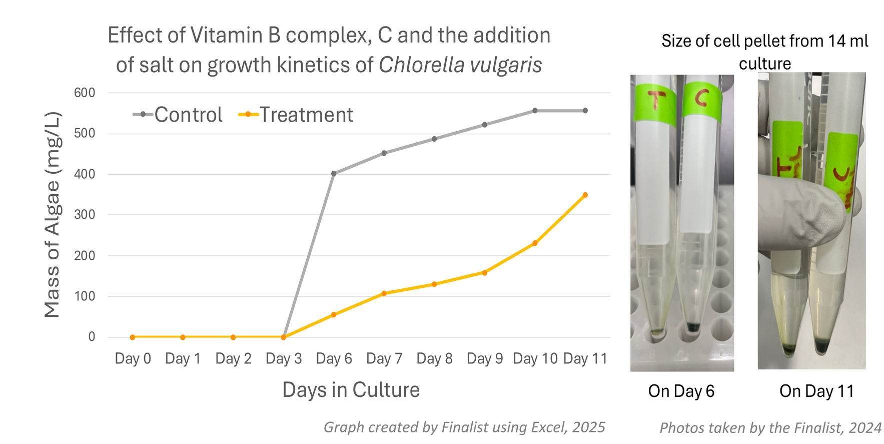


Figure 4b: Effect of Vitamin B complex, C, and the addition of salt simultaneously on lipid production by *Chlorella vulgaris*

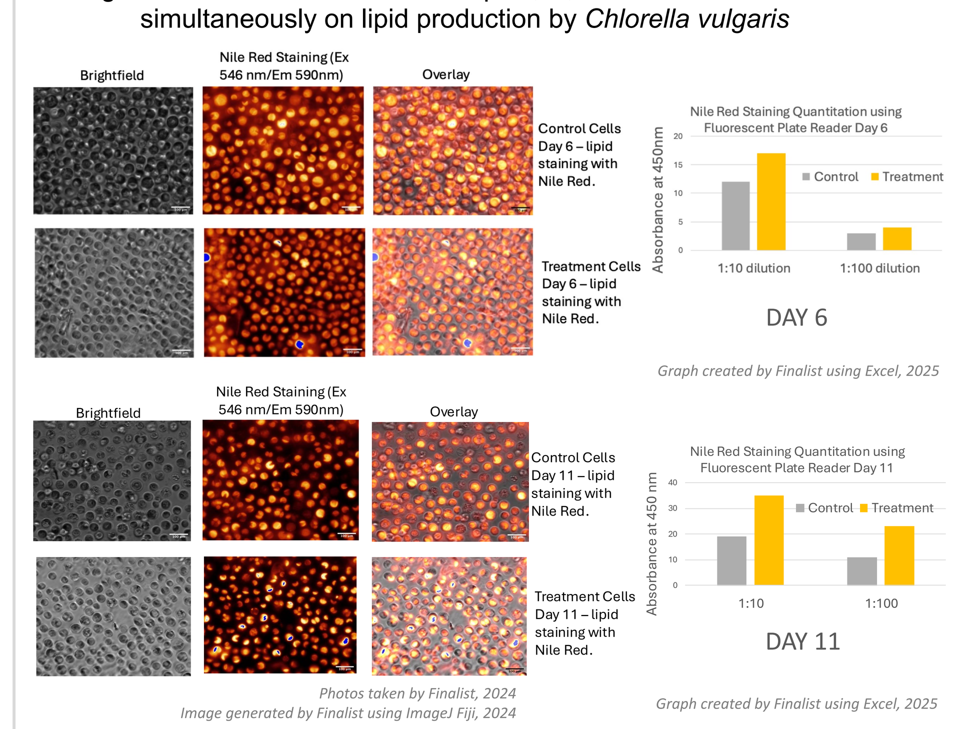


Figure 5a: Effect of Vitamin B complex, C, and the addition of salt sequentially on the growth of *Chlorella vulgaris*

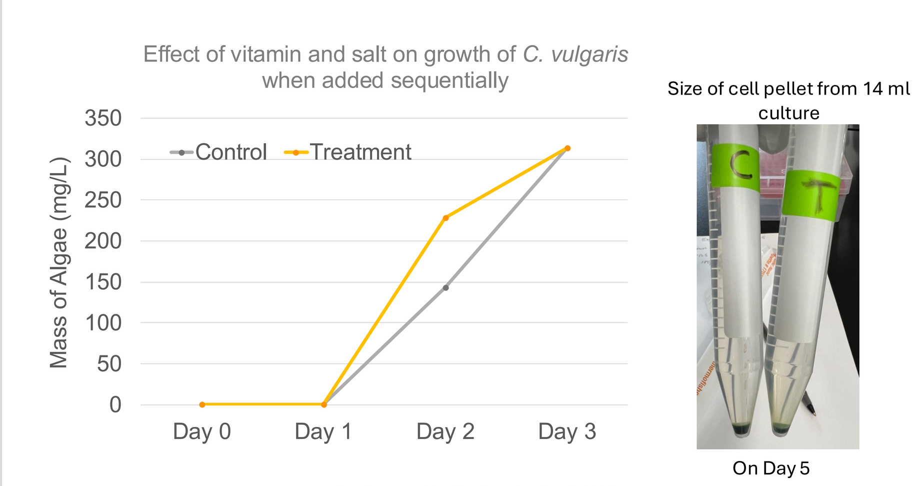
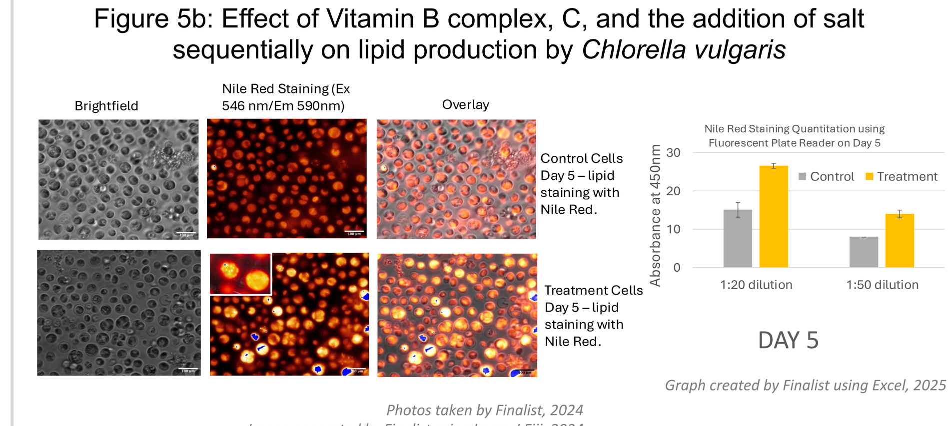


Figure 5b: Effect of Vitamin B complex, C, and the addition of salt sequentially on lipid production by *Chlorella vulgaris*

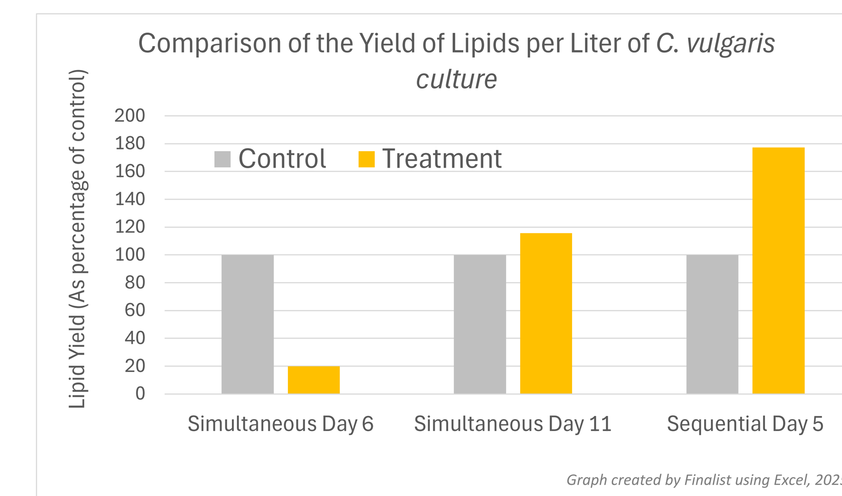


Results

- Results from this study showed that *Chlorella vulgaris* and *Nannochloropsis* have high growth rates.
- The addition of vitamins B complex and C increased the rate of photosynthesis, and co-treatment of vitamins and plant hormone IAA increased biomass yield of *Nannochloropsis* and *Chlorella vulgaris* by 6% and 7%, respectively.
- Treatment with salt and vitamins simultaneously lowered biomass yield, however, increased lipid production by ~84% per mg of microalgae by Day 11.
- Treatment with vitamins for first three days followed by salt stress for next two days did not lower the biomass yield, however, it increased production of lipids by 77% per mg of microalgae by Day 5.

Conclusion

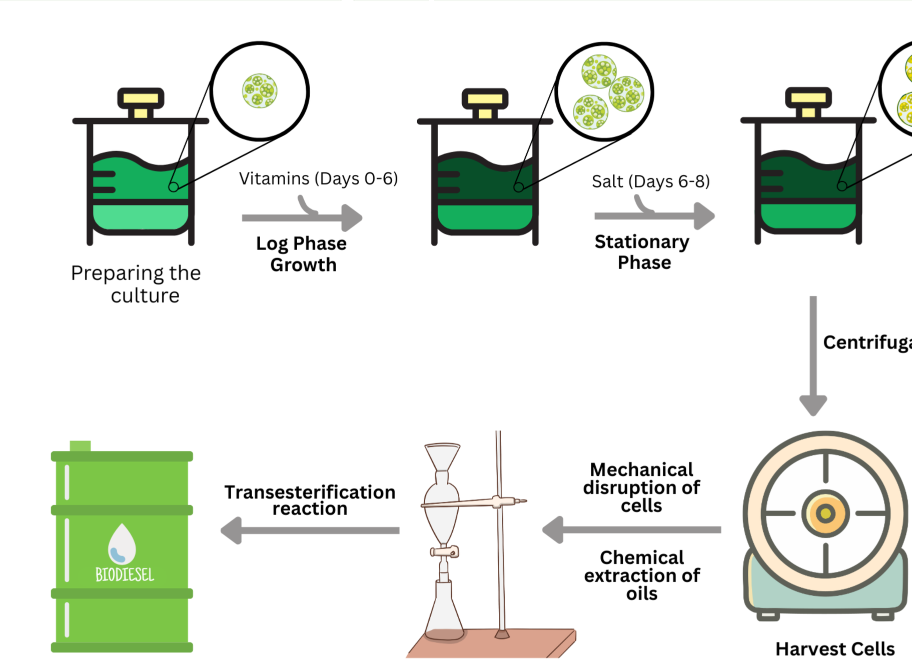
- Based on the results, it can be concluded that vitamins and high salt stress help increase algal growth and lipid production in a positive way.
- If vitamins and salt are added simultaneously, even though there is an increase in lipid production per cell, the amount of biomass will be less, resulting in low total lipid yield in the culture.
- The better way would be a two-step process in which the addition of vitamins come first for three days, followed by the addition of salt. This would result in the highest total lipid yield in the culture.



Future Direction

- Scaling up the 2-step process to a manufacturing scale.
- Optimization of Step 1 (vitamin treatment) and Step 2 (salt stress) to be done based on the optimal duration for each stage in the microalgal life cycle in a bioreactor:
  - Lag phase (adaptation): about 1 day
  - Exponential growth phase (vitamin treatment): 4 to 7 days depending on species and conditions.
  - Stationary phase (addition of salt): around day 6 to 8.
  - Harvesting process: Can vary from 1 hour to a day.
  - Cleaning/sterilization and preparation for the next cycle: Can take up to multiple days depending on size of the bioreactor.
- Test if low salt stress increases oil production by marine microalgae *Nannochloropsis*.

Scaling Up for Production



Flowchart created by Finalist using Canva, 2025
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PATENT PENDING