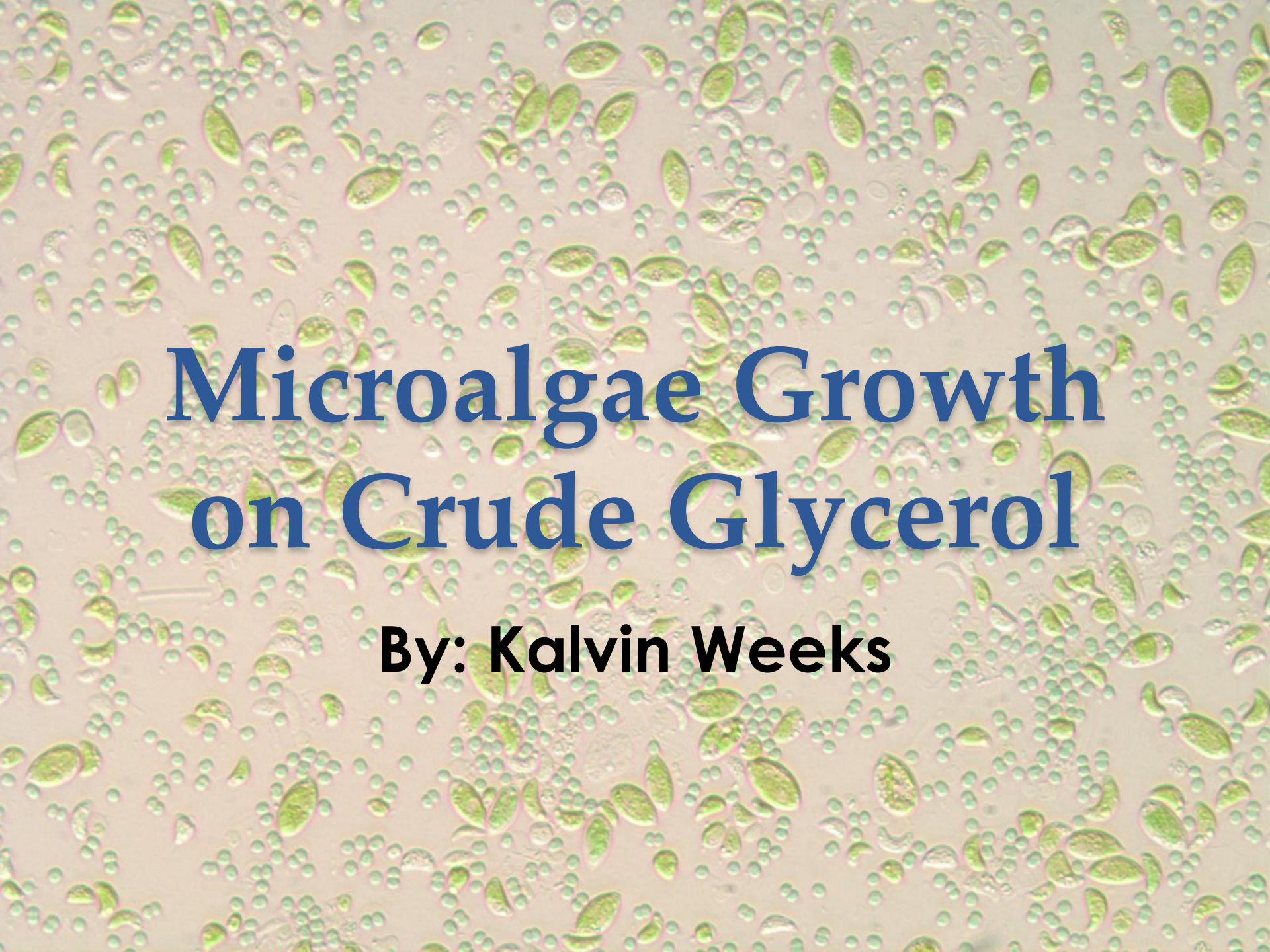


Microalgae Growth on Crude Glycerol

By: Kalvin Weeks

What are microalgae?

- Microalgae are unicellular organisms, usually autotrophic, capable of growing in the most diverse environments found across the earth

- Responsible for petroleum deposits
- Vast number of species estimated to be in the millions
- Characteristics comparable to autotrophs and heterotrophs

Microalgal Advantages

- Can be grown on un-arable land, grown in salt water bodies (oceans and bays), and grown on water unfit for human consumption or agriculture

- These organisms can reach high densities per unit area and produce many marketable products such as:
  - Biomass (protein feedstock for animals and humans)
  - Lipids (used as a feedstock for production of biofuels)
  - Health Additives (omega-3 fatty acids)

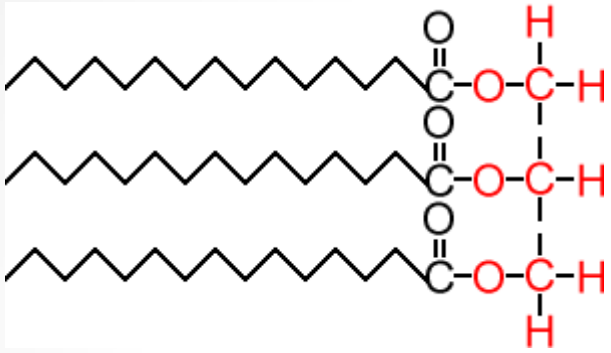
Crude Glycerol

- Crude Glycerol or bottoms is a co-product of biodiesel production

- Formed when methanol replaces glycerol, the 3 chain carbon backbone of triglycerides (oils).

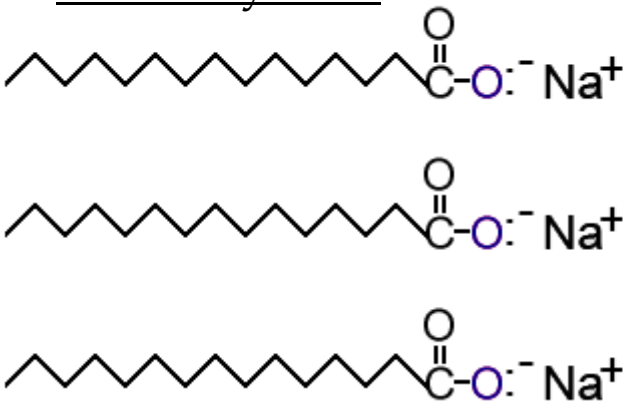
  - High energy density
  - Low cost
  - Contains impurities (methanol and soaps)

Triglyceride

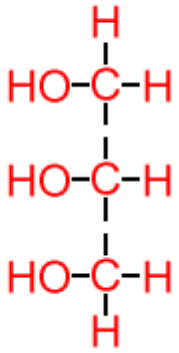


Biodiesel Process

Free Fatty Acid



Glycerol



Hypothesis

- Algae can be grown on crude glycerol to take advantage of this low-cost, energy dense carbon source.

- Mixotrophic growth using crude glycerol will result in higher growth rates and cell densities.

Objectives

- Evaluate crude glycerol as a source of carbon for algal growth which will result in:
  - Enhanced algal growth
  - Enhanced lipid production
  - Economical source of energy dense carbon

Methodologies

- Algae were grown under laboratory conditions
  - Autotrophically and mixotrophically grown with 12 hours light/ 12 hours dark
  - Heterotrophically grown in black flasks to prevent light penetration
  - Temperature kept constant in both containers at 30°C
  - Aerated and shaken daily

Variables

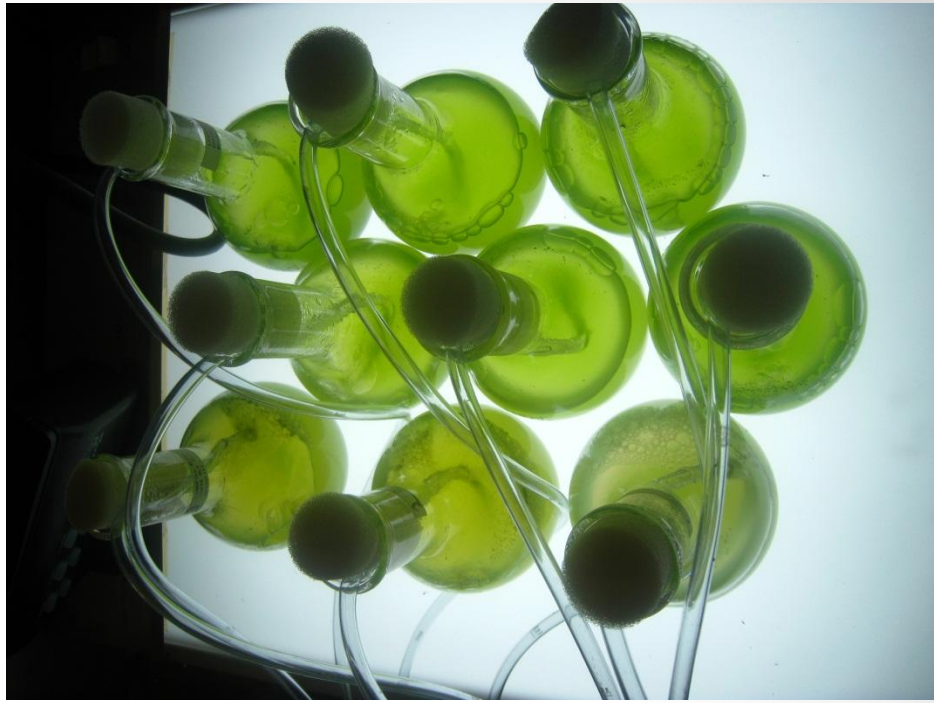
Carbon sources:

- Crude Glycerol
- Pure Glycerol

Concentrations:

- .1 g/L (mixotrophic)
- 1 g/L (heterotrophic)

Controls were an autotrophic triplicate with no additives, and a heterotrophic triplicate with no additives

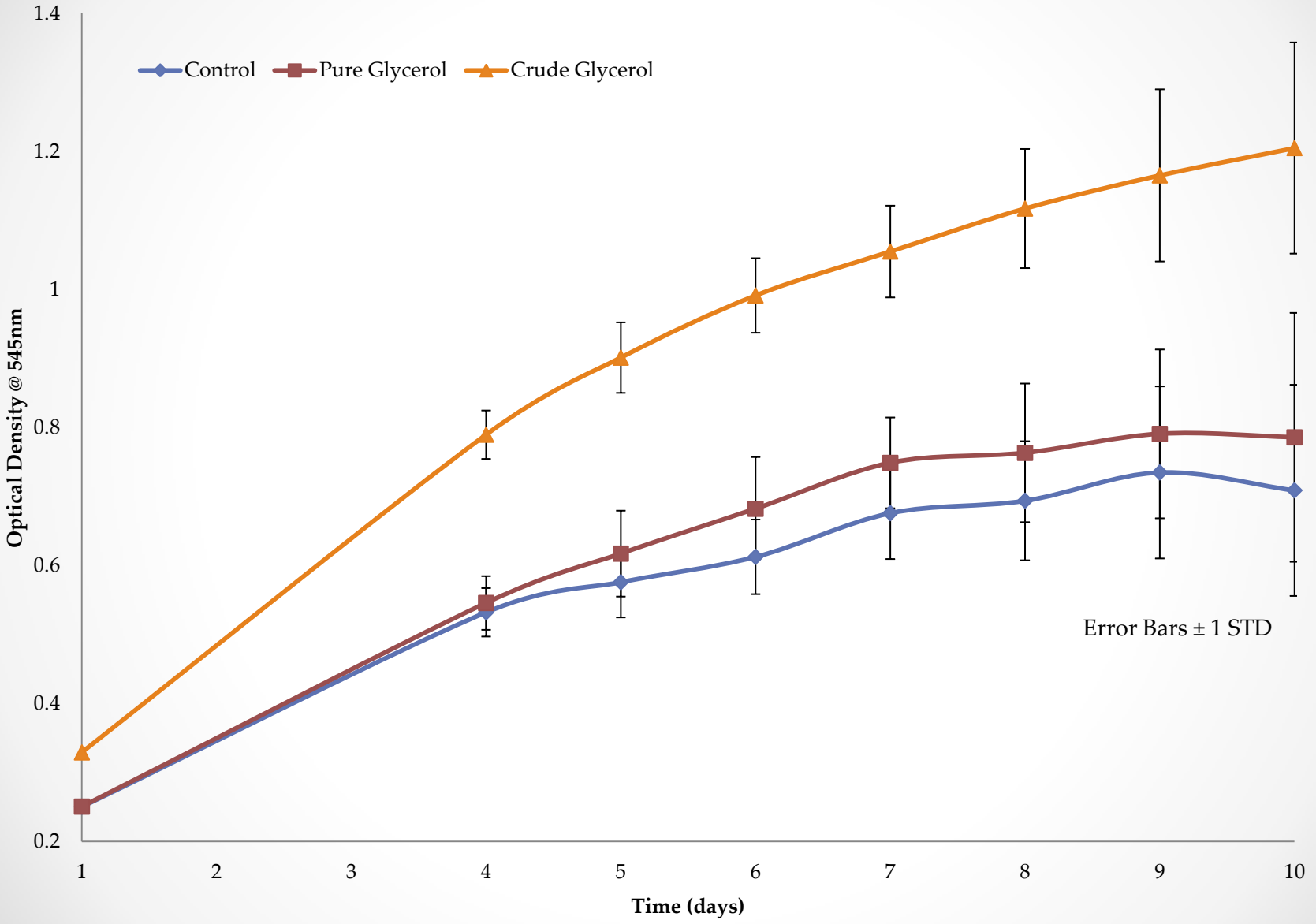


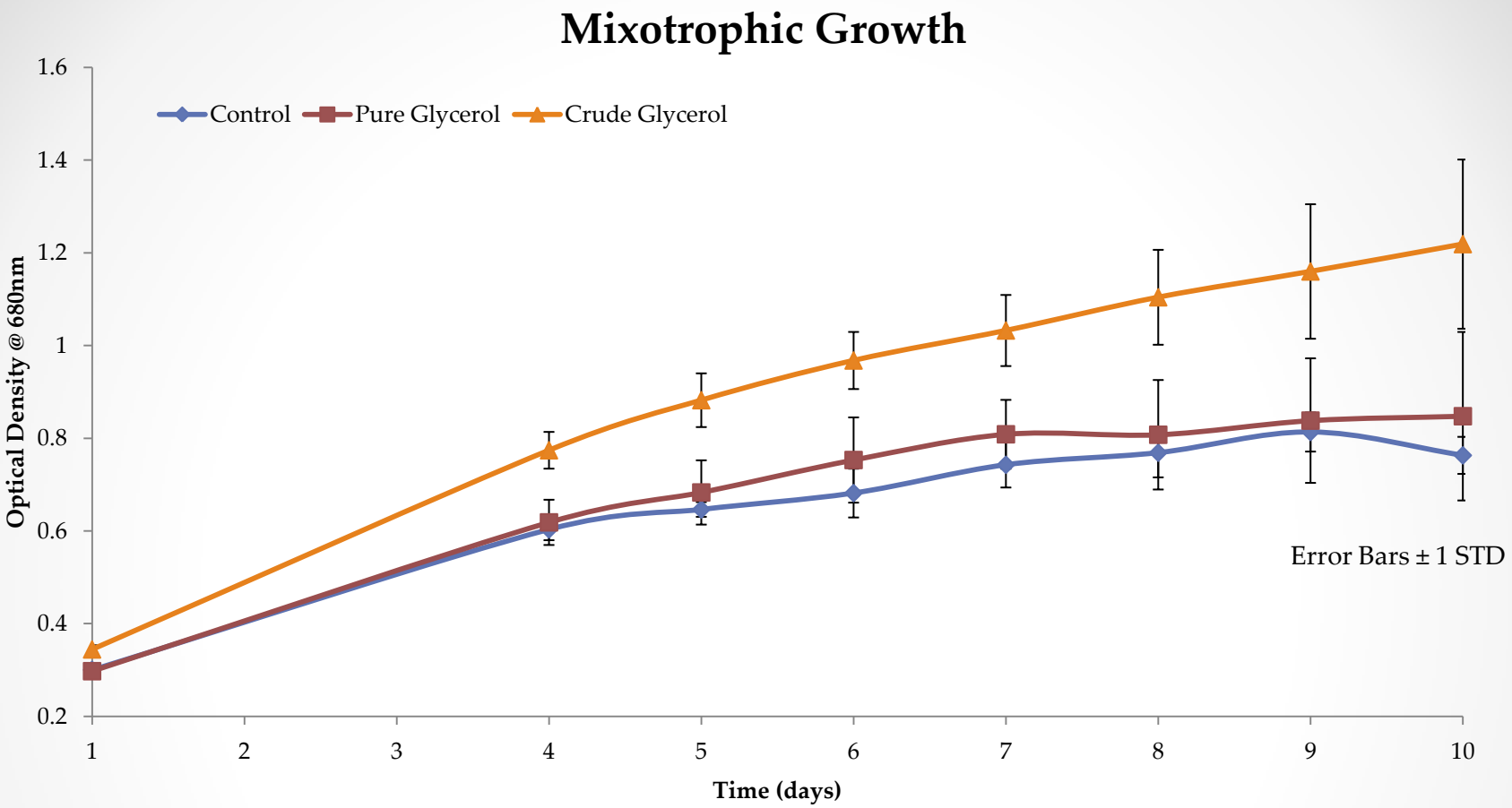
1st Triplicate	2nd Triplicate	3rd Triplicate	4th Triplicate	5th Triplicate	6th Triplicate
Autotrophic Control	Mixotrophic Pure Gly.	Mixotrophic Crude Gly.	Heterotrophic Control	Heterotrophic Pure Gly.	Heterotrophic Crude Gly.
200mL Algae	200mL Algae	200mL Algae	300mL Algae	300mL Algae	300mL Algae
No additions	.1 g/L Pure Glycerol	.1 g/L Crude Glycerol	No additions	1 g/L Pure Glycerol	1 g/L Waste Glycerol

Measurements

- Optical Density
  - Measured at 545nm and 680nm
- pH
- Lipid stain analysis
  - Used Nile Red stain dissolved in acetone
  - Fluoresces yellow under UV illumination

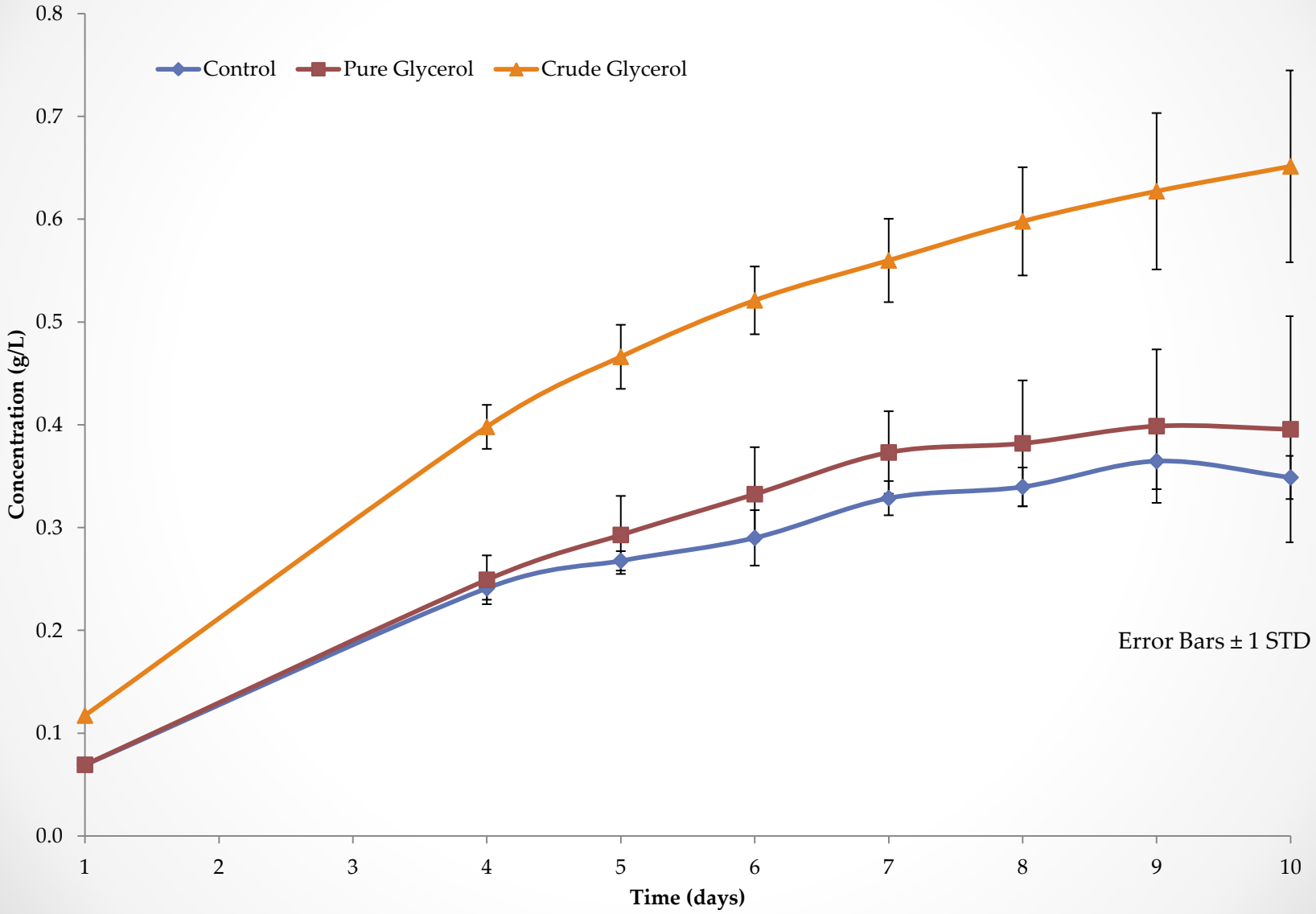
Mixotrophic Growth



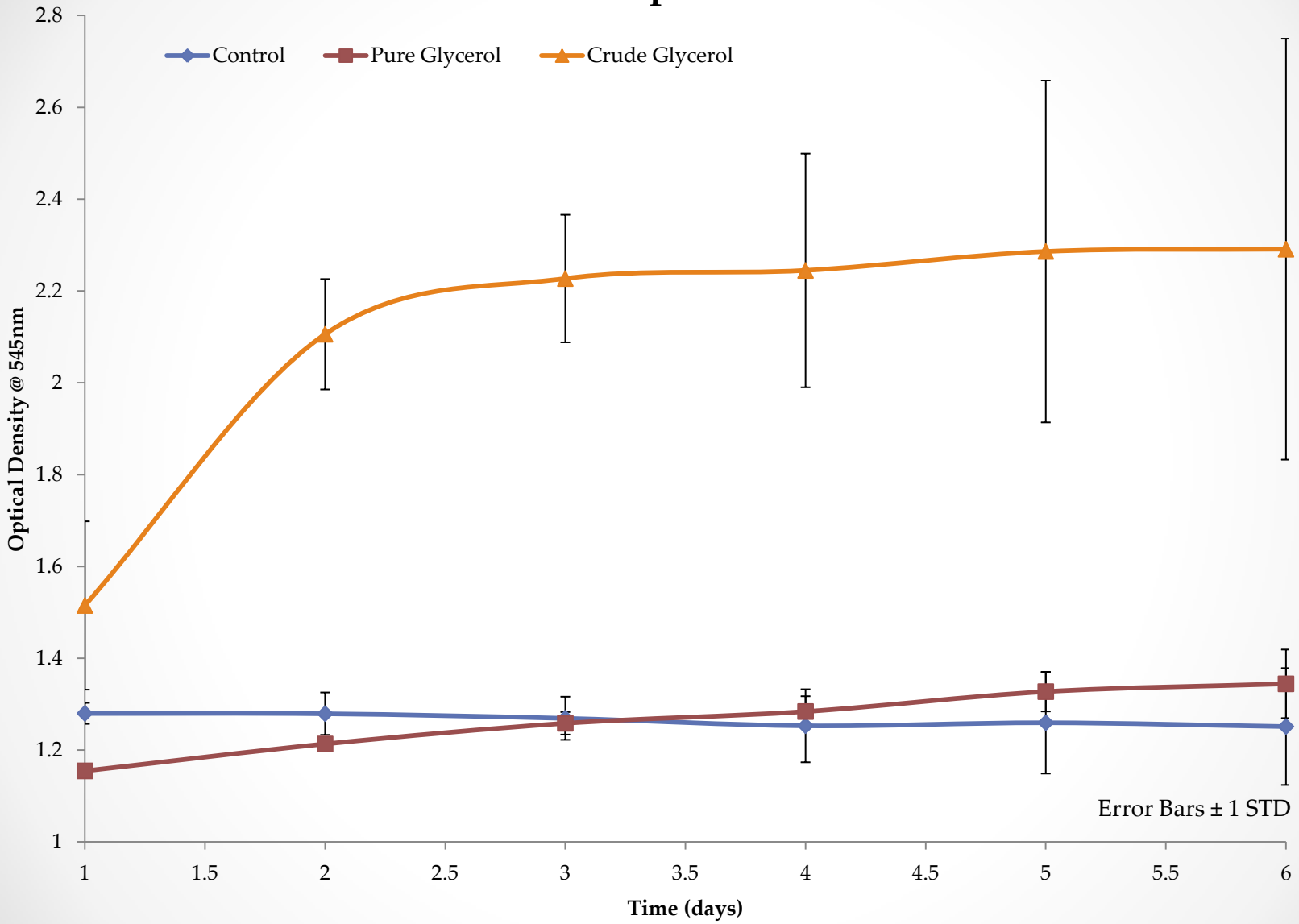


- Crude Glycerol grew to higher densities than both the control and pure glycerol
    - Fascinating find since impurities show to result in increased growth

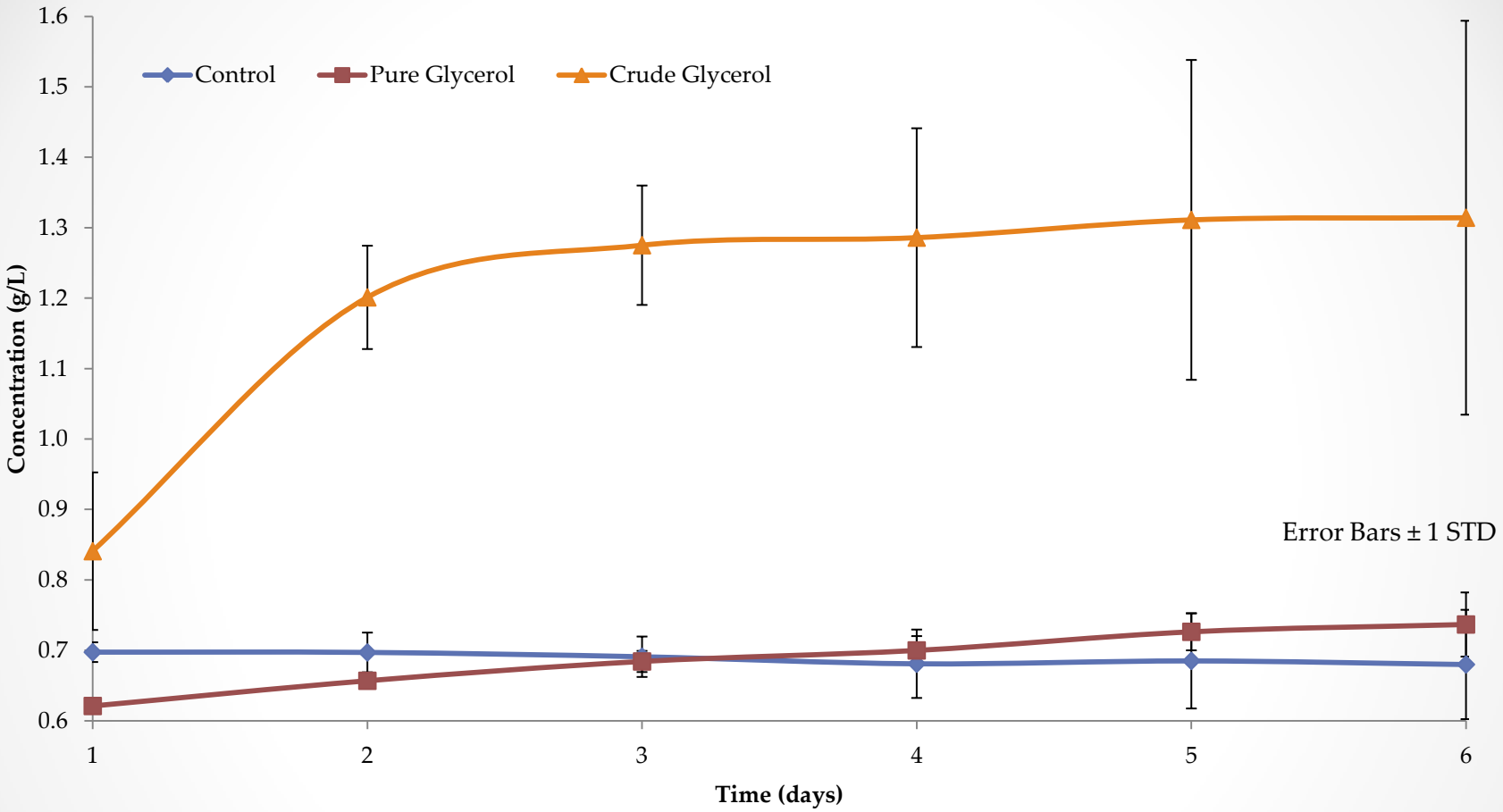
Mixotrophic Concentration (g/L) from OD @ 545nm



Heterotrophic Growth

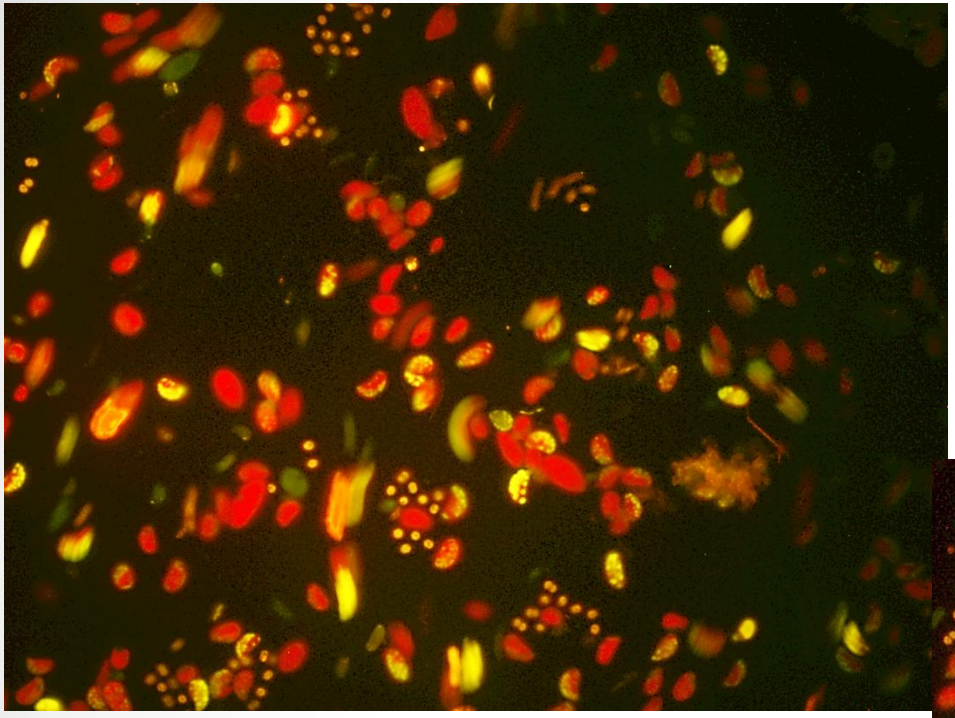


Heterotrophic Concentration (g/L) vs. Days from OD @ 545nm



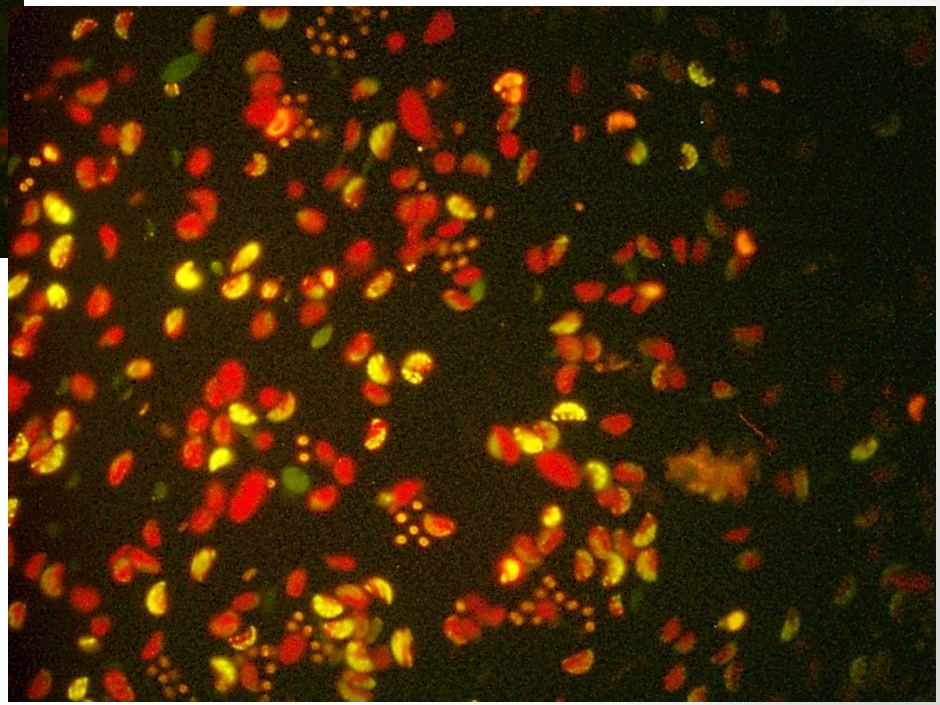
In heterotrophic experiments, crude glycerol performed better at cell growth rate and reached higher cell densities.

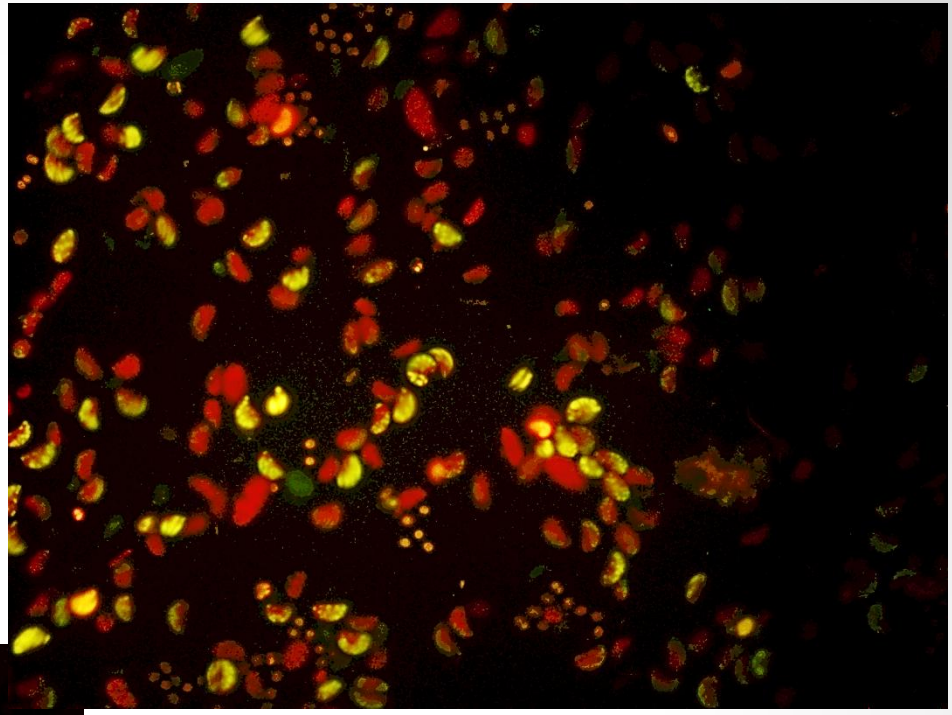
Lipid Stain



Mixotrophic Crude Gly. – 4 days into experiment

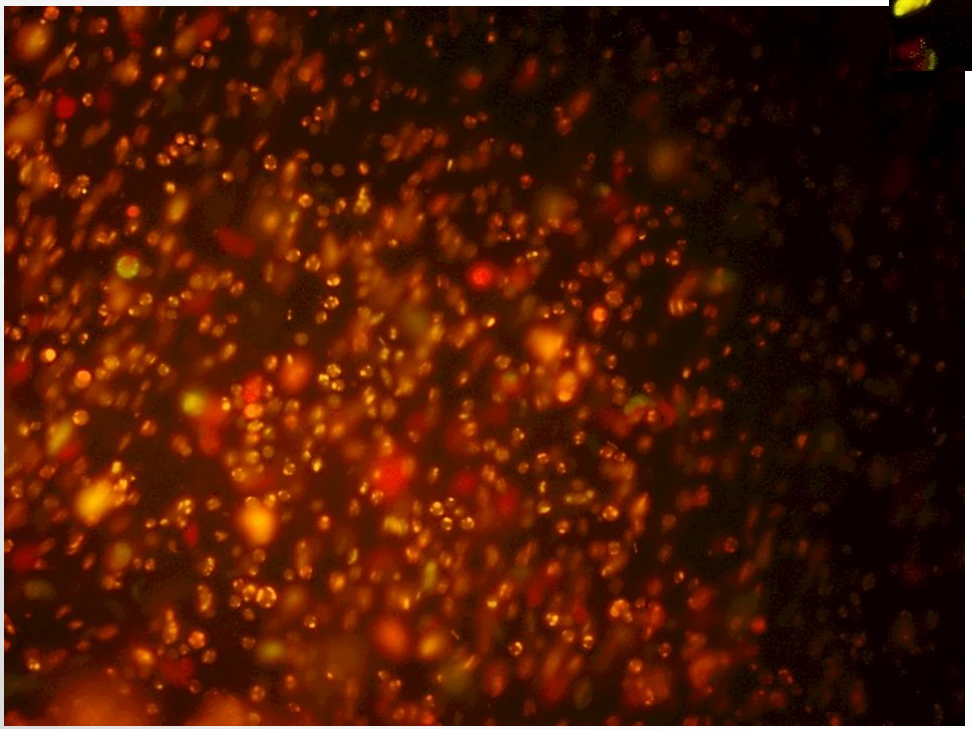
Mixotrophic Crude Gly.– 4 days into experiment

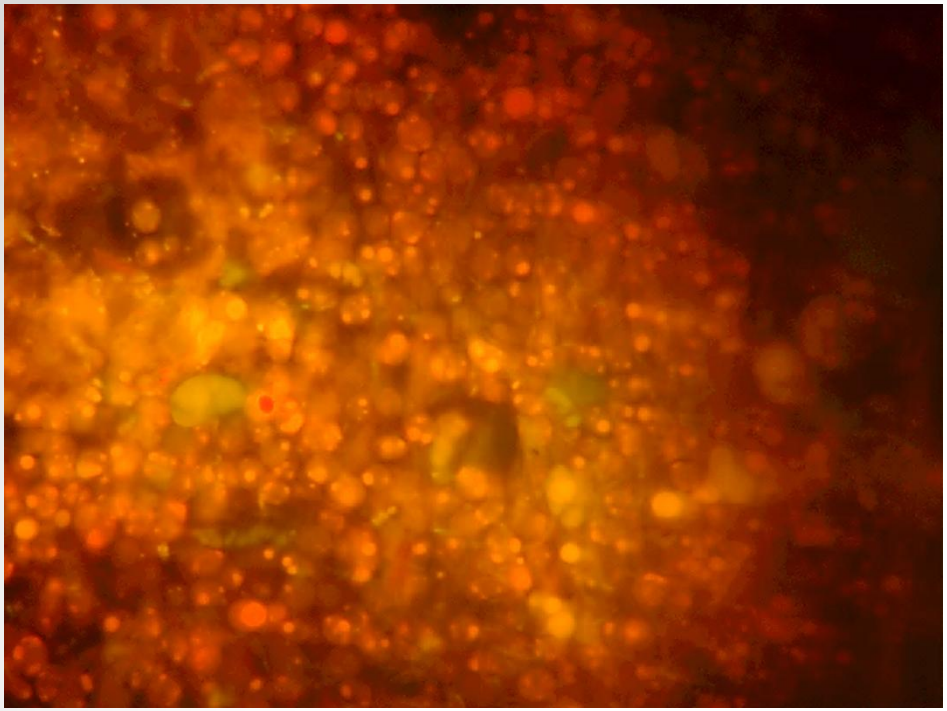




Mixotrophic Crude Gly. – 4 days into experiment

Heterotrophic Crude Gly. – Final Day





Heterotrophic Crude Gly. – Final Day

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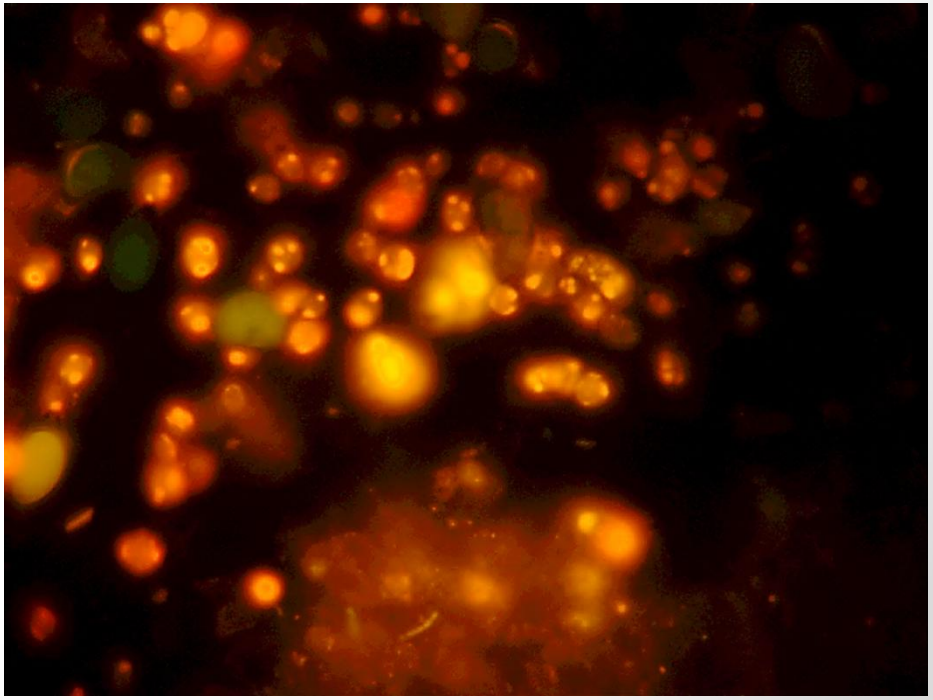

Results

- Crude Glycerol outperforms both control and pure glycerol in both mixotrophic and heterotrophic trials

- **Growth Rate:** (calculated by (day 2 – day 1)+ (day 3 day 2)….etc. and then divided by total days)

Autotrophic Control	Mixotrophic Pure Gly.	Mixotrophic Crude Gly.	Heterotrophic Control	Heterotrophic Pure Gly.	Heterotrophic Crude Gly.
.040 g/L/day	.047 g/L/day	.076 g/L/day	-.003 g/L/day	.023 g/L/day	.095 g/L/day
		1.9x the growth rate of control			4.1x the growth rate of pure gly.

- Growth rates for crude glycerol is higher than all other trials
  - Initial Growth rates for heterotrophic trials were much higher than average growth rates: 0.36 g/L/day
- Studies of optical densities shows a higher cell density present in flasks with crude glycerol
- The use of crude glycerol promotes lipid accumulation
  - Excess carbon is used up and stored in oils

Conclusions

- Algae can grow on crude glycerol
  - Crude glycerol resulted in higher growth rates and cell densities
- Growth on crude glycerol resulted in lipid synthesis
- Heterotrophic growth conditions show a more rapid growth rate

What does this mean?

- Algae can first be grown to a maximum cell density with natural sunlight and mediums (Landfill Permeate)

- Collected into a mixotrophic system
  - Crude glycerol is added
  - Algae will grow on both sunlight and surplus crude glycerol

- Mixotrophic system transformed to Heterotrophic
  - Algae become stressed and immediately absorb crude glycerol and store in the form of lipids

<u>Results</u>

- Increased cell density
- Increased volume of lipids per unit volume of algae
- Cultivation system is more optimized, more efficient, resulting in a more economical production system

- This algae is cheaper to process and contains more marketable items per unit weight

- Further steps will include processing the lipids into biodiesel, which will result in more crude glycerol which can be recycled back into the cultivation system

- Once a burden, crude glycerol is an economical choice for algal production