Omega-3 Fatty Acid Production Using Crude Glycerol

Daniel Nelson, Sridhar Viamajala, Ronald C. Sims
Biological and Irrigation Engineering Department, Utah State University
Contact: daniel.nelson@aggiemail.usu.edu

SERC SUSTAINABLE ENERGY RESEARCH CENTER AT UTAH STATE UNIVERSITY

UtahState UNIVERSITY

Introduction

Production of omega-3 fatty acids, high value nutritional supplements, is being investigated using microbial cultures. Crude glycerol, a major co-product of biodiesel production, is a low value carbon source that could potentially be used for growing organisms that accumulate omega-3 fatty acids. *Schizochytrium limacinum SR21,* a marine fungus, is of interest because it accumulates docosahexanoic acid (DHA). Current analytical methods for omega-3 fatty acids are time consuming and require multiple solvents. During this research, we have developed new methods for fatty acid extraction and analysis and have compared results with tests using existing methods. The new methods are simpler and have the potential to be scaled-up for effective extraction and conversion of multiple lipid types into fatty-acid alkyl esters that could be used as biofuel (e.g. biodiesel).

Objectives

- Observe cell growth and lipid accumulation differences between glucose and glycerol when used as carbon/energy source.
- Develop simpler methods for *in-situ* trans-esterification of triglycerides for fatty acid analysis.

Lipid Recovery & Analysis Methods

- Solvent extraction: Dry Cells, Hexane, Chloroform, THF → Sonication → Gas Chromatography (GC) Analysis
- Base-catalyzed extraction and transesterification: Dry Cells, Saponification Reagent (base) → 100°C for 30 minutes → Methylation reagent → 80°C for 10 minutes → Hexane → GC Analysis
- Acid-catalyzed *in-situ* transesterification: Cells, 5% H_2SO_4 in Methanol → 100°C for 60 minutes → GC Analysis
- Microwave assisted Acid-catalyzed *in-situ* transesterification with methanol: Dry Cells, Methanol, H_2SO_4 → Microwave 16 minutes → Water, Chloroform → GC Analysis
- Microwave assisted Acid-catalyzed *in-situ* transesterification with Butanol: Dry Cells, Butanol, H_2SO_4 → Microwave 16 minutes → Neutralize, Hexane → GC Analysis

Acknowledgements

- USTAR Biofuels Initiative
- Dr. Brett Barney, USU; Dr. Zhiyou Wen, Virginia Tech

Cell Growth Results

The cell growth was compared for two substrates glucose and glycerol. In culture, cells agglomerate and are difficult to separate. Microscopy shows this interaction.

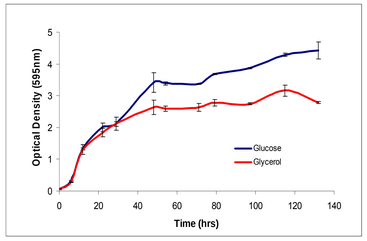


Figure 1: Cell cultures grown on 5% glucose and 5% glycerol. Biomass was harvested after 5 days and lyophilized.

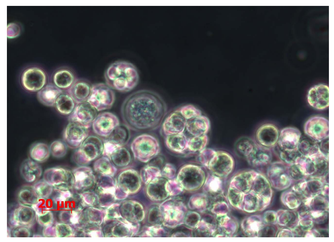


Figure 2: 1000X microscopy agglomerated cells in culture.

Lipid-class Analysis Results

Lipid accumulation in cells was analyzed using the Solvent extraction method. Preliminary GC analysis suggests lipid accumulation is greater with glycerol.

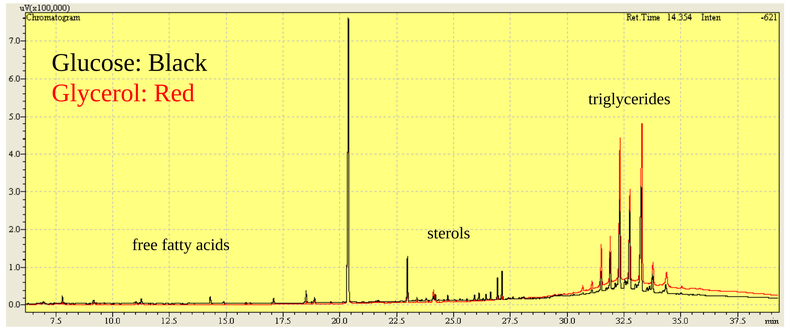
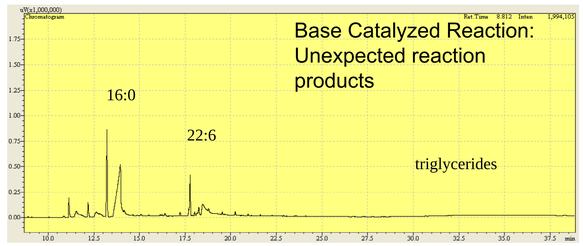


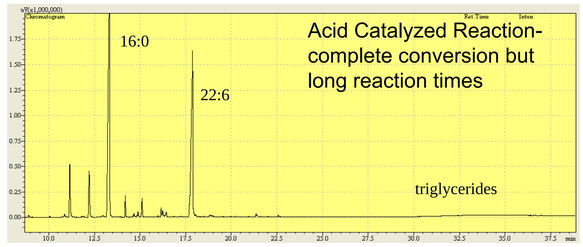
Figure 3: Overlay comparing total lipid production on glucose and glycerol. The areas of interest are triglycerides, sterols, and free fatty acids. It can be seen that triglycerides are the main lipid-class produced, with only minor amounts of free fatty acids or sterols.

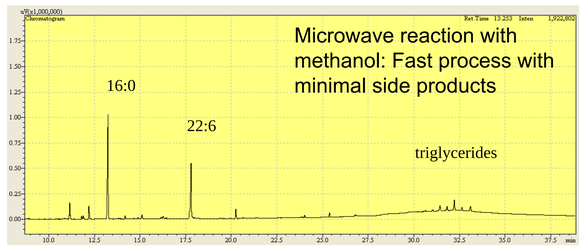
Future Work

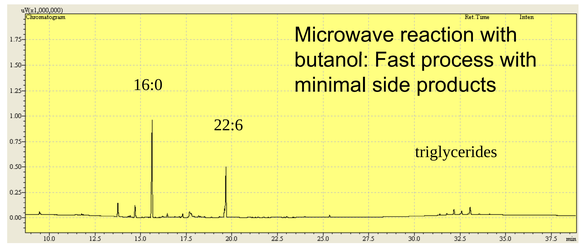
- Determine lipid profiles and kinetics of lipid accumulation in SR21 during growth on glycerol.
- Quantify rates of *in-situ* acid catalyzed transesterification of lipids using SR21 as a model organism.

Transesterified Lipid Analysis Results









Conclusions

- Specific lipid content is higher in SR21 cells grown on glycerol in comparison to cells grown on glucose.
- Microwave assisted *in-situ* transesterification is rapid and can potentially be applied for recovery of lipids from dry and wet biomass.