Scale up of Bioplastic (PHB) Production from Algae Based Media

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ABSTRACT

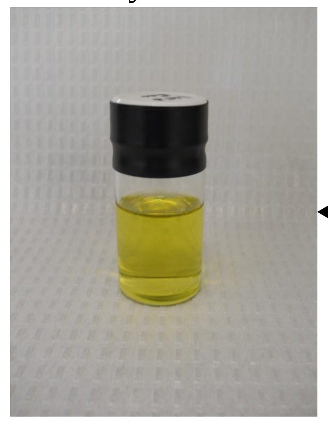
As crude oil consumption continues petroleum reserves are being depleted, driving up energy and fuel prices as well as the cost of producing synthetic plastics. The non-biodegradability of synthetic plastics has led problems with pollution and the significant buildup of synthetic plastic material in landfills. An alternative to the use of petroleum based plastics is the use of biodegradable plastics that are produced using raw materials that are biologically derived. One class of biodegradable plastics is polyhydroxyalkanoates (PHAs), of which polyhydroxybutyrate (PHB) is the most studied. PHB is biopolymer that is synthesized by many organisms and is naturally produced in certain organisms as an energy storage molecule. PHB has properties similar to traditional plastics such as polystyrene and polypropylene. However, PHB is not produced at commercial scales due to the cost associated with processing and producing large quantities of PHB. The focus of this project has been to scale up the production of PHB by utilizing a novel strain of E. coli capable of secreting PHB, making use of waste material, specifically from algae biofuels processing, and different extraction techniques to aid in lowering PHB production and processing costs.

INTRODUCTION & BACKGROUND

Microalgae can be a significant source of renewable biomass for the production of various bio-products including biofuels and bio-based materials. As algae grow they take up nutrients such as nitrogen and phosphorous, compounds that require removal from wastewaters such as municipal wastewater. Therefore, coupling wastewater remediation with the production of algal biomass has tremendous potential to generate large quantities of renewable biomass. This biomass can be grown and harvested using rotating algal biofilm reactors, a technology developed at Utah State University. Once harvested the algal biomass can be processed, using an Utah State University procedure also developed to produce feedstock material for the production of various bio-based products.

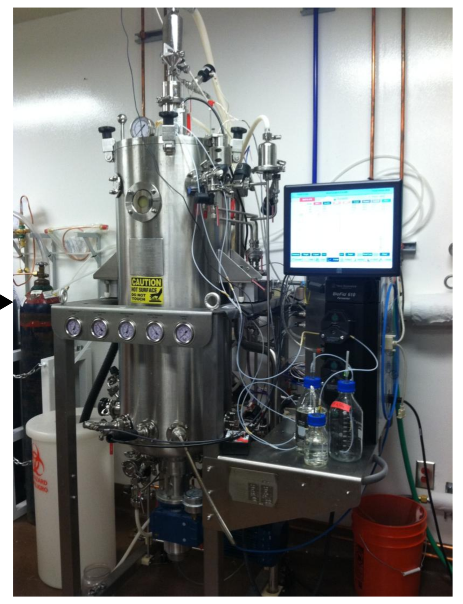
ABE Fermentation:
Hydrolyzed algal biomass is used as a substrate for ABE fermentation to produce acetone, butanol, and ethanol.

Algal Biodiesel Production:
Algal lipids, or oils, can be extracted and isolated through this procedure. These oils can be directly converted to fatty acid methyl esters or biodiesel..



Wet Lipid Extraction Process

Bioplastic PHB Production:
An aqueous phase generated from the acid and base hydrolysis of algal biomass is used as a media for the growth of recombinant *E. coli.*



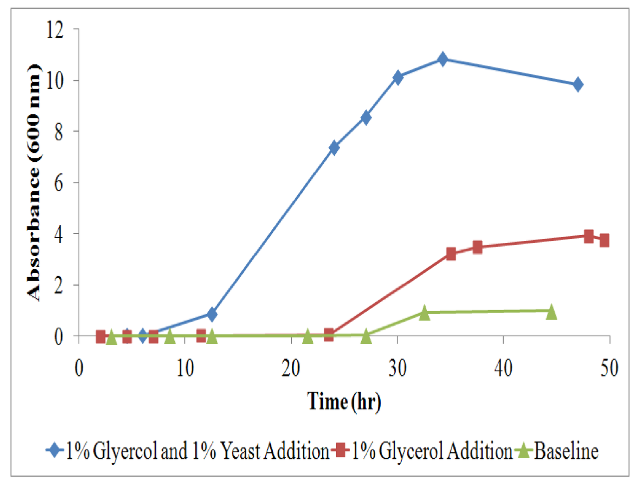
Algal Biomass Growth and Harvest:
A rotating algal biofilm reactor (RABR) can be used to remediate wastewater (removal of nitrogen and phosphorous) through the growth of algae. This system promotes growth of algae in biofilm form providing a straightforward means of harvesting large quantities of algae.

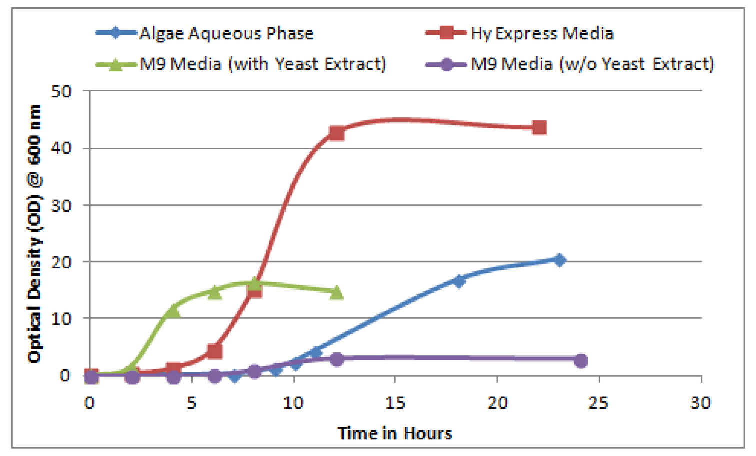
GROWTH OF *E. coli*

Growth experiments using recombinant *E. coli* began at the shaker flask scale (100 – 200 mL) cultures. Objectives of the initial experiments focused on testing the ability of the algae aqueous phase in supporting growth of *E. coli* and determining if PHB can be produced by *E. coli* grown on the aqueous phase. Growth studies were performed to improve the growth of *E. coli* in the aqueous phase and Once it was determined that the aqueous phase showed the capability to sustain growth by itself and/or with supplementation, the scale of fermentation was increased to test the feasibility of using the aqueous phase at larger scales.

Shaker flask to 10 L Fermentor

10 L to 125 L Fermentor





Media	Maximum OD:
Glucose Based (M9)	13.2 (1% Glucose and YE)
Glycerol Based (M9)	10.8 (1% Glycerol and YE)
Algae Based w/Glycerol	17.0 (1.5% Glycerol, 0.2% Glucose, and 1% YE)

PHB PRODUCTION AND EXTRACTION

Production of PHB using recombinant *E. coli* was quantified using gas chromatography. *E. coli* grown on various medias and scales was harvested and evaluated for the amount of PHB produced. In addition to measuring the amount of PHB generated by the bacteria, several methods were also tested to extract and collect the PHB contained in the *E. coli* biomass. Chloroform is the most commonly used solvent. However, chloroform is toxic and difficult to scale up. Therefore, additional methods were tested that have potential for scaling.

Media	PHB Yield by dry cell mass:	Scale
Glucose Based (M9)	35%*/16-20%	Shaker flask/ Up to 100 L
Glycerol Based (M9)	16-20%	Up to 100 L
Aqueous Phase with Glycerol	13%	Shaker Flask

Sample of PHB extracted from *E. coli* biomass using Propylene Carbonate.

1 2 3 4 5

1. Dried *E. coli* biomass
2. Propylene carbonate extracted PHB (raw PHB)
3. Propylene carbonate extracted PHB with Acetone wash
4. PHB refined using chloroform and methanol
5. Sheet of PHB: Extracted and refined using chloroform and methanol.

SUMMARY OF PROJECT

Growth of *E. coli* has been achieved at various scales using different medias, including the algae aqueous phase. Extraction of PHB from the harvested biomass has also been performed at varying scales. However some methods evaluated were not scalable or were not feasible to scale up.

Media base/ Ext Method:	Flask Level:	5 L/10 L Level:	100 L Level:
Glucose in media:	✔	✔	✔
Glycerol in media:	✔	✔	✔
Algae Aqueous phase:	✔	✔	✔
Chloroform Extraction:	✔	N/a	N/a
NaOH Extraction:	✔	Failed at this point	N/a
Propylene Carbonate Ext:	✔	✔	✔

FUTURE WORK

In moving forward with this project there are a number of areas that require further attention to improve the production of PHB from *E. coli* grown in algae based media:

- **Continue to characterize/analyze and optimize the aqueous phase media for bacterial growth.**
- **Improve PHB yields from the secreting strain *E. coli* via genetic improvements.**
- **Optimize and continue to scale up extraction process of PHB, specifically the Propylene Carbonate method.**
- **Submit research as peer reviewed academic publications.**

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