Bioplastic Production from E. Coli grown in a Waste Stream Generated from Algal Biodiesel Production

Abstract

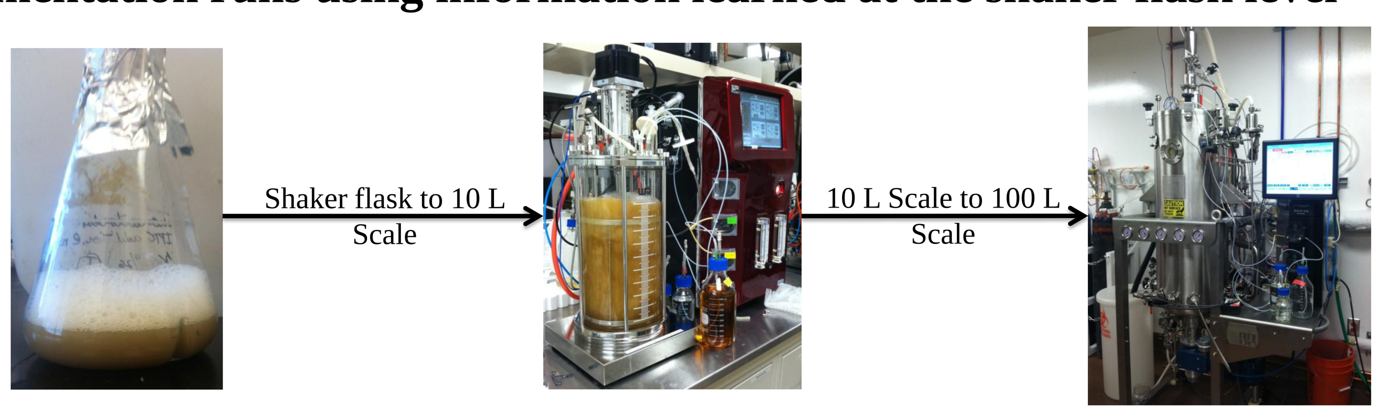
Poly-3-hydroxybutyrate (PHB), a subset of polyhydroxyalkanoates (PHA), can be generated in recombinant *Escherichia coli* (XL1BLUE). PHAs can be expensive to produce and purify with the carbon source and media being some of the major costs during fermentation. In order to reduce production costs, a waste product of algal biodiesel production was evaluated as a medium for bacterial growth. This aqueous waste stream is generated through acid/base hydrolysis of algal biomass and is rich in soluble nutrients. The aqueous phase was utilized as a media in shaker flasks (500 mL volume) to grow the bacteria. Additional supplementation used an inexpensive carbon source (glycerol) and yeast extract which resulted in high biomass production in comparison to no supplementation. In addition, gas chromatography results indicate the production of PHB from the *E. coli* grown on the aqueous phase. These results indicate that the aqueous waste stream generated from algal biodiesel production can be utilized for PHB production to help lower the cost of biodegradable bioplastic production.

Background

As crude oil reserves decrease throughout the world, petroleum based plastics are becoming less economical and environmentally friendly as they are non-degradable and are currently filling up landfills each year in the United States. New sources of biodegradable plastics are being researched to replace petroleum derived plastics. PHAs are a naturally occurring bioplastic generated by certain organisms as a carbon and energy storage molecule. Algal biomass grown and harvested from the Logan Lagoons wastewater treatment facility can be processed to generate the aqueous phase to use as a growth medium for *E. coli*.

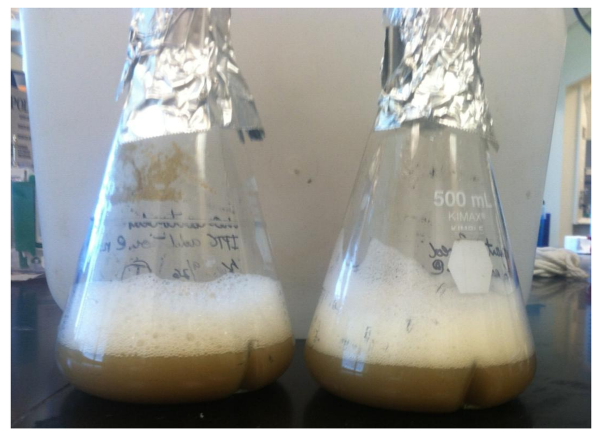
Objectives

- Assess the capability of the aqueous phase, generated from algal biomass, in supporting the growth of *E. coli*
- Determine if *E. coli* grown in the aqueous phase are still able to generate PHB
- Begin to evaluate deficiencies in nutrients in the aqueous phase by supplementing nutrients and measuring changes in *E. coli* growth
- Start characterization of the aqueous phase using various analytical methods
- **Scale up the growth of *E. coli* from shaker flasks up to 100 L scale fermentation runs using information learned at the shaker flask level**



Materials and Methods

Supplementation was approached with several different additives to find the best combination with the aqueous phase for the maximum growth. These additives included 1% glycerol, 1% trace elements (micronutrients), 4% Nitrogen-Phosphorus-Sulfur (NPS) solution, and 1% yeast extract. Each supplemented culture was grown in 125 mL volume and in a 500 mL shaker flask and housed in an incubator at 37˚C for 48 hours. Every test included an autoclaved solution and a non-autoclaved solution.



500 mL shaker flasks growing 125 mL of XL1BLUE in aqueous phase.

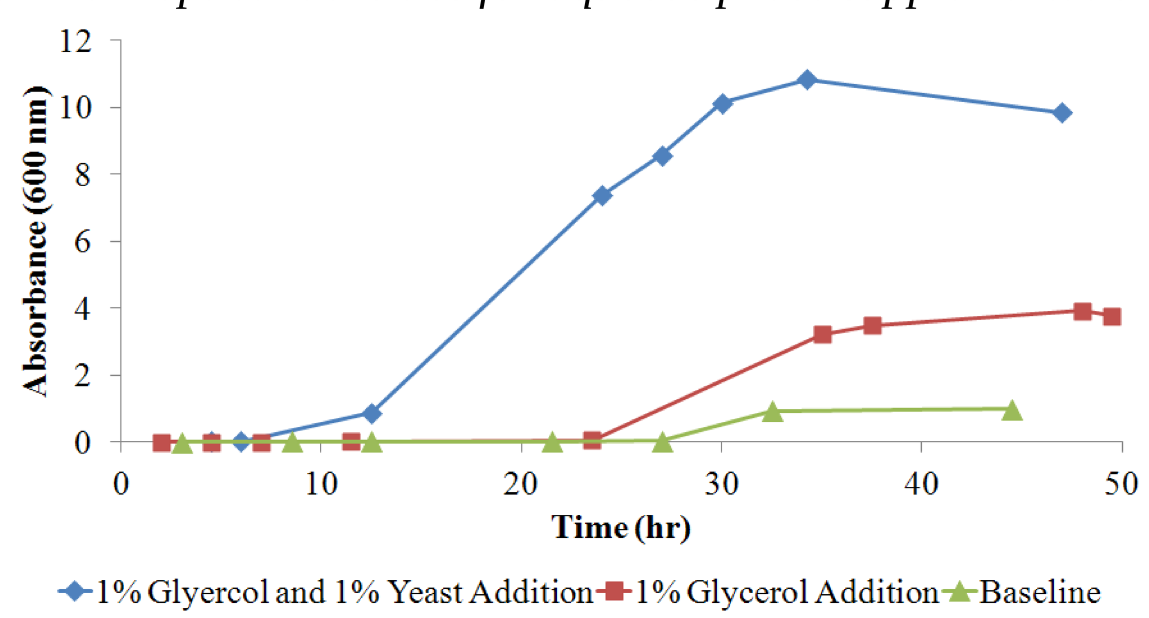
Results

A preliminary supplementation study was conducted to determine significantly limiting nutrients, such as carbon:

- A negative control that did not include any aqueous phase did include deionized water with 1% glycerol and 1% yeast extract
- Aqueous phase media supplementation produced the most biomass with the addition of 1% glycerol and 1% yeast extract whose optical density reached ~11
- The next highest combination, which was 1% glycerol and 4% NPS, was only half the optical density of the glycerol/yeast extract supplementation

	Autoclaved	Not Autoclaved
Baseline	0.92	1.06
1% Glycerol	3.54	3.93
1% Glycerol and 1% Trace	3.91	4.71
1% Glycerol and 4% NPS	3.69	5.97
1% Glycerol and 1% Yeast Extract	9.28	10.86
Negative Control (no Aqueous Phase) 1% Glycerol and 1% Yeast Extract	2.15	2.78

Maximum optical densities for aqueous phase supplementation.



Comparison of non-autoclaved media in baseline aqueous phase with no supplementation, 1% glycerol supplementation only, and 1% glycerol and 1% yeast extract supplementation.

Characterization of Aqueous Phase

Elements: (mg/L)	From Aqueous Phase:	M9 Media:
Nitrogen	2092	260
Phosphorous	30	2,160
Calcium	261	2.5
Cobalt	0.012	-
Copper	0.0307	-
Potassium	87.3	860
Magnesium	< 6	49
Manganese	0.22075	-
Sodium	13,600	2,400
Nickel	0.1364	-
Zinc	0.32915	-
Iron	1.051	-

Elemental analysis for the aqueous phase determined by inductively coupled plasma (ICP) analysis (Utah Water Research Laboratory)

Scale up of E. coli growth in the Aqueous Phase Media

Media:	Shaker Flask:	5 L/10 L:	100 L:
Aqueous Phase	13%	~3%	4%
Maximum OD Observed	10.86	22	16-20

Conclusion

- PHB is attainable through growth of recombinant *E. coli* in aqueous phase media generated as a waste by-product of algal biodiesel production
- The *E. coli* strain XL1BLUE grows best when the aqueous phase media is supplemented with 1% glycerol and 1% yeast extract
- Additional supplementation will be based on the preliminary characterization performed (ICP). The bioplastic yield of this media supplementation is 13% and has the potential to be scaled up
- The results of this study show the feasibility of bioplastic production using algae as a nutrient source in order to help decrease the cost of bioplastic production
- In the future, improvement of PHB yield will be done through scale up methods

Acknowledgments/References

Biological Engineering and Logan City Environmental Departments
Asif Rahman for providing the recombinant XL1BLUE strain of *E. coli*
Utah Water Research Laboratory (Joan McLean and Tessa Guy for their help in ICP and Nitrogen and Phosphorous analysis of the aqueous phase)

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SBI Science & Technology Review

1/29/2013