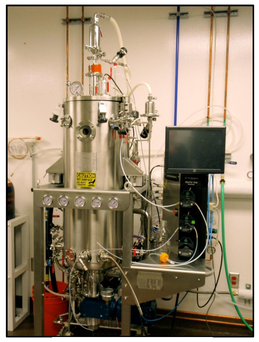
Propylene Carbonate Extraction for Recovery of Poly-3-hydroxybutryate

Brian Smith, Jordan Morely, Ashik Sathish,
Asif Rahman, Ronald C. Sims, Charles D. Miller

Abstract

Poly-3-hydroxybutryate (PHB) is a biopolymer produced as a storage molecule for energy and carbon under poor growth conditions in certain organisms. PHB is a class of polyhydroxyalkanoates (PHAs), which have similar properties to plastics derived from petroleum. PHB provides an environmentally friendly alternative to petroleum-based plastics due to its biodegradability and method of production. It can be produced by recombinant *Escherichia coli*, harvested from the cell, and recovered. One of the major expenses of producing PHB is the extraction of the PHB from the biomass. This study focused on evaluating different methods of PHB extraction for their ability to effectively extract PHB from *E. coli* biomass and the feasibility of scaling up those processes. These processes provide a way to produce PHB from growing *E. coli* on a waste product generated from algae processing.



125 L Bioreactor

Poly-3-hydroxybutryate Extraction Techniques

There are several procedures published to extract PHB from biomass of different organisms. Some procedures use toxic solvents recovering high yields and purities, but use large quantities of hazardous solvents making it difficult to scale up to handle larger quantities of biomass. During the course of this project various extraction procedures were tested for their ability to extract PHB from the biomass, safety of the chemicals being used, the capability of the procedure to extract wet biomass, and finally the feasibility of scaling up the procedure being evaluated.

Chloroform
Failed:
Solvent is toxic
Difficult to scale

→ **Sodium Hydroxide:**
Failed:
Difficulty Scaling
Loss of PHB

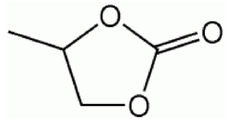
→ **Acetone**
Failed:
Low extraction yield

→ **Propylene Carbonate**
Successful:
Non-toxic solvent
High recovery/purity
Can use wet biomass

Evaluation of Different PHB Extraction Methods

Method of Extraction:	Are materials toxic?	Can use wet biomass	Is Solvent Required?	Solvent to Process 1 kg Biomass
Chloroform	Yes	No	Yes	10 L
Sodium Hydroxide	No	Yes	No	1 L
Acetone	No	No	Yes	10 L
Propylene Carbonate	No	Yes	Yes	725 mL

Propylene Carbonate



Propylene Carbonate is an organic solvent produced by combining propylene oxide with carbon dioxide. It is considered a polar aprotic solvent (no proton to donate) Due to its characteristics (low vapor pressure, stability, and high polarity) it is used in many applications. These include production of fibers and textiles, dyeing, personal care agents and cosmetics, and a component of some adhesives. It is also considered non-toxic making it more suitable to use at larger scales.

Propylene Carbonate Extraction Procedure

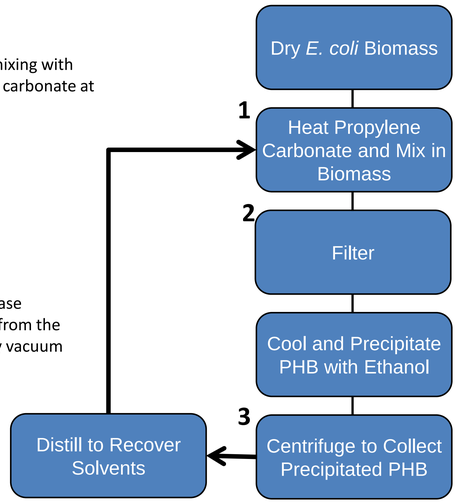
Propylene Carbonate was first tested at the test tube scale, using 0.5 g to 1 g of dried *E. coli* biomass per sample. Confirmation that the Propylene Carbonate procedure was extracting PHB from the biomass and isolation of the PHB was possible was achieved by gas chromatography.

Once confirmed, the Propylene Carbonate extraction procedure was scaled up to run 40 g of dry *E. coli* biomass per run. The procedure is described in the figure and pictures below:

1 Biomass mixing with Propylene carbonate at 130°C

2 Solvent phase separated from the biomass by vacuum filtration

3 Solvent phase collected (Propylene Carbonate), cooled, and mixed with Ethanol to precipitate PHB



PHB extract after removing Ethanol and Propylene Carbonate

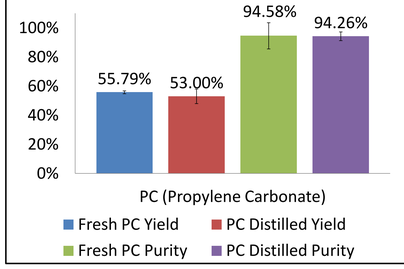
Distillation

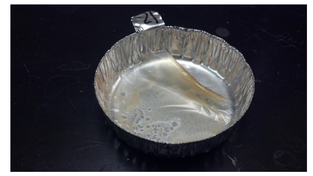
Distillation apparatus to recover Propylene Carbonate and Ethanol from waste stream

- Waste solution recovered after PHB precipitation consisted of Propylene Carbonate and Ethanol
- Through distillation, both the Ethanol and Propylene Carbonate have been recovered.
- Recovered 70% Propylene Carbonate and nearly all Ethanol
- Preliminary experiments show no difference in the yield of PHB extracted with distilled Propylene Carbonate

Results

The Propylene Carbonate extraction method was evaluated to test its effectiveness at recovering PHB from dried *E. coli* biomass. This was done using 0.5 g of *E. coli* biomass containing approximately 20% PHB (predetermined by GC). Yield was based on the mass of PHB recovered and the purity was based on results obtained from the GC (ratio of the mass of PHB determined by GC to mass of extract analyzed). Both fresh Propylene Carbonate and Distilled Propylene Carbonate were tested.





PHB extract after removing solvents

Future work

Optimize Propylene Carbonate Extraction parameters for *E. coli* biomass
- Digestion time with Propylene Carbonate and temperature
- Possible Pretreatments
  - Temperature
  - pH
- Precipitation techniques

Energy and Mass Balance
- Determine the overall effectiveness of this extraction procedure in relation to scale up.

Acknowledgements

Biological Engineering Department
Logan City Environmental Department