UROP project:
Biosynthesis of PHB-b-PHV block-copolymer from CO2, O2, H2 by using Ralstonia eutropha.

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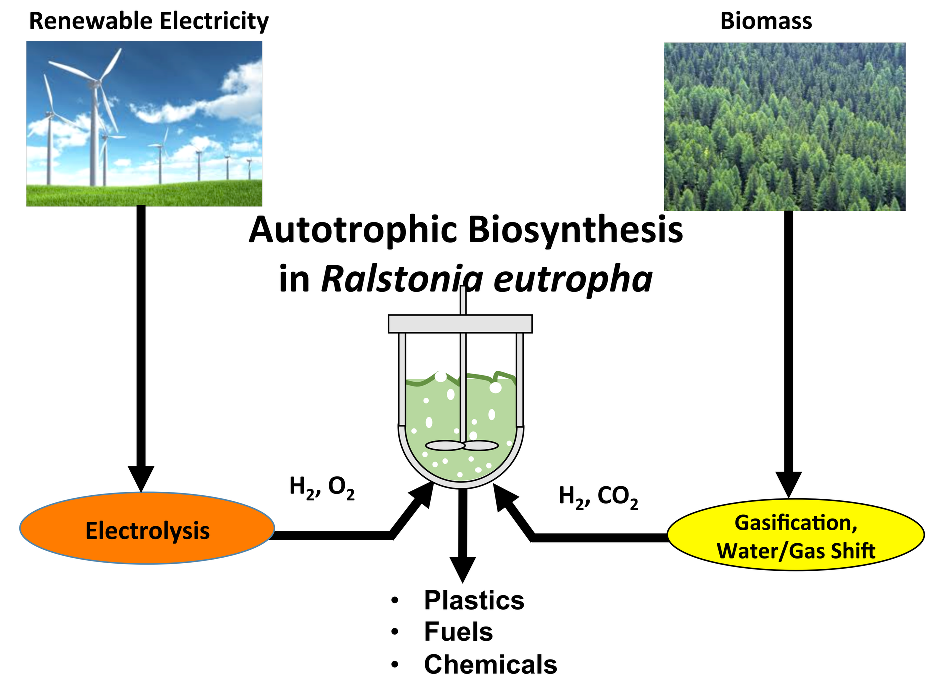
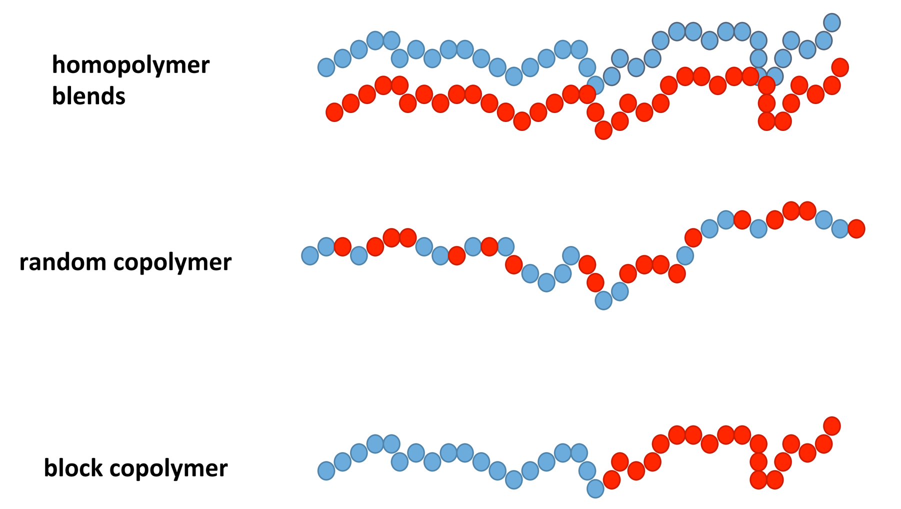
1.Goal of the project

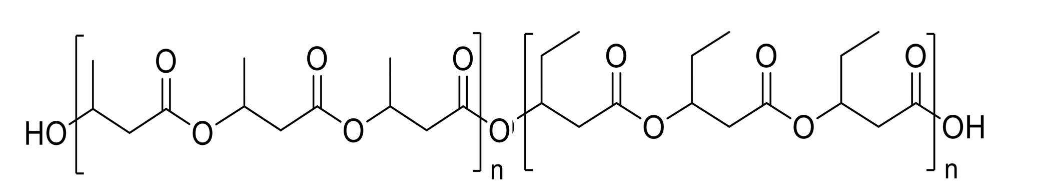
The goal of our project is to produce bio-degradable PHB-b-PHV block-copolymer in an environmentally friendly way. The polymer is synthesized in a bacteria named Ralstonia *eutropha* during its growth. Traditional process of growing this bacteria involve organic carbon sources such as fructose or glucose. However, our team managed to use CO2 instead of these carbon sources to produce PHB. By introducing valeric acid in the culture, the cells will be able to synthesize PHB-co-PHV copolymer, which can alter polymer properties. By carefully controlling valeric acid addtions we can control the polymer microstructure to make block-copolymers which are known to have greater strength than random copolymers.





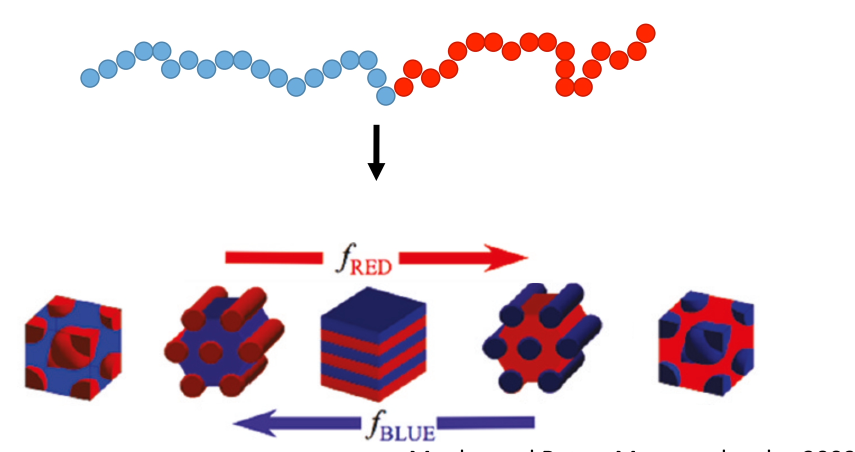
The more controlled structure of a block copolymers increases its toughness and flexibility

Desired Product:



polyhydroxybutyrate (PHB) Polyhydroxyvalerate (PHV)

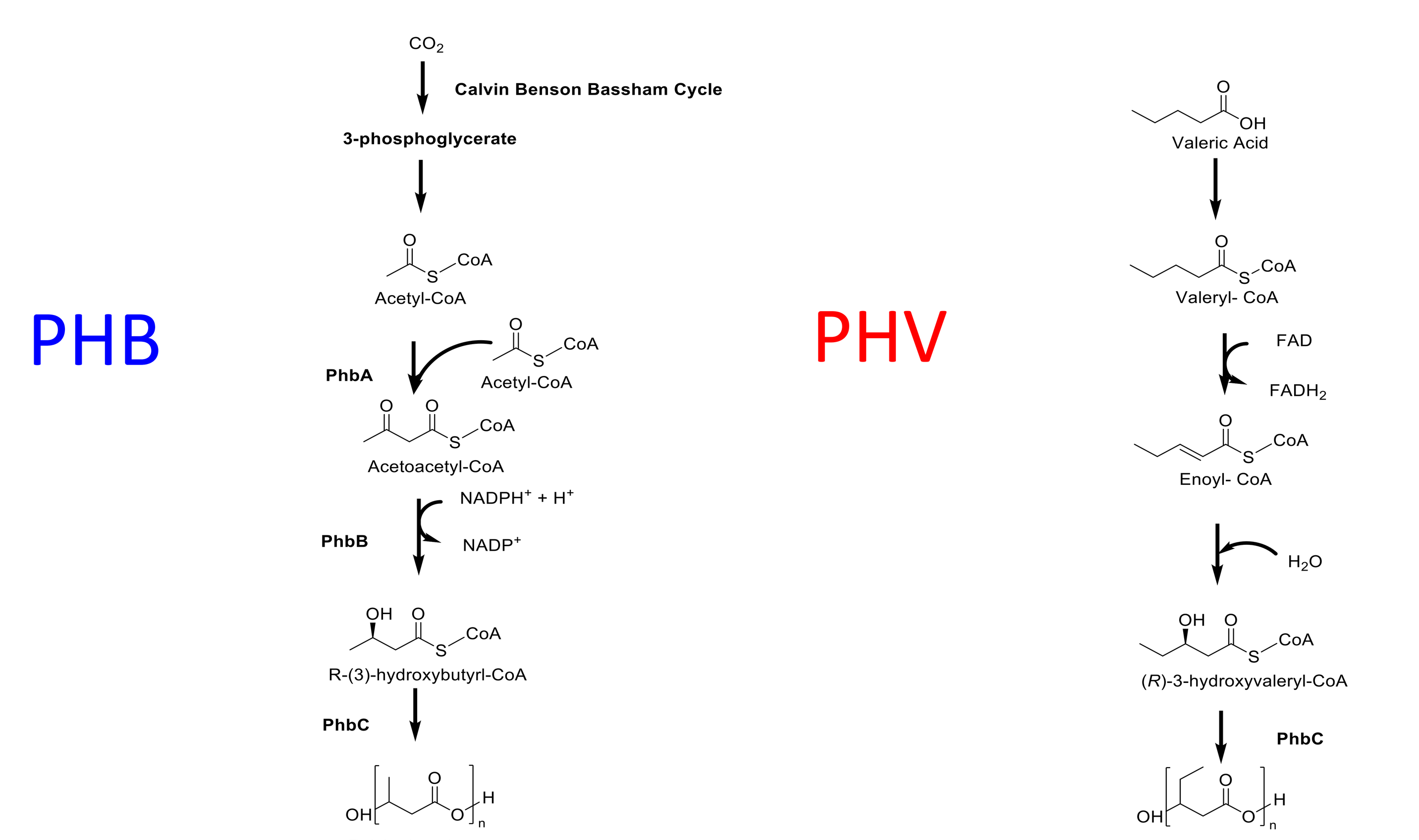
Block Copolymers undergo Self-Assembly



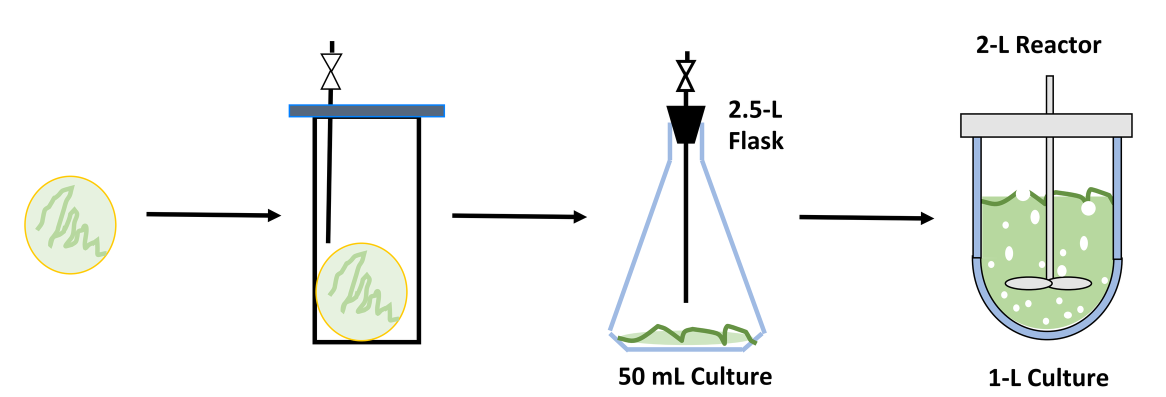
Mueler and Bates, Macromolecules 2009.

Minimum Free Energy Configuration, G

2. Metabolic pathways involved



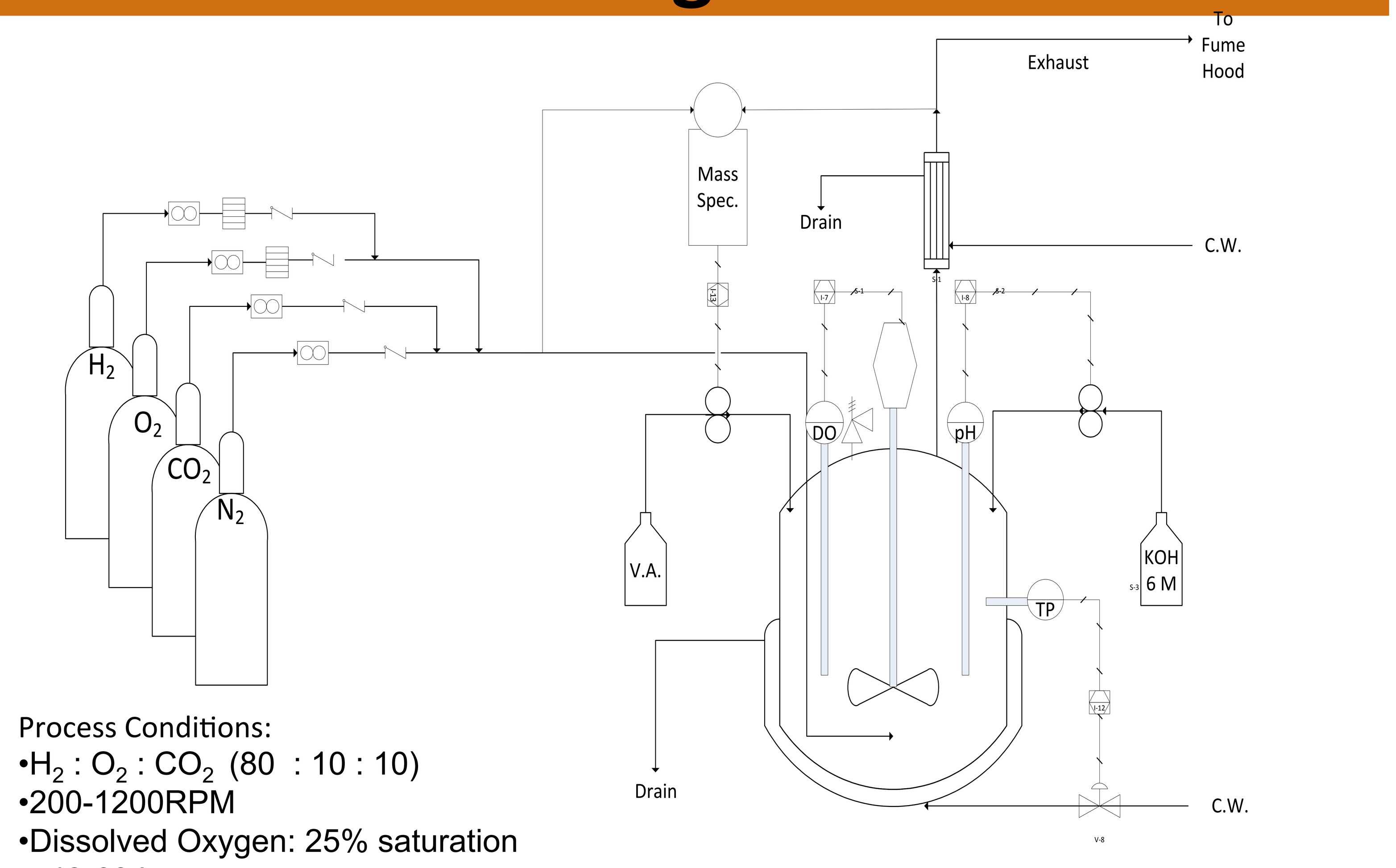
3. Process Seed Train



Cold Storage (Gas Plate)	Gas Plate	Shake Flask	Stirred Tank Reactor
•4°C	•H_2 : O_2 : CO_2 70 : 20 : 10	•H_2 : O_2 : CO_2 80 : 10 : 10	~48-60 hrs
	•~24 hrs	•250 RPM	
		•~18-21 hrs	

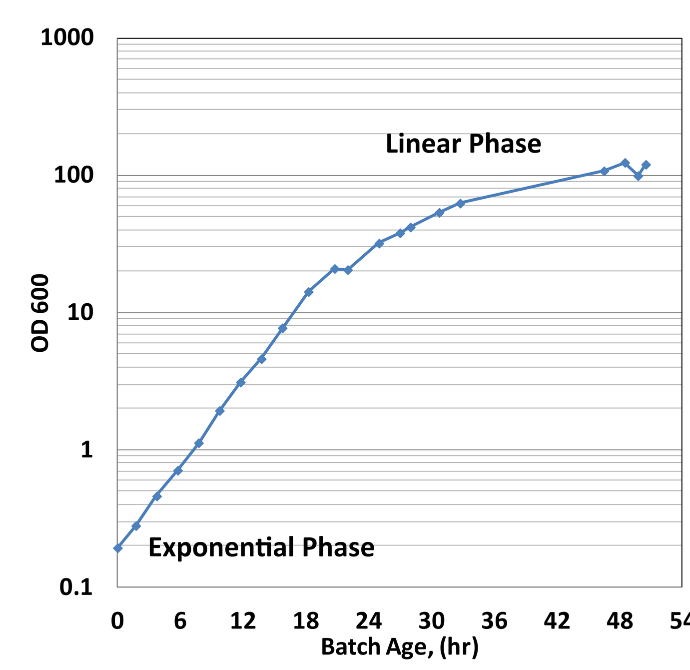
- Minimal media w/ no carbon source
- All cultivations at 30 °C

4. Process Flow Diagram



Process Conditions:
- H_2 : O_2 : CO_2 (80 : 10 : 10)
- 200-1200RPM
- Dissolved Oxygen: 25% saturation
- ~48-60 hrs

5. Results

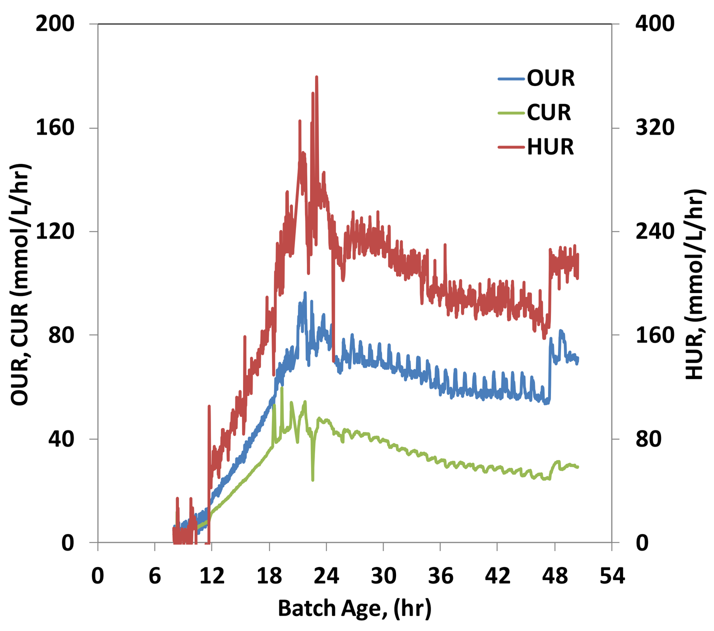


Growth of R. *eutropha*

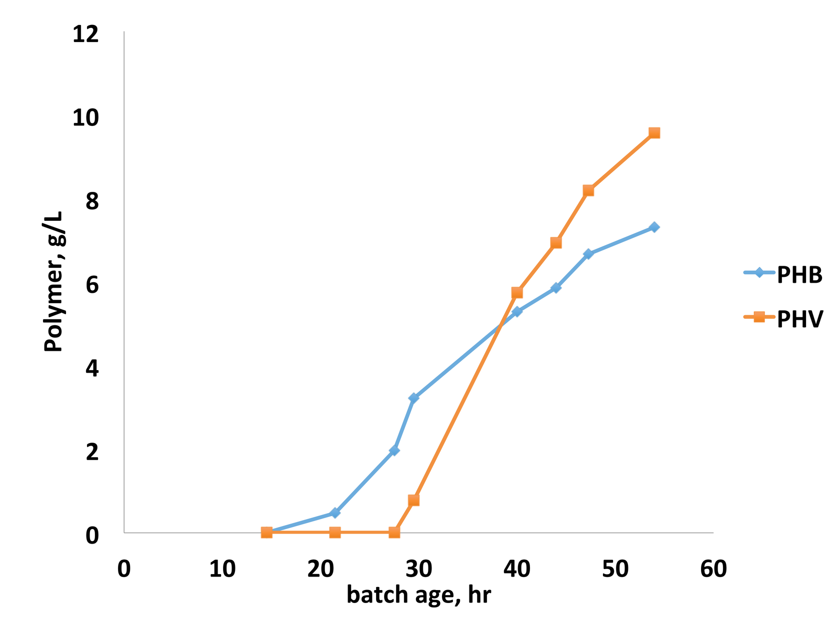
Just like any other cells, the growth of R. *eutropha* can be described by three phases, lag, exponential, and linear. The whole process takes up to 48-60 hours. Its specific growth rates during its exponential phase are generally around 0.2 hr^{-1}, which is measured by spectroscopy under wavelength 600nm. Polymers are produced during the linear phase, which starts around 20 hours of its growth

Controlling Polymer Synthesis

By adjusting valeric acid injection, we were able to produce 4 different polymers. Pure PHB was produced by completely excluding valeric acid from the feed; PHB-b-PHV (13%) was produced by providing the culture with one valeric acid shot per during cells linear growth range : PHB-c-PHV (13%) and PHB-co-PHV (50%) were produced by continuously feeding valeric acid during the cells linear growth range. The consumption of valeric acid was monitored by using mass spectroscopy analysis on fermentation off-gas. Consumption of valeric acid is seen in the small spikes in carbon dioxide and oxygen uptake rates (CUR and OUR).

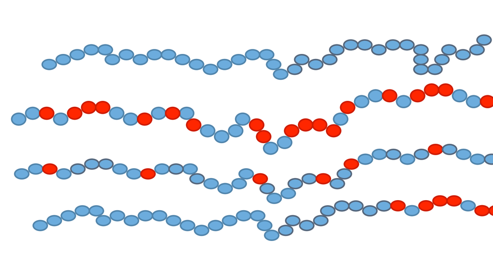


Mass spectroscopy data of gas uptake rates



Polymer production during cell growth

Physical and Mechanical Properties of Purified Polymers



Polymer	Structure	Molecular Weight (kDa)	Polydispersity Index	Strength	Toughness
PHB	Pure	80	3.0	ϕϕϕ	ϕ
PHB-co-PHV (50% PHV)	random	149	3.0	ϕϕ	ϕϕϕ
PHB-co-PHV (13% PHV)	random	112	3.7	ϕ	ϕ
PHB-b-PHV (13% PHV)	block	170	3.6	ϕϕϕ	ϕϕ

Pure PHB is strongest of all the polymers but is brittle due to its high crystalinity. A random copolymer containing over 50% presence of PHV has greatly enhanced toughness and flexibility. Even though the fraction of PHV is only 13% within the block-copolymer we produced, its carefully controlled structure makes the local PHV content close to 50% in the PHV domain. Thus it shows toughness while retaining strength. Whereas for the random co-polymer, PHV is randomly distributed in its structure and the PHV monomers break the integrity of the PHB polymer crystals, so the random co-polymer turns out to be weak and brittle.

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