Feasibility Analysis of Poly-β-Hydroxybutyrate (PHB) Extraction from Hybrid Poplar Leaves

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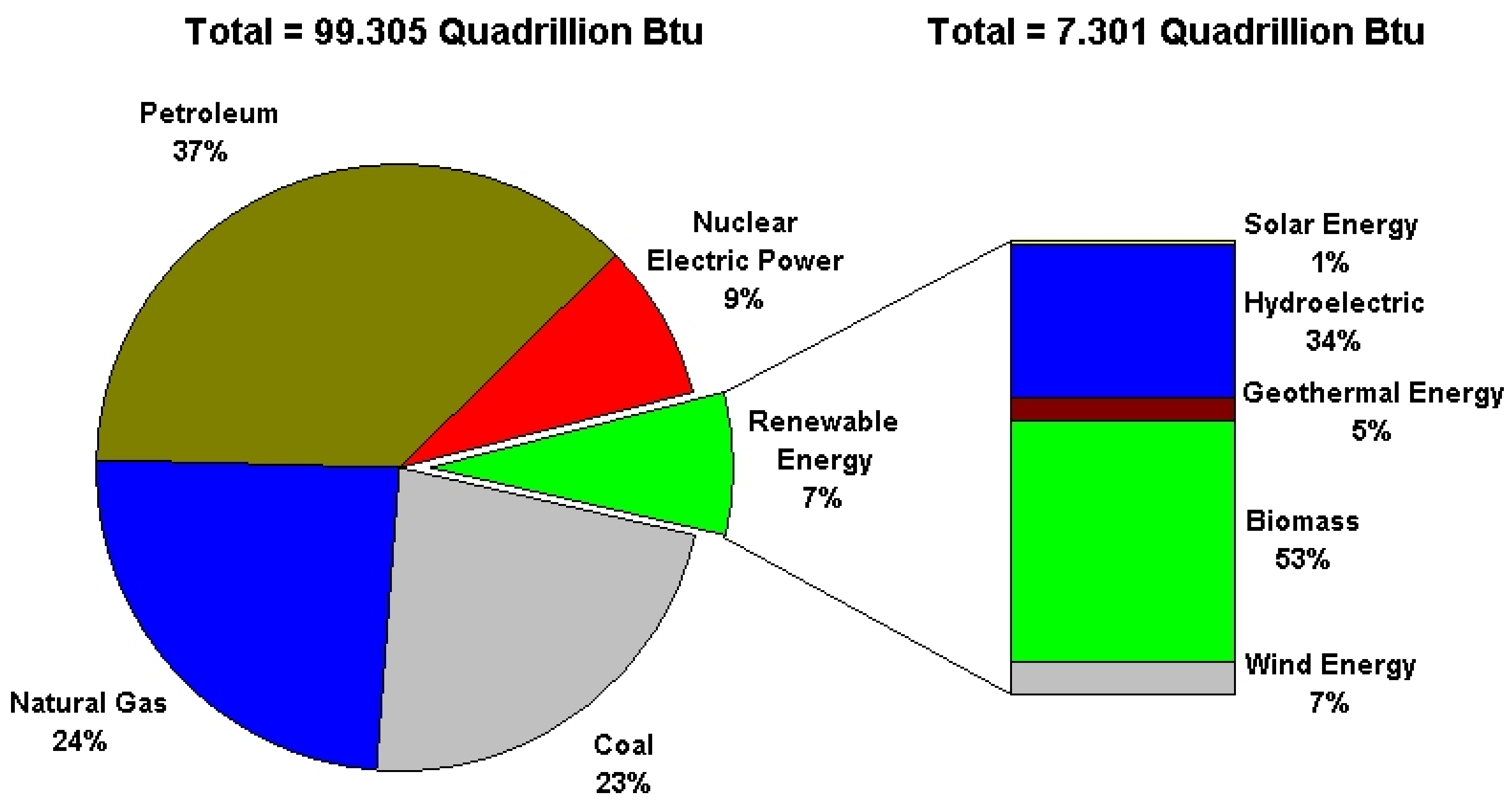
US Energy Scenario

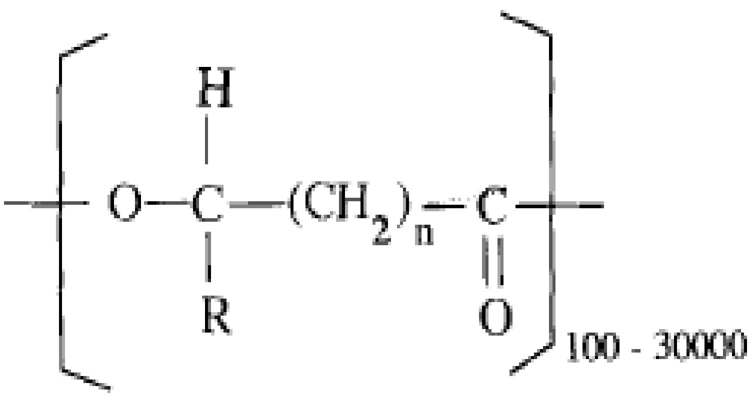


Source: http://www.eia.doe.gov/cneaf/alternate/page/renew_energy_consump/figure1.html

Data: Renewables in global energy supply. IEA Report, 2008.

PolyhydroxyAlkanoates (PHA)

- Biodegradable plastics.
- Occur widely in nature and produced by microorganisms.
- Used as a energy storage molecule similar to starch.
- Poly (3) hydroxybutyrate (PHB) is the most common PHA produced by microorganisms.



		Polymer
n = 1	R = hydrogen	Poly(3-hydroxypropionate)
	R = methyl	Poly(3-hydroxybutyrate)
	R = ethyl	Poly(3-hydroxyvalerate)
	R = propyl	Poly(3-hydroxyhexanoate)
	R = pentyl	Poly(3-hydroxyoctanoate)
	R = nonyl	Poly(3-hydroxydodecanoate)
n = 2	R = hydrogen	Poly(4-hydroxybutyrate)
n = 3	R = hydrogen	Poly(5-hydroxyvalerate)

Source: Lee, 1996

Poly (3) Hydroxy Butyrate (PHB)

- Biodegradable plastic similar to polypropylene.
- Soil bacteria are the most common source of PHB.
- Heterotrophic growth under nutrient deficient conditions could produce up to 70% cell mass as PHB.

PHB Production in Higher Plants

- One of the advantages attributed to PHB production in plants is direct conversion of sunlight and CO_2 into biodegradable plastics.
- PHB concentration of 2.5×10^{-3} to 0.18 % DW in transgenic alfalfa (Saruul et al. 2002) and 1.88 % DW in sugarcane (Petrasovits et al. 2007) have been reported.

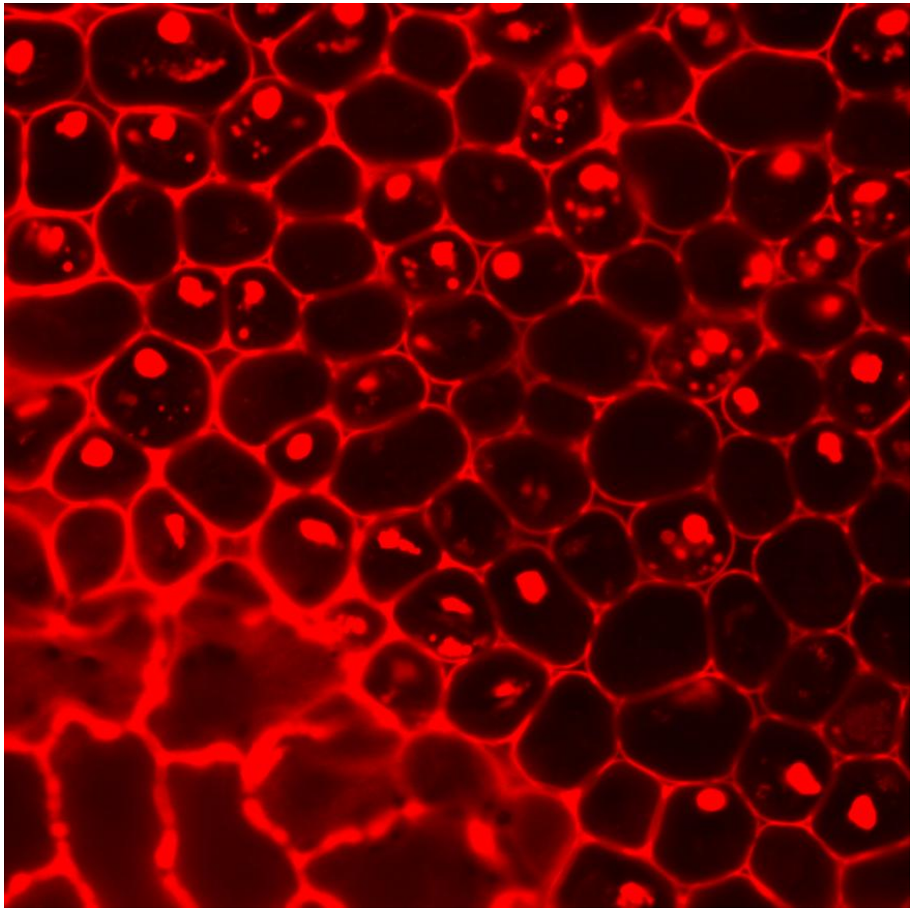
Poly (3) Hydroxy Butyrate (PHB) Production in Poplar

- Hybrid poplar (*Populus* spp.) is one of the fastest growing hardwood deciduous species, commercially grown in many parts of the world for wood production.
- Hybrid poplar is one of the potential bioenergy crops for production of liquid transportation fuels.
- Prof. Steve Strauss group at OSU has genetically modified hybrid poplars (*Populus tremula x alba)* and induced PHB production.

Objectives

- Determine PHB concentration in genetically modified hybrid poplar leaves.
- Develop a process model for extraction of PHB from hybrid poplar leaves.
- Conduct a feasibility analysis for PHB production in hybrid poplars.

Visualization of PHB in Poplar Leaf Tissue



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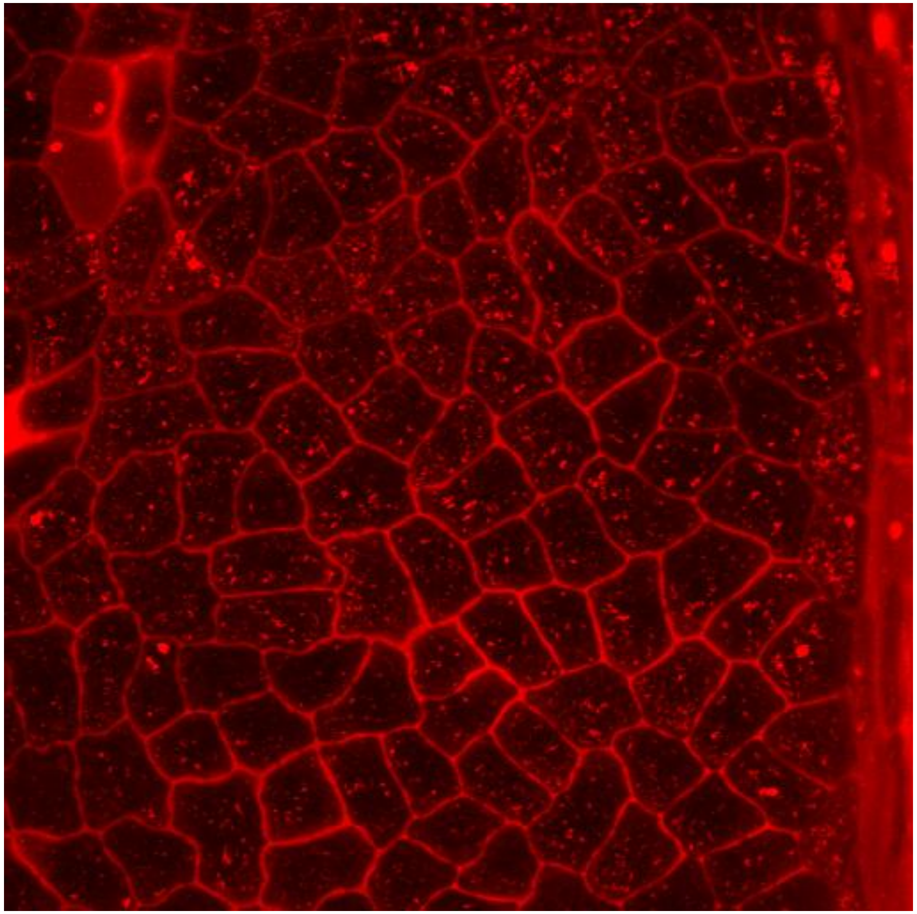
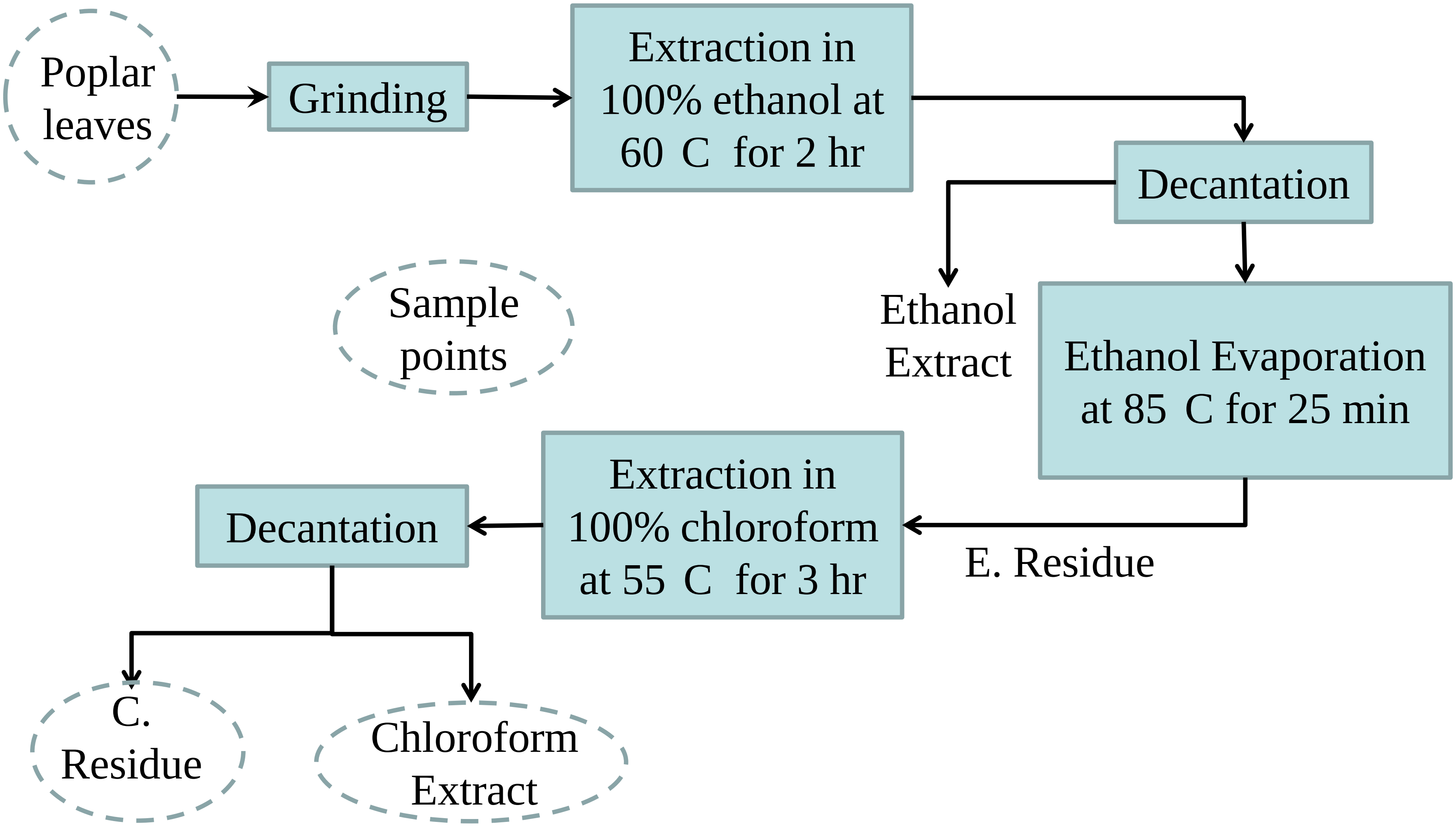
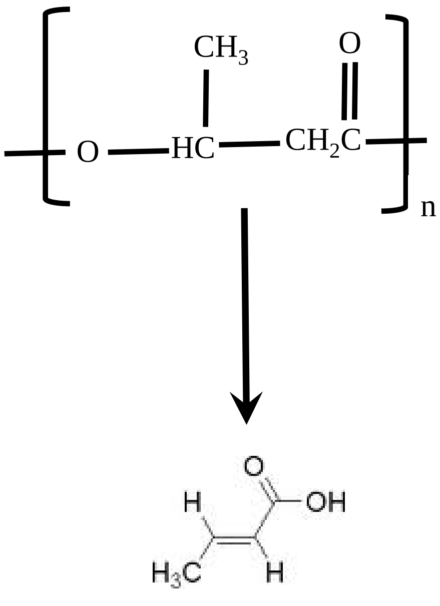


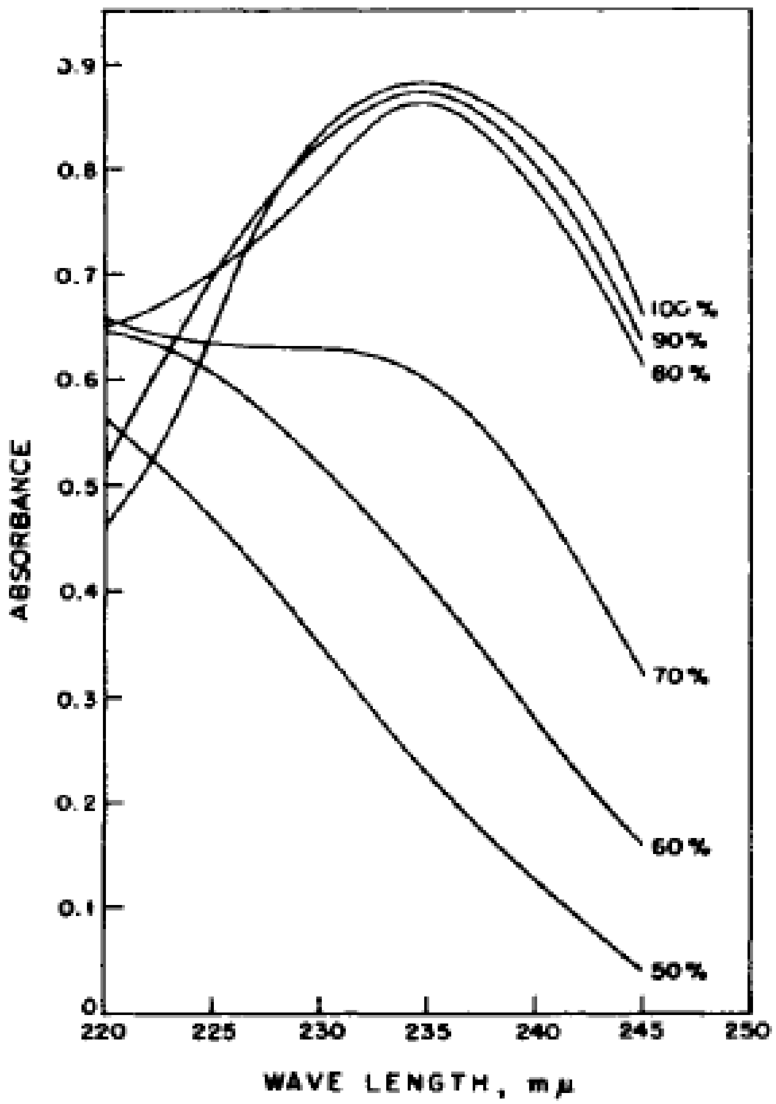
Control Nile Blue

PHB Quantification



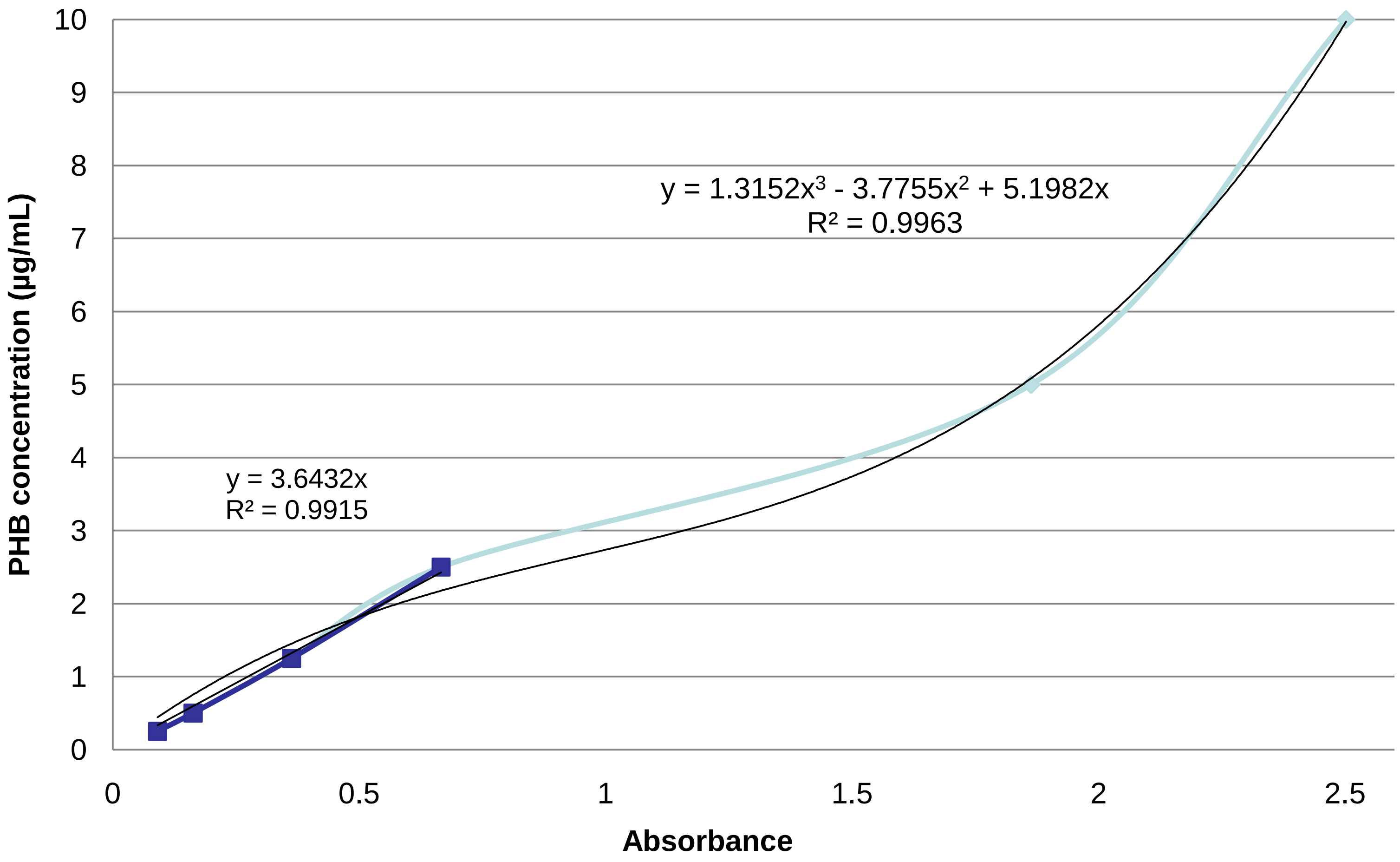
PHB Quantification





Source: Slepecky and Law, 1960

PHB Calibration Curve



Sieve Analysis of Poplar Leaves

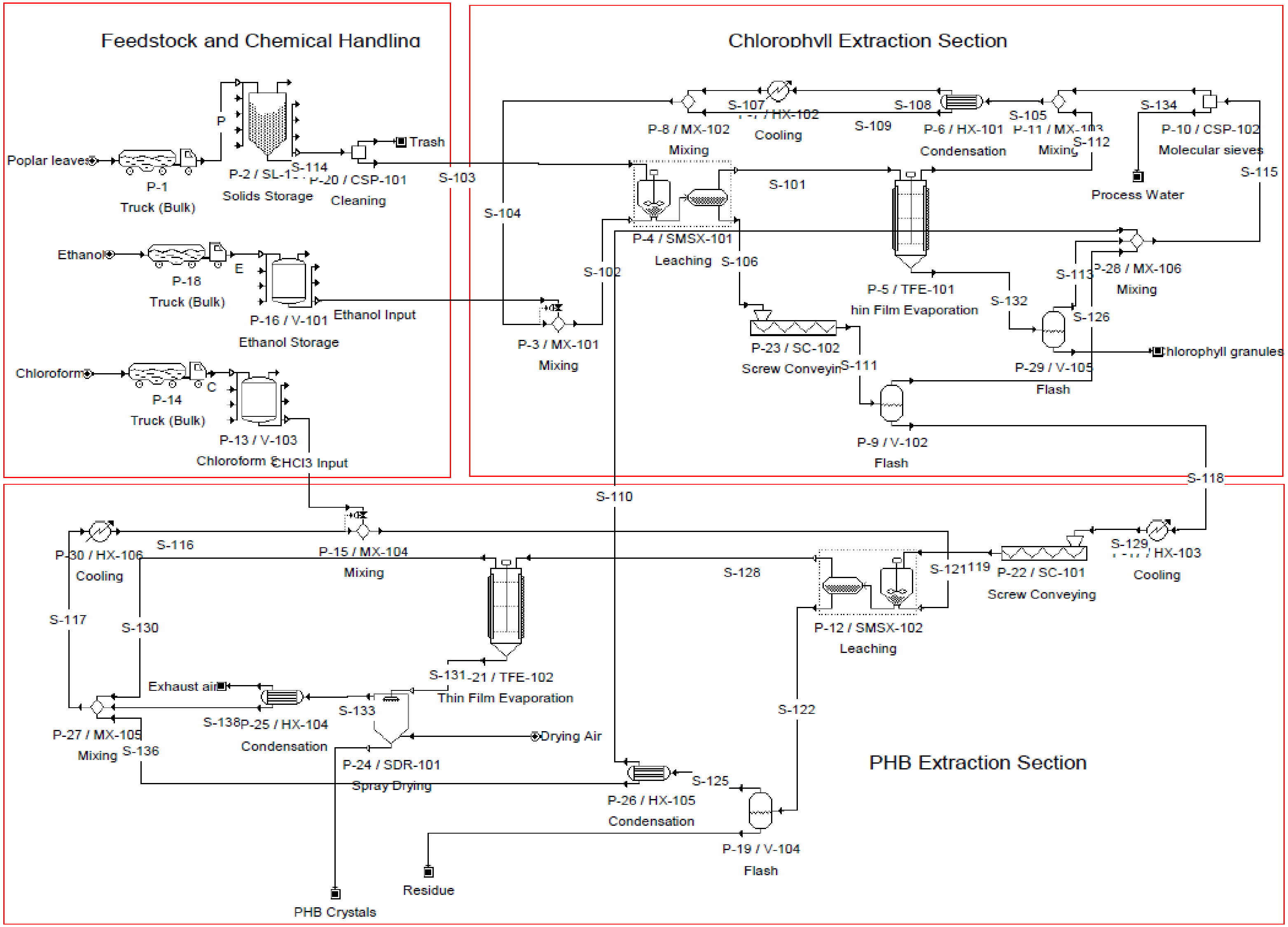
Sieve	Sieve opening (µm)	Weight retained (%)	Cumulative Retained (%)
10	2000	0	0
20	850	5.48	5.48
40	425	13.36	18.84
60	250	28.08	46.92
80	180	13.36	60.28
100	150	11.64	71.918
Pan	-	28.08	100

Composition of Poplar Leaves

Solids (%)	Ash (%)	Nitrogen (%)	Carbon (%)	Source
93.35±0.56	8.43±0.13	2.68±0.15	42.9±3.04	This work
-	-	1.94±0.18	39.4±0.60	Singh and Behl (1991)
-	-	-	42.9 ±1.09	Fang and Tang (2007)

Sample	PHB in 10g sample (g)	PHB (%)
Replicate 1	0.085	0.845
Replicate 2	0.069	0.693
Replicate 3	0.075	0.750
Average		0.763±0.076

Processing Poplar Leaves for Poly (3) HydroxyButyrate (PHB)



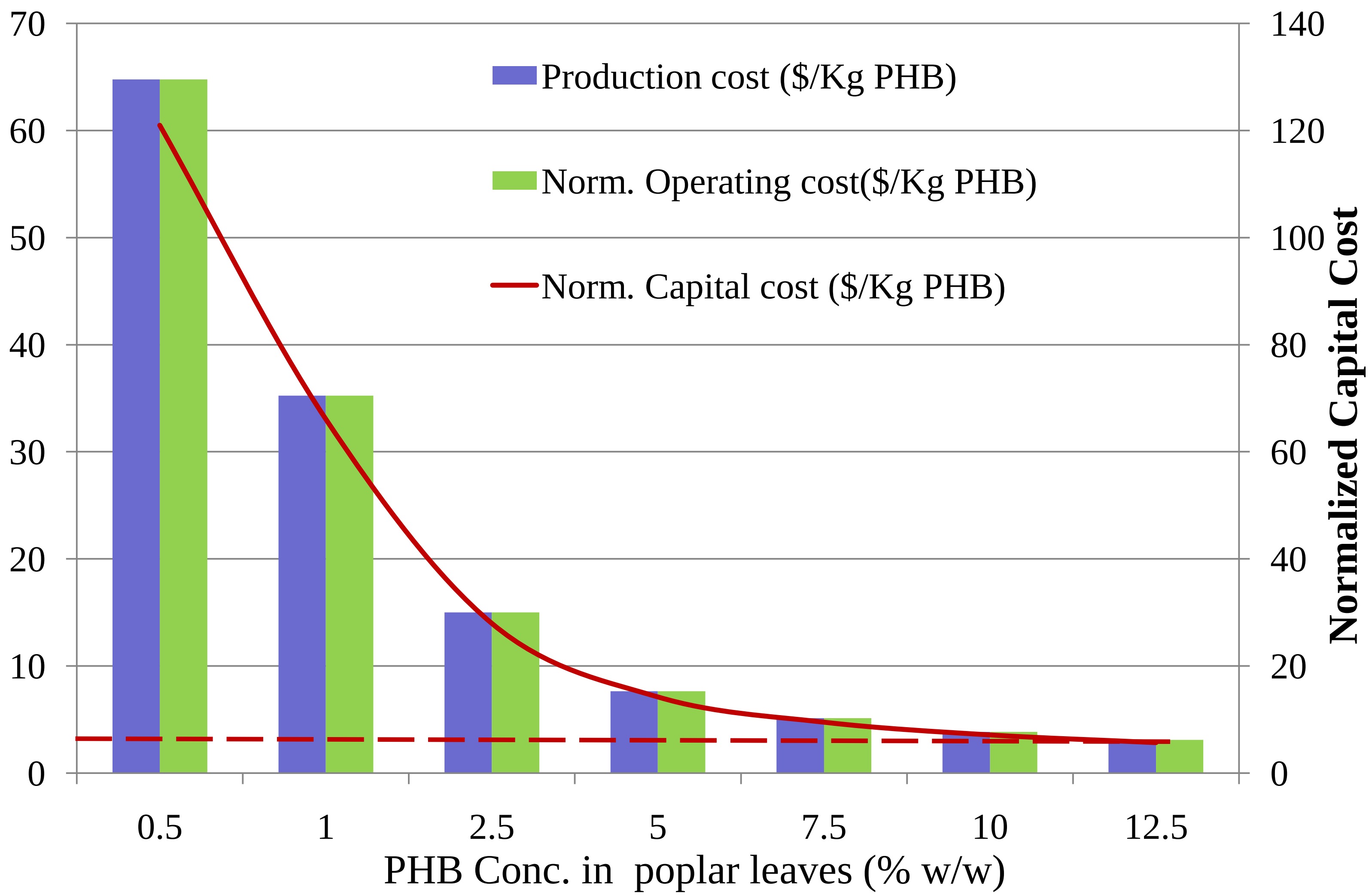
Process Economics: Raw Material Inputs

		PHB conc. in poplar leaves =0.5% (w/w)			PHB conc. in poplar leaves =12.5% (w/w)		
Material	Unit Cost ($/kg)	kg/yr	Annual Cost ($)	Cost (%)	kg/yr	Annual Cost ($)	Cost (%)
Poplar Leaf	0.04	7,920,000	348,480	95.91	7,920,000	348,480	96.17
Debris	0.00	396,000	0	0.00	396,000	0	0.00
Ethanol	0.75	7,731	5,798	1.60	7,859	5,894	1.63
Chloroform	1.01	8,973	9,062	2.49	7,891	7,970	2.20
Air	0.00	6,650,446	0	0.00	6,839,472	0	0.00
Total		14,983,149	363,340	100.00	15,171,222	362,345	100.00

Process Economics: Overall Economics

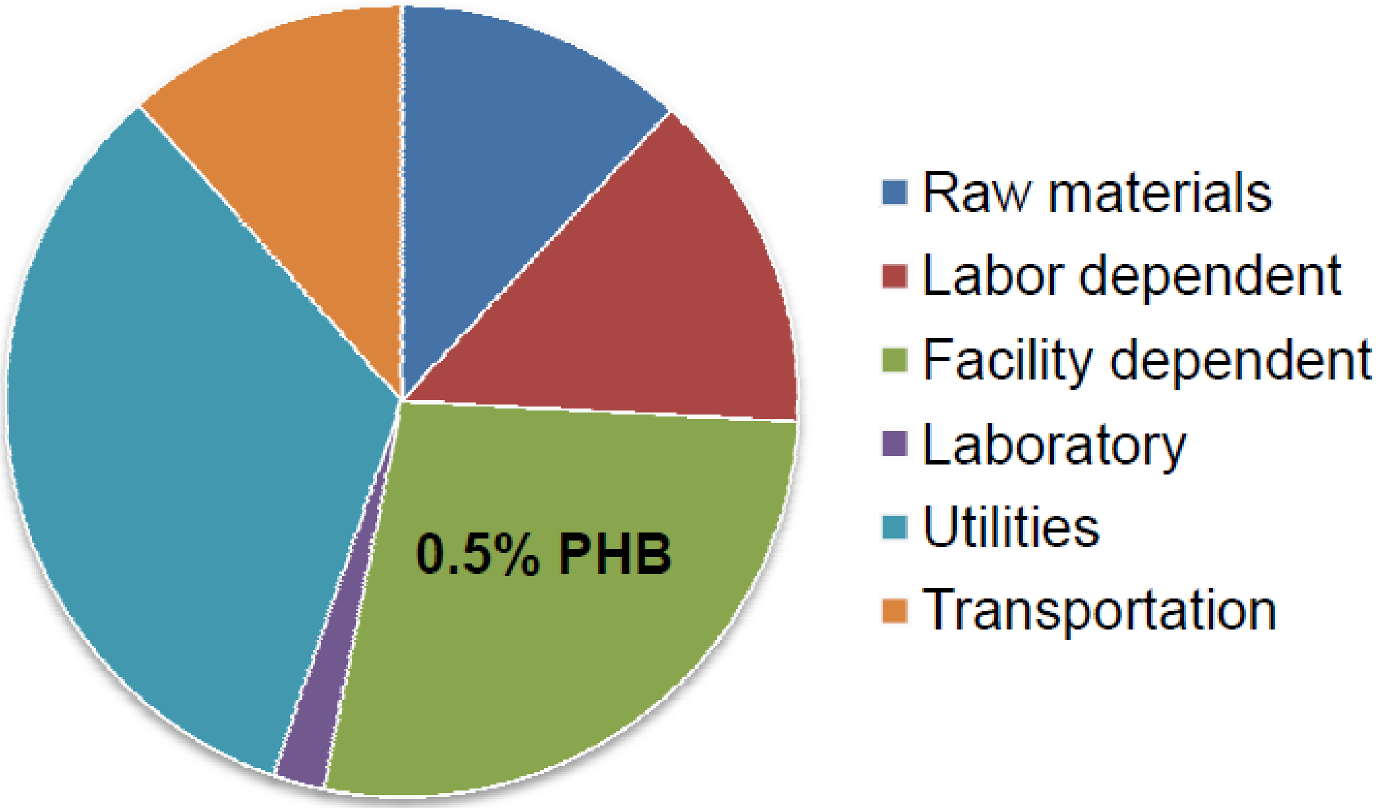
PHB conc. in poplar leaves (% w/w)	1.0	10	12.5
A. Direct Fixed Capital ($)	5,277,000	5,206,000	5,187,000
B. Working Capital ($)	173,000	175,000	175,000
C. Startup Cost ($)	264,000	260,000	259,000
D. Up-Front R&D ($)	0	0	0
E. Up-Front Royalties ($)	0	0	0
F. Total Investment (A+B+C+D+E) ($)	5,714,000	5,641,000	5,621,000
G. Investment Charged to This Project ($)	5,714,000	5,641,000	5,621,000
H. Revenue/Credit Stream Flowrates			
PHB Crystals (Main Revenue) (Kg/yr)	86,451	791,439	987,269
Leaf residue (Credit) (Kg/yr)	7,299,978	6,595,020	6,399,198
Chlorophyll granules (Coproduct)	22,195	21,589	21,421
I. Annual Operating Cost			
Actual AOC ($/yr)	3,047,000	3,054,000	3,052,000
Residue ($/yr)	146,000	132,000	128,000
Net AOC ($/yr)	2,901,000	2,922,000	2,924,000
J. Product Unit Cost			
Actual PHB Crystals ($/kg)	35.25	3.86	3.09
Net PHB Crystals ($/kg)	33.56	3.69	2.96

Process Economics: Effect of Poplar Leaf PHB Concentration



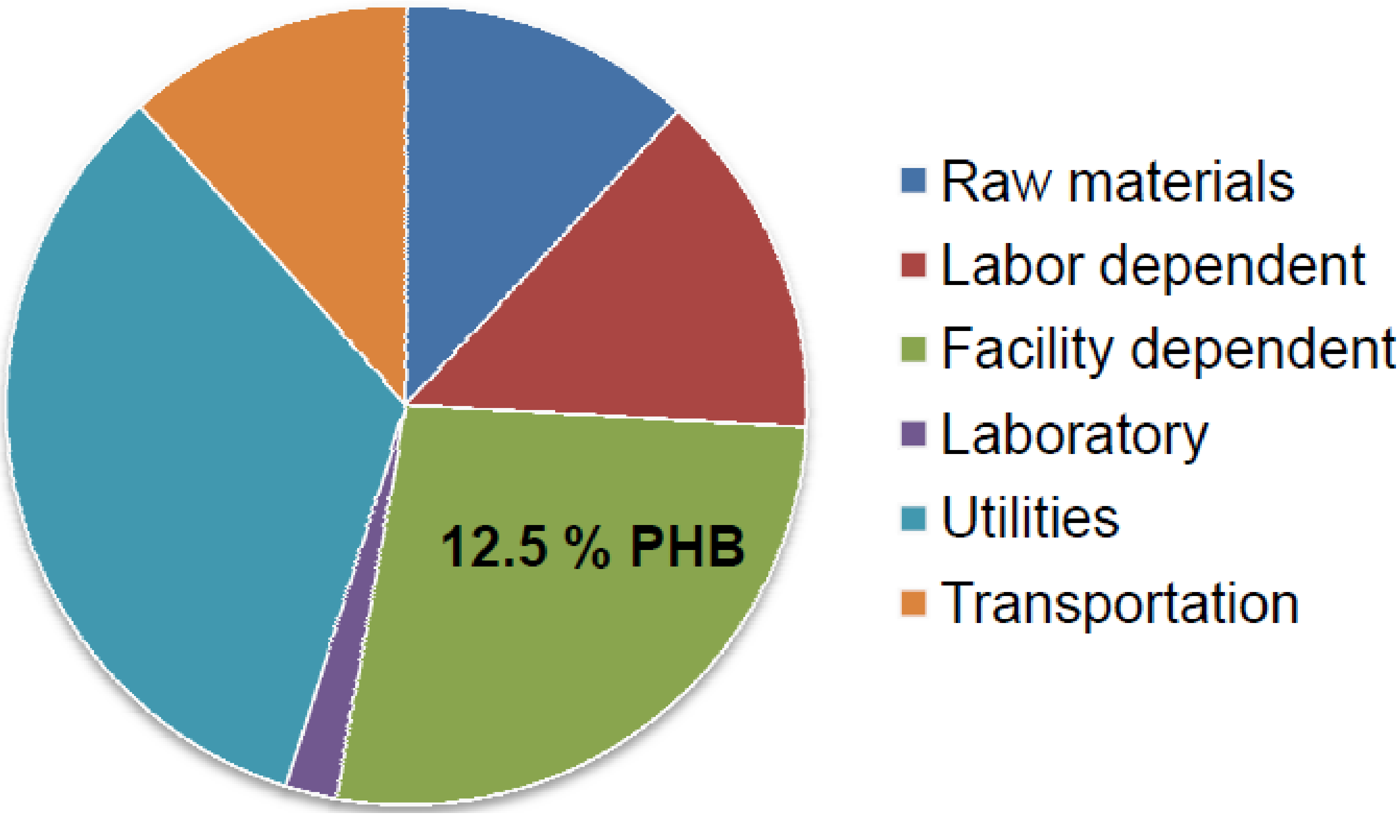
Process Economics: Effect of Poplar Leaf PHB Concentration

Distribution of operating costs for PHB production (0.5% PHB)



Process Economics: Effect of Poplar Leaf PHB Concentration

Distribution of operating costs for PHB production (12.5% PHB)



Conclusions

- A modified chloroform method was developed to quantify PHB in poplar leaves. The absorbance vs. PHB concentration was linear in the range of 0-2.5 µg/mL PHB concentrations.

- Particle size analysis of ground poplar leaves indicated a bimodal distribution.

Conclusions

- Composition analysis of poplar leaves indicates a solids, ash, nitrogen and carbon content of 93.35±0.56, 8.43±0.13, 2.68±0.15 and 42.9±3.04 respectively.

- PHB production cost decreases from $33.56/Kg to $2.96/Kg as the PHB concentration in poplar leaves increases from 1 % (w/w) to 12.5% (w/w).

Reducing the overhead costs and increasing the PHB content of poplar leaves to >12.5% (w/w) could make PHB economically competitive against petroleum based polymers such as polypropylene.

Funding for this project was provided by Western Sun Grant Center and Dept. of Transportation.

Thank you

Visualization Protocol

- •Extract chlorophyll with ethanol.
- •Cut the leaf disks into thin sections and stripes.
- •Clear with Sodium Hyperchloride (Bleach).
- •Stain with Nile Blue A.
- •Wash several times in water and 8% Acetic Acid.
- •Excitation 488 and 543 nm, Emission LP 560