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WBS # 4.2.1.4**CID# ID 14214****Final Report**

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Recipient: Metabolix, Inc.
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Cambridge, MA 02139

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Subcontractors: Cornell University, University of Massachusetts Lowell,
University of Massachusetts Amherst, University of
Tennessee, University of Central Florida, University of
Akron, University of Minnesota, University of Illinois
Chicago, Michigan State University

Other Partners: Oak Ridge National Laboratories,

Project Contact(s): Dr. Oliver Peoples, CSO, Metabolix, Inc. Phone: (617)
583-1712
e-mail address: peoples@metabolix.com

Ben Locke, Metabolix Inc. Phone: (617) 583-1741
e-mail address: locke@metabolix.com

DOE Project Team: DOE-HQ contact – Mark Decot, mark.decot@ee.doe.gov
DOE Field Project Officer – Fred Gerdeman, 303 275-
4935, fred.gerdeman@go.doe.gov
DOE Contract Specialist – Margo Gorin, 303-275-4737,
margo.gorin@go.doe.gov
DOE Project Monitor – Janice Ford, 303-275-4935,
janice.ford@go.doe.gov

DOE Analysis: [This item to be filled out by DOE Project Officer or Project Monitor]

Final Report

Project Title: Biomass Biorefinery for Production of Polymers and Fuel

Project Objectives: The project objectives are to create a biorefinery for the production of energy and a new family of biobased products, polyhydroxyalkanoates (PHAs). Uniquely, these biobased products are produced as finished polymers in a range of wild-type and genetically engineered bacteria and can be produced directly in the green tissue of transgenic plants. This fully integrated multidisciplinary program involved 3 Universities and 1 National Laboratory over a 5 year 9 month period, addressing fundamental questions across the value delivery chain. Success in these endeavors will enable the sustainable economic production of energy and a family of biobased materials with functional properties adequate to replace over half of the 150,000 million tons per year of petroleum based plastics used today. In the first two years the project was focused on plant science efforts to directly produce PHA bioplastics directly in transgenic Switchgrass. Following a decision by Department of Energy (DOE) Energy Efficiency and Renewable Energy Office (EERE) to exclude plant science project from funding the remainder of the program was re-focused on key downstream activities which included biorefinery processes and polymer science and engineering. The overall biorefinery concept was to extract the bioplastic and use the residual biomass as a source of surplus energy to produce electricity and transferred to the power grid or for other biorefinery processes. The combined polymer and fuel production from the transgenic biomass crops establishes the basis of a biorefinery operation that has the potential to reduce the nation's dependence on foreign oil imports for both the feedstocks and energy needed for plastic production. A major part of the original objectives in Years 3-6 of the project as originally funded involved an expanding effort on polymer recovery, polymer science and polymer processing. In year 2 of the program following a major change in direction by the Office of the Biomass Program which effectively terminated the plant science effort and resulted in a refocusing of the project at the end of Year 2 on the development of polymer recovery systems from fermented biomass, and polymer science and polymer processing.

Overall this program achieved a great deal of success and some of the highlights include:

- Robust gene transformation system for switchgrass and ultimately polyhydroxybutyrate (PHB) production in switchgrass
- Improved PHB copolymer recovery technology validated in pilot plant
- Fundamental understanding of PHA copolymer properties
 - Including model to predict melt rheology
 - Improved processing aids for PHB copolymers
- Demonstration of PHB resin processing in industrial equipment meeting line speed and end use application needs
- Complete LCA of PHA production based on Clinton commercial plant

Although not an objective, during the course of this project Metabolix formed a JV to commercialize PHAs by fermentation with Archer Daniel Midland Company and the first production plant is under construction in Clinton Iowa. The work done under this joint program was instrumental in demonstrating the validity of this business opportunity.

Initial Program tasks and Goals

ID Number	Task / Milestone Description	Planned Completion	Comments
1	Nuclear Transformation of Plastid Targeted PHB Enzymes into Switchgrass (U. Tennessee)	10/31/06	
2	Development of Plastid Encoded PHB Production Systems (U. Central Florida)	10/31/03	
3	Development of Inducible PHB Production Systems	10/31/03	
4	Introduction of Plastid Encoded PHB Production Systems into Switchgrass	10/31/03	
5	Development of PHA Copolymers	10/31/03	
5.1-5.3	multi-gene expression constructs for medium chain length PHA formation in <i>Arabidopsis</i> chloroplasts	5/31/03	
5.4	Regulatory programs	10/31/03	
5.5	Analysis of Copolymer Rheological Properties (U. Akron)	12/31/03	
5.6	Ester Interchange Programs (Cornell University)	2/28/05	
5.7	PHA Copolymer Processing	10/31/05	
5.7.1	Crystallization behavior of PHAs (Oak Ridge National Lab)	2/28/05	
5.7.2	Impact of additives on crystallization behavior of PHAs	2/28/04	
5.7.3	Predictive models for PHA composition/properties (U. Illinois, Chicago)	2/28/05	
5.8	PHB Synthesis with <i>Saccharomyces</i> (U. Minnesota)	4/30/03	
6	Development of Optimized Recovery Processes for PHAs in a Biorefinery context	10/31/06	
7	Life Cycle Analysis	10/31/06	
8	Investigation of Applications for PHAs	10/31/06	

ID Number	Task / Milestone Description	Planned Completion	Comments
8.1	Use of PHB oligomers for polyurethane production (U. Massachusetts, Amherst)	10/31/03	
8.2	Use of PHB as a precursor for green solvents	10/31/03	
8.3	Development of improved polymer blends and processing (U. Massachusetts, Lowell)	10/31/05	
9	Management and Reporting	10/31/06	
9.1	Final Report	10/31/06	

In response to a DOE Program Office change in priorities in November 2002, the work plan for the plant science effort (Tasks 1 – 4 and 5.1) was revised to focus on essential activities necessary to obtain clear, successful endpoints prior to the end of Year 2 of the program (October 31, 2003). The revised work plan, agreed to by DOE, focused on the following key plant science milestones.

- Y1-2 Task 1: Demonstration of PHB production in switchgrass.
- Y1-2 Task 2: Demonstration of tobacco producing 2-5% PHB using plastid-encoded expression cassettes.
- Y1-2 Task 3: Demonstration of an inducible PHB production system in *Arabidopsis*.
- Y1-2 Task 4: Development of a methodology for plastid transformation of switchgrass.
- Y1-2 Task 5: Demonstration of a metabolic pathway for medium chain-length PHA production in *Arabidopsis*.

This section of the report summarizes progress of DOE funded work on these tasks and provides an update of the status of these projects as of Q2, 2007.

Y1-2 Task 1: Demonstration of PHB production in Switchgrass: (Work during the DOE funded portion of this program was performed in collaboration with Professor Bob Conger's laboratory at the University of Tennessee. Work since the DOE program has been performed exclusively at Metabolix).

Switchgrass is an ideal crop for production of polymers and other industrial products since it is a non-food crop, grows on marginal land with minimal inputs, and is a high biomass producer. Residual biomass remaining after isolation of the desired products can be used to produce energy. At the start of this project, the transformation of switchgrass with simple constructs encoding a visual and a selectable marker via particle bombardment procedures had been described¹. During the first year of our research, a second publication detailing *Agrobacterium* transformation methods was published². Other than this pioneering work, no attempts to express multi-gene pathways in switchgrass had been performed and very little was known regarding gene

¹ Richards et al., 2001, Plant Cell Rep., 20, 48-54

² Somleva et al., 2002, Crop Sci., 42, 2080-2087.

expression. The principal objective of Task 1 was the nuclear-encoded expression of genes encoding plastid-targeted PHB biosynthetic enzymes in switchgrass, a strategy which previously yielded high PHB levels in the model plant *Arabidopsis* as well as in maize and rapeseed³.

A series of genetic constructs were designed to express the three enzyme activities necessary to convert substrate acetyl-CoA to PHB (Figure 1). In one construct (Figure 1b), a novel thiolase-reductase fusion was used to reduce the total number of promoters and 3' non-coding regions required for expression of the PHB pathway. The genetic sequences of the PHB genes in all of the constructs were modified to enable plastid targeting of the PHB enzymes. Constructs were transformed into *Arabidopsis* as a preliminary test and levels of polymer were quantified. *Arabidopsis* plants transformed with the three gene construct (Figure 1a) produced up to 11.5% dwt PHB whereas plants transformed with the fusion construct (Figure 1b) produced 6.4% dwt PHB. The ability of the fusion construct to enable PHB synthesis, although at levels lower than the three gene construct, suggests the AB fusion is a good target for improvement via gene shuffling. Results of the *Arabidopsis* study were published in Plant Biotechnology Journal⁴.

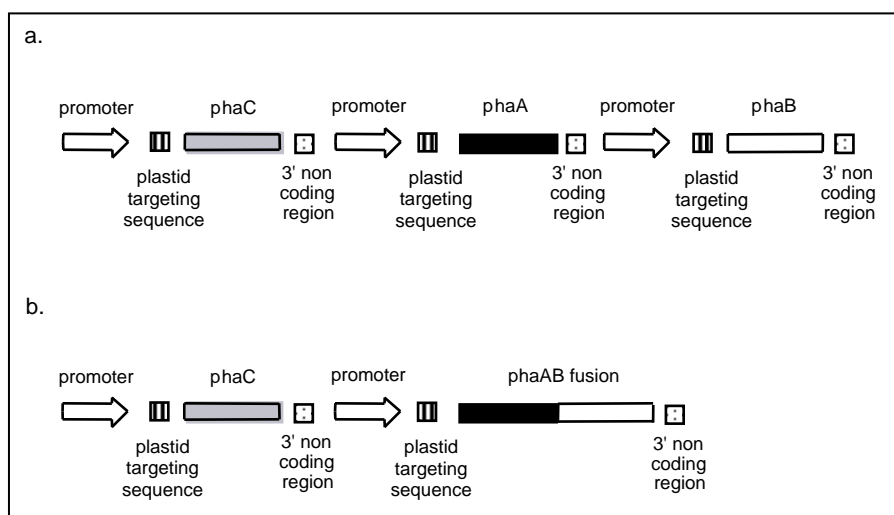


Figure 1. Genetic constructs for expression of *phaC*, *phaA* and *phaB*.

³ Poirier & Gruys, 2002, Production of polyhydroxyalkanoates in transgenic plants. In: *Biopolymers: Polyesters I - Biological Systems and Biotechnological Production*, vol. 3a. pp. 401-435.

⁴ Kourtz et al., 2005, Plant Biotechnol., 3, 435-447.

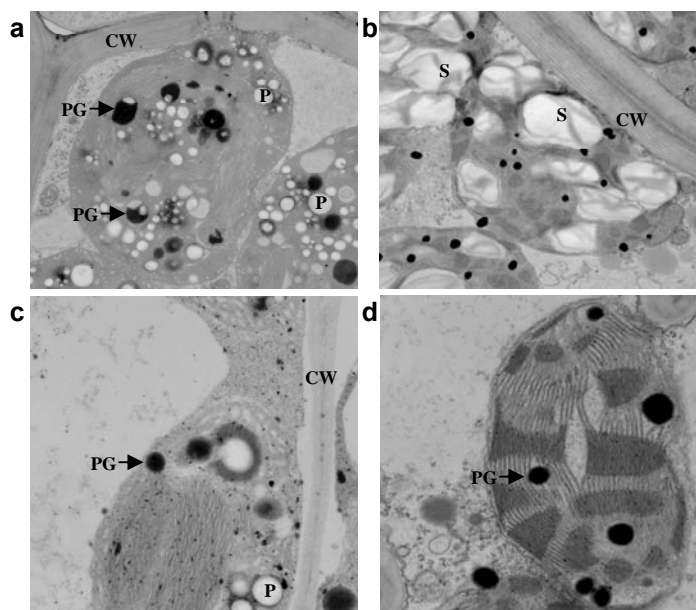


Figure 2. Transmission electron micrographs (TEM) of plastids from PHB producing and wild-type switchgrass plants **(a)** A bundle-sheath chloroplast with PHB granules; **(c)** A mesophyll chloroplast from the same transgenic plant with PHB granules; **(b)** A bundle-sheath chloroplast from a wild-type plant; **(d)** A mesophyll chloroplast from a wild-type plant. *P* PHB granules; *S* starch granules; *CW* cell wall; *PG* plastoglobules.

Initial attempts to transform switchgrass with these constructs via published particle bombardment procedures¹ yielded low transformation efficiencies even with vector controls. At the end of the DOE funded period of this research, one plant containing the PHB genes had been isolated but did not produce detectable levels of polymer. Subsequent research efforts, a portion of which were funded by a USDA/DOE grant, succeeded in optimizing the *Agrobacterium*-mediated transformation protocol² such that very high transformation efficiencies can be obtained, even with complex multi-gene constructs. Over 400 transgenic switchgrass plants carrying the PHB genes have been isolated and fully characterized by polymerase chain reaction (PCR) and their polymer levels have been quantified by Gas Chromatography and Mass Spectrometer (GC/MS) procedures. Many of these plants contain PHB in their plastids (Figure 2) with levels up to 3.72% dwt PHB in leaf tissues and 1.23% dwt in whole tillers observed. These levels are a significant and promising first step towards producing a plant feedstock that can be used in a biomass biorefinery for production of plastics and energy. A manuscript describing this study has been submitted to Plant Biotechnology Journal.

Y1-2 Task 2: Demonstration of Tobacco producing 2-5% PHB using plastid encoded expression cassettes. (Work during the DOE funded portion of this program was performed in collaboration with Professor Henry Daniell's laboratory at the

University of Central Florida. Work since the DOE program has been performed exclusively at Metabolix).

The goal of this task was to develop highly efficient plastid-encoded PHB production systems using tobacco as a model biomass crop. Plastid-encoded gene expression in tobacco has been successful in producing high levels of foreign proteins⁵ and provides a method to significantly reduce or eliminate transgenic pollen flow since plastid DNA is maternally inherited in most plant species.⁶ Constructs for plastid-encoded PHB production were generated and transformed into tobacco during the first year of the project. At the end of the DOE funded period of research, Metabolix had obtained transplastomic tobacco lines in which regions of leaf tissue were producing up to 1.5% dwt PHB.

Subsequent research, funded in part by a USDA/DOE grant, allowed the isolation of transplastomic lines producing up to 6.3 % dwt PHB in soil grown plants. During detailed characterization of the lines, it was discovered that the design of the transgene expression constructs promoted rearrangements within the tobacco plastome. This is an important finding since many plastid transformation constructs reported in the literature are designed in a similar way. We have since redesigned the constructs in an attempt to increase the stability of the expression cassettes within the plastome. These new constructs have been transformed into tobacco and transgenic plantlets are in the process of being isolated for characterization. A manuscript describing the PHB production and rearrangement in transplastomic PHB producers is being prepared for publication.

Y1-2 Task 3: Demonstration of an inducible PHB production system in *Arabidopsis*.

Previous studies with plastid-targeted PHB production in the model plant *Arabidopsis* produced significant levels of PHB in plants⁷, however growth of the plants was impaired. Task 3 was designed to develop inducible systems for PHB production in an attempt to alleviate the stunted phenotype often observed in high producers. Plants could be grown in the field to near maturity allowing the normal production of plant biomass. Subsequent treatment with an approved agricultural chemical would switch on PHB synthesis. The ecdysone induction system⁸ was chosen since it utilizes chemicals that are commercially available and approved for field use.

During the DOE funded period of the program, multi-gene constructs containing inducible expression cassettes for plastid-targeted enzymes of the PHB pathway were prepared and transformed into *Arabidopsis*. Transgenic plantlets were isolated and screened for PHB production after application of inducing agent. Several lines were isolated where PHB production was controlled by application of inducing agent. These plants possessed fairly normal phenotypes and produced levels of up to 14% dwt PHB. The research funded by the DOE program laid the ground work for publication of the inducible PHB system in Transgenic Research⁹.

⁵ De Cosa et al., 2001, Nat. Biotechnol. 19, 71-74.

⁶ Daniell, 2002, Nat. Biotechnol. 20, 581-586.

⁷ Bohmert et al., 2000, Planta, 211, 841-845.

⁸ Martinez et al., Plant Journal, 1999, 19, 97-106.

⁹ Kourtz et al, 2007, *Chemically inducible expression of the PHB biosynthetic pathway in Arabidopsis*, Transgenic Research, in press. DOI 10.1007/s11248-007-9067-1.

Y1-2 Task 4: Develop a methodology for plastid transformation of Switchgrass. (Work during the DOE funded portion of this program was performed in collaboration with Professor Henry Daniell's laboratory at the University of Central Florida. Work since the DOE program has been performed exclusively at Metabolix).

Since plastid DNA is maternally inherited in switchgrass, location of the PHB gene expression cassettes on the switchgrass plastome would significantly reduce or eliminate transgene flow via pollen providing a method of gene containment. To date successful plastid transformation protocols for monocots have not been reported. Success in switchgrass would likely provide a broadly enabling transformation technology for grasses and perhaps other monocots. During the first year of the DOE program, a series of constructs for switchgrass plastid transformation trials were prepared in the laboratory of Henry Daniell and transformation trials were initiated. Tissue culture systems required for regeneration were transferred from Professor Conger's laboratory to the laboratory of Professor Daniell. Pilot tissue culture studies in the Daniell lab identified an agent that can be used for selection of transgenic switchgrass in regeneration experiments by preventing the growth of wild-type plants. In test chloroplast transformation experiments for switchgrass, plantlets resistant to selection provided by the selectable marker on the transformation vector were obtained. Preliminary indications suggested that the marker genes were inserted into the chloroplast genome. At the end of the plant science portion of the DOE program, funding to the Daniell Laboratory was terminated.

Y1-2 Task 5.1-5.3 Demonstration of a metabolic pathway for medium chain-length PHA production in Arabidopsis.

This task was designed to test a novel biosynthetic pathway for producing PHAs containing medium chain length 3-hydroxyacid monomers in the plastids of the model plant *Arabidopsis*. Success in this program would enable the production of a range of PHA copolymers from the Biomass Biorefinery, by combining this pathway with the PHB pathway, significantly expanding the applications that can be targeted with PHAs.

During the first year of the DOE program, a genetic construct designed to express a novel biosynthetic pathway for medium chain length PHA formation in plants was created. This pathway was developed previously at Metabolix in *E. coli* using genes encoding the proteins PhaG, AlkK and PhaC. Expression of these genes in *E. coli* grown in the presence of glucose allows production of medium chain length PHAs from intermediates of fatty acid biosynthesis. This Task was not progressed due to re-focusing of the effort.

Y1-2 Task 5.4: Regulatory Programs. This Task focused on biodegradation testing of PHB copolymer resins and products and is reported under Y3-5 Task 4.1

Y1-2 Task 5.5: Analysis of Copolymer Rheological Properties. This program continued throughout the project and is reported in Y3-5, Task 4.1

Y1-2 Task 5.6: Ester Interchange Programs. This program was continued and is reported as Y3-5, Task 4.7

Y1-2 Task 5.7: PHA Copolymer Processing. This program was continued for the length of the project and is reported as Y3-5, Task 4.5

Y1-2 Task 5.7.1 - 5.7.3 Fundamentals of PHB Copolymers. Work continued and is reported as Y3-5, Tasks 4.1 - 4.4

Y1-2 Task 5.8 PHB synthesis with *Saccharomyces cerevisiae* (Professor Friedrich Srienc, University of Minnesota). The objective of this project is to establish a simple eukaryotic system for rapid screening of new gene constructs for producing new PHB copolymers and for identifying genes, mutations or metabolic shifts beneficial to increasing PHB levels in eukaryotic cells. There is a wealth of genomic, proteomic and physiological information available for *S. cerevisiae* which is well suited to high throughput screening systems simply not possible in plants. In this project a novel technique for measuring the PHB content in yeast cells was developed. Initial studies used a galactose inducible expression system to express the three genes, bacterial PHA pathway.

Quantification of PHB in single cells: To facilitate the isolation of yeast strain variants that are able to accumulate large amounts of PHB we developed a novel flow cytometry staining protocol. Previous studies used either light scattering or fluorescence after staining with Nile red to detect the presence of PHB in individual cells¹⁰. Nile red also stains the lipids present in the cell resulting in large amounts of background staining. In order to minimize the background, other staining methods that are more specific for the PHB granules than Nile red are desired. One stain that may be an alternate stain to Nile red for PHB analysis is BODIPY 493/503¹¹, which is known to be more specific for cellular lipid droplets than staining with Nile red. In this study a method for quantitatively determining the quantity of PHB in engineered *S. cerevisiae* using flow cytometry after staining with Nile red and Bodipy 493/503 was developed.

A Becton-Dickinson FACSCalibur flow cytometer with a 15-mW Ar laser with a wavelength of 488 nm was utilized to determine the fluorescence intensity of the cells stained with Nile red or BODIPY 493/503. Nile red fluorescence was measured using a 580±42 nm band pass filter. BODIPY 493/503 fluorescence was measured using a 530±30 nm band pass filter. Data was collected using linear amplification. At least 30,000 cells were measured in each sample, and the average channel number of the height of each pulse was utilized to determine the mean fluorescence of each sample. Automated flow cytometry was done using the system described by Abu-Absi et al. (in press.)

Figure 1 illustrates the cytograms that were produced by analysis of cells with different amount of PHB by cytometry after treating the cells with Nile red. It was found

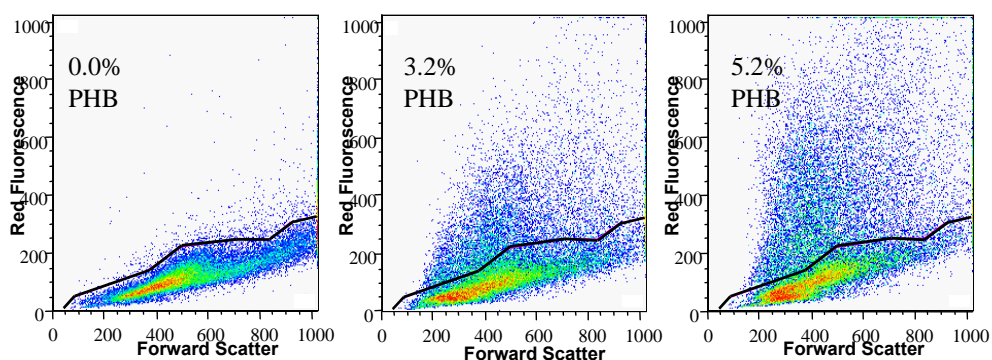


Figure 1: cytograms of *S. cerevisiae* stained with Nile red stain and analyzed by flow cytometry. The cytograms indicate data from cells containing 0, 3.2 and 5.2 percent PHB as determined from gas chromatography analysis.

that the fluorescence of the cells increased when the PHB content of the cells increased.

¹⁰ Degelau A, Scheper T, Bailey JE, and Guske C. 1995. Applied Microbiology and Biotechnology 42:653-657.

Muller S, Losche A, Bley T, and Scheper T. 1995. Applied Microbiology and Biotechnology 43:93-101.

¹¹ Gocze P and Freeman, D. A. 1994. Cytometry 17: 151-158.

Also, it was found that the size of the cells contributed to the background fluorescence as indicated by the increase of fluorescence of the cells in the control sample as the forward scatter or size of the cells increased. A distinct population of cells containing PHB was not found. However, the number of cells producing PHB could be estimated by drawing a gate around the population of cells in the control sample. In samples containing PHB, the percentage of cells that fell outside this gate was considered to contain PHB. As the average PHB content of the cells increased from 0 percent to 3.2 percent to 5.2% PHB, the percentage of cells containing PHB increased from 2 percent to 27 percent to 43 percent respectively indicating that the fraction of cells with detectable amounts of PHB increased with overall PHB content. The above trends were also seen when the cells were treated with BODIPY 493/503.

In order to make the stain quantitative, the results from the flow cytometer had to be compared to the results obtained from gas chromatography. The average channel number of the fluorescence was determined by taking the average of the channel number from all of the cells. By plotting the average channel number against the PHB per cell as determined by GC analysis, a linear calibration curve for the flow cytometer

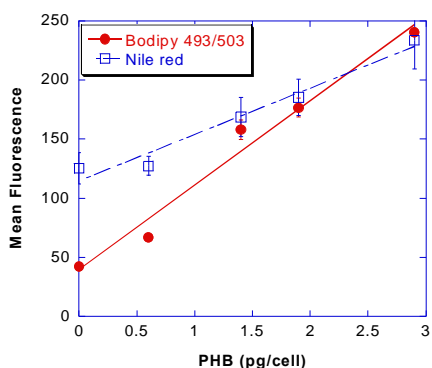


Figure 2: A comparison of the calibration curves determined by staining cell samples with different quantities of PHB with Nile red and BODIPY 493/503. The correlation for the Nile red data was 0.982 with a slope of 39.1 and an intercept of 114. The correlation for the BODIPY 493/503 data was 0.988 with a slope of 71.5 and an intercept of 39.5. The increase in the slope of the calibration curve indicates an increase in the sensitivity of the stain.

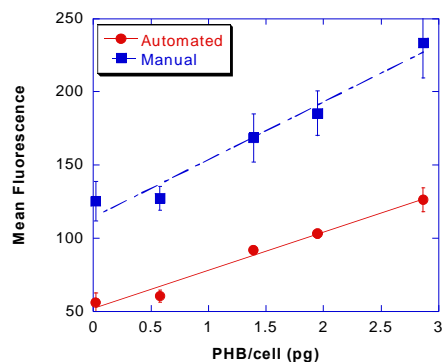


Figure 3: A comparison of the calibration curves created by on-line and off-line staining of *S. cerevisiae* containing PHB with Nile red. The on-line measurements were made using a flow injection flow cytometer set up described by Abu-Absi et al. (in press) and the off-line measurement was made by manually staining 1 mL of cell suspension with 10 μ L of 100 μ g/mL Nile red. The best linear fit of the on-line samples produced a line with a slope of 26.1, an intercept of 52.0, and a correlation R: 0.990. The best linear fit of the off-line samples produced a line with a slope of 31.1, an intercept of 97.7, and a correlation R: 0.974.

can be created as seen in Figure 2. Although both the Nile red and Bodipy 493/503 stain produced a linear calibration curve, the slope of the calibration curve of the cells stained with Bodipy 493/503 had a slope and smaller intercept indicating that the Bodipy stain is more specific for PHB than Nile red. In addition, the standard deviation of the cells that were analyzed after staining with Bodipy (as indicated by the error bars in Figure 2) was smaller than the standard error associated with the analysis of cells after staining with Nile red.

It is also important to develop a rapid automated system to quickly measure the content of PHB in a culture. One way to accomplish this is to use a flow injection flow cytometer device such as the one described by Abu-Absi (in press). Five samples of *S. cerevisiae* with various amounts of PHB were stained using the above device. A

calibration curve as described above was constructed and compared to the previous curve constructed using manual staining (Figure 3). The automated method produced a linear correlation, as did the manual method. However, the linear correlation was higher for the automated stain and the error associated with each measurement was smaller for the automated stain.

In conclusion it was found that by staining cells containing PHB with Nile red or Bodipy 493/503 and analyzing the fluorescence of these cells using flow cytometry, one can quantitatively measure the PHB content of the cells. Furthermore, the PHB content of the cells can be determined at the single-cell level. Because the cells remain viable after staining, this technique allows for the isolation and characterization of mutant or recombinant cells that produce a high level of PHA can be isolated using fluorescence activated cell sorting.

This program was terminated following the decision by DOE to discontinue funding for plant science related Tasks.

Y1-2 Task 6: Development of Optimized Recovery Processes for PHAs in a Biorefinery Context: This Task was continued and is reported as Y3-5 Tasks 2 and 3.

Y1-2 Task 7: Lifecycle Analysis: This task was continued and is reported in Y3-5 task 3.

Y1-2 Task 8: Investigation of Additional Applications for PHAs.

Subtask 8.1: Evaluation of PHA oligomers for Polyurethane Production (Collaboration with Professor Shaw Lin Hsu, University of Massachusetts, Amherst): Processes for the hydrolytic degradation of polyhydroxybutyrate (PHB) to produce oligomers and other derivatives for testing in urethanes were developed and samples shipped to U. Mass for evaluation. Results of these studies demonstrated that cured films made using a formulation containing the PHB derivatives exhibited unusual properties. This project was terminated.

Subtask 8.2: PHB as a precursor for green solvents: Several 3HB esters were identified as potential solvents/coalescing solvents. Basic characterization data and synthetic routes for manufacture directly from the polymer were generated. Discussions were initiated with DOD regarding the potential use of 3HB esters as environmentally friendly cleaning solvents. ICI Paints, UK expressed interest in higher boiling point 3HB esters for use as coalescing solvents in architectural paints. Initial screening showed that the esters have acceptable compatibility with the vinyl acetate and acrylate copolymer emulsions and good low temperature film forming properties. A further series of esters has been submitted for testing under DIN 55649-2000. If the esters have longer retention times than tetradecane, as measured by GC, then they can be considered VOC exempt under the German Blue Angel code of practice. Currently there are no coalescing solvents commercially available that meet the Blue Angel requirements. These esters are currently being screened by ICI Paints in a number of aqueous architectural paint formulations (gloss, semi-gloss, satin, matt).

Internal administrative issues relating to testing protocols delayed the Army Materials Command Cleaner Compatibility Evaluation Program. The program will designate each solvent as an Army tested solvent for procurement purposes.

Year 3 - 6 Revised Goals

Based on extensive discussions with the Office of the Biomass Program following the decision to terminate funding of plant science programs by EERE, the program was refocused on the following Tasks for the remainder of the contract. In 2006 the program was extended for another year.

- **Y3-6- Task 1: Polymer Extraction from Plant Systems**
- **Y3-6- Task 2: PHA Recovery:**
- **Y3-6- Task 3: Integrated Biomass Biorefinery for Producing PHAs.**
- **Y3-6- Task 4: Fundamentals of PHA Polymer Properties/Processing**
- **Y3-6- Task 5: Production of PHA samples**
- **Y3-6- Task 6. Life Cycle Analysis – Consolidated with Task 3**
- **Y3-6- Task 7: Management and Reporting.**

The work, results and decisions for each of these Tasks are described independently for the remainder of the program under these Task headings.

Y3-6-Task 1: Polymer Extraction from Plant Systems

Task 1.1 Evaluate non-PHA Extractables.

Task 1.1 Deliverables and Resources			
Year 3	Year 4	Year 5	Resources
-Identify potential extractants from solvents identified in Task 2. -Identify solvent system which achieves less than 0.2% extractables in final polymer	-	-	Metabolix

Task 1.1 Decision Points: This Task was deferred in Year 4 and eliminated in year 5 to focus remaining resources on Tasks 2- 6 in order to complete these critical Tasks leading to commercial production in 2008.

Task 1.2 Optimization of PHA extraction from biomass

Task 1.2 Deliverables and Resources			
Year 3	Year 4	Year 5	Resources
Obtain polymer extracted with >70% efficiency using solvents identified in Tasks 1.1 and 2.1	Increase recovery efficiency to 85%	Increase recovery efficiency to >90%	Metabolix
	Obtain initial regulatory opinion On solvent systems	Obtain final regulatory approval of solvent process	Regulatory consultant

Task 1.2 Decision Points: In year 3 of the program in Q1, a database of solvent candidates for PHA recovery from plant crops was developed and characterized in terms of solubility parameters and key processing characteristics of the solvents (e.g. physical and thermodynamic properties as well as safety, regulatory and environmental criteria).

The solvents were ranked in terms of preference and likely applicability to the range of PHA polymer compositions expected to be produced in crops. Three solvent candidates which can be used for all PHA compositions likely to be produced in plants were identified.

Solvent 1, Solvent 2 and blends of these two solvents with aliphatic solvents were identified as having the best characteristics of PHA solubility, boiling point, flash point, density, water solubility, low toxicity, PHA solution viscosity, Hansen solubility parameters and solvent availability and cost. Laboratory-scale solvent extraction was performed on a small quantity of *Arabidopsis* (model crop) containing a low level of PHA (approximately 10%). It was confirmed that the current solvent extraction system, extracted the polymer at acceptable purity albeit at relatively low efficiency (70% recovery). This Task was eliminated in year 4/5 to focus remaining resources on Tasks 2- 6 in order to complete these mission critical Tasks leading to commercial production in 2008.

Task 2: PHA Recovery:

Task 2.1 Determine Optimal Solvent Systems.

Task 2.1 Deliverables and Resources			
Year 3	Year 4	Year 5	Resources
Identify 3 additional solvent candidates for PHA extraction from plants with a recovery cost less than \$0.25/lb PHA	-	-	Metabolix

Task 2.1 Decision Points: The decision point is to select the best solvents for Task 1.

Task 2.1 Progress Year 3: The goal was to qualify additional solvents that will be suitable to extract the likely range of PHA compositions that will be produced in crops and specifically optimize the selection with reference to the likely impurities that could be co-extracted.

The traditional solvents (e.g. Solvent 1 below) used for extraction of copolymers from fermentation broth were found not to be suitable for the low co-monomer polymer grades that would typically be produced in plants. An extensive literature search was completed and a database developed summarizing key properties of alternative solvents. These included solvency parameters (Hansen parameters), flammability, toxicity, physical properties and commercial availability.

One of the most important parameters impacting the recovery cost is the viscosity of the PHA solution. High viscosity results in high pumping, separation and processing costs. Viscosity profiles of Solvent 1 solutions were developed as a function of temperature and concentration as depicted in Figures 2.1a and 2.1b below.

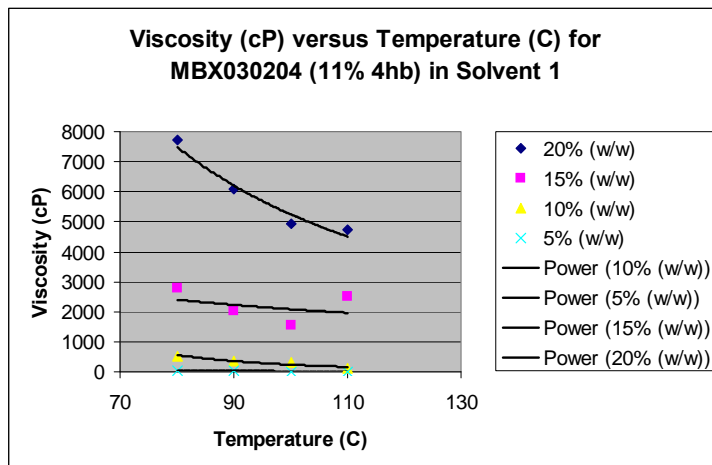


Figure 2.1a. Viscosity of PHA solutions in Solvent 1 vs. temperature. The benefit of elevated temperature is greatest for more concentrated solution above 20% w/w.

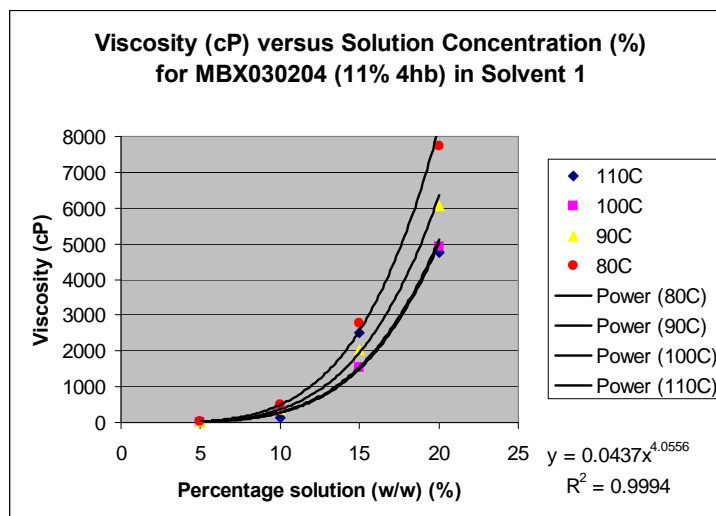


Figure 2.1b. Viscosity of PHA solutions in Solvent 1 as a function of concentration. Viscosity follows fourth power law: $\text{Viscosity} = \text{Constant} \times \text{concentration}^4$

After evaluation of many different solvent in the laboratory, an alternative solvent was identified (Solvent 2) that extracts copolymers and homopolymer very efficiently to high concentration. Viscosity measurements reported for the Solvent 1 above were repeated to confirm that the new solvent would have acceptable extraction performance. The viscosity of both Solvent 1 and Solvent 2 solutions fit the following power function:

$$Y \text{ (cP)} = a (b)^X,$$

in which b is percent solution (%) (w/w) and is a constant dependent on temperature. The exponent x varies between 3.0 and 3.5 depending on solvent. Although Solvent 1 has a viscosity benefit for high co-monomer content polymers, the new solvent performed better across the composition spectrum. It was confirmed that Solvent 2

extracted homopolymer at concentrations exceeding 4% while Solvent 1 was limited to 0.5%.

During the last quarter focus shifted to investigating selectivity of the new solvent systems as it relates to impurity rejection. A large batch of polymer was extracted into the solvent and precipitated to yield a filtrate that successively concentrated under vacuum to yield a small volume (< 5 mL) of concentrated, viscous yellow liquid that was essentially solvent free. Analysis confirmed that a significant fraction of the impurities derived from biomass lipids as confirmed by matching the fatty acid profile. Thin-layer chromatography (TLC) analysis indicated presence of phospholipids as well as neutral lipids. In addition, the TLC confirmed the presence of unsaturated neutral lipid components.

Based on the analytical characterization, the major impurities were identified as phospholipids, free fatty acids released during the extraction step and additionally an intense color body yet to be identified but suspected to be a highly conjugated membrane bound co-factor. In order to facilitate further process development a GC-MS method and library was developed to quantify the lipid content at various points in the process and in the final product.

Based on the impurities identified, process schemes were developed to target removal of the impurities with the aim to improve color and odor. These included:

- Pre-treatment of the biomass post fermentation but prior to solvent extraction to remove interfering impurities prior to solvent extraction.
- Selective use of chemicals during the process to convert color and odor bodies to components that can be separated more readily
- Optimizing the washing process after polymer precipitation.

A summary of the results are presented in Table 2.1a. During year 3 the individual options were investigated to establish benchmark performance. During the subsequent project years various combinations of the best options were evaluated to guide selection of the ultimate integrated process scheme for optimal purity. In order to compare the efficacy of different treatment regimes with respect to polymer quality (specifically color) it is essential to quantify the measurement of color. A review of available instrumentation and ASTM methods pointed to the HunterLab instrument as the industry standard for polymer color measurement. An on-site evaluation was performed with the vendor and this formed the basis for the acquisition of an in-house instrument.

Table 2.1a: Impact of pre-treatment and washing options on polymer color.

Treatment	PHA Dissolution	PHA FFA content	HunterLab YI
<i>Aqueous pre-treatments prior to solvent extraction (Polymer directly dried from solution)</i>			
Control	99.7%	1.9%	10.4
Chemical pre-treatment	89.3%	1.4%	6.6
Enzyme pre-treatment 1	74.6%	2.7%	7.3
Enzyme pre-treatment 2	90.8%	1.5%	6.5
Enzyme cocktail pre-treatment	90.1%	2.8%	5.1
<i>Post extraction and precipitation washing efficiency (no pre-treatment)</i>			
Precipitated polymer (as is)		N/A	7.3
Precipitated polymer (solvent 1)		N/A	6.2
Precipitated polymer (solvent 2)		N/A	5.3

Reference market material	2.3
Key: FFA: Free Fatty Acids YI: Yellowness Index	

Although the individual treatments did not meet the color of the reference material, the results showed enough promise to justify further study of integrated options to achieve the final desired purity.

Year 4: Task 2.1

Task 2.1 Optimize solvent systems and improve polymer quality.

Task 2.1 Deliverables and Resources		
Year 4	Year 5	Resources
Identify flowsheet options to eliminate residual color and odor. Control compositional distribution to optimize polymer processing characteristics.	-	Metabolix

Task 2.1 Decision Points: The decision point is to develop a process that eliminates color and odor as evaluated by a taste and odor panel for food contact applications. The process should also produce polymer that can be fully processed on current polymer processing equipment.

Task 2.1 Progress Year 4: The overall objective of this task during Year 4 was to optimize the product quality from the solvent recovery process by optimizing the the process followed by demonstration at pilot scale. The key parameters that were identified during Year 3 to be optimized during Year 4 included:

- Pre-digestion of biomass impurities.
- Extraction solvent formulation and viscosity optimization.
- Solvent polishing post dissolution to remove impurities.
- Post precipitation polymer washing regime.

During Q1 the pre-digestion of impurities using both chemical and biological routes were investigated in addition to different washing regimes post polymer precipitation.

During Q2 extensive shelf life studies were conducted to confirm that pre-digested material could be stored for a period of time and still produce polymer with molecular weight (Mw) > 750 kD as required for most final applications.

A comprehensive cost analysis was completed and indicated that a two-solvent process based on precipitation of the polymer from solution using a precipitant such as heptane is was preferred from a cost, flexibility and purification perspective and work commenced on the design of a dedicated pilot facility on this basis. Year 3 pilot recovery operations. The final solvent system was selected based on a review of cost, availability, safety considerations and solvency power and selectivity. Typical comparative data for different solvents investigated are summarized in Table 2.1.b.

Table 2.1b. Comparative data for different solvents for PHA recovery from fermentation broth. Solvent 2 offers the best overall balance of cost and process performance, coupled to an ability to dissolve a wide range of polymer compositions including homopolymer.

PHBco-monomer (11%) Copolymer Extraction Performance					
Solvent System	PHB solubility (w/w %)	Solubility (w/w %)	Viscosity 3.5% (w/w) solution (cP)	Color of final product	Impurities (MeOH extractables)
Solvent 1	< 2.5% (gels)	> 5% at 80°C	5-10 cP	Good	0.1%
Solvent 2	> 5.0%	> 5% at 60°C	20-30 cP	Fair	0.3%
Solvent 3	> 3.5%	> 5% at 80°C	10-20 cP	Good	0.1%

Solvent 1 does not dissolve the homopolymer and Solvent 3 was found to be hydrolytically unstable. Solvent 2 displayed the best overall balance of performance with the key disadvantages being higher solution viscosity as well as lower impurity rejection. This resulted in a structured program to address various options to eliminate color and odor from the final precipitated polymer focusing on the options previously identified.

During Q3 work focused on optimizing the selected solvent system of to achieve the best overall purity. Formulation of the solvent system enabled viscosity reduction of 50% and this improved purity significantly. Viscosity is a key determinant of separation efficiency and hence purity.

It was also determined that the lowest dissolution temperature that avoids gelling should be used as this minimized co-dissolution of impurities for best purity.

In order to remove final trace impurities various solution (separated solvent with dissolved polymer) polishing techniques were evaluated. Several adsorbents and wash liquids with and without chemical additives were evaluated with the best solution being simple liquid back-washing. The results were dramatic as indicated in Figure 2.1c.



Back wash liquid 1

Back wash liquid 2

Back wash liquid 3

Figure 2.1c Films demonstrating the impact of back washing of the polymer solution with various formulated liquids. Back wash liquid 1 was most effective and adopted.

During Q4, different treatment regimes were evaluated during both extraction and the centrifugal solution polishing stages. Chemical dosage rate, time and temperature effects were optimized and kinetics of color removal as well as Mw degradation were quantified. The color removal processes can reduce the Mw of the polymer under certain conditions and therefore conditions must be carefully controlled. It was found that in general harsher treatments for longer periods improved color at the expense of Mw as indicated in Figure 2.1d.

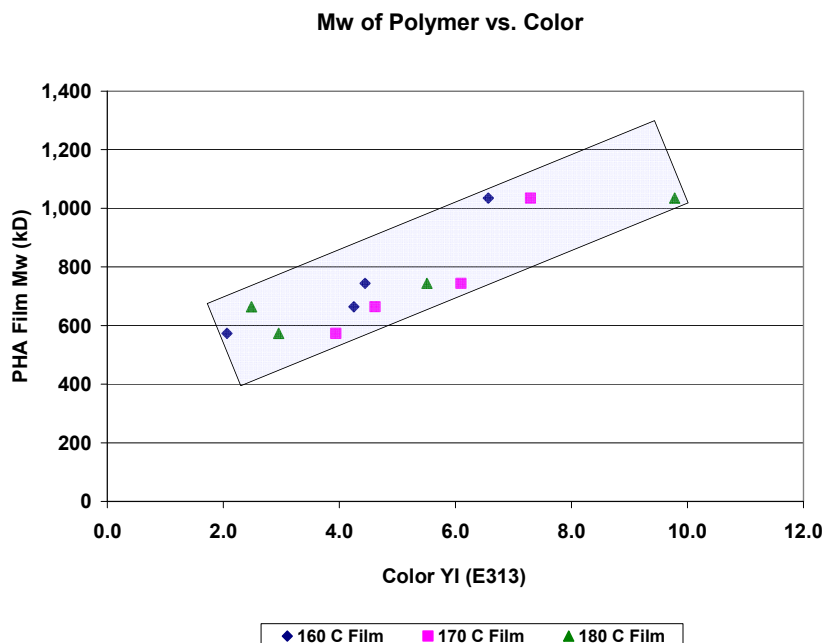


Figure 2.1d General trade-off between improved color and polymer Mw.

It was established that the inevitable Mw loss as a result of chemical dosage during the polymer extraction and polymer solution back-extraction stages can be minimized by limiting the contact time.

After polymer recovery in the extraction plant, plastic pellets are produced that are processed to final goods in conventional plastics processing equipment. This includes extruders, injection molding, cast film, blown film and other similar unit operations where the polymer is melt processed at temperatures of the order of 160 C. Any color removal process must ensure adequate melt stability of the polymer. Polymer recovered via the chemical dosage and back extraction process does not display adequate melt stability.

It was found that a simple washing step applied to the polymer after precipitation effectively restored and even improved melt stability compared to the control. Different unit operations to perform the washing operation of the precipitated polymer were evaluated and a specification for the pilot plant developed. Suitable control strategies for large-scale operation were developed and specified for validation at the pilot plant.

Elimination of the last trace of color from the recovered polymer remained a key objective as this would allow access to much broader applications and markets. The focus during Year 5 was set as the implementation of the color removal process at pilot scale such that further learning and optimization can be performed in more representative of industrial processing conditions.

During Q4 a pilot plant with capacity of 10 tpm was commissioned incorporating all the key unit operations developed during the project. The pilot plant was constructed with ADM and Metabolix funds, not DOE funds. Many of the washing protocols developed during the year could not be implemented at the time of start-up as the processes were only defined after the pilot plant design was frozen. Additional capital was approved to implement the advanced wash protocols during Year 5.

Year 5: Task 2.1***Task 2.1 Optimize solvent systems and improve polymer quality.***

Task 2.1 Deliverables and Resources		
Year 4	Year 5	Resources
Identify flowsheet options to eliminate residual color and odor. Control compositional distribution to optimize polymer processing characteristics.	Implement process with reduced color and odor in the recently completed pilot plant. Resolve all scale-up issues to deliver product suitable for applications targeted in Task 4	Metabolix

The pilot plant commissioned during the last quarter of Year 4 was equipped with scaleable extraction and product recovery equipment. At the same time work continued in the laboratory to characterize the polymer produced during the pilot recovery with particular emphasis on color, odor and Mw stability.

Temporary arrangements were made to implement the wash protocol on the precipitated polymer. This was successful in producing polymer of acceptable quality for all intended applications in addition to improving melt Mw stability as required for large-scale compounding and extrusion of polymer.

The necessary engineering design data for the washing unit operation was completed by performing both equilibrium and kinetic studies. The design was finalized and a capital project was approved by the Company to modify the pilot plant to allow for improved washing and stabilization of the polymer.

At this stage it was concluded that the process has been developed to the point that acceptable quality can be produced for all significant markets. Further effort would be focused on finalizing unit operations design, approving and implementing a project to install the selected unit operations at the pilot plant and then producing high quality product on a reproducible basis.

A summary of the pilot production during the overall project is presented below.

Table 2.1c. Pilot Production Summary during Project

Year	Pilot Production (kg)
2003	1,473
2004	1,290
2005	5,812
2006	38,340
2007	94,450
Total	141,365

Task 2.2 Develop Process to Produce a Biorefinery Product, PHA latex.

Task 2.2 Deliverables and Resources			
Year 3	Year 4	Year 5	Resources
Optimize cell lysis and digestion of non-PHA cellular material (achieve >95% dissolution)	Optimize latex stabilizer package and purification of latex (removal of > 95% of non-PHA contaminants)	Optimize latex process economics in a biorefinery process (target = \$1.00/lb PHA polymer)	Metabolix, Inc.

Task 2.2 Decision Points: By the end of year 1, the technologies for cell lysis and digestion of non-PHA cellular material along with the impact on the polymer should be completed. The method giving the most cost-effective digestion at lowest cost with least effect on the PHA polymer will be selected as the basis for the work in the following year. In Year 4, if we cannot effectively purify and stabilize the latex in a cost-effective manner then we will review alternative approaches including a solvent dissolution and dispersion approach. This approach will build on the results of Task 2.1.

PHA polymers have a unique quality in that they can be used to produce an amorphous aqueous latex that forms a coherent film upon drying and does not require any coalescing solvent (VOC free), yet produces a scrub resistant film upon curing. The key steps in developing a latex are 1) cell lysis and non-polymer impurity digestion, 2) stabilization of the latex, 3) latex purification and 4) product formulation.

The overall aim of this task was to develop volatile organic compound (VOC) free latex from a biorefinery based fermentation cell paste. The product needs to film form at room temperature and this generally requires an average particle size of less than 1 micron. The process as envisaged has three major steps:

1. Digestion and dissolution of cellular impurities (target is > 95%) to yield a polymer dispersion of sufficient purity on a suspended solids basis.
2. Separation of the polymer dispersion from the dissolved impurities to remove sufficient impurities (target is > 95%) to provide a latex of sufficient quality.
3. Stabilization of the resulting latex by optimizing the additive package to ensure that particles remain suspended, do not agglomerate and prevents degradation during storage.

The standard Metabolix fermentation was used to produce a cell paste containing 73.2% polymer on a washed solids basis. PHA with 25% co-monomer was used for the initial studies. The first step comprised washing of the cell paste to remove dissolved fermentation impurities using centrifugation. The cells were then lysed followed by further digestion of cellular impurities to release a sub-micron PHA latex dispersion. Chemical and enzyme dosage and sequence of addition were optimized as were pH and temperature conditions.

After final enzyme treatment, the cells could easily be disrupted using a simple hand-held homogenizer. A sub-micron latex was produced that had good film forming characteristics after separation of soluble impurities using centrifugation. However, testing indicated that the polymer was too sticky during processing. Work was therefore refocused on lower co-monomer compositions to formulate a latex that still has good film forming characteristics at 80 °C but with less stickiness.

As indicated in Table 2.2a below, it was possible to dissolve 79% of the impurities and produce a final latex with 94% PHA purity on a dry solids basis. This is sufficient for the particular application and close to the Task target of >95% purity of the final latex. At

the same time expensive high pressure homogenization was avoided and the whole cells could be washed in conventional equipment, solving a challenging process hurdle.

Table 2.2a. Impurity digestion profile and PHA purity in resulting latex as a function of latex process steps.

Step	TS %	TDS %	WDC g	TDS after subtracting for solids added g	Polymer g	% dissolution of non-polymer biomass	Polymer/WDC apparent purity
Broth after washing	13.33	0.14	272	2.5	225	0	82.6%
After chemical digest	13.00	1.11	260	19	225	35%	86.5%
After enzyme digestion	13.00	2.22	239	39.9	225	79%	94.1%

During the third quarter an alternative surfactant was used as it is known that the standard surfactant interferes with membrane filter washing of the latex formulation. Using the same enzymatic digestion as for previous samples, 100% of the non-polymer biomass impurities were digested. Membrane filtration was attempted to purify the latex but unfortunately more than 80% of the impurities were retained (i.e. did not pass through the membrane). Centrifugation using a Sorvall laboratory centrifuge was successfully used to remove the impurities. The cell suspension was then homogenized using the microfluidizer to break up the remaining cells releasing a latex with a d50 of 2um. The resulting material was relatively pure but did not film form below 80°C because of unusual thermal characteristics of the polymer. At the time it was assumed to be related to the particular fermentation broth but subsequent work points to the change in surfactant as the major contributor to this effect.

A second latex batch was prepared using a fermentation batch with a composition of 18% co-monomer and containing 78.7% polymer on washed dry biomass. The batch was subjected to the same process as previous samples reverting back to the original surfactant. The digestion of non-polymer biomass was less efficient but the resulting latex, although low in purity (93%), still film formed @80°C.

A third preparation used fermentation broth with 18% co-monomer content and 78% polymer on washed dry biomass. The process was very similar to the previous with two small deviations. A surfactant was added during the enzyme treatment because it is known to increase the activity of the particular enzyme by 230%. The resulting latex was high in purity (99.4%) and the digestion of non-polymer biomass was 98%. The d-50 was 2.3um and film formation was good @60 and 80°C. This latex sample was provided to a potential commercial partner for further studies of film forming characteristics.

Year 4: Task 2.2

Task 2.2 Decision Points: Work during the previous periods developed a suitable semi-pure latex formulation that film forms at 60-80°C for agricultural applications. The goal during Year 4 was to investigate the market opportunity for semi-pure latex in agricultural applications with a potential partner and reach a decision regarding commercialization.

Task 2.2 Progress Year 4: Given the inherent limitations of the aqueous latex recovery process developed to previously (low purity and inability to produce a latex that

will film form to high crystallinity), the focus was shifted to solvent and melt dispersion of the purified PHA resin into water with a suitable stabilization package.

During Year 4 some success was achieved dispersing 11% co-monomer polymer solutions (using a water miscible solvent) into water and stripping off the solvent at 65°C. A stable dispersion with sub-micron sized particles was achieved but film formation was poor due to premature crystallization of the polymer during the solvent stripping process. Good film formation requires preservation of the amorphous character of the particles.

A review identified melt dispersion of the polymer as a very attractive route to a PHA latex provided a number of process challenges could be overcome:

- Mw stability of the polymer when contacted with water under high temperature melt conditions.
- Suitable design of extruder/kneader to meet the very high shear requirements inherent in melt dispersion processes.
- Avoiding boiling or flashing of the water after dispersion.
- Finding a suitable surfactant that is stable under melt conditions and forms an effective surfactant after dilution.

In order to thoroughly evaluate this process, significant additional physical resources would be required with an estimated cost of \$350,000.

An interested industrial partner was identified for latex via the melt dispersion route. The partner is a leading supplier of industrial surfactants and would contribute this aspect of the technology. A joint proposal was developed that would be considered during Year 5.

Year 5: Task 2.2

Task 2.2 Develop Process to Produce a Biorefinery Product, PHA latex.

Task 2.2 Deliverables and Resources		
Year 4	Year 5	Resources
Evaluate solvent and melt dispersion techniques to produce high purity latex using resin as starting material	Optimize latex process economics in a biorefinery process	Metabolix, Inc.

Task 2.2 Decision Points: Work during the previous periods developed a suitable semi-pure latex formulation that film forms at 60-80°C for agricultural applications.

Task 2.2 Progress Q1, Year 5: Given the inherent limitations of the aqueous latex recovery process developed to date (low purity and inability to produce a latex that will film form to high crystallinity), the focus was shifted to melt dispersion of the purified PHA resin into water with a suitable stabilization package.

An interested industrial partner has been identified for latex via the melt dispersion route. The partner approved funding and support for this program and they also committed to provide expertise related to surfactants.

A internal budget was prepared for the capital equipment and additional resources required to pursue this technology. After extensive internal review it was decided that this project could not be funded as it would compete with limited resources focused on near-term commercial opportunities of the full-scale plant under construction focused on more conventional thermoplastic applications (injection molding, sheet extrusion and blown film). The latex project was therefore discontinued during Year 5.

Task 3: Integrated Biomass Biorefinery for Producing PHAs.

Task 3.1 Develop Flowsheets and Lifecycle Analysis for the PHA Biomass Biorefinery.

Task 3.1 Deliverables and Resources			
Year 3	Year 4	Year 5	Resources
Identify base-case conversion of biomass residue to energy in CHP case and co-firing case Provide a report summarizing flowsheets, mass/energy balance, capital costs and operating costs	-	-	Metabolix

In year 1 of the project a base case model for the potential output from a switchgrass PHA biorefinery in which the switchgrass biomass residue post PHA extraction was used in a combined heat and power scenario for steam and electricity was developed. This was the original task 7 goal of the project. This analysis was reviewed in discussions with Tracy Carole of DOE which resulted in the correction and revisions to the energy and CO₂ impact numbers presented in the original DOE proposal. The energy content calculations are based on the corn stover model as presented in Table 5 of "Greenhouse Gas Profile of a Plastic Material Derived from a Genetically Modified Plant", Journal of Industrial Ecology, Vol. 4, No. 3¹². The relative consumption of steam and electricity was identified as a key parameter in terms of calculating the surplus energy available from biomass combustion due to different conversion efficiencies. The ratio as used in the abovementioned paper was thus compared with a solvent process for microbial biomass. It is believed that much of the latter process once optimized will be transferable to PHA solvent recovery from switchgrass.

Energy Impact of PHA Production The original DOE Proposal projected a net energy credit related to PHA production from switchgrass of 9,750 BTU/lb of PHA. The subsequent Benefits Data Analysis submitted to the Idaho Operations Office indicated an electricity credit of 18,730 million kWh for 20 billion lb PHA (equivalent to 3,194 BTU electricity/lb PHA). These calculations have been revised after discussion with the DOE. The revised estimate is an overall fuel credit of 44,250 BTU/lb PHA, equivalent to 11,052 BTU electricity/lb PHA at a conversion efficiency of 25%. The difference is primarily related to the fraction of the total process energy required as electrical energy compared to steam

The calculations are summarized in Table 7.1.

Table 7.1: Revised Energy and CO₂ Calculations

Description	Units	Value	Notes
Process Energy Required for PHA Recovery			
PHA recovery steam	kg/kg	12.5	J. of Ind. Ecology (Table 5)
Recovery electricity	MJ/kg	8.8	J. of Ind. Ecology (Table 5)
Compounding electricity	MJ/kg	2.4	J. of Ind. Ecology (Table 5)
Total electricity	MJ/kg	11.2	
Total recovery and	MJ/kg	37.4	2100 kJ/kg steam and electricity

¹² Kurdikar et al., 2001, Journal of Industrial Ecology, 4, 107-122.

compounding energy	BTU/lb	16,075	expressed as MJ electric power
Electricity as % of total		30%	Metabolix process for microbial biomass indicates 25% ²
Fuel conversion efficiency	Electricity Steam	25% 90%	Agreed with DOE
Overall PHA primary fuel energy required	MJ/kg BTU/lb	74.0 32,150	Overall conversion factor is 1.98 or 50% (70%/0.9 + 30%/0.25)
Biomass Farming and Transportation Energy			
Recoverable PHA		7.5%	10% in Switchgrass, 75% recovery
Diesel use in farming	BTU/lb	1,314	217,230 BTU/ton switch grass (dry)
Chemical and fertilizers	BTU/lb	2,000	330,578 BTU/ton switch grass (dry)
Transportation energy	BTU/lb	1,085	179,300 BTU/ton switch grass (dry)
Total feedstock energy	MJ/kg BTU/lb	10.2 4,400	
Energy Available from Switchgrass			
Switchgrass available	kg/kg PHA	11	Assume 90% recovery of 92.5% (7.5% PHA is recoverable)
Switchgrass fuel value	MJ/kg	17	
Fuel energy available per unit of PHA	MJ/kg BTU/lb	187 80,422	
Net Fuel Energy Available and Bio-Power Generated			
Net energy credit per PHA	MJ/kg BTU/lb	102.8 44,250	Expressed as fuel energy
Bio-power generated (as electricity)	MJ/kg BTU/lb	25.7 11,052	Assume 25% conversion efficiency
CO₂ credit per unit PHA	g CO₂/lb	1,905	As per Proposal

Note 1: The feedstock energy was based on an estimated 10.7 kg N/acre and 25% of fuel and electricity for planting and harvesting (based on 4 year cycle) the feed

Note 2: A higher electricity factor was used as this is conservative. It is reasonable to expect higher electricity load for crop based PHA recovery as the PHA content is lower requiring more solids processing.

If a combined heat and power cycle (CHP) is implemented every 100 units of biomass energy would generate 30 units of electricity and 50 units of steam. Based on the switchgrass energy content of 80,422 BTU/lb PHA available this represents 40,210 BTU steam/lb PHA and 24,125 BTU electricity/lb PHA. The steam represents 350% of the process demand. If this low grade heat could be utilized the excess bio-power available would be 19,325 BTU/lb PHA (after subtracting 11.2 MJ/kg PHA process electricity). This aspect will be further investigated during the comprehensive LCA to be conducted in year 2 in conjunction with further scooping of extraction processes (Task 6).

Corrections and Clarifications to the DOE Proposal Data: Based on the revised energy numbers the Energy Impact Table on p. 11 of the DOE proposal has been revised as detailed in Table 7.2 (the change to the linear low density polyethylene (LDPE) numbers are the result of a simple error in the arithmetic).

Table 7.2. Revised Energy Impact Table (DOE Proposal p. 11)

	Year 10	Year 20
Polymer (million lbs)	2,000	20,000
PHA energy million BTU fuel value	-88,500,000	-885,000,000

(based on 44,250 BTU fuel value/lb PHA)		
LDPE energy required per unit polymer (based on 33,270 BTU fuel value/lb)	+66,540,000	+665,400,000
Energy benefit of PHA relative to LDPE (quad)	0.155	1.55

The original Benefits Data Analysis submitted to the Idaho Operations Office indicated the consumption of 559,200 million ft³ and 107 million barrels of oil for the production of 20 billion lbs of LDPE plastic. This was an error as the total fossil equivalent required is 107 million barrels of oil. Using a conversion ratio of 5600 ft³ gas/barrel yield and equivalent quantity of gas of 599,200 million ft³ (note difference in digits). The actual usage can be a mix of the two fuel sources in any proportion. The total energy figure of 33,270 BTU/lb LDPE agrees with the DOE estimate.

Conclusions:

1. The energy benefit related to PHA production was reviewed with DOE and the current estimate is that surplus electricity of 11,052 BTU/lb PHA will be produced compared to 9,750 BTU/lb PHA as presented in the DOE Proposal. This figure is based on a split steam and power cycle.
2. If a Combined Heat and Power (CHP) cycle is used the surplus electricity generated would be 9,325 BTU/lb PHA. The steam would be 350% of that required for PHA production. This would favor consideration of alternative sinks for low grade steam such as might be useful for the production of other potential biorefinery targets: ethanol, lactic acid/PLA, succinic acid, 1,3-propanediol.
3. The net fossil energy benefit of producing PHA relative to LDPE at the level of 20 billion lbs is estimated at 1.55 quads.
4. PHA farm input and transportation is estimated at 4,400 BTU/lb and process energy is estimated at 32,150 BTU/lb (88% of energy is process related). This clearly confirms the importance of optimizing both the recovery process as well as the PHA content in the switchgrass.

Consistent with the proposal extensive negotiations with Ecobalance were held to utilize their expertise in Lifecycle analysis for the PHA biomass biorefinery. Due to restrictions put on Ecobalance by their parent company accounting firm Pricewaterhouse Coopers relating to the use and disclosure of the results we were unable to agree on a contract. Discussions with Dr. John Sheehan (formerly of National Renewable Energy Laboratory (NREL) in Golden Colorado) progressed with the intention to establish a project to be carried out in year 2 and complete this task for the switchgrass system.

Task 3.2 Evaluate the Conversion of Biomass Residue

Task 3.2 Deliverables and Resources		
Year 4	Year 5	Resources
Determine capital, operating costs and LCA footprint of key modules (corn syrup fermentation, cellulose hydrolysis, cellulose fermentation, PHA extraction from crops, crop residue hydrolysis and crop residue energy recovery).	Combine modules to develop case studies of different biorefinery scenarios that are modeled as combinations of modules.	Metabolix NREL

Task 3.2 Decision Points: During Year 3, the basis for conducting this task was agreed with NREL and a work plan was agreed. At the request of DOE Metabolix established a CRADA with NREL so that this task could be undertaken. However, due to staffing limitations NREL was unable to undertake this study in a timeframe consistent with completing the project. Following discussions with the DOE program manager Metabolix proceeded to engage Professor Bruce Dale at Michigan State University to undertake a cradle to factory gate analysis of the production of PHA by fermentation using corn wet mill glucose as the feedstock. This analysis was based on the actual flowsheet and engineering for the PHA fermentation facility being constructed at Clinton Iowa. Details of this flowsheet are not included here for confidentiality reasons.

The results of this study will be presented by Prof. Dale at conferences beginning in May, 2008 and the detailed study is expected to be published in a peer-reviewed journal.

The study carried out a life cycle assessment (LCA) study on the Polyhydroxyalkanoates (PHA) product system to estimate the environmental performance of PHA derived from corn grain, particularly renewable energy consumption and greenhouse gas emissions. The system boundary includes processes from agricultural production to the PHA fermentation and recovery process. Thus this study estimates 'cradle to gate' nonrenewable energy and greenhouse gas emissions associated with PHA. The functional unit in this study is one kg of PHA.

County level agricultural information (e.g., corn yield, tillage practices, soil properties, climate information, etc.) is used in estimating the environmental burdens associated with both corn grain and stover production. Corn farming counties are specified. Four counties in Iowa (Boone, Cedar, Clinton and Jones counties) supply corn grain to a wet milling plant located in Clinton County. These counties, except for Boone County, also supply corn stover to a cogeneration power plant, which provides electricity and steam to the corn wet milling and PHA fermentation and recovery processes. County level soil organic carbon dynamics, nitrate losses due to leaching, and nitrogen oxide and nitrous oxide emissions are predicted by the DAYCENT model. The current tillage practices are applied to corn culture simulations in the DAYCENT model. Corn stover is assumed to be collected in corn culture under the conservation tillage practices.

The environmental burdens associated with products in multi-output processes are estimated by the system expansion approach, in which alternative product systems for co-products are introduced. An alternative product system delivers an equivalent function fulfilled by a co-product. The environmental burdens associated with corn stover are assumed to be the incremental effects of harvesting corn stover. The effects include changes in soil organic carbon level, nitrogen related emissions (e.g., N_2O , NO_x , NO_3^-) and phosphorus loss, yield reduction in the subsequent growing season due to removal of nutrients, and fuel used in harvesting corn stover. The alternative product systems for corn gluten meal (CGM) and corn gluten feed (CGF) in the wet milling process are corn grain and nitrogen in urea used as animal feeds, and the alternative product system for corn germ is soybean based on oil production rate. Fermentation residues in the PHA fermentation and recovery process are used as fuel in a cogeneration power plant, assuming coal is replaced. Thus, the alternative product system for fermentation residues is a coal-fired boiler system.

Most of energy used in the corn wet milling and PHA fermentation and recovery processes is generated from a cogeneration power plant, in which corn stover is burned to generate electricity and steam. Off-site power used in the PHA fermentation and recovery process is purchased from a wind power plant.

PHA consumes 2.5 MJ kg⁻¹ of nonrenewable energy (see Figure 1). Corn grain and PHA fermentation and recovery process consume large amounts of nonrenewable energy. Over 70 % of nonrenewable energy consumption in corn grain is associated with agrochemicals, and about 50 % of nonrenewable energy consumption in the PHA fermentation and recovery processes is associated with ammonia input. The largest nonrenewable energy credits are associated with the utilization of fermentation residues as fuel in the PHA fermentation and recovery process.

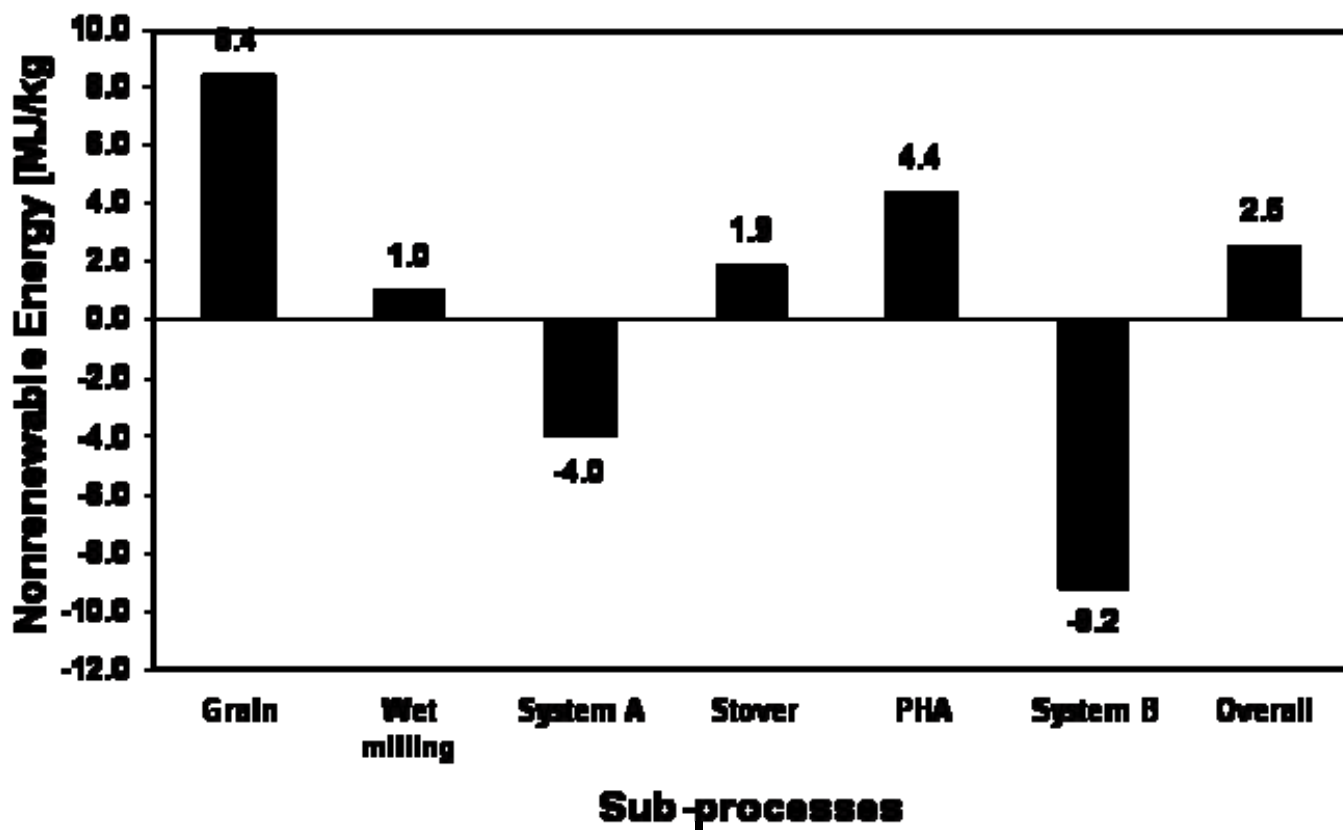


Figure 1 Nonrenewable energy consumption associated with one kg of PHA [System A: alternative systems for co-products in the wet milling process; PHA: PHA fermentation and recovery process; System B: alternative systems for fermentation residues in the PHA fermentation and recovery process]

PHA offers greenhouse gas credits, approximately -2566 g CO₂ eq. kg⁻¹ (see Figure 2). Greenhouse gas emissions include not only greenhouse gas emissions from processes and energy used, carbon sequestered by soil, and N₂O emissions from soil but also carbon dioxide credit associated with the carbon content in PHA. Carbon content in PHA (55.8 %) produces - 2046 g CO₂ eq. kg⁻¹ as greenhouse gas credits. N₂O emissions from soil are the dominant greenhouse gas emissions in the PHA product system except for the greenhouse gas credits described above.

Biomass production (i.e., corn grain and stover) is the dominant sub-process influencing nonrenewable energy consumption and greenhouse gas emissions associated with PHA production.

Nonrenewable energy consumption in most petroleum based polymers is 69 – 124 MJ kg⁻¹, and greenhouse gas emissions of petroleum based polymers range from 1994 to 7646 g CO₂ eq. kg⁻¹ (see Figure 4). Therefore, PHA is a more environmentally friendly polymer than any petroleum based polymer as measured by nonrenewable energy consumption and greenhouse gas emissions.

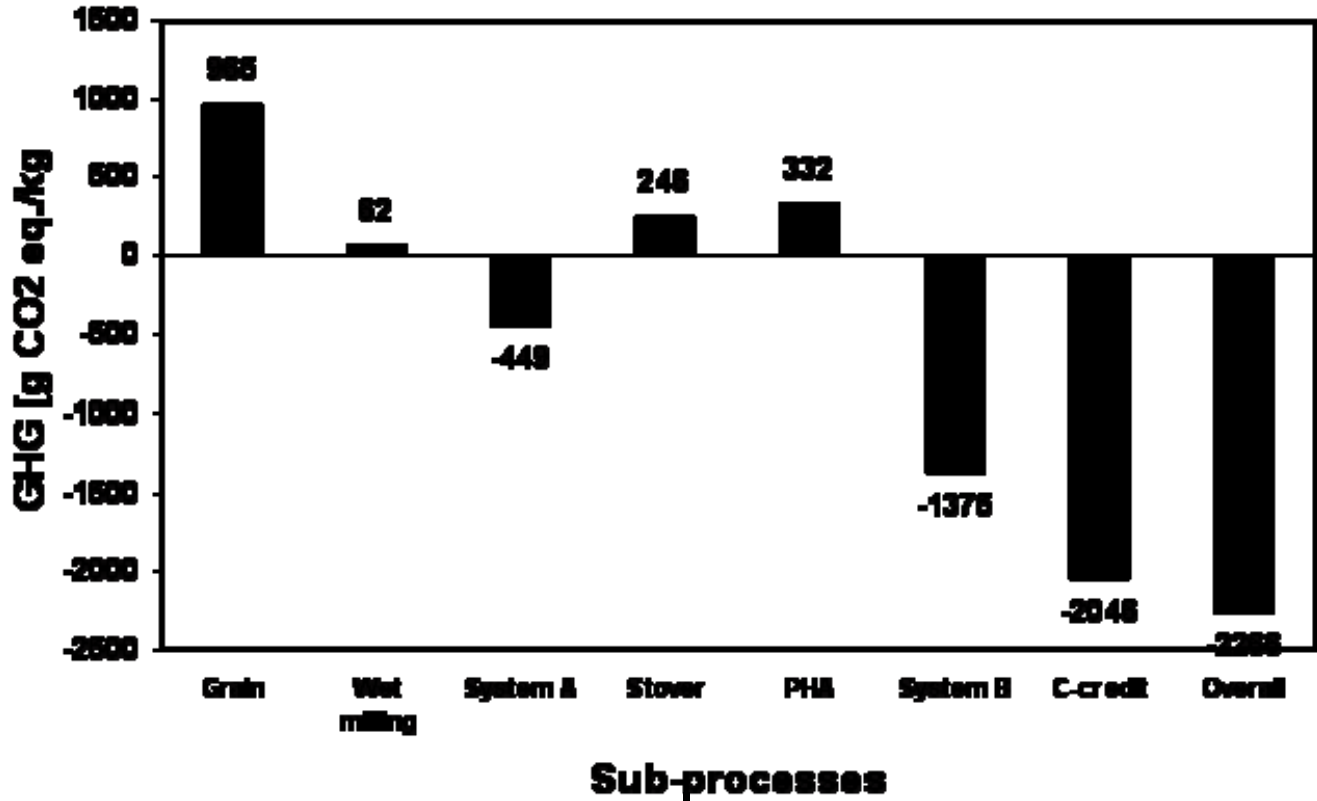
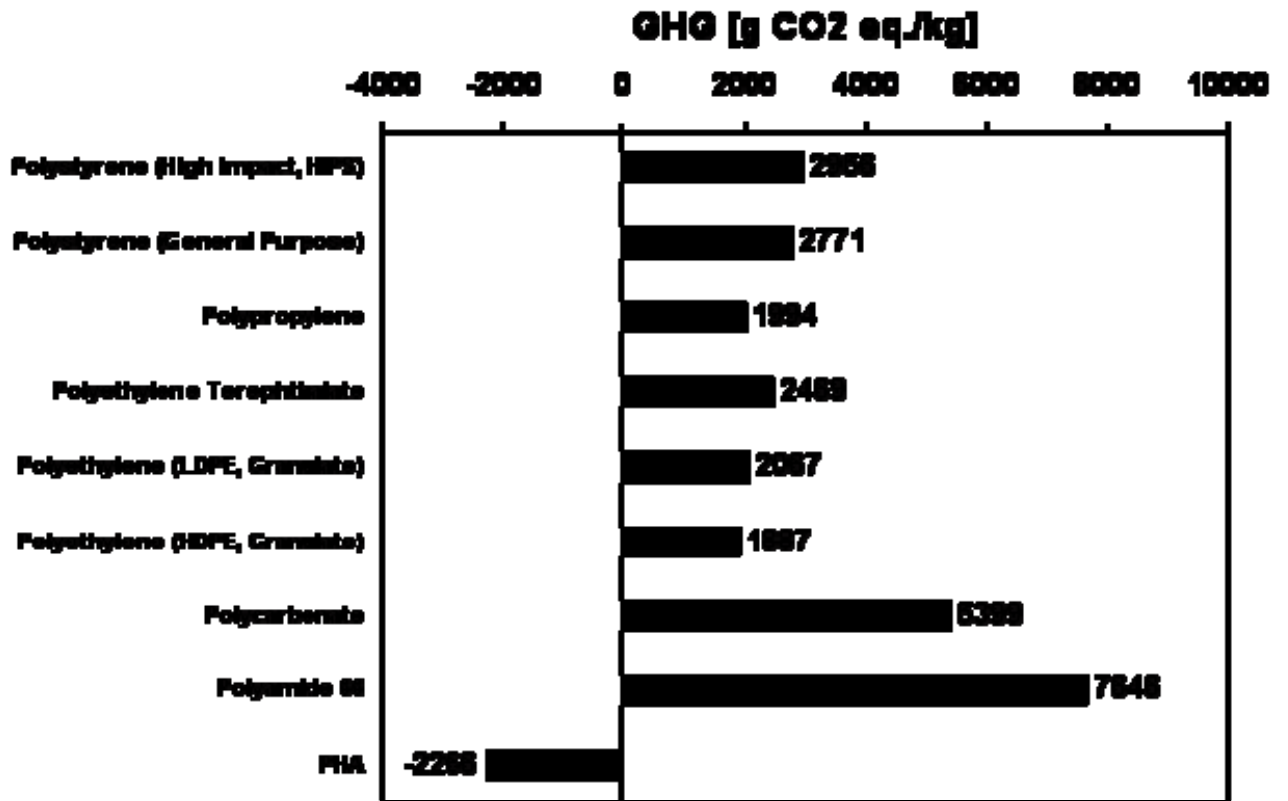


Figure 2 Greenhouse gas emissions associated with one kg of PHA [System A: alternative systems for co-products in the wet milling process; PHA: PHA fermentation and recovery process; System B: alternative systems for fermentation residues in the PHA fermentation and recovery process; C-credit: carbon dioxide credit due to carbon content in PHA]



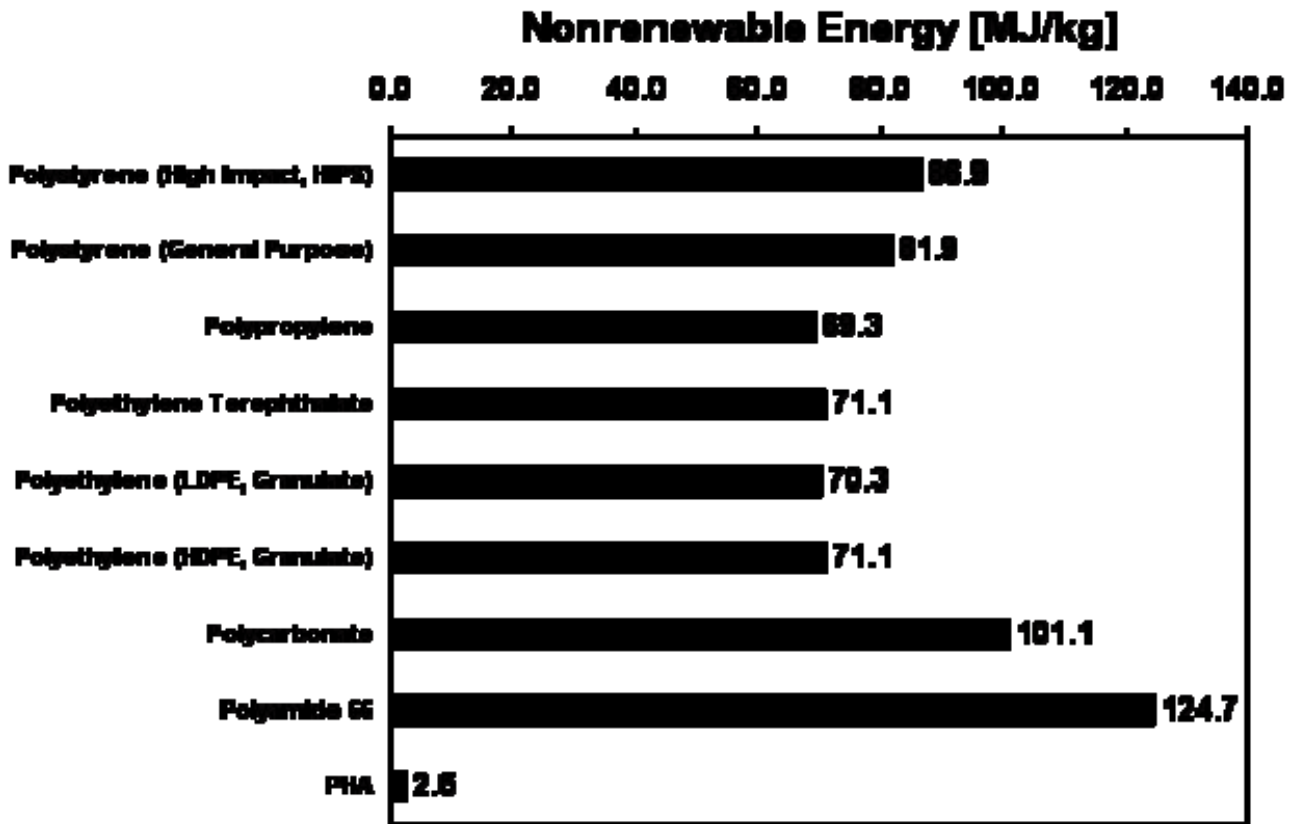


Figure 3 Greenhouse gas emissions and nonrenewable energy of polymers

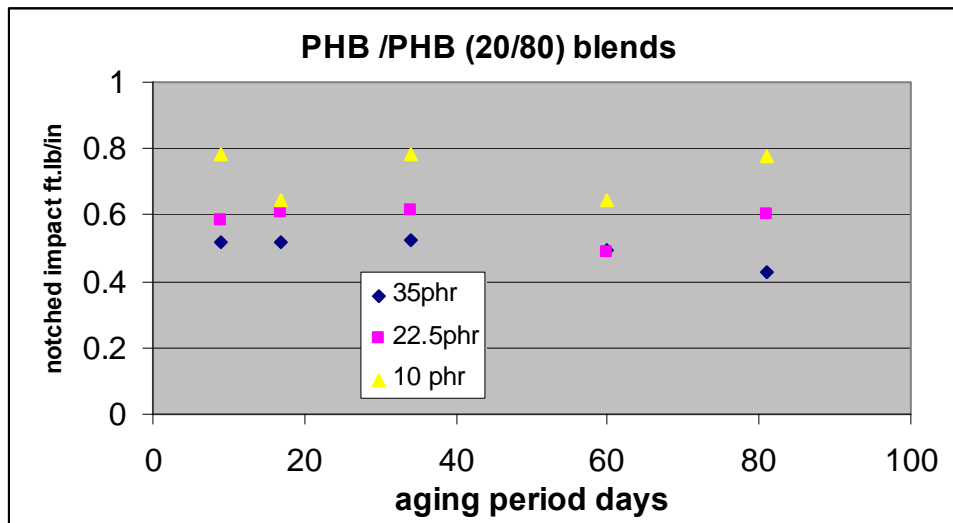
Y3-6- Task 4: Fundamentals of PHA Polymer Properties/Processing

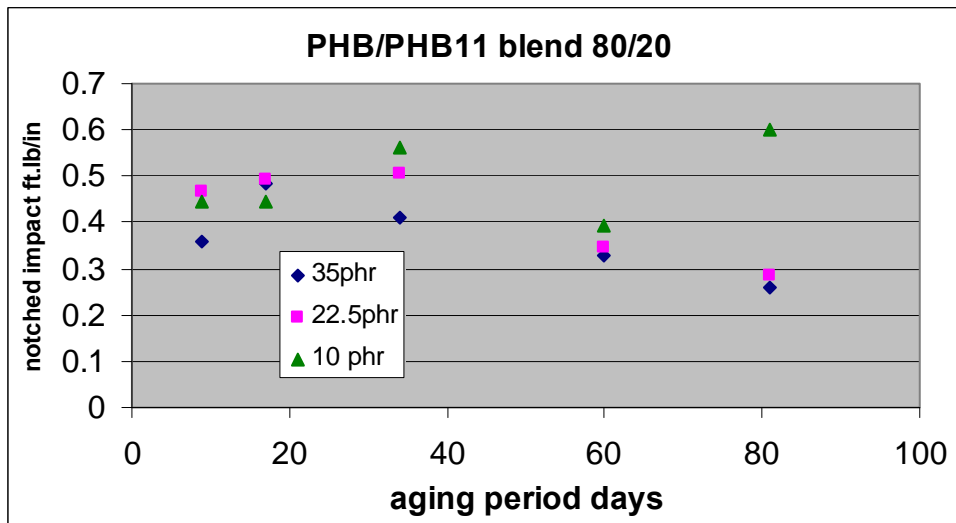
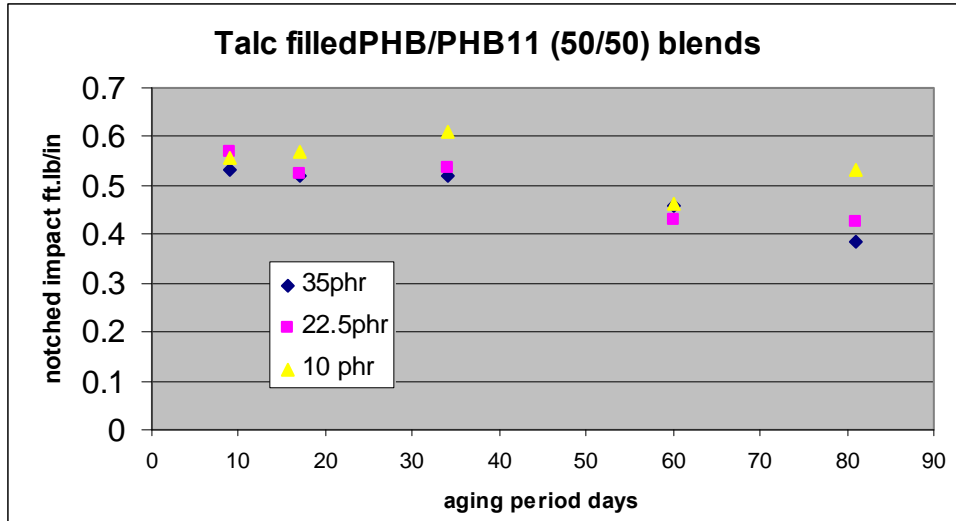
Task 4.1 Predictive Modeling of PHB Copolymer Properties. (U. Illinois, Chicago, Prof Chudnovsky).

In Year 3, procedural information for preparing compression molded films has been transferred to University Illinois, Chicago. Measurement of tensile and true stress strain versus temperature by Professor Chudnovsky's group was carried out. This data was incorporated into the model for predicting properties of PHB copolymers.

True stress strain measurements on PHB compositions containing 13.5%, 20% and 26% co-monomer were completed and molecular weight determinations on the compression molded samples carried out. Further samples produced by cast sheet extrusion of formulated compositions containing nucleating agent NA1 and a rubber impact modifier have been sent to U. Illinois for assessment. The results from these studies were incorporated into a model which can predict PHB copolymer properties. This model is proprietary to Metabolix and so is not disclosed here.

Additional work in this area in Years 4 and 5 were carried out by Metabolix and UMass, Lowell. In particular the role of PHB in blends was investigated in more detail as this can contribute to both changes in crystallization rate and modulus properties (final crystallinity). It is known historically that high levels of crystallinity in the final PHB composition can contribute to aging embrittlement; this has been published for PHB and PHBV compositions. Measurement of notched impact has been used to monitor the changes in performance with time. The following three charts show the effect of changing the PHB/PHB11 resin and filler loading on the aged notched impact performance, all bars were molded with a mold temperature of 60C:

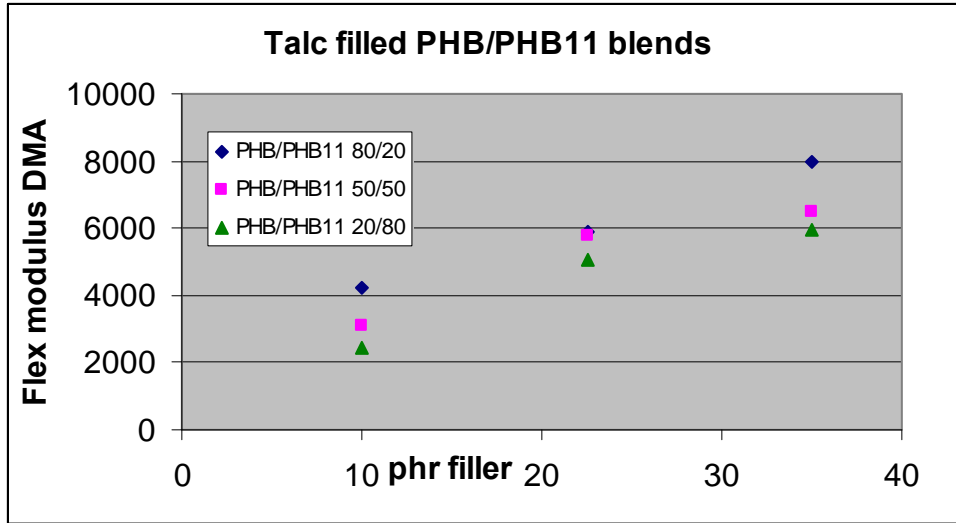




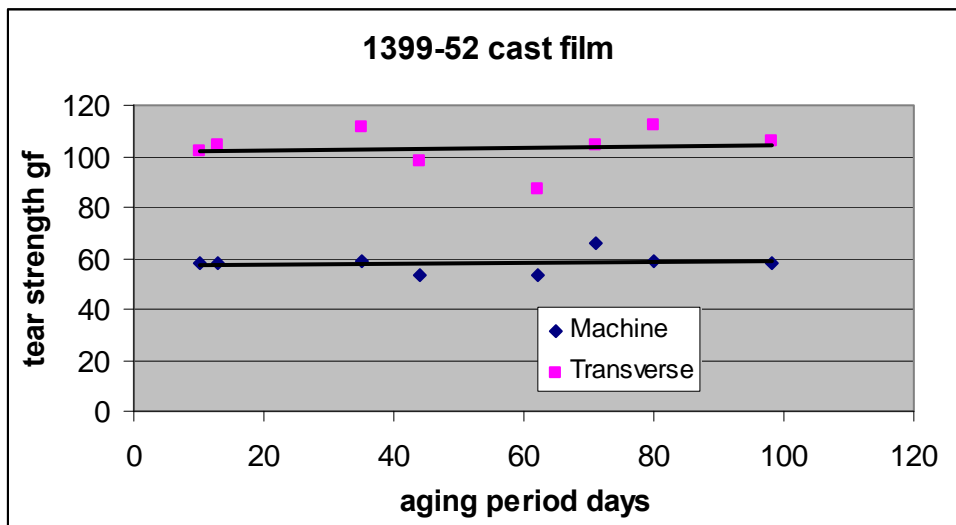
Several general observations can be made:

1. impact strength falls with increasing filler loading
2. the level of filler loading has an effect on the aged impact profile with increasing filler having a higher tendency to embrittle on aging
3. the impact performance of 10phr talc loading does not appear to change with time
4. increasing PHB loading reduces impact strength
5. increasing PHB loading also tends to promote aging embrittlement

The following table shows the correlation with DMA flexural modulus (dual cantilever, 1Hz frequency, 20C) for the same compositions; modulus did not change significantly over this aging time period.



For softer products such as 60 micron cast film, tear properties are a better parameter for studying the aging processes. The following graph shows the effect of aging on a PHB/PHB11 (20/80) blend which has been rubber modified and no change in properties was observed.



Biodegradability Testing

This activity which was carried out to confirm the biodegradability of the PHA resin formulations developed during this project. Metabolix has also submitted a number of samples to OWS (Organic Waste Systems, Belgium) for independent biodegradation studies.

To overcome the need for a constant resubmission of new formulations for testing to ASTM D6400 and EN 13432, a period which takes over 6 months, Metabolix has adopted a protocol for submission which will encompass all its anticipated polymer compositions. Metabolix intends to produce polyhydroxyalkanoate polymers varying from about 65% crystallinity to about 5% crystallinity, these being the extremes of co-monomer in the copolymers; all these polymers will contain a proprietary nucleating agent

packaging. Further Metabolix anticipates that these compositions will contain from 0% to about 30% of inorganic mineral filler. Based on these assumptions the following compositions have been submitted for full testing to ASTM D6400 and EN 13432:

- polyhydroxyalkanoate polymer with ~ 65% crystallinity, nucleated, 0% filler
- polyhydroxyalkanoate polymer with ~ 65% crystallinity, nucleated, 30% filler
- polyhydroxyalkanoate polymer with ~ 5% crystallinity, nucleated, 0% filler
- polyhydroxyalkanoate polymer with ~ 5% crystallinity, nucleated, 30% filler

These samples have been submitted to OWS (Organic Waste Systems, Belgium) an independent, world recognized, test facility for biodegradation testing. Metabolix and OWS believe that by testing these four points in a matrix, all compositions contain blends of nucleated polyhydroxyalkanoate polymers having an average crystallinity between 65% and 5% and filler levels from 0% to 30% would be covered under the proposed testing protocol.

Test Protocol

ASTM D6400 and EN 13432 involves three testing procedures namely:

- aqueous aerobic respirometric biodegradation testing to ISO 14851 (equivalent to ISO 9408, OECD 301C, ASTM D.5271-92, EN 29408, DIN V54900-2)
- mechanical disintegration under composting conditions to ASTM D6400, EN 13432 and ISO 16929 (all equivalent)
- ecotoxicity test including heavy metal content and plant testing

Test Data – Respirometric

Normal testing is carried out at 55-60C to simulate hot composting conditions, however OWS tested the respirometric degradation at 21C which is also indicative of cold composting, soil burial and aquatic degradation conditions.

Nucleated polyhydroxyalkanoate polymer with ~ 65% crystallinity showed that after 70 days 85.4% +/- 6.9% of the organic carbon had been converted to carbon dioxide.

Nucleated polyhydroxyalkanoate polymer with ~ 5% crystallinity showed that after 70 days 82.3% +/- 19.7% of the organic carbon had been converted to carbon dioxide.

Both degradation rates were comparable to the cellulosic control material.

Test Data – Pilot Scale Composting (pressed sheet samples)

PHA crystallinity	Thickness mm	Observation
~ 65% unfilled	0.4	Disappeared after 6 weeks
~ 65% unfilled	1.0	Falling apart after 12 weeks
~ 65% unfilled	3.0	Some surface erosion after 12 weeks
~ 65% filled	0.3	Disappeared after 8 weeks
~ 65% filled	0.9	Disappeared 8-12 weeks
~ 65% filled	3	Some surface erosion after 12 weeks
~ 5% unfilled	0.6	Disappeared 2-4 weeks
~ 5% unfilled	1.15	Disappeared 4-6 weeks
~ 5% filled	0.95	Disappeared 2-4 weeks
~ 5% filled	1.4	Disappeared 4-6 weeks

Test Data – Full Composting Study

Only unfilled materials were used for the full composting study. The biodegradable polymer samples were made up of ground pellets and pressed sheet samples:

Nucleated ~65% crystalline polyhydroxyalkanoate polymer at a maximum thickness of 0.82mm resulted in >90% breakdown. in the required 12 week period.

Nucleated ~5% crystalline polyhydroxyalkanoate polymer at a maximum thickness of 1.6mm resulted in >90% breakdown. in the required 12 week period.

Test Data – Cress Growth Trials

Germination of cress in 1/3 Metabolix compost/reference substrate was not significantly different from the germination in the 1/3 mixture of blank compost/reference substrate.

Germination of cress in 1/1 Metabolix compost/reference substrate was not significantly different from the germination in the 1/1 mixture of blank compost/reference substrate.

This data is sufficient for Metabolix to self certify the compliance the PHA compositions to ASTM D6400 and EN 13432; it also allows Metabolix to further claim cold composting, soil burial and aquatic degradation for it range of products.

Metabolix is also working with the US Army Laboratories at Natick to have the product range approved for ASTM D7081 the Marine Degradation of Dense Compositions. Preliminary work has already shown that Metabolix PHAs degrade at a comparable rate to the cellulosic control.

Biodegradability Testing: OWS has now completed all biodegradability testing on the Metabolix resins and have test data that allow Metabolix to claim the following compliance results:

- ASTM D6400 hot/municipal composting
- EN 13432 packaging
- cold/domestic composting
- soil burial

Biodegradability Testing:

OWS Have completed the ecotoxicity testing to ASTM D6400 /EN13432 for compost material. Both ABC 100 and ABC 001 nucleated and with 1 phr slip additive met the required plant growth requirements.

The composting study indicated that ABC 100 at a maximum thickness of 0.9mm degraded >95% within the 90 day required whilst for ABC 001 the thickness was increased to 1.4mm. The difference in thickness can be explained through the level of crystallinity with the polymers.

At this time Metabolix has sufficient information to self certify compliance to ASTM D6400 and EN 13432 for all the anticipated formulation blends.

Task 4.2 Analysis of Copolymer Rheological Properties: (Professor Shi-Qing Wang, University of Akron) During Year 1 a scoping study was initiated with Professor Wang to investigate the rheology of polyhydroxyalkanoate polymers. As a model system, a series of PHB copolymers with different co-monomer levels were provided by Metabolix for analysis. The polymers were prepared by microbial fermentation and solvent purified. Table 4.2.1 details the basic properties of these polymers:

Table 4.2.1 Basic Properties of PHB Copolymers

Grade	% co-monomer	Mw	Mn	PD
1606-048	27	196,000	105,400	1.86
1606-061	14	133,000	69,600	1.91
1606-078	31	386,000	177,900	2.17

1606-100	20	412,000	190,700	2.16
1606-255	33	583,000	259,100	2.25

Table 5.5

The following graphs indicate the rheological data generated for the copolymers using both controlled stress and controlled rate rheometers.

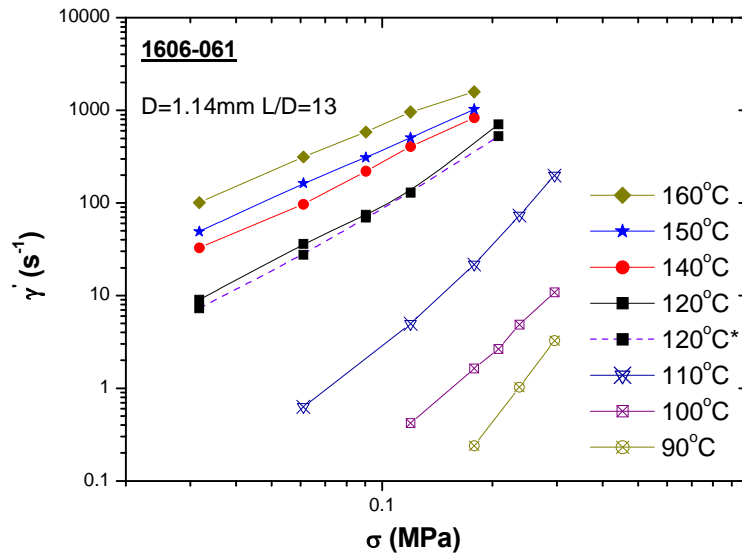


Figure 4.2.1

Figure 4.2.1.1 Illustrates the typical rheological traces expected for thermoplastic polymers. It further shows that all the polyhydroxyalkanoate polymers can be melt processed below the DSC measured T_m , in this case 124°C . This provides an extension of the normal temperature processing window considered for thermoplastic polymers. Typically one looks at melt processing of polymers at temperatures $T_m + 50^\circ\text{C}$. Because of the limited thermal stability observed for some PHAs this would seriously constrict some thermoplastic applications. Being able to process below T_m greatly increases the opportunities for PHAs and complements the work by Professor McCarthy (see Task 4.5) who demonstrated that poly 3-hydroxybutyrate co 3-hydroxy valerate polymers with T_m in the range $150\text{-}165^\circ\text{C}$ (measured by DSC) could be injection molded at temperatures as low as 130°C ; at these temperatures no loss in molecular weight was observed.

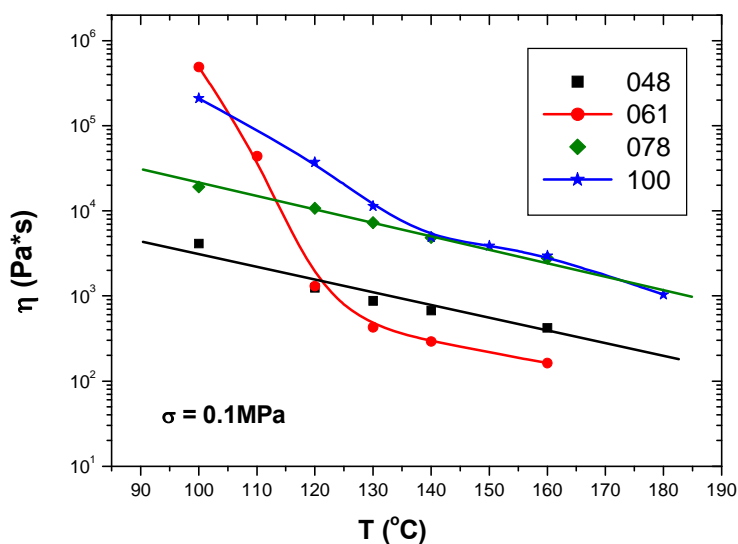


Figure 4.2.2 Viscosity - Temperature Profiles at 0.1MPa

Figure 4.2.2 demonstrates that copolymers containing 14% and 20% co-monomer have different temperature dependence profiles compared with higher co-monomer compositions. This is indicative of crystallization in the melt of these polymers. Following the change in viscosity of these polymers as a function of time under isothermal conditions may provide an insight into the early crystallization behavior of these polymers. These rates are too slow to easily be followed by normal DSC techniques

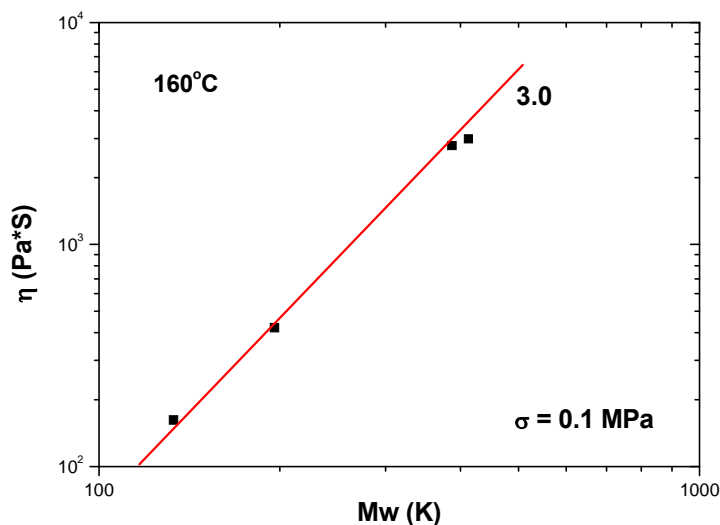


Figure 4.2.3 Viscosity versus Mw at 0.1MPa at 160°C for 4 samples.

Figure 4.2.3 shows that a plot of log viscosity against log molecular weight (160C) over the composition range 14-30% co-monomer is linear with a slope of 3.0. This implies that co-monomer content has a very low impact on the rheological properties of these copolymers and molecular weight is the dominant feature.

Conclusion: The preliminary program has indicated that the rheology of polyhydroxyalkanoate polymers may be complex. These polymers can be processed

below T_m thus expanding the potential processing window for these polymers. Overlying the normal thermoplastic properties is the potential for rheological changes as a function of induced melt crystallization and possible degradation mechanisms.

Typically entanglement chain lengths for polymers are measured by plotting log zero shear viscosity against log molecular weight to determine the point where the slopes change from around 3.4 (above entanglement chain length) to 1 (below entanglement chain length). This involves generating a number of polymer samples with different molecular weights. An alternative technique is to measure the plateau modulus (M_e value) characteristics of the polymer (see Figure 4.2.4). The curve below provides a M_e value of 4,650 for an essentially amorphous PHB copolymer; this compares with values of around 1,200 for polyethylene and 13,000 for a different PHB copolymer composition PHBH.

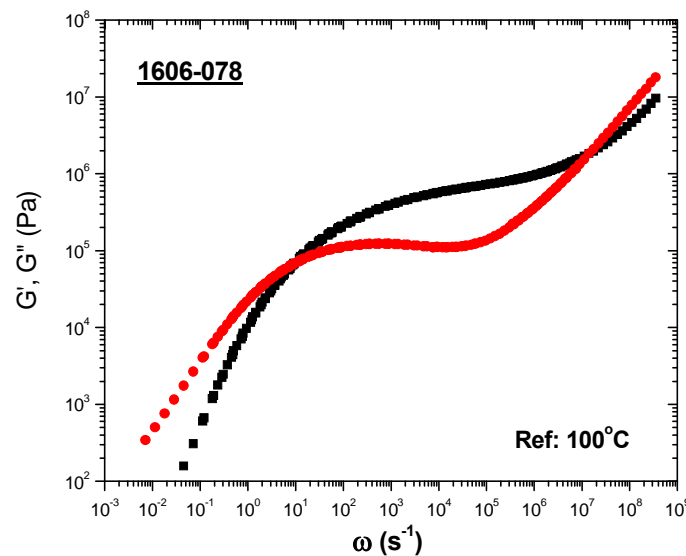


Figure 4.2.4 Plateau Modulus for 1606-078

As a rule of thumb for polymers to realize the maximum benefit from chain entanglement the polymer molecular weight should be around 40 times the value of M_e . This equates to 50,000 for polyethylene as compared to 180-200,000 for PHB copolymers.

In Year 3 of the program two approaches to controlling the low shear viscosity of PHA resins were pursued. In the first, the impact of molecular weight distribution on the rheological properties of PHAs was evaluated and in the second approach the impact of introducing chain branching was evaluated. To evaluate the impact of molecular weight distribution, batches of PHB copolymers were processed to create blends of high and low

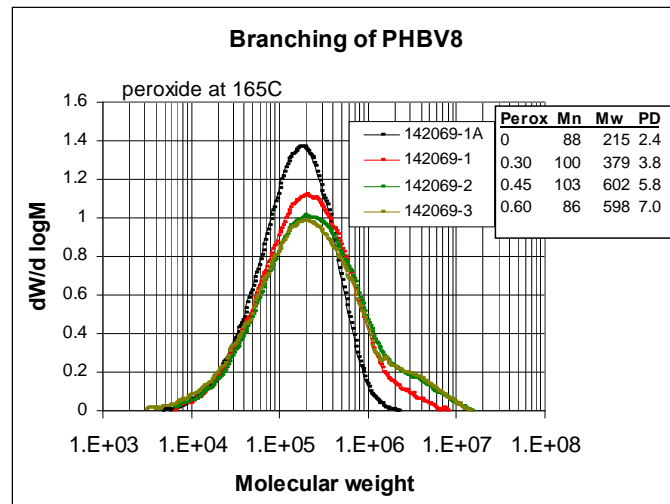


Figure 4.2.5 Numerical Calculation of Mw

molecular weight materials such that the polydispersity of the composition was raised. Parallel plate rheology measurements at 160C under an applied strain of 2% was carried out and demonstrated that increasing the polydispersity has a significant impact on the low shear rheological properties. This is very useful for applications such as injection molding where flow under shear is extremely important to fill the cavity under low pressure and short time scales. For other applications such as blown film, this approach provides a route to increasing the low shear melt viscosity which results in increased melt elasticity an important rheological characteristic necessary for improving bubble stability and blowing thin films. The data obtained from this series of experiments was used to produce a mathematical model for predicting the rheology PHA blends.

A second approach to increasing polymer melt elasticity is the incorporation of long-chain branching in the polymer structure, a method routinely used for conventional synthetic polymers. A variety of chemical methods can be used to incorporate branching in a polymer. The most effective choice depending on a variety of factors including the structure of the polymer and the temperature at which the branching chemistry can be carried out. Peroxide branching, was previously demonstrated for PHAs by Monsanto, but this approach lacked reproducibility due to the selection of the peroxide initiator and reaction conditions.

In Year 3 of the program, an efficient PHA branching technology was developed by Metabolix. This technology is currently in the process of being patented and will be reported in full when the patent application is formally filed. As a prototype molecule, PHB-co-8% 3HV was melt processed at 165C with an organic peroxide. Changes in the molecular structure were followed by gel permeation chromatography. Figure 4.2.5 shows the changes in molecular weight distribution for different levels of peroxide. As the level of peroxide is increased, there is the formation of a high molecular weight tail. This tail is characteristic of the incorporation of long-chain branching. The data shown in the box in Figure 4.2. shows the numerical calculation of Mw, the weight average molecular weight. Mw steadily increases with increasing peroxide level while the Mn remains relatively constant.

Confirmation of this branching is best assessed by rheological measurements. In a dynamic rheological test, the melt viscosity and melt elasticity of a polymer is measured as a function of frequency at a constant temperature. Melt elasticity, G' , is a measure of polymer branching. Linear polymers, such as as-made PHAs have low melt elasticity. Polyethylenes have different inherent levels of branching as a consequence of the different catalyst systems used in their production.

Figure 4.2.6 illustrates the successful introduction of chain branching in a PHB co 11% copolymer reacted with two levels peroxide at 165C as determined by measuring the melt rheology. As the level of peroxide is increased, the low shear (0.1 sec^{-1}) elasticity increases by over an order of magnitude. Also shown in the Figure are characteristic melt elasticities of three polyethylenes, LLDPE, LDPE and HDPE. LLDPE is a linear polymer that is well known to have low melt elasticity and

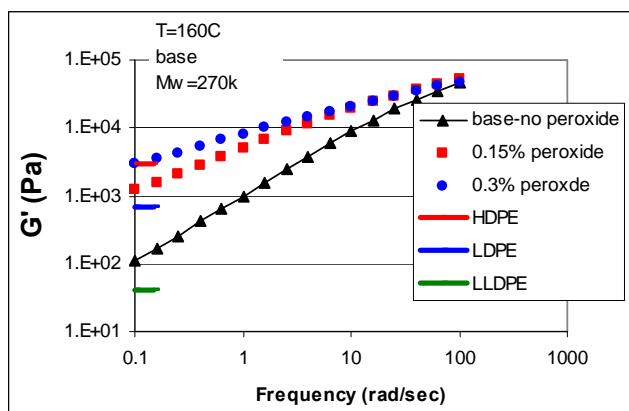


Figure 4.2.6. Effect of Peroxide on Melt Elasticity

behave poorly in film blowing. Both HDPE and LDPE can be easily blown into films. The use of peroxide branching in PHAs increases melt elasticity to useful levels. This result appears to be independent of the particular co-monomer used in the polymer.

In conclusion Year 3 saw 2 major step changes in our ability to improve the low shear viscosity and hence melt elasticity of PHA resins. The branching technology has allowed PHA formulations to be developed having good melt strength within the range of that for LDPE, the 'gold standard' for film blowing, to HDPE which has excess melt strength and requires special equipment for film blowing. This technology proved invaluable for achieving the results on the specific targeted applications, in particular paper coating and blown film, which are reported in Task 4.5.

The branches introduced into the polymer result in significant changes to the rheology of PHAs as measured by oscillatory melt rheometry. Storage modulus (G') measured at 160C and low shear rates (0.1 sec^{-1}) have been increased by two orders of magnitude. The increase in melt elasticity can also be assessed by measuring changes in the high molecular weight portion of the molecular weight distribution. We have carried out studies to define the efficacy of peroxides used for branching and process optimization.

Metabolix purchased an AR 2000 rheometer from TA Instruments to expand the rheological characterization work at Metabolix whilst allowing U Akron to focus on assessing the rheological effects of seed nuclei in the melt. To further compliment the work in polymer branching Metabolix also purchased a Viscotek GPC unit with triple detector system (refractive index, viscosity and light scattering) and appropriate software. This unit allows for more fundamental understanding of the chain branching characteristics of the polymers.

The branched polymers have been previously reported to exhibit high melt strength as measured by oscillatory melt rheometry. Additionally, branching can affect the molding behavior of polymers. This is conventionally manifested in surface appearance of molded parts near gates. In molding applications, die swell characterization is of value. To this end, the branched PHA polymers were characterized by capillary melt rheometry. In this test, the polymer is extruded through a capillary. The diameter of the extruded fiber is compared to the diameter of the capillary.

Three samples (linear, low branching, high branching) were analyzed by capillary rheometry. The linear and low branched samples showed low levels of die swell. The highly branched sample showed very high levels of die swell. Having identified these extremes of material rheological behavior, screening studies on their effects on molding could be carried out. Rheology work was continued in house at Metabolix to develop methods for characterizing the rheology of highly crystalline PHAs.

Previous work, mostly at U. Akron, focused on PHA copolymers with low (<160C) melting points. As a result, rheology could be studied at 160C, a temperature at which the rate of molecular weight loss of PHA is quite slow. However, commercially attractive PHA formulations have wider and broader melting temperature ranges which obviates relevant measurements at that temperature. The rheological behavior of blends of PHB with a PHB copolymer is more challenging because the blend must be heated to about 175oC to achieve full melting which results in polymer molecular weight loss which then impacts melt rheology. A methodology was developed in which the polymer is placed in the rheometer at 175oC for 30 seconds then rapidly cooled to 160oC. In this approach, longer time span rheological measurements are possible. Experiments demonstrate that this approach mimics the behavior of PHA in melt processing equipment.

Task 4.3: Crystallization Properties of PHAs (ORNL)

PHA polymers are semi-crystalline and as such understanding the crystallization behavior of these materials is key to maximizing not only polymer processing

compositions to produce molded goods, coatings films and fibers but is also crucial to optimizing the material properties of final product forms.

A set of samples covering a range of polymer compositions, including PHB and PHB copolymers, was received from Metabolix, as shown in Table 4.3.1. These polymers are being used as basic copolymer structures to begin to validate the laboratory equipment.

Table 4.3.1 Samples Received from Metabolix

Date Rcvd	MBX Designation	Co-monomer mole%	Mn	Mw	PD
7/15/2003	030202	9	5.52E+05	9.65E+05	1.7
	030302	9	5.71E+05	9.80E+05	1.7
	030402	18	4.50E+05	9.02E+05	2.0
	030101	26	1.31E+05	3.17E+05	2.4
9/15/2003	142005	0	3.09E+05	6.18E+05	2.0
	MBX 0606-061	14	0.74E+05	1.44E+05	2.0
	L57/5	0	1.96E+05	5.38E+05	2.7
10/10/2003	142037-4	26	3.98E+05	7.04E+05	1.8
	142037-1	0	1.64E+05	4.28E+05	2.6
	142037-2	7	2.64E+05	4.98E+05	1.9
	142037-3	11	2.54E+05	4.96E+05	2.0

The DEA analyzer has been run through a preliminary set of studies with PHA to explore the breadth of phenomena observable with these systems, some of which extend beyond the routine matter of relaxation into details of crystallization and phase analysis (e.g. crystallinity).

Preliminary DEA data suggest that all the phenomena for which DEA was thought be helpful in these systems has been observed, and data have been collected from at least -100°C to beyond the melting point. This includes new observations not previously utilized in semi-crystalline polymer research. The expected observations of T_g , nucleation, melting phenomena have been observed. The DEA spectroscopy combined with X-ray analysis is expected to provide more detailed insights into the nature of the nucleation and crystallization processes. DEA, in particular, will aid in understanding the early stages of nucleation and crystallization processes. When combined with other spectroscopic methods, the nature of PHA copolymer crystallization and relaxation may be directly observed. The balance of these two processes is important in understanding the roles of polymer structure, nucleating agents, and polymer orientation on crystallization during processing and subsequent post-processing crystallization (physical aging) of PHA copolymers. The understanding of these processes will play an important role in the design of polymer formulations able to be processed as moldings, films and fibers at commercially viable speeds.

Pairs of nucleated and un-nucleated PHAs were examined at two structural length scales using Waxd and polarized light microscopy for clues to the origins of the embrittlement phenomenon, post-crystallization. In the first, Waxd was used to examine if any changes in the crystallites occurs during the key period of annealing occurring during the first day. That work revealed clearly that the embrittlement phenomenon does not result in any changes in the (average) crystal structure with time, or the crystallinity during the study period. This was surprising, as there are models of polymer

crystallization kinetics and annealing mechanisms which would indicate otherwise. If related to crystals, per se, then minor phase changes, or changes that occur in going from T_c to room temperature must be operating.

In the second case, we looked for morphological clues, while examining a broad region of under-cooling, in each sample. In spite of no mention in the literature, we readily found clues to the origins of the embrittlement phenomena by using multiple microscopy methods and sample preparation techniques. The results indicate that *it is morphology development during crystallization processes that causes the embrittlement*.

The same two length scales were used to examine the effects of added nucleating agent on the structure. The changes that are observed will be related to the effect of the nucleating agents to cause the solidification to occur at lower under-coolings. These data will be related to the findings in the morphology studies (related to under-cooling effect). We have found subtle, but measurable differences in the Waxd of the crystallites as demonstrated by studies at different effective under-coolings.

Installation of Polarized Light Microscopy and Depolarized Light Photometry (PLM, DLP) together with Small Angle Laser Light Scattering (SALLS) both coupled to a computer controlled hot stage controller have been established. Within just a few weeks, these systems were already yielded important information, including:

1. Crystallization:
 - a. Growth front velocities (GFV) and the max velocity temperature (MVT)
 - b. Bulk Growth Rate kinetics via DLP
 - c. Nucleation behavior as a $f(T)$ and relation to MVT
2. Morphology
 - a. Establishing that PHAs have unique growth morphology, which may not be strictly spherulitic, and this changes at temperature related to the MVT
 - b. Linking annealing / embrittlement in PHAs with morphology development during crystallization
 - i. Modes of embrittlement may be different on either side of the MVT
 - c. Peculiar observations of melt structure and how it relates to morphology development above the MVT (or, generally?)
3. Hv-SALLS
 - a. Surprisingly, in the few cases where spherulites are small enough to yield good patterns, they did not (either high under-coolings, or presumably with nucleating agent). This reinforces the view that the textures are not spherulites (anisotropic spheres of randomly oriented crystallites) as found with microscopy
 - b. We have observed an unusual correlation in the Hv scattering of a few samples. This may be important to phase discontinuities developed during embrittlement, or some new element of phase structure in between the crystallite and the "spherulites"
 - c. Morphologies on the mm level from un-nucleated samples are too big for spherulitic scattering to be seen. In these cases, however, we have observed light diffraction from the banding patterns in the "spherulites" which can be related to under-cooling.

This project ended once the allocated funds were used up and we decided not to provide additional funding because this issue was largely resolved by the development of the new nucleating technology described in Section 4.4.

Task 4.4 Development of Nucleating Agents for PHAs (Subtask 5.7.2 Impact of Additives on Crystallization of PHA Copolymers)

Key to meeting the Task 4.5 milestones, in particular achieving industrial processing line speeds in the targeted applications is improving the rate of crystallization of the PHA polymer resins. This is typically done by identifying and optimizing nucleating agents. The ability to accelerate the crystallization behavior of PHAs is extremely important in processing applications such as injection molding, cast film extrusion and fiber. While the crystal growth rate is comparable to that of polyethylene and polypropylene, the nuclei density is several orders of magnitude lower. Typically, nucleating agents and clarifying agents (no loss in transparency) are used. Talc has been shown to have very little effect on nucleation efficiency for PHAs. Up until the studies reported here Boron nitride was still the most efficient nucleating agent to date with 0.5% being the optimum level.

In the first year of this project Metabolix showed that processing temperature has a major impact on the re-crystallization behavior of PHAs. Differential Scanning Calorimetry (DSC) was used to investigate the crystallization behavior. The DSC protocol involves heating the sample to a set temperature above T_m and isothermally holding the sample for 3 minutes, the sample is then cooled at 10C/minute to $-30C$ during which time the crystallization behavior of the polymer can be observed. A second heating cycle at 10oC/minute to 200°C is then carried out to observe any secondary crystallization and melting behavior. For example a copolymer of comprising 89% 3-hydroxybutyric acid and 11% co-monomer if conditioned at 170C shows a re-crystallization peak on cooling of 84oC with a heat of crystallization of 23J/g. However if the conditioning temperature is raised to 185oC, then the re-crystallization peak is lowered to 46oC and the heat a crystallization reduced to $\sim <2J/g$. Thus processing from 185oC would extend the cycle times required for injection molding by a factor of 2 or 3.

In order utilize the broad range of polymer properties achievable by using different PHB copolymer compositions we also need to understand the impact of co-monomer ratio on crystallization. We have shown that the half life of crystallization at 65C increases rapidly with increasing level of co-monomer. 65oC was selected from previous programmed DSC cooling crystallization runs as an optimum temperature for crystallization during pelletization.

Historical work has shown that boron nitride was the most efficient nucleating agent for PHB and PHBV copolymers. In DSC programmed cooling experiments from an isothermal conditioning temperature of 200oC PHB exhibits a peak crystallization temperature of 63C; with boron nitride this was increased to 96.8C. We investigated the use of boron nitride as a nucleant for a series of different PHB copolymers and found boron nitride to be virtually ineffective in our system.

In a separate external effort a consultant to Metabolix identified a new nucleant candidate for PHB copolymers and due to the apparent high efficacy of this new nucleant designated NA1 we used this nucleant as the basis for all additional work on PHB copolymer crystallization. As a first step we evaluated the crystallization impact of the nucleant NA1 on a series of different PHB copolymers using Isothermal DSC crystallization studies.

As a screening tool, the DSC is used to determine the peak crystallization temperature (T_{cr}) during cooling from the fully molten state. A large increase in temperature (of nucleated vs un-nucleated polymer) is due to large increases in crystallization rate

Table 4.4.1 Nucleation of PHB Copolymers by MBX-NA1

<i>PHA Polymer</i>	Tcr (un-nucleated)	Tcr (nucleated)
PHB	63.1	118.4
PHB-co-7%co-monomer	48.5	91.4
PHB-co-11%co-monomer	none**	69.7
PHB-co-8%3HV	66.9	102.3
PHB-co-5%3HH	49.7	99.0
PHB co-monomer	10.5	15.6

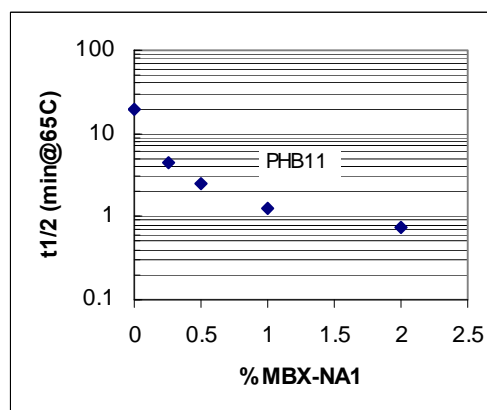
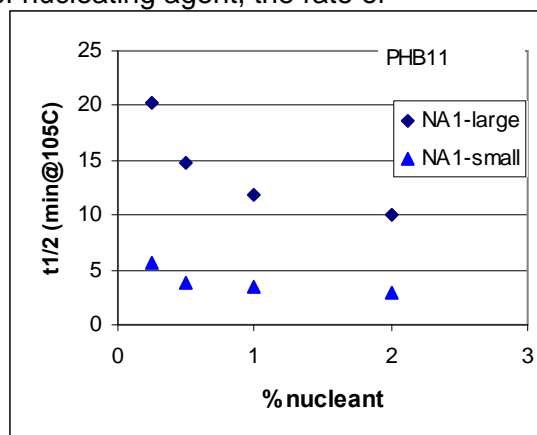
(note: ** - crystallization so slow that not observed at DSC cooling rate of 10C/minute)

Table 4.4.1 shows the effect of MBX-NA1 on a variety of PHB copolymers that includes co-monomers of interest such as 4-hydroxybutyric acid, 3-hydroxyvaleric acid, and 3-hydroxyhexanoic acid. In addition, the homopolymer of 4-hydroxybutyric acid, the co-monomer in PHB-co-monomer copolymers, is included. In all cases, MBX-NA1 provides effective nucleation.

The quantification of the increased rates of crystallization requires isothermal crystallization measurements. These measurements are made by heating a sample to the melt (200C for PHB-based polymers) and quench cooling to the crystallization temperature to observe the rate of crystallization. The characteristic time for crystallization is represented by the crystallization half-time, the time for the polymer to attain 50% of its crystallinity. Figure 4.4.1 shows the crystallization half-times for PHB-co-11% co-monomer at 65C, the approximate maximum of the rate of crystallization. With no nucleant, PHB11 has a half-time of crystallization of about 20 minutes. This time is certainly much too long for commercially practical use of these polymers. The addition of NA1 dramatically increases the rate of crystallization to less than 1 minute, a commercially useful crystallization rate.

It was noticeable that at increasing levels of nucleating agent, the rate of crystallization continually increases. This implies that the effective particle size of the nucleating agent is not small enough to saturate the system with nuclei. This is an important since increased particle size and loading will have adverse effects on transparency, permeability, and physical properties.

Upon nucleation with 1% of particles of about 25 micron, the $t_{1/2}$ decreases to 2 from about 20 minutes. Using wet milling techniques, we reduced the particle size of NA1 to several microns. At this particle size,

**Figure 4.4.1** Effect of MBX-NA1 on Crystallization Rate**Figure 4.4.2** Effect of NA1 particle size

the rate of crystallization is too fast to be measured at 65C. Thus, data here is reported at a crystallization temperature of 105C, where kinetics are in a more measureable range. Under these conditions, un-nucleated copolymer has a $t_{1/2}$ of about 40 minutes. Figure 4.4.2 shows the effect of using smaller sized particles. At a typical loading of 1%, the $t_{1/2}$ decreases to several minutes from about 12 minutes. At 65C, where the rate of crystallization is about 3 times faster than at 105C, the $t_{1/2}$ is below a minute. This is in the range of commercial processing speeds for processes like injection molding. The decrease in $t_{1/2}$ between 1 and 2% loadings is less significant than in the case of larger particles. This suggests that this smaller particle size is approaching the point of nuclei saturation. It is desirable to have the plateau concentration be as low as possible. From practical considerations and experience in other crystallizable polymer systems, a loading of 1 percent is considered adequate.

Based on these very promising results an extensive development program was undertaken to develop not only the optimum NA1 nucleant but also scaleable processes for producing PHA nucleant masterbatches suitable for industrial scale compounding and extrusion work. Due to the fact that much of this information is being protected as highly confidential trade secret know-how it is not reported in detail here.

As an example however, masterbatches were made with a different screw design. At a compounding rate of 95 lbs/hour on a 27mm twin screw extruder, and good molecular weight PHB copolymer. This routinely provides good dispersion of nucleating agent. The masterbatch was run at scales over 3,000 lb scale and masterbatch compounding was carried out on a 27mm twin-screw extruder. This quarter, we have further scaled the masterbatch process to a 50mm twin-screw extruder. Compounding was carried out to successfully make about 400 lb of 33% masterbatch. This provides enough nucleating agent for 12,000 lbs of PHA. These nucleating agent masterbatches were instrumental in achieving the developments in key polymer processing areas discussed in Task 4.5.

Task 4.5 Demonstrate PHA Processing for Large Volume Applications

Task 4.5 Deliverables and Resources			
Year 3	Year 4	Year 5	Resources
Demonstrate PHA resin formulation suitable for high speed injection molding			Metabolix Inc.
Demonstrate PHA resin formulation suitable for extrusion coating of paper	Demonstrate economic line-speeds for extrusion coating of paper. Produce PHA coated paper cups		University of Massachusetts at Lowell
Demonstrate PHA resin formulation for blown film	Demonstrate economic line-speeds for blown film		
	Demonstrate PHA resin formulations for fiber production	Demonstrate economic PHA fiber production	

Success in Task 4.5 was based on the combination of learning from Tasks 4.1 – 4.4 with the ultimate successful resin formulations being blends of different PHB copolymers with the nucleating agent package and fillers as appropriate. Initial efforts were begun in collaboration with U. Mass Lowell to develop basic extrusion capability for the PHB copolymer resins (some of this work was carried out under Task 8.3 in years 1-2 of the project). This work was then carried out largely through collaborations with numerous external third party plastics converter companies to validate the resin performance for each targeted conversion process. Many of these companies are major global brand owners and are working with Metabolix under confidentiality agreements. For this reason the no company names are provided. Out of necessity this was an iterative process involving feed back from extrusion trials and analysis of the material properties of the various products produced, leading to improvements in resin formulations and processing conditions.

In the first two years of the program work was focused around the compounding and processing expertise at the University of Massachusetts, Lowell and was designed to develop basic processing conditions for the PHB copolymers into resin pellets suitable for extrusion studies.

Low Temperature Processing of PHAs From previous work carried out by ICI and Monsanto, the recommended processing temperature for high 3-hydroxybutyrate copolymers was 180-190C due to the fact that the T_m for pure PHB is around 175-180C. This processing temperature would allow complete melting of the crystallites. Figure 4.5.1 shows the melting behavior of a PHB composition at 180C

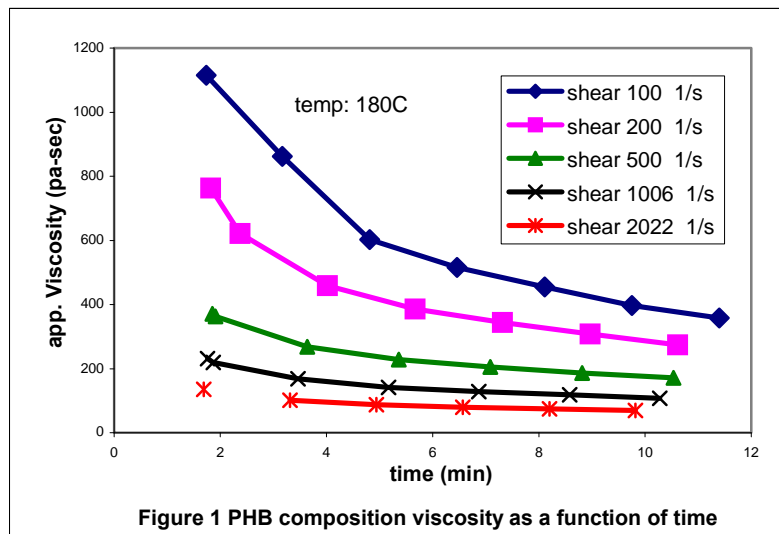
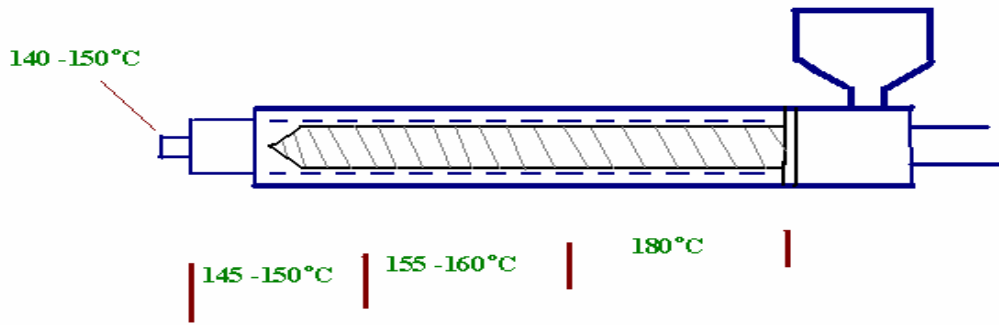


Figure 4.5.1

Figure 4.5.1 shows that the viscosity continues to fall as a function of time, indicating molecular weight degradation. The mechanism for thermal degradation of poly 3-hydroxyalkanoates is via beta elimination and hence the use of conventional stabilizers that are not effective. The other alternative is to lower the processing temperature. However there is still a need to melt the crystalline polymer, hence the use of a reverse temperature profile was conceived. The following diagram illustrates the type of screw profile that can be used for both extrusion and injection molding operations.



Injection Molding - Temperature Profile

By connecting a pressure transducer at the die head, the rheology of the composition at the die can be measured, which is an operation that cannot be carried out in a normal capillary rheometer. Figure 4.5.2 shows the rheology of a formulated PHBV composition which has a T_m of 165°C when measured by DSC.

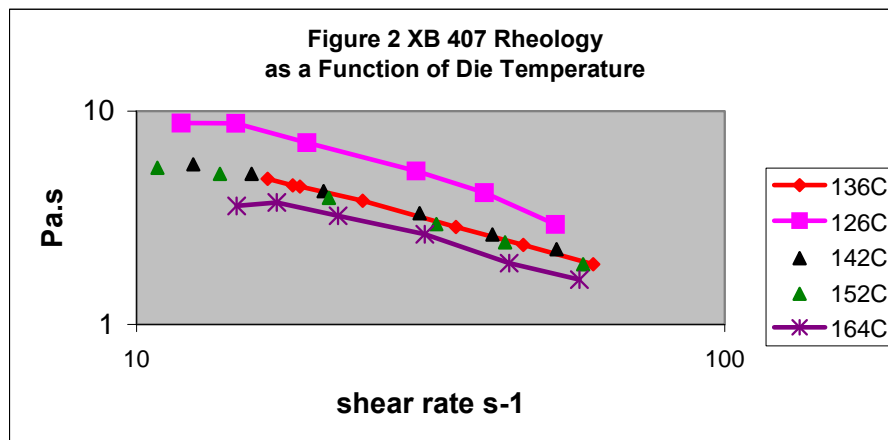


Figure 4.5.2

By molding at lower processing temperatures the impact resistance of the product was also improved. Typically, the molecular weight of the polymer produced by microbial fermentation ranges from 800,000 to several million. This range is much higher than traditional petrochemical polymers and may not be suitable for some industrial applications that require low melt viscosities and acceptable processing temperatures (e.g. adhesives or paper coating). A program was introduced to understand the effect of temperature, time and residual trace elements from the fermentation process and copolymer composition on rate of change in polymer molecular weight. Figure 4.5.3 clearly shows that co-monomer content has a significant impact on the thermal stability of 3-hydroxybutyrate copolymers as measured by onset of weight loss.

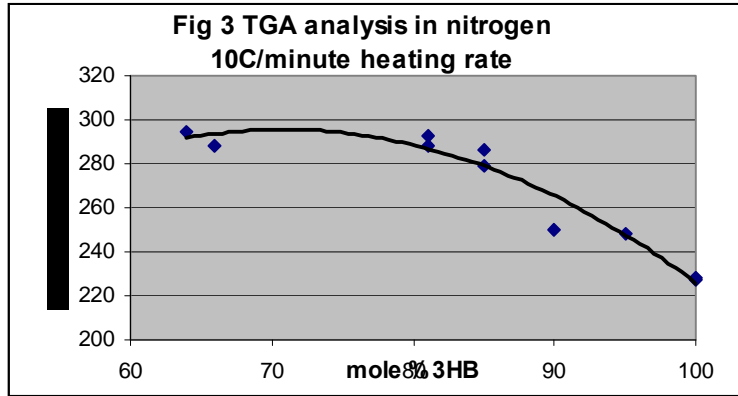


Figure 4.5.3

This data, together with small isothermal studies, was used to generate data for use in a continuous thermolysis model using single and twin screw extruders where residence time (rpm) and temperature were the major parameters. Figure 4.5.4 shows the change in molecular weight as a function of temperature for a fixed residence time.

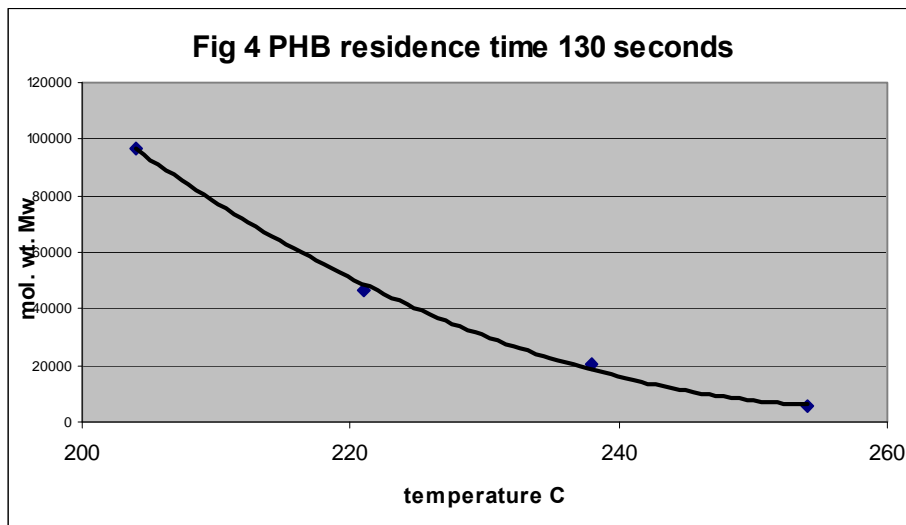
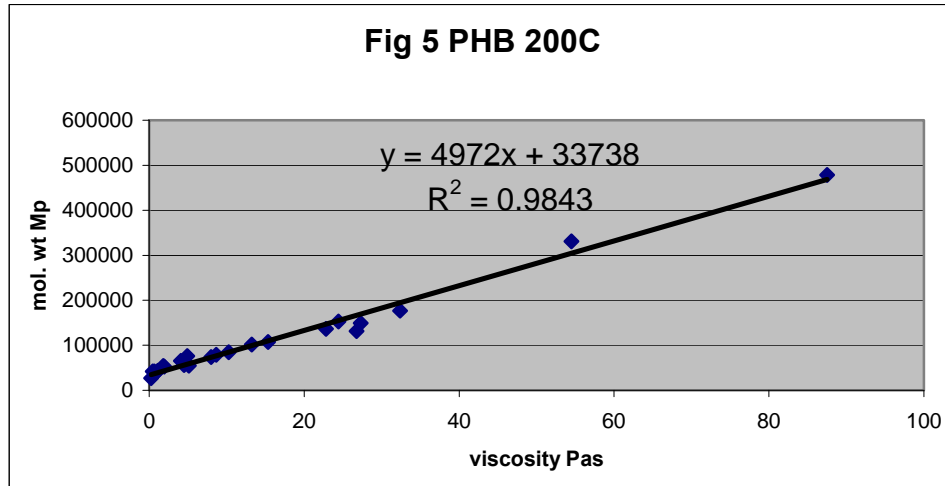


Figure 4.5.4

For these systems there was a strong linear correlation between viscosity and molecular weight (see Figure 4.5.5).

**Figure 4.5.5**

By using these correlations it is possible to provide a control loop system for an extruder that would allow the optimum residence time/temperature parameters to be determined. By incorporating a pressure transducer at the die head one could simultaneously measure the melt viscosity and provide a control loop to modify the residence time to maintain a constant molecular weight product.

With the possible exception of major converting operations (e.g. cast film, blown film and fiber) the majority of polymers are blended with fillers, process additives, plasticizers, other polymers etc to meet the desired performance characteristics. A preliminary study was initiated to investigate the effect of various additives on PHAs for molding applications. The evaluation of talc and calcium carbonate as fillers showed some interesting trends. Talc both provides improved stiffness but also acts as a nucleating agent for PHB homopolymers and copolymers with efficiency of nucleating being determined by particle size of the filler. Results of some typical compounding studies are shown in Table 4.5.1.

	Flex modulus MPa	Tensile Strength MPa	Elongation @ break %
60 phr calcium carbonate	2522	18.5	2.1
75 phr calcium carbonate	2792	17.9	2.0
90 phr calcium carbonate	3182	18.2	1.9
75 phr calcium carbonate + 10phr plasticizer C4	1807	16.3	3.8
75 phr talc grade A	3500	25.1	2.7
75 phr talc grade A + 5phr plasticizer C4	1904	23.4	4.8
75 phr talc grade A + 10phr plasticizer C4	1252	19.5	5.5
75 phr talc grade A + 15phr plasticizer C4	1056	13.9	5.4

Table 4.5.1

The majority of PHAs for commercial applications need to be supplied in pellet form. Hence the recovered polymer from either fermentation or plant crops will need to be extruded and pelletized. For small quantities strand pelletizing is the preferred route, although as volumes increase die face pelletizing will be needed. Fast crystallizing

polymers are relatively easy to pelletize because surface tack rapidly diminishes after extrusion and a high modulus material is more easily cut using conventional cutting techniques. Conversely, slow crystallizing polymers are considerable more difficult to pelletize because the strands stick together in the water bath and at the cut faces. Using rheological data generated in section 4.1-2 work we can predict the molecular weight – temperature – shear rate dependence on surface melt fracture and hence optimize the extrusion temperature profile to provide smooth uniform strands at the lowest acceptable processing temperatures. This will preserve the optimum number of nuclei for subsequent crystallization. DSC crystallization studies (section 5.7.2) are then used to predict the optimum time – temperature residence time in the water bath that will allow the strands to be cut. Process aids such as slip and anti-block agents are being investigated. Whilst much information is available on slip and anti-block agents for polyolefins, these may not be as effective in PHA polymer systems.

Strand pelletizing of polymers is one of the most convenient processes for the small scale conversion of polymer reactor crumb to pellets suitable for injection molding, film and fiber extrusion. It is critical to understand the crystallization behavior of PHAs so that the optimum process conditions can be identified. Typically in strand pelletizing conventional thermoplastic polyolefins, polyesters or polyamides cold water is used in the waterbath, thus allowing the strand to become rigid for cutting. If PHAs are processed under these conditions the resultant strand is extremely soft and rubbery, and will not cut. Work was carried out to design the correct geometry for strand pelletization of PHA polymers. A 3" Welex single screw extruder was used to model large scale extrusion. The extruder temperature profile was:

Feed port 170C
Zone 1 165C
Zone 2 160C
Zone 3 160C
Die 155C

The water bath was configured to have a triple pass with a path length of 24 feet. Temperature controllers were added to maintain the water bath temperature at 65+/-2C. The strand was passed through and an air knife used to remove residual water and then directly strand pelletized using a standard rotary cutter. Using this new process, PHA polymer pellets suitable for injection molding, cast sheet/paper coating and film applications were produced.

In years 3-5 of the program work focused on developing large volume extrusion applications for PHB copolymer resins. Much of the effort focused on injection molding as this application can be done with small quantities of pilot materials. The key objectives were to improve the line speed and ductility of the final product as well as to explore additional mold geometries. In Year 3 we made the extensive progress on injection molding line speeds based on the use of the new nucleating agent NA1 and single cavity molding systems in laboratory or pilot environments. In Year 4 we began to explore molding of items under more industrial conditions to understand the sensitivity of PHAs to a range of molding regimes typical of the current plastics and products made by this industry. This included investigating a range of mold geometries and investigating the effects of other processing parameters such as mold temperature, melt temperature, hot/cold runner systems, roll temperatures and melt rheology to maximize physical properties of the final molded items. For paper coatings, there has been a significant body of work related to coating paper with PHA and indeed we have samples of coated cups in hand which we obtained from the Biopol acquisition. Paper coating is essentially a cast film process in which the film is cast onto a paper surface, so there is significant

overlap with other cast film applications, including cast sheet for thermoforming. For the paper coating the key hurdles relate to developing PHA formulations which eliminate the need for a sacrificial layer of polyethylene to prevent “sticking” of the PHA surfaces, have economic line speeds, and eliminate the phenomena known as “necking” from the extruder which is common to all polyesters. This task is therefore one of continuous improvement. For blown film, this has never been accomplished with PHA polymers. Key first step is to be able to create a “bubble” from the extruder and then expand that bubble to down gauge the film thickness.

Injection Molding : The key issues which were addressed during these efforts include:

- Fast mold cycle time
- Elimination of aging embrittlement providing finished parts with good ductility
- Range of mold geometries demonstrated
- Range of multi-cavity molding operations demonstrated
- Solution to the flashing problem
- Meet or exceed the physical property requirements of finished product

These achievements were a consequence of integrating the copolymer properties developed in Task 4.1-4 with real industrial extrusion operations for a variety of injection molded end products. Only a summary and range of examples is presented here. More detail was provided in the quarterly and annual reports submitted to DOE. The two mission critical factors for developing an economic injection molding process are to 1) have a PHA resin formulation which can be molded in standard processing equipment at high speed and 2). Disposable plastic utensils were initially chosen to validate this application as they combine the need for fast cycle time with good flow properties in relatively complex shapes. Additional molded product forms include golf tees and cell phone housings, which provide a means to explore different mold geometries and product performance criteria. In each case Metabolix worked with an injection molding company to optimize the use of resources and gain access to relevant equipment, molds and expertise.

Injection Molding : Utensils

The major effort in year 2 of the program focused on developing PHA polymer formulations suitable for high speed injection molding. In particular we focused on the developing the technology to enable the production of flatware which meets all of the functional requirements in use as specified by the military and yet is biodegradable allowing it them to be readily disposed. Success in this effort can only be achieved through combining the knowledge generated from the basic polymer property tasks. For example we have found that increasing the level of co-monomer improves ductility of the product but the mold cycle time decreases due to the slower crystallizing behavior of the copolymer. This slower crystallization can result in the modulus of the composition increasing over time (4-6 weeks) changing the mode of failure from ductile to brittle fracture. The aging embrittlement as a function of processing conditions and composition was studied using tensile and flexural modulus (three point bend and DMA). Molecular weight of the polymers was shown to have a significant impact on rate of embrittlement, with high molecular weight grades showing less aging effect. A balanced composition has been developed that allows reasonable mold cycle times and good ductility. Samples of the resulting flatware were supplied to the DOE project manager. Demonstration that PHA can be converted into flatware meeting all of the functional

specifications is a great first step. However the process had to be further optimized to achieve appropriate economics.

Work continued to optimize injection molding cycle times using “utensil” molds to the point where we achieved cycle times for single cavity utensil molds in the region of 30-45 seconds for PHAs compared with 8-9 seconds for polypropylene. Other biodegradable compositions being evaluated for use in injection molded biodegradable flatware e.g. starch and PLA have cycle times in the range 35-60 seconds. Currently the cost model for polypropylene and high impact polystyrene utensils is 30% raw material cost and 70% molding cost. Hence there is a strong driver to develop PHA polymer formulations and processing conditions, which enable fast molding cycle times. Our goal for this Task going forward is to achieve injection molding cycle times comparable to polypropylene.

We therefore investigated blends of different PHB copolymers in combination with the nucleating agent NA1 (see task 4.3). A formulated composition with a mean molecular weight around 450,000 appears to have the right rheological characteristics. By optimization of the nucleating package NA1, selection of a high crystallinity PHA and the use of talc as a heat transfer additive, mold cycle times of 8 seconds have been achieved. Figure 4.5.6 shows the progressive improvement of cycle time during the course of this program.

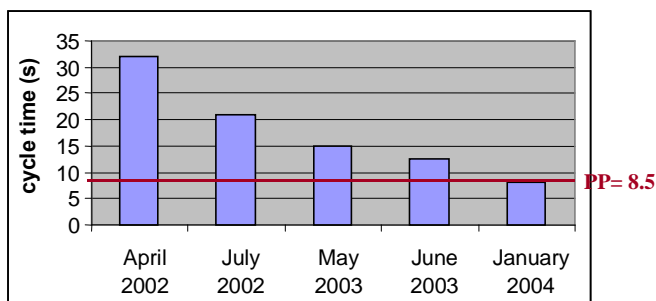


Figure 4.5.6

For polypropylene and polystyrene utensils the molding cost is approximately 70% of the total manufacturing cost, typically around 0.7cents/utensil. Therefore by reducing the mold cycle time of the PHA from 32 seconds to 8 seconds representing a significant cost saving to the end user and a key requirement for the broad adoption of these PHA resins.

It was also important to continuously monitor the impact of formulations developed for faster cycle time on the material properties of the molded parts and this was done routinely during the development effort. For example, Table 4.5.2 illustrates the effect of different resin formulations (e.g. PHB copolymer compositions, level of PHA additive, filler loading etc the actual formulations are proprietary) on the mechanical properties of molded tensile bars. Tensile bars for these experiments were produced using the following processing parameters:

- Melt temperature – 320F
- Screw speed 200 rpm
- Mold temperature 150F
- Cooling cycle – 30 seconds

Formulation	Tensile strength MPa, rt aged	Elongation @ break % rt aged	Youngs modulus MPa rt aged	Tensile strength MPa, 70C aged	Elongation @ break % 70C aged	Youngs modulus MPa 70C aged
062804-1	24.5	3.2	1632	25.4	2.8	2235

062804-2	18.8	6.1	1671	20.1	6.9	1715
062804-3	21.7	4.4	1882	22.4	6.2	1989
062804-4	22.0	4.1	1911	21.5	5.9	1920
062804-5	20.5	5.1	1779	21.3	6.2	1803
062804-6	18.3	6.6	1554	19.3	8.8	1556
062804-7	22.4	6.1	1471	22.3	5.2	1814

Table 4.5.2

Work continued to explore the composition space for both lightweight (3.5g) and heavyweight (6.5g) utensil designs using single cavity prototype molds. The design makes it a challenge for optimum stiffness/ductility from the same compositions. Because of these criteria, the formulation was focused around a PHB copolymer system rather than the previous PHB system. By increasing the mold temperature from 110F to 160F the cooling cycle has been reduced from 20 seconds to 5-10 seconds. The melt temperatures were also reduced from 330F to 290-300F, including the hot runner tip, bringing the final total cycle time to 12-15 seconds. After molding the stiffness of the molded utensil continues to increase due to continuing crystallization of the polymer, this is typical for other semi-crystalline polymers such as polypropylenes. Aging studies were carried out to establish the final stiffness/ductility balance.

Flexural ductility of the light weight forks compositions were assessed by placing a 5 lb load on the end of the tines whilst clamping the handle to the edge of the bench and the results are shown in Table 4.5.3.

Table 4.5.3

Formulation	Barrel temperature F	Hot runner temperature F	Mold Temperature F	Cooling time sec	1 day flex	14d flex
062804-1	340	360	100	20	ductile	Brittle
	340	340	150	20	ductile	Brittle
	320	340	150	20	ductile	Brittle
	320	320	150	20	ductile	Brittle
	320	320	150	50	ductile	ductile
	300	320	150	20	ductile	ductile
	300	320	100	20	brittle	Brittle
	300	320	100	50	ductile	brittle
062804-3	340	360	100	20	ductile	brittle
	340	340	150	20	ductile	brittle
	320	340	150	20	ductile	ductile
	320	320	150	20	ductile	ductile
	320	320	150	50	ductile	ductile
	300	320	150	20	ductile	ductile
	300	320	100	20	ductile	ductile
	300	320	100	50	ductile	ductile
	300	300	165	10	ductile	ductile
	300	300	165	20	ductile	ductile
	300	300	160	10	ductile	ductile
	300	300	160	5	ductile	brittle

This demonstrates the importance of minimizing the melt temperature exposure with mold temperatures designed to maximize crystallization behavior.

100 sets of heavyweight knife, fork and spoons (Figure 4.5.7) were successfully molded at Innovative Mold Solutions.



Figure 4.5.7

We have also successfully demonstrated the molding of first generation and second generation cell phone housings in cold runner molds used for polycarbonate. The compositions met impact and heat distortion requirements.

Key to the next stage of development of this application is to eliminate the flashing problem observed with molds built to handle traditional petrochemical resins such as polypropylene (PP), polystyrene (PS), high impact PS (HIPS) and polycarbonate (PC, this resin is used extensively in housings for electronic goods such as cell phones, computer housings etc.). All these systems have parting line vents to accommodate high viscosities during the final stage packing of the mold. The high viscosities achieved with traditional thermoplastic polymers are achieved through low mold temperatures for amorphous polymers such as polystyrene or high impact polystyrene or through rapid viscosity increase due to crystallization of the polymer such as polypropylene. Whilst the development of NA1 nucleating agent technology has dramatically increased the crystallization behavior of PHB copolymers, for many resin formulations required to obtain final product performance, they are still slower than traditional thermoplastics such as polyethylene and polypropylene. It should be noted that this optimization of crystallization rates with different resin formulations is of necessity an iterative process. Further the optimum crystallization temperature for PHAs is around 55-65C. Since the crystallization temperature is only 100C lower than the melt processing temperature (typically some 200C for more for traditional thermoplastic polymers) the rate of viscosity increase as a direct function of temperature is lower. There is therefore a need to increase the crystallization behavior of PHAs even further. This have been achieved by the blending PHA grades to increase the sequence distribution length of the 3HB block as well as recent improvements in the efficiency of NA1 nucleating agent. Below we summarize a few of the specific molding targets including golf tees and phone housings.

Injection Molding : Golf Tees

Initial studies on this application used resin 032405 series of PHB copolymer compounds designed for golf tees. A molding study was carried out in a prototype mold at IMS where the production of the Epoch golf tee is carried out. Table 4.5.4 shows the properties obtained from testing impact bars and also golf tees:

	032405-1	032405-2	032405-3	032405-4	032405-5	032405-6
cycle time s	16	14.1	17	15.2	15	25.1
golf tee unnotched impact ft-lb/in	0.38	0.57	1.59	0.70	0.72	0.60
Izod notched impact molded 60C	0.48	0.513	0.792	0.547	0.713	0.575
Izod notched impact molded 45C ft-lb/in	0.471	0.5	0.793	0.501	0.713	0.54
tee deflection (1.4 kg load) in	0.3884	0.4142	0.933	0.5242	0.5182	0.442

Table 4.5.4

This compared very favorably with a competitive starch/synthetic polyester compound with regard to stiffness and impact resistance. The cycle time for the starch/polyester was around 23 seconds making it significantly slower to mold. Under high humidity conditions (eg 75% relative humidity 85F) the starch based tees became extremely pliable and difficult to force into the ground, whilst the PHA based tee compositions remained unchanged.

Golf tees are good test parts for developing injection molding because they require high stiffness to penetrate into the soil and good impact resistance so that they can be used for multiple shots without breaking. Because neither notched nor unnotched impact was providing adequate information regarding the impact performance of the golf tees, a new test procedure was adapted whereby a golf tee is positioned in the Izod impact machine. Figure 4.5.8 shows provisional data correlating golf tee impact strength with number of actual golf swings to failure:

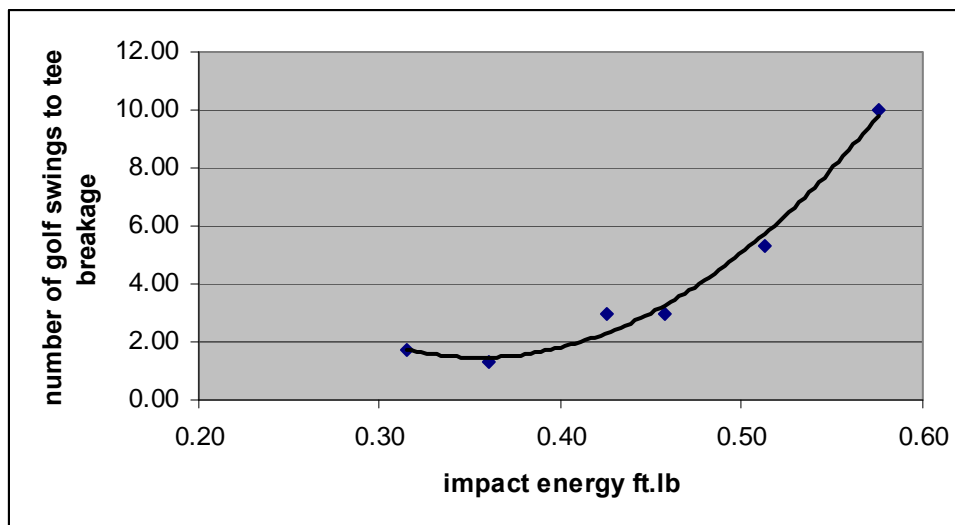


Figure 4.5.8

This is now being used to complete final optimization of the PHA tee resin composition. Single cavity molding operations have cycle times in the range of 14-16s compared with 23s for the incumbent material.

Because of the unique head design the tee is molded through a hot runner gate just below the head and ejected from the point (usual to eject from the head). This places significantly more constraints on the polymer composition because of the thickness of the tee shaft and need for high ejection forces to remove the molded piece. Cycle times for this geometry are typical 20-25 seconds to avoid "punch through". Here again the balance of stiffness and ductility is important so that the golf tee is stiff enough to be positioned in the ground whilst withstanding several strikes with a "wood" club before failure.

A new stiffness test on golf tee was designed using a DMA mode and measuring the force required for a given deflection; nylon 6,6 was selected by Evolve Golf as the benchmark in stiffness for degradable compositions. Figure 4.5.9 and 4.5.10 show that compositions can be developed which have similar stiffness characteristics to nylon 6,6:

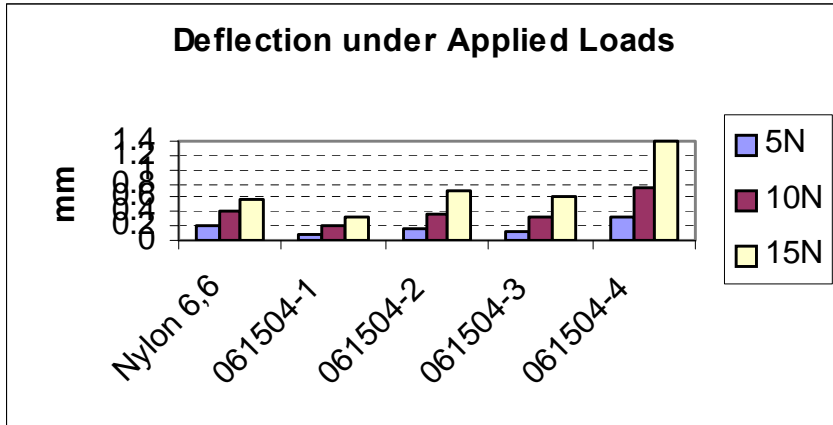


Figure 4.5.9

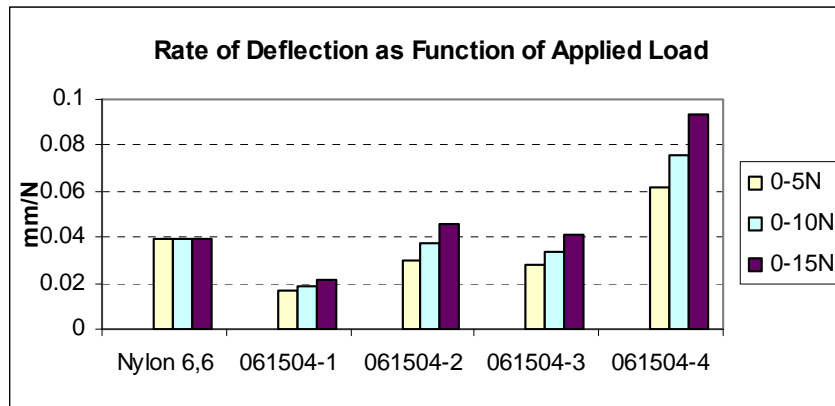


Figure 4.5.10

Injection Molding : Cell Phone Casings

For cell phone casings the traditional material is polycarbonate, one of the most expensive petroleum based plastics. This work was done in collaboration with two major cell phone company's under confidentiality agreements and so the names have been replaced with CellCo1 and CellCo2. The objective of the work was to optimize the balance of impact properties versus stiffness in a cell phone application. This follows on from the previous quarters work in looking at the effect of rubber phase content and filler loading. Figure 4.5.11 show the correlation between impact strength, flexural modulus and tensile properties for formulations containing 30% rubber phase (high comonomer PHB copolymer with low Tg), 10phr plasticizer and variable filler content.

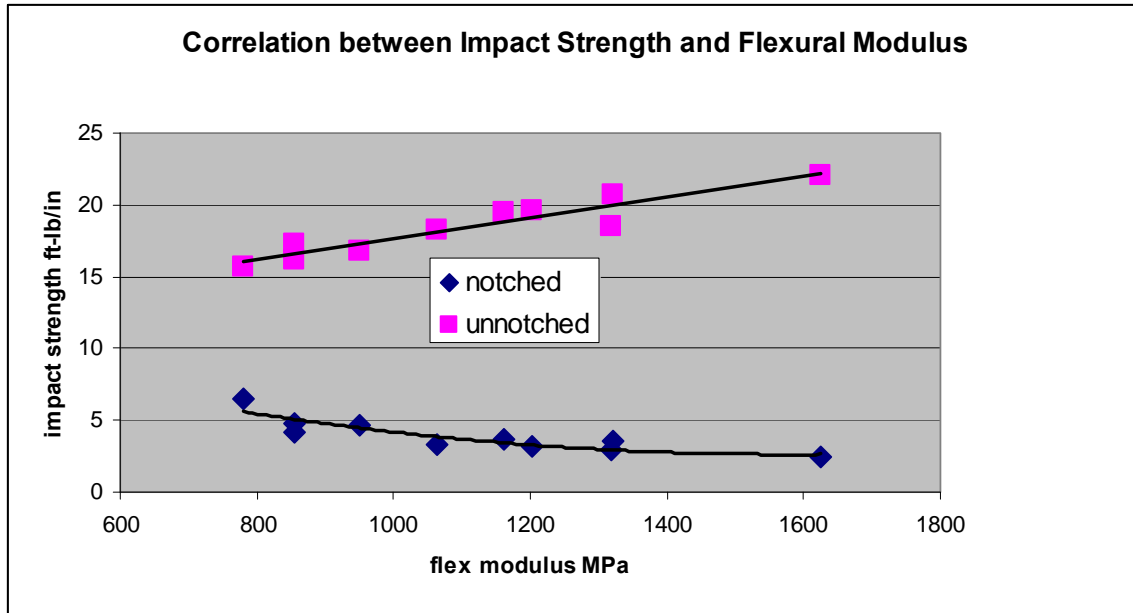


Figure 4.5.11

The notched impact data shows the expected reduction with increasing stiffness of the composition. The unnotched impact data follows the reverse trend which initially may be counter intuitive. On examination all the unnotched samples did not fail during the impact test and hence the impact data reflects the force required to distort the sample under the pendulum action. Based on this data a molding trial was carried out using a CellCo1 production clam shell cell phone on a Toshiba EC 180 injection molding machine. Polycarbonate is the preferred resin used by CellCo1 for cell phone housings. In the above four cavity hot runner molds the cycle time for polycarbonate was around 26 seconds whilst for the Metabolix PHA composition a cycle time of 18.8s was achieved and could potentially be reduced even further. A second trial was run for CellCo2 at their facilities in Europe (toll molder for CellCo2) using an old prototype cell phone design. CellCo2 selected on older cell phone mold because of prior problems in molding PLA. See photo below for example of molded phones.



These parts were produced on a Roboshot 100 ton electric injection molding machine - machine with small (22mm) extrusion barrel. Because the mold was relatively old and had significant wear on the matched faces some slight flashing was observed, however the cycle times were faster than polycarbonate in the same cavity. Industrially satisfactory molding characteristics of the PHA composition and fast cycle times were

achieved. Subsequent evaluations by both CellCo1 and CellCo2 indicated that the stiffness of the compositions needed to be improved.

Injection Molding : Soil Stabilization Stakes

Uzet, Ireland has designed a new stake for soil stabilization which has higher holding power for both turf and erosion control netting. These stakes have are typically 4 – 6 inches and have a complex mold shape. Work is being carried to develop a suitable composition for the stakes which provide sufficient stiffness for the stakes to be driven into a variety of soil compositions but also not to break during either the driving action of if they hit a rock. Hence the compositions need to have different physical characteristics to those being developed for golf tees which require higher impact performance. Fast cycle times are required for the stakes to be economic. A number of formulations were identified and screened for flexural modulus using a DMA three point bending mode and Izod notched impact strength using simple bar modeled test pieces.

Four compositions were then selected and stakes molded and the Table 4.5.5 provides the optimum molding conditions for the different formulations:

		1399-70B	1399-70B	1399-90A	1399-90B	PS
		initial	final	final	final	final
feedport	F	330	330	330	330	410
zone 1	F	325	325	325	325	420
zone 2	F	320	320	320	320	430
nozzle	F	315	305	320	320	445
mold	F	120	120	130	130	78
injection speed	in/s	2.5	2.5	3	2.5	3.5
shot size	in/s	1.8	1.8	1.72	1.72	2.75
screw speed	rpm	200	200	260	80	250
V-P position	in/s	0.12	0.12	0.2	0.2	0.2
max injection pressure	psi	20,000	20000	13800	11800	12800
decomp dist	in/s	0.15	0.15	0.2	0.2	0.2
decomp velocity	in/s	0.1	0.1	0.2	0.2	0.1
back pressure	psi	250	251	400	350	802
hold 1 pressure	psi	3000	3002	5500	3810	3002
hold 1 time	s	2	5	2	5	5
hold 2 pressure	psi	4000	4002	3000	3000	
hold 2 time	s	1	3	1.5	1	
ejector forward	in/s	1.5	1.5	1.5	1	1.1
ejector forward velocity	in/s	5	5	1.5	1.5	5
injection time	s	0.82	0.82	0.66	0.77	0.85
peak pressure	psi	13721	13287	13874	12137	7620
pressure @ VP	psi	14213	12763	14235	14277	75540
cool	s	20	20	0	20	8
cycle time (total)	s	24	22	16.9	26.4	22.2

Table 4.5.5

All formulations molded extremely well and field trials are being carried on on each composition. Formulation 1399-90A had the fastest cycle time and 1000 stakes were produced in a 2 cavity mold in just less than 3 hours. This was noticeably faster than stakes produced in the same mold with a non-degradable polystyrene composition where the cycle time was 22.2 seconds. Four batches of 1MBX 1399-93C resin were produced using different batches of Metabolix recovered PHA grades. These were used

to mold 4000 stakes which have been used for full scale testing on the holding capability of the stakes for soil stabilization and turf laying operations. The PHA stakes met all the test requirements. The partner company is in the process of having a 32 cavity 6" stakes mold and a 36 cavity 4" stake mold constructed for production of stakes in the USA. Larger scale pilot production trials in the commercial molds were successfully completed at which time all activity on this target under the program was terminated. Small scale limited commercial launch of stakes was subsequently undertaken. See Figure 4.5.13.



Figure 4.5.13

Injection Molding : Bottle Caps Closures

CapCo1 has shown interest in evaluating biodegradable compositions for oil cap closures. Their process is based on a compression molding operation whereby the molten plastic is extruded into a rod which is immediately cut into the desired length sections and dropped into a compression mold where the cap is formed. The cap is removed from the mold using a push ram which forces the cap over the threads in the mold. Key attributes of the product are the need to easily release from the metal mold surface (more of a challenge with a polar polyester compared with HDPE) and high green elasticity to allow short term deformation of the cap during demold without permanent distortion. See Figure 4.5.14.

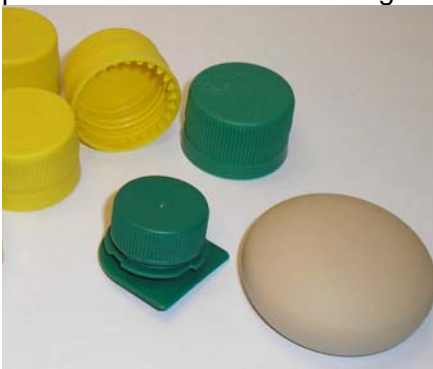


Figure 4.5.14

Further modifications to the resins were carried out on a more challenging mold design with 6x2 cavities with hot runner, small runner with very small sub gates. PP has to be run at 300-310C to avoid blocking of the tips.

- Molding conditions
- Barrel : 175C/170C/165C (feed to nozzle)
- Nozzle 180C
- Hot runner 170C
- Hot tips 180-190C (depending on position)

Injection speed reduced from 55mm/m to 15mm/minute to eliminate flash. Cycle times were comparable to the current PP composition, however the energy to mold the parts in PHA was estimated to be about 50-60% that of the PP because of the lower melt temperature for the polymer and running the mold at 60C compared to 15-20C for PP (requires larger volumes of cold water to keep the mold cold).

Injection Molding : Cosmetic Containers

Cosmetic companies have shown interest in biodegradable cosmetic cases and selected a lipstick case as a means of assessing the performance of current Metabolix compositions. Lipstick molding trial were carried out at R P C Cresstale, UK the Europe.

- Molding machine – Battenfeld BA 1000CDK
- Mold – hot runner 3x4 cavity set up, direct gating, hot tip, hot runner
- Normal product for mold – PP with melt processing temperature of 270C, mold temperature 20C, cycle time (robotic mode) 26.3s (can run 22s but distortion of base during demolding)
- Barrel capacity – 5/6 shots

These were run in automated mold with robotic removal of cap base – cycle time 26.3 s (same as PP) to produce industrially acceptable parts.

Injection Molding : Large Part Molding:

Metabolix identified a company which has developed a unique approach for denitrification of static water areas in ornamental gardens and golf courses. The high level of fertilizers used in these environments leads to a build up of nitrates in the ponds and also high algae growth. The interested company has developed a bacterial composition for reducing nitrates and algae but require a biodegradable container for their products, currently they use a perforated sphere in polystyrene which needs to be removed after use.

Two parts were molded, a 11" diameter hemisphere weighing 420g and a 6" diameter hemisphere weighing 60g; both parts being substantially larger than any parts previously molded in PHAs. Substantial savings in cycle time were realized and there are still options for further improvement. Spheres were sent to the end user for evaluation in degradation studies in a pond environment.

Papercoating/Cast Sheet and Film:

As an example of the type of work done to develop paper coating grades, two batches of paper coating resins, 1399-84A and 1399-84B were produced on the 2.5" Welex single screw extruder at UMass, Lowell. The data was extremely consistent during the run and the target Mw goals were met. The key difference between the two compositions was in crystallization behavior. Rheological profiles for the two compositions were generated to understand if the difference in crystallinity had an impact on rheology at low processing temperatures. See Figure 4.5.15.

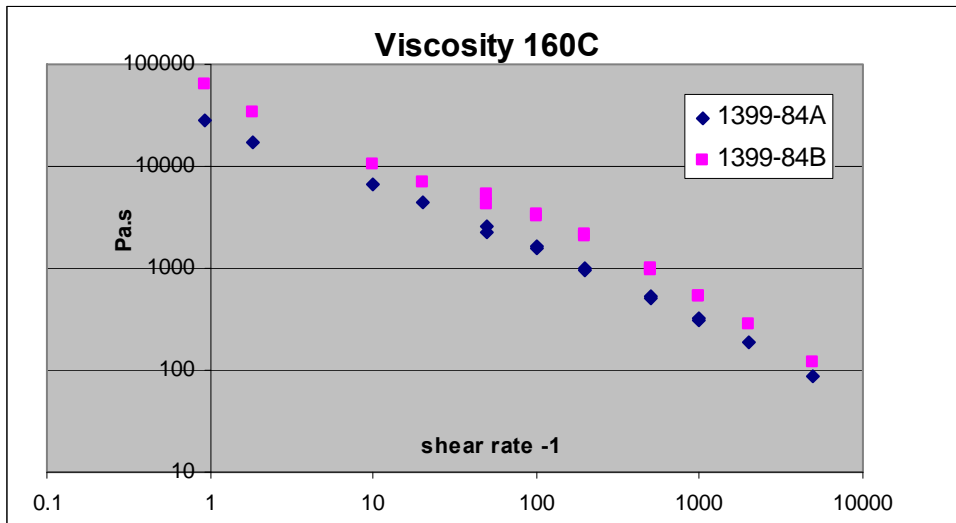


Figure 4.5.15

1399-84B does show a slightly higher viscosity profile which would not have been expected for the molecular weight differences. Both resins were evaluated in paper coating and the coating line conditions are detailed in the Tables 4.5.6 and 4.5.7 together with the effect of line speeds:

Barrel temperature profile C	1399-84A	1399-84B
feed	100	100
zone 1	170	170
zone 2	165	165
zone 3	165	165
zone 4	160	160
nozzle	160	160
extruder torque Nm	18	14.5
Screw speed rpm	70	70
Die gap mm	0.5	0.5
chill roll	polished	chrome

Table 4.5.6

grade	chill roll	substrate	comments
1399-84A	65C	PET	no sign of sticking to chill roll up to 19.4m/minute
			good film quality 15-150 micron
			easy removal from PET film
			melt instability (necking oscillation) starting at 18m/minute
			melt instability with oscillation at 19.4m/minute
	65C	paper	very slight edge transfer to chill roll at 19.4m/minute
			good film surface at 18m/minute
	45C	PET	slight snapping delamination from chill roll 11.6m/minute
			slightly louder snapping delamination from chill roll 15m/minute
			noisy delamination from chill roll 19.4m/minute but no transfer
	45C	paper	start of paper delamination at 6.8m/minute
			no paper delamination at 3.5m/minute
1399-84B	65C	PET	no sign of sticking to chill roll up to 19.4m/minute
			good film quality 15-150 micron
			easy removal from PET film
			melt instability (necking oscillation) starting at 18m/minute
			melt instability with oscillation at 19.4m/minute
	65C	paper	no sign of sticking to chill roll up to 19.4m/minute
			good film quality 15-150 micron
	45C	PET	no sign of sticking to chill roll up to 19.4m/minute
			good film quality 15-150 micron
	45C	paper	no sign of sticking to chill roll up to 19.4m/minute
			good film quality 15-150 micron

Table 4.5.7

This trial conclusively showed that the maximum line speed was directly related to crystallization half life and chill roll temperature with 60C being the preferred conditions.

Scoping work was further pursued using the PHA resin formulation using a small 6" wide cast film extrusion unit at Lowell and an improved composition for paper coating identified. A paper coating trial using 14 point SBS cupstock paper was successfully completed at Fortifiber, a local paper-coating company. Melt temperature was controlled at 340F and the molecular weight adjusted to match the rheology of the traditional polyethylene paper coating grade MFI 7 at 620F. Lines speeds up to 300'/minute (limit of 26" wide pilot line equipment) were run with no signs of roll blocking. A range of coating thicknesses was prepared.

- Single side 0.5mil
- Single side 1mil
- Single side 2mil
- Double sided 1mil
- Double sided 2mil

This represented a true step change in PHA paper coating technology as all previous attempts on this application required the use of a sacrificial layer of polyethylene in order to prevent the PHA coated paper blocking i.e. sticking to itself as the coated paper was rolled up.

The coated paper samples were tested for moisture barrier properties at 73F and 50% humidity and the results are presented in Figure 4.5.6 a. Based on these results over 1000 ft of paper was coated for evaluation in the production of hot cups (single side coated). The rolls were successfully cut to produce widths suitable for cup forming trials. Cup forming trials were carried out at Clovernook Center for the Blind and Partially Sighted, Memphis, TN.

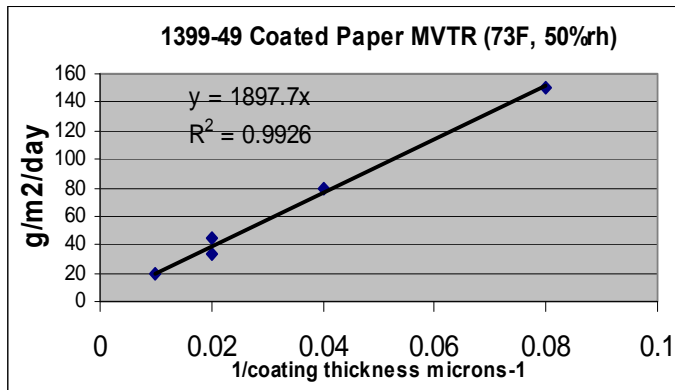


Figure 4.5.16



Figure 4.5.17

Cups (Figures 4.5.16 and 4.5.17) were produced at a rate of 157/minute with a side sealing temperature of 850F and a base sealing temperature of 540F; these were identical conditions to those for polyethylene (PE) coated paper. Cups were immediately tested for their ability to hold hot water at 160F (simulate coffee and tea dispensing) and passed this test. Metabolix continued to run papercoating trials at Black Clawson with line speeds for 100 to 450 fpm being observed for different grades. Additional technology was developed to reduce the adhesion between the PHA film and the roll surface.

The coated paper produced for cup forming trials was shipped to a cup manufacturer for slitting into rolls to produce 10 oz cups:

Side stock = 9-13/16" (9.8125")

Bottom stock = 2-15/16" (2.9375")

Bottom stock was double sided coated with PHA resin. Cup forming trials were carried out at Clovernook Center for the Blind and Partially Impaired. A PMC 1200 cup forming machine was used to produce the cups at a rate of 150 cups/minute (maximum continuous production rate for the unit). Using a side heat sealing temperature of 850F (hot air) and base sealing temperature of 900F cups were produced to comparable quality of commercial polyethylene coated cups. When tested the cups produced deep paper fiber tear on all the sealed areas and withstood hot coffee (90% cup fill) with no leakage. During the trial nearly 60,000 cups were produced. A portion of these cups were used for a pulping trial with the US Navy and to further validate the marine biodegradation of the cup. The Navy proposes to develop a process for the maceration of the food waste and biodegradable containers followed by discharge into the sea. These tests are to validate the robustness of the maceration unit prior to submission for MARPOL approval. In conjunction with the US Navy PHA coated (1mil) coffee paper (18mil) cups have been evaluated for marine degradation. Both the coating and paper had completely degraded within 70 days.

Cast Sheet/Film: This subtask is an extension of the paper coating work which is essentially a case film process. Historically PHB and PHBV materials have shown aging embrittlement over relatively short time periods (1-2 months). The aging properties of a 60 micron film (1399-72) produced on a 600mm wide cast film line using a resin based

on the PHB copolymer has been studied for aging properties and the results based on measuring tear strength are shown in Figure 4.5.18.

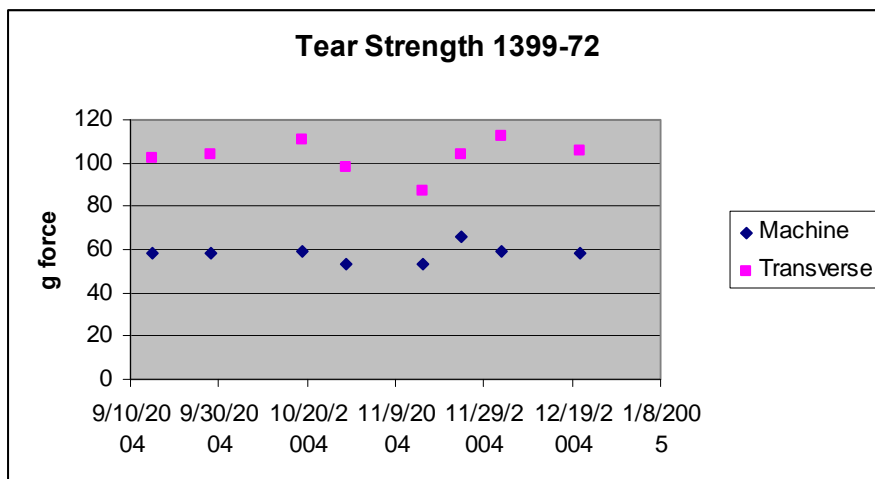


Figure 4.5.18

The film shows no change in tear properties over a three month period.

Cast Sheet/Film: This effort is an extension of the paper coating effort, which is essentially a cast-film process. Large scale cast sheet extrusion trials were carried out to generate sheet samples 12" wide and 1.6mm thick.

100m samples of 60 micron and 90 micron films were produced on a 600mm wide pilot coating line. The necking of the film reduced the width from 600mm to 550mm without the use of air pinning techniques, this was comparable to PE cast film.

Table 4.5.8 gives the characteristic parameters for the polymer compositions and cast film.

	thin	thick
microns	50-60	90-100
Mw	413258	374525
Mn	212880	202472
PD	1.98	1.85

Ramp crystallization from 185C

onset crystallization	99.36	99.36
peak crystallization	88.22	88.44
end crystallization	52.16	57.2
J/g	27.95	26.56
Tm onset	93	89
Tm Peak 1		117.67
Tm peak 2	136.35	136.63
Tm end	164	164
J/g	27.76	27.88

Isothermal crystallization 105C

t zero	5.92	5.92
t peak	10.75	10.27
t end	17.55	17.17
half life minutes	4.83	4.35
total minutes	11.63	11.25

Table 4.5.8

The first trial was designed to produce films with properties close to those of HDPE and the resulting properties of these films are shown in Table 4.5.9.

Lowell Data

Tensile summary 7 days	50-60 micron	90-100micron	50-60 micron	90-100micron
	mean	mean	sd	sd
parallel to machine direction				
tensile strength MPa	17.7	18.0	3.96	4.1
elongation @ break %	410	430	119	49
Modulus MPa	385	410	87.5	52
perpendicular to machine direction				
tensile strength MPa	15.9	16.3	1.9	5.2
elongation @ break %	333	361	77	36
Modulus MPa	456	494	135	136

Table 4.5.9

The tear properties of this formulation (1399-55) tended to deteriorate slightly on aging, reflecting some level of post crystallization behavior. The product was reformulated (1399-72) to speed up the rate of crystallization and reduce the modulus even further and, the properties of the resulting film, is shown in Table 4.5.10.

Tensile - machine	ISO 527	
strength	N/mm ²	24.12
elongation@ yield	%	668
Elongation @ break	%	677
Modulus	Mpa	214
Tensile - diagonal		
strength	N/mm ²	21.6
elongation@ yield	%	698
Elongation @ break	%	705
Modulus	Mpa	247
Tear Graves	ISO 53515-A	
machine	N/mm	81.9
diagonal	N/mm	59.7
Puncture	F max N	4.46

Table 4.5.10

Thermoforming: Thick sheet both 1.5mm and 0.6mm thick has been produced using both different PHB copolymer compositions. Both could readily be extruded with no signs of roll sticking provided the roll temperatures were maintained at 50-55C. If the temperature was reduce to ambient or sub ambient conditions (normally used for PS and PP systems) then severe sticking to the rolls was observed. A range of shapes were thermoformed demonstrating the versatility of the different compositions. Good sharp profiles were obtained with no weak spots. All exhibited good ductility in deflection, this is a major problem observed with PLA thermoformed components. The thermoformed samples were exposed to aging at 60C for 48h with signs of blocking of loss in shape.

Thicker (300 micron) films have been produced at Bixby International for thermoforming applications, in particular coffee cup lids for papercups. A small thermoforming unit was purchased so that work can be carried out at Metabolix with minimal material wastage. Currently molds are being built so that standard cup lids can be replicated.

Blown Film:

For polymer to be capable of being processed through extrusion blown film lines, they need high melt strength under low shear conditions to provide bubble stability. Development of blown film applications also requires much larger samples of resin and therefore these studies got underway once the new pilot plant was up and running. Only small scale studies on laboratory equipment are reported here but in the interim period since the end of the DOE program this technology has been scaled up considerably and although there are additional issues to be resolved it is clear that they can be resolved with further process improvements to make commercial PHA film.

Conventional polymers e.g. polyethylene with narrow molecular weight distributions are unsuitable for film blowing applications, branched polymers are typically used commercially. The development of an improved branching technology to improve the melt strength of PHB copolymers is described in detail in Task 4. Samples were tested for blown film capability using a small 1" extruder with a 3" ring extrusion diameter. The sample containing chain branching exhibited significantly better "bubble stability" than the sample with no branching. Preliminary blown film trials were carried out on an external pilot blown film line (2" extruder, 2" ring, 1.2mm gap, Natick labs). The trial confirmed the fast crystallization behavior of formulated PHBV and PHBco-monomer blends using nucleating agent NA1 but limitations in blowing the bubble greater than 1.2x. Further work was identified to improve the dispersibility of NA1 and also to improve melt strength. Preliminary work at the Department of Defense Natick Laboratories confirmed the larger scale trial and showed that there were limitations in trying to process narrow polydispersity PHAs. This is analogous to early problems in processing LLDPE that had similar narrow polydispersity. The solution for LLDPE was to introduce small quantities (5-20%) of highly branched LDPE. Scoping work by Metabolix scientists using the facilities at UMass, Lowell for processing and UAkron for rheological characterization has identified some new technology that can reproducibly generate consistent levels of branching. Figure 4.5.19 shows a successful blown film process at Natick laboratories where a blow up ratio of 1.6x was achieved. This is a first for PHA processing and a major breakthrough accomplishment.



Figure 4.5.19

Blown films with 3x die to bubble diameter have been produced using the incorporation of branched polymers. Film thickness was limited to 50 microns because of

particulate contaminants in the polymer composition as a result of residual biomass from fermentation/solvent recovery and poor dispersion of the nucleating agent.

Only a small amount of work has been carried out in this area during Q4. This was due to material availability and access to pilot equipment capable of processing only 10's kg per hour. We have demonstrated the ability to increase bubble stability using branched PHAs at low levels. We were not able to demonstrate a reduction in film thickness because of dispersion issues with our current stock of NA1 nucleating agent. A separate program to reduce the particle size of NA1 is in progress and we will return to blown film when we can accomplish a dispersion with no NA1 particles above 2-3 microns.

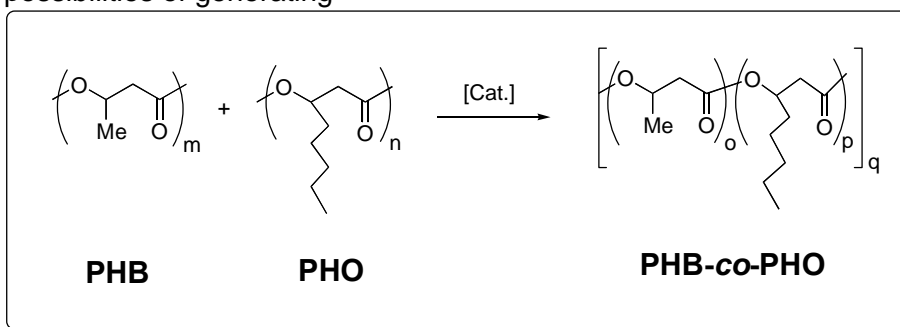
Task 4.6 PHA Blends

Task 4.6 Deliverables and Resources			
Year 3	Year 4	Year 5	Resources
Develop PHA blend compositions with other biobased polymers (PLA and starch)	Deliver PHA/PLA blend fibers	Deliver PHA-starch blend films	Metabolix, Inc. University of Massachusetts at Lowell

Work on this particular Task was carried out only on a limited time available basis and where work was done it was integrated with Task 4.5.

Task 4.7. Chemical Modification of PHB, Ester Interchange Programs Prof. Geoffrey W. Coates and Dr. Joseph A. R. Schmidt, Department of Chemistry, Cornell University.

Polyhydroxyalkanoate (PHA) bioplastics are biologically derived materials which form as perfectly isotactic polymers of high molecular weight inside living cells. The basic properties of some PHAs have been studied and, in general, functionally compare favorably with today's high volume resins. Although these plastics have wide utility, various properties must still be improved upon. The most serious drawbacks of PHB include brittleness and a propensity to decompose at the temperatures required for melt processing. On the other hand, polyhydroxyoctanoate (PHO) is hindered by its very low melting point. For these reasons, we have undertaken a research project exploring the possibilities of generating

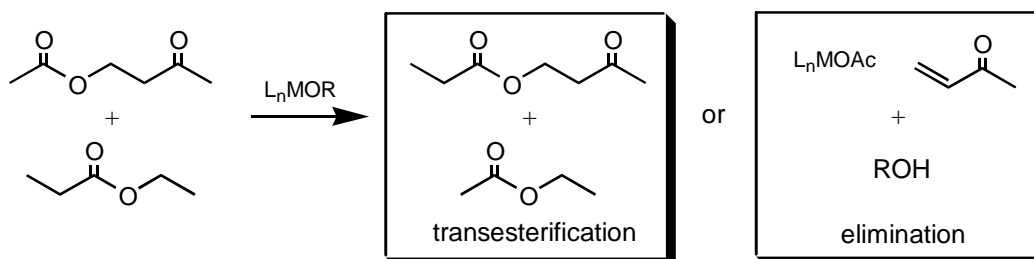


Scheme 1: Proposed transesterification reaction of PHB and PHO.

PHB/PHO random copolymers (co-PHB/PHO, Scheme 1), in an effort to produce materials with optimal properties, while avoiding prohibitively high-cost methods. These targets were chosen because of the availability of PHB and PHO from Metabolix.

A two-pronged approach to this problem, employing combinatorial methods, as well as traditional catalyst design techniques was taken. In the first case, we have initiated the combinatorial screening of a wide array of chemicals previously cited as transesterification catalysts in other systems, as well as compounds closely related to these materials. As a model system, allowing for rapid screening, we have studied the reaction of ethyl propionate with 4-acetoxy-2-butanone, both of which can be acquired commercially (Scheme 2). This system was chosen due to the strong similarity between

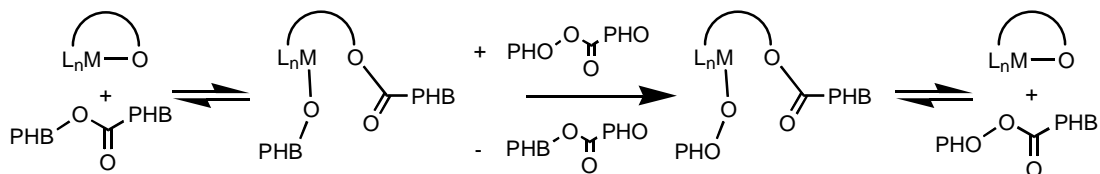
Scheme 2



4-acetoxy-2-butanone and the repeat units of the PHA polymers. The most challenging aspect of this transesterification involves the acidity of the protons on the methylene group adjacent to the carbonyl moiety, resulting in a competition between the desired transesterification reaction and an unwanted elimination side reaction (Scheme 2). With this in mind, it becomes apparent that a successful catalyst for this system must be strongly nucleophilic, while being only mildly basic. Screening of these reactions can be readily accomplished using gas chromatography, allowing quantitative product determination. Catalyst screening focused primarily on metal alkoxides, but was also expanded to include other materials such as oxides and acetates.

The second approach to this transesterification problem involved the design of new zinc catalysts for the creation of block copolymers of PHB and PHO. The catalyst designs are based on previous studies in the Coates laboratory and involve a beta-diketiminato ligand tethered to an alkoxide unit. The advantage of these ligands is that block copolymers formed using these species will result in complete incorporation of both biologically derived PHA polymer chains without degradation of the molecular weight of the final copolymer (Scheme 3).

Scheme 3



To date, we have developed routes to make a series of beta-diketiminato ligands with alkoxide tethers and successfully isolated some of their zinc complexes. The aluminum, magnesium, and tin analogues employing this betadiketiminato ligand were also synthesized. These metals traditionally give better transesterification conditions and may lend more utility to this system.

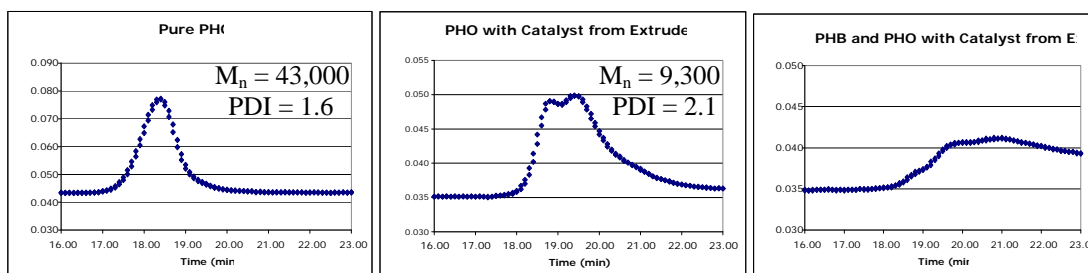
From this two-pronged approach numerous catalyst systems were found that catalyze the elimination reaction and only one catalyst (named Catalyst 1) was identified that resulted in a preference for transesterification. The viability of Catalyst 1 as a

transesterification catalyst was evaluated further initially using a model system, which measured the reaction of PHAs with ethyl propionate before focusing the efforts on the application of Catalyst 1 to the actual target polymers (PHB and PHO). Requirements for achieving success included developing an analytical capability to determine that transesterification is taking place and the development of experimental approaches to carry the actual transesterification reaction given the vastly different behavior and solubilities of the two starting polymeric materials. Initial studies used PHB synthesized by a chemical polymerization process and analytical methods were developed including ^{13}C NMR and GPC techniques.

To observe how the polymers behaved under melt conditions, samples containing PHB, PHO, and a mixture of PHB and PHO were run through a micro-extruder at 150°C for varying lengths of time. As expected, in the absence of catalyst no changes to the molecular weight or polydispersity index (PDI) of PHO were observed by GPC (we were unable to obtain these data for PHB), and no mixing of the two polymers was evident. However, when PHO was subjected to Catalyst 1 for 10 minutes in the extruder, a bimodal GPC trace was observed with a reduction in the molecular weight from 43,000 to 9,300 as well as an increased PDI. These results are consistent with the catalyst chopping the polymer chains into shorter chains of varied length. Furthermore, when PHB and PHO were mixed together with Catalyst 1 in the extruder, the GPC trace showed a very broad molecular weight distribution (Figure 4.7.5), and the polymer sample was soluble in THF indicating the likely incorporation of PHO into the PHB chains.

For better control of reaction conditions, subsequent reactions were performed in vials on a hot plate. This method allowed for a precise and reproducible measurement of polymer and catalyst, which was difficult with the micro-extruder, as well as the ability to maintain an inert atmosphere in setup and throughout the transesterification reaction. The first attempt with this method using PHO and 5 mol % catalyst produced a surprising outcome. The resulting yellow polymer sample was completely insoluble in THF, despite the high solubility of the starting PHO polymer in the solvent. Furthermore, a series of new peaks were observed in the ^1H NMR which cannot be accounted for by PHO or Catalyst 1 alone. This could account for the unexplained absence of the PHO from the ^{13}C NMR spectrum of the materials from transesterification of PHB/PHO. This result also caused us to examine our catalyst loading, which was determined to be much higher than necessary. With a catalyst loading of just 0.1 mol %, the molecular weight of the PHO

Figure 4.7.5: GPC traces of polymer samples from the micro-extruder.



sample was reduced by more than half in one hour at 100°C (turnover frequency of 1150 h^{-1}), and the ^1H NMR spectra showed only traces of the unidentifiable side products.

Interestingly when the reaction time was extended up to 8 hours at the same catalyst loading and temperature, no variations in molecular weight or PDI were observed. While several unanswered questions remain regarding the behavior of Catalyst 1 as a catalyst for the transesterification of PHB and PHO, work completed in the fourth quarter seems to demonstrate the catalyst's effectiveness in melt reactions as well as in solvent. We are working to overcome some logistical difficulties in working with PHB in order to incorporate both PHO and PHB into our trials. We are also exploring transesterification using much lower molar equivalents of the catalyst. When copolymer production is better understood we hope to be able to generate a variety of copolymers of chosen composition.

Before incorporating PHB into the reactions, reaction rates for pure PHO were determined. Last quarter it was noted that PHO was nearly halved in molecular weight within one hour, however no further reduction was observed at times up to 8 hours. This quarter, shorter reaction times were examined, which gave a clearer picture of reaction progress (Figure 4.7.6). While it is difficult to determine if the higher molecular weights were due to a lack of transesterification or reactions between longer polymers, it is encouraging that PHO was never degraded beyond 20,000 g/mol, even at unnecessarily long reaction times.

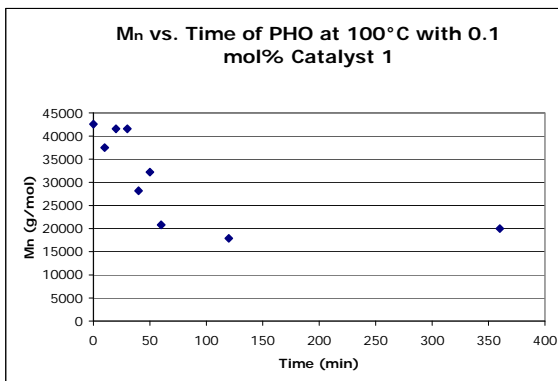
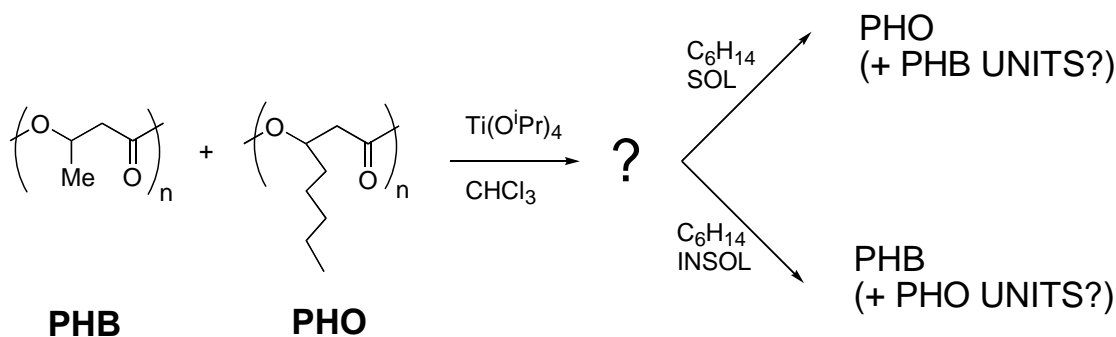


Figure 4.7.6: Graph of PHO molecular weight vs. reaction time with Catalyst 1, measured by GPC.

Due to the insolubility of PHB in most solvents, the first transesterification reactions with PHB and PHO were conducted neat with Catalyst 1, similar to the melt conditions of the micro-extruder experiments previously attempted. At 100°C PHB did not melt, and temperatures around 150°C melted both polymers, however PHB was too viscous to be stirred with a magnetic stir bar. Not surprisingly, GPC data from these reactions, after filtration of unreacted PHB, were comparable to reactions without PHB.

Transesterification reactions performed in solvent produced more consistent results than the neat reactions. Diglyme was used as the solvent, as it is a high-boiling solvent in which PHB is soluble at elevated temperatures. A series of reactions were conducted at 120°C for five hours keeping polymer ratios constant and varying catalyst loadings. Samples prepared at higher catalyst loadings were, interestingly, completely soluble in THF at room temperature, and were therefore characterized by GPC. Unfortunately, the products consisted exclusively of very low molecular weight material around 4000 g/mol. When lower catalyst loadings were used, insoluble material persisted so these samples were characterized by high temperature GPC. The data, however, did not show evidence of incorporation of high molecular weight PHB. Instead, the molecular weight of the samples decreased with higher catalyst loading, mimicking the trend observed in reactions without PHB.

Due to the unreliability of GPC data for samples containing PHB, a new method has been developed to characterize reaction mixtures by ^1H NMR that takes advantage of the solubility difference between PHO and PHB. Both polymers are soluble in hot chloroform, however upon addition of hexane, insoluble polymer precipitates out of solution. After filtration, ^1H NMR confirmed the separation of PHB from PHO. Using this technique, samples can be screened for successful transesterification by separating soluble and insoluble material. Long-chain alkyl groups observed in the precipitated polymer, or likewise PHB units in the soluble phase, would support a successful transesterification reaction. We are now investigating whether, upon solvent fractionation, we can detect PHB units in PHO polymers, or PHO units in PHBs (Scheme 2).



Scheme 2. Transesterification and Solvent Extraction.

While evidence of copolymer production remained elusive, several obstacles in working with PHB were overcome and we developed a relatively simple method for the characterizing of reaction samples.

Y3-6- Task 5: Production of PHA samples

Task 5 Deliverables and Resources			
Year 3	Year 4	Year 5	Resources
Provide 50 –100 kg of different PHA compositions in support of Tasks 2 and 4 (\$500/kg)	Deliver 250 - 500 kg batches of different PHA compositions in support of Tasks 2 and 4 (\$250/kg)	Deliver 1 – 10 tons of PHA polymer (~\$40/kg) to support Task 4. If the pilot facility is increased to 100 tpm then the cost will come down to \$5/kg.	Metabolix, Inc.

Task 5 Decision Points: This task is a supporting task and the only decision points relate to when and which polymer samples to make in support of Tasks 2 and 4. Because of the results achieved in Task 4.5 polymer production requirements were continuously re-evaluated. In addition based on progress in Task 2.2 capital projects were approved and the pilot plant capacity was increased significantly.

Pilot Production Summary of PHB Copolymers during Project

Year	Pilot Production (kg)
2003	1,473
2004	1,290
2005	5,812
2006	38,340
2007	94,450
Total	141,365

Task 6. Life Cycle Analysis

Task 6 Deliverables and Resources			
Year 3	Year 4	Year 5	Resources
-	-	Report of the Lifecycle analysis of the PHA biomass biorefinery	Metabolix

This task was completed for the PHA fermentation facility being constructed at Clinton Iowa and is reported in Y3-6, Task 3. PHA produced from corn wet mill sugar at the Clinton site and based on many of the technology improvements developed in this DOE program has a very favorable energy and greenhouse gas profile.

Y 1-7-Task 7: Management and Reporting.

A 6 month extension for the project was requested in order to accomplish these milestones. The new project end date would then be April 30, 2007, this change is reflected in the chart below.

Updated Gantt/Milestone Charts NOTE: This information is also presented in the new format,

ID Number	Task / Milestone Description	Planned Completion	Actual Completion	Comments
Task 1	PHA Polymer Extraction from plant biomass			
1.1	Evaluation of non-PHA extractables	10/31/04		Task is eliminated
1.2	PHA extraction	10/31/06		Task is eliminated
Task 2	PHA Recovery	4/30/07		
2.1	Optimize solvent systems and improve product quality	10/31/04		Completed.
2.3	Development of Process to produce biorefinery product-latex	4/30/07		Task delayed indefinitely.
Task 3	Integrated Biomass Biorefinery	4/30/07		
3.1	Develop biorefinery flowsheets	10/31/04		Task completed. Base case biorefinery documented based on co-firing with coal.

ID Number	Task / Milestone Description	Planned Completion	Actual Completion	Comments
3.2	Evaluate conversion of biomass residue	4/30/07		Abandoned
3.3	Develop engineering concept for integrated biorefinery			Abandoned in favor of completing LCA of Clinton plant
Task 4	Fundamentals of PHA Polymer properties	4/30/07		Complete
4.1	Predictive Modeling of PHA Copolymer Properties		2/28/07	Complete
4.2	Analysis of PHA rheological properties	12/31/03		Complete
4.3	Crystallization Properties of PHAs (ORNL)	2/28/05		Project completed
4.4	Develop nucleating agents for PHAs	2/28/05	4/30/07	The use of NA1 has been optimized for commercial systems. Complete
4.5	Demonstrate PHA processing for large volume applications	4/30/07		Complete
4.6	Development of PHA blends with other biobased polymers	4/30/07		Complete
4.7	Demonstrate reactive processing of PHAs	3/31/05		Project completed
5.0	Production of PHA Samples	4/30/07		Completed
Task 6	Lifecycle Analysis	4/30/07		
6.1	Initial life cycle analysis assessment for Switchgrass PHA production	6/31/03	6/31/03	Completed in-house analysis as reported.
6.2	Final life cycle analysis assessment	10/31/06	12/2007	Completed for Clinton PHA fermentation plant
Task 7	Management and Reporting	4/30/07		Completed
7.1	Final Report	6/31/07	3/2008	Completed

Patents: Nothing to report this quarter.

Publications/Presentations:

Progress in Quarter 1:

Publications/Presentations:

Metabolix has participated in trade show, presenting a report on progress and characteristics of PHA Natural Plastics. These included the Sustainable Packaging Coalition meeting in Portland, Oregon, the Packaging Strategies meeting in Philadelphia, The Intertech Conference on Commercializing Bioresins in Atlanta, the BIO Pacific Rim Conference on Biodegradables, the Biodegradables conference in Frankfurt, Germany

among others. Tabletop displays were set up at several of these conferences as well. Articles and announcements also appeared in industry publications. The building of a greenhouse and hiring of additional staff not covered by the Biomass Research and Development Initiative are progressing. The original work is continuing through the joint USDA/DOE "Biomass Research and Development Initiative" solicitation (Grant Award No. 68-3A75-3-142, Program Manager Dr. Mark Peters, Tel.# 301-504-2226).

Plans are being made to attend the National Plastics Exposition (NPE) conference in June in Chicago. Metabolix will also be attending the Global Plastics Environmental Conference (GPEC) conference in March. The company was notified that it would receive the environmental award at this conference as well.

Progress in Quarter 2:

Metabolix has participated in trade shows which included the Sustainable Packaging Coalition meeting in Atlanta, Georgia, the GPEC, also in Atlanta where Metabolix received an environmental award, and the BIO Conference in Chicago. Tabletop displays were set up at several of these conferences as well. Articles and announcements also appeared in industry publications.

Metabolix will have a booth in the New Materials Pavilion at the NPE Plastics conference in June in Chicago and a booth at PackExpo in November.

Progress in Quarter 3:

Metabolix has participated in trade shows which included the National Plastics Exposition at the end of June in Chicago and the Sustainable Packaging Coalition meeting in Atlanta. Articles and announcements mentioning Metabolix also appeared in industry publications as well as in Forbes Magazine. The company will be participating in additional conferences in the US, Canada and Europe in the remainder of the year. Metabolix filed an S-1 with the SEC announcing its intention to file for a public offering later this year.

Metabolix will have a booth in the New Materials Pavilion at PackExpo in November in Chicago, will participate in two conferences on biodegradables in Europe, will present at a biodegradables conference in September and will have a tabletop display at the Sustainable Packaging Forum in Minneapolis-St. Paul, MN in September.

Progress in Quarter 4:

Metabolix has participated in trade shows which included the National Plastics Exposition at the end of June in Chicago and the Sustainable Packaging Coalition meeting in Atlanta. Articles and announcements mentioning Metabolix also appeared in industry publications as well as in Forbes Magazine. The company will be participating in additional conferences in the US, Canada and Europe in the remainder of the year. Metabolix filed an S-1 with the SEC announcing its intention to file for a public offering later this year.

Metabolix will have a booth in the New Materials Pavilion at PackExpo in November in Chicago, will participate in two conferences on biodegradables in Europe, will present at a biodegradables conference in September and will have a tabletop display at the Sustainable Packaging Forum in Minneapolis-St. Paul, MN in September.

Metabolix won the ICIS award for its innovative technology in November and the Bioplastics Award, given at the Biodegradables Conference in Frankfurt, Germany in December.