DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

Renewable Enhanced Feedstocks for Advanced Biofuels and Bioproducts (REFABB)

Date: March 24th, 2015

Technology Area Review: Feedstock Supply & Logistics

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Organization: Metabolix, Inc.

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Goals and Technology Fit

Goal Statement

The goal of the REFABB project is to reduce the capital intensity of biomass processing and logistics costs inherent in biomass transport, provide for high value co-products that are scalable as the technology is proven with the demand for biofuel, and achieve superior financial returns for biorefinery operations.

Technology Area Fit

The REFABB project fits the Bioenergy Technology Office's Feedstocks Supply, Logistics and Conversion goals by creating a value-added feedstock which can be used to produce both bioproducts and biofuels.

Relevance and Tangible Outcomes for The United States

The REFABB program will enhance prospects of switchgrass as a commercially viable biomass crop for the production of biofuels, bioproducts, and biopower with additional revenue from multiple product streams. This will contribute to US economic and energy security by reducing foreign energy dependence.

Quad Chart Overview

Timeline

- Project start date: 9/1/2011
- Project end date: (Actual 9/30/2015 *through no-cost extension*; planned 9/30/2014)
- Percent complete: 85%

Budget

	Total Costs FY 10 –FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date
DOE Funded	$1,994,049	$1,558,589	$1,109,018	$1,338,345
Project Cost Share	$1,116,257	$1,119,794	$ 942,020	$ 767,231

Barriers Addressed

- Feedstock Logistics (Ft-L, densified biomass for transporting)
- Feedstock Supply (Ft-c, genetics and development)
- Creates cost effective, integrated biomass conversion technology for biofuels and bioproducts

Partners

- Interactions:
  - Kentucky Bio-processing (2 %)
  - University of Massachusetts (<1%)
- Collaborations:
  - USDA-ARS (6 %)
  - Michigan State University (<1%)

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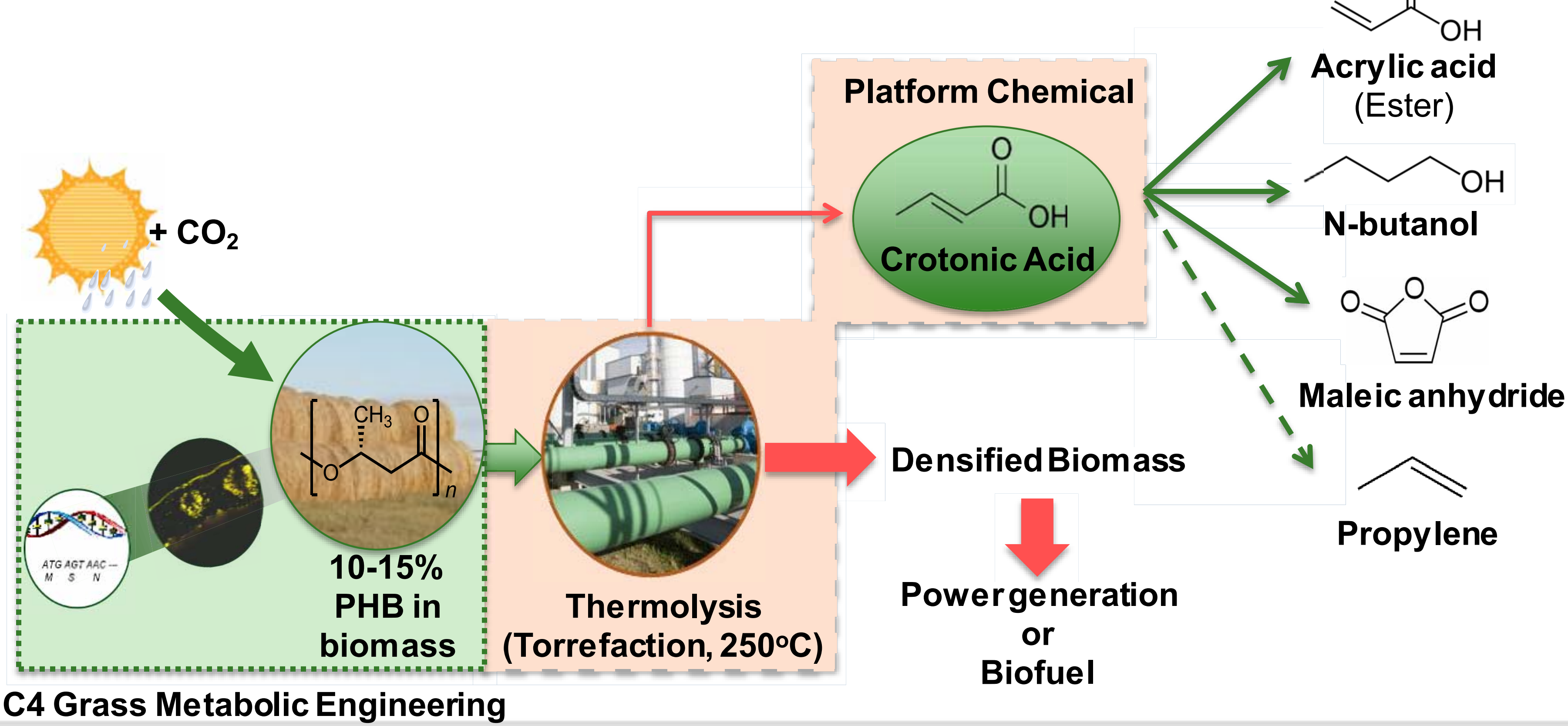
1 - Project Overview

Opportunity: *A disruptive technology to enable economic renewable biomass energy production by co-producing commodity chemicals at low cost with net CO_2 fixation*

Project highlighted in MIT Technology Review, 2013, 116, p. 84.



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2 – Approach (Technical)

- Task 1. Core plant science activities for producing high levels of PHB in switchgrass
  - Develop model system *Setaria* for high throughput transformations *(discontinued, Go/No-Go decision)*
  - Increase carbon flow to polymer production
  - Develop novel gene containment technology in switchgrass
  - Increase transgene expression via alternative plastid transformation strategies
  - Scale up PHB producing switchgrass lines to supply feedstocks for Task 2
- Task 2. Develop and validate key process technologies for an integrated biorefinery
  - Develop and optimize torrefaction process and crotonic acid recovery
  - Develop catalyst technology to convert crotonic acid to existing large volume commodity chemicals
  - Develop process engineering package
- Task 3. Complete a lifecycle analysis of the integrated biorefinery concept based on data from Task 2

2 – Approach (Management)

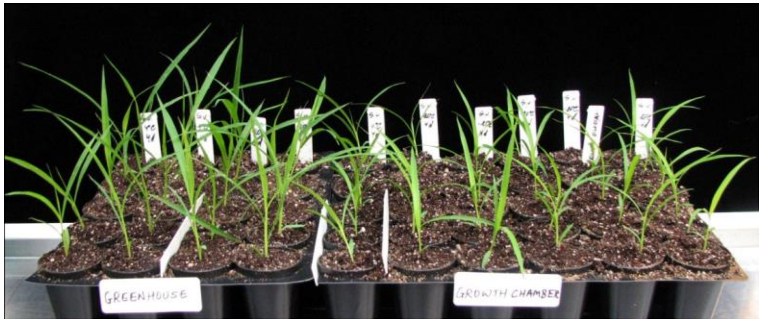
- Critical success factors
  - Achieve 10% PHB in switchgrass
  - Achieve >90% yield of crotonic acid from thermolysis of PHB producing switchgrass
- Top 2-3 potential challenges
  - Timelines for crop engineering remain challenging
  - Must get to greater than 90% recovery of crotonic acid from PHB biomass
- Metabolix is a milestone driven organization that is actively managing this project as described in the Project Management Plans submitted yearly to DOE. This has resulted in decisions to terminate specific sub-projects and re-focus on alternative approaches to achieve better outcomes throughout the course of the program.

3 - Technical Accomplishments, Progress & Results

Task 1. Core plant science activities for producing high levels of PHB in switchgrass

<u>Task 1.1.</u> Development of model system for high throughput transformation

- *Setaria viridis*, a self-pollinating annual diploid C_4 grass (NADP-ME subtype)
  - Plant size: 10–15 cm, life cycle: 6–9 weeks, ~13,000 seeds per plant
  - Broad interest from academia and industry at the time of REFABB program initiation
  - <u>Significance</u>: potential to lower cost of crop development and accelerate discovery of gene systems for enhancing PHB levels as well as the production and conversion of bioenergy crops in general

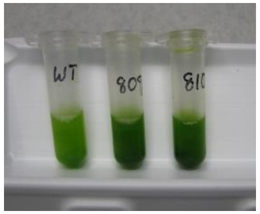


- Program status. Subtask terminated (Go/No Go Decision)
  - ***Timelines and ease of transformation are less efficient than switchgrass.***

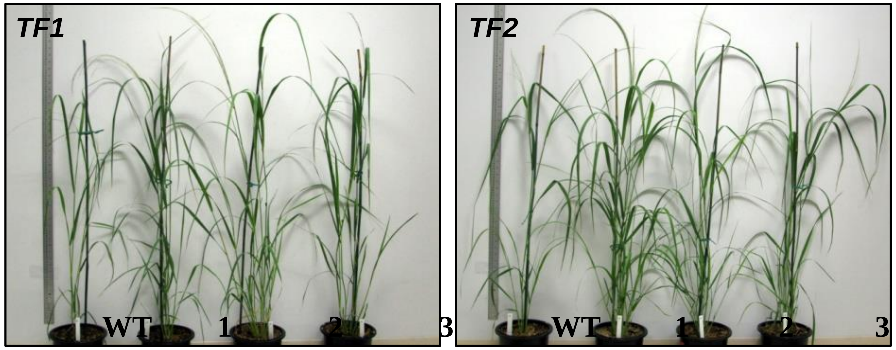
Task 1.2. Increasing carbon flow to PHB production

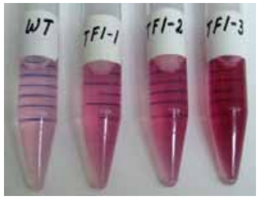
Progress.

- Overexpression of key genes in switchgrass results in improved > 60% increase in biomass production
  - Up to 4 x increase in starch content; 4 x increase in soluble sugars
  - Up to 2.5 x increase in total chlorophyll; 2.5 x increase in carotenoids



Chlorophyll assay





Starch assay

- Co-expression of PHB genes with yield enhancing genes in progress. Early results in young plants, increased polymer production.
- <u>Significance</u>: Potential for lower cost feedstocks, increased PHB levels and higher fermentable sugar content

Task 1.3. and 1.4

- Task 1.3. Gene containment technology
  - Progress: Site specific recombinase-mediated transgene excision, clear demonstration of excision of transgenes in pollen achieved
  - Results published: Somleva et al., *BMC Biotechnol, 2014,* ***14, 79.***
  - Challenges: Achievement of complete excision will require higher levels of expression of the recombinase

- Task 1.4. Increasing transgene expression via plastid transformation
  - Progress: Plastid insertion achieved, currently working to obtain homoplasmy
  - Challenges: Achievement of homoplasmy.

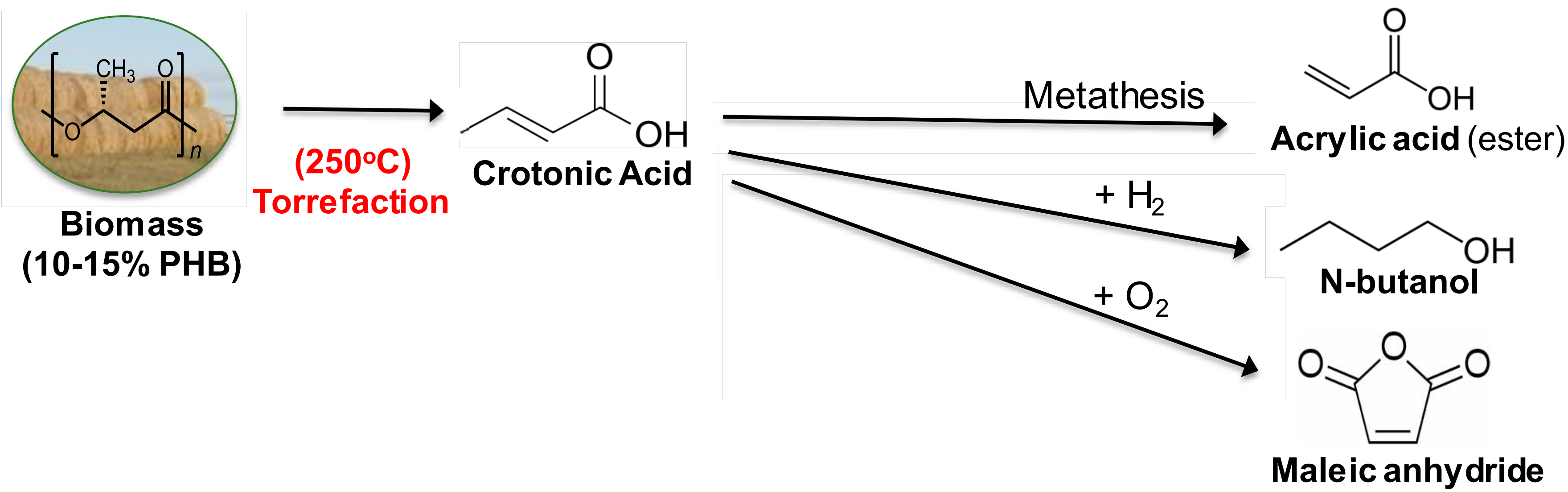
- Significance: Potential breakthrough technologies reducing cost and timelines for production of engineered bioenergy crops and achievement of commercial levels of PHB

Task 1.5. Scale up PHB producing switchgrass lines

- Scale up objective is to provide PHB biomass based on PHB lines previously developed at Metabolix having 4-6% PHB in leaf for thermolysis testing
- Growth and biomass harvest of existing lines
  - >200 plants transported and established at Kentucky Bioprocessing (KBP)
  - Summer conditions in greenhouse were found not to be favorable for switchgrass (too hot, poor survival)
  - Transferred PHB lines to U. Mass to provide better growth conditions
  - Lines growing well at U. Mass
- Challenges: Ongoing challenges with transporting GMO perennial biomass
- Significance: potential to generate thermolysis data directly using PHB switchgrass

Task 2. Develop and validate key process technologies for an integrated biorefinery

Task 2.1. Develop and optimize torrefaction process and crotonic acid recovery - *Eastern Regional Research Center of USDA-ARS* (ERRC)



Task 2.2. Develop catalyst technology to convert crotonic acid

- Metathesis (*Metabolix*) to acrylic
- Hydrogenation (*ERRC*) to butanol
- Oxidation (*ERRC*) to maleic anhydride

Task 2.1. Develop and optimize torrefaction process and crotonic acid recovery

- Performed in cooperation with ARS initially using pure PHB mixed with switchgrass biomass to develop and refine the process
- Initial results: Achieved a conversion yield of up to 45% at lowest operating temperature of the ARS unit

  (Mullen et al., *Mild pyrolysis of P3HB/switchgrass blends for the production of bio-oil enriched with crotonic acid. J Anal Appl Pyrol, 2014,* ***107, 40***)
- Challenges: Need to achieve over 90% recovery of PHB in biomass to crotonic acid
  - Optimization of the ARS unit ongoing (limited by high operating temp)
  - Lab scale optimization ongoing at MBLX (not limited by high operating temp)
- Significance: Potential breakthrough technology for biomass biorefineries enabling a distributed wheel and spokes model with high value chemical co-products and consolidation of densified biomass at centralized site for power or biofuel production

Task 2.2. Convert crotonic acid to large volume chemicals

- Task 2.2.1 Conversion of crotonic acid to acrylic acid via metathesis
  - Clear demonstration of metathesis of ethyl crotonate to ethyl acrylate
  - The ester of crotonic acid is the preferred substrate and ethylene is preferred co-substrate
  - Turnover number >500 achieved *(Highest reported in literature for similar reactions is <10)*

$$TON = {yield\ \%}/{mol\ \%\ catalyst}$$

  - Results published in peer-reviewed journal

    Schweitzer & Snell, *Acrylates via metathesis of crotonates. Org Process Res Dev, in press, in press, DOI: 10.1021/op5003006, 2015.*

- <u>Challenges</u>: More development/improvement of catalysts needed. Heterogeneous catalysts (immobilized) required.
- <u>Significance</u>: Clear demonstration of the feasibility of producing acrylic acid from PHB biomass via the thermolysis product crotonic acid

Task 2.2. Convert crotonic acid to large volume chemicals

- Task 2.2.2 Conversion of crotonic acid to butanol
  - Performed in collaboration with ARS
  - Hydrogenation of the ester of crotonic acid is a better option than using the acid directly
  - During continuous hydrogenation, 90% molar yield of butanol was achieved
  - Results published in peer-reviewed journal

  Schweitzer et al., *Bio-based n-butanol prepared from poly-3-hydroxybutyrate: optimization of the reduction of n-butyl crotonate to n-butanol. Org Process Res Dev, in press, DOI: 10.1021/op500156b., 2015.*

- Significance: Clear demonstration of the feasibility of producing butanol from PHB biomass via the thermolysis product crotonic acid

Task 2.2. Convert crotonic acid to large volume chemicals

- Task 2.2.3. Conversion of crotonic acid to maleic anhydride using partial oxidation
  - Performed in collaboration with ARS
  - Yields of up to 35% achieved in initial work.
  - Process optimization ongoing

Progress on other tasks

- Task 2.3. Complete an engineering package for the integrated biorefinery based on the results form Task 2
  - Data for the engineering package is being collected during the experimental process.
- Task 3. Complete a lifecycle analysis of the integrated biorefinery concept based on data from Task 2
  - This Task will be carried out once we have completed Task 2.3

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4 - Relevance

Relevance to Goals and Objectives of Biomass Program Multi-Year Program Plan

- The REFABB project is developing technology relevant to both Feedstock Supply R&D and Biochemical Conversion R&D outlined in the multi-year program plan
  - Production of densified biomass for use in biofuel or biopower generation
  - Production of commodity chemicals (butanol, acrylic acid, maleic anhydride) from biomass
- The REFABB project will enable PHB switchgrass as a commercially viable biomass crop for the production of biofuels, bioproducts, and biopower with additional revenue from multiple product streams
- Expected outputs of the REFABB project are the deployment of distributed biomass thermolysis plants processing ~500,000 tpy PHB switchgrass harvested within a ~100,000 acre growing area
- Densified biomass from ~ 5 thermolyis units will be consolidated at large scale centralized biorefineries for biofuel production

5 - Future Work
(no cost extension until 9/30/2015)

★ Milestone

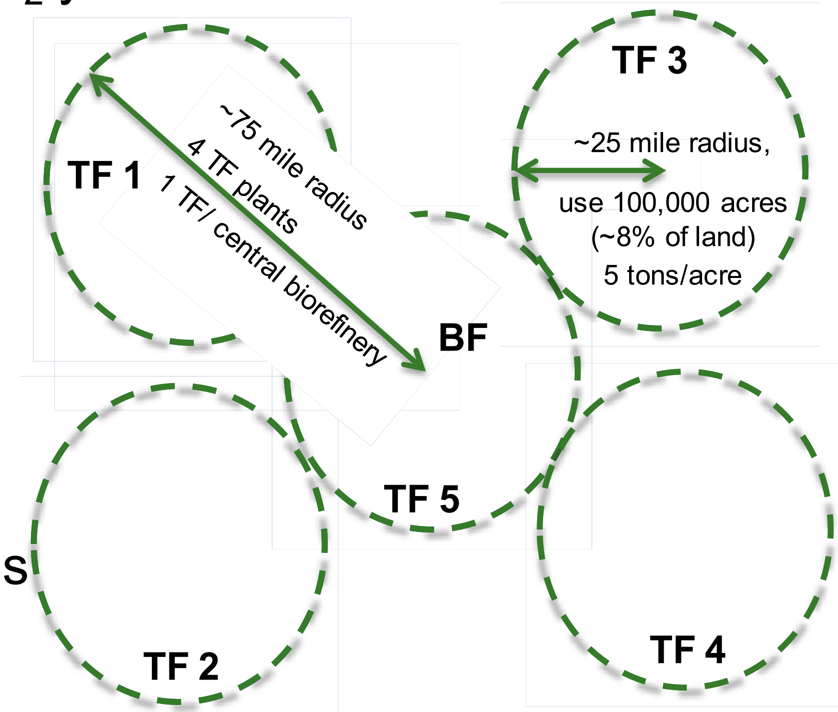
Task 1: Core Plant Science Activities	2015
Achieve 10% PHB in switchgrass	★
Develop plastid transformation for switchgrass	★
Scale up switchgrass growth to provide feedstocks for thermolysis	★

Task 2: Develop & Validate Key Process Technologies for Integrated Biorefinery	2015
Optimize crotonic acid recovery from thermolysis reactions	★
Optimize oxidation catalysts for production of maleic anhydride	★
Develop process engineering package	★
Life cycle analysis	★

Summary
Project Overview

Each Centralized Biorefinery Saves or Eliminates:

Greenhouse gases	~ 3.5 million tons CO_2/yr
Fossil fuel	~ 2.75 million bbl/yr
or Electricity	~ 300 Mw_e
Petrochemicals	340,000 ton/yr



Biobased Chemical	Annual Capacity (mm tpy)	CO_2 emissions (mm tpy)
Butanol	3	~5
MAN*	2	~3
Acrylic acid	4	~8
Propylene	65	~50

* maleic anhydride

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Summary

- **Approach:** Developing a disruptive technology to enable economic renewable biomass energy production by co-producing commodity chemicals at low cost
- **Technical accomplishments:** Significant progress made with crop engineering, thermolysis technology, and development of chemistries for conversion of crotonic acid to commodity chemicals
- **Relevance:** The REFABB project will enhance prospects of switchgrass as a commercially viable biomass crop for the production of biofuels, bioproducts, and biopower with additional revenue from multiple product streams
- **Critical Success factors and challenges:** Yields of PHB achieved in switchgrass, and efficient conversion of PHB to crotonic and crotonic to chemicals are key success factors. These tasks are ongoing.
- **Future Work:** Work will continue to meet the milestones and Go/No Go decisions as outlined in the REFABB Project Management Plan

Progress since 2013 review

Task 1: Core Plant Science Activities

2013 task estimate | Revised projection | ★ Milestone | ▲ Go/No Go

Development of model system	*Task terminated, system less efficient than switchgrass*
Achieve 10% PHB in switchgrass	*Progress ongoing*
Complete gene containment system	*Completed ahead of schedule; paper published*
Develop plastid transformation for switchgrass and apply to PHB production	*Difficulties with 1st round of vectors; Go/NoGo decision to produce new ones, promising results being obtained*
Scale up switchgrass growth to provide feedstocks for thermolysis	*Task continued to produce more biomass*

← 2013 → ← 2014 → ← 2015 →

Progress since 2013 review

Task 2: Develop & Validate Key Process Technologies for Integrated Biorefinery

2013 task estimate | Revised projection | ★ Milestone | ▲ Go/No Go

Optimize crotonic acid recovery from thermolysis reactions	*Optimization ongoing*
Optimize metathesis reactions for yield and catalyst turnover	*Task completed; paper published*
Optimize hydrogenation reactions for production of butanol	*Task completed; paper published*
Test and optimize oxidation catalysts for production of maleic anhydride	*Task started late; Optimization ongoing*
Develop process engineering package	*Data from extended tasks still being collected*
Life cycle analysis	*Data from extended tasks still being collected*

← 2013 → ← 2014 → ← 2015 →

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Additional Slides

Publications, Presentations, Commercialization

- Presentations:
  - Biomass 2011. Washington, DC. July 26th, 2011
  - Biomass Research and Development Technical Advisory Committee. Washington, DC. November 15th, 2012. http://www.biomassboard.gov/pdfs/snell_november2012_tac.pdf.
  - Plant Bio-Industrial Oils Workshop. Saskatoon, Saskatchewan. March 7th, 2013.
  - Plant Biotechnology USA. Raleigh, North Carolina. August 6th-8th, 2013.
  - The BioEnterprises and I-95 Rural Economic Development Summit. Clemson Pee Dee Research & Education Center, Florence, SC. September 19th-20th, 2013.

Publications, Presentations, Commercialization

Publications

- Schweitzer et al., *Bio-based n-butanol prepared from poly-3-hydroxybutyrate: optimization of the reduction of n-butyl crotonate to n-butanol. Org Process Res Dev, in press, DOI: 10.1021/op500156b., 2015.*
- Schweitzer & Snell, *Acrylates via metathesis of crotonates. Org Process Res Dev, in press, DOI: 10.1021/op5003006, 2015.*
- Mullen et al., *Mild pyrolysis of P3HB/switchgrass blends for the production of bio-oil enriched with crotonic acid. J Anal Appl Pyrol, 2014,* ***107, 40***
- Somleva et al., *Transgene autoexcision in switchgrass pollen mediated by the Bxb1 recombinase. BMC Biotechnol, 2014,* ***14, 79.***
- Somleva et al., *PHA bioplastics, biochemicals, and energy from crops. Plant Biotechnol J, 2013,* ***11, 233.***

Press

- Project highlighted in MIT Technology Review *(Talbot, D., Plastic from Grass, Engineers seek a cheaper biodegradable polymer. MIT Technology Review,* 2013, ***116, 84***.)

Publications, Presentations, Commercialization

- Commercialization
  - Patent application submitted on yield enhancing genes (Ambavaram, M. and M.N. Somleva, *Transcriptional regulation for improved plant productivity. Patent Application WO/2014/100289)*
  - Work to increase the levels of PHB in switchgrass using these yield enhancing genes alone, and in combination with additional PHA pathway genes whose expression alone is detrimental to plant health, are ongoing and look very promising.
  - Metabolix has initiated discussions with the major Ag biotech companies to determine their interest in novel yield enhancing genes for major food crops such as corn. Assuming the yield results achieved in switchgrass can be replicated even in part in these crops, then this will have a positive impact on the food vs fuel debate in addition to opening up commercial licensing discussions.

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