2013 DOE Bioenergy Technologies Office (BETO) Project Peer Review

Renewable Enhanced Feedstocks for Advanced Biofuels and Bioproducts (REFABB)

Date: May 23rd 2013

Technology Area Review: Feedstock Supply & Logistics

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Organization: Metabolix

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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.

Quad Chart Overview

Timeline

- Project start date: 9/1/2011
- Project end date: 9/30/2014
- Percent complete: 50%

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

Budget

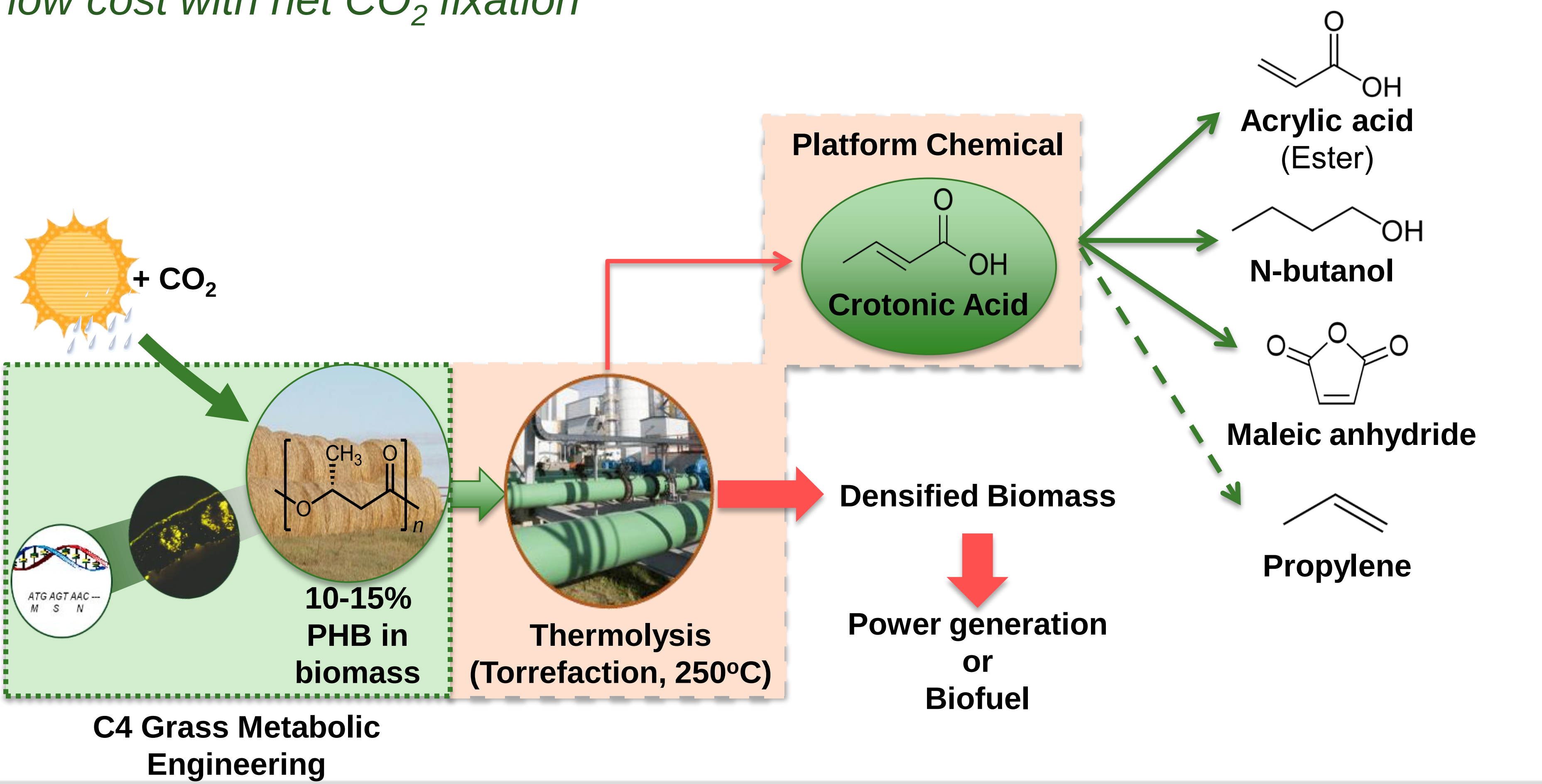
- Funding for FY11:
  (DOE $1,406,040/Cost share $937,360)
- Funding for FY12:
  (DOE $2,300,000/Cost share $1,533,333)
- Funding for FY13:
  (DOE $2,293,960/Cost share $1,529,307)
- Years of funding: 1.5
  Avg. annual funding: $2mm/yr

Partners

- Interactions: Kentucky Bio-processing
- Collaborations: USDA-ARS
- Project Management: Metabolix

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*



1 - Approach

- Task 1. Core plant science activities for producing high levels of PHB in switchgrass
  - Develop model system *Setaria* for high throughput transformations
  - 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 - 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

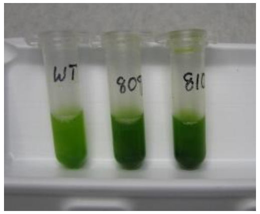
- *Setaria viridis*, a self-pollinating annual diploid C_4 grass (NADP-ME subtype)
  - Plant size: 10–15 cm, life cycle: 6–9 weeks, ~13,000 seeds per plant
  - Intensifying interest from academia and industry
- Program status
  - Growth, development, and *in vitro* response of 16 accessions characterized
    - Two different transformation methods evaluated
    - Transgene transmission to T_3 generation evaluated

Effect of photoperiod on *Setaria* growth

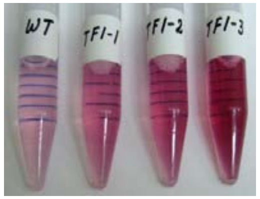
- Significance: potential to lower cost of crop development and accelerate discovery of gene systems for enhancing PHB levels as well as the production and conversion of bioenergy crops in general

Task 1.2. Increasing carbon flow to PHB production

- Overexpression of key genes in switchgrass results in improved biomass production
  - > 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

- Challenges: main concern is to develop a complete picture of these results over the full lifecycle of the transgenic lines
- Significance: Potential for lower cost feedstocks, increased PHB levels and higher fermentable sugar content

Task 1.3. and 1.4

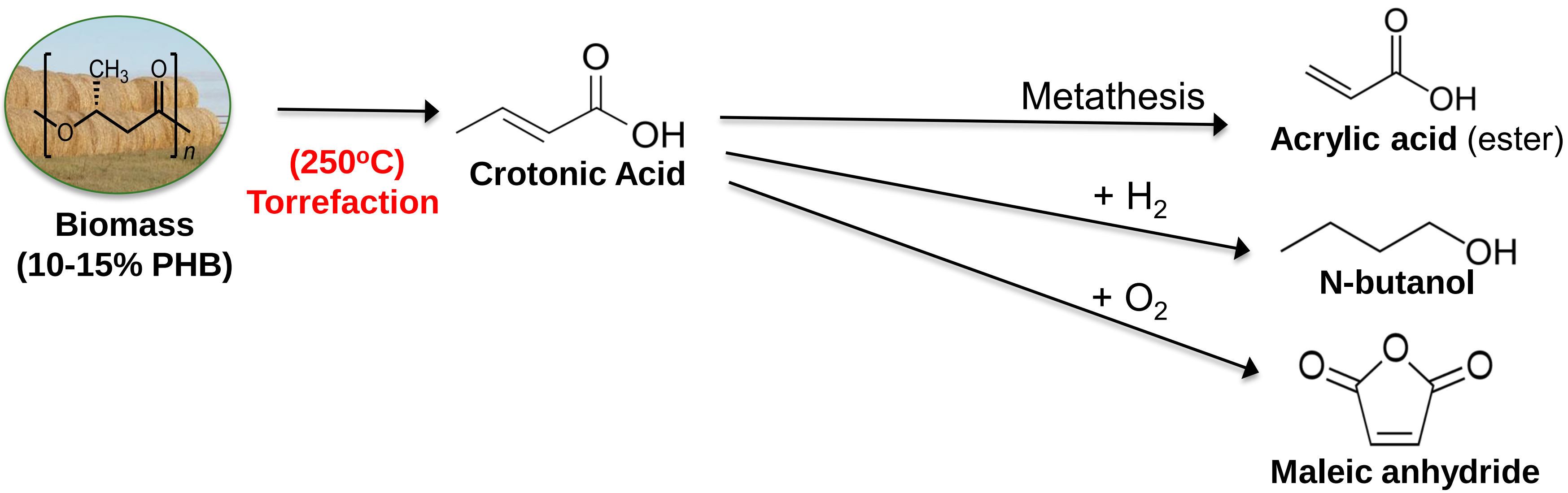
- Task 1.3 Gene containment technology
  - Approach 1: site specific recombinase-mediated transgene excision
    - Clear demonstration of excision of transgenes in pollen achieved
  - Approach 2: Manipulation of ROS production for cell/tissue ablation
    - Lines with reduced pollen viability identified
- Task 1.4 Increasing transgene expression via plastid transformation
  - A platform vector for switchgrass plastid transformation was developed and cloning of the PHB operon in progress
  - Highly embryogenic callus cultures were generated and a novel selection strategy designed
- Challenges: Better promoters for gene containment. Plastid transformation of monocots
- 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)
  - Growth conditions adjusted, best performing lines bulked up, biomass harvested
  - PHB production is at expected levels even with growth condition issues
- Challenges
  - Problems with plant survival due to unfavorable growth conditions at KBP
  - Ongoing challenges with transporting GMO perennial biomass
  - Transferred PHB lines to U. Mass to provide better growth conditions
- 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 microbial PHB biomass to develop and refine the process
- Initial results: Achieved a conversion yield of ~ 56% at lowest operating temperature of the unit
- 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)
  - Add additional activities based on recent successful large scale PHA thermolysis activities at Metabolix
- 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 >250 achieved *(Highest reported in literature for similar reactions is <10)*

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

- Task 2.2.2 Conversion of crotonic acid to butanol
  - Performed in cooperation 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
- Significance: Clear demonstration of the feasibility of producing acrylic acid and butanol from PHB biomass via the thermolysis product crotonic acid

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Progress on other tasks

- Task 2.2. Conversion of crotonic acid to maleic anhydride using partial oxidation
  - This task is just getting underway
- 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

3 - 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
- Densified biomass from ~ 5 thermolyis units will be consolidated at large scale centralized biorefineries for biofuel production

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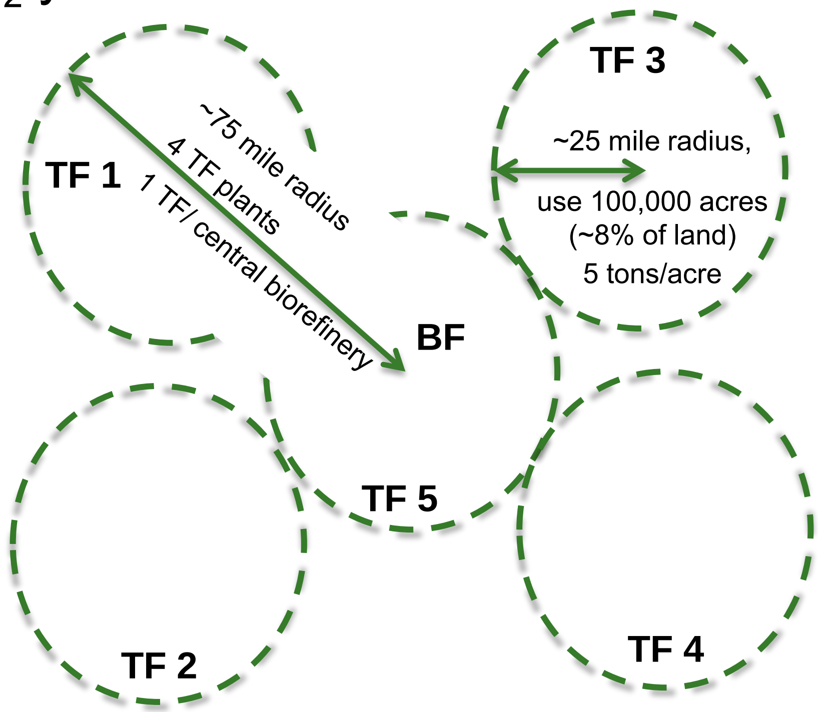
Project Overview

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

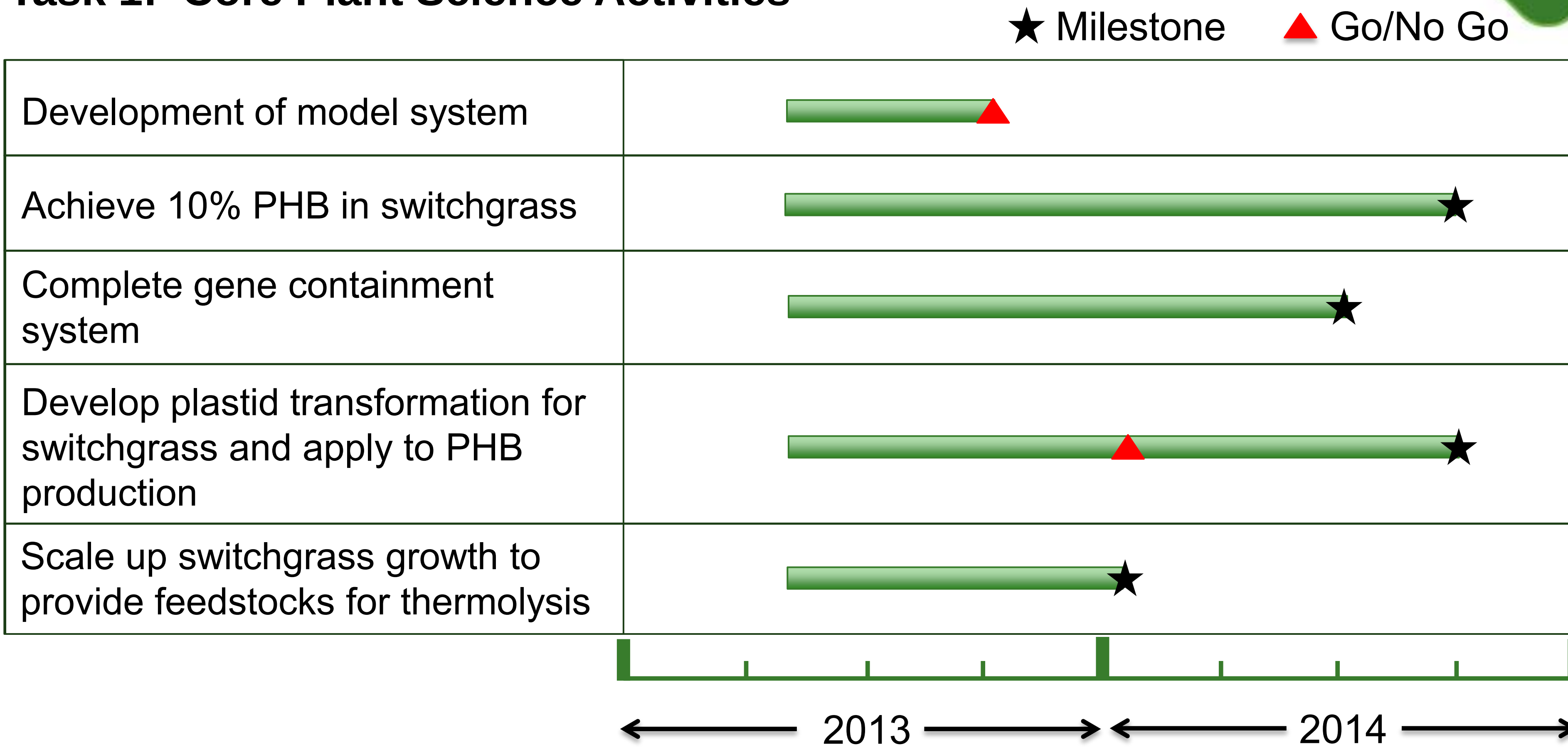
* maleic anhydride

4. Critical Success Factors

- Critical success factors
  - Achieve 10% PHB in switchgrass
  - Achieve >90% yield of crotonic acid from thermolysis of PHB producing switchgrass
  - Complete the activity on chemical conversion of crotonic acid
- Top 2-3 potential challenges
  - Timelines for crop engineering remain challenging
  - Must get to greater than 90% recovery of crotonic acid from PHB biomass
- Impact on commercial viability of biomass and/or biofuels
  - Success with REFABB program will provide technology for the economic production of a platform chemical, crotonic acid, from crops and its conversion to commodity chemicals (butanol, acrylic acid, maleic anhydride)
  - Low CapEx of thermolysis facilities enables direct grower participation in plants and value creation thereby enabling the industry
  - Densified biomass produced upon thermolysis is readily transportable and stored solving critical logistical issues with biomass
  - Production of value-added chemical co-products and densified biomass increases the commercial viability of biomass and/or biofuels

5 - Future Work

Task 1: Core Plant Science Activities



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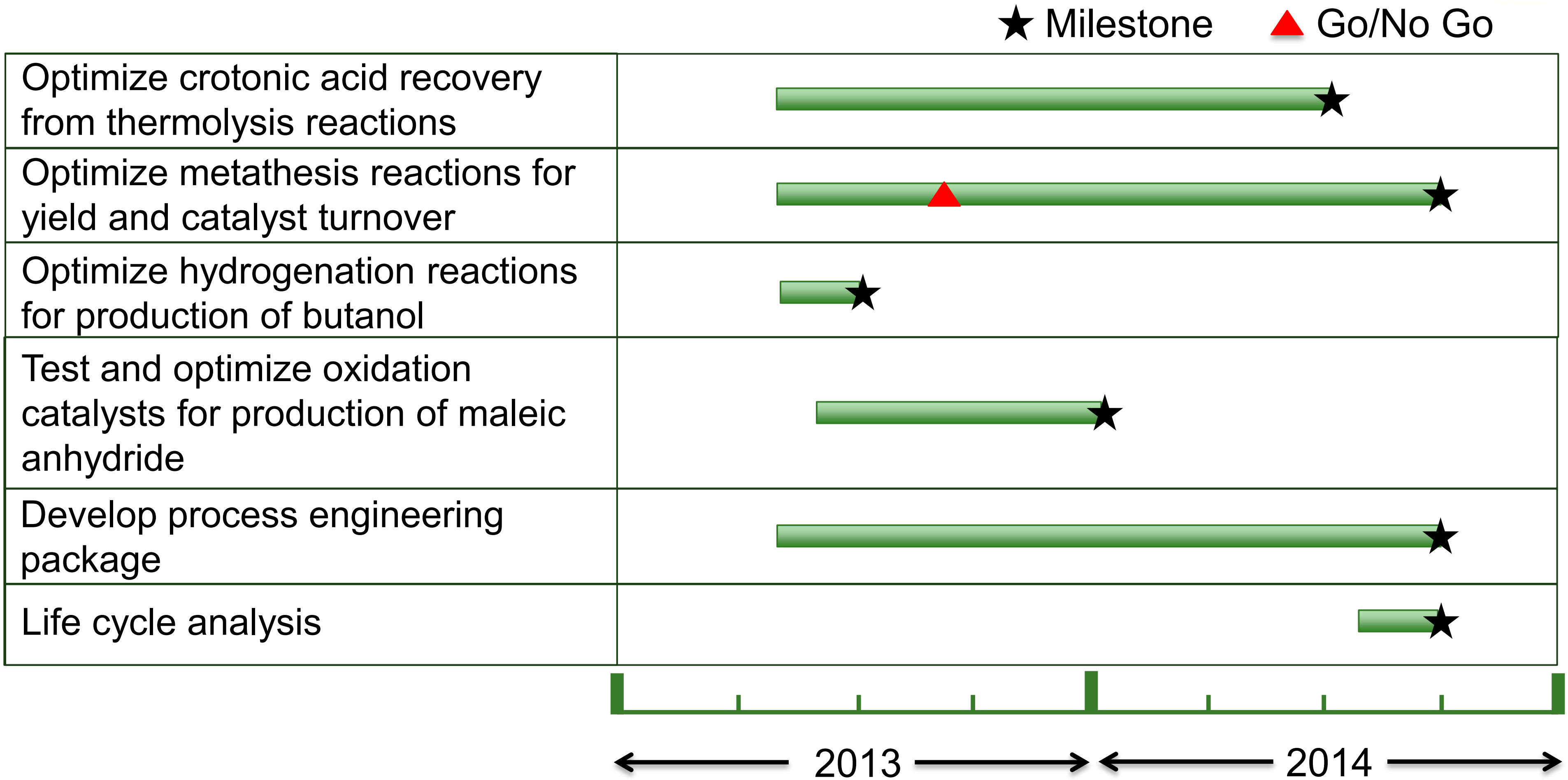
5 - Future Work (cont.)

Task 2: Develop and Validate Key Process Technologies for an Integrated Biorefinery



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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
- **Future Work:** Work will continue to meet the milestones and Go/No Go decisions as outlined in the REFABB Project Management Plan
- **Technology transfer:** Metabolix agressively protects its intellectual property and intends to pursue commercialization of this technology

Additional Slides

Publications, Presentations, Commercialization

- Presentations:
  - 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.
- Commercialization efforts
  - Although it is premature to initiate commercialzation efforts on the REFABB technology, Metabolix has begun to describe this technology to prospective industrial and financial partners. In general the potential for a disruptive breakthrough in biomass is very attractive, the main concern remains risks associated with the timelines for crop science and potential regulatory risk associated with GMO crop. Ongoing technical efforts in the current program can significantly reduce both risks