# DOE Bioenergy Technologies Office (BETO) 2017 Project Peer Review

## US-India Consortium For Development of Sustainable Advanced Lignocellulosic Biofuels Systems

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Feedstock Supply and Logistics Technology Area


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Partner institutions: University of Missouri, Montclair State University, Virginia Tech, Texas A&M University

# Goal Statement

1. Develop sustainable production systems for switchgrass and biomass sorghums on low-productivity lands.
   - Flood plains of major rivers -- Missouri
   - Soil with low fertility and/or low water retention capacity – Florida and Missouri
   - Areas prone to drought where irrigation is not practical – Florida and Missouri
2. Develop microbial biocatalysts that can convert fermentable sugars derived from cellulosic feedstocks to butyrate at high titer. Butyrate can be chemically converted to butanol (a drop-in biofuel).
3. Develop process flowsheet diagrams for (pre-)commercial scale production of biofuels and chemicals to aid techno-economic analyses for different feedstocks and fuels.
4. Demonstrate recovery and utilization of biorefinery 'waste' residues.
   - Stillage
   - Lignin
5. Life cycle, economic and supply chain analysis of feedstocks and biofuels.

*The use of underutilized land in the US for the production of advanced biofuels offers the potential for energy production with minimal impacts on food/feed production, and minimal environmental impacts on soil and water.*

*Through interactions with colleagues in India, this project also addresses the R&D priority area of the US – India Joint Clean Energy Research and Development Center.*

# Quad Chart Overview

## Timeline

- Project start date: 9/18/2012
- Project end date: >9/17/2017
- Percent complete: 60% by scope

## Budget

| | Total Costs FY 12 – FY 14 | FY 15 Costs | FY 16 Costs | Total Planned Funding (FY 17-Project End Date) |
|---|---|---|---|---|
| DOE Funded | $1,412,174 | $931,742 | $1,206,019 | $2,663,025 |
| Project Cost Share (Comp.)* | $1,868,285 | $833,452 | $1,049,414 | $2,462,704 |

Note: Minimum cost share required for this project is 50% of total project costs, on an invoice-by-invoice basis

## Barriers

- **Ft-B Sustainable Production**: Existing data on the productivity and environmental effects of biomass feedstock production systems and residue collection are not adequate to support lifecycle analysis of biofuels.
- **Bt-J Catalyst Development**: There is a need for biological or chemical catalysts that can convert the sugar mixture and inhibitors in the hydrolysate broth derived from biomass pretreatment and hydrolysis for the production of advanced biofuels, bioproducts, and fuel intermediates. Improvement in the robustness of catalysts, e.g. bacterial, fungal, algal, or chemical, and their ability to perform in hydrolysate broths can lead to significantly lower capital costs.
- **Bt-K Biological Process Integration**: Process integration remains a key technical barrier hindering development and deployment of biochemical conversion technologies.

## Partners

- University of Florida, University of Missouri, Virginia Tech, Montclair State University, Texas A&M University, Green technologies, Tiger Energy Solutions (and institutions and industry in India)

# 1 - Project Overview

## Overall Goal

- To address the second-generation biofuel R&D priority area of the US-India Joint Clean Energy Research and Development Center.
    - Sponsored by DOE Office of International Affairs.
    - Partnerships related to alternative energy between US and Indian research teams with mutual interests.

## Technical Goals of the US team

To develop and demonstrate commercially scalable, advanced ligno-cellulosic biofuels through:

1. Sustainable feedstock cultivation on low-productivity lands.

2. Use of novel microbial biocatalysts for advanced biofuel production.

3. Environmentally and economically sustainable practices through optimized feedstock production and conversion processes and maximum utilization of biorefinery waste stream.

# 2.1 – Approach (Technical)

## Feedstock Development

University of Florida [UF],
University of Missouri [UM]

- Crop production on low-productivity lands will require suitably adapted feedstocks.
- Sorghum is a robust crop that can withstand a variety of abiotic stresses, including heat, drought, and flooding.
- Sorghum breeding traditionally has focused on crop production on well-managed lands.
- This project aims to identify sorghum genes that confer flooding tolerance.
  - [UM/UF] Screening of sorghum lines in a field-based flood laboratory (‘channels’).
  - [UF] Lines with contrasting flood tolerance phenotypes used to develop genetic mapping populations.
  - [UM] In parallel, transcriptomics analyses identified genes differentially expressed during flooding.
- [UF] Sorghum breeding efforts are aimed at development of high-biomass sorghums that perform well in hot conditions with limited irrigation.
  - Similarities in climate between Florida and India
- [UM] Switchgrass is a native prairie grass with a limited history of breeding.
- [UM] Screening of 15 different accessions at UM has indicated variation in tolerance to flooding. Switchgrass cultivar ‘Alamo’ performed best.
- [UM] Transcriptomics studies like those for sorghum will be considered pending funding. Switchgrass genome sequencing efforts are essentially complete as part of other initiatives.

Genetic variation in sorghum can be exploited to enhance flooding tolerance

# 2.1 – Approach (Technical)

## Feedstock Production

University of Missouri [UM], University of Florida [UF]

- Crop production on low-productivity lands will require fine-tuned management that balances crop needs, production costs, and environmental stewardship.
- Identify soil and environmental criteria to ensure a commercially successful advanced feedstock production system.
  - [UM] Establish switchgrass research plots on several private farm sites
    - 'Real-life' data from low-productivity land (six sites of 6 acres each), instead of optimally managed research sites on prime production land.
  - [UM] Subject the plots to different fertilizer treatments and mowing regimens to determine effects on biomass yield, soil quality, and ground water quality.
  - [UM] Examine N-use efficiency in switchgrass using $^{15}N$ labeled fertilizer to determine how much of the fertilizer is taken up by the plant vs. stays in the soil vs. seeps into the ground water.
  - [UF] Grow different biomass sorghums on irrigated vs. rain-fed land and determine biomass yield and yield deficit due to limited water supply. Identify high-yielding genotypes and genotypes with a limited yield deficit



# 2.1 – Approach (Management)

- Sorghum genetics experiments are coordinated between UF and UM
  - Initial flooding tolerance screening of sorghum germplasm at UM
  - Validation experiments at UF and UM
  - Transcriptomics at UM
  - Mapping population and screening tools developed at UF
  - Phenotyping flooding response of mapping population at UM
  - Sorghum breeding at UF

- Switchgrass genetics experiments are conducted in Missouri
  - UM manages university research sites and greenhouses
  - Flooding tolerance evaluation of switchgrass
  - Long-term evaluations of switchgrass and multiple other crops and management strategies at dedicated site at UM
  - Contract with commercial switchgrass farmers at six sites in Missouri

- Communications between the UF and UM teams occur regularly via phone, email and in-person visits
- Communication with the farmers via in-person visits
- Several joint publications in progress

# 2.1 – Approach (Management)

- Contracted farmers must be willing to accommodate research plots on their land to enable ‘real-life’ evaluations – *special individuals*.
- Accept and manage inherent risk of crop losses due to *expected* adverse conditions (flood, drought, poor soils).
- Recognize the potential for poor crop performance on low-productivity land, or attempt to mitigate the risk by applying high levels of fertilizer to ensure good performance. The latter approach reduces the economic viability and GHG benefits of the crop, and likely affects groundwater.
- *Through the use of multiple sites representing different environmental conditions, the risk of losing all plantings is small.*
- *The availability of multiple sites also enables the establishment of crop management protocols for different soil conditions.*
- *Detailed management studies (representing many management strategies) have provided the necessary information to recommend best management practices for a variety of site types.*

# 2.2 – Approach (Technical)

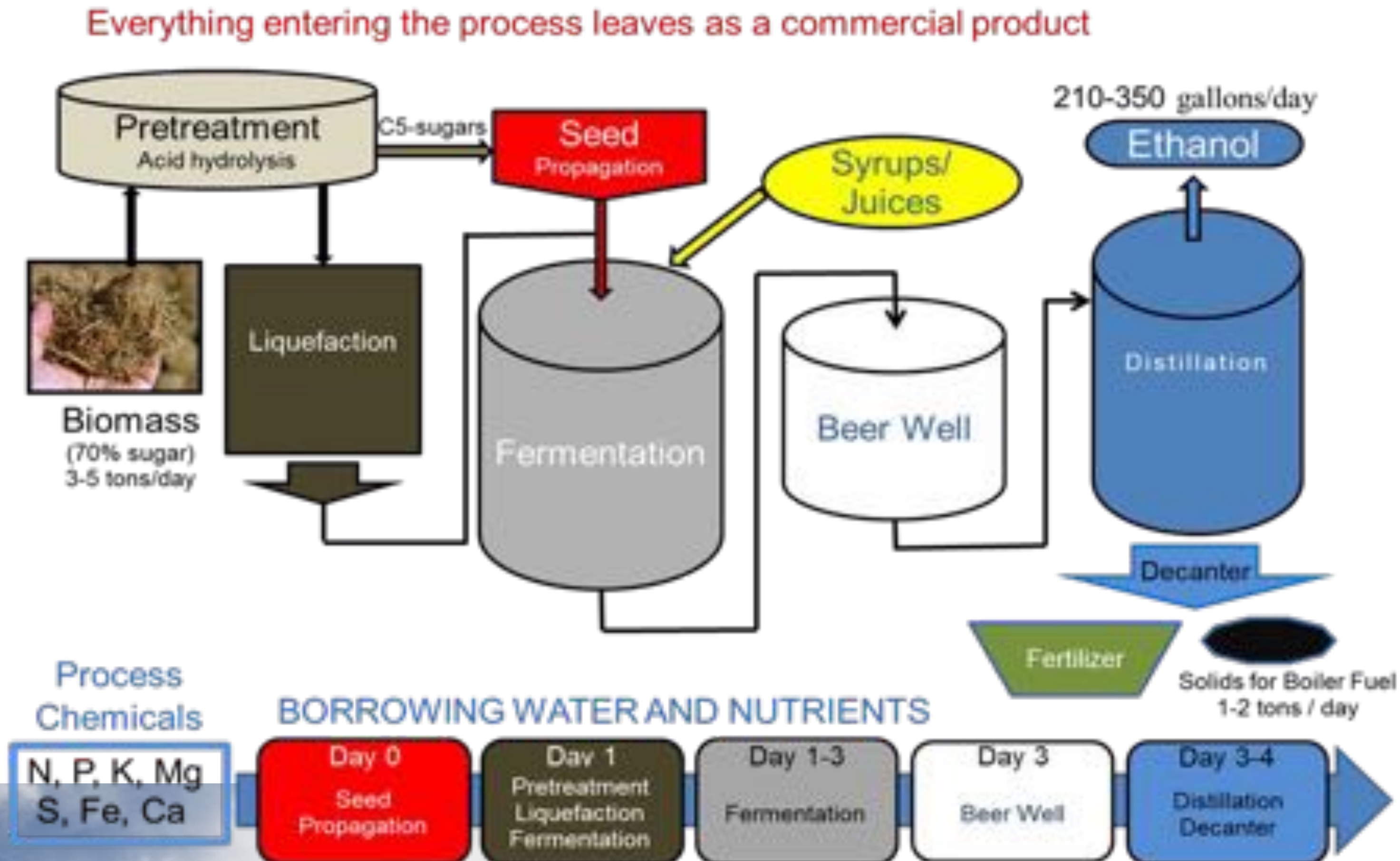
## Conversion Technologies I

University of Florida [UF]

- Butanol is a more desirable fuel than ethanol, but butanol production via ABE fermentation is inefficient due to its toxicity to the biocatalyst organism.
- Butyrate is tolerated at higher titers than butanol, and can be easily converted via chemical means to butanol with high efficiency using a strong reducing agent.
- [UF] Microbial biocatalysts able to produce butyrate as their sole fermentation product at high titer and yield are under development.

- No biorefinery production data exist for the conversion of switchgrass to butanol.
- [UF] Pretreatment and saccharification of sorghum bagasse on a pilot scale (10 kg) followed by fermentation to ethanol (100 L).
- [UF] Large-scale (6 tons) pretreatment and saccharification of sorghum bagasse followed by fermentation to ethanol (12,000 L fermentor).
- [UF] Pretreatment and saccharification of switchgrass at pilot scale (10 kg) followed by fermentation to ethanol (100 L).
- [UF] Pretreatment and saccharification of sorghum bagasse on a pilot scale (10 kg) followed by fermentation to butyrate (100 L).
- [UF] Pretreatment and saccharification of switchgrass at pilot scale (10 kg) followed by fermentation to butyrate (100 L).
- [UF] Development of process flowsheet diagrams to model large-scale conversion of switchgrass and sorghum bagasse to butyrate.

# The UF Stan Mayfield biorefinery pilot facility




# 2 – Approach (Management)

- Optimization of large-scale (12,000 L) conversion of switchgrass to butyrate is expensive due to the high cost of shipping multiples of 6 tons of switchgrass to Florida.
- The UF pilot biorefinery was closed in summer 2016 due to lack of operating funds and commitment from companies.
  - Consequence of the repeal of State RFS ( in Fall 2013) and low price of oil and natural gas that together undermined additional state and private investments
- *We have purchased 6 tons of switchgrass from a farm in Virginia*
- *The use of smaller-scale (100 L) switchgrass-to-butyrate conversions, coupled with detailed techno-economic models from large-scale sorghum bagasse-to-ethanol conversion, and pilot-scale experiments with switchgrass-to-ethanol will be used to model large-scale processing of switchgrass and sorghum to butyrate (and then butanol).*

# 2.2 - Approach

## Conversion Technologies II

University of Florida [UF]

- Biorefineries generate waste that is difficult to dispose of, but that contains potentially valuable chemicals.
- The use of the biorefinery waste stream will improve the environmental and economic balance sheets for advanced lignocellulosic biofuel production.
- [UF] Anaerobic digestion of organic components in stillage as a way to generate methane gas (co-product).
- [UF] Develop a pre-commercial-scale system for recovery of plant nutrients from stillage.
  - Focus on phosphate, an essential plant nutrient
  - Originates from the plant biomass and the phosphoric acid pretreatment.
- [UF] Studies on land application of these residuals as a fertilizer.
- [UF] Development of lignin-based nano-composites with properties that enable production of high value co-products that can help to offset biorefinery operational costs.
  - Improved mechanical/thermal/UV-tolerance properties

# 2.2 – Approach (Management)

- This part of the project is conducted at UF and is carried out in a number of autonomous components that have minimal interdependence.

# 2.3 – Approach (Technical)

## Sustainability, Marketing and Policy Analyses

▫ Virginia Tech [VT], Montclair State [MSU], University Texas A&M University [TAMU]

- Integration of feedstock production and bioconversion in techno-economic and life cycle assessment (LCA) models is critical for the development of a sustainable biofuel production industry.
- Being able to demonstrate that a sustainable biofuel production system is feasible will help convince industry and financial institutions to invest.
- [MSU] Developing standards and protocols.
    - Aggregated index approach (literature review, stakeholder inputs and surveys, assess feasibility of standards and protocols).
- [VT, MSU, TAMU] Energy, emissions, and economic analyses.
    - LCAs for different production-conversion-distribution pathways.
        - SimaPro software and Eco-invent database.
    - Other environmental impacts (e.g. land productivity) through literature review
    - Feasibility analyses (techno-economic and capital budgeting model).
    - Region wide socio-economic and distributional impact analyses (Input/Output Analysis, Social Accounting Matrix and CGE approach).
- [VT] Supply chain management analyses.
    - Biomass supply chain optimization model, assess drivers and barriers through survey of supply chain actors.

# 2.3 – Approach (Management)

- This part of the project has so far involved three institutions (VT, TAMU, MSU), but the individual investigators know each other very well and have a history of working together.
- The other project components (feedstocks, conversion) have now generated the data that can be fed in the models
- The energy landscape is constantly subject to change, with the current low prices of natural gas and petroleum undermining the success of existing biorefineries, and delaying the construction of new ones.
- Incentives, mandates, and policies aimed at rural economic development can support bio-economy initiatives

- *Initial efforts were based on literature reviews and model development, which are relatively immune to economic challenges faced as a result of the current low energy prices.*
- *Models can be adjusted for the production of bio-chemicals, rather than fuels. The prospects for chemicals are better than for biofuels.*

# 3 – Technical Accomplishments/ Progress/Results I

- A sorghum mapping population has been developed that will be used to study the genetic basis of flooding tolerance.
- A newly developed rapid screening protocol for the quantitative assessment of flooding damage will be used to phenotype the mapping population
- Transcriptome profiling of sorghum roots of contrasting genotypes under flooding vs. well-watered conditions has shown that large-scale metabolic changes occur when roots are flooded, that these responses change over time, and that the responses differ in root tips vs. root base, and that tolerant genotypes respond differently
- A separate mapping population has been developed to identify resistance genes against the sugarcane aphid, a newly emerged (2014) and devastating pest of sorghum.
- Six new disease-resistant, drought-tolerant, high-biomass sorghum cultivars with enhanced biomass processing characteristics have been developed.
- Ten promising tall, low-input hybrid energy sorghums have been developed; the parents of these hybrids are short and can be harvested with a combine
- Screening of 15 switchgrass accessions resulted in the identification of ‘Alamo’ as the most tolerant to flooding (F. Fritschi, UM).

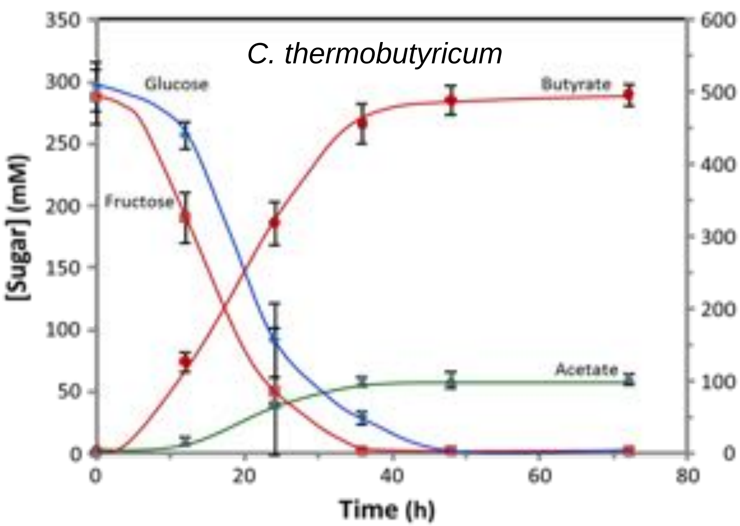
# 3 – Technical Accomplishments/ Progress/Results II

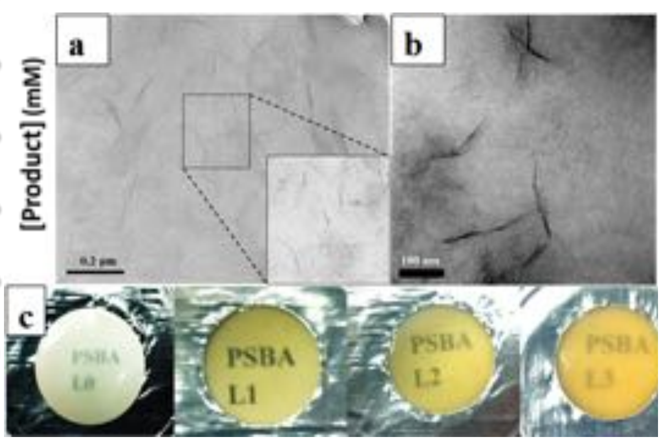
- Six commercial switchgrass fields in different sites in Missouri have been subjected to different management strategies (mowing and fertilizer regimens) over multiple seasons to determine yield trends and environmental impacts
- This study showed that a single late harvest after a killing frost consistently resulted in better quality biomass regardless of N management and site conditions. This includes higher energy content and higher ethanol yield parameters.
- Annual N inputs equal or greater than 67 kg $ha^{-1}$ improved ethanol production per unit area of land.
- Life cycle and  economic analyses (WP3) will need to determine if this practice is environmentally and economically sustainable

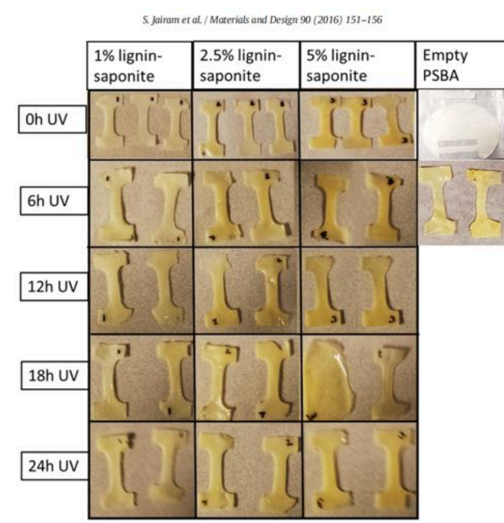



# 3 – Technical Accomplishments/ Progress/Results III

- [UF] Optimized culturing conditions of *Clostridium thermobutyricum* have improved butyrate titers three-fold (from 15 g/L at project inception to 45 g/L).
- [UF] A recombinant *E. coli* is able to produce butyrate at 22 g/L. This is the highest reported yield in a recombinant organism. Further improvements continue to be made.
- [UF] Synthesis protocols were developed for the production of polymeric films containing lignin as co-polymer. The presence of lignin in polystyrene-co-butylacrylate polymers substantially improved the mechanical properties of the polymer: Young's modulus doubled and tensile strength increased 500-fold. This co-polymer was highly resistant to UV-exposure, including levels experienced in outer space.
- An efficient, computer-controlled anaerobic digester system has been designed that generates clean methane gas from the biorefinery stillage. The phosphate and ammonia in the sludge can be recovered as struvite, which can serve as a fertilizer.

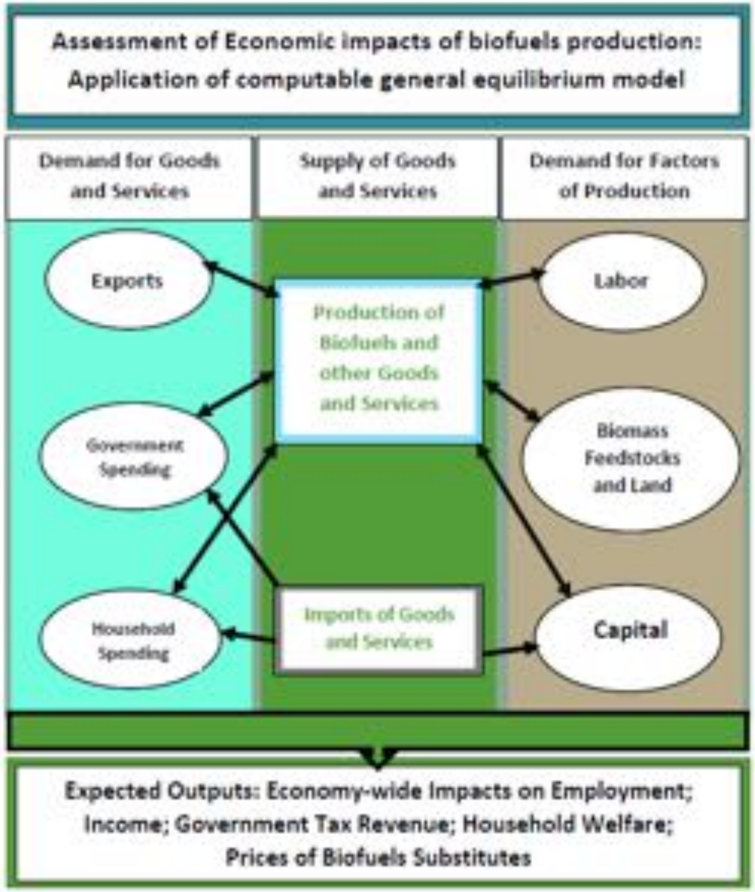


# 3 – Technical Accomplishments/ Progress/Results IV

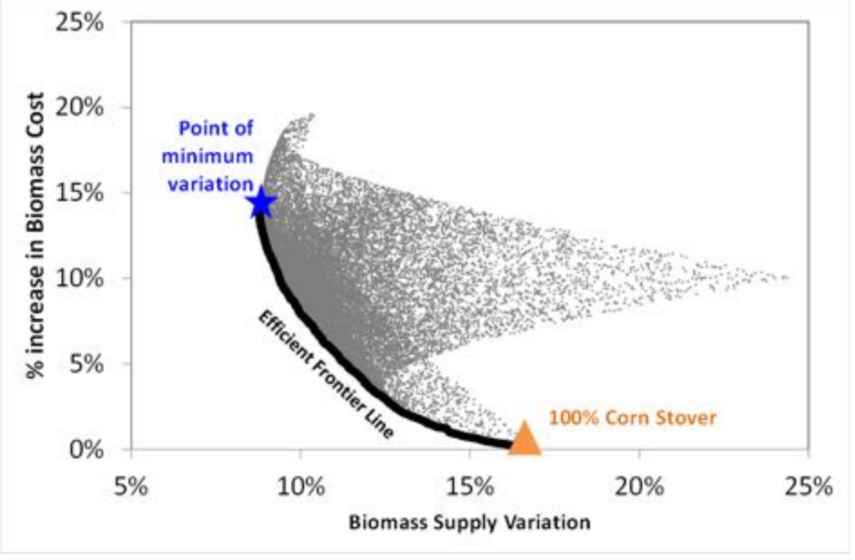
- Development of a Computable General Equilibrium (CGE) model to assess regional economic impacts of bioenergy production.
  - a class of economic impact models that use economic data to estimate how an economy might react to changes in policies, markets, technologies, or other similar factors.




General structure of the CGE model for switchgrass as a bioenergy crop

# 3 – Technical Accomplishments/ Progress/Results V

- The year-to-year supply of corn stover varies in the US from 20-30% , undermining the potential of using stover for biofuel production
- Mixing switchgrass with corn stover in the feedstock portfolio can lessen the overall feedstock supply variation, reducing cost and increasing efficiency.
- The 'efficient frontier' was developed to determine the optimal feedstock portfolio




The Efficient Frontier (black curve) indicates Pareto efficient, lowest biomass cost for a given supply risk (variation). The triangle represents 100% corn stover. The star represents the point of the minimum supply variation through diversification of corn stover with switchgrass and wheat straw (approx. at the ratio 2:1:1). The grey area is simulation output (Golecha and Gan, 2016)

# 4 – Relevance

- **Ft-B Sustainable Production**: Feedstock improvement and development of best management practices will assist in sustainable production of bioenergy crops.
  - Use of low-productivity lands for crop production will not compete with food/feed production.
  - Lower land costs will keep crop production costs low.
  - Management practices on low-productivity lands may add to production costs.

- **Bt-J Catalyst Development**: Novel microbial biocatalysts able to produce high yields of butyrate and improved, recyclable cellulolytic enzyme systems will make production of advanced lignocellulosic fuels more cost-competitive.

- **Bt-K Biological Process Integration**: Scale-up experiments at the pre-commercial scale using inputs from the different work packages enables assessment of their relevance in commercial settings. This information is then used for economic and life cycle analyses and expected to enable development of supply chains.

- This project covers all aspects of a biofuel production value chain. The goal is to develop and demonstrate at farm and pilot-scale a production and conversion system that could be implemented on a commercial scale on low-productivity land in the US (and in India).

- Tech transfer opportunities: enhanced germplasm; microbial biocatalysts; recyclable cellulolytic enzymes; phosphate recovery and re-utilization system.

# 5 – Future Work

- Determine the flooding tolerance of the lines in the sorghum mapping population to identify genomic regions associated with flooding tolerance. Connect these results with the transcriptomics data.
- Determine the sugarcane aphid resistance in the second sorghum mapping populations to identify genomic regions associated with aphid resistance.
- Determine the anatomical/morphological basis of flooding tolerance in switchgrass.
- Continued improvement of engineered butyrate-producing microorganisms with a final target of 100 g/L.
- Small-scale conversion of switchgrass to ethanol and butyrate to generate data for ASPEN models
- Continue the development of ASPEN model for process flow sheets, especially for large-scale conversion of sorghum and switchgrass biomass to butyrate.
- Optimize the use of biorefinery residues for methane production and soil amendment.
- Evaluate performance of the newly developed lignin-based polymers (durability, compatibility)
- Development of additional novel lignin composites and applications.
- Economic and life cycle analyses of the production and conversion processes developed in this project.
- Focus groups and interviews with industry to identify/mitigate market barriers

# Summary

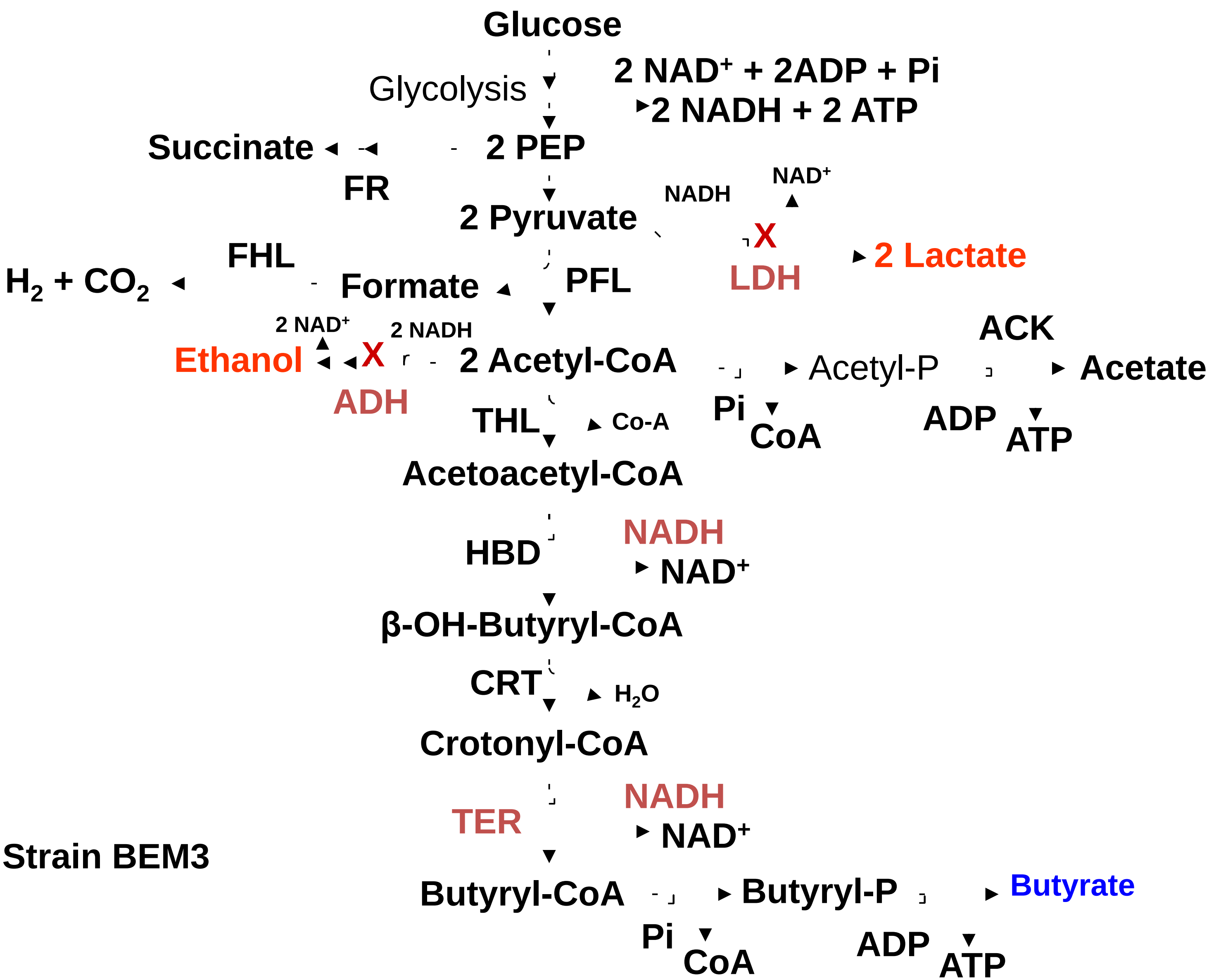
- This project addresses all aspects of biofuel production and supply.
  - Feedstock production, biochemical conversion, and economic & environmental sustainability assessments.
- The focus is on the sustainable production of switchgrass and biomass sorghum on low-productivity land, i.e. with low fertility, and prone to flooding or drought, as a way to produce biomass without competing with food/feed production on prime farm land.
- Genetic improvement of the feedstocks is improving yield potential and reduces production risk on low-productivity land.
- A novel approach to butanol production via microbially produced butyrate is expected to enable much higher butanol yields than currently feasible.
- Implementation of feedstock development and biorefinery technologies at pre-commercial scale is expected to facilitate scale-up to commercial-scale facilities.
- Use of biorefinery waste residues can enhance economic returns and reduce environmental concerns.
- Development of standards and certification protocols, life cycle and supply chain analyses for cellulosic butanol are expected to assist in market deployment.

# Additional Slides

# Responses to Previous Reviewers' Comments

- This project was reviewed in March 2015, two months after a major reorganization and streamlining of the project, that was necessary in part due to one of the industrial partners unable to meet its original commitment. This restructuring also included a change in PI (to the current PI)
- A concern raised by the review team at the time was that the program was too ambitious (with too many different components) and had too many high-risk elements.
- With the streamlining of the project at the beginning of 2015, clearly defined tasks that were achievable within the time frame of a year were formulated, progress was reviewed quarterly, and adjustments made as appropriate.
- At this time, the majority of objectives are on track, and results are being disseminated via peer-reviewed journal publications.
- New sorghum germplasm will be evaluated in replicated yield trails and can be released for commercial use
- Some of the high-risk projects have generated useful and relevant data (e.g. commercial switchgrass sites), while several others that were experiencing challenges are being discontinued (e.g. establishing switchgrass on a flood plain).

# Fermentation pathway of Butyrate-producing *E. coli*



# Eliminating co-products in Butyrate-producing *E. coli* Strain BEM8