DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

US-India Consortium for Development of Sustainable Advanced Lignocellulosic Biofuels Systems

24 March 2015

Feedstock Supply and Logistics Technology Area

Dr. Wilfred Vermerris
University of Florida

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: 25% by scope

Budget

	Total Costs FY 10 – FY 12	FY 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date
DOE Funded	--	$321,357	$829,394	$5,049,249
Project Cost Share (Comp.)*	--	$113, 516	$1,058,833	$5,112,113

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, 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

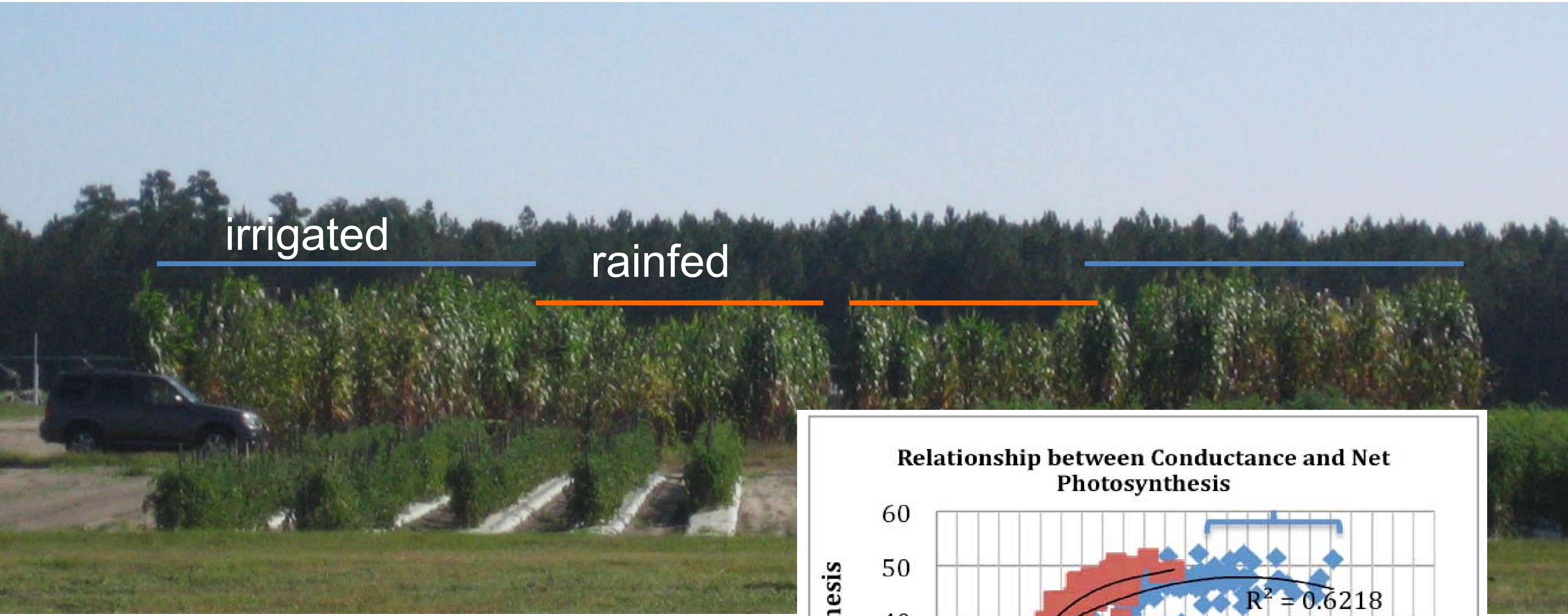
To develop and demonstrate commercially scalable, advanced ligno-cellulosic biofuels through:

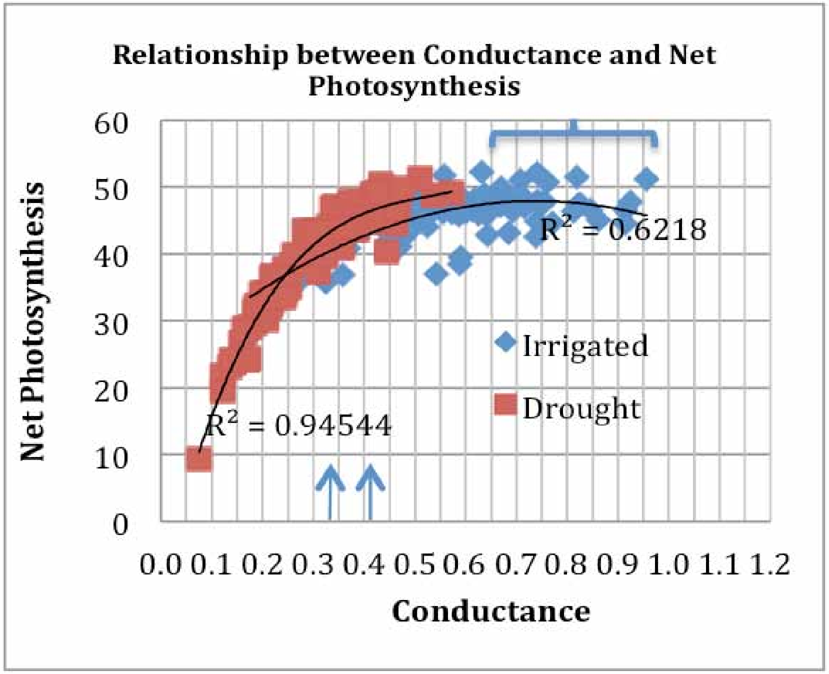
1. Sustainable feedstock cultivation on low-productivity lands.
2. Use of novel microbial biocatalysts for advanced biofuel production.
3. Environmentally and economically sustainable practices through optimized feedstock production and conversion processes and maximum utilization of biorefinery waste 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 to identify 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 make germplasm exchange feasible.
- [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 if the sorghum experiments are successful. Switchgrass genome sequencing efforts are underway as part of other initiatives.





Genetic variation in sorghum can be exploited to enhance flooding tolerance and drought tolerance

2.1 – Approach (Management)

- Switchgrass experiments in Missouri were coordinated by a commercial partner, Show Me Energy Cooperative [SMEC], which also provided substantial amounts of cost-share funds.
- In Spring 2014, financial constraints at SMEC made continued cost-sharing of funds untenable.
- UM terminated the agreement with SMEC as of 1 July 2014.
- No acceptable alternative commercial partner has been identified to replace SMEC.
- In the meantime, the original project PI did not apply for continuation of funding (budget period 2) in a timely manner.
- A letter of non-compliance was issued by DOE on 10 December 2014

As a consequence, the following changes have occurred:

- A change of PI.
- A revised BP2 budget has been prepared, which reflects the reduced amount of cost-share available at UM.
- A revised plan of work for BP2 has been prepared, which reflects the lack of a commercial partner at UM and the reduced overall budget.
  - A reduced number of commercial sites for evaluation of management practices, at a reduced total cost to meet cost-share requirements

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)

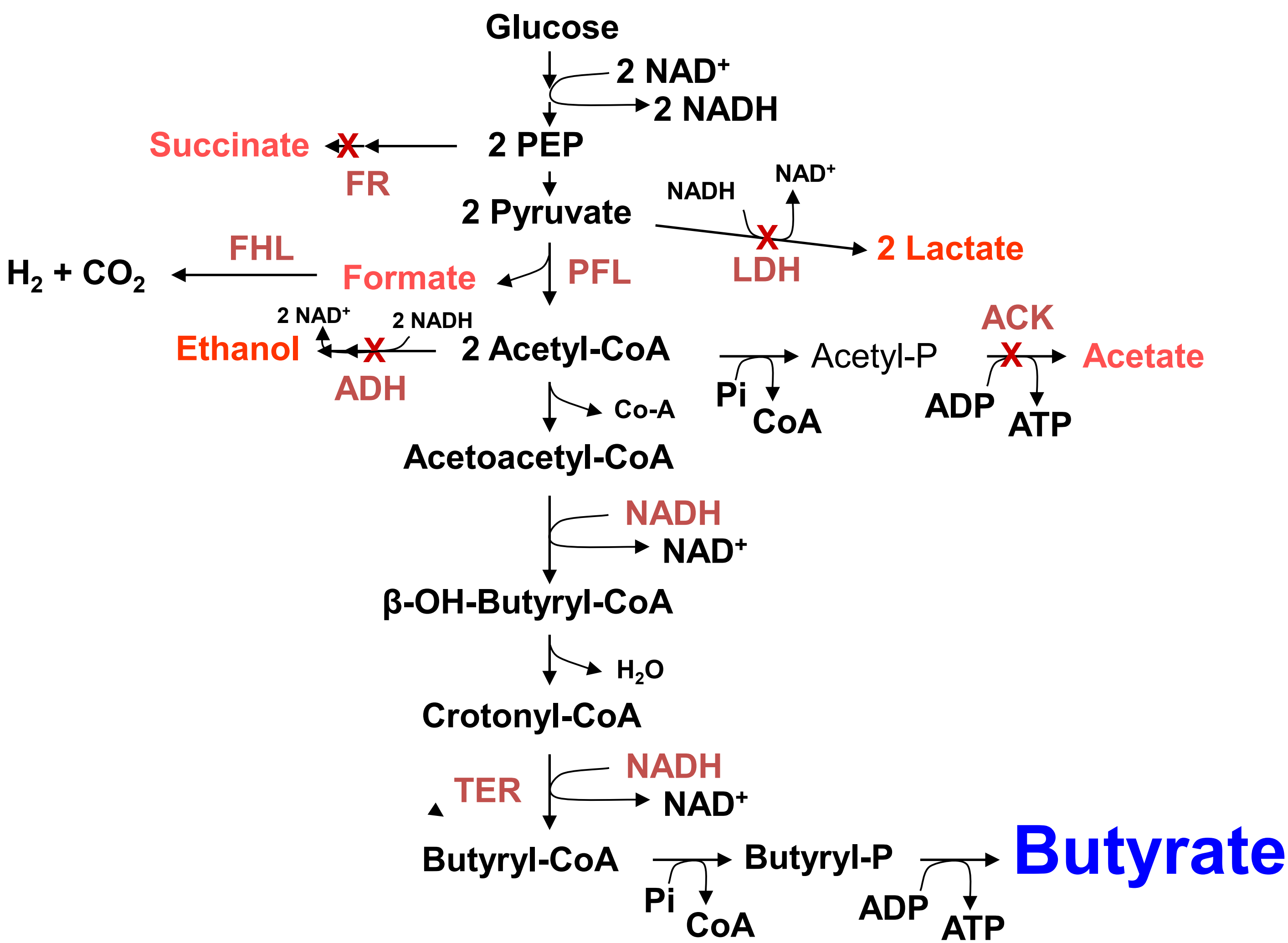
- Contracted farmers must be willing to accommodate research plots on their land to enable ‘real-life’ evaluations – *special individuals*.
- Accept and manage inherent risk of crop losses due to *expected* adverse conditions (flood, drought, poor soils).
- Recognize the potential for poor crop performance 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.*
- *Continuation of research is dependent on good establishment of switchgrass; this was accomplished on most, but not all sites. The unsuccessful commercial sites will be abandoned for financial reasons. A separate 20-acre site at a UM research farm will be replanted with switchgrass.*
- *Detailed management studies (representing many management strategies) are expected to provide the necessary information to recommend best management practices for a variety of site types.*

2.2 – Approach (Technical): Conversion Technologies

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.
- Microbial biocatalysts able to produce butyrate as their sole fermentation product at high titer and yield are under development.
    - Compare the efficiencies of butyrate fermentation to that of butanol fermentation by naturally occurring *Clostridium* isolates.

Eliminating co-products in butyrate-producing *E. coli* strain BEM8

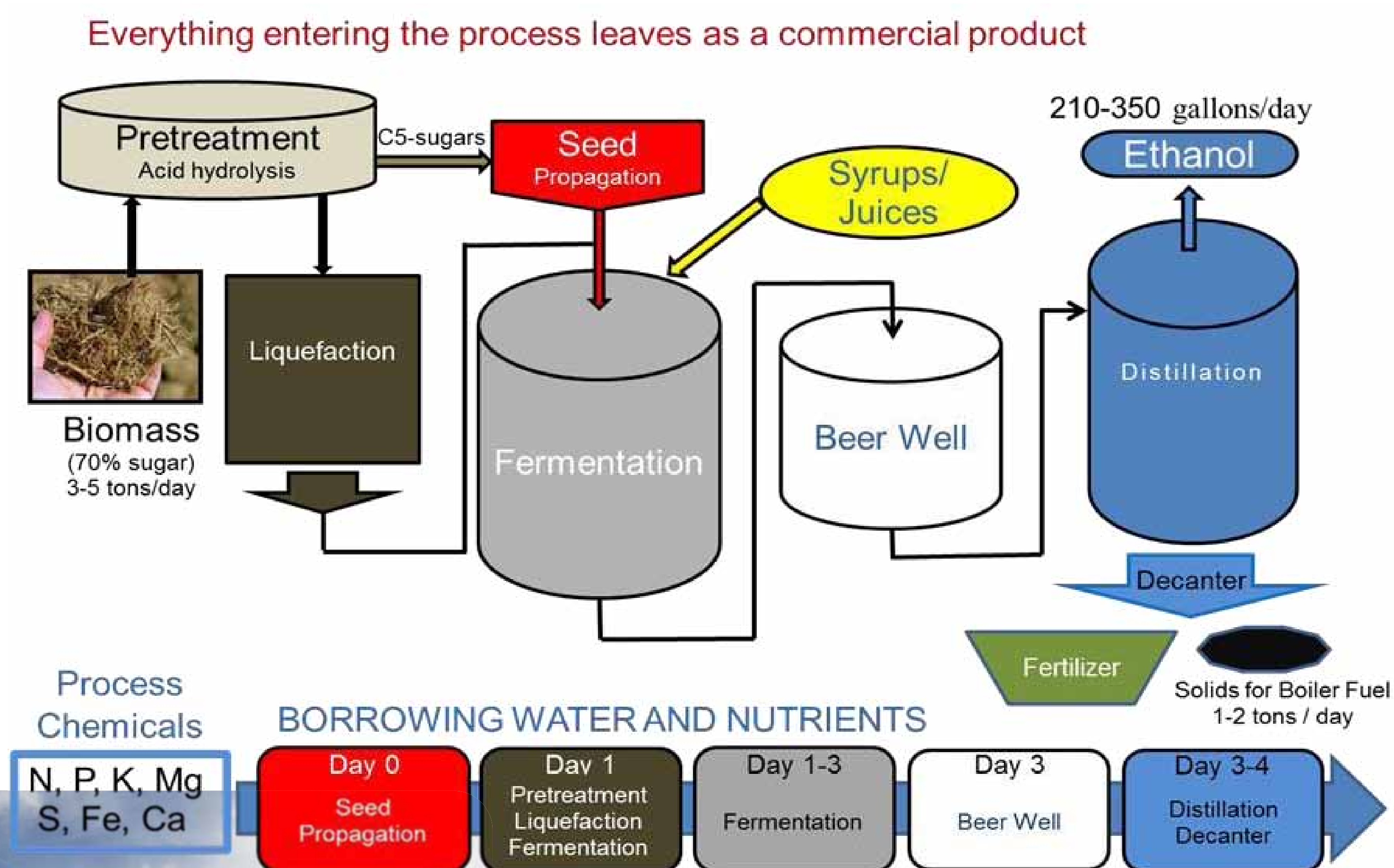


2.2 – Approach (Technical): Conversion Technologies

University of Florida [UF]

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

The UF Stan Mayfield biorefinery pilot facility



2 – Approach (Management)

- The original project proposal was based on switchgrass-to-ethanol conversion, but based on DOE preference, the focus was switched to butanol.
- 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 use of smaller-scale (100 L) switchgrass-to-butyrate conversions, coupled with detailed techno-economic models from sorghum bagasse will be used to streamline large-scale processing schemes for switchgrass.*

- Shipping switchgrass from Missouri is expensive due to the long distance
- *We are planning on purchasing switchgrass from Tennessee*

2.2 – Approach: Conversion Technologies

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.
- Anaerobic digestion of organic components in stillage as a way to generate methane gas (co-product).
- 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.
- Studies on land application of these residuals as a fertilizer.
- 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)

- The installation of the commercial equipment to recover phosphate from the biorefinery waste stream on an industrial scale is delayed by a year. This equipment is donated; the delay originates with the manufacturer.

- *There are enough other ongoing activities (including phosphate recovered with lab-scale devices), that this delay is not holding up progress.*

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)

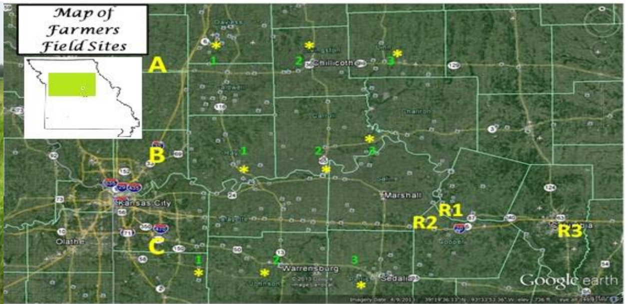
- The energy landscape is constantly subject to change, with the current low prices of natural gas and petroleum undermining the success of existing biorefineries, and delaying the construction of new ones.

- *Surveys and other feasibility studies will provide valuable information for the development of potential policies that would facilitate implementation of bioenergy production systems under different economic scenarios.*
- *Initial efforts are based on literature reviews and model development, which are relatively immune to economic challenges faced as a result of the current low energy prices. Petroleum prices will likely increase over time, when production data from the project will be available.*

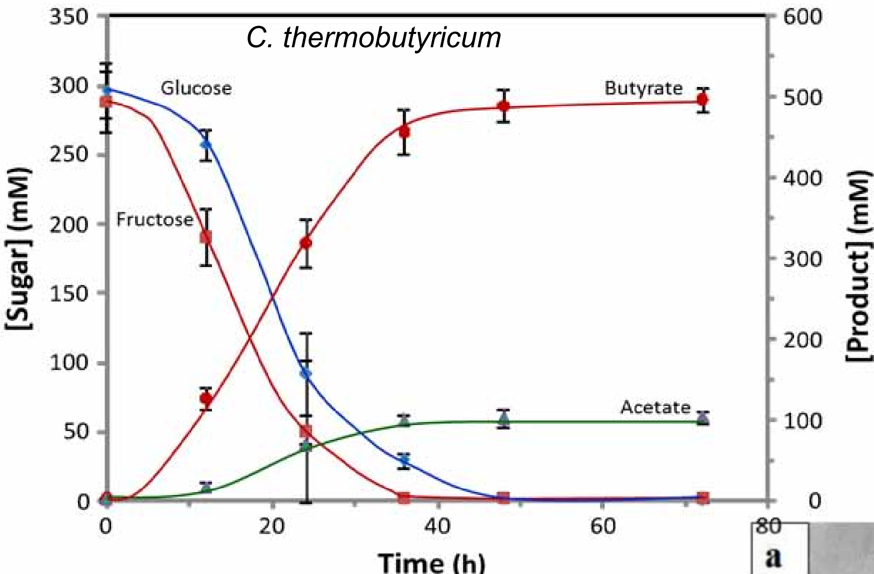
3 – Technical Accomplishments/ Progress/Results

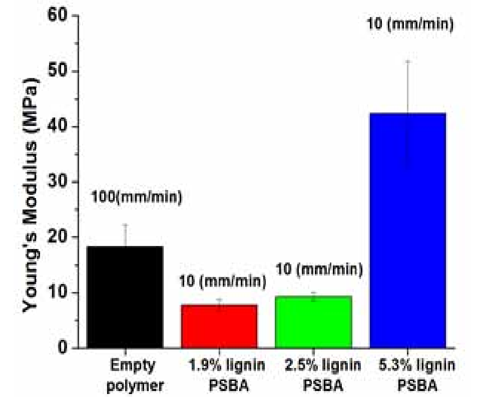
- Screening of 240 genetically diverse sorghum accessions (‘mini-core’) resulted in the identification of 6 flooding-tolerant and 6 flooding-sensitive lines, which were used to initiate mapping populations to study the genetic basis of flooding tolerance.
- Selection of disease-resistant sorghum inbred lines as potential parents for new, drought-tolerant biomass hybrids.
  - Hybrids cannot be propagated via self-pollination, which offers a convenient way to protect intellectual property
  - Yields tend to be higher due to hybrid vigor
- Selection of disease-resistant, drought-tolerant, high-biomass sorghum cultivars.
  - Sorghum cultivars are easier and faster to produce than hybrids.
- Screening of 15 switchgrass accessions resulted in the identification of ‘Alamo’ as the most tolerant to flooding (F. Fritschi, UM).
- Establishment of six 6-acre experimental sites on commercial farms, and three experimental sites on University of Missouri farms to determine best management practices for switchgrass grown <u>on different low-productivity sites</u> in terms of fertilizer applications and mowing regimens.

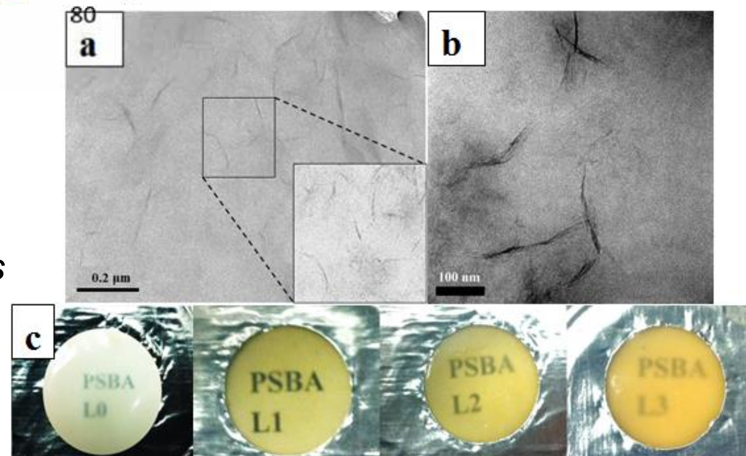


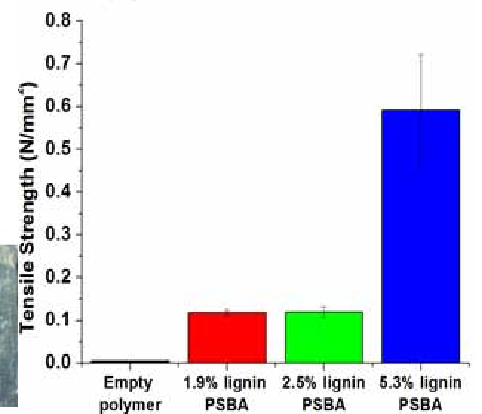



- [UF] Optimized culturing conditions have improved butyrate titers three-fold (from 15 g/L at project inception to 45 g/L).
- [UF] Synthesis protocols were developed for the production of polymeric films containing lignin as co-polymer. The presence of lignin in polystyrene-co-butylacrylate polymers substantially improved the mechanical properties of the polymer: Young’s modulus doubled and tensile strength increased 500-fold. Tolerance to UV-exposure is expected to be enhanced.



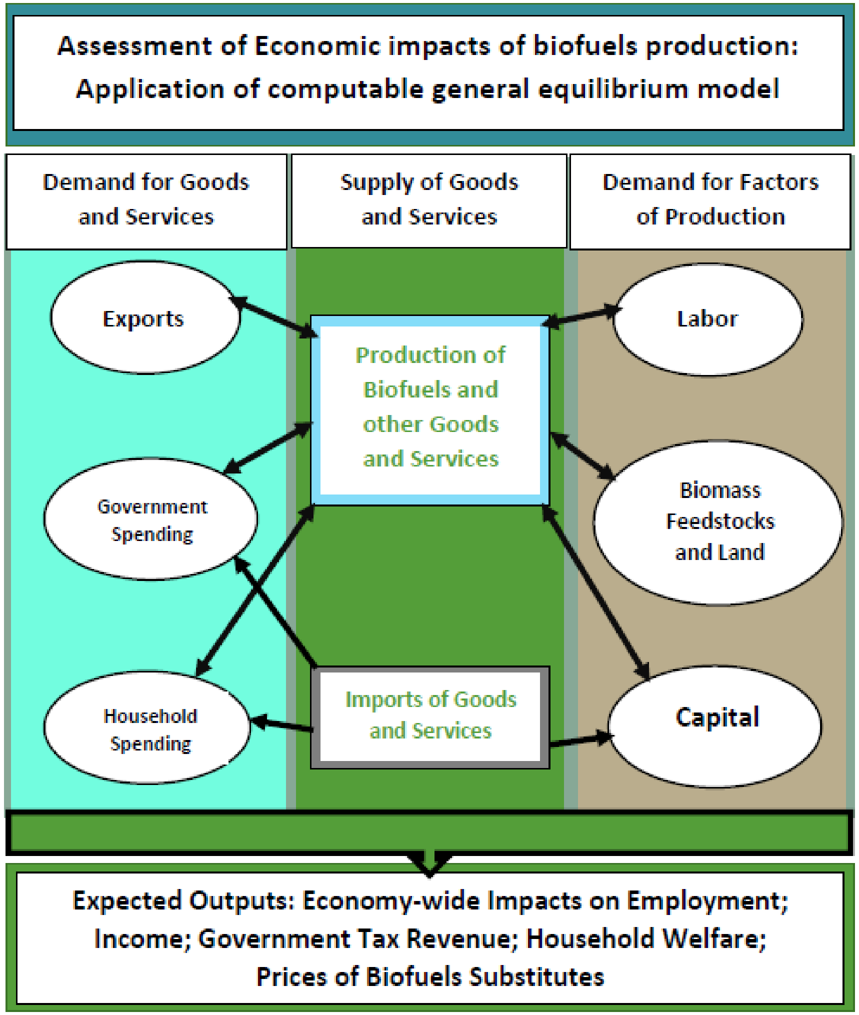






The effects of addition of different proportions of lignin on PSBA co-polymers indicated improved mechanical properties, at the expense of optical properties

- 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

4 – Relevance

- **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).

- **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.

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

5 – Future Work

- Generation-advancement of sorghum mapping populations. We will generate at least 150 progeny per population.
- Selection of high-biomass sorghums from 15 breeding populations (>1,000 individual plants each) and generation of hybrid seed for 50 entries to be evaluated during summer 2015 growing season.
- Generate cDNA libraries of sorghum subjected to flooding vs. well-watered (control) conditions for high-throughput gene expression profiling to identify genes conferring flooding-tolerance.
- The six experimental switchgrass sites will be used to develop best management practices (fertilizer, mowing) based on yield and impact on soil and ground water.
- Continued improvement of engineered butyrate-producing microorganisms with a final target of 100 g/L.
- Development of ASPEN model for process flow sheets.
- Installation of equipment to recover phosphate from conversion waste stream.
- Evaluation of use of biorefinery residues for soil amendment.
- Evaluation of UV-absorbance properties of novel lignin composites.
- Development of models for economic and life cycle analyses of the production and conversion processes developed in this project.

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 expected to enhance yield potential and reduce 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.
- *Progress has been made in the identification of flooding-tolerant sorghum and switchgrass accessions; 6 research sites have been established on commercial farms; the development of improved microbial catalysts and nano-composites; the development of a model that describes the regional economic impact of biofuel production.*

Additional Slides

Responses to Previous Reviewers' Comments

- This project was reviewed by a biochemical review panel in 2013 , less than one year from project inception. The main criticisms were:

1. **The project is too ambitious.**

- **RESPONSE**: Certain aspects were indeed too ambitious *per se*, or too ambitious for the available budget. The scope has been adjusted by removal of several high-risk aspects (switchgrass genetics, switchgrass-to-butanol conversion at the pre-commercial scale) and reduced number of experimental switchgrass sites at commercial farms. This will bring better focus to the project and increase the chances of success.
  On the other hand, progress on the butyrate producing organism has been excellent, despite concerns expressed in 2013.

2. **Lack of novelty on the agricultural experiments.**

- **RESPONSE**: The use of flood-prone land for agricultural production is challenging, and successful, commercially viable implementation has not yet been reported in the literature. The development of stress-tolerant crops is also challenging and has received little attention from the large agri-businesses due to limited market potential.

3. **This project is challenging to manage; teamwork is essential.**

- **RESPONSE**: This is a valid observation that was admittedly seriously underestimated by the original PI. A more active management approach has been implemented so that the project can move forward successfully.

Fermentation pathway of Butyrate-producing *E. coli*