U.S. Department of Energy (DOE) Bioenergy Technologies Office (BETO) 2017 Project Peer Review

PRocess Intensification for the reduced CommErcial CAPEX of Biofuels Production (PRICE CAP) using Dynamic Metabolic Control

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Biochemical Conversion

Michael D. Lynch
Duke University

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Goal Statement

- The goal of this project is to develop scalable and cost-effective next generation semi-continuous fermentation based processes for biofuels reducing commercial scale capital costs 5-10 fold.
- This will be accomplished by achieving unprecedented volumetric rates (>25g/L) and titers (> 500g/L) leveraging advanced dynamic 2-stage microbial strains having Synthetic Metabolic Valves (SMVs) which convert growing cells into active stationary phase catalysts which can be concentrated and recycled.

Quad Chart Overview

Timeline

- 10/1/2016
- 9/31/2018
- 12.5%

Barriers

- Ct-J: Process Intensification
- Ct-H: Catalytic Upgrading of Sugars

Budget

	Total Costs FY 12 –FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17-Project End Date)
DOE Funded	0	0	0	**$1,691,595**
Project Cost Share (Duke)	0	0	0	$154,459
Project Cost Share (DMC)				$35000

Partners

- Duke University
- DMC Limited

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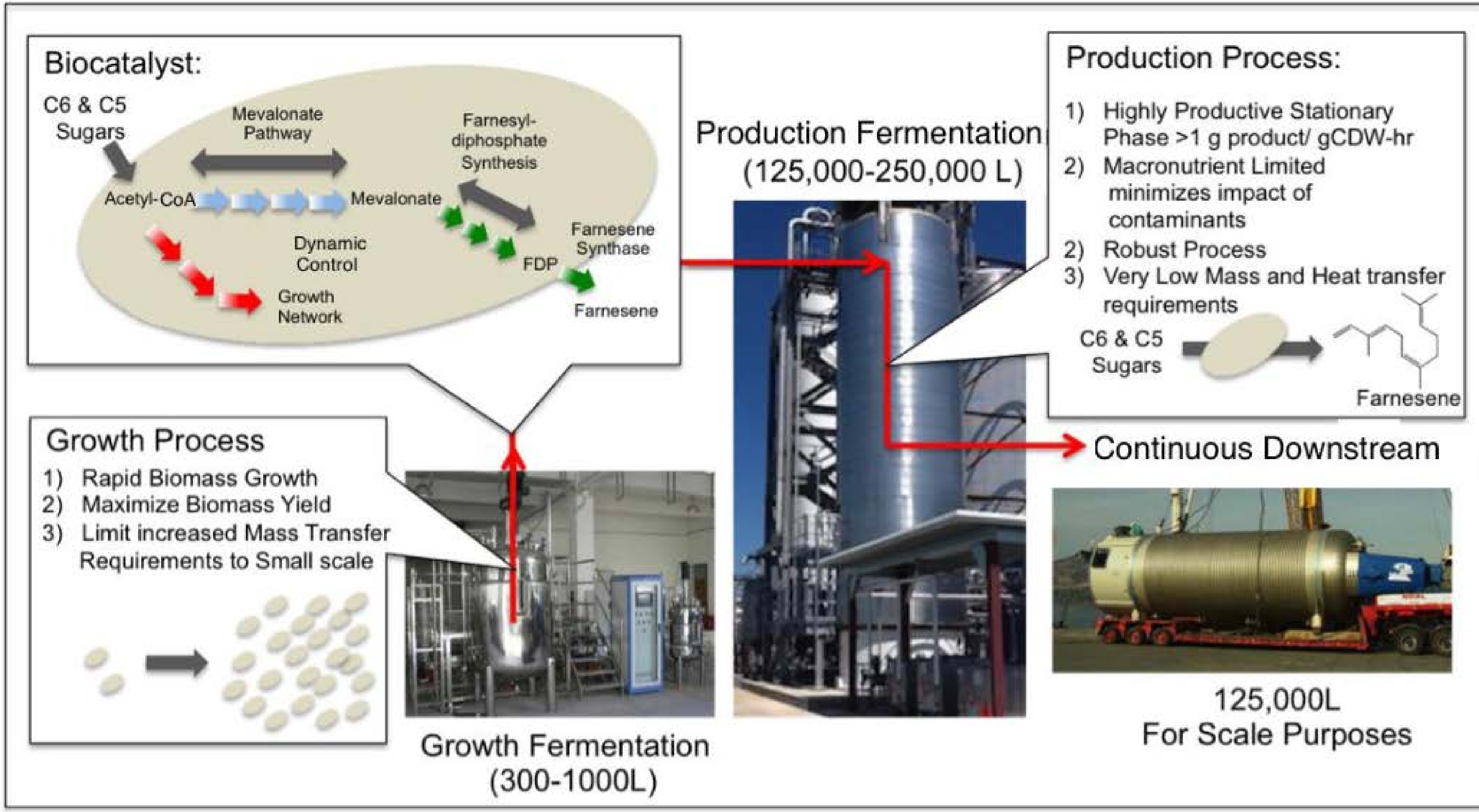
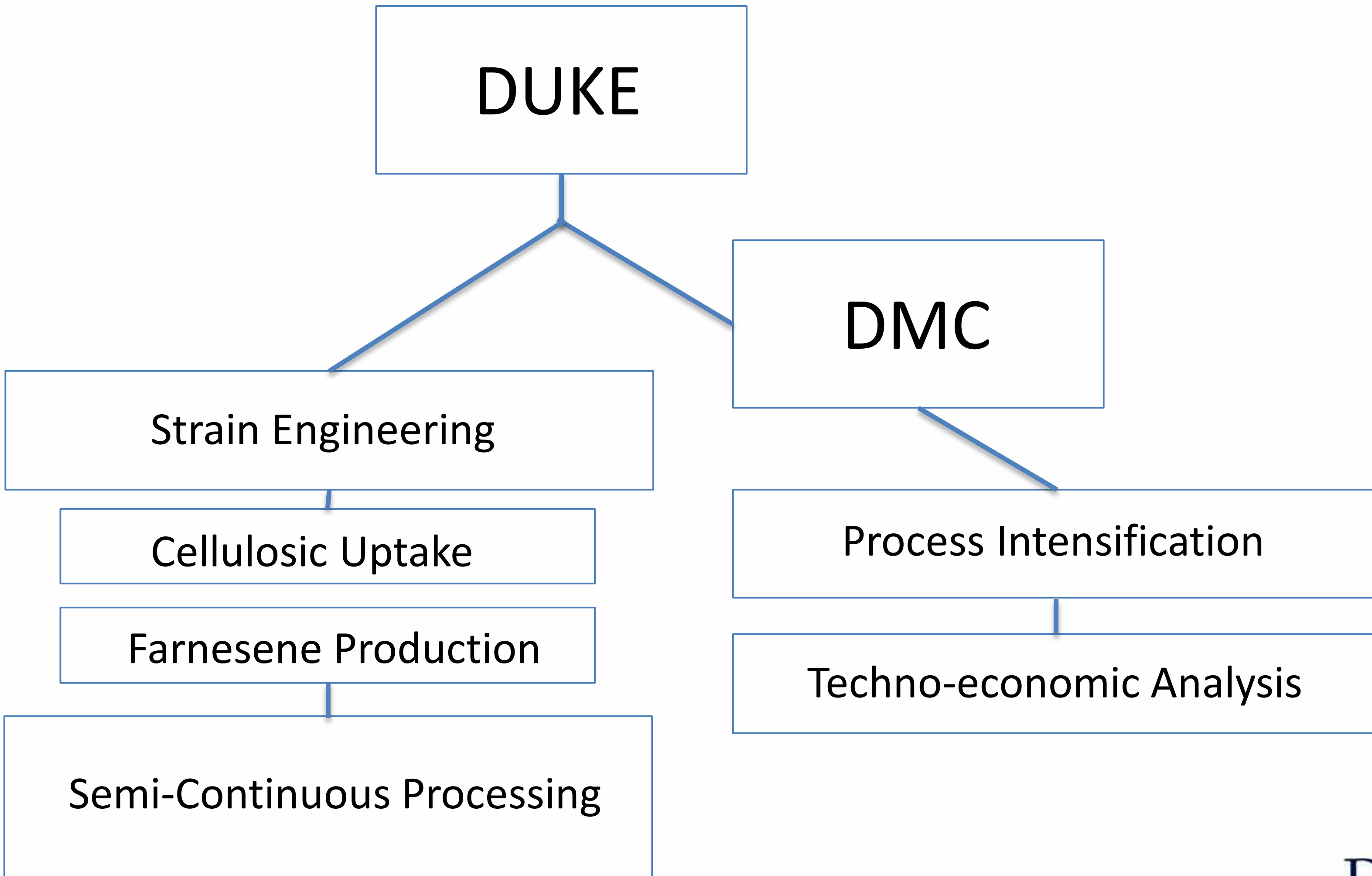
1 - Project Overview



2 – Approach (Management)



2 – Approach (Technical)

Task 1 Strain Optimization (M13-M24)

SubTask 1.A Farnesene Biosynthesis (M13-M24): Biosynthesis of farnesene production will be engineered to meet target specific production rates of > 0.75g/gDCW-hr. ***Demonstrating High Specific Rates***

SubTask 1.B Cellulosic Sugar Utilization (M1-M12): Critical to the success of our proposed semi-continuous process is the simultaneous consumption of all cellulosic sugars. Toward this goal the utilization pathways for key cellulosic components including sugars and acetate will be engineered to exceed those needed to achieve target specific production rates.

2 – Approach (Technical)

Task 2 Semi-continuous Process Development (M13-M24)

SubTask 2.A High Cell Density Seed Generation (M1-M12) High cell densities in a small, well aerated seed vessel (upwards of 100gCDW/L) fermentations.

SubTask 2.B Biomass Recycling (M13-M24): Effective recycling of the biomass, while preserving productivity is essential to achieving the high yields targeted for our semi-continuous process

SubTask 2.C Multi-Stage Process Development (M12-M24) Integration of seed and recycling into a functional semi-continuous process achieving > 25g/L –hr rates

Task 3 Process Modeling & TEA (M11-M24)

SubTask 3.A Baseline Process Analysis

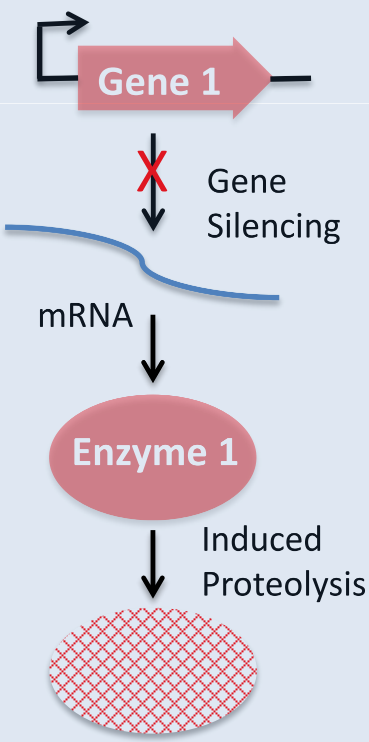
SubTask 3.B Evaluation of Cost Reduction Options (M13-M24)

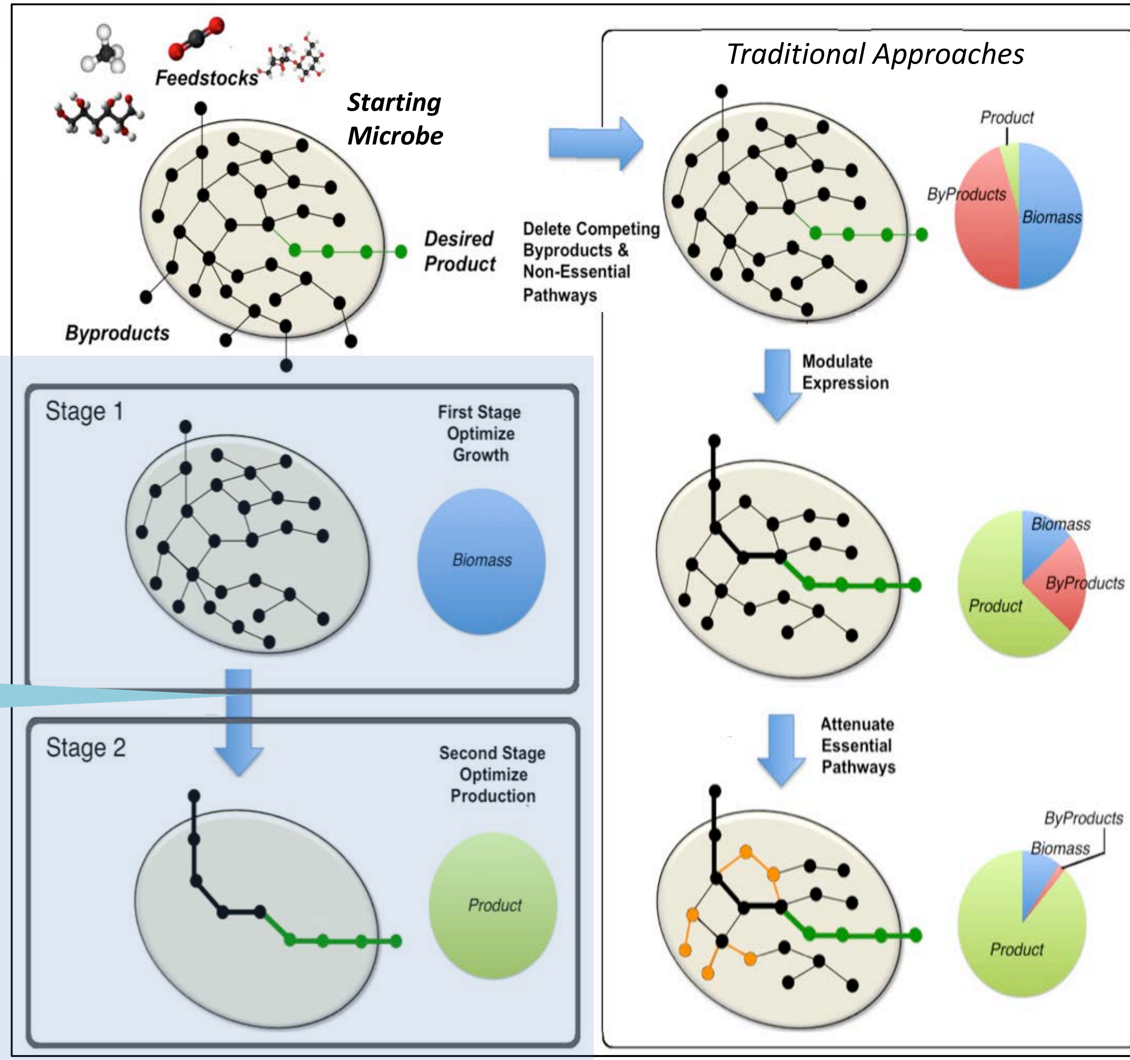
Using a combination os Aspen models and Spreadsheets with actual supplier quotations

2 – Approach (Technical) – DMC

Approach

Dynamic Metabolic Control enabled by gene silencing and controlled proteolysis





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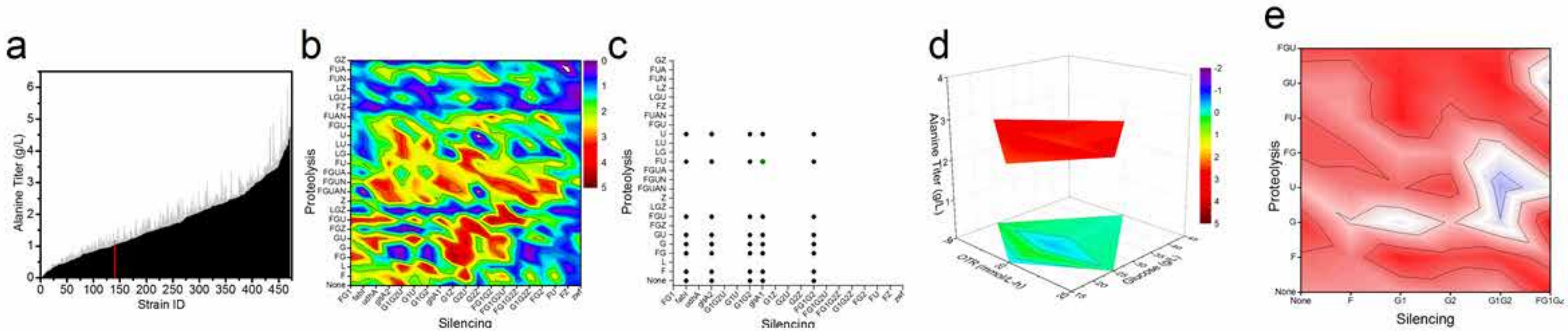
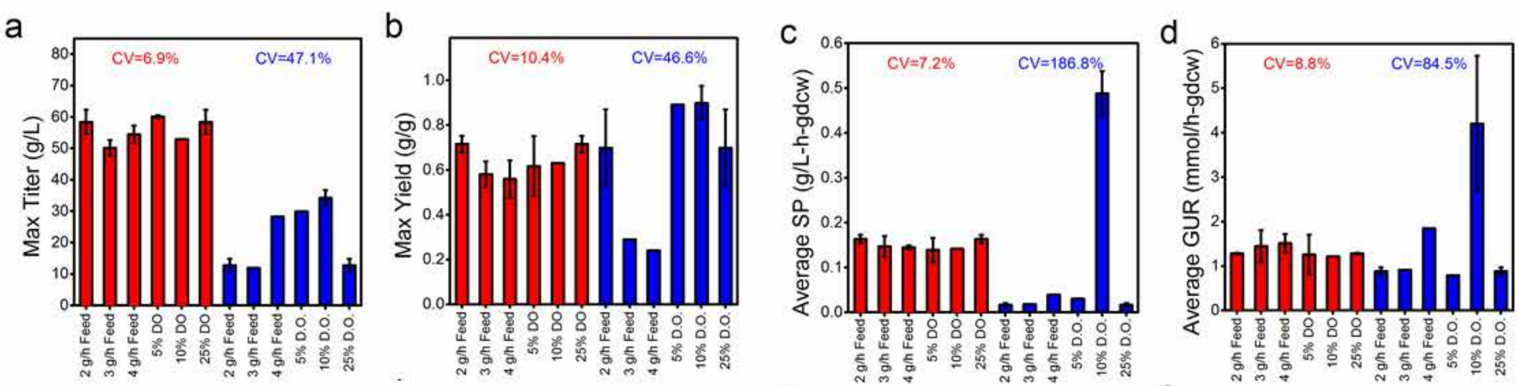
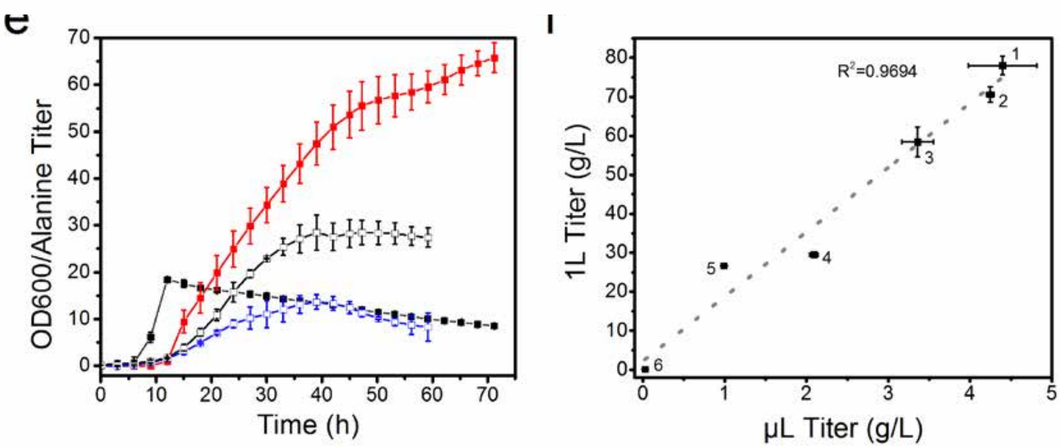
2 – Approach (Technical) – Scalability







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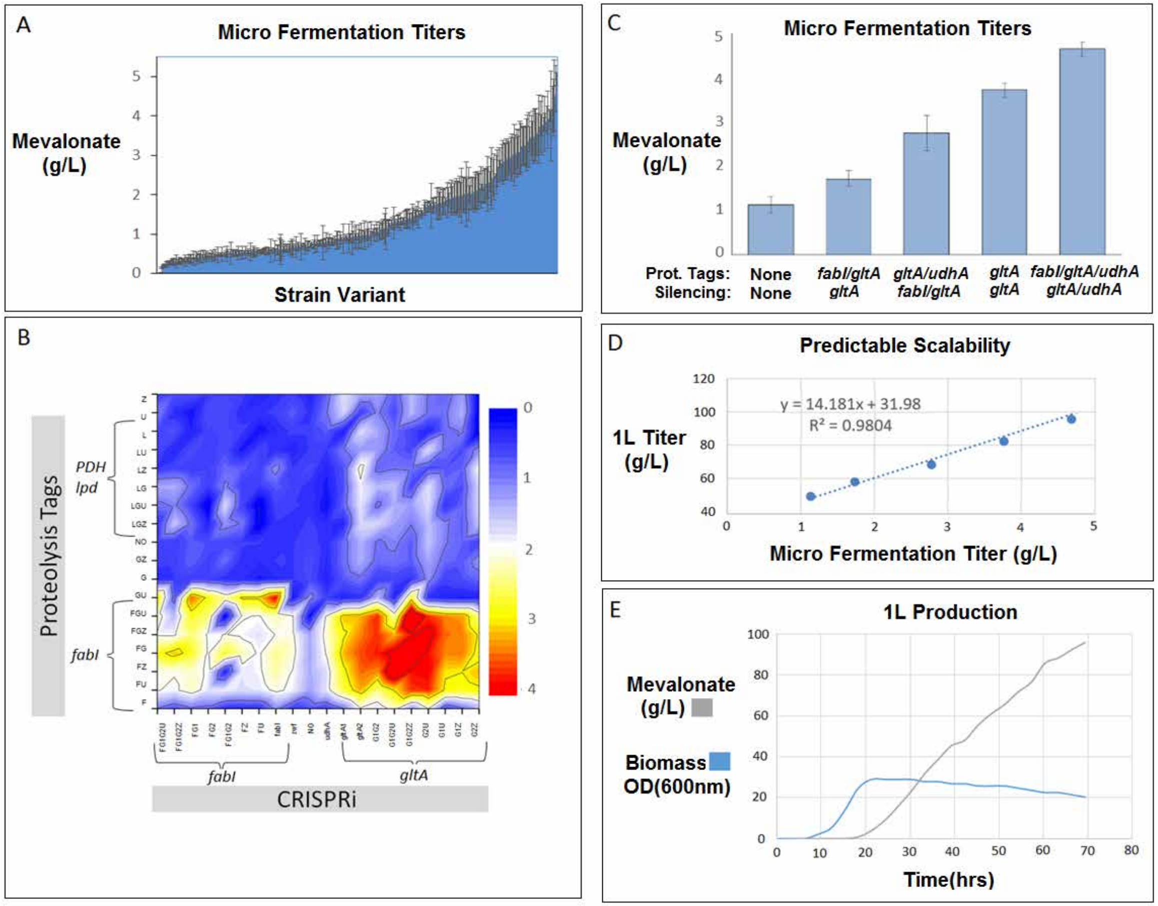
3 – Technical Accomplishments
SubTask 1.A Farnesene Biosynthesis

- Robust Process for Mevalonic Acid (a precursor to Farnesene developed) > 100g/L
- Farnese Production Demonstrated @ screening Scale



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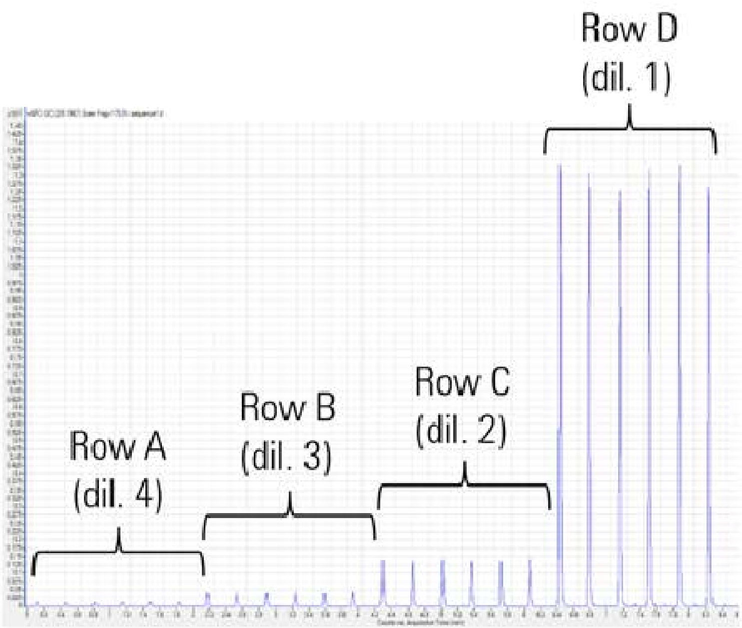
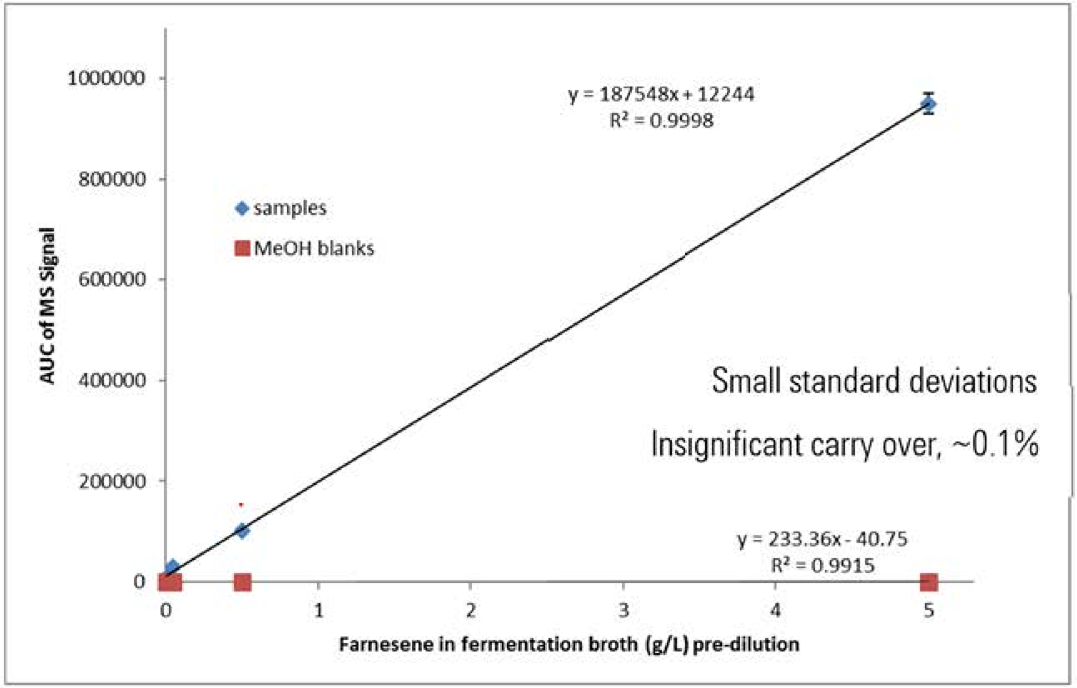
3 – Technical Accomplishments
SubTask 1.A Farnesene Biosynthesis

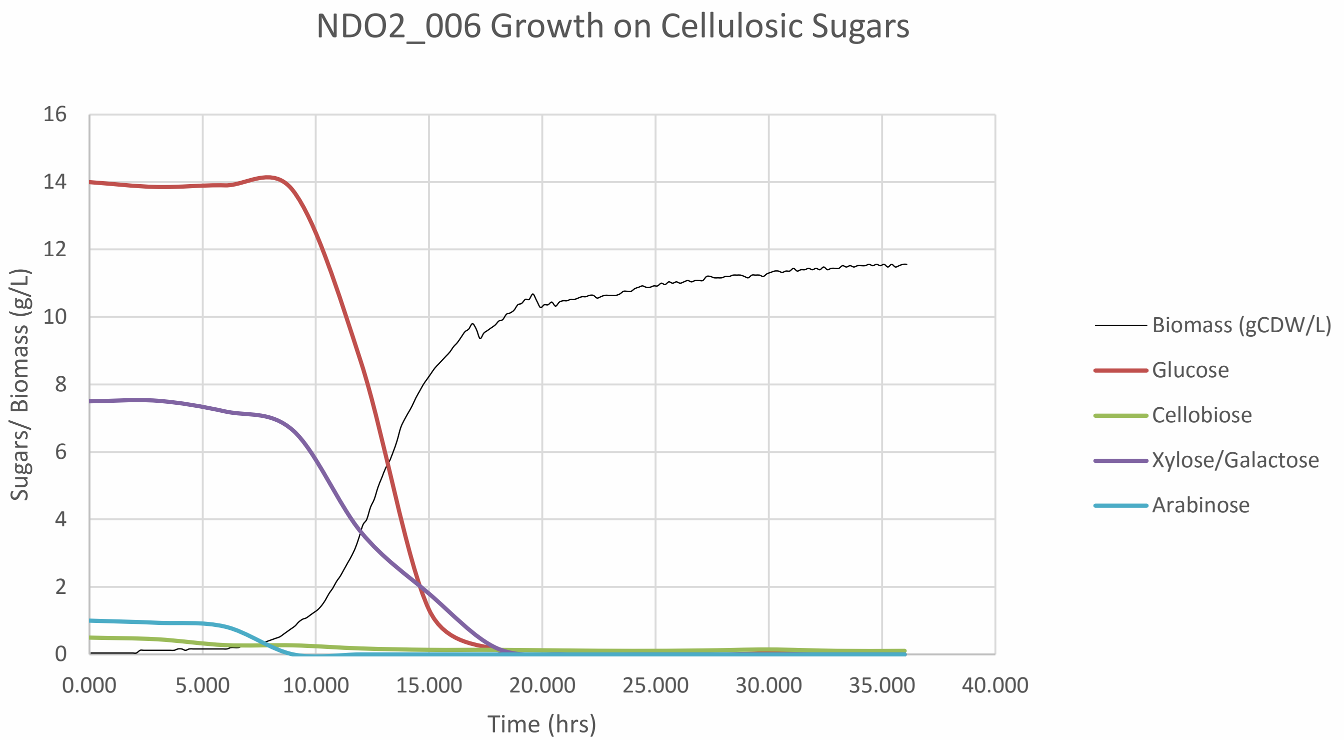
- High throughput MS Method for Farnesebne Quantification Developed





3 – Technical Accomplishments
SubTask 1.B Cellulosic Sugar Utilization

- Co-utilization of glucose, galactose, xylose, arabinose and cellobiose has been demonstrated in 1L fermentations with synthetic cellulosic feedstocks.



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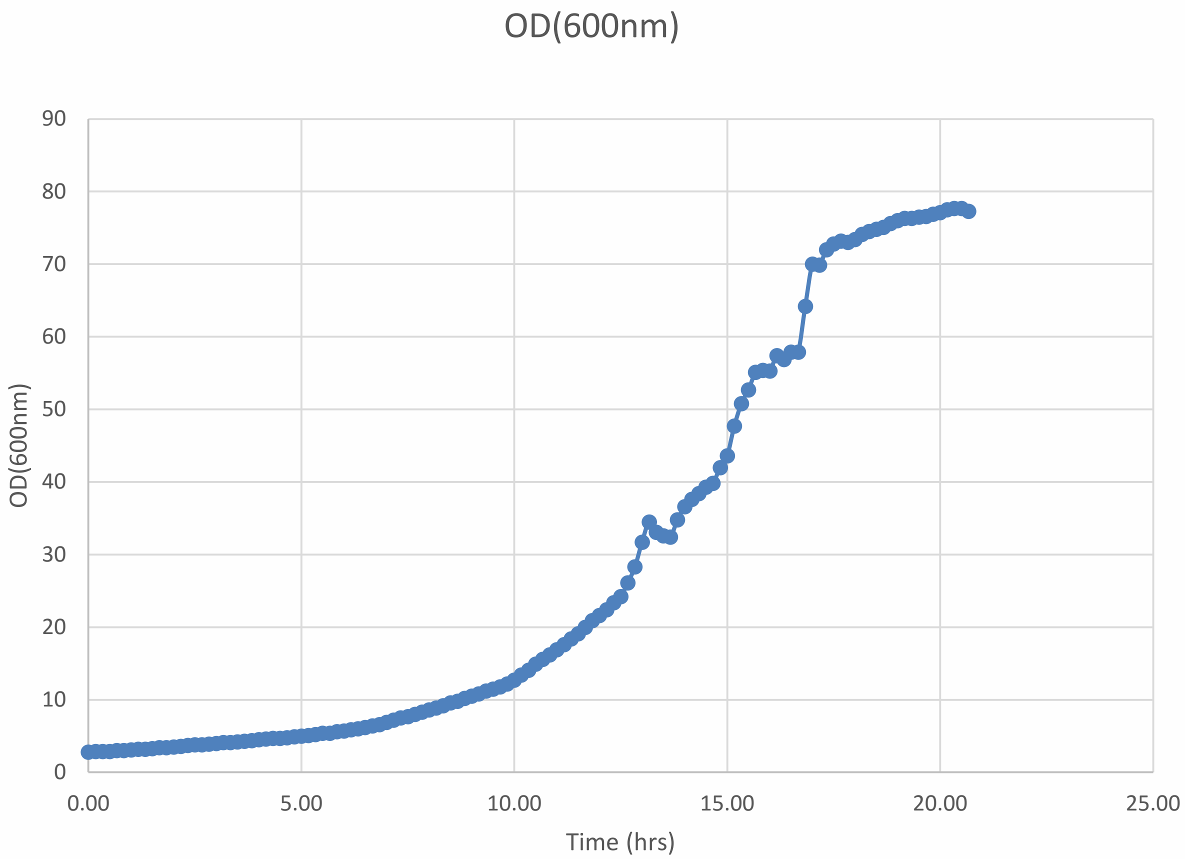
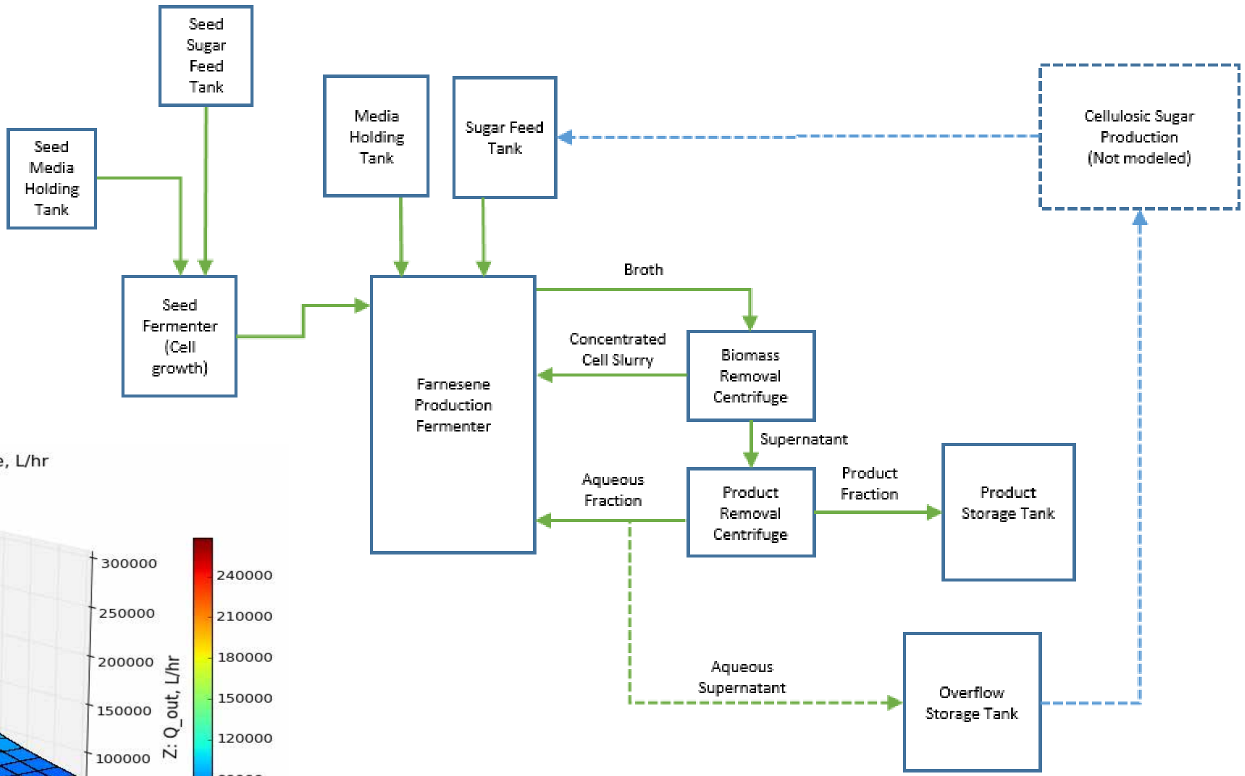
3 – Technical Accomplishments
SubTask 2.A High Cell Density Seed

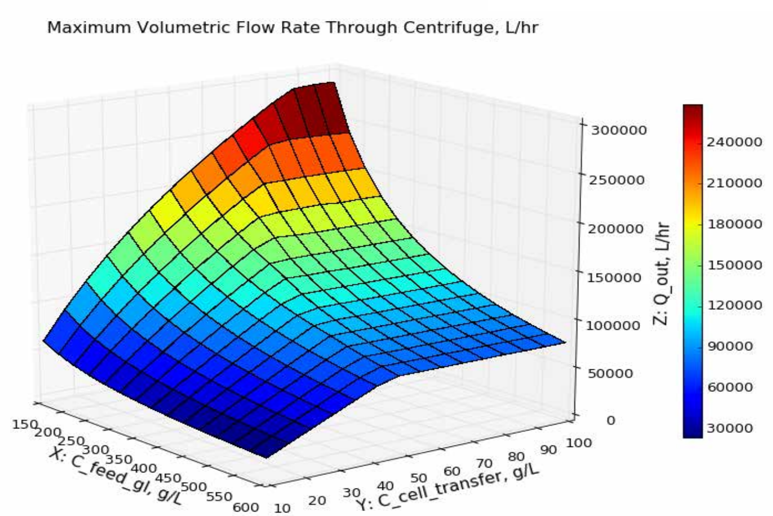
- Yield Coefficients determined, Seed cultures demonstrated to achieve > 30gCDW/L [OD(600nm)> 75)] in < 24 hrs



3 – Technical Accomplishments
SubTask 3.A Baseline Process Model

- Model Developed, Cellulosic Sugar Concentration identified as a primary design factor.





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4 – Relevance

- *Develop semi-continuous fermentation technology to greatly reduce capex requirements for bio-based products commercialization*
  - *Potential 5-10 fold reduction in capex ,*
  - *Commercial plants for the cost of current demonstration plant*
  - *Reduce Risk, and funding hurdles in new process scale up*

- *High rate farnesene biosynthesis and cellulosic sugar utilization are broadly applicable to the space.*
  - *Farnesene is a great initial candidate molecule*
  - *Facile separation from water*
  - *Can be funneled into fuels and higher value chemicals.*

- *Early project successes support feasibility and potential impact of technology after project completion.*

5 – Future Work

- *Continue to optimize strains for farnesene production and cellulosic sugar co-utilization to reach rate and titer targets*
  - *Leverage High throughout strain construction and robust evaluation tools*
  - *Leverage High throughput analytical capabilities*
  - *Evaluate from 2,000 up to 10,000 strain variants per week*
  - *Evaluate farnesene production and sugar co-utilization*
  - *Confirm best performers in controlled fermentations*

- *Demonstrate semi-continuous process for fuel production with real world cellulosic sugars at lab scale.*
  - *High cell density Catalyst Generation*
  - *Catalyst recycling simultaneous with fuel separation*
  - *Engineer prolonged usable catalyst life > 100 hrs*
  - *20 L lab reactor can generate >50 L of fuel in a 100 hr time scale*

- *Develop process Model to drive R&D directions needed to meet capex cost reduction targets.*

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Summary

- A 2-Stage semi-continuous fermentation process for high rate farnesene production is under development
- We have made significant progress in demonstrating high rate cellulosic sugar co-utilization and developing the R&D infrastructure for semi-continuous 2-stage farnesene fermentation
- Approach Broadly applicable to numerous bio-based products
- Potential to greatly reduce capital costs for biobased processes reducing a major hurdle to commercialization.

Additional Slides

Responses to Previous Reviewers' Comments

- If your project is an on-going project that was reviewed previously, address 1-3 significant questions/criticisms from the previous reviewers' comments (refer to the 2015 Peer Review Report, see notes section below)
- Also provide highlights from any Go/No-Go Reviews

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.

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Publications, Patents, Presentations, Awards, and Commercialization

- List any publications, patents, awards, and presentations that have resulted from work on this project
- Use at least 12 point font
- Describe the status of any technology transfer or commercialization efforts

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