DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

SynTec

Synthetic Biology for Tailored Enzyme Cocktails

Biochemical Conversion
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Novozymes, Inc

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GOAL STATEMENT

ACCELERATE INNOVATION IN ENZYME DISCOVERY

REDUCE COSTS FOR PRODUCING CELLULOSIC SUGARS

- Deliver a screening platform which can be used to reduce discovery time required for tailoring enzymes to process specific contexts,
- Demonstrate use of screening tool to achieve reduction in net production costs for cellulosic sugars

- Relevance and tangible outcomes for the United States: Generalizable improvements to enzyme screening technology leads to lower biomass to sugar biochemical conversion costs

QUAD CHART OVERVIEW

TIMELINE

- Start Aug, 2013
- End Sep, 2015
- 80% complete

BARRIERS ADDRESSED

- Bt –G (Cellulase enzyme loading)
- Bt-K (Biochemical conversion process integration)
- Others: Hemicellulase enzyme loading

BUDGET

	Total Costs FY 10 – FY 12	FY 13 Costs	FY 14 Costs	FY 14 Total Incurred Costs	Total Planned Funding (FY 15-Project End Date
DOE Funded	0	0	$ 665,230	$ 1,377,298	$ 1,834,770
Project Cost Share Novozymes	0	0	$ 268,688	$ 719,279	$ 1,155,483
Project Cost Share MBI	0	0	$ 50,920	$ 50,920	$ 16,694
Project Cost Share NZNA	0	0	$ 6,349	$ 6,349	$ 6,349

PARTNERS

- MBI (19%)
- Novozymes North America (1%)

PROJECT OVERVIEW

ACCELERATE ENZYME DISCOVERY-TO DELIVER LOWER COSTS FOR BIOMASS SUGAR PRODUCTION

- Develop faster, combinatorial technologies for mining natural diversity-
  - What are the best “parts” out there that can be harnessed to deconstruct biomass?
  - Past natural diversity screening at Novozymes- slow steps are **purifying and quantifying** enzymes once they’ve been cloned and expressed
  - Identify potential synergism between domains in primary screens- in past, limited combinations of domains from nature are screened for synergy
  - Investigate potential for improving synergy by “proximity” effects: cellulosome paradigm

- Rapid tailoring of enzyme cocktails for unique feedstock types and unique processes
  - AFEX-PCS as a test case: enzymatic conversion of hemicellulose fraction
  - “Clean sugar” concept- avoid salts from pH adjustment
  - Goal: engineer an enzyme cocktail that performs better than commercial cocktail benchmark, and without need for pH adjustment prior to hydrolysis

TECHNICAL APPROACH- OVERVIEW

Faster screening pipeline delivered through:

- Streamlining enzyme purification
- Streamlining enzyme dose normalization

"de-bottlenecking"

Better likelihood of uncovering synergistic components:

- Exploit automation to create large numbers of combinations - look for hydrolysis that is "greater than sum of the parts"
- Test whether bringing parts together in a complex can improve performance (vs. "free" domains)

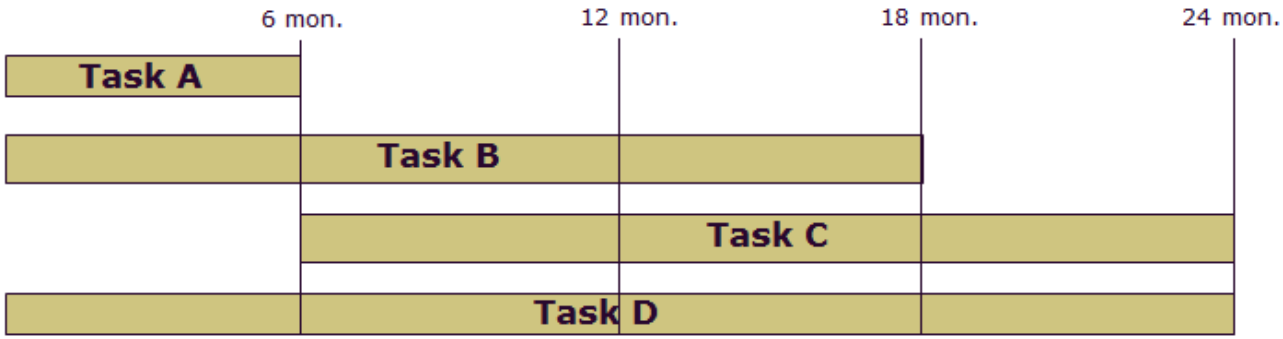
Quantify improvements through technoeconomic modeling:

- **Critical Success Factor:** Achieve a 2.4 x reduction in net sugar production costs *vs.* costs with CTec3/HTec3 benchmark enzyme
- Cost reduction through improved enzyme performance, assayed without pH adjustment, ~pH 6.2; corresponds to a 1.5X reduction relative to CTec3/HTec3 measured at benchmark optimal pH of 5.0

Challenges

- Rapid construction of a novel screening platform
- Short enzyme screening time frame

MANAGEMENT APPROACH



Task A: Establish SynTec Platform

- Go/No-go decision point: HTP cloning strategy (no-go)
- Task A was extended through 12 mo. due to delay in engineering a thermostable scaffold

Task B: Identify hemicellulases for AFEX PCS

Task C: Identify cellulases for AFEX PCS

Task D: Technoeconomic modeling of accomplishments

- Reproduce enzyme improvements (at MBI), validate with DOE/NREL
- Target a **2.4 x reduction in net sugar production cost** *vs.* cost with CTec3/HTec3 benchmark assayed without pH adjustment, ~pH 6.2; corresponds to a 1.5X reduction relative to CTec3/HTec3 measured at its optimal pH of 5.0
- Assumptions: lab scale assay; cost reductions through enzyme dose reductions & conversion level improvements.
- MBI's technoeconomic model (based on modified NREL model)

TECHNICAL APPROACH

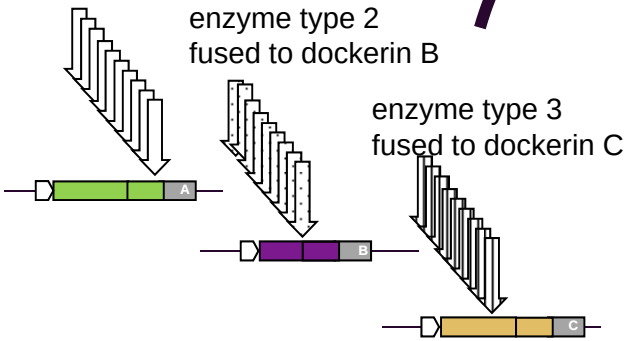
LEVERAGING PROTEIN: PROTEIN INTERACTIONS TO BUILD A STREAMLINED ENZYME SCREENING PIPELINE

1) Gene modules grouped by enzyme activity: each group with specific “tag”

enzyme type 1
fused to dockerin A

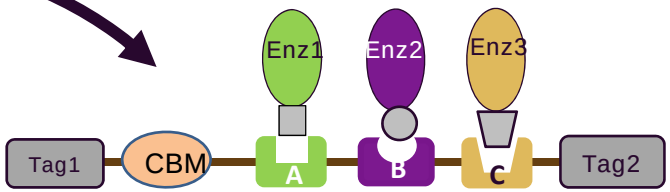
enzyme type 2
fused to dockerin B

enzyme type 3
fused to dockerin C



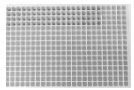
2) Expression of tagged modules

3) Automation to create enzyme mixtures (Enz 1,2,3)



4) Add Scaffold. Tags mediate *in vitro* assembly into a macromolecular complex

5) Isolate complexes (tag-based purification, dose normalization)

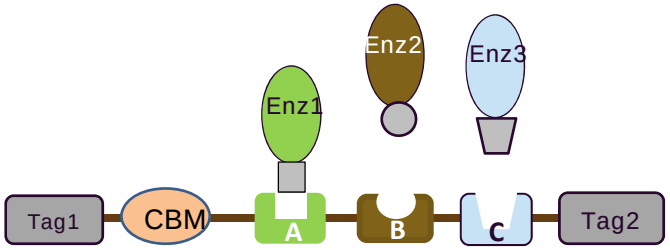


6) Assay various combinations of modules on a pretreated biomass feedstock (Output: glucose xylose)

7) Hits get included in next screen

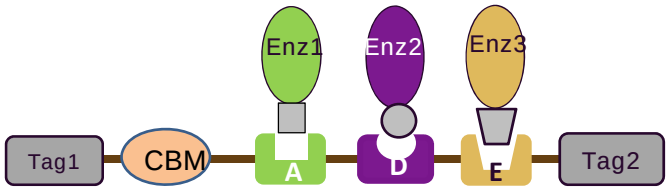
TECHNICAL ACCOMPLISHMENTS:

ENGINEERING A THERMOSTABLE SCAFFOLDIN



1st generation scaffoldin

Unable to maintain all 3 dockerin:cohesin pairs at 55°C

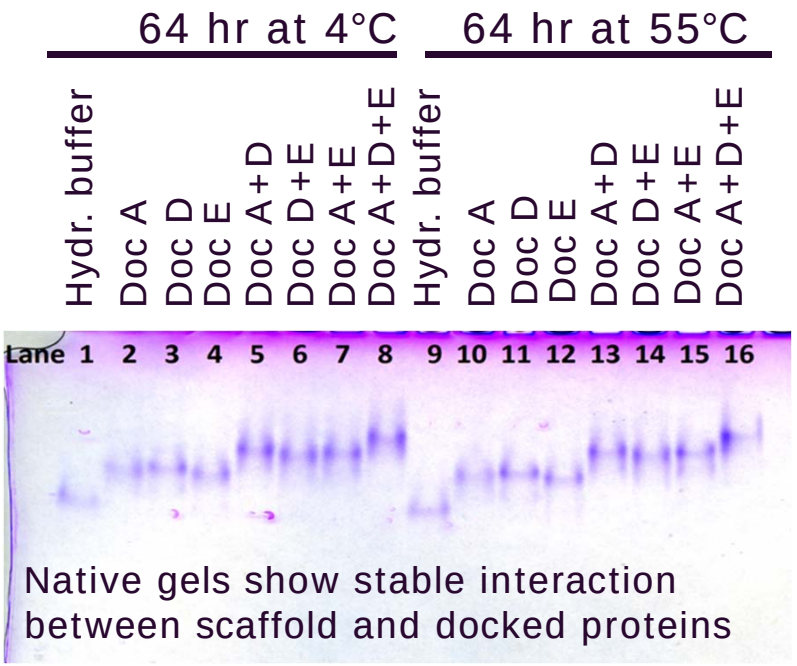


2nd generation scaffoldin

All dockerin:cohesin pairs are stable at 55°C

Task A, Key Achievement:

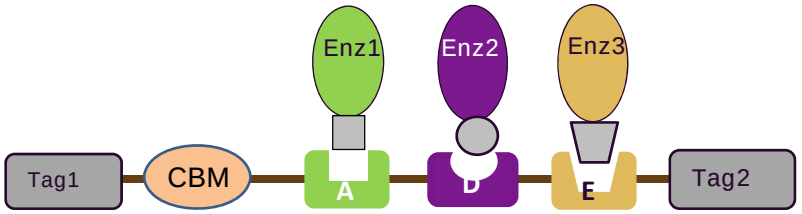
- Stable dockerin:cohesin interactions throughout hydrolysis allows us to screen for intramolecular synergy between domains

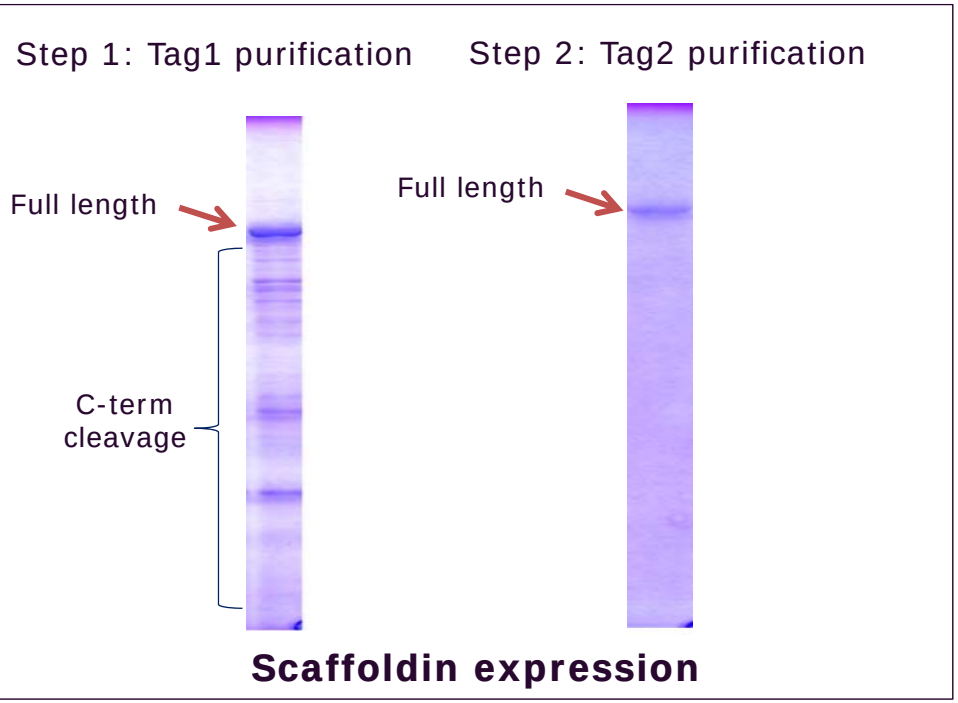


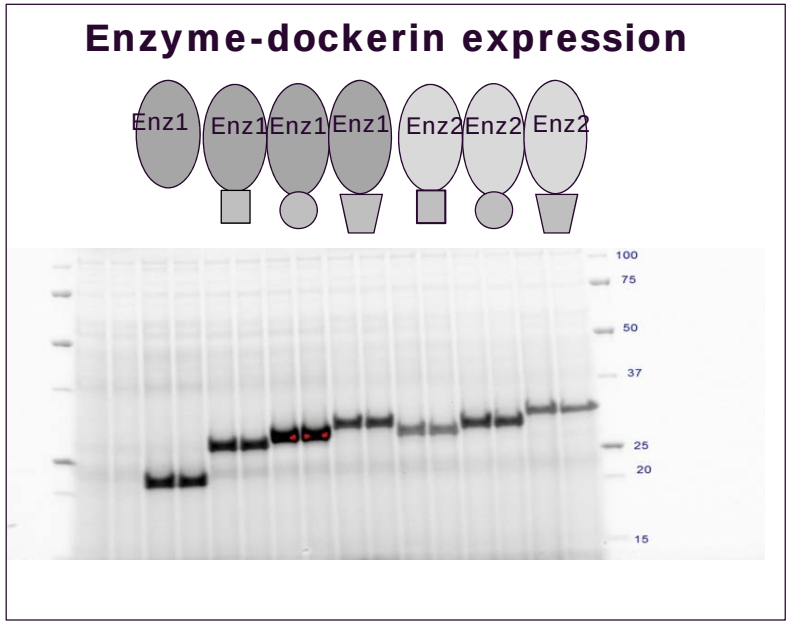
Native gels show stable interaction between scaffold and docked proteins

TECHNICAL ACCOMPLISHMENTS:

SOLVING EXPRESSION ISSUES- SCAFFOLD AND LIBRARY ENZYME DOCKERIN FUSIONS





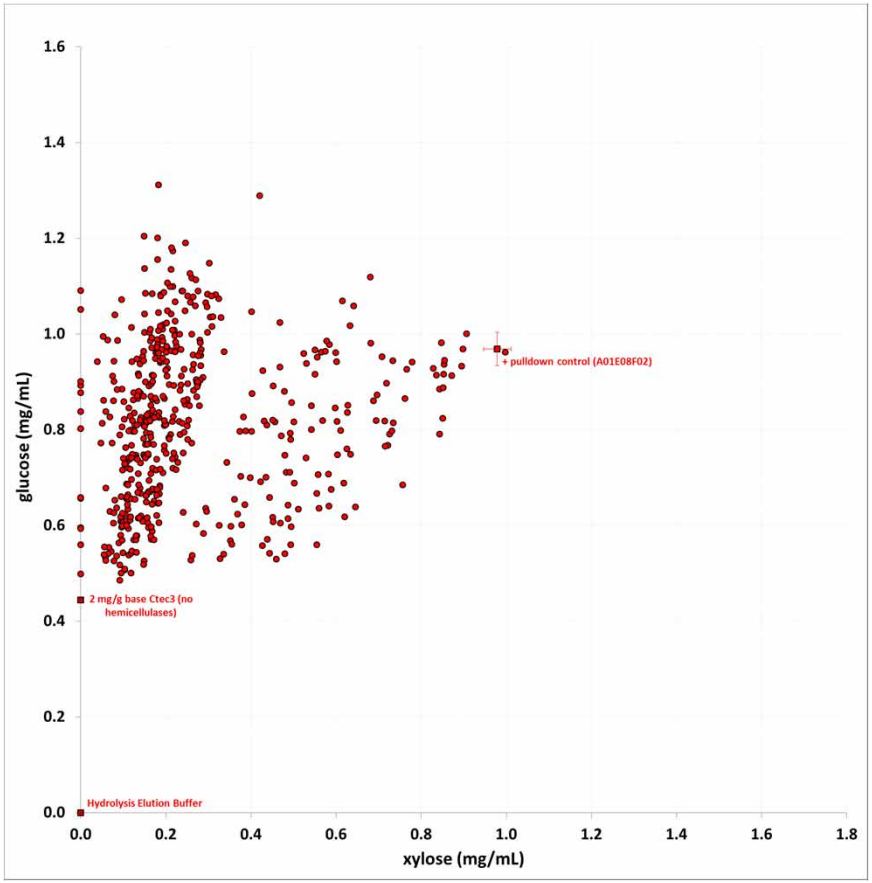


Task A, Key achievements:

- Two-tag purification of scaffold gives highly pure screening reagent
- Selection of dockerin tags to allow for effective expression

TECHNICAL ACCOMPLISHMENTS:

IMPROVED SCREENING PLATFORM ESTABLISHED



- ≤5% CV for pull-down or hydrolysis controls
- Consistent ranking of top candidates among independent screens
- 384 well high throughput screens minimize need for protein input

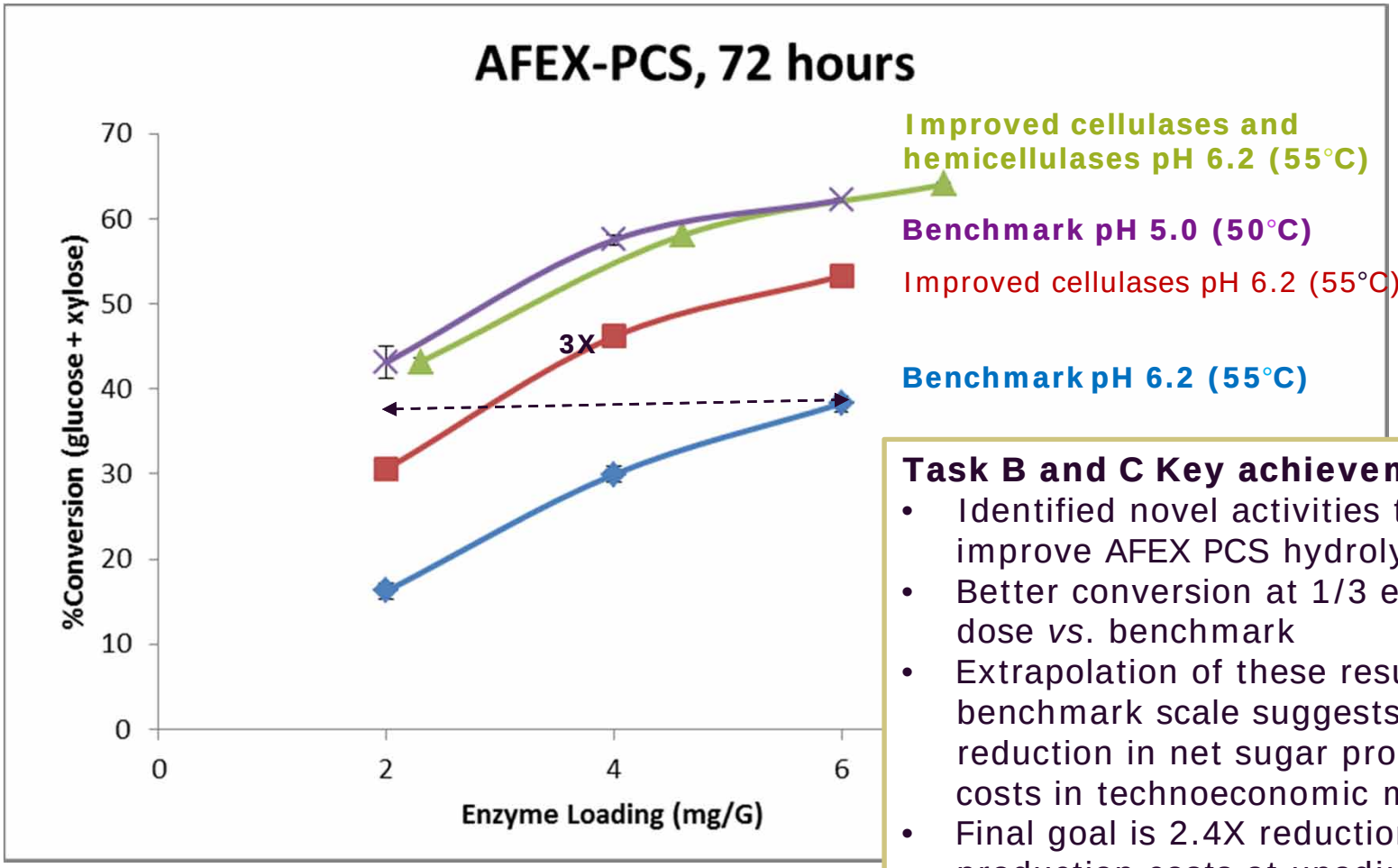
Task A, Key achievement:

- Optimized method for combinatorial screening of enzyme modules from nature

Example of raw screening data.
Each dot from a unique mixture of hemicellulases, added to a base cellulolytic cocktail. Output glucose and xylose are plotted (low %TS)

TECHNICAL ACCOMPLISHMENTS:

IMPROVED HEMICELLULASES AND CELLULASES FOR AFEX-PCS HYDROLYSIS AT UNADJUSTED pH (~6.2)



Small scale assay, low %TS
Benchmark cocktail: CTec3/HTec3

Task B and C Key achievements:

- Identified novel activities that improve AFEX PCS hydrolysis
- Better conversion at 1/3 enzyme dose *vs.* benchmark
- Extrapolation of these results to benchmark scale suggests a 1.5X reduction in net sugar production costs in technoeconomic model
- Final goal is 2.4X reduction in sugar production costs at unadjusted pH

RELEVANCE

- SynTec contributes to Conversion R&D in the BETO MYPP. Overall goal: "…*develop commercially viable technologies for converting biomass feedstocks into ….chemical intermediates…*"
  - Technology will accelerate enzyme discovery
  - Reduction of enzyme dose required for production of sugars from biomass

- Application in emerging biomass industry:
  - Provides a means to quickly tailor enzyme cocktails to better match upstream and downstream unit process steps
  - AFEX example: rapid discovery of new hemicellulases that are not part of benchmark cocktail
  - ~pH 6.2 example: optimize cocktail to match process pH from pretreatment

- Relevance to BETO, emerging industry, and market place: accelerating rate of enzyme cost reduction through technology innovation.

FUTURE WORK

Final goal is 2.4X reduction in net sugar production costs at unadjusted pH

- Only a few cellulases (Task C) have been screened to date. As we identify improved cellulases, we expect to further reduce enzyme costs

Investigate intramolecular synergy

- Among current top hits, we have not yet seen benefit of including novel domains in a macromolecular complex. “Free” enzyme combinations perform similarly to same domains loaded onto the scaffold.
- We will continue to assess intramolecular synergy for new hits

Scale up top candidates and validate performance at MBI, verify assay results and technoeconomic modeling with DOE/NREL

SUMMARY

KEY POINTS:

1. Overview Developed a faster, superior method for screening modules from nature for application in biomass conversion to sugars

2. Approach SynTec screening allows us to streamline bottlenecks in purification and quantitation, and allows for broader screening of potentially synergistic modules *vs.* previous methods

3. Technical Accomplishments

- Greater than 3X dose reduction at ~pH 6.2 for production of mixed sugars *vs.* CTec3/HTec3.
- Identified novel activities that improve AFEX PCS hydrolysis

4. Relevance faster discovery of enzyme cocktails tailored for specific conversion processes

5. Future work Continue to improve enzyme performance through cellulase diversity screening; scale up and validate performance at MBI to achieve target 2.4X reduction in net sugar production cost based on technoeconomic model

Additional Slides

SynTec Team

Novozymes:
Technical project leader: Janine Lin
Johnnie Hahm, Paul Harris, Sumati Hasani, Ian Haydon, Tia Heu, Aubrey Jones, Michael Lamsa, Fang Liu, Ronald Mullikin, Ani Tejirian, Carly Todd, William Widner, Elizabeth Znameroski, James Broering, Grace Cooley, and Kurt Creamer

MBI:
Farzaneh Teymouri
Bryan Bals

ACRONYMS

- AFEX PCS: Ammonia fiber expansion pretreated corn stover
- CBM: Carbohydrate binding module
- SynTec: Synthetic biology for tailored enzyme cocktails
- Enz: Enzyme
- CV: Coefficient of variation. Obtained by dividing the standard deviation by mean of the data
- MBI: Michigan Biotechnology Institute
- NZNA: Novozymes North America

PUBLICATIONS, PATENTS, PRESENTATIONS, AWARDS, AND COMMERCIALIZATION

Presentations

- Johnnie Hahm, Paul Harris, Sumati Hasani, Ian Haydon, Tia Heu, Aubrey Jones, Michael Lamsa, **Janine Lin**, Fang Liu, Ronald Mullikin, Ani Tejirian, Sarah Teter, Carly Todd, William Widner, Elizabeth Znameroski, James Broering, Grace Cooley, and Kurt Creamer. **Discovering optimally tailored enzyme cocktails using a synthetic screening tool.** Invited talk**, 37th Symposium on Biotechnology for Fuels and Chemicals,** April 27-30, 2015, San Diego, CA.
- **Johnnie Hahm**, Paul Harris, Sumati Hasani, Ian Haydon, Tia Heu, Aubrey Jones, Michael Lamsa, Janine Lin, Fang Liu, Ronald Mullikin, Ani Tejirian, Sarah Teter, Carly Todd, William Widner, Elizabeth Znameroski. **Development and application of a synthetic cellulosome-based screening platform for enhanced enzyme discovery**. Accepted for poster presentation at **11th Carbohydrate Engineering Meeting**, May 10-13, 2015, Espoo, Finland.
- **Ian Haydon**, Johnnie Hahm, Elizabeth Znameroski, Fang Liu, Tia Heu, Sumati Hasani, Michael Lamsa, Aubrey Jones, William Widner, Ronald Mullikin, Paul Harris, Sarah Teter, Janine Lin. **Development of modular, self-assembling synthetic protein complexes as screening platforms for enhanced enzyme discovery**. Poster submitted to **AIChE / SBE 2nd Synthetic Biology Conference**, June 10-13 2015, Boston, MA

Patent Applications

- Two patent applications on novel enzymes pending.