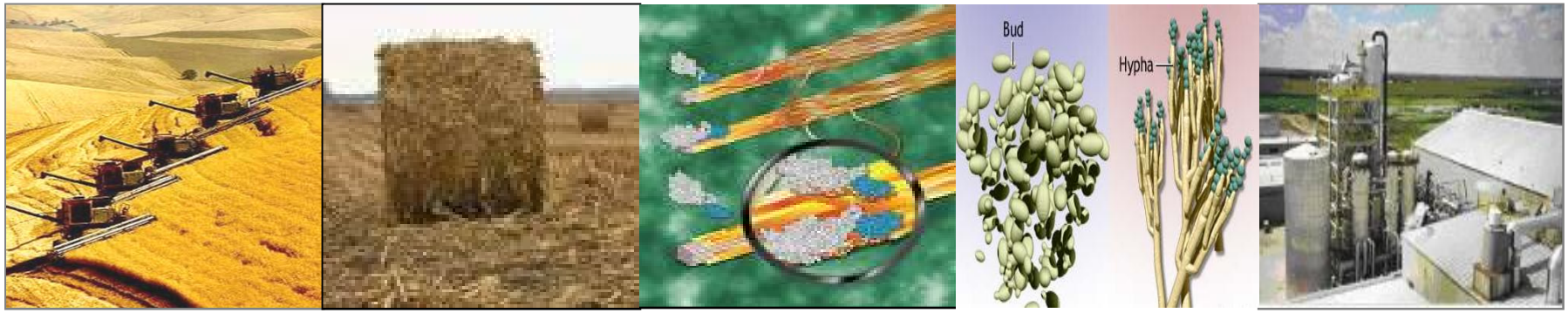
Development of a Commercial Enzyme System For Lignocellulosic Biomass Saccharification



Feb.,14th, 2011
Biochemical Platform Review

Dr. Manoj Kumar
Director Science & Technology
Global New Business Development
DSM Bio-based Products & Services

Unlimited. DSM

DSM: Company Profile 2009
Life Sciences and Material Sciences Company

- **Global top 30 chemical industry**
- Net sales : $10.8 billion
- Net earnings : $455 million
- **22,738 employees**
  - of which in R&D: approx. 2,130
  - of which in the Netherlands: approx. 7,300
  - of which in the US: approx. 4,000
- >200 locations on 5 continents
- **No 1 or 2 in Dow Jones Sustainability Index** from 2004 to 2010
- **Strong technological toolbox**:
  Integrated use of biotechnology, biocatalysis, organic chemistry, chemical and polymer technology, materials sciences

Member of Dow Jones Sustainability Indexes 06
Member of Dow Jones Sustainability Indexes 05
Member of Dow Jones Sustainability Indexes 04

World Business Council for Sustainable Development

DSM Enzymes for Cellulosic Feedstocks:

Leveraging DSM's Enzyme Technology

- DSM has a large collection of both commercial and pre-commercial hydrolases (cellulase, hemicellulase, mannanase, pectinase) along with fermentation processes developed for various business segments
- Several existing DSM *in house* enzyme products of value for cellulose processing
- **US DoE Partnership with DSM for commercial development of cellulosic enzymes for *lignocellulosic* feedstocks**

Cellulose Enzymes

A. niger sequence*:* Pel *et al.* (2007) Nature Biotechnology **25**, 221

P. chrysogenum sequence: van den Berg *et al.* (2008) Nature Biotech. **26**, 1161

DSM Project Overview

Timeline

- October, 1st 2008
- September, 30th 2012
- 56% Complete

Budget

- $21.25 MM
  - 28.7% DoE; FFRDC 4.7%
  - 66.6% DSM
- DoE Funding received in FY08: 0
- DoE Funding for FY09: $3.5 MM
- DoE Funding for FY10: $1.13 MM
- DoE Funding for FY11-12: $1.74MM

Barriers

- Barriers addressed
  - BT-F: Cellulase Production Cost
  - BT-G: Cellulase Enzyme Loading
  - BT-H: Enzyme Biochemistry
- Technical targets (WBS 2.2.2)
  - achieving high sugar and ethanol conversion rates and yields in the core processing step of enzymatic hydrolysis and thereby reducing cost
  - developing higher-activity enzyme mixtures and lower-cost enzyme production processes

Partners

- ABNT/SNL/LANL
- Project Management by DSM

Stage

- Bench Scale; Stage 2-3 of R&D as per DoE Biomass MYPP 2010

Project Participants & Partnerships

- DSM (Delft & Belvidere site)

- Abengoa Bioenegry http://www.abengoabioenergy.com/
  - Mr. Quang Nguyen & Dr. Bob Wooley
- Sandia National Lab http://www.sandia.gov/mission/ste/index.html
  - Dr. Ling Quin, Dr. Ken Sales
- Los Alamos National Lab http://www.lanl.gov/orgs/b/b8/b8index.shtml
  - Dr. Andrew Bradbury, Dr. Pankaj Soni, Dr. Devin Close

- US Department of Energy: Biomass Program

DSM Project Goals

- Evolution and development of current *in-house* cellulolytic enzymes system to reach enzyme efficiency mandates set by DoE Biomass MYPP.

- Production of cellulase enzymes on pilot plant scale supporting a cost structure commensurate with *an integrated production* model by combining the following improvements
  - *in secreted enzymes expression titers,*
  - *doubling the specific activity of the cellulolytic enzymes*
  - *reducing the dosage needed for LC biomass saccharification.*

- Demonstration of yield performance on C6 monomeric sugars at or above 90%

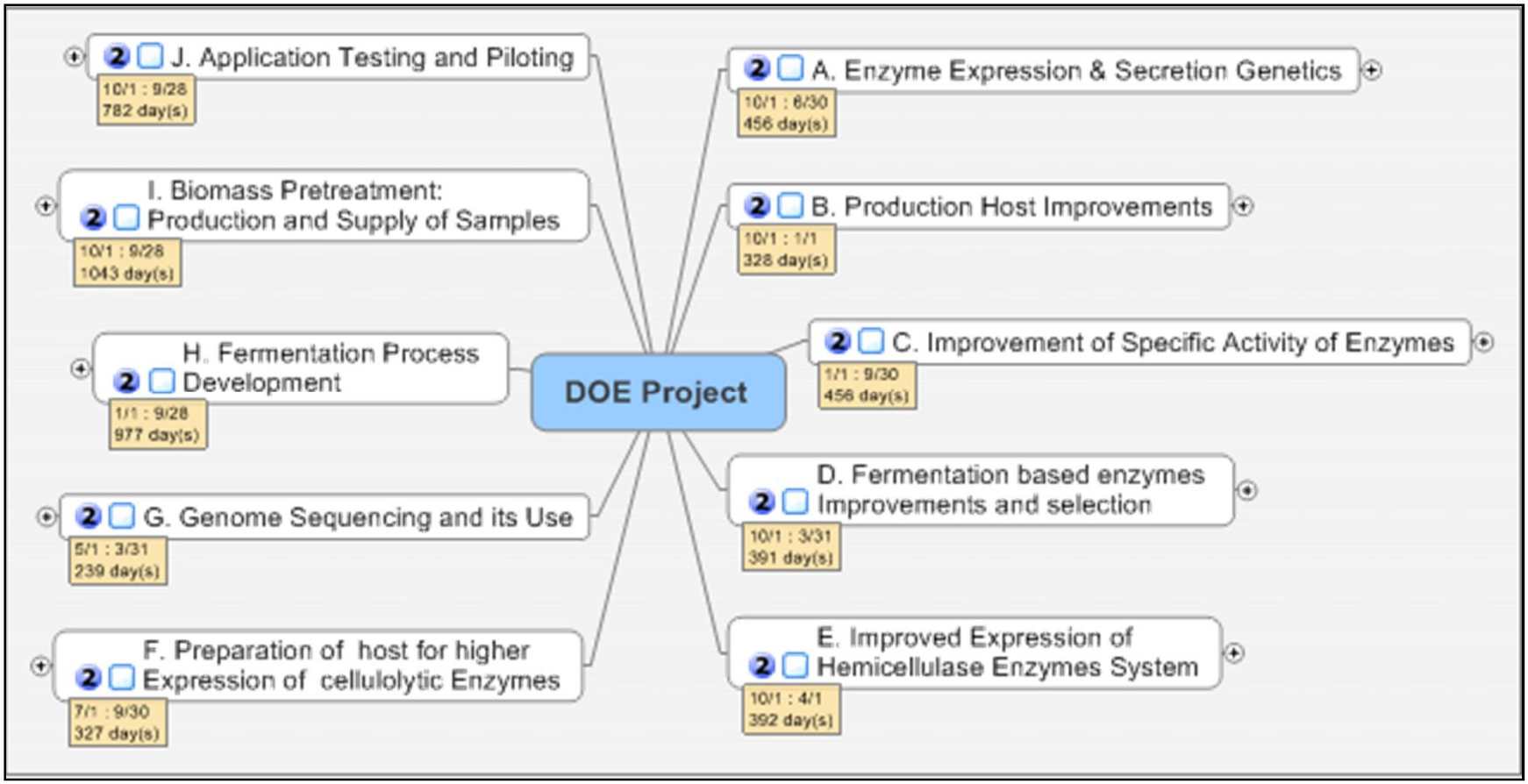
- Successful application test with the evolved LC hydrolyzing enzymes system on a pilot plant scale

- Relevance of DSM project to the Biomass Program, the market, and alignment with MYPP goals
  - M 4.5: Demonstrate and validate ag residue saccharification to produce mixed, dilute biomass sugars (priority High); Stage 2; Agricultural Residue Processing Pathway BC/IB
  - Alignment with MYPP Milestone M 4.5.1 C Validate cellulase enzyme cost
  - Market Relevance: Cost of Production

DSM Project Objectives (SOPO)

- The use of a proprietary dilute acid pretreated and conditioned wheat straw biomass (ABNT) as a means of optimizing induction of cellulolytic enzyme cascade systems.
- Use of advanced proprietary fungal host systems for homologous and heterologous production of cellulolytic enzyme activities for commercially viable saccharification of biomass feed-stocks.
- Further enzyme efficiency improvements of LC saccharifying enzymes by employing metabolic engineering, directed evolution and protein engineering.
- Demonstrate the in-process feasibility of the enzyme system's performance at a commercially viable cost structure in a pilot plant configuration.
- Demonstrate the production of LC saccharifying enzyme systems at a commercially viable cost structure in lab and small scale pilot plant configurations.

DSM Project



- *DSM Project Management Approach:*
  - *Quarterly progress monitoring, milestone driven*
  - *PMP & Gantt Chart, Go/No Go based project management with plan B*

DSM Project Approach

- Use of DSM's unique and long standing expertise in *fungal* physiology, genetics, molecular biology, metabolic engineering, fermentation, process development, and manufacturing.

- DSM's broad knowledge and application base in biotechnology, chemistry, polymer science, fermentative production and animal nutrition will facilitate the development of integral biorefinery production systems. This will make optimal use of feed-stocks and utilities in order to generate the highest added value from output products.

- Abengoa: an established biofuels company bringing in technology development for biomass pretreatment, feed-stock position, application know-how, and functioning biomass processing pilot plant.

- Sandia National Labs: Protein structure determination using computational aids, microscopy, and crystallography to determine the structure and substrate-enzyme interactions.
- Los Alamos National Labs: Protein engineering for improved enzymes.

Technical Progress (Oct 2008-Dec 2010)

- 8-10x improved enzyme expression and cellulolytic activity in the fermentation broth by CSI campaigns, protein engineering, and Fermentation process development
- Reduction in enzyme dosage based on optimization in application
- Successful over-expression of multiple native cellulases in heterologous and homologous host
- Creating a beachhead IP portfolio with >20 patent applications
- Crystal structure of all major cellulases to help us in protein engineering effort at SNL
- Successful protein engineering of CBH1
- Genome sequenced, annotated, and gene chip usage in enzyme production improvements

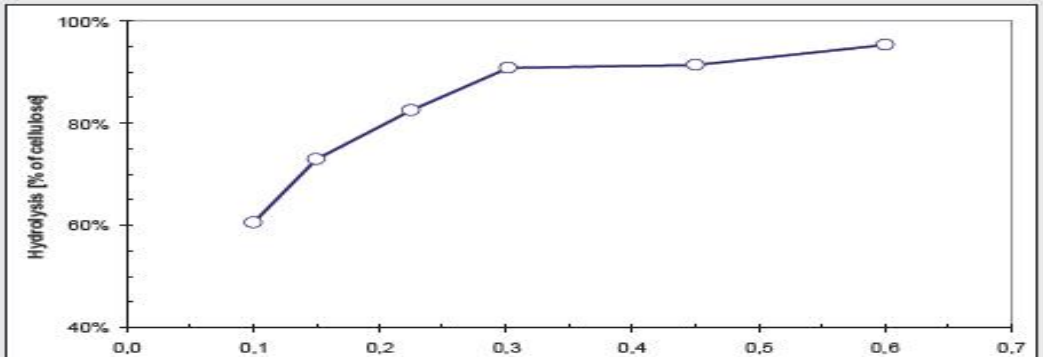
  Thermostability improvement LANL

DSM Thermostable Cellulases:

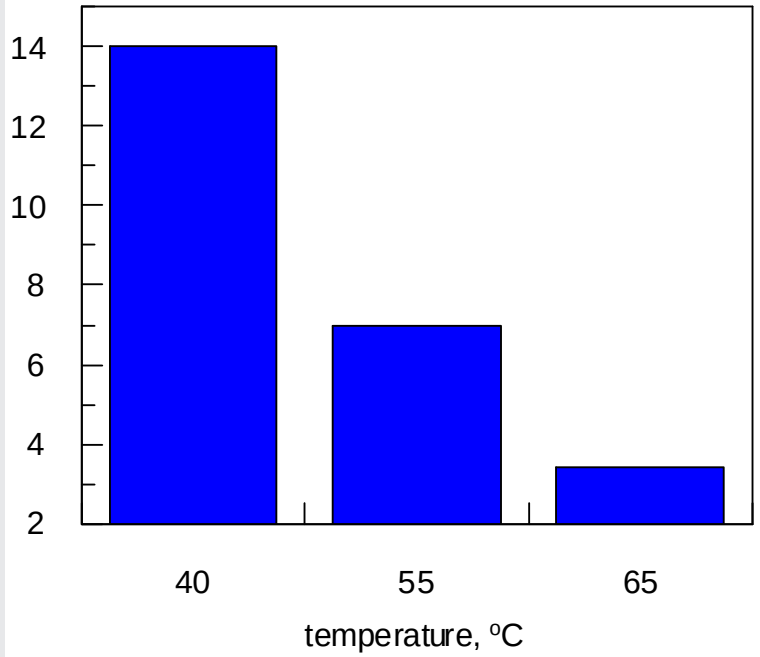
- Increased rate of cellulase activity, less energy cost for cooling, higher DS loading, and decreased risk of contamination.
- Can be cloned and over-expressed at high levels in *fungal hosts*.
- Ease of DSP/Formulation since one can introduce heat step to precipitate *fungal host* proteins.
- Biotransformation reactions can be carried out at higher temperatures where accessibility to substrates gets better and lignin is less tightly associated with cellulose.
- Enzymes are more robust to exposure of inhibitors and product ethanol.
- More resistant to proteolysis.

DSM Cellulosic Enzyme System Performance

Washed pWS before (left) and after (right) hydrolysis.



Enzyme Dosage



DSM enzymes work faster at higher temperatures

Lowering both Capital & Operating Cost

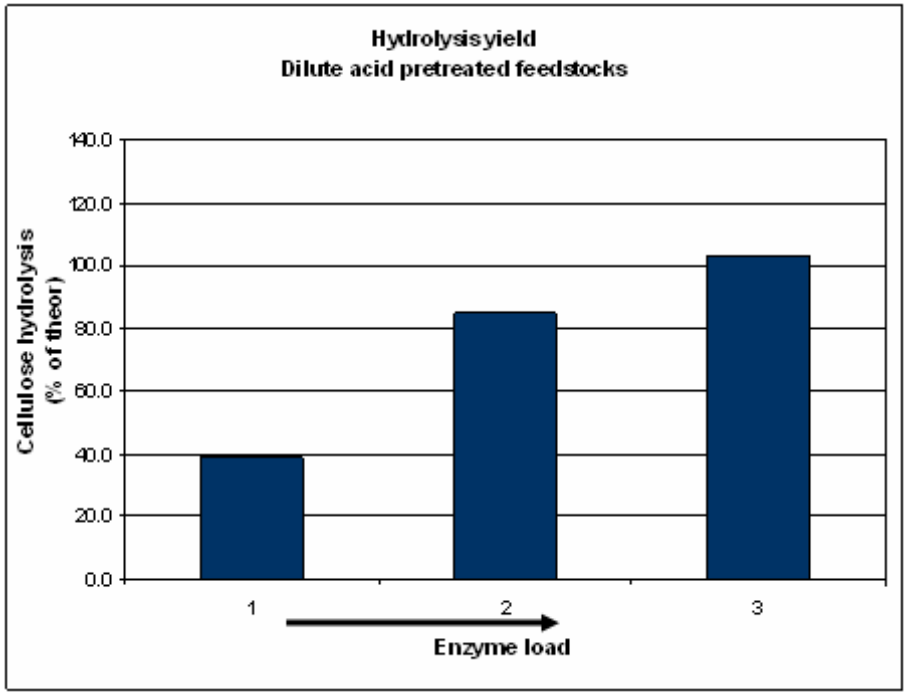
Dosage

- Enzyme dosage is a key parameter for its cost contribution in making cellulosic ethanol.
- Lets compare DSM Enzymes at 50 and 60 °C
  - Application tests:
    - 20 % TS pWS (unwashed)
    - 72 h hydrolysis (50°C and 60°C)
    - 72 h fermentation (33 °C) with C6 yeast

- Experimental results obtained at 50 & 60 °C suggest 20-24% reduction in DSM enzyme cocktail dosage when operating at 60C vs 50 C to achieve same hydrolysis yield.

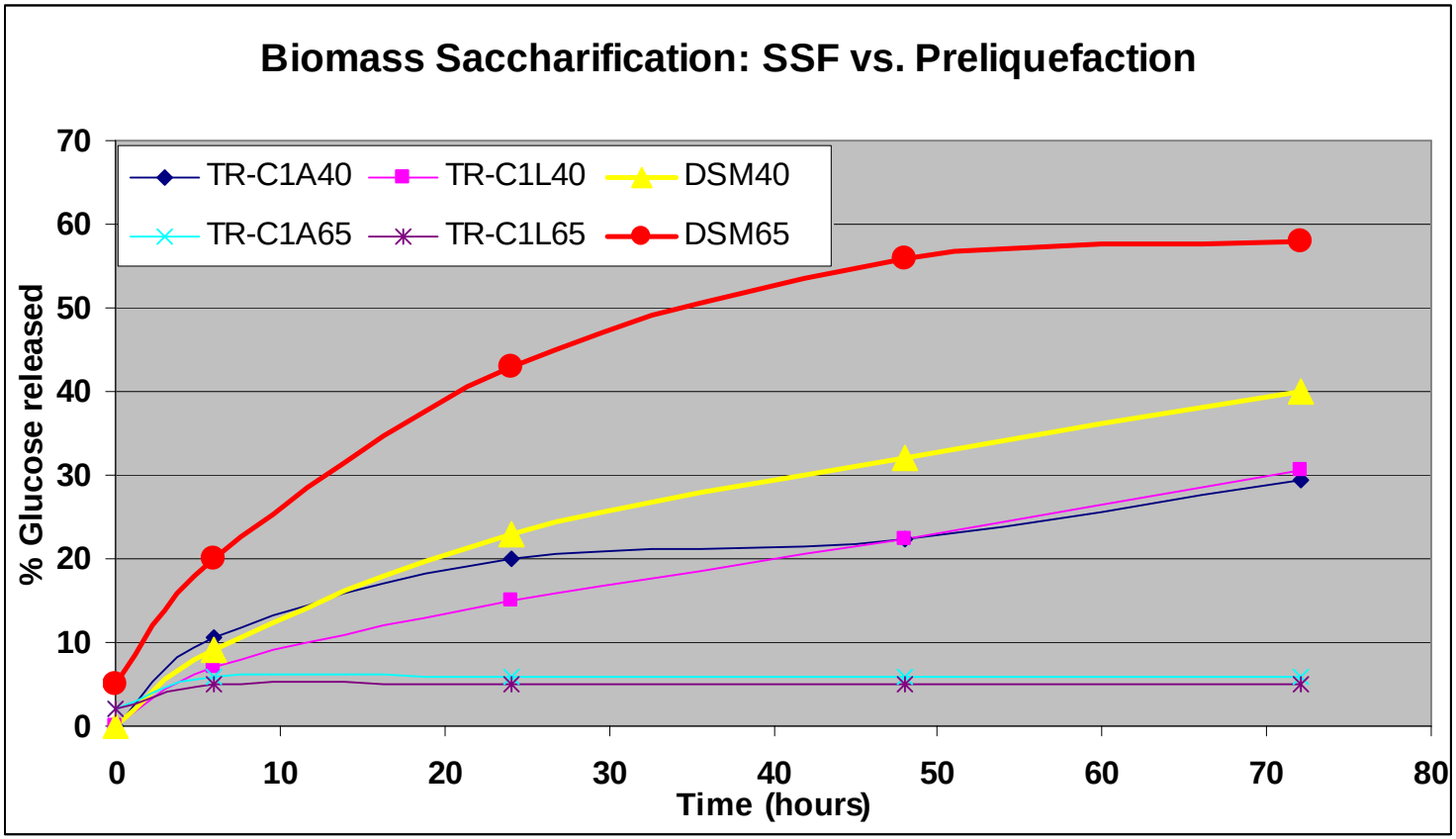
Proprietary DSM Enzymes Applied in Lignocellulose Hydrolysis

- Proprietary Dilute acid pretreated lignocellulosic feedstock converted at 10% dry matter
- DSM cellulase added and incubated to reduce viscosity and produce monomeric sugars for fermentation
- DSM *C6*-Yeast in Simultaneous Saccharification and Fermentation



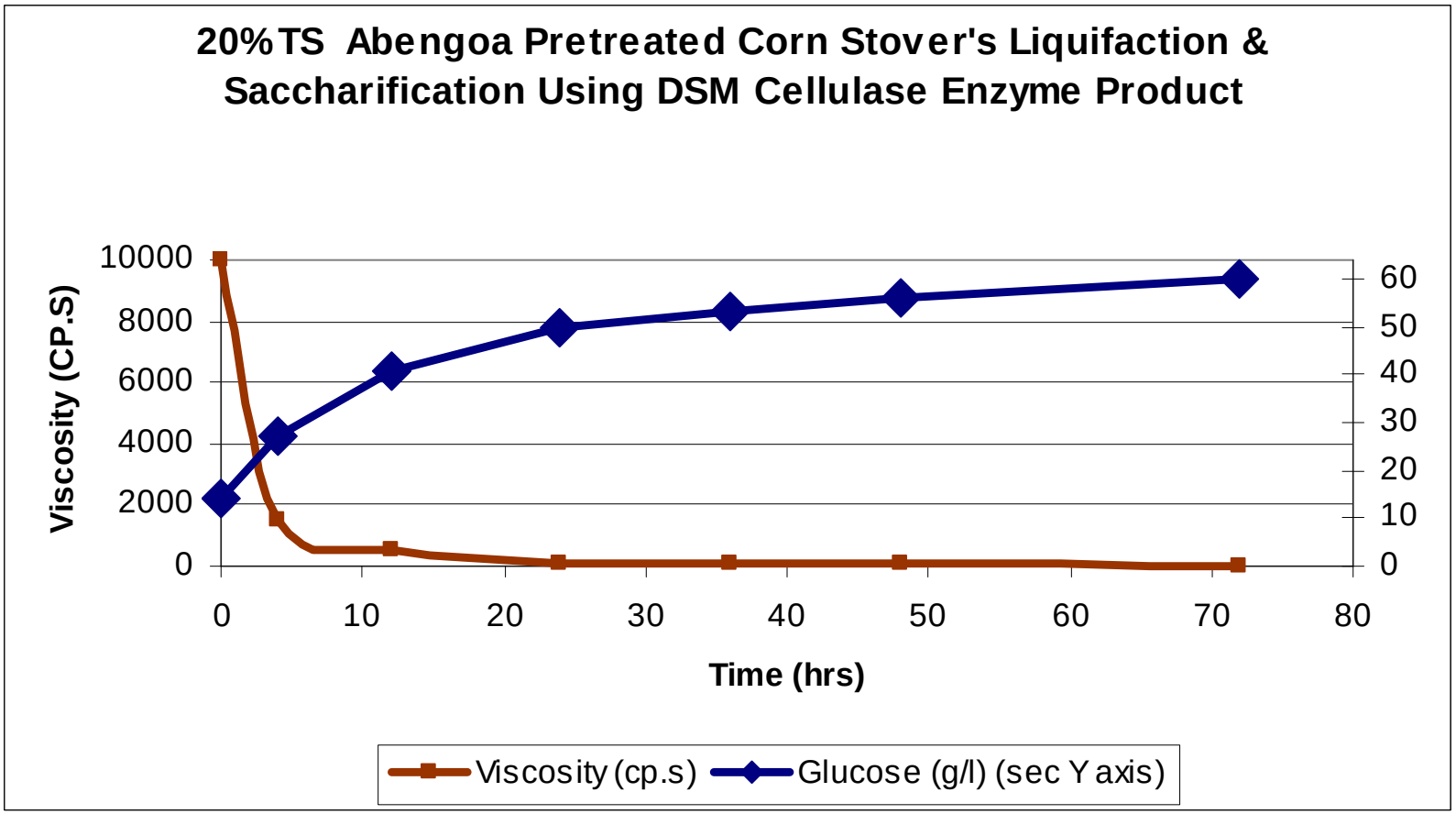
Complete cellulose conversion in Simultaneous Saccharification and Fermentation (SSF)

DSM Cellulase Enzyme Cocktail Performance



- ***Lower viscosity and avoid bacterial contamination***

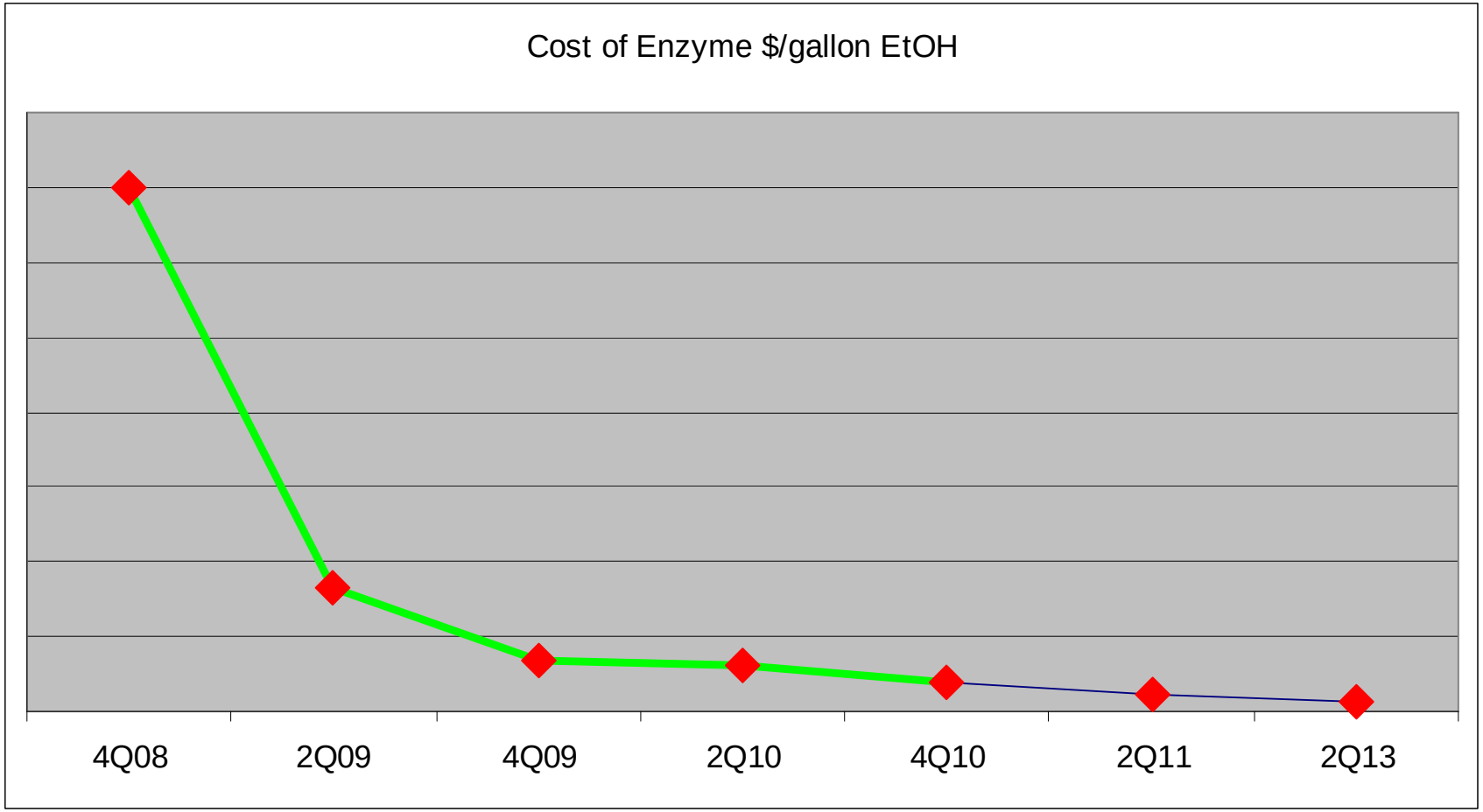
Pilot Scale (2000 liter) Test of DSM Enzyme @ ABNT York Site



Stability, More Robust, Ease to Use

- DSM whole broth enzyme cocktail has a long storage stability of so far minimum of 3 months at RT, potentially related to the thermo-stability.
- Whole broth enzyme stability is essential for building the *on-site* manufacturing and supply chain reliability.
- Cost of shelf, warehousing, transport, and inventory maintenance minimized
- DSM enzyme retained its full activity during SSF/SHF operation using 20% DS unwashed pCS (400h at 60 °C).
- Easy to use from an operation point of view.

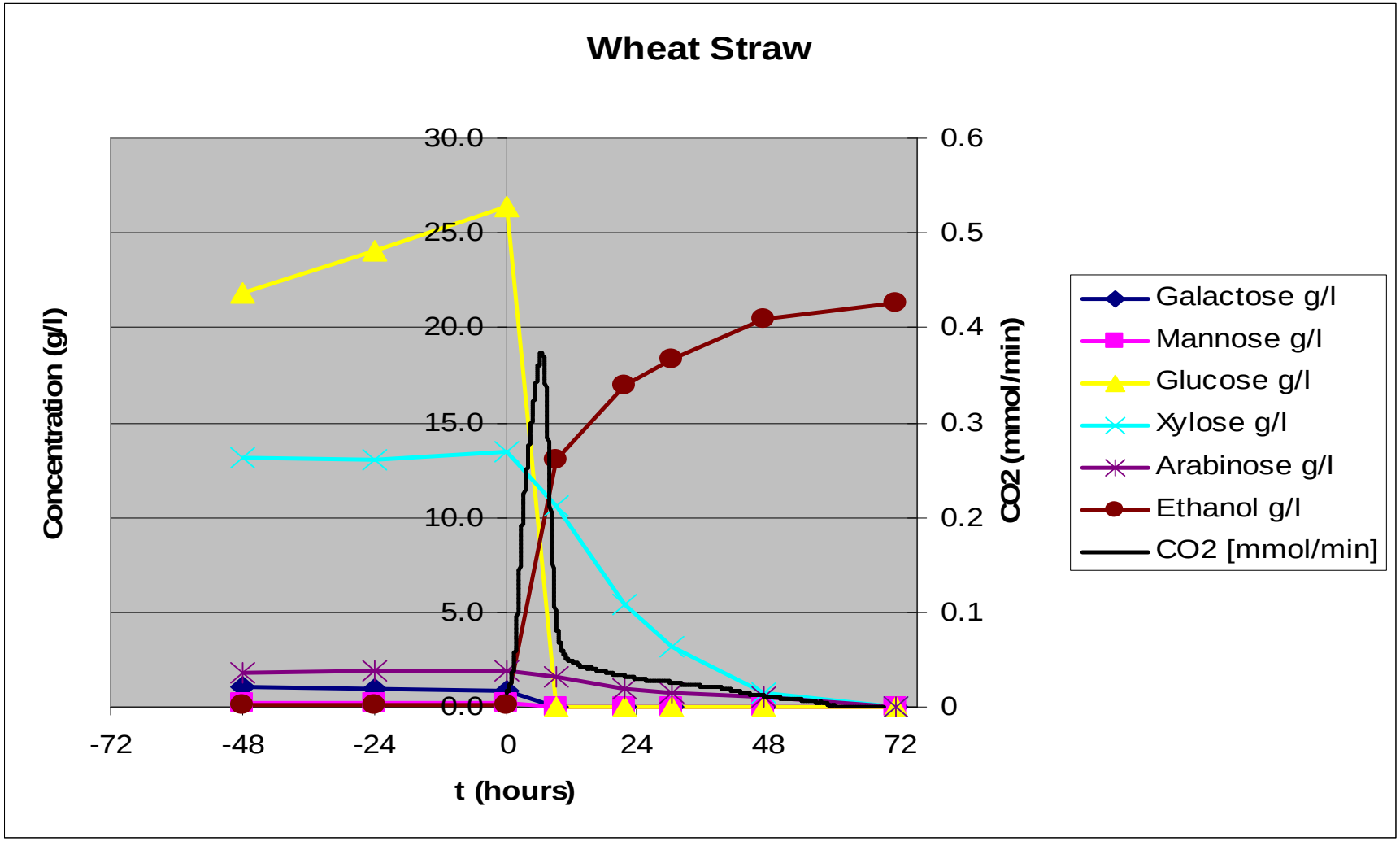
Enzyme Cost Reduction: On Target



DSM Engineered Cellulases Solution

- Differentiated and Tailor made for Biomass saccharification
  - Peers enzymes based on *Trichoderma* technology basically originated for textile and paper industry
- Thermostable enzymes suited to work at 65C vs. 40-50C for *Trichoderma*
  - Lower dosage, no contamination, higher dry solid loading
- DSM enzyme system efficient for SSF, SHF, SHCF
- Fast viscosity reduction allowing higher DS via fed-batch
- No interference with yeast growth
- Insignificant inhibition (glucose) up to 6% w/w
- No Inhibition (ethanol) up to 8% w/w
- On-site manufacturing/ whole broth: provides enough nutrients for yeast growth

Integrated Performance: *10% TS Wheat-straw (low severity dilute acid PT)*



Pretreatment conditions were150'C, 20 min residence time, acid loading of 1% v/v H2SO4 on total slurry.

Technical Progress (Oct'08 – Dec'10)

- ***Technical Accomplishments***
  - ***NREL validation of chosen commercial but new cellulase enzyme technology***
  - ***8-10x improvement in enzyme expression achieved at lab scale***
  - ***Addressing MYPP technical Barrier BT-F***
  - ***Relevance to MYPP Milestone M 4.5.1***
  - ***Tasks A, B, C, D, F, G, H, and I contributed to project deliverables and progressing as planned***
  - ***All tasks except task J are currently executed***

Success Factors and Challenges

- ***Critical Success Factors***
  - ***Technical***
    - ***Reaching the targeted enzyme cocktail expression titer and efficacy***
    - ***Vertical integration of upstream and down stream technology***
    - ***Availability of cost effective feed-stock***
    - ***Low to no cost down-stream processing***
  - ***Market***
    - ***No market exists for cellulase enzymes today for biofuels***
    - ***Enzyme manufacturing investments only expected when demand is foreseen and rest of the technology segments are integrated***
  - ***Business***
    - ***DSM business model is based on an integrated biorefinary platform where biochemical conversion technology pieces are vertically aligned.***
    - ***Integrated production of cellulase enzyme cocktail production on site of a biorefinery***

Success Factors and Challenges

- ***The top 2-3 potential challenges***
  - ***Technical***
    - ***Successful vertical integration of cellulase enzyme cocktail developed with pretreatment technology and ethanologen in a cost effective manner overcoming inhibitors issues at the demo and production scale.***
    - ***Higher gravity saccharification and fermentation.***
    - ***Multi component enzyme cocktail optimization for both efficacy and cost.***
  - ***Non-technical***
    - ***High risk R&D investment***
    - ***Trained and experienced work-force in biomass R&D***
    - ***World oil and commodity pricing***

Success Factors and Challenges

- ***Window of opportunity to develop this technology @ DSM***
  - ***High- business model developed based on vertical integration of technology pieces and provider of full package of biochemical conversion technology i.e. both ethanologen c5/c6 yeast and cellulase enzyme cocktail.***
  - ***DSM envisions to bring this technology to the market in the time frame aligned with completion and going critical of biomass based biofuel demo plants. This is similar to DoE MYPP goals and vision.***

- ***the biorefinery industry***
  - ***This technology aims to provide better integration with up-stream and down stream technology pieces and gives new and better alternatives to similar technology solutions and thus will have excellent opportunity to be developed within broader biorefinary industry.***

- ***the market***
  - ***Market for cellulosic biofuels exists today based on government mandates. However, it is waiting for an integrated technology solution and building of commercial scale cellulosic ethanol plants. This provides a good umbrella to develop DSM technology.***
  - ***DSM, the largest fermentation based chemicals product company has high stakes in the creation of LC feed-stock that will support and sustain its biotech based business opportunities.***

Future Work

- **Remaining work plan (Sep 2012)**
  - **Continue to improve the titer of cellulase cocktail**
  - **Develop robust and cost effective fermentation process**
  - **Improve enzyme efficiency**

- **Upcoming key milestones**
  - **Selection of final fermentation production host for enzyme**
  - **Development of fermentation process to deliver the cost**
  - **3x enzyme application testing at small pilot scale**

- **Decision points and issues resolution**
  - **Result based decision and milestones are in place in PMP and including back up plans instituted for alternate strategy deployment to meet the targets.**

Summary

- **Relevance**
  - **DSM's development of thermally stable and acidic pH cellulase cocktail for multiple benefits**
- **Approach**
  - **Use of DSM's decades of experience in industrial biotechnology**
    - **Largest company with fermentation-based product revenue ($1.5 billion)**
- **Technical accomplishments**
  - **Validation of chosen commercial but new cellulase technology platform**
  - **In two years improved cellulase production titer by 8-10 folds**
- **Success factors and challenges**
  - **Vertical integration and efficacious enzyme system**
  - **Cost effective development of multi-component cellulase cocktail**
- **Near term future work**
  - **Selecting the final enzyme production host**

DSM: *creating better life everywhere*

Responses to Previous Reviewers' Comments

- **1. Relevance to overall Program objectives and market need**
  - Relatively late entry into cellulose deconstruction area.
    - This project has made significant progress towards its deliverables in short period of two years in comparison to its peers. Given that there is no current commercial cellulosic biofuel plant in operation today but expected to come online by 2012, DSM is confident its *"Development of a Commercial Enzyme System for Lignocellulosic Biomass Saccarification" project will be able to deliver a competitive product to this market.*
- **2. Approach to performing the Research, Development and Demonstration (RD&&D)**
  - Still developing host fungal production systems. General technology for all protein production, not focused on cellulose-degrading enzymes.
    - PI Response: Choice of fungal host is made and development of host fungal is nearly complete. Focus on thermostable cellulose-degrading enzymes is made for various economical and technical reasons as explained in this presentation.
- **3. Technical Progress and Accomplishments**
  - Standards for comparison do not appear to be the industrial standards. An incorporation of other commercial enzymes would provide further validation of progress.
  - It is not clear how the results of the enzyme improvement compare to others already available. Their comparisons are all with in-house variants.
  - Not clear if DSM enzymes are significantly better than those from other suppliers
    - PI Response: Comparative enzyme analysis with commercial enzymes are now completed and is incorporated in this review.
- **4. Critical Success Factors and Showstoppers**
  - High-throughput assay not yet developed.
    - PI Response: HTP Assay has now been developed and routinely used
  - still trying to develop assays, this is essential - also still working on hosts for enzyme production, this could be a limiting factor in doing the rest of the proposal
    - PI Response Enzyme production host selection is in place now and fermentation process development using this host is in full pace.
- **5. Proposed Future Research approach and relevance (as defined in the project).**
  - Will have to validate with use on NREL corn stover please publish results, make publically available
    - Validation with NREL corn stover is completed by both internally and by NREL it self.

Publications and Presentations

- **Multiple presentations have resulted from work done under this project**
  - **2010 & 2009 Bio world Congress on Industrial Biotechnology**
  - **2010 Fuel Ethanol Workshop**
  - **2010 SIM meeting on Fuels and Chemicals**
  - **2009 and 2010 ACS Annual Meetings**
  - **A manuscript is under preparation for publication**