Highly Efficient Thermostable DSM Cellulases: Why & How?

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Director Science & Technology NA
DSM Bio-based Products & Services

1st World Congress of Bioenergy
Dalian, PRC

April 26, 2011

HEALTH · NUTRITION · MATERIALS

DSM Key Figures

Net sales € 8.2 billion (2010); ~21,911 employees worldwide

Life Sciences and Materials Sciences Company

Strong Technological Toolbox

In Top 30 Global Chemical Companies

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DSM Innovation in Biotechnology

Today's Technology Built on Historical Strengths

CLASSICAL BIOTECH (1870's onward)

- Yeast
- Ethanol
- Yeast extracts
- Vitamins
- Penicillin
- Enzymes
- Citric acid

MODERN BIOTECH (After 1980's)

- Recombinant enzymes *(e.g. Chymosin)*
- Metabolic engineering *(e.g. Vitamin B2,Cephalexin)*
- Biocatalysis *(e.g. Pharma intermediates)*
- Cell culture *(e.g. Per·C6TM cell line)*

INDUSTRIAL BIOTECH (Since 2005)

- Biomass conversion technologies
- Biosuccinium™ bio-based succinic acid
- Emerging portfolio

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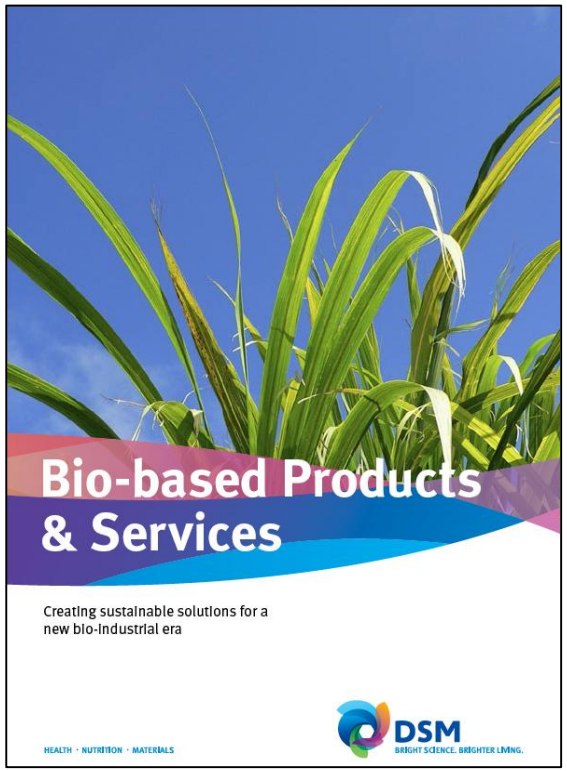
Bio-based Products & Services

Creating sustainable solutions for a new bio-industrial era



- A dedicated group within DSM
  - Biogas
  - Bio-based Chemicals & Products
  - Bio-based Chemicals & Polymers
  - Bio-Conversion Solutions
- Bio-Conversion Solutions
  - Delivers a technology package to enable the production of 2G biofuels and bio-based chemical
  - Our current primary application target is bioethanol based upon the co-development of cellulase enzymes and advanced yeast

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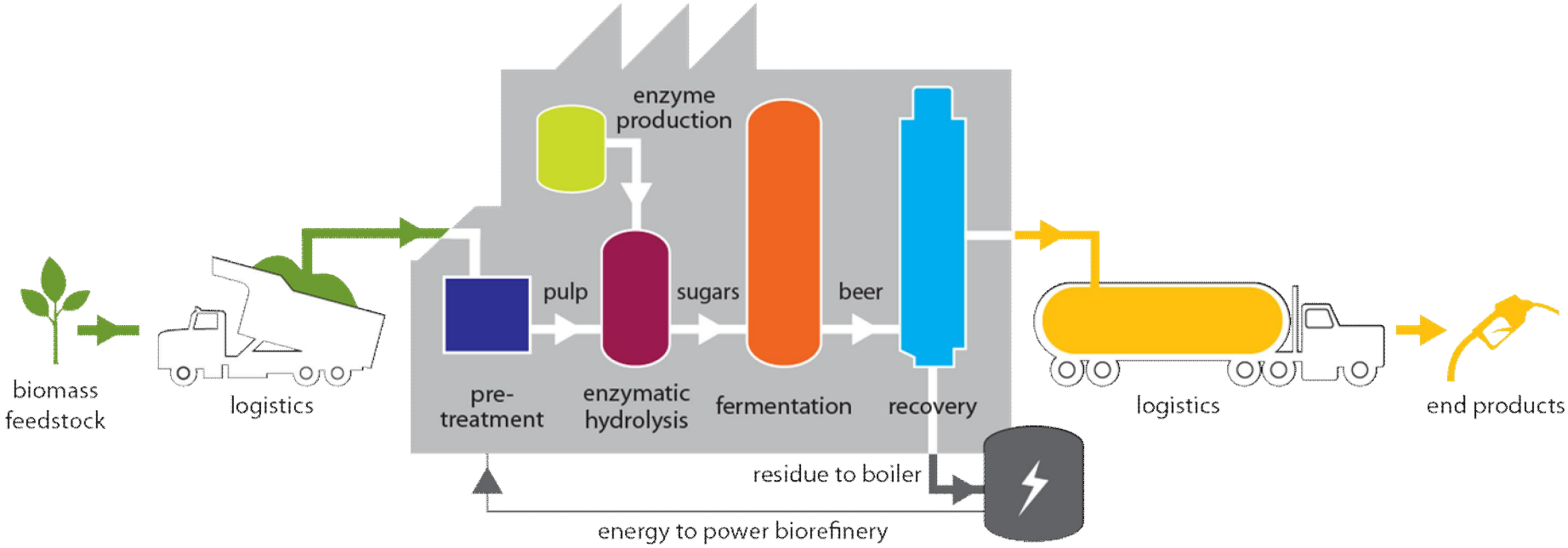
Biorefinery for 2G Bio-ethanol

Process promising but requires substantial innovations



- Yeast and Enzymes are crucial for 2G Bio-ethanol
- DSM's yeast and enzyme technologies is unique combination

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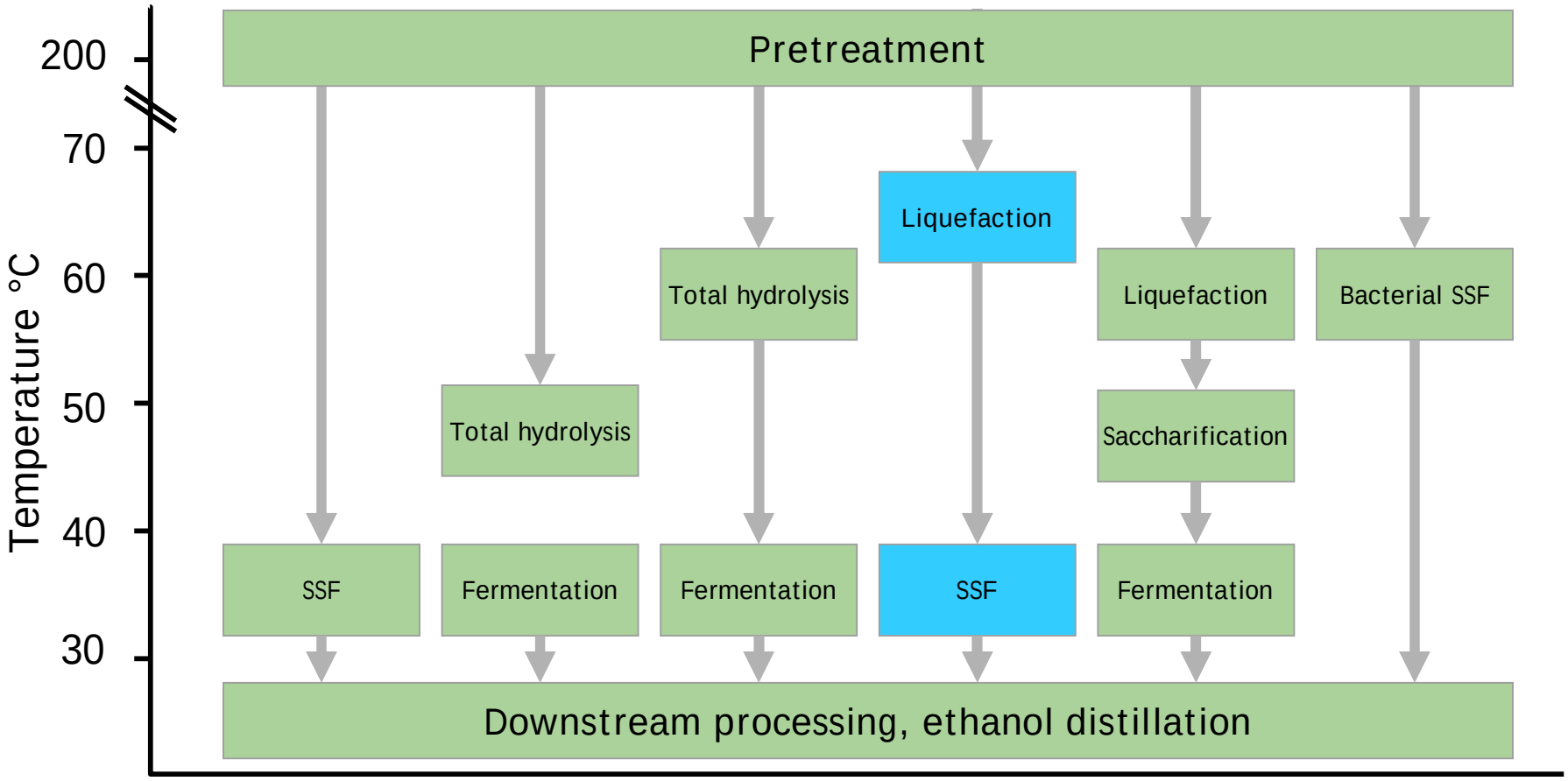
Configuration of Various Hydrolysis and Fermentation Processes



Ref: Liisa Viikari, 2007, Adv Biochem Eng. Biotech

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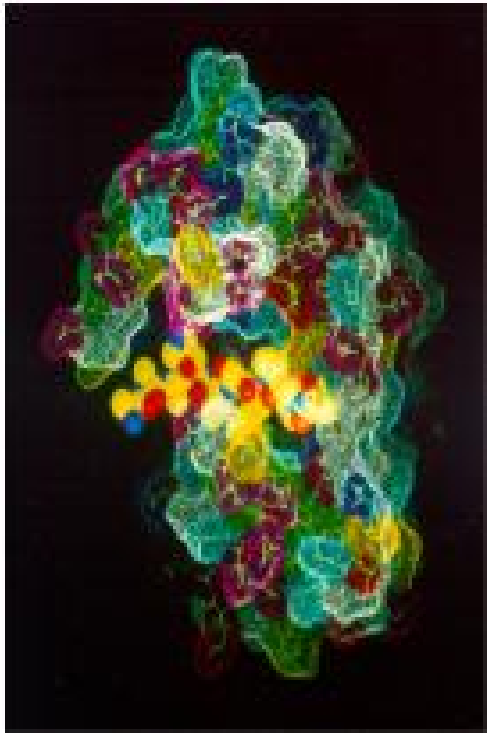
Enzymes: *A Core Bio-based Building Block Platform for DSM*



- DSM possesses a strong knowledge base within enzyme research, application and manufacturing
- DSM holds hundreds of patents based on enzyme technology including biomass deconstruction enzymes
- DSM sells $300MM worth of enzymes in baking, food & fruit processing, brewing, wine, dairy, animal feed, antibiotics, flavors, and biocatalysis
- US DoE Partnership with DSM for commercial development of enzymes for lignocellulosic feed-stocks saccharification.
- DSM has a global enzyme business

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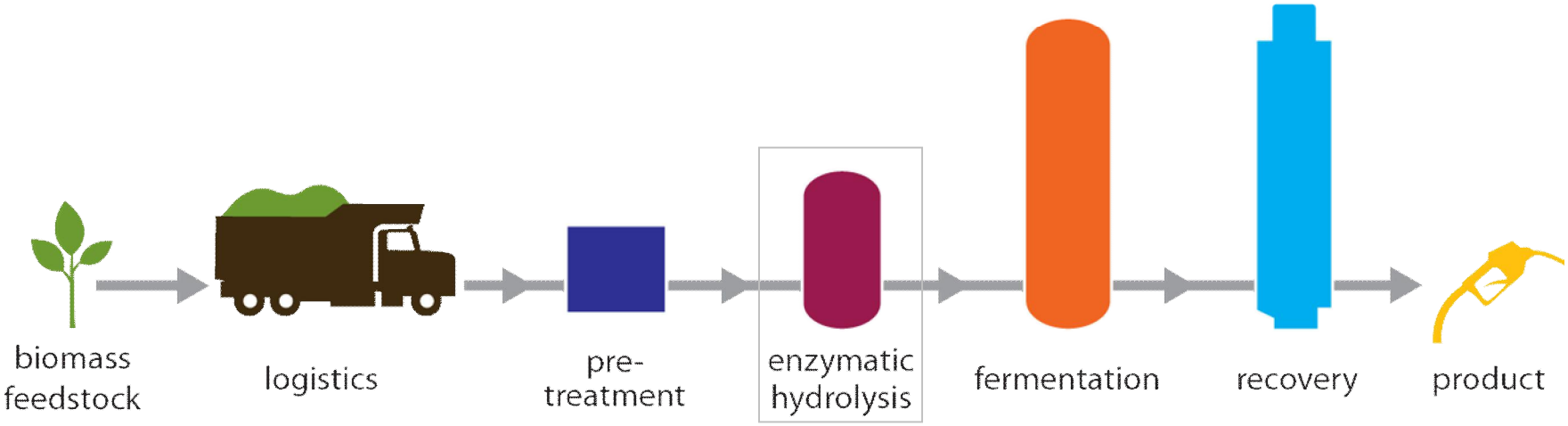
Choosing the Right Enzymes



- DSM has searched its collection of food enzymes for suitable thermophilic, acid stable enzymes for application in 2G bioethanol
- These enzymes have been optimized for 2G bioethanol production

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Thermostable Cellulases: *Why ?*

- 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.

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Viscosity Reduction

- Positive effect of temperature on liquifaction of the solids.
  - At 65 C the solids become faster gelatin-like in comparison with 40C when treated with DSM enzyme.
    - higher absorption of water to the polymers.
  - Faster gelatinizing resulting in easier accessibility of the substrate by the enzymes
- The effect of higher temperature (65°C in comparison to 50 °C) on viscosity
  - sugar concentrations ~100 g/L. (approx equal to complete hydrolysis of 20 % wheat straw)
    - 50 °C: 70 Pascal.sec
    - 65 °C: 60 Pascal.sec
- Higher throughput in all process steps
- Reduction in $/gallon capital installed having
  - lower capital and fixed costs, less evaporation energy per gallon produced.

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Prevention of Contamination

- Operating at higher temperature during hydrolysis will result in a *'more sterile'* hydrolysate.
  - Hydrolysate obtained at 65°C will have a significantly lower bio-burden than hydrolysate of 45-50°C.
  - If hydrolysis time is extended and thereby fermentation time is shortened, *the risk on contamination during fermentation will also be significantly reduced.*

- An important advantage of DSM enzyme cocktail. A loss of 5% due to contamination problems is *not uncommon* for this type of industrial operations.
  - At 60 $/T feedstock and 80 gal/T ethanol yield, a 5% loss = 0.0375 $/gal.
  - At 3 $/gal installed capital and 10% depreciation = 0.015 $/gal
  - At 0.15 $/gal fixed costs = 0.0075 $/gal

- So the cost of contamination is then 0.06 $/gal.

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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.

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Energy Costs Saving

- The energy cost savings are related to the delta temperature between the unit operations during the whole process.
  - When fermentation is done at a higher temperature, cooling costs can be significantly lower.
- The advantage of thermostable enzymes
  - can be added in an earlier stage (higher temperature) after pretreatment thus reduced chilling cost to start liquefaction/saccharification/biomass hydrolysis
  - Reduction in overall process time.
  - Energy cost reduction significance depends on scale, process, and equipment.
- Reduction of energy cost on mixing during hydrolysis.
  - Adding enzymes in an earlier stage of the process also results in an earlier liquefaction which makes mixing easier
- Earlier liquefaction allows reduction in cooling capacity:
    - heat transfer is better in a mixed liquid than a static solid (or slurry).
- DSM thermostable cellulase cocktail is fully active between 60-65C.
  - cooling rate reduction during hydrolysis
  - reduction in cooling capacity

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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.

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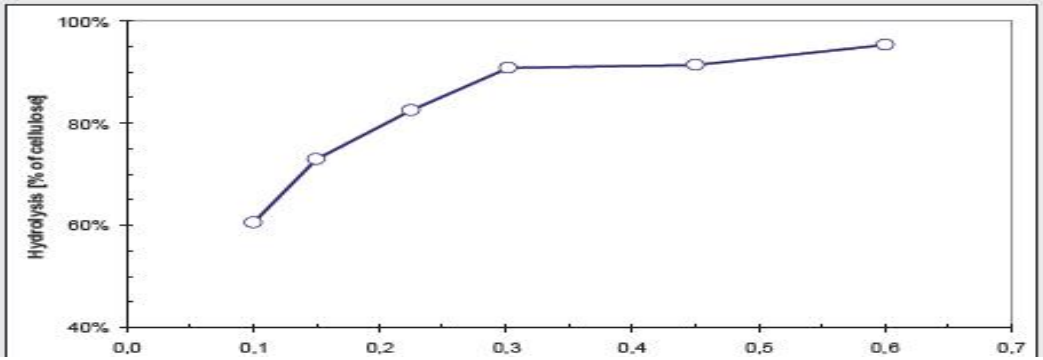
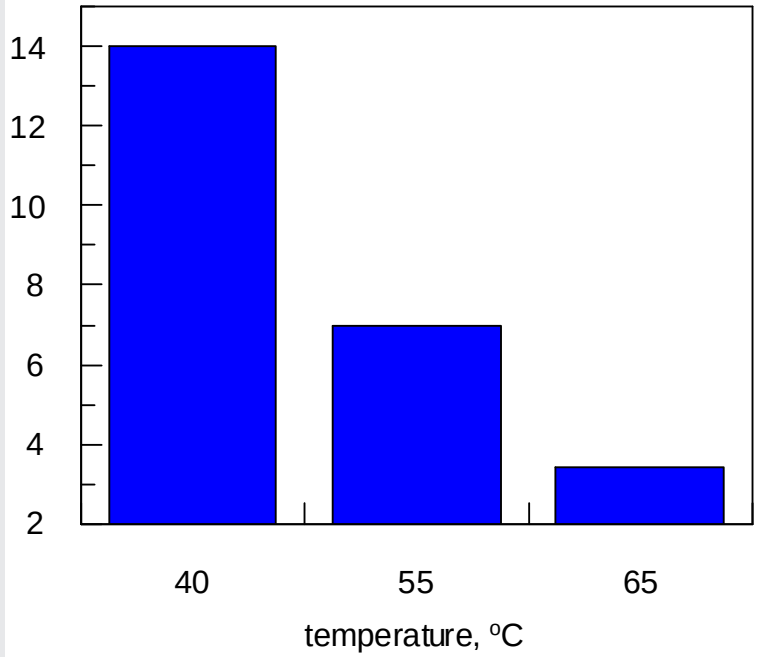
DSM Cellulosic Enzyme System Performance

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



Enzyme Dosage



DSM enzymes work faster at higher temperatures

Lowering both Capital & Operating Cost

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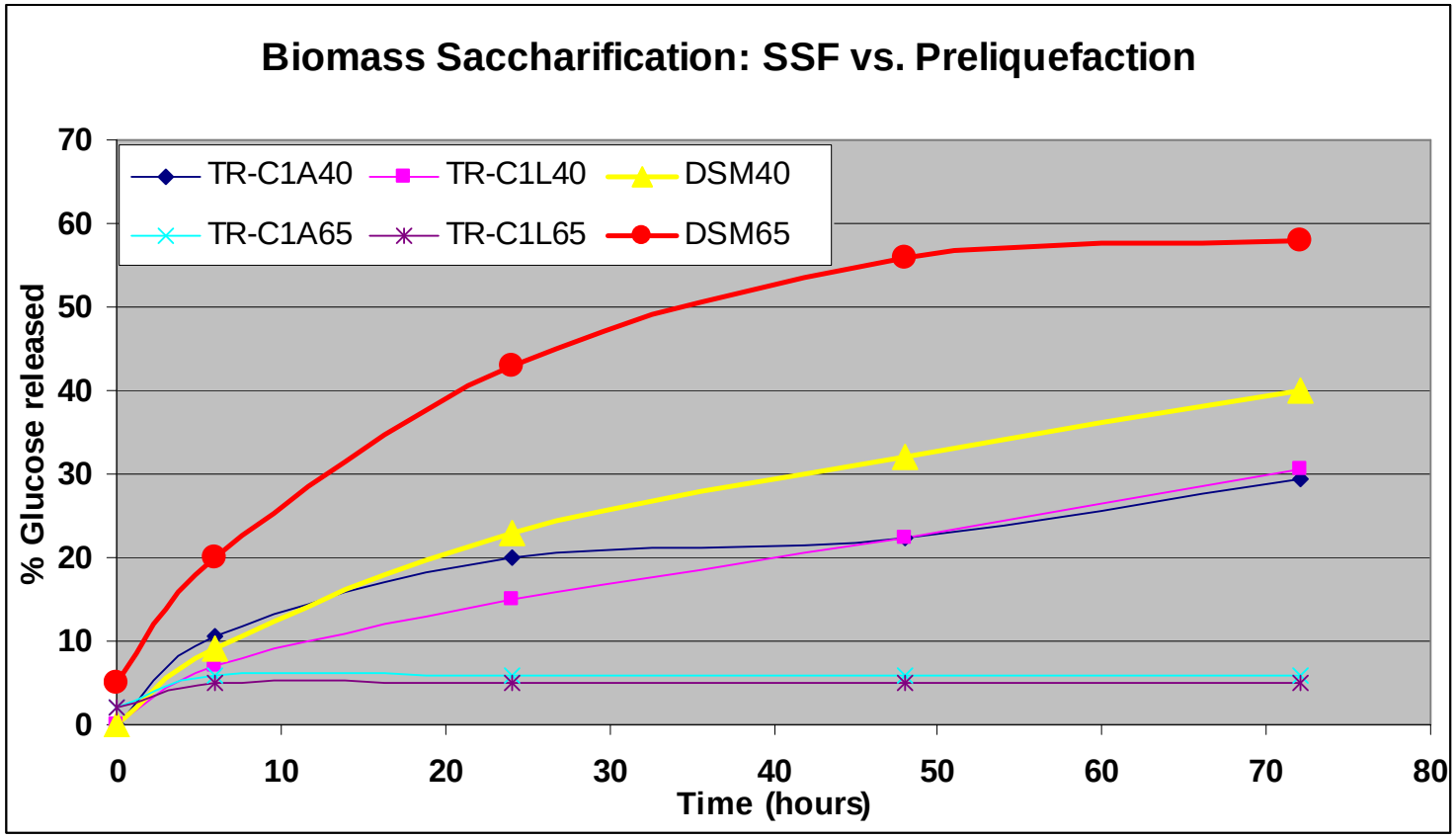
DSM Cellulase Enzyme Cocktail Performance



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Successful Feed-Stock Tests with DSM Enzyme Cocktail

- Corn fiber (dilute acid; hot water; steam explosion)
- Corn stover (dilute acid)
- Wheat straw (dilute acid; hot water; steam explosion)
- Spruce (SO_2 catalyzed steam explosion)
- Switch grass (dilute acid)
- Poplar (dilute acid)

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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

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Thermostable Cellulases - *How?*

- Diversity
  - Large libraries of (bio)catalysts required
  - Access to genomes and meta-genomes
  - Directed evolution to optimize biocatalysts
- Screening Power
  - Automation and miniaturization
  - High through-put screening
  - Fast and reliable analysis
- Protein Engineering
  - Directed Evolution
  - Rational Design
  - Molecular biology assisted variants generation

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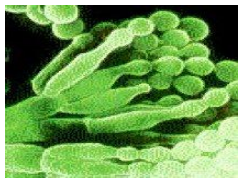
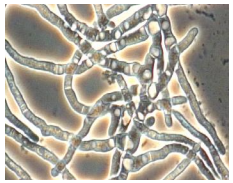
DSM pluGbug® : *Key to Industrial Process Development*

- Limited number of enzyme production organisms - focus
- Overexpression: higher activity per cell than natural isolates
- Higher cell density in less time as compared to isolates
- Higher volumetric productivity (activity / fermentation volume)
- Less interfering enzymatic activities (known background activities)

- Lower *Enzyme* costs & shorter development times





- pluGbug® technology for rapid and cost effective *Biocat* development
- *In-house* production with pluGbugs® provides maximum flexibility

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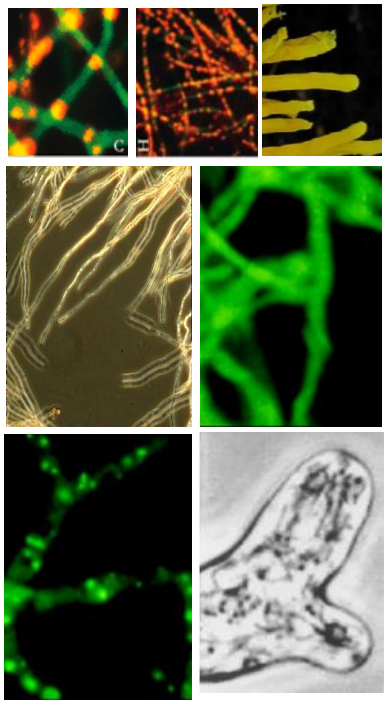
DSM Industrial Strain Development:
"Design & Build" concept

- 'Developing genetic tools and procedures to generate genetically pre-defined and stable rDNA production strains, improved for protein production, which possess finally no selection marker-gene at all'
  - (DSM Patents)
- Selection marker-gene free system
  - genetic tool for repeated modification of host properties
  - important legislation issue
- Modified *A.niger* pluGBug™ host
  - (Key Enzyme negative / protease poor / etc…..)
- Genetically entirely (pre-)defined using amplicons
  - all expression cassettes targeted in 'empty' *key enzyme* loci – controlled construction
  - prevents change in fermentation behavior – defined protocol
  - higher enzyme expression levels
  - early registration



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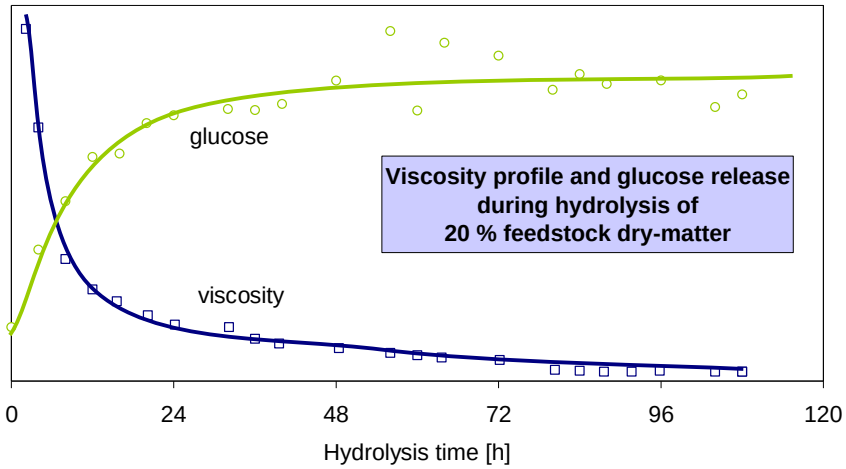
Summary: DSM Thermophilic Cellulases

Enzymes active above 60°C

- Fast liquefaction
- Fast viscosity reduction enabling high dry matter level
- Better control of microbiological contamination
- Resistant to higher temperature
- High tolerance for inhibitors
- Fit for different application methods



- Advantages of DSM Cellulases
  - Low enzyme dosing
  - Lower process costs

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DSM and Bio-Conversion Solutions

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