# Integrated cellulsoic enzymes hydrolysis and fermentative advanced yeast bioconversion solution ready for biomass biorefineries

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DSM Bio-based Products & Services

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HEALTH · NUTRITION · MATERIALS

# Our Team

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| Funding | | ***US Department of Energy*** | |

# 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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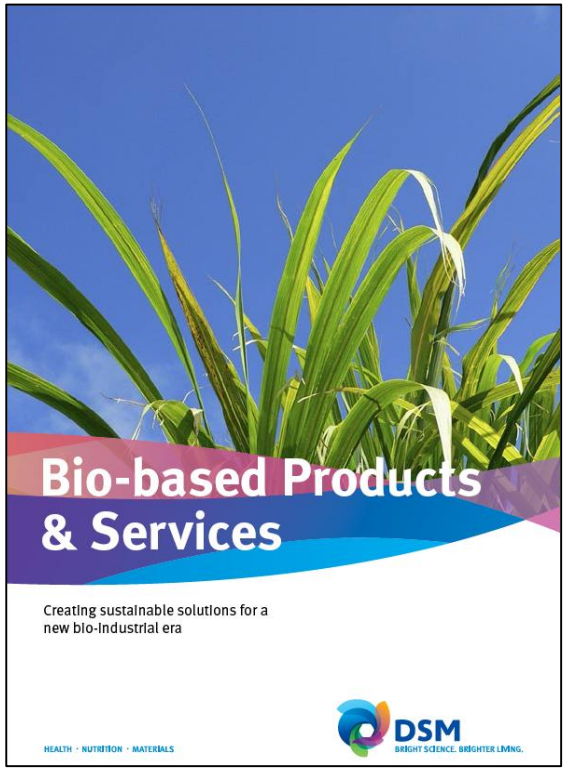
# Bio-based Products & Services

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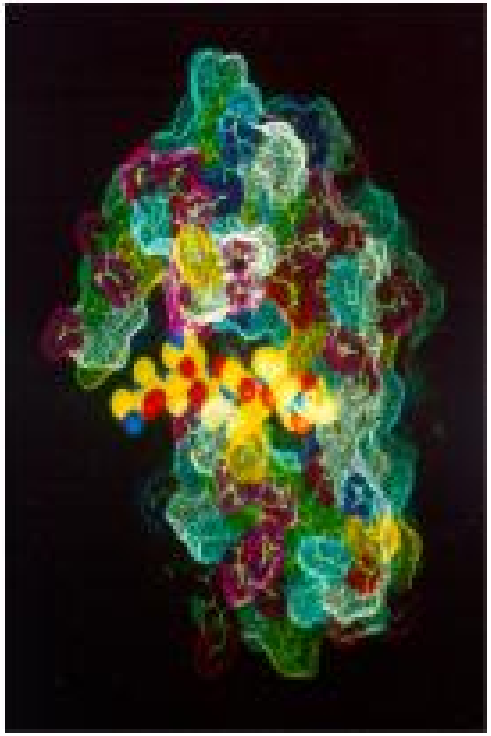
# Enzymes: *A Core Bio-based Building Block Platform for DSM*



- 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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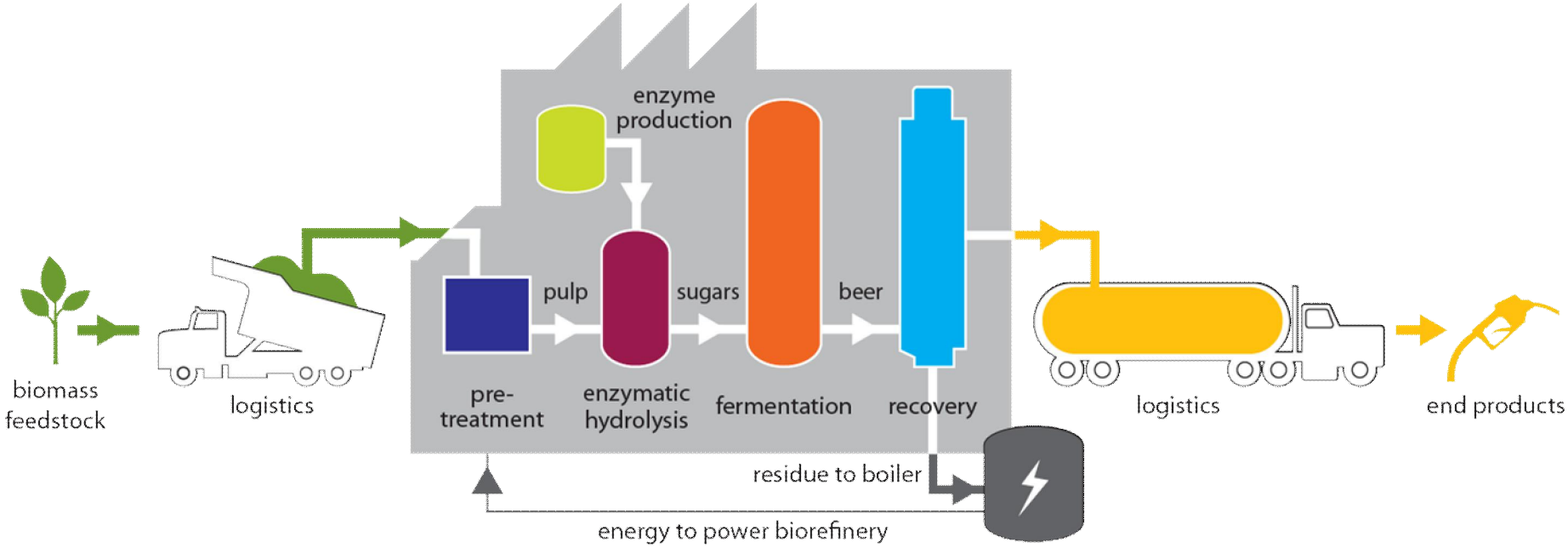
# Biorefinery for 2G Bio-ethanol

## 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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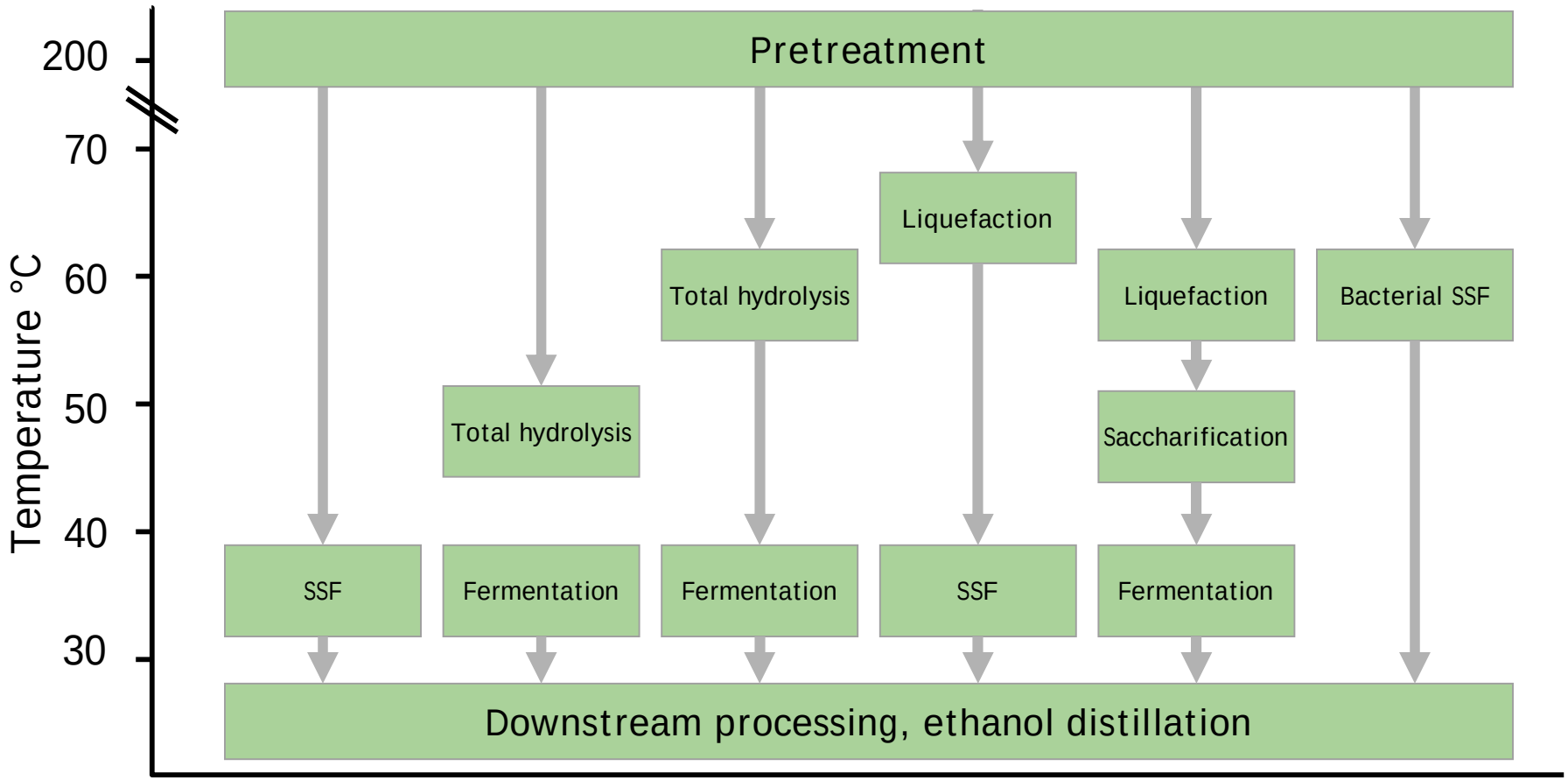
# Configuration of Various Hydrolysis and Fermentation Processes




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

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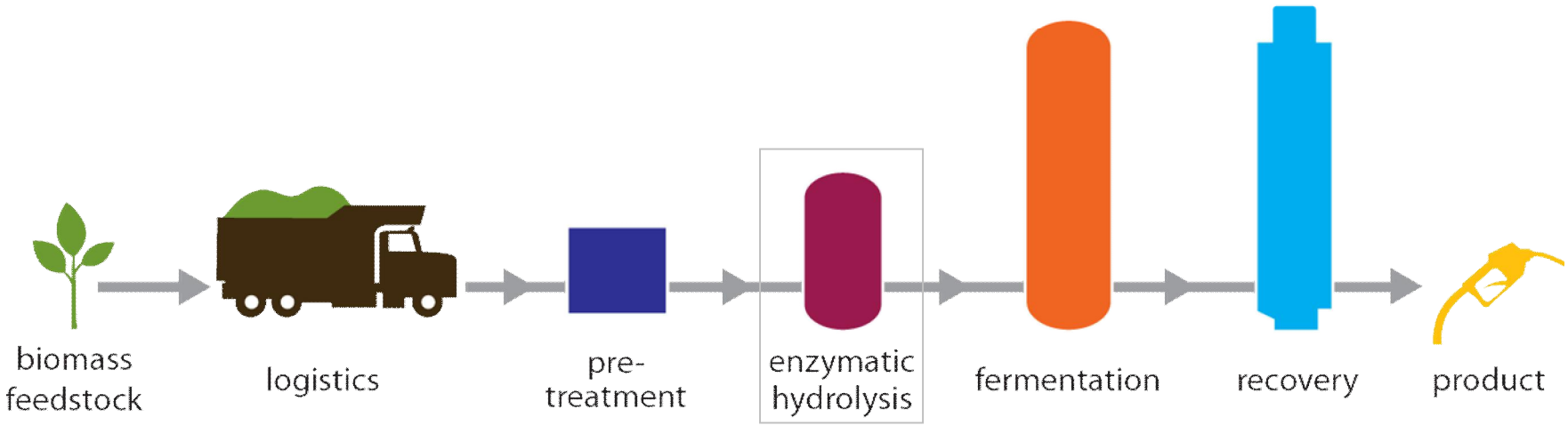
# Choosing the Right Enzymes




- 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 or No DSP 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 concentration ~100 g/L. (approx equal to complete hydrolysis of 20 % wheat straw)
    - 50 °C: 70 Pascal.sec
    - 65 °C: 60 Pascal.sec
    - *Lower viscosity = higher dry solids =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 = 0.04 $/gal.
  - At 6 $/gal installed capital and 10% depreciation = 0.03 $/gal
  - At 0.2 $/gal fixed costs = 0.01 $/gal

- So the cost of contamination is then 0.08 $/gal.

*NREL/TP-5100-47764 Biomass to Ethanol Process Design Report: $0.34/gallon enzyme cost*

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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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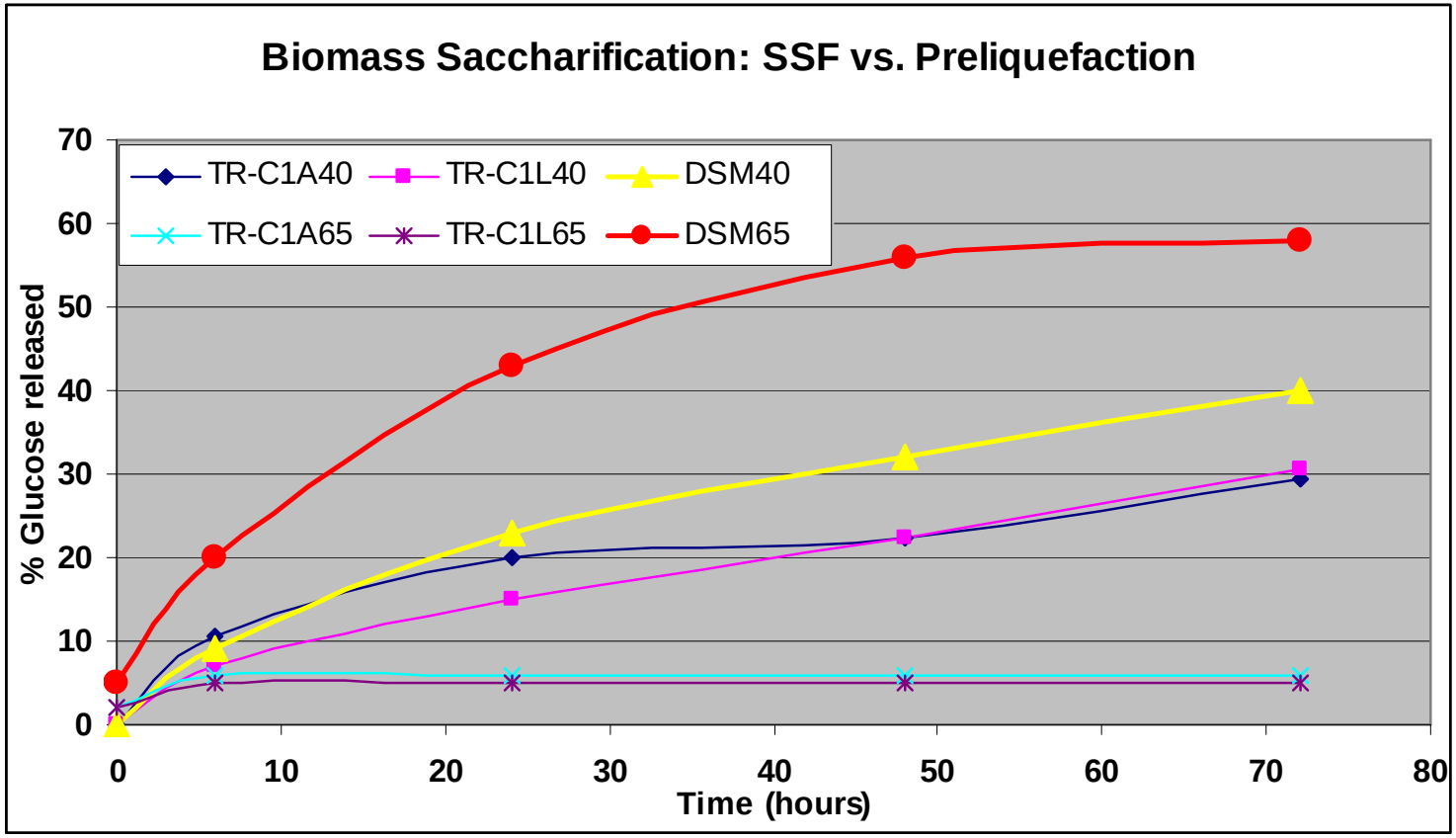
# DSM Cellulase Enzyme Cocktail Performance

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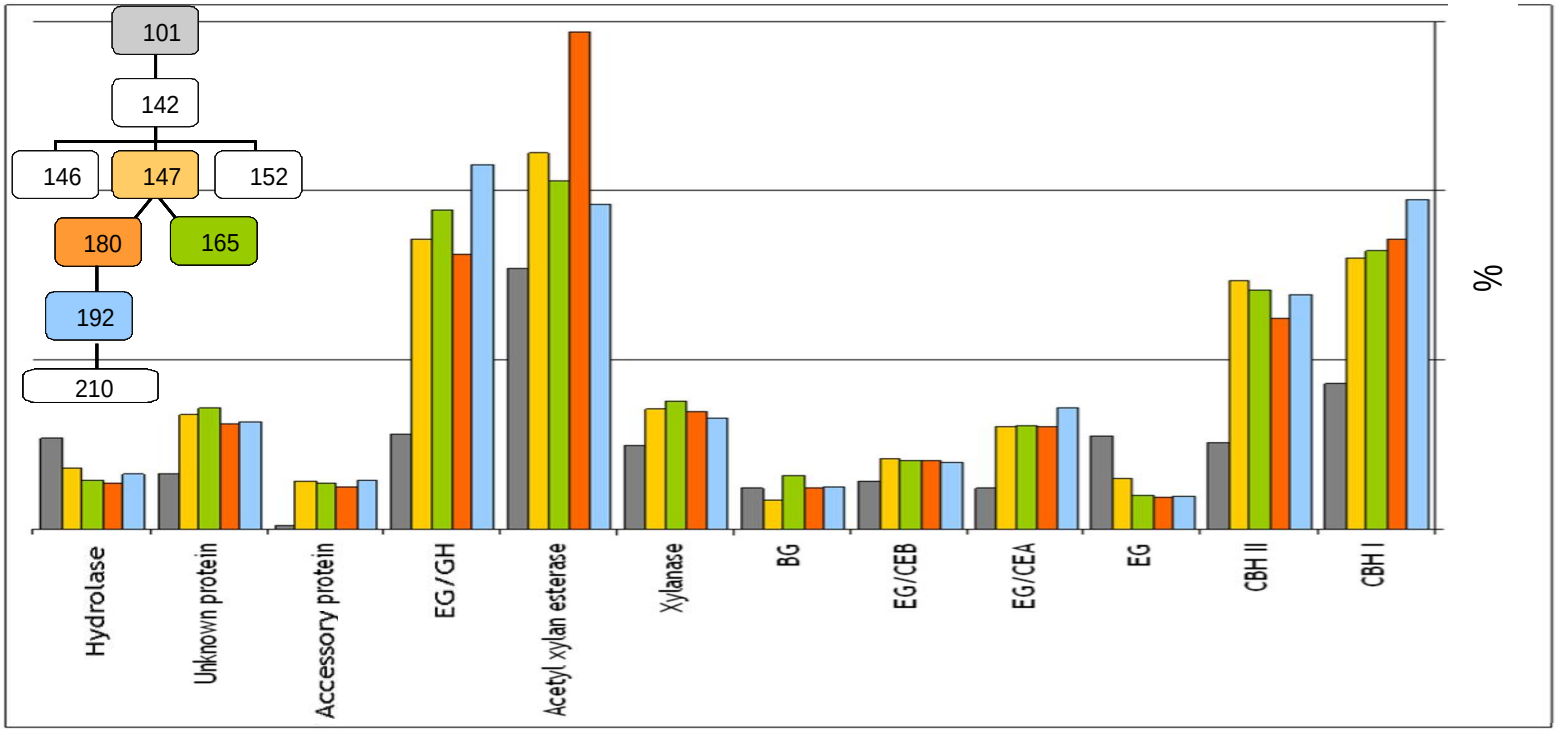
# DSM Thermophilic Enzyme Cocktail: Development Journey




Proteomic analysis of key cellulases from few DSM fungus mutants and wild-type strain.
The most abundant proteins were identified and their levels quantified. Differences in protein levels are given as the percent.

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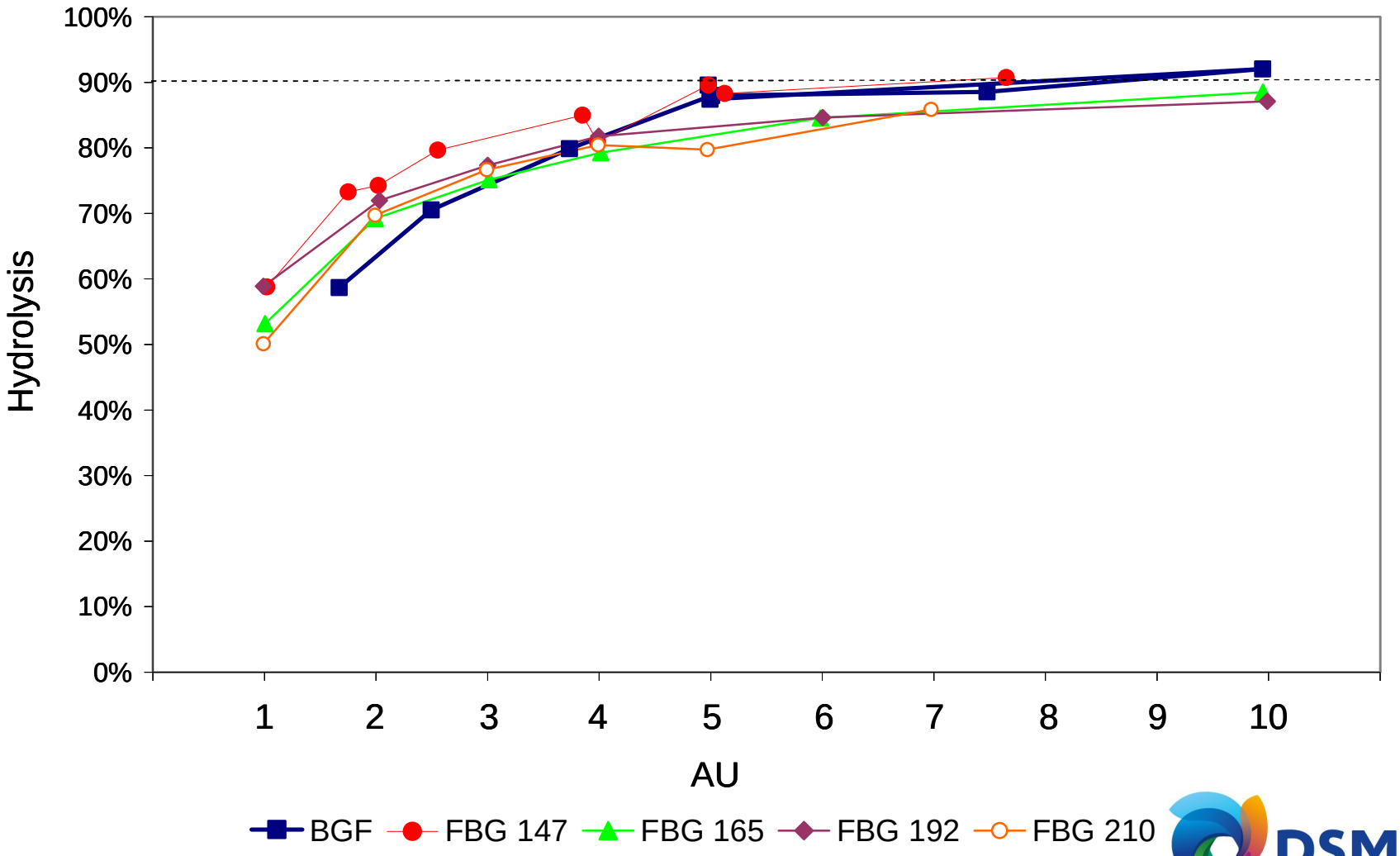
# DSM Thermophilic Enzyme Cocktail: Development Journey

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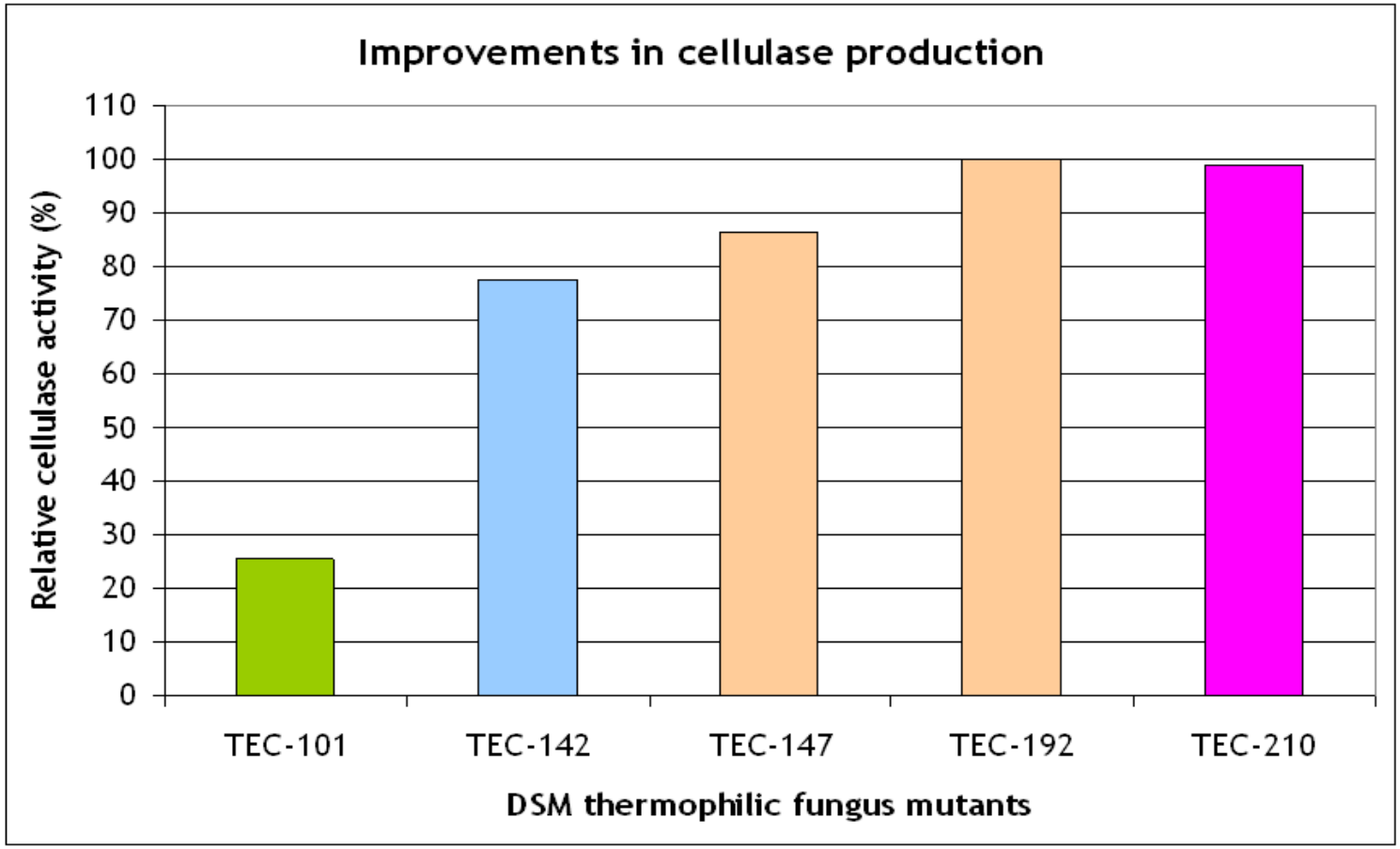


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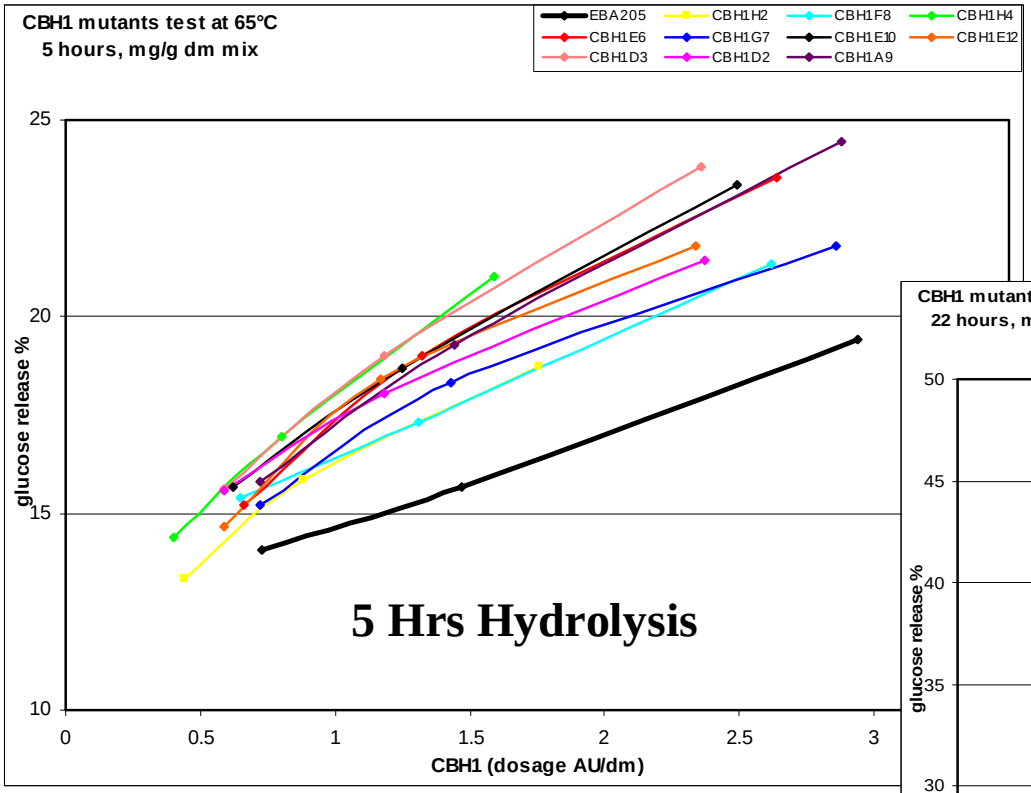
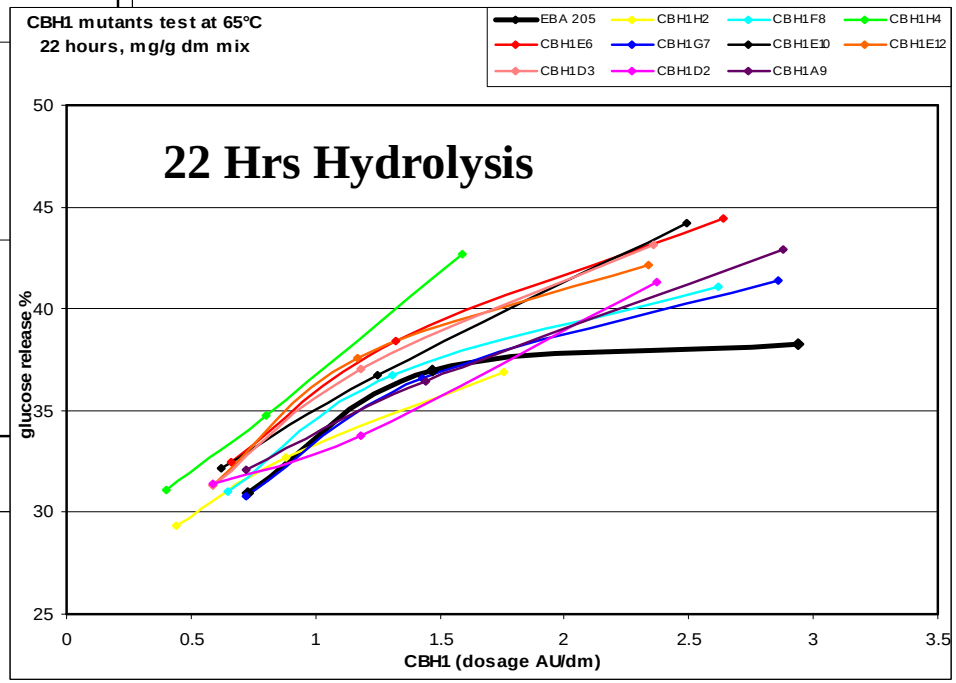
# DSM Thermophilic Enzyme Cocktail: Development Journey

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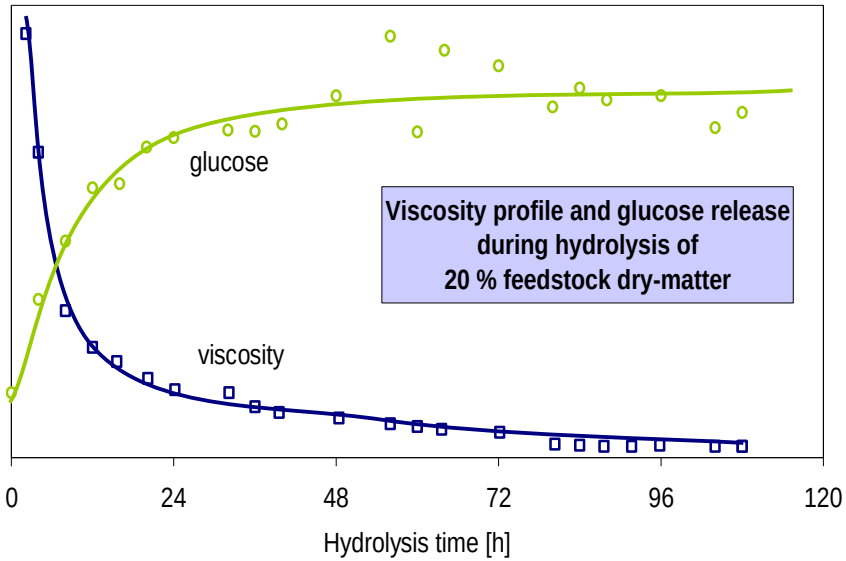
# Pilot Scale Performance: DSM Thermophilic Cellulases

## Enzymes active above 60°C

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




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

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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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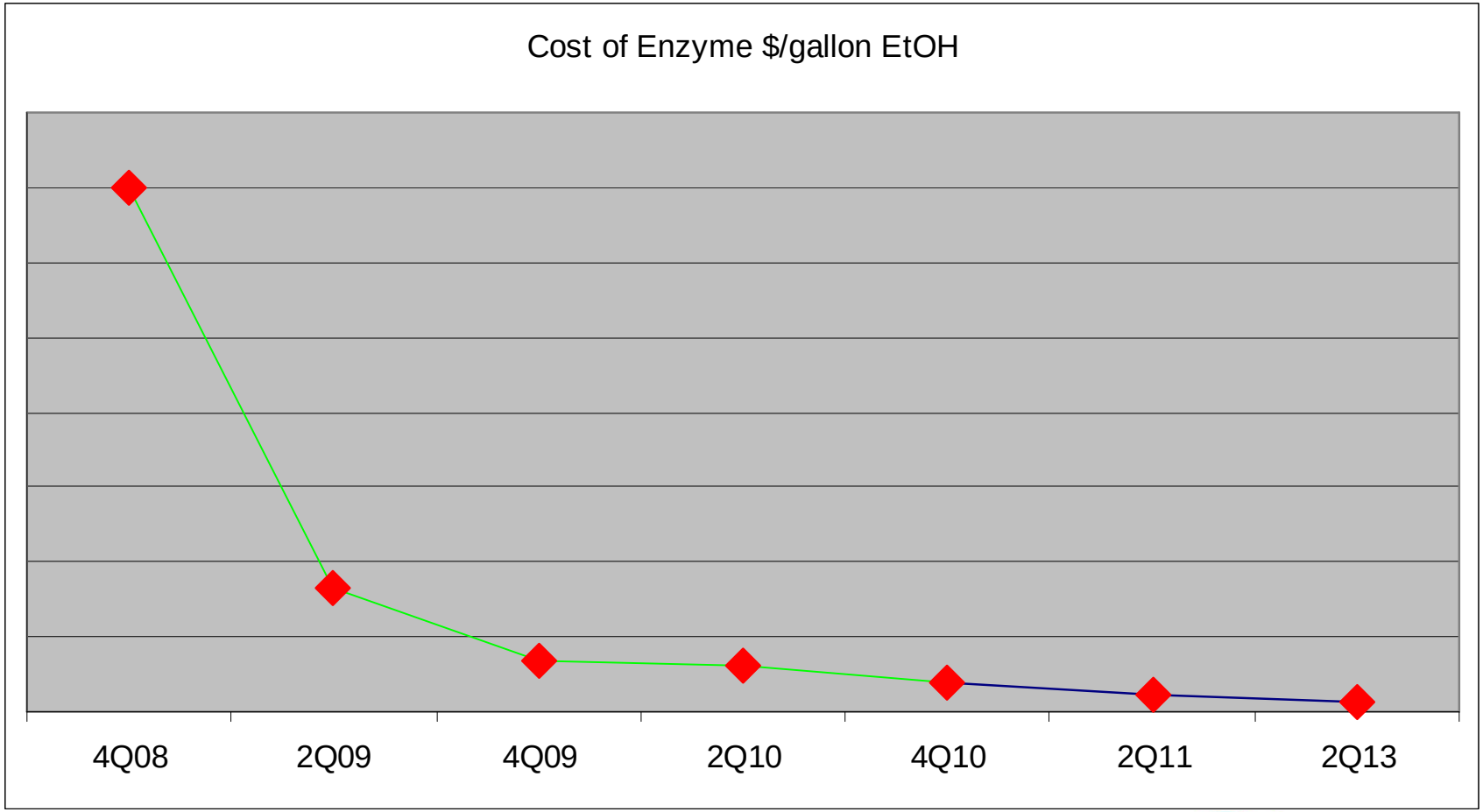
# Enzyme Cost Reduction

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# Recap: 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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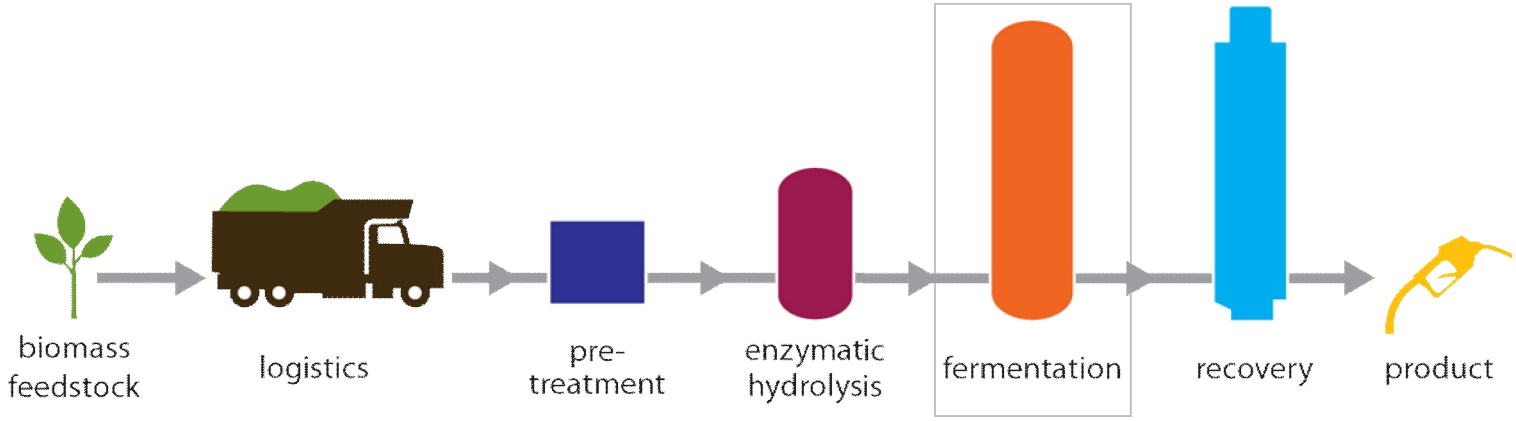
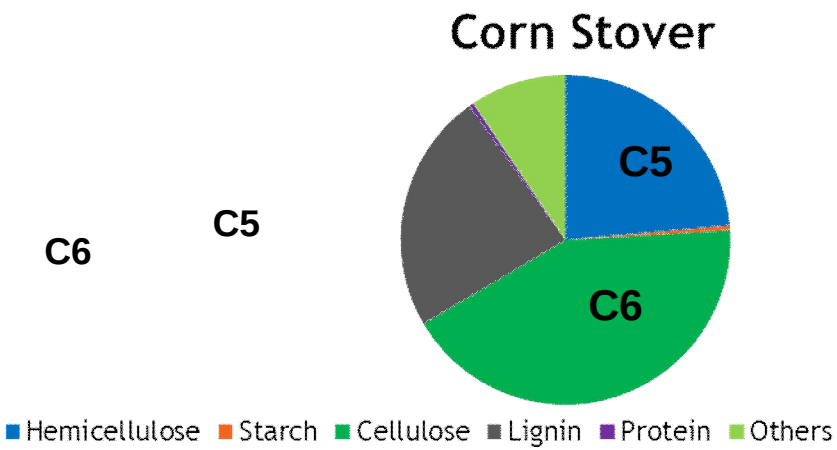
# DSM has Developed the Right Yeasts




**Hemicellulose 20-55% (C5); Cellulose 20-45% (C6)**

Challenge:
convert both C6 & C5 sugars in cellulosic biomass

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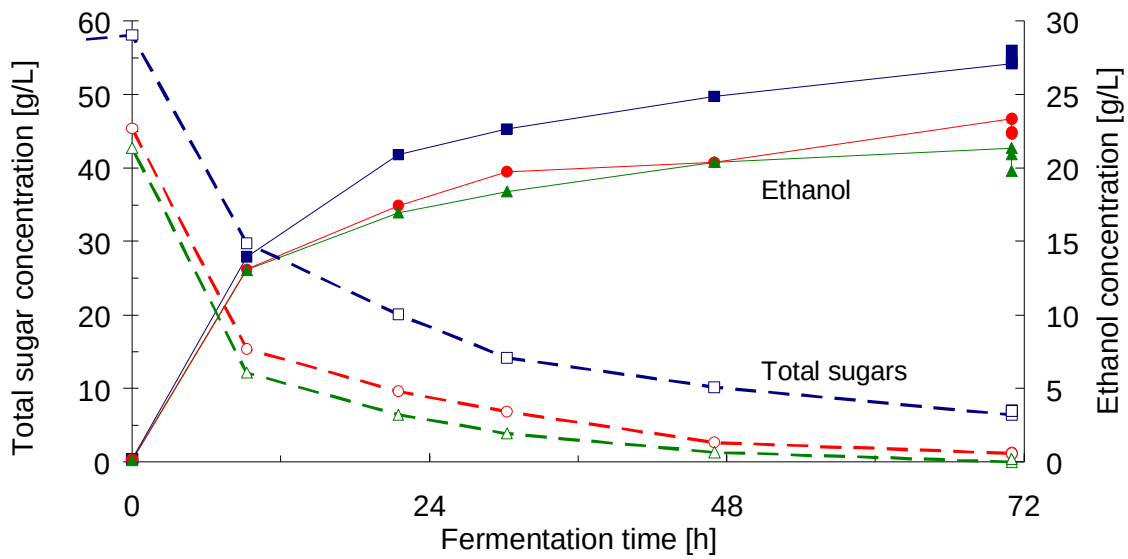
# Application Tests with Real Hydrolysates

## High ethanol yields with different feedstocks




Corn fiber — Wheat straw — Corn stover —

| | Corn fiber | Wheat straw | Corn stover |
|---|---|---|---|
| Total sugar*) (g/l) | 58.1 | 42.7 | 45.4 |
| Ethanol yield (g EtOH/g sugar) | 0.47 | 0.48 | 0.50 |
| Ethanol yield (gallons/ton) | 68 | 72 | 81 |

*) released, monomeric sugars at the start of the fermentation

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# Separate versus combined C5 & C6 sugar fermentation

| Separate fermentation | Combined fermentation |
| --- | --- |
| Diluted C5 stream needs to be concentrated by evaporation | No need for concentration of C5 stream leads to energy and water savings |
| Concentration of C5 stream will increase inhibitor level | No concentration therefore no increase in inhibitor levels |
| 2 different fermentation organisms require a more complex process design (2 inoculation systems, 2 propagation lines, etc) | 1 fermentation organism can do C5&C6 fermentation keeping simple process design |
| Part of (oligomeric) C5 sugars remain unconverted, additional C5 enzymatic hydrolysis needed | 1-step enzymatic hydrolysis of both oligomeric and monomeric C5 sugars |

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# DSM Advanced Yeast

## Designed for usage in 2G biorefineries

- Ethanol conversion of both C5 and C6 sugars
- Fast conversion of sugars (72hrs)
- High ethanol yield (98%) with low/no byproduct formation
- Commercially available strain (stable, marker-free)
- High inhibitor tolerance

Oral Presentation by Panos Sarantinopoulos in *Session 17-03*

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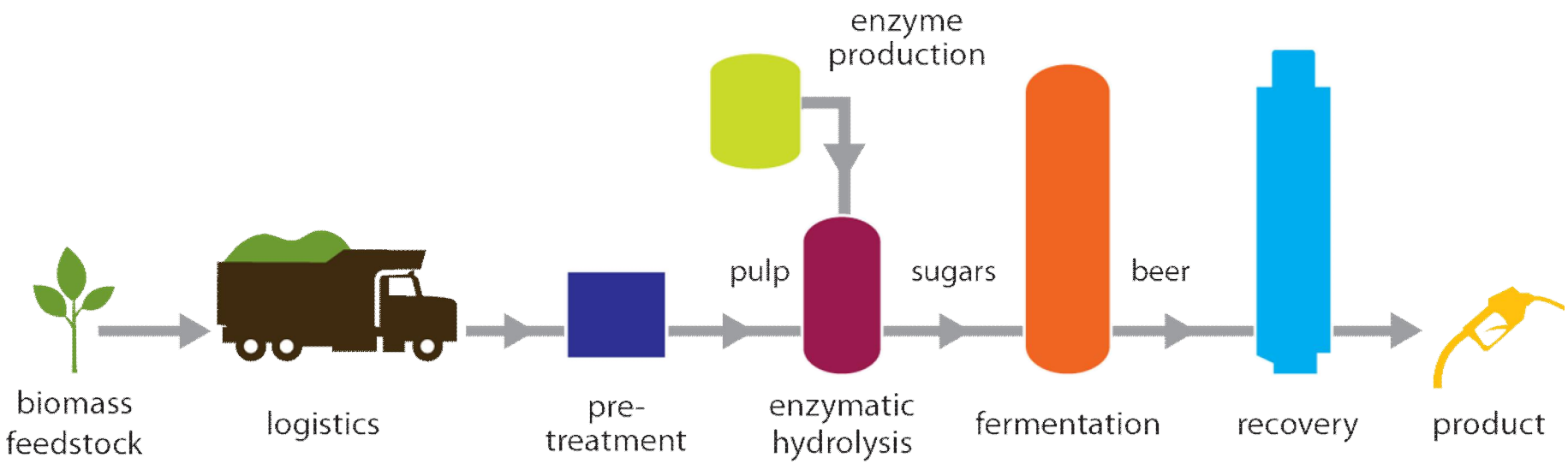
# Integrated Technology Solution Provider




An integrated approach is essential because:

- The optimal technology solution is a combined effort throughout the whole value chain
- One shared goal: maximizing the value in the 2G ethanol production

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# DSM's Integrated Technology Solution Package

- Application Platform development
- Partnerships throughout the value chain
- Public private partnerships
- In- and Out- Licensing models
- On Site Manufacturing concept

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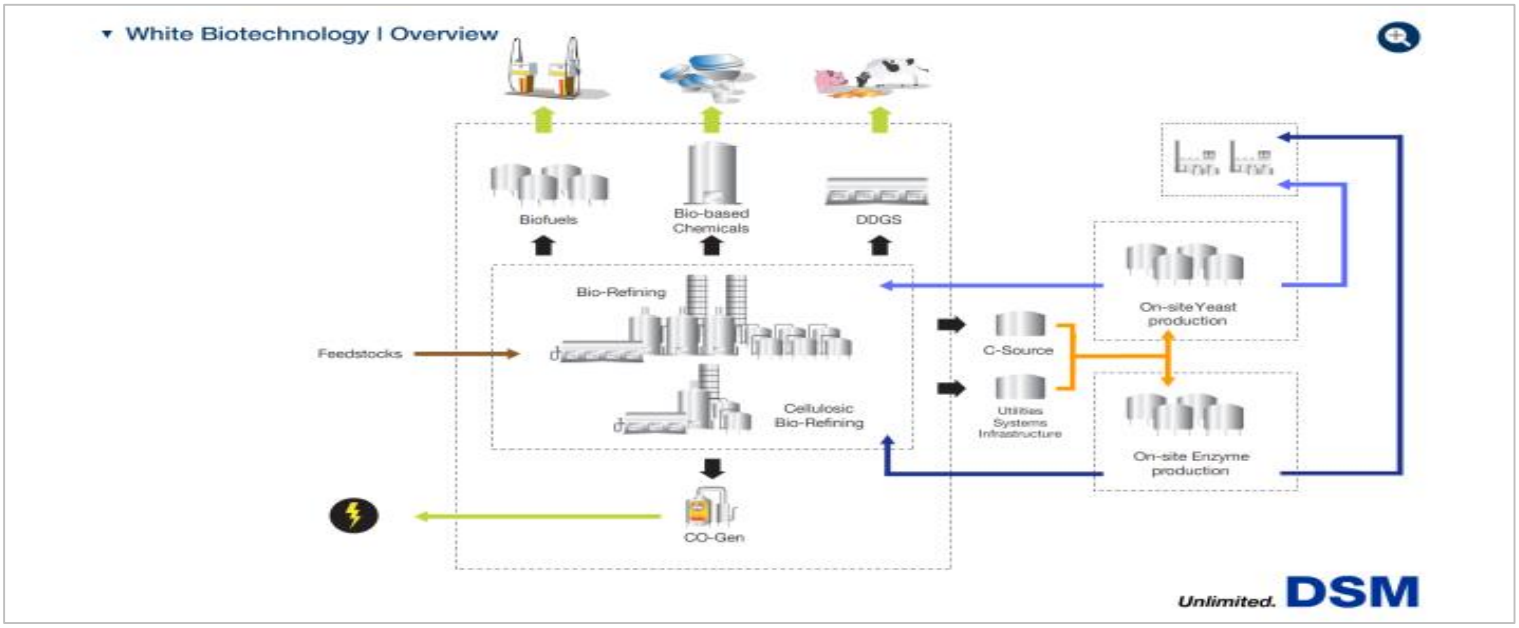
# DSM On-Site Manufacturing solution

## Complete Stand Alone Unit




- A specialized enzyme production plant supplying a region with clarified and concentrated enzyme solution.
- The plant is fully self-supporting in all of its functions.

*MAY 2011 Biomass to Ethanol Process Design Report NREL/TP-5100-47764*

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# DSM On-Site Manufacturing solution

## Advantages of OSM versus remote site

- Supply chain consequences
- Superior in LCA comparison
- Enabling process optimization and control
- Improved process economics

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# DSM and Bio-Conversion Solutions

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