Boosting the cellulytic degradation of steam pretreated barley straw

Lisa Rosgaard Novozymes Inc.
Starch R&D

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Unlocking the magic of nature

Babilafuente Bioethanol Project (2003-2007)

- Develop the technology to include lignocellulose from barley and wheat husks and straw in a fuel ethanol pilot plant concomitantly utilising barley and wheat grain
- Production capacity is aimed at 200 ML/year of which 195ML will obtained from barley grain and 5ML from lignocellulosic material (barley and wheat husks and straw)
- Supported by the EU (5th Framework programme)

Babilafuente, Salamanca, Spain

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Project partners

Pretreatment of barley straw and SSF

LUNDS UNIVERSITET

REPSOL YPF

- Vehicle testing and marketing of EtOH/gasoline blend

Project coordinator

ABENGOA BIOENERGY R&D, INC.

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Evaluate and provide enzymes

Ciemat

Pretreatment of wheat straw and SSF

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Key research areas at Novozymes

- Cellulose conversion
- Hemicellulose conversion
- Enzyme efficiency
- Substrate loading
- Lignin-enzyme interaction

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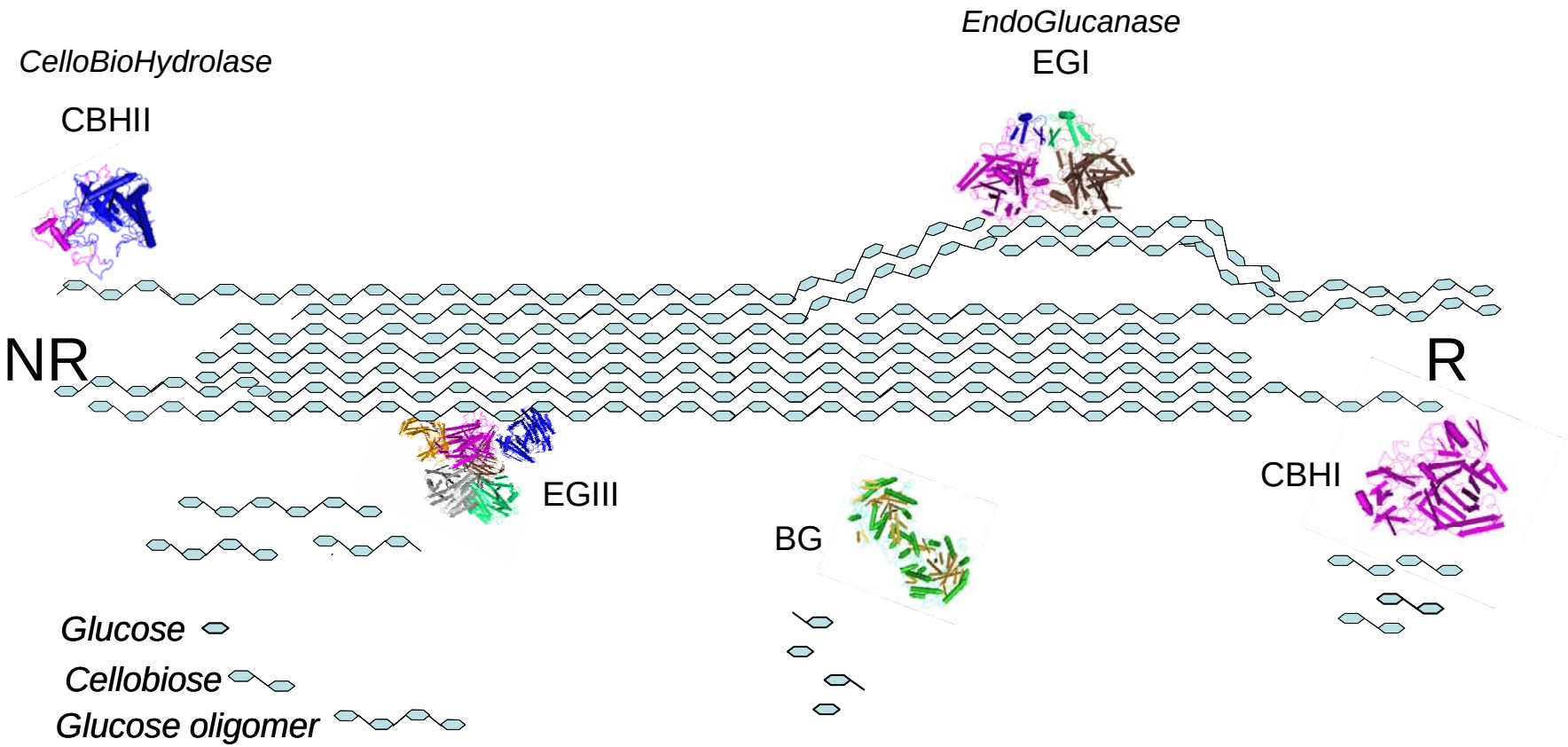
Degradation of cellulose



Structures modified from NCBI MMDB (Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler-Bauer A, Marchler GH, Mazumder R, Nikolskaya AN, Rao BS, Panchenko AR, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA,YinJJ,BryantSH,"*MMDB: Entrez's 3D-structure database*", Nucleic Acids Res. 2003 Jan; 31(1): 474-7)

Hypothetical structure of lignocellulose

if only
si

J. Bidlack, M. Malone and R. Benson. Okla. Acad. Sci., 1992, Vol. 72.

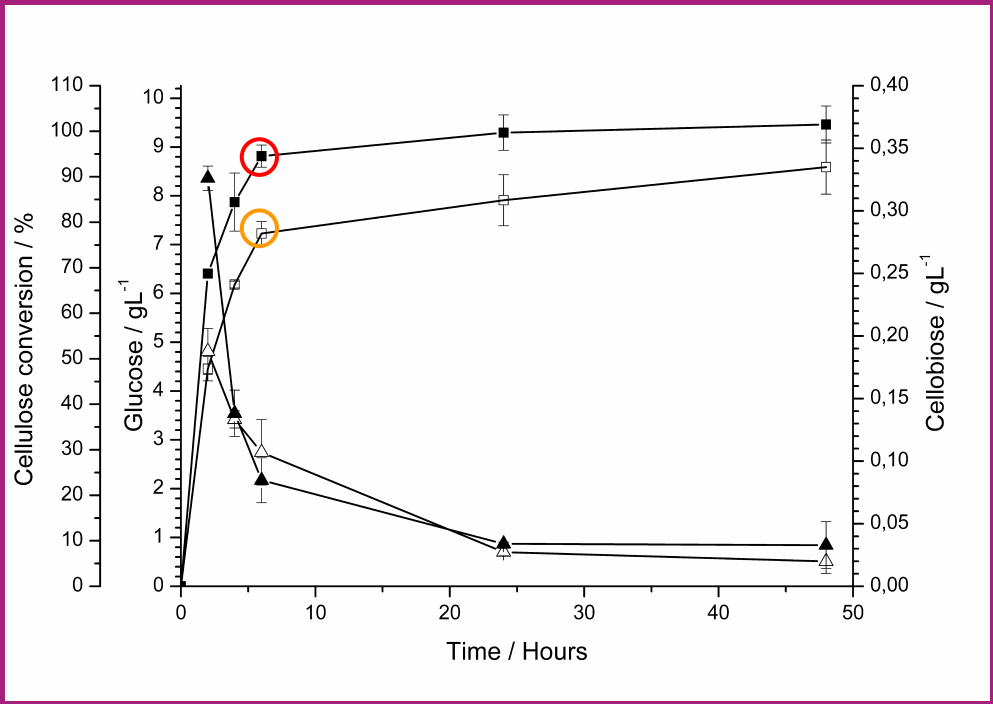
Testing fermentation broths on lignocellulose

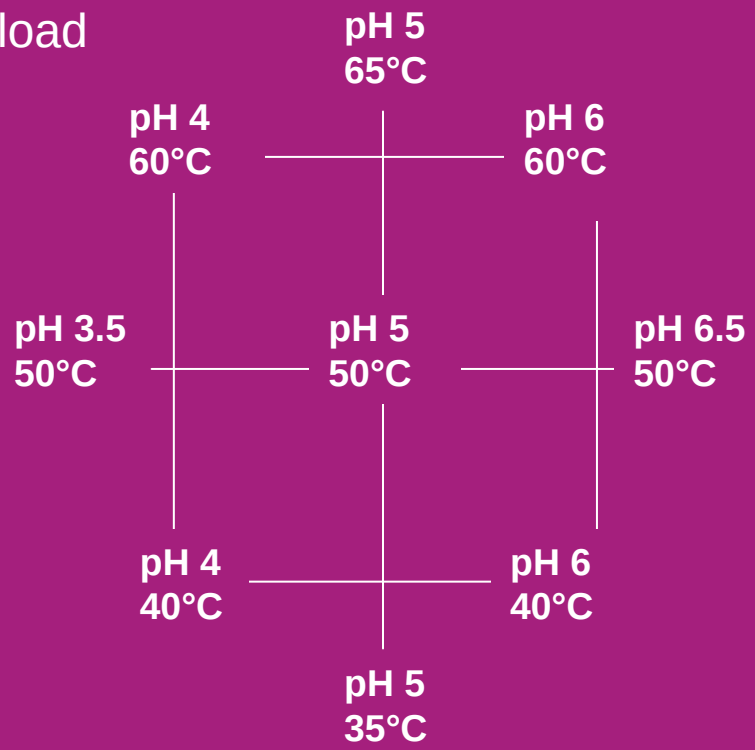
Small scale testing (2g scale at 2% dry matter)
6 hours incubation

Benchmark: Celluclast:NS188 = 2.3: 1mg enzyme per g reaction ~8FPU: 13CBU per g solids

0.035mg broth protein per g reaction

Corresponding to approx. 10% of the total protein load





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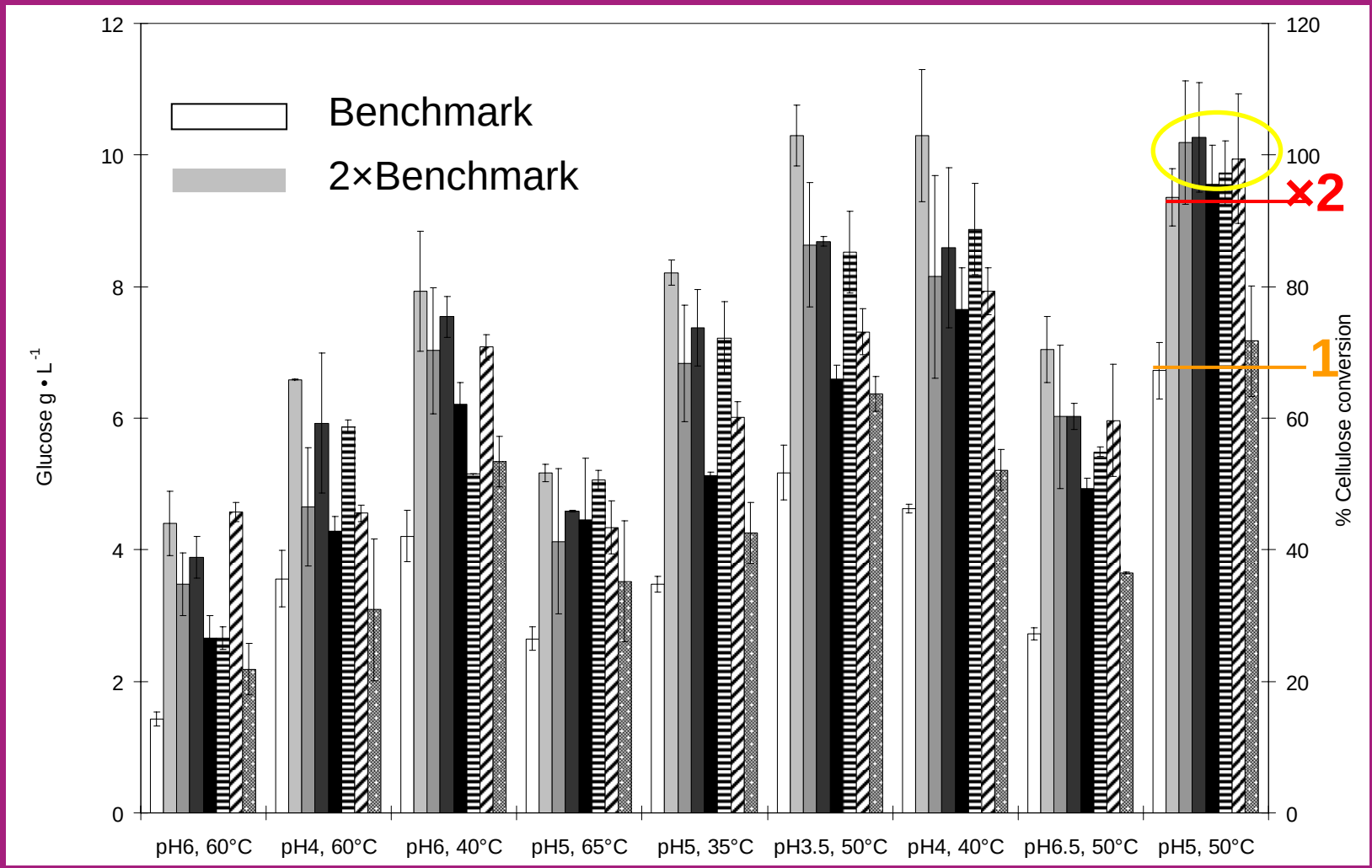
L. Rosgaard, S. Pedersen, J. R. Cherry, P. Harris, A. S. Meyer. Biotechnology Progress

Testing fermentation broths on lignocellulose-2



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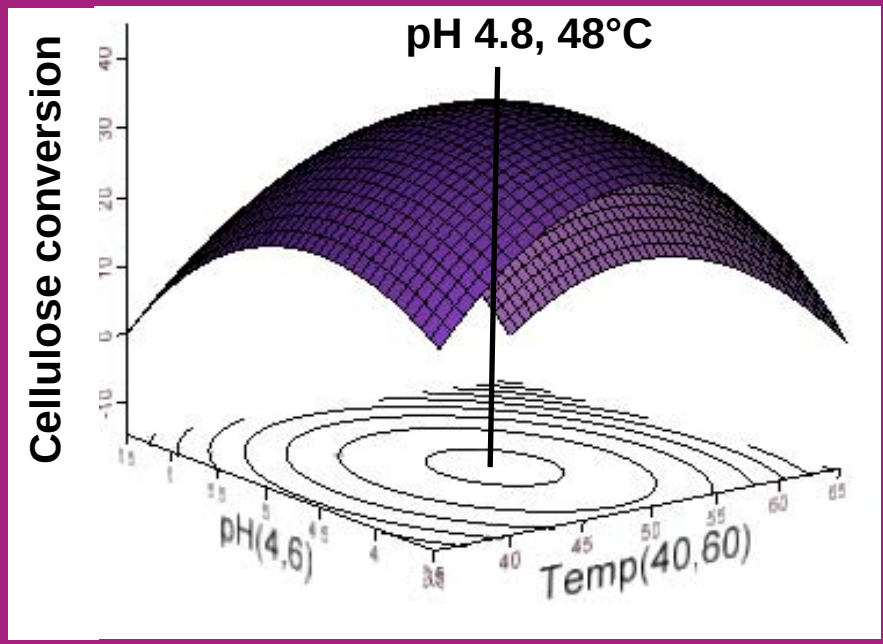
Testing fermentation broths on lignocellulose- 3

Chaetomium thermophilum -
Thielavia terrestris +
Thermoascus aurantia +
Corynascus thermophilus +
Myceliophthora thermophila +
Penicillum funiculosum +

+ = all optimum at pH 4.8-5, 48-50°C and compare to Celluclast/NS188 on lignocellulose



Conclusions

- The tested fermentation broth samples significantly boosted the cellulose conversion when benchmarked against Celluclast® + Novozym 188.
- The data also demonstrated that spiking, rather than substitution, of Celluclast® + Novozym 188, may be a feasible way forward in industrial, large scale cellulose conversion

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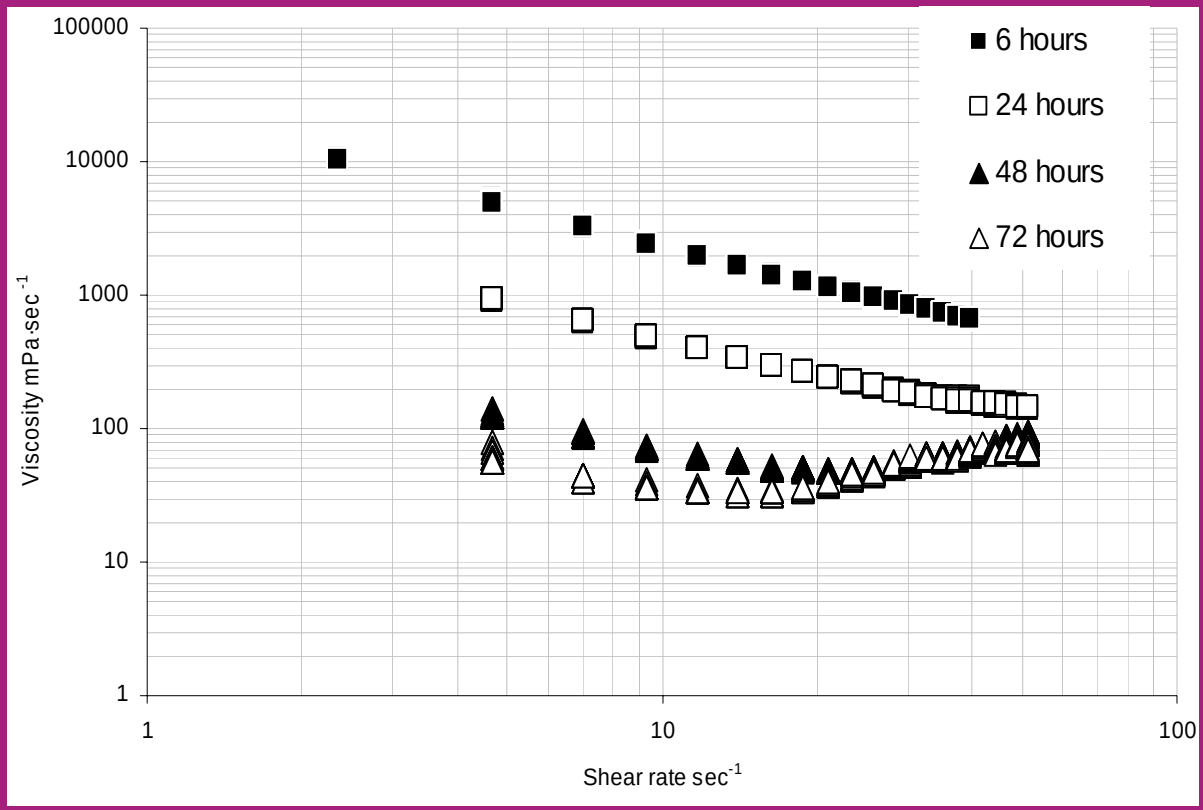
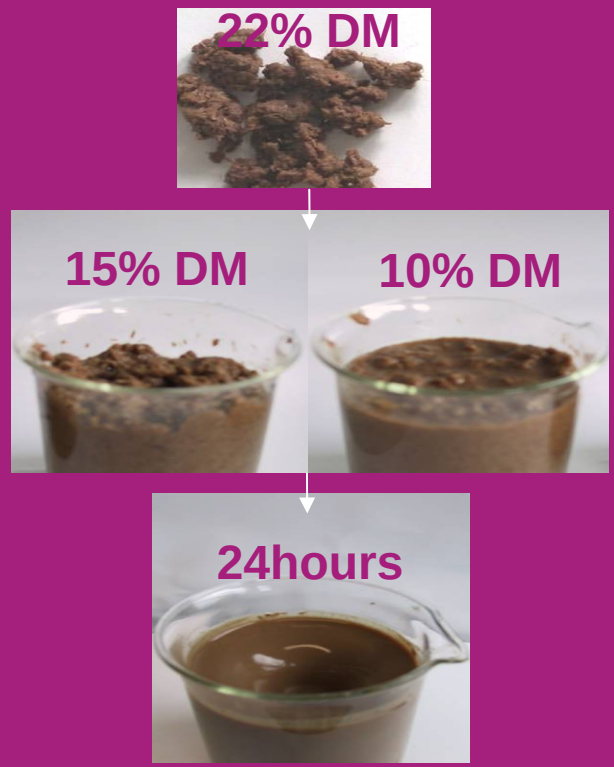
Increasing substrate loading

- The viscosity of the slurry decrease rapidly within the first hours of hydrolysis. This gives the opportunity to load additional substrate





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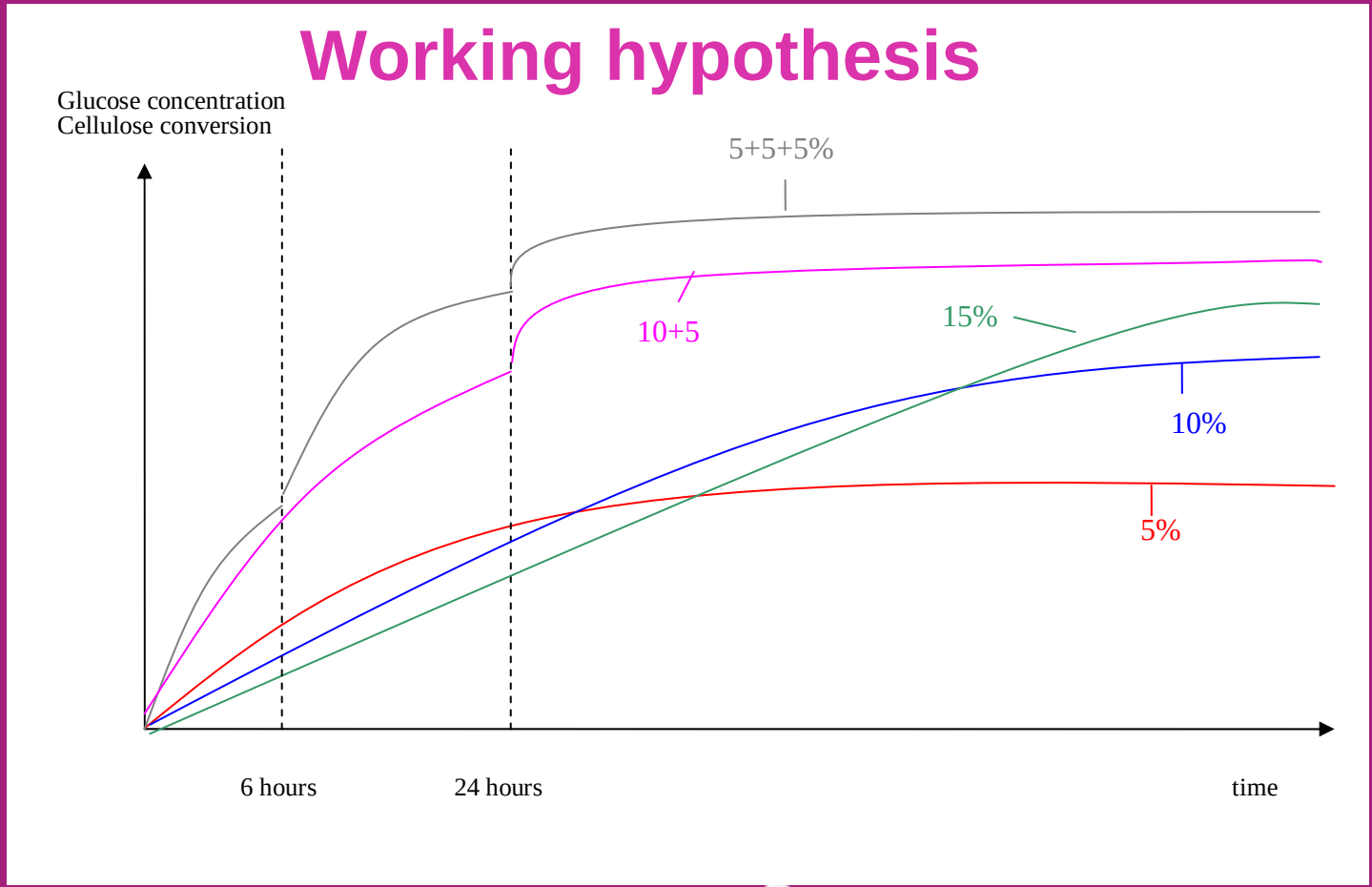
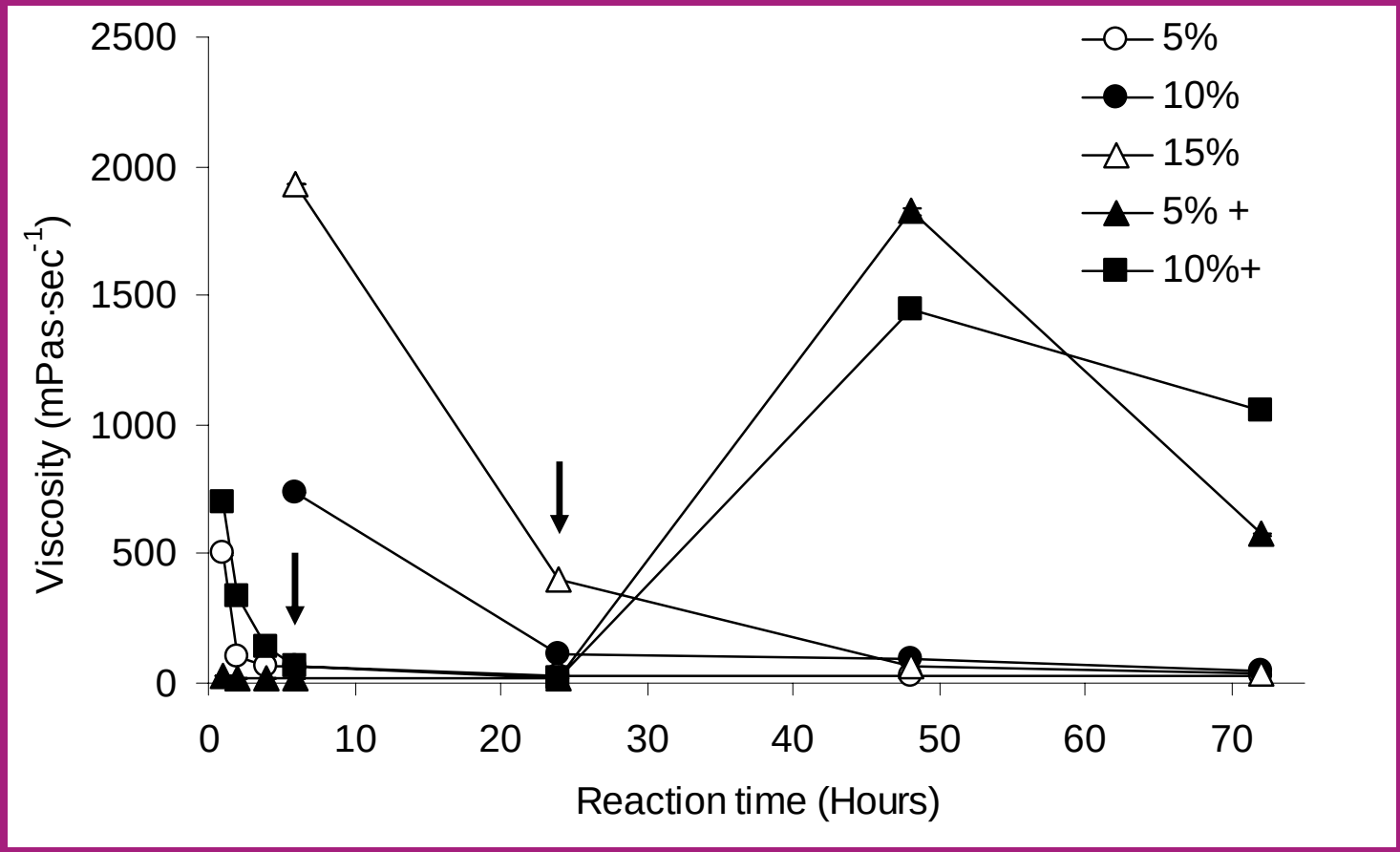
L. Rosgaard, P. Andric, K. D. Johansen, S. Pedersen, A. Meyer. In prep. for Applied Biochemistry and Biotechnology

Increasing substrate loading-2

- Reaction conditions: whole slurry of dilute acid hydrolysed barley straw. pH5, 50°C. Full enzyme loading at the start of reaction.



Increasing substrate loading-3



- Viscosity decrease is rapid but does not decrease when additional substrate is added

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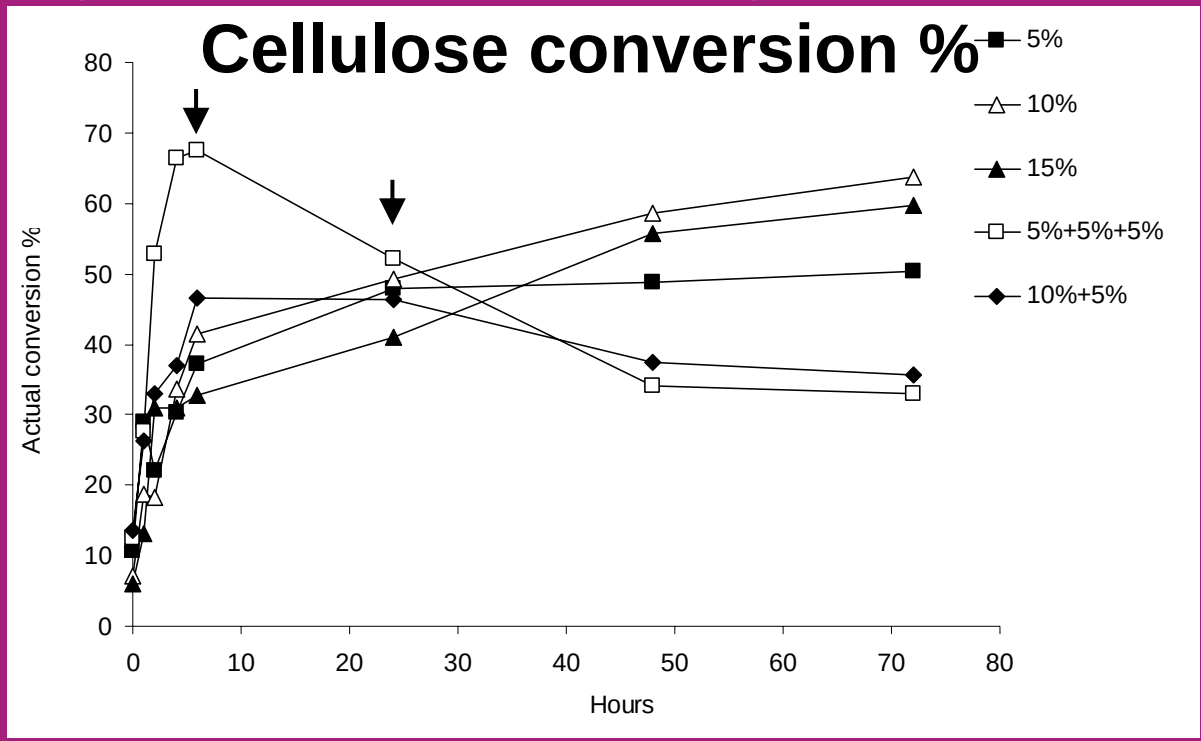
Increasing substrate loading 4



- Additional substrate loaded does not appear to be efficiently hydrolysed

Conclusion

- There was no net benefit of gradually increasing the substrate loading with respect to cellulose conversion or in relation to viscosity. However it is speculated that mixing of substrate and enzyme as well as timing of adding additional substrate is crucial.

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Outlook- Novozymes Biotechnology, Davis, CA.

- Enzyme discovery
- 2001: US Department of Energy (DOE) and National Renewable Energy Lab (NREL) collaboration to bring down the cost of enzymes for ’biomass to bioethanol’ production.
- 2005: Target was met and surpassed by a 30-fold reduction in costs to 0.1-0.18$ per gallon.
- Extensive research and engineering of cellulases has led to a new generation cellulase products for bioethanol production.

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