Biofuels and Bioenergy: Challenges and Opportunities,
Vancouver, BC, August 29th, 2006
Session 2C: Biofuels Production Issues, Oral Presentation #2C-2

Enzyme Compositions for Lignocellulose-Based Biorefineries: AN OVERVIEW

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Vancouver, BC, Canada

Outline

I - FOREST PRODUCTS BIOTECHNOLOGY GROUP (FPB)

II - ENZYMES FOR LIGNOCELLULOSE-BASED BIOREFINERIES

III - FPB RECENT ACHIEVEMENTS (2003-2006)

CONCLUSIONS

ACKNOWLEDGEMENTS

FPB Units

Biorefinery of Lignocellulose into Biofuels & Co-Products (Special Focus on Softwoods)

1. Process Development Unit (PDU)
2. Wood Chemistry Unit
3. Molecular Biology and Protein Research Unit

I – Forest Products Biotechnology Group (FPB)

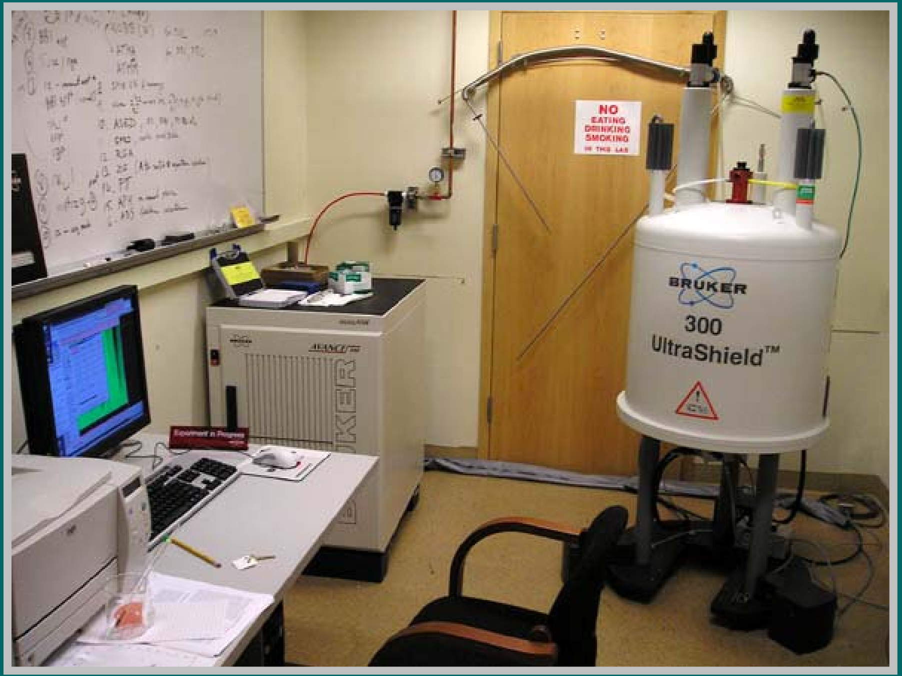
Process Development Unit (PDU)

I – FPB – Wood Chemistry Unit

ANALYTICAL EQUIPMENT - HPLC/MS

I – FPB – Wood Chemistry Unit

ANALYTICAL EQUIPMENT - SPECTROMETRY



I – FPB – Wood Chemistry Unit

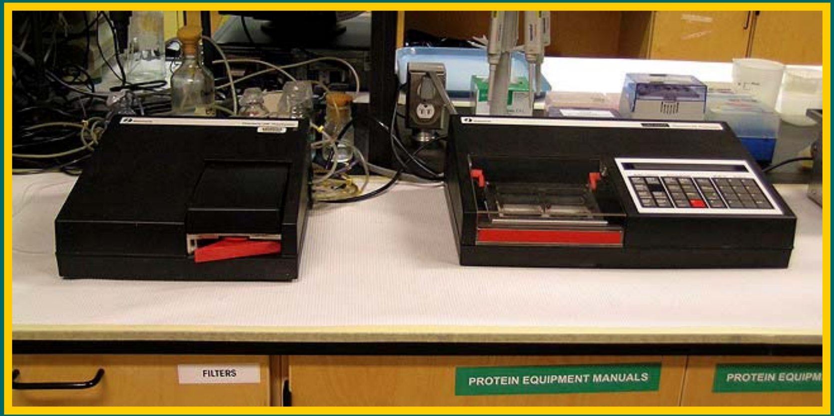
BIOPOLYMERS PROPERTIES ANALYSIS

I – FPB – Wood Chemistry Unit

ANALYTICAL EQUIPMENT - Miscellaneous

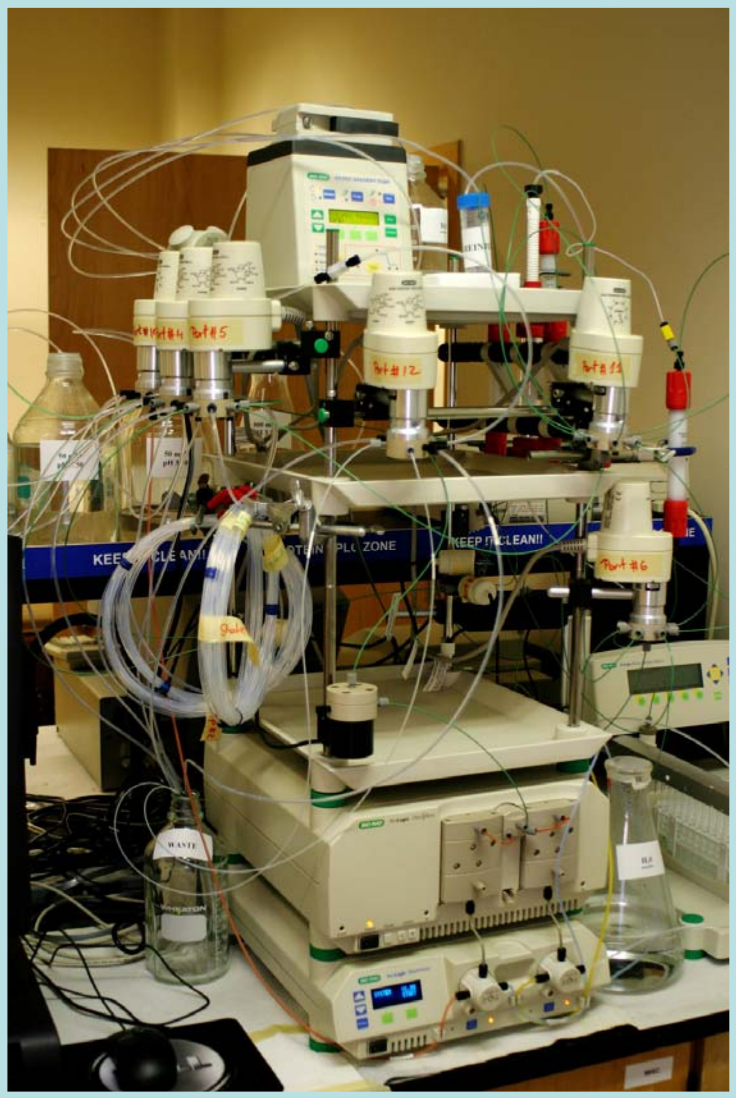
I – FPB – Molecular Biology and Protein Research Unit

1D-2D-EF AND PCR EQUIPMENT



I – FPB – Molecular Biology and Protein Research Unit

Automated Multidimensional Preparative FPLC



Enzymatic Hydrolysis

Lignocellulose-Based Biorefinery: is a facility that integrates lignocellulosic biomass conversion processes and equipment to produce renewable fuels, power, and chemicals.

Core Processes of a Modern Lignocellulose-Based Biorefinery

BIOMASS PRETREATMENT → ENZYMATIC SACCHARIFICATION (HYDROLYSIS) → FERMENTATION

Biomass Main Components:

lignin, cellulose, and hemicellulose

Enzymatic Hydrolysis – Technology Background

Enzymatic hydrolysis converts the cellulose and hemicellulose biomass fractions into fermentable sugars (mainly glucose and xylose). This process is performed by the synergistic action of a complex of enzymes including: cellulases, hemicellulases, β-glucosidases, etc.

Filamentous fungus *Trichoderma reesei* <1996 (*Hypocrea jecorina >1996*) is currently the most important industrial strain for production of lignocellulose degrading enzymes (~100 g/L protein).

Enzymatic Hydrolysis – Brief History

Earliest publications

Pringsheim Hans, Über den fermentativen Abbau der Cellulose, Hoppe-Seyler´s Zeitschrift für Physiologische Chemie, Strassburg, 1912, 78, 266-91

Pringsheim Hans, Über den fermentativen Abbau der Hemicellulosen, Hoppe-Seyler´s Zeitschrift für Physiologische Chemie, Strassburg, 1912, 80, 376-82

Über den fermentativen Abbau der Cellulose.[1])

Von

Hans Pringsheim.

(Aus dem chemischen Institut der Universität Berlin.)
(Der Redaktion zugegangen am 18. März 1912.)

Der rein hydrolytische Abbau der Cellulose durch Fermente ist bis auf den heutigen Tag ein ungelöstes Problem

"The hydrolytic degradation of cellulose by ferments is so far an unresolved problem…"

Enzymatic Hydrolysis – Brief History

Latest publications

Zhang P. Y.-H., Lynd L.R., 2004, *Toward and Aggregated Understanding of Enzymatic Hydrolysis of Cellulose: Noncomplexed Cellulase Systems*, Biotechnology and Bioengineering, Vol. 88, No. 7, p. 807

"(…) there is much about enzymatic hydrolysis that is not yet fully understood (…)"

Enzymatic Hydrolysis – Brief History

Questions Partially Answered

A Microbiological Process Report

Enzymatic Hydrolysis of Cellulose

Elwyn T. Reese

Pioneering Research Division, Quartermaster Research and Development Center, Natick, Massachusetts

Received for publication August 25, 1955

Applied Microbiology 1956, 4, pp. 39-45
(The C_1-C_x Hypothesis)

Question #1:

Multiple components in cellulolytic systems. One of the most important questions being resolved is "Are there several cellulases, or is there only one type?"

Answer #1: multiple components acting synergistically are responsible for effective hydrolysis of lignocellulose including CBHs, EGs, XYNs, MANNs, B-Gs, NON-CATALYTIC PROTEINS

Enzymatic Hydrolysis – Brief History

Question #2:

Intermediate products of cellulose hydrolysis. A second problem receiving increased emphasis is "What are the products of the enzymatic hydrolysis of cellulose?"

Applied Microbiology 1956, 4, pp. 39-45

Answer #2: mono- and cellooligosaccharides. There is also information available on the products of hemicellulose hydrolysis (mono- and xylooligosaccharides, mainly).

Enzymatic Hydrolysis – Brief History

Question #3: Enzyme Specificity

Applied Microbiology 1956, 4, pp. 39-45

TABLE 3. *Comparison of enzyme and acid hydrolysis of cellulose*

Substrate	Amount of Agent to Give Unit Activity*	
	Trichoderma viride enzyme†	Hydrochloric acid
	ppm	*ppm*
Carboxymethylcellulose (C)	2	>200,000
"Walseth" cellulose (W)	6	>200,000
Cotton fibers	1	64,000

* Concentration required to give unit activities: C = 0.40 mg reducing sugar/ml/hr/ 50 C. W = 0.50 mg reducing sugar/ml/2hr 50 C. Cotton = 50 mg increase in swollen wt/hr 50 C.

† Derived from data of Gilligan and Reese (1954).

COMPARISON OF ENZYMATIC WITH OTHER HYDROLYSES

Specificity of cellulolytic enzymes. Enzymes are more efficient agents of hydrolysis than are acids (table 3). A comparison of activities at 50 C on three cellulosic substrates shows that 100,000 times as much acid is required to bring about the same degree of hydrolysis.

Answer #3: enzymes are extremely specific. They hydrolyze specific bonds in the substrate while acids can hydrolyze any of them.

Enzymatic Hydrolysis – Brief History

Some of the questions yet to be answered

1. The role of the lignocellulosic substrate properties (both pretreated substrates and untreated substrates) in determining the effectiveness of lignocellulose degrading enzymes at all stages of the process.

2. The role of enzyme-substrate interactions in the effectiveness of cellulose hydrolysis.

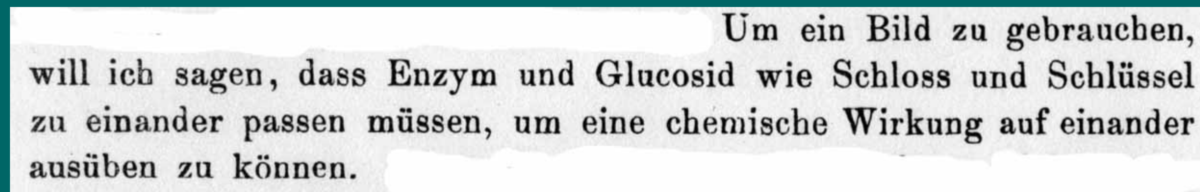
3. Principles for customization of enzyme compositions to effectively degrade a broad range of lignocellulosic substrates with maximum of added value (not always complete degradation into monosaccharides such as glucose and xylose!).

II - Enzymes for Lignocellulose-Based Biorefineries

All these questions lead us to an old hypothesis…

Hermann Emil Fischer (1852-1919)

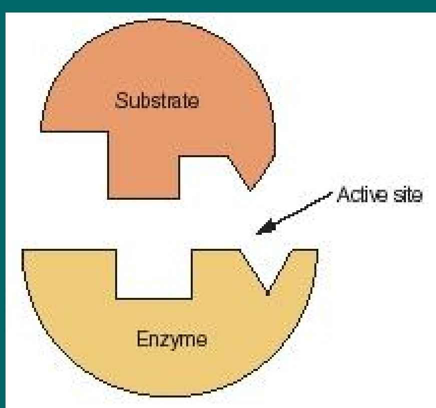
The Key & Lock Hypothesis (1894)



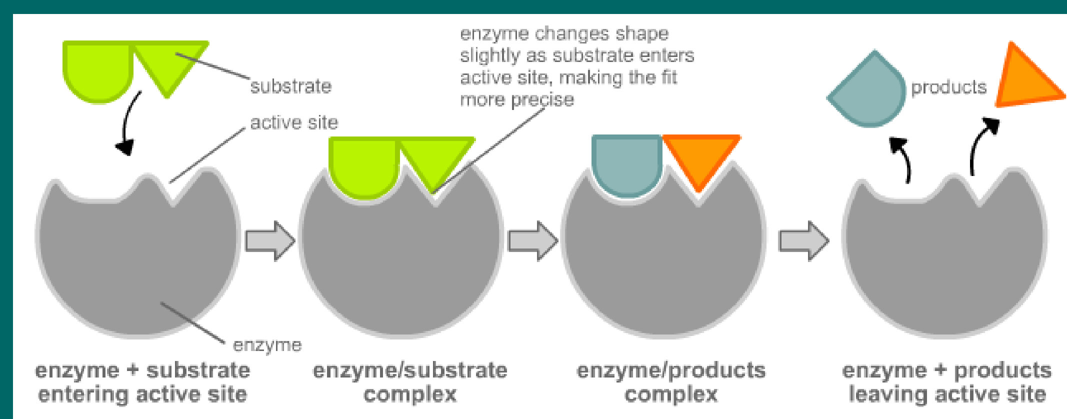
Um ein Bild zu gebrauchen, will ich sagen, dass Enzym und Glucosid wie Schloss und Schlüssel zu einander passen müssen, um eine chemische Wirkung auf einander ausüben zu können.

"To use a picture, I would like to say that enzyme and glucoside have to fit to each other like a lock and key in order to exert a chemical effect on each other"

Source: ***Berichte der Deutschen Chemischen Gesellschaft (Berlin, 1894), Einfluss der Configuration auf die Wirkung der Enzyme, Vol. 27, p. 2992***

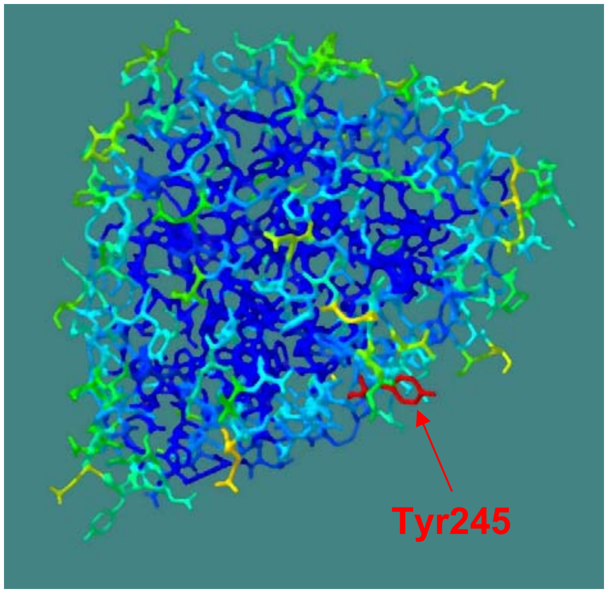


Induced Fit Model (Koshland, 1959)



II - Enzymes for Lignocellulose-Based Biorefineries

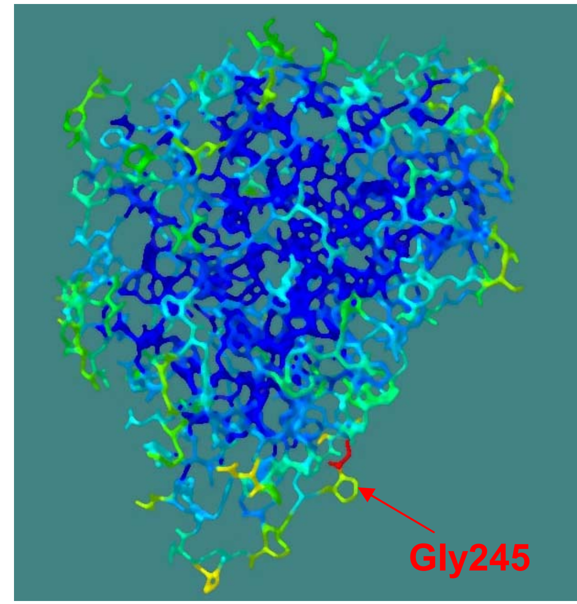
Significance – Example: Reduction of Enzyme Inhibition



Native catalytic domain Cel5A Endocellulase Cel5A *Acidothermus cellulolitycus* (PDB#: 1ECE)

~1500% inhibition reduction

40% increase biomass conversion



Y245G Mutant catalytic domain Cel5A Endocellulase Cel5A *Acidothermus cellulolitycus* (PDB#: 1VRX)

Source: ***Baker J.O., et al. (2005), Catalytically enhanced endocellulase Cel5A from Acidothermus cellulolyticus, Applied Biochemistry and Biotechnology, 121-124, pp. 129-48***

Recent Significant Advance

DOE/NREL CONTRACT (2001-2004)

FOR REDUCTION OF PRODUCTION COSTS OF BIOMASS CONVERTING ENZYMES

(DILUTE ACID PRETREATED CORN STOVER – MAIN FOCUS)

NOVOZYMES & GENENCOR

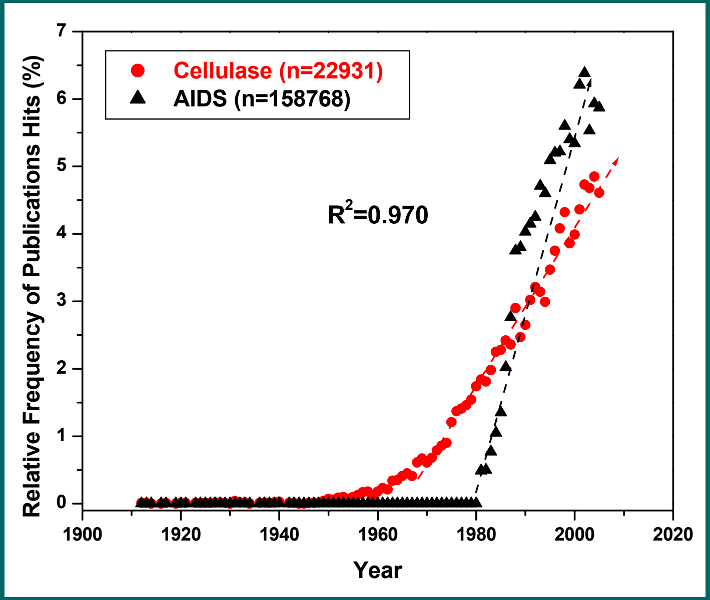
ANNOUNCED 30-FOLD REDUCTION IN THE COST OF ENZYMES NEEDED TO CONVERT BIOMASS TO ETHANOL

(~6-FOLD IMPROVEMENT OF CATALYTIC PERFORMANCE)

Source: DOE/NREL Press releases (2004-2005)

II - Enzymes for Lignocellulose-Based Biorefineries

Significance – The interest in this enzymes is great



"America is addicted to oil"

February 1, 2006
President's G.W. Bush State of the Union Speech, Washington D.C., USA

Source: ***SciFinder Scholar, 2004, American Chemical Society***

The "cure" for this "oil addiction", today is as important as learning how to treat AIDS. Wide implementation of lignocellulose biorefineries could be an effective "treatment" to such "addiction".

III – FPB Recent Achievements (2003-2006)

I. Development of a Unique Collection of Lignocellulosic Substrates

UNTREATED LIGNOCELLULOSIC SUBSTRATES:

Softwood (DF, HS, LPP)
Hardwood (YP, HP, RM)
Agricultural Residues (WS, RS, CS)

PRETREATMENT TECHNOLOGIES:

Steam Explosion (SE) – SO_2 or acid/base-free
Ethanol Organosolv (OS)
Dilute Acid (DA)

PRETREATMENT CONDITIONS:

Severity factor: 3.20 – 4.50 Log Ro
H-Factor: ~500 – 10,000 (for OS)

SUBSTRATE COLLECTION:

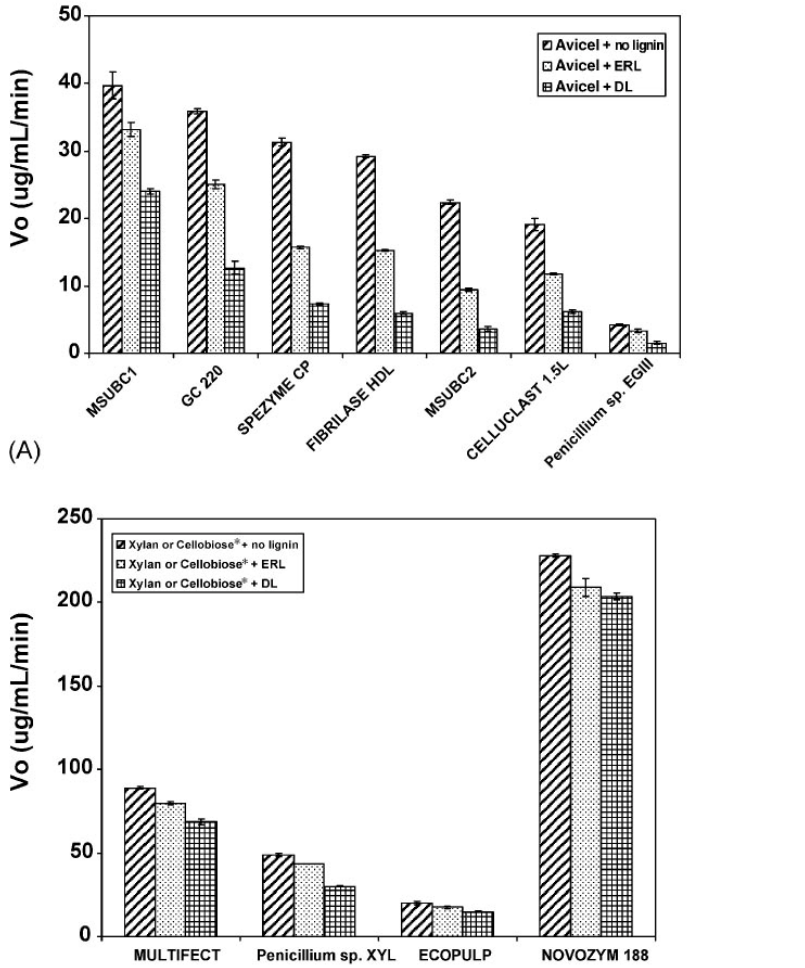
30+ fully characterized substrates
Xylan content: ~0-20%
Glucan content: ~50-90 %
#reactions: 400/handsheet

~60,000 reactions total

Alex Berlin et al. (2006) Boosting glucan bioconversion in lignocellulose by enrichment of a *T. reesei* preparation with an endo-β-1,4-xylanase. *Biotechnology Progress* (in progress)

III – FPB Recent Achievements (2003-2006)

IV. Identification of enzymes will low susceptibility to lignin inhibition



*Cellobiose was the substrate for Novozym 188, for the rest it was birchwood xylan

Sources:

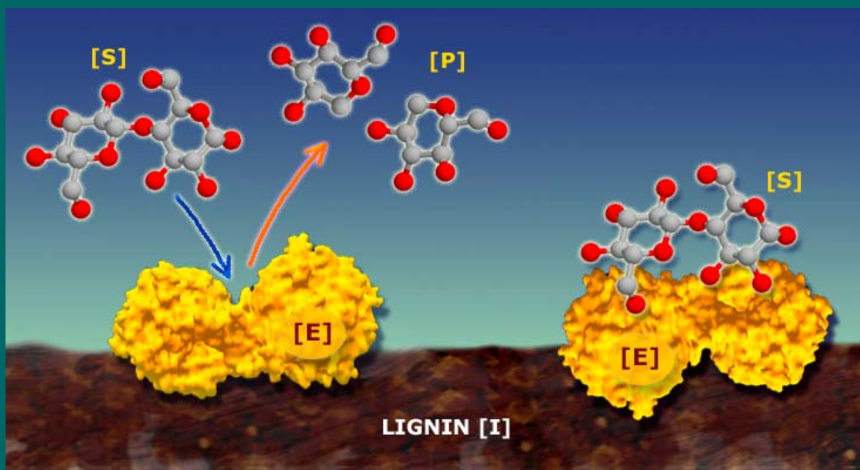
Alex Berlin et al. (2005), Weak lignin-binding enzymes: a novel approach to improve activity of cellulases for hydrolysis of lignocellulosics. Appl. Biochem. Biotechnol. Spring;121-124:163-70.

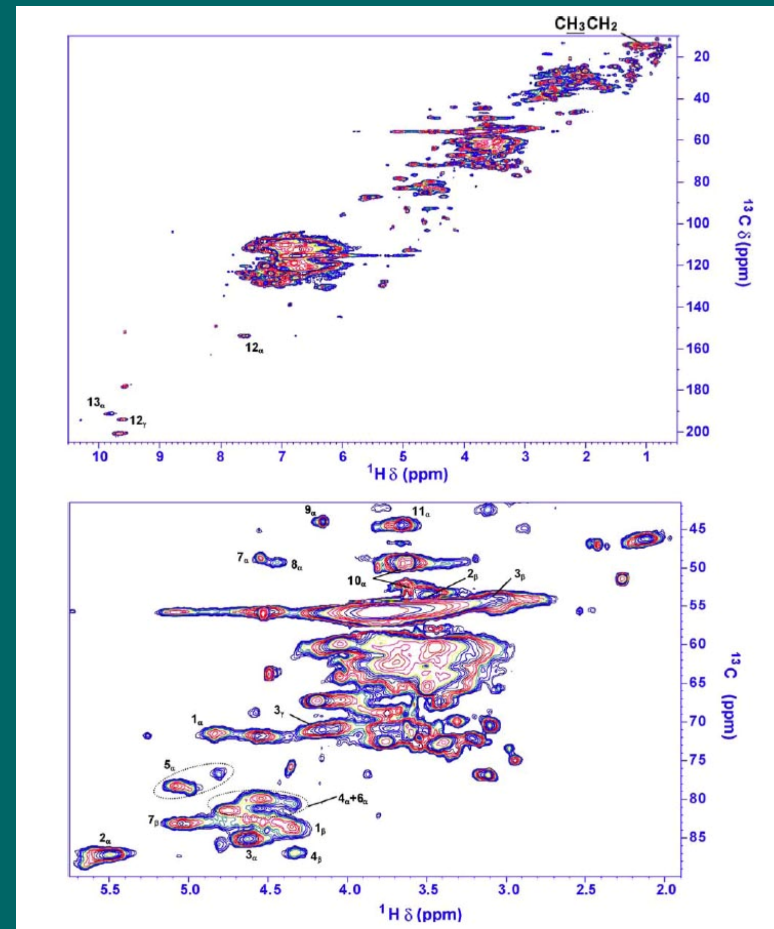
Alex Berlin et al. (2006), Inhibition of β-glucosidase, xylanase and cellulase activities by softwood lignin preparations, *Journal of Biotechnology*, Vol. 125, pp. 198-209

III – FPB Recent Achievements (2003-2006)

V. First attempts to elucidate the mechanism of lignin inhibition

Beta-glucosidase – Lignin binding options

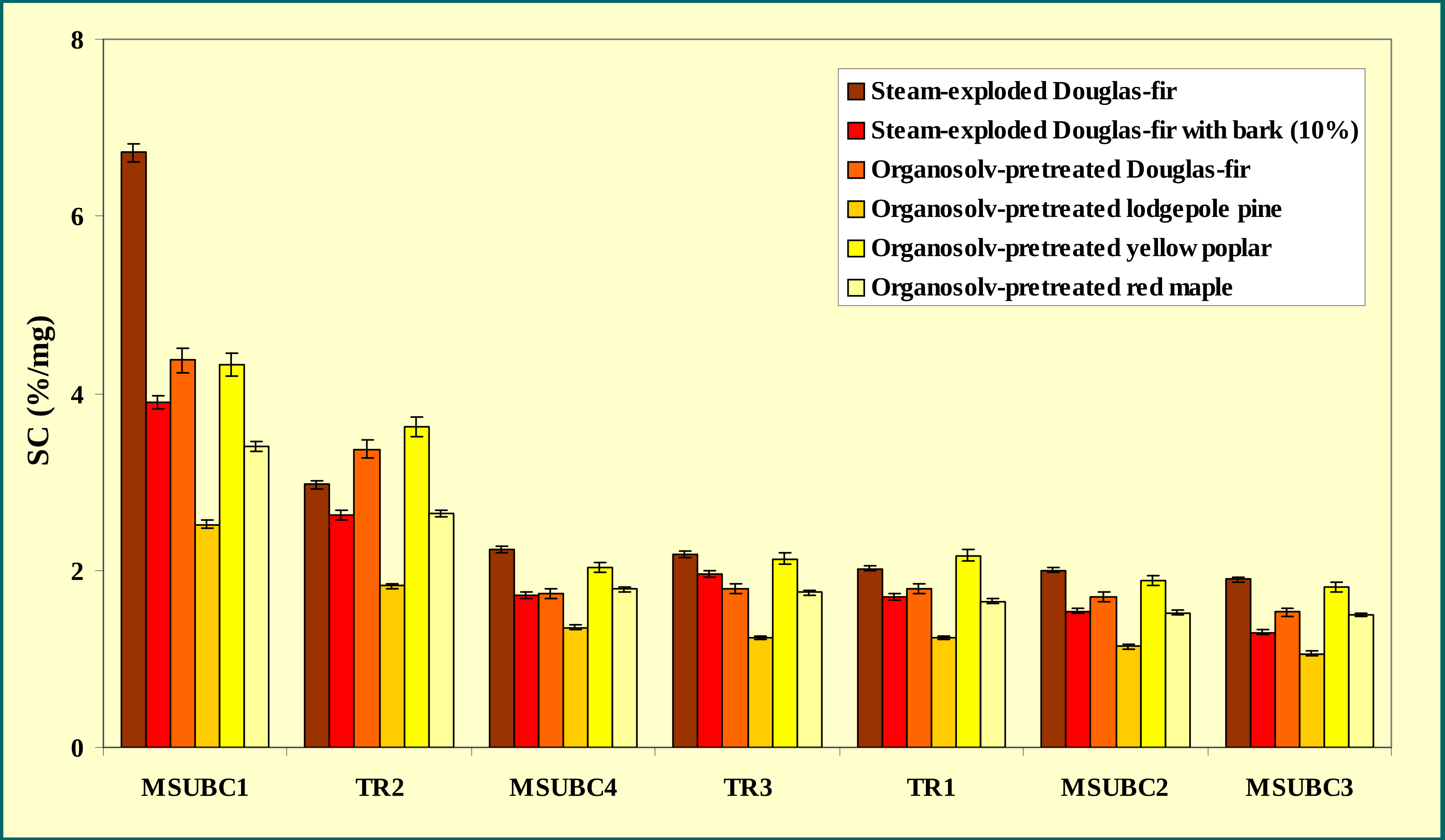




Sources: *Alex Berlin et al. (2006), Inhibition of β-glucosidase, xylanase and cellulase activities by softwood lignin preparations, Journal of Biotechnology, Vol. 125, pp. 198-209; ACS Meeting (2004).*

III – FPB Recent Achievements (2003-2006)

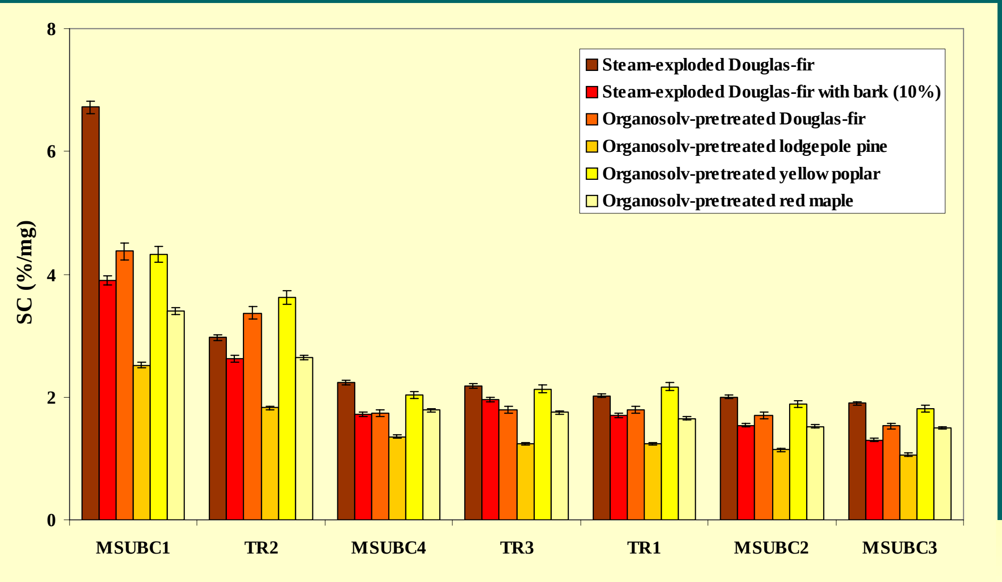
VI. Identification of novel enzyme compositions with hydrolytic properties comparable or superior to the *T. reesei* enzyme complexes.

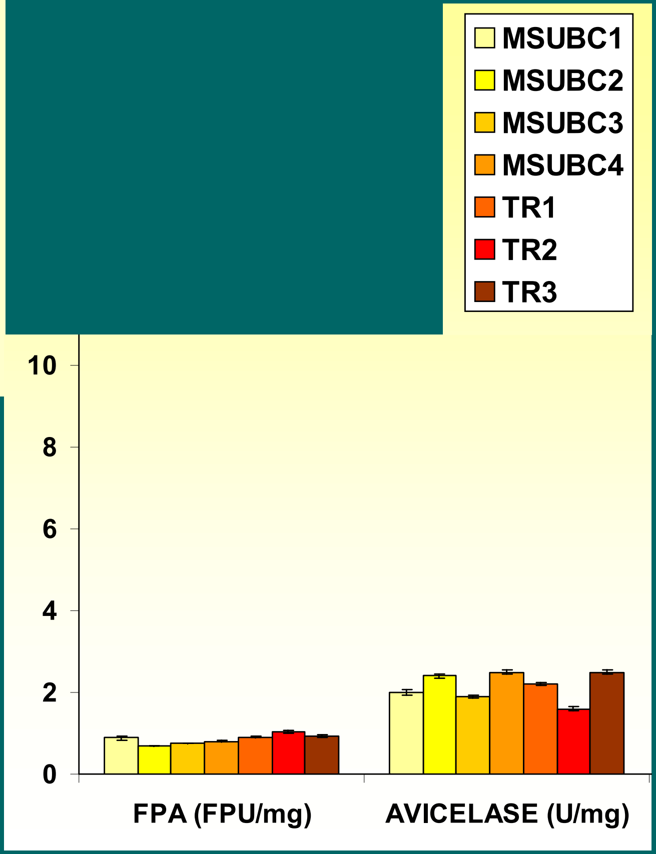


Source: Evaluation of cellulase preparations for hydrolysis of hardwood substrates. Appl. Biochem. Biotechnol. 2006 Spring;129-132:528-45

III – FPB Recent Achievements (2003-2006)

VII. Poor correlation between standard assays to evaluate enzyme activity and the performance of these enzyme on lignocellulosic substrates.





Sources:

Alex Berlin et al. (2005) Evaluation of novel fungal cellulase preparations for ability to hydrolyze softwood substrates – evidence for the role of accessory enzymes. Enzyme and Microbial Technology, Volume 37, Issue 2, 1 July 2005, Pages 175-184

Alex Berlin et al. (2006) Evaluation of cellulase preparations for hydrolysis of hardwood substrates. Appl. Biochem. Biotechnol. 2006 Spring;129-132:528-45

Conclusions

1. **This century will be characterized by the transition from petrochemicals to carbohydrate-based commodities.**
2. **Emerging research areas, such as Biocommodity Engineering, will demand from lignocellulose degrading enzyme compositions to be fine tuned to "extract" the maximum value from biomass not just to convert its constituents into monosaccharides for ethanol production but also for production of other substances with high added value such as bioactive oligosaccharides.**
3. **While our understanding of the mode of action of "lignocellulases" has improved, we have much more to learn before we can efficiently develop enzyme cocktails with increased and more specific activities.**
4. **Rational engineering of these highly-specific and effective enzyme compositions must be based on a comprehensive understanding of the biomass chemistry and the interactions between biomass components and lignocellulose degrading enzymes.**

Acknowledgements

National Sciences and Engineering Research Council of Canada (NSERC)

Natural Resources Canada (NRCAN)

Novozymes, Genencor, NREL

Colleagues from Forest Products Biotechnology, UBC

Predictions…

Sometimes we go to the newspapers for our predictions. According to a report in the *New York Times*, by the year 2000, wood will be used only for food. If true, an appreciable role in the conversion will belong to microorganisms.

Source: ***Reese E.T., 1956, A Microbiological Process Report – Enzymatic Hydrolysis of Cellulose, Applied Microbiology, Vol. 4, p. 44***

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