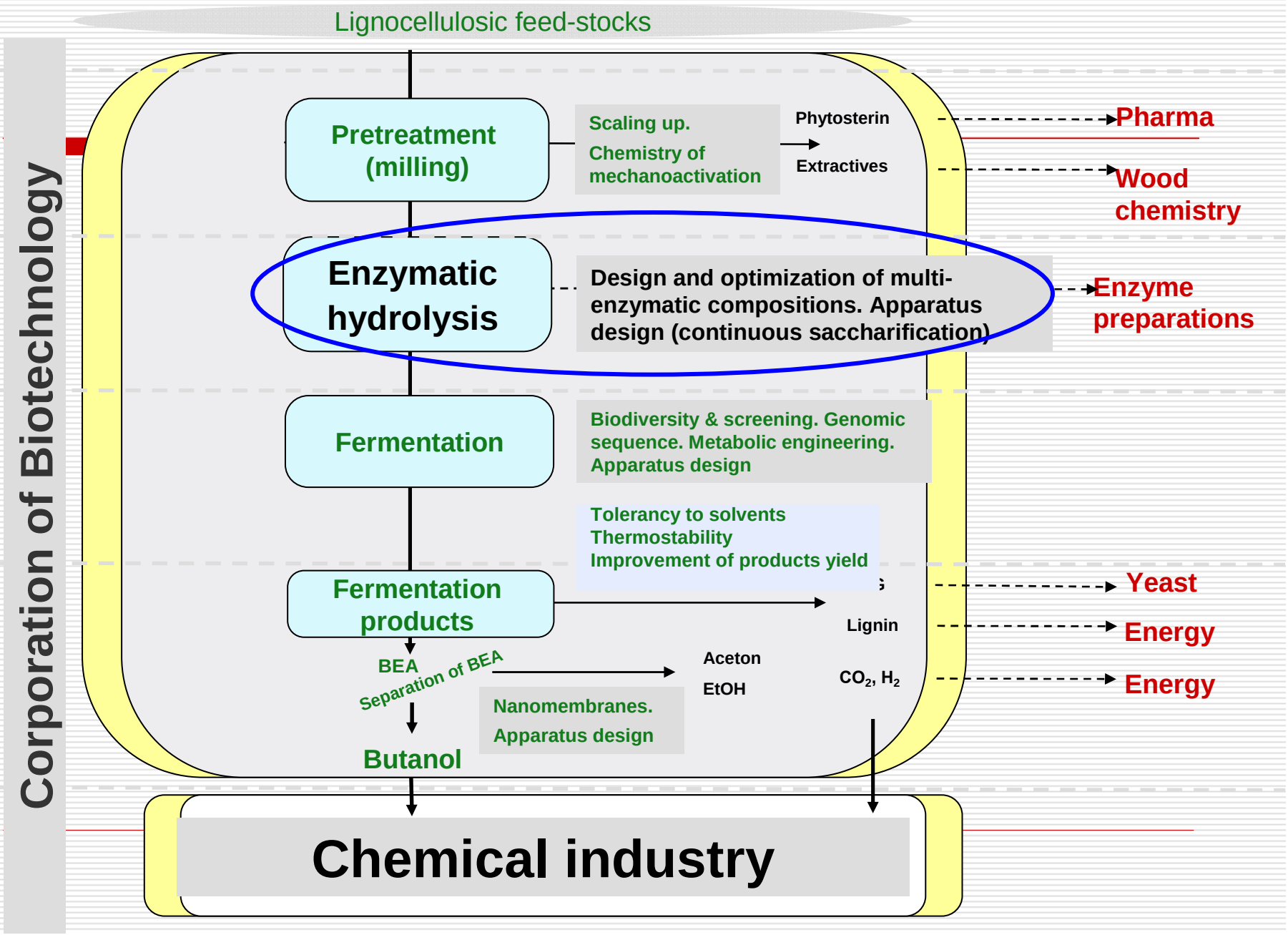
Enzyme complex for highly efficient saccharification of lignocellulosic feedstocks

Prof. Arkady P.Sinitsyn

A.N.Bach Institute of Biochemistry,
Russian Academy of Sciences

German-Russian Forum Biotechnology
At the BIOTECHNICA 2011, Hannover
10.10.2011

Concept of Pilot Plant for complex utilization of lignocellulosic feed-stocks



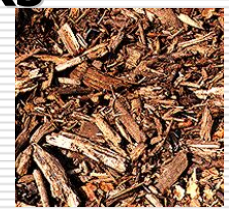
Selection of enzymes for saccharification of different lignocellulosic feed-stocks

DDG

Grain hulls

Corn stalks & stower

Straw

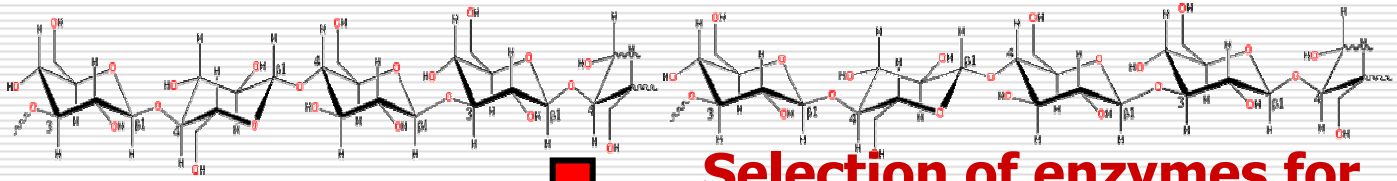


Woody materials

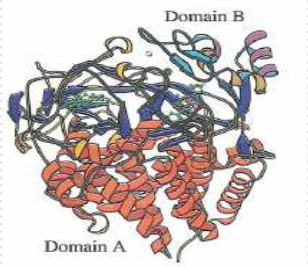
Bagasse

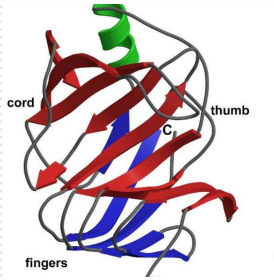
Grass

Pretreatment



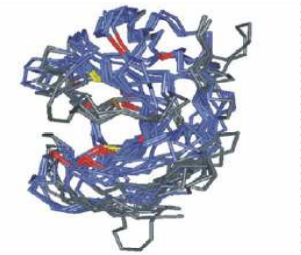
Selection of enzymes for hydrolysis of polysaccharides



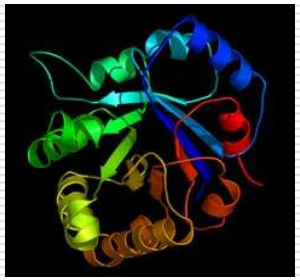


Eg-s

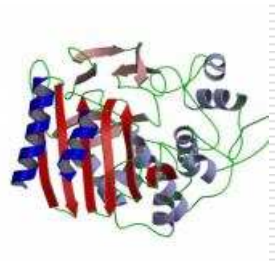
CBH-s



β-G



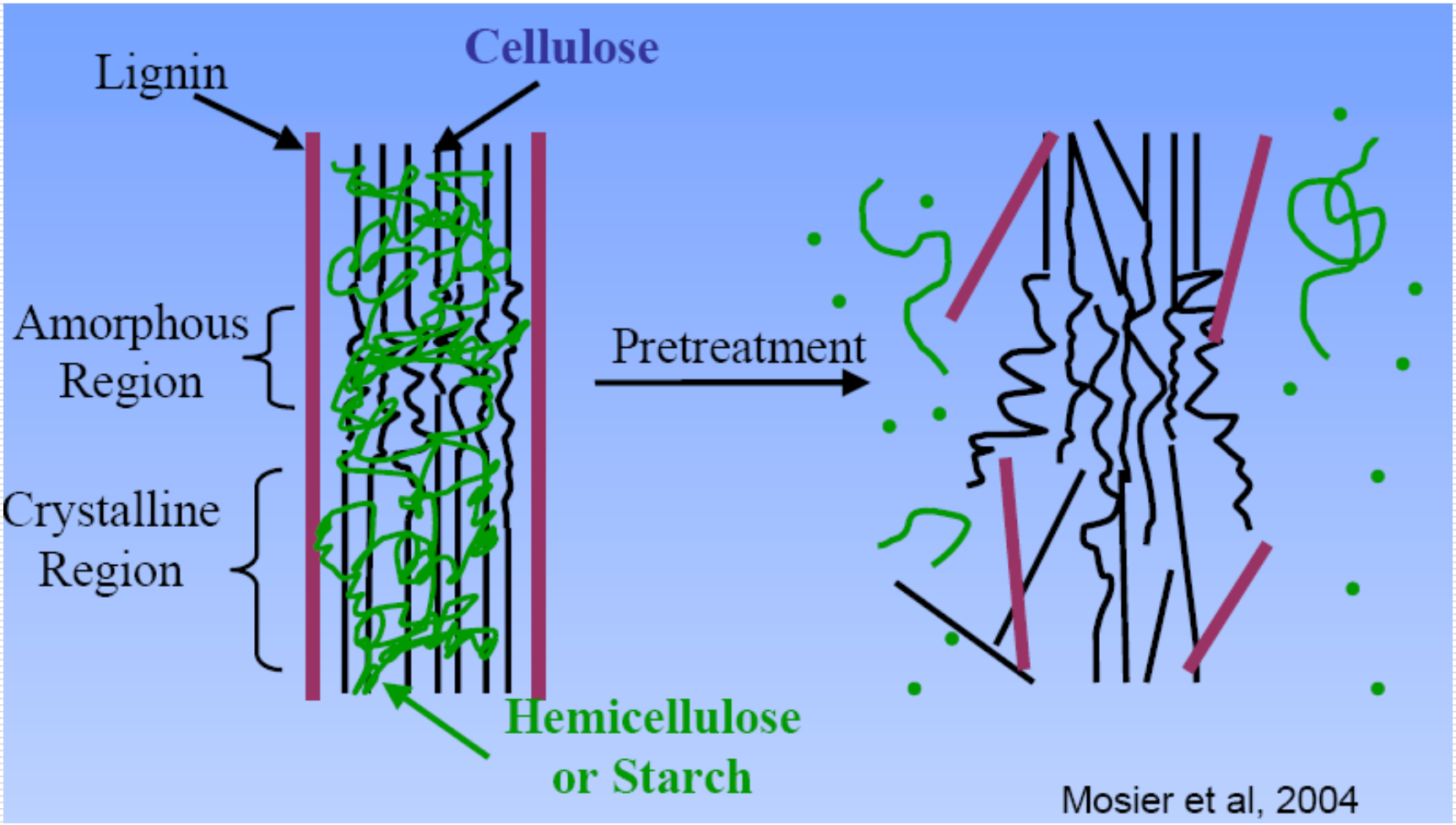
Xylanases
Mannanases



Accessory enzymes

Fermentable sugars (C6 and C5)

Pretreatment leads to destruction of a crystalline structure of cellulose and/or to lignin degradation



Different dry mills

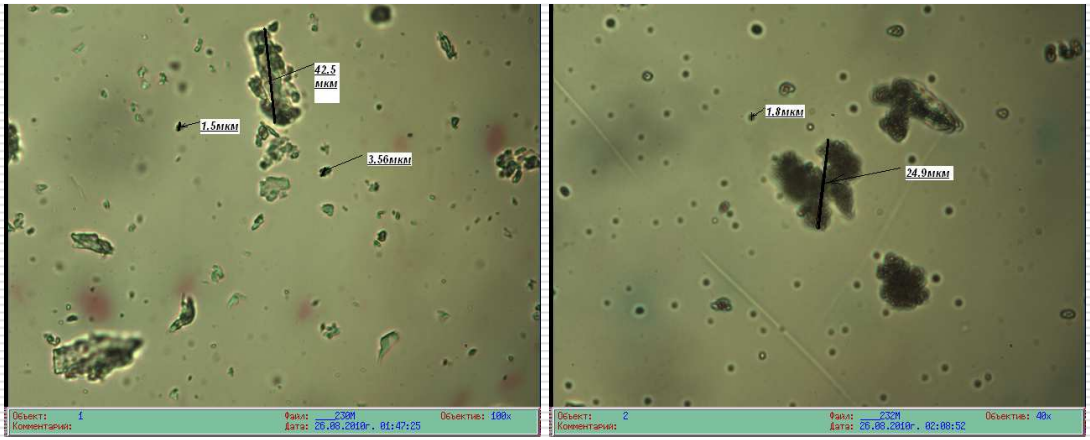
Impeller mill IM450

Ball mill-activator AGO-2

Efficiency of milling on the Impeller mill IM450

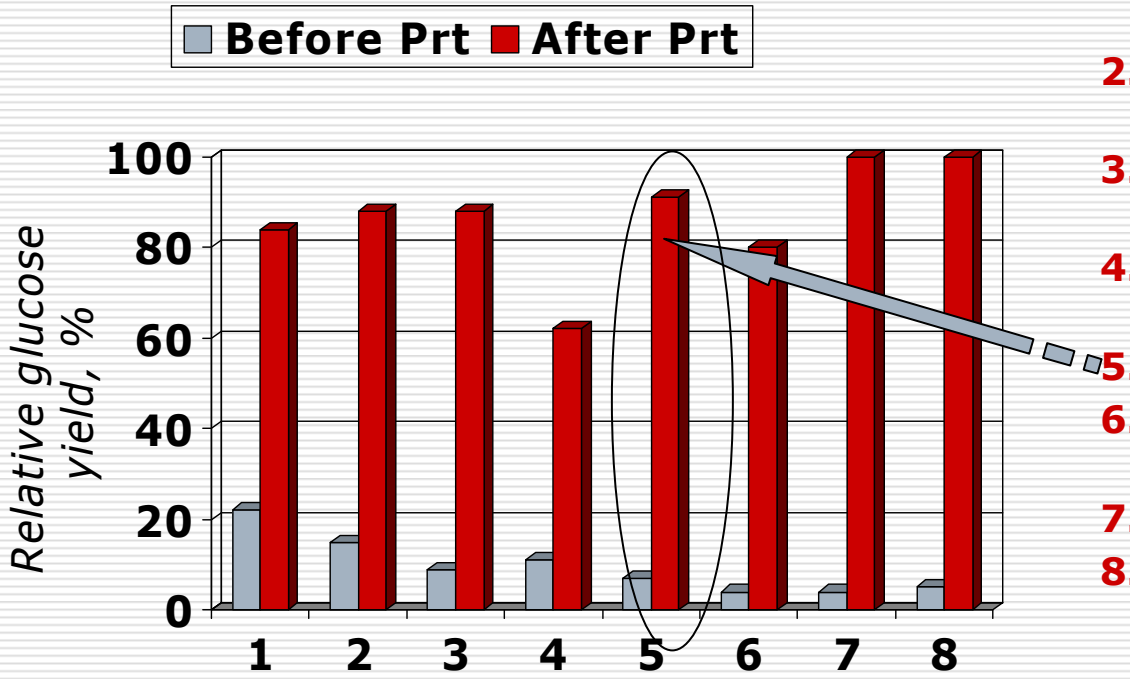
Initial aspen chips

Aspen after milling



Aspen particles after milling (light microscopy)

Comparison of reaction ability of different pretreated lignocellulosic feed-stocks



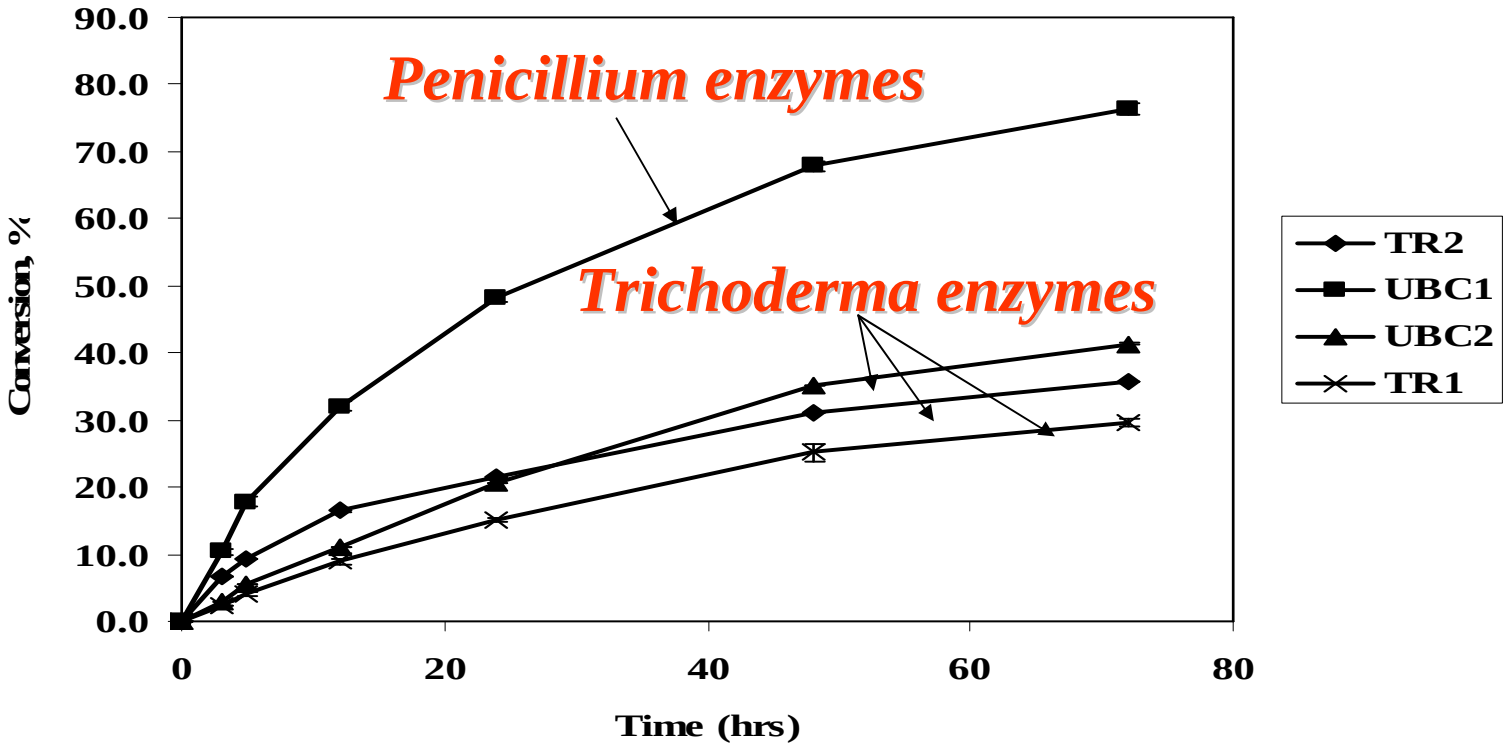
1. Bagasse / steam explosion
2. Wheat straw / steam explosion
3. Oat hulls / steam explosion
4. Rice hulls / steam explosion
5. **Hard wood / dry milling**
6. Soft wood / steam explosion
7. Soft wood / organosolv
8. High kappa cellulosic wood pulp

Saccharification by cellulases, 10 FPU/g + 20 bGU/g, pH 5, 50°C, 24 h

Novel strains producers of cellulases, hemicellulases and related enzymes available in INBI-MSU-Fermtech

- Mutants of *Penicillium sp.*:
  - Strain B221-151
  - Strain B221-6
- Mutants of *Trichoderma sp.*:
  - Strain TW-1
  - Strain TW-307

Selection of unique cellulolytic enzymes: hydrolysis of steam-exploded Douglas Fir by different cellulases

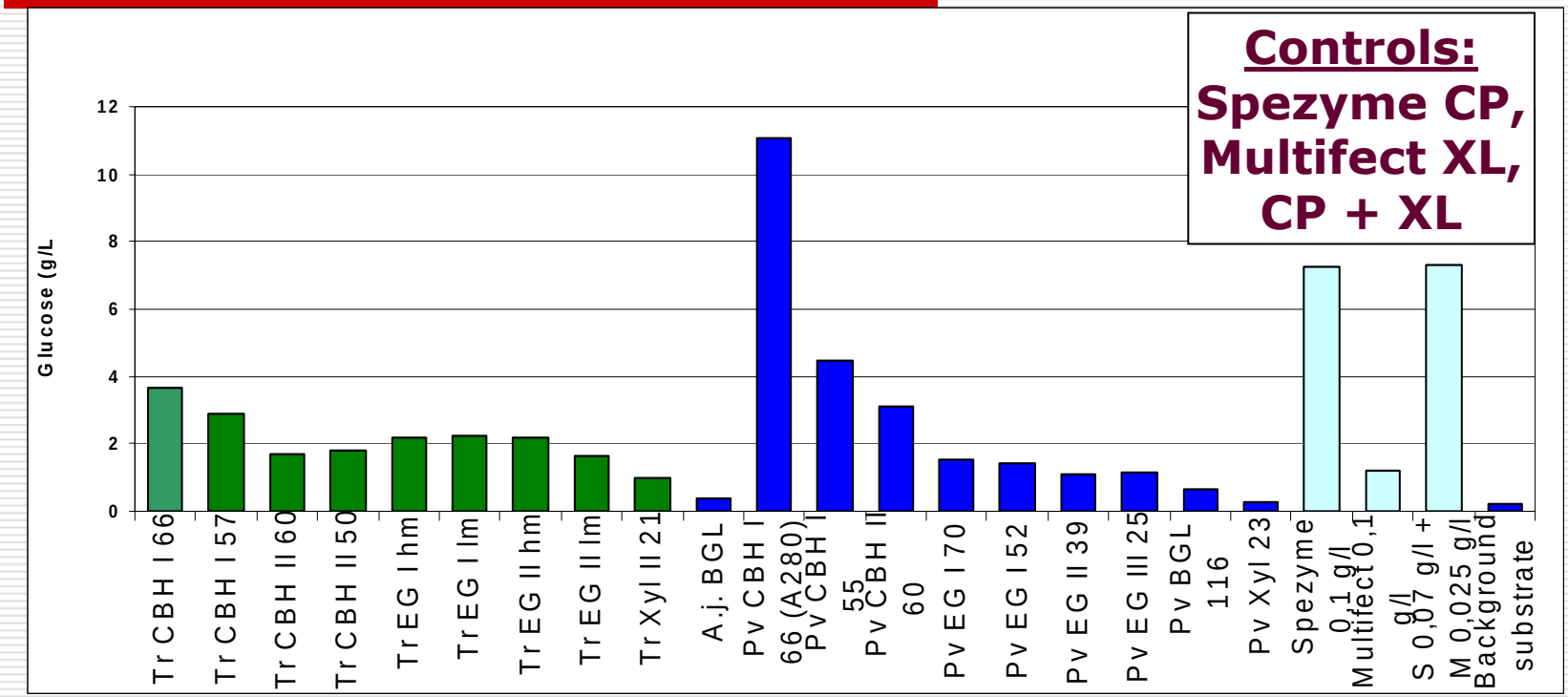


10 FPU per 1 g of substrate, [S] = 5%, pH 5, 50°C

Sinitsyn et al, *Appl.Biochem.Biotechnol.*, 2005, v.121-124, p.219

Glucose yield by action of individual enzymes

Pretreated Corn Cobs, pH5, 50°C, 72 hours.
Total protein concentration – 0.2 mg/ml,
cellobiase – 0.1 U/ml.



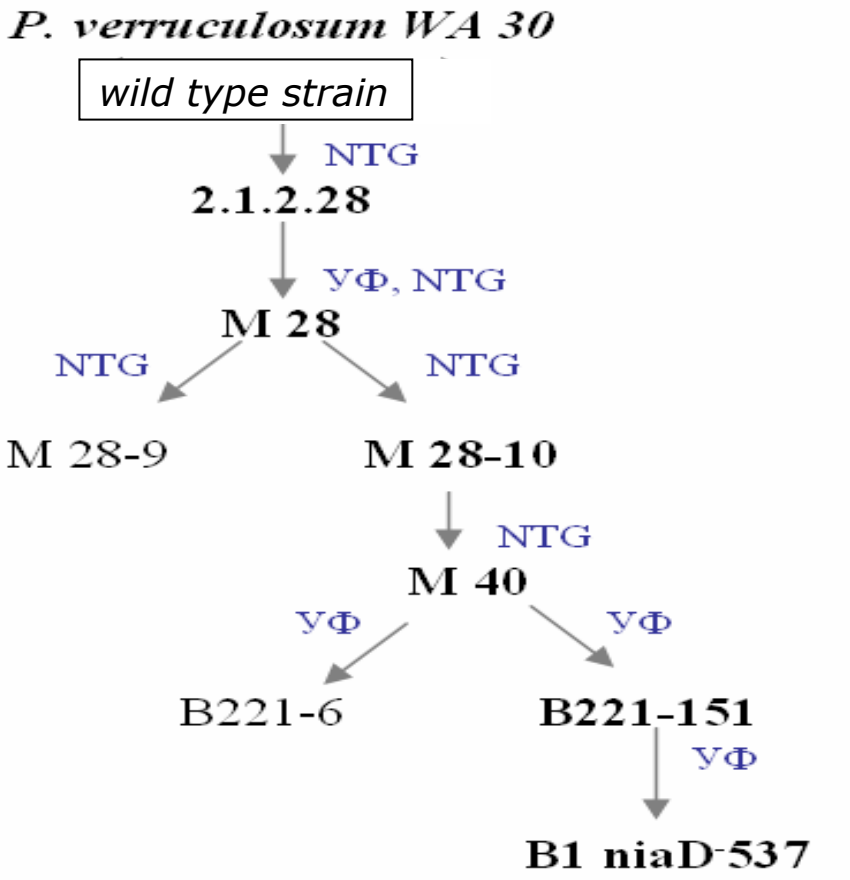
***Trichoderma* individual enzymes**

***Penicillium* individual enzymes**

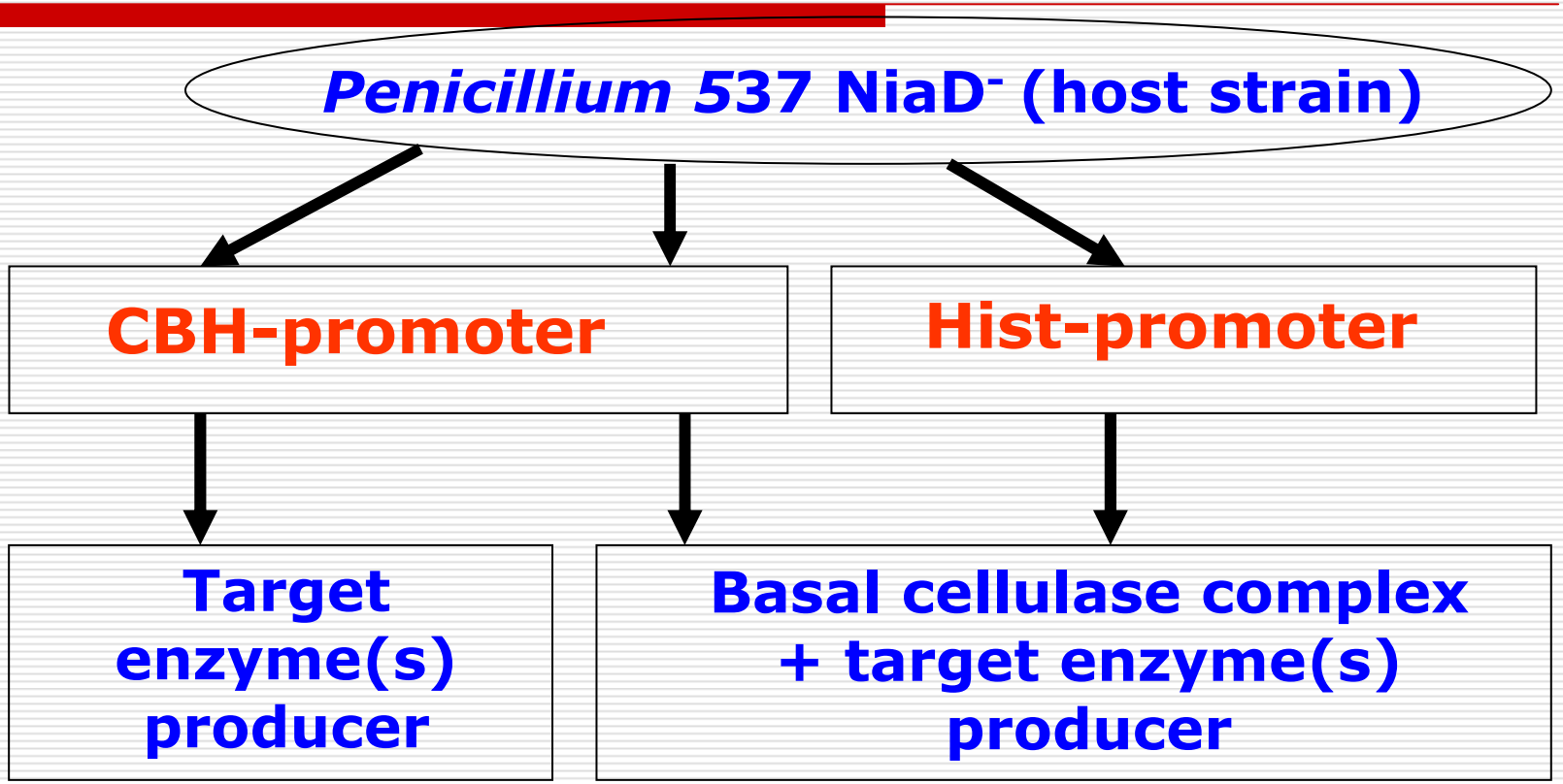
Penicillium sp. **strain – producer of cellulase complex with remarkably better saccharification ability than** ***Trichoderma sp.***

- Possible driving forces of *Penicillium* product:
  - High specific activity of different cellulases
  - Diverse and efficient enzymatic cocktail
  - Production of β-glucosidase (cellobiase)
  - Less inhibition by lignin and lignin residue
  - Less unproductive adsorption of cellulose

Results of classical mutagenesis of *Penicillium verruculosum* strain *(x5)*



Strategy for creation of recombinant strain – producers of enzymes for saccharification of lignocellulosic feedstocks. GENETIC ENGINEERING APPROACH

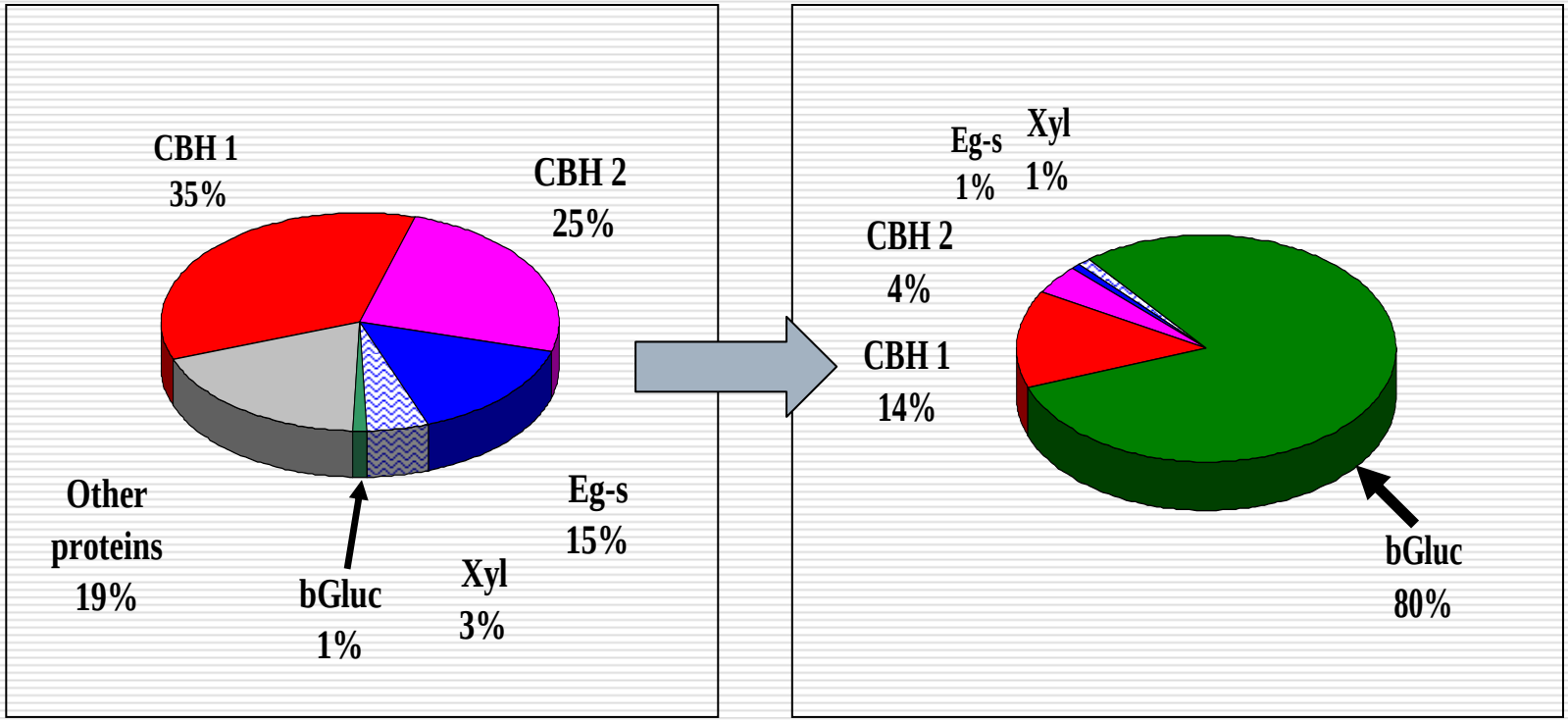


Example of high level expression of single target enzyme by recombinant *Penicillium* strain using *cbh1* promoter

(heterologous β-glucosidase)

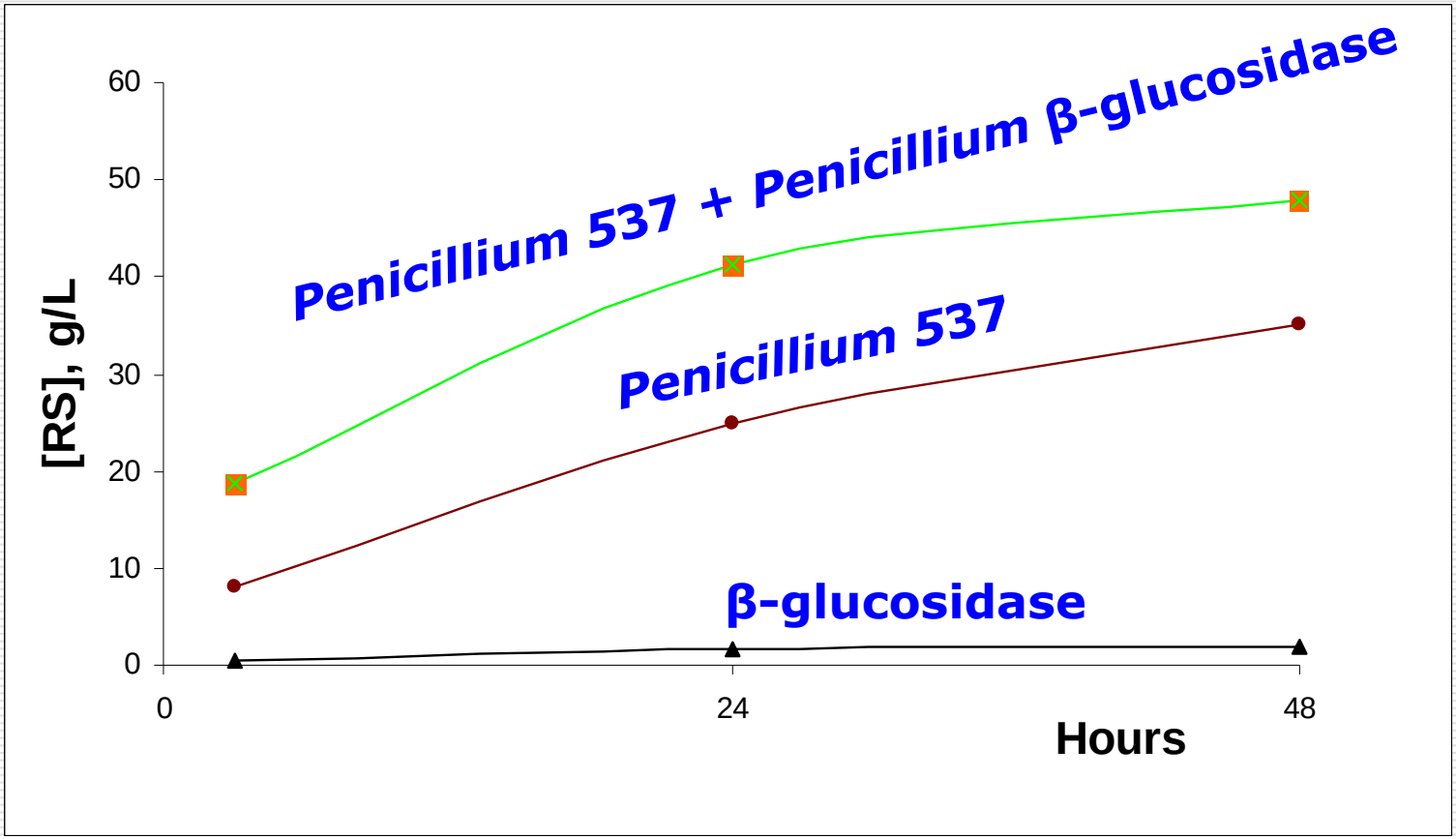
***Penicillium 537* host strain**

***Penicillium* strain – producer of β-glucosidase (CBH-promoter)**



Results of saccharification of milled softwood

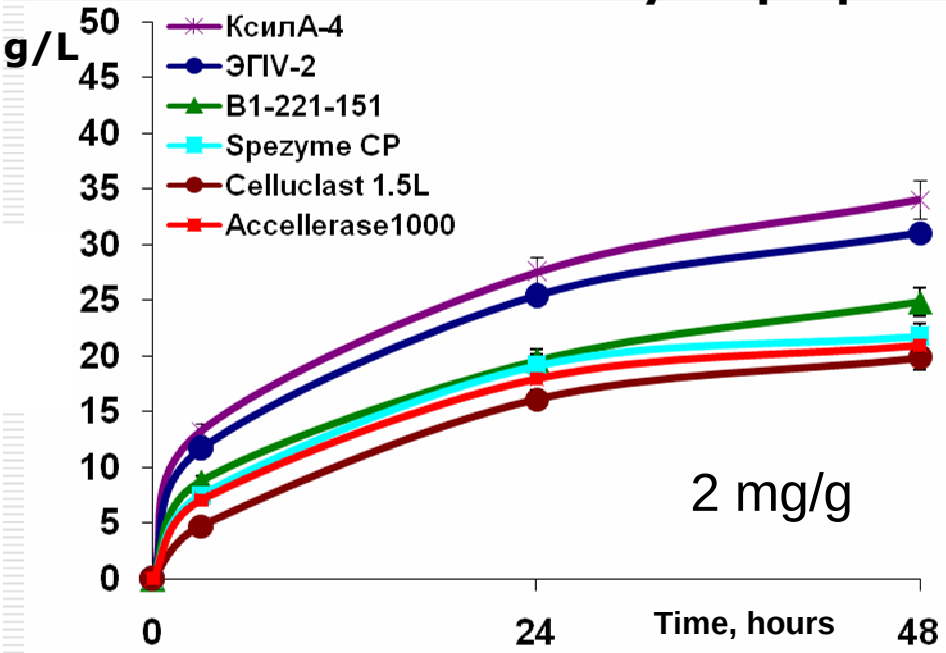
[S] = 10%, pH 5, 50°C

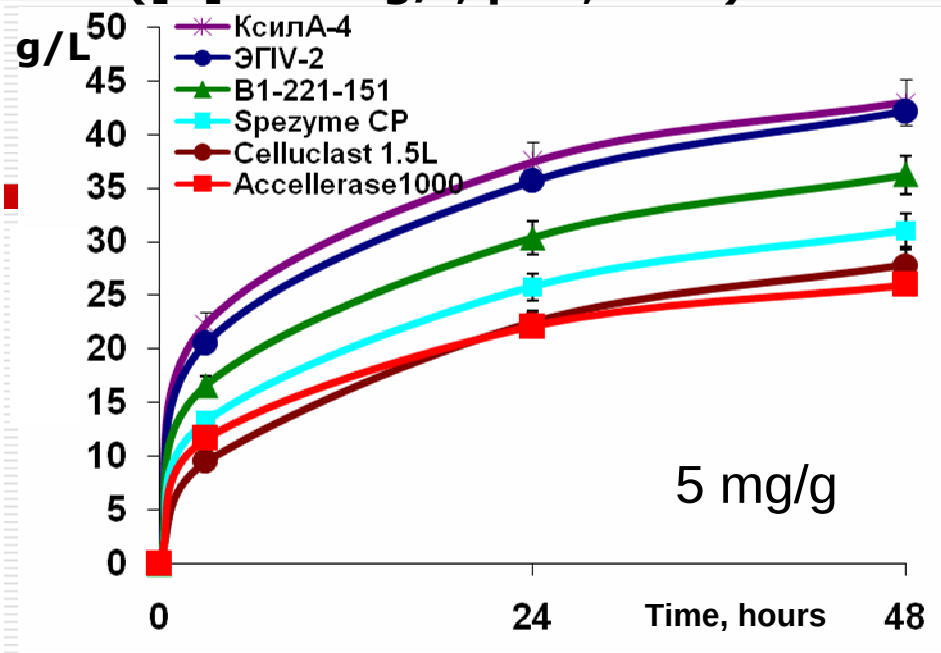


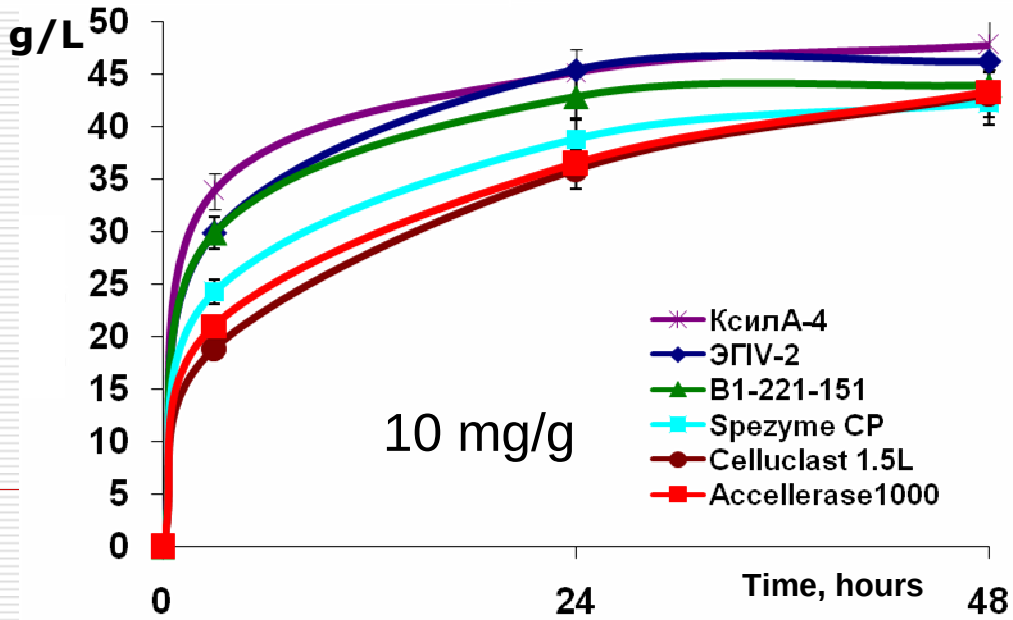
Cloning & expression of different genes using *cbh1* promoter

Strain	Gene	Enzyme
T.reesei and *P.verruculosum*	*cbh1* *cbh2*	Cellobiohydrolase 1 Cellobiohydrolase 2
T.reesei and *P.verruculosum*	*egl1* *egl2*	Endoglucanase 1 Endoglucanase 2
T. reesei	*egl4*	Endoglucanase 4
P. canescens	*xylA*	Xylanase A
T. reesei	*xylIII*	Xylanase 3
T. reesei	*manB*	Mannanase B

Dosage dependence (in mg of protein per 1 g of substrate) on reducing sugars yield in the course of saccharification of dry milled hardwood by recombinant enzyme preparations ([S]= 100 g/l, pH5, 50°C)



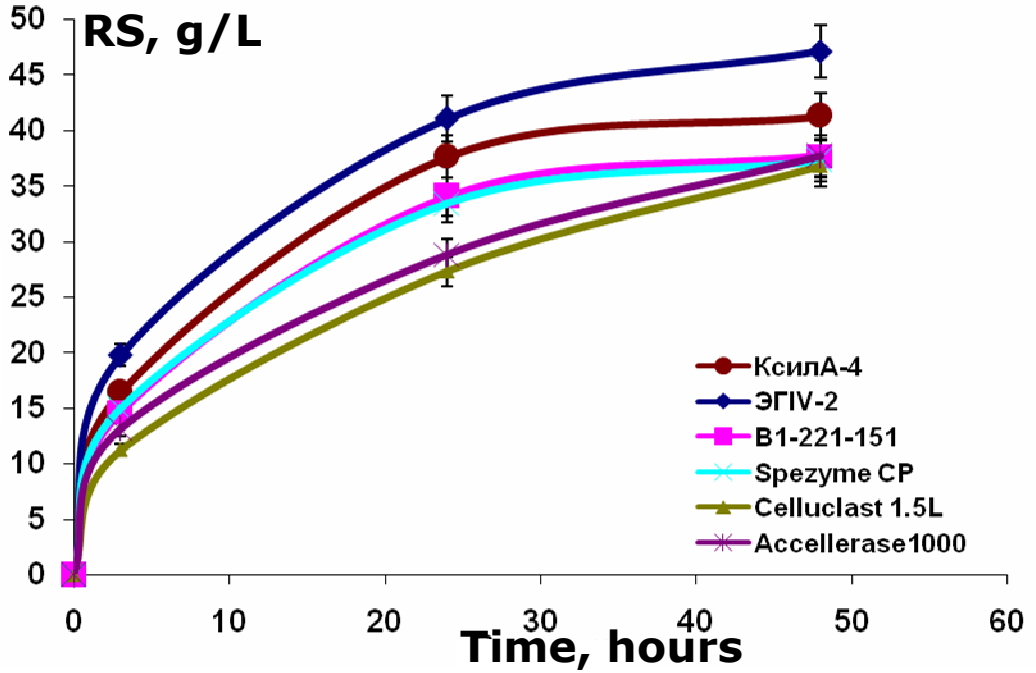




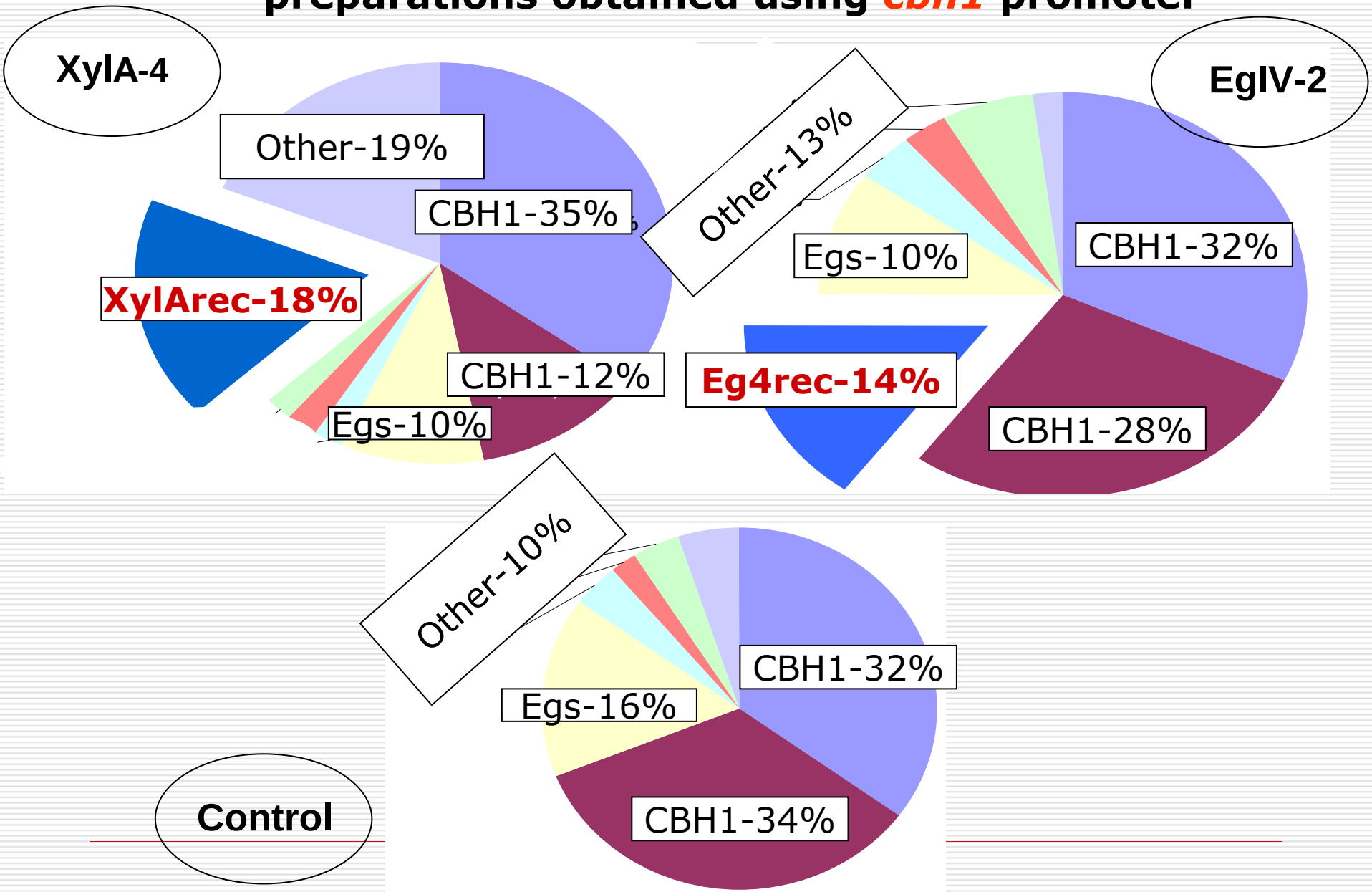
Results of saccharification of dry milled bagasse by recombinant enzyme preparations (*cbh1* promoter)

RS release in the course of saccharification of milled bagasse by XylA-4, EgIV-2 and reference enzyme preparations

[E]= 5 mg/g, [S]= 100 g/l, pH 5, 50˚C



Composition of the most efficient recombinant enzyme preparations obtained using *cbh1* promoter

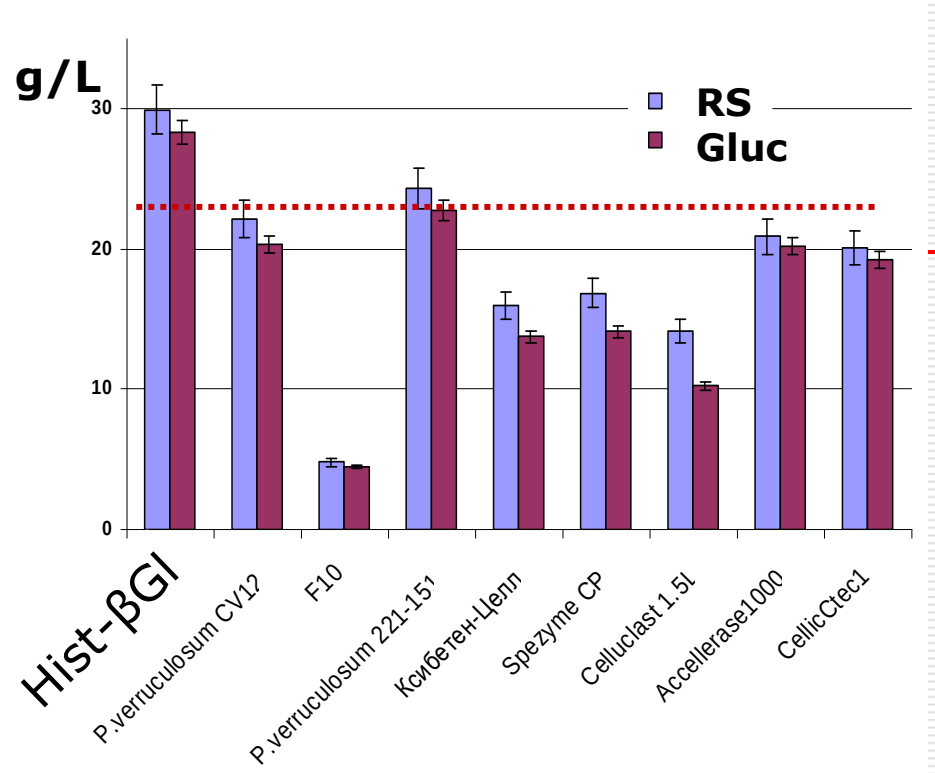


Cloning of different genes using *hist4* promoter

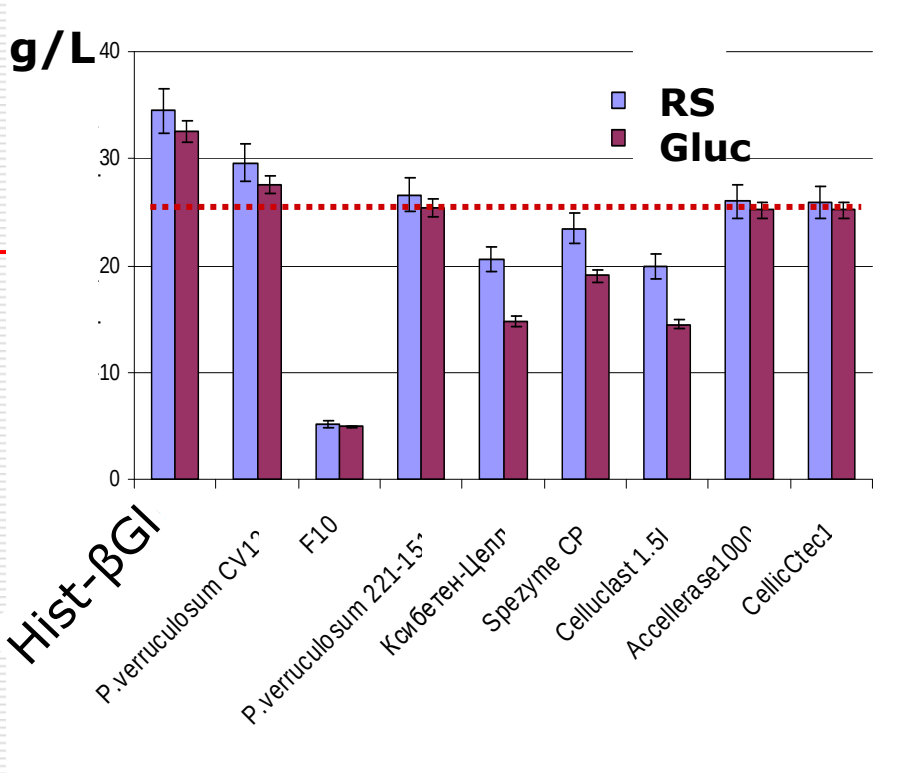
Strain	Gene	Enzyme
A.niger	*bgl* (AN: AF302657)	**β-glucosidase**
P.canescens	*xylA* (AN: AY756109)	**Xylanase A**
P.verruculosum	*egl2*	**Endoglucanase 2**

Results of screening of recombinant enzyme preparations obtained by *hist4* promoter by saccharification ability, dry milled hardwood and bagasse

[E]= 5 мg/g, [S]= 100 g/l, pH 5, 50^{0}C, 24 hours



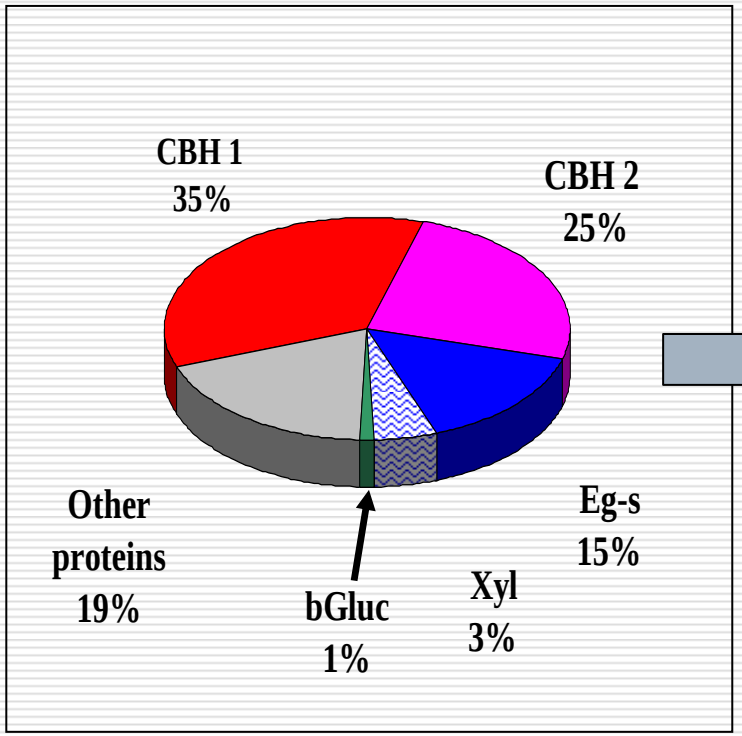
Dry milled hardwood



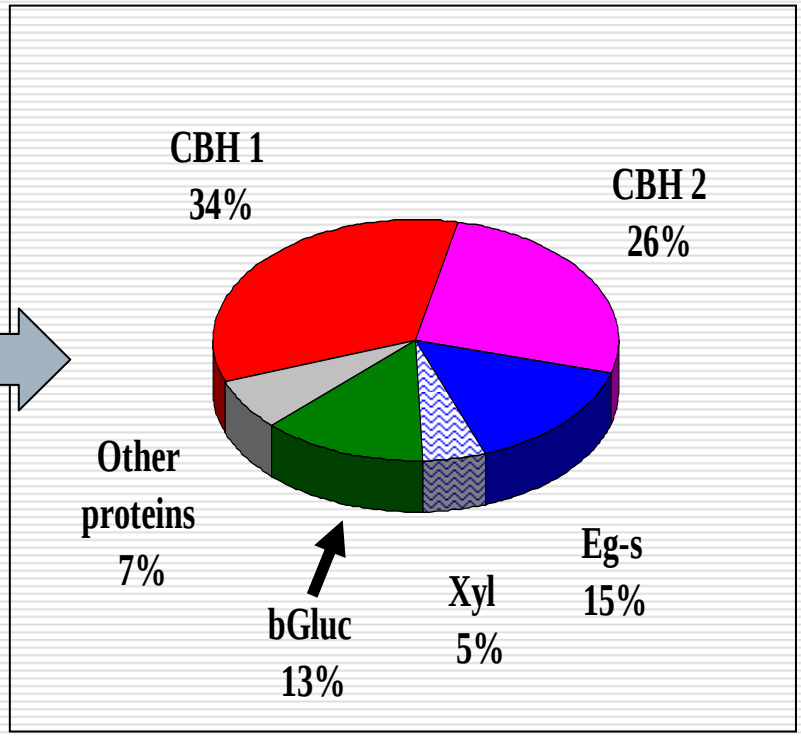
Dry milled bagasse

Composition of the most efficient recombinant enzyme preparation obtained using *hist4* promoter (heterologous β-glucosidase)

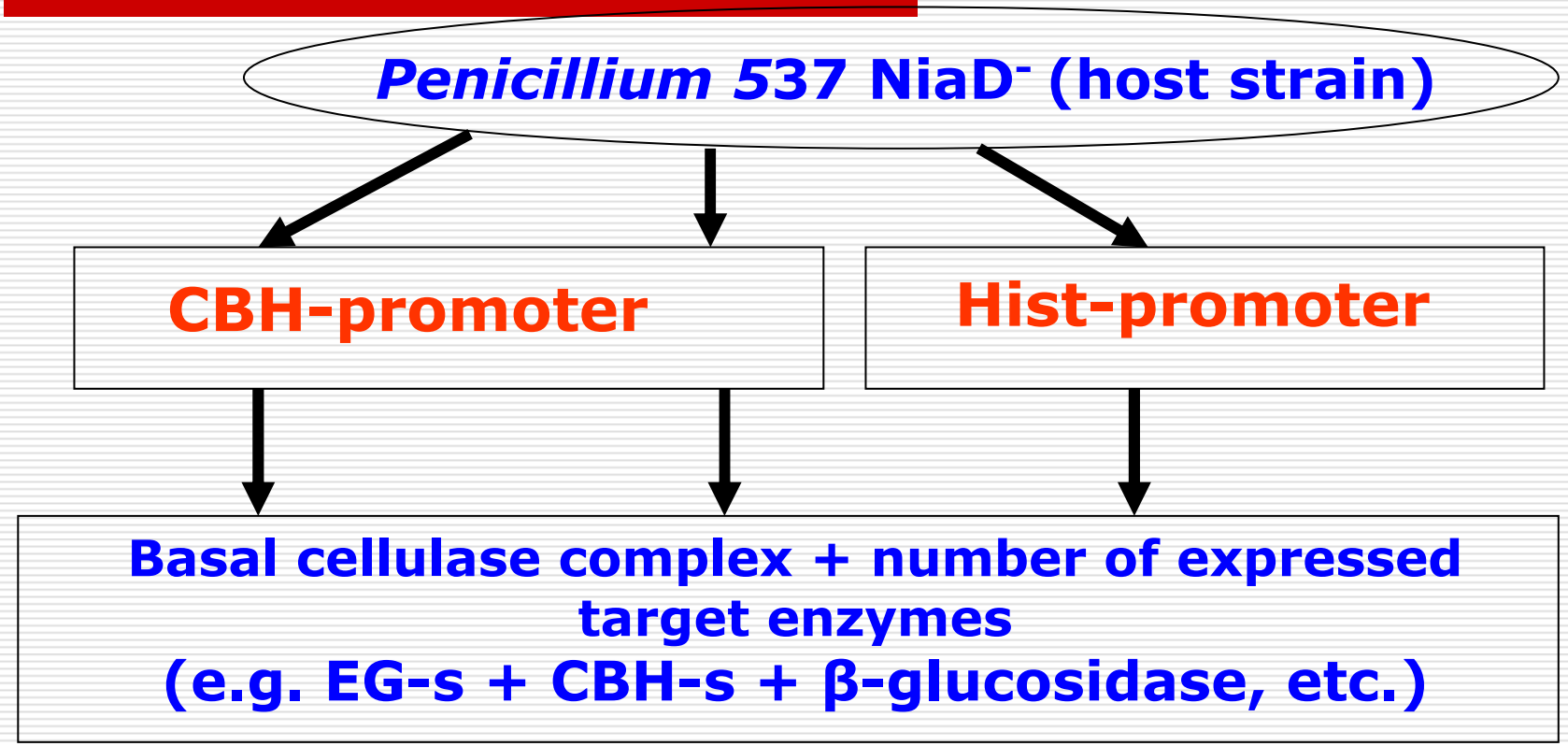
***Penicillium 537* host strain**



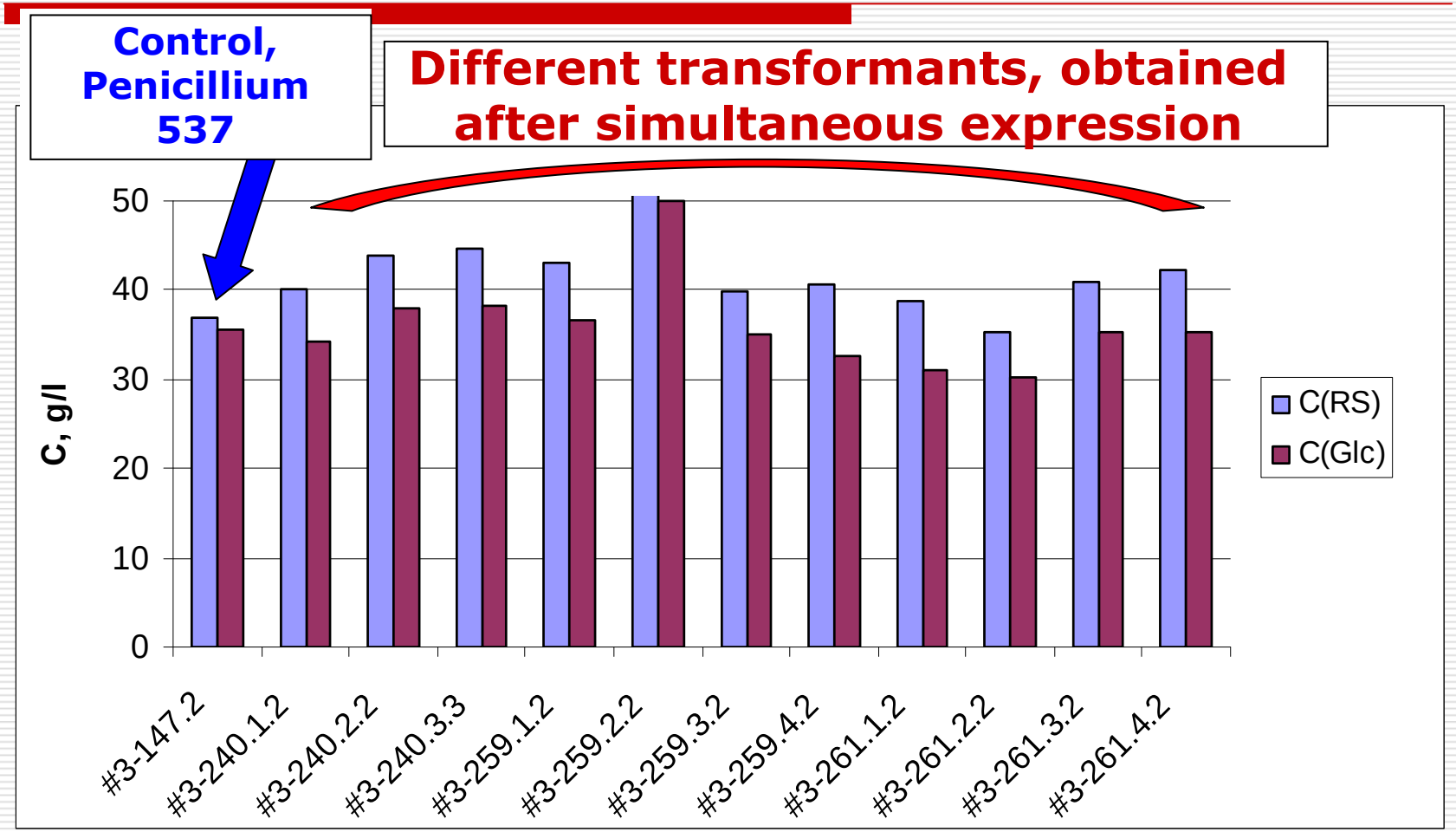
***Penicillium* strain – producer of cellulase complex enriched by β-glucosidase (hist-promoter)**



Creation of recombinant strain – producers of efficient enzyme complex for saccharification of lignocellulosic feedstocks by simultaneous expression of several target genes



Results of hydrolysis of dry milled bagasse by enzyme preparations obtained by recombinant *Penicillium* strains after simultaneous expression of different target enzymes (EG-s, CBH-s, bG)



5 mg/g of protein loading, [S] = 10%, pH 5, 50°C

Thank you !

apsinitsyn@gmail.com

www.inbi.ras.ru

+7 (495) 939-5966
+ 7 (916) 611-4857