Penicillium Hosts as the Platform for the Development of New Recombinant Strains Producers of Carbohydrolases and Related Enzymes

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Grain

Grain hulls

Corn stalks & stower

Straw

Woody stocks

Bagasse

Grass

Pretreatment

Biotransformation

Biobased chemicals

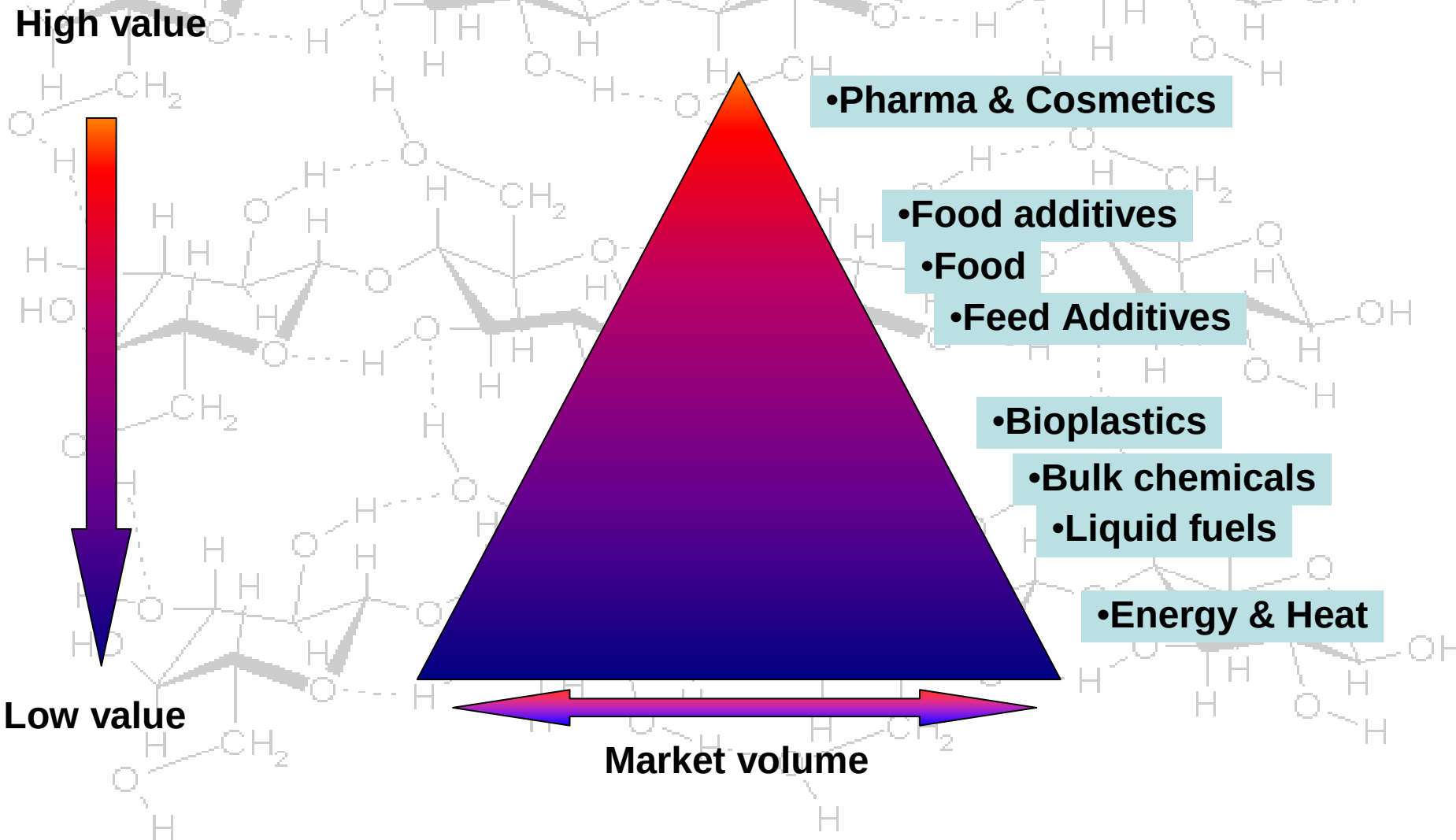
Pharma products

Biobased fuels

New animal feeds

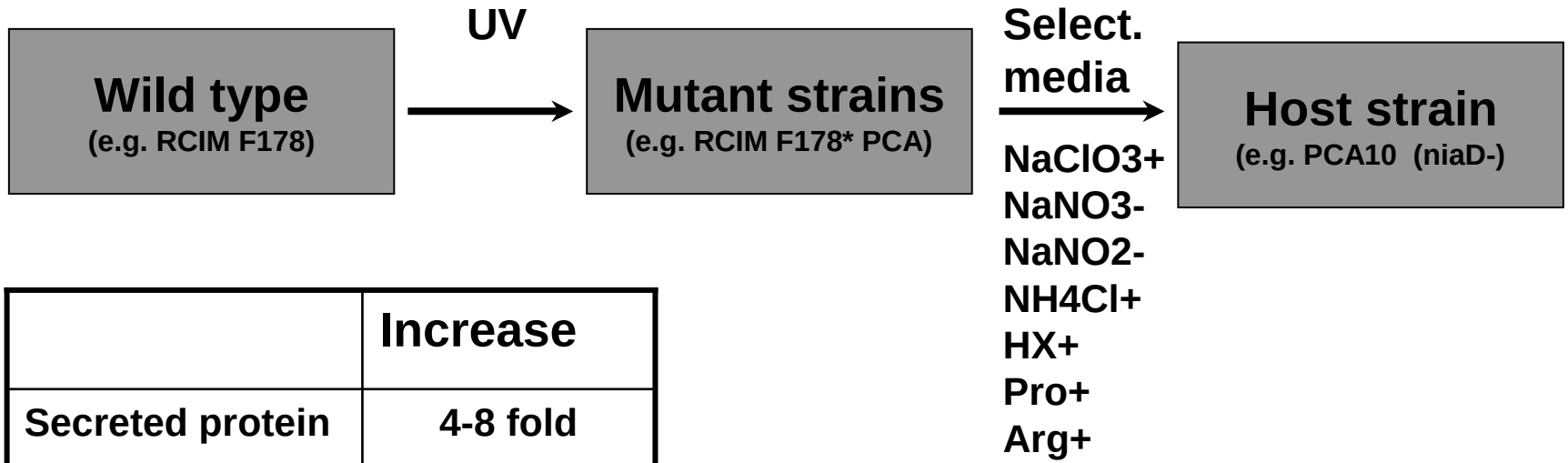
Heat and electricity

Value added from biomass



Penicillium host strains. Stage 1

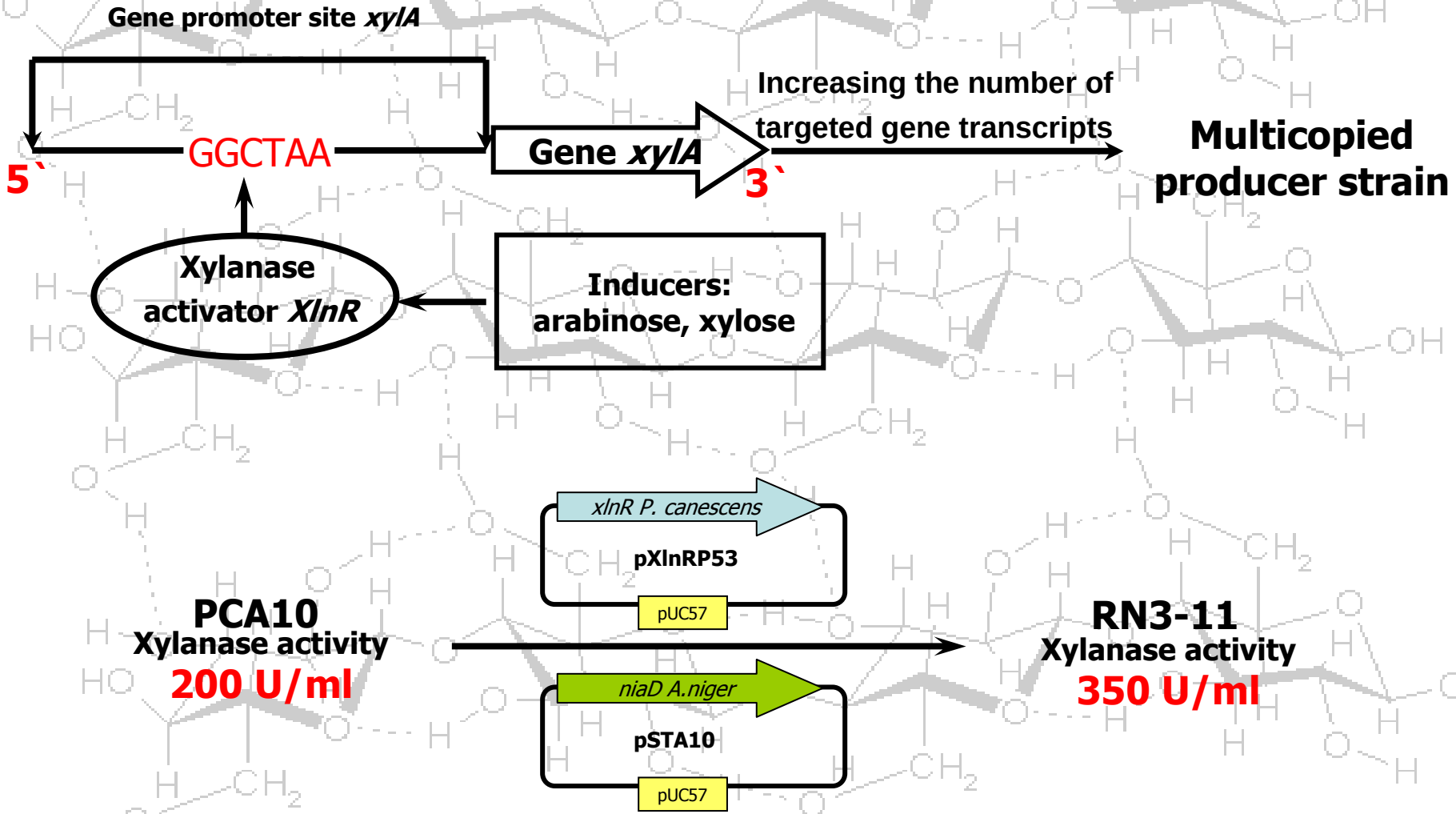
- **UV mutagenesis of the wild type *Penicillium* strains**



	Increase
Secreted protein	4-8 fold
Xylanase activity (total)	5-8 fold
β-galactosidase activity (total)	~5 fold
Cellulase (MCC) activity (total)	4-6 fold

Penicillium as the host. Stage 2

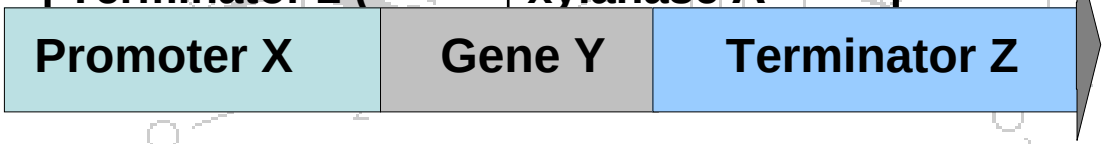
- Cloning of xylanase gene transcription activator XlnR

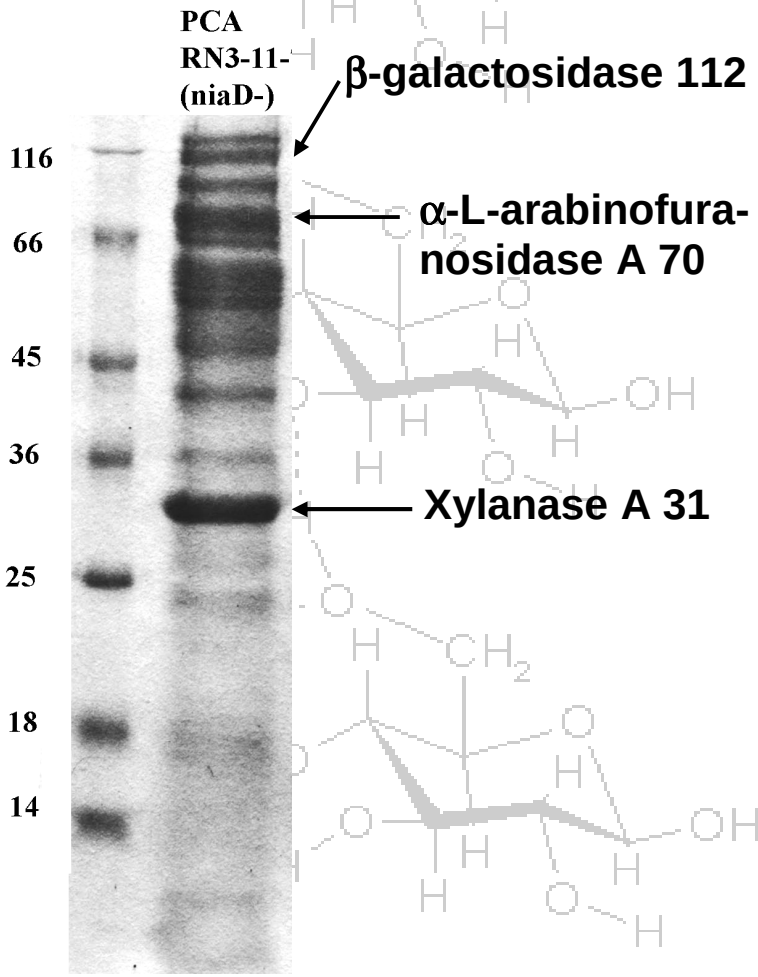


Penicillium as the recipient strain. Stage 3

- Cloning the regulatory gene fragments of major enzymes

Regulatory elements	The structural part of the encoding gene
Promoter 1 (~2200 bp)	β-galactosidase (*bgal*)
Promoter 2 (~ 1700 bp)	xylanase A (*xylA*)
Promoter 3 (~ 889 bp)	arabinofuranosidase A (*abfA*)
Terminator 1 (~ 2000 bp)	β-galactosidase
Terminator 2 (~	xylanase A





Enzymes for Industrial Biotechnology

endo-1,4-β-glucanase,
endo-1,4-β-xylanase
phytase, pectinase

→ **Agriculture/Animal feeds**:
Feeds additives

pectinase,
cellobiohydrolase,
endo-1,4-β-glucanase
β-glucosidase,
α-galactosidase,
β-galactosidase,
inulinases

→ **Food Industry and Processing**:
Clarification of fruit juices,
Food Industry by-products treatment

endo-1,4-β-glucanase

→ **Textile**:
Fabric treatment, Denim wash

endo-1,4-β-xylanase

→ **Pulp-and-paper:**
Non-chlorine paper bleaching

Cellulases, β-glucosidase,
hemicellulases, α-amylase,
glucoamylase

→ **Bioalcohols / Biofuels:**,
Ethanol and butanol from Starch
and Lignocellulose

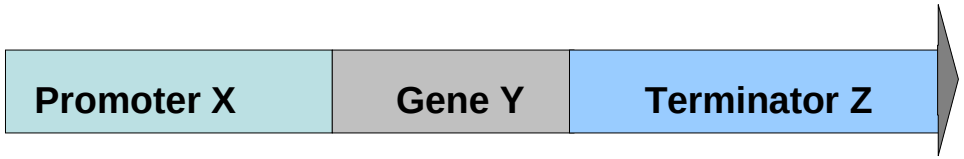
The Main Goal:

Strain development and obtaining of enzyme preparations with attractive biotechnological properties on base of *Penicillium* recombinant strains

Targets:

- Targeted genes cloning into *Penicillium* (ΔniaD) host strains.
- Screening and selection of transformants with targeted activities.
- Optimization of fermentation schemes and conditions (joint cultivations, inducers, batch/feed-batch et al) and enzyme preparation production for further evaluations.

Targeted Genes, Expressed in the *Pen.canescens* strain



Homologous genes

Promoter X	Gene Y	Secreted Enzyme
bgaS	*abfA*	α-L-arabinofuranosidase A
bgaS	*aglA*	α-galactosidase A
xylA	*faeA*	ferruloil esterase A
bgaS	*xegA*	xyloglucanase A
bgaS	*xylA*	xylanase A
bgaS	*pelA*	pectinlyase A
bgaS	*phyA*	phytase A
bgaS	*rglA*	rhamnogalacturonanlyase A
bgaS	*abfB*	arabinofuranosidase B
abfA	*bgal*	β-galactosidase

Targeted Genes, Expressed in the *Penicillium* strains

Penicillium sp.

egl2 - endo-1,4-β-glucanase II

egl3 - endo-1,4-β-glucanase III

cbhI - cellobiohydrolase I

cbhII - cellobiohydrolase II

Trichoderma sp.

manB - mannanase B

xyl3 - xylanase III

Heterologous genes

Aspergillus sp.

aglC - α-galactosidase C

inu1 - exo-inulinase

inuA - endo-inulinase

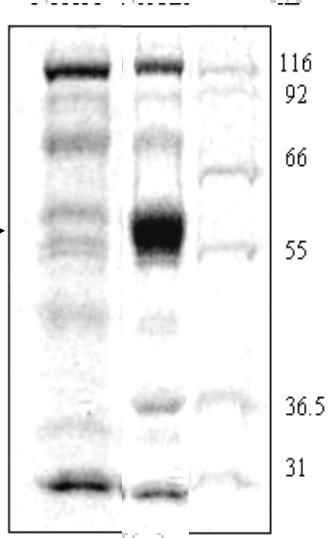
bgl1 - β-glucosidase

phyA - phytase

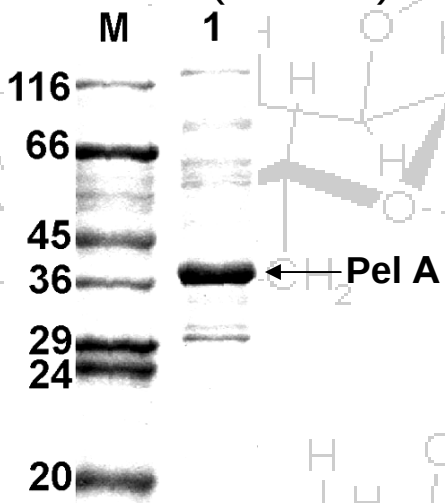
Lipases, esterases, oxidases and more…

Targeted Genes, Expressed in the *Pen.canescens*

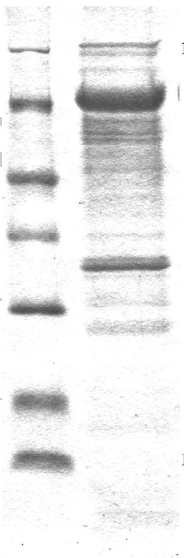
α-galactosidase *P.can* (60 kDa)



Pectinlyase *P.can* (40 kDa)



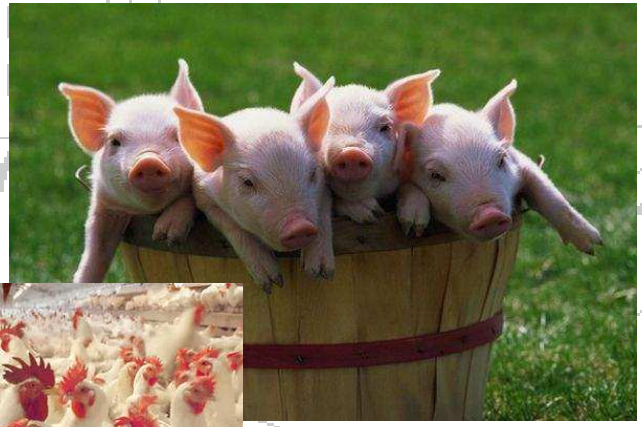
Cellobiohydrolase I
P.verruculosum (66 kDa)

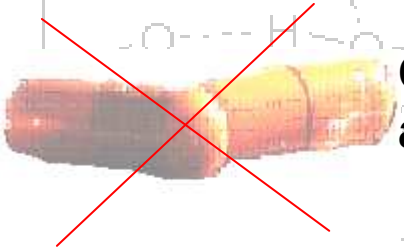


Target Activities in Recombinant Strains

Preparation	Substrate	Targ.activity, U/ml	Increase,fold
Abf6	p-NPh-α-L-arabinofuranoside	**50-60**	**>10**
AglA33	p-NPh - α-Gal	**1200-1400**	**>500**
AglC4	p-NPh - α-Gal	**140-160**	**>15**
FAE9	p-NPh – butyrate	**50-70**	**>30**
XG9	Xyloglucan	**60-80**	**>20**
PhPl29	Phytin, pectin	**220, 180**	**>10, >90**
PhPlAgl9	Phytin, pectin, p-NPh -α-Gal	**280, 120, 325**	**>15, >60, >400**
Phy215	Phytin	**400**	**>150**
PEC23	Pectin from citrus	**190**	**~100**
Eg2	Carbohymethylcellulose	**1200-1500**	**>200**
pBGL-32	p-NPh -β-Glc	**800-1000**	**>500**
CBHI	Avicel	**7-10**	**>5**
CBHII	Avicel	**7-10**	**>5**
INU1	Inulin	**2500-3000**	**>800**
MANB	Galactomannan	**35**	**>30**
XYLIII	Birch xylan	**500-600**	**>10**

Enzymes in Animal Feeds





Improved digestibility and energy value of feeds

Reduction of «antinutritional» factors in feeds

Improvement of the physiological state of animals

Growth and productivity acceleration

Feed components and enzymes used:

Wheat, rye, barley, corn – xylanases, β-glucanases, phytase

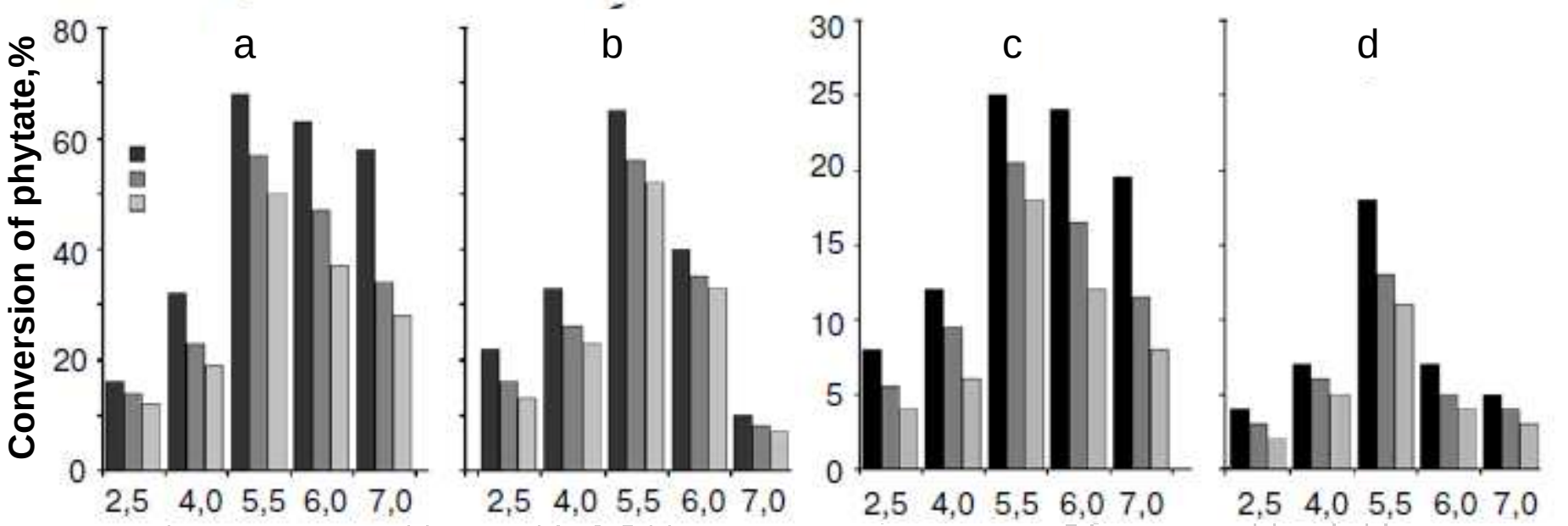
Soy, peas, lupin – α-galactosidases, protease

Sunflower – protease, hemicellulases

Protein-containing substrates (feather, trimming flour) – (endo)protease

Phytate Conversion in Animal Feeds

Soybean mill **Corn mill**

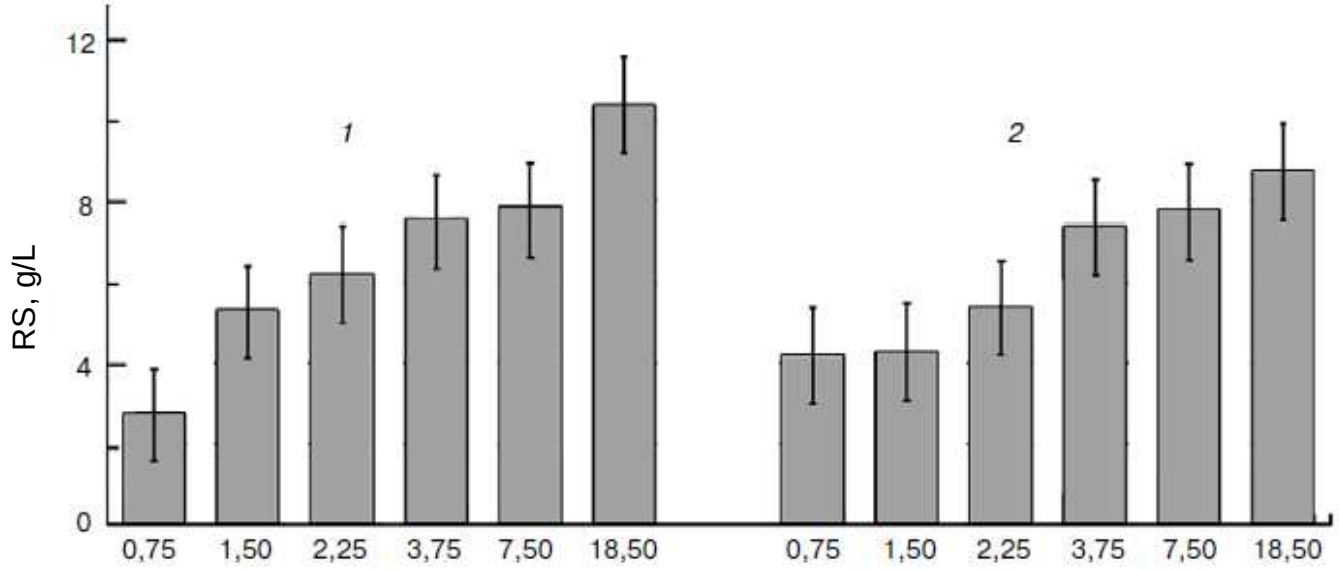


PHY 215 **Commercial Phytase *Asp.niger*** **PHY 215** **Commercial Phytase *Asp.niger***

Load, U of phytase activity
black - 150,
gray - 100,
light gray - 75

in vitro Feed Test.

α–Gal A *Pen. canescens* Commercial preparation



p-NPh-Gal activity dosage on 1 g of soy mill

Reducing sugars yield from soybean mill upon enzyme dosage
1- α-GalA *P.canescens*,
2- commercial preparation α-D-galactosidase/Amano/10

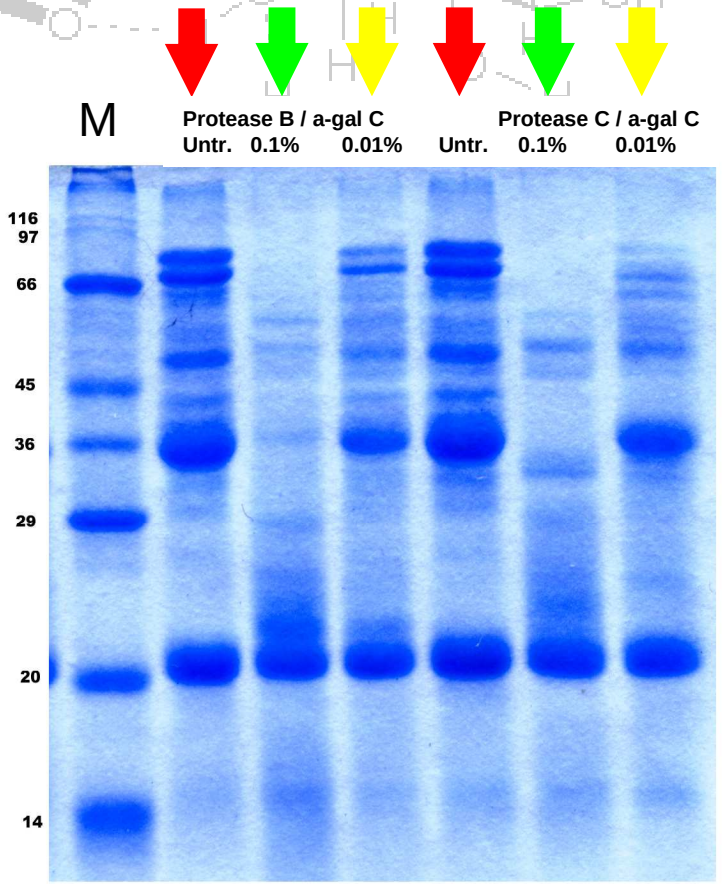
Protein digestibility from different sources

Untreated

- Egg white : 100 (taken as 100%)
- Whole milk proteins: 91
- Beef/trimming: ~80
- Casein: 77
- Soybean: 74
- Wheat gluten: 64

Hydrolysates:

- Trimming : 99-102
- Soybean hydrolysate: 95-98



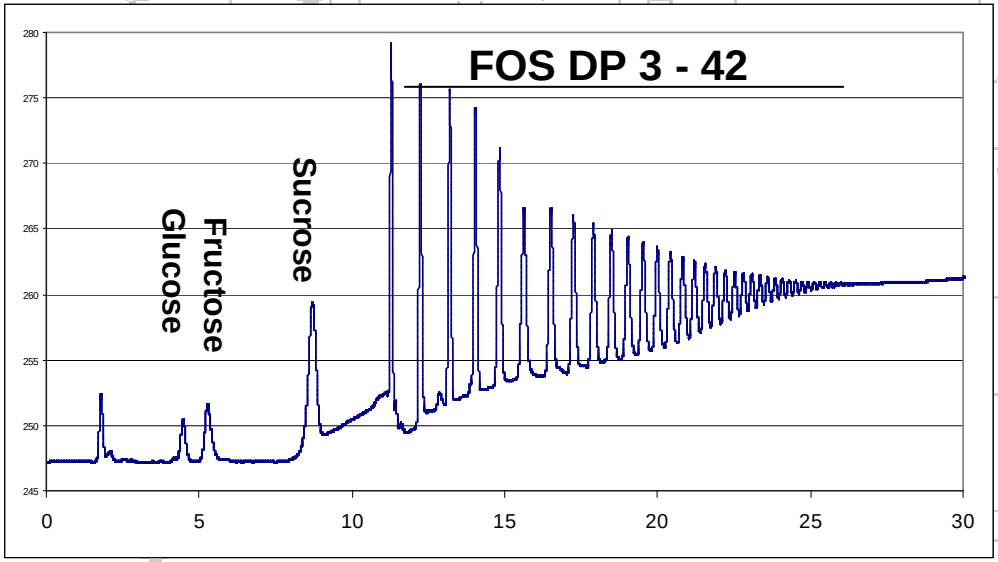
SDS-PAGE of soluble soy proteins with and w/out protease/a-galactosidase treatment

Applications in Food Industry

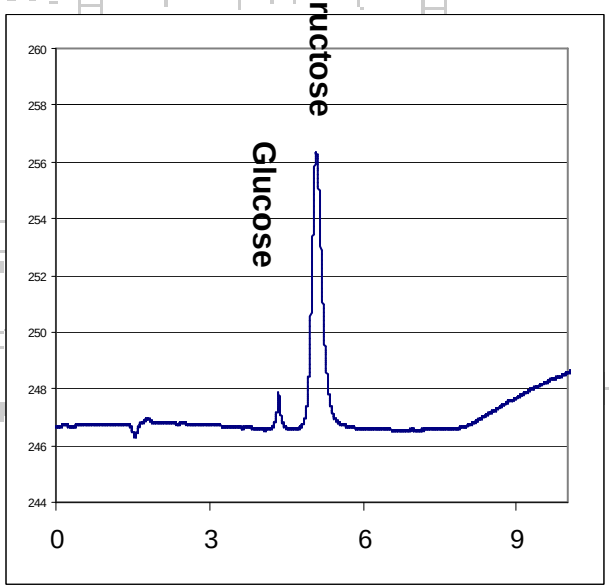
Fruit and berry juices and purees

Increase of juice yield from fruits and berries with the help of pectinases

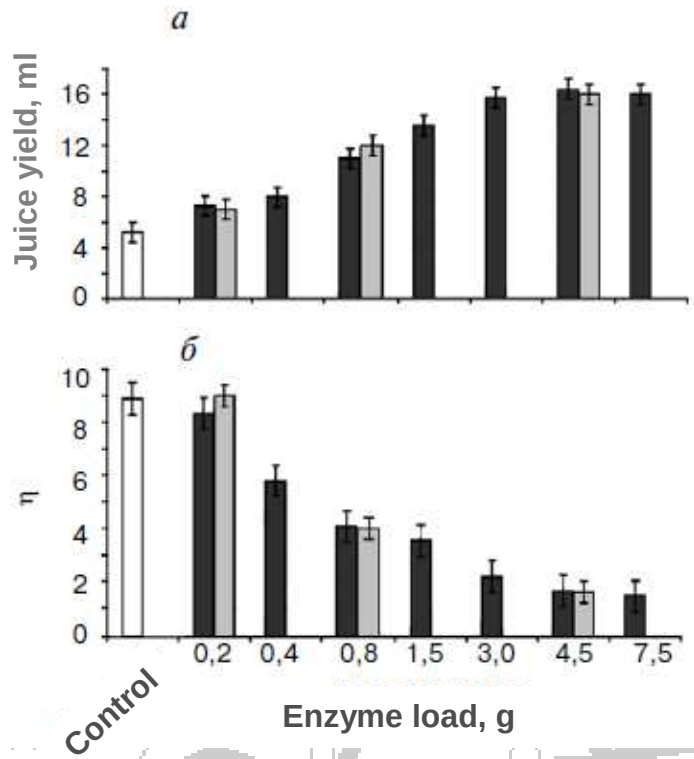
Jerusalem artichoke syrup processing



Inulinases



Cranberries Juice Production and Clarification



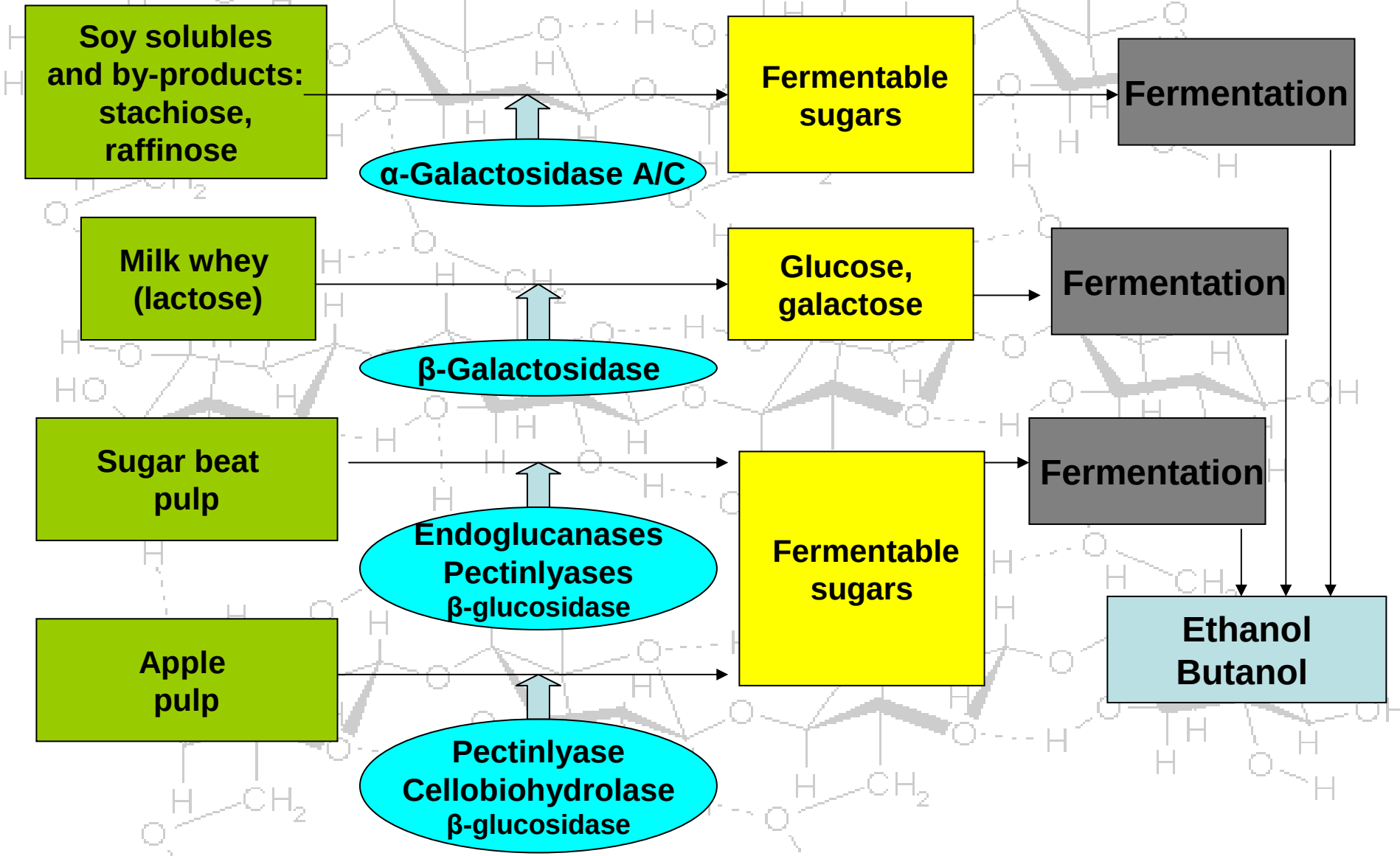
Yield of cranberry juice

Viscosity of cranberry juice

PelA preparation from *Pen. can.*
Enzyme dosage – grams per ton

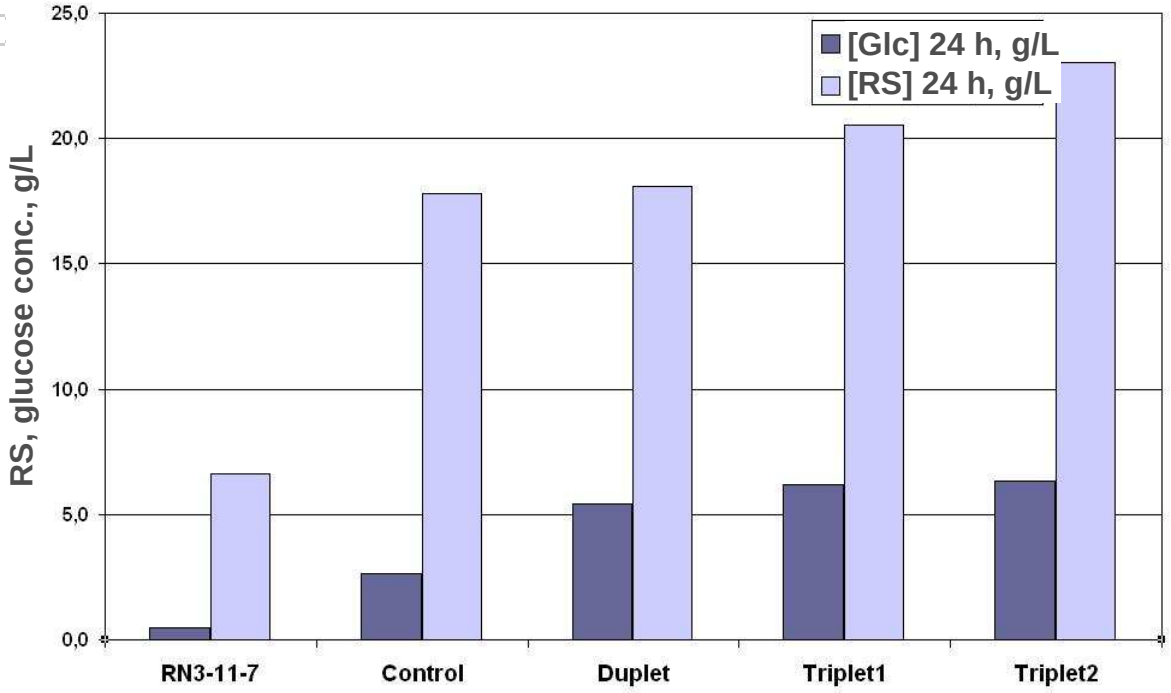
Control: Commercial Preparation
“Rapidase Press” (70 ml per 1 ton of berries)

Value added from food industry by-products (e.g. in biofuels production process)

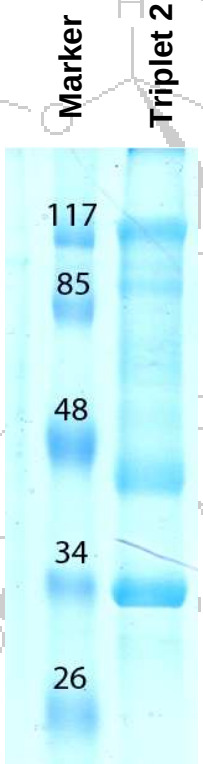


Bioconversion of Food Industry by-products

Apple Pulp after Juice Extraction



Duplet: PelA, β-G
Triplet1: EG II, PelA, β-G
Triplet2: EG II, PelA, β-G



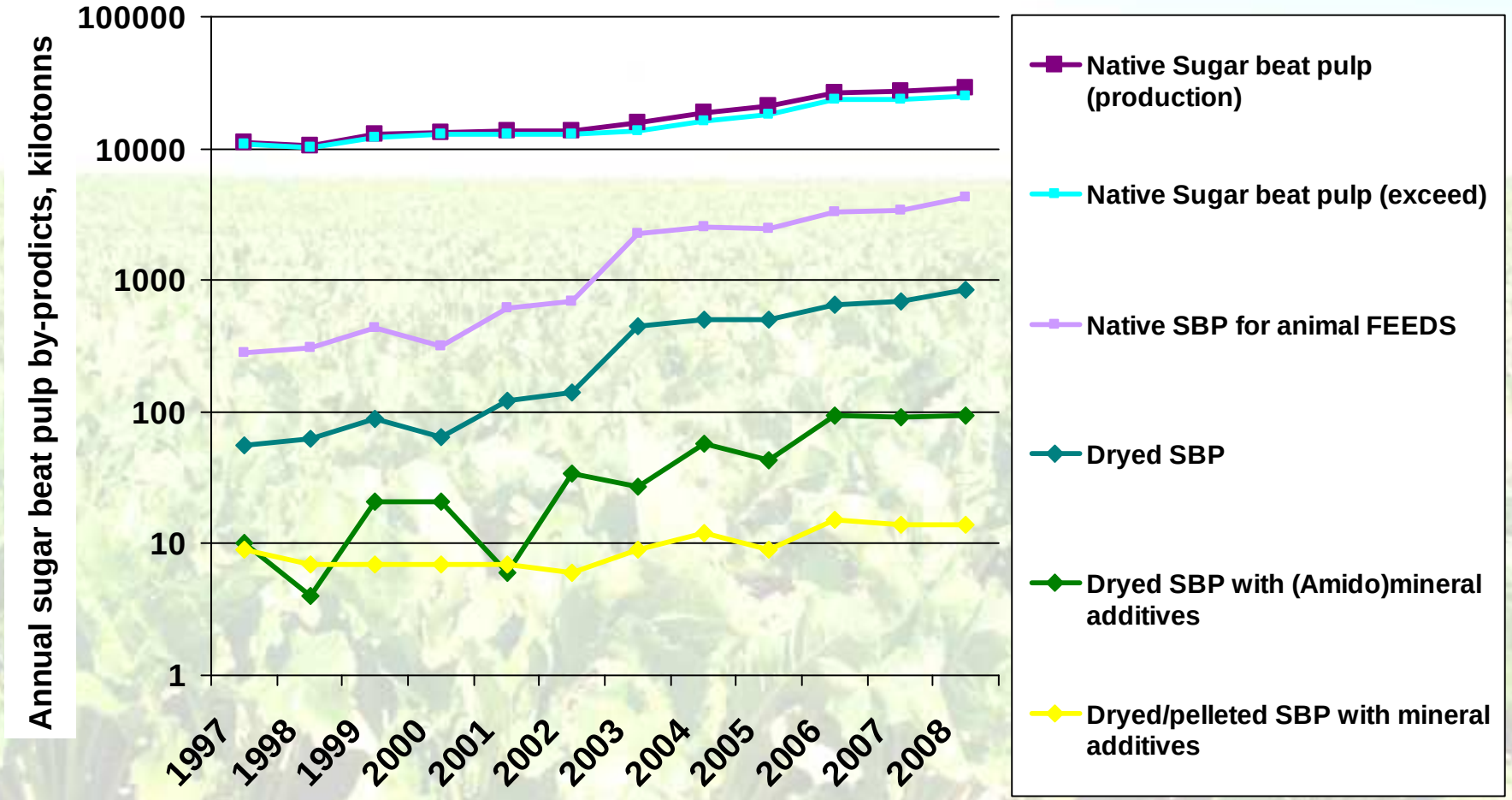
← β-G 116kDa

← Pel A 40kDa

← EG II 33kDa

Reducing sugars and glucose yield after the hydrolysis of apple pulp, 24 hours, 50°C,
Control – enzyme preparation after co-cultivation of single enzyme strains – β-G, Pel A, and EG II
RN3-11-7 – initial *Penicillium* host strain

Sugar beat pulp balance in Russia

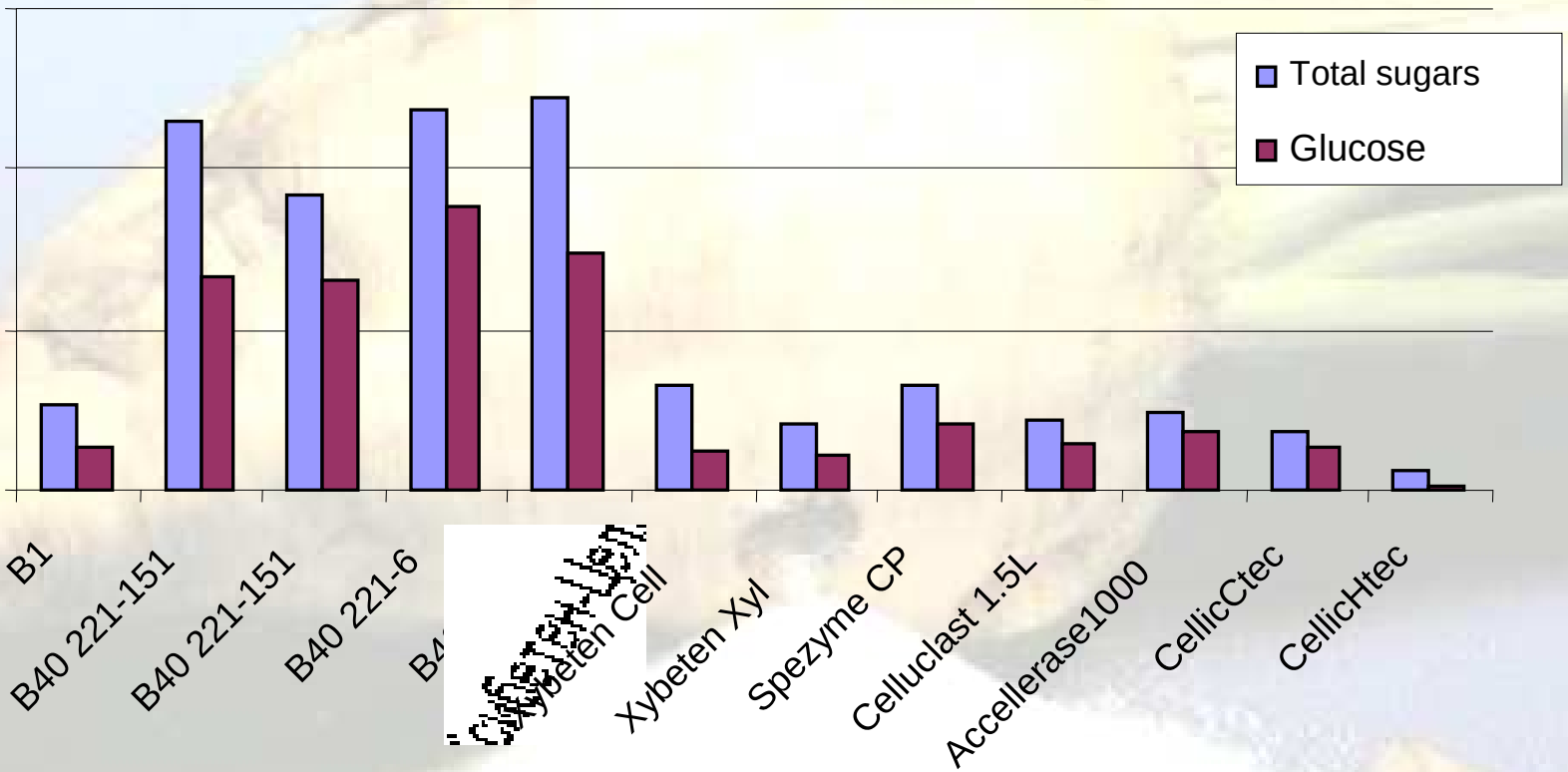


Existing by-products can be processed to get valuable feeds and bio-based bulk chemicals

Hydrolysis for sugarbeat pulp by laboratory and commercial enzyme preparations,

100 g/L S, 50°C, 5 mg E / 1g S, 24 hours

Sugars yield,

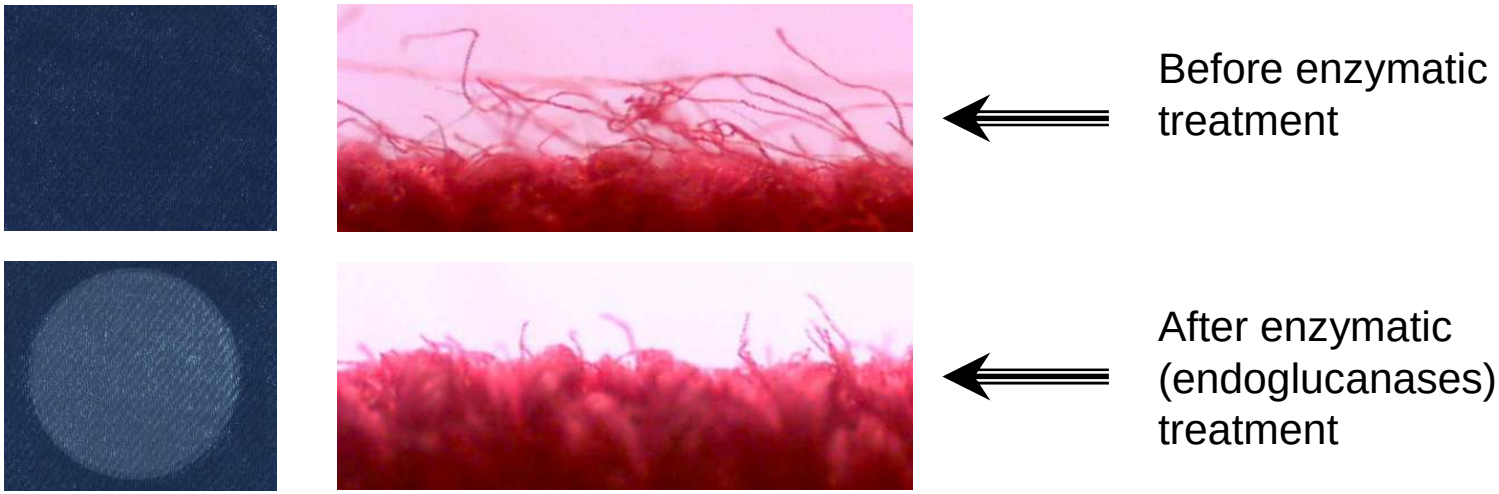


Laboratory preparations

Commercial enzyme preparations

Textile Processing

Application of topolytic endoglucanases
for biopolishing of fabric and denim wash processes



Before enzymatic treatment

After enzymatic (endoglucanases) treatment

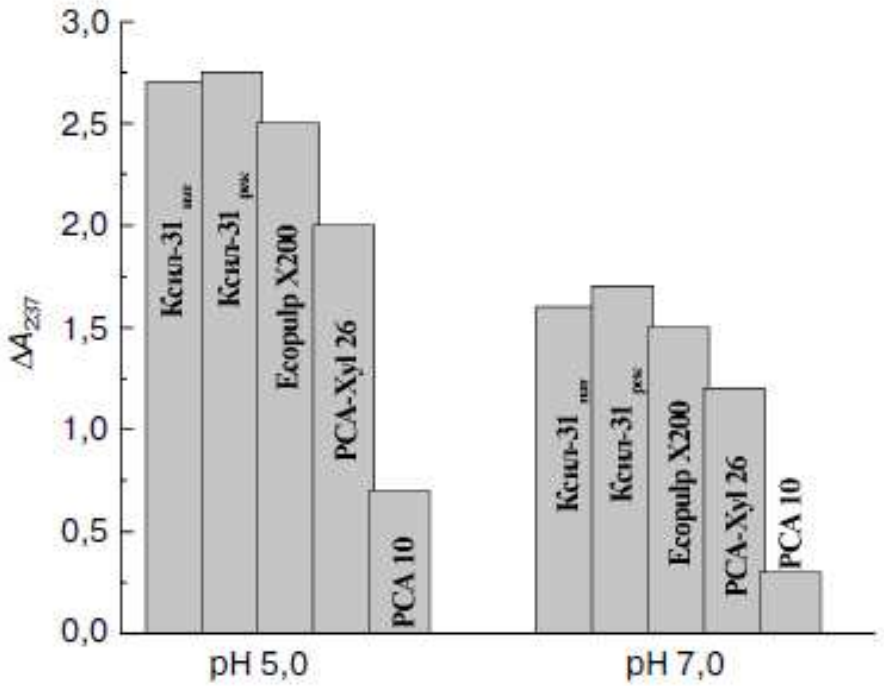
Pulp-and-Paper

Xylanases in biobleaching of craft pulp

w/out xylanases

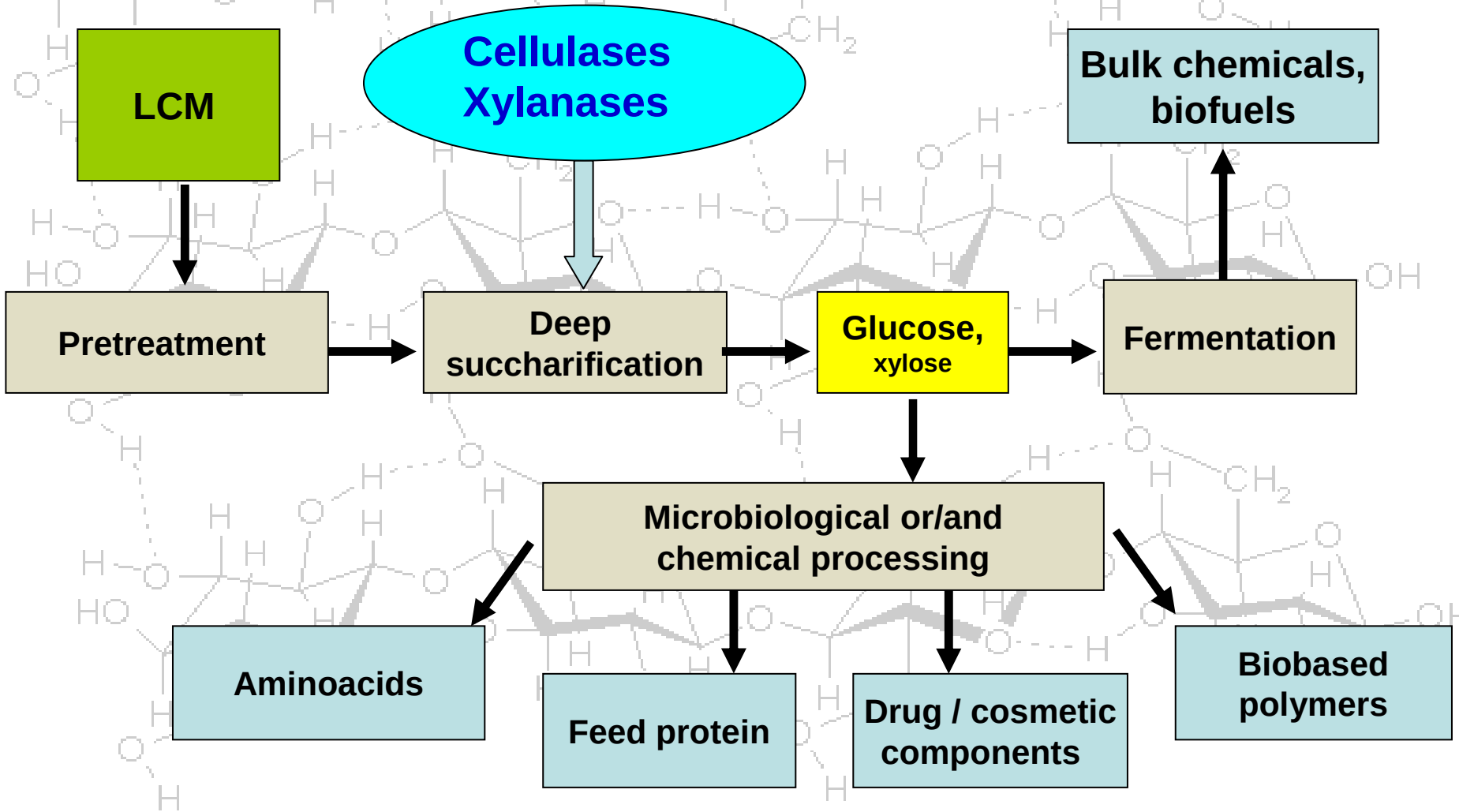
after xylanase treatment

Biobleaching of Cellulose Pulp

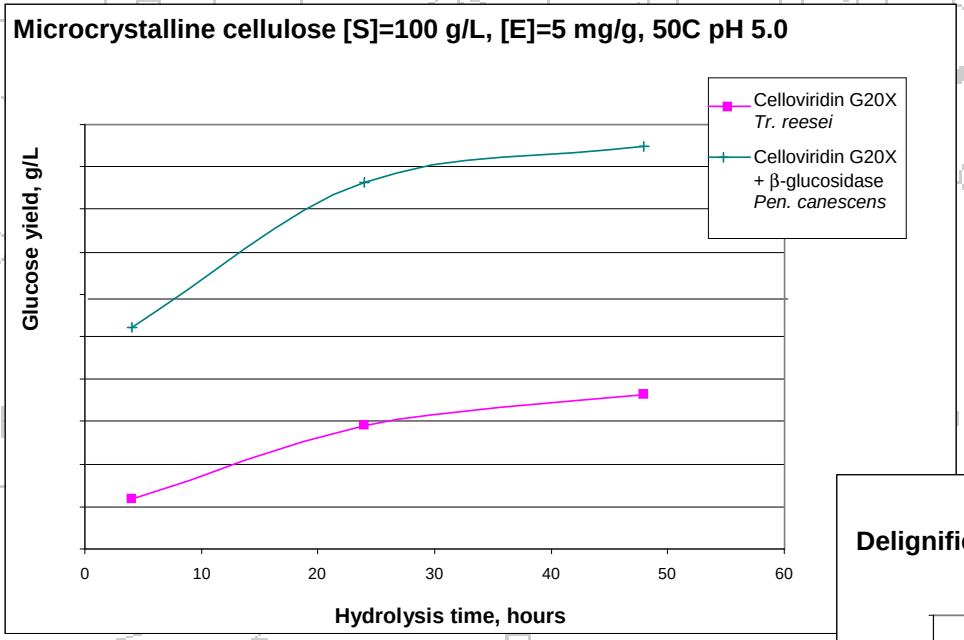


The efficiency of cellulose pulp biobleaching with enzymes Xyl31-nat and Xyl31-rec and enzyme preparations. Protein load 0,005 mg/ml, 50^{o}C, 200 rev/min

Bioconversion of (ligno)cellulosic materials

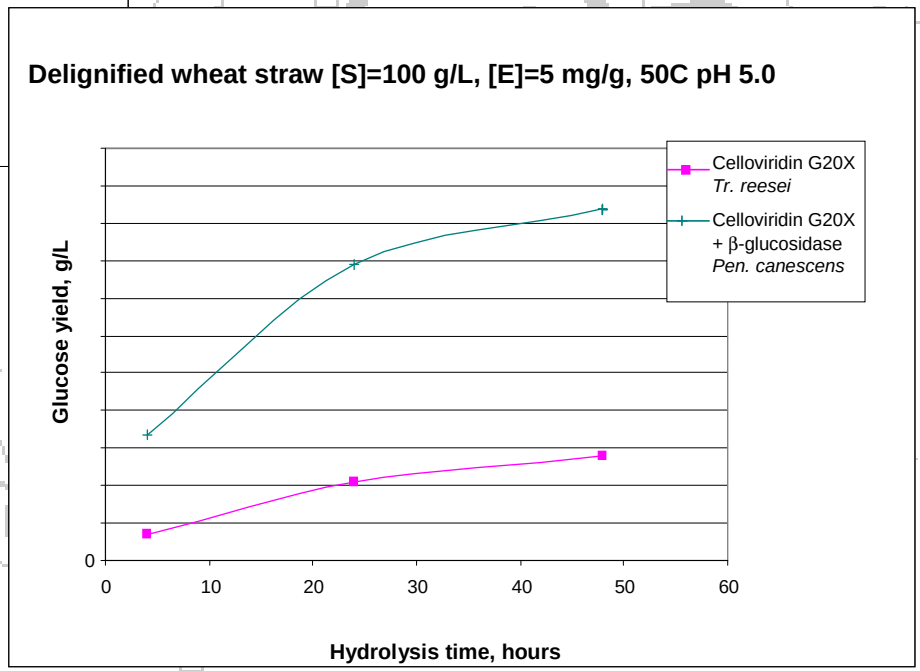


Hydrolysis of Cellulose-containing Substrates



Microcrystalline cellulose

Delignified wheat straw



Results

- **Universal gene expression systems based on fungal *Penicillium* strains were developed**
- **Recombinant strains and enzyme preparations with promising properties were obtained**
- **The enzyme preparations were tested for various industrial applications**

CONTRIBUTORS

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