OUTLINES
Wednesday 24th June

TO Kim Anh (SBFT) ~60min
Screening cellulosic fungi in term of hydrolysis ability-secretom.

COMA Veronique(LCPO) ~60min
Intermediate compounds during hydrolysis: identification and quantification.

Lunch time (CEMES)

FILLAUDEAU L., NGUYEN Tien Cuong & LE Tuan (LISBP)
Hydrolysis of pretreated lignocellulosic matrixes: focus on physical insight ~90min

LISBP LABORATOIRE D'INGÉNIERIE DES SYSTÈMES BIOLOGIQUES ET DES PROCÉDÉS
INRA SCIENCE & IMPACT
cnrs
INSA TOULOUSE
Université de Toulouse

BIO-Asie

HTMS BioAsie project

Bioprocess intensification

Challenges related to transfer limitation

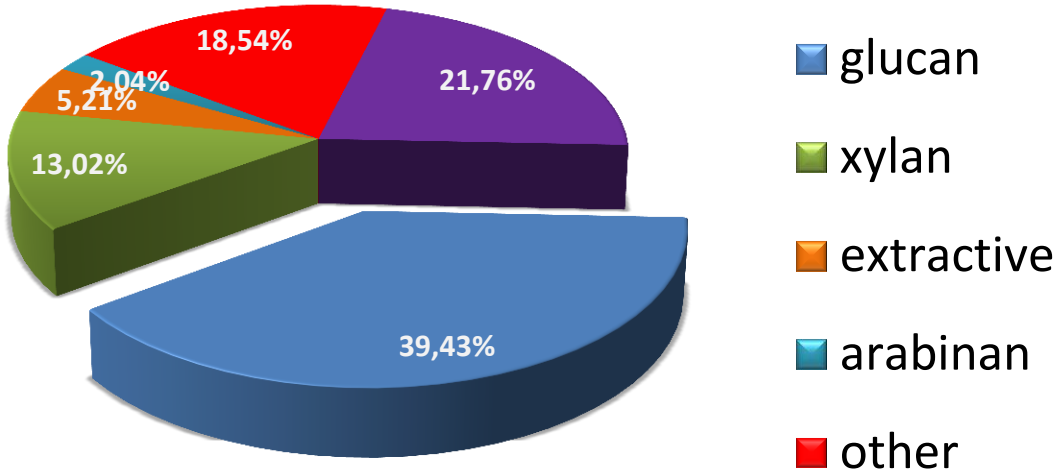
1st Workshop at LISBP (Toulouse, FR), 23-26th June 2015

Exploring of biodiversity for screening cellulosic fungi

To Kim Anh, Pham Tuan Anh, Le Tuan, Cao Xuan Bach, Nguyen Dang Khoa,

Hanoi University of Science and Technology, Vietnam

Agricultural lignocellulose by-product as materials



- The by products are available, and diversified
- Handling issue

Biorefinery concept

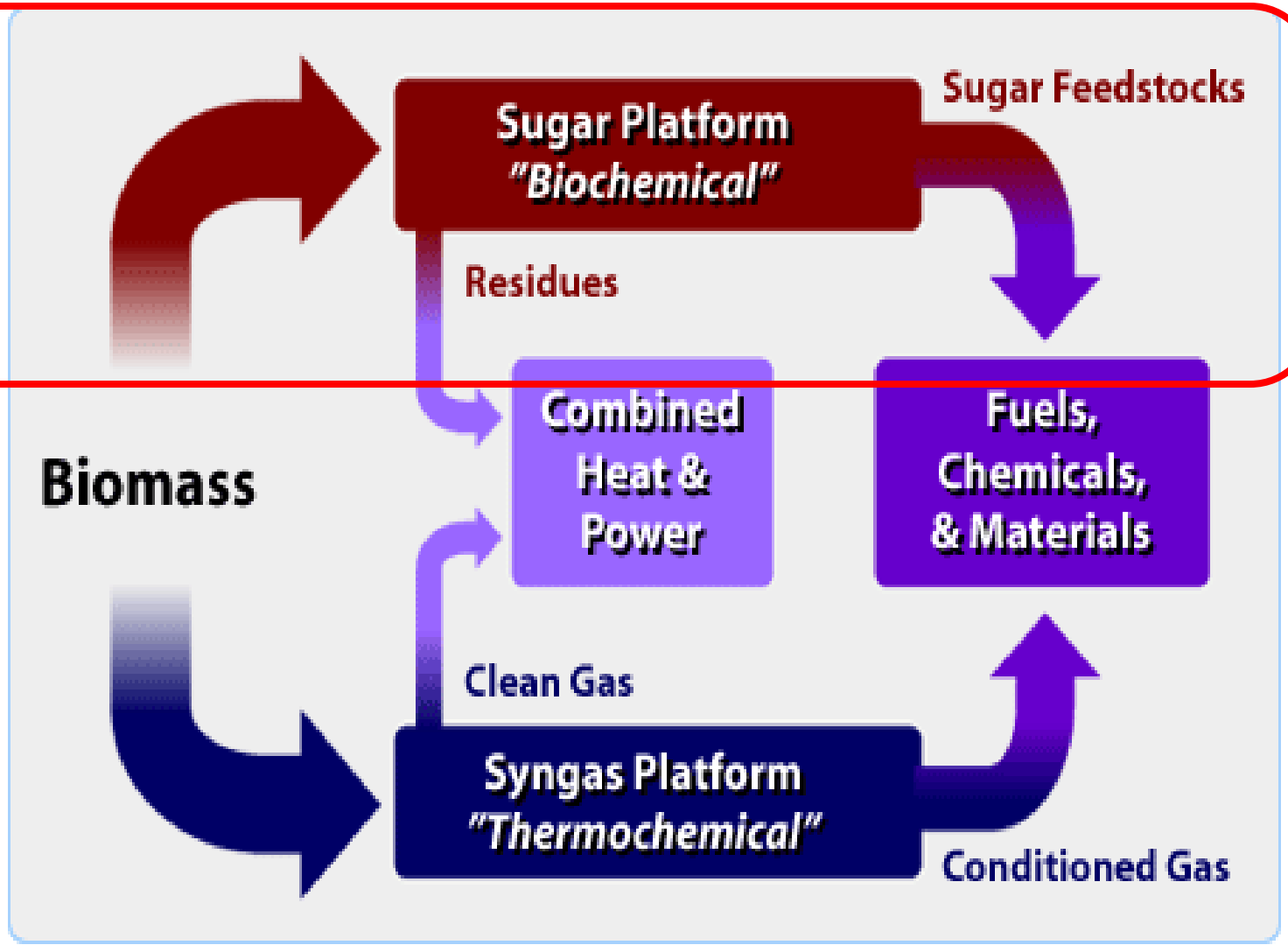
- NREL: A biorefinery is a facility that integrates biomass conversion processes and equipment to produce fuels, power, and value-added chemicals from biomass. The biorefinery concept is analogous to today's petroleum refinery, which produce multiple fuels and products from petroleum. en.wikipedia.org/wiki/Biorefinery
- Cluster of biobased industries producing chemicals, fuels, power, products, and materials. www.biorenew.iastate.edu/resources/glossary.php
- US-DOE: A biorefinery is an overall concept of a processing plant where biomass feedstocks are converted and extracted into a spectrum of valuable products
- NL: The separation of biomass into distinct components which can be individually brought to the market either directly after separation or after further (biological, thermochemical/chemical) treatment(s)

Ed de Jong, Rene van Ree, http://www.biorefinery.nl/

Biorefinery platforms

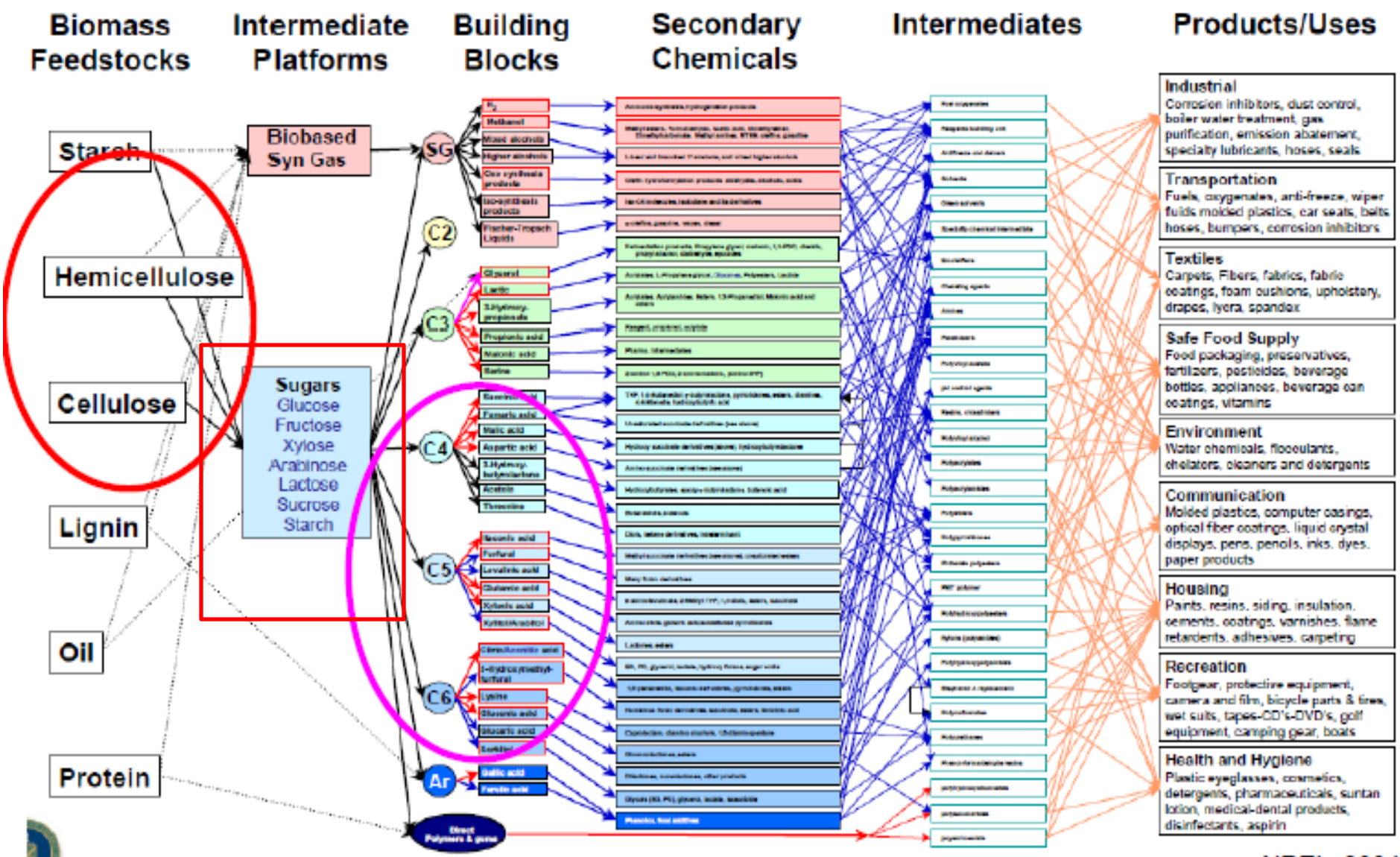
“Sugar platform”

«Syngas platform"



NREL

Potential products by biorefinery



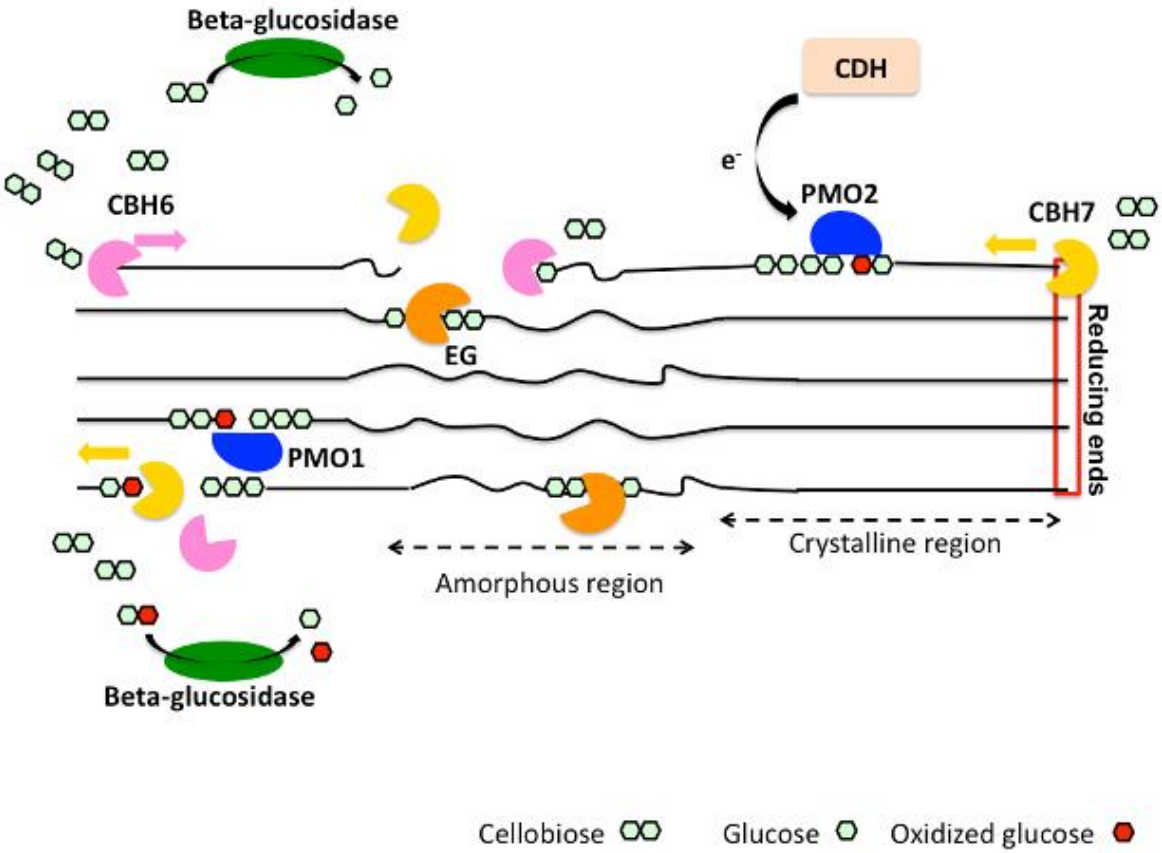
NREL, 2004

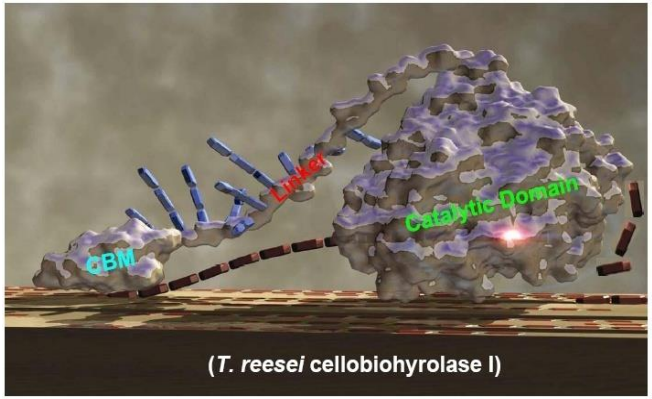
Enzymes involving in lignocellulose conversion

CAZy : http://www.cazy.org/

- Glycoside hydrolases (GHs)
- Glycosyl transférases (GTs)
- Polysaccharide lyases (PLs)
- Carbohydrate esterase (CEs)
- Auxiliary Activities (AAs)
  - Lignin degradation enzymes
  - Lytic polysacchide Monooxxydase (LPMO)

Cellulase vs cellulose





Himmel *et al.*, *Science* 315: 804 (2007)

- CBM protein linked to enzyme
- Fixed to substrate, increasing enzyme concentration on substrate

- There is need to pretreatment of the substrate before enzyme reaction

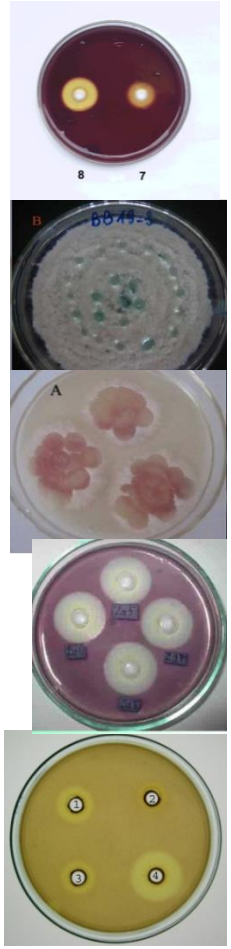
Decay wood

Decay rice straw

Hot spring

Rain forest

Soil



Screening lignocellulose enzymes

Laccase

LiP perroxydase

Mn peroxydase

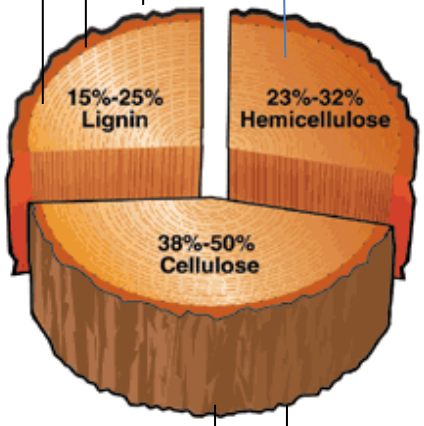
Bioethanol

Functional food

Textile

Pulp

Biopolymer



Xylanase

Mananase

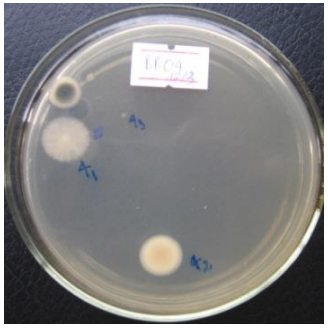
Hazadous waste and waste treatment

Glucanase

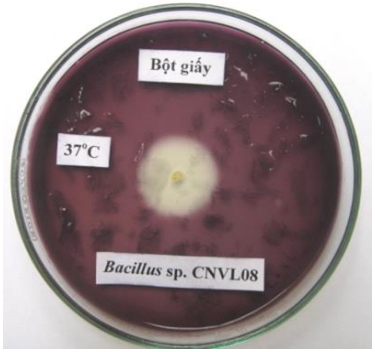
B-glucosidase

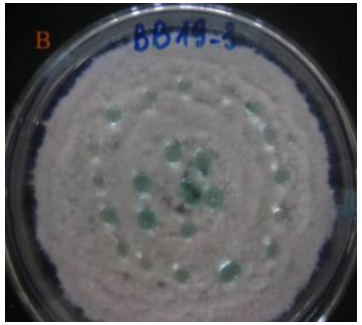
Fungi Isolation

Samples



PDA (30^{0}C, 3-5days)

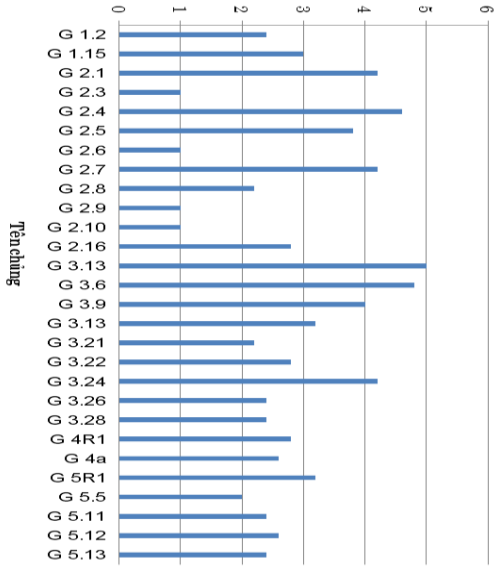




Isolation on indicated agar (avicel, CMC, xylan and ABTS…)

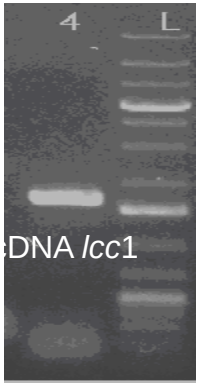
Liquid medium +inducer

Verify enzyme activity

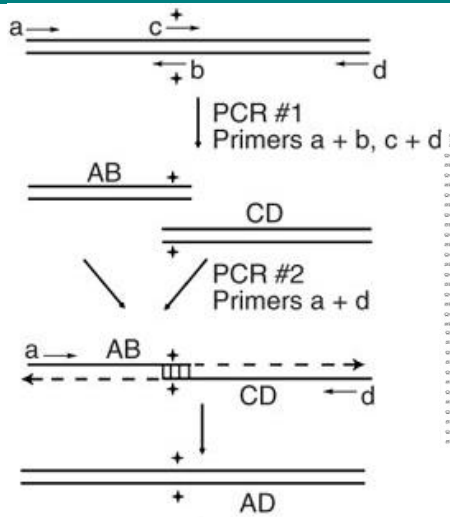


Tách dòng và biểu hiện laccase *T. versicolor*

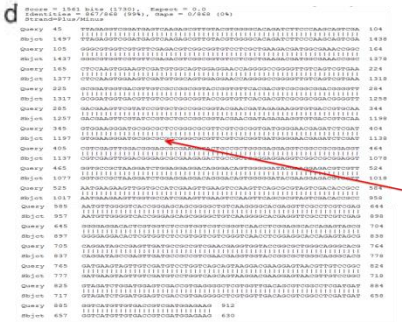
T. versicolor



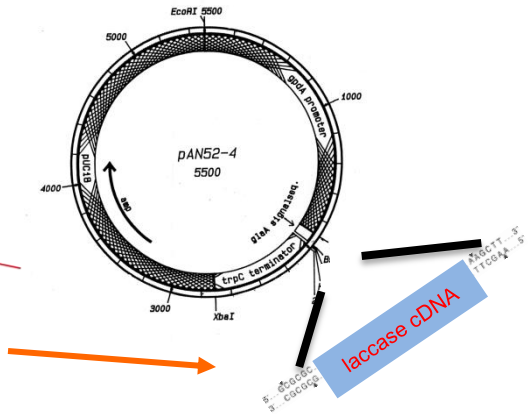
Dòng gen *lcc1*: 1,5 kb, 99% tương đồng *lcc1 T. vesicolor* X84683



Đột biến điểm cDNA loại trừ điểm cắt *Bss*HII

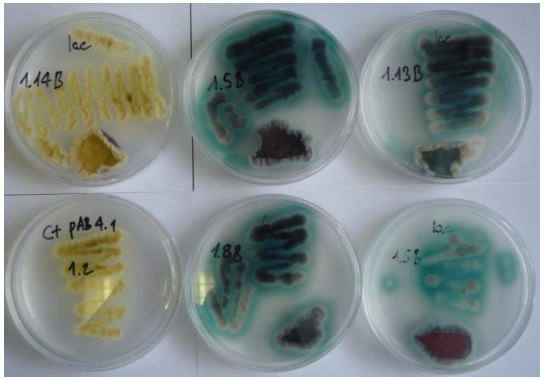


cDNA *lcc1* đột biến điểm

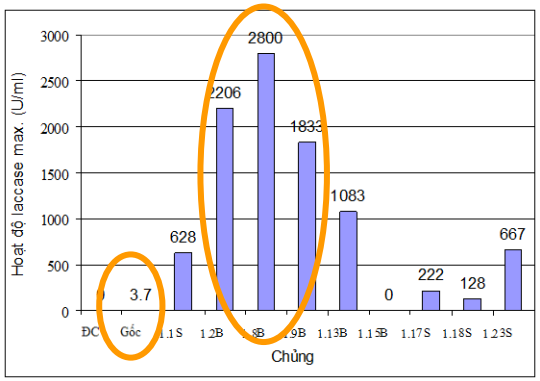


Vectơ pAN52-4 biểu hiện laccase trong *A. niger* D15#26

Đối chứng Chủng dương tính

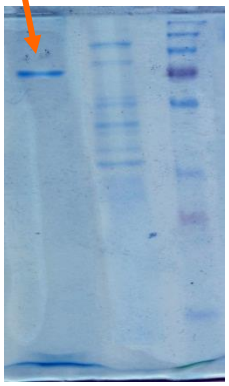


Laccase chuyển hóa ABTS thành sản phẩm có màu xanh đặc trưng



Chọn dòng A. niger D15#26-pAN52-4 biểu hiện *lacc*1

70 kDa



Điện di đồ laccase tái tổ hợp

Elaborate an enzyme cocktail

Recombinant endoglucanase

Recombinant exoglucanase

Recombinant β-glusidase

Recombinant laccase

Recombinant xylanase

→ Enzyme Cocktail

Quy trình thủy phân bã mía (SHF)

Hydrolysis

Alkaline pretreated sugarcane bagasse, 10% NaOH, S/L:= 1:10

pH 4,8

Hydrolysis, 50^{0}C, pH 4,8, 48h

5 CMCase/10, 41 FPU/30 U betaglucosidase/g bã mía

Detoxification , 37^{0}C, pH 4,8, 1h

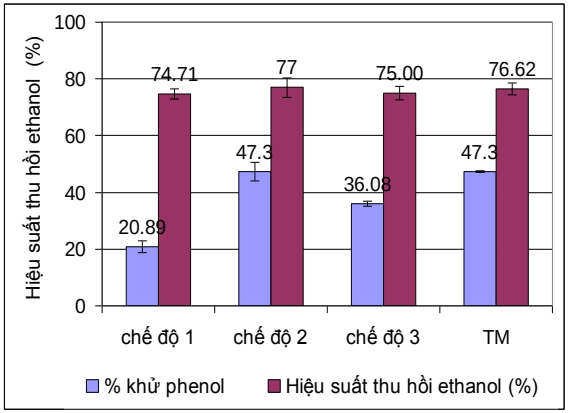
Laccase 70U/g BM

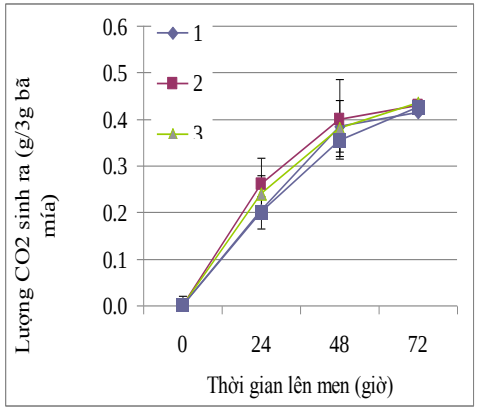
Fermentation , 72h, 37^{0}C

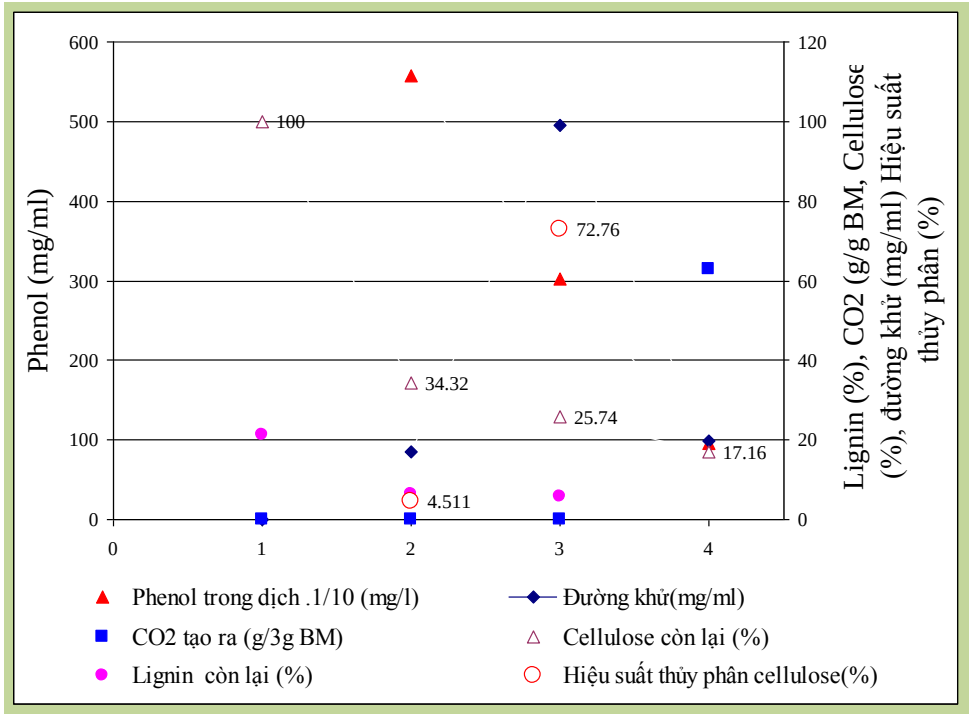
10^{6}CFU/m

_____ 1.
====, 2
…..,.. 3

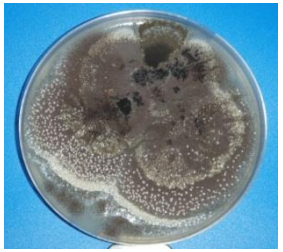
- Ethanol efficiency 72.76%
- Difficult to optimize







Selection of secretom from isolates on lignocellulosic substrate



Fungus

↓

Subculture

↓

Liquid culture

↓

Centrifugation

↓

Stock at -20°C

↓

Hydrolysis of substrate

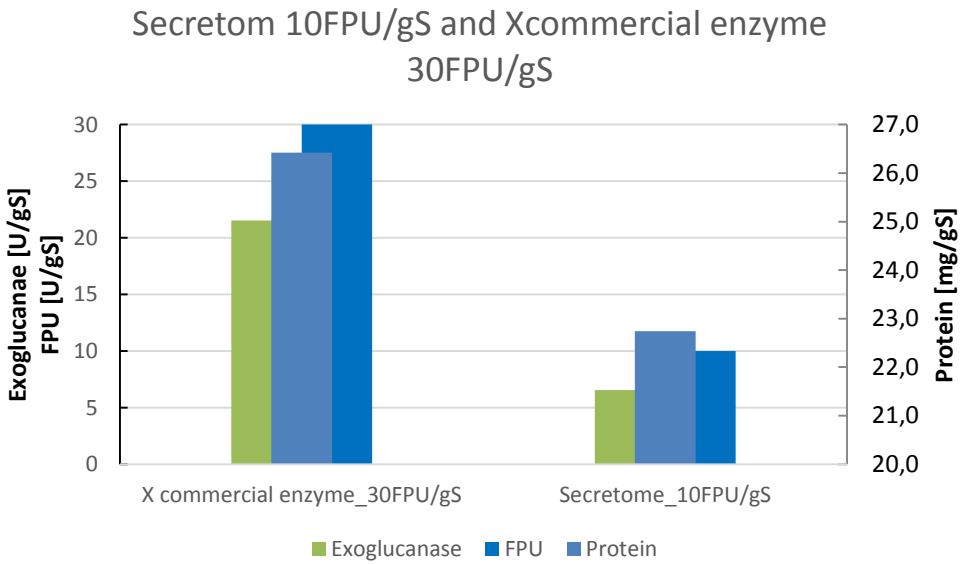
- $2x10^6$ spore/mL
- Pretreated substrate
- Culture condition (pH, substrate content)

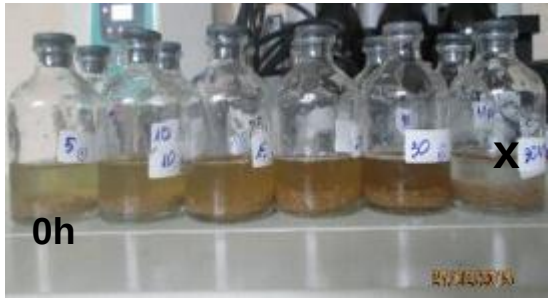
- Pretreated substrate
- Enzyme dose
- 50 ^{0}C, 150 rpm, 48 h

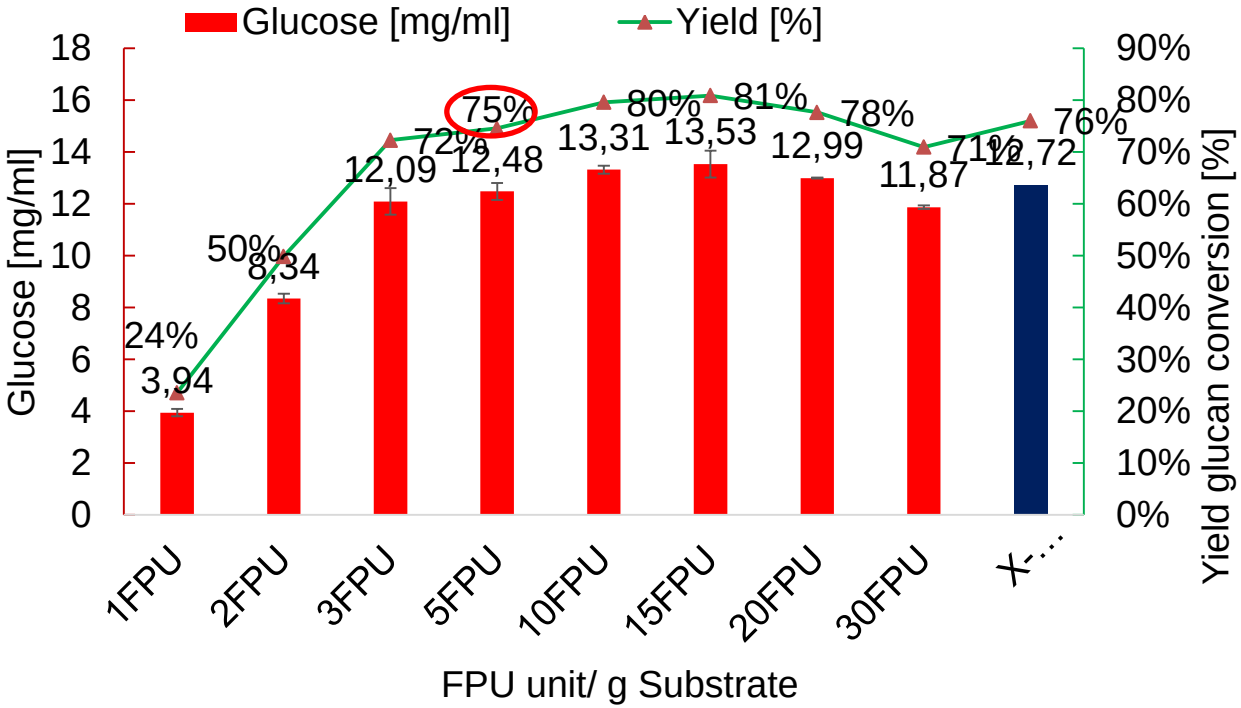
Analysis for:

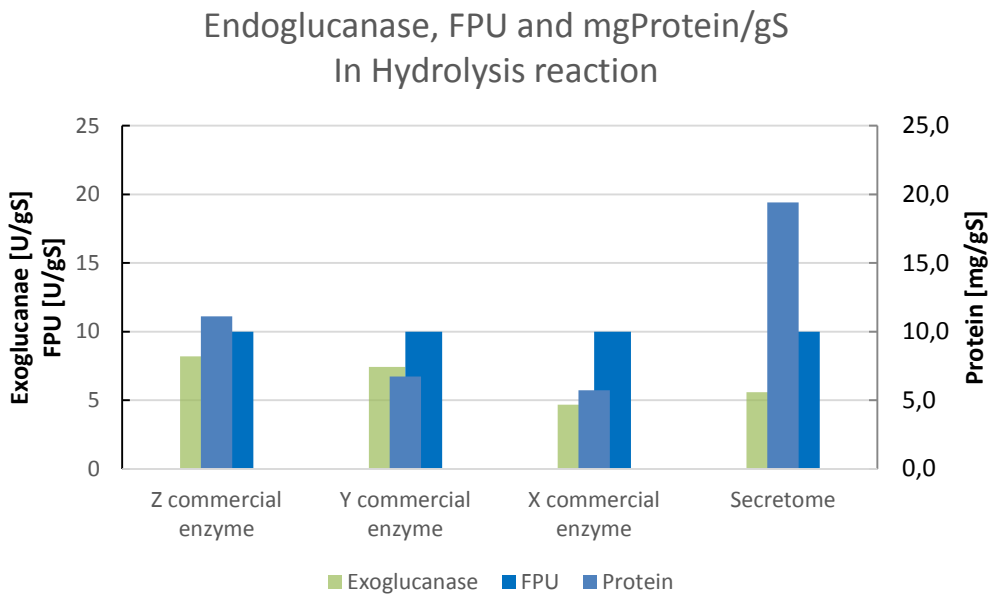
- Enzyme activity
- Protein profile
- Hydrolysis yield

Hydrolysis of SBFA by secretom

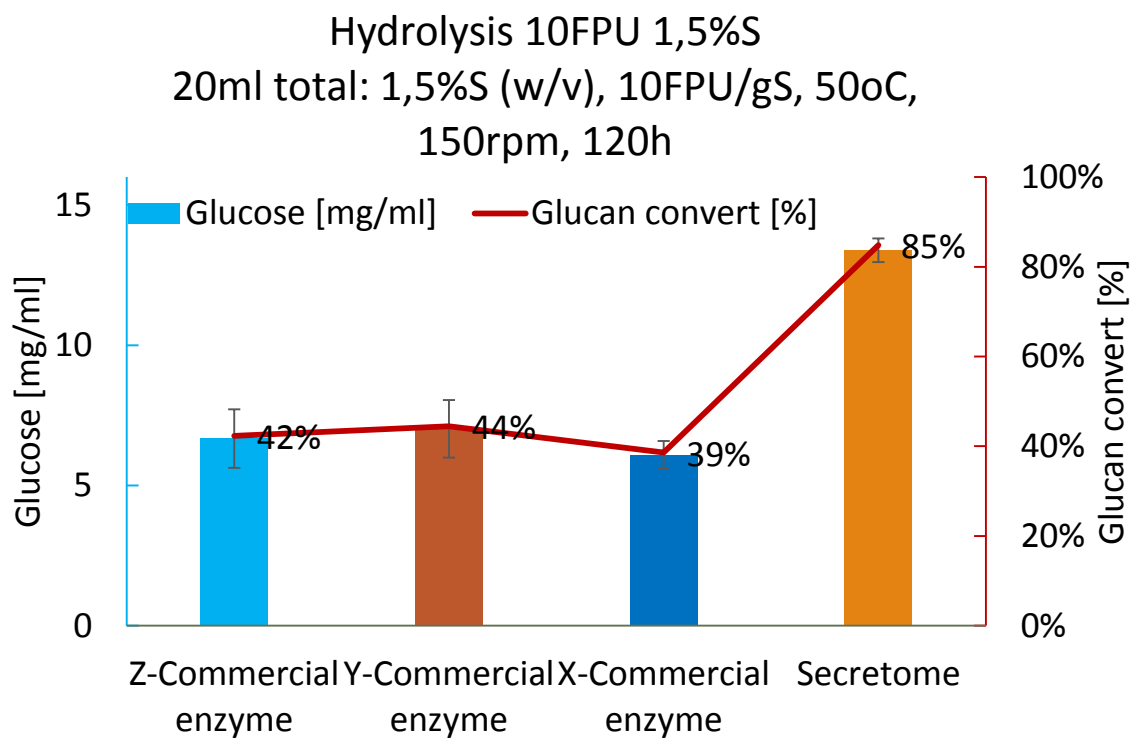






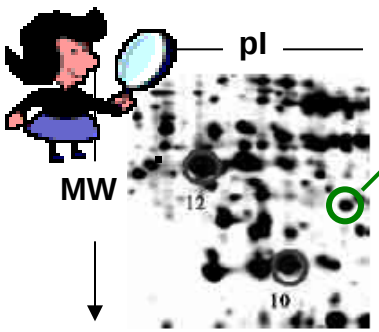


Enzyme pattern of the secretom???



Analysis for secretome enzyme profile (under investigation)

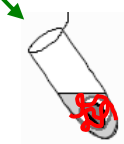
Image analysis
Of gel



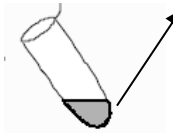
select an unknown
purified protein

MGQGWATAGLPSFRPEPYKCYGHPVP
SQEASQQVTVKTHGTSSQATTSSQK…

Trypsin digestion
Of protein



Isolated spot +Endoproteinase
⇒cut the aa chain
into peptides (every K and R aa)

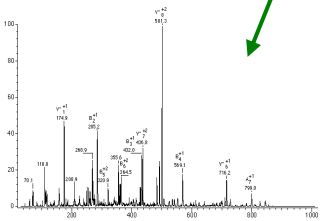


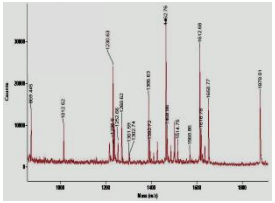
Peptides
in solution

MGQGWATAGLPSFR
PEPYK
CYGHPVPSQEASQQVTVK.

MALDI-TOF
Mass spectrometry

select a peptide
& fragmentation





MS/MS Analysis
Measure the mass of the fragments
⇒ sequence information

MS Analysis
Measure the mass of the peptides
(peptide mass fingerprint of the
protein)

MG
MGQ
MGQGWA
WAT
WATA
...

Protein database
searches

Improvement of enzyme activity by *helper* enzyme (Projected)

HelperEnzym /associate protein

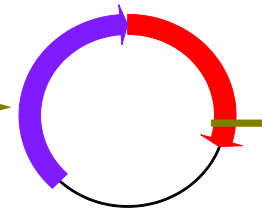
Secretom

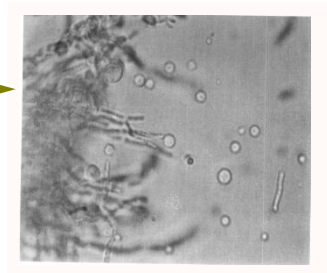
Helper enzym gene coding

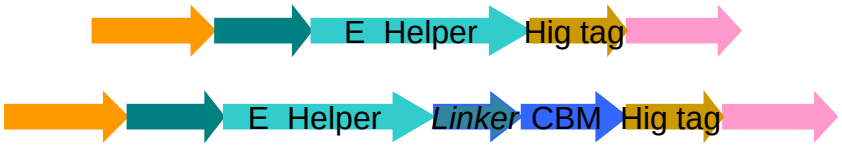
CBH -A. *niger*

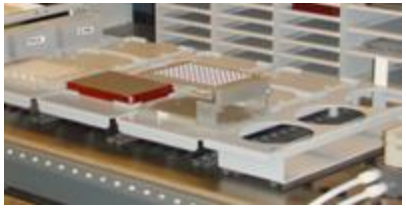
CBM

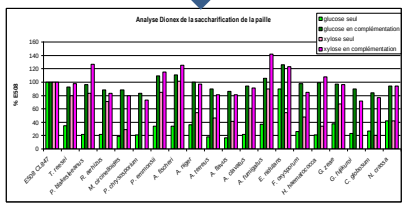
Heterologue expression











- Helper enzyme to enzyme cocktail
- Increase of activity by increase enzyme fixed on the substrate surface (CBM-enzyme)

Conclusion

- Microbial lignocellulase complex is the enzyme system of interest in biorefinery of lignocellulose
- Enzyme engineering for biorefinery
- Cooperation in enzyme exploitation is needed

Thank you very much
for your kind attention