

# Integrative Biorefinery research@IBL



Conversion of renewable plant biomass to fuels and chemicals:  
Bio-process

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Integrative Biorefinery Laboratory  
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# Bio-based economy & Biorefineries

Biotechnology plays an increasing importance on key industrial sectors in production of commodity and specialty products for everyday life.

Bioresources are explored as alternative feedstocks and for finding novel microbes and enzymes for development of greener production processes and establishment of the prospective biorefinery industry.

[http://www.wallcoo.net/human/SZ\\_206\\_Entionment\\_03\\_city\\_Photo\\_manipulation/html/wallpaper29.html](http://www.wallcoo.net/human/SZ_206_Entionment_03_city_Photo_manipulation/html/wallpaper29.html)



Fuels:  
ethanol , biodiesels & BTL



Feed:  
feed supplement, prebiotic



Chemicals:  
commodity & specialty



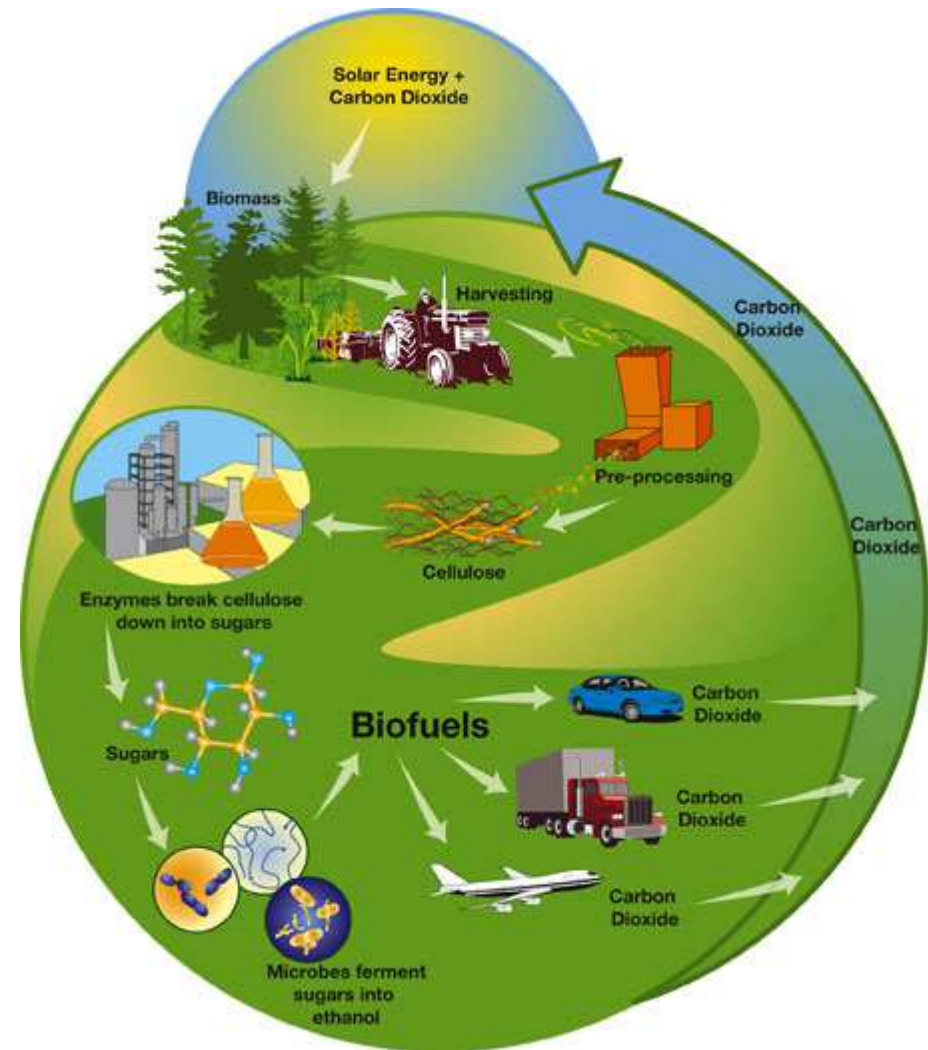
Materials:  
bioplastics & biomaterials

# Biorefinery industry

Biorefinery is a promising industry related to the production of **biofuels, biomaterials, and chemicals** from renewable or renewable bio-resources.

- sustainable production of biofuels, bio-materials and chemicals
- carbon-neutral process
- Food V.S. Feed
- efficient waste utilisation and management
- improved local economics

**Sustainable alternative production platform to the current petroleum resource**

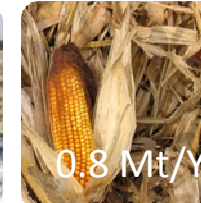
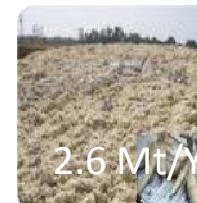
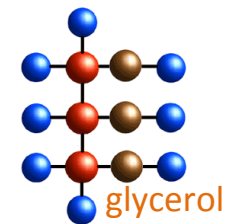
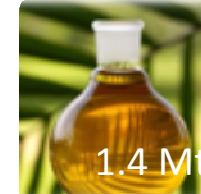
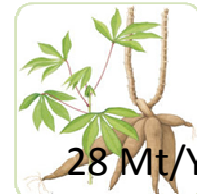




# Thailand as a regional hub for biorefineries

## Agricultural platform:

Diverse conventional 1° feedstock & 2° lignocellulosic agricultural residues



## Industry platform:

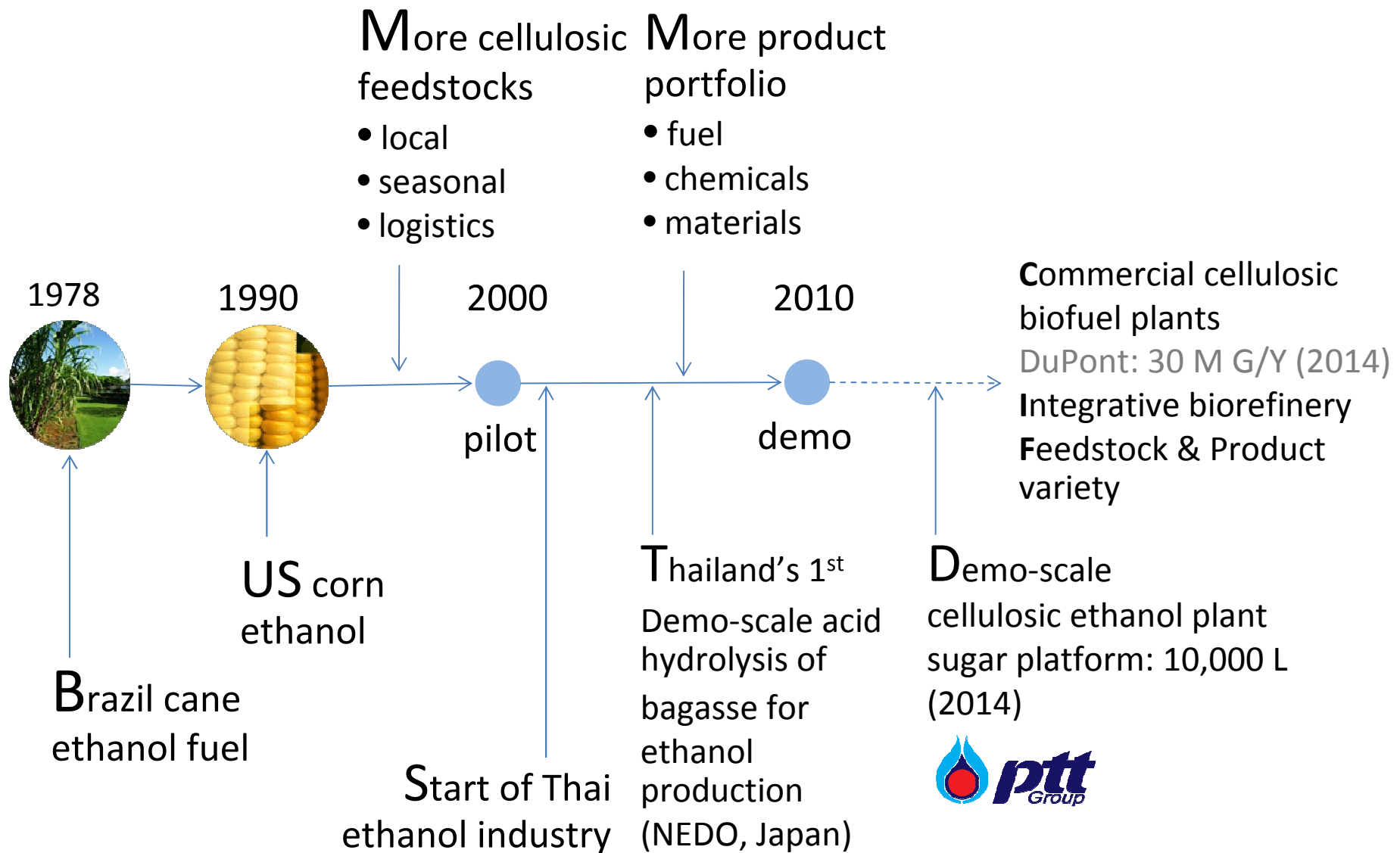
- Strong upstream agro-industry and downstream petrochemical platform
- Asia's leader in biofuel and bioplastic industries

## Technology platform:

Wide-ranging R&D activities in governmental and industrial sectors on biorefinery industry



# Integrative biorefinery: Global & Thailand progress



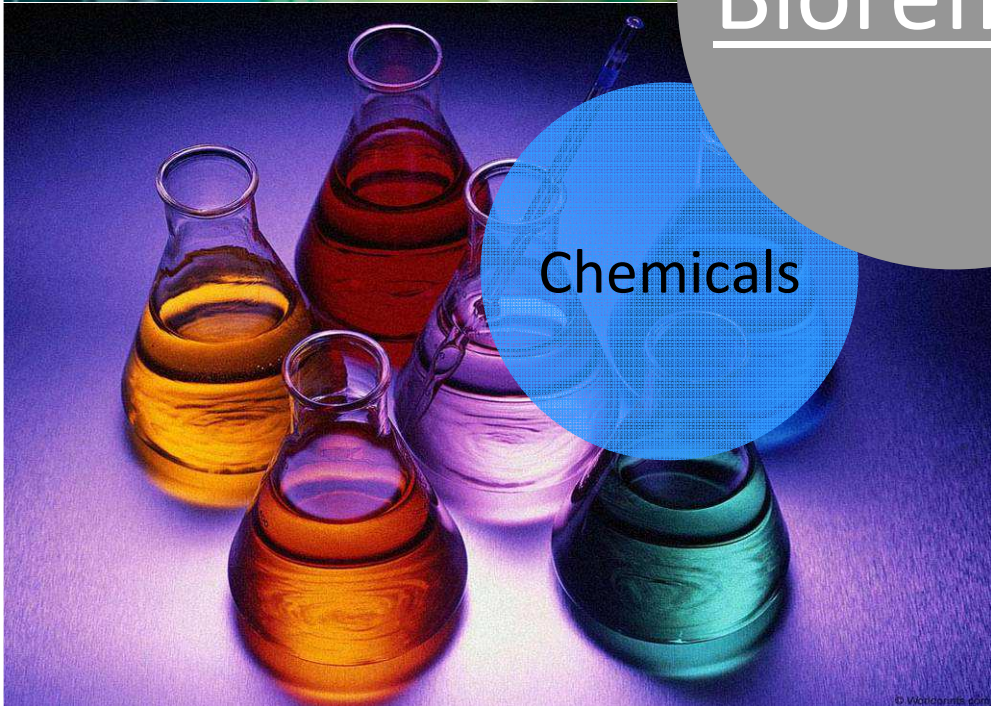


Bio-plastics



Bioethanol

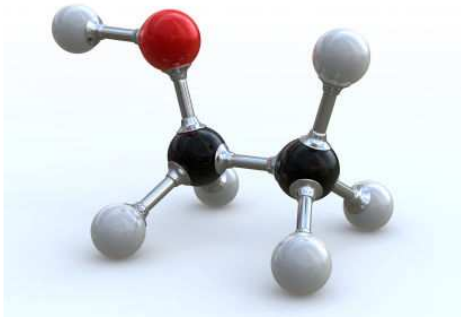
Biorefinery



Chemicals



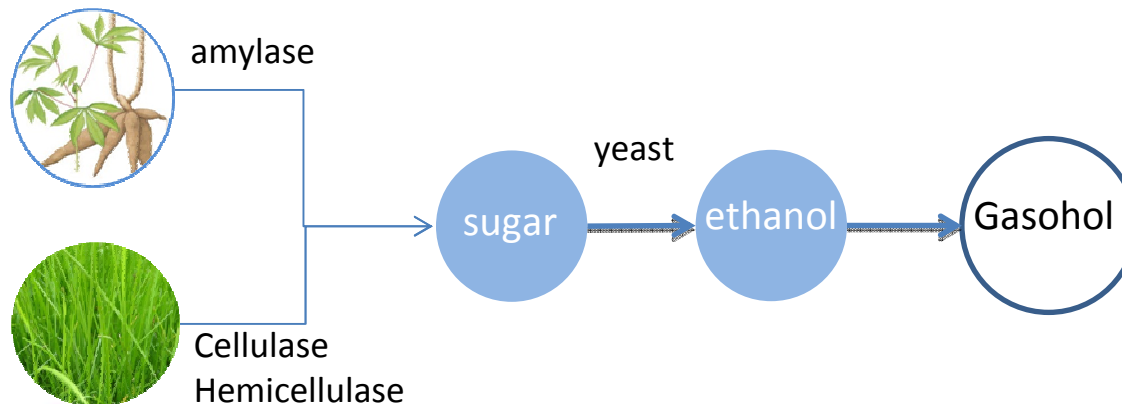
Biodiesel



# Bioethanol

Renewable energy from fermentation of sugars to ethanol

- Currently, there are 18 major ethanol plants across the country with total production capacity of 2.75 M liters/day (2010)
- Feedstock from molasses/cassava/sugarcane juice
- Available as **E10**, **E20**, **E85**



BlueInnovation	
วันที่ 07.03.13 08:17	
บาท / ลิตร	
BlueGasoline 91	45.75
BlueGasoline 95	48.25
BlueGasohol 91	37.38
BlueGasohol 95	39.83
BlueGasohol E20	34.58
BlueGasohol E85	22.98
BlueDiesel	29.99
บาท / กิโลกรัม	
PTT MGX	10.50



# Biodiesel

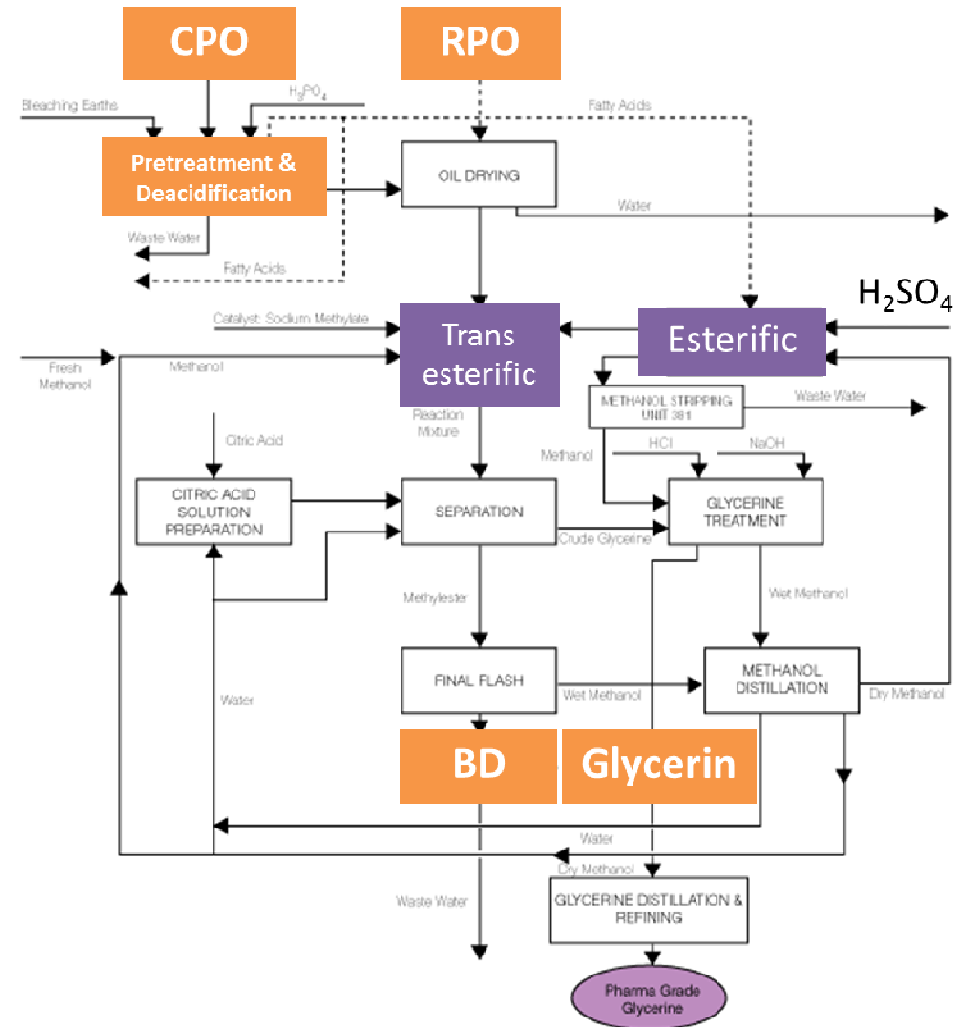
Renewable biofuel from chemical modification of vegetable/waste oils

- Currently, there are 13 biodiesel plants in the country with total actual production of 1.3 M L/d mainly from palm oil feedstock.
- Industrial-grade (B5)/ Community biodiesel production
- Fatty acid methyl ester (FAME)

TAG +  
MtOH



**BIODIESEL PRODUCTION PLANT  
BASE PLANT CONFIGURATION**







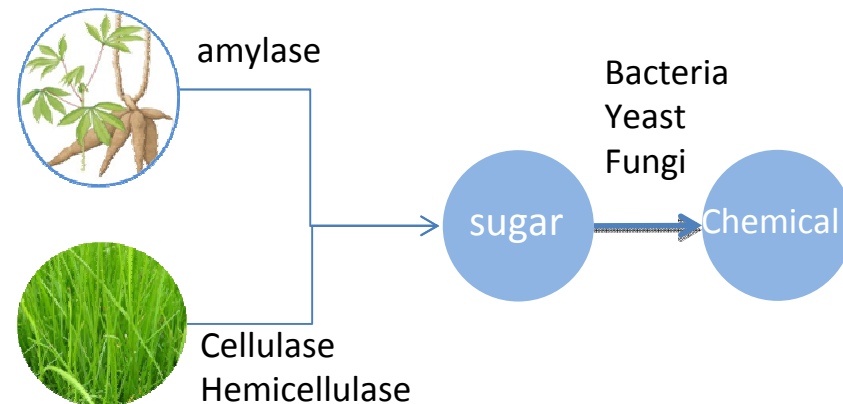
# Bio-chemicals

Commodity and specialty chemicals from fermentation

- Acetic acid:** 14,000 tons/year
- Citric acid:** 24,000 tons/year
- Glutamic acid:** 90,000 tons/year
- MSG:** 270,000 tons/year
- Lysine:** 50,000 tons/year
- Bio-succinic (PTT)**



- Food
- Animal feed
- Polymer
- Chemical
- Pharmaceutical

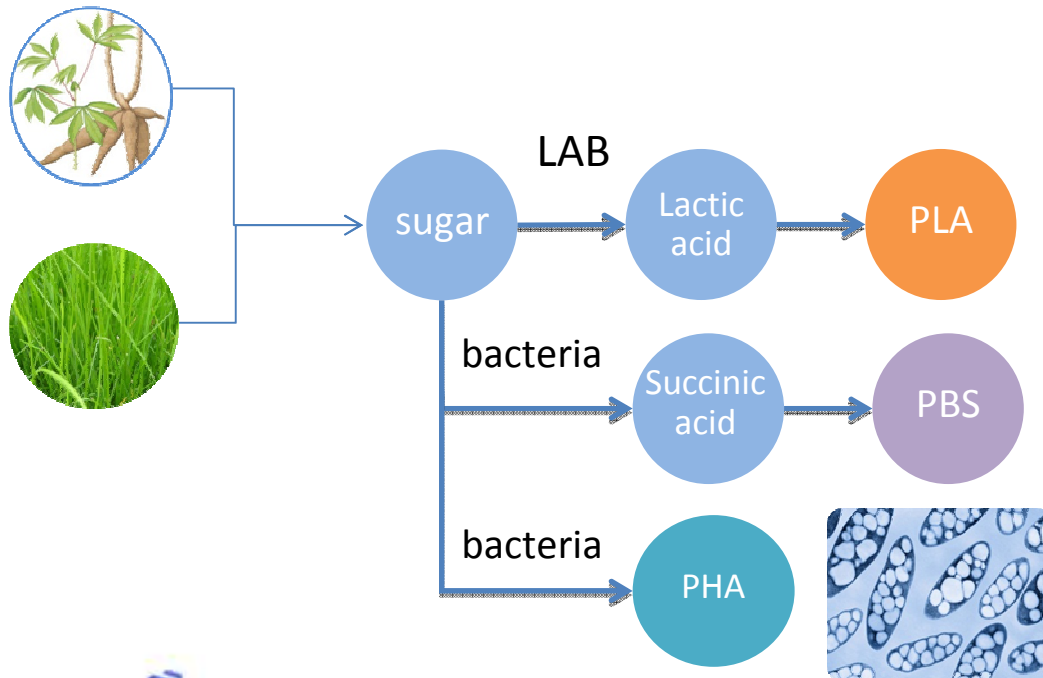


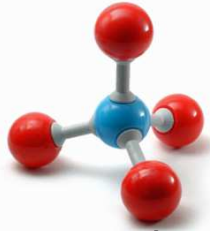


# Bio-plastic

Alternative environmentally friendly materials from bio-based feedstock

- NIA has initiated the national strategic plan for bioplastic industry aiming for positioning the country as the “bioplastic hub”
- Primary target: PLA, PHA, and PBS



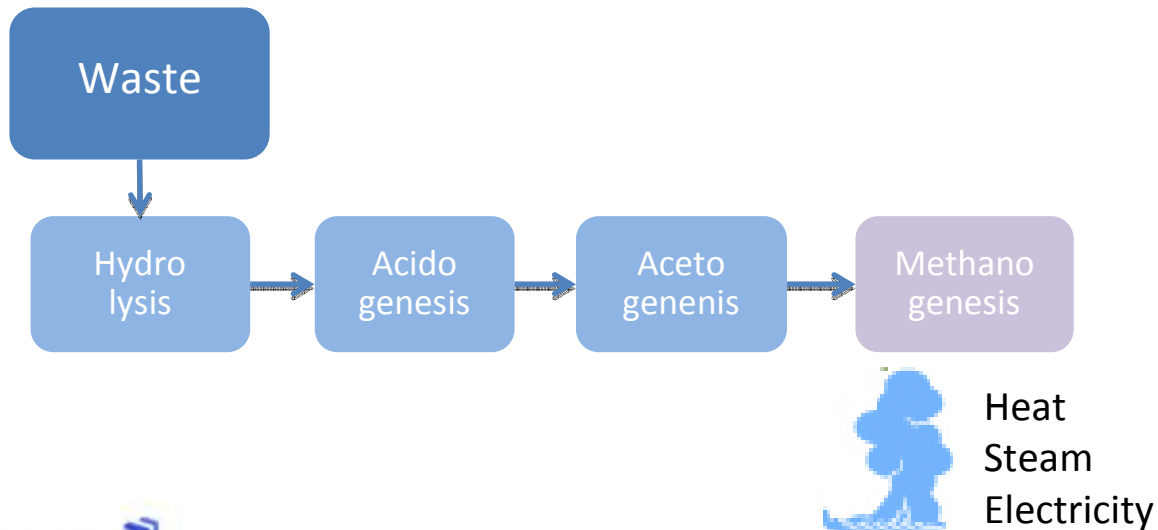


# Biogas

Methane and biohydrogen from anaerobic digestion

## Commercialisation of high rate biogas system in farms and industries

- 2,300 biogas plants
- 380 million m<sup>3</sup> (equivalent to 188 ktoe)
- Cassava waste water (53%), manure (39%) with potential substrates e.g. municipal wastes, POME, and glycerol



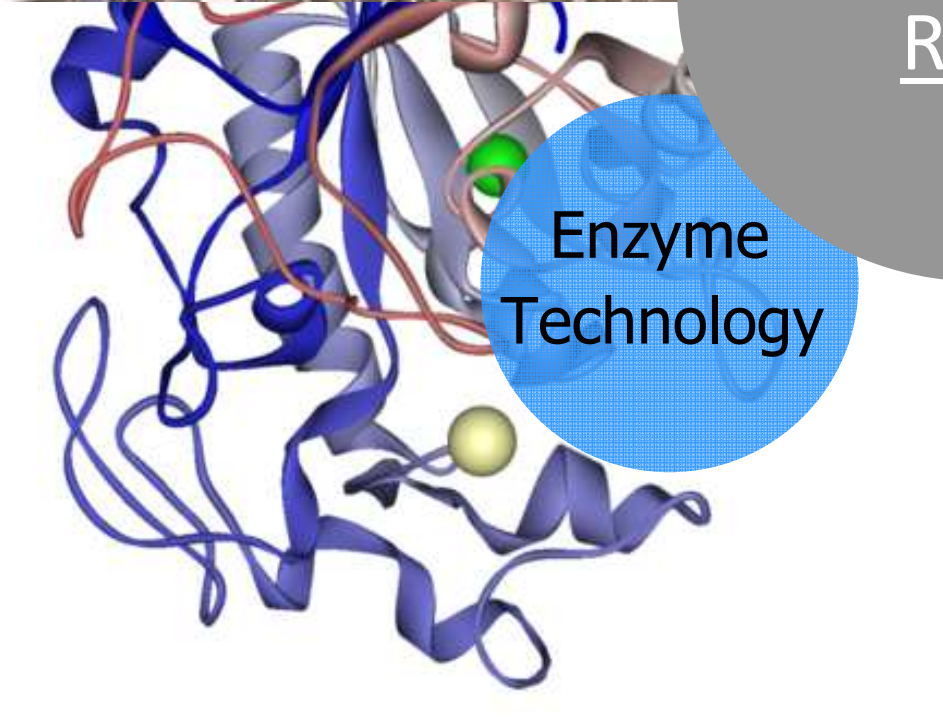


Bio-process

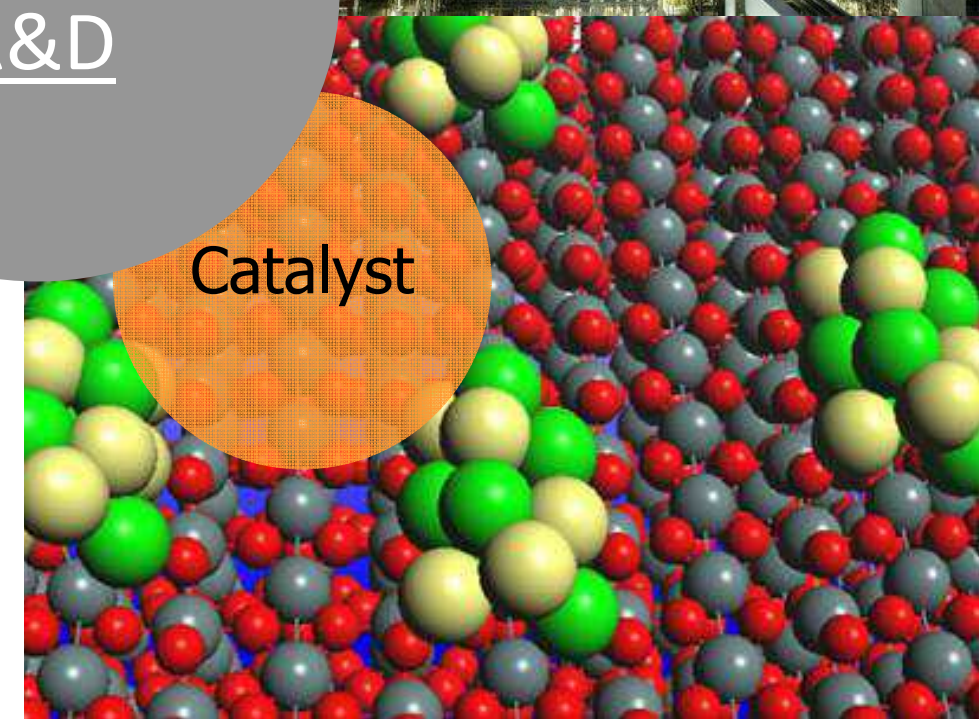


Thermochemical process

Multidisciplinary  
R&D

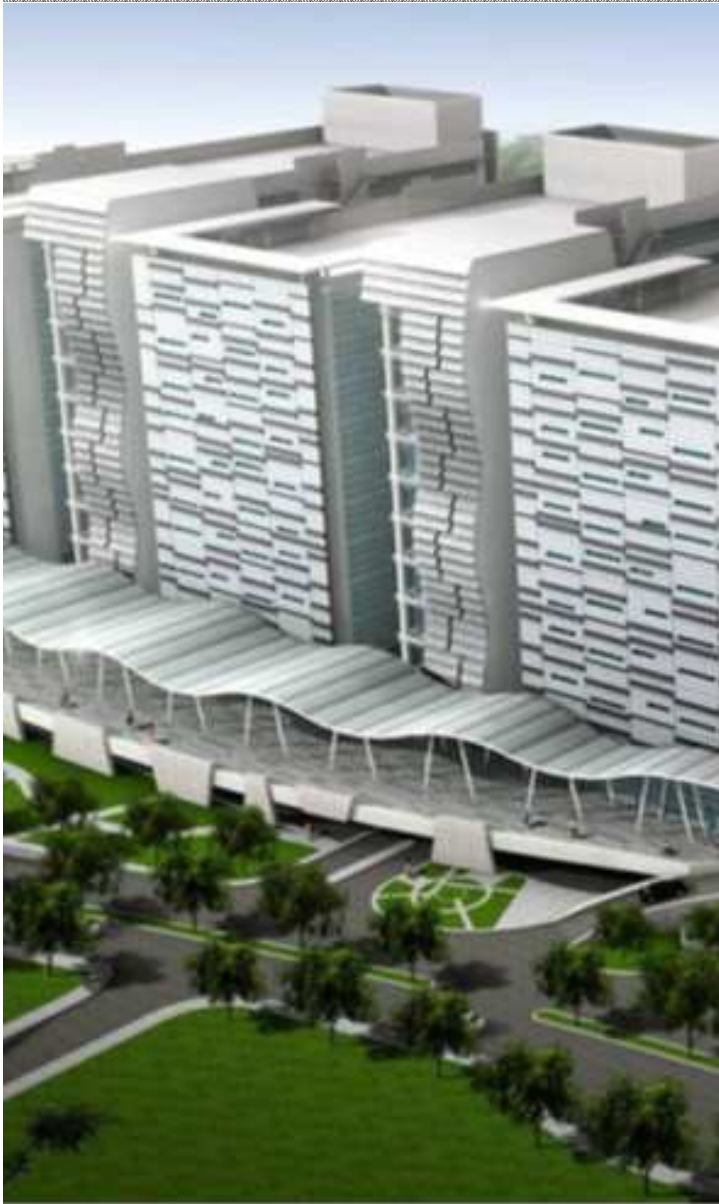


Enzyme Technology



Catalyst

# NSTDA-JGSEE Integrative Biorefinery Laboratory (IBL)

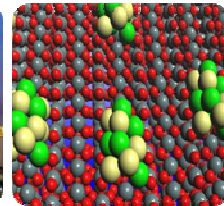


**IBL** is established as a strategic multi-disciplinary R&D center as a **1<sup>st</sup> focal point** for biorefinery research aiming to strengthen the country's platform technology and accelerating commercialisation of biorefinery processes in industrial sectors.

- Allied strength from BIOTEC & JGSEE and their network
- Platform technology & Industrial-directed translational R&D
- Multi-disciplinary integrative R&D



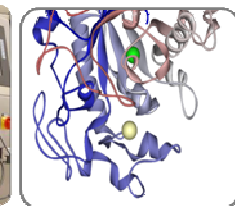
Thermal



Chemical &  
Catalytic

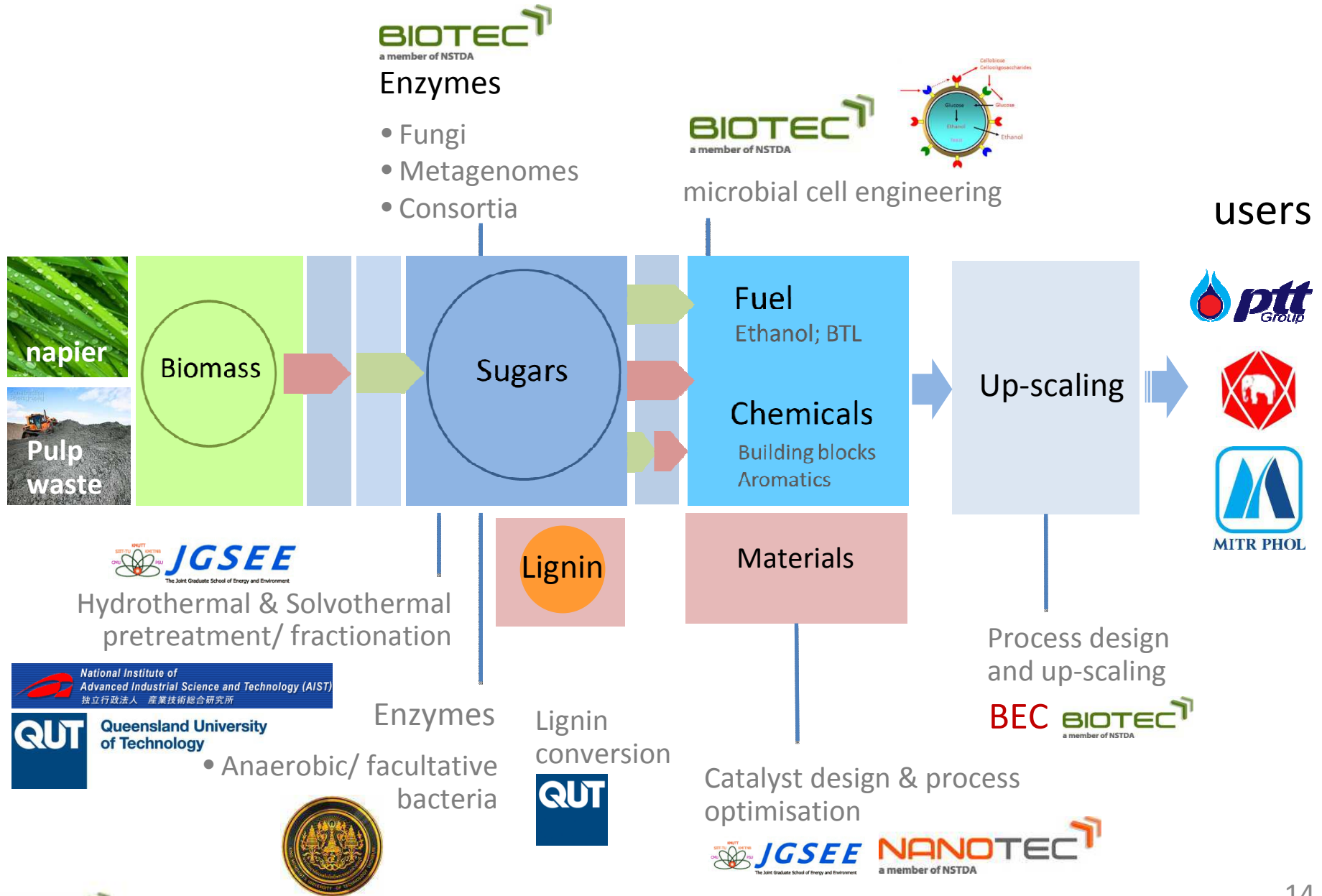


Bio-process



Enzymes &  
Biocatalytic

# Integrative biorefinery laboratory: **network**



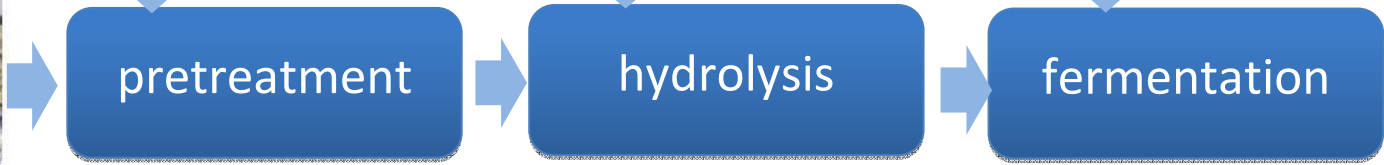
# Integrative biorefinery: Sugar platform



- Chemical pretreatment
- Thermal pretreatment
- Reaction design

- Enzyme screening
- Enzyme formulation
- Process optimisation

- Strain selection
- Genetic engineering
- Co-fermentation



- High biomass digestibility
- Low chemicals
- Energy efficient
- Up-scaling capability

- Synergistic enzyme action
- Higher efficiency
- Low cost on-site production

- Lignocellulolytic enzyme expression
- Consolidated process
- C6/C5 utilisation





# Lignocellulosic biomass

**D**egradation of plant lignocellulosic biomass is a key process on recycling of organic carbon in the global biogeochemical cycle.

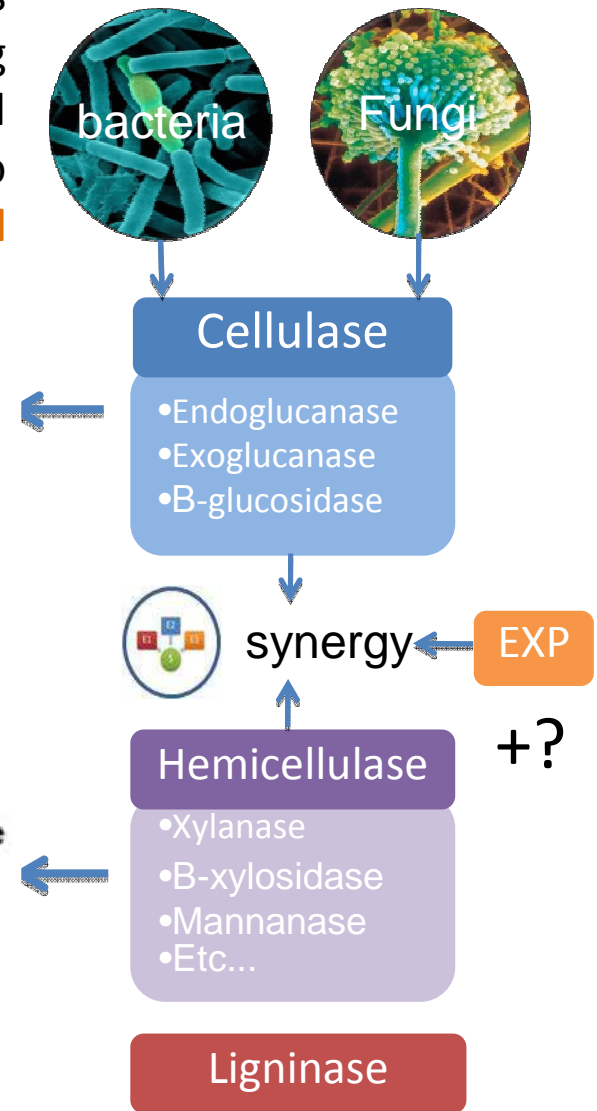
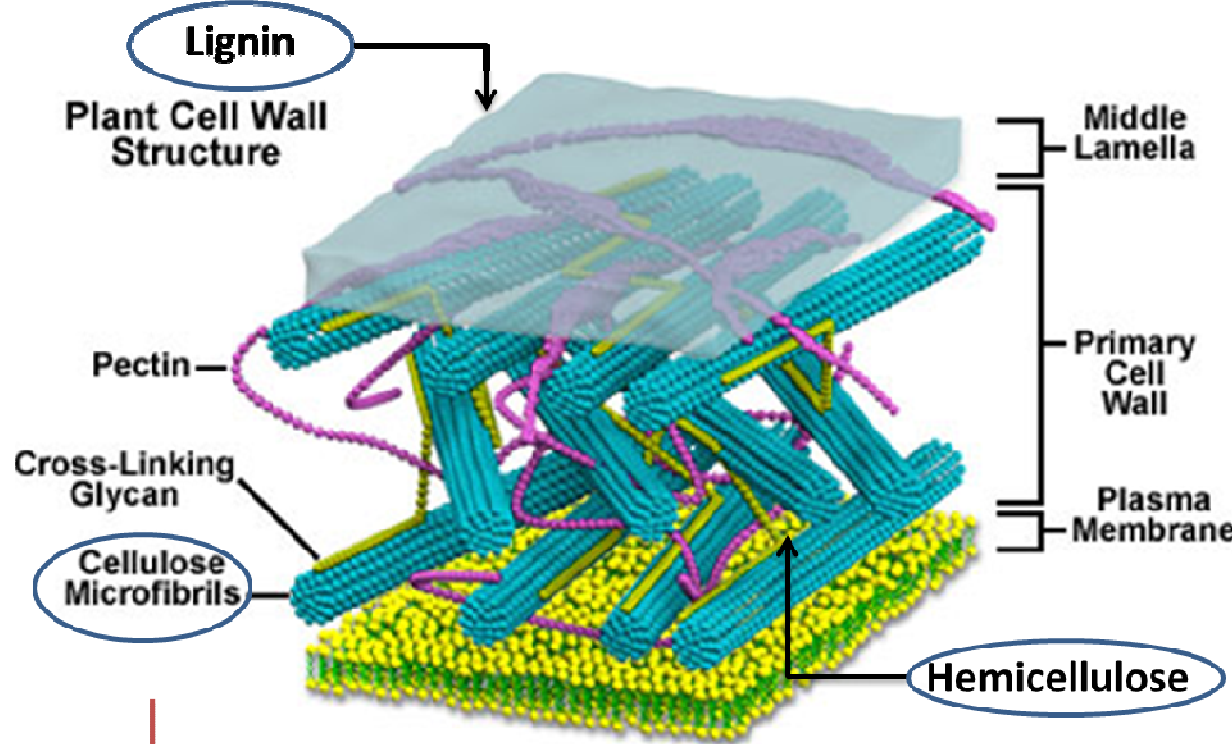
**R&D** aim to understand the basis on lignocellulose degradation by means of complex microbial and enzymatic processes. This can be achieved through exploring the basis of lignocellulose degradation in nature, which will provide the platform for our further application of potent microbes and enzymes on sustainable bio-industries.





# Lignocellulose composition & decomposition

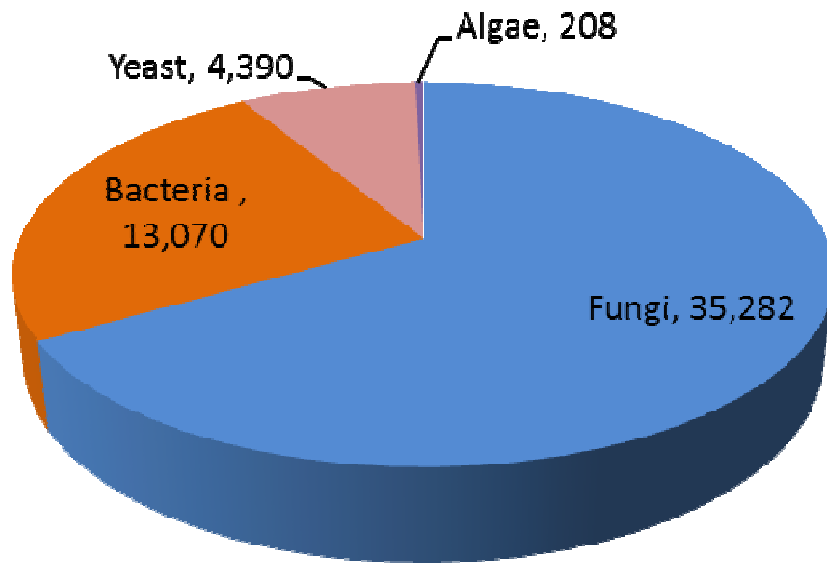
Due to its complex structure, degradation of lignocelluloses involved various biomass degrading microbes, forming microcosms producing various enzymes working specifically and synergistically. Sugars are then subsequently converted to various products providing the concept of **consolidated bioprocessing**



# Enzyme discovery from bioresources

## BIOTEC Culture collection

Screening of microbial isolates producing target enzymes



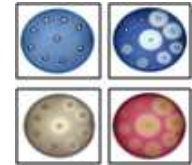
**BIOTEC Culture Collection**

## Environmental metagenomes



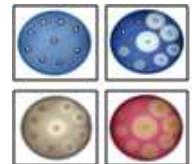
Jae Sorn Hot Spring

Plasmid: 200 Mb



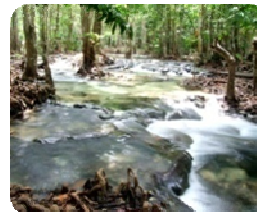
Termite gut

Fosmid: 2 Gb



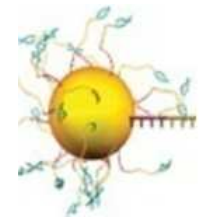
Microbial consortium

Fosmid: 4 Gb



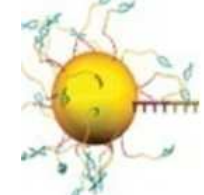
Peat swamp forest

Fosmid: 2 Gb

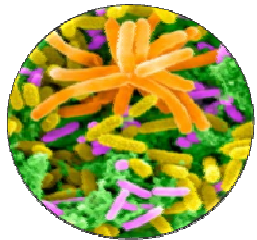


Industrial bagasse collection site

Fosmid: 4 Gb



# Cellulases and Hemicellulases from microbial bioreources



Symbiotic stable consortia



Cultured microorganism



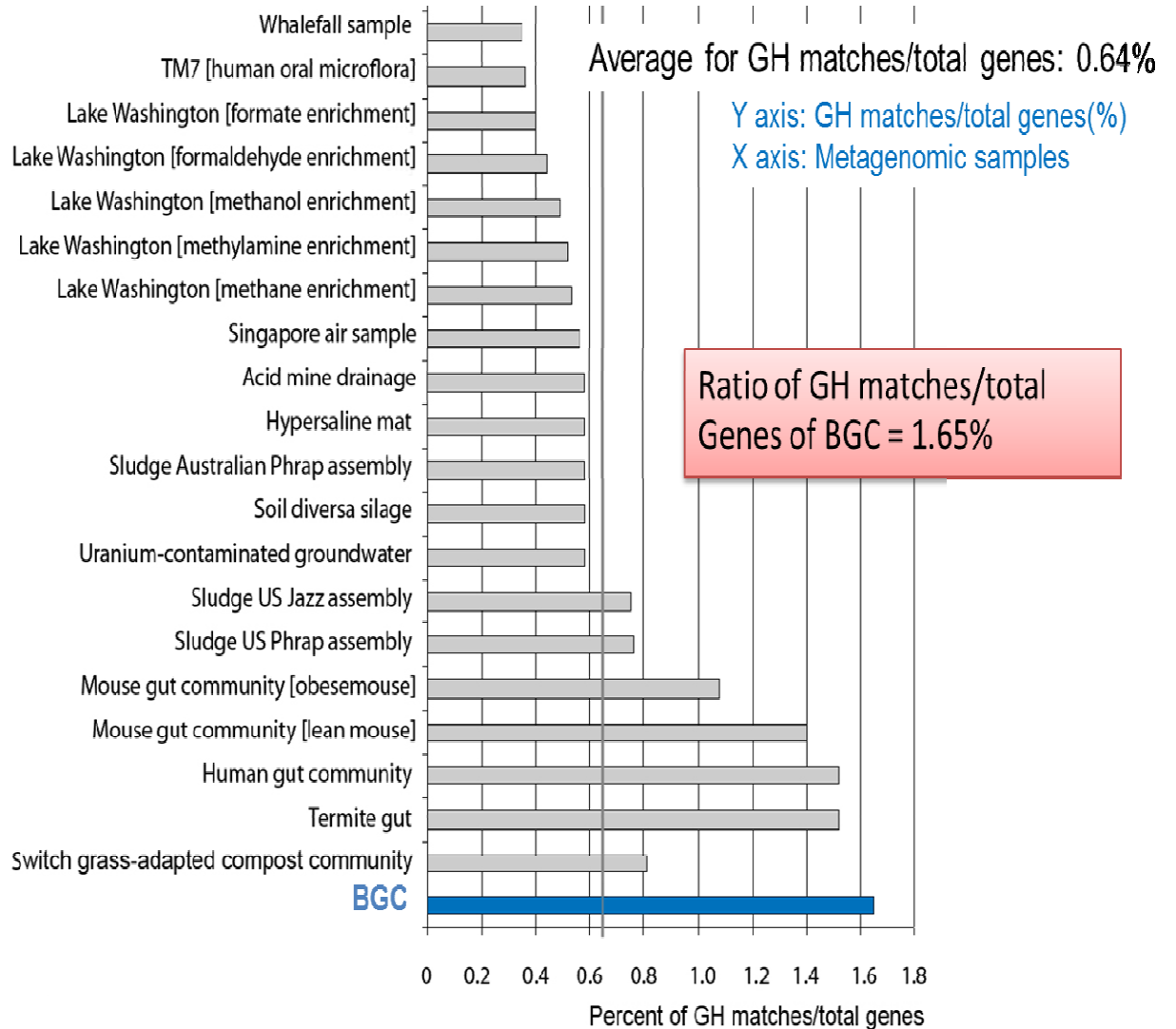
**BGC:**  
Bagasse



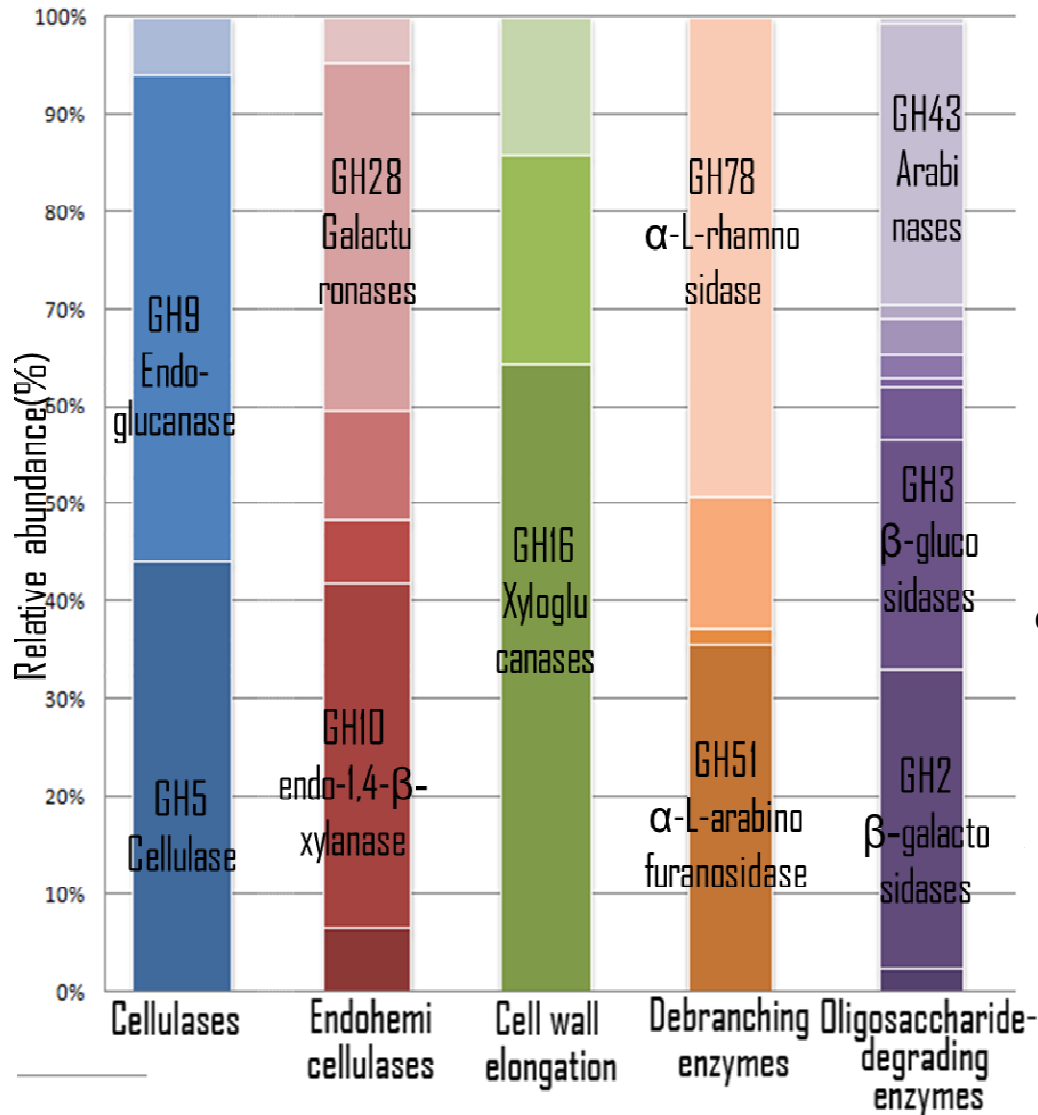
**CRC:**  
Cow rumen



**ASC:**  
Pulp AS



# Identification of lignocellulolytic enzymes from biomass degrading consortium BGC metagenome shotgun pyrosequencing



Wongwilaiwalin et al. 2013 Appl. Microbiol. Biotechnol. (in-press)



BGC



Termite

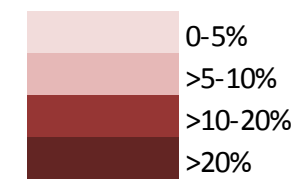


Bovine rumen



Wallaby foregut

	BGC	Termite	Bovine rumen	Wallaby foregut
Cellulase	0-5%	>20%	0-5%	0-5%
Endohemicellulase	0-5%	>20%	0-5%	0-5%
Cell wall elongation	0-5%	0-5%	0-5%	0-5%
Debranching enzyme	0-5%	0-5%	>10-20%	0-5%
Oligosaccharide deg	>20%	>20%	>20%	>20%

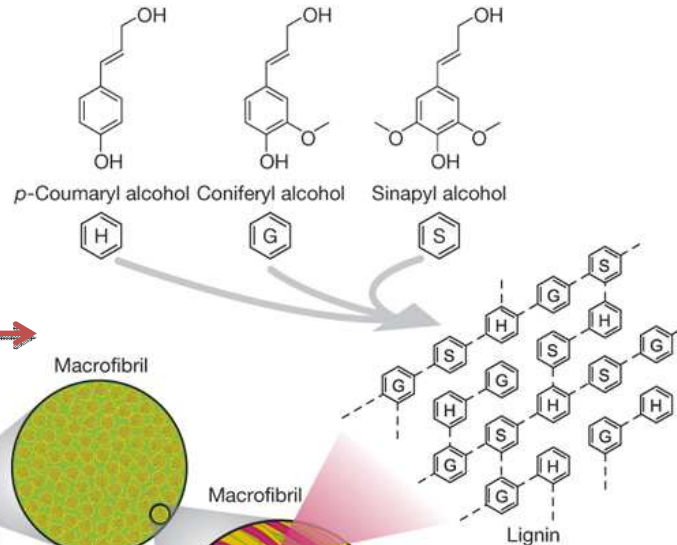


- 957 ORFs were annotated to GH in 69 families
- > 50% were classified into 5 families of the biomass degrading enzyme

# Development of biomass-degrading enzyme system

## Ligninase

- Lignin peroxidase
- Mn-Peroxidase
- Laccase



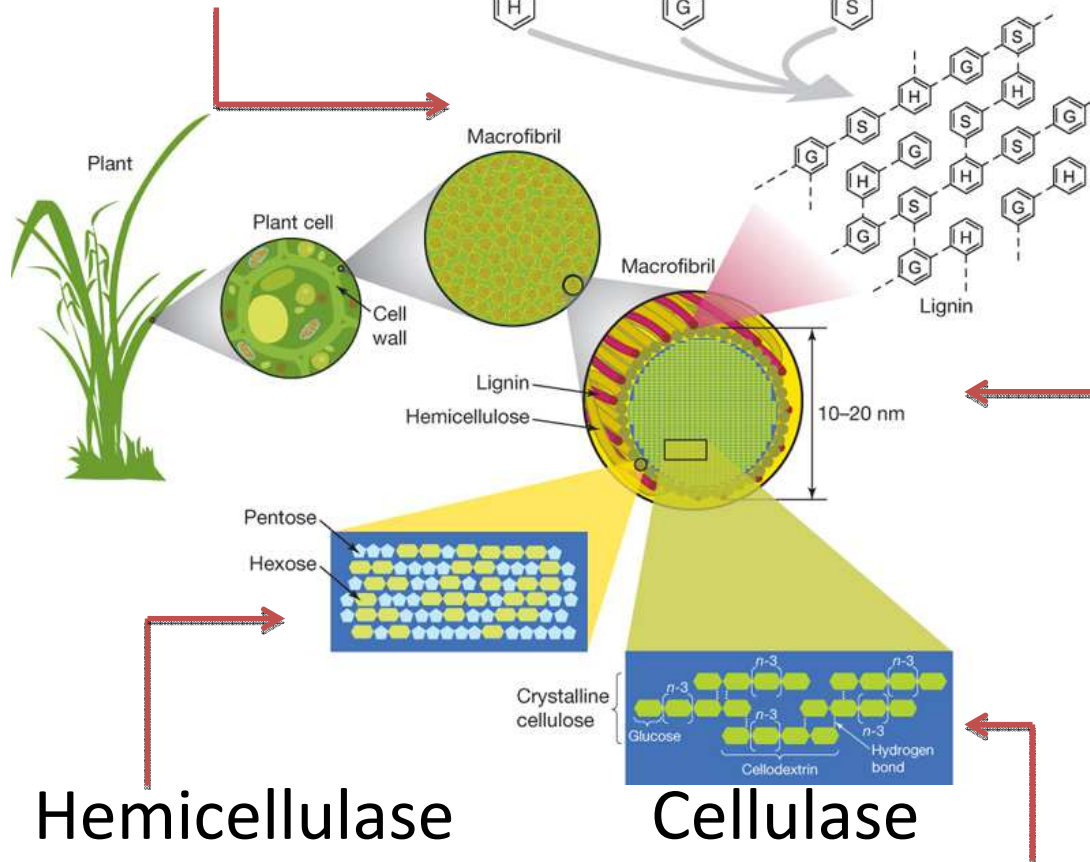
BIOTEC Culture Collection



Microbial consortia



Metagenome



## Accessory enzymes & proteins

- Esterase
- Expansin

## Hemicellulase

- Xylanase
- Mannanase

## Cellulase

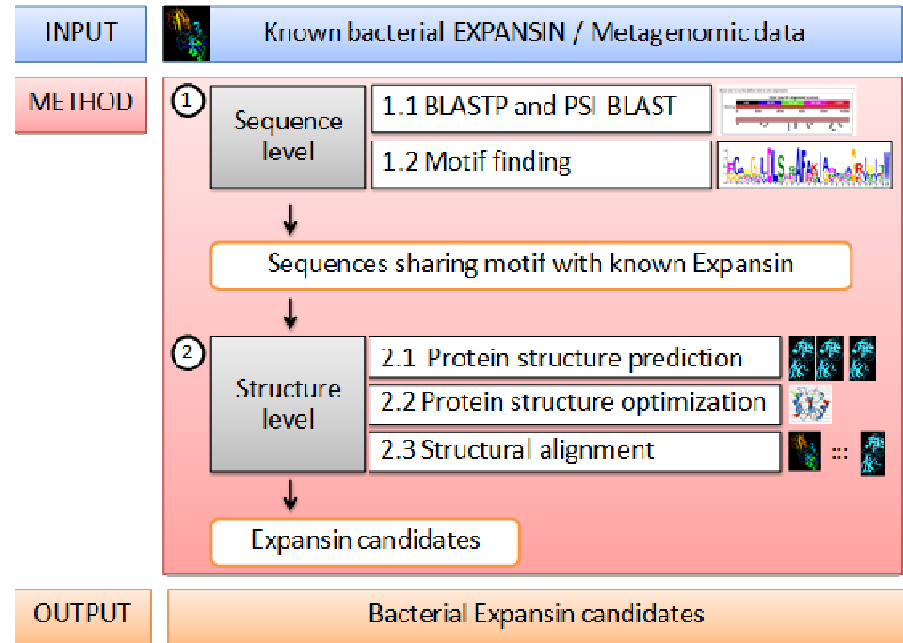
- Endoglucanase
- Exoglucanase
- $\beta$ -Glucosidase

# Biomass-degrading enzyme discovery

Accessory enzyme from BCC

Expansin from bioinformatics

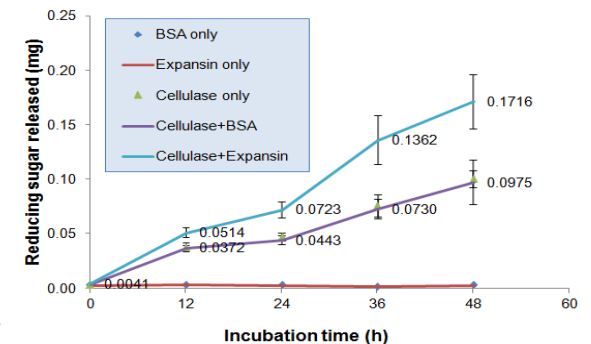
[www.biotec.or.th/enzymecatalog](http://www.biotec.or.th/enzymecatalog)



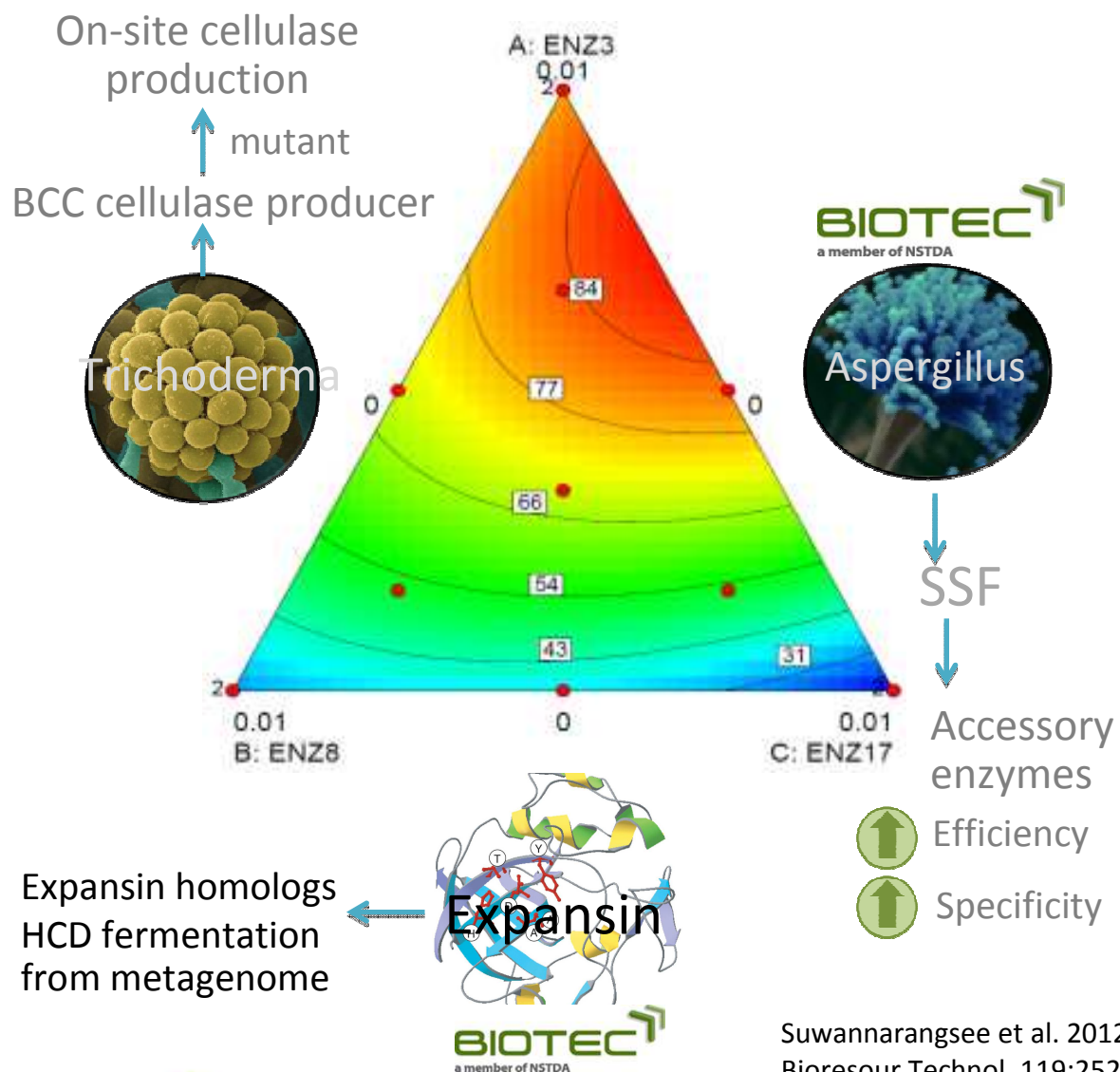
Pick up the top candidates and test their expression and function

Synthesised gene

Expression



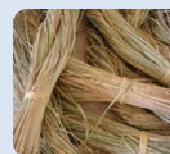
# Synergistic lignocellulolytic enzyme system for hydrolysis of local agricultural biomass



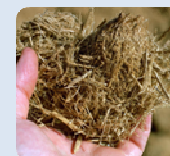
## Ternary enzyme mixture

Celluclst™:BCC199:Expansin  
41.4:37.0:21.6

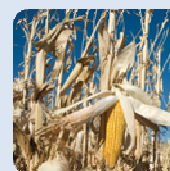
- Synergy = 1.7
- 965.4 mg R.S./g (2-fold)
- 356.4 mg R.S./FPU (258%)
- 60% reduction of Celluclast™



Rice straw



Bagasse



Corn stover

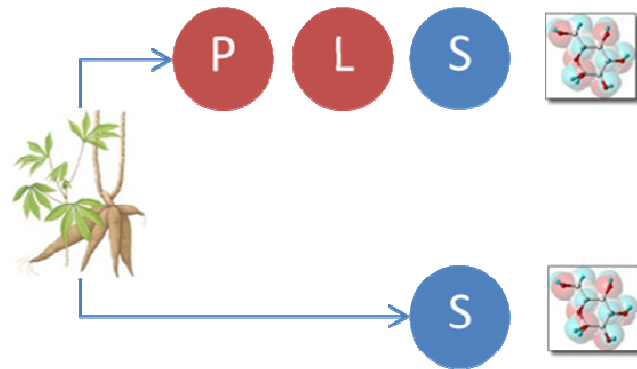
Suwannarangsee et al. 2012  
Bioresour Technol. 119:252-61

# Aspergillus aculeatus

# Enzyme for cassava processing

A multi-enzyme producing fungal strain isolated from soil in Thailand.

- Multi-activity plant polysaccharide degrading enzymes: RSD amylase, cellulases, hemicellulases and pectinases
- Versatile: high growth rate on various substrates both in SmF and SSF



*A. aculeatus*



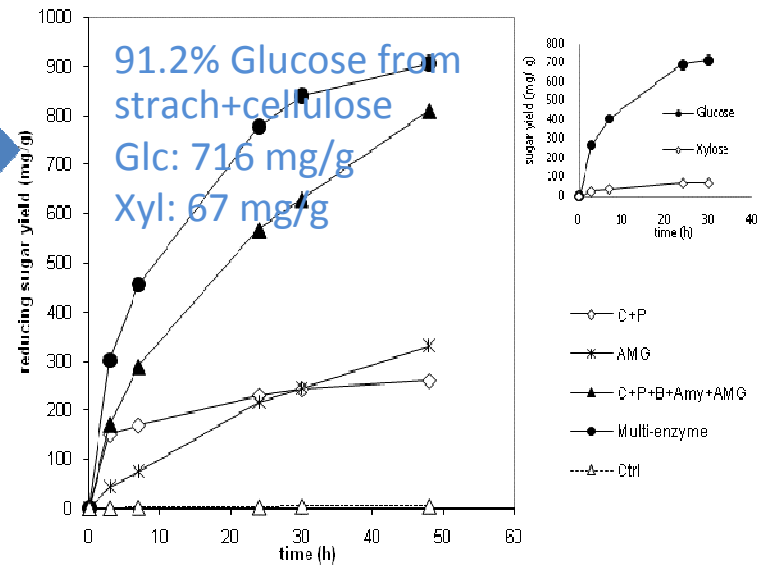
DoE (SSF)



25 kg  
Up-scaling production



Pilot-scale production by SSF & downstream processing

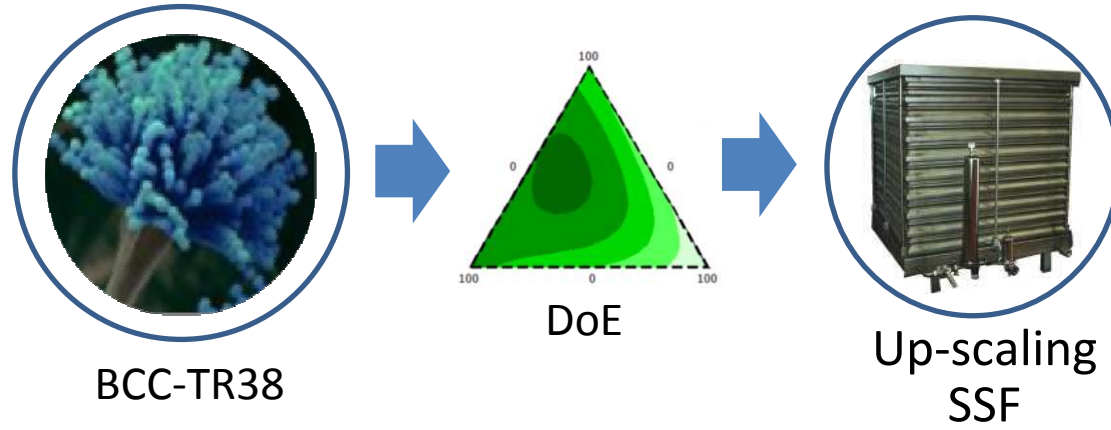


Rattanachomsri et al. (2009)  
J Biosci Bioeng 107; 488-493





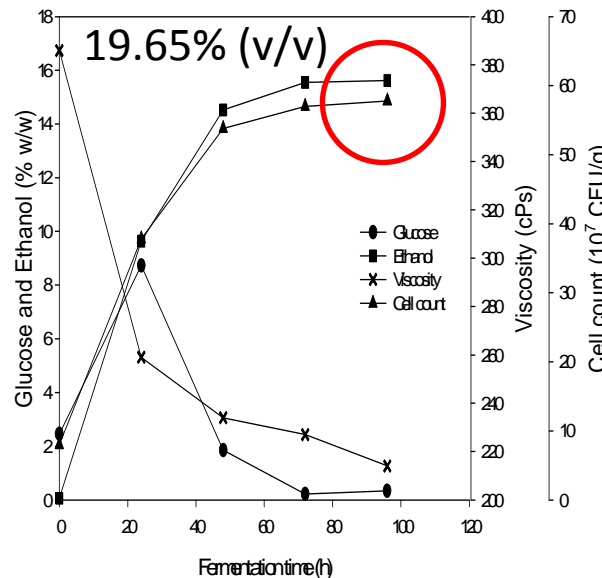
# Viscosity reduction in very high gravity fermentation



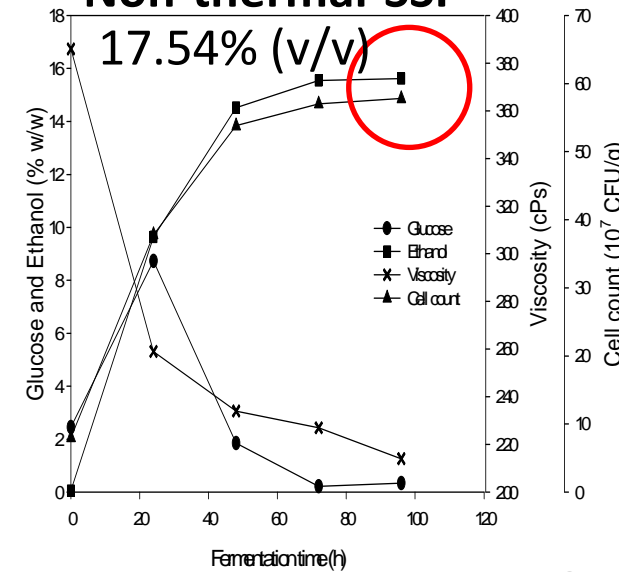
Viscosity reduction in VHG



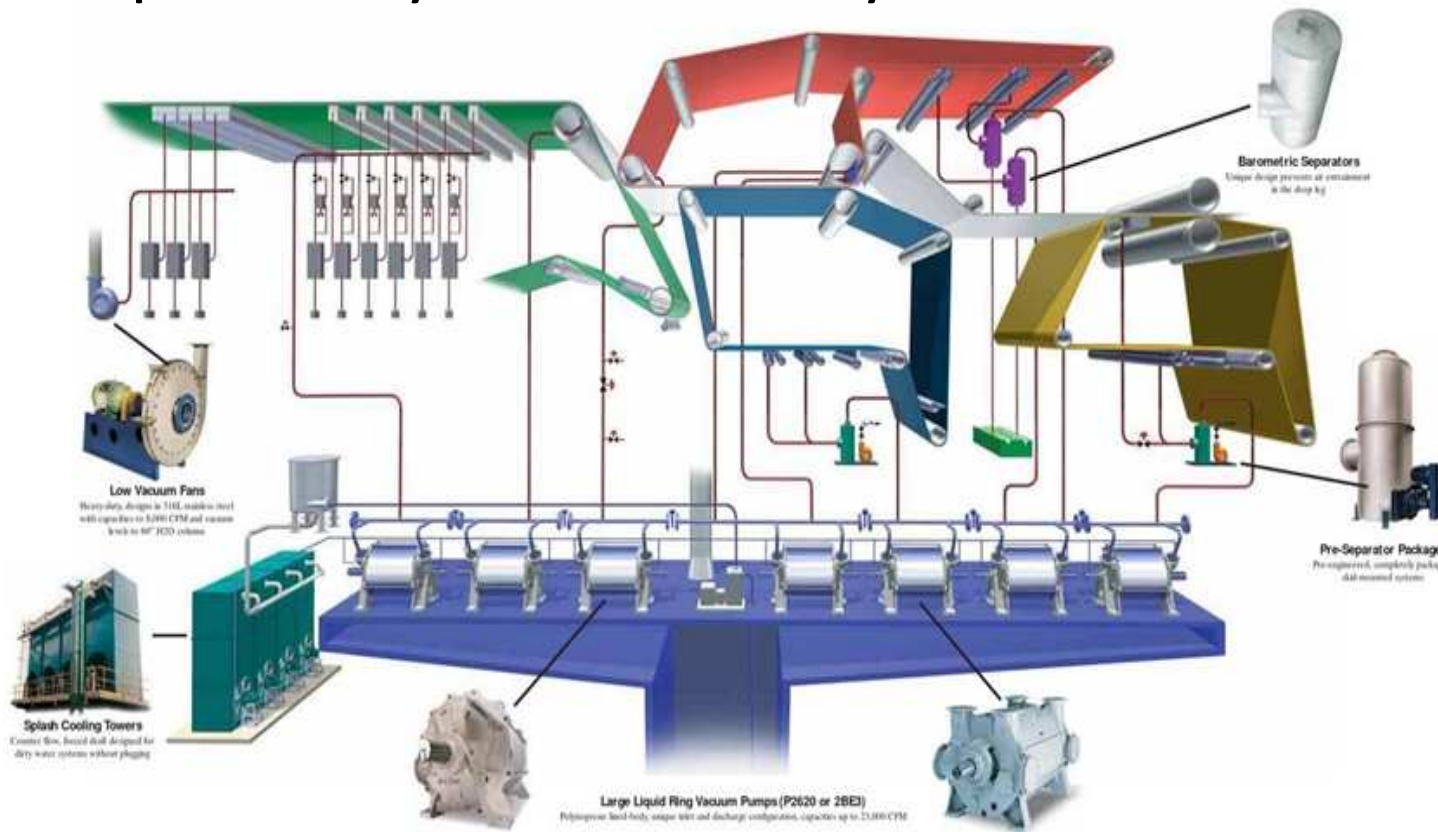
## Thermal-SSF



## Non-thermal-SSF



# Pulp industry for biorefinery



- ➔ Pulp
- ➔ Commodity chemicals
- ➔ Energy



# Conclusion & Future prospects

**C**ombined biochemical, molecular metagenomic, and bioinformatic tools have been used for exploration of microbial bioresources for various biotechnological applications with the focus on the prospective biorefinery industry.

**F**uture **R&D** will be focused on integration of enzyme-based biocatalytic and catalytic processes for conversion of biomass to valorised fuels, chemicals, and materials in greener and more sustainable bio-industries.



# Biorefinery for sustainability of nature and society

## Acknowledgement

- National Center for Genetic Engineering and Biotechnology
- National Science and Technology Development Agency
- Enzyme Technology & Microbial Cell Factory Laboratory members

**Thank you..**