Verawat Champreda
Microbial Biotechnology and
Biochemicals Research Unit

From Biodiversity to Bioindustry

Development of enzymes for green industry

Bioeconomy

Biobased economy refers to all economic activity derived from activity focused on biotechnology.

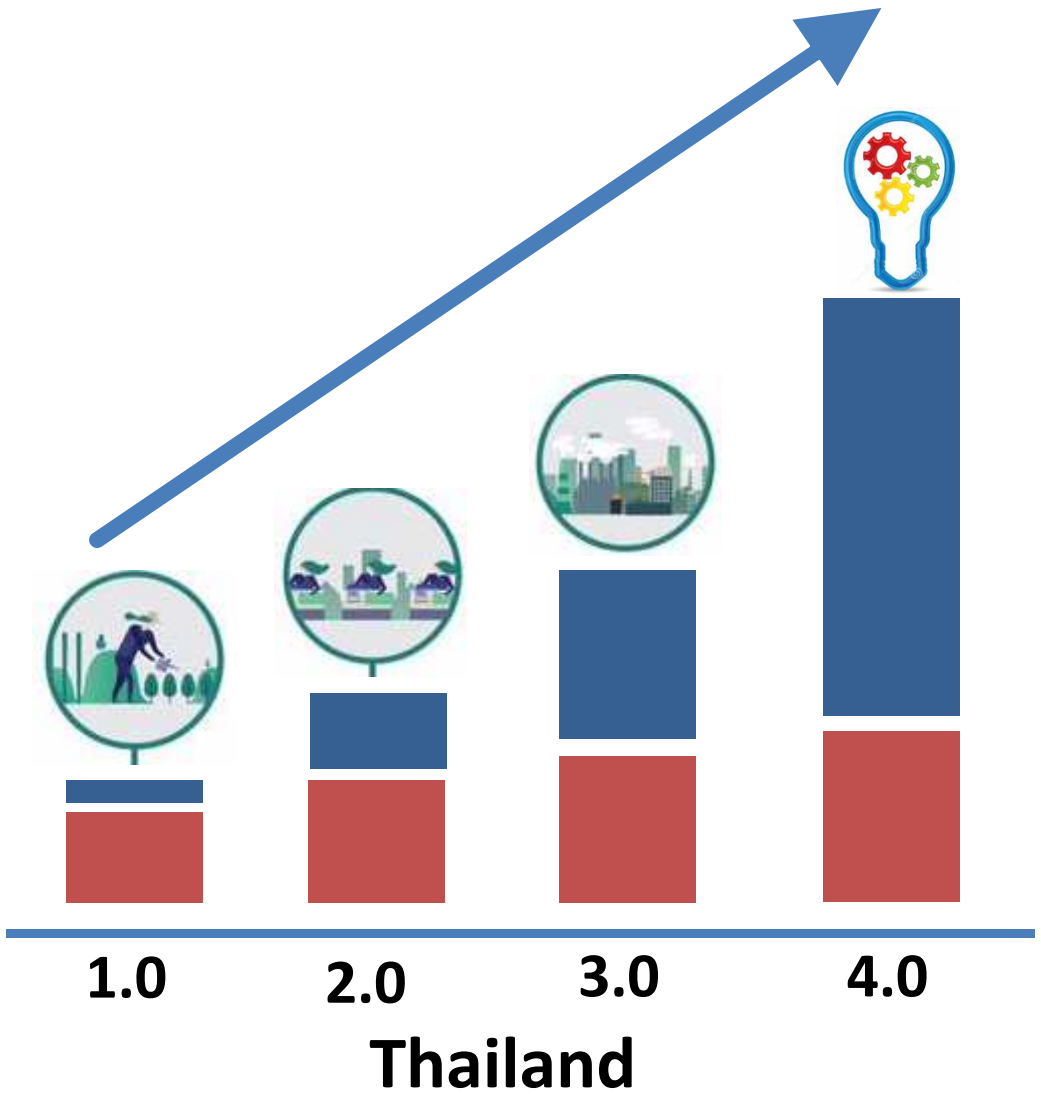
In other words, understanding mechanisms and processes at the genetic and molecular levels and applying this understanding to creating or improving industrial processes.

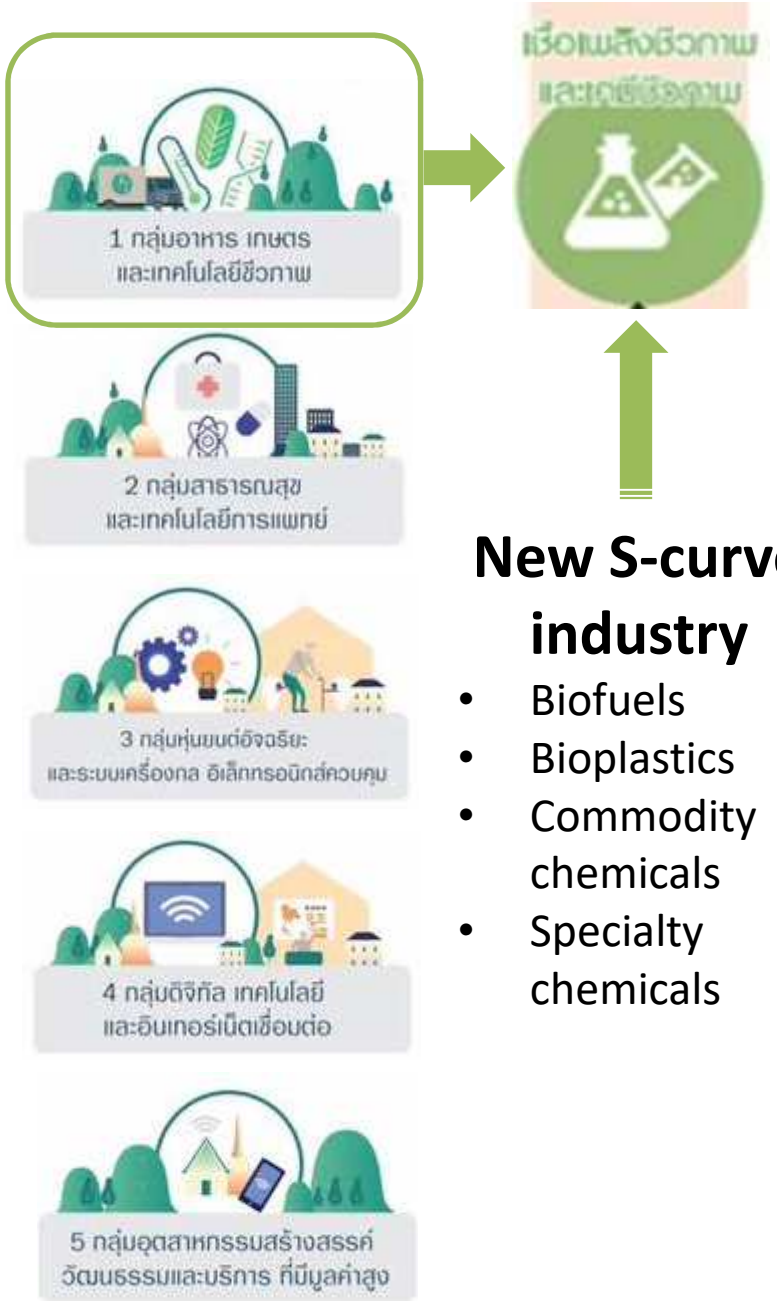
- **Renewable resource**
- **Green & Clean processing**
- **Environmental & Economic sustainability**

Bio-economy as a global trend

http://www.bio-step.eu

Bio-economy as a driving force for Thailand 4.0

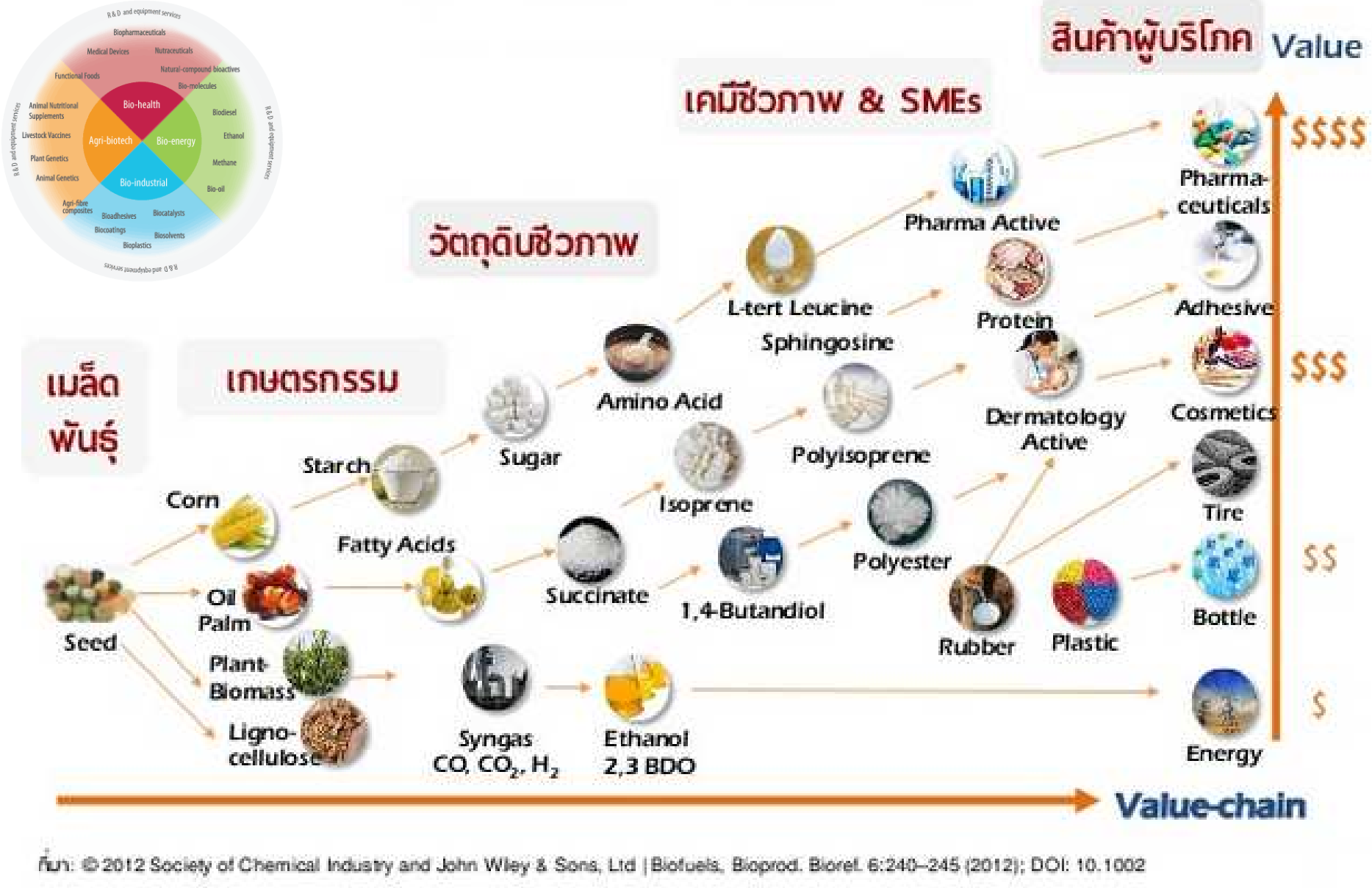




New S-curve industry

- Biofuels
- Bioplastics
- Commodity chemicals
- Specialty chemicals

Value-chain for bio-based industry



ที่มา: © 2012 Society of Chemical Industry and John Wiley & Sons, Ltd | Biofuels, Bioprod. Bioref. 6:240–245 (2012); DOI: 10.1002

http://www.slideshare.net/htk999/20160224-bioeconomy-242559

Enzyme & Bio-based economy

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

Bioresources are explored as a genetic source for novel microbes and enzymes for the prospective bio-industry.

- C — Crude SSF/ SmF enzymes
- T — Technical enzyme: Crude/ Recombinant formulation
- P — High-grade recombinant enzyme

Food & Feed

Pre-biotics/ supplements

Biofuels & Chemicals

Saccharification/ processing

Green processing

Pulp/ Textile/ Detergent

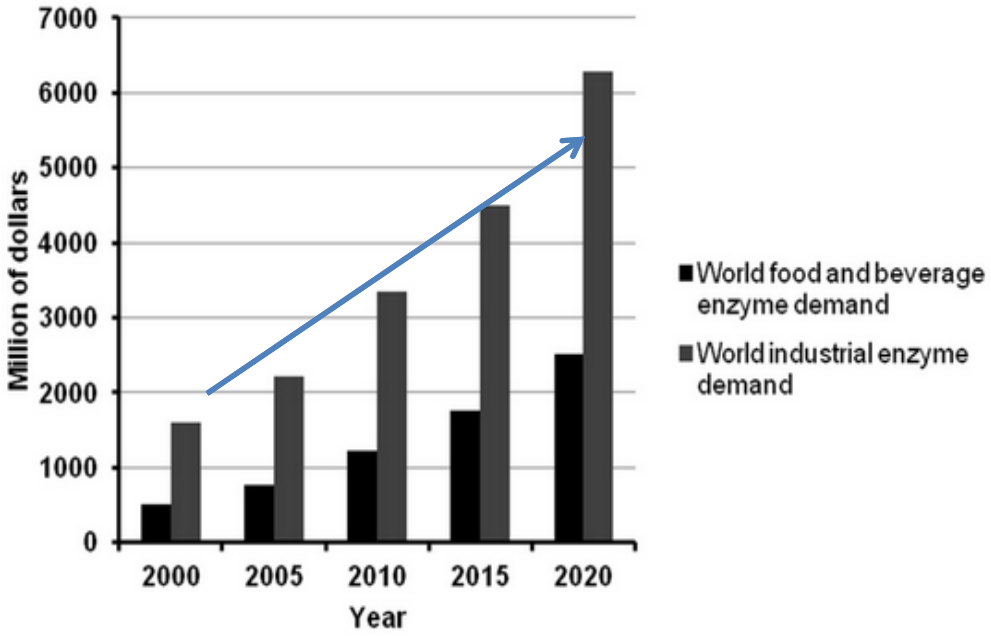
Healthcares & Pharmacueuticals

Specialty high value enzymes

Enzyme: a key to bio-industry at global scale

Enzymes are indispensable components in commodity and specialty products.

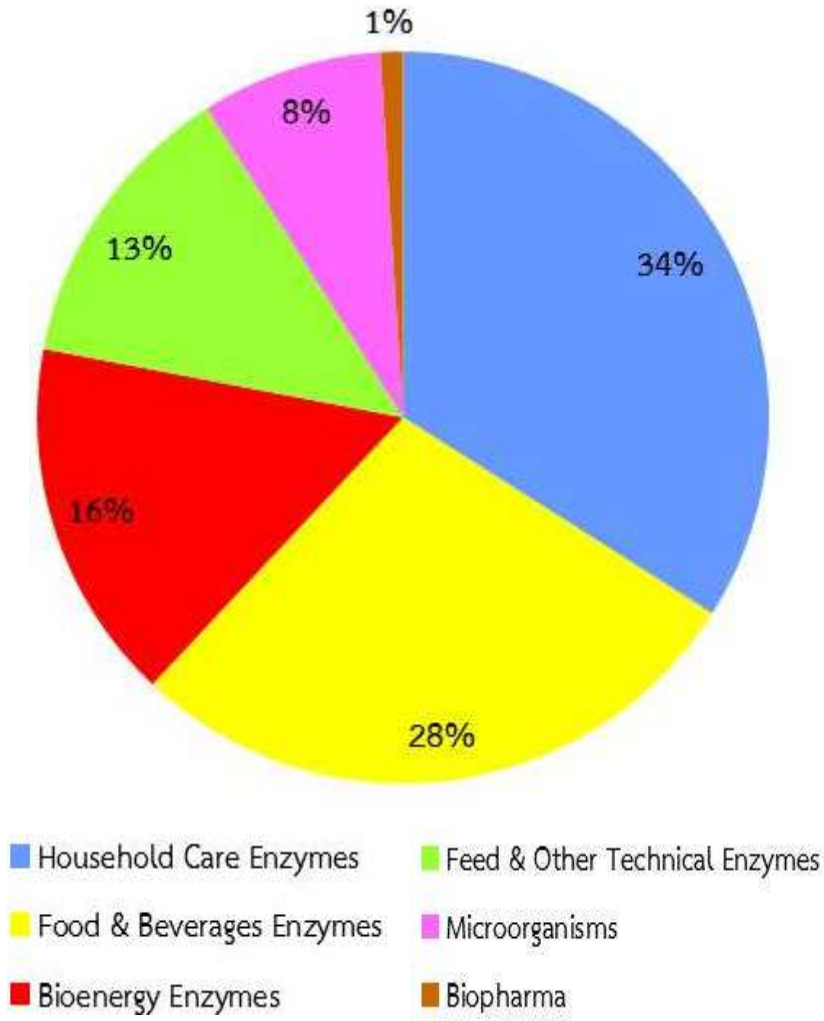
- Biocatalysts in bio-processes
- Additives in products for desirable properties



Global enzyme market: 9,000 M $ (2020) with the growth rate of 6.8%/year

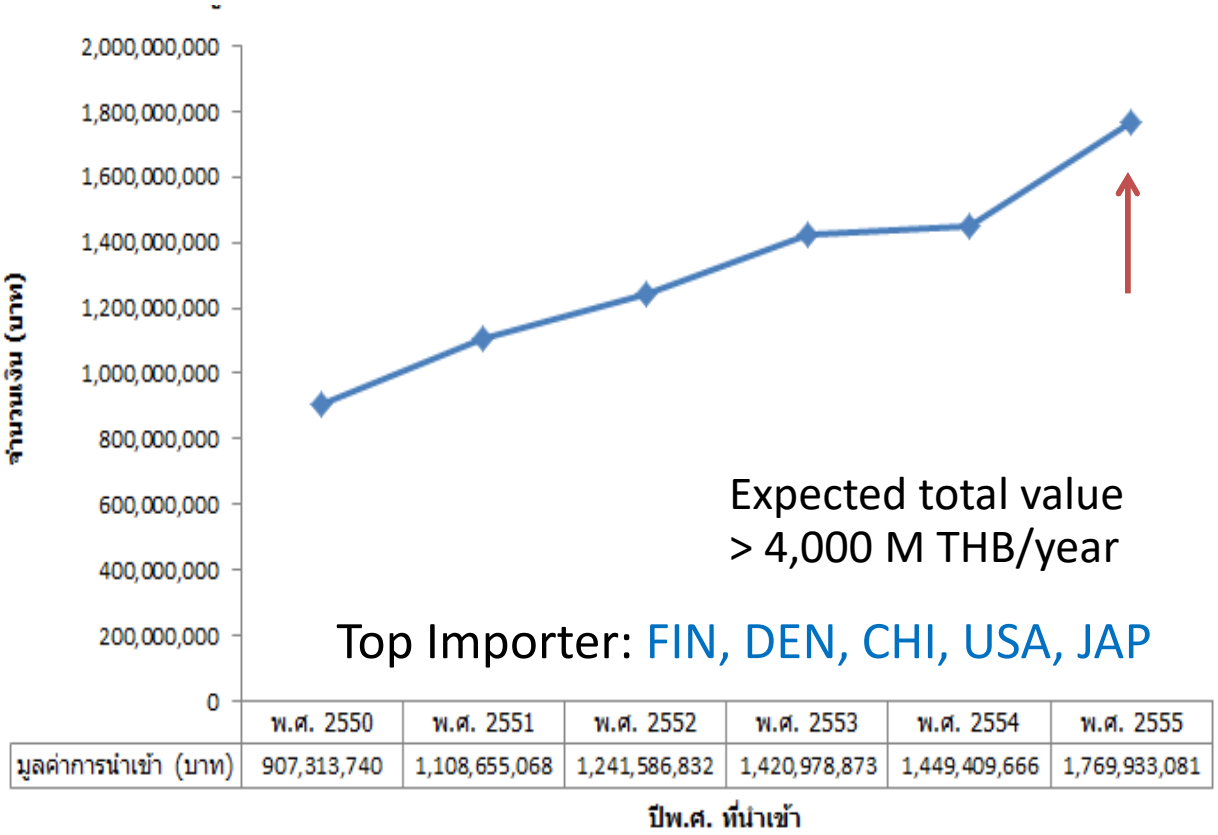
Source: Freedonia Group, 2011

2012 Enzyme business and bio-business sales by industry



Enzyme & Bio-business in Thailand

Enzyme import value: 2550-2555



	พ.ศ. 2550	พ.ศ. 2551	พ.ศ. 2552	พ.ศ. 2553	พ.ศ. 2554	พ.ศ. 2555
มูลค่าการนำเข้า (บาท)	907,313,740	1,108,655,068	1,241,586,832	1,420,978,873	1,449,409,666	1,769,933,081

Expected total value
> 4,000 M THB/year

Top Importer: FIN, DEN, CHI, USA, JAP

SWOT Enzyme & Bio-industry

Strength

- ความหลากหลายทางชีวภาพและของจุลินทรีย์
- งานวิจัยที่เข้มแข็งและต่อเนื่องทางด้านเอนไซม์และผลิตภัณฑ์จุลินทรีย์ (lab-scale)
- อุตสาหกรรมการหมักที่เข้มแข็งในการใช้จุลินทรีย์เพื่อการผลิตอาหาร อาหารสัตว์ สารเคมี และพลังงาน

Weakness

- ขาดความสามารถในการต่อยอดงานวิจัยสู่ระดับขยายขนาด
- ไม่มี Demonstration plant เพื่อ OEM/ODM
- ข้อจำกัดในการประเมิน feasibility เพื่อถ่ายทอดเทคโนโลยี

Opportunity

- แผน New S-curve industry ด้าน
- อุตสาหกรรมชีวภาพ
- การลงทุนที่เพิ่มขึ้นของภาคเอกชนในอุตสาหกรรมชีวภาพ
- Niche market: ความจำเพาะต่อวัตถุดิบตั้งต้น สภาวะในกระบวนการผลิต และรูปแบบผลิตภัณฑ์ในประเทศ
- ศักยภาพในการเป็น Bio-industry hub ใน AEC

Threats

- การแข่งขันในตลาดเอนไซม์ระดับโลก
- เอนไซม์ทางการค้าและเทคโนโลยีทางเลือก (non-bio process)
- การ license เทคโนโลยีโดยตรงจากต่างประเทศ

ความจำเป็นในการพัฒนาเทคโนโลยีโรงงานต้นแบบที่ประกอบด้วยระบบการผลิต (process system) และองค์ความรู้ในการขยายขนาดการผลิต (process knowhow) ซึ่งเป็นสิ่งสำคัญต่อการก้าวข้าม bottle neck ของงานวิจัยทางด้านเทคโนโลยีชีวภาพของประเทศ

Enzymes for green industry

From diversity to industry

Environmental metagenome

Culture collection

Enzyme library

Multi-activity

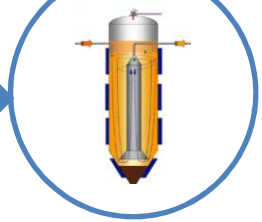
W.T./Mutant

Recombinant

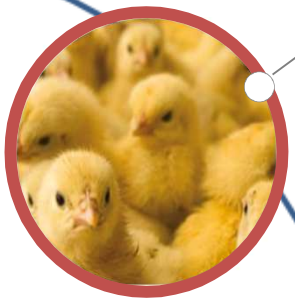
Expression systems

Single activity

- *E. coli*
- *P. pastoris*
- *O. thermomethanolica**
- *Aspergillus*



Lab-to-pilot Production & Formulation

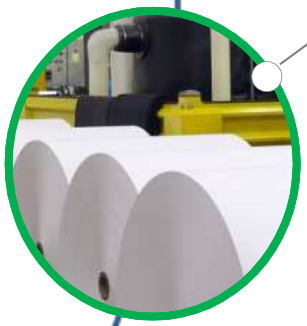


Animal Feed

Enhancing nutrition/ digestibility

Biofuels & Biorefinery

Sugar platform conversion



Green processing

Pulp/Textile

Reducing chemicals and energy

Specialty enzymes

Healthcare products

Local enzyme market = **2,500 M** THB

Global market = **4.4 Billion** USD

http://www.bccresearch.com

Thailand Bioresource Research Center (TBRC)

TBRC

THAILAND BIORESOURCE RESEARCH CENTER

The TBRC Network facilitates the coordination of exchange of biological information and resources and develops mechanisms enabled by information technologies to broaden access to biomaterials to the public and scientific community.

A focal microbial bank network for microbial utilization in ASEAN

- >10,000 isolates (+properties) of bacteria, yeast, and fungi

THAI SCIENCE BIODIVERSITY

Health and Medicine

Bioactive cpds
Food supplement
Pro-/Pre-biotics
Cosmetics

Agriculture

Biofertilizer
Biocontrol
Plant growth promoter

Food and Feed

Food/Feed
Pro-/Pre-biotics

Environment

Waste treatment
Bioremediation

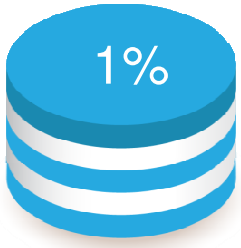
Energy

Biofuels
Bioenergy

Other Industries

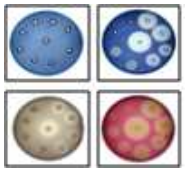
Green processing agents: paper, textiles

Cultured microbes



BCC: 80,000 strains

TBRC
THAILAND BIORESOURCE RESEARCH CENTER



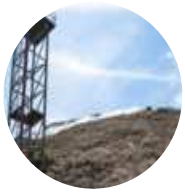
Strain improvement
- Mutant
- Transgenic

Uncultured microbes

Termite gut
Fosmid: 2 Gb

Peat swamp forest
Shotgun: 13.5 Mb
454 Pyrosequencing

Stable lignocellulolytic microbial consortium
Shotgun: 34.9 Mb
Titanium pyrosequencing



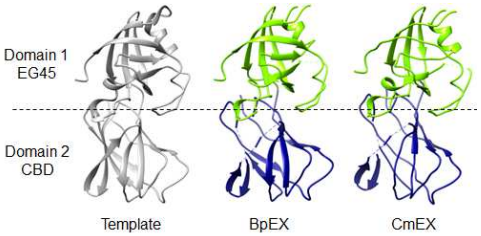
Industrial bagasse collection site
Shotgun (31.9 Mb)/
Fosmid (428 Mb)
Ion Proton/ Ion PGM

Diversity/Genes/Pathways

Public database

Genome
Metagenome
[NCBI/SRA]

Bioinformatic tools
- Sequence
- Structure



Enzyme production platform

Solid state fermentation (SSF): simple bulk fermented products

Microbes

Fungi
Bacilli

Rotary drum fermenter

Tray fermenter

Downstream processing

Products

Microbes
Fermented products
SSF enzyme

Submerged fermentation (SmF): enzymes & high valued products

Microbes

Bacteria
Yeast
Fungi
Recombinant

Stirred Tank Fermenter

- Batch
- Fed-batch
- Continuous

Downstream processing

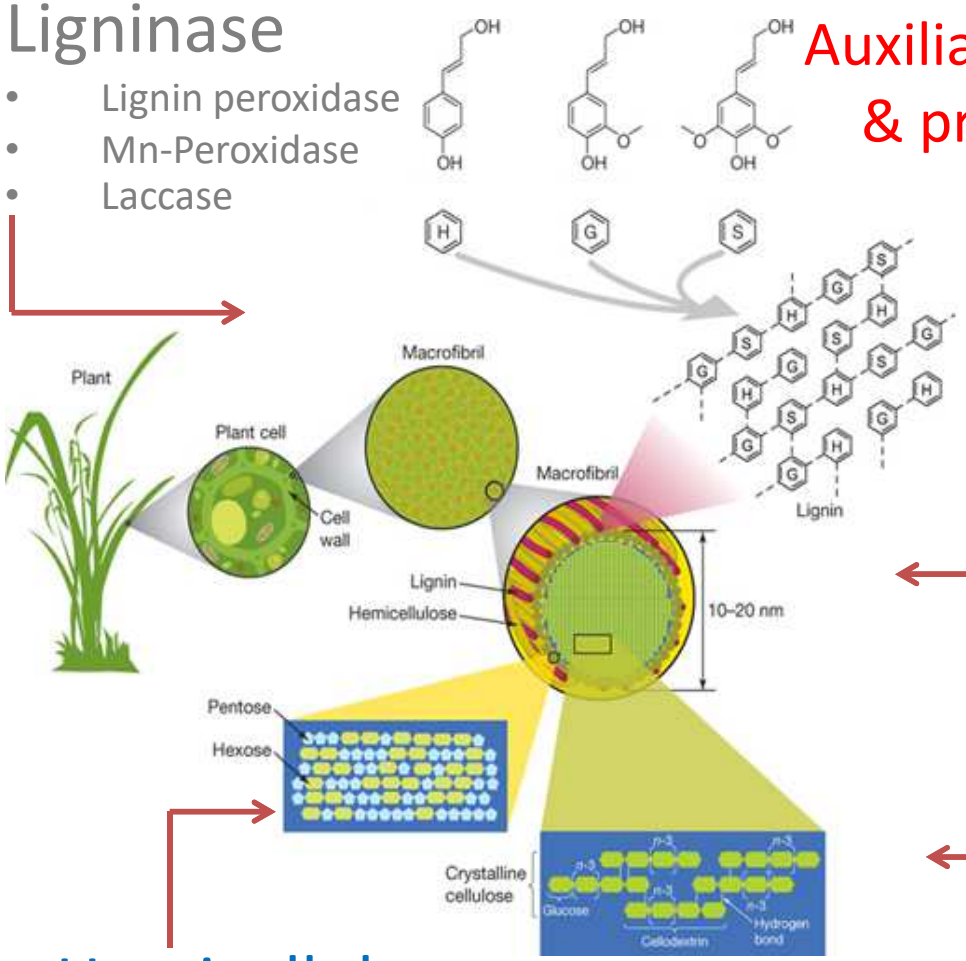
Products

Enzymes
Microbial agents
Bioactive cpds
Biopolymers

Lignocellulose structure & decomposition

Ligninase

- Lignin peroxidase
- Mn-Peroxidase
- Laccase



Auxiliary enz & proteins

Hemicellulase

- Endo-acting: Xylanase/Mannanase
- Exo-acting/debranching

Cellulase

- Endoglucanase
- Exoglucanase
- β-Glucosidase

Bagasse

Rice straw

Corn stover

Cellulose 33-51%

Hemicellulose 19-34%

Lignin 21-32%

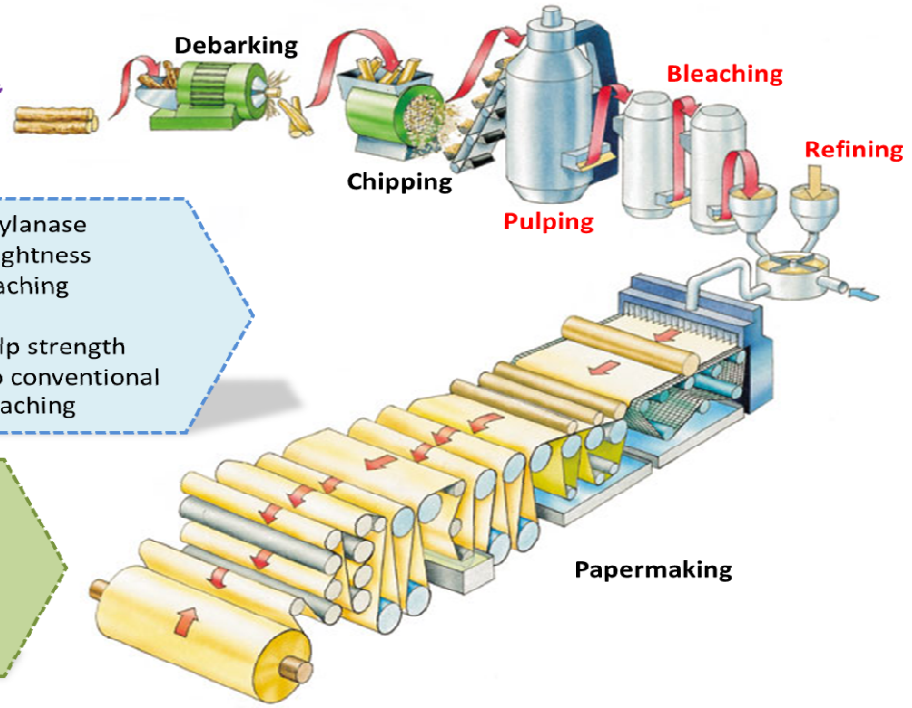
Modified from Rubin (2008), Nature 454; 841-845

Pulp & Paper industry

Pulping: Cellulase
•Reduce energy and chemicals in pulping

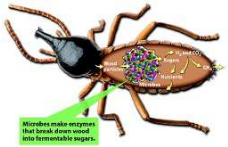
Bleaching: Xylanase
•Increase brightness
•Reduce bleaching chemicals
•Increase pulp strength compared to conventional chemical bleaching

Refining: Cellulase
•Reduce refining energy
•Increase fibrillation and pulp stregth

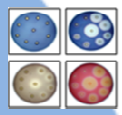


ENZBleach: Alkaliphilic xylanase from termite gut metagenome

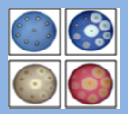
Environmental metagenomic gene library

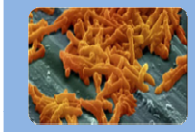


Jae Sorn Hot Spring
Plasmid: 200 Mb
Thermophilic enzymes

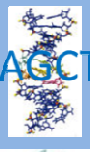


Termite gut
Fosmid: 2 Gb
Alkalophilic lignocellulolytic enzymes





Microbial consortium
Fosmid: 4 Gb
Clostridial cellulosomes & secreted cellulases



Peat swamp forest
Fosmid: 2 Gb
Lignocellulolytic enzymes

Industrial bagasse collection site
Fosmid: 4 Gb
Accessory enzymes

Metatranscriptomics
Metaproteomics

Recombinant expression in *E. coli*

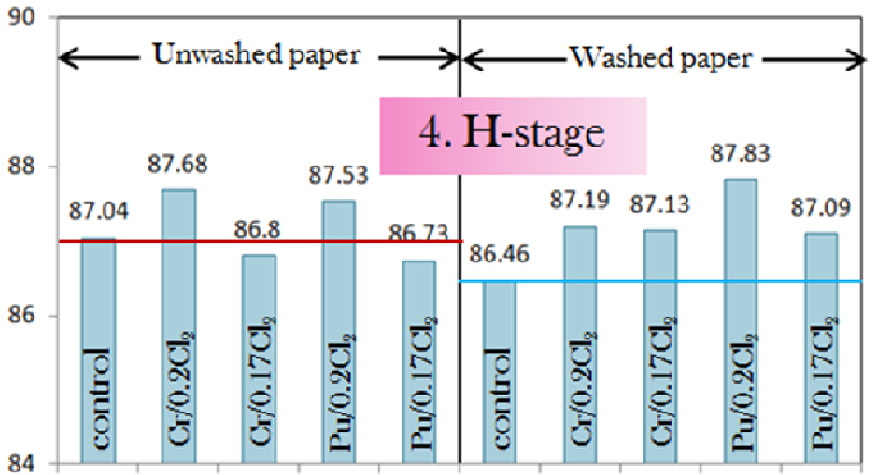
DoE Optimisation
HCD Fermentation

Pilot-scale industrial process

ENZ bleach

Brightness
Chlorine

Chemical
Energy
Waste water



Pulp brightness (%)

Textile industry

Eco-friendly textile processing model

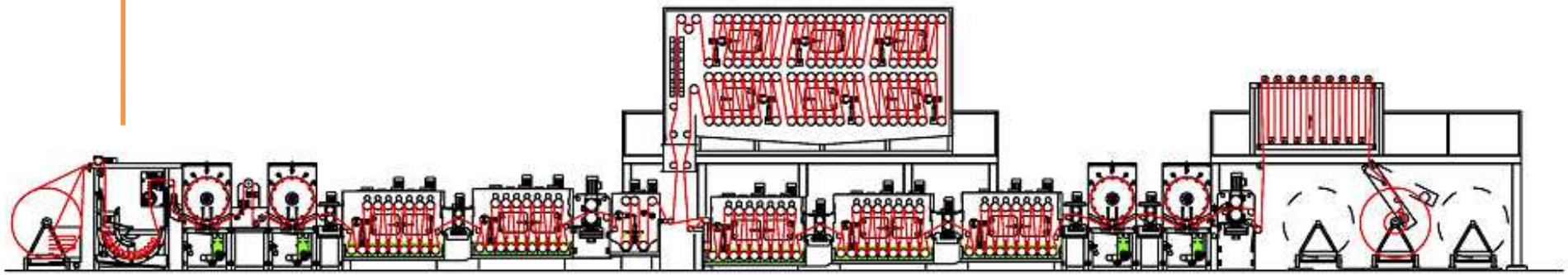
Singeing: Cellulase

Energy ↓

Bleaching: Xylanase

Energy ↓

Chemical ↓



Energy ↓

Chemical ↓

Desizing: Amylase

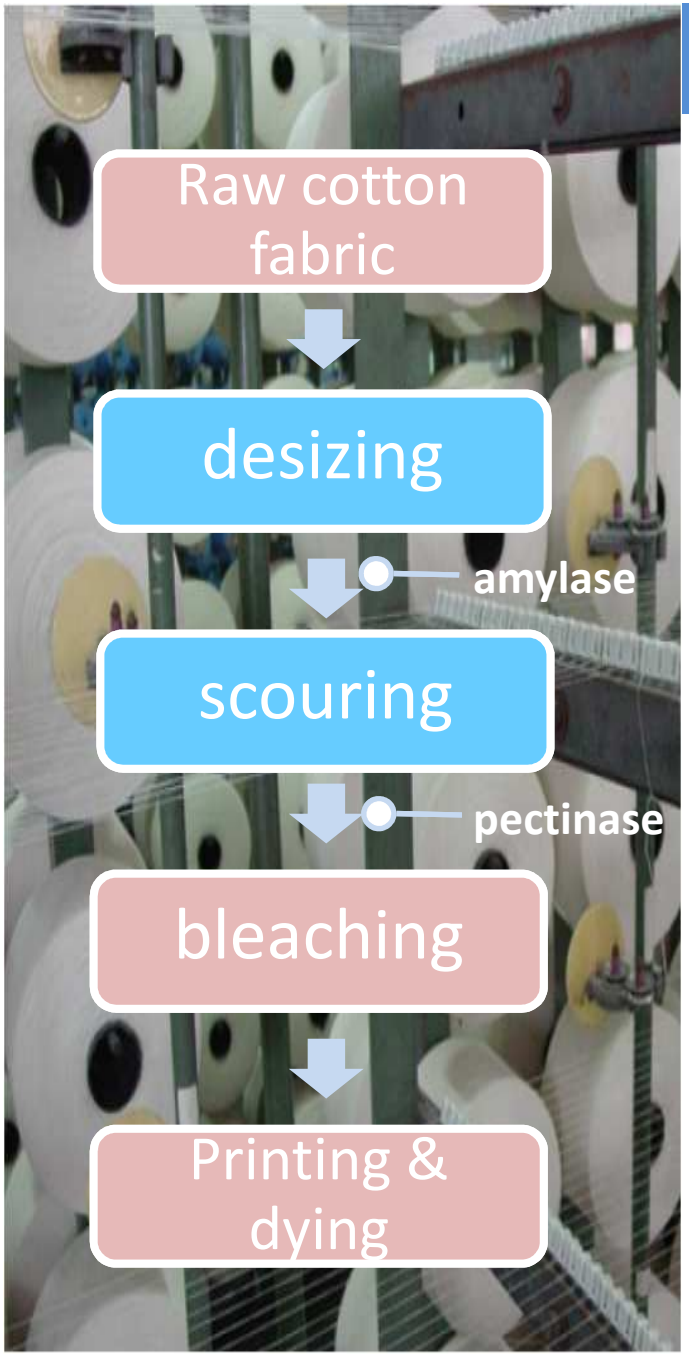
Chemical ↓

Scouring: Pectinase

Environment ↑

Waste water treatment: bioremediation

ENZEase: Dual action enzyme for textile industry



A. aculeatus

DoE (SSF)

Pre-pilot-scale production by SSF & downstream processing

Enzyme for simultaneous starch removal and surface wax cleaning

THANAPAISAL

Desizing: amylase

Tegawa scale 6-7

Scouring: pectinase

Water permeation < 30 s

Animal feed industry

Enzymes for animal feed & products

Screening of fiber digesting enzymes for increasing digestibility of animal feedstuff

- Optimal @40°C, pH 4-5
- Stable @80-90°C for 1-5 min

DoE (SSF)
Cel/Xyl/Man

Pre-pilot SSF

Phytase gene

Rec *Pichia pastoris*

Pre-pilot SmF

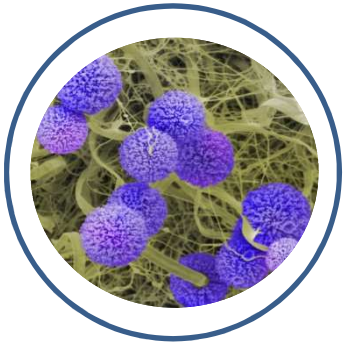
Encapsulation & formulation

NANOTEC

***in- vitro* digestibility test:**

- fiber
- inhibitor
- nutrient

ENZBoost: Thermotolerant high-activity mannanase (WT/Rec)



A. niger
BCC4525

DoE (SSF)
•Mannanase

Pilot-scale
production by SSF

Encapsulation &
formulation

in- vitro
digestibility test:

•fiber

•inhibitor

•nutrient

Feed additive

Recombinant
P. pastoris

Highest activity of mannanase from W.T. fungi previously reported
Optimal activity @80°C with wide T range (40-80°C)

Bioethanol from cassava

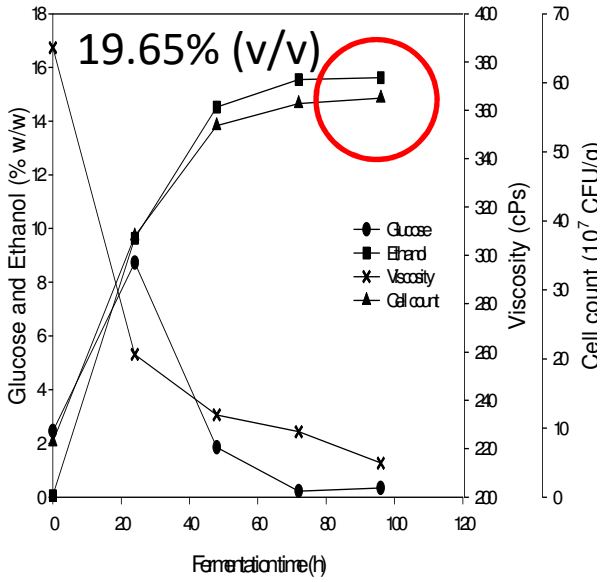
Viscosity reduction in very high gravity fermentation

Root: 12,841 cPs ➡ 383 cPs

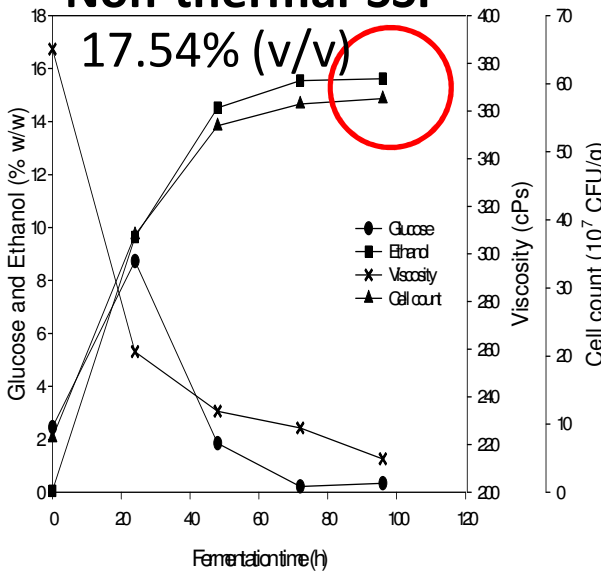
Chips: 821 cPs ➡ 421 cPs

Pulp: 3,084 cPS ➡ 498 cPs

Thermal-SSF



Non-thermal-SSF



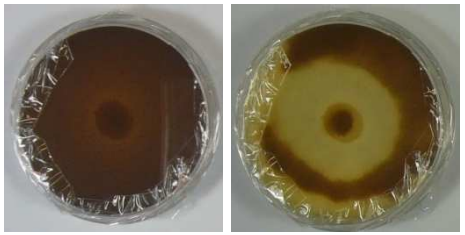
VHG fermentation of cassava root mash (32% solid) pretreated with 0.5% ME-II at 45°C for 2 h and ferment with Thermosac at 32°C, pH 4.5 for 96 h.

Enzymes for healthcare and pharmaceuticals

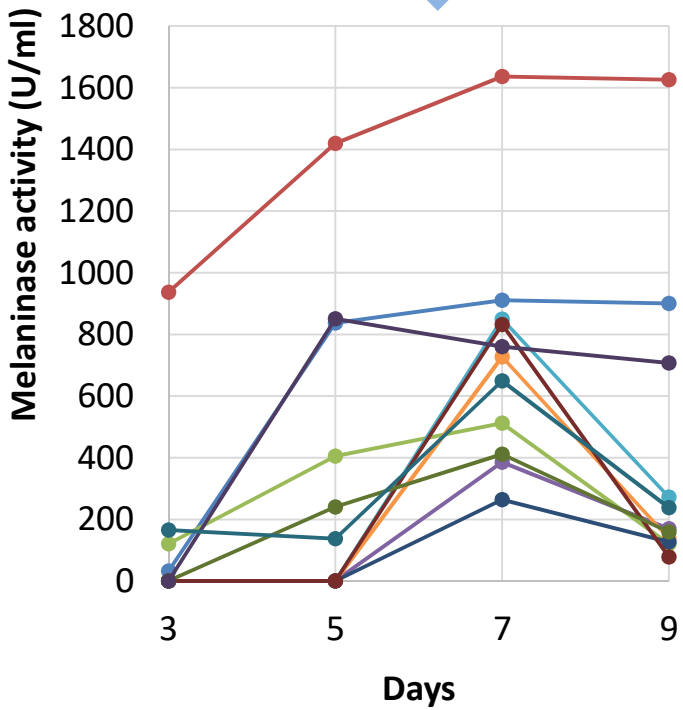
Screening of melanin hydrolyzing enzyme from fungal origin and its use in cosmetic industry



Sample collection at mountain area with shady grove park in Thailand



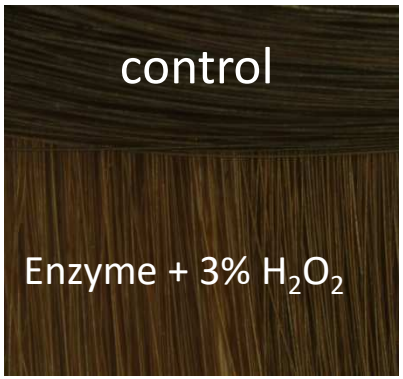
Melanin medium agar plate



Large-scale production (10-20L)

Skin lightening test in 3D model

Hair bleaching test



Some important therapeutic enzymes and their application

Enzyme	Application	Sources
L-Asparaginase	Anti-tumour	*Pseudomonas acidovorans* *Acinetobacter* sp.
L-Glutaaminase	Anti-tumour	*Beauveria bassiana* *Vibrio costcola*
L-Tyrosinase	Anti-tumour	*Trichoderma reesei* *Streptomyces ingrifaciens*
Superoxide dismustase	Anti-oxidant, anti-inflammatory	*Mycobacterium* sp.
Penicillin acylase	Synthetic antibiotic production	*Penicillium* sp.
Collagenase	To treat burns skin ulcers	*Clostridium histolyticum*
Lipase	Digest lipids	*Aspergillus oryzae*
Streptokinase	Anti-coagulant	*Serratia marcescens*
Urokinase	Anti-coagulant	
Laccase	Detoxifier	*Trametes versicolor*
α-galactosidase	Treatment of α-galactosidase deficiency (Fabry's disease)	
Chitinase	Anti-fungi and anti-bacteria	
Lysozyme	Anti-bacteria	

Garung *et al.,* 2013, Sabu, 2003; Vellard, 2003, Zaidi *et al.,* 2014

Microbial bio-products: from lab-to-industry: Gii project

Research

Engineering

Enzyme discovery
Recombinant system development
[+++ Bio-active cpd
Bio-polymer
Valourised chemicals]

Product formulation (Lab scale)
- Performance
- Stability
- Shelf-life

Process optimization (5-20 L)
- Fermentation
- Downstream proc.

Product formulation
- Performance
- Stability
- Shelf-life

Transition-scale process optimization (200 L)
- Fermentation
- Downstream proc.
- Formulation

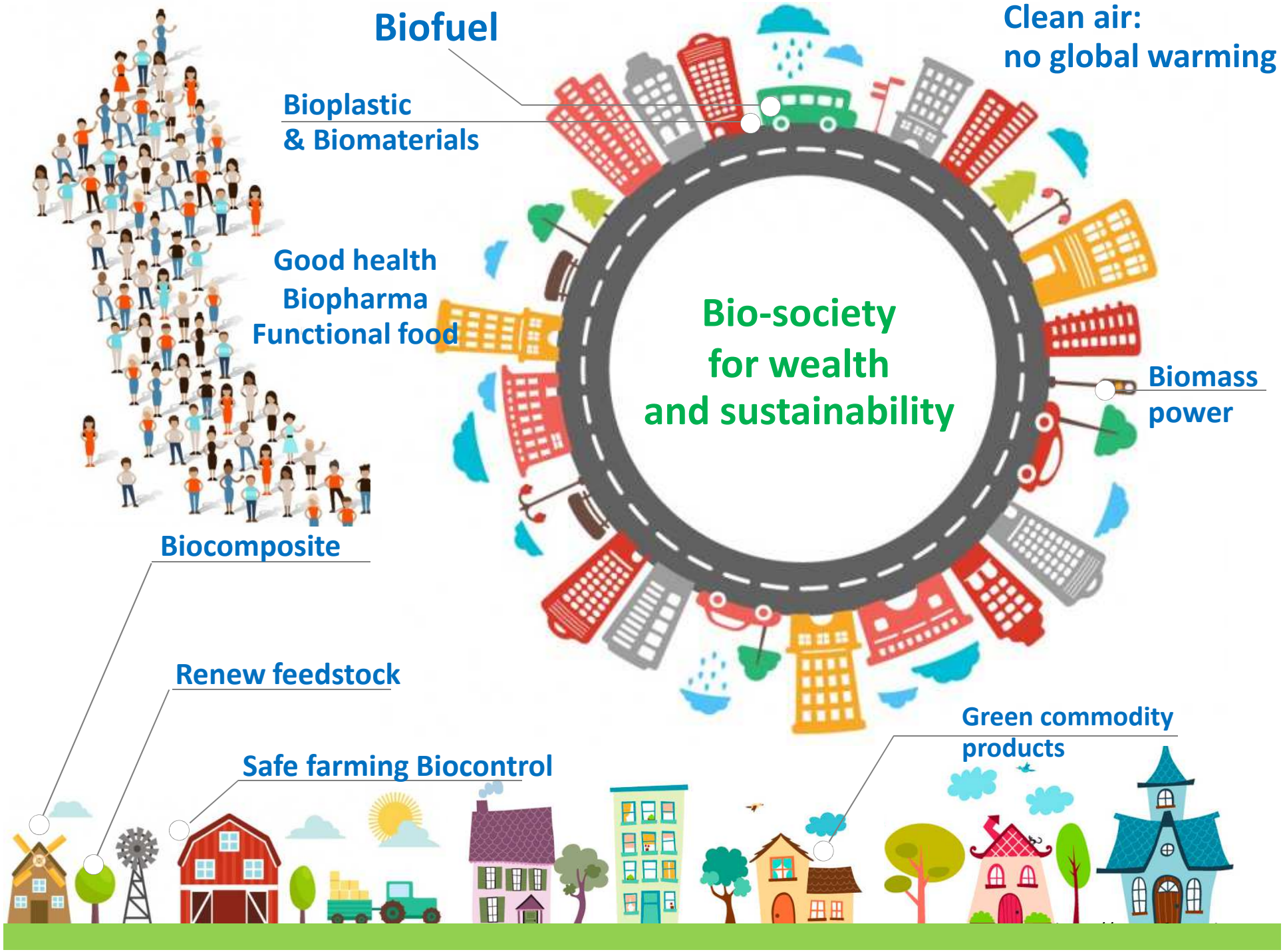
Technical/Economic

Proc. Design & Engineering

Pilot-scale optimization & production (500-5000 L)
- Field/market study

BIOTEC
a member of NSTDA

BIOTEC
a member of NSTDA


Biofuel
Clean air:
no global warming
Bioplastic
& Biomaterials
Good health
Biopharma
Functional food
Bio-society
for wealth
and sustainability
Biomass
power
Biocomposite
Renew feedstock
Safe farming Biocontrol
Green commodity
products