Integrative Biorefinery research@IBL

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

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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.

Fuels: ethanol, biodiesels & BTL

Feed: feed supplement, prebiotic

Chemicals: commodity & specialty

Materials: bioplastics & biomaterials
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

**More cellulosic feedstocks**
- local
- seasonal
- logistics

**More product portfolio**
- fuel
- chemicals
- materials

1978
- **US** corn ethanol
- **Brazil** cane ethanol fuel

1990
- Start of Thai ethanol industry

2000
- Commercial cellulosic biofuel plants
  - DuPont: 30 M G/Y (2014)
- Integrative biorefinery Feedstock & Product variety
- More cellulosic feedstocks

2010
- Thailand’s 1st Demo-scale acid hydrolysis of bagasse for ethanol production (NEDO, Japan)
- More product portfolio
- Demo-scale cellulosic ethanol plant sugar platform: 10,000 L (2014)
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
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)

\[
\text{TAG} + \text{MtOH} \xrightarrow{\text{Lipase/Thermal}} \text{FAME} + \text{Glycerol}
\]

\[
\text{NaOH} \quad \text{H}_2\text{SO}_4
\]

\[
\text{CPO} \quad \text{RPO} \quad \text{Pretreatment & Deacidification} \quad \text{OIL DRIVING} \quad \text{Water} \quad \text{Fatty Acids} \quad \text{Oil Draining} \quad \text{Water} \quad \text{Fatty Acids}
\]

\[
\text{Trans esterific} \quad \text{Esterific} \quad \text{BD} \quad \text{Glycerin}
\]

\[
\text{Glycerol Treatment} \quad \text{Malathion Staining} \quad \text{Methanol Distillation} \quad \text{Water}
\]

\[
\text{Final Flash} \quad \text{Wet Methanol} \quad \text{De-Methanol}
\]

\[
\text{Glycerine Distillation & Methanol} \quad \text{Pharmaceutical Glycerine}
\]
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

Diagram:
- Sugar → Lactic acid → PLA
- Sugar → Succinic acid → PBS
- Bacteria → Succinic acid
- Bacteria → PHA
Biogas

Methane and biohydrogen from anaerobic digestion

Commercialisation of high rate biogas system in farms and industries

• 2,300 biogas plants
• 380 million m³ (equivalent to 188 ktoe)
• Cassava waste water (53%), manure (39%) with potential substrates e.g. municipal wastes, POME, and glycerol

Waste → Hydrolysis → Acido genesis → Aceto genesis → Methano genesis → Heat, Steam, Electricity
IBL is established as a strategic multi-disciplinary R&D center as a 1st 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
Integrative biorefinery laboratory: network

Hydrothermal & Solvothermal pretreatment/ fractionation

Enzymes
- Fungi
- Metagenomes
- Consortia

Biomass

Sugars

Fuel
- Ethanol; BTL

Chemicals
- Building blocks
- Aromatics

Lignin

Materials

Lignin conversion

Catalyst design & process optimisation

Up-scaling

Process design and up-scaling

users

BEC

14

napier Pulp waste

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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

Degradation 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.
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

- **Yeast**: 4,350
- **Bacteria**: 13,070
- **Algae**: 208
- **Fungi**: 35,282

**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

Environmental community

Symbiotic stable consortia

Cultured microorganism

BGC: Bagasse
CRC: Cow rumen
ASC: Pulp AS

Average for GH matches/total genes: 0.64%

Y axis: GH matches/total genes (%)
X axis: Metagenomic samples

Ratio of GH matches/total genes
Genes of BGC = 1.65%
Identification of lignocellulolytic enzymes from biomass degrading consortium BGC metagenome shotgun pyrosequencing

- 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

Hemicellulase
- Xylanase
- Mannanase

Cellulase
- Endoglucanase
- Exoglucanase
- β-Glucosidase

Accessory enzymes & proteins
- Esterase
- Expansin

Metagenome

BIOTEC Culture Collection

Microbial consortia
Biomass-degrading enzyme discovery

Accessory enzyme from BCC

Expansin from bioinformatics

www.biotec.or.th/enzymecatalog
Synergistic lignocellulolytic enzyme system for hydrolysis of local agricultural biomass

Ternary enzyme mixture

Celluclast™: 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™

**Aspergillus aculeatus**

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

Pilot-scale production by SSF & downstream processing

**Enzyme for cassava processing**

- **A. aculeatus**
- DoE (SSF)
- Up-scaling production

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

91.2% Glucose from starch+cellulose
Glc: 716 mg/g
Xyl: 67 mg/g

http://www.thaigoodview.com/node/49942
http://www.sciencephoto.com/media/10618/enlarge

Pilot-scale production by SSF & downstream processing

NSSF  VHG  GLH
Viscosity reduction in very high gravity fermentation

VHG: > 300 g/L solid
High product conc.
Lower processing cost

- BCC-TR38
- DoE
- Up-scaling SSF

Viscosity reduction in VHG
root chip pulp

Thermal-SSF
19.65% (v/v)

Non-thermal-SSF
17.54% (v/v)
Pulp industry for biorefinery

- Pulp
- Commodity chemicals
- Energy
Conclusion & Future prospects

Combined 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.

Future 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

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