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1st International Symposium on Integrated Biorefinery (ISIBio) 2014:

Utilization of Biomass and Biodiversity of Microbes for The Production of Biofuels and Bioproducts

Bogor, September 25th, 2014

Chair of Organizing Committee

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Proceedings

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1st International Symposium on Integrated Biorefinery (ISIBio) 2014

"Utilization of Biomass and Biodiversity of Microbes for The Production of Biofuels and Bioproducts"

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Concurrent Session :

Foreword from the Project Leader of Indonesian Side JST-JICA SATREPS Biorefinery - Innovative Bio-Production Indonesia (iBioI)

On Behalf of Innovative Bio-Production Indonesia (iBioI) member and the International Symposium on Integrated Biorefinery (ISIBio2014) committee we are pleased to hold the 1st ISIBio Symposium on Utilization of Biomass and Biodiversity of Microbes for the Production of Biofuels and Bioproducts. This event is supported by JST-JICA SATREPS-Biorefinery Project with high contribution of *Innovative Bio-Production Kobe* (IBioK), *Integrated Bio-refinery Indonesia* (IBioI), Indonesian Institute of Sciences (LIPI), and Kobe University (Japan).

In order to achieve of the establishment of biorefinery concept in Indonesia, the objective of this symposium are to improve and developing globalization of bio-refinery project for facing the world challenges renewable energy from scientists, research professionals, young researchers, industrial delegates and educational communities. Collaboration of related researchers from different expertise such as microbiology, biology molecular, protein engineering, biochemistry, agriculture, biochemical engineering, fermentation, machine engineering and so on will be created an integrated core-research on bio-process engineering and bio-refinery in Indonesia.

Organizing committee is very grateful and honored to have 12 abstracts from invited speaker and 19 fullpapers from participant. Those are delivered from Research Center for Biotechnology-LIPI, Research Center for Biology-LIPI, University of Indonesia, University of Kebangsaan Malaysia, School of Government and Public Policy-Indonesia, Sepuluh Nopember Institute of Technology, Surabaya, Technical Implementation Unit for Development of Chemical Engineering Processes-LIPI, Andalas University, Syarif Hidayatullah State Islamic University, Banten, Center for Innovation –LIPI.

I would like to give my high appreciation to all institutions that supporting us and every kind person who have contributed and to all organizing committee of the 1st ISIbio 2014. Finally, I would like to wish you all the best toward the success of 1st ISIBio 2014 as a continuously symposium in the coming years.

Cibinong, April 2015

Prof. Dr. Bambang Prasetya
Project Leader of Indonesian Side JST-JICA SATREPS Biorefinery

Perspectives of bio-refinery in Indonesia and Japan

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Abstract—Bioenergy represents the utilization of biomass as starting material for the production of sustainable fuels and chemicals. Environmental concerns and the depletion of oil reserves have resulted in governmental actions and incentives to establish greater energy independence by promoting research on environmentally friendly and sustainable biofuels like bioethanol and biodiesel. Ethanol is the most widely used biofuel either as a fuel or as a gasoline blends. The world ethanol production has reached about 51000 million litres, being USA and Brazil the first producers. The fuel ethanol can be obtained directly from sucrose or from starchy and lignocellulosic biomass. The complexity of the process depends on the type of feedstock. The spectrum of designed and implemented technologies ranging from simple conversion of sugars by fermentation, To the multi-stage conversion of lignocellulosic biomass to ethanol. Among the new research trends in this field, process integration by means of developing yeast strain has the ability to do simultaneous saccharification and fermentation is the key factor for reducing costs in ethanol and other chemicals production. In the recent year, there is considerable research has been carried out on the cell surface engineering of saccharomyces. *Saccharomyces cerevisiae* for the production of bioethanol from various biomass resources.This paper will discuss about the perspectives of a possibility of bio fuel and chemicals production with cell surface engineering yeast and degradation enzymes from lignocellulosic biomass and the enzymatic.

The Potential of Biorefinery Development in West Sumatra

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Abstract—A biorefinery is a facility that integrates biomass conversion processes and equipment to produce value-added products such as fuels, chemicals, feed, pharmaceuticals, materials and energy from biomass. The objective of a biorefinery is to optimize the use of resources and minimize wastes, thereby maximizing benefits and profitability, enhancing the energy efficiency and material recovery. Biorefineries consist of various processing facilities such as digestion, fermentation, pyrolysis, gasification, etc. This work presents a potential of bioenergy development in West Sumatra based on the availability of raw material for feedstocks such oil palm, cacao, gambier, rice, forest waste, etc. Key challenges in developing biorefinery commercialization is also reviewed.

Bioproduction of Compatible Solute Under High Salinity Environment using The Moderate Halophile Halomonas Elongata as a Super Cell Factory

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Abstract—In order to deal with high salinity environments, halophiles have evolved to biosynthesize organic compatible solute such as ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid; ECT) and glycine betaine (N,N,N-trimethylglycine; GB). Industrial interests in the properties of compatible solutes are getting higher because the properties are useful in various applications such as macromolecules protection (cell membranes and enzymes), therapeutic use (cell, skin, and various diseases), toiletry products (cosmetics, shampoo, rinse), feed additives (animals), improved biomass production (plants), increased fermentation yields (microorganisms), etc.

In the moderate halphileHalomonas elongata OUT30018, ECT is *de novo* biosynthesized as a major osmolyte from biomass derivative simple carbon sources such as glucose, xylose, arabinose and glycerol. Another compatible solute,GB, however, is not biosynthesized from simple carbon sources but from environmental choline, while extracellular GB is efficiently taken up into the cells as a compatible osmolyte. Therefore, we aimed to engineer a system for de novo biosynthesis of GB from simple carbon sources via a three-step glycine methylation pathway in *H. elongata*.

First, we generated an ECT-deficient mutant *H. elongata* strain KA1 by disrupting the ECT-biosynthetic ect AB Coperon. Subsequently, the artificial GB-biosynthetic operon containing four genes comprising an mCherry reporter gene, two synthetic codon-optimized methyltransferase genes, HeGSMT and HeDMT, which encode glycine/sarcosinemethyl tarnsferase and dimethyl glycine methyl tranfere from Apha nothecehal ophytica, and agenomic SAM synthetase gene of H. elongata OUT30018, HeSAMS, were introduced at the ECT biosynthetic loci in genome of H. elongata KA1 to generate H. elongata strain KA1.3. As a result, instead of ECT, GB was successfully biosynthesized and the amount of biosynthesized GB was increased when H. elongata KA1.3 was subjected to high salinity stress (8% NaCl). We also found that addition of methionine to the medium further enhanced GB biosynthesis and salt-stress tolerance of H. elongata KA1.3.

This result suggests that biosynthesis of methionine, a precursor of SAM that serves as methyl groups donor for glycine methylation, is a rate-limiting step for the de novo GB biosynthesis in H. elongata KA1.3. De novo GB biosynthesis from biomass-derived simple carbon sources by H. elongata KA1.3 would open the gate for the development of a novel biorefinery process for GB bioproduction. Currently, H. elongatamutator strain was generated as a tool fordirected evolution of the H. elongata strain with improved GB bioproduction.

Conversion A Liquid Waste Biomass for Hydrogen Production

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Abstract—A prototype system for hydrogen gas production from a biological system of facultative fermentation has been applied for electricity power supply. The prototype was designed for application in remote or isolated areas in Indonesia. The fermentation system, which was designed to be as simple as possible, includes the preparation of the microbial seed, the substrate material, the vessel and other required equipment, gas capture and purification, a converter, and transportation. The model experiment in the field undergoes several modifications depending on the biomass sources in the actual location, i.e., some areas have agroforestry, sugarcane, soy sauce and palm sugar wastes. The light intensity and temperature followed the natural conditions. The results indicated that a cultivation scale of 5-25 litres per substrate does not affect the result, i.e., a hydrogen production of approximately 60-70% of the total gas produced. The hydrogen gas produced was converted into electricity sources to power fans and house lamps. However, the hydrogen power is not yet sustainable due to the batch fermentation system, the biomass supply and the local electrical system, which is conventional (not a grid system). We propose to merge the electrical system in those areas, i.e., combining the source of electrical power from wind, solar, biomass, ocean current and fossil fuel-based generators. The model of the electricity pool system is important for Indonesia because, geographically, Indonesia consists of more than seventeen thousand islands, where the electricity supply remains unstable.

The Potential of Biorefinery Development in West Sumatra

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Abstract—A biorefinery is a facility that integrates biomass conversion processes and equipment to produce value-added products such as fuels, chemicals, feed, pharmaceuticals, materials and energy from biomass. The objective of a biorefinery is to optimize the use of resources and minimize wastes, thereby maximizing benefits and profitability, enhancing the energy efficiency and material recovery. Biorefineries consist of various processing facilities such as digestion, fermentation, pyrolysis, gasification, etc. This work presents a potential of bioenergy development in West Sumatra based on the availability of raw material for feedstocks such oil palm, cacao, gambier, rice, forest waste, etc. Key challenges in developing biorefinery commercialization is also reviewed.

I. Introduction

The world has realized that the time had come for the community to develop a sustainable industry that shifts our dependence on petroleum to the utilization of renewable resources. In this case there is no doubt that biomass from agriculture and forestry will play a key role and a foundation for the development of biomass-based economy. Integrated biomass utilization is a key technology in a sustainable society. Agricultural land can no longer be seen merely as a medium for the growth of crops for food and feed. Now it has much bigger roles and multi-functions as a supplier of biomass from energy crops, industrial crops and by-products from the food and feed production. Thus, its functions are not only in terms of land use but also in the case with regard to the full utilization of biomass harvested (Nel, 2009).

Renewable resources can be converted to biomass-based products using physical, chemical, and biological (including enzymes and microorganisms) processes to make products such as the chemicals, pharmaceuticals, food and feed, paper and pulp, textiles, energy. Indonesia has abundant of potential biomass feedstock for biorefinery process. It includes empty fruit buch from oil palm, cocoa pod from the cocoa plantation, straw from rice production, and waste from logging and wood processing industries, baggase, etc. However, this paper limits the discussion on the potential of biomass existing in West Sumatra province.

Biorefinery Concept

A biorefinery is a term used to refer to the manufacturing facility using biomass as feedstock to produce fuels, power, and chemicals. Biomass is any organic matter that are available on a renewable or recurring basis including "dedicated energy crops and trees, agricultural food and feed crop residues, aquatic plants, wood and wood residues, animal wastes, and other waste materials" (Ohara 2003). As it is renewable and abundant, biomass is potential to replace fossil fuels and petrochemicals (Yang and Yu 2013).

Biomass mostly used for biorefinery is generally classified into "four main categories depending on their origin: energy crops, agricultural residues and waste, forestry waste and residues, and industrial and municipal wastes" (de Lasa *et al.,* 2011). The energy crops are, crops that are commonly planted densely and have high-yielding and short rotation. They are low cost crops and usually need low maintenance. These crops are intendedly planted or grown to supply a large number of consistent-quality biomass for biorefinery. Woody energy crops are hardwood trees that grow fast and normally harvested within 5–8 years of plantation. The short rotation woody energy crops are traditionally used for manufacture of paper and pulp. Unlike agriculture crops and perennial grasses, the productivity of woody biomass is little affected by seasonal variations (de Lasa *et al.,* 2011).

Agricultural waste and residues mostly consist of stalks and leaves which are usually not harvested from fields for commercial porposes. The forestry waste and residues are biomass that usually not harvested from logging sites in commercial hardwood and softwood stands. Biomass resulted from forest management operations like thinning of young stands and removal of dead and dying trees are also included in the forestry residues.

Industrial and municipal wastes include municipal solid waste (MSW), sewage sludge and industrial waste. Residential, commercial and institutional postconsumer waste usually contains good amounts of plant which are derived from organic materials which can be used as potential source of biomass. Black liquor, the waste product generated during wood pulping, is an example of industrial waste (de Lasa *et al.*, 2011).

There are many agricultural waste and food processing wastes of the current agricultural and food industries that have little use. They, however, can be converted to higher-value fuels and chemicals. Accordingly, the traditional agricultural processing industry should incorporate the integrated biorefinery concept to minimize the negative impact of biofuel production on food supply while maximizing its revenues.

A reliable supply of feedstock and processing technologies are needed in developing the biomass resource base for industrial sector applications. Now, numerous biorefinery technologies for processing lignocellulosic materials are in the development in many parts of the the world. In order to achieve efficient conversion of the raw material, a mixture of mechanical, biocatalytic and chemical treatments needs to be combined.

There are several advantages for current bio-processing industries to reinvent themselves as biorefineries through the production and marketing of multiple products (Pye, 2005). The advantages includes:

- The enhanced revenue from a single facility that utilize its infrastructure and raw material resource to the maximum possible extent, and the additional revenues from high value co-products that could reduce the selling price of the primary product.
- A lower financial risk, since the profitability of the facility will be less dependent on fluctuations in the selling price of, and market for, a single commodity product.
- A reduction in waste generation and the lower disposal and treatment costs, since more of the raw material is converted to saleable products.
- The opportunity for more efficient operations through greater process integration and utilities optimization.
- A possible degree of operating flexibility through changes in the ratio of the different products that can be made in response to changes in markets and raw materials

The key to successful development of biorefineries will ultimately depend on the progress in three areas (Nel 2009): (i) low energy milling of bio-feedstocks to its components, (ii) efficient bioconversion of mixed sugars to products and (iii) the utilisation of by-products. These improvements will require integration of biomass conversion technologies with all major areas of industrial biotechnology such as novel enzymes and microorganisms, functional genomics, pathway engineering, protein engineering, biomaterial development, bioprocess design, product development and applications (Nel 2009).

A biorefinery is not a completely new concept. Many of the traditional biomass converting technologies such as sugar, starch, and pulp and paper industries use aspects connected with this approach. The combinations that involve raw materials, conversion or technology processes, and final products are almost not limited in the biorefinery concept. The final decision as to what product will be prioritized in a biorefinery will much depend on the availability of raw materials, technological knowledge, public policies, regulations, and market dynamics (Yang and Yu 2013)

Potential Raw material for feedstocks in West Sumatra

Current commercial biorefineries are using traditional sugar- and starch-based feedstocks like corn, soybeans, and sugarcane to produce value-added products for food and feed applications, and fuel ethanol and specialty chemicals (Yang and Yu 2013). The potential raw material for biorefinery feedstock from West Sumatra are discussed in this section.

Cocoa pod

West Sumatra has approximately 137,355 hectares of cocoa plantation with potential production of dry weight cocoa bean between 900 kg/ha/year–2500 kg/ha/year. Cacao is an industrially important crop since cocoa beans and its processed products are the main ingredients of chocolate, one of the world's most popular foods. However, in the production of the cocoa beans, waste in the form of cacao pod is also generated. Every ton of dry cacao beans generates 10 tons of wet cacao pod (Mansur *et al.*, 2014). Proper usage of the husks could provide economic advantages and decrease their environmental impact. Figure 1 illustrates the part of cocoa fruit and chemical composition cocoa pod potential for ethanol and pectin production. Proposed integrated cocoa pod biorefinery for ethanol and pectin production is illustrated in Figure 2.

Empty Fruit Bunch of Oil Palm

West Sumatra has approximately 357,079 hectares of oil palm plantation. Average Productivity of oil palm plantations is 16 tonnes of Fresh Fruit Bunch (FFB) per ha for smallholder plantation, while the potential for using superior seed in intensive farming could reach 30 tons FFB /

ha. Every tonne of FFB generates 250 kg of Empty Fruit Bunch (EFB). Proper usage of the EFB provide economic advantages and decrease their environmental impact.

Rice Straw

West Sumatra has approximately 236,874 hectares rice farming. Table 1 illustrates the potential ethanol production from rice straw in West Sumatra with assuming the production of 6 ton/ha

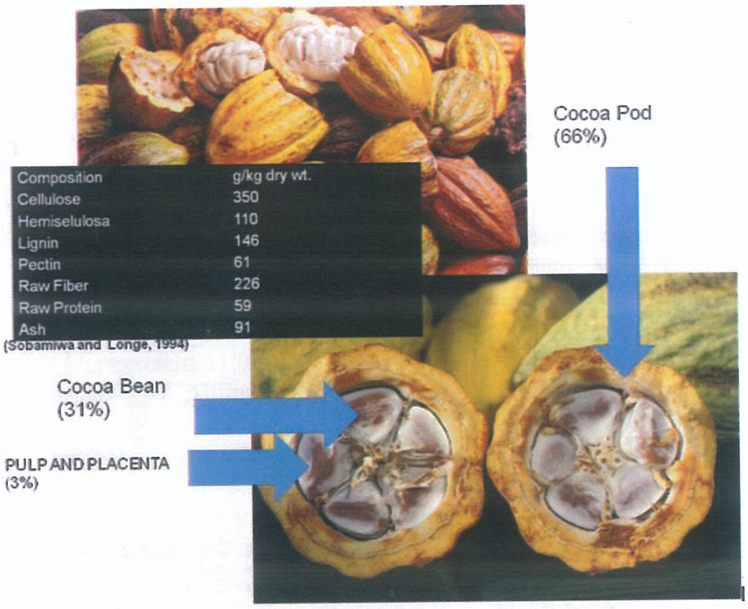

Figure 1 Part of cocoa fruit and chemical composition cocoa pod potential for biorefinery

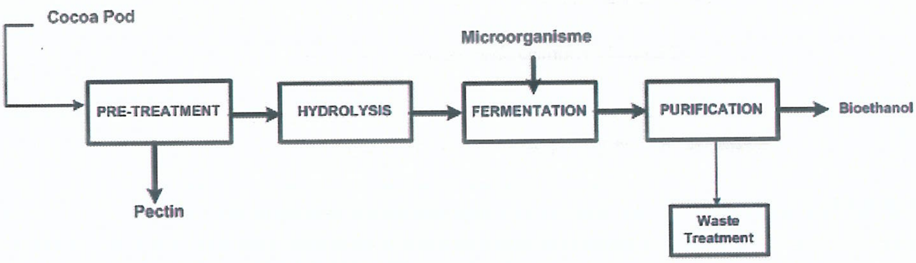

Figure 2. Integrated cocoa pod biorefinery for ethanol and pectin produc

Gambier

Gambir (*Uncaria gambir* Roxb.) is one of the important non timber forest products in West Sumatra. It is traditionally used for numerous purposes such as medicine, batik and leather industry. West Sumatra has approximately 21,412 hectares of gambier production. Gambier processing in West Sumatra begins with the extraction of gambier juice from boiled leaves, leaving behind biomass waste around 7,000 kg/ha/year. This biomass is potential for biorefinery. Figure 3 illustrates the biomass from gambier extraction industry.

Sugarcane

West Sumatra has approximately 7,364 hectares of land in sugarcane production.. Sugarcane processing in West Sumatra begins with the extraction of juice and leaving behind bagasse. This fibrous material is potential lignocellulosic source for producing ethanol. Most of the sugar juice is used to produce brown sugar which is produce by boiling the sugar juice until very thick. It is then left to dry in the molding.

Challenges of biorefinery Development

The diversity of feedstock available.

The physico-chemical properties and chemical compositions and cost of feedstock vary considerably depending on the types, sources and collection logistics. This diversity creates challenges to develop replicable biomass supply systems and specialized conversion technologies to bio-power or bio-fuels for various types of biomass waste.

Collection and transportation logistics problem and cost.

The collection and transportation of biomass from distant field to biorefinery location is costly.

The viability of market.

The integrated biorefinery must optimize the use of biomass to create products matched perfectly with market demands and economically competitive with fossil fuels (Maity 2014).

Sustainability.

Understanding of economic, environmental and social impacts of biorefinery must receive special attention.

Budget al.,located in research and development.

The government, researchers and industries contribute significantly to the development of feedstock and technologies to foster growth of biorefinery. Many of these technologies need on-going and consistent supports to achieve profitable manufacturing processes.

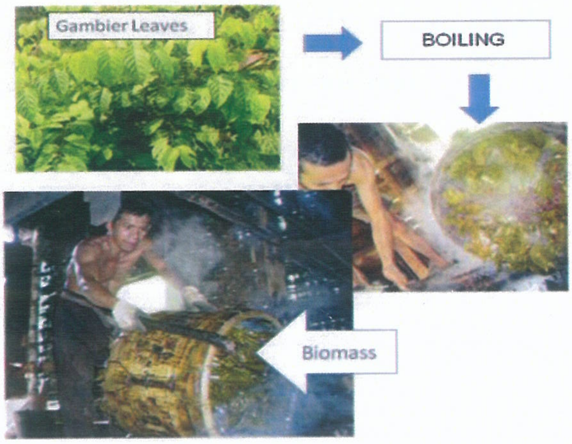

Figure 3. Biomass from Gambier Extraction Industry

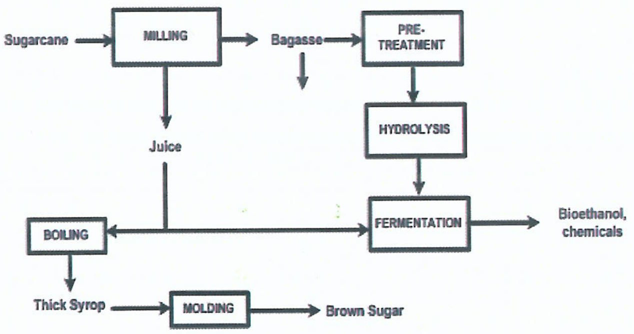

Figure 4. Proposed integrated biorefinery from sugarcane processing industry

II. CONCLUSIONS

West Sumatra has numerous raw materials potential for biorefinery feedstock especially for bioenergy. However, the utilization of the material is still low. Technical challenges (such as variable quality of feedstock, immature technology), commercial (such as high investement cost, expensive production cost) and strategic challenges (such as regulation, consumer acceptance) and also sustainability challenges (Reduction of GHG emission requirement in biorefinery products life cycle) have to be solved and need multidisciplinary studies. Andalas University would be the best host for any collaboration on accelerating biomass utilization in West Sumatra.

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