Japan-ASEAN Science, Technology and Innovation Platform (JASTIP)

Report of JASTIP-Net Activity

Date: 2017 / 11 / 30

1. Research partner	Affiliated Organization		
	Name	Dr. Euis Hermiati	
	Affiliation	Research Center for Biomaterials - LIPI	
	Position	Researcher	
	Address	Jl. Raya Bogor Km 46 Cibinong. Bogor 16911 Indonesia	
2. Collaborative research	Collaborative research theme	**Headquarters** ☐ To Develop Operational Linkages and Human Resources among Academic Sector, Government Agencies, and Private Sectors in ASEAN countries and Japan. ☐ To Introduce Various STI Collaborations for Effectively and Efficiently into the Society based on the three joint laboratories' activities. **Energy & Environment Joint Lab** ☐ Studies on Rural/Community Renewable Energy. ☐ Development of Renewable Energy Technology adapted to the ASEAN region. ☐ Studies on Energy Policy/Security in the ASEAN region.	

		Bioresources & Biodiversity Lab ☐ Studies on Biodiversity in the ASEAN Region Contributing to the Improvement of Identification, Collection and/or Information. ☐ Sustainable Utilization of Bioresources for Biorefinery, Bioremediation, Wood Construction, Food or Medicine. ☐ Plant Improvement for Agroforestry Systems and Carbon Sequestration Contributing to the Mitigation of and/or Adaptation to Climate Change. **Disaster Prevention Joint Lab** ☐ Innovative Ideas on Disaster Prevention, Mitigation and Recovery Technologies and Policies Peculiar to Each ASEAN Country. ☐ How to Cope with Trans-Boundary Disasters in the ASEAN Region Such as Tsunami, Flood, Drought and Haze. ☐ Understanding and Quantitative Evaluation of Disaster Risks Peculiar to ASEAN Countries.
	Collaborative research title	Development of integrated process for conversion of sugarcane trash to bioethanol and value-added chemicals
	Host core-researcher	Prof. Dr. Takashi Watanabe

3. Members

Name	Euis Hermiati		
Affiliation	Research Center for Biomaterials - LIPI	Position	Researcher
Name	Widya Fatriasari		
Affiliation		Position	Researcher
Name	Triyani Fajriutami		
Affiliation	Research Center for Biomaterials - LIPI	Position	Researcher
Name	Sita Heris Anita		
Affiliation	Research Center for Biomaterials - LIPI	Position	Researcher
Name	Muhammad Ghozali		
Affiliation	Research Center for Chemistry - LIPI	Position	Researcher
Name	Raden Permana Budi Laksana		
Affiliation	Research Center for Biomaterials - LIPI	Position	Researcher Assistant

Name	Verawat Champreda		
Affiliation	National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA)	Position	Principal researcher/ Lab head Enzyme Technology Laboratory

Name	Pattanop Kanokratana		
Affiliation	National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA)	Position	Researcher/ Enzyme Technology Laboratory
Name	Pornkamol Unrean		
Affiliation	National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA)	Position	Senior researcher/ Enzyme Technology Laboratory
Name	Benjarat Bunterngsook		
Affiliation	National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA)	Position	Postdoctoral researcher/ Enzyme Technology Laboratory
Name	Aphisit Poonsrisawat		
Affiliation	National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA)	Position	Researcher assistant/ Enzyme Technology Laboratory

Name	Takashi Watanabe		
Affiliation	Research Institute for Sustainable Humanosphere	Position	Professor, Dr.
Name	Hiroshi Nishimura		
Affiliation	Research Institute for Sustainable Humanosphere	Position	Assist. Professor, Dr.
Name	Satoshi Oshiro		

Affiliation	Research Institute for Sustainable Humanosphere	Position	Researcher, Dr.
Name	Masato Katahira		
Affiliation	Institute of Advanced Energy, Kyoto University	Position	Professor, Dr.
Name	Takashi Nagata		
Affiliation	Institute of Advanced Energy, Kyoto University	Position	Assoc. Professor, Dr.
Name	Keiko Kondo		
Affiliation	Institute of Advanced Energy, Kyoto University	Position	Researcher, Dr.
Name	Hideaki Ohgaki		
Affiliation	Institute of Advanced Energy, Kyoto University	Position	Professor, Dr.

4. Report of activities

4.1. Research activities and major findings

4.1.1. Prtetreatment of sugarcane trash using microwave hetaing and dilute acids

The objective of this study was to evaluate the effects of severeness during pretreatment on sugars obtained in the soluble fraction and in the hydrolysate after enzymatic hydrolysis. The organic acids used were maleic and oxalic acids of different concentrations, but they were adjusted to certain pH values (1, 1.5 and 2). Phosphoric acid was also used for comparison. All pretreatments were conducted at 180 °C for 5 mins. Functional groups, crystallinity index, and morphological properties of untretaed and pretreated biomass were also analyzed. Results showed that sugarcane trash exposed to the same severeness (combined severity factor of 2.05, 1.55 or 1.05) during pretreatment resulted in similar pulp recovery (67.78-64.66% at pH 1, 93.18-97.62 at pH 1.5, and 96.60-100 at pH2). However, different acids of the same pH resulted in different sugars and inhibitors concentrations in the soluble fractions with the highest concentration produced in samples pretreated by maleic acid. The sugar yields obtained after enzymatic hydrolysis of those biomass also showed that different acids of the same pH produced different sugar yields. For example, at pH 1 maleic acid pretreatment resulted in relatively higher reducing sugar yield (37.38% per initial biomass) than did oxalic and phosphoric acids pretreatments (31.83 and 26.71% per initial biomass, respectively). It can be concluded that microwave-assisted pretreatment of sugarcane trash with acids of the same value of combined severity factor (CSF) gave the different effects on the characteristics of soluble fraction and enzyme susceptibility of pretreated sugarcane trash.

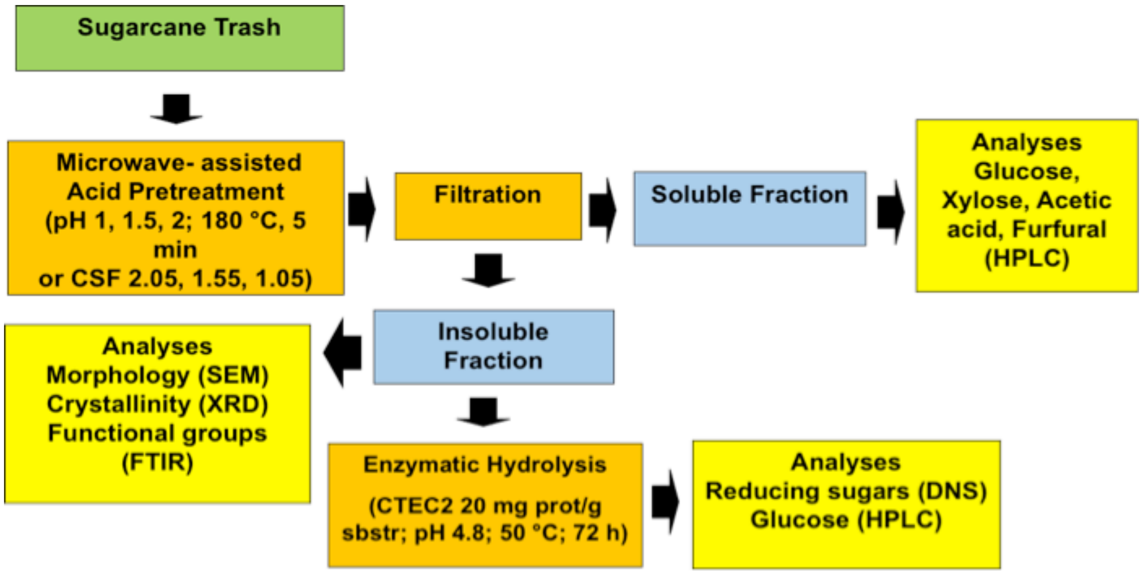

Figure 1. Microwave-assisted acid pretreatment of sugarcane trash

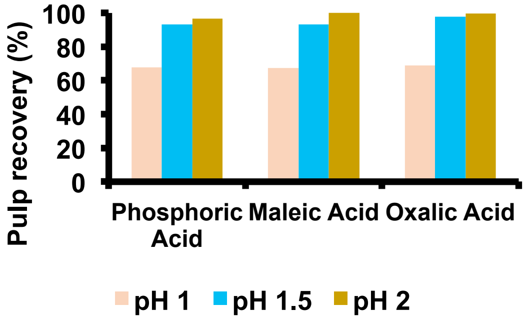

Figure 2. Pulp recovery of sugarcane trash after microwave and acid pretreatment

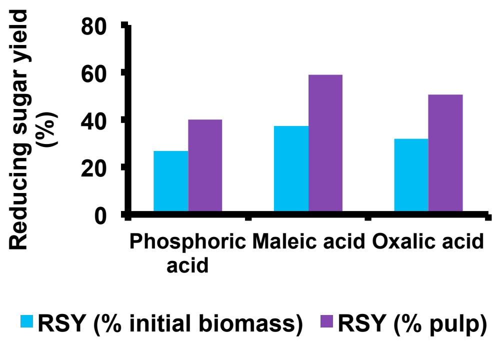

Figure 3. Reducing sugar yield after enzyamatic saccharification of sugarcane trash pretreated by microwave and acids of pH 1

At BIOTEC, we also explore the potential on pretreatment by batch glycerolysis in Parr reactor (GLY).

The biomass was pretreated at 150 and 170 °C in aqueous-glycerol in the presence of 0.3 M oxalic acid with varying glycerol concentration. The highest cellulose digestibility was achieved at 170 °C, which led to the glucose yield of 636 mg/g pretreated biomass. This corresponded to the highest lignin recovery of 90.3%. The glucose and lignin obtained can be further converted to value-added products either by fermentation (i.e. glucose to ethanol) and catalytic conversion (i.e. lignin to phenolics).

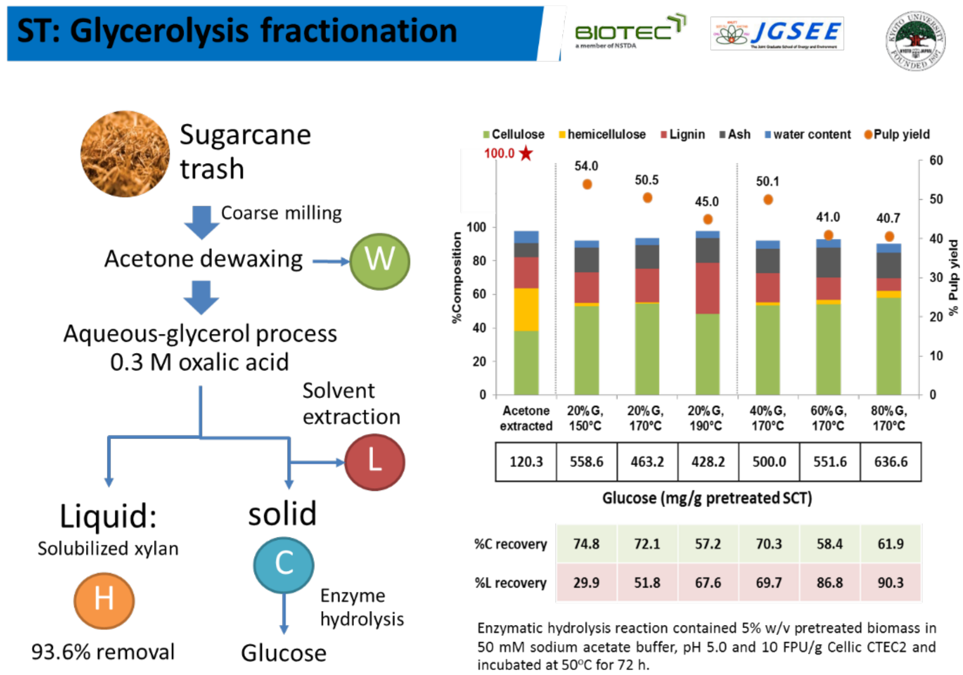

Figure 4. Pretreatment of sugarcane trash by batch glycerolysis

4.1.2. Development of an effective enzyme system for saccharifying the pretreated cellulosic fraction

Lytic polysaccharide monooxygenases (LPMOs) are auxiliary enzymes catalyzing oxidative cleavages of cellulose chains in crystalline region, resulting in increasing accessibility of the cellulosic substrates to the hydrolytic enzyme counterparts. In this study, a novel auxiliary protein family 9 lytic polysaccharide monooxygenase (BgAA9) has been identified from metagenomic library derived from a thermotolerant microbial community in bagasse collection site. The enzyme showed closed similarity (94%) to a family 61 glycoside hydrolase from *Chaetomium thermophilum*. The recombinant BgAA9 expressed in *Pichia pastoris* cleaved cellohexaose (DP6) into shorter cellooligosaccharides (DP2, DP3 and DP4). Supplementation of 1-5 mg/g BgAA9 to a commercial cellulase, Accellerase® 1500 showed strong additive effect on saccharification of celluloses (Avicel® PH101, decrystallized cellulose and filter paper) and alkaline-pretreated sugarcane bagasse, resulting in 1.6-2.8 folds and 0.4-0.5 folds increase in the total reducing sugar yield, respectively,after incubation at 50°C for 72 h. The highest total fermentable sugar yield was obtained from 75%:25% of Accellerase®1500:BgAA9 (555.98 mg/g) equivalent to 9.8% higher than individual Accellerase®1500 (506.24 mg/g). The enzyme is a potent auxiliary component for enhancing efficiency on saccharification of agricultural wastes in biorefinery.

Moreover, BgAA9 exhibited strong synergistic effect with Accellerase® 1500 on saccharification of wet sugarcane trash pretreated by microwave heating supplemented with diluted oxalic acid developed by LIPI, resulted in 53% increasing of total glucose and xylose yield when supplementation of 5 mg/g biomass of BgAA9. However, minor synergistic effect was found on wet sugarcane trash pretreated by microwave heating supplemented with diluted maleic acid and phosphoric acid. This could be due to different chemical compositions and structures of the biomass pretreated by different methods.

However, when compare the synergistic effect of BgAA9 on dried sugarcane trash pretreated by 3 different methods: glycerolysis, steam explosion and microwave heating with maleic acid (highest saccharification yield comparing to oxalic acid and phosphoric acid), BgAA9 showed additive effect on dried sugarcane trash pretreated by microwave heating with diluted maleic acid with 15% increasing of total glucose and xylose yield at 5 mg/g biomass BgAA9 loading. Unfortunately, no significant additive effect was found from sugarcane trash pretreated by glycerolysis and steam explosion.

 Visit RISH, Kyoto University	 Workshop at Kyoto University	 JASTIP Annual Meeting, Bangkok
 Visit BIOTEC - NSTDA	 Visit BIOTEC - NSTDA	 International Symposium for Sustainable Humanosphere, Bogor

4.2. Academic and social implications towards achieving SDGs

The research aimed to develop the use of sugarcane trash, a potential bioresource, for producing bioethanol, a kind of renewable energy. Some technology innovations related to pretreatment and enzymatic hydrolysis of this potent biomass have been studied. The reserach is a collaboration among ASEAN countries, Indonesia and Thailand, which have abundant with tropical bioresources, with Japan, a country with leading technology in the conversion of lignocelullosic biomass. This work provides a platform forincrease capacity building of human resources in the the three countries, especially in the development of biofuels from lignocellulosic biomass. This project also strengthens the collaboration in science and technology between Japan and other ASEAN countries beside Indonesia and Thailand in the future. Therefore, the research contributes towards achieving key targets of SDGs with potential for future collaboration with extended network.

- Affordable and clean energy
- Sustainable cities and communities
- Climate action
- Life on land
- Partneship for the goals

5. List of publications

6. List of oral presentations

Hermiati E, Fatriasari W, Fajriutami T, Anita SH, Ghozali M, Laksana RPB, Champreda V, Kanokratana P, Unrean P, Bunterngsook B, Poonsrisawat, A, Watanabe T, Nishimura H, Oshiro S, Katahira M, Nagata T, Kondo K, Ohgaki H. Development of integrated process for conversion of sugarcane trash to bioethanol and value-added chemicals. The 2nd JASTIP Bioresources and Biodeversity Lab Workshop and Humaosphere Asia Research Node Workshop, Kyoto, Japan, 23 January 2017.

Hermiati E, Fatriasari W, Fajriutami T, Anita SH, Ghozali M, Laksana RPB, Champreda V, Kanokratana P, Unrean P, Bunterngsook B, Poonsrisawat, A, Watanabe T, Nishimura H, Oshiro S, Katahira M, Nagata T, Kondo K, Ohgaki H. Development of integrated process for conversion of sugarcane trash to bioethanol and value-added chemicals. JASTIP 3rd Symposium, Bangkok, Thailand, 5 February 2017.

Bunterngsook B, Kanokratana P, Oshiro S, Watanabe T, Eurwilaichitr L, and Champreda V.

Identification and characterization of lytic polysaccharide monooxygenase (AA9) from bagasse metagenome The 7th International Symposium for Sustainable Humanosphere, Bogor, Indonesia, 1-2 November 2017.

Kanokratana P and Champreda V. Integrated utilization of sugarcane waste for biofuel and chemical production. 3rd JASTIP Bioresources and Biodiversity Workshop Bogor, Indonesia, 1-2 November 2017-11-28

Bunterngsook B, Kanokrattana P, Watanabe T, Oshiro T, Eurwilaichitr L, Champreda V. Identification and characterization of lytic polysaccharide monooxygenase (AA9) from bagasse metagenome (manuscript in prep)