UPM
UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

ENVIRONMENTAL BIOTECHNOLOGY RESEARCH GROUP

RESEARCH REPORT 2012

EB GROUP

Environmental Biotechnology Research Group (EB Group), Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia was officially started in 2005. Currently, EB Group focuses on four research themes which are, Biocompost, Biomaterial, Bioproduct, and Bioenergy. There are 5 principal researchers with 1 Post-doctoral fellow, 15 PhD students, 25 Masters students and 5 Research Assistants. We aim to be a high performance research group conducting research on oil palm biomass and other renewable raw materials in Malaysia into valuable green products with our tagline "EB for 3P" (Environmental Biotechnology - for Profit, People and Planet). We conduct collaborative research in close collaboration with other academic institutions and industries locally and internationally, such as FELDA, SIRIM, Kyutech (Japan), AIST (Japan) and CES (Korea) ■

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RESEARCH REPORT 2012 - ENVIRONMENTAL BIOTECHNOLOGY RESEARCH GROUP

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Students

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MESSAGE FROM THE EB GROUP LEADER

PROFESSOR DR. MOHD ALI HASSAN

AlhamduLillah, praise to ALLAH for His generous favours and blessings for us all. I am very happy that over the years, our Environmental Biotechnology Research Group (EB Group) has gone from strength to strength. I am glad to share with you our research report for 2012. We use renewable resources, particularly biomass from the oil palm industry, and conduct research in the areas of bioenergy, bioproduct, biomaterials and biocompost. We continue to collaborate with Kyushu Institute of Technology (Kyutech), FELDA Palm Industries Sdn. Bhd., Advanced Institute of Science and Technology (AIST) Japan, Malaysian Technology Development Corporation (MTDC), Yayasan Pelajaran Johor and Ajinomoto Corporation Japan. In addition, we have extended our cooperation with Ministry of Housing and Local Government – National Solid Waste Management Division, Subang Jaya Municipal Council (MPSJ), Malaysian Agricultural Research and Development Institute (MARDI), Ministry of Agriculture, Forestry and Fisheries (MAFF) Japan, and recently with CES Company, Incheon Korea. We have 5 academic staff, with 1 Post-doctoral fellow, 18 PhD students, 20 Masters students, 5 Research Assistants and 15 undergraduate students, both at the Faculty of Biotechnology and Biomolecular Sciences, Institute of Bioscience and Faculty of Engineering UPM. Our PhD students are also involved in the split PhD program with Kyushu Institute of Technology. Currently, we have more than RM3,000,000 in R&D grants, not including matching grants from Japan and Korea. In terms of output, we successfully published 28 research papers in 2012, with a total of more than 47 Impact Factors. We also filed 2 patents.

I appreciate the hard work from all EB members in maintaining our high-performance culture. May ALLAH give us the strength to continue the good work and contribute to the university, the ummah and the nation.

God bless. Wassalam.

Professor Dr. Mohd Ali Hassan

EB GROUP
BIG PICTURE

Anaerobic Treatment

WATER

FRESH FRUIT BUNCH

MILL

Palm Oil Mill Effluent (POME)

- Empty Fruit Bunch
- Mesocarp Fiber
- Oil Palm Frond

Crude Palm Oil

Palm Kernel Oil

Kernel

Cellulose

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Sludge

Carbonization

Activated Carbon

Lignin

Organic Acid

Biosugar

BIOCOMPOST

BIOCHAR

BIOABSORBENT

BIOFUEL

BIOCOMPOSITE

BIOVANILLIN

BIOPLASTIC

BIOETHANOL

BIOBUTANOL

BIOHYDROGEN

SERDANG BIOMASS

INPUT

Used cooking oil

Empty fruit bunch

Lanscaping waste

Food waste

Sludge

UPM
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Kyutech

TOWN BIG PICTURE

OUTPUT

- BIOCOMPOST
- BIOCHAR
- BIODIESEL
- BIOGAS

Prof. Dr. Mohd Ali Hassan

Specialisation:

Bioprocess Engineering and Environmental Biotechnology.

Current research interest:

Treatment and utilization of biomass for the production of bio-based products, bioremediation and reduction of greenhouse gases.

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Academic Qualification:

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3. MSc (Food Engineering), University of Leeds, U.K. (1982)
4. BSc (Honours)(Chemical Engineering), University of Leeds, U.K. (1980)
5. 'A' Levels (Math., Chem., Physics), Oxford College Further Edu., U.K. (1977)
6. Post-graduate Diploma (Islamic Studies), University Kebangsaan Malaysia (1985)

Selected Publications :

Mohd Ali Hassan, Lian-Ngit Yee, Phang Lai Yee, Hidayah Ariffin, Abdul Rahim Raha, Yoshihito Shirai, Kumar Sudesh. 2012. Sustainable Production of polyhydroxyalkanoates from renewable oil palm biomass. *Biomass & Bioenergy*. 50,1-9.

Mitra Mohammadi, Mohd Ali Hassan, Yoshihito Shirai, Hasfalina Che Man, Hidayah Ariffin, Lian-Ngit Yee, Tabassum Mumtaz, Mei-Ling Chong, Lai-Yee Phang.Separation and purification of polyhydroxyalkanoates from newly isolated *Comamonas* sp. EB172 by simple digestion with sodium hydroxide. *Separation Science and Technology.* Volume 47, 534-541 pp.

Mior Ahmad Khushairi Mohd Zahari, Mohd Rafein Zakaria, Hidayah Ariffin, Mohd Noriznan Mokhtar, Jailani Salihon, Yoshihito Shirai and Mohd Ali Hassan. Renewable sugars from oil palm frond juice as an alternative novel feedstock for value added products. 2012. *Bioresource Technology.* Volume 110, 566-571 pp.

Mitra Mohammadi, Mohd Ali Hassan, Phang Lai Yee, Hidayah Ariffin, Yoshihito Shirai, Yoshito Ando. 2012. Recovery and purification of intracellular polyhydroxyalkanoates from recombinant *Cupriavidus necator* using water and ethanol. *Biotechnology Letters*, 34 (2), pp. 253-259.

Lian-Ngit Yee, Jo-Ann Chuah, Mei-Ling Chong, Lai-Yee Phang, Abdul Rahim Raha, Kumar Sudesh, and Mohd Ali Hassan. 2012. Molecular characterisation of phaCAB from *Comamonas* sp. EB172 for functional expression in *Escherichia coli* JM109. *Microbiological Research*. 167(9):550-7

Saleha Shamsudin, Umi Kalsom Md Shah, Huzairi Zainudin, Suraini Abd-Aziz, Siti Mazlina Mustapa Kamal, Yoshihito Shirai, Mohd Ali Hassan. Effect of steam pretreatment on oil palm empty fruit bunch for the production of sugars. (2012) *Biomass and Bioenergy*, 36, pp. 280-288.

Mitra Mohammadi, Mohd Ali Hassan, Lai-Yee Phang, Yoshihito Shirai, Hasfalina Che Man, Hidayah Ariffin, Amirul Al-Ashraf, Siti NorSyairah. (2012). Efficient polyhydroxyalkanoate recovery from recombinant *Cupriavidus necator* by using low concentration of NaOH. *Environmental Engineering Science*. 29(8): 783-789.

Norjan Yusof, Mohd Ali Hassan, Phang Lai Yee, Meisam Tabatabaei, Mohd Ridzuan Othman, Minato Wakisaka, Yoshihito Shirai. 2011. Nitrification of high-strength ammonium landfill leachate with microbial community analysis using fluorescence in situ hybridization (FISH). *Waste Management and Research,* Vol. 29 (6), 602-611 pp.

Meisam Tabatabaei, Raha Abdul Rahim, Norhani Abdullah, André-Denis G. Wright, Yoshihito Shirai, Kenji Sakai, Alawi Sulaiman and Mohd Ali Hassan. 2010. Importance of the methanogenicarchaea populations in anaerobic wastewater treatments. *Process Biochemistry,* Vol. 45 (8), 1214-1225 pp.

Yung-Hun Yang, Christopher J. Brigham, Charles F. Budde, Paolo Boccazzi, Laura B. Willis, Mohd Ali Hassan, Zainal Abidin Mohd Yusof, ChoKyun Rha and Anthony J. Sinskey. 2010. Optimization of growth media components for polyhydroxyalkanoate (PHA) production from organic acids by *Ralstonia eutropha*. *Applied Microbiology and Biotechnology*, Vol. 87 (6), 2037-2045 pp.

Patent (2012) :

Polyhydroxyalkanoate recovery. Mohd Ali Hassan, Mitra Mohammadi, Phang Lai Yee, Hidayah Ariffin, Yee Lian Ngit, Yoshihito Shirai. 2012. PI 2012700848. Filed.

Selected Publications :

Mohd Huzairi Mohd Zainudin , Nor'Aini Abdul Rahman, Suraini Abd-Aziz, Masamitsu Funaoka, Takanori Shinano, Yoshihito Shirai, Minato Wakisaka and Mohd Ali Hassan. (2012). Utilization of glucose recovered by phase separation system from acid-hydrolysed oil palm empty fruit bunch for bioethanol production. *Pertanika Journal of Tropical Agricultural Science*.35 (1): 117 – 126.

Ezyana Kamal Bahrin, Mohamad Faizal Ibrahim, Mohamad Nafis Abdul Razak, Suraini Abd-Aziz, Umi Kalsom Md. Shah, Noorjahan Alitheen and Madihah Md Salleh. (2012). Improved cellulase production by *Botryosphaeria rhodina* from OPEFB at low level of moisture condition through statistical optimization. *Preparative Biochemistry & Biotechnology.* 42 (2): 155 – 170.

Nurul Asyifah Mustapha, Suraini Abd-Aziz, Phang Lai Yee and Azlian Mohamad Nazri. (2012). Nutrient composition, non-starch polysaccharides and true metabolisable energy value of brown rice as poultry feedstuff. *Journal of Applied Poultry Research.* 21:103-110.

Mohamad Faizal Ibrahim, Suraini Abd-Aziz, Mohamad Nafis Abdul Razak, Phang Lai Yee and Mohd Ali Hassan. (2012). Oil Palm Empty Fruit Bunch as Alternative Substrate for Acetone-Butanol-Ethanol Production by *Clostridium butyricum* EB6. *Applied Biochemistry & Biotechnology*. 166:1615–1625.

Siren Linggang, Phang Lai Yee, Helmi Wasoh and Suraini Abd-Aziz. (2012). Sago Pith Residue as Alternative Cheap Substrate for Fermentable Sugars Production. *Applied Biochemistry & Biotechnology.* 167:122–131.

Mohamad Nafis Abdul Razak, Mohamad Faizal Ibrahim, Phang Lai Yee, Mohd Ali Hassan and Suraini Abd-Aziz. (2012). Utilization of oil palm decanter cake for cellulase and polyoses production. *Biotechnology and Bioprocess Engineering.* 17: 547-555.

Nurul Kartini Abu Bakar, Zuraidah Zanirun, Suraini Abd-Aziz, Farinazleen Mohd Ghazali and Mohd Ali Hassan. (2012). Production of fermentable sugars using oil palm empty fruit bunch using crude cellulase cocktails from *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2. *BioResources.* 7(3), 3627-3639.

Ezyana Kamal Bahrin, Piong Yeau Seng and Suraini Abd-Aziz. (2011). Effect of oil palm empty fruit bunch (OPEFB) particle size on cellulase production by *Botryosphaeria* sp. in solid state fermentation. *Australian Journal of Basic and Applied Sciences.* 5(3): 276-280.

Norhayati Ramli, Suraini Abd-Aziz, Mohd Ali Hassan, Noorjahan Alitheen, Kamarulzaman Kamaruddin and Zoolhilmi Ibrahim. (2011). Molecular cloning and extracellular expression of cyclodextrin glycosyltransferase gene from *Bacillus* sp. NR5 UPM. *African Journal of Microbiology Research.* 5(21): 3475-3482.

Nurul Kartini Abu Bakar, Suraini Abd-Aziz, Mohd Ali Hassan and Farinazleen Mohd Ghazali. (2010) Isolation and Selection of Appropriate Cellulolytic Mixed Microbial Cultures for Cellulases Production from Oil Palm Empty Fruit Bunch. *Biotechnology*. 9(1): 73 – 78.

Prof. Dr. Suraini Abd. Aziz

Specialisation:

Biochemical Engineering and Enzyme Technology

Current Research Interest:

Utilization of lignocellulosic biomass for bioenergy and bioproduct

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Dr. Hidayah Ariffin

Specialisation:

Bioprocess Engineering and Environmental Biotechnology.

Current research Interest :

Utilization of oil palm biomass for the production of bio-based chemicals, biopolymers and biocomposites; Recovery and chemical recycling of biopolymers.

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Academic Qualification :

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3. BEng (Process and Food) Universiti Putra Malaysia (2004)

Selected Publications :

Mitra Mohammadi, Mohd Ali Hassan, Lai-Yee Phang, Yoshihito Shirai, Hasfalina Che Man, Hidayah Ariffin. 2012. Intracellular polyhydroxyalkanoates recovery by cleaner halogen-free methods towards zero emission in the palm oil mill. *Journal of Cleaner Production*. 37 : 353-360pp.

Mior Ahmad Khushairi Mohd Zahari, Hidayah Ariffin, Mohd Noriznan Mokhtar, Jailani Salihon, Yoshihito Shirai and Mohd Ali Hassan. 2012. Factors affecting poly(3-hydroxybutyrate) production from oil palm frond juice by *Cupriavidus necator* (CCUG52238T). *Journal of Biomedicine and Biotechnology*. Volume 2012, Article ID 125865, 8 pages.

Mior Ahmad Khushairi Mohd Zahari, Mohd Rafein Zakaria, Hidayah Ariffin, Mohd Noriznan Mokhtar, Jailani Salihon, Yoshihito Shirai and Mohd Ali Hassan. 2012. Renewable Sugars from Oil Palm Frond Juice as an Alternative Novel Fermentation Feedstock for Value-Added Products. *Bioresource Technology*. Volume 110, 566-571 pp.

Hidayah Ariffin, Haruo Nishida, Yoshihito Shirai and Mohd Ali Hassan. 2010. Highly selective transformation of poly[(R)-3-hydroxybutyric acid] into trans-crotonic acid by catalytic thermal degradation. *Polymer Degradation and Stability*. Vol. 95 (8), 1375-1381pp.

Hidayah Ariffin, Haruo Nishida, Mohd Ali Hassan and Yoshihito Shirai. 2010. Chemical recycling of polyhydroxyalkanoates as a method towards sustainable development. *Biotechnology Journal*. Vol. 5, 484-492pp.

Haruo Nishida, Hidayah Ariffin, Yoshihito Shirai and Mohd Ali Hassan. 2010. Precise Depolymerization of Poly(3-hydroxybutyrate) by Pyrolysis. *In: Biopolymers*. Ed. Magdy M. Elnashar. 369-386 pp.

Hidayah Ariffin, Haruo Nishida, Yoshihito Shirai and Mohd Ali Hassan. 2009. Anhydride Production as an Additional Mechanism of Poly (3-hydroxybutyrate) Pyrolysis. *Journal of Applied Polymer Science*, Vol. 111, 323-328 pp.

Hidayah Ariffin, Haruo Nishida, Mohd Ali Hassan and Yoshihito Shirai. 2009. Chemical recycling of polyhydroxyalkanoates as a method towards sustainable development. *Journal of Bioscience and Bioengineering*, Vol. 108, s79p.

Hidayah Ariffin, Haruo Nishida, Yoshihito Shirai and Mohd Ali Hassan. 2008. Determination of Multiple Thermal Degradation Mechanisms of Poly(3-hydroxybutyrate). *Polymer Degradation and Stability*, Vol. 93, 1433-1439 pp.

Hidayah Ariffin, Mohd Ali Hassan, Umi Kalsom Md Shah, Norhafizah Abdullah, Farinazleen Mohd Ghazali and Yoshihito Shirai. 2008. Production of bacterial endoglucanase from pretreated oil palm empty fruit bunch by *Bacillus pumilus* EB3. *Journal of Bioscience and Bioengineering*, Vol. 106 (3), 231-236 pp.

Seminar presented (2012) :

15th International Biotechnology Symposium (IBS 2012), Daegu, South Korea. Efficient utilization of oil palm frond for green chemicals and 1 biobased products. Oral presentation.

Selected Publications :

Yee Lian Ngit , Tabassum Mumtaz, Mitra Mohammadi, Phang Lai Yee, Yoshito Ando, Raha Abdul Rahim, Kumar Sudesh, Mohd Ali Hassan, Hidayah Ariffin and Mohd Rafein Zakaria. (2012) Polyhydroxyalkanoate synthesis by recombinant *Escherichia coli* JM109 expressing PHA biosynthesis genes from *Comamonas* sp. EB172. *J Microbial and Biochemical Technol*. 4: 103-110

Mohamad Firwance Basri, Shahrakbah Yacob, Mohd Ali Hassan, Yoshihito Shirai, Minato Wakisaka, Mohd Rafein Zakaria and Phang Lai Yee. (2010) Improved Biogas Production from Palm Oil Mill Effluent by a Scaled-down Anaerobic Treatment Process. *World J Microbiol Biotechnol* 26: 505-514

Mohd Rafein Zakaria, Meisam Tabatabaei, Farinazleen Mohamad Ghazali, Suraini Abd-Aziz, Yoshihito Shirai, Mohd Ali Hassan. (2010) Polyhydroxyalkanoate production from anaerobically treated palm oil mill effluent by new bacterial strain *Comamonas* sp. EB172. *World J Microbiol Biotechnol*. 26: 767-774.

Mohd Rafein Zakaria, Hidayah Ariffin, Noor Azman Mohd Johar, Suraini Abd_Aziz, Haruo Nishida, Yoshihito Shirai, Mohd Ali Hassan. (2010) Biosynthesis and characterization of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer from wild type *Comamonas* sp. EB172. *Polym Degrad Stab*. 95: 1382-1386

Meisam Tabatabaei, Mohd Rafein Zakaria, Raha Abdul Rahim, André-Denis G. Wright, Yoshihito Shirai, Norhani Abdullah, Mehdi Shamsara, Kenji Sakai, Mohd Ali Hassan. (2012) Comparative Study of Methods for Extraction and Purification of Environmental DNA from Wastewater Sludge. *Afr J Biotechnol*. 31:4926-4937

Dr. Mohd Rafein Zakaria

Specialisation :

Environmental Biotechnology

Current research Interest:

Bioconversion of biomass into value-added products, Study of *Pseudomonads*: A key microorganism for wide range of product formation and application

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Research Assistant

2012

UNIVERSITI PUTRA MALAYSIA
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Nurul Asyifah Mustapha

Highest qualification with EB Group:
Master Science of Environmental Biotechnology, UPM, Malaysia

Former Supervisor:
Prof. Dr. Suraini Abd. Aziz

Current Position:
Research Assistant

Dr. Ahmad Amiruddin Mohd Ali

Highest qualification with EB group :
PhD Environmental Engineering (Kyushu Institute of Technology, Japan)

Former Supervisor:
Prof. Dr. Yoshihito Shirai

Current Position:
Postdoctoral Researcher

Dr. Ezyana Kamal Bahrin

Highest qualification with EB group:
PhD

Former Supervisor:
Prof. Dr. Suraini Abd Aziz

Current Position:
Contract Staff at Biotech Research Center, MARDI

Dr. Mitra Mohammadi

Highest qualification with EB group:
PhD

Former Supervisor:
Prof. Dr. Mohd Ali Hassan

Current position:
Assistant Professor in Kheradgarayan Motahar Institute of Higher Education.

Dr. Norhayati Ramli

Highest Qualification:
Ph.D. (Microbial Biotechnology), UPM (2008-2012)

Former supervisor:
Prof. Dr. Suraini Abd Aziz

Current position:
Research Assistant, Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, UPM

Mohd Najib Ahmad

Highest qualification with EB group:
MSc in Bioprocess Eng.

Former Supervisor:
Prof. Dr. Mohd Ali Hassan

Current position:
Research officer in MPOB

Siren Linggang

Highest qualification with EB Group:
Master Science of Environmental Biotechnology,
UPM, Malaysia

Former Supervisor:
Prof. Dr. Suraini Abd. Aziz

Current Position:
Research Assistant

EB Group 2012

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OUTBOUND

Participants	Program	Research Theme	Host/Location	Duration	Sponsor
Nor Hashimah Abdul Rahman	Student Exchange Support Program	Student Exchange Program on Ecological and Life Technologies	MAEDA Laboratory, Life Science and Systems Engineering, Kyushu Institute Technology, Kitakyushu, Japan	**3 months** 1 September 2012 - 1 December 2012	Japan Student Services Organization (JASSO)
Nur Ain Zamzuri	Student Exchange Support Program	Student Exchange Program on Ecological and Life Technologies	MAEDA Laboratory, Life Science and Systems Engineering, Kyushu Institute Technology, Kitakyushu, Japan	**6 months** 1 June 2012 - 27 December 2012	Japan Student Services Organization(JASSO)
Yee Lian Ngit	Student Exchange Support Program	Student Exchange Program on Ecological and Life Technologies	MAEDA Laboratory, Life Science and Systems Engineering, Kyushu Institute Technology, Kitakyushu, Japan	**3 months** 1 September 2012 - 1 December 2012	Japan Student Services Organization(JASSO)
Noor Ida Amalina Ahamad Nordin	Research Attachment	Biocomposite Production from Oil Palm Mesocarp Fiber	Prof. Dr Yoshihito Shirai lab/ Associate Prof Dr Yoshito Ando, Kyushu Institute of Technology (Kitakyushu campus)	**11 Days** 14 November 2012 - 25 November 2012	Kyushu Institute of Technology (Kyutech), Fukuoka, Japan.
Ahmad Amiruddin Mohd Ali / Mohd Ridzuan Othman	Research Training	Biodiesel Training	Prof. Dr. Yoshihito Shirai and Hidemasa Co. Ltd., Japan	**2 days** 8 August 2012 - 9 August 2012	Kyushu Institute of Technology (Kyutech), Japan
Hidayah Ariffin	Research Meeting	Application and Development of Palm Biomass	Assoc. Prof. Dr. yoshito Ando / Kyushu Institute of Technology, Japan	**4 days** 4 December 2012 - 8 December 2012	Kyushu Institute of Technology (Kyutech), Japan
Mohd Rafein Zakaria	Research Attachment	Improve Pha Biosynthesis by Transposon Mutagenesis	Assoc. Prof. Toshinari Maeda Kyushu Institute of Technology (Kyutech), Japan	**2 months** Febuary 2012 - April 2012	Kyushu Institute of Technology (Kyutech), Japan

INBOUND

Participants	Program	Research Theme	Host/Location	Duration	Sponsor
Mr. Syvang Xayyavong	Visiting Researcher	Quality control and standardization of biodiesel	Prof. Dr. Yoshishito Shirai		
Prof. Dr. Mohd Ali Hassan		Ajinomoto Cost Center		21 October 2012 - 17 November 2012	
Maria Del Mar Palmeros Parada	PhD student		Prof. Dr. Mohd Ali Hassan	22 Oktober 2012 - 25 November 2012	Ajinomoto Cost Center
Masaharu Fukuzaki	MSc student		Prof. Dr. Mohd Ali Hassan	30 Oktober 2012 - 27 November 2012	Ajinomoto Cost Center

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Renewable sugars from oil palm frond juice as an alternative novel fermentation feedstock for value added products

By: Mior Ahmad Khushairi Mohd Zahari, PhD student

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Renewable sugars from oil palm frond juice as an alternative novel fermentation feedstock for value-added products

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ABSTRACT

In this paper, we report that pressed juice from oil palm frond (OPF) contained renewable sugars such as glucose, sucrose and fructose. By using a simple sugarcane press, 50% (wt/wt) of OPF juice was obtained from fresh OPF. The glucose content in the juice was 53.95 ± 2.86 g/l, which accounts for 70% of the total free sugars. We have examined the effect of various OPF juice concentrations on the production of poly (3-hydroxybutyrate), P(3HB) by *Cupriavidus necator* CCUG 52238[T]. The cell dry mass in shake flask experiment reached 8.42 g/l, with 32 wt.% of P(3HB) at 30% (v/v) of OPF juice, comparable with using technical grade sugars. The biopolymer had a molecular mass, M_w of 812 kDa, with a low polydispersity index of 1.61. This result indicates that OPF juice can be used as an alternative renewable carbon source for P(3HB) production and has potential as a renewable carbon source.

1. Introduction

The use of renewable resources such as agricultural and agro based-industry wastes as raw materials for the production of fermentable sugars can help to reduce the production cost and the dependence on the food crops. Bioconversion of agricultural waste into sugars through lignocellulosic route has been widely studied (Nguyen et al., 2010; Tengerdy and Szakacs, 2003; Saddler and Mackie, 1990). In Malaysia, oil palm plantation and the palm oil industries are the main contributors to the generation of agricultural waste. Studies have been done to utilize the waste efficiently, for example to convert the oil palm empty fruit bunch (OPEFB) into fermentable sugars (Kader et al., 1999; Ariffin et al., 2006; 2008a ; Roslan et al., 2011), utilization of the OPEFB as substrate for enzyme production (Umikalsom et al., 1997; Ariffin et al., 2008b) and pulp preparation from OPEFB (Rushdan, 2002). OPEFB is a lignocellulose which contains about 70–80% of holocellulose. Due to the high content of polysaccharides, it has great potential to be converted to fermentable sugars for the production of value-added products such as bioethanol, biobutanol, lactid acid and bioplastics.

However, the main problem with OPEFB is that it is difficult to be hydrolyzed due to its natural lignin component which is recalcitrant to degradation. Various pretreatments such as mechanical, chemical and steam pretreatments are needed in order to loosen up the lignocellulose structure. In addition to that, enzymes need to be used in order to convert the holocellulose into sugars. The overall process not only contributes to the high cost of sugars production, but it also causes the need of proper wastewater treatment system since chemicals are being used in the pretreatment.

Apart from OPEFB, research has been done on sugars production from oil palm trunk (OPT). Recently, Kosugi et al. (2010) found that OPT contains high amounts of readily available sugars. Characterization of the sap from the inner part of the OPT revealed that a large amount of glucose (85.2 g/l) and some sucrose and fructose were present in the sap. In 2009, about 15.2 and 17.5 million tonnes (wet weight) of OPT and OPEFB were generated in Malaysia. However, the most abundant biomass from oil palm plantation is not OPEFB or OPT. The most generated oil palm biomass is oil palm frond (OPF), which amounted to 83 million tonnes (wet weight) annually (MPOC, 2010). OPF is obtained during pruning for harvesting fresh fruit bunch (FFB), therefore it is available daily. OPF is currently under-utilized as the plantation owners believe that all the OPF is necessary for nutrient recycling and soil conservation

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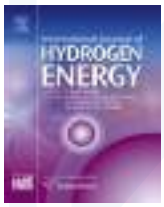

Kinetic analysis of biohydrogen production from anaerobically treated POME in bioreactor under optimized condition

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ABSTRACT

In this study, the biohydrogen production from POME was performed under mesophillic conditions by mixed culture in a 2 L bioreactor using the optimized conditions obtained previously. The effect of controlling pH initially or throughout the fermentation was also examined. The fermentation performance was monitored by comparing P, R_m, λ, and P_s in both systems. In this present study, the reactor system showed higher hydrogen production potential values with the utilization of pH control. Hydrogen production potential was increased two folds when the reactor system was equipped with pH control rather than just fixed the initial pH at 5.8. The biohydrogen production under controlled pH occurred after 7 h fermentation resulting in maximum Ps and Rm of 1.32 L/L POME and 0.144 L/L.h, respectively.

1. Introduction

Considering that the hydrogen gas is one of the alternatives to fossil fuels, there are numerous ways to produce hydrogen [1]. Hydrogen must be made from renewable resources if its use to impact global CO_2 levels. The carbohydrate-rich crops and food industry wastes are considered as suitable substrates for dark fermentative hydrogen production [2]. Palm oil mill effluent (POME) has a good potential for biohydrogen production when being treated anaerobically. Utilization of biohydrogen gas, produced from anaerobic treatment of POME can be beneficial to the palm oil industry and the nation due to the energy availability. Besides, methane gas which is known to be greenhouse gases could be minimized and thereby reduce the environmental impact.

Biohydrogen production is a complex process and is greatly influenced by many factors such as substrate specificity, substrate concentration, reactor configuration, hydraulic retention time (HRT), organic loading rate (OLR), pH, temperature, oxidation-reduction potential and nutritional requirement [3]. Moreover, the optimization of fermentation conditions, particularly nutritional and environmental parameters are of primary importance for bioprocess development [4]. Previously, factors affecting hydrogen production from POME during anaerobic digestion in batch fermentation by anaerobic mixed cultures were optimized using Response

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Uncharacterized *Escherichia coli* proteins YdjA and YhjY are related to biohydrogen production

By: Mohd Zulkhairi Mohd Yusoff, PhD student

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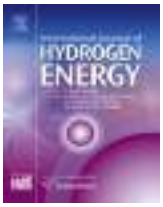

Uncharacterized *Escherichia coli* proteins YdjA and YhjY are related to biohydrogen production

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ABSTRACT

Biohydrogen has gained importance as an alternative energy source, and advances in molecular biology and biotechnology have raised the quality and efficiency of biohydrogen production from various microorganisms and substrates. Here, *Escherichia coli* proteins YdjA and YhjY have been identified as essential in biohydrogen production from glucose. The mutations *ydjA* and *yhjY* reduced biohydrogen productivity compared to the parent strain from 40 to 4 and 29 μmol/mg protein, respectively. Through transcription analysis, it was determined that YdjA and YhjY are positive effectors of the FHL complex since their inactivation repressed *fhlA*. In addition, the FHL expression of the repressor gene, *hycA*, increased for the *ydjA* mutant, so YdjA reduces transcription of the HycA repressor. Hence, two new proteins have been identified that are important for biohydrogen production.

1. Introduction

The merits of biohydrogen as a fuel source remain undisputed due to its higher energy content compared to hydrocarbon fuels [1,2]. The necessity of replacing fossil fuels also has been discussed extensively since petroleum prices have increased dramatically and there is continued anxiety about the level of green house gases (GHG) in the atmosphere [3]. Critically, Kim et al. reported that only water vapor is produced once biohydrogen is combusted, and no pollutants evolve which may contribute to the GHG phenomena [4].

Many methods and sources are available for biohydrogen production through physical, chemical or biological approaches [1]. Haijun has reported chemical and physical approaches such as partial oxidation of fossil fuels and steam reforming of natural gas are producing large amounts of hydrogen, however these processes have created environment pollution and increase the cost of hydrogen production [5]. On the other hand, hydrogen also has been produced through the water–gas shift reaction and as a by-product of petroleum refining, gasification of coal, and electrolysis of water which are grouped as chemical or electrochemical methods. Indeed, methods that have been mentioned have high costs since some of them require high temperature >850 °C (physical properties) [1,6]. Another studies have reported that the addition of a specific chemical to the fermentation process by

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Effect of steam pretreatment on oil palm empty fruit bunch for the production of sugars

By: Saleha Shamsuddin, PhD student

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Effect of steam pretreatment on oil palm empty fruit bunch for the production of sugars

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ABSTRACT

Lignocellulose into fuel ethanol is the most feasible conversion route strategy in terms of sustainability. Oil palm empty fruit bunch (EFB) generated from palm oil production is a huge source of cellulosic material and represents a cheap renewable feedstock which awaits further commercial exploitation. The purpose of this study was to investigate the feasibility of using steam at 0.28 MPa and 140 °C generated from the palm oil mill boiler as a pretreatment to enhance the digestibility of EFB for sugars production. The effects of steam pretreatment or autohydrolysis on chemical composition changes, polysaccharide conversion, sugar production and morphology alterations of four different types of EFB namely fresh EFB (EFB1), sterilized EFB (EFB2), shredded EFB (EFB3) and ground EFB (EFB4) were evaluated. In this study, the effects of steam pretreatment showed major alterations in the morphology of EFB as observed under the scanning electron microscope. Steam pretreated EFB2 was found to have the highest total conversion of 30% to sugars with 209 g kg^{-1} EFB. This production was 10.5 fold higher than for EFB1 and 1.6 fold and 1.7 fold higher than EFB3 and EFB4, respectively. The results suggested that pretreatment of EFB by autohydrolysis using steam from the mill boiler could be considered as being a suitable pretreatment process for the production of sugars. These sugars can be utilized as potential substrates for the production of various products such as fuel ethanol.

1. Introduction

Currently more than 46 900 km^2 of oil palm are cultivated in Malaysia, the world's largest exporter of palm oil [1]. As one of the biggest exporters of palm oil and palm oil products, the palm oil industry in Malaysia generates huge quantities of biomass in the form of oil palm empty fruit bunch (EFB), oil palm shell (OPS) and oil palm fibers (OPF). The potentials of these biomasses are yet to be exploited. Out of these biomasses, EFB generated during the processing of palm oil, can be considered as a primary feedstock for the production of sugars which can be further used as carbon source for ethanol production by yeast. Ethanol production from biomass consists of four basic steps namely pretreatment, hydrolysis, fermentation and distillation. Pretreatment is the crucial step in which the biomass can be broken down into sugars through enzymatic hydrolysis enhancing the yield of saccharification.

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Acetone-Butanol-Ethanol production by *Clostridium acetobutylicum* ATCC 824 using Sago pith residues hydrolysate

By: Siren Linggang, Master student

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Acetone–Butanol–Ethanol Production by *Clostridium acetobutylicum* ATCC 824 Using Sago Pith Residues Hydrolysate

Siren Linggang · Lai Yee Phang · Helmi Wasoh · Suraini Abd-Aziz

Published online: 30 September 2012

Abstract Sago pith residues (58 % starch, 23 % cellulose, 9.2 % hemicellulose, and 4 % lignin) are one of the abundant lignocellulosic residues generated after starch extraction process in sago mill. In this study, fermentable sugars from enzymatic hydrolysis of sago pith residues were converted to acetone–butanol–ethanol (ABE) by *Clostridium acetobutylicum* ATCC 824. With an initial concentration of 30 g/L of concentrated sago pith residues hydrolysate containing 23 g/L of glucose and 4.58 g/L of cellobiose, 4.22±0.17 g/L of ABE were produced after 72 h of fermentation with yield and productivity of 0.20 g/g glucose and 0.06 g/L/h, respectively. Results are in agreement when synthetic glucose was used as a carbon source. Increasing sago pith residue hydrolysate to 50 g/L (containing 40 g/L glucose) and supplementing with 0.5 g/L yeast extract, approximately 8.84±0.20 g/L of ABE (5.41±0.10 g/L of butanol) were produced with productivity and yield of 0.12 g/L/h and 0.30 g/g glucose respectively, providing a 52 % improvement.

Keywords Sago pith residues hydrolysate · Fermentable sugar · Acetone–butanol–ethanol · *Clostridium acetobutylicum* ATCC 824

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Abbreviations

ABE	Acetone–butanol–ethanol
DNS	Dinitrosalicylic acid
FID	Flame ionization detector
HMF	Hydroxymethyl furfural
HPLC	High-performance liquid chromatography
OPEFB	Oil palm empty fruit bunch
RCM	Reinforced Clostridial Medium

Introduction

The increasing expansion of agro-industry has led to the accumulation of large quantities of lignocellulosic residues, such as oil palm wastes, rice straw and sugarcane bagasse as well as sago pith residues. Sago pith residues are one of the abundant lignocellulosic residues available in the state of Sarawak, Malaysia [4]. Approximately 7.2 thousand tons of sago pith residues are generated annually from sago mills [6] and usually discharged into nearby rivers together with sago effluent without proper treatment. Sago pith residues, the final waste product generated during extraction process of sago starch in sago mills, is starchy lignocellulosic. According to Ozawa et al. [26], sago pith residues contain 58 % starch, 23 % cellulose, 9.2 % hemicelluloses, and 3.9 % lignin. The presence of lignocellullosic materials in sago pith residues becomes a strong pollutant to the surrounding soil and water due to its slow degradability in the environment [1, 4, 8]. Therefore, it is necessary to develop an alternative utilization of sago waste to minimize the cost of waste treatment and to solve environmental problems.

Previously, sago pith residues have been used as medium for edible mushroom cultivation [7, 12] and substrate for the production of laccase enzyme using *Pleurotus sajor-caju*

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Intracellular polyhydroxyalkanoates recovery by cleaner halogen-free methods towards zero emission in the palm oil mill

By : Mitra Mohammadi, PhD student

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Intracellular polyhydroxyalkanoates recovery by cleaner halogen-free methods towards zero emission in the palm oil mill

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ABSTRACT

In this study, the effect of extraction process, bacterial strain and cell PHA content on halogen-free recovery methods using sodium hydroxide (NaOH) and water as cleaner and more environmental friendly processes were examined for polyhydroxyalkanoates (PHA) recovery. The bacterial strains used were local isolate wild type *Comamonas* sp. EB172 and recombinant *Cupriavidus necator* (*C. necator*), with 30% and 50% of PHA content. It was found that the PHA was efficiently recovered from bacterial cells by the non-halogenated methods. Although the PHA purity obtained by water recovery method was lower than alkaline treatment, it does not have any adverse effect on the polymer molecular weight. The performance of the halogen-free methods depends on the microbial strain which is due to the difference in the thickness of the cell membranes. The intracellular PHA content also affected the effectiveness of the extraction methods for PHA recovery as higher amount of polymer in the cell increased the quantity of PHA recovered. The halogen-free methods using NaOH and water can serve as promising alternatives for conventional industrial halogenated solvent extraction process, since these new methods are environmentally more benign, effective and simple in operation. Finally, the potential of integrating PHA production and halogen free recovery system with the palm oil industry is discussed. It is expected that the proposed integrated system herein provides a more attractive proposition to utilize biomass as renewable resource for the production of value-added bioproducts like PHA, which will make the palm oil industry more sustainable and profitable, and promotes zero-emission at the mill.

1. Introduction

There has been much public and scientific interest regarding the development of bio-based plastics such as polyhydroxyalkanoates (PHA)[1] to reduce the harmful effect of petrochemical-based plastic usage on the environment (Alvarez-Chavez et al., 2012; Glew et al., 2012). PHAs as environmental friendly green thermoplastics are biodegradable and biocompatible, which can be produced from renewable resources such as lipids, carbohydrates, alcohols and organic acids under imbalanced growth conditions by several microorganisms (Loo and Sudesh, 2007; Yang et al., 2011). Nevertheless, the main obstacle for commercial production and application of PHAs in consumer products is their high cost compared to synthetic plastics (Khanna and Srivastava, 2005). The recovery of PHA as an intracellular product, significantly affects the overall economics, and therefore, developing a clean, simple and efficient process for PHA extraction from source materials at a useful level of quality and purity is a remarkable proposal (Ghatnekar et al., 2002). The selection of suitable PHA extraction methods depends on several process parameters such as concentration of chemicals, reaction time, recovery temperature, pH and etc. In addition, the choice of recovery system, PHA-producing bacteria, composition of the growth medium, presence of certain chemical compounds in the environment, the intracellular PHA content, length of PHA granules, cell wall structure and economics of process are considered as the most important external factors affecting the extraction

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[1] Sodium hydroxide (NaOH); polyhydroxyalkanoates (PHA); *Cupriavidus necator* (*C. necator*); sodium dodecyl sulphate (SDS); *Escherichia coli* (*E. coli*); non-polymer cellular material (NPCM); gas chromatography (GC); gel permeation chromatography (GPC); palm oil mill effluent (POME); poly(3-hydroxybutrate) [P(3HB)].

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Factors affecting poly(3-hydroxybutyrate) production from oil palm frond juice by *Cupriavidus necator* (CCUG52238^T)

By: Mior Ahmad Khushairi Mohd Zahari, PhD student

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Research Article

Factors Affecting Poly(3-hydroxybutyrate) Production from Oil Palm Frond Juice by *Cupriavidus necator* (CCUG52238^T)

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Factors influencing poly(3-hydroxybutyrate) P(3HB) production by *Cupriavidus necator* CCUG52238^T utilizing oil palm frond (OPF) juice were clarified in this study. Effects of initial medium pH, agitation speed, and ammonium sulfate $(NH_4)_2SO_4$ concentration on the production of P(3HB) were investigated in shake flasks experiments using OPF juice as the sole carbon source. The highest P(3HB) content was recorded at pH 7.0, agitation speed of 220 rpm, and $(NH_4)_2SO_4$ concentration at 0.5 g/L. By culturing the wild-type strain of *C. necator* under the aforementioned conditions, the cell dry weight (CDW) and P(3HB) content obtained were 9.31 ± 0.13 g/L and 45 ± 1.5 wt.%, respectively. This accounted for 40% increment of P(3HB) content compared to the nonoptimized condition. In the meanwhile, the effect of dissolved oxygen tension (DOT) on P(3HB) production was investigated in a 2-L bioreactor. Highest CDW (11.37 g/L) and P(3HB) content (44 wt.%) were achieved when DOT level was set at 30%. P(3HB) produced from OPF juice had a tensile strength of 40 MPa and elongation at break of 8% demonstrated that P(3HB) produced from renewable and cheap carbon source is comparable to those produced from commercial substrate.

1. Introduction

Poly(3-hydroxybutyrate), P(3HB) is a biodegradable thermoplastic polyester accumulated intracellularly by many microorganisms under unfavorable growth conditions [1]. The high production cost of P(3HB) can be decreased by strain development, improving fermentation and separation processes [2–4], and/or using a cheap carbon source [5]. In P(3HB) production, about 40% of the total production cost is contributed by the raw material, whereby the cost of carbon feedstock alone accounts for 70 to 80% of the total raw material cost [6, 7]. Therefore, the utilization of renewable and sustainable substrates for the production of P(3HB) has become an important objective for the commercialization of bioplastics. A lot of research have been carried out to discuss and propose the utilization of renewable biomass to replace commercial sugars as carbon source in order to reduce the production cost of P(3HB) [8–12].

Recently, we reported on the use of oil palm frond (OPF) juice as the novel and renewable feedstock for the production of P(3HB) [13]. We demonstrated that OPF juice is a good substrate for the production of P(3HB) from wild-type *Cupriavidus necator* (CCUG52238^T), with better yield of product formation in comparison to technical grade sugars. This can be explained by the presence of minerals and nutrients in the OPF juice which are essential for bacterial growth

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Molecular characterisation of *pha*CAB from *Comamonas* sp. EB172 for functional expression in *Escherichia coli* JM109

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Molecular characterisation of *phaCAB* from *Comamonas* sp. EB172 for functional expression in *Escherichia coli* JM109

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ABSTRACT

In this study, PHA biosynthesis operon of *Comamonas* sp. EB172, an acid-tolerant strain, consisting of three genes encoding acetyl-CoA acetyltransferase (*phaA*$_{Co}$ gene, 1182 bp), acetoacetyl-CoA reductase (*phaB*$_{Co}$ gene, 738 bp) and PHA synthase, class I (*phaC*$_{Co}$ gene, 1694 bp) were identified. Sequence analysis of the *phaA*$_{Co}$, *phaB*$_{Co}$ and *phaC*$_{Co}$ genes revealed that they shared more than 85%, 89% and 69% identity, respectively, with orthologues from *Delftia acidovorans* SPH-1 and *Acidovorax ebreus* TPSY. The PHA biosynthesis genes (*phaC*$_{Co}$ and *phaAB*$_{Co}$) were successfully cloned in a heterologous host, *Escherichia coli* JM109. *E. coli* JM109 transformants harbouring pGEM′-phaC$_{Co}$AB$_{Re}$ and pGEM′-phaC$_{Re}$AB$_{Co}$ were shown to be functionally active synthesising 33 wt.% and 17 wt.% of poly(3-hydroxybutyrate) [P(3HB)]. *E. coli* JM109 transformant harbouring the three genes from the acid-tolerant *Comamonas* sp. EB172 (*phaCAB*$_{Co}$) under the control of native promoter from *Cupriavidus necator*, *in vivo* polymerised P(3HB) when fed with glucose and volatile mixed organic acids (acetic acid:propionic acid:n-butyric acid) in ration of 3:1:1, respectively. The *E. coli* JM109 transformant harbouring *phaCAB*$_{Co}$ could accumulate P(3HB) at 2 g/L of propionic acid. P(3HB) contents of 40.9% and 43.6% were achieved by using 1% of glucose and mixed organic acids, respectively.

Introduction

Polyhydroxyalkanoates (PHA) are biodegradable, water-insoluble polyesters that are accumulated intracellularly as carbon storage compounds in the cytoplasm (Steinbüchel and Füchtenbusch 1998; Li et al. 2009). PHA can be synthesised by numerous prokaryotes in response to stress conditions such as limitations of nitrogen, phosphate, oxygen and other elements essential for growth (Anderson and Dawes 1990). PHA has plastic-like properties and has therefore attracted much attention as the next generation bio-based and biodegradable plastics (Sudesh and Iwata 2008). In addition, the biocompatibility and non-toxic properties of PHA have created niche applications in the medical, packaging, agriculture and cosmetics fields as substitutes for petrochemical synthetic plastics (Braunegg et al. 1998; Sudesh et al. 2007; Bhubalan et al. 2011). However, the high PHA production cost is mainly from fermentation and recovery processes which have hindered the commercialisation process of PHA. In order to overcome the high production cost, researchers have extensively investigated the usage of recombinant microbial strains, mixed cultures, cheap carbon substrates, efficient fermentation and recovery/purification process (Khanna and Srivastava 2005; Verlinden et al. 2007; Li et al. 2007).

PHA biosynthesis in bacteria mainly involves three basic enzymatic steps (Naik et al. 2008). These enzymatic steps are involved in the generation of PHA monomer units that are eventually polymerised by PHA synthase. The genes responsible for the biosynthesis of PHA are those encoding the PHA synthase (PhaC), acetyl-CoA acetyltransferase (PhaA) (also referred to as β-ketothiolase or simply thiolase) and acetoacetyl-CoA reductase (PhaB) enzymes, which may be clustered in a single operon. The enzymes responsible for the monomer supply are thiolase and reductase (Steinbüchel and Lütke-Eversloh 2003; Rehm 2003). The pathway for the synthesis of P(3HB) begins with the condensation of two molecules of acetyl-CoA to acetoacetyl-CoA by β-ketothiolase, which is encoded by the *phaA* gene. Acetoacetyl-CoA reductase, a product of the *phaB* gene, reduces the acetoacetyl-CoA to (*R*)-3-hydroxybutyryl-CoA, and finally PHA

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Sago pith residue as an alternative cheap substrate for fermentable sugars production

By: Siren Linggang, Master student

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Sago Pith Residue as an Alternative Cheap Substrate for Fermentable Sugars Production

S. Linggang · L. Y. Phang · M. H. Wasoh · S. Abd-Aziz

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Abstract Sago pith residue is one of the most abundant lignocellulosic biomass which can serve as an alternative cheap substrate for fermentable sugars production. This residue is the fibrous waste left behind after the starch extraction process and contains significant amounts of starch (58%), cellulose (23%), hemicellulose (9.2%) and lignin (3.9%). The conversion of sago pith residue into fermentable sugars is commonly performed using cellulolytic enzymes or known as cellulases. In this study, crude cellulases were produced by two local isolates, *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus*, UPM2 using sago pith residue as substrate. *A. fumigatus* UPM2 gave the highest FPase, CMCase and β-glucosidase activities of 0.39, 23.99 and 0.78 U/ml, respectively, on day 5. The highest activity of FPase, CMCase and β-glucosidase by *T. asperellum* UPM1 was 0.27, 12.03 and 0.42 U/ml, respectively, on day 7. The crude enzyme obtained from *A. fumigatus* UPM2 using β-glucosidase as the rate-limiting enzyme (3.9, 11.7 and 23.4 IU) was used for the saccharification process to convert 5% (w/v) sago pith residue into reducing sugars. Hydrolysis of sago pith residue using crude enzyme containing β-glucosidase with 23.4 IU, produced by *A. fumigatus* UPM2 gave higher reducing sugars production of 20.77 g/l with overall hydrolysis percentage of 73%.

Keywords Sago pith residue · Crude enzyme · *Trichoderma asperellum* UPM1 · *Aspergillus fumigatus* UPM2 · Saccharification

Introduction

Lignocellulosic biomass comprising of cellulose, hemicellulose and lignin has been recognized as a major renewable resources and also the most abundant source of organic component in high amounts on earth. Due to its abundance and renewability, there is a great

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Oil Palm Empty Fruit Bunch as Alternative Substrate for Acetone–Butanol–Ethanol Production by *Clostridium butyricum* EB6

Mohamad Faizal Ibrahim · Suraini Abd-Aziz · Mohamad Nafis Abdul Razak · Lai Yee Phang · Mohd Ali Hassan

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Abstract Acetone–butanol–ethanol (ABE) production from renewable resources has been widely reported. In this study, *Clostridium butyricum* EB6 was employed for ABE fermentation using fermentable sugar derived from treated oil palm empty fruit bunch (OPEFB). A higher amount of ABE (2.61 g/l) was produced in a fermentation using treated OPEFB as the substrate when compared to a glucose based medium that produced 0.24 g/l at pH 5.5. ABE production was increased to 3.47 g/l with a yield of 0.24 g/g at pH 6.0. The fermentation using limited nitrogen concentration of 3 g/l improved the ABE yield by 64%. The study showed that OPEFB has the potential to be applied for renewable ABE production by *C. butyricum* EB6.

Keywords *Clostridium butyricum* · Acetone butanol ethanol (ABE) · Oil palm empty fruit bunch (OPEFB) · Anaerobic fermentation · Biomass

Introduction

In recent years, growing attention has been paid to the production of fine chemicals from biomass. Acetone–butanol–ethanol (ABE) fermentation by Clostridia has attracted many

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Recovery and purification of intracellular polyhydroxyalkanoates from recombinant *Cupriavidus necator* using water and ethanol.

By: Mitra Mohammadi,
Alumni PhD student

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ORIGINAL RESEARCH PAPER

Recovery and purification of intracellular polyhydroxyalkanoates from recombinant *Cupriavidus necator* using water and ethanol

Mitra Mohammadi · Mohd Ali Hassan · Lai-Yee Phang · Hidayah Ariffin · Yoshihito Shirai · Yoshito Ando

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Abstract A new halogen-free and environmental-friendly method using water and ethanol is developed as an alternative for the recovery of polyhydroxyalkanoates (PHA) from recombinant *Cupriavidus necator* in comparison to the established chloroform extraction method. After optimisation, our results showed that the halogen-free method produced a PHA with 81% purity and 96% recovery yield, in comparison to the chloroform extraction system which resulted in a highly pure PHA with 95% yield. Although the purity of the PHA using the new method is lower, the molecular weight of the extracted PHA is not compromised. This new method can be further developed as an alternative and more environmental-friendly method for industrial application.

Keywords Bioplastic recovery · *Cupriavidus necator* · Halogen-free method · Polyhydroxyalkanoates

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Introduction

Polyhydroxyalkanoates (PHA) are biodegradable, biocompatible, thermoplastic and piezoelectric polymers which are accumulated in various microorganisms as intracellular carbon and energy storage materials during unfavorable growth conditions (Yang et al. 2011). However, PHA has not been successfully commercialized due to its high costs as compared to conventional plastics. As an intracellular product, the recovery of PHA contributes significantly to the overall economics. Therefore developing an inexpensive and less-polluting PHA recovery method to

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PRODUCTION OF FERMENTABLE SUGARS FROM OIL PALM EMPTY FRUIT BUNCH USING CRUDE CELLULASE COCKTAILS WITH *TRICHODERMA ASPERELLUM* UPM1 AND *ASPERGILLUS FUMIGATUS* UPM2 FOR BIOETHANOL PRODUCTION

Nurul Kartini Abu Bakar,[a] Zuraidah Zanirun,[a] Suraini Abd-Aziz,[a] Farinazleen Mohd Ghazali,[b] and Mohd Ali Hassan [a]

Utilization of oil palm empty fruit bunch (OPEFB) for bioethanol production with crude cellulase cocktails from locally isolated fungi was studied. Enzymatic saccharification of alkaline pretreated OPEFB was done using different cellulase enzyme preparations. Crude cellulase cocktails from *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2 produced 8.37 g/L reducing sugars with 0.17 g/g yield. Production of bioethanol from OPEFB hydrolysate using Baker's yeast produced approximately 0.59 g/L ethanol, corresponding to 13.8% of the theoretical yield. High reducing sugars concentration in the final fermentation samples resulted from accumulation of non-fermentable sugars such as xylose and cellobiose that were not consumed by the yeast. The results obtained support the possible utilization of OPEFB biomass for bioethanol production in the future.

Keywords: Oil palm empty fruit bunch (OPEFB); Crude cellulase enzymes; Lignocellulosic bioethanol; Locally isolated fungi

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INTRODUCTION

Bioethanol was first produced from edible feedstocks containing readily fermentable sugars, such as sugarcane, corn, and starch (Goh *et al*. 2010). However, production of bioethanol from food-based material has triggered conflicts between food production and fuel production, which has resulted in an expensive bioethanol selling price (Goh *et al*. 2010; Badger 2002). Driven by its favorable potential economics, exploitation of lignocellulosic biomass as feedstock to bioethanol production has prompted the initiation of many research projects. To date, bioethanol has successfully been produced in the United States and Brazil using wood, switchgrass, wheat straw, sugarcane bagasse, and municipal solid waste (MSW) (Badger 2002). In Malaysia, palm oil is a huge and profitable agricultural commodity. In 2005, approximately 55.73 million tones of oil palm biomass were produced (Shuit *et al*. 2009). The palm oil industry generates enormous quantities of biomass ranging from palm fiber, palm kernel cake (PKC), decanter cake, fronds, trunks, shells, and oil palm empty fruit bunch (OPEFB) (Shuit *et al*. 2009; Yusoff 2006; Joseph 2010). OPEFB is obtained from the process of stripping off fruit from fresh fruit bunches (Law *et al*. 2007; Umikalsom *et al*. 1997). OPEFB contains approximately 54.4% of cellulose, 28% hemicelluloses, and 17.6% lignin

Physicochemical property changes and enzymatic hydrolysis enhancement of oil palm empty fruit bunches treated with superheated steam

By: Ezyana Kamal Bahrin, PhD student

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PHYSICOCHEMICAL PROPERTY CHANGES AND ENZYMATIC HYDROLYSIS ENHANCEMENT OF OIL PALM EMPTY FRUIT BUNCHES TREATED WITH SUPERHEATED STEAM

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The effect of superheated steam treatment on oil palm empty fruit bunches (OPEFB) was investigated in terms of physicochemical property changes and enzymatic hydrolysis enhancement. The experimental treatment was carried out at different temperatures (140-210°C) and durations (20-90 min). Results showed that as the superheated steam temperature and time increased, the size distribution also changed, resulting in more small particles. Analysis on the surface texture, color, and mechanical properties of the treated OPEFB also showed that significant changes resulted due to the superheated steam treatment. In support to this, Fourier Transform Infrared (FTIR) spectroscopy and thermogravimetric (TG) analyses showed that solubilization and removal of the hemicelluloses component also took place. As a result of this phenomenon, higher total sugar and glucose yield was achieved once the treated OPEFB was subjected to enzymatic hydrolysis. This suggests that superheated steam treatment could enhance OPEFB structure degradation for the preparation of a suitable substrate in order to produce a higher glucose yield in the enzymatic hydrolysis process.

Keywords: Superheated steam; Oil palm empty fruit bunch; Physicochemical properties; Enzymatic hydrolysis

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INTRODUCTION

Currently, oil palm plantations contribute to the largest solid biomass generation in Malaysia (Baharuddin et al. 2010). From 55.73 million tons of oil palm biomass generated in Malaysia, oil palm empty fruit bunches (OPEFB) contributed 17.0 million tons, or 30.5 percent, and this is increasing year by year (Shuit et al. 2009). Currently OPEFB is the largest source of lignocellulosic material that can be obtained from the palm oil industry. OPEFB is a renewable material and can be obtained at a very low cost directly from the mill, thus making it a very suitable candidate as a potential feedstock for various applications such as biosugar, compost, and bioethanol production (Baharuddin et

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RESEARCH PAPER

Utilization of Oil Palm Decanter Cake for Cellulase and Polyoses Production

Mohamad Nafis Abdul Razak, Mohamad Faizal Ibrahim, Phang Lai Yee, Mohd Ali Hassan, and Suraini Abd-Aziz

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Abstract The abundance of oil palm decanter cake (OPDC) is a problem in oil palm mills. However, this lignocellulosic biomass can be utilized for cellulase and polyoses production. The effectiveness of chemical and physical pretreatment in reducing the lignin content was studied by saccharification using a Celluclast 1.5 L and scanning electron microscope. Physicochemical pretreatment of OPDC with 1% (w/v) NaOH and autoclaving at 121°C for 20 min increased potential polyoses produced to 52.5% and removed 28.7% of the lignin content. The optimized conditions for cellulase production by a locally isolated fungus were a time of 120 h, a substrate of untreated OPDC, a spore concentration of 1×10^7 spore/mL, a temperature of 30°C, and a pH between 7.0 and 7.5. *Trichoderma asperellum* UPM1 produced carboxymethylcellulase (CMCase), β-glucosidase and filter paper activity (FPase) in the following concentrations: 17.35, 0.53, and 0.28 U/mL, respectively. *Aspergillus fumigatus* UPM2 produced the CMCase, β-glucosidase and FPase in the following amounts: 10.93, 0.76, and 0.24 U/mL. The cellulases from *T. asperellum* UPM1 produced 2.33 g/L of polyoses and the cellulases from *A. fumigatus* UPM2 produced 4.37 g/L of polyoses.

Keywords: pretreatment, oil palm decanter cake (OPDC), *Trichoderma asperellum* UPM1, *Aspergillus fumigatus* UPM2, cellulase, polyoses

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1. Introduction

The Malaysian Economic Planning Unit estimated that 6.96 million hectares of Malaysia is covered by oil palm plantations, and these plantations have contributed RM17.0 billion to the Gross Domestic Product (GDP), of which RM49.6 billion was export revenue [1]. Ironically, the amount of pollutants produced during palm oil extraction is higher than the palm oil produced. Palm oil mill effluent (POME) is the most significant pollutant making up 50% of the waste generated [2]. The conventional method of treating POME is by creating a series of low cost ponds to hold the effluent. However, this requires a large area of land and a long retention time, and releases a huge amount of methane gas into the environment [3]. The use of the decanter system is an effective solution to meet the zero emission target in palm oil mills. According to Chavalparit *et al.* [4], the decanter system with oil recovery is the best practice in clean technology and is widely used in Thailand. The usage of the decanter system is essential for waste reduction in mills.

The three phase decanter system separates sludge from oil clarifier into oil, sludge and oil palm decanter cake (OPDC). The sludge which contains a low concentration of pollutant can be easily treated and recycled into the mill for fresh fruit bunch extraction. In practice, the mills neglect and leave the OPDC for natural degradation. According to the waste to wealth through biotechnology concept, OPDC, which is composed of cellulose and hemicellulose can be utilized as the substrate for production of cellulase, polyoses (lignocellulosic biosugar), biofuel and fine chemicals. Currently, researchers use the OPDC in a mixture in biocompost and animal feed [4,5].

Basically, hydrolysis of lignocellulosic material into simple sugars is performed *via* enzymatic, chemical or

Separation and purification of polyhydroxyalkanoates from newly isolated *Comamonas* sp. EB172 by simple digestion with sodium hydroxide

By: Mitra Mohammadi, Alumni PhD student

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Separation and Purification of Polyhydroxyalkanoates from Newly Isolated *Comamonas* sp. EB172 by Simple Digestion with Sodium Hydroxide

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A simple, mild, and effective process for the recovery of intracellular polyhydroxyalkanoate from a newly isolated gram-negative wild-type bacteria *Comamonas* sp. EB172 was developed using sodium hydroxide. Various parameters such as sodium hydroxide concentration, digestion time, and temperature were examined for their effect on polyhydroxyalkanoate recovery. The results showed that polyhydroxyalkanoate with 88.6% purity and 96.8% recovery yield were obtained by incubating the dried cells with 0.05 M sodium hydroxide at 4°C for 1 h, followed by purification steps using ethanol and water. Removal of non-polymeric cellular materials from the *Comamonas* sp. EB172 was increased under alkaline solution as a result of enhanced cell wall permeability. In addition, the presence of glycerol in the polymer suspension proved that saponification of the lipid layer in the bacterial cell wall occurred due to sodium hydroxide reaction.

Keywords *Comamonas* sp. EB172; downstream processing; NaOH digestion; polyhydroxyalkanoates; purification; recovery

INTRODUCTION

Nowadays, there has been considerable interest for usage of biodegradable polymers such as polyhydroxyalkanoates (PHAs) to address concerns over the petrochemical-based plastic waste accumulation. However, extensive application of PHA is hindered mainly by their high production cost compared to conventional plastics (1,2). It has been reported that the upstream and downstream processing of bacterial PHA are the key cost factors in the fermentation system (3). Therefore, application of a suitable strain which can utilize the low-cost carbon sources can significantly decrease the PHA production cost. Towards the development of zero discharge strategy for the palm oil industry, it was indicated that organic acid derived from acidogenic fermentation of palm oil mill effluent (POME) is a promising and potential cheap substrate for PHA production (4). In order to lower down the PHA production cost, the development of a local strain which is able to produce the copolymers from mixed organic acid, would be an attractive proposition. As an effort, newly isolated *Comamonas* sp. EB172 was found to be a suitable microbe for industrial PHA production because of its capability to accumulate poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HB)] copolymers from mixed organic acids (5). Over the fermentation process itself, downstream processing also has a significant effect on the economics of PHA production. Therefore, it is important to develop a novel, efficient, and less polluted process for PHA recovery with high purity and recovery yield from microorganism cells.

Different methods are available in literature to recover the PHA. However, the selection of a suitable extraction method depends on factors such as the cell type, the composition of the growth medium, the presence of certain chemical compounds in the environment, the length of

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Brown rice as a potential feedstuff for poultry

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Primary Audience: Researchers, Nutritionists, Feed Manufacturers

SUMMARY

Rice, especially brown rice, has the potential to replace corn as a feedstuff for poultry. It is an inexpensive local feed source with high availability and low production and processing costs. Two local varieties of brown rice, MR239 and MR257, were investigated for use as feedstuffs in the poultry industry, including their composition and TME values (using the force-feeding technique). The MR239 and MR257 varieties of brown rice contained nutrients such as CP, fat, ash, and carbohydrates. The energy content and amino acid profile of MR239 and MR257 are reported. The nonstarch polysaccharides in MR239 and MR257 consisted of CF, NDF, ADF, and acid detergent lignin. The β-glucan and arabinoxylan contents in MR239 and MR257 were determined. Both varieties of brown rice were found to be potential sources of feed for poultry.

Key words: brown rice, nonstarch polysaccharide, nutrient composition, poultry, true metabolizable energy

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DESCRIPTION OF PROBLEM

Poultry is one of the most advanced industries in the livestock sector because it involves processes such as the production of poultry meat, eggs, and feeds; feed formulation; the feeding process; and the import and export of poultry products or the poultry themselves. This industry is among the most efficient food commodity groups supplying the fast-growing human population, especially through the consumption of chicken. To produce poultry products, the feedstuff must first be produced as an energy and protein source for poultry. Natural resources from plant materials are the most significant ingredients in poultry feedstuffs because they are excellent sources of protein for monogastric animals. The feedstuffs available for poultry include corn [1], soybeans [2], barley [3], wheat [4], and rice or rice by-products [5]. However, the choice of feedstuff is dependent on its availability, quality, and overall cost of production.

To provide complete nutrition for poultry, feed compounds are mostly based on a mixture of corn and other ingredients. In Malaysia, a search for local sources of poultry feed has been underway to replace expensive imported feedstuffs, mainly corn and soybeans. The average amount of corn imported for the Malaysian feed industry was reported to be 2.879 million tons per year from 2002 to 2007 [5]. Alternatively, corn has been produced locally. However, corn production has been unsuccessful because of

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Bioconversion of glycerol for bioethanol production using isolated *Escherichia coli* SS1

By: Sheril Norliana Suhaimi, Master student

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BIOCONVERSION OF GLYCEROL FOR BIOETHANOL PRODUCTION USING ISOLATED *ESCHERICHIA COLI* SS1

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ABSTRACT

Bioconverting glycerol into various valuable products is one of glycerol's promising applications due to its high availability at low cost and the existence of many glycerol-utilizing microorganisms. Bioethanol and biohydrogen, which are types of renewable fuels, are two examples of bioconverted products. The objectives of this study were to evaluate ethanol production from different media by local microorganism isolates and compare the ethanol fermentation profile of the selected strains to use of glucose or glycerol as sole carbon sources. The ethanol fermentations by six isolates were evaluated after a preliminary screening process. Strain named SS1 produced the highest ethanol yield of 1.0 mol: 1.0 mol glycerol and was identified as *Escherichia coli* SS1 Also, this isolated strain showed a higher affinity to glycerol than glucose for bioethanol production.

Key words: Glycerol; anaerobic fermentation; bioethanol; *Escherichia coli* SS1

INTRODUCTION

Glycerol is also known as 1,2,3-propanetriol or glycerin. Glycerol has a wide range of applications, including those in the paint, cosmetic, food and pharmaceutical industries, in addition to its use as feedstock for the production of several chemicals. Glycerol can be produced by microbial fermentation and chemical synthesis (26). In addition, it is produced as a by-product during both soap manufacturing and biodiesel production. Recently, the low price of glycerol has been reported due to an abundance of glycerol being generated from the biodiesel industry, and because of this industry's rapid growth, the glycerol generation is also expected to increase (7). Excess glycerol may subsequently result in higher biodiesel production cost if this by-product is not properly handled or disposed of (9).

In response to the increased availability of glycerine in the commercial market, the bioconversion of glycerol into valuable

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ORIGINAL ARTICLE

Efficient Polyhydroxyalkanoate Recovery from Recombinant Cupriavidus necator by Using Low Concentration of NaOH

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Abstract

An efficient method for recovering polyhydroxyalkanoate (PHA) from bacterial cell was developed by using a low concentration of sodium hydroxide (NaOH). In this study, the effectiveness of low-concentration NaOH on PHA recovery from recombinant Cupriavidus necator was investigated by testing several NaOH concentrations, in relation to digestion time and reaction temperature. Gas chromatography (GC) analysis showed that PHA with more than 96% purity and recovery yield can be achieved after the recovery process which involved treatment of lyophilized cells with 0.05 M NaOH at 4 °C for 3 h. The GC analysis was supported by transmission electron microscope images and associated with considerable release of protein after NaOH addition. The recovery process developed herein was found effective in recovering PHA even from cells with low PHA content, with only 13% reduction in molecular weight (M_w). Ultimately, the present method could be an alternative to the PHA recovery by organic solvents, with added values such as being simple, nontoxic and environmental friendly.

Key words: Polyhydroxyalkanoate; bioplastics; recombinant Cupriavidus necator; NaOH recovery; downstream processing

Introduction

As an effort to prevent the harmful environmental effect posed by nonbiodegradable, petrochemical-based plastics, the development of environmental friendly biodegradable plastics such as polyhydroxyalkanoate (PHA) has drawn much attention throughout the world (Choi and Lee, 2004; Jung et al., 2005). However, their production at industrial scale is limited by the high cost compared with conventional petroleum-based plastics. Anton et al. (1995) proposed some key strategies to reduce the PHA production cost; for example, by improving reactor productivity, employing cheaper substrates, and also developing economical purification and recovery schemes. Therefore, developing a process that allows a simple, efficient, and less polluted recovery of PHA could be an attractive proposition (Chen et al., 1999).

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PHA recovery using organic solvents, chemical reagents, or surfactants has the drawbacks of high cost and serious pollution. These methods are, therefore, difficult to be commercialized (Xuejun, 2006). Thus, developing a cleaner recovery system without the use of solvent is essential to eliminate the usage of halogenated solvents such as chloroform. It has been found that some of the chemical recovery treatments such as alkaline pH shock, anionic detergents such as sodium dodecyl sulfate (SDS), and EDTA permit the partial release of intracellular products (Harrison et al., 1991). Strazzullo et al. (2008) reported a simplified and effective method for direct PHA recovery from humid biomass of Halomonas campaniensis using SDS. Apart from SDS, NaOH is also used to recover PHA. NaOH causes saponification of the lipid layer in the cell wall and increases the cell permeability that helps release the non-polymeric protein material without rupturing the cells (Harrison et al., 1991; Jacquel et al., 2008). Choi and Lee (1999) reported the digestion of a non-PHA biomass of recombinant E. coli in 0.1 M NaOH at 30 °C for 1 h. More than 90% purity and recovery yield was obtained from the cells with 77% polyhydroxybutyrate (PHB) content. A highly pure PHA can be obtained using a concentrated alkaline

Improved cellulase production by *Botryosphaeria rhodina* from OPEFB at low level of moisture condition through statistical optimization

By: Ezyana Kamal Bahrin, PhD student

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IMPROVED CELLULASE PRODUCTION BY *Botryosphaeria rhodina* FROM OPEFB AT LOW LEVEL MOISTURE CONDITION THROUGH STATISTICAL OPTIMIZATION

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□ *The response surface method was applied in this study to improve cellulase production from oil palm empty fruit bunch (OPEFB) by* Botryosphaeria rhodina. *An experimental design based on a two-level factorial was employed to screen the significant environmental factors for cellulase production. The locally isolated fungus* Botryosphaeria rhodina *was cultivated on OPEFB under solid-state fermentation (SSF). From the analysis of variance (ANOVA), the initial moisture content, amount of substrate, and initial pH of nutrient supplied in the SSF system significantly influenced cellulase production. Then the optimization of the variables was done using the response surface method according to central composite design (CCD).* Botryosphaeria rhodina *exhibited its best performance with a high predicted value of FPase enzyme production (17.95 U/g) when the initial moisture content was at 24.32%, initial pH of nutrient was 5.96, and 3.98 g of substrate was present. The statistical optimization from actual experiment resulted in a significant increment of FPase production from 3.26 to 17.91 U/g (5.49-fold). High cellulase production at low moisture content is a very rare condition for fungi cultured in solid-state fermentation.*

Keywords cellulase, optimization, response surface methodology, solid-state fermentation

INTRODUCTION

Cellulases have great potential and broad applications to be employed in the industrial sector. Cellulases have been used extensively in the textile

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Utilization of Glucose Recovered by Phase Separation System from Acid-hydrolysed Oil Palm Empty Fruit Bunch for Bioethanol Production

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ABSTRACT

Oil palm empty fruit bunch (OPEFB) is one the most abundant lignocellulosic wastes produced throughout the year in the palm oil industry. A new process of separating lignocellulose components after acid hydrolysis (known as phase separation system) has been previously developed, by which lignin and carbohydrate can be completely and rapidly separated in 60 minutes between 25 and 30°C. In this process, cellulose is completely hydrolyzed to oligosaccharides and remains in the acid phase. The maximum glucose yield of 53.8% was obtained by hydrolysis, with 4% acid after autoclaving at 121°C for 5 minutes. This work focused on the separation of monosaccharide (glucose) from cellulose fraction, which was subsequently used as a substrate for ethanol production. For this purpose, different types of nitrogen sources were evaluated, with yeast extract as the best nitrogen source (93% of theoretical yield) as compared to palm oil mill effluent (POME) and sludge powder for the growth of acid tolerant *Saccharomyces cerevisiae* ATCC 26602. Batch and repeated batch fermentation of *S. cerevisiae* ATCC 26602 using OPEFB hydrolysate gave 0.46 g glucose g ethanol^{-1}, representing 87% of theoretical yield with a productivity of about 0.82 g^{-1} l^{-1} h^{-1} and 0.48 g glucose g ethanol^{-1},representing 89% of theoretical yield with productivity of about 2.79 g^{-1} l^{-1} h^{-1}, respectively.

Keywords: Bioethanol, oil palm empty fruit bunch, phase separation system, acid hydrolysis, glucose

INTRODUCTION

Lignocellulose waste material, such as oil palm empty fruit bunch (OPEFB), is well known for its potential as a renewable resource for the production of food, feed and fine chemicals. Approximately 15 million tonnes of OPEFB is generated annually by palm oil mills in Malaysia (Rahman *et al*., 2006). In practice, OPEFB is burnt in incinerator to obtain bunch ash or is dumped for mulching in the oil palm plantation. With technologies such as diluted acid hydrolysis and enzymatic hydrolysis, cellulose from waste

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Evaluation of factors affecting polyhydroxyalkanoates production by *Comamonas* sp. EB172 using Central composite design

By: Noor Azman Mohd Johar, Master student

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Evaluation of Factors Affecting Polyhydroxyalkanoates Production by *Comamonas* sp. EB172 Using Central Composite Design

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ABSTRACT

Aims: Statistical approach, central composite design (CCD) was used to investigate the complex interaction among temperature (25-37 °C), initial medium pH (5-9), inoculum size (4-10 % (v/v)), concentration of $(NH_4)_2SO_4$ (0-1 g/L) and concentration of mixed organic acids (5-10 g/L) in the production of polyhydroxyalkanoates by *Comamonas* sp. EB172.
Methodology and Results: Mixed organic acids derived from anaerobically treated palm oil mill effluent (POME) containing acetic:propionic:butyric (ratio of 3:1:1) were used as carbon source in the batch culture of *Comamonas* sp. EB172 to produce polyhydoxyalkanoates (PHAs). The analysis of variance (ANOVA) showed that all five factors were significantly important in the batch fermentation by shake flask with a P value of less than 0.001. The optimal temperature, initial medium pH, inoculum size, concentration of $(NH_4)_2SO_4$ and concentration of mixed organic acids were 30 °C, 7.04, 4.0 % (v/v), 0.01 g/L and 5.05 g/L respectively.
Conclusion, significance and impact of study: Optimization of the production medium containing mixed organic acids has improved the PHA production for more than 2 folds. Under optimal condition in the shake flask fermentation, the predicted growth is 2.98 g/L of dry cell weight (DCW) with 47.07 wt % of PHA content. The highest yield of PHA was 0.28 g of PHA per g mixed organic acids.

Keywords: optimization, central composite design, *Comamonas* sp. EB172, polyhydroxyalkanoate, response surface methodology

INTRODUCTION

Studies on biodegradable plastics derived from microbes have been carried out for many years. However, the production cost is still a barrier to the use of biodegradable plastics, eg. polyhydroxyalkanoates (PHAs). Hence, the solution will lie upon low-cost options such as using cheaper carbon sources, efficient fermentation, and economical recovery process for PHA production (Grothe *et al.* 1999, Patwardhan *et al.* 2004). The cost for substrate may contribute 30 – 60 % of the overall PHAs production cost (Zakaria *et al.* 2010a). Therefore, there have been several studies on the utilization of industrial by-producrs and agricultural wastes as alternative carbon sources for PHAs production (Hassan *et al.* 1997, Mumtaz *et al.* 2008). Utilization of biomass or renewable resource for PHAs production would be viable depending on the availability of the biomass and the technology involved in converting the complex materials into PHAs.

Palm oil mill effluent (POME) has been the most abundant and polluting agricultural wastewater in Malaysia (Alam *et al.* 2008). Hassan *et al.* (1997) studied on the production of organic acids from partial anaerobically treated POME, and it was revealed that the organic acids derived from POME could be use as carbon source for PHAs production by *Rhodobacter sphaeroides*. There were some other reports on the use of organic acids as substrate for PHA production by single and mixed culture (Chakraborty *et al.* 2009, Albuquerque *et al.* 2011). The feeding strategies of organic acids in the fermentation contributed to the variations of PHAs accumulation in the cell. Apart from feeding strategy, PHA accumulation can also be triggered under nutrient-limited conditions such as limited nitrogen, oxygen, sulphur, magnesium or phosphorus in the presence of excess carbon (Annuar *et al.* 2007; Sharma *et al.* 2007). Besides, the ration of carbon to nitrogen (C/N ratio) in medium formulation is an important factor with respect to the nutritional needs for both microbial biomass and PHA accumulation. . The effects of other factors such as temperature, initial medium pH, inoculum size and concentration of $(NH_4)_2SO_4$ on PHAs production by using various types of

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Economic analysis of biogas and compost projects in a palm oil mill with clean development mechanism in malaysia

By: Tatsuya Yoshizaki, PhD student

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CASE STUDY

Economic analysis of biogas and compost projects in a palm oil mill with clean development mechanism in Malaysia

Tatsuya Yoshizaki · Yoshihito Shirai · Mohd Ali Hassan · Azhari Samsu Baharuddin · Nik Mustapha Raja Abdullah · Alawi Sulaiman · Zainuri Busu

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Abstract This article is a case study to compare the economic viabilities of biogas generation and compost projects in a palm oil mill in Malaysia with and without clean development mechanism (CDM). Biogas is captured from anaerobic ponds or digester tanks treating palm oil mill effluent (POME) and converted to green renewable electricity for grid connection, while compost is produced from the shredded empty fruit bunch and raw untreated POME. The both technologies were compared by considering the changes of the materials flow and energy balances. A palm oil mill with a capacity of 54 t fresh fruit bunch per hour has the potential to produce either 6.9 GWh of electricity from biogas or fertilizer equivalent to 488 t of nitrogen, 76 t of phosphorus and 1,065 t of potassium per year. The economic analysis for 10 years project term analysis indicated that CDM gave a significant impact and ensured economic viability for both projects with 25 % of internal rate of return (IRR), RM 12.39 million of net present value (NPV) and 3.5 years of payback period (PBP)

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Enhanced biogas production from palm oil mill effluent supplemented with untreated oil palm empty fruit bunch biomass with a change in the microbial community

By: Ahmad Amiruddin Mohd Ali,
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Cited Journal

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Note

Enhanced Biogas Production from Palm Oil Mill Effluent Supplemented with Untreated Oil Palm Empty Fruit Bunch Biomass with a Change in the Microbial Community

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The biogas and biomethane production in a 50 litre closed stirred tank anaerobic bioreactor treating palm oil mill effluent (POME) supplemented with oil palm biomass in the form of oil palm empty fruit bunch (OPEFB) under mesophilic condition was evaluated. With OPEFB supplementation, the biogas and biomethane generation increased by 63% and 52%, respectively. During this process, we found changes in the OPEFB morphology and microbial community through microbiota analysis using 16S rRNA gene clone library method, after OPEFB was added, suggesting that the increased biogas and biomethane production would be due to enhanced lignocellulosic biomass degradation.

Key words: Biogas, biomethane, microbial community, oil palm empty fruit bunch (OPEFB), palm oil mill effluent (POME)

1. Introduction

Biogas and biomethane fermentation of lignocellulosic biomass have previously been attempted but with varied success due to the rate limiting hydrolysis of the materials which affects both fermentation rate and extent [1]. Without any recourse to pretreatments, the presence of lignin is one of the major drawbacks in lignocellulosic fermentation, as it renders the lignocellulose resistant to biological and chemical degradation, thus hampering biogas and biomethane productivity [2, 3]. The palm oil industry represents the largest agro-economic sector in Malaysia. The industry produces abundant biomass wastes with 19.8 million tonnes of oil palm empty fruit bunch (OPEFB) and more than 50 million tonnes of palm oil mill effluent (POME) being generated from over 400 mills in Malaysia annually [4, 5]. Biogas production from anaerobic fermentation of POME has been extensively studied [6–10]. Recently, co-digestion of POME with OPEFB pretreated with alkali, acid and steam were reported to increase biogas productivity in thermophilic POME fermentation [11, 12]. However, presently no study has been carried out to identify the changes in additional OPEFB and microbial consortia related to increased biogas and biomethane productivity in such systems. This study deals with the biogas and biomethane enhancement by OPEFB supplementation as well as the changes in the OPEFB morphology and microbial community during the fermentation.

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2. Materials and methods

2.1 POME

Raw POME was collected from Seri Ulu Langat Palm Oil Mill, Dengkil, Selangor, Malaysia, and preserved in < 4℃ prior to use.

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Review

Sustainable production of polyhydroxyalkanoates from renewable oil-palm biomass

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ABSTRACT

With rapid industrialization and increasing per-capita consumption of conventional plastics, there is a growing need for the development of bio-based materials from renewable resources to reduce the environmental footprint of plastic production. Oil palm biomass, which is the largest biomass in Malaysia, has tremendous potential as a primary or secondary feedstock for polyhydroxyalkanoate (PHA) production. PHA production can be made more competitive and sustainable by using oil palm biomass effluent and residues that are available at the factories. The oil palm biomass as non-food biomass from the mill is a great strategy towards zero discharge in palm oil industry by combining wastewater treatment system for mixed organic acids production together with PHA production from the clarified organic acids. Hence, several oil palm biomass have been explored and considered as sustainable promising sources for PHA production in future. Solid waste such as oil palm frond and oil palm empty fruit bunch can be used as sugar based substrate. In addition, palm oil mill effluent can be effectively converted to mixed organic acids and glycerol as wastes from palm oil based biodiesel processing plant are suitable for PHA production. The successful bioconversion and utilization of oil palm biomass can reduce the production costs of PHAs and minimize greenhouse-gas emissions. This article provides an overview of various types of biomass generated by the palm oil industry and describes their bioconversion into PHAs by various PHA producers. Future perspectives and challenges for the commercialization of PHAs produced using oil palm biomass are also discussed.

1. Introduction

Bioplastics are plastics derived from renewable resources; however, not all bioplastics are biodegradable. Table 1 lists the commercially available types of bioplastics and biodegradable plastics. PHAs are more favorable when compared with chemically synthesized polymers and starch-based polymers, due to their inherent biodegradability, sustainability and

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Improved Economic Viability of Integrated Biogas energy and Compost Production for Sustainable Palm Oil Mill Management

By: Tatsuya Yoshizaki, PhD student

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Improved economic viability of integrated biogas energy and compost production for sustainable palm oil mill management

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ABSTRACT

This paper proposes a new approach for integrated technology of biogas energy and compost production for a palm oil mill. This study evaluated the economic viability based on the changes of materials flow and energy balance when a palm oil mill introduces this approach. A palm oil mill processing 54 tonnes fresh fruit bunch (FFB) per hour has the potential to produce 8.2 GWh per year of electricity using biogas captured during anaerobic treatment of palm oil mill effluent (POME). Compost production using shredded empty fruit bunch (EFB) and POME anaerobic sludge obtained from the anaerobic digester is equivalent of 579 tonnes, 151 tonnes and 761 tonnes per year of nitrogen, phosphorus and potassium respectively. The integrated technology is a more attractive solution compared to the case when the palm oil mill installs either biogas energy or compost technology individually. The result of economic analysis suggests that this integrated approach is the most economically effective in comparison to the other two cases. Interestingly, even without Clean Development Mechanism (CDM), the integrated technology can still be economically viable, which can be a good solution for sustainable palm oil industry management in the near future.

1. Introduction

Palm oil industry is well known as a significant agricultural industry in terms of economic benefit for several tropical countries. In Malaysia, palm oil industry is the fourth largest contributor to the national economy, and currently accounts for Ringgit Malaysia (RM) 1889 which is 8 percent of the national gross national income (GNI) per capita (PEMANDU, 2010). However, palm oil mills have created environmental problems due to large quantities of polluted waste materials which are discharged to the environment. Palm oil mill effluent (POME) is highly polluted industrial waste water with a high organic content with 113,000 ppm of chemical oxygen demand (COD) and 35,000 ppm of biochemical oxygen demand (BOD) (Baharuddin et al., 2010a). POME has been recognized as a main contributor of green house gas (GHG) emission which consists of methane from open pond or tank treatment system (Shirai et al., 2003). Empty fruit bunch (EFB) is the residue after the milling process of fresh fruit bunch (FFB). It is a common practice to dispose the EFB into plantation for nutrients recycling, however it leads to pollution problems such as eutrophication and an increase of toxicity in the soil (Stichnothe and Schuchardt, 2010).

Over the past decade, the palm oil industry has developed proper utilizations of these by-products in order to ensure sustainable economic growth, but they are still inefficient in terms of cost (Mumtaz et al., 2010; Tan et al., 2010). Clean Development Mechanism (CDM) launched by Kyoto protocol under the United Nations Framework Convention on Climate Change (UNFCCC) is one of the platforms which aims to reduce GHG emission through an international corporation to ensure the project's economic feasibility with certified emission reduction (CER) credit trading. There are CDM projects such as utilizing biogas from POME as renewable energy (UNFCCC, 2008a), and composting of EFB with raw POME (UNFCCC, 2007). The introduction of either technology individually is not feasible without CDM at project design document (PDD). Meanwhile, there is no economic evaluation on the integration of biogas energy and compost technologies with POME

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Biovanillin from agro wastes as an alternative food flavour

Nur Ain Zamzuri and Suraini Abd-Aziz*

Abstract

This review provides an overview of biovanillin production from agro wastes as an alternative food flavour. Biovanillin is one of the widely used flavour compounds in the foods, beverages and pharmaceutical industries. An alternative production approach for biovanillin as a food flavour is hoped for due to the high and variable cost of natural vanillin as well as the limited availability of vanilla pods in the market. Natural vanillin refers to the main organic compound that is extracted from the vanilla bean, as compared to biovanillin, which is produced biologically by microorganisms from a natural precursor such as ferulic acid. Biovanillin is also reviewed as a potential bioflavour produced by microbial fermentation in an economically feasible way in the near future. In fact, we briefly discuss natural, synthetic and biovanillin and the types of agro wastes that are useful as sources for bioconversion of ferulic acid into biovanillin. The subsequent part of the review emphasizes the current application of vanillin as well as the utilization of biovanillin as an alternative food flavour. The final part summarizes biovanillin production from agro wastes that could be of benefit as a food flavour derived from potential natural precursors.

Keywords: biovanillin; bioconversion; agro wastes; ferulic acid; food flavour

INTRODUCTION

Vanillin is a plant secondary metabolite and the main constituent of natural vanilla, which acts as an important flavouring and aromatic component used worldwide. It is extracted from vanilla beans obtained from the tropical vanilla orchid, principally *Vanilla planifolia* and, to a lesser extent, *V. tahitiensis* and *V. pompona*. Based on the worldwide vanillin market, of 10 000 tons of vanillin per year less than 0.5% is isolated from vanilla pods.[1] The remainder is fulfilled by chemically synthesized vanillin derived from lignin or fossil hydrocarbons like guaiacol.[2] Environmental friendly routes have been proposed for obtaining vanillin through microbial bioconversion by bacteria and fungi using eugenol and ferulic acid as substrates.[3–5] According to the Food and Agriculture Organization of the United Nations,[6] the main producers of vanilla are Indonesia, Madagascar and China, as summarized in Table 1. Traditionally, vanilla flavour in ice cream, yoghurt and cakes comes from vanillin extracted from the pods of a tropical orchid. It is an expensive flavouring compound and has a strong demand; thus the food industry has been searching for alternative sources. Recently, biotechnology application was found to be a potential source for the bioproduction of vanillin, which is called biovanillin. This is due to the increasing demand for bioflavours that are regarded as natural by FDA and European legislation; thus production of vanillin from raw materials via biotechnology routes creates a great potential.[7,8]

Through biotechnology, a strain of soil bacteria has been developed by the Institute of Food Research in the UK, which can make vanillin from a material found in agricultural waste involving the *Pseudomonas* bacterium genus. It has the ability to transform ferulic acid from agricultural waste into biovanillin. Currently, a synthetic vanilla flavour synthesized chemically dominates the market for about 80% of total vanillin production. Researchers now have hopes for the new biovanillin that can be regarded as natural, involved in a non-chemical process and which can be further utilized in the near future.[1]

BIOVANILLIN

Biovanillin refers to vanillin produced from natural resources of biobased materials. Price variation and high consumer demand for natural flavours have moved towards vanillin production from natural resources. Literature reports are available on the production of natural flavours using various biotechnological techniques.[9–13] Vanillin production by applying biotechnological techniques such as microbial bioconversion of substrates like eugenol or ferulic acid is considered an alternative and economically feasible way of obtaining vanillin. Thus it has gained much interest in recent years due to European and US legislation already classifying the product as 'natural'.[1,12,14–16] The main factors that stimulated a trend towards production of bioflavours derived from natural precursors are the high variation between prices of natural and synthetic vanillin as well as the increase in demand for natural and healthy flavours. As a result, the bioproduction of flavours and fragrances has been critically analysed by respective researchers.[9,11–13,17]

COMPARISON BETWEEN NATURAL VANILLIN, SYNTHETIC VANILLIN AND BIOVANILLIN

Vanillin (4-hydroxy-3-methoxybenzaldehyde) with the molecular formula $C_8H_8O_3$, as shown in Fig. 1, is an important organic compound for the characteristic flavour and smell of vanilla. The functional groups of vanillin are aldehyde, ether and phenol, and

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Recovery of glucose from residual starch of sago hampas for bioethanol production.

By: Dayang Salwani Awang-Adeni, PhD Student

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Research Article

Recovery of Glucose from Residual Starch of Sago Hampas for Bioethanol Production

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Lower concentration of glucose was often obtained from enzymatic hydrolysis process of agricultural residue due to complexity of the biomass structure and properties. High substrate load feed into the hydrolysis system might solve this problem but has several other drawbacks such as low rate of reaction. In the present study, we have attempted to enhance glucose recovery from agricultural waste, namely, "sago hampas," through three cycles of enzymatic hydrolysis process. The substrate load at 7% (w/v) was seen to be suitable for the hydrolysis process with respect to the gelatinization reaction as well as sufficient mixture of the suspension for saccharification process. However, this study was focused on hydrolyzing starch of sago hampas, and thus to enhance concentration of glucose from 7% substrate load would be impossible. Thus, an alternative method termed as cycles I, II, and III which involved reusing the hydrolysate for subsequent enzymatic hydrolysis process was introduced. Greater improvement of glucose concentration (138.45 g/L) and better conversion yield (52.72%) were achieved with the completion of three cycles of hydrolysis. In comparison, cycle I and cycle II had glucose concentration of 27.79 g/L and 73.00 g/L, respectively. The glucose obtained was subsequently tested as substrate for bioethanol production using commercial baker's yeast. The fermentation process produced 40.30 g/L of ethanol after 16 h, which was equivalent to 93.29% of theoretical yield based on total glucose existing in fermentation media.

1. Introduction

In recent years, there has been an increasing trend towards more efficient utilization of agro-industrial by-products for conversion to a range of value-added bioproducts, including biofuels, biochemicals, and biomaterials [1]. As an initiative, this study was formulated to utilize sago hampas as an alternative substrate for glucose production, which will be used as feedstock for bioethanol production. Sago hampas is a starchy lignocellulosic by-product generated from pith of *Metroxylon sagu* (sago palm) after starch extraction process [2]. *Metroxylon sagu* Rottb. is an increasingly important socioeconomic crop in Southeast Asia whereas New Guinea is believed to be its center of diversity [3]. In Malaysia, the state of Sarawak is recognized as the largest sago-growing areas, which is currently the world's biggest exporter of sago starch, exporting annually about 44,000 t of starch mainly to Peninsular Malaysia, Japan, Singapore, and other countries [4]. The isolation of sago starch involves debarking, rasping, sieving, settling washing, and drying [2]. However, the mechanical process currently employed to extract sago starch is inefficient and often fails to dislodge residual starch embedded in the fibrous portion of the trunks [3]. On dry basis, sago hampas contains 58% starch, 23% cellulose, 9.2% hemicellulose, and 4% lignin [5]. Approximately, 7 t of sago hampas is produced daily from a single sago starch processing mill [6]. Currently, these residues which are mixed together with wastewater are either washed off into nearby streams or deposited in the factory's compound. These circumstances, in time, may potentially lead to serious environmental problems.

Several studies on the utilization of sago hampas as animal feed, compost for mushroom culture, for hydrolysis to confectioners' syrup, particleboard manufacture, and as substrate for local microbes to produce reducing sugars and

In Press

Polyhydroxyalkanoate synthesis by recombinant *Escherichia coli* JM109 expressing pha biosynthesis genes from *Comamonas* sp. EB172.

By: Yee Lian Ngit, PhD student

Impact Factor 2.040

Microbial & Biochemical Technology

Yee et al., J Microb Biochem Technol 2012, 4:4
http://dx.doi.org/10.4172/1948-5948.1000079

Research Article Open Access

Polyhydroxyalkanoate Synthesis by Recombinant *Escherichia coli* JM109 Expressing PHA Biosynthesis Genes from *Comamonas* sp. EB172

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[4]*Ecobiomaterial Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia*
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Abstract

Recombinant *Escherichia coli* JM109 harbouring the polyhydroxyalkanoate (PHA) biosynthesis gene (phaCABco) of *Comamonas* sp. EB172, an acid tolerant microbe, was examined for the production of PHAs from various carbon sources. The study demonstrated that the recombinant *E. coli* JM109 had the potential to utilize both sugar- and acid-based carbon sources, for the biosynthesis of both poly(3-hydroxybutyrate) P(3HB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) P(3HB-co-3HV) copolymers. In the shake flask experiments, the strain was capable of producing P (3HB-co-3HV) copolymer from mixed organic acids, and higher productivities were obtained using glucose compared to mixed acids. However, PHA accumulation was found to be similar, regardless of the carbon source used. Nitrogen supplementation in the medium was found to improve the cell dry weight, but negatively affected the 3HV formation in copolymer production. Maximum 3HV monomer (3 mol%) was obtained with C/N 42.1, using mixed acids as the carbon source. In the 2L bioreactor, the productivity and yield based on substrate utilization coefficient were found to be 0.16 g PHA/(L.h) and 0.41 g PHA/g substrate under C/N around 75, using 20 g/L glucose and 0.5 g/L ammonium sulphate, respectively. The polymer produced by the recombinant strain had molecular weight in the range of 8.5 x 105 to 1.4 x 106 Da. Overall, the ability of the recombinant *E. coli* JM109 to utilize both glucose and mixed acids, has widened its substrate selection for fermentation, including the opportunity to use renewable biomass.

Keywords: Recombinant; PhaCABco; Mixed acids; Polyhydroxyalkanoate; *Comamonas* sp. EB172

Introduction

Polyhydroxyalkanoates (PHAs) are energy storage, hydrophobic granules that can be accumulated by many microorganisms [1-4]. PHAs are biodegradable, biocompatible thermoplastics, and hence, these biopolyesters are not only the potential alternative candidates for recalcitrant synthetic plastics, but also present long-term benefits for environmental pollution issues. However, the high cost for PHA production compared to the availability of low-cost petroleum-based plastic, is the major obstacle to commercialize these biosynthesized PHAs [5,6]. The most significant factor for the high production cost of PHAs is the fermentation process, which is mainly due to the cost of raw material as well as the recovery process [7]. A great deal of effort has been made to reduce the production cost by employing superb microbial strains, as well as, developing fermentation and recovery process with cheap carbon sources and non-halogenated solvents, respectively [7-9].

Economic biotechnological PHA formation largely depends on the choice of productive microorganisms and their culture condition. Recently, in our continuous effort of utilizing mixed organic acids derived from palm oil mill effluent (POME) for PHA production, we have produced several reports on the isolation, biosynthesis and characterization of both P(3HB) and P(3HB-co-3HV) by a local, acid tolerant strain of *Comamonas* sp. designated as *Comamonas* sp. EB172 [10-14]. While developing a suitable fermentation strategy to feed mixed acids in its original form (keeping identical ratio as obtained), our focus was also to develop a non-halogenated PHA recovery system, at the end of fed-batch fermentation [10,15,16]. However, *Comamonas* sp. EB172 is known to utilize only fatty acids but not glucose or fructose [13]. Therefore, the three genes involved in the biosynthesis of PHAs by *Comamonas* sp. EB172 were cloned and characterized [17]. Meanwhile, the PHA biosynthesis genes of *Comamonas* sp. EB172 had also been cloned and heterologously expressed for its functionality, to demonstrate the ability of the isolated biosynthesis gene on PHA production in *E. coli* JM109 host.

Recombinant *E. coli* has commonly been employed for PHA production due to its convenience for genetic manipulation, fast growth, high cell density cultivation and ability to utilize inexpensive carbon sources. The strain has been reported to produce short-chain-length (scl) polyesters containing C4 or C5 monomers, such as P (3HB), poly(3-hydroxyvalerate) P(3HV), poly(4-hydroxybutyrate) P(4HB) homopolymer, or the P(3HB-co-3HV) copolymer [18] and P(3HB-co-4HB) [19]. Nevertheless, recombinant E. coli, containing the phaC1 gene from *Pseudomonas aeruginosa*, was able to produce medium-chain- length (mcl) PHAs, having C6 to C14 monomers including homopolymers of 3-hydroxyhexanoate (3HHx), 3-hydroxydodecanoate (3HDD) and terpolymer poly(3-hydroxybutyrate-co-3-

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Title : Polyhydroxyalkanoate Recovery

Inventor: Mohd Ali Hassan, Mitra Mohammadi, Phang Lai Yee, Hidayah Ariffin, Yee Lian Ngit, Yoshihito Shirai.

Abstract:
The present invention is relates to a method of recovery of polyhydroxyalkanoates (PHA) from biomass material, which would be simple and an environmental friendly process for generation of biodegradable polymers/plastics, from biomass materials. More specifically, the present invention is directed to a freezing and thawing based method for intracellular PHA recovery from bacterial cells that is comparable to other known PHA recovery methods in terms of its recovery yield and purity attained and advantageously thus, is further directed to providing for clean and purified PHA while keeping the recovery method reproducible, free from harsh halogenated solvents and importantly also energy efficient. The process of the present invention does not adversely affect the quality of recovered PHA and thus has potential to serve as an effective alternative to the conventional solvent extraction processes for PHA recovery in being environmental friendly, non-toxic, effective, simple and easy to operate that circumvents the use of any specified or advanced equipment/machine for its accomplishment

IP Status: Pending Patent
Filing Date: 30/10/2012
Application No: PI 2012700848
Country Filing: Malaysia
Applicant: Universiti Putra Malaysia (UPM)

Title : Oil Palm Biomass Powder and Method For Producing The Same and Biomass Composite Molded Article and Method For Producing The Same

Inventor: Mohd Ali Hassan, Yoshihito Shirai.

Abstract:
The present invention relates to a technique of utilizing oil palm biomass. Biomass generally consists of cellulose, hemicellulose, and lignin. Among these main components, hemicellulose is most likely to be decomposed at a low temperature. Degradation temperature of hemicellulose is in the range of 180 to 320°C, and this temperature range overlaps with the melt-molding temperatures for common thermoplastics. Hence, if biomass is blended with a thermoplastic at the temperature around 200°C, the hemicellulose component is decomposed and will decrease the physical properties of a blend. In this invention, a non-chemical superheated steam (SHS) was applied to treat oil palm biomass aimed at removing hemicellulose component. SHS is used to treat a raw material with steam of 170°C to 250°C for 10 minutes to 6 hours, and then the treated samples were grind. SHS-treated sample has no peak in the temperature range of 180°C to 320°C (contribute by removal of hemicelluloses component) and a peak was plotted in the temperature range of 300°C to 400°C (contribute by cellulose component) in a differential thermogravimetric curve. Ground sample has 50% by mass or more size of fiber in range of 1 µm to 500 µm, which is suggested suitable for biocomposite production.

IP Status: Pending Patent
Filing Date: 2012
Application No: JP2011-256880
Country Filing: Japan
Applicant: Kyushu Institute of Technology (Kyutech), Japan

EB GROUP

Conference and Workshop Attendent

MAY

- **22 – 23 May 2012**
  Workshop on Scanning Electron Microscopy of Biomaterials, Amrec, Sirim Bhd., Kedah
  Mohd Nor Faiz Norrrahim, Yee Lian Ngit, Mohd Ridzuan Othman and Mohamad Faizal Ibrahim

JUNE

- **24 – 26 June 2012**
  Asian Federation of Biotechnology (AFOB) Delegate Meeting and Regional Symposium, Tainan, Taiwan
  Prof. Dr. Suraini Abd. Aziz

SEPTEMBER

- **27 September 2012**
  Joint Seminar Of Globally Aware Young Researchers from Kyutech And Sister Universities, Graduate School Of Life Science And Systems Engineering, Kyushu Institute Of Technology, Japan
  Nor Hashimah Abdul Rahman, Nur Ain Zamzuri and Yee Lian Ngit

- **10 – 11 September 2012**
  International Palm Oil Sustainability Conference, Putrajaya Marriot Hotel, Putrajaya
  Prof. Dr. Mohd Ali Hassan, Dr. Rafein Zakaria, Ammar Asbi, Ahmad Amiruddin Mohd Ali, Ahmad Muhaimin, Juferi Idris and Mohd Ridzuan Othman

- **16 – 21 September 2012**
  15th International Biotechnology Symposium (IBS 2012), Daegu, South Korea
  Prof. Dr Suraini Abd. Aziz, Prof. Dr. Mohd Ali Hassan, Dr. Hidayah Arifin

- **3 – 5 September 2012**
  Batch/ Bioprocess Modelling, Scheduling & Optimisation, International Islamic University Malaysia (IIUM), Kuala Lumpur
  Nur Falia Shazana Manja Binti Farid and Noor Ida Amalina Binti Ahamad Nordin

OCTOBER

- **23 – 24 October 2012**
  International Conference on Biomass for Biofuel and Value Added Product, Double Tree by Hilton, Kuala Lumpur
  Dr. Rafein Zakaria, Mohd Huzairi Mohd Zainudin and Mior Ahmad Khushairi Mior Zahari

- **30 October 2012**
  Workshop on Zero-Discharge form Palm Oil Mill Industry & Creation Of New Green Profitable Business, Sabah Hotel, Sandakan
  Mohd Ridzuan Othman, Juferi Idris, Ammar Asbi, Ahmad Ahmad Amiruddin Mohd Ali,

- **26 October 2012**
  The 5th Japan-Korea Joint Symposium on Bio-Microsensing Technology, Tobata Campus, Kyushu Institute of Technology, Japan
  Nor Hashimah Abdul Rahman And Nur Ain Zamzuri

EB GROUP

Conference and Workshop Attendent

OCTOBER

- **23 – 24 October 2012**
  International Conference on Biomass For Biofuels And Value-Added Products (ICBBVAP) 2012, Double Tree By Hilton Hotel, KL
  Prof. Dr. Mohd Ali Hassan

- **29th – 31st October 2012**
  2nd ASEAN Sago Symposium, Kuching, Sarawak
  Prof. Dr Suraini Abd. Aziz, Siren Linggang

NOVEMBER

- **26 – 27 November 2012**
  International Conference On "Agricultural and Food Engineering For Life", Palm Garden Hotel, Putrajaya
  Prof. Dr. Mohd Ali Hassan, Dr Hidayah Arifin and Dr. Rafein Zakaria

- **26 – 28 November 2012**
  International Conference on Agricultural and Food Engineering 2012 (CAFEi 2012), Palm Garden Hotel IOI Resort, Putrajaya
  Prof. Dr. Mohd Ali Hassan and Noor Ida Amalina Binti Ahamad Nordin

- **29 – 30 November 2012**
  International Conference on Green Biotechnology : Renewable Energy and Global Care, Uban Ratchathani Univercity, Thailand
  Mohamad Faizal Ibrahim

DECEMBER

- **12 – 13 December 2012**
  Advanced Material Conference, Bayview Hotel, Langkawi
  Mohd Nor Faiz B. Norrrahim and Elmy Nahida Binti Othman

- **5th – 8th December 2012**
  The Agricultural Policy Seminar on "Capacity Building through Information Sharing About Utilisation of Biomass Energy In Rural Area And Agriculture Communities In ASEAN Countries", Bali, Indonesia
  Prof. Dr. Suraini Abd. Aziz

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Conference and Workshop Attendent

SRI INNOVATION AND COMMERCIALIZATION READINESS TRAINING PROGRAMME

Venue : UPM, Residence Hotel at UNITEN & Avillion Admiral Cove Hotel, Port Dickson.
Date : 30 January – 10 February 2012

ASIAN FEDERATION OF BIOTECHNOLOGY (AFOB) DELEGATE MEETING AND REGIONAL SYMPOSIUM

Venue : Tainan, Taiwan
Date : 24 – 26 June 2012

15TH INTERNATIONAL BIOTECHNOLOGY SYMPOSIUM AND EXHIBITION (IBS 2012)

Venue : Daegu, Korea
Date : 15 – 22 September 2012

EB GROUP

Conference and Workshop Attendent

2ND ASEAN SAGO SYMPOSIUM

Venue : Kuching, Sarawak
Date : 29 – 31 October 2012

BIOTECH MINI SYMPOSIUM 2012

Venue : BioTech, UPM
Date : 30 November 2012

THE AGRICULTURAL POLICY SEMINAR ON "CAPACITY BUILDING THROUGH INFORMATION SHARING ABOUT UTILISATION OF BIOMASS ENERGY IN RURAL AREA AND AGRICULTURE COMMUNITIES IN ASEAN COUNTRIES"

Venue : Bali, Indonesia
Date : 5 – 8 December 2012

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STUDENT

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Oil palm frond as a novel and renewable non-food feedstock for biomaterials and bioenergy

Ahmad Muhaimin Roslan

PhD Environmental Biotechnology (Semester 2)

emin85@yahoo.com

Supervisor

Prof. Dr. Yoshihito Shirai

Objectives

1. To produce good biocomposites from oil palm frond biomass using superheated steam treatment.
2. To produce high concentration of bioethanol from oil palm frond using thermophilic saccharification and fermentation.
3. To produce nanofibers from oil palm frond.

Research Summary

Oil palm frond (OPF) is being produced locally at a staggering amount of over 70 million tons per year. It is being underutilized as the current practice is to leave it on site as soil cover and for nutrient recycling. However recent studies showed that OPF have huge potentials as a feedstock for conversion to other materials.

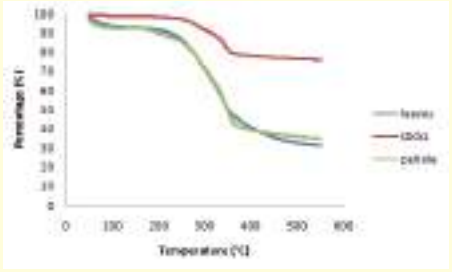
As the leaves of oil palm tree, OPF is the place where the photosynthesis takes place. It is where the carbon dioxide are trapped and converted to glucose occurs with the help of sunlight. Glucose produced is then transferred throughout the plant from the leaves through the stem and petiole of the OPF.

Given the circumstances, that is why glucose concentration is very high in stem and petiole. Since petiole is generally very high in moisture content, glucose can be easily pressed out using simple pressing machine. Upon pressing, up to 50% of the moisture collected, and can be used for other value added product conversion, and leaving petiole biomass which is already mechanically pretreated.

The petiole biomass still contain another 50% moisture which is rich in sugars, as well as lignocellulosic materials. By using superheated steam treatment, the lignocellulosic materials can be converted into powdered form. Powdered biomass can be used to produce biocomposite with a suitable application.

Meanwhile, by using enzymatic saccharifications, lignocellulosic materials can be converted into sugars, and collected together with existing sugars trapped in the biomass. These high sugars hydrolyzate can further be used as feedstock for bioconversion by fermentation to produce bioethanol, biobutanol, polyhydroxybutanoic etc.

The fermentation waste can then be sterilized prior to use as the feedstock for animal feed or composting. Overall, the theoretical potential of OPF is endless eventhough studies have not yet ended.



3 TG of OPF components

2 OPF colour upon SHS

1 Super-Heated Steam Equipment for pretreatement

The feasibility of sago hampas as a feed stock for bioethanol production

Research Summary

Sago hampas hydrolysate (SHH) contain about 85-90% (w/w) of glucose after hydrolysis process by dextrozyme enzyme. Higher amount of glucose in SHH gives extra advantage as it can be served as a substrate for bioethanol production by commercial baker's yeast. However SHH also contains dextrin and maltose, as well as other inorganic compound due to the complex structure of hampas. Although hydrolysate has been separated from solid particles, some impurities that exist together with glucose might influence the capability of SHH as a susbtrate for ethanol fermentation. Hence, the fermentation process utilizing 80 g/L of glucose from SHH was initially carried out. All trials were conducted in 100 ml working volume, with temperature of 30°C and initial pH of 5.5. The agitation was set at 100 rpm throughout the process. On top of that some preliminary study on the pregerminate time of commercial baker's yeast as well as its growth profile was observed. From the result obtained the yeast was not able to grow in the SHH media although glucose was the main component in the hydrolysate.

Hence, the SHH was then mixed with yeast extract, peptone, KH2PO4, MgSO4.7H2O and NH4SO4, the media which usually used for culturing S. cerevisiae. The same media was then used for control, but for carbon source it was replaced with commercial glucose (CG). Efficient fermentation was then observed in SHH media with the theoretical conversion yield about 98%, comparable with the media containing CG. To ensure higher ethanol concentration (v/v: >10%) which meet industrial needs, the fermentation should be fed with higher glucose concentration. Thus to accommodate with this criteria three different concentration (g/L: 100,150 and 200) glucose in SHH were initially tested. From all trials, the 100 g/L of initial glucose shows higher yield with glucose has been completely consumed after 16 – 18h of fermentation process.

However for 150 g/L and 200 g/L glucose, the fermentation process took more than 24h to complete, thus affect the volumetric productivity, product yield and fermentation efficiency. Some by-products such as glycerol, lactic acid and acetic acid were also produced. Higher glycerol (9 – 10g/L) was observed in the media of 150 and 200 g/L of glucose, revealed that excess amount of NADH which produced during synthesize of biomass and organic acids have been oxidized to NAD+.

In order to improve the fermentation process utilizing high glucose concentration (150 and 200 g/L), the studies on the effect of nitrogen sources usable by yeast was carried out. Three different nitrogen sources (NH2SO4, urea and yeast extract) were studied on their effect on the ethanol production, ethanol yield and ethanol productivity. Overall, urea supplemented medium has shown the capability to influence better ethanol production compared to NH2SO4 and yeast extract in glucose concentration of 150 and 200 g/L. Thus, the successful of ethanol fermentation process from glucose of SHH together with urea as nitrogen source has creates another economical source of C and N, for production of value added product in our country.

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Objectives

1. To pretreated the sago 'hampas' for glucose production using enzymatic hydrolysis
2. To study bioethanol production from hydrolyzed sago 'hampas' through batch fermentation system utilizing commercial baker's yeast
3. To optimize the bioethanol production from hydrolyzed sago 'hampas' by response surface

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Supervisor

Dr. Hidayah Ariffin

Objectives

1. Characterization of low to medium molecular weight polyhydroxyalkanoates hydrolyzed by saturated steam.
2. Determination of polyhydroxyalkanoates hydrolysis kinetics and mechanim in saturated steam
3. Proposition of kinetics and mechanism involved in superheated steam hydrolysis of polyhydroxyalkanoates
4. Evaluation of superheated steam hydrolysis for chemical recycling and surface modification of polyhydroxyalkanoates

Controlled depolymerization of polyhydroxyalkanoates by steam hydrolysis

Research Summary

Polyhydroxyalkanoate (PHA) has unique characteristics of thermoplastic, biodegradable and biocompatible biopolymer that can be produced intracellularly by microorganism and some plant species. This promising biopolymer has been researched since its discovery in 1920s, remarkably starts the green revolution of non-petrochemical aliphatic polyester that is producible via fermentation biosynthesis and has been commercialized as early as in 1962. The constituent of PHAs, polyhydroxybutyrate acid (PHB) is biocompatible with human as it is also a built compound of blood, made this producible biopolymer able to contribute very significantly in the biomedical applications especially in tissue engineering. This biodegradable carbon reserve plays important role in the environmental carbon storage. Cascade utilization of polymers could be introduced before they are finally being released to the environment. Single use of bioplastics does not support the sustainability of the carbon cycle; therefore a process to depolymerize polymers is needed. Nevertheless, biological production of PHA sometime able to produced ultra-high molecular weight to suit variation of thermo-mechanical properties needed. On the other hand, medium to low-molecular weight PHA are important for slow release and short term coating applications. Medium to low-molecular-weight materials are suitable feedstock for blending and re-polymerization process. Several methods have been used to depolymerize PHA, namely pyrolysis and hydrolysis. Pyrolysis, abiotic hydrolysis and enzymatic hydrolysis of PHA have been extensively studied; however steam hydrolysis of PHA is yet to be studied. Controlled depolymerization of PHA in this study, involved with the concept of the material conversion to molecules that built up of the original material or lowering of its origin molecular weight. This research is aimed at recovering low to medium-molecular weight polymers with hydroxyl and carboxyl chain-ends from polyhydroxyalkanoates (PHA) by steam hydrolysis. These low to medium- molecular weight polymers can be used as feedstock for re-polymerization and other applications. Degradation of PHA by steam hydrolysis will be controlled by several parameters, namely; temperature and retention time. The experiments will be conducted using saturated and superheated steam input. The hydrolysis products will be characterized and the effect of controllable parameters toward the target product formation will be investigated in details. The analysis of molecular weight will be carried out using gel permeation chromatography (GPC) and the depolymerization is projected theoretically to follow autocatalytic random degradation mechanism with the identification of critical point, hydrolysis rate constant and activation energy based on relative molecular weight of polystyrene as the standard. The hydrolyzed products will be characterized by using ^{1}H NMR and ^{13}C NMR, FTIR, DSC, XRD, XPS and AFM. Mass balance for the PHAs hydrolysis will also be studied. At the end of this study, it is expected that the depolymerization mechanisms and kinetics for PHA hydrolysis can be proposed, with the selective formation of targeted products for cascade utilization of PHA

Combustion of empty fruit bunch in a pilot scale carbonization reactor

Research Summary

Biomass char is a promising feedstock of renewable energy,, contributing around 10 – 15% which is approximately 45 EJ of world energy use currently. It is a primary candidate because of being the only renewable source of fixed carbon, which is essential in the production of conventional hydrocarbon liquid transportation fuels and many consumer goods. Thus, it is an attractive research area to be explored which gives a significant effect to the economy as well as the environment.

The utilization of biomass fuel could help to reduce problem of massive emissions of carbon dioxide (CO2) and its climate impact particularly on issues related to greenhouse gases (GHG) or global warming. The emissions of CO2 had increased rapidly over the past few decades and as a result of human activities, almost 30 billion tonnes of CO2 enters the atmosphere.

Being the largest palm oil producer in the world, Malaysia has the potential to produce renewable energy from biomass for clean energy emission with approximately 362 palm oil mills, processing 71.3 million tons of fresh fruit bunch per year and producing an estimated 19 million tons of crop residues annually in the form of empty fruit bunch, fibre and shell.

The design of the combustion reactor, which converts biomass to bio-oil, char and gases of the fuel is an important consideration in achieving high thermal degradation of materials. Combustion process in a complete absence or inadequate presence of oxygen in achieving a high thermal fuel known as pyrolysis technology is one of the promising technologies for biomass utilization.

To date, very little work on the combustion of empty fruit bunch in medium scale (30 - 50kg capacity) using fabricated furnace for biochar production is available in the published literature. As such, combustion study for such bio char production would be useful in the palm oil industry intending to minimize CO2, residues and environmental pollutants during combustion process.

The first and second objectives of this study is to evaluate the combustion of empty fruit bunch in a pilot scale carbonization reactor and to evaluate the effect of air flow rate and particle size on bio-char quality from combustion process. The third objective involving the effect of air flow rate and particle size during combustion process on biochar surface area is also investigated,

Biochar medium scale reactor

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Supervisor

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Objectives

1. To evaluate the combustion of empty fruit bunch in a pilot scale carbonization reactor
2. To evaluate the effect of air flow rate and particle size on biochar yield quality from combustion of empty fruit bunch
3. To evaluate the effect of air flow rate and particle size on pore developement in empty fruit bunch based activated carbon

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Objectives

1. To characterize and evaluate the potential of the extracted OPF juice as a novel, renewable and sustainable carbon substrate for fermentation process
2. To optimize pH, agitation speed and ammonium sulfate, $(NH_4)_2SO_4$ concentration which affecting the cell growth and P(3HB) production by using OPF juice as the sole renewable carbon source in shake flasks and 2 L bioreactor
3. To improve P(3HB) production from OPF juice in 2 L fed-batch bioreactor by using *Cupriavidus necator* NCIMB11599 (mutant strain of *R. eutropha* H16)
4. To scale-up the biosynthesis of P(3HB) using OPF juice from 2 to 20 L stirred tank fermenter based on constant k_La

Oil palm frond juice as a novel and renewable substrate for the production of poly (3-hydroxybutyrate)

Research Summary

Poly(3-hydroxybutyrate), P(3HB) is a biodegradable thermoplastic polyester accumulated intracellularly by many microorganisms under unfavorable growth conditions. In P(3HB) production, about 40% of the total production cost is for raw material. Thus, the use of a cheaper carbon source is required in order to reduce the high production cost of P(3HB). Malaysia is the world's second largest producer of crude palm oil after Indonesia. In 2010, Malaysia exported 16.7 million tons of palm oil, worth 59.8 billion ringgit, a 5% increased compared to the export in 2009. Apart from its contribution to economic growth, palm oil industry also supplies a renewable biomass which can be further utilized to produce other value added product such as bioplastic. One of the major renewable biomass generated abundantly from palm oil industry is the oil palm frond (OPF). With 83 million tonnes of OPF being generated from 4.7 million ha of total planting area in the year 2009, it is estimated that 4.65 million tonnes of renewable sugars can be produced from Malaysian palm oil industry. In addition, based on the total planting area for oil palm in 2009, approximately 1 tonne/ha of renewable sugars could be produced from fresh OPF generated in Malaysia, which can be further utilize as a non-food, non-cellulosic, renewable and sustainable fermentation feedstock to support the biotechnology industries in the long term. Based on these findings, an attempt has been made to produce P(3HB) from OPF juice by using wild type and mutant strain of Cupriavidus necator. This research study was divided into four different stages which are extraction and characterization of juice from OPF, followed by, production and optimization of P(3HB) in shake flask fermentation, scaling up of the fermentation process from 2 L to 20 L (15 L working volume) bioreactor and finally, enhancement of P(3HB) production from OPF juice in fed-batch fermentation by mutant strain of Cupriavidus necator NCIMB 11599.

Prior to fermentation, OPF juice was extracted by using conventional sugar cane pressing machine. By simple pressing, 50% (wt/wt) of OPF juice was obtained from the basal part of freshly chopped OPF petiole, while the remaining part was left in the plantation for soil erosion control and nutrient recycling. The glucose content in the juice was 53.95 ± 2.86 g/l, which accounts for 70% of the total free sugars. Furthermore, the OPF juice is rich in minerals and nutrients which are essential for bacterial growth during fermentation. In order to study the potential of OPF juice as a renewable carbon source for fermentation, we have examined the effect of different sugars concentration in the OPF juice on the production of P(3HB) by using wild type strain of C. necator CCUG52238T. The highest cell biomass and the P(3HB) accumulation were recorded for the experiment supplemented with 30% (v/v) of OPF juice, with 32 wt.% of P(3HB) content in the cell. It is observed that the cell biomass and the P(3HB) content were slightly higher compared to that obtained from a mixture of technical grade sugars. Results from the preliminary study also showed that there was no inhibition on cell growth and product formation in P(3HB) fermentation using the OPF juice as substrate. As for optimization study, we further investigate the effects of different initial pH, agitation speed and ammonium sulfate, (NH4)2SO4 concentration on the production of P(3HB) in shake flasks experiments using OPF juice as the sole carbon source. Under the optimal conditions, the highest cell weight was 9.31 ± 0.13 g/L with 45 ± 1.5 wt.% of P(3HB) contained in the cells, accounts of 40% increment for P(3HB) content compared to the non-optimized condition. Cultivation in a 2-L bioreactor with 30 % DOT yielded P(3HB) content of 44 wt.% with some improvement of cell growth i.e. 11.37 g/l. The molecular mass, elongation to break and tensile strength of the polymer produced from OPF juice were comparable to those reported in literature and suitable for commercial utilization. The production of P(3HB) was then scale up to 20 L (15 L working volume) bioreactor based on constant kLa. An almost similar CDW and P(3HB) production profile was obtained in 20 L bioreactor study compared to 2 L bioreactor within the similar cultivation time indicating the successful of the scaling up process.

By culturing the mutant strain of C. necator NCIMB11599 using OPF juice, further enhancement on P(3HB) production was recorded. In fed-batch cultivation supplemented with concentrated OPF juice, we managed to obtain higher cell dry mass of 40 g/l with 75 wt.% of P(3HB) accumulation. Images of bacterial cells taken at 60 h of cultivation period with a Transmission Electron Microscope (TEM) showed the vast majority of microbial cells contained many P(3HB) granules with a few cells autolysis occurred, indicating the suitable time for cells harvesting. Thus, this study shows that OPF juice can be used as an alternative renewable carbon source for P(3HB) production and has potential as a renewable carbon source for other value added products.

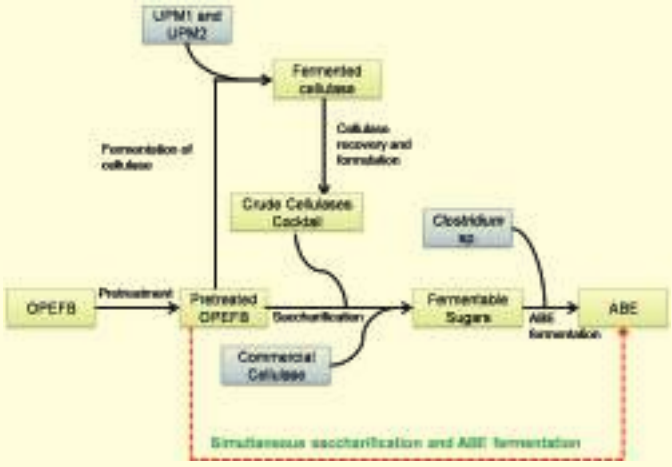
Acetone–butanol–ethanol fermentation using oil palm empty fruit bunch as substrate

Research Summary

Biological production of acetone-butanol-ethanol (ABE) is currently under demand for the extraction of biobutanol. Biobutanol represents the next significant change required to meet the growth in demand for environmentally responsible and renewable fuels for transportation. Biobutanol ($C_4H_{10}O$) or butyl alcohol is an alcohol that can be used as a solvent or fuel produced from biomass by a microbial fermentation. It has low vapor pressure, can be easily blended with gasoline, contain much energy as gasoline, better adapted to be used in the present distribution system, less corrosive, and can be used in existing vehicles. These criteria of biobutanol have become a great renewable energy source if the production of butanol can be produce at lower cost.

In the recent years, growing attention has been devoted on the production of ABE from lignocellulosic biomass as an alternative renewable energy. Oil palm tree is one of the most planted for edible oil in tropical countries such as Malaysia and Indonesia. As a leading industry in world's oil production, palm oil industry has leaved behind huge amount of biomass from its plantation and milling activities as compared to other type of agriculture biomass. However, its lignocellulosic residues have not been effectively used; it disposed of by mulching and dumping at plantation. In fact, the oil palm empty fruit bunch (OPEFB) contents high amount of carbon source in the form of cellulose and hemicellulose, a polymer structure made by sugar monomers. This polymer is able to be converted into sugar monomers through hydrolysis process using biological catalyst known as cellulase.

Cellulase is a great product in which works synergistic in the bioconversion of lignocellulosic materials into fermentable sugars. Many technologies have been developed in order to obtain higher efficiency of hydrolysis process and produce cellulase at low cost. The study was successfully developed the crude cellulase cocktail produced by *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2 using pretreated OPEFB as substrate for subsequently used in the bioconversion of pretreated OPEFB into sugars. The sugars obtained were further used for the production of ABE by *Clostridium* sp. in anaerobic fermentation system. The ABE fermentation was further improved by conducting the simultaneous enzymatic saccharification and ABE fermentation at the same time.



Process overview of ABE fermentation using OPEFB as substrate

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Objectives

1. To obtain fermentable sugars from pretreated oil palm empty fruit bunch using crude cellulases cocktail produced by *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2.
2. To obtain acetone–butanol–ethanol using sugars from pretreated oil palm empty fruit bunch by locally isolated *Clostridium butyricum* EB6.
3. To obtain acetone–butanol–ethanol using sugars from pretreated oil palm empty fruit bunch by *Clostridium acetobutylicum* ATCC 824.
4. To profile the acetone–butanol–ethanol from from pretreated oil palm empty fruit bunch through two step and simultaneous enzymatic saccharification and ABE fermentation.

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Objectives

1. Identification of microbiota associated with degradation of lignocellulose oil palm empty fruit bunch (OPEFB) during composting.
2. Identification and Functional Characterization isolated thermophilic lignocellulose-degrading bacteria on OPEFB degradation.
3. Effect of isolated bacteria on the enhancement of co-composting of OPEFB and palm oil mill effluent anaerobic sludge.
4. Construction of metagenomic library for isolation of genes encoding for cellulase and xylanase from OPEFB compost and characterization of expressed cellulase and xylanase.

Isolation and functional characterization of gene encoding for lignocellulose enzymes from microbial diversity of oil palm empty fruit bunch compost

Research Summary

Malaysia has become the largest oil palm producer with the production of about 18 million tones per year and about 47% of world's supply. Besides producing oil, it has generates abundant of waste such as Palm oil mill effluent (POME), Empty fruit bunch (EFB), Mesocarp fiber and Palm kernel shell. EFB is one the largest waste produce in the mill. Previously, EFB has been dumped for soil mulching in the plantation area. One way to promote the zero-discharge system and creating value added product from these waste are through the co-compositing of EFB and POME anaerobic sludge.EFB compost is manageable product which can be use as soil amendment and organic fertilizer. Composting is a natural biological process which is controlled under aerobic condition. In composting, the breakdown of organic materials into simpler substance such as lignocellulose is driven by existence of microorganism, mainly by aerobic thermophilic bacteria. The effectiveness of composting process is dependent on the environmental condition such as temperature, oxygen, moisture, material used, size and activity of microbial populations.

In this study, determination of microbial populations was done through microbiota approach using culture-independent analysis of the 16s rRNA gene amplified directly the DNA extracted from compost. The amplified 1500-bp 16S rRNA PCR products were used for the construction of clone library. The insert DNA of each clone in the library was determined by using DNA sequencer. The sequence similarity to closest relative was done through the sequence match program in National Centre for Biotechnology Information (NCBI) and Ribosomal Database Project II (RDPII). Sequence with >97% similarity were selected and were grouped to Operational Taxonomical Unit (OTU). Subsequently, with regard to the results obtained from microbiota analysis, screening and isolation of bacteria producing cellulase and xylanase was done. Several microbes that are capable to express cellulase and xylanase have been isolated. The enzymes involved in biomass degradation from microbes were identified and through the analysis of partial amino acid sequencing of target enzymes. The microbial consortium analysis was done through combination of those cellulase and xylanase-producing microbes for effective lignocellulose EFB degradation. In order to identify potential cellulase and xylanase enzyme from unculturable microbes, metagenomic of EFB compost was done. Metagenome involves the extraction of metagenomic DNA from EFB compost. The 36-40kbp DNA is subsequently cloned into vector (fosmid) for constructing metagenomic library. From the metagenomic library, the genes encoding cellulase and xylanase were screened and identified by polymerase chain reaction or activity screening.

The findings of this study helped to understand the microbial population throughout the composting process and identify the microbes that have the capability of producing cellulase and xylanase. It is also helped to find the lignocellulosic enzyme by isolating genes encoding cellulase and xylanase which was screened from metagemonic library. Hopefully in the future, these finding will provide the good enzyme to improve the feasibility of lignocellulose biomass conversion.

Positive clone of metagenomic library of EFB compost

Future alternative energy: biohydrogen and microbial fuel cells are the solution

Research Summary

Renewable energy supply has gained importance consideration as an alternative energy sources to overcome fossil fuels depletion. Advances in molecular biology, biochemistry and biotechnology have raised the quality and efficiency of biofuels evolution from various microorganisms and substrates. Biohydrogen and Microbial Fuel Cells (MFC) could be classified as potential biofuel prospects that have earned huge coverage due to their eco-friendly and alternative renewable energy solution. The merits of biohydrogen as a fuel source remains undisputed due to its higher energy content compared to hydrocarbon fuel (Mohd Yusoff et al., 2012). The necessity of replacing fossil fuels also have been discussed extensively since petroleum prices have increased dramatically and there is continued anxiety about the level of green house gases (GHG) in the atmosphere. Escherichia coli was extensively used in this study since E.coli known as a robust bacterium for research development due to its well characterized proteins and the accessibility of its complete genome (Abo-Hashesh et al., 2011; Blattner et al., 1997). Additionally, genetic and protein engineering have been used to enhance biohydrogen production in E. coli (figure 1).

Fig. 1 Glucose fermentation for biohydrogen production using mutant strain

In addition, rises of MFC would promise a sustainable wastewater treatment and management toward efficient and sustainable energy sources. Furthermore, research and development in MFCs just enter an initial stage of their complex understanding application due to biological process, especially in the anode compartment. MFC is an outstanding device that expeditiously converts biochemical energy especially from degradation of organics into electrical energy by the catalytic reaction of microorganisms. In this study, indigenous microbial community from sewage wastewater sludge was used as inoculum while sludge and treated sludge have been opted as a substrate during MFC process. Soluble chemical oxygen demand has been discovered as essential consumable substances contribute to the electricity generation through the process. Two kinds of sludge treatment process have been done namely ozonation and microwave digestion . Maximum voltage produced has approached up to 190-200 mV from microwave digested sludge relatively higher compared to ozonation treated sludge. On the other hand, untreated sludge as a control 160-180 mV and 100-120 mV, respectively. Exoelectorgens community analysis has been performed sing 454 pyrosequencing technique, in order to attain beneficial information and relationship towards electricity generation. The information of mechanism between biological and biochemistry interrelation would lead for better enhancement of electricity generation and microbial competency for further research.

Microalgae research as a supplement materials for bacteria butrient in MFC

In conclusion, integration of bioprocess technology and molecular biotechnology is important to enhance the productivity and efficiency of the process mechanism. Industrial application and utilization of biological materials (biomass, sludge, microalgae, industrial effluent and so on) also one of necessity objectives should be consider as finest goal of the study.

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Objectives

1. To improve biofuels production through molecular biotechnology approaches
2. To produce electricity from sludge using microbial fuel cell
3. To apply microbial fuel cell and E. coli engineered strain for industrial application

Recent publications:

Mohd Yusoff, M.Z., Maeda, T., Sanchez-Torres, V., Ogawa, H.I., Shirai, Y., Hassan, M.A., Wood, T.K. 2012. Uncharacterized Escherichia coli proteins YdjA and YhjY are related to biohydrogen production. International Journal of Hydrogen Energy, 37(23), 17778-17787.

Mohd Yusoff, M.Z., Hu, A., Feng, Maeda, T., C.-P., Shirai, Y., Hassan, M.A., and C.-J., Yu. Influence of Pretreated Activated Sludge for Electricity Generation in Microbial Fuel Cell Application. Submitted to Bioresource Technology (December 2012).

Sanchez-Torres, V., Mohd Yusoff, M.Z., NAKANO, C., Maeda, T., Ogawa, H.I., and Wood, T.K. Influence of Escherichia coli hydrogenases on hydrogen fermentation from glycerol. International Journal of Hydrogen Energy (Accepted galley proof 5 January 2013)

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Objectives

1. Characterization of superheated steam treated mesocarp fiber in comparison with untreated mesocarp fiber.
2. Production and characterization of superheated steam treated OPMF / PP biocomposite with respect to the effect of fiber loading, particle size and compatibilizer.
3. The effect of fiber treatment and chemical composition on characterization of PP reinforced treated mesocarp fiber.
4. Evaluation of SHS treatment as an alternative pretreatment method in comparison to chemical pretreatment and steam explosion.

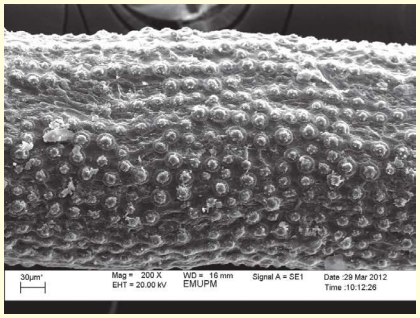
Superheated steam treatment of oil palm mesocarp fiber for biocomposite production

Research Summary

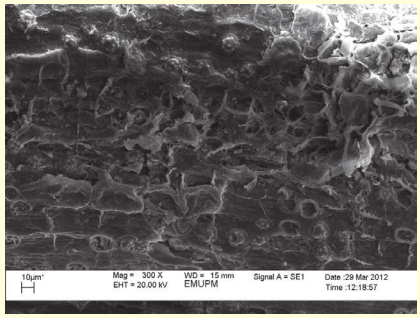
Lignocellulosic material has been widely tested as reinforce material in biocomposite. However, high hygroscopicity and incompatibility with hydrophobic polymer are among the disadvantages of lignocellulosic materials for biocomposite production. The problems arise due to the natural characteristics of the fiber which is hydrophilic due to the presence of many hydroxyl groups from holocellulose component. One approach to overcome these shortcomings is by modifying the nature of the lignocellulosic materials to make it more hydrophobic. This can be accomplished by extracting hemicellulose from natural fiber. In this study, a non-chemical superheated steam (SHS) was applied to treat oil palm mesocarp fiber (OPMF) aimed at removing hemicellulose component. The OPMF were treated with SHS at two different temperatures: 200 and 230 °C for 100 min under atmospheric pressure.

The SHS-treated OPMF was characterized for its chemical composition by FT-IR and chemical analysis. Thermal stability was analysed using TGA and particle size distribution was done by sieving analysis. Overall, treated OPMF exhibited higher ratio of cellulose and lignin to hemicellulose, had increased thermal stability and improved hydrophobicity compared to the untreated OPMF. Samples treated at 230 °C for 100 min showed the highest removal of hemicellulose, their content dropped from 34 to 10 %, which accumulated to 70.6 % of hemicellulose removal. FTIR spectrum revealed that signals for O-H and C=O absorption at around 3338 cm-1 and 1737 cm-1, respectively, were decreased suggesting that partial removal of hemicellulose occurred after SHS treatment. Furthermore, thermal stability test by TGA showed that T5% of treated OPMF was shifted to higher temperature by nearly 30 °C compared to the untreated OPMF. Removal of highly hydrophilic hemicellulose from OPMF through SHS treatment led to the elimination of hydroxyl groups in the fiber and hence, less hydrophilic OPMF can be obtained. SHS treatment also provided OPMF fiber with smaller particle size, which is an important factor in improving fiber dispersion in the polymer.

Overall, the findings from this study suggested that SHS treatment can be a simple, environmental friendly method for chemical and physical modification of lignocellulose, to improve its compatibility and interfacial adhesion with the polymer in biocomposite production.

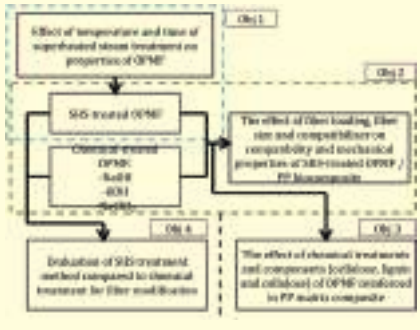


untreated oil palm mesocap fiber viewed under SEM



OPMF image after undergoes SHS treatment (SEM image).

SHS machine use to treat OPMF



Research overflow

Improved recovery and purification of polyhydroxyalkanoates from renewable resources

Research Summary

Polyhydroxyalkanoates (PHA) is a complete biodegradable, biocompatible, microbial thermoplastic which has potential to replace petroleum-derived thermoplastics. PHA is an excellent plastic option; a clean energy alternative with no emissions of greenhouse gases, which helps in addressing the challenge of global climate change. PHA are synthesised when bacteria are exposed to a surplus of carbon and limited for vital nutrients such as nitrogen, phosphorus and sulphur. Under these conditions, cells cannot grow but they do accumulate carbonbased polyesters. PHA production by using Comamonas sp. will be conducted using mixed organic acids obtained by anaerobically treated palm oil mill effluent (POME) which replaced the costly conventional carbon substrate. The objectives of this study are to develop an alternative process recovery of intracellular PHA, to simulate and model the optimise recovery techniques and to develop mass and energy balances for the complete PHA recovery. PHA from Comamonas sp. will be recovered by using methods sodium hydroxide, since it can reduce operation cost and also reduce the solvent d a m a g e to health and environment.

Moreover to get a higher purity, a purification step could be added to the process. In this study, different initial conditions, washing step, optimization on recovery, optimization on g force and develop the energy mass balance will be done using Design Expert software. The properties of PHAs are highly dependent upon their recovery techniques; hence, biodegradable polymer having a wide range of properties. The micrograph, chemical, mechanical and thermal properties are investigated using high pressure chromatography (HPLC), gas chromatography (GC), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), molecular weight (GPC), scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

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Objectives

1. To develop an alternative, environmental friendly recovery process using chloroform, sodium hydroxide and enzyme
2. To characterize the recovered PHA
3. To develop mass and energy balance for the recovery process

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Objectives

1. To screen, isolate and identify potential biovanillin producing bacteria.
2. To isolate the functional genes for biotransformation of ferulic acid into biovanillin and further construct genetically engineered *E.coli.*
3. To produce biovanillin in one step fermentation using genetically engineered *E.coli.*

One-step biotransformation of ferulic acid into biovanillin using genetically engineered *Escherichia coli*

Research Summary

Vanillin is the major component of natural vanilla and a secondary metabolite of plant which is an important aromatic component as well as flavouring compound in the industry of food and personal products. It is derived from the tropical Vanilla orchid by the extraction from vanilla beans. Natural vanillin extracted from vanilla pods has a very high price and limited supply in the market due to it involved a time-consuming process which required intensive cultivation, pollination, harvesting and ripening of pods. It is also very dependable on the suitability of soil and climate conditions. Thus, current market demand for vanillin is fulfilled by the chemically synthesized vanillin. However, this artificially derived vanillin flavour could not be referred as a natural product.

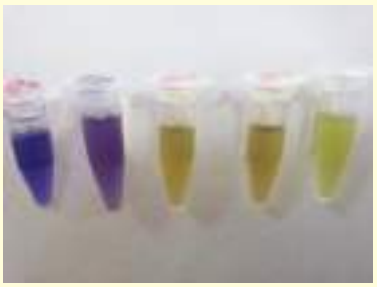
Therefore, the recent increasing demand for natural flavours and the problem of vanillin derived from Vanilla plant is relatively expensive has move the trends towards investigation of other biotechnological routes to produce vanillin. As a result, vanillin production through biotransformation of potential precursor by microorganism has been proposed towards a sustainable and environmental friendly process. The researchers are now investigating the potential biovanillin production through biotechnological approach which can be regarded as natural product to replace the natural vanillin extracted from vanilla pod as well as the chemically synthesize vanillin in the near future. Microbial or enzymatic transformation route has been reported as the most promising way to produce vanillin from the precursors like ferulic acid, vanillic acid, eugenol or isoeugenol. The bacterial biotransformation pathway was compared in order to screen and select the functional genes for biovanillin to be produced efficiently.

In this study, ferulic acid will be used as biovanillin precursor due to the chemically close relationship to vanillin, low cost, and readily available. It has been reported that the bacterium from 'Pseudomonas' family have the ability to produce vanillin via biotransformation process involving ferulic acid obtained from biomass. Through the investigation of biotechnological routes for biovanillin production from ferulic acid, the main concern is on the vanillin further oxidation into vanillic acid which resulted to the poor yield of vanillin. Based on the common pathway of bacteria for biovanillin production, vanillin will be further oxidized into vanillic acid due to oxidation of vanillin was easily occurred in compared to ferulic acid. As a result, vanillin as an intermediate was nearly undetectable at the end of the fermentation process.

Thus, the aim of this study is to develop a methodology for biovanillin production using genetically engineered E.coli by one step pathway without further oxidation of vanillin into vanillic acid. From this study, bacteria named as Pseudomonas sp. AZ10 UPM has been successfully isolated as a potential biovanillin producer using ferulic acid as sole carbon source. By using this strain, isolation of functional genes for biovanillin production can be carried out using DNA walking strategy and later can be cloned and expressed into pUC19 vector. The construction of genetically engineered E.coli containing biovanillin functional genes is expected to produce biovanillin in one step fermentation without further oxidation of vanillin into vanillic acid.

Selection plate for transposon mutagenesis of vdh gene

Plasmid extraction for vdh gene inactivation



Colorimetric rapid screening for isolation of biovanillin producer

Development of a recovery system for biovanillin

Research Summary

IIn fermentation processes, the products formation is either intracellular (biopolymers, inclusion bodies) or extracellular (antiobiotics, enzymes). The recovery of these bioproducts is considered technically difficult and expensive. It typically comprises of an extensive sequence of steps, and in each step, one or more unit operations are required. Figure 1 illustrates a generalized block diagram for bioseparation. In the whole processing chain of manufacturing bioproducts, the downstream section always consumes the biggest chunk of the financial resources. The bioseparation costs increase for a reduction in final yield of the bioproducts. Therefore, there is always a trade-off between the costs and the purity of the final product.

As for this work, adsorption technique is selected as the main technique for vanillin recovery. Adsorption is categorized under low-resolution (or selectivity) and high-throughput (or productivity). Nonetheless, one of the advantages of adsorption is its ability to recover solutes or adsorbates from dilute solutions. This is very much applicable to biovanillin production via fermentation because the highest known reported production was 19.2 g/L as reported by Hua et al. (2007). In their work, they used 8% (wet w/v) of resin DM11 incorporated into the culture of Streptomyces sp. Another interesting point in the proposed recovery strategy for the study of biovanillin recovery is the cost of the selected resin. Resin H103 is priced at only RM360 per kilogram. The next best resin, out of seven resins tested, is Sepabeads SP207. The performance of Sepabeads SP207 is slightly better than resin H103, but the price is almost five times higher, at RM1,785 per kilogram. The selection of resin H103 would be reducing the overall cost of biovanillin production and recovery. Furthermore, initial characterizations show that resin H103 could adsorb more than 98% of the total vanillin.

The selected resin can be applied in two different modes of in situ recovery. The first choice is by passing the fermentation broth into a column packed with adsorbent resin; either fixed bed or fluidized bed. Prior to loading into a fixed bed column, it is recommended to place a filter up front in order not to clog the bed with cell. Although this part requires the use of membrane, the cost of the membrane would be much lower as compared to the method of using membrane as the main separation technique. The remaining solution is then recycled back to the bioreactor and the fermentation process could be prolonged with the addition of fresh substrate. On the other hand, the fermentation broth could be loaded directly to a fluidized bed column, as the column is sparged with air and hence deterring the cell to clog the column. Figure 2 depicts the experimental setup for in situ product recovery configurations, and the explained technique is visualized by option 3.

Figure 1: Generalized block diagram for bioseparation (Harrison et al., 2003)

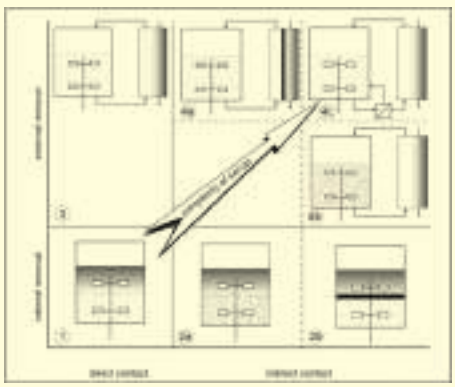


Figure 2: In situ product recovery configurations (Stark & von Stockar, 2003).

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Objectives

1. To determine the suitable resins on the absorption capacity of vanillin in term of its kinetics and reaction parameters
2. To elucidate vanillin adsorption behaviour in fixed bed column via dynamic adsorption capacity and rate constant
3. To perform scale-up analysis of vanillin adsorption onto fixed bed resin H103

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Objectives

1. To obtain the most favorable condition of high pressure steam pretreatment that increase OPEFB digestibility to biosugars production
2. To obtain high sugars concentration from steam pretreated OPEFB by Acremonium cellulase using Response Surface Methodology
3. To obtain high ethanol yield by Saccharomyces cerevisie using Response Surface Methodology

Cellulosic bioethanol from steam pretreated oil palm empty fruit bunch

Research Summary

In the palm oil mill, about 10% of the total dry biomass produced by the palm is the oils;the other 90% of the palm represents a further huge source of fiber and cellulosic materials which await a further commercial exploitation.The availability of this excess energy sources at the mill and its utilization, could helps to minimize the cost of palm oil production in overall. One of alternative ways in further use of these waste materials is the utilization in the area of pretreatment for bioethanol production.

Oil palm empty fruit bunch (OPEFB) is a large amount of byproduct produced from oil palm plantations and palm oil mills. It can be used as raw material to produce bioethanol due to it contains of cellulose and hemicellulose that can be degraded into fermentable sugars through enzymatic hydrolysis. In order to obtain high fermentable sugars, the structure of OPEFB has to be altered or removed by a suitable or ideal pretreatment in the production of bioethanol. This crucial process is primarily to make the biomass easily broken down into sugars by it opened structure through enzymatic hydrolysis.

Steam pretreatment has been chosen as the most favourable pretreatment of OPEFB. This pretreatment has to be thought to have relatively moderate energy cost production due to the steam is already generated as part of the mill operation for electricity and sterilising the fruit. Besides that, all raw materials to initiate the pretreatment (water,OPEFB, fiber and shells) are available in the mill

This in overall will enhance the sustainability of oil palm plantations. Furthermore steam pretreatment is suitable to be implementing in the palm oil mill as the OPEFB can immediately be processed and saccharified to the biosugars for subsequent bioethanol production. High pressure steaming is considered one of the most successful options for fractionating wood into its three main components. Heating biomass in the presence of saturated steam of 190oC and 220oC and pressure 1.2 to 4.1 MPa normally is efficient in partially hydrolysed hemicelluloses, modified the lignin, increase in accessible surface area, decrease of the cellulose crystallinity and its degrees of polymerization. Therefore,in this study the high pressure steam of 0.8 to 2.3 MPa will be studied on the effect of enzymatic hydrolysis for biosugars production.

The steam pretreatment is believed to convert the pretreated lignocellulosic biomass to more than 80% of sugars yielded from enzymatic saccharification. In this study, a variety of operation temperatures, pressures and residence times to be applied to the OPEFB have to be tested. In general, these parameters however are different depending on the pretreatment strategy as well as on the type and physical of the raw material used to make the pretreatment successfully, effectively and had a positive impact on the overall process.

Efficient bioethanol production from oil palm frond juice

Research Summary

Currently, there is tremendous world-wide interest in the second generation bio-ethanol (SGB) due to drawback of the first generation bio-ethanol (FGB). With respect to Malaysia situation, they have a great potential to develop SGB due to abundant availability feedstock biomass. In 2010, Malaysia's palm oil industry produced almost 80 million dry tonnes of solid biomass p.a. This volume is projected to increase to 85–110 million dry tonnes by 2020. Most of the solid biomass is found in the plantations, as fronds and trunks account for about 75 percent of the biomass volume. The remaining 25 percent is generated in the mills during the extraction of palm oil (National Biomass Strategy 2020, 2012). This project is focusing on the utilization of one of the abundant oil palm biomass which is the frond.

Oil palm fronds offer a promising sugar feedstocks for fermentation as it is available throughout the year and regularly cut during harvesting of fresh fruit bunches (FFBs) and pruning of the palm trees. To date, the oil palm frond (OPF) is not fully exploited due to the lacking of collection infrastructure system. Once the potential of this biomass is being revealed, a sizeable investment should be put to set up a supply system for OPF collection, storage and transport. An alternative route to derive biofuels and biobased chemicals from oil palm biomass is to squeeze sugar juice from the basal part of the oil palm frond. In general, 54% w/w of juice can be extracted from fresh OPF petiole by using conventional pressing machine (Zahari et al., 2012). From the material balance, 1 tonne of fresh OPF could produced 400 kg of sugars by breaking down the cellulosic components, compared to only 40 kg of sugars from the oil palm frond juice alone. The use of sugar juice is unlikely to be the most economically viable in the long term, but it could provide a stepping stone for the industry until the lignocellulosic route becomes technically feasible (National Biomass Strategy 2020, 2012). The good thing of OPF juice as sugar feedstock for ethanol fermentation is because the pretreatment steps of lignocellulosic can be minimized.

This research is focusing on the bio-ethanol from the oil palm frond juice due to its high sugar content. However, there is still lack of research focusing on the exploitation of oil palm frond to produce ethanol. A substantial amount of sugars (76.09 ± 2.85 g/l) can be obtained simply by pressing the OPF where 70% of the free sugars obtained was glucose, 26.9% sucrose and 2% fructose. Furthermore, the OPF juice is rich in minerals and nutrients which are essential fermentation process by most microorganism (Zahari, M.A.K.M. et al., 2012).

There are several issues should be looked into this research before the fermentation process could be optimized to achieve efficient production of ethanol. It is a bit challenging to maintain aseptic condition during the pressing process due to environmental condition at the mill. Hence, a possible method should be developed to maintain the sterility and chemical composition of the OPF juice after pressing without using heat sterilization. This is because the heat may encourage the formation of Maillard reaction products which will inhibit the microbial growth in fermentation process. Juice pressed from fresh OPF is perishable because it is nutritionally rich and its high moisture is suitable for bacterial growth. In order to produce ethanol from OPF juice, the juice must be stored for several days while it is transported from plantations and mills to an ethanol production plant. Considering the tropical climate change in this country, some changes would occurred to the OPF juice composition during the transportation process. Finally, after all the above issues have been resolved, the fermentation process could be carried out to produce ethanol from OPF juice.

oil palm frond petiole

Shake flask fermentation of ethanol from OPF

3 years old oil palm tree

Oil palm frond juice

Concentrating OPF juice by rotary evaporator

Glucose analysis by glucose assay kit

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Objectives

1. Study the kinetic of sugar degradation in OPF juice upon concentration and storage at mild temperature.
2. Investigate the effect of chemical treatments and concentration on the sugar content, total bacteria, yeast and mould count in OPF juice.
3. Optimize the ethanol production parameters from treated OPF juice by Saccharomyces cerevisiae through batch fermentation.

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Objectives

1. To analysis economic performance of integrated technology of biogas and compost production in palm oil mill.
2. To evaluate energy balances of a palm oil mill under conventional practice and using biogas collected from anaerobic treatment.
3. To analysis economic viability of integrated zero-discharge system in palm oil mill.

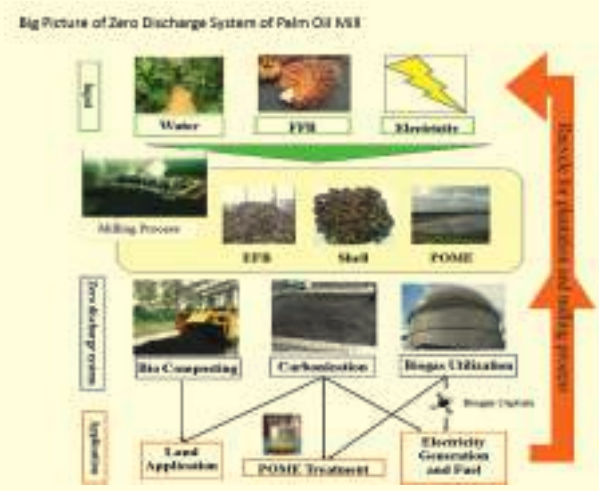
Zero-discharge of palm oil industry

Research Summary

Palm oil industry is known as a significant agricultural industry in terms of economic benefit for Malaysia. However, palm oil mills have created environmental problems due to huge quantities of polluted waste materials. Palm oil mill effluent (POME) is highly industrial waste water with high organic content. It has been recognized as a main contributor of green house gas (GHG) emission especially consisted of methane from open pond and tank treatment system. Empty fruit bunch (EFB) is a residue after milling fresh fruit bunch (FFB). Its common practice is to disposal into plantation as nutrients recycle, however it leads to pollution problems such as eutrophication and an increase of toxicity in the soil. Although the palm oil industry has developed proper utilizations of these byproducts over the past decade, it is less research to ensure the economic viability by introducing these technologies.

The idea of zerodischarge is to introduce several technologies including biogas from POME as renewable energy, composting EFB through mixing with POME anaerobic sludge, and carbonizing EFB. These technologies should be integrated and support each other. This integrated system can provide a good solution for palm oil mill to utilize its byproducts actively. Furthermore, this demonstration can improve surround environment by avoiding improper treatment of POME and inefficient disposal of EFB. This study is applied in Sandakann, Sabah, under Bornean Biodiversity and Ecosystems Conservation (BBEC) Program with Japan International Company Agency (JICA).

Biogas Reactor at Felda Serting Hilir



Development of a recombinant *Escherichia coli* for the production of polyhydroxyalkanoates

Research Summary

Polyhydroxyalkanoates (PHA) are biodegradable polymers synthesized by a variety of microorganisms as an intracellular carbon and energy storage materials. Recombinant Escherichia coli is one of the main bio-factory used for production of PHA. The nature of E. coli, which is devoid of PHA-degrading enzymes, and the extensive genome studies on this microbe that can be easily manipulated and improved have led it to be a powerful host for PHA accumulation. In this study, functionality of recombinant E. coli harbouring isolated PHA biosynthesis genes from a local isolate, Comamonas sp. EB172, a high acid-tolerant strain isolated from POME sludge in the open digester, was evaluated through PHA production from glucose and mixed organic acids as carbon sources. The results of recombinant E. coli harbouring isolated genes were then compared with recombinant E. coli harbouring Cupriavidus necator PHA biosynthesis genes as a control, both in shake flask and 2 L bioreactor.

The unknown PHA biosynthesis genes of Comamonas sp. EB172 were successfully amplified. The amplified genes sequences consisted of 1182 bp acetyl-CoA acetyltransferase (phaACo), 738 bp acetoacetyl-CoA reductase (phaBCo) and 1694 bp PHA synthase, class I (phaCCo) genes. It was found that the isolated phaACo and phaBCo genes were positioned downstream of the phaCCo gene and clustered together as phaCCo-phaACo-phaBCo. The sequence analysis of the isolated phaACo, phaBCo and phaCCo genes revealed that they shared 85.5%, 89.4% and 69.3% identities, respectively, with orthologs from Delftia acidovorans SPH-1 and Acidovorax ebreus TPSY. The isolated phaCABCo also showed the high nucleotide sequences similarity to PHA biosynthesis gene of Alcaligenes latus with 63.1% similarity (63.1% for phaC, 81.9% for phaA and 75.5% for phaB).

The new phaCABCo gene fragments were successfully cloned into a modified pGEM' vector under the control of promoter from C. necator, and transformed in E. coli JM109 for functional expression. Approximately 41% of PHA content was detected in recombinant E. coli JM109 harbouring pGEM'-phaCABCo and it was comparable to that recombinant E. coli JM109 containing phbCABRe with 46% PHA content (as a control). The functionality of phaCABCo was further evaluated with regards to its PHA production ability in which 33 wt% and 17 wt% of poly(3-hydroxybutyrate) were produced by recombinant E. coli JM109 harbouring pGEM'-phaCCoABRe and pGEM'-phaCReABCo, respectively. The phaCABCo gene exhibited the ability for PHA metabolic synthesis either by expression of PHA synthesis gene from Comamonas sp. EB172 or co-expression of phaCCo and phaABCo genes with PHA synthesis gene from C. necator. Hence, the effect of carbon and nitrogen on PHA accumulation by selected E. coli JM109 transformants harbouring pGEM'-phaCABCo was evaluated.

The PHA production by recombinant E. coli JM109 harbouring pGEM'-phaCABCo was carried out in shake flask using glucose and mixed organic acids, respectively. The use of glucose can increase cell growth and PHA accumulation with nitrogen supplementation. However, mixed organic acids failed to increase cell growth but the PHA accumulated in the cell can be improved by supplying the nitrogen. The recombinant E. coli successfully achieved 0.8 – 2.3 g/L CDW and PHA content were 34 – 46% when cells grown in medium with 10 – 20 g/L glucose. Nevertheless, the addition of nitrogen to 10 g/L mixed organic acids decreased the cell growth from 0.5 – 0.2 g/L but increased the PHA accumulation from 32 – 37% of PHA content. However, E. coli JM109 transformant harbouring pGEM'-phaCABCo successfully polymerised P(3HB-co-3HV) when fed with mixed organic acids without nitrogen source compared to only P(3HB) from glucose. Overall, supplementation of nitrogen source in the medium improved the cell dry weight with glucose as carbon source, and increased the 3HV monomer for the polymer produced from mixed organic acids. The supplementation of nitrogen source led for the changes of 3HV fraction. The results showed 3HV monomer was obtained using lower nitrogen in the range of 0 - 0.5 g/L in shake flask. Nonetheless, 3HV monomer decreased by increasing the nitrogen concentration from 0.5 – 1.0 g/L. In order to develop a proper strategy from shake flask to bioreactor, batch fermentation was carried out. The highest productivity, 0.16 g PHA/(L.h), was obtained when 20 g/L glucose with 0.5 g/L (NH4)2SO4 was used in 2 L batch fermentation. When 10 g/L mixed organic acids was used, only 0.003 g PHA/(L.h) can be obtained. Although both glucose and mixed organic acids could be utilised for PHA accumulation, the cell growth was quite low when mixed organic acids was used as carbon source in batch fermentation. Fed-batch fermentation not only improved the cell growth using 20 g/L glucose and 1 g/L (NH4)2SO4, but also improved the productivity using mixed organic acids for P(3HB-co-3HV) copolymer production. The productivity with 0.1 g PHA/(L.h) was achieved using 10 g/L mixed organic acids as feeding carbon source. The 20 g/L glucose in the culture medium at the start of the fed-batch fermentation increased the cell growth from 0.1 - 4.5 g/L after 16 h cultivation. The constant feeding of 10 g/L mixed organic acids triggered 3HV monomer formation started after 16 h and enhanced PHA content to more than 70% and P(3HB-co-3HV) copolymer with about 2 mol% 3HV monomer. The fed-batch cultivation increased the cell growth, PHA accumulation and improved the molecular weight of polymer in the range of 850 - 1490 kDa.

The overall results in this study indicated that the isolated PHA biosynthesis genes from wild type bacteria can serve as PHA production system using glucose and mixed fatty acids. Although glucose is still the best carbon source for recombinant E. coli JM109, the supplementation of mixed organic acids is essential for co-polymer accumulation and better PHA accumulation. This finding can contribute towards the accumulation of PHA using natural or renewable carbon sources. The high molecular weight of PHA produced can serve as alternative polymer instead of the conventional synthetic plastic.

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Objectives

1. Isolation of the PHA biosynthesis genes from Comamonas sp. EB172.
2. Heterologous expression of phaC and phaAB genes from Comamonas sp. EB172 in *Escherichia coli* JM109.
3. Determination of the functionality of the recombinant *Escherichia coli* JM109 for the production of PHA.

Compost hybrid modeling of organic waste

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Objectives

1. Compost process modeling and simulation
2. Composting kinetics model sensitivity analysis of degradation organic wastes
3. Compost modeling of organic waste : A hybrid model approach

Research Summary

Malaysia is the largest palm oil producer.Waste generated from oil palm plantation are 60% from POME, 23% from EFB, mesocarp fibre (12%), shell (5%) for every tonne fresh fruit bunches processed in the mills. In 2011 it is estimated we produced 93.8 million fresh fruit bunches.So it is relatively abundant amount accumulated in mills.In order to avoid EFB being burned to produce ash(substitute potash) since we practice zero burning practice. Same occurs to municipal solid waste where Municipal solid waste (MSW) in Malaysia which are currently elevate its generation due to development of urban area such as housing area and most municipal council depends on landfills as their main option on solid waste management. Currently, landfills and open dumping are main method of waste disposal treatment thus becomes not effective when it's depend on one type of treatment(Manaf, Samah, & Zukki, 2009). It is reported by Tarmudi, Abdullah, & Tap (2009) with increased MSW waste rate generation address the need to change into efficient management system. In order to justify suitable treatment process the authors suggested that characteristics of municipal solid waste also plays important roles. Base on 9th Malaysia Plan 2006-2010 report, the average components of MSW are quite similar with the largest categories consisting of food waste (45%), plastic (24%) followed by paper (7%), iron (6%) and lastly 3% for glass and others. Therefore, another type of treatment recommended as alternative of MSW disposal need to be initiate. Similarity of two type waste generated are organic and both in solid form. Therefore suitable treatment for both is composting which do the recycle process and preferable waste management practice.

In the past few decades, various large-scale composting systems were proposed, Roger (1993). However, many of them have failed from an economic or environmental aspect. More complex technology has been introduced to control the composting process, resulting in higher treatment costs compared to other methods .Most of the system designers are suffered from lack of systematic approach for overall system design. In general, designing and operation planning are based on trial-and-error tests, which cause increasing an overall cost and failure to obtain an optimum solution due to budget constraint.

Mathematical models serve as an essential tool for evaluating reactor performance and analysis, optimization and scale up predictability. Due to the complexity of the composting process, a generalized mathematical model does not exist; thus, these models are specific to the substrates used and the reactor systems in which degradation occurred. Furthermore, it is challenging to obtain kinetic parameters for composting processes and this often differentiates the methods used to model different composting systems. The starting point for many models is to place the system in a thermodynamic framework where an energy balance may be performed on the system. However, base on current trends of compost model it is time to include biochemical and biological process model into the system.

Production of fermentable sugars from oil palm empty fruit bunch using crude lignocellulolytic enzyme cocktail

Research Summary

Malaysia is currently the second largest producer of oil palm as it defeated by Indonesia with approximately 50% of world palm oil volume since 2007. However, Malaysia is the largest exporter of palm oil in the world with 11% of oils and fats production and 27% export trade of oils and fats. There are 362 palm oil mills currently operating in Malaysia with the processes of about 82 million tonnes of a fresh fruit bunch and approximately producing 33 million tonnes of residue in the forms of empty fruit bunch, shell and fiber. Among the agricultural commodity being planted in Malaysia, oil palm industry also generates the largest fraction of agricultural wastes. Oil palm empty fruit bunch (OPEFB) is one of the residues that remained in a large amount. In line with government strategy to fully utilized agricultural wastes into value added product, OPEFB has been in a great potential feedstock as it always available in bulk.

In general, OPEFB consist of 3 major components of lignin, cellulose and hemicellulose. Lignin is the most recalcitrant properties to be degraded. As lignin was the outer layer of the OPEFB, the hydrolysis of cellulose and hemicellulose was very limited due to the blocking of enzyme attack by lignin. Hydrolysis process usually was catalyzed by cellulases enzymes and in order for a hydrolysis process to take part, lignin should be removed by either biological, chemical and physical ways. To date, there are many chemical and physical approaches available in industries to remove the lignin. However, it had raised several drawbacks such as environmental impact and higher energy consumption in the mill. Thus, biological approach combining the crude of ligninolytic and cellulolytic enzymes was seen promising to produce fermentable sugars. The use of fungi or its enzymes to degrade lignin should lead to the reduction in manufacturing cost as well as pollution.

The main extracellular enzymes participating in lignin degradation are lignin peroxidase, manganese peroxidase and laccase. Whilst, the hydrolysis steps of cellulose involved three major classes of cellulases enzymes namely endoglucanase often called carboxymethylcellulases, cellobiohydrolase or exoglucanase and ß-glucosidase. Cellulases are capable of transforming lignocellulosic materials into fermentable sugars such as glucose, xylose, arabinose and mannose which is the crucial steps to many potential usages of biomass. Therefore, economic production of ligninolytic and cellulolytic enzymes are critically important to biomass utilization.

In this study, preliminary isolation and screening of locally isolated fungus producing ligninolytic enzyme will be conducted to obtain the 3 components of ligninolytic enzymes and the optimum lignin degradation efficiency will be carried out using several parameters such as mediator, pH and substrate percentage. As for the cellulase production, Trichoderma asperellum UPM1 and Aspergillus fumigatus UPM2 were used as it demonstrated to have a remarkable cellulase enzyme production. Improvements of cellulases production were made by the cultivation on different substrates and mode of fermentation. Finally, mixing of ligninolytic and cellulolytic enzyme at different ratios, reaction time and supply mode (simultaneous and one to one) will be conducted to obtain higher yield of fermentable sugar for any applications in value creation.

Decolourization of dye on plate agar as one of the screening method to select the fungi producing ligninolytic enzyme

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Objectives

1. To delignify OPEFB using crude ligninolytic enzymes from locally isolated fungi
2. To improve cellulases enzyme production by T*richoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2
3. To optimize fermentable sugar produced by one step enzymatic degradation and hydrolysis using crude lignocellulolytic enzyme cocktail from OPEFB as substrate

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Objectives

1. To determine the enzymes system involves in the bioconversion of ferulic acid into biovanillin from oil palm empty fruit bunches (OPEFB) alkaline hydrolysate by the action of *Aspergillus niger* and *Phanerochaete chrysosporium.*
2. To compare the enzymatic pathway involves in the bioconversion of ferulic acid into biovanillin from oil palm empty fruit bunches (OPEFB) alkaline hydrolysate and synthetic ferulic acid.

Determination of enzymatic pathways during the bioconversion of ferulic acid into biovanillin from oil palm empty fruit bunches alkaline hydrolysate

Research Summary

Vanillin is an important flavouring and aromatic component (3-methoxy-4-hydroxybenzaldehyde), that is generally obtained from the bean or pod of the tropical orchid *Vanilla planifolia*, and in less extent of *Vanilla tahitiensis* and *Vanilla pompon*. It is widely known as "vanilla". Each year, there are more than 12,000 tonnes of vanillin is produced but only less than 1% are made of natural vanillin from Vanilla. Natural vanillin is less in the market contributed by its high price. High price of natural vanillin is due to the limited availability of vanilla pods, combined with increasing customer demand for natural flavour has led to existence of many biotechnological methods for vanillin production from other natural sources (Priefert et al., 2001).

Ferulic acid or 4-hydroxy-3-methoxycinnamic acid, is a phenolic compound which is ester-linked to polysaccharide compound and extremely abundant hydrocinnamic acid in plant cell wall. It is commonly distributed in higher plants and plays crucial roles in plant cell walls as it control the extensibility of cells walls and growth and strengthen the cell wall by cross linking pentosan chains, arabinoxylans and hemicelluloses, making these components less preferable to hydrolytic enzymes during germination. Besides, ferulic acid provides protein protection against pathogen invasion in plant cell wall (Fry, 1982). As a component of lignin, ferulic acid is a precursor in the manufacture of other aromatic compounds such vanillin (Zheng et al., 2007). Several studies have reported the occurrence of vanillin as an intermediate of microbial degradation of ferulic acid (Rosazza et al., 2003; Priefert et al., 2001).

The bioconversion of several substrates to vanillin normally produces relatively low yields. In this case, ferulic acid from oil palm empty fruit bunches (OPEFB); a biomass from palm oil industry is used as precursor for biovanillin production as it can be considered as the most promising substrate. Alkaline hydrolysis is used to treat OPEFB in order to release ferulic acid. The hydrolysate is then used as substrate for fermentation process using *Aspergillus niger* ATCC6275 and *Phanerochaete chrysosporium*. Enzyme assays was done to determine the enzyme system involved during the bioconversion of ferulic acid into biovanillin. Through this analysis, the production trend of the enzymes is observed during the bioconversion process in order to enhance the ferulic acid production as biovanillin precursor.

Sample analysis using HPLC

Sample collection at palm oil mill

Dried OPEFB

HPLC

Development of a recombinant *Escherichia coli* for the production of polyhydroxyalkanoates

Research Summary

Demand on hydrogen production has increased considerably due to the facts that it is ideal, clean and sustainable energy source because of its high conversions and non-polluting nature. At present, hydrogen is mainly produced from fossil fuels, biomass, and water using chemical or biological processes.

Biohydrogen can be derived from biomass or bio-waste waste that is obtained from plants and animals and also include their by products. An example of biomass is agriculture waste. It has been reported that billions of tons of agricultural waste are generated each year in the developing and developed countries.

Sago palm industry is one of the major contributions in agricultural waste producer in Malaysia. Since Malaysia is one of three leading sago world producer, the production of sago and export value are keep increasing. Thus, it will leads to the increment number of waste from the processing. The residue could lead to serious environmental pollution if it is being discharged into drains or nearby streams.

Biological hydrogen production involves various types of microorganisms that able to produce hydrogen gas including strict or facultative anaerobic bacteria, aerobic bacteria, photosynthetic bacteria, blue green and green algae. Bacteria that belongs to genus Clostridium sp. would be most preferable because of their rapid metabolism, high hydrogen potential yield and also easy to handle by heat treatment due to their spore forming characteristic. Clostridium butyricum EB6 is an example of efficient biohydrogen producer belongs to Clostridium sp. that was successfully isolated from anaerobic digested POME sludge.

A specific environment needs to be created to support the growth of hydrogen producer. Biohydrogen production requires certain essential micronutrients for bacterial metabolism, growth and activity. Many different nutrients formulation have been used in studies on biological hydrogen production, resulting various production effectiveness. However, most of the nutrients formulations have not yet been optimized in order to maximize the anaerobic hydrogen production.

Gas chromatography for hydrogen analysis

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Supervisor

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Objectives

1. To exploit nutrient formulation for biological hydrogen production using sago wastes
2. To enhace biohydrogen production using fed-batch system by *Clostridium butyricum*EB6

Fermentable sugars from oil palm frond petiole

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Dr. Hidayah Ariffin

Objectives

1. Effect of OPF petiole storage period and characteristics of fermentable sugars from OPF petiole.
2. Improved storage stability of fermentable sugars from OPF petiole by water removal.

Research Summary

Oil palm frond (OPF) is one of the major crop residues which are produced annually in Malaysia. Current strategies of managing the residues are by formulating it into ruminant feed. Ongoing research found that the OPF juice contain high sugar composition that can be developed for another rising industries like fermentation, biohydrogen and other high end products. Depolymerizing cellulose using enzyme is a known way to extract sugars. Rather using enzyme for extraction, the sugars is obtained directly from the oil palm frond petiole by using conventional sugarcane press machine.

Storage evaluation was also been done to examine the critical sugar degradation in OPF prior to pressing. Besides, this study research was proposed to optimize the production of the juice extraction with the means of treatments as well as techniques to preserve the juice after extraction. The treatment involve including shredding, direct pressing with pressurized machinery and applied selective storing methods for preserving the juice. This study is part of a growing research on oil palm field and economically beneficial to the country. It will explore the potential available on fermentation industry.

Oil Palm Frond at Plantation

Research Workflow

Fresh OPF juice

Microbe Counting Using concentrated OPF juice

Alkaline hydrolysis of oil palm empty fruit bunch fibres for ferulic acid release

Research Summary

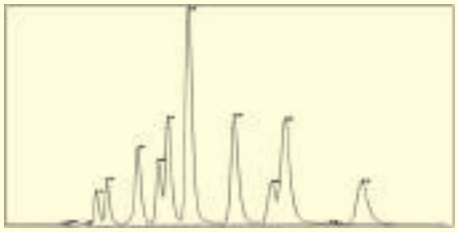
Palm oil industry in Malaysia has been a great strength over half centuries, especially in term of economic development. As oil palm plantations cover a majority of planting land, the vast production and processing result in abundance of by-products. Oil palm empty fruit bunch (OPEFB) is the major palm oil industry's waste.

Agricultural wastes contain ferulic acid, a hydroxycinnamic acid of the phenolic group that can be used as a biological precursor to be fermented into biovanillin. Various biomass had been utilized before to generate biovanillin like rice bran oil, maize bran, corn cobs, sugar beet pulp and others through biotechnological route. In that case, vanillin, the active ingredient of vanilla flavour, could also be generated from OPEFB fibre, where this actually meets the requirement and demand by the consumers on natural flavours, importantly to substitute the synthetic flavour used in food and beverages industries.

OPEFB was examined for ferulic acid release using alkaline treatment. This is because alkaline treatment is well known for its delignification efficiency and breaking up ester bonds attaching ferulic acid to lignin and hemicelluloses. Different types of alkali and a range of concentrations were examined on OPEFB for ferulic acid release. Other chemical and physical parameters tested were effect of sodium bisulfite concentrations, reaction time and temperature on ferulic acid release.

The untreated and treated (physically and chemically) OPEFB were analysed using ATR-FTIR analysis and chemical contents analysis (lignin, cellulose andhemicellulose) for comparisons. OPEFB alkaline hydrolysate was analysed for eight phenolic acids, namely, 2-metoxyhydroquinone, vanillyl alcohol, p-hydroxybenzoic acid, syringic acid, p-hydroxybenzaldehyde, vanillic acid, vanillin, p-coumaric acid, syringaldehyde and ferulic acid (in order as shown in the HPLC chromatogram).

Ground oil palm empty fruit bunch fibres



HPLC chromatogram of phenolic acids

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Supervisor

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Objective

- To identify appropriate alkaline treatment strategies and conditions for ferulic acid release from oil palm empty fruit bunch fibres.

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Objectives

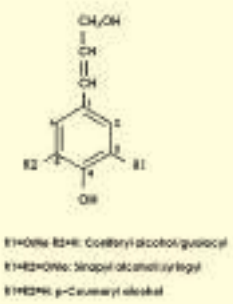
1. To determine the ligninolytic enzyme activities produced during the growth of commercial mushroom (*Pleurotus florida* and *Pleurotus sajor-caju*)
2. To optimize the production of ligninolytic enzymes of selected mushroom

Optimization of ligninolytic enzymes from commercial mushroom (*Pleurotus florida* and *Pleurotus sajor-caju*) using OPEFB as substrate

Research Summary

Lignin is the second most abundant biological material on the planet, plant – originated polymer having complex three – dimensional network comprised of monomethoxylated, dimethoxylated and non - methoxylated and comprises 15-25% of the dry weight of woody plants. This macromolecule plays a vital role in providing mechanical support to bind plant fibers together. Lignin also decreases the permeation of water through the cell walls of the xylem, thereby playing an intricate role in the transport of water and nutrients. Finally, lignin plays an important function in a plant's natural defense against degradation by impeding penetration of destructive enzymes through the cell wall.

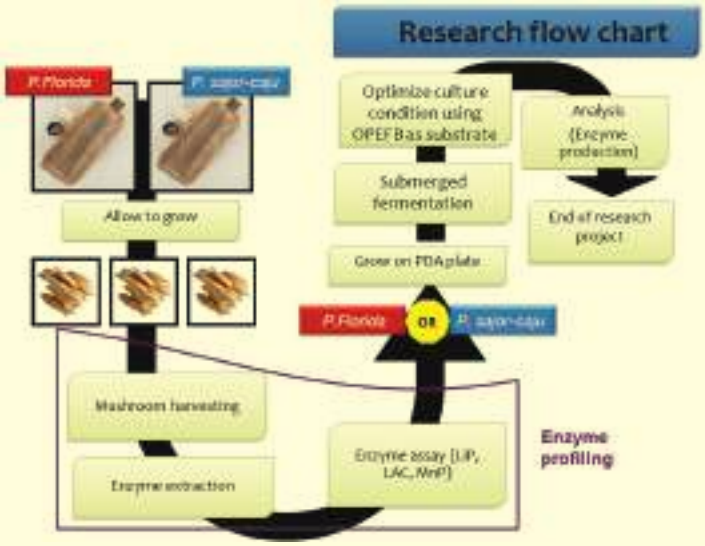
Because lignin protects cell wall polysaccharides from microbial degradation, thus imparting decay resistance, it is also one of the most important limiting factors in the conversion of plant biomass to pulp or biofuels. It is also undesirable in most chemical papermaking fibers and is removed by pulping and bleaching processes. Removal of lignin from plant cell wall contributes enormously towards many industrial sectors but the resistance of lignin to breakdown is a major obstacle. Therefore the aims of this study are to determine the ligninolytic enzyme activities produced during the growth of commercial mushroom (*Pleurotus florida* and *Pleurotus sajor-caju*) before optimize the production of ligninolytic enzymes of selected mushroom.



Building block of lignin

Pleurotus florida

Pleurotus sajor-caju



Research flow chart

Isolation and expresnon of novel cellulase genes from mixed microflora in oil palm biomass compost through culture independent approach

Research Summary

Oil palm biomass compost is a material with abundant lignocellulosic waste produced in palm oil industry. Palm oil industrial waste (include the frond (OPF), trunks, empty fruit bunch (EFB), shells (PKS), fibre (MF) and the liquid waste POME) is a potential material for the mixed microflora compost development thus for the isolation of lignocellulosic enzymes including cellulase aroducing microorganims. However, majority (>99%) of microorganisms from the environment resist cultivation in the laboratory (Barer and Harwood, 1999) and most of the cellulase may contributed by many "uncultured" microorganisms causing the isolation is limited through culture-dependent technique.

In this study, the isolation of cellulase genes is targeted through culture-independent technique. This study will be focusing on extracting the DNA directly from the mixed microflora oil palm biomass compost sample for the isolation of novel cellulase genes. The first part of this research study is the identification of novel cellulase genes through pyrosequencing technology in Kyushu Univercity, Japan. The second part is the metagenomic technique which will be carried out for the novel cellulase and genes expression in *E. coli*. The aims of this study is to provide evidence for the diversity of novel cellulase in the mixed microflora oil palm biomass compost and also to isolate novel cellulase which would offer higher efficiency for industrial applications.

Blended OPEFB

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Objectives

1. To screen and identify novel cellulase genes from mixed microflora oil palm biomass compost through pyrosequencing.
2. To express the novel cellulase genes via transformation of *E. coli* through cloning

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Objectives

1. To determine the appropriate pretreatment of OPDC as suitable substrate for fermentable sugar (polyoses) production.
2. To characterize the production of crude cellulase cocktail using OPDC by *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2.
3. To optimize biobutanol production using OPDC hydrolysate by response surface methodology (RSM) approach.

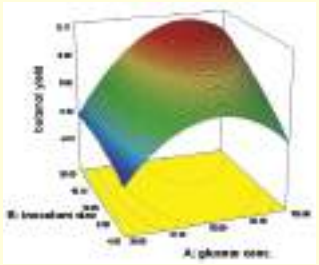
Biobutanol production as sustainable biofuel using oil palm decanter cake

Research Summary

Oil palm decanter cake which were produced by 3-phase decanter system for oil recovery was shown high potential biomass resources for the production of crude cellulase cocktail, polyoses and consequently to biobutanol as sustainable biofuel. The effectiveness of various chemical and physical pretreatment (NaOH, HCl and HNO_3) to alter lignin content will be evaluated. The presence of lignin and hemicellulose decrease the accessibility of cellulase enzymes to cellulose, thus reducing the hydrolysis efficiency. Appropriate pretreatment is important to reduce high amount lignin content and retain the amount of cellulose and hemicellulose in OPDC. The microscopic change after the appropriate pretreatment was observed by scanning electron microscope (SEM).

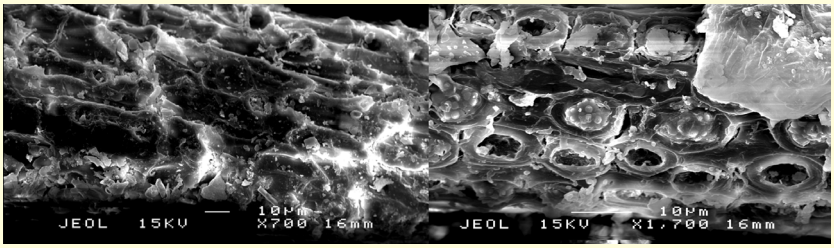
Production of cellulase was done by liquid (submerge) fermentation. Cellulase production using single microorganisms may not efficient in the hydrolysis process. Trichoderma sp. species produced high activity of FPase and CMCase but low in ß-glucosidase activity. The formulation of crude cellulase cocktail may solve the deficiency of ß-glucosidase in saccharification process by addition of cellulase from *Aspergillus* sp. In this study, the crude cellulase cocktail are produce by *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2. The optimum chemical and physical conditions such as type of biomass (substrate), pH, temperature, incubation period and inoculum size will be investigate to increase the cellulase activity.

Optimization of biobutanol production using OPDC hydrolysate by *Clostridium acetobutylicum* ATCC 824 will be evaluate statistically using response surface methodology (RSM). The parameters involve are initial substrate pH, temperature, inoculum size and substrate ratio. The analysis of variance (ANOVA) using 2-level factorial use to screen significant variables that influence the biobutanol yield. The central composite design (CCD) use to determine the optimum value to each significant parameter and obtain high concentration of biobutanol. The yield and productivity of biobutanol production will be calculated.



Interaction inoculum size and glucose concentration on biobutanol yield

Oil palm decanter cake as substrate for production of cellulases and polyoses



Raw and pretreated OPDC

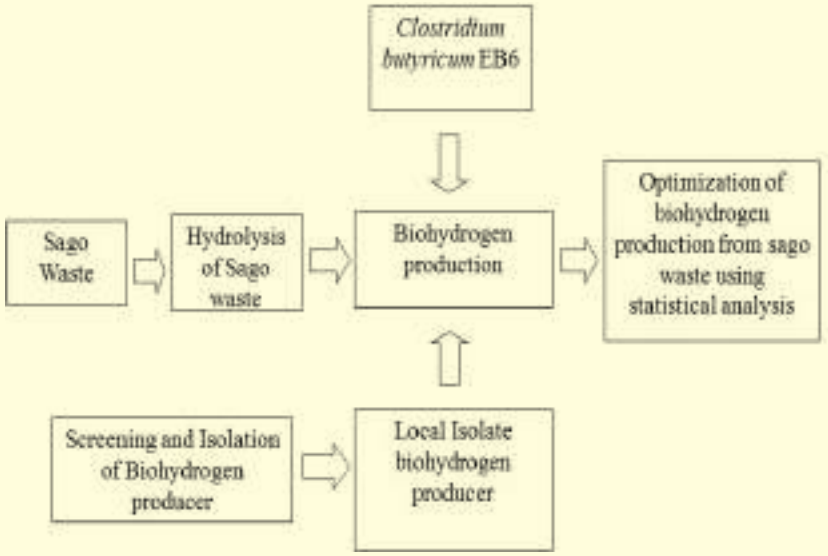
Hyvolution: Biohydrogen production from sago biomass by *Clostridium Butyricum* A1

Research Summary

Biomass is one of the most abundant and renewable resources that can be converted into value added product. The agricultural activity is one of the biggest activities that lead to the abundantly of biomass especially in Malaysia. In Sarawak, the sago mill has produced significant amount of post-processing waste and residue. The final waste product in the extraction of starch compound from sago palm is the starchy fibrous pith residue or known as sago 'hampas'. It is usually washed off into drains or nearby streams together with wastewater which contribute to pollution load and serious environmental problems. The treatment of sago 'hampas' has produced the solid residue known as sago pith residue (SPR), which is lignocellulosic material. Therefore, it can be used as carbon source for production of biofuel through fermentation. Biofuels are liquid or gaseous fuels for power plants and transport sectors that are produced from renewable resources such as biomass (Demirbas, 2007). Hydrogen is one of the biofuels that has been found to be well suited as it is clean and has a high calorific value fuel.

In this study, the effect between sago 'hampas' and sago pith residue on the biohydrogen production by local isolate, which are *Clostridium butyricum* strain EB6 and strain A1 will be studied. Basically, the hydrolysis of sago 'hampas' and SPR will be produced the fermentable sugar which can be used as carbon source in the fermentation. On the other hand, the *Clostridium* sp. will be employed since it is known as good biohydrogen producer. Furthermore, the resource that gives the significant effect to the biohydrogen production will be further optimizing using statistical analysis.

All in all, this research is expected to give better understanding on the searching an alternative to replace the non-renewable fuel nowadays.



Experimental design for biohydrogen production from sago waste by local isolate2

Solid residue from sago mill effluent dumped into nearby river

Biohydrogen production

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Supervisor

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Objectives

1. To study the effect of different types of sago waste on the biohydrogen production by local isolate.
2. To optimize the production of biohydrogen from sago waste by local isolate using statistical analysis.

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Objectives

1. To investigate the effect of various OPF bulking size in composting of POME under different FFB sterilization process
2. To investigate the feasibility of biocompost production from co-composting of oil palm EFB with raw POME

Improvement in co-composting process of pressed-shredded empty fruit bunch and raw POME from continuous sterilizer system

Research Summary

The continuous sterilizer system is a high performance system in palm oil extraction. In future trend, more and more mills in Malaysia will installing such fresh fruit bunch (FFB) processing system. However, The non-ponding system in continuous sterilization system has generated huge amount of empty fruit bunch (EFB) and raw POME that had create problem to the mills.

Therefore, composting of continuous sterilizer EFB with the addition of raw POME was an option to solves wastes accumulation problems in the mills. Currently composting technology on empty fruit bunch (EFB) and raw POME still in the infrant stages.

The decomposition of empty fruit bunch (EFB) in acidic condition may inhibit microbial decomposition rate.

Therefore, further study regarding microbial decomposition on empty fruit bunch (EFB) was important for utilizating continuous sterilizing empty fruit bunch (EFB). In order to get better understanding on physicochemical in continuous sterilizing empty fruit bunch, detail study on chemical and structural properties has been conducted.

The scanning electron microscopy (SEM) and transmission electron microscopy (TEM) give a full picture on structural disruption under sterilization. For composting process, microbial seeding method play an important role in effective composting since raw POME lack of microbes. Hence, effectively utilizing microbial seeding was a key for successful composting process. This study was targeting to deliver a good composting process for raw POME and empty fruit bunch (EFB) for industrial application.

Assessing the potential of the blend polyethylene with poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) as a food packaging plastics and its recyclability

Research Summary

Public are aware that the polyethylene (PE) is not biodegradable and non-renewable. However, people tend to use it due to its usefulness. This study brings the potential of the blends between PE and poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) as food packaging plastics. PHBV is a biodegradable polymer which is very suitable to be applied as food packaging plastics due to its excellence barrier properties. Previous research reported the PHBV has low oxygen transfer rate as compared to the other polyolefin which could enhance the product shelf life. However, PHBV is not practical to be applied as commodity plastics due to its brittleness and narrow processing window. Therefore, by blending the PHBV with PE should provide a solution for this problem and simultaneously reducing the use of PE. In this study, the properties of the blend include mechanical, morphological, thermal, and barrier properties were evaluated and its recyclability by thermal degradation. The results obtained herewith were compared with those of commercial plastics such as grocery, ziplock, detergent, and garbage bags. Besides that, the crotonic and pentanoic acid produced by thermal degradation are useful to be applied in other fields such as cosmetic, hair styling product, herbicides, hydrogel and flavouring. Overall, due to the interesting characteristics of the PE/PHBV polymer blends, the use of the polymer blends could be an option in reducing the use of non biodegradable petroleum-based PE with the additional value added to the plastic.

Nuclear Magnetic Resonance

Research Work Flow

Collected pyrolyzates

Glass Tube Oven

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Supervisor

Dr. Hidayah Ariffin

Objectives

1. Characterization of PE/PHA blend and its suitability as food packaging plastics
2. Separation and Recyclability of PE/PHA blend by thermal degradation.

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Dr. Hidayah Ariffin

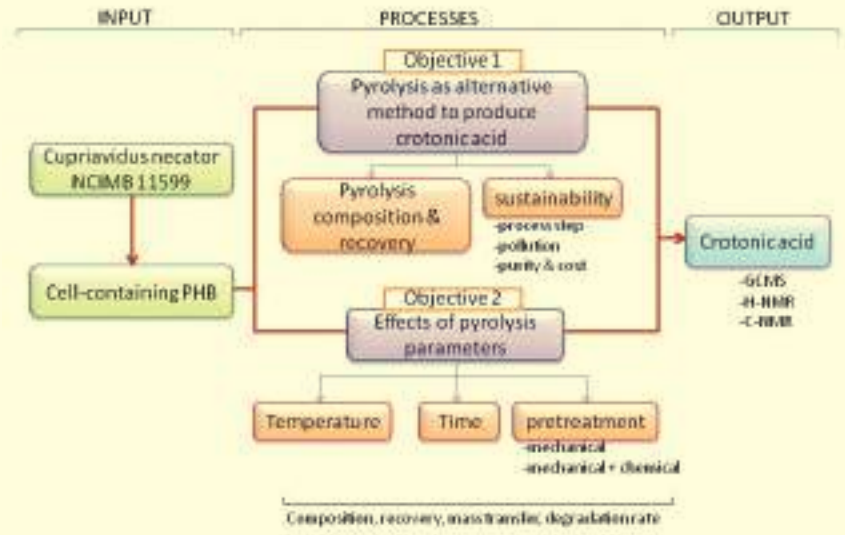
Objectives

1. Environmental friendly method for crotonic acid production via pyrolysis of polyhydroxybutyrate (PHB)-containing bacterium
2. Effect of pyrolysis parameters on the composition and recovery of crotonic acid

Alternative route to crotonic acid production by pyrolysis of polyhydroxybutyrate - containing bacterium

Research Summary

Crotonic acid is a short chain unsaturated carboxylic acid. Crotonic acid and its derivatives have various specific applications; for example as a component in dental materials, cosmetics, hair styling products, plasticizers, herbicides, compatibilizers, paints and hydrogels. Current production of crotonic acid is via petrochemical synthesis. However, it has several drawbacks. The chemical synthesis of crotonic acid involves many steps. Furthermore, purification of crotonic acid by crystallization may contribute to the environment pollution as it causes the formation of about one ton of highly contaminated effluents per ton of processed crotonic acid. This is accompanied b about 1500m3 of contaminated air per ton of crotonic acid from the drying process. Moreover, the crystallization process also causes product losses. The present proposed research provides an alternative route to crotonic acid production which involves biological synthesis and eco-friendly methods. This can be done by the use of polyhydroxybutyrate(PHB)-producing bacterium. This bacterium accumulates PHB as energy reserve materials under suitable conditions during fermentation. PHB can later be converted to its dehydrated monomer which is crotonic acid via thermal degradation. In this research, recovery of crotonic acid from PHB-producing bacteria will be conducted by the mean of pyrolysis. Pyrolysis process will be conducted in a glass tube oven, and the pyrolyzates will be collected and characterized by Gas Chromatography-Mass Spectrometry (GC-MS). The geometric isomerism of crotonic acid produced will be determined by 1H-NMR. This study is expected to contribute to new method for green route for crotonic acid production.



Glass tube oven used for isothermal pyrolysis

Analysis of pyrolysis composition by GC-MS

Appropriate treatment of palm oil mill final discharge wastewater as recycled water

Research Summary

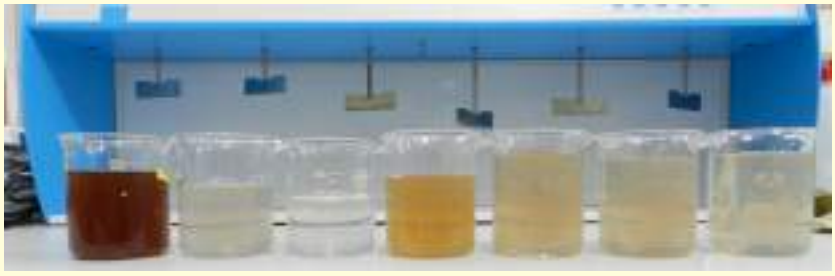
In palm oil industry, huge amount of water have been utilized for palm oil sterilization and extraction process. The processing system has been applying widely in Malaysia for year. It has been estimated that around one tonne of fresh water was needed for processing every tonne of fresh fruit bunch (FFB). As a return, huge amount of wastewater has been generated, treated and discharged to river every day. Current treatment system applying in oil palm industry is using river water, treated and use for mill.

In this study, the effect of chemical coagulant and activated carbon application as appropriate treatment of palm oil mill final discharge wastewater have been evaluated in order to recycled water for the mill to achieve zero discharge. Current chemical treatment used at the mill will be used to treat final discharge wastewater. Activated carbon or biochar is used as absorbent material due to its large number of cavernous pores that provide a large surface area relative to the size of the actual carbon particle and its visible exterior surface.

A jar test method is used to stimulate the coagulation and flocculation process that encourage the removal of COD and suspended solids in final discharge wastewater which can lead to turbidity, color, odor and taste problem. In this research, jar test is used to determine the optimum operating conditions for final discharge wastewater by optimizing dosage of coagulant and activated carbon, mixing and sedimentation time and pH value of existing treatment system to reduce capital expenditure on new treatment system.

Granular activated carbon

Palm Oil Mill Final discharge



Sampel after treatment using chemical coagulant and activated carbon

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MSc Bioprocess Engineering
(Semester 4)

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Supervisor

Prof. Dr. Mohd Ali Hassan.

Objectives

1. To study the effectiveness of chemical coagulants for the treatment of palm oil mill final discharge wastewater.
2. To investigate the potential of biochar to polish treated palm oil mill final discharge wastewater.

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Supervisor

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Objectives

1. Optimization for production of Polyhydroxyalkanoate (PHA) by *Comamonas* EB172 using Response Surface Methodology (RSM)
2. Develop a simple kinetic model for production of PHA in 2 liters bioreactor

Optimization of polyhydroxyalkanoates production by an acid-tolerant bacterium, *Comamonas* sp. EB 172 using response surface methodology

Research Summary

Mixed organic acids derived from anaerobically treated palm oil mill effluent (POME) containing acetic:propionic:butyric (ratio of 3:1:1) were used as carbon source in the batch culture of Comamonas sp. EB172 for producing polyhydoxyalkanoates (PHAs).

Statistical approach, central composite design (CCD) was used to investigate the complex interaction among temperature (25-37 oC), initial medium pH (5-9), inoculum size (4-10% (v/v)), concentration of (NH4)2SO4 (0-1 g/l) and concentration of mixed organic acids (5-10 g/l). The analysis of variance (ANOVA) showed that all of these five factors were significantly important in the batch fermentation by shake flask with the P value less than 0.001. The optimal temperature, initial medium pH, inoculum size, concentration of (NH4)2SO4 and concentration of mixed organic acids were determined at 30oC, 7.04, 4.0% (v/v), 0.01 g/l and 5.05 g/l respectively. Optimization of the production medium containing mixed organic acids improved the PHA production more than 2 fold. Under optimal condition in the shake flask fermentation, prediction for the growth was at 2.98 g/l of dry cell weight (DCW) with 47.07 wt.% of PHA content.The highest yield of PHA was 0.28 g of PHA per g mixed organic acids.

For the second objective for this study was to determine the kinetic of the PHA production and the mixed organic acids consumption in the 2 L bioreactor with the optimal condition obtained from the first objective with simultaneous considerations of substrate inhibition, cell growth, maintenance and product formation were explored. Results showed that growth of of Comamonas sp. EB172 was inhibit ed under initial-sufficient conditions. From the experimental results verify that the model established in this work was able to describe the PHA production from mixed organic acids by Comamonas sp. EB172.

Bio-based polyester from palm oil catalyzed

Research Summary

Petrochemical-based polymers became popular in the 1940's, and have been used in a wide range of products since then. It is because of their favourable mechanical and thermal properties including strength, lightness, durability and resistance to degradation, they have been part of the contemporary life. However, because of the depletion of petroleum fossil source, plus with the accumulation of petrochemical-based polymer in the environment, it is necessary to develop new type polymer especially from renewable resource. Fatty acids are some of the most promising candidates for the production of polymeric materials, because they occur widely and abundantly in plants, fish, animals and microbes in nature. In Malaysia, palm oil industries play an essential role and deemed one of the major contributions in agro-industries, beside of other commercial crops, like rubbers and cocoas, thus also shown great potential as renewable raw material for the polymeric industry.

In this work, the model reaction is first developed by the reactions involving commercial fatty acids. The commercial fatty acids will be converted into dicarboxylics acids by fermentation using *Candida tropicalis* ATCC20962. The reaction is proceeding with the modification of dicarboxylic acids by epoxidation, hydrogenation and hydroxylation. Then, the reaction is followed by condensation polymerization of the monomers to produce bio-based polyester. Immobilized lipase from Candida antartica will be used in the condensation polymerization step. Then, the overall reactions are repeated by using palm oil since that palm oil is rich with various types of fatty acids.

The aim of this work is to develop new bio-based polyester from palm oil that compost of various saturated and unsaturated fatty acids as an alternative to the petroleum-based polyester. In developing such a product, it is essential to find out the key variables affecting the performance of the production process. Therefore, the objectives of this research were; 1) to develop a model reaction from commercial saturated fatty acid (Palmitic Acid and Stearic Acid) and unsaturated fatty acid (Oleic Acid and Linoleic Acid) into bio-based polyester. 2) to characterize the bio-based polyester produced from palm oil and compare with the bio-based polyester produced from commercial fatty acids.

Big picture

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Supervisor

Dr. Hidayah Ariffin

Objectives

1. To develop a model reaction from commercial saturated fatty acid (Palmitic Acid and Stearic Acid) and unsaturated fatty acid (Oleic Acid and Linoleic Acid) into bio-based polyester.
2. To characterize the bio-based polyester produced from palm oil and compare with the model reaction produced from commercial fatty acids

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Supervisor

Dr. Nor'Aini Abdul Rahman

Prof. Dr. Suraini Abd-Aziz

Objectives

1. To determine microbial populations from oil palm plantation soils for lignin-degrading bacteria strains by using 16s rRNA clone library approach
2. To isolate, characterize and optimize the ligninolytic bacteria strains for production of ligninolytic enzymes

Biodegradation of lignin by isolated ligninolytic bacterial strains from oil palm plantation soils

Research Summary

Ligninolytic enzymes are very important for their essential role in the biodegradation of lignin and the potential use in biotechnology industrial processes such as bioremediation, biopulping and food industry. Ligninolytic enzymes which are mainly consists of lignin peroxidase, manganese peroxidase and laccase have showed efficient biodegradation ability of lignin wastes. Recent published works imply that a range of soil bacteria which are normally aromatic-degrading bacteria are able to produce ligninolytic enzymes and have wider tolerance of temperature, pH and oxygen limitation than fungi. In this study, the potential lignin-degrading bacteria strains were screened and isolated from oil palm plantation soils and were measured for the production of ligninolytic enzymes activities.

In this study, bacterial communities in the soil samples can be obtained by using independent culture-based techniques of 16s rRNA gene library approach. Based on the lists of the total microbial community, isolation of potential ligninolytic bacteria strains were conducted and classified according to phylogenetic analysis. Bacillus antracis, Ochrobactrum antropi and Leucobacter komagatae were selected for kraft lignin (KL) and oil palm empty fruit bunch (OPEFB) lignin degradation in submerged fermentation.

Hence, this research is also done to study the effects of pH and temperature in order to determine the optimum conditions which significantly influence the production of ligninolytic enzymes. The improvement of ligninolytic enzyme activity was done by protoplast fusion approach between the best ligninolytic enzyme producers

Isolation of lignin degrading bacteria

Fermentation for ligninolytic enzyme assays

Pure colony of ligninolytic bacteria

Ligninolytic bacteria

Improved recovery of bio-based crotonc acid

Research Summary

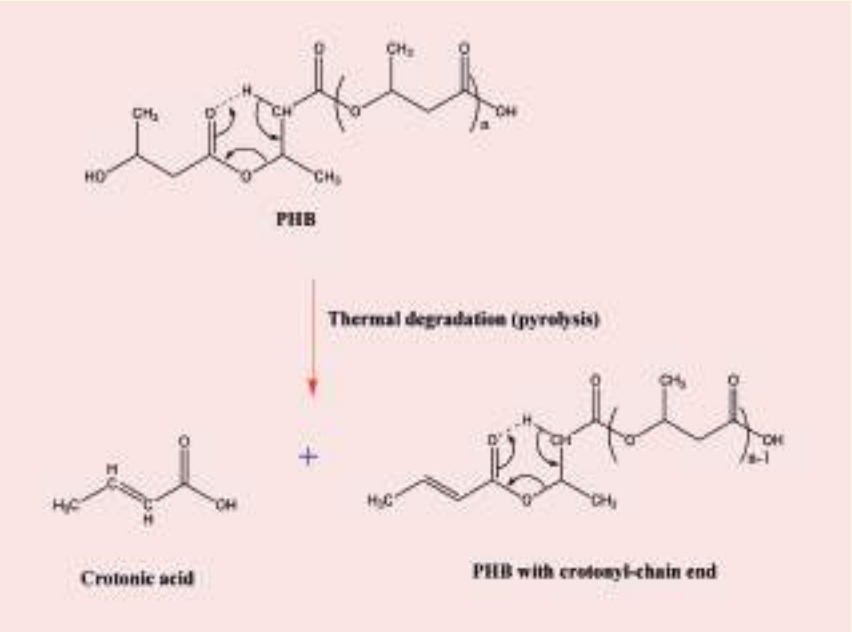
Crotonic acid is dehydrated monomer of Polyhydroxybutyrate (PHB). Crotonic acid can be obtained by thermal degradation of PHA under controlled temperature and retention time. It is a short and unsaturated chain carboxylic acid, which is also known as 2-butenoic acid or 3-methyl-acrylic acid. There are many applications of crotonic acid and its derivates in industries such as cosmetic ingredients, dental materials, hair styling products and etc.

Currently, crotonic acid is chemically synthesized which is not only involving multi-steps, but also producing toxic air and wastewater as byproducts. An alternative to chemical production of crotonic acid can be done by biologically produce the PHB, which is the hydrated polymer of crotonic acid, and subsequently pyrolyze the cell containing PHB to obtain crotonic acid.

Fermentation of PHB in in 2L STR

However, current study in laboratory shows that direct pyrolysis of cell containing PHB gives low purity crotonic acid, which is about 70 - 75 % purity. This could be contributed by the presence of other organic compounds in the cell that may volatile together with the crotonic acid during pyrolysis and collected as pyrolysis products. In order to improve the crotonic acid production, it is proposed that recovery of PHB need to be done prior to pyrolysis.

In this study, PHB will be produced by Cupriavidus necator NCIMB11599 utilizing glucose through fed-batch fermentation. This is followed by recovery of PHB by mild alkaline method as suggested by Mohammadi et al. (2012). After recovery, the PHB will be pyrolyzed in a glass tube oven to obtain crotonic acid (scheme 1). The pyrolyzates will be collected and analyzed using GC-MS and 1H-NMR to determine its purity and recovery yield.



Scheme 1: PHB degradation into crotonic acid during pyrolysis

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Objectives

1. Effect of PHB recovery on the characteristics of PHB pyrolysis products.
2. Effect of alkali earth compounds on the recovery and diastereoselectivity of the crotonic acid formed via pyrolysis

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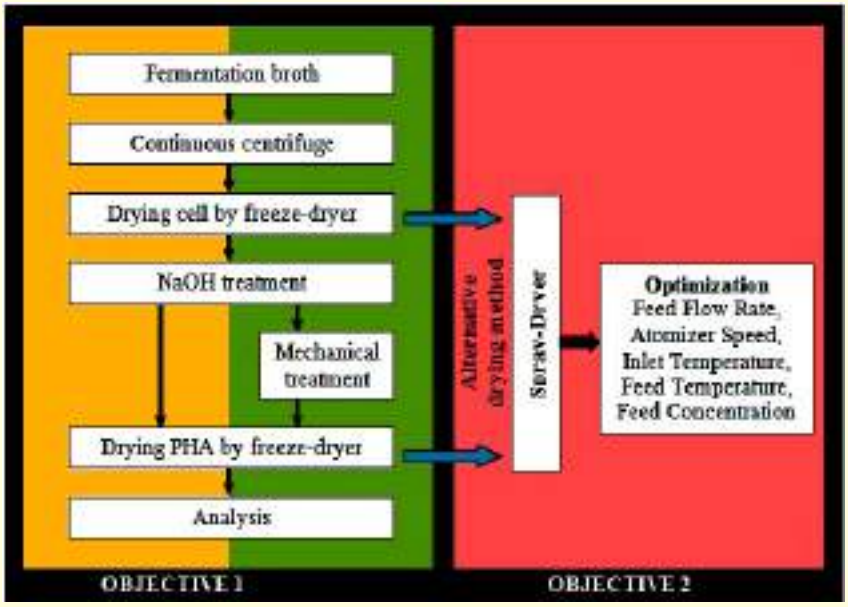
Objectives

1. To improve the method of PHA recovery from *Comamonas* sp. EB172 by mechanical-chemical hybrid treatment.
2. To optimize spray-drying condition for PHA recovery process.

Improved recovery of polyhydroxyalkanoate from *Comamonas* sp. EB172

Research Summary

Comamonas sp. EB 172 is a potential candidate for polyhydroxyalkanoate (PHA) bioplastic production due to its ability to utilize wastewater-derived acids for the production of PHA. However, previous study has shown that recovery of PHA from wild type *Comamonas* sp. EB172 by NaOH treatment resulted in low purity PHA. This is due to the nature of *Comamonas* sp. EB172, an acid tolerant bacterium which has higher impermeability towards treatment by NaOH, due to its high monounsaturated composition in the cell wall. This study proposes that recovery of PHA from *Comamonas* sp. EB172 can be improved by applying mechanical pre-treatments (bead mill and homogenizer) towards the polyhydroxyalkanoate containing biomass prior to NaOH treatment. Furthermore, previous study used freeze-drying as drying method without the use of protective agent and this is suspected to aid in PHA recovery due to sudden changes of the cell's environment during the process, which might disrupt the bacterial cell wall. Therefore, this study is also done to compare the characteristics and purity of PHA recovered from freeze-dried and spray-dried cells in order to know the effect of drying method on recovery as well as to clarify the unspontaneous mechanical treatment by freeze-drying. Optimization of PHA drying process using spray dryer will also be conducted in order to propose a sustainable drying method for large scale PHA recovery. PHA characteristics and purity will be determined by GC-MS, SEM and TEM. Overall, this study is expected to provide new information for large scale PHA recovery using NaOH.



Overview of the study

Characterization of phaZ gene of *Comamonas* sp. EB172 by transposon mutagenesis and over production of polyhyroxyalkanoates

Research Summary

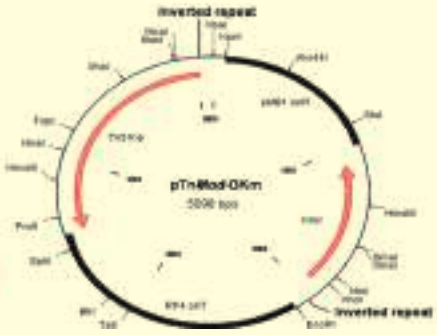
The accumulation of petrochemical plastic waste in the environment is an increasing problem and it also affecting the potential survival of many species. According to Snell and Peoples in 2009, they predicted from year 2006 to 2009, quantity of synthetic plastics used is increasing from 25 million tonnes to 230 million tonnes. The natural environment is continuously polluted by these hazardous plastics, to overcome this problem development and production of environmental-conserved biodegradable plastics is rapidly expanding in order to reduce our reliance on synthetic plastics.

Researchers have developed biodegradable plastics, such as polyhydroxyalkanoates (PHAs) and the PHAs extracted from bacterial cells show material properties that are similar to polypropylene. Polyhydroxyalkanoates are polyesters of hydroxyalkanoates (HAs) and it was first discovered in 1926. The microorganisms accumulating PHA are easily recognized by using staining either with Sudan black or Nile blue (Ostle and Holt, 1982; Schlegel et al., 1970). According to Byrom in 1994, the stored PHA can be degraded by intracellular depolymerases and metabolized as carbon and energy source as and restored the supply of the limiting nutrient.

Comamonas sp. EB172 is a new local isolate obtained from POME sludge. The bacterium were rod–shaped, Gram negative, non-pigmented, non-spore-forming and non-fermentative. It showed superior growth profile on organic acids and this bacterium was able to synthesize PHA when supplied with organic acids. But under unbalanced conditions especially when nitrogen sources are limited in the medium, the bacterium will accumulates up to 60-70% of PHA in their cell bodies. Therefore, when the carbon sources are in depletion, an enzyme known as phaZ gene is triggered and the PHA is consumed back by the bacterium as a carbon and energy sources. Thus by removing the phaZ gene, higher accumulation of PHA is expected and molecular weight of the polymers improved.

The aim of this project is to remove the intracellular depolymerase or known as phaZ gene and this can be done by using transposon mutagenesis or known as jumping genes. The application of this transposon mutagenesis will delete the phaZ gene and improve the PHA production. The transposon used in this experiment is a minitransposon or recognized as plasposon which is specialized transposons which arrange the related transposase outside of the transposon's inverted. The unique features of minitransposons and self-cloning transposons have been combined to construct new Tn5-based minitransposons that are very useful for the rapid genetic analysis of gram-negative bacterial genomes. The basic minitransposon has been modified to include a conditional origin of replication and exchangeable antibiotic resistance determinant and modular arrangement of the new TnMod minitransposons allows for different combinations of antibiotic resistance determinants and high- or low-copy-number origins of replication (Dennis and Zylstra, 1998).

Overall big picture of my project



Plasposon used in the experiment, pTnMod-oKm

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Objectives

1. To isolate and identify the depolymerase gene (phaZ) from *Comamonas* sp. EB172 by transposon mutagenesis.
2. To over produce of PHA from *Comamonas* sp. EB172 mutants in shake flask and 2 L fermenters.
3. To characterize the mechanical and thermal properties effects of PHA produce from *Comamonas* sp. EB172 mutants.

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Objectives

1. To isolate potential glycerol-fermenting bacteria for ethanol production
2. To optimize bioethanol production by using response surface methodology

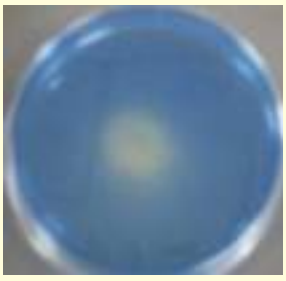
Bioethanol production from glycerine wastes using locally isolated bacteria

Research Summary

Bioconverting glycerol into various valuable products is one of glycerol's promising applications due to its high availability at low cost. Many microorganisms are known to naturally utilize glycerol as their sole carbon and energy sources. Various v aluable chemicals can be produced from glycerol via biological conversion. Bioethanol is one of the interests that can be produced from glycerol conversion. The type of end products from microbial conversion of glycerol is depending on the types of microorganism used and fermentation conditions. Hence, the suitable microorganism capable to convert glycerol into bioethanol and appropriate condition for fermentation process should be considered in order to allow high production of ethanol. Thus, the objectives of this study were to isolate potential glycerol-fermenting bacteria for bioethanol production and to optimize the fermentation conditions for bioethanol production from crude glycerol using Response Surface Methodology (RSM). The screening procedure was modified to isolate glycerol-consuming ethanol producer. The potential ethanol producer obtained was identified as Escherichia coli sp with ethanol yield of 1.0 mol/mol glycerol, hydrogen as byproduct with the yield of 0.24 mol/mol glycerol and minor amounts of organic acids such as succinic, lactic and acetic acid. This isolated strain has greater affinity to glycerol as compared to glucose as substrate for ethanol production. Furthermore, fermentation conditions such as substrate concentration, temperature, pH, nitrogen source, trace element solution and salt content were optimized. The obtained results were subjected to two-level factorial design where only four paramaters showed significant result, except temperature and trace element solution. The optimized conditions obtained from Central Composite Design (CCD) were 35.8 g/L of substrate, pH at 7.8 and nitrogen sources at 6.8 g/L with 17.05 g/L of ethanol produced. The validation was carried out by using crude glycerol as substrate in 1 L bioreactor.

Crude glycerol obtained from palm oil-based båiodiesel

Glycerol fermentation in serum bottle



Decolorize zone against blue dye shows the presence of ethanol

Inoculum preparation in anaerobic flask

Co-composting of municipal sewage sludge with landscaping wastes

Research Summary

Malaysia is becoming one of the developing countries, which is underutilizing renewable energy as the new sources of energy for human uses. Among of various sources of renewable energy that been used, biomass seems to be the most promising option for Malaysia due to the generated biomass and by-products in abundant masses, which may become major concern for peoples as well as the industry itself.

Supposing landscaping wastes or yard wastes and can be categorized as a part of biomass by-product that been produced daily. Landscaping waste is defined as the accumulation of biodegradable waste (normally plant materials, including leaves, grass clippings, pruning, branches, brush, garden material) as the result of care of the landscape area. Normally, peoples will throw the landscape wastes into their waste bin, directly be disposed into the landfill sides, or sometimes they burn them in open burning. On the other hands, municipal sewage sludge (MSS) is the wastes generated during treatment of domestic sewage (primary, secondary or advanced wastewater treatment) before being released back into the nature, usually in form of solid, slurry, or liquid residue. MSS usually consists of 90 - 99% of water content and accumulation of settleable solids from wastewater treatment processes, mainly organic components that good for plant and soil sauna. Researches that been carried out lately in lab scales is focusing on utilization of both wastes as raw material in producing such a good product such as fertilizer which suitable be used in natural environment.

The aim in this research is to enhance the utilization of agricultural biomass produced from landscape area, which is landscaping wastes as a suitable substrate to produce the high value-added product such as biofertilizer by using biotechnology approaches. This research is focusing more on the composting process of landscaping wastes and MSS that been carried out in the 10m3 composter, which can be considered as semi-pilot scale for producing the compost products in the industry. Other than that, this research is done to produce good quality of biofertilizer from landscape waste using MSS as the seed culture for microorganism degradation activities, rather than introducing any effective microorganism (EM) on the compost process..

Municipal sewage sludge (MSS)

Landscaping wastes

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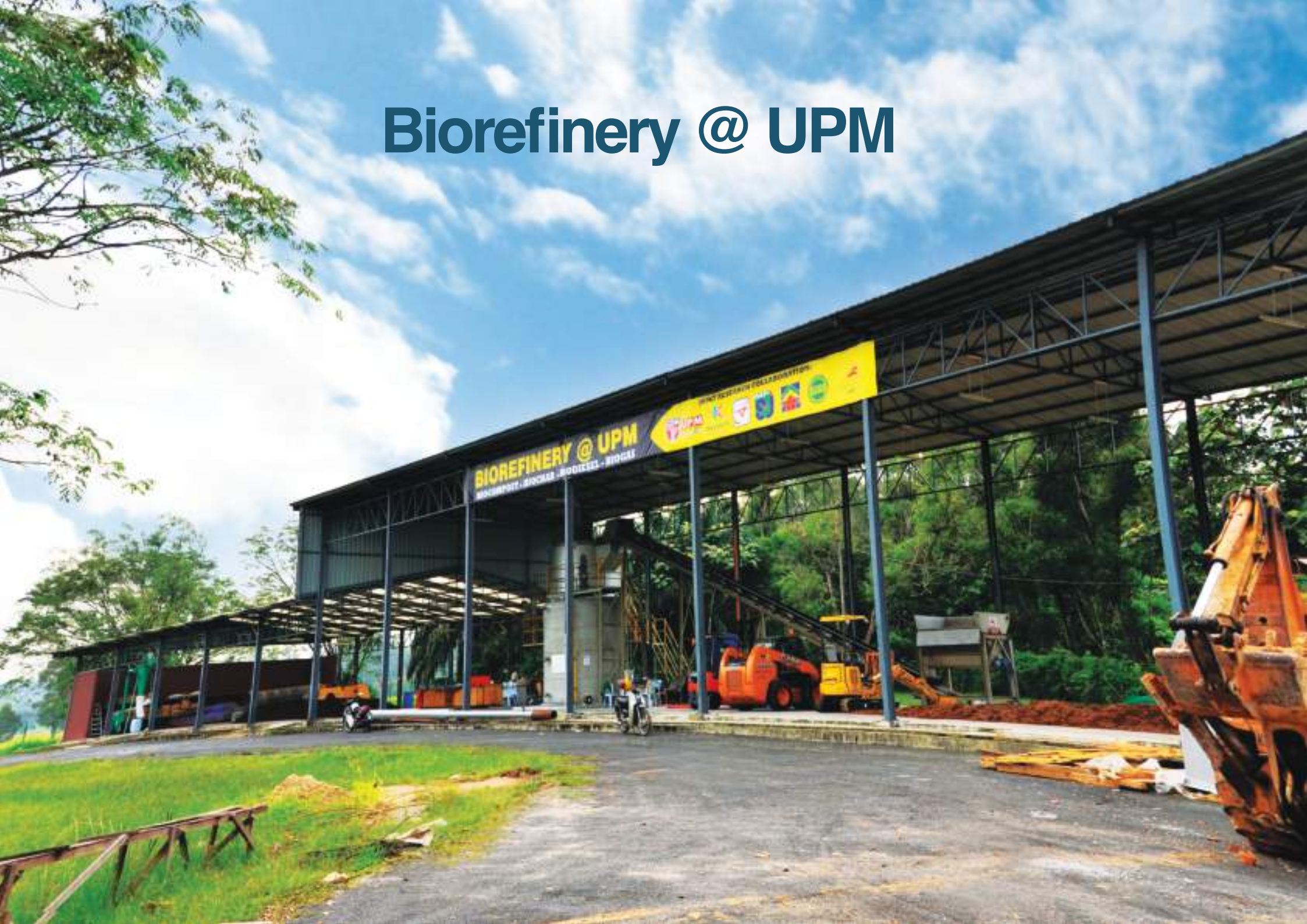
Objectives

1. To obtain the optimize condition for production of biofertilizer from landscaping wastes and municipal sewage sludge.
2. To evaluate for the potential of biofertilizer from landscaping wastes and municipal sewage slidge on the growth performance of ornamental plant.

Photographs

Biorefinery @ UPM



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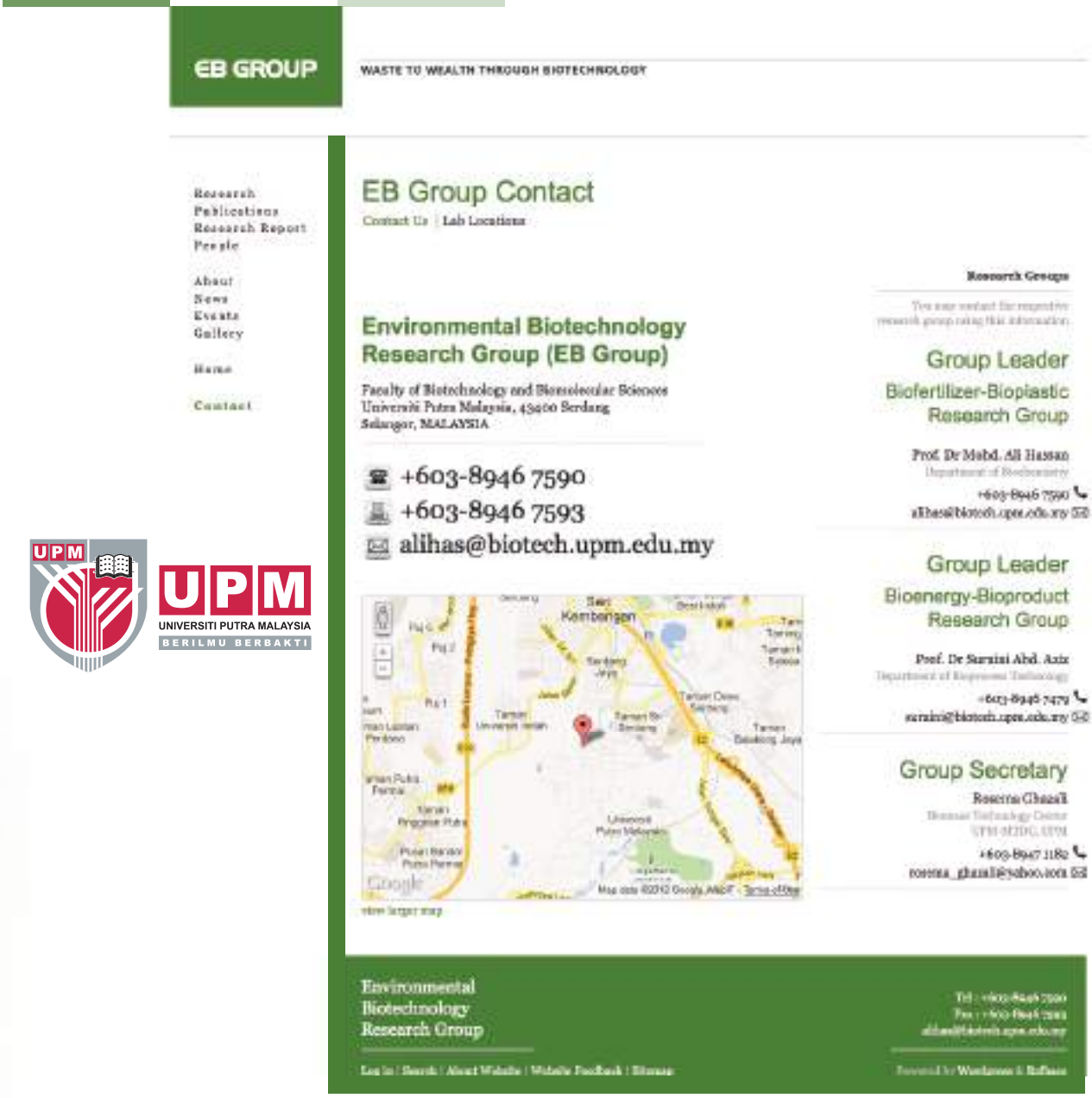

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