UPM

UNIVERSITI PUTRA MALAYSIA

BERILMU BERBAKTI

ENVIRONMENTAL BIOTECHNOLOGY RESEARCH GROUP

RESEARCH REPORT 2011

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 consists of four subgroups; Biocompost, Biomaterial, Bioproduct, Bioenergy. Biocompost and Biomaterial are led by Professor Dr Mohd Ali Hassan, whereby the other two groups are led by Professor Dr Suraini Abd Aziz. There are 6 principal researchers in the group and 33 students including Masters and PhD. Our aim is to be a high performance research group conducting research on the utilization of oil palm biomass and other renewable raw materials in Malaysia for valuable green products. Most of our research are conducted in close collaboration with other academic institutions and industries locally and internationally, such as FELDA, Kyushu Institute of Technology (Kyutech, Japan), and National Institute of Advanced Industrial Science and Technology (AIST, Japan). ■

On the cover

69 1st Bioplastic Pilot Plant in Malaysia

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RESEARCH GROUP

46 BIOENERGY BIOPRODUCT

66 BIOMATERIAL

82 BIOFERTILIZER

► Bioplastic Pilot Plant Holding Tank

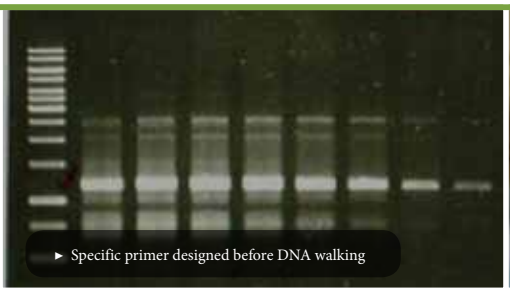
MESSAGE FROM THE EB GROUP LEADER

AlhamduLillah, praise to ALLAH for His generous favours and blessings for us. I am very happy that over the years, our Environmental Biotechnology Research Group (EB) has gone from strength to strength. I am glad to share with you our research report for 2011. We now have four sub-groups comprising of bioenergy, bioproduct, biomaterials and biofertiliser. We continue to collaborate with Kyushu Institute of Technology, 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 6 academic staff, 14 PhD and 17 MS students at the Faculty of Biotechnology and Biomolecular Sciences, Institute of Bioscience and Faculty of Engineering UPM. In addition we also have 5 PhD students on the split program with Kyushu Institute of Technology. In total, we have RM 2.76 million in R&D grants. In terms of output, we successfully published 13 research papers in 2011, with a total of 11.353 Impact Factors, with another 9 papers which are currently in press. We also filed 4 patents and won 2 gold medals at 22nd International Invention, Innovation and Technology Exhibition (ITEX 2011).

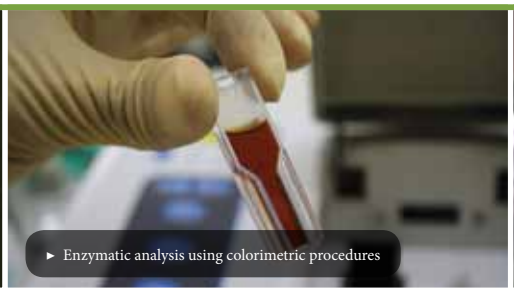
I appreciate the hard work from all EB members in maintaining our high-performance culture. May ALLAH give us the strength to continue the good work and contribute to the university, the ummah and the nation.

God bless. Wassalam.

PROFESSOR DR MOHD ALI HASSAN



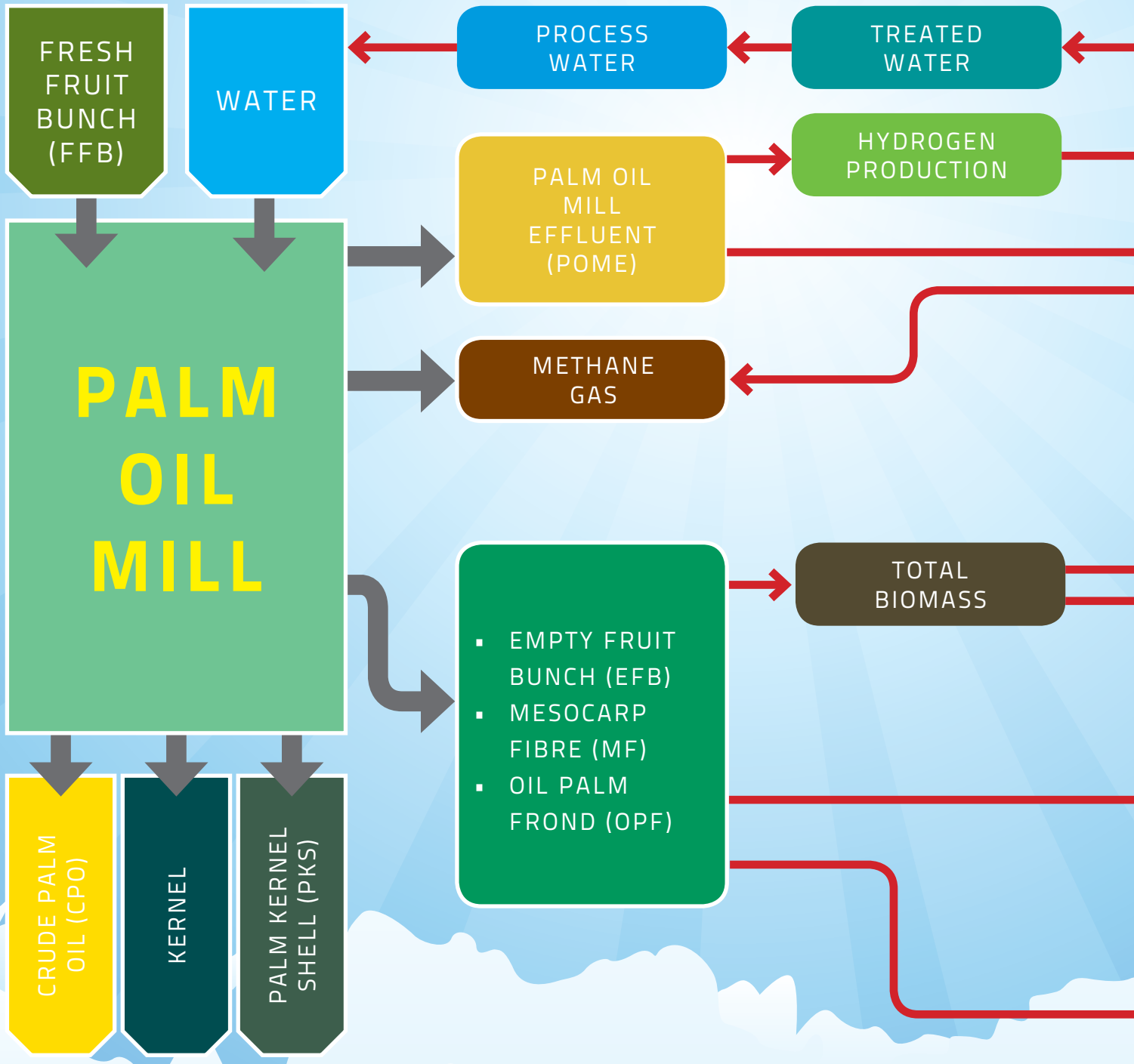
► Specific primer designed before DNA walking



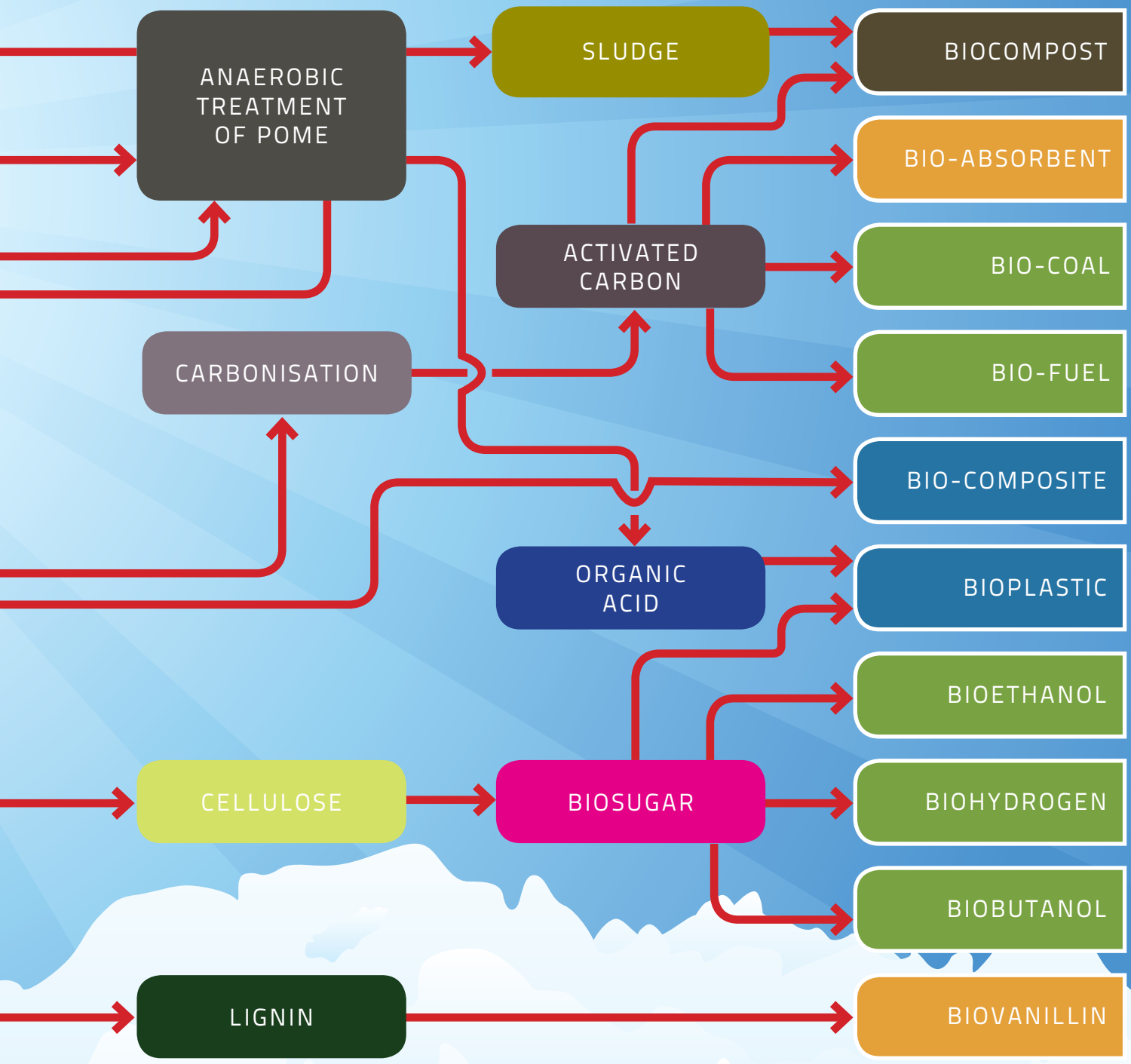
► Enzymatic analysis using colorimetric procedures

► Lignin-degrading bacteria

EB GROUP BIG PICTURE

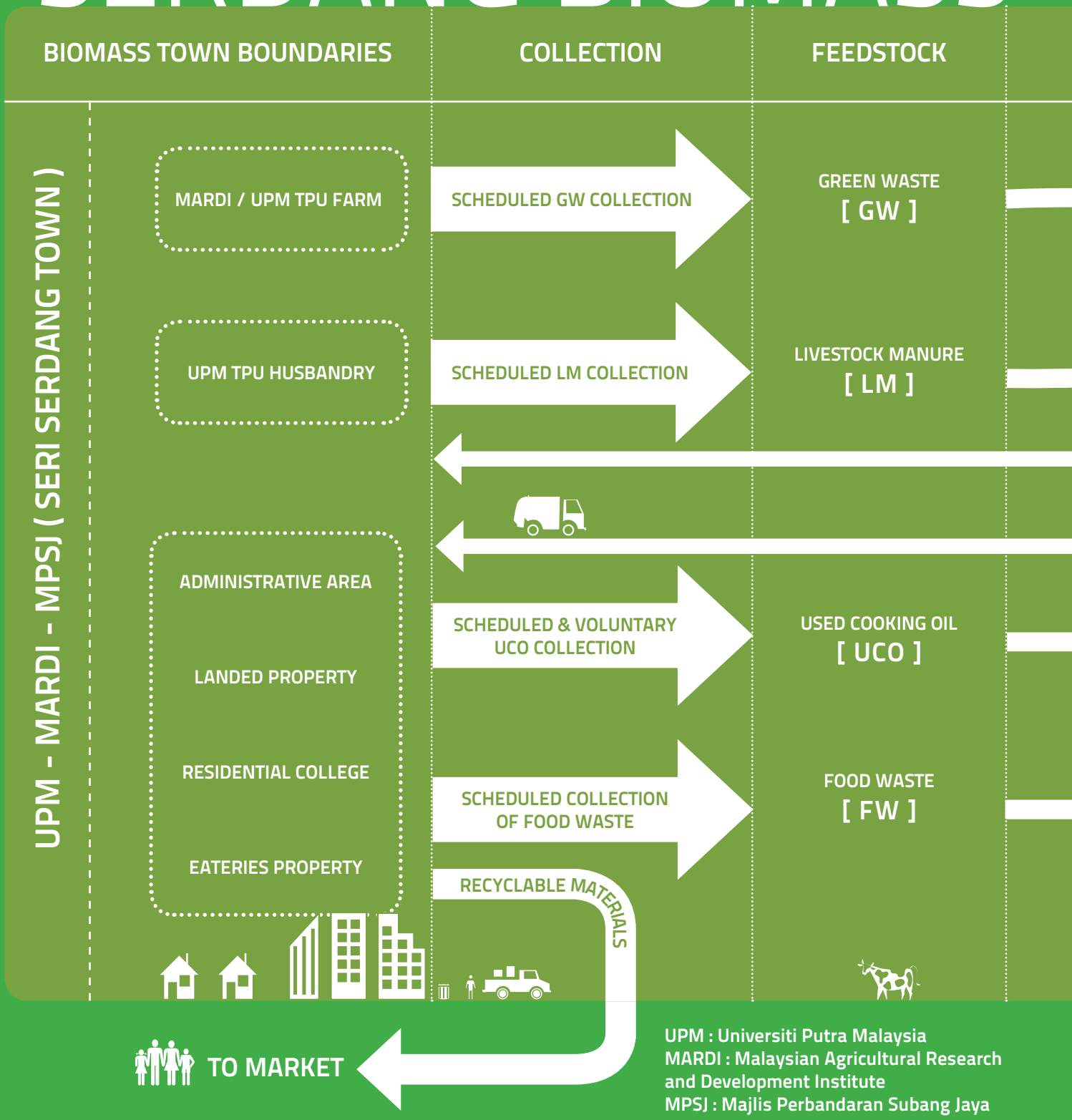


TRADITIONAL TECHNOLOGY



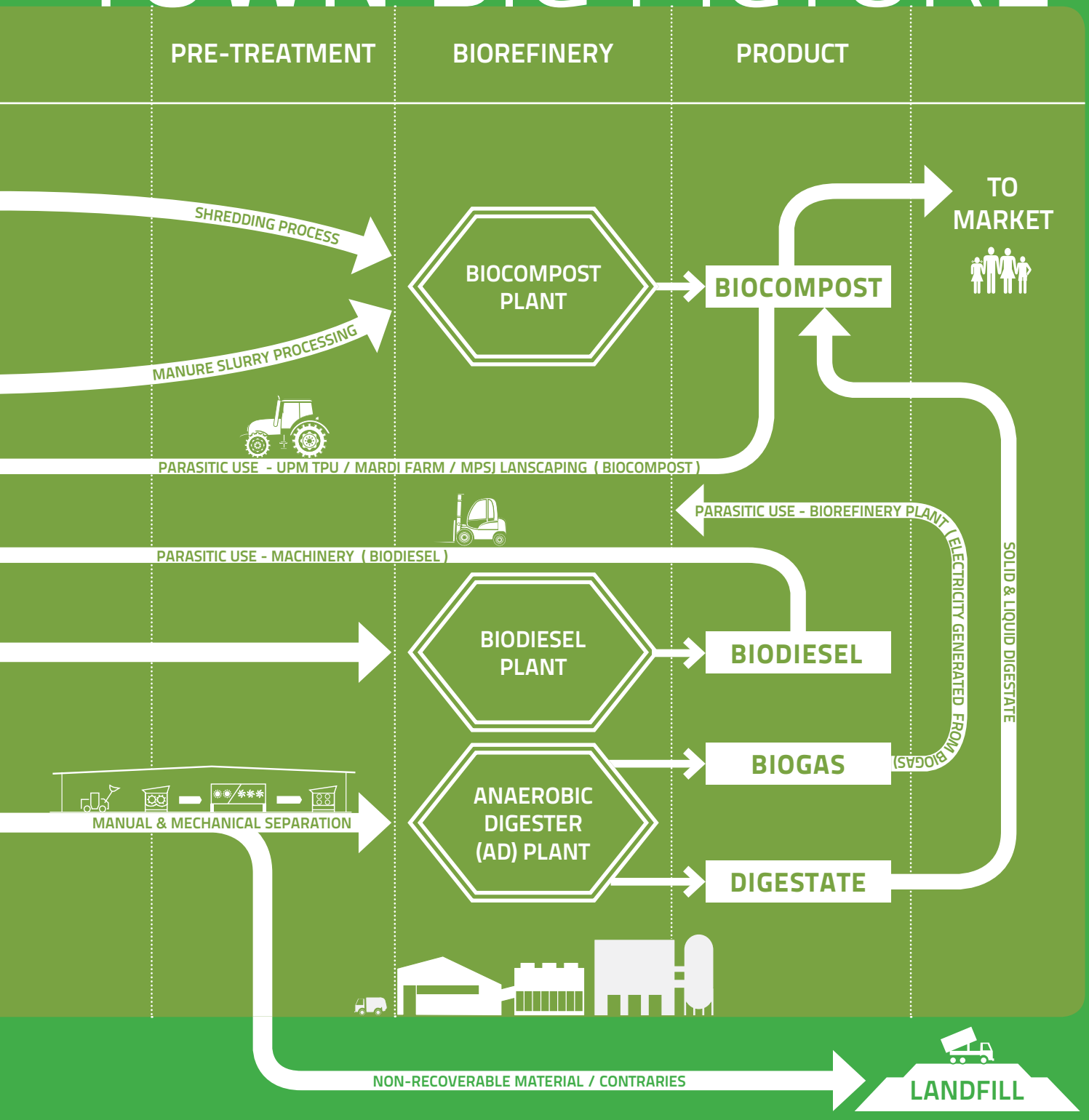
EMERGING TECHNOLOGY

SERDANG BIOMASS

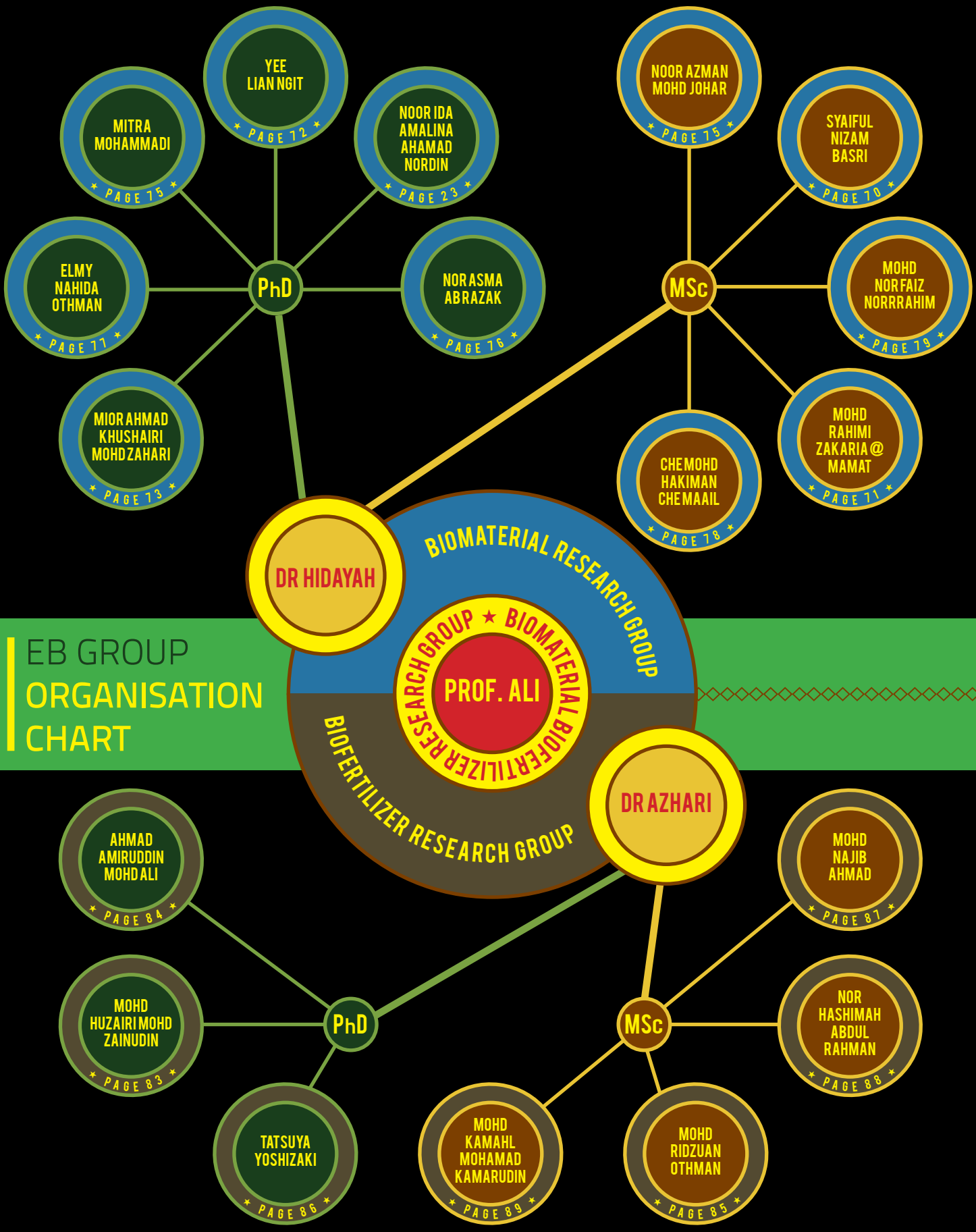


UPM : Universiti Putra Malaysia
MARDI : Malaysian Agricultural Research and Development Institute
MPSJ : Majlis Perbandaran Subang Jaya

TOWN BIG PICTURE

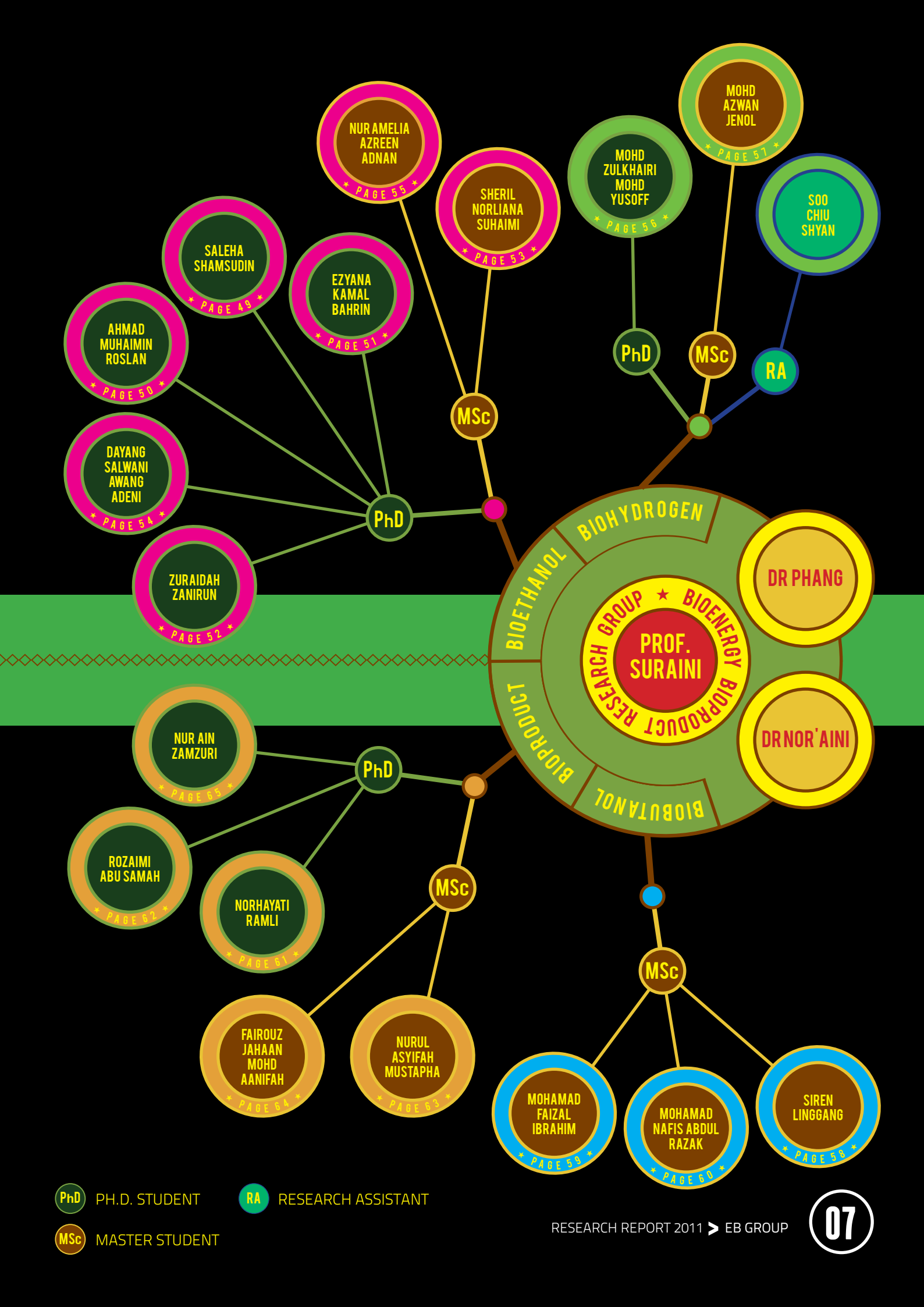


EB GROUP ORGANISATION CHART



EB GROUP KEY //

- GROUP LEADER
- PRINCIPAL RESEARCHER


NUR AMELIA AZREEN ADNAN
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PhD
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DR NOR'AINI
NUR AIN ZAMZURI
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MOHAMAD NAFIS ABDUL RAZAK
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SIREN LINGGANG
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PhD PH.D. STUDENT
RA RESEARCH ASSISTANT
MSc MASTER STUDENT

EB GROUP RESEARCHER

Professor Dr Mohd Ali Hassan

SPECIALISATION

Bioprocess Engineering & Environmental Biotechnology

CURRENT RESEARCH INTEREST

Treatment and utilization of biomass, wastes and effluents for the production of bioproducts, bioremediation and reduction of greenhouse gasses

h INDEX: 14

ACADEMIC QUALIFICATION

PhD (Environmental Biotechnology),
University of Okayama, Japan (1997)
M. Phil. (Chemical Engineering),
University of Birmingham, U.K. (1990)
M.Sc. (Food Engineering),
University of Leeds, U.K. (1982)
B.Sc. (Honours) (Chemical Engineering),
University of Leeds, U.K. (1980)

CONTACTS

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RECENT PUBLICATIONS

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.

Tabassum Mumtaz, Suraini Abd-Aziz, Nor'Aini Abdul Rahman, Phang Lai Yee, Helmi Wasoh, Yoshihito Shirai, Mohd Ali Hassan. 2011. Visualization of core-shell PHBV granules of wild type *Comamonas* sp. EB172 in vivo under transmission electron microscope. International Journal of Polymer Analysis and Characterization, Vol. 16 (4), 228-238 pp.

Isnazunita Ismail, Mohd Ali Hassan, Nor'Aini Abdul Rahman, Chen Sau Soon. 2011. Effect of retention time on biohydrogen production by microbial consortia immobilised in polydimethylsiloxane. African Journal of Biotechnology, Vol. 10 (4), 601-609 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.

Mohd Rafein Zakaria, Hidayah Ariffin, Noor Azman Mohd Johar, Suraini Abd-Aziz, Haruo Nishida, Yoshihito Shirai and Mohd Ali Hassan. 2010. Biosynthesis and characterization of poly(3-hydroxybutyrate-co-3- hydroxyvalerate) copolymer from wild-type *Comamonas* sp. EB172. Polymer Degradation and Stability, Vol. 95 (8), 1382-1386 pp.

Norjan Yusof, Mohd Ali Hassan, Phang Lai Yee, Meisam Tabatabaei, Mohd Ridzuan Othman, Masatsugu Mori, Minato Wakisaka, Kenji Sakai and Yoshihito Shirai. 2010. Nitrification of ammonium-rich sanitary landfill leachate. Waste Management, Vol. 30 (1), 100-109 pp.

2011 PATENTS

Mohd Ali Hassan, Hidayah Ariffin, Mior Ahmad Khushairi Mohd Zahari, Mohd Rafein Zakaria, Jailani Salihon, Mohd Noriznan Mokhtar, Yoshito Shirai. 2011. Renewable Sugars From Oil Palm Waste. PI2011004440

Mohd Ali Hassan, Azhari Samsu Baharuddin, Alawi Sulaiman, Minato Wakisaka, Haruo Nishida,Yoshihito Shirai, Mohd Zulkhairi Mohd Yusof, Ezyana, Noriznan Mokhtar and Lim Siong Hock. 2011. A Method for Treating Oil Palm Biomass. Patent pending: PI 2011000731

Mohd Ali Hassan, Azhari Samsu Baharuddin, Alawi Sulaiman, Ahmad Amiruddin Mohd Ali and Yoshihito Shirai. 2011. System for Evaporating Final Discharge Wastewater generated in the Palm Oil Mill. PI2011005385

EB GROUP RESEARCHER

Professor Dr Suraini Abd Aziz

SPECIALISATION

Biochemical Engineering / Enzyme Technology

CURRENT RESEARCH INTEREST

Utilization of lignocellulosic biomass for bioenergy and bioproduct

h INDEX: 10

ACADEMIC QUALIFICATION

PhD (Biochemical Engineering),
University of Wales, Swansea,
United Kingdom (1997)
MSc. (Biochemical Engineering),
University of Wales, Swansea,
United Kingdom (1993)
BSc (Hons) (Clinical Biochemistry),
Universiti Kebangsaan Malaysia (1992)

CONTACTS

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suraini.aziz@gmail.com

RECENT PUBLICATIONS

Norhayati Ramli, Suraini Abd-Aziz, Mohd Ali Hassan, Noorjahan Banu 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.

Ahmad Muhaimin Roslan, Phang Lai Yee, Umi Kalsom Md. Shah, Suraini Abd-Aziz and Mohd Ali Hassan. (2011). Production of bioethanol from rice straw using cellulase by local *Aspergillus* sp . International Journal of Agricultural Research. 6(2): 188 – 193.

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.

Tabassum Mumtaz, Suraini Abd-Aziz, Nor'Aini Abdul Rahman, Phang Lai Yee, Helmi Wasoh, Yoshihito Shirai and Mohd Ali Hassan. (2011). Visualization of core/shell PHBV granules of wild type *Comamonas* sp. EB 172 in vivo under transmission electron microscope. International Journal of Polymer Analysis and Characterization. 16 (4): 228-238.

Norhayati Ramli, Suraini Abd-Aziz, Mohd Ali Hassan, Noorjahan Banu Alitheen and Kamarulzaman Kamaruddin. (2010). Potential Cyclodextrin Glycosyltransferase Producer from Locally Isolated Bacteria. African Journal of Biotechnology. 9(43): 7317-7321.

Mohd Rafein Zakaria, Meisam Tabatabaei, Farinazleen Mohd Ghazali, Suraini Abd-Aziz, Yoshihito Shirai and Mohd Ali Hassan. (2010). Polyhydroxyalkanoate production from anaerobically treated palm oil mill effluent by new bacterial strain *Comamonas* sp. EB172. World Journal of Microbiology and Biotechnology. 26(5): 767 -774.

Dayang Salwani Awg-Adeni, Suraini Abd-Aziz, Kopli Bujang and Mohd Ali Hassan. (2010). Review: Bioconversion of Sago Residue into Value Added Products. African Journal of Biotechnology . 9(14): 2016 – 2021.

Mohd Rafein Zakaria, Hidayah Ariffin, Noor Azman Mohd Johar, Suraini Abd-Aziz, Haruo Nishida, Yoshihito Shirai and Mohd Ali Hassan. (2010). Biosynthesis and characterization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer from wild type *Comamonas* sp. EB172. Polymer Degradation and Stability. 95(8): 1382-1386.

Tabassum Mumtaz, Nor Amanlina Yahaya, Suraini Abd-Aziz, Nor'Aini Abdul Rahman, Phang Lai Yee, Yoshihito Shirai and Mohd Ali Hassan. (2010). Turning Waste to Wealth - Biodegradable Plastics Polyhydroxyalkanoates (PHA) from Palm Oil Mill Effluent (POME). Journal of Cleaner Production. 18(14): 1393-1402.

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.

2011 PATENTS

Suraini Abd-Aziz, Mohd Ali Hassan, Mohd Sukri Ismail, Razali Sarbini, Nurul Kartini Abu Bakar, Mohd Faizal Ibrahim, Mohd Nafis Abdul Razak and Phang Lai Yee. 2011. Crude Cellulase Cocktail for Lignocellulosic Materials Degradation. PI2011002674.

EB GROUP RESEARCHER

Dr Phang Lai Yee

SPECIALISATION

Environmental Biotechnology

CURRENT RESEARCH INTEREST

1. **Bioconversion of glycerol into bioethanol using local isolate**
2. **Bioethanol production from glycerol using immobilized cells**
3. **Upstream and downstream processing for bioplastic production**
4. **Upstream process for hydrogen and ethanol co-production using biomass resources**

h INDEX: 06

ACADEMIC QUALIFICATION

PhD (Environmental Biotechnology),
Kyushu Institute of Technology, Japan (2004)
MSc. (Environmental Biotechnology),
Universiti Putra Malaysia (2001)
BSc. (Biotechnology),
Universiti Putra Malaysia (1998)

CONTACTS

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SELECTED PUBLICATIONS

Ahmad Muhaimin Roslan, Phang Lai Yee, Umi Kalsom Md. Shah, Suraini Abdul Aziz and Mohd Ali Hassan. (2011). Production of Bioethanol from Rice Straw using Cellulase by Local *Aspergillus* sp. International Journal of Agricultural Research. 6(2): 188 – 193.

Farah Nadia Omar, Nor Aini Abdul Rahman, Halimatun Saadiah Hafid, Tabassum Mumtaz, Phang Lai Yee, Mohd Ali Hassan. (2011). Utilization of kitchen waste for the production of green thermoplastic polyhydroxybutyrate (PHB) by *Cupriavidus necator* CCGUG 52238. African Journal of Microbiology. 5 (19):2873-2879.

Yoshihito Shirai and Mohd Ali Hassan. (2008). Pilot-scale recovery of low molecular weight organic acids from anaerobically treated palm oil mill effluent (POME) with energy integrated system. African Journal of Biotechnology, 7, 3900-3905.

Ahmad Muhaimin Roslan, Mohd Ali Hassan, Suraini Abd-Aziz and Phang Lai Yee. (2009). Effect of POME Supplementation on Cellulase Production from Rice Straw by Local Fungi Isolates. International Journal of Agricultural Research.

Mohamad Firwance Basri, Shahrakbah Yacob, Mohd Ali Hassan, Yoshihito Shirai, Mohd Rafein Zakaria and Phang Lai Yee. (2010). Improved biogas production from palm oil mill effluent by a scaled-down anaerobic treatment process. World Journal of Microbiology and Biotechnology, 26, 505-514.

Tabassum Mumtaz, Noor Amalina Yahaya, Suraini Abd-Aziz, Nor'Aini Abdul Rahman, Phang Lai Yee, Yoshihito Shirai and Mohd Ali Hassan. (2010). Turning waste to wealth-biodegradable plastics polyhydroxyalkanoates from palm oil mill effluent – a Malaysian perspective. J. Cleaner Prod.

Phang Lai Yee,Minato Wakisaka,Yoshihito Shirai andMohd Ali Hassan. Freezing and thawing technique for the removal of suspended solids and concentration of palm oil mill effluent (POME). Journal of Chemical Engineering of Japan, 35, 1017-1019 (2002).

Phang Lai Yee, Mohd Ali Hassan, Yoshihito Shirai, Minato Wakisaka and Mohamed Ismail Abdul Karim. Continuous production of organic acids from palm oil mill effluent with sludge recycle by the freezing-thawing method. Journal of Chemical Engineering of Japan, 36, 707-710 (2003).

Phang Lai Yee, Minato Wakisaka, Yoshihito Shirai andMohd Ali Hassan.Effects of single food components on freeze concentration by freezing and thawing technique. Japan Journal of Food Engineering, 4, 77-82 (2003).

Phang Lai Yee, Minato Wakisaka, Yoshihito Shirai and Mohd Ali Hassan.Effect of sodium chloride on freeze concentration of food components by freezing and thawing technique.Japan Journal of Food Engineering, 5, 97-102 (2004)

2011 PATENT

Suraini Abd-Aziz, Mohd Ali Hassan, Mohd Sukri Ismail, Razali Sarbini, Nurul Kartini Abu Bakar, Mohd Faizal Ibrahim, Mohd Nafis Abdul Razak and Phang Lai Yee. Crude Cellulase Cocktail for Lignocellulosic Materials Degradation. (Filed in June 2011; PI2011002674).

EB GROUP RESEARCHER

Dr Hidayah Ariffin

SPECIALISATION

Bioprocess Engineering and Environmental Biotechnology

CURRENT RESEARCH INTEREST

1. **Utilization of oil palm biomass for the production of bioplastics (PHA) and biobased chemicals**
2. **Chemical recycling of PHA**
3. **Direct recovery of crotonic acid from PHA-producing bacteria by pyrolysis**

h INDEX: 04

ACADEMIC QUALIFICATION

PhD (Environmental Engineering), Kyushu Institute of Technology, Japan. (2009)
MSc. (Bioprocess Engineering), Universiti Putra Malaysia (2006)
Bachelor of Engineering (Process and Food), Universiti Putra Malaysia (2004)

CONTACTS

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SELECTED PUBLICATIONS

Hiroshi Nonaka, Hidayah Ariffin and Masamitsu Funaoka. 2011. Basic Characteristics of Cellulase Immobilized on Lignophenol. Kobunshi Ronbunshu. Vol. 68 (5), 315-319 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.

Hidayah Ariffin, Haruo Nishida, Yoshihito Shirai and Mohd Ali Hassan. 2008. Non-random degradation behavior of Poly (3-hydroxybutyrate) in pyrolysis. Polymer Preprints, Vol. 49 (2), 451p.

2011 PATENT

Mohd Ali Hassan, Hidayah Ariffin, Mior Ahmad Khushairi Mohd Zahari, Mohd Rafein Zakaria, Jailani Salihon, Mohd Noriznan Mokhtar, Yoshihito Shirai. 2011. Renewable Sugars From Oil Palm Waste. PI2011004440

2011 SEMINAR PRESENTED

Asian Congress on Biotechnology 2011 (ACB 2011), Shanghai, China. 11 – 15 May 2011. Title: Improved physical properties of polyhydroxyalkanoates by different fermentation strategies and blending with bio-based materials

EB GROUP RESEARCHER

Dr Nor'Aini Abdul Rahman

SPECIALISATION

Environmental Biotechnology

CURRENT RESEARCH INTEREST

Bioconversion of palm oil waste/ biomass and food waste into value-added products e.g. polyhydroxyalkanotes, organic acids, biofuel and biocompost

h INDEX: 03

ACADEMIC QUALIFICATION

PhD (Metabolic Engineering),
Kyushu Institute of Technology, Japan (2004)
MSc (Environmental Biotechnology),
Universiti Putra Malaysia (2000)
BSc. (Biochemistry and Microbiology),
Universiti Putra Malaysia (1996)

CONTACTS

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SELECTED PUBLICATIONS

Nazlina Haiza Mohd Yasin, Nor'Aini Abd Rahman, Hasfalina Che Man, Mohd Zulkhairi Mohd Yusoff and Mohd Ali Hassan. 2011. Microbial quantification of hydrogen producing bacteria at different pH from fermented food waste. International Journal of Hydrogen Energy 36: 9571-9580.

Farah Nadia Omar, Nor Aini Abdul Rahman, Halimatun Saadiah Hafid, Tabassum Mumtaz, Phang Lai Yee, Mohd Ali Hassan. Enhanced production of polyhydroxyalkanoates by Cupriavidus necator CCUG 52238 utilizing organic acids from kitchen waste. African Journal of Microbiology Research. 5(19): 2873-2879.

Siti Balkhis Ibrahim, Nor'Aini Abdul Rahman, Rosfarizan Mohamad and Raha Abdul Rahim. 2010. Effects of agitation speed, temperature, carbon and nitrogen sources on the growth of recombinant Lactococcus lactis NZ9000 carrying domain 1 of the aerolysin gene. African J of Biotechnology 9(33): 5392-5398.

Halimatun Saadiah Hafid, Nor 'Aini Abdul Rahman, Farah Nadia Omar, Phang Lai Yee, Suraini Abd-Aziz, Mohd Ali Hassan. 2010. A comparative study of organic acids production from kitchen wastes and simulated waste. Australian Journal of Basic and Applied Sciences 4(4): 639-645.

Fadzillah Ismail, Nor'Aini Abdul Rahman, Suraini Abd-Aziz, Chong Mei Ling, Mohd Ali Hassan. 2009. Statistical optimization of biohydrogen production using food waste under thermophilic condition. The Open Renewable Energy Journal 2: 124-131.

Zatilfarihiah Rasdi, Nor Aini Abdul Rahman, Suraini Abd-Aziz, Phang Lai Yee, Mei Ling Chong and Mohd Ali Hassan. 2009. Optimisation of biohydrogen production from palm oil mill effluent by natural microflora using response surface methodology. Open Biotechnology Journal. 8: 79 – 86.

Sim Kean Hong, Yoshihito Shirai, Nor 'Aini Abdul Rahman and Mohd Ali Hassan. 2009. Semi and Continuous Anaerobic Treatment of Palm Oil Mill Effluent for the Production of Organic Acids and Polyhydroxyalkanoates. Research Journal of Environmental Sciences. 3(5): 552 – 559.

Nazlina Haiza Mohd Yasin, Nor Aini Abdul Rahman, Fadzillah Ismail, Mohd Zulkhairi Mohd. Yusof and Mohd. Ali Hassan. 2009. Effect of different temperature, initial pH and substrate composition on biohydrogen production from food waste in batch fermentation. Asian Journal of Biotechnology. 1(2): 42-50.

Farah Nadia Omar, Nor'Aini Abdul Rahman, Halimatun Saadiah Hafid., Phang Lai Yee, Mohd Ali Hassan. 2009. Separation and Recovery of organic acids from fermented kitchen waste by an integrated process. African Journal of Biotechnology. 8(21): 5807-5315. ISSN 1684-5315

2011 SEMINAR PRESENTED (POSTER)

Nor Aini Abdul Rahman and Zatilfarihiah Rasdi. Biohydrogen production from palm oil mill effluent under control and uncontrol pH. The 3rd International conference on fuel cell & hydrogen technology. 2011. 22-23 Nov 2011. Kuala Lumpur

EB GROUP RESEARCHER

Dr Azhari Samsu Baharuddin

SPECIALISATION

Biocompost Engineering &
Food and Agro waste utilization

CURRENT RESEARCH INTEREST

1. **Tropical biomass technology and utilization**
2. **Biofertiliser production from organic, municipal and oil palm waste**
3. **Zero emission system for palm oil industry**

h INDEX: 02

ACADEMIC QUALIFICATION

PhD (Bioprocess Engineering),
Kyushu Institute of Technology, Japan (2010)
MSc (Bioprocess Engineering),
Universiti Putra Malaysia (2006)
B.Eng (Chemical Engineering),
Universiti Kebangsaan Malaysia (2002)

CONTACTS

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SELECTED PUBLICATIONS

Azhari Samsu Baharuddin, Nor' Aini Abdul Rahman, Umi Kalsom Md Shah, Mohd Ali Hassan, Minato Wakisaka, and Yoshihito Shirai, (2011). Evaluation of pressed shredded empty fruit bunch (EFB)-palm oil mill effluent (POME) anaerobic sludge based compost using Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) analysis. African Journal of Biotechnology 10(41): 8082-8089

Mohd Najib Ahmad, Noriznan Mokhtar, Azhari Samsu Baharuddin, Lim Siong Hock, Suraini Abd-Aziz, Mohd Ali Hassan and Yoshihito Shirai (2011). Microbial diversity and physicochemical changes in oil palm frond composting with POME Anaerobic Sludge. BioResources, 6(4): 4762-4780

2011 CONFERENCE / SEMINAR PRESENTED

Azhari Samsu Baharuddin, Mohd Ali Hassan, Kenji Sakai, Haruo Nishida and Yoshihito Shirai compost production from empty fruit bunch (EFB) and palm oil mill effluent (POME) sludge and their recycle. International Symposium on Biomass Refinery in Palm Oil Industry 2011, Fukuoka, Japan

Azhari Samsu Baharuddin, Mohd Ali Hassan and Yoshihito Shirai. "Zero discharge system (in palm oil mills)" & "Strategy for creating business opportunities through zero discharge system". Conference on Green Tech for SMEs in Palm Oil and Palm Biomass, 28th July 2011, Sandakan, Sabah

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2011 PATENTS

Mohd Ali Hassan, Azhari Samsu Baharuddin, Alawi Sulaiman, Minato Wakisaka, Haruo Nishida,Yoshihito Shirai, Mohd Zulkhairi Mohd Yusof, Ezyana Kamal Bahrin, Noriznan Mokhtar and Lim Siong Hock (2011). A Method for Treating Oil Palm Biomass. Patent pending: PI 2011000731

Mohd Ali Hassan, Azhari Samsu Baharuddin, Alawi Sulaiman, Amir Mohd Ali and Yoshihito Shirai (2011). System for Evaporating Final Discharge Wastewater generated in the Palm Oil Mill. PI 2011005385

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EB GROUP ATTACHMENT

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DR HIDAYAH ARIFFIN	Collaborative Research	Development of novel bio-based polymeric composites	ASSOC. PROFESSOR DR YOSHITO ANDO Kyushu Institute of Technology, Japan	10 days 24 Oct ~ 3 Nov 2011	Kyushu Institute of Technology (Kyutech), Japan
NOOR IDA AMALINA BINTI AHAMAD NORDIN	Program for local graduate students to undertake overseas research attachment	Preparation and characterization of biocomposite from polypropylene reinforced mesocarp fiber	PROFESSOR DR YOSHIHITO SHIRAI Kyushu Institute of Technology, Japan	33 days 02 Jul ~ 4 Aug 2011	Kyushu Institute of Technology (Kyutech), Japan
	Collaborative Research	Characterization of mesocarp fiber treated with super heated steam treatment.		60 days 12 Oct ~ 10 Dec 2011	
ELMY NAHIDA BINTI OTHMAN	Collaborative Research	• Steam and super heated steam hydrolysis of polyhydroxyalkanoates for chemical recycling • Characterization of steam hydrolyzates using GPC, NMR and FTIR	PROFESSOR DR HARUO NISHIDA Eco-Town Collaborative R&D Center for the Environmental and Recycling, Kyushu Institute of Technology, Japan	83 days 10 Feb ~ 10 May 2011	Kyushu Institute of Technology & Universiti Kuala Lumpur
MOHD RIDZUAN OTHMAN	Research Attachment / Training	Biomass carbonization from bamboo by using pond	PROFESSOR DR YOSHIHITO SHIRAI & YAMAMOTO COMPANY Shimane, Japan	36 days 28 Jan ~ 04 Mac 2011	Kyushu Institute of Technology (Kyutech), Japan

INBOUND

PARTICIPANTS	POSITION	RESEARCH THEME	PARTICIPANTS	POSITION	RESEARCH THEME
DR. HARUO NISHIDA	PROFESSOR Kyushu Institute of Technology, Japan	Chemical recycle	DR. YOSHIHITO ANDO	ASSOCIATE PROFESSOR Kyushu Institute of Technology, Japan	Chemical synthsis of polymer
DR. TOSHINARI MAEDA	ASSOCIATE PROFESSOR Kyushu Institute of Technology, Japan	Hydrogen fermentation	KOICHI NAGATA	PH.D STUDENT / STAFF Kyushu Institute of Technology, Japan	Environment economics
TATSUYA YOSHIZAKI	PH.D STUDENT Kyushu Institute of Technology, Japan	Zero discharge in palm oil industry	KOTARO WATANABE	PH.D STUDENT Kyushu Institute of Technology, Japan	Chemical synthsis of polymer
ZHANG BEI	MASTER STUDENT Kyushu Institute of Technology, Japan	Biomass composite	TAKAAKI MAEDA	MASTER STUDENT Kyushu Institute of Technology, Japan	Chemical synthsis of polymer
HIROKI ARIYOASHI	MASTER STUDENT Kyushu Institute of Technology, Japan	Utilization of *Escherichia coli*	YUKI YOSHIMIZU	MASTER STUDENT Kyushu Institute of Technology, Japan	Inhibition of periodontal pathogen

2011 EB GROUP PUBLICATIONS

19.963
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PUBLISHED IMPACT FACTOR **11.359**

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IMPACT FACTOR JOURNALS

CITED JOURNALS
03

04
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⌘ PUBLISHED

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IF 0.573 ■ Effect of retention time on biohydrogen production by microbial consortia immobilised in polydimethylsiloxane. PAGE 25

IF 0.533 ■ Utilization of kitchen waste for the production of green thermoplastic polyhydroxybutyrate (PHB) by *Cupriavidus necator* CCGUG 52238. PAGE 28

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IF 1.418 ■ Microbial diversity and physicochemical changes in oil palm frond composting with POME Anaerobic Sludge. PAGE 21

IF 0.814 ■ Visualization of core/shell PHBV granules of wild type *Comamonas* sp. EB 172 in vivo under transmission electron microscope. PAGE 24

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CITED ■ Production of Bioethanol from Rice Straw using Cellulase by Local *Aspergillus* sp. International Journal of Agricultural Research. PAGE 39

CITED ■ Effect of oil palm empty fruit bunch (OPEFB) particle size on cellulase production by *Botryosphaeria* sp. in solid state fermentation. PAGE 40

CITED ■ Bioconversion of Restaurant Waste into Polyhydroxybutyrate (PHB) by Recombinant *E.coli* through Anaerobic Digestion. PAGE 41

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Microbial characterization of hydrogen-producing bacteria in fermented food waste at different pH values

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ABSTRACT

An anaerobic fermentation of food waste was conducted in a 0.5 L bioreactor incubated at a thermophilic temperature of 55 °C to evaluate the effects of different controlled pH values (5.0, 5.5 and 6.0) on biohydrogen production. Effective biohydrogen production was found at controlled pH 5.5 and 6.0 corresponding to lower lactic acid production compared to pH 5.0. It was demonstrated that biohydrogen production from food waste was pH-dependent with hydrogen yields of 79, 76 and 23 mmol H_2/L-media/d for pH 5.5, 6.0 and 5.0, respectively. Specific microbial determination for *Clostridium* sp. and total bacteria quantification were carried out by the fluorescent *in-situ* hybridization (FISH) technique. The number of *Clostridium* sp. for acclimatized sludge, fermentation broth at pH 5.0, 5.5 and 6.0 were 2.9×10^8, 3.6×10^8, 7.8×10^8 and 5.4×10^8 cells/ml, respectively. The quantification analysis showed that 92% of the total bacteria belonged to *Clostridium* sp. from clusters I and XI from the sample at controlled pH 5.5. The denaturing gradient gel electrophoresis (DGGE) bands of the sample after heat-treatment, acclimatization and during fermentation indicated the presence of *Bacteroidetes*, *Caloromator australicus* sp. and *Clostridium* sp.

1. Introduction

Hydrogen can be considered as an ideal, clean and sustainable energy carrier. It contains the largest gravimetric energy

household solid waste [9] as a substrate contributing to waste reduction [1,3,10]. The average amount of municipal solid waste generated in Malaysia was 0.5–0.8 kg/person/d in the rural areas while it was 1.7 kg/person/d in urban areas [11,12].
n of commercial areas
48% food/organic
8.58%), iron and
rs (4.51%) [12].
lopment and
of food waste

NAZLINA HAIZA MOHD YASIN

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CHANGES IN PHYSICOCHEMICAL AND MICROBIAL COMMUNITY DURING CO-COMPOSTING OF OIL PALM FROND WITH PALM OIL MILL EFFLUENT ANAEROBIC SLUDGE

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The aims of this study were to investigate the physicochemical changes and microbial population during co-composting of 1 ton oil palm frond (OPF) with 1,000 L palm oil mill effluent (POME) anaerobic sludge. In the first 30 days of composting, the temperature of the composting piles was observed in the thermophilic phase, within a range of 50 - 56°C. Meanwhile, the oxygen level, moisture content, and pH profiles of the compost were maintained at 2.0 to 12%, 60 to 70%, and 7.9 to 8.5, respectively, throughout the composting process. The total bacteria count was estimated to be about 55 x 10^{10} CFU/mL in the mesophilic phase, and then it increased up to 66 x 10^{10} CFU/mL in the thermophilic phase, and finally decreased to 9.0x10^{10} CFU/mL in the curing phase. The initial C/N ratio, 64, decreased to 18 after 60 days of composting process, indicating the maturity of compost product from OPF-POME anaerobic sludge. The diversity of the bacterial community was investigated using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. The results suggested that the co-composting process of OPF with POME anaerobic sludge was dominated by *Pseudomonas sp.*

Keywords: Oil palm frond; Palm oil mill effluent (POME) anaerobic sludge; Composting; DGGE

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INTRODUCTION

Malaysia is one of the largest producers of palm oil, of which around 75.5 million metric tons (t
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MOHD NAJIB AHMAD
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Research Article

wm&r

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Nitrification of high-strength ammonium landfill leachate with microbial community analysis using fluorescence in situ hybridization (FISH)

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Abstract

Nitrification of mature sanitary landfill leachate with high-strength of $N-NH_4^+$ (1080–2350 mg L^{-1}) was performed in a 10 L continuous nitrification activated sludge reactor. The nitrification system was acclimatized with synthetic leachate during feed batch operation to avoid substrate inhibition before being fed with actual mature leachate. Successful nitrification was achieved with an approximately complete ammonium removal (99%) and 96% of $N-NH_4^+$ conversion to $N-NO_3^-$. The maximum volumetric and specific nitrification rates obtained were 2.56 kg $N-NH_4^+$ m^{-3} day^{-1} and 0.23 g $N-NH_4^+$ g^{-1} volatile suspended solid (VSS) day^{-1}, respectively, at hydraulic retention time (HRT) of 12.7 h and solid retention time of 50 days. Incomplete nitrification was encountered when operating at a higher nitrogen loading rate of 3.14 kg $N-NH_4^+$ m^{-3} day^{-1}. The substrate overloading and nitrifiers competition with heterotrophs were believed to trigger the incomplete nitrification. Fluorescence in situ hybridization (FISH) results supported the syntrophic association between the ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria. FISH results also revealed the heterotrophs as the dominant and disintegration of some AOB cell aggregates into single cells which further supported the incomplete nitrification phenomenon.

Keywords

Nitrification, high-strength ammonium wastewater, mature landfill leachate, biological nitrogen removal, FISH

Date received: 6 September 2010; accepted: 19 December 2010

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1.222

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Full Length Research Paper

Elephantopus scaber induces cytotoxicity in MCF-7 human breast cancer cells via p53-induced apoptosis

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Accepted 12 September, 2011

Plants have not only been consumed as food but have also been adopted as folk medicine for centuries. *Elephantopus scaber* Linn, a herb from the Asteraceae family, has traditionally been taken as decoction or tea to cure various ailments and diseases throughout the world. Recent studies had also suggested that this plant posses various bioactivities such as anti-bacterial, anti-inflammatory, hepatoprotective as well as anti cancer properties. In this study, the cytotoxic effect of an ethanolic extract of *E. scaber* on a breast cancer cell line, MCF-7 and the underlying cell death mechanism was examined. *E. scaber* showed cytotoxic effect towards MCF-7 cells with an IC_{50} value of 15 µg/mL. In comparison to the untreated control, the extract triggered cell death with increased phosphatidylserine externalization, DNA breaks and significant morphological apoptotic characteristics in the MCF-cells. Furthermore, we also found that expression of the tumor suppressor p53 protein was up-regulated in response to the treatment. In conclusion, these results suggested that the ethanolic extract of *E. scaber* may be a potential anti cancer agent for human breast cancer cells by the induction of p53-dependent apoptosis.

Key words: Ethanol extract, MCF-7, tumor suppressor protein, DNA fragmentation, phosphatidylserine externalization.

INTRODUCTION

Breast cancer is one of the leading causes of cancer mortality worldwide every year. Despite the availability of treatments in the form of surgery, radiation therapy, chemotherapy, hormonal therapy and biologic therapy for breast cancers (American Cancer Society, 2007; World Health Organization, 2011), most of the therapeutic means are associated with some drawbacks such as high cost of treatment and adverse side effects after prolonged exposure. For instance, tamoxifen, the oldest and most-prescribed selective estrogen receptor modulator (SERM) for treating hormone-receptor-positive breast cancer, had been proven to reduce the risk of developing invasive and non-invasive breast cancer among women (Fisher [illegible]) cases had also witnessed the susceptibility of women who underwent tamoxifen therapy for more than five years to have relatively higher risks for stroke, cataracts, cardiac arrhythmia or atrial fibrillation, hypertriglyceridemia, deep-vein thromboembolic events and even death (Fisher et al., 2005; Veronesi et al., 2007). There is indeed a need to search for more easily available and much more reliable therapeutic sources into overcoming the problems associated with current breast cancer treatments.

Natural products have been used for a long time in folk medicine to treat a great variety of diseases. In ethnopharmacology, plant-derived products are not only [illegible]

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VISUALIZATION OF CORE-SHELL PHBV GRANULES OF WILD TYPE *Comamonas* sp. EB172 *IN VIVO* UNDER TRANSMISSION ELECTRON MICROSCOPE

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Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) granule formation* in vivo *in wild type* Comamonas *sp. EB172 grown in mixed organic acids under nitrogen limitation were visualized under transmission electron microscope (TEM). The early stages of PHBV production revealed dark-stained mediation elements near the center of the cell supporting the third model of granule formation. The native granules revealed characteristic core-shell structure in which white PHB cores were surrounded by darker PHBV shells. In vitro, the copolymer showed bimodal molecular weight distribution and exhibited two melting peaks in the differential scanning calorimeter (DSC) thermogram, supporting the formation of block copolymer.

Keywords: *Comamonas* sp.; EB172; PHBV granules; TEM

INTRODUCTION

Production of economically competitive polyhydroxyalkanoates (PHAs) and PHAs with new properties has been the impetus for many research groups to study the biosynthesis, degradation, and homeostasis of PHAs in microorganisms.[1] Biosynthesis of PHAs involves transforming soluble substrates, such as hydroxyalkanoate coenzyme A esters, into insoluble inclusions during polymer elongation; these

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Full Length Research Paper

Effect of retention time on biohydrogen production by microbial consortia immobilised in polydimethylsiloxane

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Accepted 14 December, 2010

An investigation on biohydrogen production from palm oil mill effluent (POME) was conducted in a continuous stirred tank reactor seeded with polydimethyl-siloxane (PDMS) immobilised mixed cultures at adjusted retention time. The hydrogen-producing bacteria obtained from an anaerobic digester used for treating POME were acclimatised and immobilised in PDMS. The immobilised cultures were assessed for their effectiveness in generating hydrogen in a continuous system. In this study, the PDMS cultures were fed with raw POME at hydraulic retention times of 6, 4 and 2 days and operated at controlled pH and temperature of 5.5 and 55°C, respectively. At hydraulic retention time (HRT) 2 days, the average hydrogen production rate per unit volume of POME was 2.1 NL/L/d. Hydrogen constituted up to 43% of the total gas produced and methane was not detected throughout the 150 days of continuous operation. The soluble carbohydrate degradation efficiency was highest at 81.2% during HRT 4 days and the concentration of soluble metabolites produced, followed the order of acetic > butyric > ethanol > propionic acid. The microbial diversity of the immobilised consortia determined by denaturing gradient gel electrophoresis (DGGE) changed at different HRTs; with increasing dominant species phylogenetically related to *Clostridaceae.*

Key words: Hydrogen fermentation, palm oil mill effluent, polydimethylsiloxane (PDMS), immobilisation.

INTRODUCTION

Dark fermentation of renewable sources have received broad attention as the process is regarded as green technology with potential in reducing greenhouse gases (GHG) impact and relatively low energy intensive compared to the typical hydrogen generation via steam methane reforming (SMR). The GHG emission of hydrogen production via the SMR process is estimated at 9.7 to 13.7 kg CO_2/kg H_2 produced (Spath and Mann, 2000; Muradov and Veziroğlu, 2008) which is one magnitude order than other known industrial polluters such as in fertiliser, steel and cement production. It is envisaged that the biomass-based hydrogen production by anaerobic heterotrophic fermentation is commercially viable by

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Abbreviations: GHG, Greenhouse gases; **SMR,** steam methane reforming; **POME,** palm oil mill effluent; **COD,** chemical oxygen demand; **EVA,** ethylene vinyl acetate copolymer; **PMMA,** polymethyl methaacrylate; **PVA,** polyvinyl alcohol; **PDMS,** polydimethylsiloxane; **PCR-DGGE,** polymerase chain reaction co… electrophoresis; **V**… retention time; … acids; **TCD,** t… **PEO,** polyeth… reactor.

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Full Length Research Paper

Enhancement of organic acids production from model kitchen waste via anaerobic digestion

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Accepted 27 July, 2011

The aim of this study was to obtain the optimal conditions for organic acids production from anaerobic digestion of kitchen waste using response surface methodology (RSM). Fermentation was carried out using 250 ml shake flask which was incubated using an orbital shaker set at 200 rpm. Fermented kitchen wastes were used as inoculums sources. The individual and interactive effects of pH, temperature and inoculum size (%) on organic acids production from kitchen waste were investigated. The highest level of organic acid produced was 77 g/L at optimum pH, temperature, inoculum size of 6.02, 35.37°C and 20% inoculum, respectively. The results indicate that the most significant parameters affecting the bioconversion of kitchen waste to organic acids were temperature and inoculum size. Verification experiment of the estimated optimal conditions confirmed that RSM was useful for optimizing organic acids production from fermented kitchen waste.

Key words: Bioconversion, model kitchen waste, anaerobic fermentation, organic acids, optimization, response surface methodology.

INTRODUCTION

Kitchen waste and other organic solid waste discharged from households, restaurant, and residue from the food industry make up about 70% of total municipal solid waste (MSW) in Malaysia (Hassan et al., 2001). Due to its large volume, the disposal of kitchen and organic waste will be a big problem. At present, dumping waste in landfill is the most common practice. Kitchen waste has high organic content such as soluble sugars, starch, proteins, cellulose and etc. (Wang et al., 2005). Since kitchen waste has high organic compound and moisture contents, anaerobic process is the most suitable method for its treatment in comparison with alternative treatments such as incineration, landfill and composting (Zinatizadeh et al., 2006). Anaerobic digestion is a complex process that involves hydrolysis, acidogenesis, acetogenesis and soluble organic products such as organic acids (Lim et al., 2008). Various organic acids produced from organic wastes especially kitchen waste has been utilized as energy and carbon sources in the production of bio-degradable plastic (Horiuchi et al., 2002). Organic acids such as lactic acid followed by acetic and propionic acids were found to be the main products of kitchen waste fermentation (Bo et al., 2007). Those organic acids are also widely used in pharmaceutical, food and beverage industries.

Apart from that this study also highlights on the variation problem of the kitchen waste during anaerobic digestion process and aims to develop a model of kitchen waste. The development of model kitchen waste was important to overcome the variation and fluctuation that later will affect the lture conditions on digestion, it has roduction was noculums size ss, pH plays a ucing bacteria uce acid. The c to bacteria.

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Full Length Research Paper

Evaluation of pressed shredded empty fruit bunch (EFB)-palm oil mill effluent (POME) anaerobic sludge based compost using Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) analysis

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Accepted 15 June, 2011

Pressed-shredded empty fruit bunches (EFB) and palm oil mill effluent (POME) anaerobic sludge from a 500 m^3 closed anaerobic digester system was utilized for the co-composting treatment. Scanning electron microscopy (SEM) analysis showed that the shredding-pressing treatment on EFB gave better results in removing the debris and silica bodies as compared to only shredding treatment. However, similar characteristics were detected in both physically-treated EFB samples by Fourier transform infrared (FTIR) analysis, mainly in the regions of 900 to 1740 and 2800 to 3400 cm^{-1}. After the anaerobic digestion of fresh raw POME, the protein origin (Amide I) band appeared in the POME anaerobic sludge. Besides, the band intensities at 2925 and 2855 cm^{-1} which attributed to the composition of fat and lipid was decreased. The maturity of the composting material after 40 days of treatment was detected by the appearance of the nitrate band at 1376 cm^{-1} and the results corresponded to the final C/N ratio of 12.4. Solid state ^{13}C CP/MAS nuclear magnetic resonance (NMR) was also used to reveal the characteristic changes of pressed-shredded EFB-POME anaerobic sludge based compost.

Key words: Empty fruit bunch, palm oil mill effluent, compost.

INTRODUCTION

The co-composting of empty fruit bunches (EFB) and palm oil mill effluent (POME) is one of the alternative ways to reduce the abundance of biomass generated at palm oil mills (Baharuddin et al., 2009). Composting is a biological process by which organic material undergoes decomposition and transformation processes. Mineralization and humification of organic matter take place during the composting process. Normally, a compost pile has two stages: an active composting stage and a curing period stage. When the temperature rises above 60°C, many microorganisms begin to die or become dormant, and after that the temperature starts to stabilize or decrease. In the curing stage, the materials degrade at a

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Abbreviations: EFB, effluent; **SEM**, transform infra crude palm oi

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Full Length Research Paper

Utilization of kitchen waste for the production of green thermoplastic polyhydroxybutyrate (PHB) by *Cupriavidus necator* CCGUG 52238

Farah Nadia Omar, Nor'Aini Abdul Rahman*, Halimatun Saadiah Hafid, Tabassum Mumtaz, Phang Lai Yee and Mohd Ali Hassan

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Accepted 30 May, 2011

Polyhydroxybutyrate (PHB) was produced by *Cupriavidus necator* CCGUG 52238 using organic acids from fermented kitchen waste. HPLC and nuclear magnetic resonance (NMR) analyses revealed that the acid comprised mainly of lactic and acetic acids. In shake flask culture, the lactic acid concentration above 10 g/L inhibited both cell growth and polyhydroxybutyrate (PHB) production. The PHB production by the strain was achieved at the highest PHB content of 52.79% in batch fermentation using the kitchen-waste derived organic acids. The PHB yield and productivity were 0.38 g/g and 0.065 g/L/h, respectively. In fed-batch culture, about 4-fold increase in PHB productivity (0.242 g/L/h) was achieved by applying intermittent feeding strategy.

Key words: *Cupriavidus necator* CCGUG 52238, kitchen waste, organic acids, polyhydroxybutyrate (PHB).

INTRODUCTION

The growing interest in the development of biodegradable plastics with properties similar to synthetic thermoplastics for the management of the existing plastic waste has directed the attention towards bacterial polyhydroxyalkanoates (PHA). The production of PHAs from agriculture, food processing waste materials is an attractive approach for not only effectively decreasing PHA production cost but also constructing a process for effective utilization of waste. Considering that PHA content and productivity are usually lower for bacteria grown in crude and inexpensive substrates, the development of efficient processes for the successful bioconversion remain a challenge to be pursued (Castilho et al., 2009)

alone accounted to 3,000 tonnes per day. This will continue to increase in coming years and is expected to reach 30,000 tons per day in 2020 (GEC). The waste usually consists of 45% food waste, 24% plastic, 7% paper and 6% iron. In the current practice of waste management, approximately 95 to 97% of waste collected is taken to landfill for disposals and less than 5% of the waste is being recycled. However, considering the landfill shortage and contamination, government aimed to have 22% of the waste recycled by 2020. While composting the kitchen refuse can recycle back the nutrients and energy to soil and reduce almost 50% of the waste; anaerobic digestion can also be applied to ic acids which are Producing PHAs the inevitable gradable PHA e market. hen waste is igestion than research has ermented food n (Hafuka et

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Full Length Research Paper

Molecular cloning and extracellular expression of cyclodextrin glycosyltransferase gene from *Bacillus* sp. NR5 UPM

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Accepted 26 August, 2011

The cloning of a polymerase chain reaction (PCR) gene fragment from *Bacillus* sp. NR5 UPM isolated from the soil in Malaysia into an *Escherichia coli* expression vector was successfully carried out. Analysis of the nucleotide sequences revealed the presence of an open reading frame of 2112 bp which encoded a protein containing 704 amino acids with a putative molecular weight of 78.6 kDa. The deduced amino acids sequence showed about 98% homology with the CGTase from *Bacillus* sp. KC201. Compared to the wild type, the CGTase that was produced in *E. coli* cells only required one-fourth of culture time and neutral pH to produce CGTase. After 12 h of cultivation, the CGTase activity in the culture medium reached 29.6 U/ml, which was approximately 2.5-fold higher than the CGTase from the parental strain. The CGTase was produced extracellularly by *E. coli* (94%) indicating the signal peptide was functional in *E. coli*.

Key words: Molecular cloning, nucleotide sequence, cyclodextrin glycosyltransferase, *Bacillus* sp. NR5 UPM.

INTRODUCTION

Cyclodextrin glycosyltransferase (CGTases, 1,4-α-D-glucopyranosyltransferase (cyclizing), EC 2.4.1.19) is an important enzyme that catalyzes the formation of α-CD, β-CD and γ-CD, containing 6, 7 and 8 glucose residues linked with α-1,4-glucosidic bonds, respectively. Due to their unique abilities to form inclusion complexes with a variety of hydrophobic materials and to entrap volatile compounds, these CDs have found extensive applications in food, pharmaceuticals, agricultural chemicals, cosmetics, industrial chemicals and others (Hashimoto, 2002).

Recently, many researchers have studied the molecular cloning of CGTase genes and analysed the genetic information in order to provide a better CGTase production method. The over expression of CGTase genes could enhance the enzyme activity, reduce cultivation time and produce less contaminating proteins compared to wild type (Charoensakdi et al., 2007). In this study, we have succeeded in isolating the CGTase gene from *Bacillus* sp. NR5 UPM. The isolated CGTase gene was cloned into an *E. coli* expression vector and over expressed to study the improved properties of the enzyme.

MATERIALS AND METHODS

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高分子論文集 (Kobunshi Ronbunshu), Vol. 68, No. 5, pp. 315—319 (May, 2011)

リグノフェノールを担体とする固定化セルラーゼの基礎的特性

野中　寛[*1]・Hidayah ARIFFIN[*2]・舩岡　正光[*1]

(受付 2011 年 1 月 31 日・審査終了 2011 年 3 月 7 日)

要　旨　セルロース系バイオマスの酵素糖化において，セルラーゼコストの低減のため，セルラーゼの回収再利用や固定化技術の進展が望まれる．固定化担体としては，化学修飾せずとも物理的吸着のみで酵素が強く固定され，かつ，酵素固定量が大きく，再生可能な担体素材の開発が期待される．ヒノキ木粉と *p*-クレゾールを原料として，相分離系変換システムによって誘導されたフェノール系高分子リグノクレゾールに，混合かくはんのみで *Trichoderma reesei* 由来の市販セルラーゼを吸着させて，固定化セルラーゼを調製した．固定化量は，セルラーゼ濃度増大とともに増加し，100 mg/g-lignocresol 以上に達した．固定化量が少ないほど単位セルラーゼ重量当たりの活性は低いが，最大でフリーのセルラーゼの約 60% の活性を示した．セルラーゼは酢酸緩衝液中でわずかに脱落したが，おおむね良好な安定性を示した．

1　緒　　言

セルロースの加水分解には，硫酸や塩酸を用いた酸糖化法とセルラーゼによる酵素糖化法がある．酸糖化は，中心が一部破壊されてしまうことが多い．また担体の再生が困難であるのも弱点である．脱落しやすいが，簡便かつ活性低下がおきにくいのは，水不溶性の担体に物理的に吸着させる方法[4),5),7),10)]である．しかし担体に何らかの化学修飾を要することが多く，化学修飾せずとも物理的吸着のみで酵素が強く固定され，酵素固定量が大きく，再生可能な担体素材の開発が望まれる．

Basic Characteristics of Cellulase Immobilized on Lignophenol

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Enzymatic hydrolysis of cellulosic biomass by cellulase has been receiving attention. Since the cost of cellulase has a big impact on the price of the products, it is important to develop cheaper alternatives. Here we report on the production of a novel immobilized cellulase. A functional lignin-based phenolic polymer "lignophenol" (*p*-cresol type : ligno-*p*-cresol) was synthesized from Hinoki wood meal and *p*-cresol through the phase separation system developed for selective separation of lignocellulosic components. Commercial cellulase derived from *Trichoderma reesei* was easily immobilized on lignophenol simply by mixing to give water-insoluble cellulase-lignophenol complex. Immobilization reached more than 100 mg/g-lignophenol with an enzymatic activity of about 60% compared to free cellulase. With lower immobilization loads, cellulase exhibited lower activity. Cellulase did not significantly detach from lignophenol by mixing with acetate buffer, indicating that the cellulase immobilized by a simple physical adsorption is stable.

KEY WORDS　Cellulase / Lignophenol / Immobilized Enzyme / Physical Adsorption / Enzymatic Hydrolysis /
(Received January 31, 2011: Accepted March 7, 2011)　[*Kobunshi Ronbunshu*, **68**, 315—319 (2011)]

化セルラーゼとして用いることを想定し，その基礎的特性として，セルラーゼ固定量と酵素活性の関係，セルラーゼの脱落性について検討した．

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Appl Biochem Biotechnol (2012)

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 • Phang Lai Yee • Mohd Ali Hassan

IN PRESS

Abstract Acetone-butanol-ethanol (ABE) production from renewable resources has been widely reported. In this study, *C. 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

IMPACT FACTOR

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IN PRESS

Biotechnol Lett
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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

Received: 22 September 2011 / Accepted: 12 October 2011

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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Y. Shirai · Y. Ando

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

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Visible light-sensitive MnO_2- and CeO_2-loaded ZrO_2/carbon cluster/Pt nanocomposite materials

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Keywords:
Carbon cluster
Metal oxide
Nanocomposite
Sensitization
Photocatalytic activity

abstract

Nano-sized ZrO_2/carbon cluster composite materials (I_c's) were successfully prepared by the calcination of $ZrOCl_2$/starch complex I. I_c's were found to reduce methylene blue under the irradiation of visible light ($k > 460$ nm). The materials obtained by calcining at 400 and 500 °C were selectively loaded with Pt particles to obtain Pt-loaded ZrO_2/carbon cluster composite materials denoted as I_{c400}Pt and I_{c500}Pt, respectively. In addition, the resultant materials were modified with MnO_2 and CeO_2 particles to achieve MnO_2- and CeO_2-loaded ZrO_2/carbon cluster/Pt composite materials denoted as I_{c400}PtMn, I_{c500}PtMn, I_{c400}PtCe and I_{c500}PtCe, respectively. The metal oxides-loaded ZrO_2/carbon cluster/Pt composite materials thus synthesized could decompose an aqueous silver nitrate solution by visible light irradiation to give Ag and O_2 with the [Ag]/[O_2] ratios of ca. 4. Visible light-irradiated water splitting examinations with I_{c400}PtMn and I_{c400}PtCe were also investigated and found to yield H_2 and O_2 with the [H_2]/[O_2] ratios of ca. 2.

1. Introduction

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ISSN: 0149-6395 print/1520-5754 online
DOI: 10.1080/01496395.2011.615788

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Taylor & Francis Group

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

Mitra Mohammadi,[1] Mohd Ali Hassan,[1,2] Yoshihito Shirai,[3] Hasfalina Che Man,[4] Hidayah Ariffin,[1] Lian-Ngit Yee,[1] Tabassum Mumtaz,[1] Mei-Ling Chong,[1] and Lai-Yee Phang[1]

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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 polyhydroxyalk-

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-hydroxybuyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3-HV)] 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

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ORIGINAL ARTICLE

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

Mitra Mohammadi,[1] Mohd Ali Hassan,[1,2,*] Lai-Yee Phang,[1] Yoshihito Shirai,[3] Hasfalina Che Man,[4] Hidayah Ariffin,[1] Al Ashraf Amirul,[5] and Siti Nor Syairah[5]

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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 nonpolymeric 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

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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NURUL ASYIFAH MUSTAPHA

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Full Length Research Paper

Isolation, characterization and identification of potential actinobacteria with antifungal activities towards chilli anthracnose

Jeffrey Lim Seng Heng[1], Umi Kalsom Md. Shah[2]* and Halizah Hamzah[3]

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Accepted 11 April, 2011

Actinobacteria from the genus of Streptomycetes have been regarded as the most potent producers of bioactive compounds in the world. In this study, a total of 132 isolates of actinobacteria were isolated from rhizospheres of various plant species planted at MARDI Langkawi Agro Technology Park, Malaysia. These isolates were screened for the ability to inhibit the growth of pathogenic fungi, *Colletotrichum capsisi* and *Colletotrichum gloeosporioides* isolated from chilli fruit. From these screening it revealed that 45 isolates of actinobacteria were able to produce antifungal activity towards *C. capsici,* while 67 isolates produced antifungal activity towards *C. gloeosporioides.* Out of these 132 isolates, 2 of the best antifungal-producer were selected and identified as *Streptomyces* spp. strain PM2 and PM4. Observation using scanning electron microscope (SEM) showed that the spore surface for both *Streptomyces* spp. strain PM2 and PM4 were rough and spiky. Physiological characterization of both strains showed their ability to grow in 1 to 4% of NaCl, growth temperature of 17 to 35°C and pH of 5 to 11. The ability of these *Streptomyces* spp. to secrete antifungal compounds may have been related to the availibility of the carbon sources. These findings suggest that *Streptomyces* spp. strain PM2 and PM4 are potential candidate for biocontrol against anthracnose disease.

Keywords: Actinobacteria, *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, anthracnose, antifungal activity.

INTRODUCTION

Colletotrichum capsici and *Colletotrichum gloeosporioides* have been known to be the casual agents for the anthracnose disease on chilli fruits world wide (Manandhar et al., 1995; Sangchote, 1999). *C. gloeosporioides* was the most dominant anthracnose causing agent with its ability to infect other crops and fruits such as yam and mango. Anthracnose attack on chilli fruits causes the fruits to be blemishes and thus, making it unmarketable. The typical symptoms of anthracnose attack on chilli fruit are characterized by the formation of sunken necrotic lession, with concentric rings of acervuli.

Soil borne actinobacteria had been known to possess the ability to produce bioactivities such as enzymes, pesticides, herbicides and also antibiotics (Parungao et al., 2007). Currently, fungicides were sprayed to the fruits to inhibit the disease, but the used of chemical fungicides would caused the development of resistance by the fungal and also the risk of polluting the environments. The used of biological control agents had been suggested as an alternative way of controlling plant diseases (Compant et al., 2005). Jeffrey (2008) has suggested that actinobacteria belonging to the genus of Streptomyces can be used as biocontrol agents for

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Preparative Biochemistry & Biotechnology

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☐ *The response surface method was applied in this study 26 to improve cellulase productio from oil palm empty fruit bunch (OPEFB) by* Botryosphaeria rhodina. *An experimental desig based on two-level factorial was employed to screen the significant environmental factors fo cellulase production. Locally isolated fungus,* Botryosphaeria rhodina *was cultivated on OPEF under solid state fermentation (SSF). From the analysis of variance (ANOVA), initial moistur content, amount of substrate and initial pH of nutrient supplied in the SSF system significantl influenced cellulase production. Then, the optimization of the variables was done using th response surface method according to Central Composite Design (CCD).* Botryosphaeria rhodin *exhibited its best performance with high predicted value of FPase enzyme production (17.9 U/g) when the initial moisture content was at 24.32 %, initial pH of nutrient was 5.96 and 3.98 of substrate. The statistical optimization from actual experiment resulted in a significan 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, solid state fermentation, optimization, response surface methodology

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0.603

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International Journal of Agricultural Research 6 (2): 188-193, 2011
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Production of Bioethanol from Rice Straw using Cellulase by Local *Aspergillus* sp.

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ABSTRACT

Cellulase production *in situ* was considered as one of the alternatives to reduce bioethanol production cost. In this study, cellulase enzyme was produced from rice straw by locally isolated *Aspergillus* sp. in solid state fermentation. The crude cellulase was measured to have activity of 6.3 FPU g^{-1} rice straw. The rice straw was pretreated by few cycles of wet disc milling prior saccharified it using the crude cellulase produced. More than 90% glucose from total cellulose was released by the saccharification. The saccharified product was subjected to fermentation by yeast. The highest bioethanol yield produced from the fermentation was 0.102 g g^{-1} rice straw which is equivalent to 62.61% of theoretical bioethanol yield. It was concluded that the use of crude cellulase from rice straw onto rice straw can lead to a good yield of bioethanol, provided an effective pretreatment was used.

Key words: Rice straw, crude cellulase, saccharification, fermentation, bioethanol

INTRODUCTION

Paddy is included as one of Malaysian major crop and producing huge amount of rice straw as solid biomass waste seasonally. The rice straw came after the stripping process of rice using machine at the field, where the rice straw was removed and left to dry. It has no further use apart from being used as fodder. Since, it exists in abundant amount, it is usual for farmers to burn the rice straw when it dried at their field, causing haze and other environmental problem (Lai *et al*., 2009). It is also usual for them to throw it away if the rice straw is wet due to rain. Both conventional elimination methods definitely cause environmental pollution. With regard to depleting fossil fuel issue, there are biotechnological approaches to utilize rice straw as alternative fuel, for example bioethanol (Binod *et al*., 2010).

Bioethanol was a term referring to ethanol produced from cellulosic biomass (Mabee and Saddler, 2010). Currently, bioethanol is commonly used as fuel additive and industrial chemical product (Hernandez and Kafarov, 2007). Biomass such as rice straw contains three major components which are cellulose, hemicellulose and lignin. Accordingly, only cellulose and hemicellulose have the ability to be converted into sugars (Moiser *et al*., 2005). Cellulose is a polymer constructed by chains of D-glucose linked by β(1-4) bond. This bond can be broken by introducing external chemical such as acid, or catalyze by enzymatic hydrolysis (Karimi *et al*., 2006a). Usage of acid for rice straw hydrolysis is considered as non-green approach since the

CITED JOURNAL

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Australian Journal of Basic and Applied Sciences, 5(3): 276-280, 2011
ISSN 1991-8178

Effect of Oil Palm Empty Fruit Bunch Particle Size on Cellulase Production by *Botryosphaeria sp.* Under Solid State Fermentation

Ezyana Kamal Bahrin, Piong Yeau Seng and Suraini Abd-Aziz

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Abstract: Locally isolated *Botryosphaeria sp.* showed the ablity to produce cellulases (FPase, CMCase and β-glucosidase) from oil palm empty fruit bunch (OPEFB) as substrate. Different particle sizes (0.25-0.3 mm, 0.42-0.6 mm, 0.84-1.0 mm and 5.0-10 mm) of OPEFB were investigated under solid state fermentation on the cellulase production. The highest production of FPase and β-glucosidase were obtained from OPEFB particle size of 0.42 – 0.60 mm with 3.261 ± 0.011 U/g and 0.115 ± 0.008 U/g, respectively. It was found that among the four different OPEFB particle sizes studied, particle size of 0.84 – 1.0 mm gave the highest activity of CMCase (8.134 ± 0.071 U/g). Highest concentration of reducing sugars produced in this experiment was 4.303 ± 0.095 mg/ml.

Key words: substrate particle size, cellulase, solid state fermentation, *Botryosphaeria sp.*

INTRODUCTION

Oil palm mills produce a large amount of biomass waste from its daily operation. Generally, 17.08 millio tones per annum of oil palm empty fruit bunches (OPEFB) have been produced continuously in 2005 (MPC 2006). Fully utilization of OPEFB can be achieved by generating value added products such as activat carbon, enzymes, citric acid and others. OPEFB is categorized as lignocellulosic feedstock since it is rich cellulose contents .Moreover the usage of OPEFB as substrate in cellulases production can reduce the operati cost since substrate cost became one of the major operational costs, representing 30-40% of total producti cost (Tanaka *et al.*, 2006; Zhang *et al.*, 2007).

Insolubility of OPEFB is one of the limitations in submerged fermentation. Solid state fermentation (SS is more capable in producing certain enzymes and metabolites that usually produced with low yield ... submerged fermentation. The bioconversion of OPEFB into polyoses by using SSF resembles the natural condition of growth for the majority of fungi. Bacteria, yeast and fungi are able to grow on solid substrate but filamentous fungi are the best adapted for SSF (Krishna, 2005). The hypha of the fungi has the power to penetrate into the solid substrate.

There are several factors involved in the selection of a suitable substrate for SSF such as macromolecular structure, particle size and shape, porosity and particle consistency (Krishna, 2005; Tao *et al.*, 1997). The substrate must be in a limited size range for an optimal production of cellulase. This process can be facilitated

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BIOENERGY BIOPRODUCT RESEARCH GROUP - BIOETHANOL

CITED JOURNAL

Bioconversion of Restaurant Waste into Polyhydroxybutyrate (PHB) by *Recombinant E.coli* through Anaerobic Digestion

Majd Khalid Eshtaya, Nor Aini Abdul Rahman, Mohd Ali Hassan

Abstract: Organic acids (lactic, acetic and butyric) are the major fermentation acids produced on acidogenesis of organic waste such as food waste. In this study, polyhydroxybutyrate (PHB) was produced from restaurant waste in a two-step process of microbial acidogenesis and acid polymerization. The effect of temperature (30°C, 37°C and uncontrolled) and initial pH adjustment at pH 7 in the anaerobic digestion process were investigated to enhance the production of organic acids from the waste. Anaerobic digestion of blended restaurant waste was carried out using a 500 mL Erlenmeyer flask agitated on an orbital shaking incubator. The highest organic acids level obtained were 39.6 g/L on the fifth day of fermentation conducted at 30°C and initial pH 7. The acids produced corresponded to 39.4% of the yield based on the initial COD of substrate. The main organic acids produced were lactic acid and acetic acid while other acids were produced in small amount. The fermentation acids were tested for polyhydroxybutyrate (PHB) production by recombinant *Escherichia coli* pnDTM2 and the results were comparable to those of mixed pure acids. Using organic acids from fermented restaurant waste, *recombinant E.coli* gave PHB concentration, PHB content and PHB productivity of 9.2 g/L, 44% w/w and 0.54 gL1h1, respectively in a pH stat fed-batch culture.

Keywords: organic acids, anaerobic digestion, restaurant waste, polyhydroxybutyrate

A METHOD FOR TREATING OIL PALM BIOMASS

Inventor :
Mohd Ali Hassan, Azhari Samsu Baharuddin, Alawi Sulaiman, Minato Wakisaka, Haruo Nishida, Yoshihito Shirai, Mohd Zulkhairi Mohd Yusof, Ezyana Kamal Bahrin, Noriznan Mokhtar and Lim Siong Hock

Abstract :
The present invention relates to a method for treating oil palm biomass using high pressure steam and/or superheated steam available at the palm oil mill which is operated at temperature range 100°C-350°C and pressure range 40-450psig (0.27-3.1MPa) for up to 180 minutes of holding time to enhance its degradation characteristics for potential use as a feedstock material for production of high value-added bioproducts such as biocompost, biogas, biohydrogen, biosugar, bioethanol and others. The oil palm biomass is such as empty fruit bunches, frond, mesocarp fibres, palm oil mill effluent (POME) solid and anaerobic sludge or any combination thereof.

IP Status : Pending Patent
Filed Year : 2011-02-17
Application No. : PI2011000731
Country Filing : Malaysia
Applicant : Universiti Putra Malaysia (UPM), FELDA Palm Industries (FPI), Kyushu Institute of Technology (KIT)

RENEWABLE SUGARS FROM OIL PALM WASTE

Inventor :
Mohd Ali Hassan, Hidayah Ariffin, Mior Ahmad Khushairi Mohd Zahari, Mohd Rafein Zakaria, Jailani Salihon, Mohd Noriznan Mokhtar and Yoshihito Shirai

Abstract :
The invention provides method for producing sugars from oil palm fronds wherein the steps comprises of (1) extracting oil palm frond juice from the oil palm fronds; and (2) treating the oil palm frond juice. The invention also provides a method of using the said oil palm frond juice for producing polyhydroxyalkanoates such as polymer of hydroxyalkanoic acid, hydroxybutyric acid, hydroxyvaleric acid, and a copolymer thereof, wherein the copolymers maybe poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV), poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB), polymers and/or copolymers of hydroxyterminated polyhydroxybutyrate (PHB-OH), heteropolymers thereof and any other polymers. In addition to that, biofuels and organic acids such as bioethanol, biobutanol, lactic acid, succinic acid, and all products that can be produced through chemical and biological synthesis from these sugars from the OPF juice. The present invention also provides a method for producing polyhydroxyalkanoate (PHA) by incubating at least one strain of PHA-producing microorganisms in a culture medium comprising of sugars and/or a derivative thereof from the oil palm frond juice. The microorganisms used can be of bacteria, mold or yeast from the group Azotobacter, Pseudomonas, Coliform, Alcaligenes, Bacillus, Lactobacillus and genetically modified form thereof preferably Azotobacter chroococcum, recombinant Escherichia coli, Alcaligenes latus, Pseudomonas oleovorans and Cupriavidus necator.

IP Status : Pending Patent
Filed Year : 2011-09-20
Application No. : PI2011004440
Country Filing : Malaysia
Applicant : Universiti Putra Malaysia (UPM)

EB GROUP PATENTS

SYSTEM FOR EVAPORATING FINAL DISCHARGE WASTEWATER GENERATED IN THE PALM OIL MILL

Inventor :
Mohd Ali Hassan, Azhari Samsu Baharuddin, Alawi Sulaiman, Amir Mohd Ali and Yoshihito Shirai

Abstract :
A system for evaporating final discharged wastewater generated in a palm oil mill comprising removal of wastewater grit, equalization and stabilization of steam, evaporation, collection of condensed steam and water vapour and, collection and distribution of clean water.

IP Status : Pending Patent
Filed Year : 2011-11-08
Application No. : PI2011005385
Country Filing : Malaysia
Applicant : Universiti Putra Malaysia (UPM)

CRUDE CELLULASE COCKTAIL FOR LIGNOCELLULOSIC MATERIALS DEGRADATION

Inventor :
Suraini Abd-Aziz, Mohd Ali Hassan, Mohd Sukri Ismail, Razali Sarbini, Nurul Kartini Abu Bakar, Mohd Faizal Ibrahim, Mohd Nafis Abdul Razak and Phang Lai Yee

Abstract :
The invention provides enzymes which are useful for degradation of lignocellulosic materials, which is obtained from Trichoderma asperellum UPM1 and Aspergillus fumigatus UPM2 and wherein the steps includes: (a) growing of the Trichoderma asperellum UPM1 and Aspergillus fumigatus UPM2; (b) carrying out fermentation process using the fungi grown from step (a) with substrates which has been treated to obtain enzymes; and (c) recovering the enzymes from fermented products obtained from step (b). The enzymes recovered include cellulases, xylaneses, pectinases or a combination of these enzymes which can be further used to hydrolyze lignocellulosic materials for fermentable sugars

IP Status : Pending Patent
Filed Year : 2011-06-10
Application No. : PI2011002674
Country Filing : Malaysia
Applicant : Universiti Putra Malaysia (UPM), AlafPutra Biowealth Sdn. Bhd.

CONSULTANCY

RESEARCH THEME	CLIENTS / INDUSTRIAL PARTNER	DURATION
Utilization of Palm Biomass as Feedstock for Biofuels and Biomaterials	Biomass Technology Research Centre, National Institute of Advanced Industrial Science and Technology (AIST) Japan & Kyushu Institute of Technology (KIT) Japan	RENEW UNTIL **MARCH 2013**
Effective Utilization of Biomass Through Biotechnology	Ajinomoto Co. Ltd, Japan	RENEW UNTIL **MARCH 2013**
Bioconversion of Palm Oil and Its Products to Polyhydroxalkanoates (PHA)	SIRIM Bhd	RENEW UNTIL **MARCH 2012**
Improved Biogas Production from Glycerine Washwater	FELDA Proctor Gamble (FPG) Sdn Bhd	**2007 ~ 2011** COMPLETED
Production of Biocompost from Oil Palm Empty Fruit Bunches (EFB) and Goat Manure	YPJ Plantation Sdn Bhd	**2009 ~ 2010** COMPLETED
Feasibility Study on CDM Biomass Projects for Renewable Energy	Tokyo Electric Power Company (TEPCO), Japan	**2008** COMPLETED
Improvement of Wastewater Treatment System in Miri Crude Oil Terminal	Petronas	**2006 ~ 2008** COMPLETED
Microbial Production of Ethanol from Glycerine Wastewater	Idemitsu Co. Ltd., Japan	**2007** COMPLETED
Production of Activated Carbon and Pyroligneous Acid from Palm biomass	NewTech Sdn. Bhd	**2005 ~ 2007** COMPLETED
Industrial Grant Scheme (IGS) on Bioplastics, Bioacids and Compost from Organic Wastes	Kasa Ganda Sdn. Bhd	**2003 ~ 2005** COMPLETED

AWARDS

INTERNATIONAL INVENTION, INNOVATION & TECHNOLOGY EXHIBITION, ITEX 2011
Kuala Lumpur Convention Centre, Malaysia

***COMAMONAS PUTRANENSIS* SP. NOV., A NOVEL BACTERIUM PRODUCING POLYHYDROXYALKANOATES FROM PALM OIL MILL EFFLUENT**

Prof. Dr. Mohd Ali Hassan,
Mr. Mohd Rafein Zakaria,
Mr. Noor Azman Mohd Johar,
Prof. Dr. Yoshito Shirai,
Dr. Hidayah Ariffin,
Prof. Dr Suraini Abd. Aziz

GOLD MEDAL

ECO-FRIENDLY NON-HALOGENATED METHOD FOR RECOVERY OF INTRACELLULAR POLYHYDROXYALKANOATES (PHA)

Prof. Dr. Mohd Ali Hassan,
Ms. Mitra Mohammadi,
Dr Phang Lai Yee, Dr. Hidayah Ariffin, Dr. Amirul Al-Ashraf Abdullah, Dr. Hasfalina Che Man,
Prof. Dr. Yoshito Shirai

GOLD MEDAL

► ITEX 2011 - judging process

SHELL INTERVARSITY STUDENT PAPER PRESENTATION CONTEST 2011 (S-SPEC 2011)

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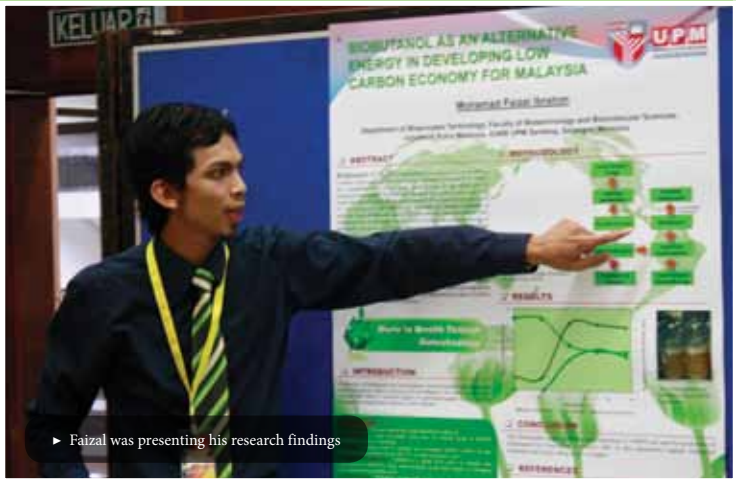
4-5 APRIL 2011, UNIVERSITY TEKNOLOGI MALAYSIA, SKUDAI, JOHOR

Competition: **Poster Presentation**
Poster Title: **Biobutanol as an alternative energy in developing low carbon economy for Malaysia**
Price: **Grand Price (First Place)**
Name: **Mohamad Faizal Bin Ibrahim**

Competition: **Paper Presentation**
Paper Title: **Production of bioethanol from rice straw using Cellulase by Local *Aspergillus* sp.**
Price: **First Runner Up**
Name: **Ahmad Muhaimin Roslan**



► Faizal was presenting his research findings

► Muhaimin's plaque

BIOENERGY RESEARCH

GROUP LEADER

Professor Dr Suraini Abd Aziz

GROUP INTRODUCTION

This research group consists of four research areas including Bioethanol, Biobutanol, Biohydrogen and Bioproduct. Our collaborative research area focused on the bioconversion of agricultural biomass such as palm oil mill effluent (POME), oil palm empty fruit bunch (OPEFB), oil palm decanter cake and sago waste residues into biobased product through biotechnology and bioprocess engineering approaches. Currently, there are nine PhD students, eight Master students and one research assistant under this group. Our research group emphasizes on developing indigenous technology for beneficial bioenergy production using

RESEARCH AREA

BIOETHANOL

■ Bioethanol is an alternative energy source and usually related to high world market demand. Recently, it has been reported that conversion of biomass into bioethanol involved physical, chemical and biological methods where the lignocellulosic material will be pretreated by thermo-chemical process, steam or mechanical grinder before it can be further hydrolyzed into fermentable sugars. The sugars obtained from hydrolysis process can be subsequently utilised for bioethanol production using local isolates from EB culture collection. ✳

BIOBUTANOL

■ Biobutanol is suitable for replacing petrol as fuel in gasoline engines. Besides, it is one of the alternative bioenergy in the next future. There are five steps involving utilization of biomass and other waste materials for biobutanol production including pretreatment of biomass, cellulases production, enzymatic hydrolysis, biobutanol production and finally is the recovery of biobutanol. In addition, substrates like oil palm empty fruit bunches (OPEFB), oil palm decanter cake (OPDC) and sago pith residue (SPR) will have to be hydrolyzed in order to biosugars and later can be further converted into biobutanol by locally isolated microorganisms from EB Research Group culture collection. ✳

► Bioethanol production using 1liter bioreactor

► Biobutanol production from OPDC hydrolysate

► Super-Heated Steam Equipment for pretreatement

BIOPRODUCT GROUP

local resources. On top of that, this research group also involved in downstream processing and basic molecular biotechnology-based. Besides, our research activity has been extended to downstream processing and basic molecular biology study using several indigenous isolates. Bioconversion of biomass materials into bioenergy and/or bioproduct can be achieved by one-step or two-step fermentation process that involves enzymatic reaction. In some cases, the biomass is pretreated chemically or enzymatically prior to actual production/fermentation process. Our industrial partners include FELDA Palm Oil Industry Sdn, Bhd, AlafPutra Biowealth Sdn.Bhd and other research institutes include SIRIM Berhad, MARDI, KIT and AIST. ⁎

■ PRINCIPAL RESEARCHER

Dr Phang Lai Yee

Dr Nor'Aini Abdul Rahman

BIOHYDROGEN

■ It has been known for many years that hydrogen can be produced from a variety of renewable resources such as agricultural or food industry wastes by using indigenous microorganisms. Our research works are focuses on the production of biohydrogen from sago waste residues as substrates. One of the important factors governing the hydrogen conversion that needs to be taken into account is the engineering process, such as bioreactor design and operating parameters. Apart from that, co-production of hydrogen and ethanol from biomass becomes one of our research focus areas too. We aim to enhance the biomass utilisation efficiency and bioenergy production through genetic modification and bioprocess control. ⁎

BIOPRODUCT

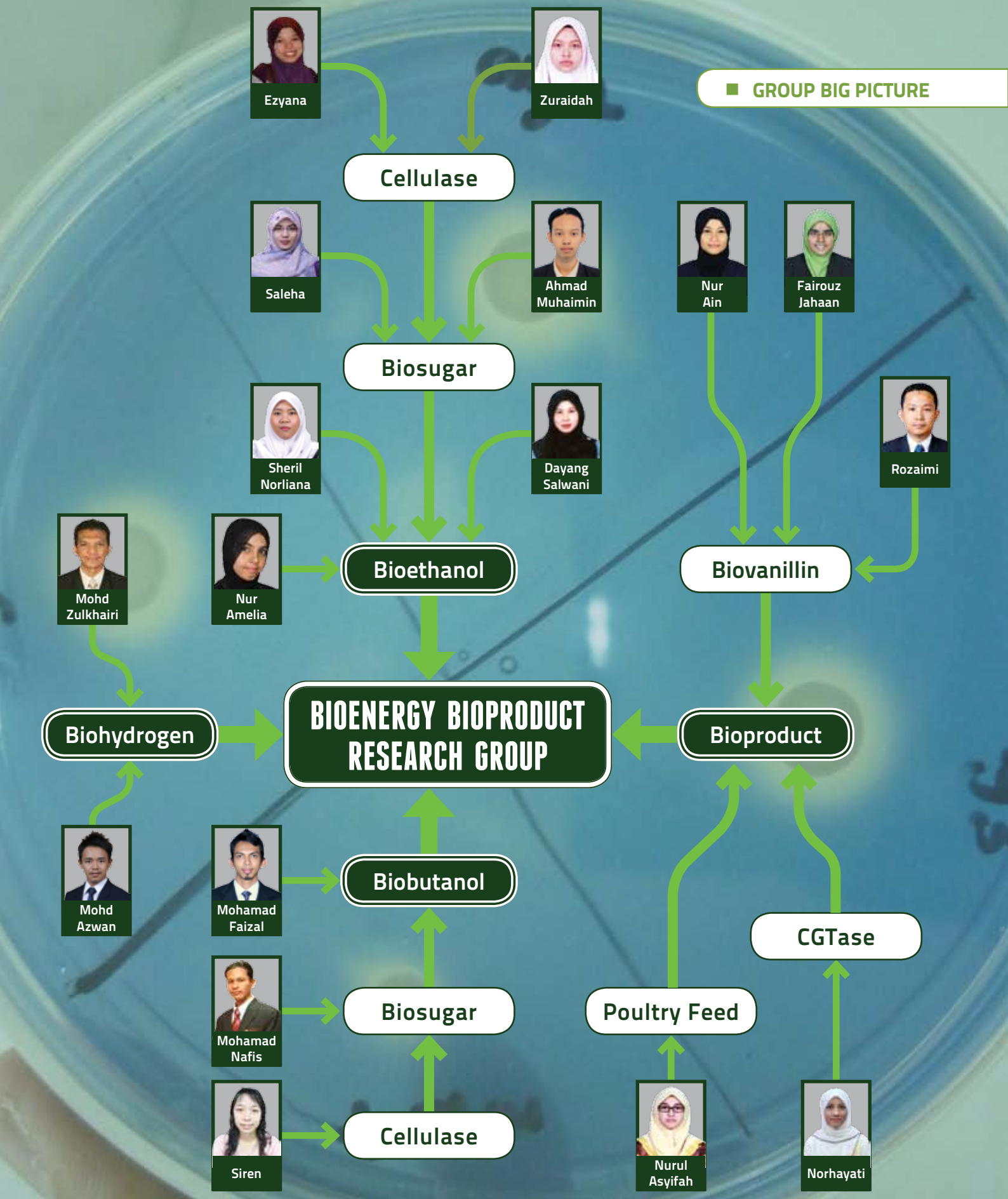
■ This research group involves in producing different biobased products by utilization of different substrates and microorganisms. All the projects apply biological pathways and microbial fermentation in order to produce beneficial enzymes and flavour compound as well as hydrolysis process for improvement of product quality. Apart from that, this group aims to achieve higher enzymatic activity in cyclodextrin glycosyltransferase production via molecular approaches, improving the nutritional value of poultry feed and production of flavour compound, biovanillin through microbial fermentation, genetic engineering as well as recovery of biovanillin. ⁎

► Submerge fermentation for production of cellulase from OPEFB

► Top view of SSF during enzyme extraction

► Crude glycerol obtained from palm oil-based biodiesel

GROUP BIG PICTURE



► Hollow zone produce by bioethanol producing bacteria in screening method for bioethanol

SALEHA SHAMSUDIN
PhD Student, Semester 8
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CELLULOSIC BIOETHANOL FROM STEAM PRETREATED OIL PALM EMPTY FRUIT BUNCH (OPEFB)

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 by-product 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. ∗

RESEARCH OBJECTIVES

To obtain the most favorable condition of high pressure steam pretreatment that increase OPEFB digestibility to biosugars production

To obtain high sugars concentration from steam pretreated OPEFB by Acremonium cellulase using Response Surface Methodology

To obtain high ethanol yield by Saccharomyces cerevisie using Response Surface Methodology

[SUPERVISOR] Professor Dr Mohd Ali Hassan

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ENHANCEMENT OF BIOETHANOL PRODUCTION AND ADDED VALUE PRODUCTS FROM PALM BIOMASS

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. In year 2002, approximately 11.9 million tones of crude palm oil were produced in Malaysia. In palm oil processing, a lot of biomass produced as a waste materials and oil palm biomass has been identified as one of the biggest resources that can be easily find in a bulk amount Meanwhile, in the palm oil processing generate large quantities of solid biomass which are rich in cellulose, hemicelluloses and lignin and can be classified as a biomass waste. Biomass is one of the materials, which can be used as fuel in energy industry for future demand, and could become largest source of domestic renewable energy. Conversion of biomass can also produce bio-fuels, which include ethanol, methanol, biodiesel and biohydrogen.
In the present situation, various attempts have been made to produce bioethanol as alternatives renewable energy from biomass. Oil palm biomass relatively has potential as a substrate for generation of bioethanol, hence, the development of an improved fermentation process for this organic waste is. Biological production of ethanol (bioethanol) is one of the compromising technique which is applicable in the current situation since the palm oil industries manage to produce lot of biological substance as a biomass.

Background of the study and problem arose

There have been a new interest to produce bioethanol simultaneously as the saccharification is running (simultaneous saccharification and fermentation, SFF). However saccharification required high temperature which will inhibit yeast growth for fermentation.
There are few things to be considered:

1. SFF technique involved mixing enzyme with microorganisms at running temperature for the enzyme which is usually high. A good thermodynamic balance must be find to stabilize microorganisms growth and products production.
2. Yeast must survive at saccharification temperature.
3. Utilization of 5-carbon sugar by yeast is very low.
4. The microorganisms used are sensitive to the by-products and to the ethanol itself. This will limit the maximum ethanol production.

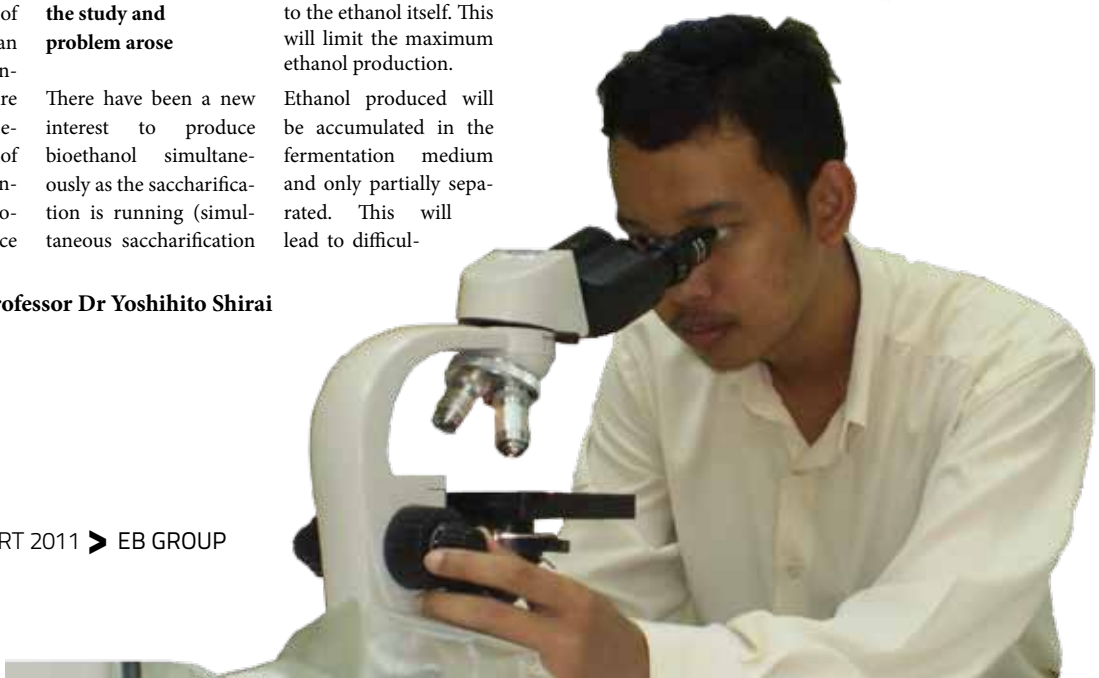
Ethanol produced will be accumulated in the fermentation medium and only partially separated. This will lead to difficulties in ethanol purification. And purification of bioethanol is costly, depending on the substrate, therefore require suitable technique.

No. (2) and (3) and (4) can be solved through metabolic engineering to create novel pathway for bioethanol production. While no. (1) and (5) require a depth study.

Important of the study

The study mends to access appraisal of molecular-based biotechnology technique to circumvent and enhance fermentation process on bioethanol production from harboring sources like oil palm biomass. Putatively, the advent of bioengineering to the microorganisms firmly explicit in to the synthetic media or well controlled condition and possess extremely higher yield of product. We are trying to elucidate the knowledge to the environmental application.
Our laboratory has demonstrated expertise in both theoretical analysis of metabolic networks as well as genetic construction of metabolically engineered organisms. Therefore, we propose to pursue the following objectives for the design and construction of an efficient microorganism for ethanol production. ∗

[SUPERVISOR] Professor Dr Yoshihito Shirai



BIOETHANOL

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CELLULASE PRODUCTION FROM OIL PALM EMPTY FRUIT BUNCH (OPEFB) BY *BOTRYOSPHAERIA RHODINA* UPM3 IN SOLID STATE FERMENTATION (SSF)

► *Botryosphaeria rhodina* was cultivated on OPEFB in solid state fermentation for cellulase production

Malaysia has a well positioned as the major producers and exporters in world's palm oil industry. Oil palm industry is currently producing the largest amount of biomass in Malaysia with 85.5% out of more than 70 million tonnes. In line with the Malaysian government approach to maximize the use of all feedstocks, byproducts and waste streams, oil palm empty fruit bunch is a potential feedstock for industry sectors since it is abundant and available throughout the year. Integration of Waste to Wealth concept is applicable to the palm oil industry in order to drive down all production costs. Value added the solid waste into useful products such as organic acid, sugars, compost, biogas and enzymes may overcome the waste disposal problem in the mill.

Bioconversion of value added product using solid state fermentation (SSF) is economical because it requires low energy consumption, low cost equipment, limited water usage and less effluent produced. Moreover, solid state fermentation conditions resemble the real cultivation of fungi in nature. Locally isolated fungus, *Botryosphaeria rhodina* is described as an endophyte which attacks woody host. The ascomycete fungus *Botryosphaeria rhodina* produces a broad range of lignocellulolytic enzymes such as laccases, pectinases, cellulases and xylanases. These complex enzymes play an important role in the degradation process of lignocellulosic materials through a synergistic action. The objective of this research is to study the effects of SSF parameters that influenced cellulase production by *Botryosphaeria rhodina*. *Botryosphaeria rhodina* exhibited its best performance on day 7 of incubation when the initial moisture content was at 20-25 %, initial pH of nutrient was 6 to 7 and with 3-5 g of substrate. Generally, fungi were cultivated at more than 50% of moisture content in solid state fermentation. However, high cellulase production at low moisture content (20-25%) is a very rare condition for fungi cultured in solid state fermentation but *Botryosphaeria rhodina* was capable to tolerate this condition.

Response surface method was applied in this study to improve the cellulase production from OPEFB by *Botryosphaeria rhodina*. An experimental design based on two-level factorial was employed to screen the significant environmental factors for cellulase production. From the analysis of variance (ANOVA), initial moisture content, amount of substrate and initial pH of nutrient supplied in the SSF system were significantly influenced the cellulase production. Then, the optimization of the variables was preceded in response surface methodology according to Central Composite Design (CCD). *Botryosphaeria rhodina* exhibited its best performance when the initial moisture content was at 26.3 %, initial pH of nutrient was 6 and 3.95 g of substrate. The model and design on the optimization of the environmental factors in this study was dependable to predict the cellulase production by *Botryosphaeria rhodina*. High cellulase production at low moisture content is very rare condition for fungi cultured in solid state fermentation. Thus, the cellulase production by this locally isolated fungus required less moisture and humidity which render a great advantage for large scale production.

In addition, fermented OPEFB by *Botryosphaeria rhodina* also was analysed according to SEM, FTIR, TG-DTA and compositional analysis to have a better understanding towards the macroscopic observation in SSF system. SEM micrographs showed a remarkable fungal growth cultivated on OPEFB for day 5 and 7. Cellulose and hemicellulose composition were gradually declined throughout the fermentation period. However, lignin content resided in the OPEFB fiber were remain unchanged until the end of the fermentation. This fact was supported with TG-DTA, FTIR and lignin determination which conclude that *B. rhodina* was unable to decompose lignin in a week. ✱

RESEARCH OBJECTIVES

To investigate the effect of environmental factors on cellulase production in SSF

To obtain an optimized condition of cellulase production by *Botryosphaeria rhodina* using Response Surface Methodology (RSM)

To study the biodegradation of fermented OPEFB by *Botryosphaeria rhodina* under SSF condition

[SUPERVISOR] Professor Dr Suraini Abd. Aziz

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PRODUCTION OF BIOETHANOL FROM OIL PALM EMPTY FRUIT BUNCH (OPEFB) USING CRUDE ENZYMES COCKTAIL PRODUCED BY *TRICHODERMA ASPERELLUM* UPM1 AND *ASPERGILLUS FUMIGATUS* UPM2

As the world largest producer and exporter of palm oil, Malaysia shared 50% of the palm oil production and 61% of exports. Palm oil industries had contributed crucial income sources for the country. Therefore the total planted area of palm oil tree increased by 4.5% to 4.69 million hectares (*http://econ.mpob.gov.my/economy/Overview_2009.pdf*). Thus, more wastes are expected to be generated in the future. Oil palm Empty fruit bunches (OPEFB) is one of the residues remained in a large amount, traditional practice of open burning would be ended with ash which then could be used as good fertiliser. However, it was not recommended as it caused an environmental problem of smoked air. Yet, strategy to address the efficient utilisation or bioconversion into various value added products such as biofuel, animal feed, chemicals and human nutrients should be explored deeply.

Recently, much attention has been given to bioethanol production from biomass resources as a consequence of a raising oil demand with unstable deliverance prices and the agricultural land competition for the food and fuel supplies. Thus, the search for an alternative of biofuel sources which is renewable and substitutable was in a great demand. A dramatic increased in bioethanol production using food based technology may not be practical (Sun and Cheng, 2002). Therefore, lignocellulosic residues of biomass (e.g OPEFB) could be utilised as an alternative sources for the production of low cost bioethanol. OPEFB is a lignocellulosic biomass which consists of 3 major components of cellulose, hemicelluloses and lignin. Hydrolysis of cellulose and hemicelluloses into fermentable sugar was the great advantage for the production of bioethanol. However, complex structures of lignin make it harder for any biological process to occur as it blocking the way of enzymes attack.

The conversion of lignocellulosic materials to bioethanol includes two processes;

1. hydrolysis of cellulose in the lignocellulosic material to fermentable sugar and
2. fermentation of sugars to bioethanol.

Hydrolysis is usually catalysed by cellulases enzyme and the fermentation is carried out by yeast or bacteria (Sun and Cheng, 2002). In order for a hydrolysis process to take part, lignin should be removed. Appropriate pre-treatment is essential to break up lignin which hinders enzyme attack. With suitable application of technology, OPEFB can be fully utilised into bioethanol as an alternative of a cheaper substrates via chemical and biological processes. The potential of wide range microorganisms utilising lignocellulosic wastes is undeniable. It involved the use of enzyme, microbes and any biological agent either in single and mixed. Hydrolysis is traditionally reliant acid process, however better environment condition favoured enzymatic hydrolysis at same cost as acid hydrolysis (Hamelinck, 2005).

Thus, one of the promising alternative for bioethanol production is the use of enzyme as it viewed by many experts to be at the very cost effective in the long term process (Hamelinck, 2005 and Sun and Cheng.,2002). Enzyme cocktail was favoured as an improved approach to increase the cooperative action of cellulases by combining different sources of cellulases particularly from *Trichoderma* and *Aspergillus* species and thus maximising it fermentable sugars production. Incorporation of overall processes by joining together several unit operations until the separation of bioethanol and fermentable sugars will developed a cost effective technology. Process integration is gaining more and more interest due to advantages related to the reduction of energy cost and decreased in the size and number of unit operation(Cardona and Sánchez,2007). The use of a process simulator (SuperPro Designer: Intelligen, Inc., Scotch Plain NJ) will greatly facilitate the process analysis, including handling the composition of raw material and product, sizing of unit operations, estimation of capital and operating costs (Kwiatkowski *et al.*,2006). ∗

RESEARCH OBJECTIVES

To enhance cellulases production by *T. asperrellum* UPM1 and *A. fumigatus* UPM2

To optimize the production of fermentable sugars from oil palm empty fruit bunch using crude cocktail of cellulases by *T. asperrellum* UPM1 and *A. fumigatus* UPM2 using Response Surface Methodology

To develop an integrated system for the production and separation of bioethanol and fermentable sugars

[SUPERVISOR] Professor Dr Suraini Abd. Aziz

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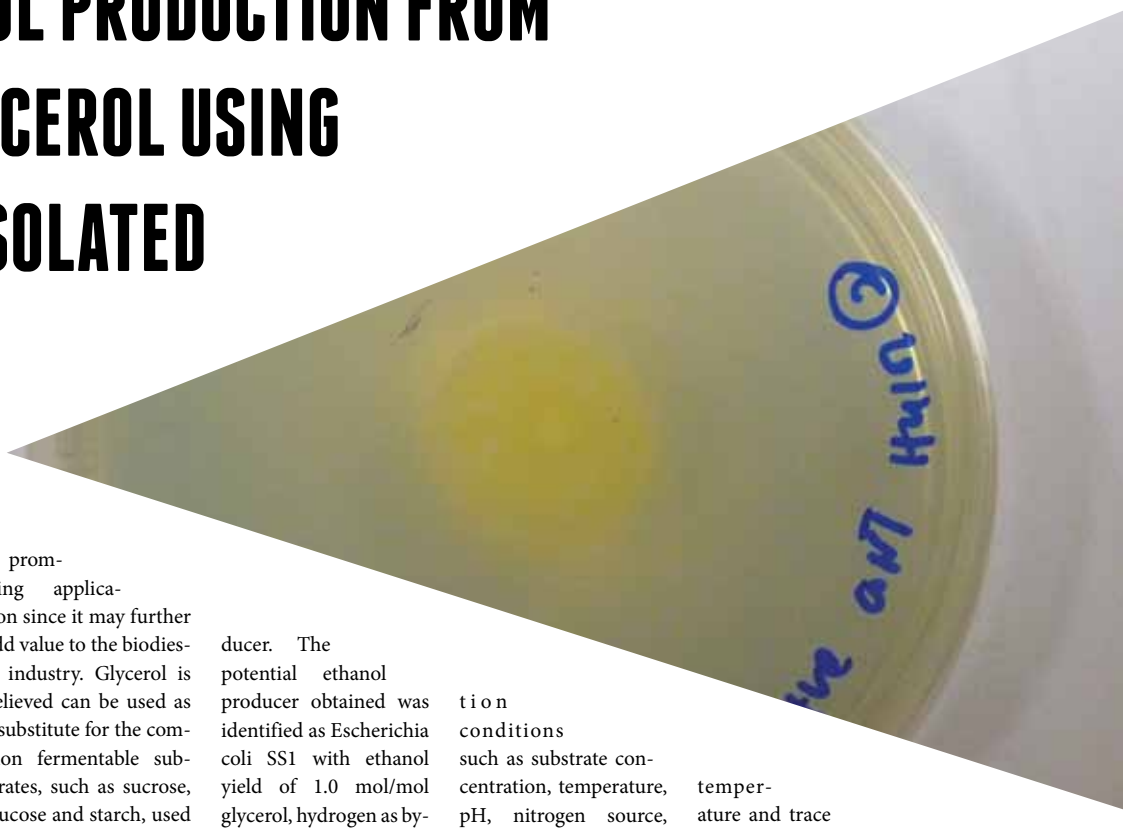
BIOETHANOL PRODUCTION FROM CRUDE GLYCEROL USING LOCALLY ISOLATED BACTERIA



Glycerol (glycerine, 1,2,3-propanetriol) is highly presence in nature since it has been used in wide range of applications. Biodiesel production creates abundant of glycerol, since the process of biodiesel generates about 10% (w/w) of glycerol as the major by-product. The increased of biodiesel production led to increase of crude glycerol in environment, which consequently rise the environmental issue due to the problem of its disposal. In response to higher availability of this compound in nature, many microorganisms are known to be naturally utilizing glycerol as sole carbon and energy source. Microbial fermentation of glycerol for valuable chemicals formation such as 1,3-propanediol, dihydroxyacetone, ethanol and succinate is considered as promising application since it may further add value to the biodiesel industry. Glycerol is believed can be used as a substitute for the common fermentable substrates, such as sucrose, glucose and starch, used in industrial fermentation processes especially in ethanol production as fuels. Glycerol used as feedstock for bioethanol production could be an alternative because it is cheap and abundance. Hence, the studies on bioconversion of crude glycerol for bioethanol production were performed by using locally isolated bacteria.

In this study, potential glycerol-consuming bacteria that have capability in producing ethanol from glycerol were isolated from environment. The screening procedure was modified to isolate glycerol-consuming ethanol producer. The potential ethanol producer obtained was identified as Escherichia coli SS1 with ethanol yield of 1.0 mol/mol glycerol, hydrogen as by-product with and minor amounts of organic acids. 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, temperature and 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 effects of the significant variables (two level factorial design) were subsequently evaluated in Central Composite Design (CCD) to develop an empirical model to determine the optimum value of each variable. The optimized conditions obtained from CCD with predicted ethanol production of 17.04 g/L. The validation was carried out by using crude glycerol as substrate in 1 L bioreactor. ✳

RESEARCH OBJECTIVES

To isolate potential glycerol-fermenting microbes for ethanol production

To optimize the microbial conversion of crude glycerin for ethanol production by statistical approach (RSM)

PICTURE

► Yellow zone surrounded bacteria colony shows the presence of ethanol

[SUPERVISOR] Dr Phang Lai Yee

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THE FEASIBILITY OF SAGO HAMPAS AS A FEEDSTOCK FOR BIOETHANOL PRODUCTION

RESEARCH OBJECTIVES

To pretreated the sago 'hampas' for glucose production using enzymatic hydrolysis

To study bioethanol production from hydrolyzed sago 'hampas' through batch fermentation system utilizing commercial baker's yeast

To optimize the bioethanol production from hydrolyzed sago 'hampas' by response surface method (RSM)

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 bakers 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 pre-germinate 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, KH_2PO_4, $MgSO_4.7H_2O$ and NH_4SO_4, 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 (NH_2SO_4, 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 NH_2SO_4 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. ✳

PICTURE

► Ethanol fermentation utilizing sago hampas hydrolysate

[SUPERVISOR] Professor Dr Suraini Abd. Aziz

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BIOETHANOL PRODUCTION FROM GLYCEROL BY IMMOBILIZED *ESCHERICHIA COLI*

In biodiesel production, glycerol will be produce as secondary by-product. These phenomena have become a problem since the production of biodiesel is increasingly in demand. Glycerol which is produce in the fermentation will be as waste and problem occur where there is no way to treat this glycerol. These will be a burden to the biodiesel manufacturer. Thus, there is a lot of interest in the production of value added products based on glycerol such as cosmetics, paint, pharmaceuticals, pulp and paper industry and also food industry.

One of the value added product that been much interest is the production of bioethanol. It has become one of the most feasible options in the area of non-conventional sources of energy. Bioethanol has the ability to reduce the greenhouse gas emission by 5-7% depend on the feedstock used which is usually derived from renewable materials for example corn, sugar beets, wheat and wood. The availability of these raw materials is a major problem for bioethanol production since these raw materials is depends on the geographic locations and season. As a result, the cost for bioethanol production keeps changing and expensive. It is also stated by previous study that ethanol from glycerol is cheaper to produce compare to ethanol from corn. Therefore by using glycerol as carbon source, it can help to reduce the bioethanol production cost.

The use of immobilized microbial cells for ethanol fermentation has become interesting in researchers due to advantages compare to traditional processes. It is known for its efficiency in improving substrate utilization and productivities of various fermentation processes. There are few advantages by using immobilized cell for example it will increase the cell density, high rate of mass transfer, intimate phase mixing, high fermentation rate, cell recycling and product inhibition will be lower. Attention has been given for fuel ethanol production using immobilized cell technology in fermentation processes since it has been known to show a significant role in bioreactor performance.

Thus the aim of this study is to enhance the bioethanol production from glycerol as carbon source by E.coli with the use of immobilized cell system which offers advantage on the bioethanol productivity. This includes investigation on the optimum condition for stability and rigidity of the alginate beads and also the performance when compare with free suspension cells. Immobilized of *E.coli* is done by entrapping the cells inside sodium alginate. The development of cell immobilization technique in regards to physical property of alginate bead and cell to alginate ratio will be examined. The effect of production media on the physical properties of alginate beads is important to make sure there is no leakage of the beads during fermentation process. Cell to alginate ratio is optimized to maintain high cell density in the fermentation system. Once rigid and stable beads are formed, it will be transferred to 2 litre bioreactor to determine the performance by using bioreactor. It is expected that bioethanol production from glycerol is enhanced in terms of productivity by using immobilized cell. ∗

[SUPERVISOR] Dr Phang Lai Yee

RESEARCH OBJECTIVES

To optimize the immobilization techniques using sodium alginate

To determine the bioethanol production by immobilized *Escherichia coli* in sodium alginate using 2 litre bioreactor

PICTURE

► Washing the beads to remove access calcium chloride

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FUNCTIONAL ANALYSIS OF ESSENTIAL GENE RELATED TO BIOHYDROGEN PRODUCTION IN *ESCHERICHIA COLI*

Biohydrogen production has become pivotal subject being discussed recently due to their eco-friendly character. Its combustion only produces H_2O and it is not one of the green house gases. Essentially, biohydrogen can be produced by various means chemical, physical and biological approaches. Biohydrogen from fermentable biomass appears an interesting idea due to low cost substrate availability. *Escherichia coli* is a robust bacterium for developmental research based on genetic engineering because its whole genome sequence is available and its metabolic pathways are relatively well-established. To date, we conducted an comprehensive search of all *E. coli* pathways for their impact on hydrogen production through screening the entire Keio mutant library (3985 isogenic mutants) using chemochromic membranes (GVD Corp., Cambridge, MA) formed by a thin film of WO_3 covered with a catalytic layer of palladium, to detect biohydrogen gas, by a colorimetric response [*figure 1*]. Several uncharacterized genes related to bacterial hydrogen production were identified and used as candidates for further investigation [*figure 2*]. The chosen strains were undergone fermentation process using respective substrate such as glucose or formate. In addition, all the experiment has adhered with reference strain, wild type BW 25113 [*figure 3*]. Biohydrogen amount generated in headspace was analyzed using gas chromatography (Agilent Technologies Inc., Santa Clara, CA) after one, two, four, and 24 hours. Metabolism pathway has been studied in response to biohydrogen production and its metabolite. Thus, in order to have better understanding of the metabolism, it's by-products was determined. Carbon balance or mass balance of the output and input during the process become essential parameters to be investigated.

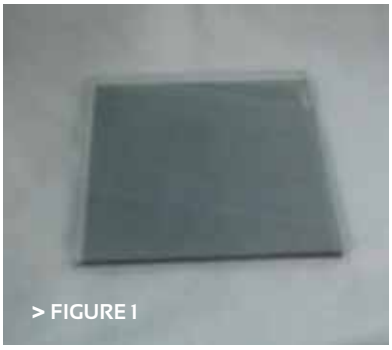
In this study, six mutants have elucidated which their genes reflected to biohydrogen production. These genes have been expended for further investigation using complex formate media and two mutants have showed significant deviation from glucose whereas it consumed formate and produce hydrogen with the yield 130.05 and 80.64 µmol H_2/mg protein respectively. The selective strains will be elected to undergo molecular level investigation using qRT-PCR in order to elucidate expression level of the gene in response to hydrogenase operon. ∗

RESEARCH OBJECTIVES

To screening responsible genes related to biohydrogen production in *E.Coli*

To elucidate expression level of related genes during biohydrogen production.

[SUPERVISOR] Assoc. Prof. Dr. Eng. Toshinari Maeda


> FIGURE 1


> FIGURE 2


> FIGURE 3

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HYVOLUTION

PRODUCTION OF BIOHYDROGEN FROM SAGO WASTE BY LOCAL ISOLATE

Hyvolution is acronym of the integrated project "non-thermal production of pure hydrogen from biomass". The concept of the hyvolution is to subject the bacteria, which is freely and efficiency in producing pure hydrogen as a product during degrading the biomass (Claassen *et al.*, 2010). The novelty of this process is where it used biomass as a substrate since there are many study has been done by wastewater. The concept of this hyvolution is based on combination bioprocess in exploiting thermophilic and phototrophic bacteria, to provide highest pure hydrogen production in efficiency and low cost. Recently, the production of biohydrogen from biomass has become a new path for hydrogen production since biomass is cheap and readily available.

Biomass is one of the most abundant and renewable resources that can be used in order to be converted to valuable products. The agricultural activity is one of the biggest activities that lead to the abundantly of biomass especially in Malaysia. Biomass provides clean, renewable energy source that could intentionally improve the economy, environment and energy cycle.

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 in the starchy fibrous pith residue or known as sago pith residue (SPR). It is usually washed off into drains or nearby streams together with wastewater. This situation will then lead to pollution load and serious environmental problems. 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 examples of biofuel that has been found to be well suited as it is clean and has a high calorific value fuel.

In the last few years, there are increasing interest in searching a new hydrogen production processes with almost no carbon emission (Balat, 2009; Holladay *et al.*, 2009; Moriaty and Honnery, 2009). Hydrogen is expected to become one of the major sources of energy to replace current energy based fossil in the future. As Malaysia sago palm industry produces large amount of sago pith residue every day, there has been greater interest in utilizing of sago residue to production of biofuel such as hydrogen. ✳

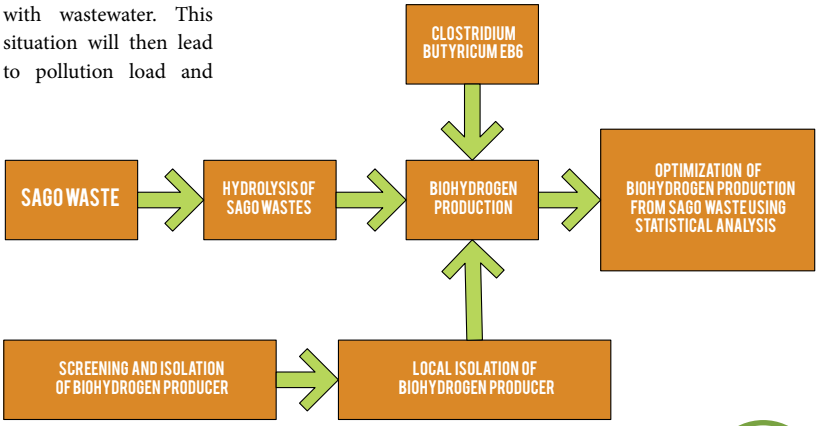
[SUPERVISOR]

Professor Dr Suraini Abd. Aziz

RESEARCH OBJECTIVES

To select suitable sago waste for biohydrogen production by local isolate

To optimize the production of biohydrogen from sago waste using statistical analysis



▸ Experimental design for biohydrogen production from sago waste by local isolate2

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BIOBUTANOL

PRODUCTION OF ACETONE-BUTANOL-ETHANOL (ABE) FROM SAGO PITH RESIDUES

The agricultural industry produces a significant amount of post-processing waste and residue. Particularly in Sarawak, sago starch industry is responsible for the production of a notable amount of residue. Sago pith residue is an abundant lignocellulosic residue that left behind after starch extraction process and contains significant amount of starch (58%), cellulose (23%), hemicelluloses (9.2%) and lignin (3.9%). This residue has a great potential as economical substrate for the production of acetone-butanol-ethanol as an alternative fuel due to their high content of cellulose and hemicellulose.

Biobutanol can be produced fermentatively from lignocellulosic residues through ABE fermentation by clostridia species. It is very energy efficient and suitable for replacing petrol as fuel in gasoline engines. In conversion of lignocellulosic biomass to biofuel, the sago pith residue needs to be treated so that the cellulose and hemicellulose in the plant fibers is exposed and more accessible to hydrolyze into simple hexose and pentose sugars. In this study, dried sago pith residue or sago hampas was ground before undergo enzymatic hydrolysis using dextrozyme. The solid part (cellulose) was separated from hydrolyzed sago hampas solution through filtration. The cellulose obtained was used as a substrate in this study. This cellulose was directly converted to fermentable sugars using cellulases produced by locally isolated fungus namely *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2. Different parameters such as substrate concentration, temperature, pH and enzyme concentration was studied to optimize the production of fermentable sugars. The fermentable sugars obtained were utilized as a carbon source for *Clostridium acetobutylicum* ATCC 824 in acetone-butanol-ethanol fermentation. The total of acetone-butanol-ethanol produced was 8.84 g/L and biobutanol of 5.41 g/L was achieved after 72 h of fermentation. ∗

RESEARCH OBJECTIVES

To produce fermentable sugars from sago pith residue utilizing local microbial enzymes

To produce acetone-butanol-ethanol from sago pith residues hydrolysate using *Clostridium acetobutylicum* ATCC 824

[SUPERVISOR]
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PRODUCTION OF ACETONE-BUTANOL-ETHANOL FROM OIL PALM EMPTY FRUIT BUNCH

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 OPEFB contents high amount of carbon source in the form of cellulose and hemicellulose, a polymer structure made by sugar monomer. 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 treated OPEFB as substrate for subsequently used in the bioconversion of OPEFB into sugar. The sugar obtain was further used for the production of ABE by *Clostridium* sp. in anaerobic fermentation system. The aim of this study are to produce crude cellulase cocktail from OPEFB by *T. asperellum* UPM1 and *A. fumigatus* UPM2 for the production of fermentable sugar and to obtain ABE from fermentable sugar produced by *Clostridium* sp. ∗

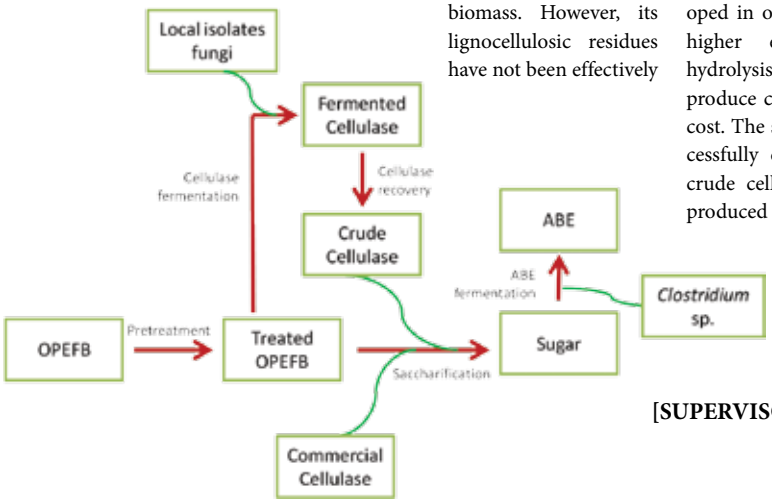
RESEARCH OBJECTIVES

To produce crude cellulase cocktail from OPEFB by *T. asperellum* UPM1 and *A. fumigatus* UPM2 for the production of fermentable sugar

To obtain ABE from fermentable sugar produced by *Clostridium* sp.

FLOW CHART DIAGRAM

► Process overview of ABE production from OPEFB



[SUPERVISOR] Professor Dr Suraini Abd. Aziz

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PRODUCTION OF BIOBUTANOL FROM OIL PALM DECANTER CAKE

The palm oil sector generates a largest amount of biomass, estimated at 80 million dry tonnes in 2010. In National Biomass Strategy 2020, government is focusing on oil palm biomass utilization to increase the Gross National Income (GNI) up to RM 30 billion by 2020. Parallel to the initiative, production of crude cellulase cocktail, polyose and butanol from oil palm decanter cake (OPDC) is a great effort to support the National Biomass Strategy. Oil palm decanter cake is a oil palm biomass which produced by 3-phase decanter system. In the system decanter system liquid from sand cyclone and the condensate of crude palm oil (CPO) vacuum were mixed together in decanter system and produce oil, oil palm decanter cake and liquid sludge.

Production of crude cellulases from oil palm decanter cake by locally isolated fungus which are *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2 studied prior the saccharification process. Pretreatment of the substrate is essential to remove the protective layer of lignin which covers the cellulose and hemicellulose structure. This barrier reduced the efficiency of cellulase to hydrolyze the cellulose. Oil palm decanter cake contains high percentage of impurities such as lignin, ash and sand. The effectiveness of various chemical and physical pretreatment (NaOH, HCl and HNO_3) to alter lignin content and increase the percentage of cellulose was studied. The effect of pretreatment to the lignocellulose structure was observed by scanning electron microscope (SEM). The treated biomass will proceed to saccharification process by commercial enzyme and crude cellulase.

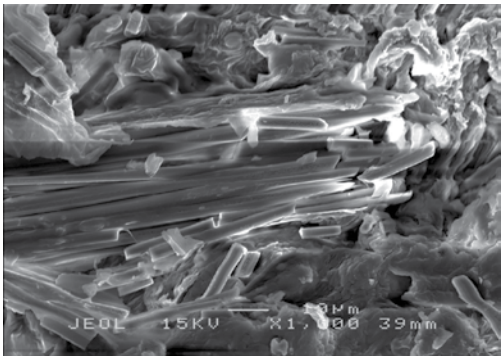
Trichoderma sp. produced high amount of exoglucanase and endoglucanase but lower in β-glucosidase activity. *Aspergillus* sp. produced high activities of β-glucosidase can be mix to the cellulase produced from *Trichoderma* sp. and produce crude cellulase cocktail. The suitable ratios of both cellulases make the optimum activities of exoglucanase, endoglucanase and β-glucosidase will be studied to increase the efficiency of hydrolysis process.

Biobutanol has superior fuel properties when compared to bioethanol which become great interest to the world scientist to produce higher amount of biobutanol from biomass. Acetone-Butanol-Ethanol fermentation using Clostridium species is the common method for the production of biobutanol. Optimization of biobutanol production by using response surface methodology approach also will be studied. The parameters are substrate concentration, pH, temperature, nitrogen content and inoculum size. Gas chromatography used to determine the concentration of butanol produced and high performance liquid chromatography (HPLC) used to determine the concentration of sugar consumption. ∗

RESEARCH OBJECTIVES

To obtain fermentable sugar from pretreated oil palm decanter cake using enzymes cocktail for biobutanol production

To optimize production of biobutanol from oil palm decanter cake polyoses by *Clostridium acetobutylicum* ATCC824



► SEM structure pretreated OPDC

[SUPERVISOR] Professor Dr Suraini Abd. Aziz

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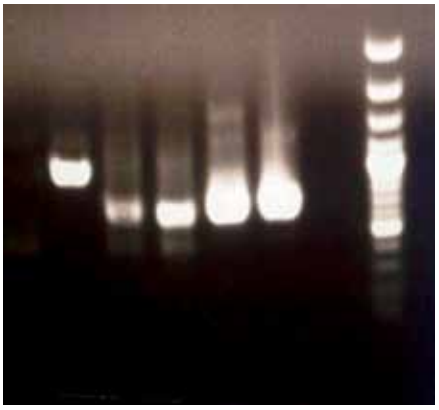
OVER PRODUCTION OF CYCLODEXTRIN GLYCOSYLTRANSFERASE (CGTASE) THROUGH MOLECULAR CLONING APPROACHES



► DNA walking results for known sequences of CGTase gene from *Bacillus* sp. NR5 UPM.

Cyclodextrin glycosyltransferase (CGTase, EC 2.4.1.19) is an important member of the amylolytic glucosylase family that catalyzes the formation of cyclodextrins (CDs) through cyclization reaction. The CGTase can be produce by several bacterial species with the major producers are belongs to *Bacillus* sp. The ability of CGTase to convert starch into CD brings a great interest to researchers. The formation of CD-inclusion complex with variety of guest molecules is advantageous as the enhancement of the physical and chemical properties of the inclusion complex formed are beneficial in biotechnology, pharmaceutical, food, cosmetic, chemical and agricultural field. However, the difficulty in producing the CD in larger scale arises as all known CGTases will produce a mixture of α-, β- and γ-CD in different ratios. Thus, complicated and tedious purification strategy is needed to purify the product. Furthermore, the low concentration of CGTase produced by wild type strain caused the major problem for CGTase production in large scale.

In this study, the CGTase-producing bacteria were successfully isolated from the soil in Malaysia. Out of 65, eleven CGTase producers has been further screened using modified Horikoshi agar type II with specific indicator. The size of halo zones formation on the indicator plate is a good qualitative measurement of CGTase producer as the biggest diameter formed is indicating the highest CGTase activity obtained. The isolates were classified using Gram staining and further identification was done at 16S rRNA level. All eleven isolates showed the characteristics of Gram positive and identified as *Bacillus* sp. The best CGTase producing bacteria, *Bacillus* sp. NR5 UPM has a probability to share the biggest market in the industrial application since it has the capability in predominantly producing β-CD as a main product and its potential in degrading the raw starch as a substrate, thus known as a raw-starch degrading enzyme producer.

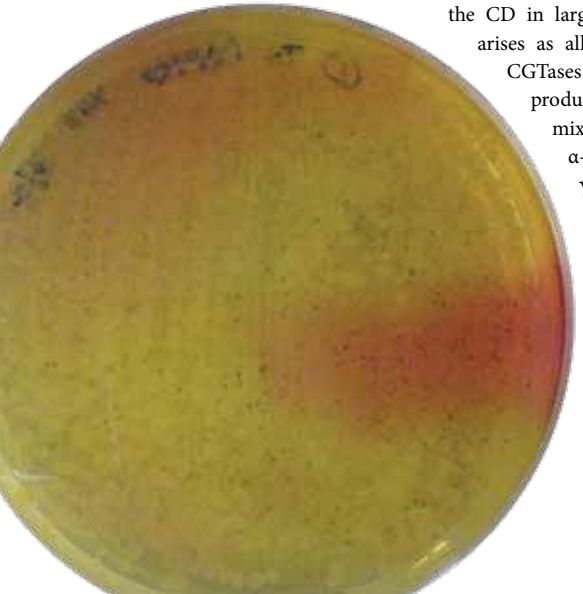
Further study on the cloning of a PCR gene fragment from *Bacillus* sp. NR5 UPM into an *E. coli* expression vector was successfully carried out. Compared to the wild type, the CGTase that was produced in E.coli cells only required one-fourth of culture time and neutral pH to produce CGTase. After 12 hours of cultivation, the CGTase activity in the culture medium reached approximately 2.5-fold higher than the CGTase from the parental strain. Interestingly, the CGTase was produced extracellularly (94%) indicating the signal peptide was functional in *E. coli*. In addition, the isolation of the promoter and transcriptional terminator from *Bacillus* sp. NR5 UPM is carried out in this study due to their important function in the transcription process. The construction of *E. coli* strain harbouring pTZCGT-BS verified the importance of the strong promoter in the expression of enzyme. The functionality of the isolated promoter in the presence of T7-vector located promoter is confirm by an increment of 3.2-fold of enzyme activity compared to the parent strain. ∗

RESEARCH OBJECTIVES

To screen, isolate and characterize CGTase producing bacteria

To isolate the CGTase genes by using primer screening technique for construction of CGTase expression system with *Escherichia coli*

To evaluate the production of enzyme by analysing the CGTase activity of the recombinant CGTases produced



► Screening of CGTase-producing bacteria with Horikoshi + phenolphthalein

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DEVELOPMENT OF A RECOVERY SYSTEM FOR BIOVANILLIN

Vanillin is one of the mostly used flavors all over the world. It is normally found in the bean of *Vanilla planifolia* (Reineccius, 2006). Presently, it is produced synthetically to meet the market demands. However, only 0.2% is extracted from beans (Priefert *et al.*, 2001). Due to these factors, extensive studies are carried out on the production of vanillin via microbial transformation from different substrates such as lignin (white rot fungi), phenolic stilbenes (*Pseudomonas paucimobilis*), ferulic acid (*Bacillus coagulans*), and eugenol (*Corynebacterium* sp.). Other than that, enzymatic routes also yield an acceptable amount of vanillin such as dioxygenase reaction on isoeugenol (Yoshimoto *et al.*, 1990), soybean lipoxygenase reaction on eugenol (Wu *et al.*, 2008) and beta-glucosidase reaction on glucovanillin (Dignum *et al.*, 2001).

The bioconversion of several substrates to vanillin normally produces relatively low yields. However, one of the mentioned substrate, ferulic acid, can be considered as the most promising substrate in the production of biovanillin. Though, it has its own drawback, in which at certain concentration of vanillin, it inhibits the enzymatic reaction as well as be oxidized further to vanillic acid (Wu *et al.*, 2008). Therefore, it is an advantage to produce biovanillin using an integrated bioprocess system which comprises of both upstream and downstream sections.

The first section of this work is biovanillin production via fermentation using *Pycnoporus cinnabarinus*. One attractive point in this research is that the substrate, ferulic acid, will be obtained from one of the most abundant by-products in Malaysia, the oil palm empty fruit bunch (OPEFB). Subsequently, the fermentation broth will undergo two different techniques in recovering biovanillin, which are cross-flow filtration and membrane-based solvent extraction. Both techniques will be evaluated mainly in term of its efficiency.

It continues with purification stage involving macroporous adsorption resin. Several resins are tested for their capabilities of adsorbing vanillin from aqueous samples. Adsorption parameters such as contact time, solute initial concentration, pH, temperature and amount of resins is screened and optimized in order to obtain the highest amount of biovanillin. Lastly, economic assessment for each separation route will be done. Separation cost and purification yield will be the pivotal points in deciding the best separation route for biovanillin. ∗

[SUPERVISOR] Professor Dr Suraini Abd. Aziz

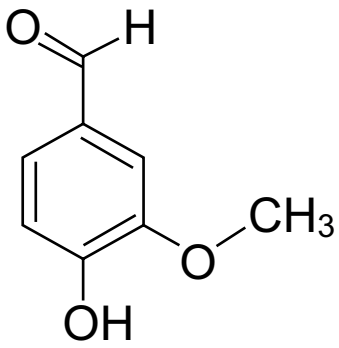
RESEARCH OBJECTIVES

To adsorb vanillin using adsorbent resin

To recover vanillin from fermentation broth via crossflow filtration

To recover vanillin from fermentation broth using membrane-based solvent extraction

To evaluate the economic feasibility for vanillin recovery from fermentation broth



► Vanillin structure

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ENZYMATIC IMPROVEMENT OF NUTRITIONAL VALUE OF BROWN RICE FOR POULTRY

The utilization of local feedstuff which is brown rice for poultry feed has been studied using few varieties. The varieties of MR239 and MR257 showed that their nutrients are not preferred for human consumption but can be obtained in high yield. The energy and nutrient composition of brown rice are similar with the maize which is the main feed used in Malaysia.

Thus, these two varieties of brown rice has been analyzed for their proximate composition to determine the protein content, fat, energy value and also the fibre content. The data obtained from varieties MR239 and MR257 has supported that local brown rice can replace maize all together or at certain rate for poultry feed. However, the presence of anti-nutrients or non-starch polysaccharides in brown rice has reduced the availability of other nutrients to be absorbed by poultry. The fibre content, cellulose, hemicellulose, arabinoxylan and beta-glucan are the components that were determined as non-starch polysaccharides in brown rice. Even though these non-starch polysaccharides presence in low percentage, a suitable treatment should be taken to remove it.

The addition of enzymes has been widely used to remove the non-starch polysaccharides and to improve the nutritional value of brown rice since poultry cannot produce endogenous enzymes to degrade these NSP. The enzymes addition can supplement or help the endogenous digestive activities of poultry, remove anti-nutritional factors and also render certain nutrients more readily available for absorption and enhance the energy value. The optimization using Response Surface Methodology (RSM) for enzymatic hydrolysis of brown rice using commercial enzymes was done in laboratory scale to produce hydrolyzed brown rice. This hydrolyzed brown rice was force f-ed to chicken to determine the true metabolisable energy (TME) value and compared it with rice without enzymes supplemented. *

RESEARCH OBJECTIVES

To determine the nutrients and anti-nutritional factors (mainly NSP) in brown rice

To optimize the enzymatic hydrolysis conditions using Response Surface Methodology (RSM) for production of hydrolyzed brown rice

To evaluate the effect of enzyme addition on poultry

► Chickens for force feeding

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BIOVANILLIN PRODUCTION FROM ALKALINE HYDROLYSATE OF OIL PALM EMPTY FRUIT BUNCH (EFB)

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. Varied 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 alkaline treated to release ferulic acid. This is because alkaline treatment is well known for its delignification efficiency and breaking up ester bonds attaching ferulic acid to lignin and hemicelluloses. A few treatment conditions are tried to achieve the best ferulic acid release from OPEFB. The alkaline hydrolysate containing ferulic acid as the precursor was fermented through two-stage fungal fermentation to produce biovanillin. The OPEFB hydrolysate was converted into vanillic acid by *Aspergillus niger* ATCC 6275 and further into biovanillin by *Phanerochaete chrysosporium*, where resin is used to trap the vanillin and avoid further conversion to vanillyl alcohol. An optimization study will also be carried out to obtain the optimal conditions of biovanillin production. The physical aspects to be optimized comprise of the following parameters; temperature, initial pH, agitation speed and initial inoculum concentration. An optimized condition of shake flask fermentation to produce biovanillin shall be achieved at the end of this study. ∗

RESEARCH OBJECTIVES

To obtain alkaline hydrolysate of oil palm empty fruit bunch containing ferulic acid

To obtain biovanillin by optimizing ferulic acid bioconversion to biovanillin through two-stage fungal fermentation using 2-level factorial design

PICTURES [FROM TOP]

- Ground oil palm empty fruit bunch
- *A. niger* ATCC 6275
- *A. niger* ATCC 6275 in alkaline hydrolysate of OPEFB
- Locally isolated *P. chrysosporium*

[SUPERVISOR] Professor Dr Suraini Abd. Aziz

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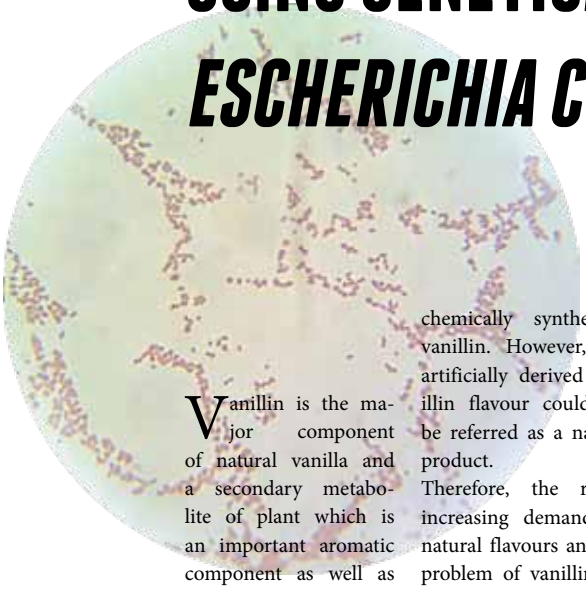
ONE-STEP BIOTRANSFORMATION OF FERULIC ACID INTO BIOVANILLIN USING GENETICALLY ENGINEERED *ESCHERICHIA COLI*



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. AZ_{10} 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. ✳

[SUPERVISOR] Professor Dr Suraini Abd. Aziz

RESEARCH OBJECTIVES

To screen, isolate and identify potential biovanillin producing bacteria

To isolate the functional genes for biotransformation of ferulic acid into biovanillin and further construct genetically engineered *E.coli*

To produce biovanillin in one step fermentation using genetically engineered *E.coli*

PICTURE

► Isolated bacteria (*Pseudomonas* sp. AZ10 UPM)

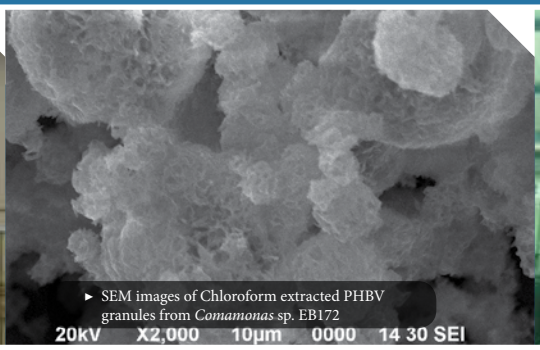
BIOMATERIAL RESEARCH GROUP

"Utilization of biomass for the production of bioplastics, biocomposites and organic acids"

■ RESEARCH SYNOPSIS

Our main research activity is to utilize biomass for the production of biomaterials such as bioplastics and biocomposites, and biobased chemicals such as organic acids and its esters. Up to date, we have successfully utilized palm oil mill effluent (POME) and municipal solid waste (MSW) fresh leachate for the production of organic acids; namely acetic, butyric, propionic and lactic acids. These organic acids can be used as the substrates to produce biodegradable plastics, *i.e.* polyhydroxyalkanoates (PHAs) and also for the production of green solvent which is ethyl lactate. Biocomposites from palm biomass are currently under development. We utilize oil palm mesocarp fiber, oil palm empty fruit bunch (EFB) and oil palm frond (OPF) petiole fiber to reinforce polymers such as polypropylene (PP) and bioplastics. We are also developing PHA blends whereby PHA is blended with other thermoplastic polymers such as polyethylene (PE) and poly(methyl methacrylate) (PMMA). Production of crotonic acid is also of our interest whereby the acid can be obtained by the pyrolysis of PHA. Apart from the development of biomaterials, our focus is also on the downstream processing whereby the environmental concern is being taken into account. We introduce an eco-friendly process for the recovery of PHA from the cells by using water as solvent, and we also introduce the chemical recycling concept for the PHA bioplastics. Recently, our group has successfully demonstrated that OPF petiole can be a good source of fermentable sugars (glucose, fructose and sucrose) whereby the sugars can be extracted from the OPF petiole by squeezing. We currently utilize the OPF juice for the production of PHA and organic acids. Due to its potential to be used as substrate for the production of bioplastics, organic acids, biofuels and biobased chemicals, the Malaysian government has included OPF juice pathway as a route to the production of biobased products, and this has been announced during the launching of National Biomass Strategy 2020, recently. ✳

► Elmy placing the sample tube in the 500-MHz JEOL JNM-ECP500 FT NMR system



► SEM images of Chloroform extracted PHBV granules from *Comamonas* sp. EB172

► Bioplastic pilot plant at SIRIM, Shah Alam

■ **RESEARCH AREAS**

STRAIN IMPROVEMENT FOR PHA PRODUCTION

Genetic engineering techniques are utilized in order to improve our locally isolated bacterium, *Comamonas* sp. EB172. We expect to develop a recombinant bacterium with higher capability of PHA production in comparison to the wild type strain.

DOWNSTREAM PROCESSING

1. PHA RECOVERY

An environmental friendly method for the recovery of PHA is used whereby the PHA is extracted from the bacterial cells with the use of non-halogenated aqueous solvents such as low concentration NaOH and water.

2. CHEMICAL RECYCLING OF PHA

Chemical recycling is aimed at converting the PHAs into monomers or low molecular weight polymers which can be reused to produce new polymer materials. So far we have tried two methods for the depolymerization of PHA, i.e pyrolysis and hydrolysis.

3. RECOVERY OF CROTONIC ACID FROM PHA-PRODUCING BACTERIA

Crotonic acid can be obtained by thermal degradation of PHA under controlled temperature and retention time. Crotonic acid and its derivatives can be used in cosmetics, plasticizers, paints, etc.

UPSTREAM PROCESSING

1. DEVELOPMENT OF BIOMASS

This includes the preparation of biomass prior to the production of biobased products, for example squeezing the OPF petiole for obtaining the sugary juice and steam treatment of palm fibers for the production of biocomposites.

2. PRODUCTION OF PHA, ORGANIC ACIDS AND ETHYL LACTATE

Fermentation is done to produce PHA and organic acids. Optimization of fermentation parameters is done in order to gain maximum yield. Ethyl lactate is produced by esterification of lactic acid with ethanol.

3. BIOCOMPOSITES AND PHA BLENDS

Biocomposites are prepared from treated palm fiber and thermoplastic polymers such as PP and PHA by melt-blending. The effect of compatibilizer on the properties of biocomposites is also studied. PHA blends are prepared by blending PHA with PE and PMMA.

► *Comamonas* EB172 culture

► Bioreactor 2 liters for production PHBV

► POME and clarified acid

BIOMATERIAL
RESEARCH GROUP

■ GROUP BIG PICTURE

PROFESSOR DR MOHD ALI HASSAN

DR HIDAYAH ARIFFIN

PRINCIPAL RESEARCHERS

SUBSTRATE

Treated POME

Kitchen Waste

Oil Palm Fronds

Syaiful Nizam

Treated POME

ACID RECOVERY

Elmy Nahida

RECYCLING OF PHA

Mohd Nor Faiz

Yee

STRAIN IMPROVEMENT

Mior Ahmad Khushairi

Cupriavidus Necator CCUG52238

PRODUCTION OF PHA

Nor Asma

PHBV RECOVERY

Mitra

Azman

MEDIA / CULTURE OPTIMIZATION

Mohd Rahimi

CROTONIC ACID PRODUCTION

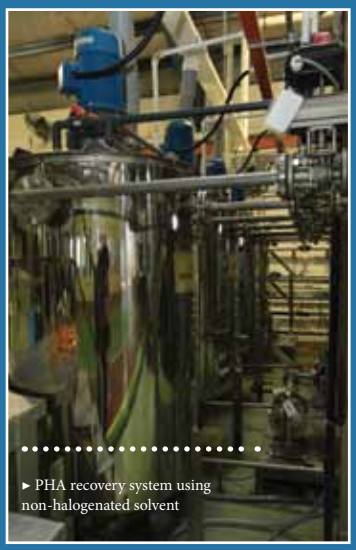
Noor Ida Amalina

BIOCOMPOSITE

Mohd Nor Faiz

THE FIRST MALAYSIAN BIOPLASTIC PILOT PLANT

The launching of the 1st Bioplastic Pilot Plant in Malaysia by the Minister of Science, Technology and Innovation in July 2011 was our biggest achievement in 2011. The pilot plant was developed under TechnoFund project, a government funded research project. Apart from UPM, other institutions involved in the research are SIRIM, USM and MIT. The research project led by SIRIM was aimed at producing PHA from palm oil and its derivatives at large scale. With the experts from the institutions involved, the team has successfully developed a pilot scale fermentation and recovery system which consists of four bioreactors, with capacity of 20, 200 (2 units) and 2000 L. The pilot plant was built under the supervision of local experts from SIRIM, using local technologies. The interesting part in this PHA pilot plant is the PHA recovery system which does not use the conventional halogenated organic solvent. Under this project, we managed to introduce an eco-friendly PHA recovery system which uses mild alkali. The pilot plant is located at SIRIM, Seksyen 15, Shah Alam. ■



► PHA recovery system using non-halogenated solvent

► Left to Right: 20, 200 and 2000L bioreactors at bioplastic pilot plant

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ANAEROBIC TREATMENT OF PALM OIL MILL EFFLUENT FOR ORGANIC ACIDS PRODUCTION IN PILOT SCALE BIOREACTOR

Malaysia is one of the largest countries that involved in palm oil industry. The total production of palm oil in Malaysia contributed 45% of the palm oil demand. With such a huge production, the palm oil industry generates large amounts of wastewater known as palm oil mill effluent (POME). POME is generated mainly from three major sources, which is sterilizer condensate, hydrocyclone waste and separator sludge. POME is thick brownish liquid which high nutrient content mainly oil and fatty acids. This nutrient content is able to support bacteria growth with the degradation of the waste to reduce its pollution strength. During the biological treatment of POME, by products recovered during anaerobic process of POME are volatile fatty acids that mainly consist of acetic, propionic and butyric acids. These acids then will be used as a substrate for polyhydroxylakanoate (PHA) production. In this research, the organic acids are produce in pilot scale acidogenic bioreactor. Hydraulic retention time, pH and agitation speed were control in order to obtained highest production of acids. Organic acids produced then will be recovered in pilot scale rotary evaporation system. With large amount of wastewater generate in palm oil industry each year, the raw material for organic acids production will be continuously exist. PHA is a biopolymer and biodegradable thermoplastics that have been produced by various types of bacteria as carbon and energy reserve materials. The high price of PHA has limited the use of these biodegradable plastic for the time being. The production of PHA based on organic acids as carbon sources will be useful to reduce the price and depending on synthetic carbon sources only. Therefore, this study will give significant contribution to new knowledge aside improve efficiency of POME treatment, reducing the cost and enhance the environment. ✳

[SUPERVISOR] Professor Dr Mohd Ali Hassan

RESEARCH OBJECTIVES

To investigate the effect of hydraulic retention time, pH and agitation speed on organic acids production at pilot scale

To study the recovery efficiency of organic acids in pilot scale rotary evaporator system

PICTURE (LEFT)

► Pilot scale rotary evaporator (50 l)

PICTURE (BACKGROUND)

► Pilot scale acidogenic bioreactor

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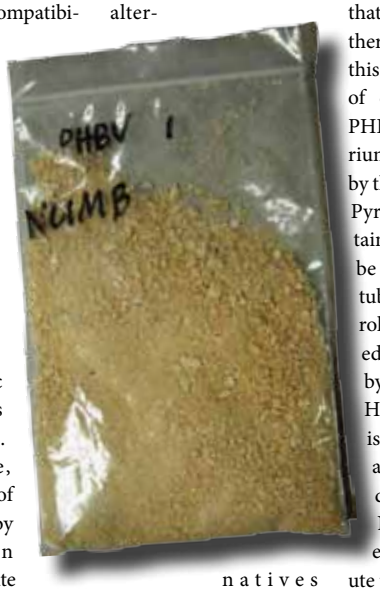
ALTERNATIVE ROUTE TO CROTONIC ACID PRODUCTION BY PYROLYSIS OF POLYHYDROXYBUTYRATE (PHB)-CONTAINING BACTERIUM

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 chemical 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 environmental pollution as it causes the formation of about one ton of highly contaminated effluent per ton of processed crotonic acid. This is accompanied by about 1500m3 of contaminated effluent per ton of crotonic acid from the drying process. Moreover, the crystallization process also causes product losses. The present proposed research provides an alternatives route to crotonic acid production which involves biological synthesis and eco-friendly method. This can be done with 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 that is crotonic acid via thermal degradation. In this research, recovery of crotonic acid from PHB- producing bacterium will be conducted by the mean of pyrolysis. Pyrolysis of PHB- containing bacterium will be conducted in a glass tube oven, and the pyrolzates will be collected and characterized by 1H-NMR, GC and HPLC. The geometric isomerism of crotonic acid produced will be determined by 1H-NMR. This study is expected to contribute to the proposal of alternative route for croytonic acid production which is more environmental friendly and involves less production steps. ✳



[SUPERVISOR] Dr Hidayah Ariffin

RESEARCH OBJECTIVES

To propose an alternative method for crotonic acid production via pyrolysis of polyhydroxybutyrate (PHB)-containing bacterium

To optimize pyrolysis conditions for enhanced recovery of crotonic acid from the PHB-containing bacterium

To analyze, quantify and determine the purity and geometric isomerism of the crotonic acidproduced from pyrolysis

PICTURE (LEFT)
► Freeze-dried cell containing PHB

PICTURE (ABOVE)
► Glass tube oven for pyrolysis experiment

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BIOMATERIAL

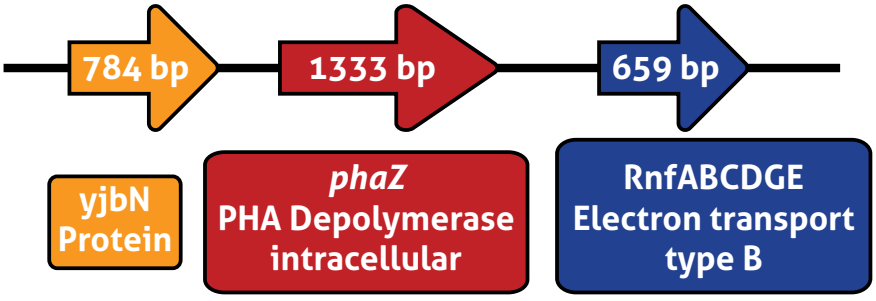
PRODUCTION OF POLYHYDROXYALKANOATES BY GENETIC ENGINEERED *ESCHERICHIA COLI* JM109

Comamonas sp. EB 172 has been identified as a novel bacterium that is able to convert organic acids derived from palm oil mill effluent, into bioplastics. In my study, PHA depolymerisation gene (*pha*Z_{Co} gene, 1333 bp) and biosynthesis operon of *Comamonas* sp. EB172 consisting of three genes encoding acetyl-CoA acetyltransferase (*pha*A_{Co} gene, 1182 bp), acetoacetyl-CoA reductase (*pha*B_{Co} gene, 738 bp) and PHA synthase, class I (*pha*C_{Co} gene, 1694 bp) were identified. The nucleotide sequences of *pha*Z_{Co}, *pha*C_{Co}, *pha*A_{Co} and *pha*B_{Co} reported here were deposited in the GenBank online database under accession numbers HM853676, JF773394, HQ650140 and HQ650141, respectively. Sequence analysis of the *pha*A_{Co}, *pha*B_{Co} and *pha*C_{Co} genes revealed that they shared more than 85%, 89% and 69% identity, respectively, with orthologs from *Delftia acidovorans* SPH-1 and *Acidovorax ebreus* TPSY. The *pha*C_{Co} gene encoded a protein of 564 amino acids, and the calculated molecular mass was 62.94 kDa. The *pha*A_{Co} gene encoded a protein of 394 amino acids with a calculated molecular mass of 40.73 kDa, while the *pha*B_{Co} gene encoded a protein of 246 amino acids with a calculated molecular mass of 26.27 kDa.

The PHA biosynthesis genes (*pha*C_{Co} and *pha*AB_{Co}) were successfully cloned in a heterologous host, *Escherichia coli* JM109. *E. coli* JM109 transformants harbouring pGEM'-pha$C_{Co}AB_{Re}$ and pGEM'-pha$C_{Re}AB_{Co}$ were shown to be functionally active synthesizing 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 (*pha*CAB_{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 *pha*CAB_{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.

Unlike shake flask fermentation, pH was maintained using NaOH (1M) and DO level can be maintained at 30% using agitation for the best growth and accumulation in batch fermentation using 2 L bioreactor. The higher concentration of glucose (20 g/L) had increased the maximum cell concentration of the recombinant *E. coli*. However, the PHA yield based on the substrate utilised coefficient (g PHA/g substrate) and PHA biosynthesis per cell (g PHA/g cell) were similar using lower amount of glucose. The increasing of carbon sources had increased the productivity of the PHA. ∗

[SUPERVISOR] Professor Dr Mohd Ali Hassan



▸ Organization of *Comamonas* sp. EB172 PHA depolymerase gene

RESEARCH OBJECTIVES

To determine the PHA biosynthesis genes from *Comamonas* sp. EB172

To study heterologous expression of *pha*AB and *pha*C genes from *Comamonas* sp. EB172 in *Escherichia coli* JM109

To determine PHA production by using constructed recombinant *Escherichia coli* JM109

BIOMATERIAL

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PRODUCTION OF POLY(3-HYDROXYBUTYRATE) FROM OIL PALM FROND

Poly(3-hydroxybutyrate) (PHB) is a biodegradable thermoplastic polyester accumulated intracellularly by many microorganisms under unfavorable growth conditions. In PHB 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 PHB. Palm oil industry in Malaysia has contributed about 52% of the total world oils and fats export in year 2006. Apart of 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. Based on these findings, an attempt has been made to ferment sap from oil palm biomass to produce PHB by using bacteria.

This research will be divided into three different stages which are extraction of sugar from oil palm fronds (OPF), followed by, PHB production through fermentation in bioreactor and finally, extraction, purification and characterization of PHB from the cell. Prior to fermentation, juice from OPF will be extracted by using simple physical separation method. At this stage, characterizations of OPF juice will also been carried out. These were including sugar composition, proximate analysis, and so forth. Further pretreatment by centrifugation, membrane filtration and sterilization will be employed to optimize the fermentable sugars production.

The next step of this study will be bioplastic fermentation, the stage in conversion of fermentable sugar to PHB by wild type of bacteria *C. necator* CCUG52238, and the mutant of *C. necator* NCIMB11599. During fermentation, several experiments will be carried out to optimize the PHB production in shake flasks. These were include; effect of substrate concentration, effect of temperature, effect of agitation and effect of initial pH will be observed by 500ml flasks as the fermentation system. During optimization in shake flasks, the profile of various physical parameters such as cell dry weight (CDW), PHB concentration and PHB content will also being studied. After parameters in the fermentation process have been optimized, the 2-L bioreactor system will be used as a tool to observe the potential of scaling up to 50-L bioreactor of the bioplastic production from oil palm biomass. At this stage, several parameters such as stirring speed, aeration, pH, C/N ratio and temperature will be optimized to increase CDW and PHB yield. The conventional method of extracting PHB from fermentation broth by chloroform and evaporation will then be applied to observe the PHB characteristic produced from oil palm biomass. ∗

▸ Poly(3-nydroxybutyrate) from oil palm frond juice

RESEARCH OBJECTIVES

To determine the appropriate sugar extraction and pretreatment method from oil palm biomass

To optimize poly(3-hydroxybutyrate) [P(3HB)] production by using sugar from oil palm biomass in shake flasks

To optimize the production of P(3HB) in 2-L bioreactor

To scale-up the fermentation process from 2L to 50L bioreactor and to optimize the process with respect to the process parameters

[SUPERVISOR] Professor Dr Mohd Ali Hassan

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OPTIMIZATION OF POLYHYDROXYALKANOATES PRODUCTION BY AN ACID-TOLERANT BACTERIUM, *COMAMONAS* SP. EB 172 USING RESPONSE SURFACE METHODOLOGY

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 °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). 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 $(NH_4)_2SO_4$ and concentration of mixed organic acids were determined at 30°C, 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 inhibited 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. ✳

► Fermentation in bioreactor for PHA production

RESEARCH OBJECTIVES

Optimization for production of Polyhydroxyalkanoate (PHA) by *Comamonas* EB172 using Response Surface Methodology (RSM)

Develop a simple kinetic model for production of PHA in 2 liters bioreactor

[SUPERVISOR] Professor Dr Mohd Ali Hassan

BIOMATERIAL

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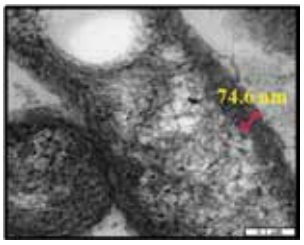
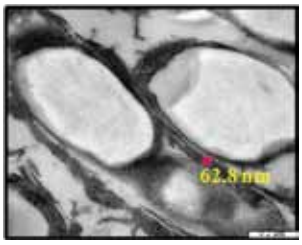
ALTERNATIVE RECOVERY METHODS OF INTRACELLULAR POLYHYDROXYALKANOATES FROM LOCAL ISOLATE *COMAMONAS* SP. EB172



Comamonas sp. EB172



Recombinant *Cupriavidus necator*

► Thickness of bacteria cell wall

RESEARCH OBJECTIVES

To evaluate the use of NaOH treatment for PHA recovery from local isolate *Comamonas* sp. EB172

To evaluate the use of chemical-free aqueous solution method for PHA recovery from local isolate *Comamonas* sp. EB172

To characterize the PHA recovered from local isolate *Comamonas* sp. EB172 using NaOH treatment and chemical-free aqueous solution method

In this study, the use of NaOH treatment and chemical-free aqueous solution for intracellular polyhydroxyalkanoates (PHAs) recovery from local isolate Gram-negative wild type bacteria *Comamonas* sp. EB172 at different NaOH concentration, treatment time, temperature and agitation is being evaluated as alternative and appropriate methods instead of halogenated solvent extraction system. These methods consist of recovery steps followed by incubation, centrifugation and purification steps using ethanol and water washing. The results of this study were compared with PHA extraction from recombinant *Cupriavidus necator* as control. The PHA recovered under the most favourable conditions was further characterized.

The chemical-free aqueous solution method as a clean process and low concentration of NaOH treatment was successfully developed for PHA extraction from wild type *Comamonas* sp. EB172. More than 88% purity and 96% recovery yield of PHA were achieved by incubating the wild type bacteria under the mild alkaline treatment using 0.05 M NaOH at 4°C for 1 h. The incubation of *Comamonas* sp. EB172 in the chemical-free aqueous solution at 30°C for 5 h could efficiently extract the PHA with more than 93% of recovery yield. Protein determination and transmission electron microscopy images as well as gas chromatography analysis proved that improvement in cell wall permeability and cell membrane breakage are the possible mechanisms of NaOH treatment and chemical-free aqueous solution method on PHA recovery which was accompanied by considerable release of protein after the extraction step. It was found that the effectiveness of chemical and non-chemical treatments depends on the microbial strain which might be due to the difference in the thickness of their cell membrane. The initial intracellular PHA content also affected the effectiveness of the extraction methods for PHA recovery. Although, the NaOH treatment could recover purer PHA as compared to the chemical-free method, both processes were able to extract the polymer with high yield. The chemical-free aqueous solution method was found to be better than NaOH treatment for PHA extraction in respect of the final polymer molecular weight, which is in fact almost double that of the chloroform-extracted PHA as control.

The overall results in this study indicated that the mild NaOH treatment and the chemical-free aqueous solution methods developed can serve as alternative recovery methods with high potential instead of the conventional halogenated solvent extraction process such as chloroform, since these new methods are environmentally more benign, effective and simple in operation. The recovery of intracellular PHA from *Comamonas* sp. EB172 cells via the recovery methods developed herein can contribute towards the sustainable process of PHA production using organic acids derived from the anaerobic treatment of palm oil mill effluent (POME). ∗

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BIOMATERIAL

IMPROVED RECOVERY AND PURIFICATION OF PHA (POLYHYDROXYALKANOATES) FROM RENEWABLE RESOURCES

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 carbon-based 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 damage 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). ∗

RESEARCH OBJECTIVES

To develop an alternative, environmentalfriendly recovery process using chloroform, sodium hydroxide and enzyme

To characterize the recovered PHA

To develop mass and energy balance for the recovery process

PICTURE

▸ Sodium hydroxide extraction

[SUPERVISOR] Professor Dr Mohd Ali Hassan

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CONTROLLED DEGRADATION OF POLYHYDROXYALKANOATES BY STEAM HYDROLYSIS FOR CHEMICAL RECYCLING

Polyhydroxyalkanoate (PHAs) 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. Dawes (1986) in his monograph emphasized that this compound acts as a reserve material and intermolecular molecule that is highly reduced and able to exert negligible osmotic pressure. 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 (Chen and Wu, 2005). It is a good alternative to plastic petroleum which is non-biodegradable and currently depleting. This biodegradable carbon reserve plays important role in the environmental carbon storage. Single use of bioplastics does not support the sustainability of the carbon cycle, therefore chemical recycling of bioplastic is proposed. Chemical recycling is a process to depolymerize polymers to low-molecular-weight materials before repolymerizing it into new polymers. By chemical recycling, cascade utilization of polymers could be introduced before they are finally being released to the environment. Several methods have been used to depolymerize PHAs, namely pyrolysis and hydrolysis. Pyrolysis and enzymatic hydrolysis of PHAs have been extensively studied; however steam hydrolysis of PHA is yet to be studied. Degradation of PHA in this study, involved with the concept of the material conversion to molecules that built up the original material or lowering of its origin molecular weight (Ariffin *et al.*, 2010) and (Ebdon and Eastmond, 1995). This research is aimed at recovering low-molecular weight polymers with hydroxyl and carboxyl chain-ends from polyhydroxyalkanoates (PHAs) by high-pressure steam (HPS) hydrolysis. These low molecular weight polymers can be used as feedstock for repolymerization and other applications. Degradation of PHAs by HPS hydrolysis will be controlled by several parameters, namely; temperature, pressure and retention time. The experiments will be conducted using autoclave as the reactor. The hydrolysis products will be characterized and the effects of the 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 degradation is projected theoretically to follow autocatalytic hydrolysis mechanism with the identification of critical point, rate constant and activation energy based on relative molecular weight of polystyrene as the standard. The hydrolyzates will be characterized by using ^{1}H and ^{13}C NMR, FTIR and DSC. Mass balance for the PHAs hydrolysis will also be studied. At the end of this study, it is expected that the degradation mechanisms for PHAs hydrolysis can be proposed, with the selective formation of targeted products for chemical recycling. ✳

[SUPERVISOR] Dr Hidayah Ariffin

BACKGROUND PICTURE

▸ Prepared hydrolyzate samples in NMR tubes for proton NMR analysis

RESEARCH OBJECTIVES

To identify the potential of PHA chemical recycling by steam hydrolysis and to characterize the hydrolysis products

To investigate the effects of high pressure and super heated steam hydrolysis parameters on the molecular weight of PHB and its characteristics

To identify the degradation behavior of PHA steam hydrolysis and to propose the mechanisms involved in steam hydrolysis of PHA

To prepare and characterize a product produced from recycled PHAs for slow release mechanism

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ENHANCEMENT OF FERMENTABLE SUGARS RECOVERY FROM OIL PALM FROND PETIOLE

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. Years back, the residues simply discarded and burnt due to the cost burden. Later, ongoing research found that the OPF juice contains high fermentation sugars such as glucose, sucrose and fructose that can be used for another rising industries. Therefore, this research study is proposed to optimize the sugars recovery during juice extraction with the means of enzymatic, mechanical pre-treatments and thermal treatment. Storage effect, prior to juice extraction will also be studied in this case. Enzymes are introduced to alter the structure of OPF petiole for better extraction. In this study, two enzymes are applied which are cellulase and pectinase. The use of the enzymes is expected to release more sugar yield better besides to get a clearer juice. Thermal treatment is done to liberate remaining sugars from the pressed fibre. Pressed fibre will be soaked in 70 °C hot water and pressed again after the first press. The mechanical pre-treatment proposed to remove the skin of OPF as the presence of the skin cause difficulties in pressing the OPF. De-skinning of OPF may aid to increase the juice release from the frond compared to without de-skin treatment. The skin of fronds actually is really tough and the presence might limit the moisture escape when pressing is done. De-skin also aid in the convenience of pressing system since the hard part has been removed. Storage of OPF prior to extraction may regulate more free sugars besides add on effect on the moisture content inside the OPF. Giving the storage period for less than 48 hours and were sampled every 6 hours, the free sugars will be analyzed and determined to see the best peak of free sugars generated. Any period longer than 48 hours will wither the frond making it hard to press besides lesser moisture will be retained. The longer of period storage will also leave the OPF prone to microbial attack which leads to dramatic decrease of sugar content. Free sugars from OPF are promising prospects for the development of fermentation industry. The reliance on this alternative source of raw material not only will be economical but contribute to the efficient management of agricultural residues in the country. Usage of OPF as sugar alternative to the fermentation definitely would not conflict to the food consumption if relatively compared to the sugar from sugar cane and any other sugar producing food plant. This study is hoped to contribute to a better OPF sugar recovery with through the convenient steps proposed. ✻

RESEARCH OBJECTIVES

To pre-treat the OPF petiole prior to juice extraction in order to improve recovery yield

To study the effect of OPF petiole storage period on juice recovery

[SUPERVISOR] Dr Hidayah Ariffin

◄ Oil Palm Frond Petiole

▲ Oil Palm Frond

Pressing Machine, OPF Juice and OPF Fibre ►

BIOMATERIAL

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IMPROVED MECHANICAL AND THERMAL PROPERTIES OF POLYHYDROXYALKANOTES BY BLENDNG WITH OTHER POLYMERS


> Hydraulic Hot Press Machine


> Instron Mechanical Analyzer


> Thermo Haake Mixer

Several excellent research done by the scientist on the bioplastics has been considered as the solution for the serious environmental problem. Good strength, low production cost, and good resistant towards chemical and biological attack are the important things need to be considered to produce bioplastics. Poly-hydroxyalkanoate (PHA) is a biodegradable polymer with some advantages of excellent biodegradability with high crystalline and good thermal properties, however it is not practical to be applied as commodity plastics due to its brittleness and narrow processing window. Blending of PHA with other polymers may enhance the mechanical and thermal properties of this biopolymer. In this study, polymers such as poly (methyl methacrylate) (PMMA), polyethylene (PE), and natural fiber is used to enhance the mechanical and thermal properties of the blends. Mesocarp fiber is selected as the natural fiber source. To the best of our knowledge, there is no research done so far on the blending of PHA with mesocarp fiber for the production of biocomposite. Several polymeric blend ratios (0-50%) will be prepared using Thermo Haake mixer and hydraulic hot press machine. The effects of plasticizer and compatibilizer incorporation in the polymer blends will be studied. Maleic anhydride and silane coupling agent will be used in this research as the compatibilizers while poly ethylene glycol (PEG) will be used as plasticizer. The miscibility of the polymer blends will be determined morphologically by using scanning electron microscopy (SEM). Besides that, thermal properties of the polymer blends will be tested by thermogravimetry (TG), differential scanning calorimetry (DSC) and thermo mechanical analysis (TMA). Mechanical properties such as, tensile strength, Young's modulus and elongation at break of the polymer blends will be determined by using Instron Mechanical analyzer. The polymer blends are expected to exhibit better thermal and mechanical properties compared to the homopolymer of PHA. It is also expected, that these polymer blends can be separated by pyrolysis after its use. In order to study the separation, thermal degradation of these polymer blends will be conducted by using glass tube oven and the pyrolyzates and result will be analysed by Nuclear Magnetic Resonance (NMR). It is hoped that the application of bioplastics and biocomposite can be extended for commodity use. *

[SUPERVISOR] Dr Hidayah Ariffin

RESEARCH OBJECTIVES

To improve the thermal and mechanical properties of PHA by blending with PMMA, PE and mesocarp fiber and to propose the applications of PHA blends based on their properties

To determine the effect of compatibilizer on the thermal and mechanical properties of PHA blends

To study on the thermal degradability of the PHA blends

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UTILIZATION OF OIL PALM MESOCARP FIBER FOR BIOCOMPOSITE

RESEARCH OBJECTIVES

To clarify effects of high pressure and super heated steam treatments on the characteristics of mesocarp fibers

To prepare and characterize biocomposites derived from steam treated mesocarp fiber and polypropylene

Depolymerization of biocomposite via thermal degradation method

Malaysia is blessed with fertile soil and abundant natural resources. Malaysia also is one of major producer in palm oil industries. According to USDA (2007), Malaysia and Indonesia are major contributor which is about 87% of world palm oil production. Despite of the economic contribution, palm oil industries generate huge amount of solid waste and effluent. In 2006, about 17.4 million tonnes empty fruit bunch, 10.7 million tonnes of mesocarp fiber, 4.3 million tonnes palm kernel shell and 53.1 million tonnes of palm oil mill effluent are generated by oil palm industries.

Oil palm biomass appears to be a very promising alternative as a source of raw materials in Malaysia. In recent years, there has been an expanding search for new materials with high performance at affordable cost and increased attention has been paid to the use of natural polymers also lignocellulosic fibers.

This study will focused on oil palm mesocarp fiber to being utilize as value added product which is biocomposites. Mesocarp is the fibrous mass left behind after oils extraction. Currently, mesocarp is burned as fuel to generate energy for mills. However, this practice creates another environmental problem due to incineration and emitted gas. Biocomposites are defined as the materials made by combining natural fiber and petroleum derived non-biodegradable polymer or biodegradable polymer. Natural fiber as filler in polymeric matrix offers several advantages such as high bending strength, good thermal and insulation properties, lower density, non-toxic, ease of recycling, flexible usage and lower cost. Mesocarp component consists of 31.9% cellulose, 35.7% hemicellulose and 32.4% lignin which →

> Hot pressed samples for tensile test

> Injection moulding samples for bending test

PICTURE (TOP)

► Biocomposites samples prepared for tensile and bending test

PICTURE (BACKGROUND)

► Abundant of mesocarp available in palm oil mill

[SUPERVISOR] Professor Dr Mohd Ali Hassan

make it a potential material for biocomposite. Only the drawbacks of natural filler are weak interaction between filler and polymer matrix and filler dispersion due to different properties of natural fibers and polymer. Natural fiber holds hydrophilic characteristic due to presence of hydroxyl group resulting poor interfacial adhesion with hydrophobic polymer matrix. In the same time also, aggregation of fiber easily can occur. Natural fibers also have high capability of water sorption due to cellulose, hemicellulose and lignin components. The hydroxyls group which presence in the fibers components leading to formation of hydrogen bonds with water. This may lead to poor physical and mechanical properties of the composite. By solving the mentioned drawback, good thermal and mechanical properties of the biocomposite can be produced.

Steam treatment will be used to improve the characteristic of the fiber in term of stability, composition, and provide a stable bond between the fiber and the polymer matrix. It is expected that the treatment can enhance the interfacial bonding strength of the composite. Two approaches will be conducted which are high pressure steam and super heated steam. The optimum treatment time, temperature and pressure will be determined. Steam treated mesocarp fibers have very low moisture content which makes grinding and mixing processes easier. By removing moisture content, it is expected that hydroxyls group is removed and promote better characteristic of mesocarp fibers. The treated sample will be grinded and sieve to desire particle size. Effect of fiber size, fiber homogeneity, fiber ratio towards thermal, mechanical and morphology properties of biocomposite will be studied. In this study, polypropylene (PP) is used to mix with untreated and steam treated mesocarp fiber. Finally, the characteristic of the biocomposites will be analysed using TGA, DSC, tensile and flexural strength and morphology by SEM. ⁎

► Cellulose mesocarp fiber

BIOFERTILIZER
RESEARCH GROUP

■ RESEARCH SYNOPSIS

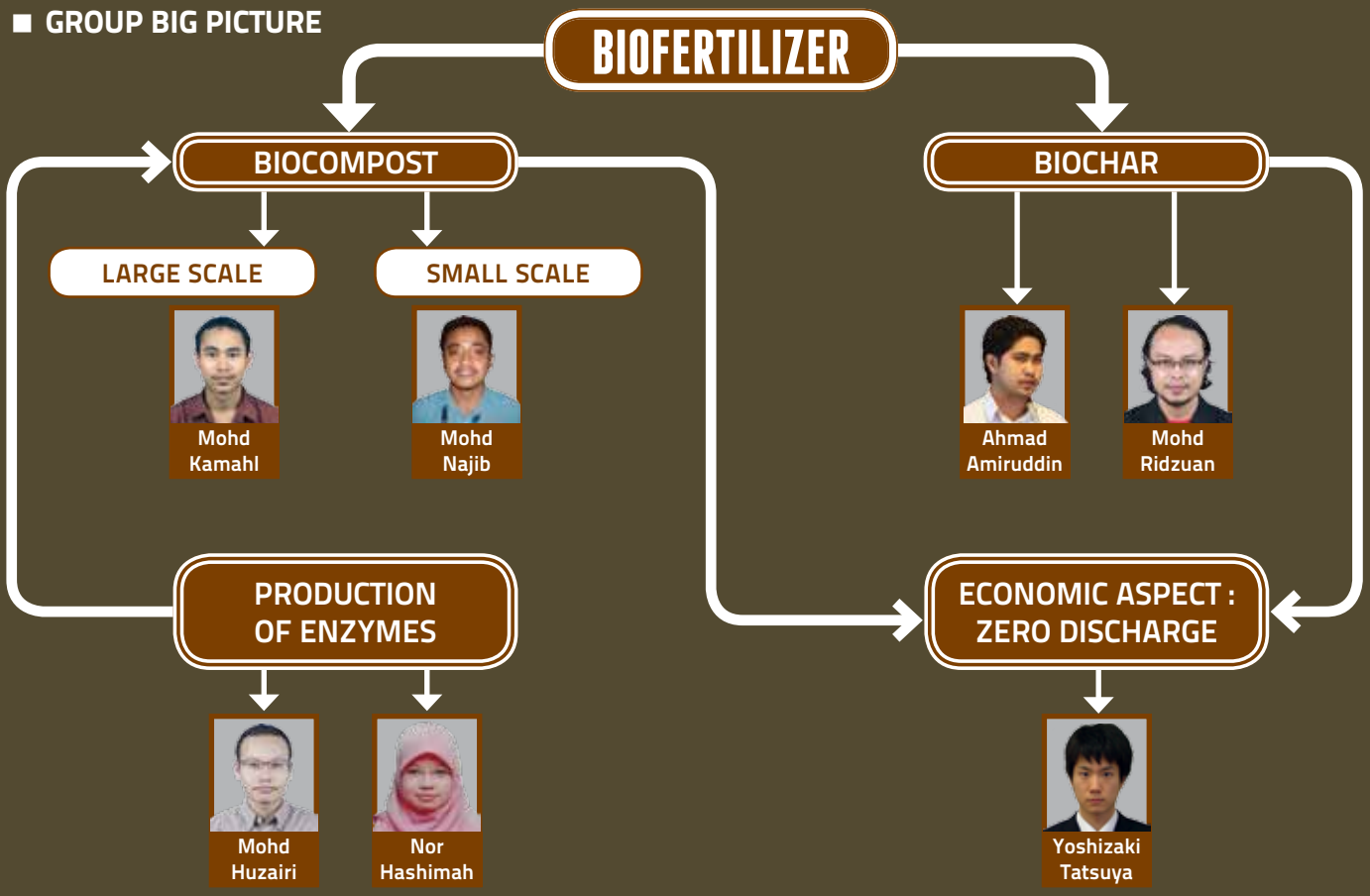
The Biofertilizer Research Group is leading in advanced composting process and waste recycling technology for agriculture purposes and wastewater treatment. The group main focus is on effective organic wastes recycling technology to convert organic waste into organic fertilizer. Currently, the researches on oil palm biomass composting and pyrolisis process are conducted in the group under the collaboration between UPM and KIT. The economic feasibility analysis also been conducted for both researches to promote zero discharge in oil palm industry. ⁎

■ PRINCIPAL RESEARCHER

PROFESSOR DR MOHD ALI HASSAN

DR AZHARI SAMSU BAHARUDDIN

■ GROUP BIG PICTURE



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ISOLATION AND CHARACTERIZATION OF CELLULASE AND XYLANASE FROM OIL PALM EMPTY FRUIT BUNCH COMPOST

RESEARCH OBJECTIVES

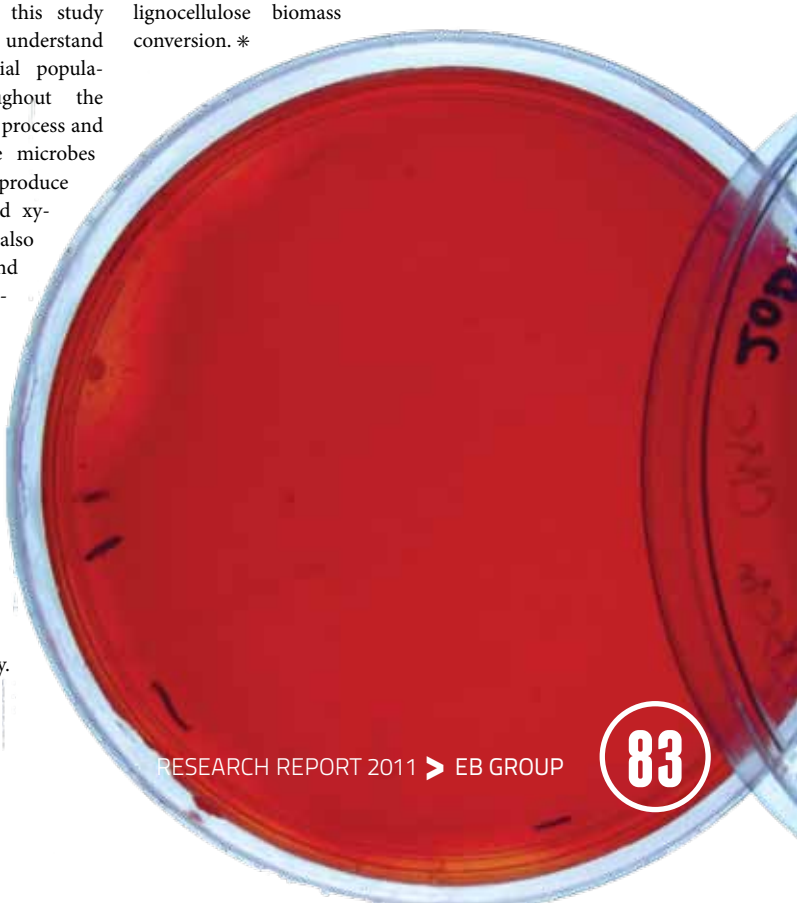
To investigate the microbial community during the oil palm empty fruit bunch compost process

To isolate and characterize the expressed cellulase and xylanase from isolated microbes

To screen and identify cellulase and xylanase gene through metagenomic approach

Malaysia has become the largest oil palm producer with the production of about 18 million tonnes 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 create value added product from these waste are through the compositing using EFB with anaerobic sludge POME. EFB compost is manageable product which can be use as soil amendment and organic fertilizer. In composting, lignocellulose material breaks down due to the existence of aerobic thermophilic bacteria. The microbial population and microbes capable of producing cellulase and xylanase was investigated. In this study, DNA was extracted and purified from EFB compost by DNA soil extraction kit. The isolated DNA was used for determining the microbial population through microbiota analysis by using culture-independent of 16s rRNA gene amplified directly from the compost. 1500-bp 16S rRNA PCR products were cloned and sequenced.

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 able to express cellulase and xylanase have been isolated. In order to identify potential cellulase and xylanase enzyme from nonculturable microbes, metagenomic of EFB compost was done. Metagenome involves the extraction of metagenomic DNA from EFB compost. The DNA is subsequently cloned into vector (fosmid) to construct the metagenomic libarary. From the metagenomic library, the genes encoding cellulase and xylanase were screened 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 can produce 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. ✳



▸ Metagenomic library of EFB compost for cellulase

[SUPERVISOR] Professor Dr Mohd Ali Hassan

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BIOFERTILIZER

ENHANCED BIOMETHANE PRODUCTION THROUGH OIL PALM BIOMASS UTILIZATION

The palm oil industry represents the largest agro-economic sector in Malaysia. However as the demand for oil palm products increase globally, more attention is being paid to the impact of huge wastes generated from the industry such as palm oil mill effluent (POME), oil palm empty fruit bunch (EFB), mesocarp fiber and oil palm fronds (OPF) towards the environment. In the conventional system, POME is treated using expansive open lagoons and/or tanks prior to discharge to the river or watercourse. The solid wastes on the other hand are being utilized as boiler fuel, mulching agent in the oil palm plantations or incinerated. Hence this study aims to utilize these oil palm biomass or residues specifically POME and EFB, which are the largest by-products of the palm oil extraction process to enhance biomethane production.

Biomethane can be converted into electricity via gas engines and utilized by the mill as its source of energy rather than using diesel as in the current practice. This will free up high calorific value biomass such as shells which are typically used as fuel source to be sold to other parties. In addition, any surplus electricity may be connected to the grid for external use and generate additional income to the mill and industry in general.

The first and second objectives of the study focus on the method for improving methane production and yield in both batch and continuous anaerobic fermentation systems of POME through utilization of added oil palm biomass.

The third objective involves the study and identification of microbes and enzymes involved in the fermentation using Scanning Electron Microscope (SEM), Denaturing Gradient Gel Electrophoresis (DGGE), Fluorescent *In-Situ* Hybridization (FISH) and microbiota analyses.

From the research, it is envisaged that better utilization of biomass resources can be achieved in the mill. In terms of potential, mills could be self-sufficient in terms of energy, capable of providing surplus electricity to the grid, as well as having unused and high value biomass such as shells available for sale. All this translates into extra revenue while simultaneously enhancing the image of the palm oil industry through more environmentally sustainable practices. ✳

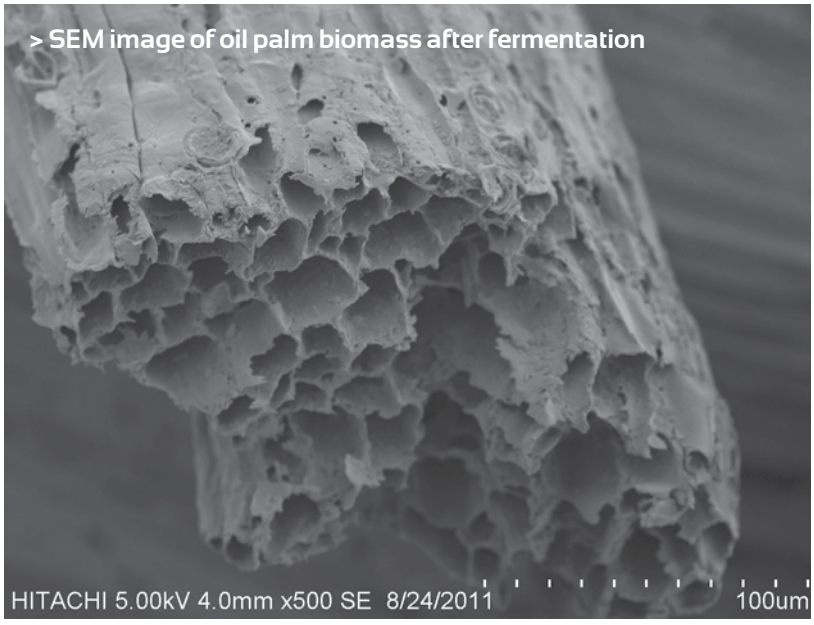
RESEARCH OBJECTIVES

To improve methane production and yield from palm oil mill effluent (POME) anaerobic batch and continuous fermentation systems by utilizing added oil palm biomass

To evaluate performance of biogas scrubbing system using mill treated river water and biochar produced from low-cost technology

To produce biochar and activated carbon from oil palm empty fruit bunch (EFB) using low-cost technology and excess steam

[SUPERVISOR] Professor Dr Yoshihito Shirai



> SEM image of oil palm biomass after fermentation

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APPROPRIATE TREATMENT OF PALM OIL MILL FINAL DISCHARGE WASTEWATER AS RECYCLED WATER FOR THE MILL TO ACHIEVE ZERO DISCHARGE

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 ton of fresh water was needed for processing every ton of fresh fruit bunch (FFB). As a return, huge amount of wastewater has been generated, treated and discharged to the river every day. Current treatment system applying in oil palm industry is using river water to use for mill. In present study, the effect of 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 replace fresh river water. Current chemical treatment used at the mill will be used to treat final discharge to achieve zero discharge. Activated carbon 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 processes that encourage the removal of COD, color, suspended colloids and organic matter in final discharge wastewater which can lead to turbidity, odor and taste problems. In this research Jar Test is used to determine the optimum operating conditions for final discharge wastewater by optimizing value of pH, dosage of coagulant and activated carbon used and mixing time to improve the performance and/or capacity of existing treatment systems and to reduce capital expenditure on new treatment systems. *

[SUPERVISOR] Professor Dr Mohd Ali Hassan

RESEARCH OBJECTIVES

To study an effective dosing of organic and inorganic coagulant usage for the treatment of palm oil mill final discharge wastewater to achieve zero discharge in the mill

To develop low cost environmental friendly methods by using organic coagulant to treat palm oil mill final discharge wastewater

▸ Zeolite for final disharge wastewater treatment

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BIOFERTILIZER

ZERO DISCHARGE ON PALM OIL INDUSTRY

RESEARCH OBJECTIVES

To evaluate economic performance of integrated technology of biogas and compost production in palm oil mill

To produce biochar and activated carbon from oil palm empty fruit bunch (EFB) using low-cost technology and excess steam

To evaluate economic viability of integrated zerodischarge system in palm oil mill

Palm oil industry is known as a significant agricultural industry in terms of economic benefit for Malaysia. However, on the other hands, 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 over the past decade, the palm oil industry has developed proper utilizations of these byproducts, 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 Sandakarn, Sabah, under Bornean Biodiversity and Ecosystems Conservation (BBEC) Program with Japan International Company Agency (JICA). *

[SUPERVISOR] Professor Dr Yoshihito Shirai

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COMPOSTING OF OIL PALM FROND BY ENHANCEMENT OF POME ANAEROBIC SLUDGE

A total of 54.44 million tons of oil palm fronds had been generated from the palm oil industry in 2008. Realizing the potential and abundance of fronds as sources of renewable raw materials, research to produce Biocompost from oil palm frond (OPF) had been initiated. Co-composting of palm biomass into microbial based biofertilizer is essential to reduce the impact of environmental pollution and generation of waste in oil palm sector and to increase palm oil productivity. In composting, providing a stable product that is high in nutrients which are easily accessible by plants is essential. The basic process control objective is to maximize microbial activity at the expense of the waste being treated. This is equivalent to maximize metabolic heat output. In the self-heating ecosystem, temperature is a function of the accumulation of heat generated metabolically and determinant of metabolic activity. The compost was entering a thermophilic phase, with temperature recorded at 52 °C after 6 days of composting. The thermophilic condition encourages the composting process with the carbon to nitrogen ratio decrease from 80 to 15.3 during 60 days of composting. Process stability is favored by moderate thermophilic temperatures via an investigation of bacterial species diversity at different composting temperatures by using denature gradient gel electrophoresis (DGGE). The result of DGGE analysis revealed that the main microbes during co-composting of chipped frond and POME anaerobic sludge belong to group of *Gammaproteobacteria*. ✳

[SUPERVISOR] Professor Dr Mohd Ali Hassan

RESEARCH OBJECTIVES

To study the microbial population during production of palm biomass biofertilizer

To develop rapid open system for oil palm fronds (OPF) composting.

BACKGROUND PICTURE

▸ Oil palm frond compost

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BIOFERTILIZER

BIODEGRADATION OF LIGNIN BY ISOLATED LIGNINOLYTIC BACTERIAL STRAIN FROM OIL PALM PLANTATION SOILS

Ligninocellulose are mainly consists of lignin, hemicellulose and cellulose (Betts *et. al.*, 1991; Sun and Cheng, 2002). Lignin is well-known for resistance to microbial degradation because of its high molecular weight and presence of various biologically stable carbon-to-carbon and ether linkages. Generally, lignin contains three aromatic alcohols which are coniferyl alcohol, sinaply and p-coumaryl and there are many problems and difficulty in dissolving lignin without destroying it and some of it subunits because of its exact chemical structure is difficult to ascertain. Lignin iss the most recalcitrant to degrade because of its highly ordered crystalline structure is more resistant to hydrolysis than hemicellulose. Thus, lignin breakdown is thought to occur by concomitant action of ligninolytic enzymes.

Microorganisms that degrade lignin through an oxidative process are fungi, actinomycetes and to a lesser extent, bacteria. In the literature review, white rot fungi have received extensive attention in research for ligninolytic enzymes because of their powerful lignin-degdrading enzymatic systems (Hatakka, 1994). Even so, fungi are unstable in practical treatment under extreme environmental and substrate conditions such as, oxygen limitation, high extractive, higher pH and lignin concentration (Nagarathnamma *et. al.*, 1999). Hence, in some studies, it shows evidence about the bacterial strains can degrade the low molecular weight portion of lignin, but are unable to depolymerize the high molecular weight backbone of the lignin polymer because the bacterial cells do not secrete lignin-depolymerizing enzymes unlike fungi which secrete extracellular enzymes called ligninases (Vicuna, 1988). Still, bacterial lignin degradation systems have ligninolytic potential because it consists of many unique and specific enzymes with the ability to catalyze the production of various useful compounds. Bacteria are important to be studied for ligninolytic potential because of their immense environmental adaptability and biochemical versatility (Lisboa *et. al.*, 2005).

There are several types of oil palm plantation soils in Felda Serting Hilir, Negeri Sembilan were chosen as samples in this project for screening and isolation of potential ligninolytic bacteria strains. The observation that several soils bacteria with the ability to degrade aromatic compounds are also able to degrade lignin provides a possible link between aromatic degradation and lignin degradation (Timothy *et. al.*, 2010).

In order to explore the full potential of ligninolytic bacteria strains contained in oil palm plantation soils, a clear and complete understanding of bacterial communities in the samples will be investigated by using culture-based techniques of microbiota analysis. Based on the lists of the total microbial community in the environmental samples, isolation of potential ligninolytic bacteria strains can be conduct and classified according to phylogenetic analysis (Fleske *et. al.*, 1998; Fritsche *et. al.*, 1999). ✳

[SUPERVISOR] Dr Nor 'Aini Abdul Rahman

RESEARCH OBJECTIVES

To screen, isolate and characterize ligninolytic bacteria strains from oil palm plantation soils

To study the effects of different temperature, pH and substrates on ligninolytic enzymes production from isolated bacteria in submerged fermentation

BACKGROUND PICTURE ▸ Pure colony of ligninolytic bacteria

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IMPROVEMENT IN CO-COMPOSTING PROCESS OF PRESSED-SHREDDED EFB AND RAW POME FROM CONTINUOUS STERILIZER SYSTEM

RESEARCH OBJECTIVES

To investigate the effect of various OPF bulking size in composting of POME under different FFB sterilization process

To investigate the feasibility of biocompost production from co-composting of oil palm EFB with raw POME

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. ∗

[SUPERVISOR] Professor Dr Mohd Ali Hassan

2011 IN PICTURES

► Prof Dr Mohd Ali, Prof. Dr Suraini and Dr Hidayah during visit to Shanghai Tauto Biotech Company

► Asian Congress on Biotechnology, Shanghai, China (10th – 16th May 2011)

► Prof. Suraini with Y.Bhg. Dato' Dr. Rosli Mohamad (Secretary General, Ministry of Education), UNESCO Representative and YAB Tan Sri Dato' Hj. Muhyiddin Hj Mohd Yassin (Deputy Prime Minister of Malaysia. High-Tea after the Hari UNESCO Malaysia 2011 on 18th November 2011.

► Prof Dr Suraini and Ms. Dayang Salwani at 10th International Sago Symposium, Bogor, Indonesia (October 2011)

► IEA Hydrogen Implementing Agreement (HIA), Task 21 Meeting, Singapore (28th February – 1st March, 2011)

► High-Tea after the Hari UNESCO Malaysia 2011 on 18th November 2011

2011 IN PICTURES

► EB Group members at International Invention, Innovation & Technology Exhibition, ITEX 2011

► Mitra and Yee at International Invention, Innovation & Technology Exhibition, ITEX 2011

► Syahinaz and Shima at Pameran Reka Cipta dan Penyelidikan (PRPI) 2011, UPM Serdang

► EB Group members at Seminar New Generation and Breakthrough Technology in GC, GCMS, GCMS-MS & ICPMS 1

► Memorial picture with other participants at Shell Intervarsity Student Paper Presentation Contest (S-SPEC 2011) at UTM Skudai

► Shima at Malaysia Technology Expo 2012

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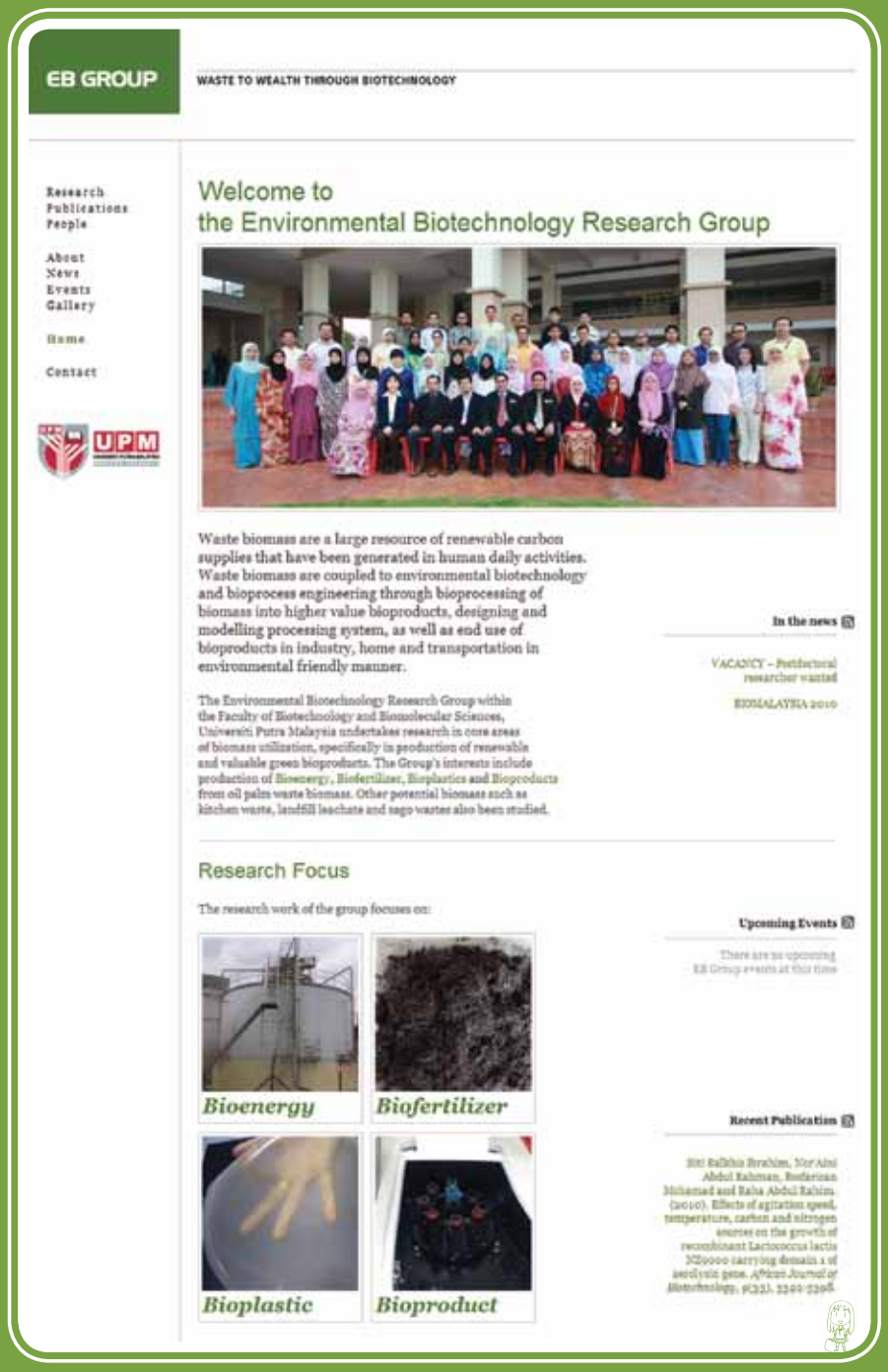

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