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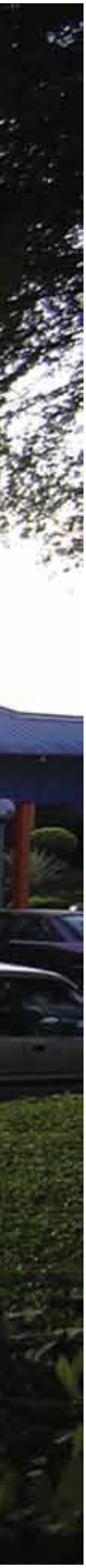
ENVIRONMENTAL BIOTECHNOLOGY RESEARCH GROUP

RESEARCH REPORT 2009

■ BIOGAS ■ BIOCOMPOST ■ BIOETHANOL ■ BIOPLASTIC ■ BIOHYDROGEN ■ BIOPRODUCT

UPM
UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

EB Group's Biomass Technology Centre Building



WASTE TO WEALTH THROUGH BIOTECHNOLOGY

CONTENTS

On the cover:
Commercial Scale Digester Tank
at Serting Hilir., Negeri Sembilan

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Kyushu Institute of Technology (Japan)

FOREWORD

MESSAGE FROM PROF. DR. MOHD ALI HASSAN

GROUP LEADER OF ENVIRONMENTAL BIOTECHNOLOGY RESEARCH GROUP

Praise to ALLAH The Al Mighty for His favours and blessings to us all. I am glad to share the first environmental biotechnology research report 2009 with you. Our research group is currently expanding into various current and important research areas such as biohydrogen, bioethanol, bioplastic, biogas and biocompost, covering a wide range of research topics on wastewater treatment, bioprocessing, enzyme technology, advanced molecular biotechnology techniques, process modelling, process design, regulatory compliance and wastewater recycling. AlhamduLillah, our group succeeded to secure various R&D grants from MOSTI (Top-down, Science Fund and Technofund), MTDC CRDF fund and increasingly from industry i.e. both local and international companies such as FELDA Palm Industries Sdn. Bhd., Yayasan Pelajaran Johor, FPG Oleochemicals Sdn. Bhd., National Institute of Advanced Industrial Science and Technology (AIST), Ajinomoto Company Inc., Tokyo Electric Power Company, Tokyo Gas, Idemitsu Kosan Co. Ltd and many more. In terms of output we are one of the top-performing research groups in UPM. The group is supported by 9 academic staffs and 41 students (PhD 17, MS 24) from Faculty of Biotechnology and Biomolecular Sciences, Faculty of Engineering and Institute of Bioscience. For the year 2009, we have succeeded to publish 31 international refereed journals with 20.87 I.F, 4 patents being filed and won several medals in exhibitions.

I appreciate the hard work and professionalism of all the group members to uplift our group to become a highly performance research group. Keep up the good work! Together let us make UPM and the nation proud of us.

God Bless. Wassalam.

"WITH KNOWLEDGE WE SERVE"

Professor Dr. Mohd. Ali Hassan
Group Leader
Environmental Biotechnology Research Group

RESEARCHERS

Professor Dr. Mohd Ali Hassan

Dean, Faculty of Biotechnology and Biomolecular Sciences

SPECIALISATION:
Bioprocess Engineering & Environmental Biotechnology

CURRENT RESEARCH INTEREST:
Treatment and utilisation of biomass, wastes and effluents for the production of bioproducts, bioremediation and reduction of greenhouse gases.

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SELECTED PUBLICATIONS:

Isnazunita Ismail, Mohd. Ali Hassan, Nor Aini Abdul Rahman, Chen Sau Soon. (2010). Thermophilic biohydrogen production from palm oil mill effluent (POME) using suspended mixed culture. Biomass and Bioenergy, 34(1): 42-47.

Mohd Rafein Zakaria, Hidayah Ariffin, Noor Azman Mohd Johar, Suraini Abd-Aziz, Haruo Nishida, Yoshihito Shirai, Mohd Ali Hassan. (2010). Biosynthesis and characterization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer from wild type Comamonas sp. EB172. Polymer Degradation and Stability (In press). doi:10.1016/j.polymdegradstab.2010.01.020

Mei-Ling Chong, Vikineswary Sabaratnam, Yoshihito Shirai and Mohd Ali Hassan. (2009). Biohydrogen production from biomass and industrial wastes by dark fermentation. International Journal of Hydrogen Energy . 34: 3277-3287.

Norjan Yusof, Akira Haraguchi, Mohd Ali Hassan, Mohd Ridzuan Othman, Minato Wakisaka and Yoshihito Shirai. (2009). Measuring organic carbon, nutrients and heavy metals in rivers receiving leachate from controlled and uncontrolled municipal solid waste landfills. Waste Management . 29(10): 2666 – 2680.

Alawi Sulaiman, Zainuri Busu, Meisam Tabatabaei, Shahrakbah Yacob, Suraini Abd-Aziz, Mohd Ali Hassan and Yoshihito Shirai. (2009). The Effect of Higher Sludge Recycling Rate on Anaerobic Treatment of Palm Oil Mill Effluent in a Semi-Commercial Closed Digester for Renewable Energy. American Journal of Biochemistry and Biotechnology. 5 (1): 1-6.

Mei-Ling Chong, Raha Abdul Rahim, Yoshihito Shirai and Mohd Ali Hassan. (2009). Biohydrogen production by Clostridium butyricum EB6 from Palm Oil Mill Effluent. International Journal of Hydrogen Energy . 34: 764-771.

Meisam Tabatabaei, Mohd Rafein Zakaria, Raha Abdul Rahim, André-Denis G. Wright, Yoshihito Shirai, Norhani Abdullah, Kenji Sakai, Shinya Ikeno, Masatsugu Mori, Nakamura Kazunori, Alawi Sulaiman and Mohd Ali Hassan. (2009). PCR-Based DGGE and FISH analysis of methanogens in an anaerobic closed digester tank for treating palm oil mill effluent. Electronic Journal of Biotechnology. 12(3).

Hidayah Ariffin, Mohd Ali Hassan, Umi Kalsom Md Shah, Norhafizah Abdullah, Farinazleen Mohd Ghazali, Yoshihito Shirai. (2009). Production of bacterial endoglucanase from pretreated oil palm empty fruit bunch by Bacillus pumilus EB3. Journal of Bioscience and Bioengineering, 106 (3): 231-236.

Hassan, M.A., Yacob, S., Shirai, Y. and Hung, Y.T. (2006). Treatment of Palm Oil Mill Wastewaters. In: Waste Treatment in the Food Processing Industry. (Eds. Wang, L.K., et al.), pgs. 101-117. CRC Press, Boca Raton, Florida, USA

Hassan, M.A., Yacob, S. and Shirai, Y. (2004). Treatment of palm oil wastewaters. In: Handbook of Industrial and Hazardous Waste Treatment. (Eds. Wang, D. et al.), pgs 719-735. Second Edition, Marcel Dekker Inc., New York.

ACADEMIC QUALIFICATIONS:

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

CONSULTANCY / PROFESSIONAL SERVICES / INDUSTRIAL LINKAGES:

- Microbial production of ethanol from glycerine wastewater (Idemitsu Co. Ltd., Japan, 2007-to date)
- Amino acids from waste glycerine by indigenous microbes (Ajinomoto Co. Ltd., Japan, 2007-to date)

INTERNATIONAL INVOLVEMENT / RECOGNITION:

2004-2006
Project Leader, Industrial Grant Scheme (IGS) Project.
UPM- MOSTI – KIT - KASA GANDA SDN. BHD.
Development of a novel process for the production of bioacids, bioplastics and compost from organic wastes.

2004-to date
Project Leader
UPM – KIT – FELDA Palm Industries Sdn. Bhd.
Utilisation of biogas and biomass from the palm oil industry for the production of new bioproducts.

2004 – 2008
Invited Speakers on Biomass and Biogas Technology in Japan.

2005- to date
Country Expert & Representative, Asia Biomass Association

2006-to date
Expert Panel on Biofuel, Syngenta Corporation (USA/UK/S'pore)

2008- to date
Joint Project Leader, AIST-UPM-KIT Biomass Technology Center

RESEARCHERS

Assoc. Prof. Dr. Suraini Abd Aziz

Faculty of Biotechnology and Biomolecular Sciences

SPECIALISATION:
Biochemical Engineering, Environmental Biotechnology and Industrial Biotechnology

CURRENT RESEARCH INTEREST:
Utilisation of lignocellulosic biomass (agrowastes & sea food wastes) for the production of biosugars, enzymes, bioethanol, biobutanol and biovanillin.

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SELECTED PUBLICATIONS:

Suraini Abd-Aziz, Christine Cheryl Fernandez, Madihah Md. Salleh, Rosli Md. Illias and Mohd. Ali Hassan. (2008). Effect of agitation and aeration rates on chitinase production using Trichoderma virens UKM1 in 2 L stirred tank reactor. Applied Biochemistry and Biotechnology. 150(2): 193-204.

Sauvaphap Ai-Noi, Suraini Abd-Aziz, Norjahan Alitheen, Osman Hassan and Mohamed Ismail Abdul Karim. (2008). Optimization of Cyclodextrin Glycosyltransferase Production by Response Surface Methodology Approach. Biotechnology. 7(1): 10-18. ISSN 1682-296X.

Sobri Mohd Akhir, Suraini Abd-Aziz, Madihah Md Salleh, Roshanida Abdul Rahman, Rosli Md Illias and Mohd Ali Hassan. (2009) Medium Optimisation of Chitinase Enzyme Production from Shrimp Waste using Bacillus licheniformis TH-1 by Response Surface Methods. Biotechnology . 8(1): 120-125. ISSN 1682-296X.

Azaliza Safarida Wasli, Madihah Md. Salleh, Suraini Abdul Aziz, Osman Hassan and Nor Muhammad Mahadi (2009). Medium Optimization for Chitinase Production from Trichoderma virens using Central Composite Design. Biotechnology and Bioprocess Engineering. 14: 781-787.

Suraini Abd-Aziz, Nor Asma Ab-Razak, Muhammad Hanafi Musa and Mohd Ali Hassan. (2009). Production of mannan-degrading enzymes from Aspergillus niger and Sclerotium rolfsii using palm kernel cake (PKC) as carbon source. Research Journal of Environmental Sciences. 3(2): 251-256. ISSN 1819-3412.

Zuraidah Zanirun, Suraini Abd-Aziz, Foo Hooi Ling and Mohd Ali Hassan. (2009). Optimisation of Lignin Peroxidase production from Pycnoporus sp. through Factorial Design. Biotechnology 8(3): 296-305. ISSN 1682-296X.

Azhari Samsu Baharuddin, Suraini Abd-Aziz, Nor' Aini Abdul Rahman, Mohd Ali Hassan, Minato Wakisaka, Kenji Sakai and Yoshihito Shirai. (2009). Characteristics and microbial succession in co-composting of oil palm empty fruit bunch and partially treated palm oil mill effluent. Open Biotechnology Journal. 3: 87-95.

Ahmad Fadhlan Hamisan, Suraini Abd-Aziz, Kamarulzaman Kamaruddin, Umi Kalsom Md Shah, Neelam Shahab and Mohd Ali Hassan (2009). Delignification of Oil Palm Empty Fruit Bunch (OPEFB) using Chemical and Microbial Pretreatment Methods. International Journal of Agricultural Research.4(8): 250 – 256.

S. Abd-Aziz, L.Y. Teoh, N. Alitheen, N. Shahab and K. Kamaruddin. (2008). Microbial degradation of chitin materials by Trichoderma virens UKM1. Journal of Biological Sciences. 8(1): 52-59. ISSN 1727-3048.

S. Abd-Aziz, G. Siew-Hung, M.A. Hassan, M.I.A. Karim and N. Samat. (2008). Indirect Method for Quantification of Cell Biomass during Solid-State Fermentation of Palm Kernel Cake based on Protein Content. Asian Journal of Scientific Research. 1(4): 385 -393.

ACADEMIC QUALIFICATIONS:

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

CONSULTANCY / PROFESSIONAL SERVICES / INDUSTRIAL LINKAGES:

- Contract research for NewTech Group Sdn. Bhd. – conducting experiments on site and determination of process parameters, sample collection and mass balance with bamboo and coconut shell as the biomass resources (2005)
- CIMB Due Diligent for Wing Lee Hing Sdn Bhd (2008)
- YPJ Plantations Sdn. Bhd - Production of Bio-Compost from Oil Palm Empty Fruit Bunches (EFB) and Goat Manure (2009)

RESEARCHERS

Assoc. Prof. Dr. Umi Kalsom Md Shah

Faculty of Biotechnology and Biomolecular Sciences

SPECIALISATION:
Environmental Biotechnology and Industrial Microbiology

CURRENT RESEARCH INTEREST:
Production of biofuel and biofertilizer from agro-biomass

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SELECTED PUBLICATIONS:

Umi Kalsom M.S., H. Nur, Norlea, and Ngaspan, S. 2006. Characterization of humic acid from humification of oil palm empty fruit bunch fibre using Trichoderma viride. J. Trop. Agric. and Food Sc. Vol 34 No 1: 165-172 .

Umi Kalsom M.S., Ismail A.B., Mohamad A., Suhaimi M., Sahilah A.M. and Abd. Zalal, M. 2006. Evaluation of lignocellulolytic fungus, Phanerochaete Chrysosporium as a potential agent for decomposition of Macaranga triloba logs in peat eco-system. J. Trop. Agric. and Food Sc. Vol 34 No 2: 365-372.

Umi Kalsom M.S. and Noor Shita D. 2007. Characterization of humic acid extracted from solid fermentation of rice straw using Trichoderma viride. J. Trop. Agric. and Food Sc. Vol 35 (2). 297-303.

M.S. Umi Kalsom, A.B. Ismail, A. Mohammad, M. Suhaimi, A.M. Sahilah, M. Abd. Zalal and M.J. Mohamad Hanif. 2007. Fungi influencing decomposition of Macaranga wood in tropical peatland ecosystem. In: Impact of Land Clearing on Peat Ecosystems: A case study at MARDI Peat Research Station, Sessang, Sarawak, Malaysia (A. Ismail, H.K. Ong, M.J. Mohamad Hanif and M.S. Umi Kalsom, ed.) pp. 19-31.

Umi Kalsom M.S., Ismail A.B., Noor Shita D., Norlea a.A. and Ngaspan S. 2008. Evaluating fungi for lignolytic enzymes activities. In: Agrobiodiversity In Malaysia (Mohd. Shukor N, Salma I. and Mohd Said S., ed.)

Umi Kalsom M.S. and Sariah M. 2008. The use of microbes for sustainable agriculture in Malaysia: Current status. In: Agrobiodiversity in Malaysia. (Mohd. Shukor N, Salma I. and Mohd Said S., ed.) pp. 70-78.

Umi Kalsom M.S. 2002. RT-PCR analysis of in vivo expression of α-galactosidase gene in Phanerochaete chrysosporium ME446 grown on solid lignocellulosic substrate. J. Trop. Agric. and Food Sc. Vol 30 No 1: 39-46.

ACADEMIC QUALIFICATIONS:

- PhD (Biotechnology) Universiti Putra Malaysia (1997)
- MSc (Microbiology) University of Kent, Canterbury, United Kingdom (1987)
- BSc. (Microbiology) Universiti Kebangsaan Malaysia (1981)

CONSULTANCY / PROFESSIONAL SERVICES / INDUSTRIAL LINKAGES:

- Allied Agri-Tech Sdn Bhd. (2007-2008)

RESEARCHERS

Dr. Hidayah Ariffin

Faculty of Biotechnology and Biomolecular Sciences

SPECIALISATION:
Bioprocess Engineering and Environmental Biotechnology

CURRENT RESEARCH INTEREST:
Production and recovery of polyhydroxyalkanoates, Chemical recycling of polyhydroxyalkanoates

CONTACTS:
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Email: hidayah_a@biotech.upm.edu.my

SELECTED PUBLICATIONS:

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. In press. doi:10.1016/j.polymdegradstab.2010. 01.018

Mohd Rafein Zakaria, Hidayah Ariffin, Noor Azman Mohd Johar, Suraini Abd-Aziz, Haruo Nishida, Yoshihito Shirai, Mohd Ali Hassan. (2010). Biosynthesis and characterization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer from wild type *Comamonas sp.* EB172. Polymer Degradation and Stability. In press. doi:10.1016/j.polymdegradstab. 2010.01.020

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, 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, Volume 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, 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, 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, 49(2), 451-452pp.

H. Ariffin, M.A. Hassan, M.S. Umi Kalsom, N. Abdullah and Y. Shirai. (2008). The effect of physical, chemical and thermal pretreaments on the enzymatic hydrolysis of oil palm empty fruit bunch. Journal of Tropical Agriculture and Food Science. 36(2), 259-268 pp.

Mohd Rafein Zakaria, Suraini Abd-Aziz, Hidayah Ariffin, Nor `Aini Abdul Rahman, Phang Lai Yee and Mohd Ali Hassan. (2008). *Comamonas sp.* EB172 isolated from digester treating palm oil mill effluent as potential polyhydroxyalkanoate (PHA) producer. African Journal of Biotechnology. 7 (22): 4118-4121pp.

H. Ariffin, M.A. Hassan, M.S. Umi Kalsom, N. Abdullah and Y. Shirai. (2006). Production and characterisation of cellulase by Bacillus pumilus EB3. International Journal of Engineering and Technology. 3(1): 47-53.

ACADEMIC QUALIFICATIONS:

- 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)

RESEARCHERS

Dr. Phang Lai Yee

Faculty of Biotechnology and Biomolecular Sciences

SPECIALISATION:
Environmental Biotechnology

CURRENT RESEARCH INTEREST:
Bioconversion of glycerol-containing waste into bioethanol, Production and recovery of PHA

CONTACTS:
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SELECTED PUBLICATIONS:

Phang, L. Y., Wakisaka, M., Shirai, Y., Hassan, M. A. 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, L. Y., Hassan, M. A., Shirai, Y., Wakisaka, M. and Abdul Karim, M. I. 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, L. Y., Wakisaka, M., Shirai, Y., Hassan, M. A. Effects of single food components on freeze concentration by freezing and thawing technique. Japan Journal of Food Engineering, 4, 77-82 (2003).

Phang, L. Y., Wakisaka, M., Shirai, Y., Hassan, M. A. Effect of sodium chloride on freeze concentration of food components by freezing and thawing technique, Japan Journal of Food Engineering, 5, 97-102 (2004).

ACADEMIC QUALIFICATIONS:

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

RESEARCHERS

Dr. Nor'Aini Abdul Rahman

Faculty of Biotechnology and Biomolecular Sciences

SPECIALISATION:
Environmental Biotechnology

CURRENT RESEARCH INTEREST:
Bioconversion of palm oil waste / biomass and food waste into value-added products e.g polyhydroxyalkanoates, organic acids, biofuel, and biocompost.

CONTACTS:
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SELECTED PUBLICATIONS:

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

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 conditions. The Open Renewable Energy Journal 2: 124-131.

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): 5807-5813.

Azhari Samsu Baharuddin, Nor Asma Abd Razak, Nor'Aini Abdul Rahman, Satiawihardja Budiatman, Yoshihito Shirai and Mohd Ali Hassan. (2009). Bioconversion of Oil Empty Fruit Bunch by Aspergillus niger EB4 under Solid-State Cultivation. Pertanika Journal of Tropical Agricultural Sciences. 32 (2): 143 – 151.

Wong Kok Mun, Nor'Aini Abdul Rahman, Suraini Abd-Aziz, Vikineswary Sabaratnam and Mohd Ali Hassan. (2008). Enzymatic hydrolysis of palm oil mill effluent solid using mixed cellulases from locally isolated fungi. Research Journal of Microbiology . 3(6): 474 – 481. ISSN 1886-4935.

Nor Aini Abdul Rahman, Kazuyuki Shimizu, Yoshihito Shirai, and Mohd Ali Hassan. (2004). Investigation on the metabolic regulation of pgi gene knock out Escherichia coli by enzyme activities and interacellular metabolic concentrations. Malaysian J of Microbiology 2(2): 24-31

ACADEMIC QUALIFICATIONS:

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

RESEARCHERS

Dr. Helmi Wasoh @ Mohamad Isa

Faculty of Biotechnology and Biomolecular Sciences

SPECIALISATION:

Food Biotechnology, Enzyme Technology and Biosensor Technology

CURRENT RESEARCH INTEREST:

1. Automation System of Direct Measurement Device Based on Nanogap Capacitor and Astable Operation for Biosensor Development to Mesure Histamine in Solution.
2. Production of Biotechnology Products using Seaweed Based Substrate Through Fermentation and Enzymatic Processes

CONTACTS:

Telephone Number: +603-8946 8096
Email: helmi@biotech.upm.edu.my

SELECTED PUBLICATIONS:

Patent: Capacitive Biosensor for detection of Histamine (PF2008-050026

ACADEMIC QUALIFICATIONS:

- PhD (Enzyme Technology-Biosensor) Universiti Putra Malaysia (2009)
- MSc. (Food Science) Universiti Kebangsaan Malaysia (2002)
- Bachelor of Food Science and Management (Hons) UKM (1999)

Dr.-Ing. Mohd Noriznan Mokhtar

Faculty of Engineering

SPECIALISATION:

Bioprocess / Biochemical Engineering

CURRENT RESEARCH INTEREST:

Bioprocess/biochemical engineering in:

1. Dynamic modeling and optimization of biological processes
2. Biological production technology (fermentation/enzymatic processes)
3. Downstream processing of biological products
4. Developing of novel bioreactor systems
5. Liquid chromatography separation (analytical/preparative column)
6. Cyclodextrins in food and bio/pharmaceutical technology

CONTACTS:

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SELECTED PUBLICATIONS:

M.N. Mokhtar and W. Zimmermann. Process for the preparation of cyclodextrins composed of more than eight glucose units. EP09153819.9 (Patent Application; 26.02.2009)

Q. Qi, M.N. Mokhtar and W. Zimmermann. Effect of ethanol on the synthesis of large-ring cyclodextrin by cyclodextrin glucanotransferase. J. Incl. Phenom. 57 (2007) 95 – 99

ACADEMIC QUALIFICATIONS:

- Dr.-Ing. (Bioprocess Engineering) Technische Universität Chemnitz, Germany (2009)
- MSc., (Biochemical Engineering) Technische Universität Dortmund (Universität Dortmund), Germany (2004)
- Bachelor of Engineering (Chemical and Process) Universiti Kebangsaan Malaysia (2000)

COLLABORATIVE RESEARCHERS

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Graduate School of Life Science and Systems Engineering (LSSE)
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STUDENTS

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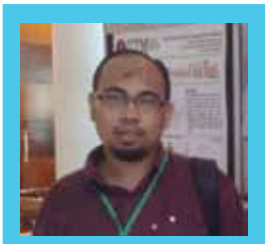
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FAIROUZ JAHAAN BT MOHD AANIFAH P60


FARAH NADIA OMAR P47


HALIMATUN SAADIAH HAFID P47


ISNAZUNITA ISMAIL P53


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MOHD KAMAHL MOHD KAMARUDIN P32


MOHD NAJIB AHMAD P31


MOHD ZULKHAIRI MOHD YUSOFF P52


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SIREN ANAK LINGGANG P41


SYAIFUL HIZAM KAMARUDDIN P54


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ZURAIDAH ZANIRUN


IMAN ABD HALIM


NOOR BAIZURA AZIZAN


AMRI IZAFFI ZAMAHSASRI

ACHIEVEMENT | PUBLICATIONS

IMPACT FACTOR JOURNAL

- Mei-Ling Chong, Raha Abdul Rahim, Yoshihito Shirai and Mohd Ali Hassan. (2009). Biohydrogen production by Clostridium butyricum EB6 from Palm Oil Mill Effluent. International Journal of Hydrogen Energy . 34: 764-771.

Impact factor : 3.452

- Mei-Ling Chong, Vikineswary Sabaratnam, Yoshihito Shirai and Mohd Ali Hassan. (2009). Biohydrogen production from biomass and industrial wastes by dark fermentation. International Journal of Hydrogen Energy . 34: 3277-3287.

Impact factor : 3.452

- Mei-Ling Chong, Nor'Aini Abdul Rahman, Phang Lai Yee, Suraini Abd Aziz, Raha Abdul Rahim, Yoshihito Shirai, Mohd Ali Hassan (2009). Effects of pH, glucose and iron sulfate concentration on the yield of biohydrogen by Clostridium butyricum EB6. International Journal of Hydrogen Energy. 34: 8859-8865.

Impact factor : 3.452

- Mei-Ling Chong, Nor' Aini Abdul Rahman, Suraini Abdul Aziz, Yoshihito Shirai and Mohd Ali Hassan. (2009). Optimization of biohydrogen production by Clostridium butyricum EB6 from palm oil mill effluent using response surface methodology. International Journal of Hydrogen Energy. 34: 7475-7482.

Impact factor : 3.452

- Norjan Yusof, Akira Haraguchi, Mohd Ali Hassan, Mohd Ridzuan Othman, Minato Wakisaka and Yoshihito Shirai. (2009). Measuring organic carbon, nutrients and heavy metals in rivers receiving leachate from controlled and uncontrolled municipal solid waste landfills. Waste Management . 29(10): 2666 – 2680.

Impact factor : 2.208

- Naz Chaibakhsh, Mohd Basyaruddin Abdul Rahman, Mahiran Basri, Suraini Abd-Aziz, Abu Bakar Salleh and Raja Noor Zaliha Raja Abdul Rahman (2009). Optimized Lipase Catalyzed Synthesis of Adipate Ester in a Solvent-Free System. Journal of Industrial Microbiology & Biotechnology. 36: 1149 – 1155. DOI 10.1007/s10295-009-0596-x.

Impact factor : 1.919

- Azaliza Safarida Wasli, Madihah Md. Salleh, Suraini Abdul Aziz, Osman Hassan and Nor Muhammad Mahadi (2009). Medium Optimization for Chitinase Production from Trichoderma virens using Central Composite Design. Biotechnology and Bioprocess Engineering. 14: 781-787.

Impact factor : 1.694

- Hidayah Ariffin, Haruo Nishida, Yoshihito Shirai and Mohd Ali Hassan. (2009). Anhydride production as a additional mechanism of poly(3-hydroxybutyrate) pyrolysis. Journal of Applied Polymer Science. 111: 323 – 328.

Impact factor : 1.306

- Meisam Tabatabaei, Mohd Rafein Zakaria, Raha Abdul Rahim, André-Denis G. Wright, Yoshihito Shirai, Norhani Abdullah, Kenji Sakai, Shinya Ikeno, Masatsugu Mori, Nakamura Kazunori, Alawi Sulaiman and Mohd Ali Hassan. (2009). PCR-Based DGGE and FISH analysis of methanogens in an anaerobic closed digester tank for treating palm oil mill effluent. Electronic Journal of Biotechnology. 12(3).

Impact factor : 1.200

- 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.

Impact factor : 0.456

- Tengku Elida Tengku Zainal Mulok, Mei-Ling Chong, Yoshihito Shirai, Raha Abdul Rahim and Mohd Ali Hassan. (2009). Engineering of E. coli for increased production of L-Lactic acid. African Journal of Biotechnology. 8(18): 4597 - 4603.

Impact factor : 0.456

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CITED JOURNAL

- Suraini Abd-Aziz, Nor Asma Ab-Razak, Muhammad Hanafi Musa and Mohd Ali Hassan. (2009). Production of mannan-degrading enzymes from Aspergillus niger and Sclerotium rolfsii using palm kernel cake (PKC) as carbon source. Research Journal of Environmental Sciences. 3(2): 251-256. ISSN 1819-3412.
- Mohd Zulkhairi Mohd Yusof, Mohd Ali Hassan, Suraini Abd-Aziz and Nor'Aini Abdul Rahman. (2009). Start–up of biohydrogen production from palm oil mill effluent under non-sterile condition in 50 liter continuous stirred tank reactor. International Journal of Agricultural Research. 4(4): 163-168. ISSN 1816-4897.
- Zuraidah Zanirun, Suraini Abd-Aziz, Foo Hooi Ling and Mohd Ali Hassan. (2009). Optimisation of Lignin Peroxidase production from Pycnoporus sp. through Factorial Design. Biotechnology 8(3): 296-305. ISSN 1682-296X.
- 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.
- Ahmad Muhaimin Roslan, Mohd Ali Hassan, Suraini Abd-Aziz and Phang Lai Yee. Effect of POME Supplementation on Cellulase Production from Rice Straw by Local Fungi Isolates (2009). International Journal of Agricultural Research. 4(5): 185 – 192.
- Fadzillah Ismail, NorAini Abdul Rahman, Suraini Abd-Aziz, Chong Mei Ling and Mohd Ali Hassan. (2009). Statistical optimization of biohydrogen production from food waste under thermophilic condition. The Open Journal of Renewable Energy. 8: 124 – 131.
- Sobri Mohd Akhir, Suraini Abd-Aziz, Madihah Md Salleh, Roshanida Abdul Rahman, Rosli Md Illias and Mohd Ali Hassan. (2009) Medium Optimisation of Chitinase Enzyme Production from Shrimp Waste using Bacillus licheniformis TH-1 by Response Surface Methods. Biotechnology . 8(1): 120-125. ISSN 1682-296X.
- Nazlina Haiza Mohd Yasin, Nor Aini 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. ISSN 1996-0700.
- Khanom Simarani, Mohd Ali Hassan, Suraini Abd-Aziz, Wakisaka Minato and Yoshihito Shirai. (2009). Effect of Palm Oil Mill Sterilization Process on the Physicochemical Characteristics and Enzymatic Hydrolysis of Empty Fruit Bunch. Asian Journal of Biotechnology 1(2): 57-66. ISSN 1996-0700.
- Azhari Samsu Baharuddin, Suraini Abd-Aziz, Nor' Aini Abdul Rahman, Mohd Ali Hassan, Minato Wakisaka, Kenji Sakai and Yoshihito Shirai. (2009). Characteristics and microbial succession in co-composting of oil palm empty fruit bunch and partially treated palm oil mill effluent. Open Biotechnology Journal. 3: 87-95.
- Ahmad Fadhlan Hamisan, Suraini Abd-Aziz, Kamarulzaman Kamaruddin, Umi Kalsom Md Shah, Neelam Shahab and Mohd Ali Hassan (2009). Delignification of Oil Palm Empty Fruit Bunch (OPEFB) using Chemical and Microbial Pretreatment Methods. International Journal of Agricultural Research.4(8): 250 – 256.
- Alawi Sulaiman, Zainuri Busu, Meisam Tabatabaei, Shahrakbah Yacob, Suraini Abd-Aziz, Mohd Ali Hassan and Yoshihito Shirai. (2009). The Effect of Higher Sludge Recycling Rate on Anaerobic Treatment of Palm Oil Mill Effluent in a Semi-Commercial Closed Digester for Renewable Energy. American Journal of Biochemistry and Biotechnology. 5 (1): 1-6. ISSN 1553-3468.
- 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. ISSN 1819-3412.
- Alawi Sulaiman, Mohd Ali Hassan, Yoshihito Shirai, Suraini Abd-Aziz, Meisam Tabatabaei, Zainuri Busu and Shahrakbah Yacob. (2009). The Effect of Mixing on Methane Production in a Semi-commercial Closed Digester Tank Treating Palm Oil Mill Effluent (2009). Australian Journal of Basic and Applied Sciences. 3(3): 1577-1583. ISSN 1991-8178.
- Azhari Samsu Baharuddin, Nor Asma Abd Razak, Nor'Aini Abdul Rahman, Satiawihardja Budiatman, Yoshihito Shirai and Mohd Ali Hassan. (2009). Bioconversion of Oil Empty Fruit Bunch by Aspergillus niger EB4 under Solid-State Cultivation. Pertanika Journal of Tropical Agricultural Sciences. 32 (2): 143 – 151.
- Lim Siong Hock, Azhari Samsu Baharuddin, Mohd Najib Ahmad, Umi Kalsom Md Shah, Nor' Aini Abdul Rahman, Suraini Abd-Aziz, Mohd Ali Hassan and Yoshihito Shirai. (2009). Physicochemical Changes in Windrow Co-Composting Process of Oil Palm Mesocarp Fiber and Palm Oil Mill Effluent Anaerobic Sludge. Australian Journal of Basic and Applied Sciences. 3 (3): 2809-2816.

NON-CITED JOURNAL

- Sharifah Soplah Syed Abdullah; Mohd Ali Hassan, Yoshihito Shirai; Masamitsu Funaoka, Takanori Shinano and Azni Idris. (2009). Effect of solvent pre-treatment on lignophenol production from oil palm empty fruit bunch fibers. Journal of Oil Palm Research. 21: 700 – 709.

ACHIEVEMENT | PATENTS

Inventor : Mohd Ali Hassan, Meisam Tabatabaei, Mohd Rafein Zakaria, Raha Abdul Rahim, Andre-Denis G.Wright, Yoshihito Shirai, Norhani Abdullah, Mehdi Shamsara, Kenji Sakai

Title : Method for Isolating DNA

IP Status : Pending Patent
Filed Date : 11/09/2009
Application No. : PCT/MY2009/000143
Country Filing : PCT
Apllicant : Universiti Putra Malaysia

ABSTRACT

The present invention provides a method for the isolation of nucleic acid from microbial cells in an environmental sample. The method includes preparing a suspension of the environmental sample, lysing the suspended sample with a buffered solution, adding sodium dodecylsufate solution to the lysed suspended sample, carrying out solvent extraction and separation to obtain an aqueous phase, reacting the aqueous phase with solvents to generate an insoluble precipitate containing nucleic acid, and isolating the nucleic acid therefrom, thereby releasing high molecular weight nucleic acid pellets from the cells.

Inventor : Mohd Ali Hassan, Voon Phooi Tee, Phang Lai Yee, Yoshihito Shirai

Title : A Method of Extracting and Purifying Polyhydroxyalkanoate Bioplastic

IP Status : Pending Patent
Filed Date : 18/12/2008
Application No. : PI20085129
Country Filing : Malaysia
Apllicant : Universiti Putra Malaysia

ABSTRACT

Polyhydroxyalkanoates (PHA) is an intracellular polymer that is biologically synthesized by microorganisms as carbon and energy reverse material. This biodegradable material has properties similar to petrochemical thermoplastics. Currently methods of PHA extraction and purification from bacterial suspension use solvent or halogenated-based approaches which are costly and pose severe environmental damage. This invention describes a combined treatment of alkaline and homogenization for extracting and purifying PHA, with higher PHA purity and recovery.

Inventor : Mohd. Ali Hassan

Title : Novel In-Vessel High Rate Composter

IP Status : Pending Patent
Filed Date : 26/06/2009
Application No. : -
Country Filing : PCT
Apllicant : Universiti Putra Malaysia; Ecobuilders Sdn. Bhd.

ABSTRACT

The present invention is an-in-vessel high rate composting apparatus for processing of oil palm biomass, organic and municipal wastes to produce compost product. The composting apparatus consists of a vertical position and cylindrical shape vessel (100) with conical bottom (2) design, materials feeding (1) and product discharge systems (3), a screw impeller (12) for axial mixing, aeration (14) and carbon dioxide (CO_2) removal systems and a leachate collection system (15). The optimum composting conditions in the vessel will be maintained by means of programming logic controller (PLC) on key parameters such as temperature, oxygen (O_2) level, moisture level and carbon dioxide (CO_2) level. The compost product could be let to mature inside the vessel or let to cure elsewhere for the completion of the compost product

Inventor : Mohd. Ali Hassan

Title : A Novel Bacterium Producing Polyhydroxyalkanoates from Palm Oil Mill Effluent

IP Status : Pending Patent
Filed Date : 29/06/2009
Application No. : PI20092761
Country Filing : Malaysia
Apllicant : Universiti Putra Malaysia; Felda Palm Industries Sdn. Bhd.; Kyushu Institute of Technology

ABSTRACT

The bacterial strain EB 172, isolated from digester treating palm oil mill effluent, was investigated by polyphasic taxanomic approach. The cells were rod-shaped, Gram-negative, non-pigmented, non-spore-forming and non-fermentative. Phylogenetic analyses usnign the 16S rRNA gene sequence showed that the strains C. terrigena (96.8%), C. koreensis (93.4%), C. composti (92.9%), and C.kerstersii (91.1%). The ability of C. putranesis to produce polyhydroxyalkanoates (PHA) when supplied with organic acids made this bacterium is unique in *Comamonas* species. The bacterial strain was clearly distinguished from all of the existing strains using phylogenetic analysis, fatty acid composition data and a range of physiological and biochemical characteristics. The DNA G+C content of the genomic DNA was 59.1 mol%. It is evident from the genotypic and phenotypic data that strain *Comamonas putranesis* represents a novel species in the genus *Comamonas*, for which the name *Comamonas putranesis* sp. nov. is proposed.

AWARDS | ACHIEVEMENT

Design, Research and Innovation Exhibition (PRPI 2009) University Putra Malaysia 21-23th June 2009

GOLD MEDAL

Appropriate Technology for Accelerated Composting of Oil Palm Biomass.
Mohd. Ali Hassan, Yoshito Shirai,, Umi Kalsom, Azhari Samsu Baharuddin, Alawi Hj. Sulaiman, Lim Siong Hock, Mohd Najib Ahmad, Chairil Anuar Dzulkornain

GOLD MEDAL

A Novel High Rate In-Vessel Composter for Oil Palm Biomass, Organic and Municipal Wastes.
Mohd. Ali Hassan, Azhari Samsu Baharuddin, Alawi Hj. Sulaiman, Lim Siong Hock, Mohd Najib Ahmad, Chairil Anuar Dzulkornain, Hj Zubair Shafie

GOLD MEDAL

A Method for Direct Isolated of High Yield, PCR - Compatible DNA from Environment Samples.
Mohd. Ali Hassan, Meisam Tabatabaei

GOLD MEDAL

PCR Based DGGE and Fish Analysis of Methanogens in an Anaerobic Closed Digester Tank for Treating Palm Oil Mill Effluent.
Mohd. Ali Hassan, Meisam Tabatabaei

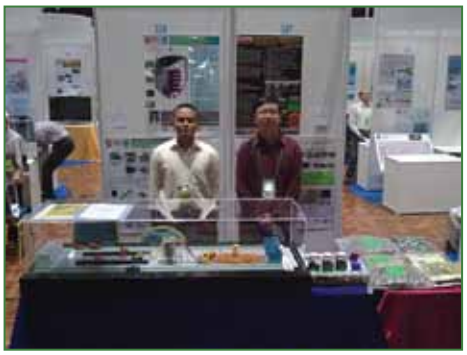
The International Exposition of Research and Invention of Institutions of Higher Learning 2009 (Pecipta 2009) Kuala Lumpur Convention Centre 8-10 October 2009

SILVER MEDAL

A Novel High Rate In-Vessel Composter for Oil Palm Biomass, Organic and Municipal Wastes.

Prof Dr. Mohd. Ali Hassan, Prof Dr. Yoshihito Shirai, Prof Dr. Kenji Sakai, Assoc. Prof Dr. Minato Wakisaka, Assoc. Prof Dr. Umi Kalsum Md Shah, Assoc. Prof Dr. Suraini Abd-Aziz, Dr. Mohd Noriznan Mokhtar, Azhari Samsu Baharuddin, Alawi Sulaiman, Lim Siong Hock, Mohd Najib Ahmad, Chairil Anuar Dzulkornain, Syaiful Hizam Kamaruddin, Mohd Fuad Sarun, Shahrudin Omar, Zubair Shafie, Hamidi Abd. Hamid

NOVEL IN-VESSEL HIGH RATE COMPOSTER
miniature model



PRPI 2009
pictures
gallery

CONFERENCE ATTENDED

BIOGAS

Yusof, N., Hassan, M.A., Phang, L.Y. Tabatabaei, M., Othman, M.R., Mori, M., Wakisaka, M., Sakai, K., Shirai, Y. Nitrification of Stabilized Landfill Leachate. Environmental Biotechnology Conference, 23-24th June 2009,Tokyo, Japan.

Meisam Tabatabaei, Mohd Rafein Zakaria, Raha Abdul Rahim, André-Denis G. Wright, Yoshihito Shirai, Norhani Abdullah, Kenji Sakai, Shinya Ikeno, Masatsugu Mori, Nakamura Kazunori, Alawi Sulaiman and Mohd Ali Hassan. PCR-Based DGGE and FISH Analysis of Methanogens in an Anaerobic Bioreactor for Treating Palm Oil Mill Effluent. Annual Conference of the Association for General and Applied Microbiology (VAAM), 2009, Bochum, Germany.

Meisam Tabatabaei, Mohd Rafein Zakaria, Raha Abdul Rahim, André-Denis G. Wright, Yoshihito Shirai, Norhani Abdullah, Kenji Sakai, Shinya Ikeno, Masatsugu Mori, Nakamura Kazunori, Alawi Sulaiman and Mohd Ali Hassan. Monitoring Methanogenic Archaea in an Anaerobic Bioreactor for Treating Palm Oil Mill Effluent Using PCR-Based DGGE and FISH Analysis. Advances In Wastewater Treatment and Reuse (AWTR), 2009, Tehran, Iran.

BIOCOMPOST

Azhari Samsu Baharuddin, Mohd Ali Hassan, Suraini Abd Aziz, Minato Wakisaka, and Yoshihito Shirai. Co-composting of Oil Palm Solid Biomass and Treated Palm Oil Mill Effluent in Pilot Scale. 5th Biomass Asia Workshop, 4th December 2008, China

Azhari Samsu Baharuddin, Mohd Ali Hassan, Minato Wakisaka, Kenji Sakai and Yoshihito Shirai. Possibility of production and CDM business of the organic fertilizer with the unused biomass of palm oil industry. Society of Chemical Engineering Japan (SCEJ) 74th Annual Meeting, 18-20th March 2009, Yokohama, Japan.

BIOPLASTIC

Pre –ICBP Workshop on Bio-Based Polymers, 10 Nov 2009 at Equatorial Hotel, Penang

2nd International Conference on Bio Based Polymer, 11-13 Nov 2009 at USM,Penang

3 Workshop MIT-SIRIM-UPM-USM, 14-15 Nov 2009 at Equatorial Hotel, Penang

Seminar on Biomass for Biofuels and Value-added Products 27-28 October 2009 at The Saujana Hotel, Kuala Lumpur.

BIOHYDROGEN

Yusoff, M. Z. M., Hassan, M. A., Zatilfarihiah R., Chong, M.-L., Abd-Aziz, S., N.A.A.Rahman. and Shirai, Y. Production Of Biohydrogen From Palm Oil Mill Effluent Under Non-Sterile Condition, 30th Symposium of Malaysian Society for Microbiology – Microbes: Biotechnology Engine for Health and Wealth Creation", from 16th – 19th August 2008 at Hyatt Regency Resort Kuantan, Pahang Darul Makmur, Malaysia.

Nazlina, H. M. Y., Nor Aini, A. R., Heidarnia, F., Yusoff, M. Z. M., & Hassan, M. A. Biohydrogen Production from Food Waste by Natural Microflora 30th Symposium of Malaysian Society for Microbiology – Microbes: Biotechnology Engine for Health and Wealth Creation", from 16th – 19th August 2008 at Hyatt Regency Resort Kuantan, Pahang Darul Makmur, Malaysia.

BIOPRODUCT

Response Surface Methodology (RSM) Workshop. Institute of Bioscience (IBS), UPM, Serdang. 12th -14th November 2008 .

Downstream Processing Workshop. Institute of Bioscience (IBS), UPM and Pall (M) Sdn. Bhd. September 2009.

30th Annual Conference of the Malaysian Society for Animal Production (MSAP). 'Maximizing Livestock Productivity through Efficient Resource Utilization'. Hyatt Regency Hotel, Kota Kinabalu, Sabah, 2-5 June 2009.

BioMalaysia 2009 Conference. 'Accelerating Commercialization in Biotechnology. Kuala Lumpur Convention Centre, Malaysia. 17th – 19th November 2009.

OSAKA Symposium


Prof. Ali at OSAKA Symposium

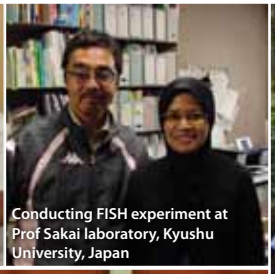

PRE –ICBP Workshop On Bio-Based Polymers


Conference Malaysian Society for Microbiology 2009

PICTURES GALLERY


Norjan's final presentation at Kyushu Institute of Technology


Dr Yano & Dr Murakami are doing lab works


Conducting FISH experiment at Prof Sakai laboratory, Kyushu University, Japan


During field work with Prof Akira Haraguchi, University of Kitakyushu, Japan


With Prof Sakai laboratory members, Kyushu University


Dr. Noriznan and Azhari


Meisam Tabatabei with Prof. Ali and wife


During EB Group's big group meeting


Farah Nadia and Halimatun during conference


Iftar 2009 group picture


Celebrating birthday with Alawi, Prof. Ali and Dr. Suraini


Prof. Yoshihito Shirai


Prof. Ali with Sobri Mohd Akhir


Institute of Bioscience laboratory's members


Ridzuan doing lab works

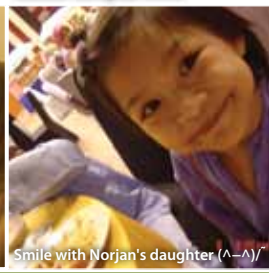

Hani Mastura doing lab works


Nazlina Haiza doing lab works


Water sampling in the river near to the active uncontrolled landfill


Alawi Sulaiman


Confocal microscope training


Smile with Norjan's daughter (^—^)/

Picture: Commercial digester tank at Felda Serting Hilir, Negeri Sembilan, Malaysia

BIOGAS RESEARCH GROUP

OVERVIEW

Our research involves in the treatment of organic rich wastewater such as palm oil mill effluent (POME) and leachate from MSW landfill for either treatment alone or production of renewable energy. The treated wastewater could be further polished to a standard that is suitable for recycling purpose so that minimal river water is needed and reduce the dependency on river water intake.

RESEARCH AREA

Currently, there are four PhD students and two Master students under this group and registered either with UPM or Kyushu Institute of Technology, Japan.

1. Improved biomethanation of palm oil mill effluent during anaerobic treatment in a semi-commercial closed digester tank.
2. Ammonium removal from municipal solid waste (MSW).
3. Microalgal and methane production from palm oil mill effluent.
4. Methane production from decantered palm oil mill effluent.
5. Advanced wastewater treatment for BOD 20 PPM in palm oil industries.
6. Appropriate treatment of palm oil mill effluent final discharge wastewater as recycled water for the mill to replace fresh river water.

PARTNERS

Universiti Putra Malaysia - Prof. Dr. Mohd Ali Hassan

Kyushu Institute of Technology - Prof. Dr. Yoshihito Shirai

FELDA Palm Industries Sdn. Bhd. - Tuan Hj. Abdul Halim Ahmad

BIOGAS RESEARCH GROUP

Improved Biomethanation of Palm Oil Mill Effluent during Anaerobic Treatment in a Semi-commercial Closed Digester Tank

Alawi B Hj Sulaiman

PhD, Semester 7
(Thesis submitted to GSO UPM)
asuitm@yahoo.com

IN THIS STUDY, ACCELERATED START-UP process, effect of higher sludge recycling rate, effect of various mixing regimes and effect of co-digesting POME with refined glycerine wash water (RGWW) were investigated. The digester performance and stability were evaluated based on the chemical oxygen demand (COD) removal efficiency, volatile fatty acids (VFA) accumulation, methane productivity, composition and yield. In the first experiment, the bottom seed sludge transfer lead to interesting results including a 24 day start-up period, stable pH condition (pH 6.8-7.2), high COD removal efficiency (>90%), satisfactory VFA to Alk ratio (<0.3), satisfactory biogas production of nearly 1.8 kg m^{-3} d^{-1} and methane composition of 50 to 60% due to the presence of high amount of methanogens in the seed sludge. In the second experiment, by increasing the sludge recycling rate, the VFA concentration was controlled below its inhibitory limit (1000 mg L^{-1}) and the COD removal efficiency recorded was above 95% which indicated good treatment performance. Two methanogens species (*Methanosarcina sp.* and *Methanosaeta concilii*) have been identified from sludge samples obtained from the digester and recycled stream. In the third experiment, the minimal horizontal mixing gave the highest methane productivity at 1.4 m^3 m^{-3} d^{-1} in comparison to natural mixing at 1.0 m^3 m^{-3} d^{-1} and minimal horizontal and vertical mixing at 1.1 m^3 m^{-3} d^{-1}. This indicates minimal mixing was required to provide good contact between substrate and microorganisms inside the digester and to release the entrapped biogas at the bottom of the digester. The vigorous mixing was discovered to inhibit the methane production process as methane was not produced at the end of the experiment and VFA concentration was also recorded high at 3700 mg L^{-1} and may have disrupted the syntrophic relationship between acidogens and methanogens and inhibited the methanogenesis. In the last experiment, once the RGWW percentage was increased to a maximum of 5.25%, COD removal efficiency remains high but the methane production rate reduced significantly down to 307 m^3 d^{-1} where the digester was already unstable with high VFA recorded of 913 mg L^{-1} and low cells concentration of 8.58 g L^{-1}. This was due to the effect of plasmolysis on the methanogens at high concentration of NaCl in the digester of nearly 4000 mg L^{-1}. ■

THE COMMERCIAL SCALE of the methane recovery project from anaerobic treatment of POME in Serting Hilir Palm Oil Mill

THE 500 M³ SEMI-COMMERCIAL SCALE closed digester tank dedicated for methane recovery for renewable energy research belongs to UPM-KIT-FELDA joint R&D collaboration.

THE RESEARCH OBJECTIVES

1. To accelerate the start-up process by using the direct transfer of seed sludge from the existing 3600 m^3 open digester tank (ODT).
2. To improve the methane production and yield by studying the effect of increasing the sludge recycling rate from the newly installed 30 m^3 settling tank.
3. To improve the methane production and yield by studying the effect of mixing using pump recirculation method on various modes of mixing.
4. To improve the methane production and yield by co-digesting POME with wastewater from the glycerine refining plant.

PUBLICATIONS

- Alawi Sulaiman, Meisam Tabatabaei, Mohd Ali Hassan, Yoshihito Shirai. 2009. The effect of higher sludge recycling rate on anaerobic treatment of palm oil mill effluent in a semi-commercial closed digester for renewable energy. American Journal of Biochemistry and Biotechnology, 5(1): 1-6.
- Alawi Sulaiman, Mohd Ali Hassan, Yoshihito Shirai, Suraini Abd-Aziz, Meisam Tabatabaei, Zainuri Busu and Shahrakbah Yacob. 2009. The effect of mixing on methane production in a semi-commercial closed digester tank treating palm oil mill effluent. Australian Journal of Basic and Applied Sciences, 3(3): 1577-1583.
- Alawi Sulaiman, Mohd Rafein Zakaria, Ali Hassan, Yoshihito Shirai, and Zainuri Busu. 2009. Co-digestion of palm oil mill effluent and refined glycerin wash water for chemical oxygen demand removal and methane production. American Journal of Environmental Sciences, 5 (5): 639-646.
- Mohd Ali Hassan, Alawi Sulaiman, Yoshihito Shirai and Suraini Abd-Aziz. 2009. Methane capture and clean development mechanism project for the sustainability of palm oil industry in Malaysia. Journal of Applied Science Research, 5(10):1568-1581.

BIOGAS RESEARCH GROUP

Nitrification of High-strength Ammonium Landfill Leachate for Improvement of River Water Quality in Malaysia

Norjan Binti Yusof

PhD, Semester 6

norjanbintiyusof@yahoo.com

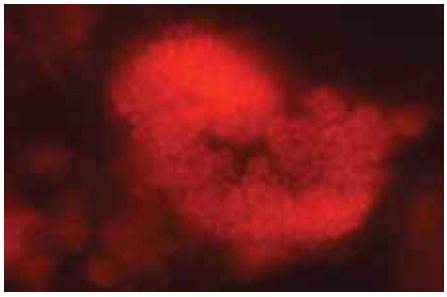
THE MATURE LANDFILL LEACHATE IS characterized by high-strength ammonium, which leads to difficulties in reducing the ammonium strength in the wastewater discharge to the permissible limit (10 mg/L) using the existing biological treatment of sequencing batch reactors (SBRs). The challenge of the nitrification of high-strength ammonium landfill leachate is substrate inhibition, particularly in the form of free ammonia (FA) and free nitrous acid (FNA) in ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). The problem is more severe, as 43% of the landfills are not well designed and not properly equipped with leachate control mechanism facilities. In particular, this type of landfill exposed the river water to the risk of ammonium contamination from the landfill leachate. Therefore, there is an urgent need to improve the existing leachate management at landfills. Prior to the nitrification study, leachate characteristics and the presence of inorganic nitrogen in the rivers receiving landfill leachate from three different types of landfills in Selangor state, Malaysia were assessed throughout a year to determine the impact of landfill leachate on river water chemistry. In response to the results of the water quality study, a nitrification-activated sludge system has been developed for high-strength ammonium landfill leachate. The system was operated under controlled conditions that favor nitrification and was started in the fed-batch mode of operation to prevent inhibitory effects of FA and FNA on nitrifiers. As the heterotrophs could also inhibit the nitrification performance, the organic carbon removal was monitored during the nitrification of mature landfill leachate. A molecular technique, fluorescence *in situ* hybridization (FISH), was used both to identify the microbial populations as well as the localization of the nitrifiers in the sludge floc complex community. ■

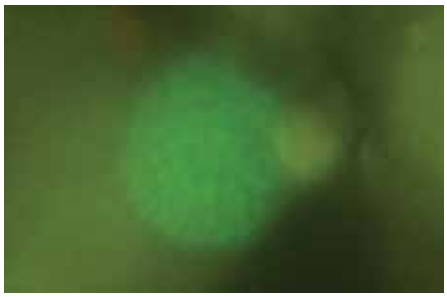
LEACHATE FROM ACTIVE UNCONTROLLED LANDFILL flows into the nearby river.

THE RESEARCH OBJECTIVES

1. To determine the presence of inorganic nitrogen, organic carbon and heavy metals in rivers receiving leachate from controlled and uncontrolled municipal solid waste landfills.
2. To determine the feasibility of obtaining complete nitrification of high-strength ammonium from mature landfill leachate.
3. To identify microbial population contributions to the nitrification of high-strength ammonium using the fluorescence *in situ* hybridization (FISH) technique.

NITRIFICATION ACTIVATED SLUDGE SYSTEM (NASS) used in nitrification of high-strength ammonium landfill leachate.





PICTURES FROM FISH MOLECULAR TECHNIQUE showing ammonia oxidizing bacteria (red) and nitrite oxidizing bacteria (green)

PUBLICATIONS

- Norjan Yusof, Mohd Ali Hassan, Phang Lai Yee, Meisam Tabatabaei, Mohd Ridzuan Othman, Minato Wakisaka, Kenji Sakai, and Yoshihito Shirai, 2010. Nitrification of Ammonium-Rich Sanitary Landfill Leachate. Waste Management, 30(1): 100-109.
- Norjan Yusof, Akira Haraguchi, Mohd Ali Hassan, Mohd Ridzuan Othman, Minato Wakisaka and Yoshihito Shirai, 2009. Measuring organic carbon, nutrients and heavy metals in rivers receiving leachate from controlled and uncontrolled municipal solid waste landfills. Waste Management, 29(10): 2666 – 2680.
- Norjan Yusof, Akira Haraguchi, Yoshihito Shirai, Mohd Ali Hassan and Minato Wakisaka, 2008. Characteristics of Leachate from Selected MSW Landfills and Relationship with River Water Chemistry. The Icfai Journal of Environmental Sciences, 2(3): 42-49.

BIOGAS RESEARCH GROUP

Appropriate Treatment of Palm Oil Mill Final Discharge Wastewater as Recycled Water for the Mill to Replace Fresh River Water

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THE OBJECTIVE OF THE PRESENT STUDY was to evaluate the effect of zeolite,activated carbon and poly-glutamate application as appropriate treatment of palm oil mill final discharge wastewater as recycled water for the mill to replace fresh river water. Zeolites are crystalline aluminosilicate materials whose compositions and nanoporous structures can be fine-tuned for applications in catalysis, adsorption and ion exchange. Activated carbon is used as adsorbent 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. (Hoehn, 1996). A Jar Test Method is used to simulate the coagulation and flocculation processes that encourage the removal of suspended colloids and organic matter in final discharge wastewater which can lead to turbidity, odor and taste problems. (Metes *et.al*,2004). In this research Jar Test is used to determine the optimum operating conditions for final discharge wastewater by optimizing value of pH,dosage of zeolite,activated carbon and poly-glutamate used,and mixing time to improve the performance and/or capacity of existing treatment systems and to reduce capital expenditure on new treatment systems. ■

THE RESEARCH OBJECTIVES

1. To compare treatment of final discharge with fresh river water using current river water treatment system at the mill.
2. Optimization of final discharge treatment using appropriate methods to achieve good quality recycled water for use in the mill.
3. Adaptation of the improved treatment system to replace current river water treatment at the mill.

Methane and Microalgae Production from Palm Oil Mill Effluent for Zero Discharge Solution

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THE MAIN FOCUS OF THE RESEARCH WORK is the treatment of palm oil mill effluent (POME). In view of this, biogas production particularly methane from anaerobic digestion of POME will be analysed in both small and large scale applications. Methods of increasing current methane yields will also be explored. The work will also include utilization of microalgae to degrade nutrients in the final stages of the existing wastewater treatment system i.e. algae ponds prior to usage of suitable polymers for further polishing. The treated effluent will then be recycled back to the mill for its operations and hence reduce if not eliminate altogether the dependency on fresh river water supply. ■

THE RESEARCH OBJECTIVES

1. To increase methane production from anaerobic digestion of POME.
2. To develop a treatment system suitable for total recycle of treated effluent using microalgae and polymers.

FINAL ALGAE POND at FELDA Serting Hilir Mill

50L BIOREACTOR METHANE FERMENTATION from POME Setup

GAS SCRUBBER SYSTEM

BIOGAS RESEARCH GROUP

Molecular Phylogeny and Characteristics of Methanogens from a Palm Oil Mill Anaerobic Tank

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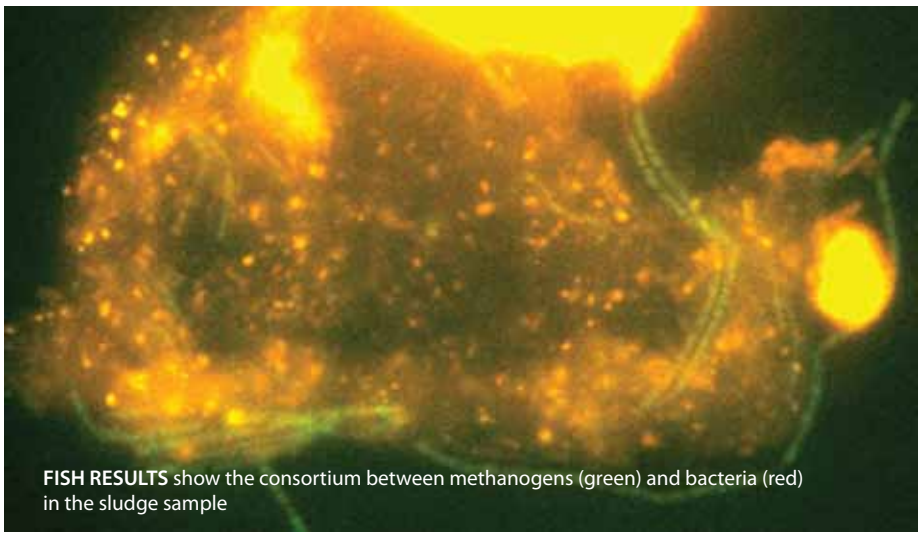
CHARACTERIZATION AND THE PHYLOGENY of the methanogenic population in anaerobic tank for treating palm oil mill effluent was investigated. In this study, environmental DNA was extracted and purified from wastewater sludge by using a simplified and less time consuming procedure (Malaysian Patent Pending Number: PI20082842 filed on 30/07/2008) and the results obtained were compared to that of other three existing protocols i.e. Ogram *et al.*, Tsai and Olson, and Jacobsen and Rasmussen methods which are normally used for environmental samples. The DNA isolated from the palm oil mill anaerobic tank in FELDA Serting Hilir, was used for determining the molecular phylogeny of methanogenic archaea by using culture-independent analysis of the 16S rRNA genes amplified directly from sludge. Restriction fragment length polymorphism (RFLP) analysis, denaturing gradient gel electrophoresis (DGGE) and fluorescence *in situ* hybridization (FISH) were also used in combination which made the present study, the first wide-scale study carried out in Malaysia. 1260-bp 16S rRNA PCR products were cloned and sequenced. Phylogenetic analysis showed the microbes were closely affiliated with known cultured methanogenic Archaea, *Methanosaeta concilii*. Based on RFLP (*Hae*III) analysis, just a few clones (clone SamaliEB; Genebank Accession Number: EU580025) seemed to be new species or at least new strains of *Methanosaeta*. This was also confirmed by DGGE analysis which showed the presence of M. *concilii* and *Methanosarcina*. sp. FISH was carried out using specifically designed 16s rRNA probes to target methanogens and bacteria. The results were in line with DGGE analysis and revealed the presence of two types of methanogens including M. *concilii* and *Methanosarcina* sp. in the anaerobic tank. Quantitative FISH showed that M. *concilii* had a population of 1.4×10^8 cell/ml of wastewater sludge, while *Methanosarcina* sp. was 2×10^5 cell/ml of wastewater sludge. This could be the reason of failing to get it cloned as for each 1000 clones of *Methanosaeta*, there was just one clone of *Methanosarcina* and therefore, the probability of picking up a clone affiliated to *Methanosarcina* was approximately 0.1%. FISH helped to elucidate the association of methanogens and bacteria together. The findings of this study helped to understand the microbial population of the anaerobic tank for treating POME in Malaysia. The results indicate that filamentous acetate-utilizing methanogens detected in the POME anaerobic tank belong to the genus *Methanosaeta* based on the cell-morphology, and the phenotypic and phylogenetic characteristics described above. The data obtained also suggest that *Methanosaeta* is the most abundant methanogen in POME anaerobic digestion and that it plays an important role in methane production from acetate and its optimum growth conditions should be considered when an attempt is made to treat POME anaerobically. In future, these findings will provide the chance to optimize the anaerobic tank conditions to increase the methane production and "carbon oxygen demand" (COD) removal. ■

PUBLICATIONS

Meisam Tabatabaei, Mohd Rafein Zakaria, Raha Abdul Rahim, André-Denis G. Wright, Yoshihito Shirai, Norhani Abdullah, Kenji Sakai, Shinya Ikeno, Masatsugu Mori, Nakamura Kazunori, Alawi Sulaiman and Mohd Ali Hassan. 2009. PCR-Based DGGE and FISH analysis of methanogens in anaerobic closed digester tank treating palm oil mill effluent (POME). Electronic Journal of Biotechnology, 12 (3). Available from Internet: http://www.ejbiotechnology.cl/content/vol12/issue3 /full/4/index.html.



FISH RESULTS show the consortium between methanogens (green) and bacteria (red) in the sludge sample

THE RESEARCH OBJECTIVES

1. To develop an improved method for extracting DNA from environmental samples (natural inhabits of methanogenic Archaea) including wastewater sludge and to compare the results obtained to that of other three existing protocols i.e. Ogram *et al.* (1987), Tsai and Olson (1991), and Jacobsen and Rasmussen (1992) methods which are normally used for environmental samples.
2. To establish the phylogenetic affiliation of unknown and uncultured methanogens present in the anaerobic tank using PCR-cloning technique.
3. To detect new species of methanogens, using Restriction Fragment Length Polymorphism (RFLP) to obtain the riboprinting of methanogens present in the tank.
4. To obtain the methanogenic profiles by using denaturing gradient gel electrophoresis (DGGE).
5. To visualize methanogens and to examine the microbial consortia, physical association between methanogens and bacteria, in the tank using Fluorescent *In Situ* Hybridization (FISH) as well as to quantify methanogens and bacteria using quantitative FISH.

Picture: Oil palm plantation in Malaysia

BIOCOMPOST RESEARCH GROUP

OVERVIEW

The Biocompost research is focusing on the utilization of organic rich biomass to produce high and consistent quality of Biocompost. Our research involves in the acceleration of the composting process by using advanced high rate composter. Meanwhile, mechanism for efficient composting process also had been studied. In microbial level, intensive study on microbial succession and degradation properties also had been carried out. The active compound production by composting microbes also had been studied to counter plant disease problem. The research outcome could highly reduce the costing in composting process as well as plant pesticide and it could offer attractive business model for SME.

RESEARCH AREA

Currently, there are two PhD and three Master students under this group. The students either registered with UPM or Kyushu Institute of Technology (KIT).

1. Appropriate technology for accelerated composting treatment of oil palm biomass, municipal and organic waste.
2. Composting of Oil palm mesocarp fiber by enhancement of POME anaerobic sludge
3. Composting of Oil Palm Frond by enhancement of POME anaerobic sludge
4. Improvement in Co-Composting Process of Pressed - Shredded EFB and Raw POME from Continuous Sterilizer System

PARTNERS

Universiti Putra Malaysia - Prof. Dr. Mohd Ali Hassan

Kyushu Institute of Technology - Prof. Dr. Yoshihito Shirai

FELDA Palm Industries Sdn. Bhd. - Tuan Hj. Abdul Halim Ahmad

BIOCOMPOST RESEARCH GROUP

Appropriate Technology of Accelerated and Controlled Composting Process for Oil Palm Biomass in Malaysia

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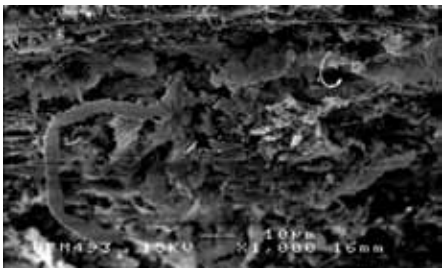
MALAYSIA IS THE LARGEST PALM OIL producer and exporter in the world. Despite high economics return to the country, the industry also generates large amount of wastes such as oil palm empty fruit bunch (EFB), mesocarp fiber, oil palm frond (OPF), palm oil mill effluent (POME) and POME sludge. Currently the solid wastes are being utilized inefficiently for soil mulching, boiler fuel and incineration whereas POME is being treated in large open pond system before safely discharged. In advanced, these wastes could be transformed into high value-added product such as bio-compost using an advanced biotechnological technique. The composting process utilizes activated POME anaerobic sludge for nitrogen source and microbial seeding and oil palm biomass as carbon source. The active microbial seeding strategy was accelerated the composting process from 100 days to only 40 days, reducing the overall operation cost and avoid the dependency on effective microbes (EM) supplementation. Furthermore, this integrated composting system produced high and consistent quality bio-compost in term of nutrients value and beneficial microbes. Based on EFB bio-compost, the final matured product comprised of satisfactory N:P:K content of 2:1:3 and considerable amount of nutrients (calcium, magnesium, sulfur, iron, manganese, zinc and copper) and final C/N ratio of 12. In addition very low level of heavy metals was detected in the compost. The bio-compost could also be fortified with other suitable wastes to increase the nutrients value. For the commercialization, this environmental-friendly technology and know-how to produce bio-compost from oil palm biomass could be transferred to the small medium industries in the rural area for wealth creation. ■

PUBLICATIONS

- Azhari Samsu Baharuddin, Minato Wakisaka, Yoshihito Shirai, Suraini Abd-Aziz, Nor' Aini Abd Rahman, and Mohd Ali Hassan, 2009. Co-composting of empty fruit bunches and partially treated palm oil mill effluents in pilot scale. International Journal of Agriculture Research, 4: 69-78.
- Azhari Samsu Baharuddin, Nakamura Kazunori, Suraini Abd-Aziz, Nor' Aini Abdul Rahman, Mohd Ali Hassan, Minato Wakisaka, Kenji Sakai and Yoshihito Shirai, 2009. Characteristics and microbial succession in co-composting of oil palm empty fruit bunch and partially treated palm oil mill effluent. The Open Biotechnology Journal, 3: 87-95

THE RESEARCH OBJECTIVES

1. To evaluate the performance of open and in-house windrow composting treatment for empty fruit bunch (EFB) and palm oil mill effluent (POME) at field scale operation.
2. To determine the microbial succession of empty fruit bunch (EFB)-palm oil mill effluent (POME) compost in conventional open and in- house windrow composting treatment by Denaturing Gradient Gel Electrophoresis (DGGE) and PCR cloning analysis.
3. To develop an accelerated and controlled composting treatment for empty fruit bunch (EFB) and palm oil mill effluent (POME) at semi commercial scale.



THE SEM IMAGE FOR EFB COMPOST. The active microbial degradation had been observed

THE FINAL PRODUCT of EFB Biocompost

Windrow Turner

BIOCOMPOST RESEARCH GROUP

Composting of Oil Palm Mesocarp Fiber by the Enhancement of POME Anaerobic Sludge

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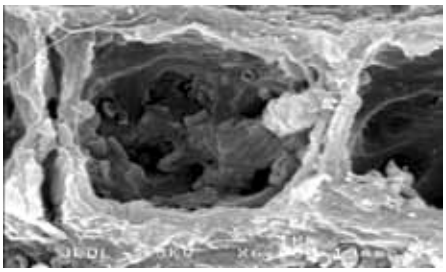
OIL PALM MESOCARP FIBER (OPMF) usually leaves as the solid wastes after oil extraction. It typically used as the burning fuel for boiler in the mills and contribute to electricity generation for mill operation. However, it has been expected that mesocarp fiber will be in excess trend in the mill due to limited boiler capacity and increasing demand in FFB processes. The composting of mesocarp fiber in return could convert this biomass into high value product. Due to cellulosic characteristic, mesocarp fiber could act as a carbon compound to serves as energy sources for microbial maintenance and growth. However, the oily properties (3 – 5%) of the OPMF could inhibit degradation by microbes. The approach in enhancement of composting process by using POME anaerobic sludge could be an appropriate ways to solves oily problem. The composting process could be accelerated with advanced processes by controlling critical parameters such as temperature, oxygen, moisture content, pH and C/N ratio. Whereas, substrates component changes in composting process could be determined by using Fourier Transform Infrared Spectroscopy (FTIR). Moreover, the microbial domination throughout composting process also been identified by using Denatured Gradient Gel Electrophoresis (DGGE) method. ■

THE RESEARCH OBJECTIVES

1. To study the physico-chemical characteristic and microbial degradation in co-composting of oil palm mesocarp fiber with POME anaerobic sludge
2. To investigate the predominant microbial succession in co-composting oil palm mesocarp fiber and POME Anaerobic sludge

PUBLICATION

- Lim Siong Hock, Azhari Samsu Baharuddin, Mohd Najib Ahmad, Umi Kalsom Md Shah, Nor' Aini Abdul Rahman, Suraini Abd-Aziz, Mohd Ali Hassan and Yoshihito Shirai., 2009. Physicochemical Changes in Windrow Co-Composting Process of Oil Palm Mesocarp Fiber and Palm Oil Mill Effluent Anaerobic Sludge. Australian Journal of Basic and Applied Sciences., 3(3): 2809-2816



THE SEM IMAGE OF MESOCARP COMPOST after 50 days. The active microbial degradation had been observed

OPMF BIOCOMPOST after 50 days of degradation

Palm Biomass Biofertilizer

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A TOTAL OF 2.82 MILLION TONES (DRY basis) of empty fruit bunch (EFB), 54.44 million tones of oil palm fronds and 4.29 million of oil palm stems were generated from the palm oil industry in 2008 and 2007-2010, respectively. Realising the potential and abundance of EFB, trunks and fronds as sources of renewable raw materials, a research to produce biofertilizer via co-composting of oil palm fronds and POME anaerobic sludge was initiated. 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. A baseline study on co-composting of oil palm fronds (OPF) and POME anaerobic sludge was undertaken at pilot scale with capacity of 1 tonnes. Different structure of OPF namely chipped and chipped-ground OPF was tested. Physicochemical changes and microbial succession of the compost product was studied successfully. ■

THE RESEARCH OBJECTIVES

1. To study the microbial population during production of palm biomass biofertilizer.
2. To develop rapid open system for oil palm fronds (OPF) composting.

BIOCOMPOST RESEARCH GROUP

Feasibility Study on Co-composting Process of Shredded-Pressed Empty Fruit Bunch (EFB) and Raw Palm Oil Mill Effluent (POME) from Continuous Sterilizer System

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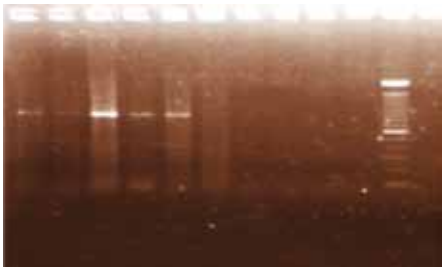
THE DEVELOPMENT OF A CONTINUOUS sterilization process was facilitated by the ability to sterilize bunches using steam at low pressure and without the use of multiple-peak cycles (Sivasothy *et al.*, 2005). Thus, this new continuous sterilization process can solve the problem of continuously transferring bunches to and from the sterilizer as being faced by conventional batch sterilization process. In the conventional milling process, fresh fruit bunches are cooked for about 70-90 min using steam at 40 psig. This process will arrest oil quality deterioration caused by enzymatic activity. It also facilitates the stripping of fruit from fresh fruit bunches and together with the extraction of kernel and oil (Mongana Report, 1955). Meanwhile, in continuous sterilization process, the fresh fruit bunches are sterilized at minimum nozzle pressure of 1.0 BAR and 80 minutes retention time at temperature 90°C as being observed in the palm oil mill which using this system. The differences in sterilizing fresh fruit bunches at difference conditions between conventional batch sterilizer system and new continuous sterilizer system expected to have some effect on the physico-chemical properties for both EFB and POME produced from both system. It is believed these differences will lead to the different co-composting process using the EFB and POME produced from both systems. In order to study the characteristic of the Shredded-Pressed EFB and POME produced from Continuous Sterilizer System, series of proximate analysis will be done which also includes (SEM, Carbon & Nitrogen & Hydrogen & Sulphur Component, Inductively Couple Plasma Test, XRD, FTIR, Cellulose, Hemi-cellulose, Lignin & Silica, Oil & Grease content, pH, DGGE and Color). The research then continues with the feasibility study on co-composting of EFB and raw POME from Continuous Sterilizer in controlled conditions with three conditions:

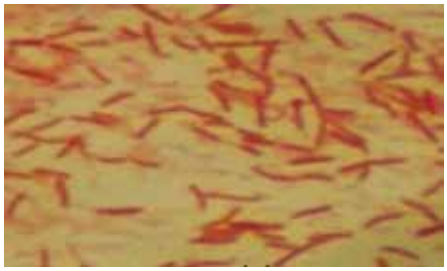
1. the composting process without any accelerant,
2. the composting process using microbial as accelerant and iii) the composting process using thick anaerobic POME sludge as accelerant. ■

THE RESEARCH OBJECTIVES

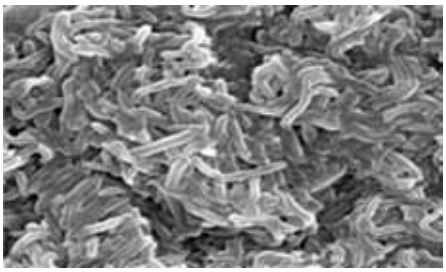
1. To evaluate the composting process using Shredded-Pressed EFB and Raw POME produced from Continuous Sterilizer mill without any accelerant as baseline.
2. To compare the effectiveness between microbial and thick anaerobic POME Sludge as accelerant to the composting process.



PROMINENT MICROBES detected in compost using DGGE



GRAM STAIN TEST of isolated bacteria



SEM MICROGRAPH of isolated bacteria

PRESSED-SHREDDED EFB

EFB COMPOST (day 20)

FINAL MATURED COMPOST

BIOCOMPOST RESEARCH GROUP

PICTURE:
VISITING BACKHUS GMBH COMPANY (DUSSELDORF, GERMANY) to observe the operation of several windrow turning machine use in large-scale composting treatment.

Picture: Bioethanol production in 1.5 L bioreactor using flocculent yeast

BIOETHANOL - BIOBUTANOL RESEARCH GROUP

OVERVIEW

The members of bioethanol group are comprised of six PhD and seven MSc students. Our research is focused on enzymes and bioethanol production from the biomass and other waste products.

Bioethanol production from renewable biomass (lignocellulosic) is an alternative way for producing energy source. Lignocellulose (rice straw, palm oil empty fruit bunches (EFB), mesocarp fiber and sago hampas) can be utilized to its full potential as an alternative cheap substrate through efficient conversion via chemical and/or biological processes. The bioconversion processes involve the use of both physical and chemical methods where the hemicellulose fraction of a lignocellulosic material will be separated by a thermo-chemical treatment prior to use of enzymes to convert the cellulose fraction into sugars.

RESEARCH AREA

The members of bioethanol group are comprised of six PhD and seven MSc students. Our research is focused on enzymes and bioethanol production from the biomass and other waste products.

1. To study the effect of lignocellulosic pretreatment to enhance the production of cellulose degrading enzymes.
2. To convert the biomass into polyoses or fermentable sugars through enzymatic hydrolysis for bioethanol production.
3. To determine and calculate the energy efficiency in a palm oil mill.

PARTNERS

AIST (Advance Institute of Science and Technology), Japan

Kyutech (Kyushu Institute of Technology), Japan

IDEMITSU , Japan

AJINOMOTO CO. INC., Japan

Yayasan Pelajaran Johor (YPJ), Malaysia

BIOETHANOL-BIOBUTANOL RESEARCH GROUP

The Feasibility of Sago Hampas as a Feedstock for Bioethanol Production

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SAGO (*METROXYLON SAGU*) 'HAMPAS' IS the solid waste produced at sago mill as a consequence of starch production. This 'hampas' contains high amount of starch, causing an environmental problem with disposal. This study emphasized on utilizing the trapped starch in sago 'hampas' as a source for glucose production via enzymatic hydrolysis which will later use as substrate for ethanol fermentation.

PUBLICATION

Dayang Salwani Awang Adeni, Suraini Abd-Aziz, Kopli Bujang and Mohd Ali Hassan, 2009. Review: Bioconversion of Sago Residue into Value Added Products. African Journal of Biotechnology, 8(23): xxxx

Initially sago 'hampas' undergo the mechanical pretreatment process - drying, ground and sieved – before used as substrate for enzymatic hydrolysis using commercial enzyme, Dextrozyme. Effect of parameters such as substrate and enzyme concentration, saccharafication reaction time, pH and temperature will be studied on their influence on glucose conversion rate and yield. Higher glucose concentration, > 80 g/L is the main targets for this study as higher substrate load for ethanol fermentation will leads to better downstream processing. Second stage of this studies is to further utilize on glucose produced from sago 'hampas' as a substrate for ethanol fermentation using local isolate yeast. Initially, batch fermentation system will be conducted using shake flask with several parameters will be examined such as temperature, pH, substrate concentration, incubation time, agitations rate and inoculum size. The production of ethanol – concentration in fermentation broth, conversion yield and productivity are the main criteria to be observed in this study. The most vital factors that affect the overall fermentation efficiency will be optimized using Response Surface Method (RSM) by Design Expert®, version 7. ■

THE RESEARCH OBJECTIVES

1. To conduct the preliminary study on the effects of enzymatic hydrolysis of sago 'hampas' for glucose production using response surface methodology (RSM)
2. To study bioethanol production from sago 'hampas' through batch fermentation system utilizing local yeast isolates (*Saccharomyces cerevisiae*)
3. To improve the recovery of bioethanol production from sago 'hampas' in 2L bioreactor

SAMPLING OF SAGO HAMPAS at the sago mill

Cellulosic Bioethanol from Oil Palm Empty Fruit Bunch (OPEFB)

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THE AIM OF THIS STUDY IS TO INVESTIGATE the feasibility of using oil palm empty fruit bunch (OPEFB) as a substrate for high cellulosic bioethanol production. In order to obtain high fermentable sugars by enzymatic hydrolysis, the structure of OPEFB has to be altered or removed using suitable pretreatment method. In this study, steaming and mechanical treatment might be combined to effectively disrupt the cellulosic structure and make it more susceptible to enzymatic hydrolysis. Therefore, the effect of heat treatment using high pressure steam (140°C for 40 psi) generated from the boiler in the mill on OPEFB for subsequent use as substrate for cellulosic bioethanol production as a pretreatment will be investigated. Enzymatic degradation of cellulose to glucose is generally accomplished using cellulases produced by *Phanerochaete chrysosporium*. Apart from that, the analysis of a detailed mass and energy balances will be done for the whole process. ■

THE RESEARCH OBJECTIVES

1. To study the effect of high pressure steam pretreatment on OPEFB for subsequent used as substrate for bioethanol production
2. To obtain high fermentable sugars concentration from enzymatic hydrolysis system produced by *Phanerochaete chrysosporium*
3. To optimize bioethanol production by local yeast isolated using Response Surface Methodology (RSM)
4. To analyse a detailed mass and energy balances for the whole process

BIOETHANOL-BIOBUTANOL RESEARCH GROUP

Cellulases Production on OPEFB using Solid State Fermentation

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LIGNOCELLULOSE CAN BE UTILIZED TO ITS full potential as an alternative cheap substrate through efficient conversion via chemical and/or biological processes. Huge amount of renewable biomass (trunks, fronds, shells, palm press fiber and the empty fruit bunches) are produced annually in palm industries. Insolubility of OPEFB is one of the limitations in submerged fermentation. SSF is capable in producing certain enzymes and metabolites that usually produced with low yield in submerged fermentation. Lignocellulose degrading fungi that has been used in this project is locally isolated *Botryosphaeria rhodina*. All these enzymes can be used to convert lignocellulosic materials into biofuels, particularly cellulosic bioethanol. The objective of this study is to monitor the cellulases production and the microbial growth on OPEFB by *Botryosphaeria rhodina* in 7 days incubations. In this research, the solid state fermentation will be characterized using the optimized condition (constant environmental condition) based on Response Surface Methodology (RSM) from previous work. ■

THE RESEARCH OBJECTIVES

1. To study the effects of substrate particle size on cellulases production by *Botryoshpaeria rhodina* in solid state fermentation
2. Optimization of cellulases production by *Botryoshpaeria rhodina* in solid state fermentation using response surface methodology (RSM)
3. To study the kinetics of cellulases production and biodegradation of OPEFB in solid state fermentation system.

Production of Xylose from Oil Palm Empty Fruit Bunch through Enzymatic Hydrolysis

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OIL PALM EMPTY FRUIT BUNCH (OPEFB) IS one the most abundant lignocellulosic waste produced throughout the year in palm oil industry. It is well known for its potential as renewable resources for the production of liquid fuel, pharmaceuticals, foods and chemical feedstock. Lignocellulose OPEFB consists of cellulose, hemicellulose and lignin. The pentose fraction, composed of D-xylose which is usually not less than 95% is the highest fraction in OPEFB (Winklehausen and Kuzmanova, 1998; Rahman *et al.*, 2006). Since, hemicellulose is more easily hydrolyzed than cellulose, it offer the possibility of producing D-xylose and xylitol. Previously, most of the biomass conversion was focused on the ethanol production. The xylitol production has always been closely related to ethanol fermentation, however, it was considered only as a by product. Nowadays, in food and pharmaceutical industries, xylitol has gained considerable attention as it has useful characteristics, including the used for insulin-independent diabetics and as a natural food sweetener (Tada *et al.*, 2004). Chemical treatment such as sulfuric acid hydrolysis has been used to extract xylose from lignocellulose. However chemical process will results in environmental problems. Therefore, the enzymatic hydrolysis is the good practice to avoid this problem. In this study, xylanase producing microorganisms will be screened and isolated from environmental sample and xylanase gene will be isolated and transformed into the *Escherichia coli*. ■

PRETREATMENT of lignocellulosic material

THE RESEARCH OBJECTIVES

1. To screen and isolate microorganism producing xylanese from EFB compost
2. To develop recombinant xylanase for in the *Escherichia coli*.
3. To study the effect of enzyme hydrolysis on biomass after disc milling pre-treatment of OPEFB

BIOETHANOL-BIOBUTANOL RESEARCH GROUP

Utilization of Palm Oil Biomass for Value Added Products

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THE RAPID GROWTH IN THE CULTIVATION of oil palm in Malaysia, resulted the increased amount of oil palm biomass such as empty fruit bunch, trunks, pruning of fronds during harvesting, palm press fiber and shell were generated. Utilization of EFB for value added products is an elegant way to dispose this biomass while generating additional income and increase the economy benefit for the industry. Study on the effect of sterilization process during mill operation was carried out to investigate the effectiveness of sterilization in an ordinary palm oil processing industry for cellulose structure alteration in promoting the hydrolysis of EFB fibers to fermentable sugar for bioethanol production. The potential of steam in the sterilizer is considered as a zero cost pre-treatment method for the effective utilization of EFB biomass to produce value-added products to the palm oil industry. The conversion of EFB into fermentable sugar by enzymatic saccharification and yeast fermentation for bioethanol production were carried out using batch and repeat batch system. Mesocarp fiber and decanter cake will be used as comparison study. The study on mass balance on processing FFB to produce CPO was also carried out. At present, most of the palm oil industries in Malaysia operate a cogeneration system using palm oil waste (fiber and shell) as fuel in a boiler to produce high pressure steam which is expanded through a steam turbine to produce electricity. The low pressure steam is used in the manufacturing process for sterilization, digestion, purification and also for temperature control. The energy conversion from residual biomass as an alternative energy source during the processing gave an advantage in the reduction of these materials before dispose to environment. The measurement of energy content on palm oil biomass for fuel boiler and assessment on the actual energy requirement and generated in palm oil mill operations were carried out to determine the potential of excess biomass for bioenergy production. The study on energy balance in the mill operation is essential to determine the potential operation system or alternative methods to save the energy. The recuperation and recycling of steam discharged from sterilizer are applicable to gain this potential energy. The use of potential recycle steam in the evaporation system of EFB hydrolysate from enzymatic saccharification for bioethanol production is an alternative in energy saving for other potential bioenergy production. Analysis of water usage in the mill operation is essential to determine the total volume of POME generated and energy requirement for generating waste from the mill. ■

THE RESEARCH OBJECTIVES

1. To conduct the preliminary study on the effects of enzymatic hydrolysis of sago 'hampas' for glucose production using response surface methodology (RSM)
2. To study bioethanol production from sago 'hampas' through batch fermentation system utilizing local yeast isolates (*Saccharomyces cerevisiae*)
3. To improve the recovery of bioethanol production from sago 'hampas' in 2L bioreactor

PUBLICATION

Khanom Simarani, Mohd Ali Hassan, Suraini Abd-Aziz, Minato Wakisaka, and Yoshihito Shirai, 2009. Effect of Palm Oil Mill Sterilization Process on the Physicochemical Characteristics and Enzymatic Hydrolysis of Empty Fruit Bunch. Asian Journal of Biotechnology, 1(2): 57 -66

Bioethanol Production from Glycerine Wastes using Locally Isolated Bacteria

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TO DATE, SURPLUS OF GLYCEROL GENERATED as byproduct of biodiesel fuel & oleochemical production become one of the environmental issue due to its disposal problem. Hence, this issue increase demand for new applications for this abundant carbon source. Many microorganisms identified to be able in utilizing glycerol as carbon source. Microbial fermentation of glycerol considered as promising way to convert glycerol into valuable products. This is due to several factors such as cheaper price, high availability, greater degree of reduction than sugars and higher yields of reduced chemicals. In this study, potential bacteria that have capability in producing ethanol from glycerol are isolated from environment. Understanding on the related metabolic pathways that involved in glycerol fermentation is important for future study to enhance production. Glycerin wastes that is used in this study is discharged from biodiesel production, theoretically containing high glycerol content, high salt content, high pH and many other impurities. Hence, the effect of these impurities on glycerol fermentation is important on the ethanol production. Optimization on the fermentation condition is needed to get the optimal condition for microbial conversion of glycerol in order to enhance ethanol production. ■

THE RESEARCH OBJECTIVES

1. To isolate potential glycerol-fermenting microbes for ethanol production
2. To optimize the microbial conversion of crude glycerin for ethanol production by statistical approach (RSM)

BIOETHANOL-BIOBUTANOL RESEARCH GROUP

Bioethanol Production from Rice Straw using Local Microorganism through Three-Phase System

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PADDY INDUSTRIES ARE EXPANDING AS the request for food increase. However, this large industry produces a massive amount of biomass waste mainly rice straw. But through biotechnology, the value of rice straw can be improved. As a biomass, rice straw possesses the ability to be degraded into monosaccharide through the usage cellulases. Rice straw also can be used as the substrate to produce cellulase enzyme. So it is like three-phase conversion in one system;

i. from rice straw to cellulase,
ii. rice straw to monosaccharide and,
iii. monosaccharide to bioethanol.

THE RESEARCH OBJECTIVES

1. To study the effects of various pretreatment on rice straw composition and structure.
2. To produce high activity crude cellulase enzyme from rice straw using local fungi isolates.
3. To produce bioethanol from rice straw in three phase system using local microorganisms.

Additional highly nutritious (for microorganisms) biomass such as Palm Oil Mill Effluent (POME) was also incorporated in this project to further enhance the conversion at the same time, reduce the cost for additional supplement. It was a hope that one day the paddy industry and farmers will obtain additional profit through selling of rice straw and most importantly save the planet by mitigating the environment pollution from rice straw burning. ■

PUBLICATION

Ahmad Muhaimin Roslan, Mohd Ali Hassan, Suraini Abd Aziz and Phang Lai Yee.2009. Effect of Palm Oil Mill Effluent Supplementation on Cellulase Production from Rice Straw by Local Fungal Isolates. International Journal of Agricultural Research, 5: 185-192.

Microbial Conversion of Oil Palm Empty Fruit Bunch to Fermentable Sugars for Bioethanol Production

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PALM OIL IS ONE OF THE MAIN agricultural products in Malaysia, and it is producing more than 50% of the world's crude palm oil which is exported all over the world as raw material for chemical industries. OPEFB is obtained by stripping off the fresh oil palm fruit bunch. However, one of the serious problems in palm fruit processing is the managing of the wastes generated throughout the processes. In the process of extraction of palm oil from oil palm fruit, a lignocellulosic material oil palm empty fruit bunch (OPEFB) biomass waste is generated annually throughout Malaysia by palm oil mills. At present, OPEFB are mainly incinerated to produce bunch ash to be distributed back to the field as fertilizers. This conventional method of burning these residues often create environmental problems as it generates severe air pollution and is prohibited by the Environment Protection Act. Therefore, as an effort to overcome this problem, extensive research has been done to convert lignocellulosic material such as OPEFB into value added products such as feedstock of activated carbon, source of pulp, fermentable sugars and biofuel. This project emphasized on conversion of OPEFB into bioethanol using locally isolated fungi as a source for cellulase enzyme. Potential cellulase enzyme producers were screened and isolated from various natural sources such as rotten oil palm bunch, dead plant and soil. From the screening and isolation process, two potential fungi were identified. Saccharification of OPEFB was done in order to evaluate the effectiveness of cellulase enzyme from fungal pure culture, mixed fungal culture and mixed cellulase enzyme. From the observation, mixed cellulase enzyme showed highest yield and concentration of reducing sugar compared to other. Reducing sugars obtained from saccharification process will be used as a substrate for bioethanol production in later stage. ■

THE RESEARCH OBJECTIVES

1. To screen, isolate and identify cellulolytic fungi from rotten oil palm bunches for production of cellulase enzymes.
2. To select appropriate cellulolytic microbial mixed culture for the production of cellulase enzymes.

PUBLICATION

Nurul Kartini, Suraini Abd-Aziz, Mohd Ali Hassan and Farinazleen Mohd. Ghazali. 2009. Isolation and Selection of Appropriate Cellulolytic Mixed Microbial Cultures for Cellulases Production from Oil Palm Empty Fruit Bunch (Submitted to Biotechnology, Manuscript no: 16667-BTC-ANSI)

BIOETHANOL-BIOBUTANOL RESEARCH GROUP

Scale-up of Cellulases Production using Oil Palm Empty Fruit Bunch (OPEFB)

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OIL PALM EMPTY FRUIT BUNCH CONSIST mainly of lignin, hemicelluloses and cellulose that made it suitable for production of bioethanol from agricultures waste. Production of bioethanol by agro waste like OPEFB not only benefit to automotive industry as alternative fuel, but also can be used in chemical industry and food industry as a substitute of current ethanol utilization. Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze the cellulolysis (or hydrolysis) of cellulose. In this study, oil palm empty fruit bunch (OPEFB) were subjected to chemical and microbial pretreatment for bioconversion of lignocellulosic biomass to fermentable sugars. After the delignification process, solid state fermentation for cellulases production by *Aspergillus fumigatus* UPM2 will be conducted.T ■

THE RESEARCH OBJECTIVES

1. Delignification of Oil Palm Empty Fruit Bunch (OPEFB) by Using Chemical and Microbial Pretreatment Method
2. Optimization of solid state fermentation for cellulases production using pretreated OPEFB by *Aspergillus fumigatus* UPM2
3. Production of cellulases using optimized condition in rotary drum bioreactor.

PUBLICATION

Ahmad Fadhlan Hamisan, Suraini Abd-Aziz, Kamarulzaman Kamaruddin, Umi Kalsom Md Shah, Neelam Shahab and Mohd Ali Hassan, 2009. Delignification of Oil Palm Empty Fruit Bunch (OPEFB) using Chemical and Microbial Pretreatment Methods. International Journal of Agricultural Research, 4: 250-256

Production of Biobutanol as Alternative Biofuels from Palm Oil Empty Fruit Bunches (OPEFB) as Main Substrate using *clostridium sp.*

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BUTANOL ($C_4H_{10}O$) OR BUTYL ALCOHOL is an alcohol that can be used as a solvent or fuel produced from biomass by a microbial fermentation. Biobutanol 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 biobutanol can be produce at lower cost. This study is focusing on the production of biobutanol from OPEFB. The OPEFB is converted into fermentable sugar by using enzymatic hydrolysis and undergoes anaerobic fermentation by locally isolated *Clostridium butyricum* (EB 5) for biobutanol production. All the samples are subjected for butanol concentration analysis using gas chromatography (GC), glucose concentration analysis using high performance liquid chromatography (HPLC) and cells concentration using optical density (OD) method together with dry cell weight (DCW). The production of biobutanol will be optimized through response surface methodology (RSM) approach. ■

THE RESEARCH OBJECTIVES

1. To produce biobutanol from oil palm empty fruit bunches (OPEFB) using *Clostridium butyricum*
2. To optimize biobutanol production from oil palm empty fruit bunches (OPEFB) by *Clostridium butyricum* using response surface methodology (RSM) approach

ETHANOL MEASUREMENT using Gas Chromatography (GC)

BIOETHANOL-BIOBUTANOL RESEARCH GROUP

Biobutanol Production as Sustainable Biofuel using Oil Palm Decanter Cake

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THE RESEARCH OBJECTIVES

1. To saccharify chemical and physical treated oil palm decanter cake using enzymes cocktail
2. To develop high productivity fermentation system for the production of biobutanol from the oil palm decanter cake polyoses using *Clostridium butyricum* (EB 5)

THE FLUCTUATING OIL PRICES AND depletion of fossil fuels initiated intensive research and commercialization activities toward the production of renewable fuels such as ethanol and butanol. Biobutanol is a liquid fuel superior to bioethanol that can be produced from renewable biomass by the acetone-butanol-ethanol (ABE) microbial fermentation process. Malaysia as world's largest producer of crude palm oil produced abundance of renewable biomass resources including decanter cake and oil palm empty fruit bunches (OPEFB). In previous researches shown that *Clostridium acetobutylicum* used greatly in the production of biobutanol from fermentable sugar in anaerobic fermentation system. Pretreatment study of lignicellulosic material is important to increase the efficiency of enzyme to hydrolyze cellulose and hemicelluloses into polyoses. Chemical and heat pretreatment can cause swelling effect and break lignin layer. Production of enzymes cocktail with high cellulases activity from locally isolated fungus to saccharify oil palm decanter cake polyoses is the intermediate step on the production biobuanol. Production of biobutanol in anaerobic fermentation by *Clostridium butyricum* (EB 5) using oil palm decanter cake polyoses also analyze. ■

STEAM STERILIZER in palm oil mill

Production of Biobutanol from Sago Pith Residue

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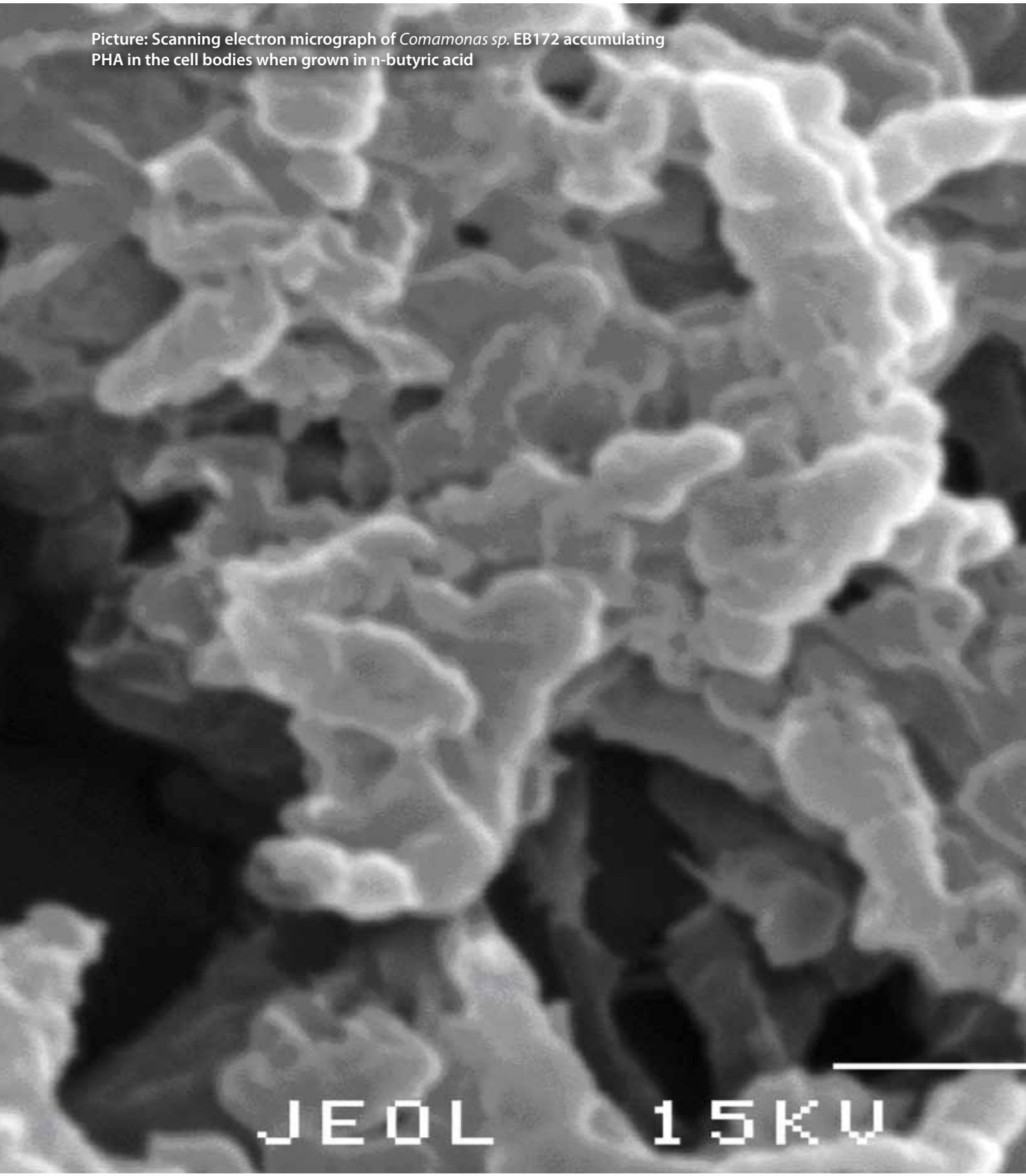
THE RESEARCH OBJECTIVES

1. To improve pretreatment stage utilizing local microbial enzyme on the conversion of sago pith residue (lignocellulosic biomass) into high yield of fermentable sugars
2. To convert the fermentable sugars obtained to biobutanol using *Clostridium acetobutylicum.*

THE AGRICULTURAL INDUSTRY PRODUCES a significant amount of post-processing waste and residue. Particularly in Malaysia, the palm oil and sago starch industries are responsible for the production of a notable amount of agro-residues. Sago pith residue is abundantly and cheaply available especially in state of Sarawak, Malaysia. This residue is the fibrous wasted that left behind after starch extraction process and contains significant amount of starch, some cellulose, hemicellulose and lignin. The presence of the lignocellullosic material (cellulose, hemicelluloses and lignin) in sago pith residue becomes a strong pollutant to our environment. However, this residue has a potential as economical substrate for the production of biobutanol as an alternative fuel. Biobutanol means butyl alcohol (butanol) that can be produced fermentatively from sugar or starch of agricultural crops and plant cellulose by *Clostridium acetobutylicum*. 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' will be grind and sieved by 2 mm screen before undergo enzymatic hydrolysis using Dextrozyme. The solid part (cellulose) obtained from the enzymatic hydrolysis using Detrozyme will be used as substrate in this study. This cellulose will be converted to fermentable sugars by celluclast and enzymes produced by locally isolated fungi. The fermentable sugars obtained will be used for biobutanol fermentation. ■



Picture: Scanning electron micrograph of *Comamonas sp.* EB172 accumulating PHA in the cell bodies when grown in n-butyric acid

BIOPLASTIC RESEARCH GROUP

OVERVIEW

Our aim is to convert wastes such as palm oil mill effluent (POME) and kitchen refuse into organic acids, before utilizing the acids in fermentation for producing more valuable products, i.e. bioplastics. From the wastes, we are able to produce mainly four types of organic acids namely acetic, butyric, propionic and lactic acids. These acids can be used to produce polyhydroxyalkanoates (PHAs) or better known as bioplastics. In the PHA production, we use our own locally isolated bacterium, *Comamonas sp.* EB172 which has been identified as PHA producer, with acid-tolerant characteristic. This bacterium has ability to accumulate up to 90% of PHA inside its body.

RESEARCH AREA

Our tagline is "**Bioconversion of organic wastes into bioacids and bioplastics**". At present, we focus on three types of organic waste residues; POME, kitchen waste, and sap from oil palm fronds. There are four main research activities in this group:

1. **Pretreatment of wastes:** This activity includes physical, chemical and biological treatments of the organic wastes in order to convert the wastes into bioacids.
2. **Strain improvement:** This involves strain improvement of our locally isolated bacterium *Comamonas sp.* EB172 by genetically engineering techniques. Strain improvement is targeted at producing recombinant bacterium which has capability in producing higher quality biopolyesters than wild type bacteria.
3. **Upstream processing:** Upstream processing involves the production of PHAs via fermentation using organic acids produced from the wastes as substrate. Lab scale fermentations are carried out in 2 and 7 L fermenters. Biopolyesters such as poly(3-hydroxybutyrate) P(3HB) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) have been successfully obtained by *Comamonas sp.* EB172.
4. **Downstream processing:** This process involves the development of free-solvent extraction methods for PHA recovery, for example by using water and sodium hydroxide. This is important to provide an environmental friendly PHA extraction and purification process.

Incorporation of PHA production with methane production from palm oil mill effluent treatment for power generation in Clean Development Mechanism (CDM) is promising in near future whereas total PHA production cost might reduce to half. This strategy could promote widely use of PHAs and better market for PHAs. The target applications for the PHAs produced are low end-material products like packaging (food container, film/ sheet in plantation) or high end-value products like suture and artificial heart valve. The application of PHA in near future could be expanded by choosing various types of microorganism and utilizing other sustainable renewable carbon sources that produces different types of polyesters with properties ranging from brittle and stiff to elastomeric rubber-like materials.

PARTNERS

Universiti Putra Malaysia, Malaysia
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Kyutech (Kyushu Institute of Technology), Japan
Standards & Industrial Research Institute of Malaysia (SIRIM), Malaysia
Massachusetts Institute of Technology (MIT), US
Lembaga Kemajuan Tanah Persekutuan (Felda), Malaysia

BIOPLASTIC RESEARCH GROUP

Scale-up Production of Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) by *Comamonas sp*. EB 172 from Palm Oil Mill Effluent

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THE RESEARCH OBJECTIVES

1. To develop process to recover and clarify volatile organic acids from anaerobically treated POME in pilot scale
2. To develop feeding strategy for the production of PHBV in 2L bioreactor
3. To scale-up the production of PHBV in 50 L bioreactor

AMONG THE BIODEGRADABLE PLASTIC polyhydroxyalkanoates (PHAs), PHBV is the most promising and has great potential as a biodegradable substitute for bulk plastics. The properties of PHBV largely depends on the ratio of 3-HB to 3-HV. A low 3-HV contents leads to hard and brittle polymers, resembling polystyrene or PVC. A high 3-HV content, on the other hand, increases the strength and flexibility of the polymer leading to thermoplastic behaviour resembling polyethylene. However, high production costs associated with substrate have limited the production of this polymer in large scale. In recent years, the use of organic wastes, in combination of anaerobic and aerobic steps are being studied as an alternative substrate for PHA production. Organic wastes having complex composition cannot be directly utilized by microbes for polymer biosynthesis. The aim of the project is to develop a recovery process for mixed organic acids from anaerobically treated POME in order to use as a substrate for PHA production. The study also aimed at developing feeding strategy using pH-stat in 2L bioreactor and upscale the process in 50-L bioreactor using locally isolated bacteria, *Comamonas sp*. EB 172. The utilization of organic acid mixtures such as acetic acid, propionic acid and butyric acid from POME as carbon and energy sources is helpful in reducing the otherwise costly disposal of the waste with the concomitant production of an environmentally friendly biodegradable plastic. Characterization of recovered plastic will also be included as part of the overall study. ■

PUBLICATIONS

- Tabassum Mumtaz, Suraini Abd-Aziz, Nor'Aini Abdul Rahman, Phang Lai Yee, Yoshihito Shirai and Mohd Ali Hassan, 2009. Fed-batch production of P(3HB-co-3HV) copolymers by Comamonas sp EB 172 using mixed organic acids under dual nutrient limitation. European Journal of Scientific Research. 33(3): 374-384
- Tabassum Mumtaz, Suraini Abd-Aziz, Nor'Aini Abdul Rahman, Phang Lai Yee, 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(21): 3900-3905

Improved Recovery of Polyhydroxyalkanoates from Renewable Resources

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THE RESEARCH OBJECTIVES

1. To study the effects of sodium hydroxide and water during extraction in PHA recovery process
2. To optimize the PHA recovery process using response surface methodology (RSM)
3. To develop an alternative process for PHA recovery by including freezing
4. To develop mass and energy balances for PHA recovery

PHAs 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. EB172 will be conducted using synthetic and mixed organic acids obtained by anaerobically treated palm oil mill effluent (POME) which replaced the costly conventional carbon substrate. PHA from Comamonas sp. EB172 will be recovered by using methods including solvent, chemical and mechanical extraction. Addition of purification step would be added to the process in order to obtain higher recovery and purity. 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 infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). ■

BIOPLASTIC RESEARCH GROUP

Metabolic Engineering of *phaZ* and *phaC* genes in *Comamonas putranensis* for Polyhydroxyalkanoates (PHAs) Production

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CURRENTLY MY RESEARCH STUDIES ARE related to genetic improvement of polyhydroxyalkanoate (PHA) producer and production of PHA using aerobic condition. The local strain, *Comamonas putranensis*, obtained from palm oil mill effluent (POME) sludge is the bacterium which was able to synthesize poly(3-hydroxybutyrate-*co*-hydroxyvalerate) (PHBV) by utilizing clarified acids from anaerobically treated POME. By isolating and sequencing the genes such as phaA, phaB, phaC and phaZ, we can study the genes involved in the biosynthesis and biodegradation of PHBV and later to produce recombinant bacteria with superior ability than wild type. Insertion and deletion techniques are the methods that I am going to apply in generating my recombinant bacteria with higher yield and productivity. ■

THE RESEARCH OBJECTIVES

1. To study heterologous expression of *phaZ* and *phaC* genes in *Escherichia coli* and *Cupriavidus necator* PHB-4.
2. To construct recombinant *Comamonas putranensis* by deleting *phaZ* gene and increasing copy numbers of *phaC* gene.
3. To determine PHA production using constructed recombinant *Comamonas putranensis.*

Appropriate Recovery Methods of Intracellular Polyhydroxyalkanoates from *Comamonas putranensis*

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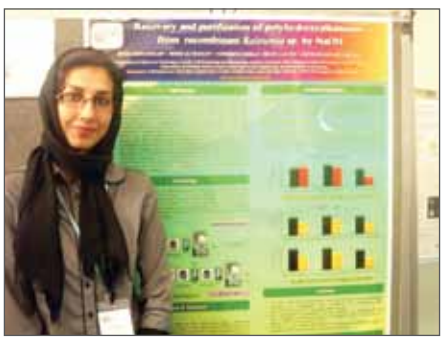
IN RESPONSE TO PROBLEMS ASSOCIATED with plastic waste and its effect on the environment, there has been considerable interest in the development and production of biodegradable plastics. Polyhydroxyalkanoates (PHAs) are a group of carbon and energy storage compounds that are accumulated during suboptimal growth by many bacteria, and intracellularly deposited in the form of inclusion bodies. Due to the similar mechanical roperties to synthetic plastics, complete biodegradability, stability and durability, PHAs ave been drawn much attention for commercial application. But their production on industrial scale is limited by the high cost compared to conventional plastics. The cost of PHAs includes mainly substrate and downstream processing cost. Therefore, developing a process that allows a simple, efficient and less polluted recovery of PHAs is an attractive proposition. Using of organic solvent extraction, chemical reagent or surfactant methods for PHA recovery have the drawback of high cost or serious pollution, and are difficult to be industrialized. It is apparent that chemical treatments such as extreme alkaline pH increase the permeability of the cell, causing partial protein release but not cell breakage. Mechanical cell disruption by high pressure homogenization is currently the general method of choice for the large scale disruption of micro-organisms. Uniform exposure of the microbial cells and a minimal exposure of solubilized protein to high shear forces, in order to obtain high yields is the goal of this method. Also, ultrasonication has been a widely used, successful method for cell disruption due to its speed, ease and cleanliness, and ability to lyses a range of cells. In other hands, combinations of chemical and mechanical methods sometimes can produce satisfactory results whereas one method alone fails. Therefore, in this study, various non-solvent materials and physical methods (high pressure homogenization and ultrasonication) will be used for recovery of different intracellular PHA content and composition from *Commamonas putranensis* in single and multiple systems. ■

THE RESEARCH OBJECTIVES

1. To study the effect of using single and mixed recovery methods on PHA recovery.
2. To study the effect of different intracellular PHA content and composition on recovery process.
3. To optimize the PHA recovery process by using the response surface methods (RSM).



BIOPLASTIC RESEARCH GROUP

Biosynthesis of Polyhydroxyalkanoates (PHA) using Organic Acids from Anaerobically Treated POME by Local Isolated Bacteria

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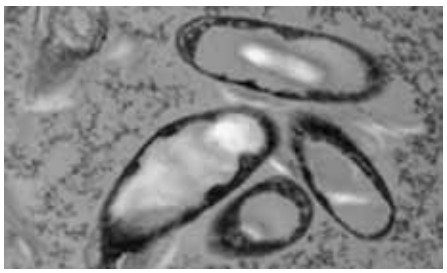
POLYHYDROXYALKANOATE (PHA) IS naturally green, biodegradable and biocompatible thermoplastics. Poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P3HB-*co*-3HV) copolymer is well studied polymers in PHA family. Their physical and thermal properties are similar to petroleum derived plastics polypropylene (PP) and polyethylene (PET). The incorporation of 3-hydroxyvalerate (3HV) in the (P3HB-*co*-3HV) copolymer is triggered when suitable precursor is added in the cultivation medium. One of the precursors for obtaining (P3HB-*co*-3HV) copolymer is when propionic acid alone or in combination with other acids is added into the cultivation medium. Bioconversion of bio-acids (acetic, propionic and butyric) from palm oil mill effluent (POME) was performed under anaerobic condition. These acids will be used for biosynthesis of PHA using locally isolated strain *Comamonas* sp. EB172. Since the toxicity caused by the acid especially propionic acid will inhibit the growth and PHA production of bacterium, search for acido-tolerant bacterium was performed using enrichment and microscopy methods. The colonies obtained from nitrogen deficient medium was stained by Nile Blue A staining and was observed under fluorescent microscope. The positive colonies containing PHA exhibited strong orange color and will undergo several tests to confirm the speculation. Biochemical test was performed using BIOLOG, and API test kits (BioMeriux, France). Other tests (cellular fatty acids composition, polymerase chain reaction, G+C content and antibiotics) were performed to determine the genus and species level of the isolated bacterium. Since the strain obtained was unique in term of biochemical, acido-tolerant and ability to produce PHA, a new name *Comamonas putranensis* sp. nov., is proposed. ■

THE RESEARCH OBJECTIVES

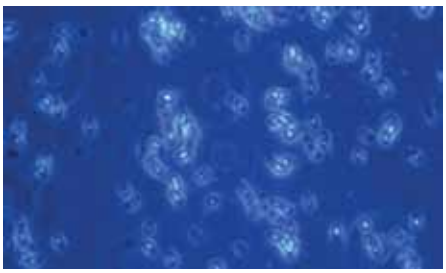
1. To isolate and screen PHA accumulating bacteria from environment using organic acids
2. To characterize the isolated strains in terms of phenotypic and genotypic approaches (polyphasic)
3. To optimize growth and PHA production in shake flasks and fermenter
4. To characterize physical and thermal properties of PHA obtained

PUBLICATIONS

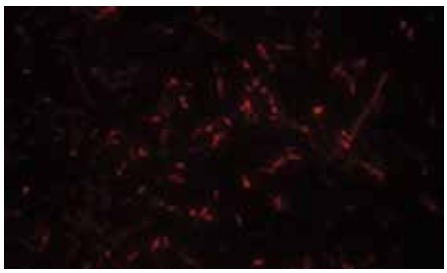
- (Patent Pending- PI- 20092761) Zakaria MR, Suraini AA, Farinazleen MG, and Hassan MA. *Comamonas putranensis* sp. nov., a novel poly(β-hydroxyalkanoates) producer isolated from digester -treated palm oil mill effluent.
- Mohd Rafein Zakaria, Suraini Abd-Aziz, Nor' Aini Abdul Rahman, Phang Lai Yee, and Mohd Ali Hassan, 2008. Comamonas sp. EB172 Isolated from Digester Treating Palm Oil Mill Effluent as Potential Polyhydroxyalkanoates (PHA) Producer. African Journal of Biotechnology, 7(22): 4118-4121
- Mohd Rafein Zakaria, Meisam Tabatabaei, Farinazleen Mohamad 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 Biotecnology, DOI: 10.1007/s11274-009-0232-y
- 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, DOI: 10.1016/j.polymdegradstab.2010.01.020



TEM IMAGE OF *COMAMONAS SP.* EB172 acculates P(3-HB-*co*-3HV) copolymers using organic acids from anaerobically treated palm oil mill effluent



PHA ACCUMULATING *COMAMONAS SP.* EB172 under phase contrast microscope.



PHA ACCUMULATING *COMAMONAS SP.* EB172 under fluorescence microscope.

BIOPLASTIC RESEARCH GROUP

Optimization of Organic Acids Production from Simulated Kitchen Waste using Response Surface Methodology (RSM)

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SIMULATED KITCHEN WASTE WAS developed for organic acids production in order to overcome the problem of kitchen waste variation composition,Simulated kitchen waste was subjected to anaerobic digestion by the fermented natural microflora. Organic acids production from simulated kitchen waste fermentation was then optimized using response surface methodology (RSM). Central composite design (CCD) was employed to determine the maximum organic acids production for process variables such as temperature, initial pH and inoculum size. The optimum temperature, initial pH and inoculums size for the acidogenic fermentation of organic acids from kitchen waste were 35°C, initial pH 6.0 and 20%, respectively. The maximum organic acid produced was 77 g/L. A satisfactory fit model was obtained in this study. Organic acids production was significantly affected by pH and temperature and the interaction between them. Organic acids produced from the simulated kitchen waste were then compared with real kitchen waste using the optimum conditions. Lactic acid showed the main organic acids detected in the kitchen waste fermentation (more than 80%) followed by acetic acid and butyric acid. The production of organic acids has potential to be used as substrates for the production of biopolymer. ■

THE RESEARCH OBJECTIVES

1. To develop simulated kitchen waste for organic acids production
2. To optimize organic acids production from kitchen waste using response surface methodology (RSM)

Recovery of Organic Acids from Food Wastes for Polyhydroxybutyrate (PHB) Production

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THE PRODUCTION OF ORGANIC ACIDS from kitchen waste by anaerobic digestion has been reported widely as well as its potential applications in industries. Currently, many researches being conducted on the uses of organic waste as an alternative substrate for polyhydroxyalkanoates (PHA) production. Industrially, the critical stage of the organic acids production is mainly downstream process including separation, extraction and purification where it consumes almost 60% of the overall production cost. Therefore, the main objective of this study is to develop the integrated recovery process for organic acids from kitchen waste. The organic acids were recovered by using the integrated method which consisted of freezing and thawing, centrifugation, filtration and evaporation. Using freezing and thawing alone, organic acids could be concentrated up to 20% and almost 66% of total suspended solids could be eliminated. The material balance for the recovery process was also developed. The recovered organic acids were then utilized by *Comamonas putranensis* for polyhydroxybutyrate (PHB) production in aerobic fermentation using shake flask. Using the acids from kitchen waste as a substrate, about 40% of PHB was accumulated in the cells ■

THE RESEARCH OBJECTIVES

1. To produce and recover organic acids from fermented food wastes in 50L bioreactor
2. To produce polyhydroxy-butyrate (PHB) utilizing the recovered organic acids from fermented food wastes using *Comamonas putranensis* in 15L bioreactor

PUBLICATION

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): 5807-5813

BIOPLASTIC RESEARCH GROUP

Production of PHA from Oil Palm Fronds (OPF)

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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 fronds (OPF) 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. This separation will include the shredding, peeling, filtering, squeezing and pressing method. At this stage, characterizations of sugar composition in the juice will also been carried out. Further pretreatment by centrifugation and filter 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 several types of bacteria such as C. *necator* CCUG52238, *Comamonas putranensis*, Bacillus sp. and Pseudomonas sp. 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 fronds. ■

THE RESEARCH OBJECTIVES

1. To determine appropriate methods for OPF pretreatment and sugars extraction from OPF
2. To optimize the production of polyhydroxyalkanoates (PHAs) using sugars from OPF in shake flasks and 2 L bioreactor
3. To scale-up the fermentation process from laboratory scale (2 L) to pilot scale (50L) and to optimize the process with respect to the process parameters

Optimization of Polyhydroxyalkanoates (PHAs) Production by *Comamonas sp.* EB172

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POLYHYDROXYALKANOATES (PHAS) ARE polyester produced naturally inside bacteria as their conserve energy. Bacteria will produce PHAs when growth in suitable media which is has excess carbon and has deficiency conditions such as limited macro elements (e.g. nitrogen, phosphorus and oxygen). One of the problems in production of PHAs is high cost that contributes from the substrate and the condition in the bioprocessing. Optimization is needed to reduce the cost by find the optimal concentration for each composition in the media and optimal condition for higher accumulation of PHAs by the bacteria. Production of PHAs will be optimizing by using response surface methodology (RSM). RSM is statistical software for optimization by interaction more than 2 parameters and determine the optimal value for each parameters. The kinetic study is to optimize the fermentation by develop the equation and simulation for production and growth of the *Comamonas putranensis*. ■

THE RESEARCH OBJECTIVES

1. To optimize the production of PHA from organic acids by *Comamonas sp.* EB172
2. To study the kinetic of PHA production by *Comamonas sp.* EB172 in 2 L bioreactor

BIOPLASTIC RESEARCH GROUP



INTERNATIONAL CONFERENCE ON BIO-BASED POLYMERS 11-13 Nov 2009 at USM, Penang

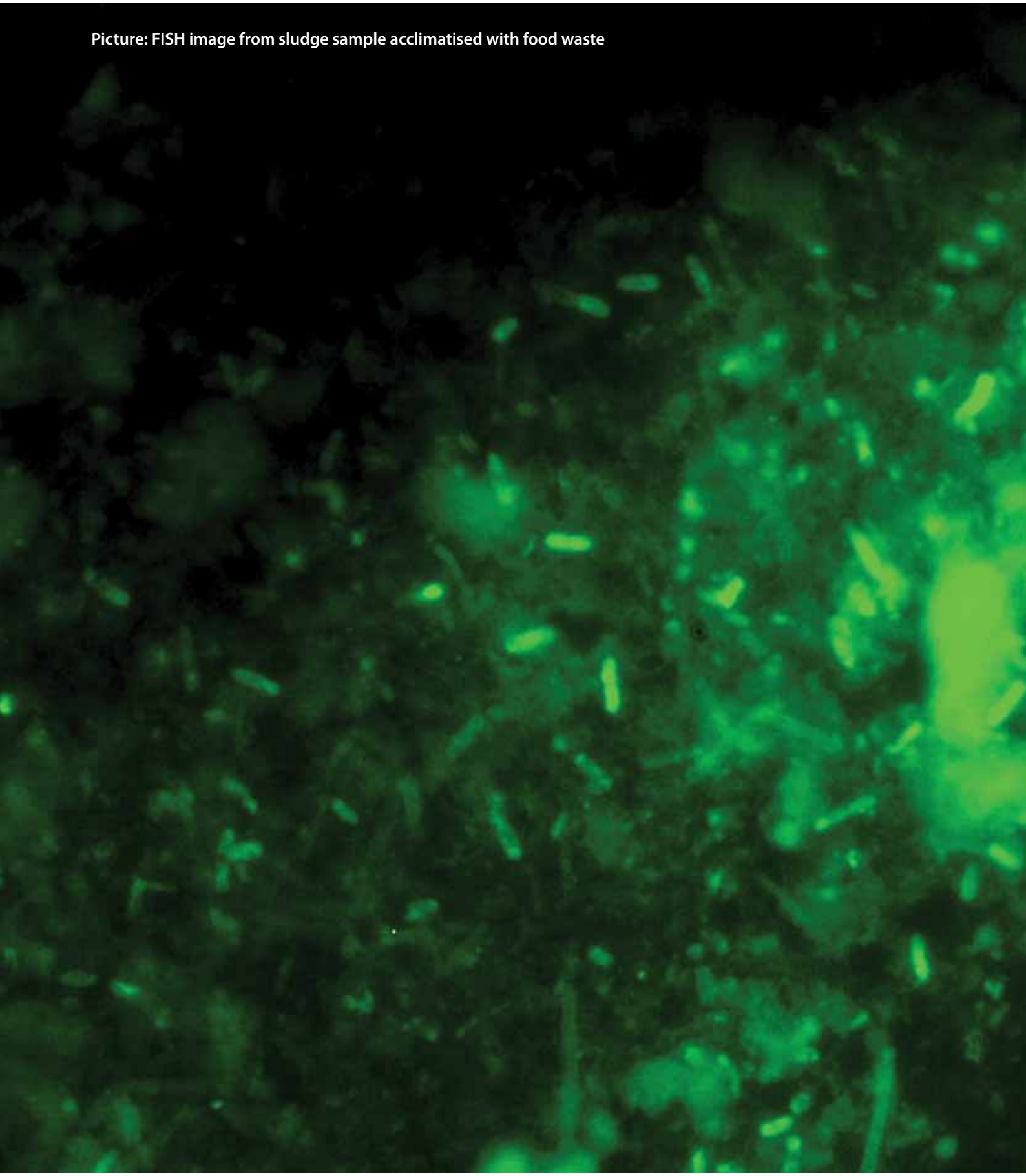
SEMINAR ON BIOMASS FOR BIOFUELS AND VALUE-ADDED PRODUCTS
27-28 October 2009 at
The Saujana Hotel, Kuala Lumpur


MALAYSIA-MIT WORKSHOP
14-15 Nov 2009 at
Equatorial Hotel, Penang, Malaysia

PHA PELLET RECOVERED using solvent extraction

ROTARY EVAPORATION SYSTEM used for PHA extraction process

20 L FERMENTER USED FOR PHA PRODUCTION using mixed acids from anaerobically treated palm oil mill effluent by *Comamonas sp.* EB172



Picture: FISH image from sludge sample acclimatised with food waste

BIOHYDROGEN RESEARCH GROUP

OVERVIEW

Biohydrogen is a viable alternative to replace fossil fuel as it is compatible with combustion process for energy conversion without producing carbon-based emissions that contribute to environmental pollution and climate change. Biohydrogen Group is a division of Environmental Biotechnology Group that conducts research on biological production of hydrogen through the application of anaerobic fermentation process. This research addressed on the application of biotechnology (anaerobic digestion) to produce biohydrogen. Two different substrates have been studied, palm oil mill effluent (POME) and food waste. We are using POME sludge as inoculum and succeed to produce biohydrogen. The study also covered on optimization of parameters such as temperature, substrate concentration, initial pH and inoculum sources using statistical tools. In our group, hydrogen producing bacteria (*Clostridium butyricum* EB6) has been successfully isolated.

RESEARCH AREA

Till date, we have succeeded in producing hydrogen gas under sterile and non-sterile condition in mesophilic and thermophilic environment using POME sludge as inoculum. Furthermore, we are seeking alternative ways of enhancing the biohydrogen production by conducting experimental work using different inocula sources. Statistical and modelling tools are also being applied as quantitative and systematic means of analysing and optimising the hydrogen yields and production rates.

Currently, there are four Master students working under the group, and they are registered either with UPM or Kyushu Institute of Technology, Japan.

PARTNERS

Universiti Putra Malaysia - Prof. Dr. Mohd Ali Hassan

Standard and Industrial Research Institute of Malaysia (SIRIM) - Dr. Chen Sau Soon

Universiti Kembangsaan Malaysia - Dr.Jamaliah

Universiti Malaya - Prof. Dr. Vikineswary Sabaratnam

Kyushu Institute of Technology - Prof. Dr. Yoshihito Shirai

BIOHYDROGEN RESEARCH GROUP

Biohydrogen Production from Palm Oil Mill Effluent under Non Sterile Condition in 50 Liter Continuous Stirred Tank Reactor

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BIOTECHNOLOGY TOOLS AND APPLICATIONS recently been introduced to the Malaysian palm oil mill industry. Proper management of the industry's by-products and their conversion to valuable products requires comprehensive integration of biotechnology principles and methodology. This work utilises the anaerobic digestion process to Palm oil mil effluent (POME) in order to produce biohydrogen gas using 50-L bioreactor. Production of hydrogen from biological processes (biohydrogen) is an important finding due to its environmental friendly process and less energy intensive as compared to electrochemical processes. It is also useful in the context of wastewater treatment especially in the palm oil industry. Feasibility study using POME sludge as the natural inoculum was conducted in 50-litre continuous stirred tank reactor (CSTR). The biogas produced was free from methane due to the heat treatment on the sludge prior to inoculation. The experimental data obtained based on the observation of significant parameters has been rather instructive for further study and development. ■

THE RESEARCH OBJECTIVES

1. To investigate the feasibility of biohydrogen production from POME in a pilot scale under non-sterile condition.
2. To study the effect of hydraulic retention time and volatile fatty acids on biohydrogen production from POME under non-sterile condition.

PUBLICATION

Mohd Zulkhairi Mohd Yusof, Mohd Ali Hassan, Suraini Abd-Aziz and Nor'Aini Abdul Rahman, 2009. Start–up of biohydrogen production from palm oil mill effluent under non-sterile condition in 50 liter continuous stirred tank reactor. International Journal of Agricultural Research, 4(4): 163-168

POME (substrate)

50-L CSTR

GAS (product)

Microbial Identification in Food Waste Fermentation for Biohydrogen Production

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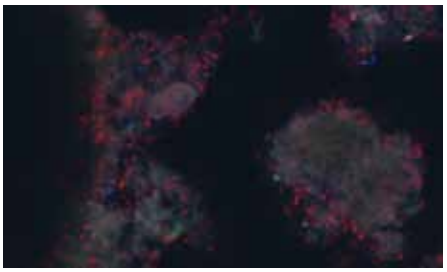
BIOHYDROGEN PRODUCTION FROM FOOD waste fermentation would provide clean and environmental friendly technology for energy generation with simultaneous waste treatment. Fermentative biohydrogen production from food waste as a carbon source is considered to be a complex process since hydrogen production was done using metabolic activity of microbes. Thus, suitable macro-environment (pH and temperature) should be considered in order to provide suitable condition for hydrogen producing bacteria (HPB) in biohydrogen production. Heat treated palm oil mill effluent (POME) sludge were used as the seed culture. The effects of different pH, temperature and substrate composition were studied. The identification of microbial profiling and colony presence during fermentation is essential to understand the process in biohydrogen production. Microbial profile will be carried out using denaturing gradient gel electrophoresis (DGGE), total bacteria and HPB will be visualized using fluorescent *in situ* hybridization (FISH). ■



FLUORESCENT *IN SITU* HYBRIDIZATION (FISH) IMAGE from sludge samples acclimatized with food waste

THE RESEARCH OBJECTIVES

1. To study the effects of different temperature, pH and substrate composition on biohydrogen production from food waste
2. To identify microbial profile using molecular techniques during biohydrogen fermentation of food waste.

PUBLICATION

Nazlina Haiza Mohd Yasin, Nor Aini 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

BIOHYDROGEN RESEARCH GROUP

Biohydrogen Production from Palm Oil Mill Effluent using Thermophilic Suspended and Immobilised Mixed Cultures

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THE MAJOR HURDLE TO HYDROGEN production from dark fermentation of complex wastewater is the lack of understanding on the conversion processes and their influence on the process. This study was to establish the know-how on generating biohydrogen from complex wastewater such as POME as knowledge gained by studying dark fermentation of carbohydrate–based substrate is insufficient to be applied for conversion of complex wastewater. Complex wastewater such as POME contained several soluble and particulates of primary substrates such as oil and grease, carbohydrate and protein. The behaviour of each component in the acidogenesis to produce hydrogen and other soluble metabolites has not been analysed. Suspended cultures are susceptible to washout at high dilution rates. Operating reactor with immobilised cells under the optimised set of conditions will mitigate the problem. However, knowledge on the physical barrier to effective conversion of complex wastewater has to be established. ■

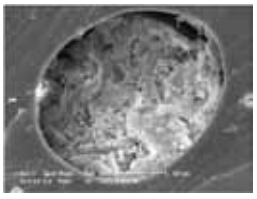
THE RESEARCH OBJECTIVES

1. To establish the potential of suspended sludge in generating hydrogen from complex wastewater under thermophilic condition.
2. To evaluate the capacity of immobilised cells in generating high volume of hydrogen under continuous condition.

PUBLICATION

Isnazunita Ismail, Mohd. Ali Hassan, Nor'Aini Abdul Rahman and Chen Sau Soon, 2010. Thermophilic biohydrogen production from palm oil mill effluent using suspended mixed cultures. Journal of Biomass and Bioenergy, 34: 42-47

COMPLETELY STIRRED TANK REACTOR (CSTR) system



IMMOBILIZED SLUDGE observed under SEM

Statistical Optimization of Biohydrogen Production from Food Waste under Thermophilic Condition

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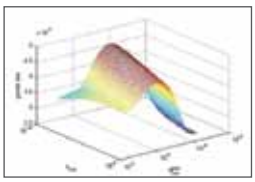
RESPONSE SURFACE METHODOLOGY (RSM), a collection of empirical models and statistical analyses, had been used to study the effects of several factors on hydrogen production rate and hydrogen production yields using food waste as a substrate. The optimization study using RSM consists of 2 parts; 2-level factorial and central composite design. The aim of 2-level factorial is to find the significant factors for the biohydrogen production from food waste. The factors which have significant effect to the biohydrogen production were proceed using central composite design to obtain the optimal condition. Each factor was analysed using ANOVA analysis in the Design-Expert software. The fermentation was conducted using serum bottle (160 mL) with 100 mL working volume. ■

THE RESEARCH OBJECTIVES

1. To optimize the operating conditions for biohydrogen production from food waste under thermophilic condition using response surface methodology
2. To study the effect of organic acids on biohydrogen production from food waste in different fermentation pH.

FERMENTATION using serum bottle



OPTIMIZATION study

APPLIED IN 3 L bioreactor

BIOHYDROGEN RESEARCH GROUP

Mathematical Modelling of Biohydrogen Production

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COMPUTATIONAL MODELLING PROVIDES a systematic approach towards a better understanding of bioreactor performance. Compared to the empirical approach in which the bioreactor behaviour is studied under practically all combinations of possible conditions of operation, the modelling approach attempts to describe both actual and probable bioreactor performance by means of models, that is, well-established theories formulated in mathematical terms. In carrying out a modelling exercise, the nature of all the important parameters of the process, their effect on the process and how each parameter can be defined in quantitative terms are critically assessed. Once formulated, the model can be solved and the behaviour predicted by the model compared with experimental data. Any differences in performance may then be used to further redefine or refine the model until good agreement is obtained. Once the model is established it can then be used, with reasonable confidence, to predict performance under differing process conditions, and it can also be used for such purposes as process design, optimization and control. ■

THE RESEARCH OBJECTIVES

1. To evaluate the predictive ability of kinetic-based and energy-based models for biohydrogen production using Palm-Oil-Mill-Effluent-based single-culture
2. To evaluate the predictive ability of kinetic-based and energy-based models for biohydrogen production using Palm-Oil-Mill-Effluent-based mixed-culture

Optimization of Biohydrogen Production from Palm Oil Mill Effluent using Mixed Microflora

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IN THIS STUDY, POME SLUDGE WAS USED AS inoculum to produce biohydrogen from POME. Preliminary screening on the effects of inocula size, heat treatment, substrate concentration and pH of incubation by using a factorial design (FD) were conducted under mesophilic condition (37°C) using a serum vial (160 mL). The experimental results from two-level FD showed that pH and chemical oxygen demand (COD) of POME significantly affected biohydrogen production. Optimizations of the specific hydrogen production (P_s) and the hydrogen production rate (R_m) were carried out by using a central composite design (CCD). The maximum P_s of 270 mL H2/g carbohydrate and R_m of 98 mL H2/h were obtained under optimum conditions of pH 5.86 and substrate concentration of 80 g/L. The optimized conditions obtained were subjected to a confirmation run and it showed a reproducible data with P_s of 282 mL H2/g carbohydrate and R_m of 137 mL H2/h. For the second part of experiment, 2-L of bioreactor was employed for the production of biohydrogen with and without pH control. The optimum conditions obtained in the serum vial were applied in the bioreactor. Based on the results obtained the biogas generated was 1.3L/L medium for uncontrolled pH. Throughout the fermentation, no methane-gas was detected. The biohydrogen yield (HY) was approximately 1L H_2/L medium, with hydrogen production rate (HPR) at 112 mL H_2/L/h. For the controlled pH experiment, pH was controlled manually every 3h at 5.86. The biogas generated from the fermentation was 2.5 L/L medium, which is almost 2-fold of biogas production from uncontrolled pH. The HY and HPR generated were 1.3L H_2/L medium with 144 mL H_2/L/h, respectively. ■

THE RESEARCH OBJECTIVES

1. To optimize the operating conditions for biohydrogen production from palm oil mill effluent under mesophilic condition using response surface methodology
2. To study the effect of controlled and uncontrolled pH on biohydrogen production from palm oil mill effluent in 2L bioreactor

PUBLICATION

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

BIOHYDROGEN RESEARCH GROUP

Biohydrogen Production from Palm Oil Mill Effluent by Bacterial Strain *Clostridium ep.* EB6

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THE STRAIN, *CLOSTRIDIUM SP.* EB6 efficiently produced biohydrogen concurrently with cell growth from palm oil mill effluent (POME). The effect of pH on hydrogen production from POME showed that the optimum pH was 5.5 with maximum hydrogen production and volumetric hydrogen production rate were 3195 mL H_2/L-medium and 1034 mL H_2/L-medium/h, respectively. For the second objective, central composite design and response surface methodology were applied to determine the optimum conditions for hydrogen production (P_c) and maximum hydrogen production rate (R_{max}) from POME. Experimental results showed that the pH, temperature and chemical oxygen demand (COD) of POME affected both the hydrogen production and production rate, both individually and interactively. The optimized conditions for the overall model were pH 6.05, 36ºC and 94 g COD/l. The hydrogen content in the biogas produced ranged from 60% to 75%. Optimization of biohydrogen production was performed via statistical analysis, namely response surface methodology (RSM), with respect to pH, glucose and iron concentration. The results show that pH, glucose concentration and iron concentration significantly influenced the biohydrogen gas production individually, interactively and quadratically ($P < 0.05$). The central composite design (CCD) results indicated that pH 5.6, 15.7 g/L glucose and 0.39 g/L FeSO4 were the optimal conditions for biohydrogen production, yielding 2.2 mol H_2/mol glucose. In confirmation of the experimental model, t-test results showed that curve fitted to the experimental data had a high confidence level, at 95% with t¼ 2.225. Based on the results of this study, optimization of the culture conditions for *C. butyricum* EB6 significantly increased the production of biohydrogen. ■

PUBLICATIONS

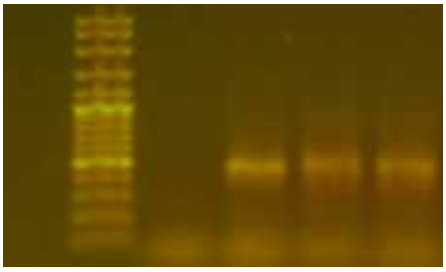
- Mei-Ling Chong, Raha Abdul Rahim, Yoshihito Shirai and Mohd Ali Hassan. (2009). Biohydrogen production by Clostridium butyricum EB6 from Palm Oil Mill Effluent. International Journal of Hydrogen Energy . 34: 764-771.
- Mei-Ling Chong, Vikineswary Sabaratnam, Yoshihito Shirai and Mohd Ali Hassan. (2009). Biohydrogen production from biomass and industrial wastes by dark fermentation. International Journal of Hydrogen Energy . 34: 3277-3287.
- Mei-Ling Chong, Nor' Aini Abdul Rahman, Suraini Abdul Aziz, Yoshihito Shirai and Mohd Ali Hassan. (2009). Optimization of biohydrogen production by Clostridium butyricum EB6 from palm oil mill effluent using response surface methodology. International Journal of Hydrogen Energy. 34: 7475-7482.
- Chong ML, Hassan MA, Suraini AA, Phang LY, Farinazleen MG, Nor'Aini AR, Shirai Y. Effect of pH, glucose and iron concentration on the yield of biohydrogen by Clostridium butyricum EB6. Submitted to Bioresource Technology (Manuscript no: BITE-D-09-01764).
- Chong ML, Raha AR, Shirai Y, Hassan MA. Biohydrogen production by overexpression of hydA in Clostridium butyricum EB6. Submitted to: International Journal of Hydrogen Energy (Manuscript no: HE-S-09-01443)

THE RESEARCH OBJECTIVES

1. To study the effect of pH on biohydrogen production from palm oil mill effluent (POME) by strain *Clostridium butyricum* EB6.
2. To study the effect of pH, temperature and chemical oxygen demand (COD) of POME on biohydrogen production by strain *Clostridium butyricum* EB6 using central composite design (CCD) and response surface methodology (RSM).
3. To study the effect of pH, glucose and iron concentration on biohydrogen production from POME by strain *Clostridium butyricum* EB6 using central composite design (CCD) and response surface methodology (RSM).

5L BIOREACTOR

5L BIOREACTOR thermophilic study



PCR AMPLIFICATION PRODUCTS for DGGE study

Picture: HPLC used for ferulic acid, vanillic acid, vanillin and vinyl alcohol determination

BIOPRODUCT RESEARCH GROUP

OVERVIEW

Our group consists of a few different research areas which intended in producing different products using different substrates and various microorganisms. However, the process applied among our projects is the same where certain biological pathways are utilized by fermenting our substrates to obtain beneficial enzymes and flavour compound or by hydrolysis to improve the quality of product. In short; our studies aim in achieving chitinase enzymes (higher enzymatic activity), cyclodextrin glycosyltransferase via molecular approaches, improving the nutritional value of poultry feed and optimizing the production of vanillin.

RESEARCH AREA

Currently, there are two PhD students, two Master students and two research assistants under this group. The research topics concentrated by the students are;

1. Scaling-up Production of Chitinase Enzyme for Shrimp Aquaculture Waste Processing
2. Over Production of Cyclodextrin Glycosyltransferase (CGTase) Through Molecular Cloning Approaches
3. Enzymatic Improvement of Nutritional Value of Brown Rice for Poultry
4. Vanillin Bioproduction from Alkaline Hydrolysates of Oil Palm Empty Fruit Bunch

PARTNERS

Standard and Industrial Research Institute of Malaysia (SIRIM), Malaysia

Strategic Livestock Research Centre, MARDI, Malaysia

BIOPRODUCT RESEARCH GROUP

Scaling-Up Chitinase Enzyme Production from Shrimp Waste using *B. Licheniformis* Th-1

Sobri b. Mohd Akhir

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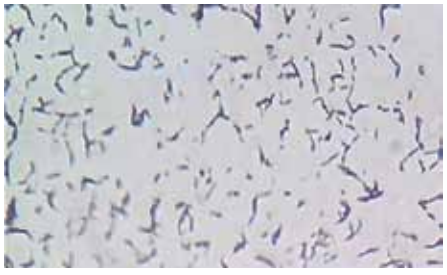
THE PRODUCTION OF INEXPENSIVE chitinolytic enzymes is an element in the utilization of aquaculture waste processing. In this study, colloidal chitin prepared by treating the chitin flakes with 85% phosphoric acid, was used as a substrate for chitinase-producing microorganism. *Bacillus licheniformis* TH-1, a strain isolated from the crude oil excreted chitinase when cultured in a medium containing chitin as major carbon source. The optimization of this growth medium was carried out using response surface methodology in order to designing or selecting the media for enhancing the microbial growth and production of chitinase enzyme. A five-level four-factor central composite design was employed to determine the maximum chitinase production at optimum levels for chitin, yeast extract, peptone, $NaNO_3$ and K_2HPO_4. The design contains a total of 20 experimental trials involving 4 replicates at the centre points. The design was employed by selecting chitin, yeast extract, peptone, $NaNO_3$, K_2HPO_4 and 3 responses of chitinase activity at 20, 22 and 24h of fermentation time. The optimal calculated values of tested variables for maximal production of chitinase were found to be comprised of chitin, 10 g/L; yeast extract, 0.5 g/L; peptone, 0.5 g/L; $NaNO_3$, 2.55 g/L and K_2HPO_4, 1.55 g/L with a predicted chitinase activity of 893 U/mL. These predicted optimal parameters were tested in the laboratory and the final chitinase activity obtained was very close to the predicted value at 900 U/mL. It is observed that the use of high concentrations of chitin and lower level of yeast extract and peptone from the medium resulted in the production of higher level of the enzyme. After statistically optimizing the medium constituents in shake flask level, the resulted medium will be further used in fermentation process to scale-up the chitinase production. For pilot-scale production system, it is essential to devise a scale-up strategy that would adopt desired level of agitation and aeration rates (in the fermenter), which in turn would give comparable or better yields relative to those obtained from shake flask study. Determination of kLa in 2L and 10L fermenter is essential to establish its aeration efficiency and to quantify the effects of operating variables on the provision of O_2. The result shows that by maintaining air flow rate at 1 vvm, the qO_2X and K_La in 10L fermenter are lower than those obtained in 2L fermenter. The reality of the effects of qO_2X and KLa in 10L fermenter needs to be further studied to determine the factors that may contribute negatively on the cells growth and enzyme production. This is necessary as it would enable one to minimize production cost and optimize the cost-effectiveness for the overall production process. ■

THE RESEARCH OBJECTIVES

1. To scaling-up production of chitinases and related chitin processing enzymes from 10 L to 50 L
2. Production and purification of chitinase enzyme for commercialization and further processing
3. To convert shrimp's waste (chitin-based materials) to high value products such as oligochitin, oligochitosan, D-glucosamine and N-acetyl glucosamine.

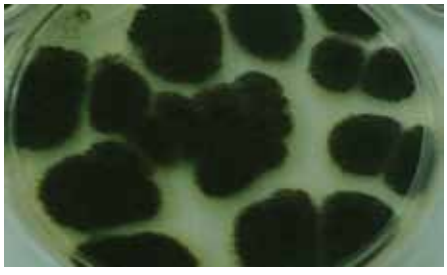
PUBLICATION

Sobri Mohd Akhir, Suraini Abd-Aziz, Madihah Md Salleh, Roshanida Abdul Rahman, Rosli Md Illias and Mohd Ali Hassan, 2009. Medium Optimisation of Chitinase Enzyme Production from Shrimp Waste using Bacillus licheniformis TH-1 by Response Surface Methods. Biotechnology, 8(1): 120-125



ISOLATED BACTERIA (Bacillus sp. NR5)
- picture from **NORHAYATI RAMLI**'s research

LOCAL VARIETY OF BROWN (RICE MR257)
- picture from **NURUL ASYIFAH MUSTAPHA**'s research



ASPERGILLUS NIGER convert ferulic acid to vanillic acid
- picture from **FAIROUZ JAHAAN MOHD AANIFAH**'s research

BIOPRODUCT RESEARCH GROUP

Over Production of Cyclodextrin Glycosyltransferase (Cgtase) through Molecular Cloning Approaches

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CYCLODEXTRIN GLUCANOTRANSFERASE (CGTase) is a multifunctional enzyme which catalyzes four related reactions: cyclizing, coupling, disproportionation and hydrolysis. By means of the cyclizing activity, CGTases convert starch and related substrates into cyclodextrins (CDs). The conversion of starch into CDs gives many benefits to industrial application. The unique hydrophobic interior cavity and hydrophilic surface of CDs enable it to be used widely in encapsulating a wide range of organic and inorganic compounds, thereby changing their physical and chemical properties, altering the stability, reactivity, and solubility of the inclusion complex formed within them. As a result, CDs find increasing application in biotechnology, food, pharmaceutical, cosmetic, agricultural, medicine and chemical industries. Genes encoding CGTase have been cloned and identified by several researchers. The molecular cloning approach using locally isolated Bacillus sp perhaps can enhance the production of CGTase, thus can be used commercially for the production cyclodextrins and maltooligosaccharides. ■

THE RESEARCH OBJECTIVES

1. To screen, isolate and characterize CGTase producing bacteria, specifically for α-CGTase, β-CGTase and φ-CGTase production.
2. To isolate the CGTase genes by using primer screening approaches, to construct CGTase expression system with Bacillus subtilis
3. To evaluate the production of enzyme by analysing the CGTase activity of the recombinant CGTases produced.
4. To scale up the CGTase production for the production of cyclodextrins and maltooligosaccharides.

Enzymatic Improvement Nutritional Value of Brown Rice for Poultry

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POULTRY CONSUMPTION ESPECIALLY chickens has been increased due to increase in human population and also the awareness to improve the poultry industry by producing better animal protein products. The increased in maize price has shifted to the use of local feedstuff which has similar energy and nutrient composition and can substitute maize all together or at certain rate. Rice is the most potential feedstuff to replace maize. Some varieties of rice can be obtained in high yield but they are not preferred for human consumption due to low eating value. The production cost of brown rice is cheaper because it does not need to undergo milling process. Brown rice that has attached bran contains high nutrients and there is also no negative effects on feed intake and there is positive effects in body weight gain of poultry. Besides, the anti-nutrients that have adverse effects towards poultry are non-starch polysaccharides (NSP) such as fibre, cellulose, hemicellulose, beta-glucan and arabinoxylan might also presence in brown rice. It will interfere with the digestibility, absorption and utilization of nutrients in the intestine, thus will affect overall animal performances. 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. Besides of improving the utilization of feedstuff, enzymes also can improve the quality of environment by reducing the output of excreta and can reduce the feed cost due to its flexibility in feed formulation. ■

THE RESEARCH OBJECTIVES

1. To determine the nutrients and anti-nutritional factors (mainly NSP) in brown rice.
2. To optimize the enzymatic hydrolysis conditions using Response Surface Methodology (RSM) for production of hydrolyzed brown rice.
3. To evaluate the effect of enzyme addition on poultry.

BIOPRODUCT RESEARCH GROUP

Vanillin Bioproduction from Alkaline Hydrolysates of OPEFB

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THIS RESEARCH IS BASED ON THE bioconversion of most abundant lignocellulosic biomass in Malaysia, oil palm empty fruit bunch (OPEFB) to obtain vanillin. The consumption of this waste material to develop into a beneficial product is a great advantage on its own. Vanillin produced is safe and environmentally benign with low content of harmful substances produced during the fermentation process. The usage of OPEFB will solves the problem of scarcity of petrochemical resources that are being used currently in the vanillin synthesis by chemical means. There might be issues on consuming the product as food flavour because of the source, waste materials. However; there are many other applications of this vanillin such as antifoaming agents, fragrance ingredient in perfumes and cosmetics, air fresheners and floor polishes. Flavour compounds, substances stimulating taste and smell, are extremely important for the food, animal-feed, cosmetics and pharmaceuticals industries. Traditionally, plants were the major sources of flavour compounds. However; they are often present at low concentrations and their isolation is thus difficult and expensive. Vanilla is the most widely used flavors in food industry. Natural vanilla extracted from the cured pods of the flowers of Vanilla planifolia has an estimated net value of more than $ 1 billion annually (Li, T. and Rosazza, J.P.N., 2000). The much higher price of the natural vanillin compared to the synthetic vanillin and strong market demand for environmental friendly products has been leading to growing interest of the flavor industry in producing it from natural sources by microorganism's bioconversion. Vanillin can be obtained by fermentation using suitable microorganism in the stationary growth phase. Ferulic acid released from agricultural residues such as oil palm empty fruit bunch (OPEFB) by a combination of physico-chemical and/or enzymatic treatments. The microbial transformation of ferulic acid is recognized as one of the most attracting alternatives to produce natural vanillin. Vanillic acid is the major product obtained by β-oxidation of ferulic acid. Thus; vanillic acid is abundant, readily available precursor for the biocatalytic synthesis of vanillin. The biotechnological process to produce vanillin from various agro by-products had been investigated using different microorganisms as biocatalysts. Here, fungi are being investigated for this bioconversion. Aspergilus niger and Phanerochaete chrysosporium are the bio-converting agent used in this research study. A. niger used, converts ferulic acid of oil palm empty fruit bunch (OPEFB) into vanillic acid and P. chrysosporium turns vanillic acid into vanillin. Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) are used to determine the presence and concentration of vanillin respectively. ■

THE RESEARCH OBJECTIVES

1. To optimize alkaline pretreatment in order to produce alkaline hydrolysates with higher ferulic acid from lignocellulosic biomass (oil palm empty fruit bunch)
2. To convert ferulic acid to vanillin through two stages fungal bioconversion
3. To optimize the fermentation product of vanillin by RSM for maximum production

Study on Bioactive Compound Produced by a Potential Indigenous Actinomycetes Strain for Biocontrol of Anthracnose in Chilli

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ACTINOMYCETES HAD BEEN WELL known as the main producer of bioactive compounds in the world. In this study we will be extracting bioactive compounds from soil borne actinomycetes for antagonistic reaction towards Colletotrichum gloeosporioides using organic solvent extraction method. The extracted bioactive compounds would then be subjected to several chromatography analyses (Silica Gel Chromatography, Thin Layer Chromatography and High Performance Liquid Chromatography) for the detection of the bioactive compounds. Fourier Transform Infrared Spectroscopy (FTIR), Mass Spectrophotometer (MS) and Nuclear Magnetic Resonance (NMR) would also be employed for the characterization and identification of the bioactive compound responsible for the inhibitory of Colletotrichum gloeosporioides as casual anthracnose causing fungus in chili if the compounds was a novel compound. By the end of this study we are expected to identify bioactive compounds obtained from actinomycetes with the ability to control anthracnose disease. ■

THE RESEARCH OBJECTIVES

1. To screen and isolate soil actinomycetes antagonist towards Colletotrichun capsici
2. To optimize fermentation of actinomycetes strains for production of bioactive compound using Response Surface Methodology (RSM)
3. To extract bioactive compound from culture filtrate of the fermentation broth using ethylacetate solvent
4. To purify the crude bioactive compound extract by using silica gel chromatography and HPLC
5. To identify the purified bioactive compound by FTIR, MS and NMR.

NOTE

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