WASTE TO WEALTH THROUGH BIOTECHNOLOGY

ENVIRONMENTAL BIOTECHNOLOGY RESEARCH GROUP

RESEARCH REPORT 2010

EB GROUP

ENVIRONMENTAL BIOTECHNOLOGY RESEARCH GROUP (EB Group), Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia was officially started in 2005. Currently EB Group consists of four subgroups; Bioenergy, Bioplastic, Biofertilizer and Bioproduct. There are eight principal researchers and 49 students including Masters, PhD and Research Assistants. We aim to be a high performance research group conducting research on oil palm biomass and other raw materials in Malaysia into valuable green products with our tagline "***Waste to Wealth through Biotechnology***". Most of our research are conducted in close collaboration with other academic institutions and industries locally and internationally, such as FELDA, KIT (Japan), AIST (Japan) and MIT (USA). ■

■ **PICTURE** TEM of *Comamonas sp* under 20,000x

EB GROUP 2010 Achievements at a Glance

24 international refereed journals

21.16 impact factor scores

03 patents filed

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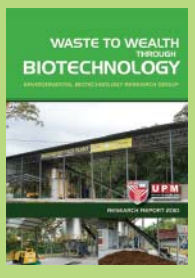


On the cover

EB Group's Biocompost Pilot Plant at Taman Pertanian Universiti (TPU), Universiti Putra Malaysia (UPM), MALAYSIA

EB GROUP

UPM UNIVERSITI PUTRA MALAYSIA BERILMU BERBAKTI

RESEARCH TITLES

BY RESEARCH GROUP

BIOENERGY

BIOHYDROGEN

BIOETHANOL

BIOBUTANOL

BIOPLASTIC

BIOFERTILIZER

BIOPRODUCT

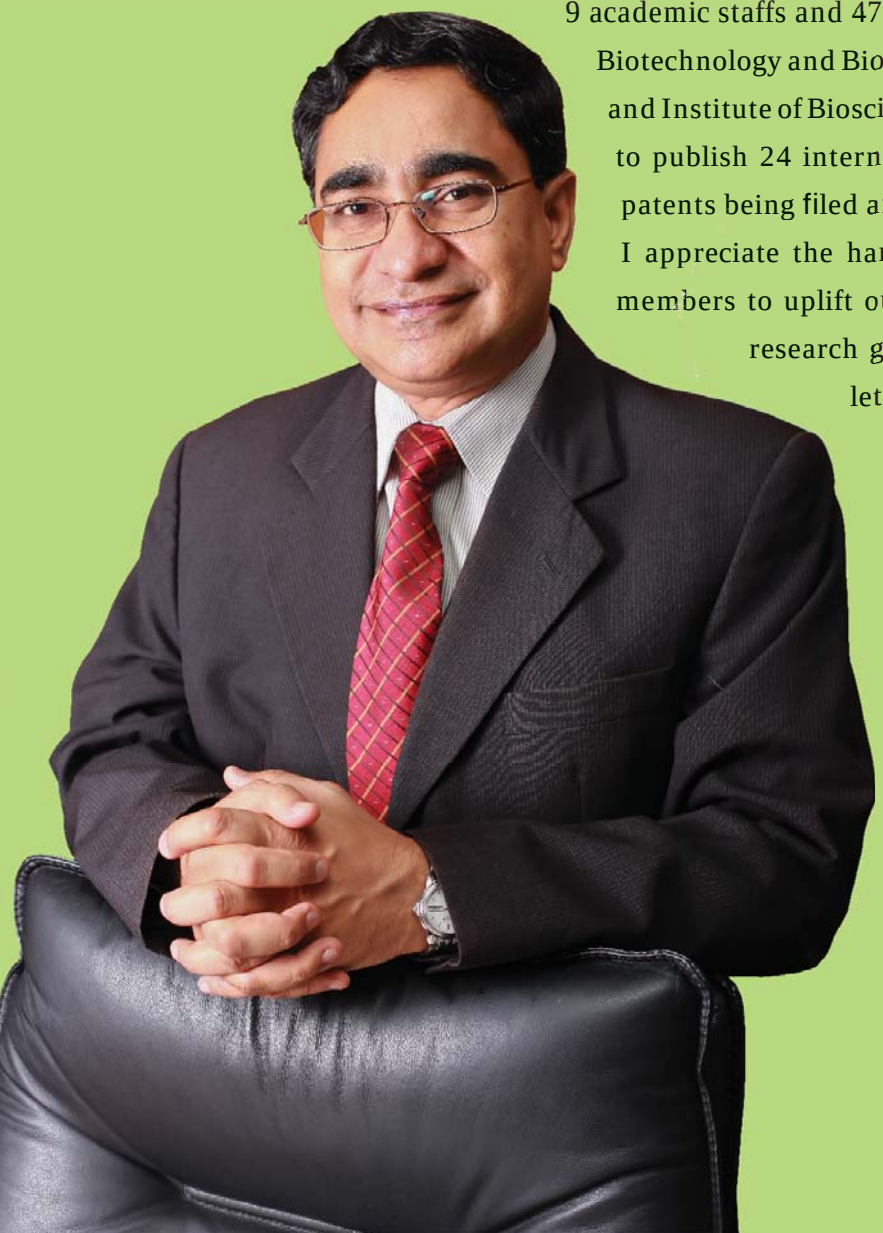
FOREWORD

AlhamduLillah, Praise to ALLAH The Al Mighty for His favours and blessings to us all. I am glad to share with you our Environmental Biotechnology Research Report 2010. Our research group is currently involved in important research areas such as bioenergy, bioplastic, bioproducts and biofertiliser, covering a wide range of research topics on wastewater treatment, bioprocessing, enzyme technology, advanced molecular biotechnology techniques, process modeling, process design, regulatory compliance, wastewater recycling and zero emission. 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 both locally and internationally. We manage to secure funding from FELDA Palm Industries Sdn. Bhd., Yayasan Pelajaran Johor, 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 47 students (PhD 20, MS 24) from Faculty of Biotechnology and Biomolecular Sciences, Faculty of Engineering and Institute of Bioscience. For the year 2010, we have succeeded to publish 24 international refereed journals with 21.16 I.F, 3 patents being filed and won several medals in exhibitions.

I appreciate the hard work and professionalism of all 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**"



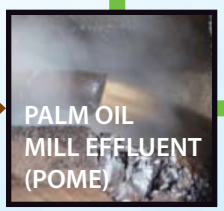
Prof. Dr Mohd Ali Hassan

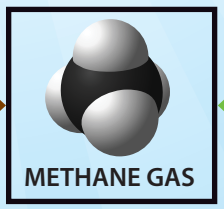
GROUP LEADER
Environmental Biotechnology
Research Group (EB Group)

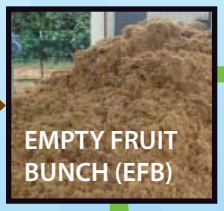
BIG PICTURE

of ENVIRONMENTAL BIOTECHNOLOGY RESEARCH GROUP

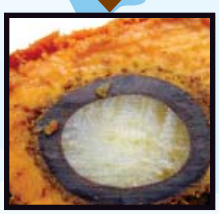
PROCESS WATER



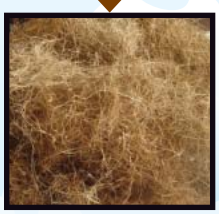




CRUDE PALM OIL (CPO)



KERNEL

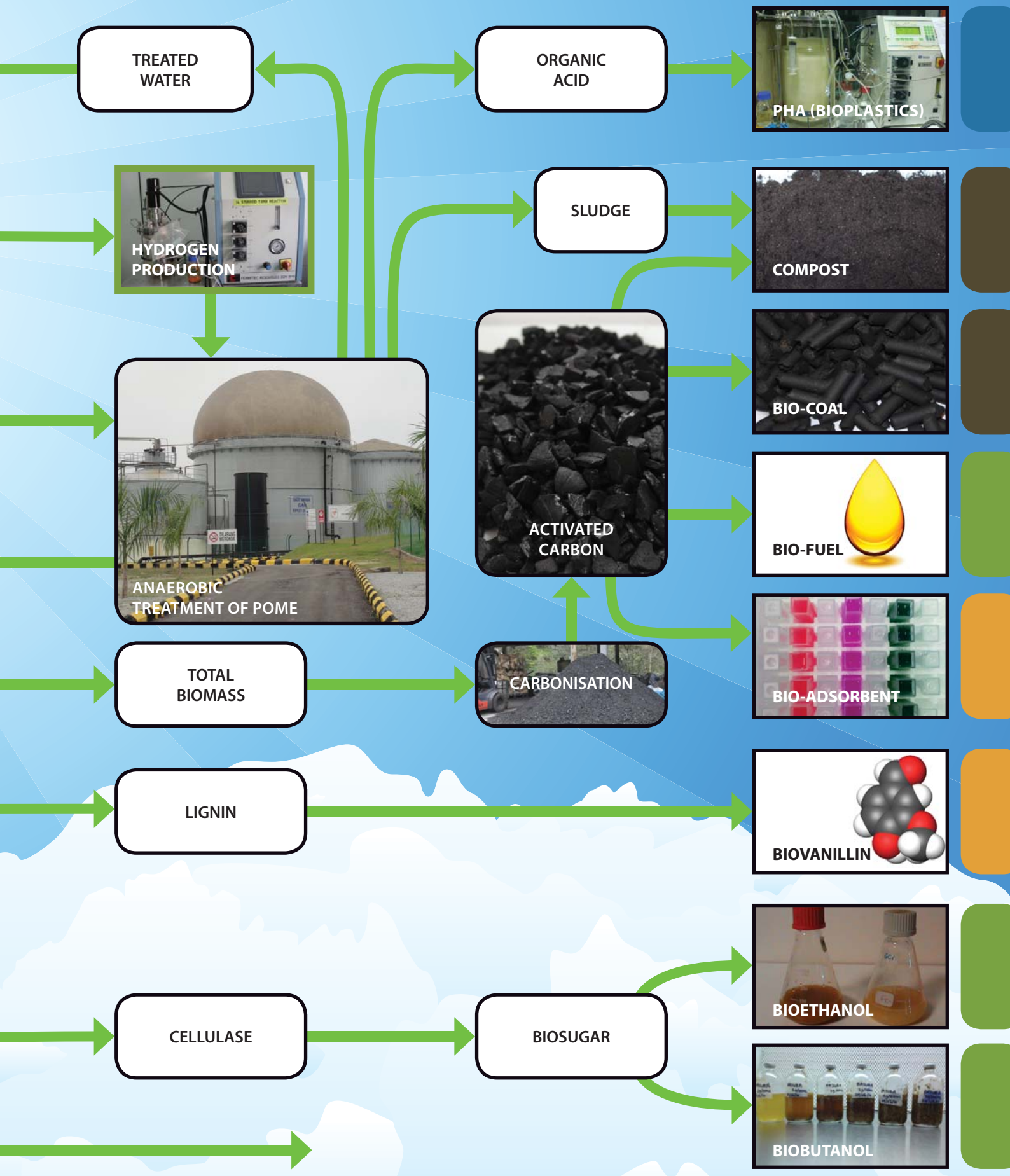


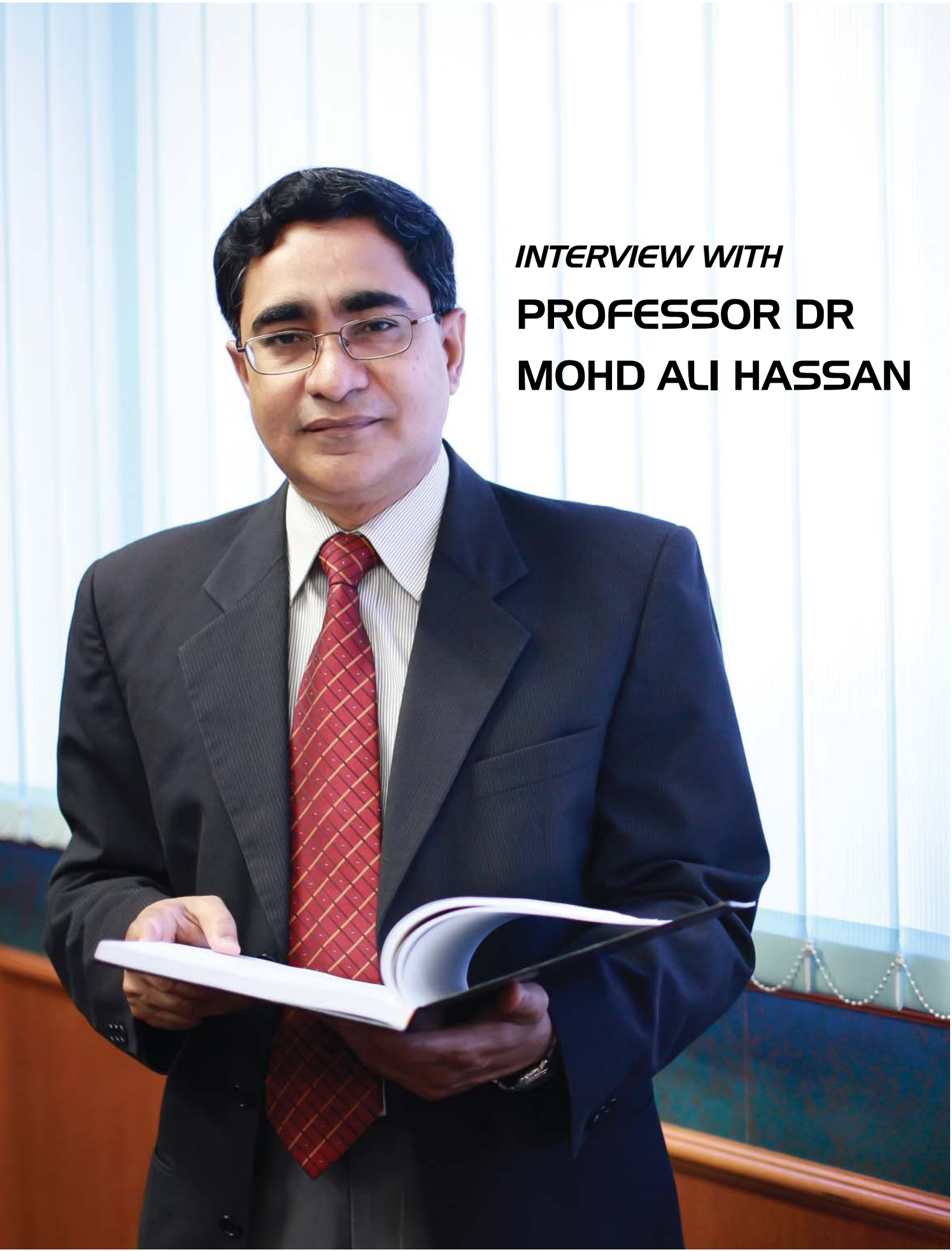
MESOCARP FIBER

PALM KERNEL SHELL (PKS)

CURRENT PROSESS

PROPOSED PROSESS


TREATED WATER
ORGANIC ACID
PHA (BIOPLASTICS)
HYDROGEN PRODUCTION
SLUDGE
COMPOST
BIO-COAL
ACTIVATED CARBON
ANAEROBIC TREATMENT OF POME
BIO-FUEL
TOTAL BIOMASS
CARBONISATION
BIO-ADSORBENT
LIGNIN
BIOVANILLIN
CELLULASE
BIOSUGAR
BIOETHANOL
BIOBUTANOL



INTERVIEW WITH

PROFESSOR DR MOHD ALI HASSAN

Set a target and work to achieve the target

When was EB group first developed and how was it started?

OVERVIEW

Officially, EB group started with the faculty, Faculty of Biotechnology and Biomolecular Sciences, in August 2004. When the faculty was opened, I was the Deputy Dean, and one of the plans was to set up focused groups for research. We came out with 6 groups and one of them is Environmental Biotechnology Research Group, which I was asked to head. Officially, the core members at that time are me and Dr. Suraini. Later, Dr. Nor'aini, Dr. Phang, Dr. Umi Kalsom, Dr. Noriznan, Dr. Hidayah and Dr. Helmi joined the group. Unofficially, we have done this EB research work with collaboration of KIT and FELDA since 1998. We signed MOU with FELDA in 2002. Before that, we had our own funding, partly from Japan, MOSTI and others. Our group becomes more structured when we had funding from FELDA. Alhamdulillah now we become more developed and we keep the momentum going.

What is your aim for EB group?

GROUP OBJECTIVE

My aim for EB group is basically a 'win-win' arrangement that symbolizes a mutual concept; everybody benefits from EB group and everyone contributes to the group. This is 'win-win' situation. However; when we say 'win-win-win', we want to develop something useful for the faculty, university, industry and country. There is an effort to benefit others besides our members and group. With this, we would not feel stressed or pressured being in the group. It's a mutual benefit; everyone gets something and does not feel being used by others.

How do you see EB group and where does EB group stand right now compared to other research groups locally and internationally?

VIEWPOINT

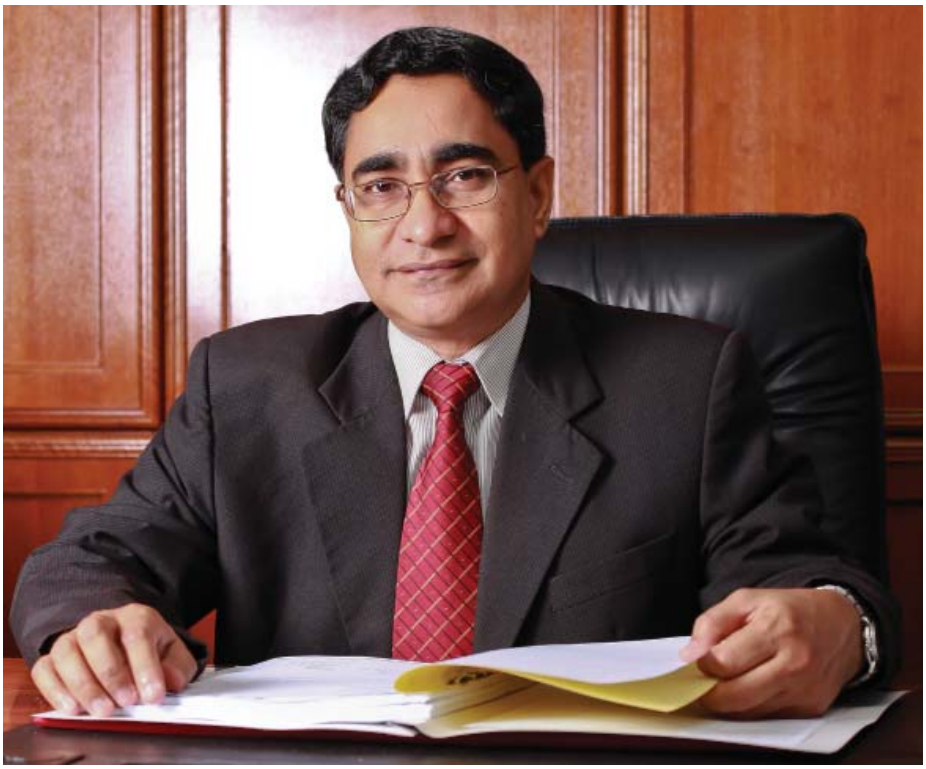
I developed this group with other lecturers based on best practice. Whatever has been practiced elsewhere, that we have seen and experienced, we try to incorporate them in our group. A very good example is our publications. Each student in our group must publish, and every objective of their research is a publication. Now we agree that publications are in ISI (impact factor) journals. The Faculty of Biotechnology and Biomolecular Sciences is a new faculty and we are the best faculty in UPM. We are creating examples for others to follow. The numbers of research groups in UPM are low. Many lecturers are working individually. Synergy is one of the principles we use. It is far better to work in a group than alone, with the collective strength we can be stronger and achieves more.

What inspired you to lead a high performance research group?

MENTORING

I was inspired by the concept of 'mentoring'.

It is different from coaching. Coaching is to instruct and teach. Mentoring is more than that, it is about inspiring and motivating. When we are motivated, we will do our work without any force. We are willing to do it, we become passionate. I have mentored others, for example, the lecturers and students in EB group. As a teacher, we want our students to be far better than ourselves. It is just like parents, who feel



"Synergy is one of the principles we use. It is far better to work in a group than alone, which gives collective strength and achieve more"

very happy if our children are doing well in their lives. I am trying to play that role in our research group. In my case, I have benefited a lot from my association with Prof. Shirai, Prof Ismail and other Professors when I did my PhD. I think they have coached and mentored me very well to become who I am now.

What is the greatest achievement of EB Group so far?

ACHIEVEMENT

Alhamdulillah, in terms of input, we have good big research grants from industries and overseas for commercialization and pilot plant studies. We are quite stable on the input side. The other side is

output. Since 2008, we have at least 20 papers or publications per year. We become excellent and in term of achievements, we have been known by many people in the scientific world, especially in the field of biomass in Malaysia. Many organizations and institutions prefer to collaborate with us, such as MIT, AIST, Ajinomoto, TEPCO, Indah Water and Alam Flora. Alhamdulillah, we are known locally and internationally. However, our biggest achievement is publications. People all around the world have cited our work in their papers as reference. This is the ultimate achievement in the scientific community.

What must EB Group do in order to sustain being a high performance group?

SUSTAINABILITY

A good system has to be in place for anything to grow and be sustainable. All the members have to work together as a team to ensure that this system works. We have developed quite a good system for EB Group. If students are well supervised, they will not waste their time doing unnecessary experiments and would not feel pressured. They are free to discuss and know what they need to do before the next discussion. A very easy way to measure the growth and sustainability of our group is by paper publications. We can't move if we do not set any benchmark. This year we achieved 50%, so how about next year? We have 40 students, therefore we should have 40 papers per year. Apart from papers, now we also consider the impact factors. Therefore we are increasingly publishing our work in higher impact factor journals.

As a leader in this group, you should have faced many challenges. What is the most challenging experience for you?

CHALLENGE

My biggest challenge is dealing with human beings. We are in a big group with different characters, expectations, backgrounds, skills and abilities. As a leader, I have to ensure that everyone performs. I can't expect the same output from everyone, which is not realistic at all. So a minimum requirement system was fixed in order to be fair to everyone. We set a minimum of one paper per student per year. Some can do more. Those who do more, we reward them more, following the carrot and stick concept Moreover, rewards are based on impact factors.

What is the major reason for your success?

SUCCESS

In one word, passion (the fire, the burning desire) is the major reason for my success. Passion to contribute to the university, to guide lecturers and students, to see those under me succeed. The passion drives me to achieve more, like our biogas project in FELDA Serting Hilir, Negri Sembilan, biocompost plant and many more. Being passionate towards my work results in enjoyment. Research and thinking are my enjoyment. I always think of myself, family, group and faculty. Try to do better and not always be at the minimum or average level. Passion is contagious, as people around us are inspired too. I need to create the atmosphere of passion among group members to build a high performing group.

As a leader and a role model, my action will be watched by others. Therefore, I have to do my best. Besides that, family support also plays a role in my success. I brought my family to Serting and Japan to show my projects, so that they can appreciate what I am doing.

"Those who do more, we reward them more, following the carrot and stick concept."

As a successful leader, researcher, dean and consultant, how do you manage your time for yourself, family and career? How do you balance your goals for your career and life?

LIFE BALANCE

Very difficult.

However, we have to understand our responsibilities and priorities. The first responsibility as a Muslim is to Allah. Then, responsibilities to my family and neighbours, as a dean, a lecturer, EB Group leader and involvement in NGO. It seems difficult to manage but I have to manage. If not, I will not succeed. Some people allocate time for their work but for me time is flexible. We have to make time for something important by removing unnecessary routines. A small pocket diary is my important tool, in which I plan my day, my week and my life.

What is your hope for EB group, next year and in the future?

FUTURE

Sustainability. I strongly believe that sustainability comes from blessing (*barakah*). We need to share and not keep everything to ourselves. The one who knows must teach others sincerely. EB group is now well known research group in the world as our website is often visited by people all over the world.

Do you have any words, messages for EB group members and other people?

MESSAGE

We have to be magnanimous, one that does not think only about oneself. All should apply the concept of '*barakah*' (blessing, the greater good derived from any act) and sincerity. ✱

"And whosoever keep his duty to Allah, Allah will appoint a way out for him, (2) And will provide for him from (a quarter) whence he hath no expectation. And whosoever put his trust in Allah, He will suffice him. Lo! Allah brings His command to pass. Allah hath set a measure for all things"

– Surah At-Talaq, verse 2-3

2010 GROUP PICTURE

MEMBERS
60

RESEARCHERS
09

POST-DOC
01

PhD STUDENTS
20

MASTER STUDENTS
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GROUP LEADER | RESEARCHER | POST-DOC | PhD STUDENT | MASTER STUDENT | RESEARCH ASSISTANT | ALUMNI | GROUP SECRETARY

Professor Dr Mohd Ali Hassan

specialisation

Bioprocess Engineering & Environmental Biotechnology

current research interest

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

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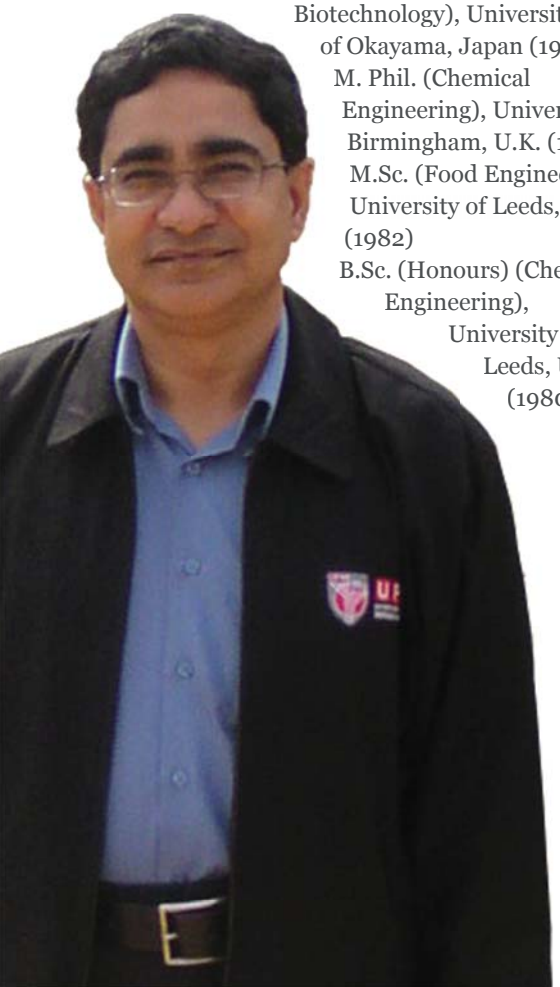
academic qualification

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



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Associate Professor Dr Suraini Abd Aziz

selected publications

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specialisation

Biochemical Engineering, Environmental Biotechnology and Industrial Biotechnology

current research interest

Utilisation of biomass for value added products (biocatalysts, biosugars, bioethanol, biobutanol, biovanillin)

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Associate Professor Dr Umi Kalsom Md Shah

specialisation

Environmental Biotechnology and Industrial Microbiology

current research interest

Utilization of microbes for production of lignocellulolytic enzymes, antibiotic, biobutanol and biofertilizer from agro-biomass

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academic qualification

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MSc. (Microbiology), University of Kent at Canterbury, U.K. (1987)
BSc. (Honours) (Microbiology), Universiti Kebangsaan Malaysia (1981)

selected publications

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.

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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

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OUTBOUND

PARTICIPANTS	PROGRAM	RESEARCH THEME
Dr Hidayah Ariffin	INTEGRATED STUDY PROGRAM ON ALTERNATIVE ENERGY PRODUCTION	Immobilization of cellulase on lignophenol and the recyclability of immobilized cellulase
	COLLABORATIVE RESEARCH	• Preparation and characterization of polyhydroxyalkanoate / oil palm empty fruit bunch (PHA/EFB) composites • Characterization of steam-hydrolyzed polyhydroxybutyrate (PHB)
Dr Helmi Wasoh	JSPS EXCHANGE PROGRAM FOR EAST ASIAN YOUNG RESEARCHERS (2010)	Degradation of Chitin from Shrimp Shell by Chitinase
Mohd Huzairi Mohd Zainudin	INTEGRATED STUDY PROGRAM ON ALTERNATIVE ENERGY PRODUCTION	Adsorption of protein on lignophenol
Mohd Zulkhairi Mohd Yusoff	YOUNG RESEARCHER OVERSEAS VISITS PROGRAM FOR VITALIZING BRAIN CIRCULATION	Hydrogen production from molecular perspective
Saleha Shamsudin	PROGRAM FOR MOHE SCHOLAR	Cellulosic Bioethanol from Steam Pretreated OPEFB
Ezyana Kamal Bahrin	JAPAN EAST ASIA NETWORK OF EXCHANGE FOR STUDENTS AND YOUTHS (JENESYS)	Pretreatment of oil palm biomass for value added product

INBOUND

PARTICIPANTS	CURRENT POSITION	RESEARCH THEME
Assoc. Prof Dr Yoshito Ando	ASSOCIATE PROFESSOR, KYUSHU INSTITUTE OF TECHNOLOGY, JAPAN	Chemical synthesis of polymer
Tatsuya Yoshizaki	PHD STUDENT, KYUSHU INSTITUTE OF TECHNOLOGY, JAPAN	Zero discharge of Palm Oil Industry
Koutarou Watanabe		Chemical synthesis of polymer

ATTACHMENT

HOST / LOCATION	DURATION	SPONSOR
PROFESSOR DR MASAMITSU FUNAOKA School of Bioresources, Mie University, Japan	27 days 14 Mac ~ 10 Apr 2010	Japan Society for the Promotion of Science (JSPS)
PROFESSOR DR HARUO NISHIDA Eco-town Collaborative R&D Center for the Environment and Recycling, Kyushu Institute of Technology, Japan	40 days 18 Nov ~ 28 Dec 2010	Kyushu Institute of Technology (Kyutech), Japan
DR. SEI-ICHI AIBA National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central, Japan	21 days 18 Aug ~ 7 Sep 2010	Japan Society for the Promotion of Science (JSPS)
PROFESSOR DR MASAMITSU FUNAOKA School of Bioresources, Mie University, Japan	27 days 14 Mac ~ 10 Apr 2010	Japan Society for the Promotion of Science (JSPS)
PROFESSOR DR HIROKI OGAWA, DR TOSHINARI MAEDA AND PROFESSOR DR YOSHIHITO SHIRAI Kyushu Institute of Technology, Japan	35 days 18 Jan ~ 23 Feb 2010	Kyushu Institute of Technology (Kyutech), Japan
PROFESSOR DR YOSHIHITO SHIRAI Kyushu Institute of Technology, Japan	52 days 30 Oct ~ 21 Dec 2010	Ministry of Higher Education Malaysia (MOHE)
PROFESSOR DR YOSHIHITO SHIRAI Kyushu Institute of Technology, Japan	6 months 1 Jun ~ 30 Nov 2010	Japan Student Services Organization (JASSO)

2010 impact factor journals

impact factor journals

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0.565 Potential Cyclodextrin Glycosyltransferase Producer from Locally Isolated Bacteria

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0.565 Review: Bioconversion of Sago Residue into Value Added Products

2010 publications

cited journals

non-cited journal

- Sahilah Abd Mutalib, Tang Sui Yan, Zaimawati Mohamed Nejis, Rosnah Hassan, Umi Kalsom Md Shah and Son Rodu. 2010. Identification and characterization of actinomycetes for biological control of bacterial wilt of *Ralstonia solanacearum* isolated from tomato. *Journal of Tropical Agriculture and Food Science.* 38(1), 103-114.

book chapters

- Mohd Ali Hassan, Shahrakbah Yacob, Cheong Weng Chung, Yoshihito Shirai and Yung-Tse Hung. (2010). Kitchen Refuse Fermentation. In Environmental Bioengineering (Vol. 11, 193-210). Handbook of Environmental Engineering. Humana Press. New York.

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

patents

- A Novel Bacterium Producing Polyhydroxyalkanoates from Palm Oil Mill Effluent
- A Method for Recovering an Intracellular Polyhydroxyalkanoates (PHA)
- Novel In-Vessel High Rate Composter

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ELSEVIER

Thermophilic biohydrogen production from palm oil mill effluent (POME) using suspended mixed culture

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ABSTRACT

A batch study was conducted to determine the fate of carbohydrate and oil that are present in palm oil mill effluent (POME) during the biohydrogen fermentation process. Sucrose and crude palm oil (CPO) were chosen as substrates and the kinetic profile indicated that mainly sucrose was metabolised by the mixed sludge. The hydrogen yield based on the COD of sucrose added was 146 $cm^3\ g^{-1}$ which is equivalent to a hydrogen to hexose mole ratio of 2.5. The free fatty acids from hydrolysed CPO were not metabolised further which render insignificant generation of hydrogen and volatile fatty acids from oil-based substrate. The average continuous biohydrogen production rate (HPR) from a unit volume of POME under thermophilic condition at 55 °C was 2.64 $m^3\ m^{-3}\ d^{-1}$ at a hydraulic retention time (HRT) of 4 days. Hydrogen constitutes up to 52% of the total biogas and methane was not detected over the 60 day continuous operation. The hydrogen yield (i.e. based on mole ratio of hydrogen to hexose) was 1.72 with an average carbohydrate conversion efficiency of 58%. These limit the potential of recovering more hydrogen energy from POME under current operating conditions.

1. Introduction

Research in dark fermentation for hydrogen (H_2) production is on the increase in recent years but many utilise typical simple sugars or starch [1,2] which are not economically feasible due to their high cost. The new strategy of market-driven research is to focus on using cheap, organic waste-based feedstocks, employing indigenous mixed cultures and improving the hydrogen production yield. Palm oil mill effluent (POME) is constantly associated with environmental burden due to the voluminous discharge of the wastewater during milling process. POME is also considered as high strength complex wastewater with total chemical oxygen demand that can reach up to 94 kg m^{-3} [3]. Many researchers have recognised the potential of harnessing hydrogen from POME [4,5] but much is still unknown on the influences of the chemical properties of POME in converting to hydrogen using mixed microflora.

Many studies have focused on manipulating the operating conditions by pre-treating either the mixed microflora or the non-sterile substrate with chemicals [6,7], sonication [8] or working at low temperatures [9] to prevent the growth of hydrogen-consuming methanogens. At the same time, treating the wastewater at high temperatures will enhance hydrogen evolution rate due to suppression of propionate formation [10] and low hydrogen partial pressure in the liquid phase. The solubility of hydrogen in water decreases with increasing temperature with minimal solubility at temperatures from 50 to 60 °C [11]. Propionate fermentation is known to produce liquid by-products without significant gas production and hydrogen is not involved in the fermentative pathway

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BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Optimization of growth media components for polyhydroxyalkanoate (PHA) production from organic acids by *Ralstonia eutropha*

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Abstract We employed systematic mixture analysis to determine optimal levels of acetate, propionate, and butyrate for cell growth and polyhydroxyalkanoate (PHA) production by *Ralstonia eutropha* H16. Butyrate was the preferred acid for robust cell growth and high PHA production. The 3-hydroxyvalerate content in the resulting PHA depended on the proportion of propionate initially present in the growth medium. The proportion of acetate dramatically affected the final pH of the growth medium. A model was constructed using our data that predicts the effects of these acids, individually and in combination, on cell dry weight (CDW), PHA content (%CDW), PHA production, 3HV in the polymer, and final culture pH. Cell growth and PHA production improved approximately 1.5-fold over initial conditions when the proportion of butyrate was increased. Optimization of the phosphate buffer content in medium containing higher amounts of butyrate improved cell growth and PHA production more than 4-fold. The validated organic acid mixture analysis model can be used to optimize *R. eutropha* culture conditions, in order

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Nitrification of ammonium-rich sanitary landfill leachate

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ABSTRACT

The nitrification of ammonium-rich wastewater is considered challenging due to the 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 feasibility of the nitrifying activated sludge system to completely nitrify synthetic stabilized landfill leachate with $N-NH_4^+$ concentration of 1452 mg/L was tested in this study. The process started with 0.4 kg $N-NH_4^+/m^3$/day of nitrogen loading rate (NLR) in a fed-batch mode to avoid any accumulation of the FA and FNA in the system followed by increasing the nitrogen loading rate (NLR) gradually. Complete nitrification was achieved with a very high ammonium removal percentage (~100%). The maximum specific and volumetric nitrification rate obtained were 0.49 g $N-NH_4^+$/g VSS/day and 3.0 kg $N-NH_4^+/m^3$/day, respectively which were higher than those reported previously for ammonium-rich removal using activated sludge system. The nitrifying sludge exhibited good settling characteristics of up to 36 mL/g VSS and a long SRT of more than 53 days which contributed to the success of the nitrification process. The coexistence and syntrophic association of the AOB and NOB was observed by using Fluorescence *in situ* hybridization (FISH) technique which supported the results on complete nitrification obtained in the system. These findings would be of prominent importance for further treatment of actual sanitary landfill leachate.

1. Introduction

More than half (60%) of the total amount of municipal solid waste (MSW) generated by the households in Kuala Lumpur city is organic waste (Kathirvale et al., 2003). Since waste is not segregated at source, most of the putrescible fraction will end up in the landfills. In a landfill, under an anaerobic condition, the organic nitrogen in the waste such as proteins is hydrolyzed into amino acids before it is further fermented to the other compounds including ammonia. The anaerobic hydrolysis of MSW containing proteins is slower than that of carbohydrates resulting in a slow release of the soluble nitrogen, i.e. ammonia (Jokela and Rintala, 2003). Thus, a high concentration of ammonia and a lower C/N are common features of the stabilized MSW landfill leachate (Fan et al., 2006; Chen, 1996) which requires a long-term after care with the ammonia removal for the landfill.

Removal of ammonia is principally important in the tertiary treatment of landfill leachate to comply with the stringent discharge limit imposed (10 mg/L for Standard A) and to mitigate the toxic effect of ammonia to the aquatic life. Currently, the treatment practiced at the stabilized sanitary landfill is a biological one using sequencing batch reactors (SBR). The leachate has a high content of ammonium in a range of 750–2430 mg/L of $N-NH_4^+$ and the treatment process applied has a difficulty in eliminating the ammonium strength effectively to the acceptable level, thus a significant amount of the inorganic nitrogen appears in the nearby stream (Norjan et al., 2008). The main difficulty associated with the treatment of a high-strength ammonium is the inhibition of free ammonia (FA or NH_3) and nitrous acid (FNA or HNO_2) in ammonia-oxidizing bacteria (AOB) and the nitrite-oxidizing bacteria (NOB) (Isaka et al., 2007; Kim et al., 2006). Moreover, the characteristics of the stabilized landfill leachate, i.e. higher temperature and pH influence the FA concentrations which its amount is a function of the temperature, pH and $N-NH_4^+$ concentration (Anthonisen et al., 1976) (Eq. (1)). The ratio of the ionization constant of the ammonia equilibrium equation and the ionization constant (K_b/K_w) of water is dependant on the temperature (Eq. (2)) (Anthonisen et al., 1976). Therefore, the FA inhibitory effect increases as the

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Biosynthesis and characterization of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) copolymer from wild-type *Comamonas* sp. EB172

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ABSTRACT

Poly(3-hydroxybutyrate) [P(3HB)] homopolymer and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] copolymer was produced by *Comamonas* sp. EB172 using single and mixture of carbon sources. Poly(3-hydroxyvalerate) P(3HV) incorporation in the copolymer was obtained when propionic and valeric acid was used as precursors. Incorporation of 3HV fractions in the copolymer varied from 45 to 86 mol% when initial pH of the medium was regulated. In fed-batch cultivation, organic acids derived from anaerobically treated palm oil mill effluent (POME) were shown to be suitable carbon sources for polyhydroxyalkanoate (PHA) production by *Comamonas* sp. EB172. Number average molecular weight (M_n) produced by the strain was in the range of 153–412 kDa with polydispersity index (M_w/M_n) in the range of 2.2–2.6, respectively. Incorporation of higher 3HV units improved the thermal stability of P(3HB-*co*-3HV) copolymer. Thus the newly isolated bacterium *Comamonas* sp. EB172 is a suitable candidate for PHA production using POME as renewable and alternative cheap raw materials.

1. Introduction

Polyhydroxyalkanoate (PHA) is a biopolymer and biodegradable thermoplastics that have been produced by various types of bacteria as carbon and energy reserve materials in their cytoplasm [1,2]. It has been reported that bacteria could accumulate up to 90% of their cell dry weight (CDW) without disruption of their osmotic pressure [1,3,4]. Poly(3-hydroxybutyrate) [P(3HB)] and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] are the most studied polyesters in the PHA family. These polymers share the physical and mechanical properties similar to petroleum derived thermoplastics polypropylene (PP) and polyethylene (PET) [5]. However, higher price of PHAs in comparison to conventional plastics has limited the wide application of these biodegradable plastics for the time being. Much attention has been spent on optimizing the PHA production process, recovery and blending with other polymers to reduce the total PHA production cost. Approximately 40% of total PHA production was derived from carbon substrates [6]. Incorporation of other substitute hydroxyalkanoic acids (HA) in the P(3HB) homopolymer resulted in improvement in their thermal stability and physical properties from stiff and brittle to elastomeric rubber-like materials [7]. Renewable and cheaper raw materials, such as palm oil mill effluent (POME), have been used as nutrient supplements for bacterial PHA production as substitutes to synthetic carbon sources [8,9]. Attempts to obtain high poly(3-hydroxyvalerate) P(3HV) units in the P(3HB-*co*-3HV) copolymers have been carried out using mixed organic acids from anaerobically treated POME by wild-type *Comamonas* sp. EB172 as an option for reducing PHA production cost [10–12]. The ability of this bacterium to adapt, grow and produce the P(3HB-*co*-3HV) under high acids concentration has encouraged us to expand our study on this bacterium.

The present study aimed to explore the ability of the strain to produce PHA from various carbon sources in one-step cultivation process. The composition of 3HV units produced can be varied by regulating the initial medium pH and propionic acid concentration. Biosynthesis of P(3HB-*co*-3HV) copolymer using mixed organic acids derived from anaerobically treated POME by *Comamonas* sp. EB172 was performed by fed-batch cultivation and the polyesters produced were further characterized.

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Highly selective transformation of poly[(*R*)-3-hydroxybutyric acid] into *trans*-crotonic acid by catalytic thermal degradation

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ABSTRACT

Highly selective transformation of poly[(*R*)-3-hydroxybutyric acid] (PHB) into *trans*-crotonic acid was achieved by thermal degradation using Mg compounds: MgO and $Mg(OH)_2$ as catalysts. Through catalytic action, not only the temperature and E_a value of degradation were lowered by 40–50 °C and 11–14 kJ mol^{-1}, respectively, but also significant changes in the selectivity of pyrolyzates were observed. Notably, $Mg(OH)_2$ showed nearly complete selectivity (~100%) to *trans*-crotonic acid. Kinetic analysis of TG profiles revealed that the catalytic thermal degradation of PHB was initiated by some random degradation reactions, followed by the unzipping β-elimination from crotonate chain-ends as a main process. It was suggested that the Mg catalysts promote the totality of the β-elimination reactions by acting throughout the beginning and main processes, resulting in a lowering in the degradation temperature and the completely selective transformation of PHB.

1. Introduction

The effective utilization of renewable resources, as an alternative to finite fossil resources, will become a subject of vital importance in the near future. Bio-based materials have already been attracting great interest from researchers, not only due to their biodegradable properties, but also because they originate from renewable resources. Recent focus has been on monomer recovery from bio-based polymers, in particular, the depolymerization behavior of poly(L-lactic acid) (PLLA) into L-lactic acid and L,L-lactide, resulting in highly stereo-selective monomer recovery leading to the reproduction of PLLA [1].

Poly[(*R*)-3-hydroxybutyric acid] (PHB) is a typical bio-based polymer stored as a reserve energy source in bacterial cells. The thermal degradation of PHB has been investigated in many previous reports, in which various kinds of degradation end-products have been detected, e.g. CO_2 [2,3], H_2O [3], propene [2,4], ketene [3], acetaldehyde [3], β-butyrolactone [3], *trans*/*cis*-crotonic acid (CA) [2,4–8], 3-butenoic acid (BA) [2], linear oligomers [4–6,8,9], and cyclic trimers [10]. It is well known that the main products from the thermal degradation are *trans*/*cis*-CAs and linear oligomers such as dimers and trimers having a crotonyl group at one chain-end [2,7]. These various kinds of pyrolyzates indicate the multiple degradation mechanisms of PHB present at high temperatures.

Crotonic acid and its copolymers may have various specific applications; for example in dental materials, cosmetics, hair styling products, plasticizers, and herbicidal [11]. Poly(crotonic acid ester)s have also been developed for specific fields such as for use in compatibilizers and paints. Geometrically-selective *trans*-CA and its esters may find future application as versatile monomers for stereo-specific or optically active polymers [12–15]. Generally, it is the properties of stereo-specificity and/or optical purity of monomeric units, which confer the fundamental advantages of bio-based materials, enabling the development of more valuable and functional polymers.

In order to transform PHB into a specific monomer, precise control of the thermal degradation is crucial. In spite of the wealth of previous studies, there are few reports thus far which focus on the selective depolymerization of PHB into the monomer. In cases of PHB pyrolysis without a catalyst, net yield of all butenoic acids (*trans*/*cis*-CAs + BA) as main pyrolysis products ranged from 39.5 wt.-% using thermal volatilization analysis [3] to 87 total ion count % (TIC-%) using pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS) analysis [4]. Recently, it was reported that ^1H NMR analysis of PHB pyrolyzates at 260 °C revealed the ratio

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Turning waste to wealth-biodegradable plastics polyhydroxyalkanoates from palm oil mill effluent – a Malaysian perspective

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ABSTRACT

Palm oil industries have been contributing significantly towards the country's economy and increase standard of living among Malaysians. However, it has also been identified as the major contributor for discharging the largest pollution load throughout the country. Owing to high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), the palm oil mill effluent (POME) cannot be discharged directly into the environment. Thus, palm oil industries are facing tremendous challenges in order to comply with environmental regulations. While anaerobic digestion has been employed by most mills as primary treatment, POME can also be a potential source of degradable organic material which can be converted into value-added products and fine chemicals. Organic acids generated during acid-phase anaerobic digestion of POME could be a potential carbon source for the production of polyhydroxyalkanoates (PHA)- a biodegradable thermoplastic material of microbial origin. This paper aims at understanding how organic acids from POME may serve as a renewable feedstock for the biosynthesis of PHA.

1. Introduction

The contribution of the oil palm industry to Malaysia's economic development has indeed been impressive. Based on the statistic obtained from the Malaysian Palm Oil Council, Malaysia currently accounts for 41% of world's palm oil production and 47% of world exports. Last year, the planted area for oil palm recorded a robust growth of 4.49 million hectares, producing 17.73 million tonnes of crude palm oil (MPOPC). Currently about 400 palm oil mills process the fresh fruit bunches (FFB) of palm. Palm oil mills with wet milling process are accounted for major production of palm oil in Malaysia, Indonesia and Thailand. The extraction of palm oil from the fruit of *E. guineensis* involves a number of processing procedures: sterilization, stripping, digestion, pressing, classification, purification, and vacuum drying for which large quantities of water are required. It is estimated that about 1.5 m^3 of water are needed to process one tonne of FFB, half of this amount ends up as palm oil mill effluent (POME). In the year 2004, more than 40 million tonnes of POME was generated from 372 mills in Malaysia (Yacob et al., 2006). It is believed that the amount of POME being produced will continuously increase in proportion to the world demand of edible oils. Owing to the ever-increasing generation of POME wastewater, disposal becomes a persistent problem and its bioconversion has been considered as an alternative for pollution abatement.

Sustainable utilization of POME wastewater involves its bioconversion into organic compounds as alternatives sources of renewable energy and/or valuable chemicals which will generate additional revenues for the industry (Wu et al., 2009). Application of POME for the production of biohydrogen (Chong et al., 2009; Thong et al., 2008), bioethanol (Alam et al., 2009), citric acid (Jamal et al., 2005), oil palm-based activated carbon (Alam et al., 2006), acetone-butanol-ethanol (Kalil et al., 2003) and compost after mixing with empty fruit bunch (EFB) (Baharuddin et al., 2009) have already been attempted.

With global warming and sustainability so firmly on the agenda, not to mention ever rising oil prices, many new types of plastics are attracting interest from both consumers and industry (Coyler, 2007). Instead of petroleum, the production of bioplastics from biorenewable sources such as waste effluents can make it more sustainable and can reduce our environmental footprint (Khanna and Srivastava, 2005). Bioplastics, regarded as the cutting edge of sustainable living, can be divided into three categories: chemically synthesized polymers such as polylactic acid (PLA), poly(ε-

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ORIGINAL PAPER

Improved biogas production from palm oil mill effluent by a scaled-down anaerobic treatment process

M. F. Basri · S. Yacob · M. A. Hassan · Y. Shirai · M. Wakisaka · M. R. Zakaria · L. Y. Phang

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Abstract This is a scale-down study of a 500-m^3 methane recovery test plant for anaerobic treatment of palm oil mill effluent (POME) where biomass washout has become one of the problems because of the continuous mixing of effluent during anaerobic treatment of POME. Therefore, in this study, anaerobic POME treatment using a scaled down 50-l bioreactor which mimicked the 500-m^3 bioreactor was carried out to improve biogas production with and without biomass sedimentation. Three sets of experiments were conducted under different conditions in terms of biomass sedimentation applied to the system. The first experiment was operated under semi-continuous mode whereas the second and third experiments were operated based on mix and settle mode. As expected, biomass retention improved the anaerobic process as the POME treatment incorporated with mix and settle system were able to operate at an organic loading rate (OLR) of 3.5 and 6.0 kg COD/m^3/day respectively, while the semi-continuous operated anaerobic treatment only achieved OLR of 3.0 kg COD/m^3/day. The highest biogas and methane production rates achieved were 2.42 m^3/m^3 of reactor/day and 0.992 m^3/m^3 of reactor/day, respectively at OLR 6.0 kg COD/m^3/day. The biomass or solids retention in the reactors was represented by the total solids measured in this study.

Keywords Palm oil mill effluent · Anaerobic treatment · Biogas production · Organic loading rate · Biomass sedimentation

Introduction

Palm oil is one of the main commodities in Malaysia. The palm oil demand increased by 10.6% to 33.17 million tonnes in year 2006. The total production of palm oil from Malaysia was 14.96 million tonnes which contributed 45% of the palm oil demand (Sumanthi et al. 2008). With such a huge production, the palm oil industry generates large amounts of wastewater known as palm oil mill effluent (POME). POME is a thick brownish liquid with average chemical oxygen demand (COD) and biological oxygen demand (BOD) values of 50,000 and 25,000 mg/l, respectively. Various treatments have been used to treat POME in order to meet the Malaysian Department of Environment (DOE) discharge standard which is BOD of 100 mg/l. Anaerobic treatment of POME is widely used because of its low operational cost. During anaerobic treatment, a large amount of biogas is produced. Biogas is a mixture of colourless flammable gases obtained by anaerobic digestion of plant based (lignocellulosic) organic waste materials and also from other types of organic waste such as cow dung, pig slurry, effluent from slaughter houses and landfill. Biogas from anaerobic decomposition consists of methane, carbon dioxide and a small amount of

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ORIGINAL PAPER

Polyhydroxyalkanoate production from anaerobically treated palm oil mill effluent by new bacterial strain *Comamonas* sp. EB172

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Abstract A new isolate designated as strain EB172 was isolated from a digester treating palm oil mill effluent and was investigated by polyphasic taxonomic approach. The cells were rod-shaped, Gram-negative, non-pigmented, non-spore-forming and non-fermentative. Phylogenetic analysis using the 16S rRNA gene sequence showed that the strain clustered with the genus *Comamonas*. Its closest neighbours were the type strains *Comamonas terrigena* (96.8%), *Comamonas koreensis* (93.4%), *Comamonas composti* (92.9%), and *Comamonas kerstersii* (91.1%). The ability of the strain EB172 to produce polyhydroxyalkanoates (PHA) when supplied with organic acids made this bacterium unique among *Comamonas* species. The bacterial strain was clearly distinguished from all of the existing strains by phylogenetic analysis, fatty acid composition and a range of physiological and biochemical characteristics. The G+C content of the genomic DNA was 59.1 mol%. The strain showed good growth in acetic, propionic and *n*-butyric acids. *Comamonas* sp. EB172 produced 9.8 g/l of cell dry weight and accumulated 59 (wt%) of PHAs when supplemented with mixed organic acids from anaerobically treated palm oil mill effluent. It is evident from the genotypic, phenotypic data and ability to produce PHAs that strain EB172 represents a new strain in the genus *Comamonas* (GeneBank accession no. EU847238).

Keywords *Comamonas* sp. · Palm oil mill effluent · Polyhydroxyalkanoates · Phylogenetic · Taxonomy

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Introduction

Palm oil mill effluent (POME) is one of the major sources of pollutant produced during oil palm processing. POME is currently treated using different approaches such as lagoon, open and closed digesters as part of the wastewater management system in Malaysia. In recent years, it has gained great attention by the research institutions and industrial sectors, due to its potential as sources of carbon and nitrogen for microbial growth. The presence of unique microflora inside the lagoon, open and closed digesters coupled with rich cellulosic and lipid waste materials in POME led to new findings in recent years. Production of organic acids (Hassan et al. 1997; Tabassum et al. 2008), methane (CH_4) (Yacob et al. 2006) and biohydrogen (Chong et al. 2009) from POME were evidences of the presence of potential carbon and nitrogen sources al well as biological biodiversity in the POME sludge. Production of mixed organic acids from anaerobicallay treated palm oil mill effluent has introduced it as a shown renewable and cheaper carbon sources for PHAs production (Hassan et al. 1997, 2002; Zakaria et al. 2008).

During the characterization of the microflora from an open digester treated-POME, we isolated a bacterium

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SYNTHESIS, CHARACTERIZATION, AND STRUCTURAL PROPERTIES OF INTRACELLULAR COPOLYESTER POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE) PRODUCED BY *COMAMONAS* sp. EB 172 FROM RENEWABLE RESOURCE

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Microbial copolymer was produced by a local isolate,* Comamonas *sp. EB 172, using mixed organic acids such as acetic, propionic, and butyric acids as carbon sources in pH-stat fed-batch fermentation. Maximum polymer production (6.59 g/L) was achieved at 50 h of fermentation when 73.64 g/L mixed acids, generated from the acidogenic fermentation of palm oil mill wastewater, were used. Accumulation of polymer in the cell was 70% (wt/wt), which was observed under transmission electron microscope. The morphological, chemical, thermal, and mechanical properties of the solvent-extracted biopolymer were determined by various techniques (SEM, GC, ^{13}C NMR, FT-IR, TGA, and tensile testing). The copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) consisted of 87 mol% β-hydroxybutyric acid (HB) and 13 mol% β-hydroxyvaleric acid (HV). With chemical properties similar to commercial PHBV and mechanical strength of around 30 MPa and 8% elongation at break, the biopolymer offers potential for industrial applications.

Keywords: *Comamonas* sp. EB 172: characterization; Fed-batch fermentation; Mixed organic acids; PHBV

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Review

Treatment of wastewater from rubber industry in Malaysia

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Presently, Malaysia is the third largest rubber producer in the world, whereby the rubber industry is an economically and socially significant industry. Rubber industry consumes large volumes of water, uses chemicals and other utilities and produces enormous amounts of wastes and effluent. Discharge of untreated rubber effluent to waterways resulted in water pollution that affected the human health. With a new global trend towards a sustainable development, the industry needs to focus on cleaner production technology, waste minimization, utilization of waste, resource recovery and recycling of water. The present work aims at highlighting various technologies that currently have been used for treatment of rubber effluent in Malaysia. The work introduces the basis of these processes including their benefits and also problems. It also adheres to the future trends of rubber effluent treatment in Malaysia by reviewing various treatment technologies for natural rubber industry implemented by Thailand, the world largest rubber producer. These new and effective effluent treatment methods would minimize environmental pollution of rubber industry and bring it to become sustainable and environmental friendly.

Key words: Rubber industry, effluent, waste management, Malaysia.

INTRODUCTION

Natural rubber is an elastic hydrocarbon polymer that is originally derived from a milky colloidal suspension, or latex of *Hevea brasiliensis*. The purified form of rubber which can also be produced synthetically is chemical polyisoprene. Natural rubber is extensively used in various applications and products (Sun, 2004). Today, 70 -80% of produced raw rubber in the world supply in south-east Asia, comes mainly from Thailand, Malaysia and Indonesia (Lonholdt and Andersen, 2005; Xiaofei, 2008). Main export of Malaysian products includes electronic equipment, petroleum and petroleum products, palm oil, wood and wood products, rubber and textile (Usa, 2007). Rubber plays an important role in Malaysian's economy and about 30% of foreign exchange revenue derived from this crop (Shacklady, 1983; Hutagalung, 2003; Anitha et al., 2007). However, some changes in land use were raised in the mid-80s and large areas of rubber field were converted for industrial, commercial and residential uses (Vijayaraghavan, 2008a, b). More tendencies were also done towards the plantation of lucrative crops likes oil palm and cocoa (Choo et al., 2003). Due to this, a gradual decrease occurred in raw natural rubber production. Therefore, It caused a decline to Malaysian's status from first to third and ranked it after Thailand and Indonesia (Vijayaraghavan, 2008a). The natural rubber statistic (2008) for Malaysia and Thailand is shown in

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Abbreviations: EQA, Environmental quality act; **UASB,** anaerobic sludge blanket reactor; **SRR,** sulphate reduction reactor; **PNSB,** purple non sulphur photosynthetic bacteria; **CW,** constructed wetland; **VF,** vertical flow; **SSF,** subsurface flow; **ORP,** oxidation-reduction potential; **BAS,** batch activated sludge; **DOE,** Department of Environment; **RRIM,** Rubber Research Institute of Malaysia; **BOD,** biological oxygen demand; **HRT,** hydraulic retention times; **COD,** chemical oxygen demand; **TDS,** total dissolved solids.

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Full Length Research Paper

Comparative study of methods for extraction and purification of environmental DNA from high-strength wastewater sludge

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DNA extraction from wastewater sludge (COD 50000 and BOD 25000 mg/l) was conducted using nine different methods normally used for environmental samples including a procedure used in this study and the results obtained were compared. The quality of the differently extracted DNAs was subsequently assessed by measuring humic acid concentration, cell lysis efficiency, polymerase chain reaction (PCR) amplification of methanogenic and eubacterial 16S rDNA. The protocol developed in this study was further evaluated by extracting DNA from various high-strength wastewater sludge samples, denaturing gradient gel electrophoresis (DGGE) and fluorescent *in situ* hybridization (FISH) analyses. The results revealed that great differences existed among the nine procedures and only a few produced satisfactory results when applied to high-strength wastewater sludge. Thermal shock alone was shown inefficient to disrupt the methanogenic cell wall to release the DNA. The method presented in this study (Procedure 9) is generally recommended because of the low concentration of contaminants and its high efficiency despite its simplicity.

Key words: High-strength wastewater sludge, DNA extraction, environmental samples, humic acids, denaturing gradient gel electrophoresis, fluorescent *in situ* hybridization

INTRODUCTION

Accurately determining the presence of microorganisms in wastewater sludge is imperative for its efficient treatment. There are numerous strategies for the detection of specific archaea and bacteria from environmental samples (Amann et al., 1995). It has been shown that conventional methods for studying microbial diversity, such as plating on selective media, are unreliable because only a small fraction of the bacterial species present in the natural habitat will grow on synthetic media (Amann et al., 1995). A newer approach is to estimate the microbial diversity by characterizing the DNA or RNA from a sample without

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Full Length Research Paper

Effects of agitation speed, temperature, carbon and nitrogen sources on the growth of recombinant *Lactococcus lactis* NZ9000 carrying domain 1 of aerolysin gene

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***Lactococcus lactis* is a Gram-positive bacterium widely used in the production of buttermilk and cheese. Recently, the bacterium becomes famous as the genetically modified organism can be used alive for the treatment of disease. In this study, different cultural conditions based on agitation speed and temperature on the growth of recombinant *L. lactis* NZ9000 harboring domain 1 of aerolysin gene (NHD1Aer) were investigated using shake flask experiment. The effect of different carbon (glucose, sucrose and lactose) and nitrogen (yeast extract, peptone, NH_4Cl, $(NH_4)_2SO_4$, and urea) sources in M17 medium on the cell accumulation were also tested. The results showed that the highest cell concentration (3.22 g/L, μ_m = 0.58 h^{-1}) was obtained when the cultivation was incubated at 27°C and at agitation of 100 rpm. The cells growth was markedly improved when utilizing glucose and peptone/yeast extract as carbon and nitrogen sources, respectively. The aerolysin gene in the cells after four generation time was extracted and then analyzed using agarose gel electrophoresis. The results obtained showed a 250 bp band amplified of domain 1 of the aerolysin gene.**

Key words: Aerolysin, *Lactococcus lactis*, fermentation, one-factor-at-a-time.

INTRODUCTION

Aerolysin is a well-known pore-forming toxin and major virulence factor that was purified from *Aeromonas hydrophila,* a human pathogen that produces deep wound infection and gastroenteritis. *A. hydrophila* is also known as fish pathogen that causes high mortality and great economic losses in freshwater fish farming worldwide. It is capable of killing target cells by forming channels in their membrane after binding to glycosylphosphati-dylinositol-anchored receptors (Abrami et al., 2000). The toxin is activated either by soluble digestive enzymes or by host endoprotease furin which remove a C-terminal fragment about 40 amino acids long. The active aerolysin will then oligomerize in order to form a stable oligomer structure on the surface of the host cells, which inserts into the membrane to form a pore (Buckley, 1992; Fivaz et al., 2001).

Lactococcus lactis is a Gram-positive lactic acid bacterium (LAB) that is generally recognized as safe (GRAS). The potential of *L. lactis* as a vaccine vector has been demonstrated in several publications and one of the report showed that *L. lactis* used as an antigen delivery vehicle is for vaccination against tetanus (Grangett et al., 2002). The gene coding for domain 1 of aerolysin gene

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Abbreviations: LAB, Lactic acid bacterium; **GRAS,** generally recognized as safe; **DNS,** dinitrosalicylic acid; **HPLC,** high performance liquid chromatography; **PCR,** polymerase chain reaction.

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Full Length Research Paper

Effects of palm oil mill effluent (POME) anaerobic sludge from 500 m^3 of closed anaerobic methane digested tank on pressed-shredded empty fruit bunch (EFB) composting process

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In this study, co-composting of pressed-shredded empty fruit bunches (EFB) and palm oil mill effluent (POME) anaerobic sludge from 500 m^3 closed anaerobic methane digested tank was carried out. High nitrogen and nutrients content were observed in the POME anaerobic sludge. The sludge was subjected to the pressed-shredded EFB to accelerate the co-composting treatment. In the present study, changes in the physicochemical characteristics of co-composting process were recorded and evaluated. The co-composting treatment was completed in a short time within 40 days with a final C/N ratio of 12.4. The co-composting process exhibited a higher temperature (60 - 67°C) in the thermophilic phase followed by curing phase after four weeks of treatment. Meanwhile, pH of the composting pile (8.1 - 8.6) was almost constant during the process and moisture content was reduced from 64.5% (initial treatment) to 52.0% (final matured compost). The use of pressed-shredded EFB as a main carbon source and bulking agent contributed to the optimum oxygen level in the composting piles (10 - 15%). The biodegradation of composting materials is shown by the reduction of cellulose (34.0%) and hemicellulose (27.0%) content towards the end of treatment. In addition, considerable amount of nutrients and low level of heavy metals were detected in the final matured compost. It can be concluded that the addition of POME anaerobic sludge into the pressed-shredded EFB composting process could produce acceptable and consistent quality of compost product in a short time.

Key words: Pressed-shredded empty fruit bunch, palm oil mill effluent anaerobic sludge, anaerobic digester, compost.

INTRODUCTION

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 empty fruit bunch (EFB) (23%), mesocarp fibre (12%), shell (5%) and palm oil mill effluent (POME) (60%) for every tonne of fresh fruit bunches (FFB) processed in the mills (Najafpour et al., 2005). In 2005, it was estimated that about 75.5 million tonnes of FFB has been processed in the country (Lau et al., 2008). Thus, the treatment of EFB and POME has gained interest from many researchers

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Full Length Research Paper

Potential cyclodextrin glycosyltransferase producer from locally isolated bacteria

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Cyclodextrin glycosyltransferase (CGTase) is one of the most important groups of microbial amylolytic enzymes that have been used for degradation of starch to yield cyclodextrin (CD) via cyclization reaction. The increasing demand for CD in industrial application has led to an extensive study about CGTase which begin with screening, isolation and characterization of CGTase-producing bacteria. The identification of CGTase producer involves the use of solid media containing phenolphthalein and methyl orange as indicators that was detected by the colour changes. The formations of clearance zone around the bacterial colony in the starch-containing medium were observed and the diameters were measured to gauge the hydrolytic efficiency of the bacteria. Out of 65 soil bacterial samples screened, *Bacillus* sp. NR5 UPM was identified as the most prolific CGTase producer, which produced highest CGTase activity (11.709 U/ml) and highest β-CD concentration (2.504 mg/ml) with α-CD:β-CD:γ-CD ratio was 0.5:91.1:8.4 when using raw soluble starch as a substrate. It also showed as the best CGTase producer when using sago starch as a substrate (15.514 U/ml). This isolate was known as a raw starch-degrading enzyme producer since it has the capability to use raw starch as a substrate. Thus, in the future, this new isolate perhaps can share the biggest market in industrial application.

Key words: Cyclodextrin, cyclodextrin glycosyltransferase, starch.

INTRODUCTION

Cyclodextrin glycosyltransferase (CGTase) (EC 2.4.1.19) 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) (Ishii et al., 2000). The production of CD that was catalysed by CGTase are in the mixture of α-CD, β-CD and γ-CD, containing 6, 7 and 8 glucose residues, respectively. However, CGTase also is capable of synthesizing other types of CDs in very small amounts, which contain nine-, ten-, eleven-, or twelve-membered rings (Szejlti, 1998). The CGTase producer came from various species such as *Bacillus, Klebsiella, Micrococcus, Brevibacterium, Thermoactinomyces* and *thermophilic archea* are the major producer. Soluble starch (Yampayont et al., 2003), potato (Avci and Donmez, 2009), corn (Kim et al., 1995, 1997), wheat starch (Gawande et al., 1999; Gawande and Patkar, 2001), etc can be used as a sole source of carbon for the production of CD with the sago starch as a potential alternative substrate (Nisanart et al., 2003; Charoenlap et al., 2004). The unique hydrophobic interior cavity and hydrophilic surface of CDs enable it to

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Abbreviations: CGTase, Cyclodextrin glycosyltransferase; **CD,** cyclodextrin; **HPLC,** high performance liquid chromatography.

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Review

Bioconversion of sago residue into value added products

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Bioconversion of the agro-residue offers the possibility of creating marketable value-added products. In this regard, sago residue which contains solid and liquid materials produced abundantly as a by-product from the sago starch processing industry. Due to its organic nature and low ash content, attempts have been made to produce several products such as fermentable sugar, enzyme, compost for mushroom, animal feed and adsorbent. Utilization of sago residue not only reduce the polluting effects from the sago processing industries, but will also provide an economic solution for waste management system at sago processing mills. This review focuses on the developments in processes and products for the value addition of sago residues through biotechnological means.

Key words: Sago palm, sago starch, sago residue, sago 'hampas', sago wastewater.

INTRODUCTION

In Sarawak, East Malaysia, agro-residues from sago starch processing industries are abundant and readily available. As stated by Bujang et al. (1996), it has been estimated approximately 7 tons (t) of sago pith waste was produced daily from a single sago starch processing mill. Currently, these residues were washed off into nearby streams together with wastewater and deposited in the factory's compound, which can lead to serious environmental problems. The problems of pollution from sago starch processing are more social and economic in nature than technological. It has been shown that sago wastewater represents high organic material ('hampas'), chemical oxygen demand (COD) and biological oxygen demand (BOD), which contravened the standard limit discharge enacted in the Environmental Quality Act, 1974 (sewage and industrial effluents regulation, 1979). According to starch processors, the installation of pollution control devices can be 20 - 50% of the total investment cost of a large-scale factory. Thus, through the exploittation of these residues from sago starch processing industry, a promising materials resources such as sago bark (peelings from initial processing), sago 'hampas' (fibrous by products from crushing and sieving) and sago wastewater can be used for global environmental conservation and sustainable development. Sago 'hampas' which contained mostly starch and lignocellulosic materials is such a good choice to be used as a substrate for solid substrate fermentation either by fungal bioconversion or by enzyme or acid hydrolysis. Sago bark which contains mostly lignin is a rigid structure, traditionally used as a base around the sago processing mill. The present review addresses the progress that has been made in each of these resources with emphasis on the bioconversion into value added products.

SAGO PALM AND ITS PROCESSING INDUSTRY

The importance of starch production by sago palm is mainly focused in the Asia-Pacific region and South East Asia (Wang et al., 1996). Sago palms are those species of the genus *Metroxylon* belonging to the Palmae family. It is a species from which useful quantities of starch-rich flour can be extracted from stem tissue by shredding and

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A Comparative Study of Organic Acids Production from Kitchen Wastes and Simulated Kitchen Waste

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Abstract: Simulated kitchen waste was developed in this study to overcome the problem of kitchen waste variation composition. The performance of organic acids production was compared between the simulated kitchen waste and original kitchen waste. Both substrates were subjected to anaerobic digestion by indigenous mixed microflora from fermented kitchen waste in a 250 mL shake flasks. The condition used were mixing at 200 rpm, adjusted pH 5 and 7 and temperature of 30°C, 37°C and 40°C. The highest organic acids produced in the both kitchen waste and model kitchen waste were 48.64g/L and 37.49g/L, respectively at pH 5 and 37°C. For both kitchen waste and simulated kitchen waste fermentation, lactic acid was dominant, 37g/L (76.2%) followed by acetic acid (17.7%) and butyric acid (6.1%).

Key words: Kitchen waste, model kitchen waste, anaerobic digestion, organic acid.

INTRODUCTION

Currently, about 15 268 ton/day of municipal solid waste (MSW) has been collected everyday in Malaysia and the average amount of waste generated is 0.5-0.8 kg/person/day (Karthivale *et al.,* 2003). About 71.6% of collected MSW consist of food and organic waste followed by plastics (13.3%), paper (5.8%), textile (2.4%), glass (2.1%), metal (2.0%), rubber and leather (0.5%), wood (0.5%) and others (1.8%). Food and organic waste has contributed to the high moisture content approximately 52.6% (Hassan *et al.,* 2001). On account of its high moisture content, these will be a challenge in order to appropriately provide waste disposal system since the organic waste will easily putrefies, resulting in groundwater contamination and odour generation (Wang *et al.,* 2005). So far, there are number of waste management technologies that have been adopted in Malaysia including landfill, and incineration. However, due to the new developments and space constrain, Malaysia has turning to incineration process especially in the cities (Hassan *et al.,* 2001). However, the incineration process is not suitable for treatment of food and organic waste due to combustion energy loss and undesirable byproduct dioxin related compounds are formed (Sakai *et al.,* 2001). Therefore, another appropriate method for stabilization and disposal of the food and organic waste need to be developed.

In MSW, food and organic waste mostly consist of uneaten food and food preparation waste especially from residences, restaurants and cafeteria known as kitchen waste (Village, 1998). Kitchen waste is characterized by a high organic content containing soluble sugars, starch, lipids, proteins, cellulose, and other compounds that are readily biodegradable, and generally contain few compounds that inhibit bacteria (Wang *et al.,* 2003b). There will be an advantage if the kitchen waste with high fraction of organic content can be utilized as a high value of carbon resource. So far, the recovery energy from methane fermentation and the production of organic fertilizers by composting using kitchen waste has been implemented (Wang *et al.,* 2001). These organic wastes also have been used for organic acids production. Production of organic acids from kitchen waste, not only can eliminate waste pollution problem but also reduce the production cost of organic acids (Wang *et al.,* 2003b). Lactic acid could be stably accumulated during kitchen waste fermentation by controlling some fermentation parameters such as temperature, pH etc (Sakai *et al.,* 2000; Wang *et al.,* 2002). Lactic acid was found to be the main fermentation products for kitchen wastes (Wang *et al.,* 2001; Wang *et al.,* 2003b). Recently, there is a need for lactic acids as it can be utilized as a raw material for polylactic acid, a polymer used as medical disposable and environmental friendly biodegradable plastics, which can replace synthetic plastics from petroleum feedstocks (Niju *et al.,* 2004).

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Accelerated Start-up of a Semi-commercial Digester Tank Treating Palm Oil Mill Effluent with Sludge Seeding for Methane Production

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Abstract: The modern closed digesters are becoming more popular for treating palm oil mill effluent (POME) and are currently being installed nationwide in Malaysia to replace the conventional open lagoons and tanks treatment system. This paper describes an accelerated start-up of the 500 m^3 semi-commercial anaerobic digester treating POME and methane gas recovery for clean development mechanism (CDM) project. Results showed that by direct seeding through the transfer of the sludge from either top or bottom of the open digester tank, the start-up period was significantly shortened. The bottom seed sludge transfer led 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) and methane composition of 50 to 60%. The presence of high amount of methanogens in the seed sludge significantly reduced the need for a long acclimatization period and the digester could be fed with POME within less than a day after the seed sludge transfer process was completed. Close examination using scanning electron microscopy (SEM) and fluorescence *in situ* hybridization (*FISH*) revealed abundant amount of bacteria and methanogens, in particular *Methanosaeta* sp., in the seed sludge samples, which are very important for successful acidogenesis and methanogenesis processes.

Key words: Palm oil mill effluent (POME) • Anaerobic treatment • Digester start-up • Biogas • Methane

INTRODUCTION

Malaysia is currently the largest producer and exporter of palm oil in the world and the industry contributes significantly to the country's economy [1]. Despite huge benefits to the Malaysian economy, the palm oil industry also generates large amounts of wastes in the form of empty fruit bunch (EFB), oil palm frond, mesocarp fiber, palm kernel shell, palm oil mill effluent (POME) and sludge from ponds and anaerobic tanks [2-3]. In general, millions of tonnes of these wastes are available each year and ready to be exploited. In the case of POME, the most popular treatment method currently is using open ponds or tanks [4]. Therefore, its utilization for higher valued products such as methane in industrial scale is rather limited despite the fact that a considerable amount of literature has been published on methane production by using anaerobic digestion technology [4-13]. However, in the last few years there has been a great concern to utilize POME for methane production via clean development mechanism (CDM) project for certified emission reduction (CER) trading [14-20].

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Research Article

Chemical recycling of polyhydroxyalkanoates as a method towards sustainable development

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Chemical recycling of bio-based polymers polyhydroxyalkanoates (PHAs) by thermal degradation was investigated from the viewpoint of biorefinery. The thermal degradation resulted in successful transformation of PHAs into vinyl monomers using alkali earth compound (AEC) catalysts. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)s (PHBVs) were smoothly and selectively depolymerized into crotonic (CA) and 2-pentenoic (2-PA) acids at lower degradation temperatures in the presence of CaO and $Mg(OH)_2$ as catalysts. Obtained CA from 3-hydroxybutyrate sequences in PHBV was copolymerized with acrylic acid to produce useful water-soluble copolymers, poly(crotonic acid-*co*-acrylic acid) that have high glass-transition temperatures. The copolymerization of CA derived from PHA pyrolysis is an example of cascade utilization of PHAs, which meets the idea of sustainable development.

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Keywords: Biopolymers · Chemical recycling · Crotonic acid · Polyhydroxyalkanoates · Thermal degradation

1 Introduction

Recently, many kinds of bio-based polymers such as polylactate (PLA), polyhydoxybutyrate (PHB) and polybutyrolactone (PBL) have been produced to replace oil-based polymers for sustainable development. The production of these bio-based polymers is an example of biorefinery, which integrates the biomass conversion processes and equipment. Sustainable polymer production should not only take into account the method used in producing polymer materials, but also the carbon cycle of the polymer. Chemical recycling of polymers, which can be defined as the conversion of polymers into low-molecular weight materials, has been an important subject in the polymer waste management. Thus, the chemical recycling of bio-based polymers will become an important subject in the field of environmental biotechnology and biorefinery.

Chemical recycling of polymers is aimed at reducing the amount of wastes, saving the material resources and reusing the recovered monomers for producing other types of polymers. At the same time, cascade utilization of polymers could be introduced before they are finally released into the environment. Refined low-molecular weight products can be reused as raw materials for the (re)production of valuable bio-based products.

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Abbreviations: 2-PA, 2-pentenoic acid; **3HB**, 3-hydroxybutyrate; **3HV**, 3-hydroxyvalerate; **AA**, acrylic acid; **AEC**, Alkali earth compound; **CA**, Crotonic acid; **DSC**, Differential scanning calorimetry; **PAA**, poly(acrylic acid); **P(CA-*co*-AA)**, poly(crotonic acid-*co*-acrylic acid); **PHA**, polyhydroxyalkanoate; **PHB**, poly(3-hydroxybutyrate); **PHBV**, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); **PHB-H/PHBV-H**, Purified PHB/PHBV; **Py**, pyrolysis; T_g, glass transition temperature; **TG**, thermogravimetry

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Isolation and Characterization of Thermophilic Cellulase-Producing Bacteria from Empty Fruit Bunches-Palm Oil Mill Effluent Compost

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Abstract: Problems statement: Lack of information on locally isolated cellulase-producing bacterium in thermophilic compost using a mixture of Empty Fruit Bunch (EFB) and Palm Oil Mill Effluent (POME) as composting materials. **Approach:** The isolation of microbes from compost heap was conducted at day 7 of composting process where the mixture of composting materials consisted of 45.8% cellulose, 17.1% hemicellulose and 28.3% lignin content. The temperature, pH and moisture content of the composting pile at day 7 treatment were 58.3, 8.1 and 65.5°C, respectively. The morphological analysis of the isolated microbes was conducted using Scanning Electron Microscope (SEM) and Gram stain method. The congo red test was conducted in order to detect 1% CMC agar degradation activities. Total genomic DNAs were extracted from approximately 1.0 g of mixed compost and amplified by using PCR primers. The PCR product was sequent to identify the nearest relatives of 16S rRNA genes. The localization of bacteria chromosomes was determined by Fluorescence *In Situ* Hybridization (FISH) analysis. **Results:** Single isolated bacteria species was successfully isolated from Empty Fruit Bunch (EFB)-Palm Oil Mill Effluent (POME) compost at thermophilic stage. Restriction fragment length polymorphism profiles of the DNAs coding for the 16S rRNAs with the phylogenetic analysis showed that the isolated bacteria from EFB-POME thermophilic compost gave the highest homology (99%) with similarity to *Geobacillus pallidus.* The strain was spore forming bacteria and able to grow at 60°C with pH 7. **Conclusion:** Thermophilic bacteria strain, *Geobacillus pallidus* was successfully isolated from Empty Fruit Bunch (EFB) and Palm Oil Mil Effluent (POME) compost and characterized.

Key words: Cellulase, thermophilic bacteria, composting, empty fruit bunch, palm oil mill effluent

INTRODUCTION

Composting can be defined as the controlled biological decomposition of organic substrates carried out by successive microbial populations combining both mesophilic and thermophilic activities, leading to the production of a final product sufficiently stable for agricultural field without adverse environmental effects (Iyengar *et al*., 2005). Composting of Empty Fruit Bunch (EFB) and Palm Oil Mill Effluent (POME) is one of the alternative ways to reduce the amount of by product and towards the zero emission programs in palm oil mill industry (Hassan *et al*., 2002; Baharuddin *et al*., 2009). The composting process typically undergoes a series of temperatures which are rapid increase in temperature, a period of sustained high temperature and followed by the slow cooling of the compost (Dees and Ghiorse, 2001).

EFB contain a high proportion of cellulosic matter which is easily decomposed by a combination of

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Isolation and Selection of Appropriate Cellulolytic Mixed Microbial Cultures for Cellulases Production from Oil Palm Empty Fruit Bunch

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Abstract: In order to construct cellulolytic fungal mixed cultures, screening and isolation of cellulolytic fungi was done using rotten oil palm fruit bunches as microorganism source. Three isolated fungi had shown the ability to degrade cellulose based on decolorization of CMC selective agar using Gram's iodine as color indicator. However, only two strains; KS1 and KS5 were selected for construction fungal mixed culture. Based on fungal interaction evaluation test done on PDA agar, both strains showed contact deadlock inhibition interaction with each other. In correlation to cellulase enzymes production, mixed cultures of strains KS1 and KS5 showed low enzymes activity compared to pure culture system. Although, the cellulase enzymes production is low, total cellulase enzymes composition was better than in pure culture system individually.

Key words: Consortia, oil palm empty fruit bunch, cellulase, submerged fermentation

INTRODUCTION

Cellulase enzymes complex is a multi-domain protein that consists of three major enzymes components which are endo-β-(1-4)-D-glucanase, exo-β-(1-4)-D-glucanase and β-glucosidase that works synergically in complex cellulose degradation (Duff *et al*., 1987). In cellulose degradation process, endo-β-(1-4)-D-glucanase or known as carboxymethyl cellulase act by randomly cleave β-(1-4) linkages of glucose chain in the amorphous region of cellulose to open up cellulose structure for subsequent attack from exo-β-(1-4)-D-glucanase (Esteghlalian *et al*., 2002). On the other hand, exo-β-(1-4)-D-glucanase (cellobiohydrolase) act to release cellobiose moiety from end of glucose chain. Finally, β-glucosidase releases glucose from cellobiose and short chain cellooligosaccharides (Krishna *et al*., 1998; Rajoka *et al*., 2004; Ikram-ul-Haq *et al*., 2005). For more than four decades, many researches have been done on cellulase enzymes either in screening and isolation of new strains, optimization processes involved or application of enzymes in industrially. Yet, by far, application cellulases industrially faced difficulties especially in total operational cost which mostly contributed from the enzymes itself compared to the raw material used. Even though various microorganisms have been reported to have the ability to produced cellulase enzymes extracellularly, most studies suggested fungi have better enzymes production compared to bacteria and yeast (Bakri *et al*., 2003). Most reported cellulase enzymes producer are from *Trichoderma* species and *Aspergillus* species (Bhat, 2000).

Trichoderma sp., was widely studied and used industrially especially in production of β-(1-4) exoglucanase and β-(1-4) endoglucanase. Compared to *Trichoderma* sp., *Aspergillus* sp., suffered low production of β-(1-4) exogulacanase and β-(1-4) endoglucanse but high in β-glucosidase enzymes (Madamwar and Patel, 1992). However, in order to obtain high degradation of cellulose material, synergistic effect of all three component of cellulase enzymes have to be achieved. Due to low production of β-glucosidase by *Trichoderma* sp., many approaches have been suggested to improve degradation of cellulosic material (Kovác *et al*., 2009). Many suggested mixed culturing between two strains and supplementation of β-glucosidase enzyme from *Aspergillus* improved total cellulase enzymes activity of *Trichoderma*. Mixed cultures is a cultivation system where two or more different microorganisms were introduce in the same fermentation condition or environment (Yang *et al*., 2003).

According to Correa *et al*. (1999), utilization of fungi mixed culture resulted in higher product yield and growth rate especially in poor nutritional agriculture residue

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Improved Anaerobic Treatment of Palm Oil Mill Effluent in a Semi-Commercial Closed Digester Tank with Sludge Recycling and Appropriate Feeding Strategy

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ABSTRACT

Anaerobic treatment of palm oil mill effluent (POME) in a semi-commercial closed digester tank with sludge recycling was studied using different feeding strategies; one fixed at every three hour and another at every six hour. The organic loading rate (OLR) was increased step-wise and stopped once inhibition on methane production occurred. The chemical oxygen demand (COD), feeding rate, hydraulic retention time (HRT), OLR, and sludge recycling ratio were measured. Performance was based on the COD removal efficiency and methane yield, while stability was assessed in terms of total volatile fatty acids (VFA) accumulation, total VFA-to-alkalinity ratio (VFA:Alk) and food-to-microorganisms ratio (F/M ratio). The feeding strategies, at every three hour and six hour, gave satisfactory COD removal efficiency of higher than 90%, but the latter feeding strategy gave a more stable process with total VFA concentration recorded below 500 mg L^{-1} and VFA:Alk ratio of less than 0.3 at maximum OLR of 6.0 kgCOD m^{-3} d^{-1}. The treatment period could also be extended up to 100 days without any obvious problems.

Keywords: Anaerobic treatment, biogas, feeding interval, methane, palm oil mill effluent, sludge recycling

INTRODUCTION

Malaysia is blessed with suitable climatic and geographical factors for the cultivation of oil palm scientifically known as *Elaeis guineensis Jacq*. The palm oil industry is very important to Malaysia and it has contributed significantly to the country's gross domestic product (GDP). The export earnings from palm oil, palm kernel oil, and its products in 1998 amounted to almost US$5.6 billion, equivalent to 5.6% of the country's GDP. Today, Malaysia is the world's largest producer and exporter of palm oil (Yusoff, 2006). However, despite the high economic returns, the generation of liquid waste or palm oil mill effluent (POME) is also huge. It was estimated that for every tonne of fresh fruit bunch processed, between 0.5 and

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The Effect of Hydraulic Retention Time and Volatile Fatty Acids on Biohydrogen Production from Palm Oil Mill Effluent under Non-Sterile Condition.

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Abstract: The effect of hydraulic retention time and volatile fatty acids produced during fermentation were investigated on biohydrogen production from palm oil mill effluent in a 50 L bioreactor. The fermentation was done in three different hydraulic retention times; HRT 5, HRT 3 and HRT 2 days. Hydraulic retention time and volatile fatty acids concentration showed a vital role in response to the biohydrogen concentration, biohydrogen rate and biohydrogen yield. The maximum biohydrogen concentration was obtained at HRT 2 days with 30% hydrogen content in biogas. The biohydrogen yield and rate were 1054 NmL/L-POME and 44 NmL/h/L-POME, respectively. The lowest biohydrogen yield and rate were observed at HRT 5 days with 557 NmL/L-POME and 5 NmL/h/L-POME, respectively. Meanwhile, the accumulation of propionic acid concentration up to 7 g/L was suggested as a factor that reduced the biohydrogen production.

Key words: Biohydrogen, palm oil mill effluent, hydraulic retention times, volatile fatty acids.

INTRODUCTION

In the present world, energy is highly demanded by the industries, power plants, offices, households, as well as individual life. The demand of the energy is expending and lead to depletion of non-renewable energy such as coal, oil, gasoline and metal cores. Since this problem has overwhelmed all over the world, a lot of researches have been carried out to utilize biomass as alternative renewable resources (Lay *et al*., 1999, Levin *et al*., 2004, Prasertsan and Prasertsan, 1996, Vijayaraghavan *et al*., 2007). Biomass is known as by-products with no or low profit from agricultural crops or industrial processes. The production of biological hydrogen (biohydrogen) from biomass has gain wide attentions since it is one of the most reliable and sustainable energy for the future (Debabrata and Veziroglu, 2001, Levin *et al*., 2004). From the overview of environmental and engineering side, utilizing biomass wastewater or solid waste as a substrate for fermentation become an essential approach since it is capable for biohydrogen production in non-sterile conditions(Valdez-Vazquez *et al*., 2006)

In Malaysia, various type of biomass generated from palm oil mill processing, consist of empty fruit bunches, palm press fiber, palm kernel cake, palm kernel shell, sludge cake and palm oil mill effluent (POME) (Prasertsan and Prasertsan, 1996). POME is one of relatively potential as a substrate for generation of hydrogen, hence, the development of an improved fermentation process for this organic waste is needed (O-Thong *et al*., 2007, Vijayaraghavan and Ahmad, 2006, Atif *et al*., 2005). POME has been generated with an average values of 25 000 mg/L biochemical oxygen demand (BOD) and 50 000 mg/L chemical oxygen demand (COD), respectively (Yacob *et al*., 2005). Owning to its characteristic with high organic content, biohydrogen production could be achieved via dark fermentation as what have been shown in the previous study (Yusoff *et al*., 2009).

During anaerobic fermentation on carbohydrate-rich substrates, volatile fatty acids (VFAs), hydrogen (H_2), carbon dioxide (CO_2) and sometimes alcohols, are simultaneously produced as demonstrated in Fig 1

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Kitchen Refuse Fermentation

Mohd Ali Hassan, Shahrakbah Yacob, Cheong Weng Chung, Yoshihito Shirai, and Yung-Tse Hung

CONTENTS

Abstract Controlled fermentation has been used for kitchen waste treatment. The most important factors affecting methane production from kitchen waste is organic loading rate and hydraulic detention time. Two main types of fermentation of kitchen waste are natural fermentation and controlled fermentation. The fermentation products are poly-3-hydroxyalkanoates (PHA) and poly-lactate (PLA).

1. INTRODUCTION

In the last century, the world had experienced various industrial revolutions, which were driven by fossil fuels such as petroleum and coal. These rapid changes also brought along serious environmental issues such as the dumping of nonbiodegradable polymers in landfills, uncontrolled release of greenhouse gases, and usage of nonrenewable energy. These concerns have sparked interest in finding alternative renewable materials such as industrial chemicals and biodegradable polymers that will reduce the environmental pollution. Despite intensive research and development in green technology and discussions by interested parties, there was

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Precise Depolymerization of Poly(3-hydroxybutyrate) by Pyrolysis

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[1]*Japan*
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1. Introduction

Poly(3-hydroxybutyrate) (PHB) is a well known microbial and biodegradable polymer, which is accumulated and stored by prokaryotic microorganisms at levels up to 90% of their cellular dry weight (Steinbüchel & Valentin, 1995). PHB has been attracting much interest from researchers not only as an environmentally compatible thermoplastic, but also as a polymeric material obtainable from renewable resources and having a high melting temperature of around 180 °C (Marchessault et al., 1981).
A major problem of PHB when used as a thermoplastic is its thermal instability during melt-processing. Therefore, intense interest has been shown in the thermal degradation of PHB and other related poly(hydroxyalkanoate)s (PHAs). Recently, it has been demonstrated that PHB is a chemically recyclable material with end products such as crotonic acid, linear oligomers having a crotonate end group (Morikawa & Marchessault, 1981), and a cyclic trimer (Melchiors et al., 1996). Melchiors et al. found that cyclic oligomers were obtained via back-biting reactions in a toluene solution with catalysts such as dibutyltin dimethoxide. However, the present review concentrates upon the thermal degradation of PHB in melt.
If plastic materials originating from renewable resources can be efficiently recycled through precise control of their thermal degradation, an ideal recycling system could be constructed for plastic products, in which the resources and production energy of the materials are minimized.

2. Accumulative results on thermal degradation behavior

The thermal degradation behavior of PHB, including other PHAs, has been discussed in many reports with the main studies listed in Table 1.

2.1 Analytical procedures

Several thermoanalytical procedures have been used to investigate the thermal degradation behavior of PHB, including thermogravimetry (TG) for the analysis of weight loss behavior (Kopinke et al., 1999; Galego & Rozsa, 1999; Li et al., 2001; He et al., 2001; Lee et al., 2001; Aoyagi et al., 2002; Carrasco et al., 2006; Kim et al., 2006; Kawalec et al., 2007; Liu et al., 2009;

A Novel Bacterium Producing Polyhydroxyalkanoates from Palm Oil Mill Effluent

Inventor:
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IP Status : Pending Patent
Filed Date : 2010-01-07
Application No. : PCT/MY2010/000004
Country Filing : PCT
Apllicant : Universiti Putra Malaysia

ABSTRACT

The bacterial strain EB 172, isolated from digester treating palm oil mill effluent, was investigated by polyphasic taxonomic approach. The cells were 5 rod-shaped, Gramnegative,non-pigmented, non-spore-forming and non-fermentative. Phylogenetic analyses using the 16S rRNA gene sequence showed that the strain was placed in the cluster of genus Comamonas; its closest neighbours were the type strains C. terrigena (96.8 %), C. koreensis (93.4 %), C. composti(92.9 %), and C. kerstersii(91.1 %). The 10 ability of C. putranensis 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 15 from the genotypic and phenotypic data that strain *Comamonas putranensis* represents a novel species in the genus Comamonas, for which the name *Comamonas putranensis* sp.nov. is proposed.

A Method for Recovering an Intracellular Polyhydroxyalkanoates (PHA)

Inventor:
Mohd Ali Hassan; Mitra Mohammadi; Nor Asma Abd Razak; Chong Mei Ling; Yoshihito Shirai; Wan Md Zin Wan Yunus; Suraini Abd. Aziz; Amirul Al-Ashraf Abdullah; Hasfalina Che Man; Siti Nor Syairah Anis; Phang Lai Yee

IP Status : Pending Patent
Filed Date : 2010-03-01
Application No. : PI/2010/000891
Country Filing : Malaysia
Apllicant : Universiti Putra Malaysia

ABSTRACT

The present invention relates to a method to recover an intracellular polyhydroxyalkanoates (PHA). The method includes the steps of (a) separating culture medium containing PHA-rich cells using centrifugation, (b) rinsing pellet obtained from setp (a) with distilled water, (c) freeze-drying pellet obtained in step (b), (d) adding distilled water to the freeze-dried sample, (e) separating non-PHA cellular material (NPCM)from PHA granules, (f) washing the pellet containing PHA from step (e) by distilled water and centrifuging for producing final pellet, and (g) freeze-drying the pellet produced from step (f).

Novel In-Vessel High Rate Composter

Inventor:
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IP Status : Pending Patent
Filed Date : 2010-12-17
Application No. : PI/2010/006055
Country Filing : Malaysia
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

CONSULTANCY

RESEARCH THEME	CLIENTS / INDUSTRIAL PARTNER	DURATION
Production of Cocktail Enzymes and Polyoses from Oil Palm Empty Fruit Bunch by Local Isolates	AlafPutra Biowealth Sdn Bhd	CURRENT
Production of Biocompost from Oil Palm Empty Fruit Bunches (EFB) and Goat Manure	YPJ Plantation Sdn Bhd	CURRENT
Amino acids from waste glycerine by indigenous microbes	Ajinomoto Co. Ltd, Japan	2007 ~ TO DATE
Microbial production of ethanol from glycerine wastewater	Idemitsu Co. Ltd., Japan	2007 ~ TO DATE
Feasibility study on CDM biomass projects for renewable energy	Tokyo Electric Power Company (TEPCO), Japan	2008 COMPLETED
Improved biogas production from glycerine washwater	FELDA Proctor Gamble (FPG) Sdn. Bhd	2007 ~ 2008 COMPLETED
Improvement of wastewater treatment system in Miri Crude Oil Terminal	PETRONAS	2006 ~ 2008 COMPLETED
Production of activated carbon and pyroligneous acid from palm biomass	NewTech Sdn. Bhd	2005 ~ 2007 COMPLETED
Industrial Grant Scheme (IGS) on Bioplastics, Bioacids and Compost from Organic Wastes	Kasa Ganda Sdn. Bhd	2003 ~ 2005 COMPLETED

2010 GRADUATION

Doctor of Philosophy

Dr Khanom Simarani

Bioconversion of Palm Oil Biomass For Value Added Products Using Internal Energy Management

Dr Norjan Yusof

Nitrification of high-strength ammonium landfill leachate for improvement of river water quality in Malaysia

Dr Chong Mei Ling

Biohydrogen Production from Palm Oil Mill Effluent by Locally Isolated Clostridium butyricum EB6

Dr Azhari Samsu Baharuddin

The Appropriate Technology for Accelerated and Controlled Composting Treatment of Empty Fruit Bunch and Palm Oil Mill Effluent

Master of Science

Mohd Huzairi Mohd Zainuddin

Production of Sugars from Oil Palm Empty Fruit Bunch through Phase Separation System

Mohd Zulkhairi Mohd Yusoff

Production of Biohydrogen from Palm Oil Mill Effluent under Non-Sterile Condition

Zatilfarihiah Rasdi

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

Design, Research and Innovation Exhibition (PRPI 2010)

PRPI10
PAMERAN REKA CIPTA, PENYELIDIKAN DAN INOVASI 2010

20th – 22nd July 2010 ~ Universiti Putra Malaysia

The Production of Biohydrogen from Palm Oil Mill Effluent towards Green Energy Development

GOLD MEDAL

Mohd Ali Hassan, Mohd Zulkhairi Mohd Yusoff, Nazlina Haiza Mohd Yasin, Nor'Aini Abdul Rahman, Suraini Abd-Aziz, Yoshihito Shirai

Pilot Scale Recovery of Mixed Organic Acids from Anaerobically Treated Palm Oil Mill Effluent (POME)

GOLD MEDAL

Mohd Ali Hassan, Tabassum Mumtaz, Suraini Abd-Aziz, Nor'Aini Abdul Rahman, Phang Lai Yee and Yoshihito Shirai.

Efficient Recovery Method for Intracellular Polyhydroxylalkanoates by Non-Halogenated Solvent System

GOLD MEDAL

Mohd Ali Hassan, Mitra Mohammadi, Nor Asma Abd Razak, Chong Mei Ling, Yoshihito Shirai, Wan Md Zin Wan Yunus, Suraini Abd-Aziz, Amirul Al-Ashraf Abdullah, Hasfalina Che Man, Siti Nor Syirah Anis, Phang Lai Yee, Hidayah Ariffin

Comamonas putranensis sp. Nov., a Novel Bacterium Producing pba from POME

GOLD MEDAL

Mohd Rafein Zakaria@Mamat, Noor Azman Mohd Johar, Hidayah Ariffin, Suraini Abd-Aziz, Mohd Ali Hassan, Yoshihito Shirai

Biohydrogen Production from Food Waste

BRONZE MEDAL

Nor'Aini Abdul Rahman, Nazlina Haiza Mohd Yasin, Mohd Zulkhairi Mohd Yusoff, Hasfalina Che Man, Mohd Ali Hassan

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Negeri Sembilan

CONFERENCE ATTENDED

2010

MAY

24 May: Seminar on High Pressure Homogenizer and Cells Disruptor. IBS Auditorium, Universiti Putra Malaysia

JULY

20-22 July: Design, Research and Innovation Exhibition (PRPI 2010) Universiti Putra Malaysia

SEPTEMBER

28 September: 2010 First Based DNA Sequencing Data Analysis and Prime Time Probes for Real Time PCR Seminar. IBS Auditorium, Universiti Putra Malaysia

OCTOBER

11-14 October: Convocation Day Exhibition 2010.
Bukit Ekspo Universiti Putra Malaysia

14-17 October: International Greentech & Eco Products Exhibition & Conference Malaysia 2010 (IGEM). Kuala Lumpur Convention Centre (KLCC) Malaysia

NOVEMBER

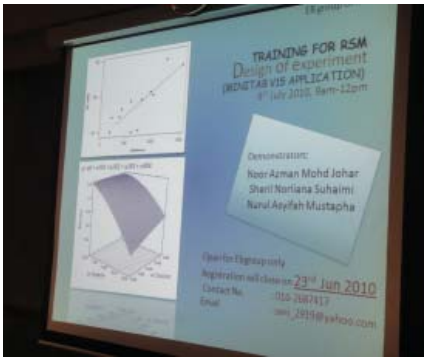
2-3 November: BioMalaysia 2010.
Kuala Lumpur Convention Centre (KLCC), Malaysia

23 -25 November: 5th Workshop on Identification and Preservation of Microorganism.
Biotech 2, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia

25th November - 5th December: Malaysian Agriculture, Horticulture and Agrotourism Show (MAHA 2010). MAEPS Mardi, Serdang, Malaysia

WRITING WORKSHOP

This workshop was organized by EB Group lecturer to improve scientific writing skill for students in this group. Prof Dr. Tan Soon Guan was invited as a speaker since he was expert in scientific writing and one of the main editors for Pertanika Journal, UPM. This workshop was held at Biotech 2.2, Faculty of Biotechnology and Biomolecular Sciences, UPM on 25th June 2010.



MINITAB WORKSHOP

This workshop was held to share the knowledge with EB Group members from students who joined Minitab workshop at USM, Penang. Mr Noor Azman, Mrs Sheril Norliana, Mrs Asyifah Mustapha and Mrs Nor Asma Razak were the instructor to taught student to used Minitab Software.


BIOENERGY
RESEARCH GROUP

This research group consist of four different sub-research areas; Bio-ethanol, Biobutanol, Biohydrogen and Biogas. Apparently, our collaborative research focusing on the utilization of agriculture residual substrates which are palm oil mill effluent (POME), food waste, oil palm empty fruit bunch OPEFB, rice straw, palm decanted cake, sago starch, or dubbed as biomass, to be converted into bioenergy product through biotechnology and bioprocess engineering.

Currently, there are six PhD students, 15 Master students and three researches assistant under this group and registered either with UPM or Kyushu Institute of Technology, Japan. The group members engaged in different research area encompasses the production of bioenergy products, pretreatment of lignocellulosic materials, celluase production, and kinetic study of cellulase producer microorganism. On other hand, the group also involved in downstream processing and basic molecular biotechnology-based.

■ PICTURE Screw cap anaerobic flask

BIOENERGY

Current studies emphasize on the feasibility of utilizing palm oil mill residues and other related biomass, for beneficial bioenergy production such as methane, bioethanol, biobutanol and biohydrogen using our own indigenous technology. The consolidated collaborative work will prove that our indigenous home-grown technology could provide alternative pathway of the bioenergy products to our doorknob in near future.

PRINCIPAL RESEARCHERS

LEFT ASSOC. PROF. DR SURAINI ABD. AZIZ
RIGHT DR PHANG LAI YEE

RESEARCH AREAS

BIOGAS

Improve Methane Fermentation from Palm Oil Mill Residuals in line with of Zero Emission Strategy

The research involves in the treatment of organic-rich wastewater such as POME (palm oil mill effluent). The activities include: biogas production from decanted POME using 50 liters closed digester tank and appropriate treatment of palm oil mill final discharge as recycled water for the mill. The treated wastewater could be further polished to a standard that is suitable for recycling purpose, and therefore the dependency on river water intake will be reduced. This integrated system is in line with our objective of zero emission for the palm oil mill. ■

BIOETHANOL

Lignocellulosic Materials: Potential Substrate for Production of Bioethanol

Our research is focusing on the utilization of biomass and other waste material for the production of biocatalyst, biosugars and finally bioethanol. Bioethanol is an alternative energy source and it is highly demanded in world market. Conversion of biomass into bioethanol involves physical, chemical and biological methods where the lignocellulosic material will be pretreated by thermo-chemical process, steam or mechanical grinder before it is being hydrolyzed into fermentable sugars. The sugar obtained from the hydrolysis is further converted to bioethanol by local isolates from EB culture collection. ■

BIOHYDROGEN

The Future Renewable Bioenergy from Waste and Biomass Materials

Hydrogen can be produced from a variety of renewable resources such as agricultural or food industry wastes by using indigenous microorganisms. Our research focuses on the production of biohydrogen from POME and food waste as substrates. One of the important factors governing the hydrogen conversion is process engineering, such as bioreactor design and operating parameters. Improving reactor design and optimizing the operating parameters, such as pH, temperature, hydraulic retention time and mixing effect, could enhance the biohydrogen conversion efficiency and obtain in-depth understanding of the process in laboratory and pilot scale, respectively. ■

BIOBUTANOL

Production of Biobutanol from Agricultural Biomass

Biobutanol is suitable for replacing petrol as fuel in gasoline engines. Besides, it is one of the alternative bioenergy for the future. Utilization of biomass and other waste materials for biobutanol production involves five main steps: pretreatment of biomass, cellulases production, enzymatic hydrolysis, production and finally recovery of biobutanol. Substrates such as oil palm empty fruit bunches (OPEFB), oil palm decanter cake (OPDC), rice straw and sago pith residue (SPR) will be first hydrolyzed to biosugars and subsequently converted to biobutanol by locally isolated microorganisms from EB Research Group culture collection. ■

BIG PICTURE BIOENERGY


Siren

Cellulases


Nooshin


Nafis

Biosugar


Firdaus


Faizal

BIOBUTANOL


Fadlan


Zuraidah


Ezyana

Cellulases


Muhaimin


Kartini

Biosugar


Sheril


Saleha

BIOETHANOL


Dayang Salwani

BIOENERGY

BIOMASS

BIOMASS

BIOHYDROGEN

Palm Oil Mill Effluent

Food waste


Zulkhairi


Isnazunita


Nazlina


Fadzillah

Biohydrogen production from palm oil mill effluent using suspended and immobilized mixed culture

Isnazunita Ismail

GENERATING biohydrogen from complex wastewater from palm oil mill industry was economically feasible also provides new strategy of market driven research. Palm oil mill effluent (POME) is constantly associated with environmental burden due to high volume discharge of water during milling process since palm oil industries were wide industry in Malaysia. The chemical properties in POME such as carbohydrate, oil, cellulose, hemicellulose and other compound were study in batch and continuous mode for the production of biohydrogen. Anaerobic degradation of POME was studied during fermentation by anaerobic fermentation under thermophilic condition. The study was conducted using suspended and immobiliezed mixed culture in continuous operation. The behavior of suspended mixed culture showed susceptible to washout at high dilution rates. To overcome this problem, immmobilezd mixed culture was done and proved that in optimize condition biohydrogen can produced well without washout occur in continuous operation.

The study on sucrose and crude palm oil (CPO) were done using immobilized mixed culture as an inoculum. The hydrogen yield based on COD of sucrose added was 146 cm3 g-1 which is equivalent to a hydrogen to hexose mole ratio of 2.5. The free fatty acids from hydrolysed CPO were not metabolized further which render insignificant generation of hydrogen and volatile fatty acids from oil-based substrate. The average continuous biohydrogen production rate (HPR) from a unit volumeof POME under thermophilic condition at 55°C was 2.64m3 m-3 d-1 at a hydraulic retention time (HRT) of 4 days. Hydrogen constitutes up to 52% of the total biogas and methane was not detected over the 60 days continuous operation. Butyrate and acetate were the predominant soluble metabolites produced during this study correspond to the hydrogen production where in theory production of biohydrogen accompanied with the production of these metabolites. The microbial community during fermentation in continuous stirred tank reactor (CSTR) was detected using denaturing gradient gel electrophoresis (DGGE). ■

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 immobilized cells in generating high volume of hydrogen under continuous condition.

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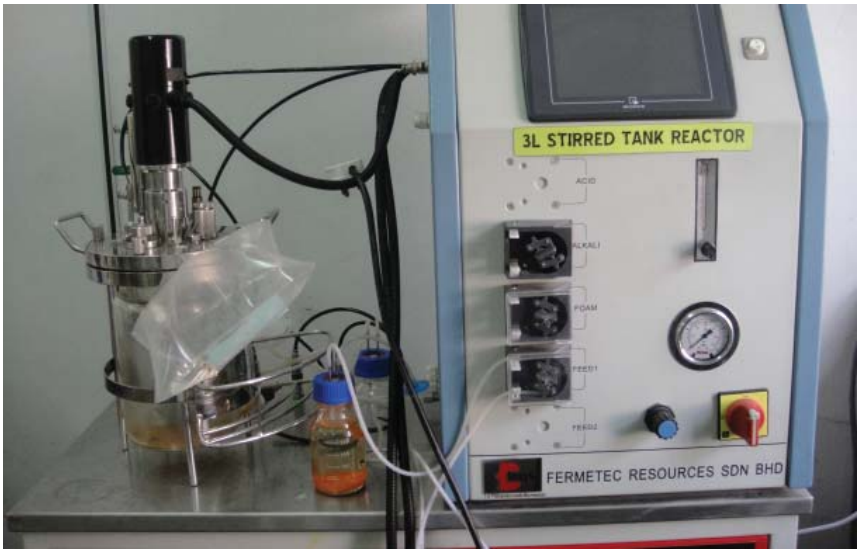
■ **RIGHT** Performing DGGE analysis for microbial identification

■ **BOTTOM** Fermentation of POME for biohydrogen production using 3 L stirred tank reactor



Waste to bioenergy, towards environmental friendly energy supply

Mohd Zulkhairi Mohd Yusoff

RESEARCH FOR alternative fuels has been extensively carried out all over the world. There are many major industry involved to produce and provide such energy supply. The important part of supplying the energy is about consistency. The consistency is come from the continuous supply of sources. Apparently, continuous supply must come along with renewable provision as well. In this study, applied research has been adopted, we are looking depth to the natural supply or any biological provision to make it renewable.

In this study, palm oil mill effluent (POME) (figure 1 and figure 2), was used as a renewable substrate for the production of alternative fuels (biohydrogen) under non-sterile conditions. POME is a potential substrate for generation of biohydrogen. However, the development of an improved fermentation process for this organic waste to biohydrogen is strongly required. POME is showed as brown in color, viscous, containing about 95–96% of water, 0.6–0.7% of oil, 4–5% of total solids and it is acidic (pH 4–5). POME also constitutes with high organic content, COD and BOD of 70 g/L and 25 g/L, respectively (Yusoff *et al* 2010). POME also contained of high nutrient content, mainly oil and fatty acids, which are important for bacterial growth (Zakaria *et al*., 2008). The POME shows acidic condition with pH 3.8-4.4 and temperature of about 84 ± 1˚C, total nitrogen content of 0.5-0.7 and oil content of 4.9-5.7 g/L, respectively.

Biohydrogen production could be achieved via dark fermentation as owning to POME's characteristic that having high organic content. The fermentation has been carried in 50 L continuous stirred tank reactor (CSTR). A few parameters have been sought to obtain the best condition to the production of biohydrogen from POME which the study has been done in 150 mL serum bottle and intensified using RSM.

In 50 L CSTR, four different hydraulic retention times has been studied (HRT 5, HRT 4 HRT 3 and HRT 2). HRT is regarded as the time for microrganisms to degrade the substrate, therefore higher HRT would increase the contact time for bacteria to react and increase the conversion rate. HRT is a vital parameter and plays an important role in order to increase biohydrogen production as biomass maintained at a certain density (Levin *et al*., 2004; Wu *et al*., 2008). HRT is also an important parameter to minimize the growth rate of the biohydrogen utilization microorganisms such as methanogen. Beside the HRT, the effect of volatile fatty acids →

RESEARCH OBJECTIVES

1. Production of bioenergy from oil palm biomass as renewable sources.
2. Utilization of oil palm biomass for other beneficial bioproducts.

2010 PUBLICATION

Mohd Zulkhairi Mohd Yusoff, Nor'Aini Abdu. Rahman, Suraini Abd-Aziz, Chong Mei Ling, Mohd Ali Hassan and Yoshihito Shirai. (2010). The Effect of Hydraulic Retention Time and Volatile Fatty Acids on Biohydrogen Production from Palm Oil Mill Effluent under Non-Sterile Condition. *Australian Journal of Basic and Applied Sciences*, 4(4), 577-587. (*See abstract at page 51*)

Main supervisor :
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FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 1 POME from mill, the temperature about 75˚C to 85˚C

FIGURE 2 Sampling POME at Seri Ulu Langat, Dengkil mill, Selangor

FIGURE 3 Heat treated POME sludge as inoculum

to the biohydrogen production has been observed. pH come one of the important during biohydrogen production, during the fermentation process, pH has been controlled at 5.5 as the optimum pH for the production of biohydrogen.

The fermentation has started using heat-treated sludge as inoculum (figure 3) from settling tank at Palm Oil Mill Serting Hilir (figure 4). During the fermentation process, the development of gas production and its composition were monitored daily until the bioreactor naturally established a steady-state before being shifted to another HRT. The gas produced mainly contains biohydrogen 30 – 40% and apart from that only CO_2 no methane gas was detected. In order to provide a high yield with high concentration of the gas, the impurities of the biogas should be recovered using scrubber to separate CO_2 and biohydrogen. Moisture is one of major parameter must in consider to utilize biohydrogen as a biofuel. The moisture should be removed from the gas by flowing the biogas through the dryer unit in order to condense the vapor in the biogas produced. The purification of biogas is important to provide high purity of hydrogen supply especially to the fuel cells.

During anaerobic degradation of POME, POME sludge was used as inoculum which contains a lot of beneficial microorganisms. Therefore, there is a good idea in order to the study on the microorganisms' community in the sludge as well as the bacteria dominant in the different stages of the fermentation. In the fore report, the different types of bacteria were dominant during different stages of fermentation (Shin *et al*., 2004; Kim *et al*., 2008; Zhang *et al*., 2003). It will benefit for the strategy to enhance the productivity of the dominant bacteria in different stages of the fermentation. The conditions might be varies of the different stages of the fermentation favor to the bacteria involved.

For the future strategy, we are strongly looking towards on modeling the purification systems for the biogas produced. In the determination of bacteria involved during the fermentation process, we have applied a molecular technique such as denaturant gradient gel electrophoresis (DGGE) (figure 5) and Fluorescence *In-situ* hybridization (FISH) (Figure 6). ■


FIGURE 4

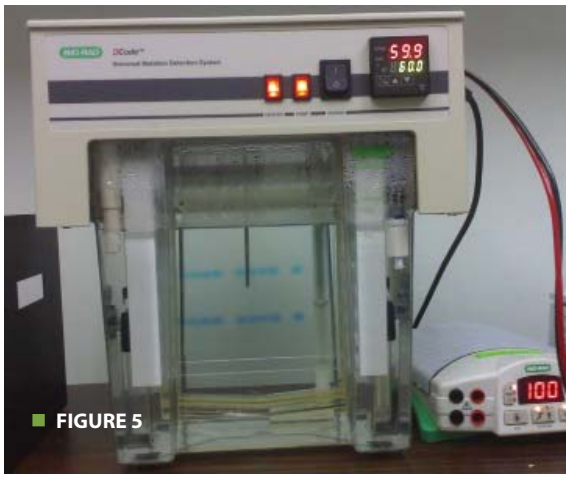


FIGURE 5


FIGURE 6

■ **FIGURE 4** Inoculum sources, settling tank at Serting Hilir mill, Negeri Sembilan, Malaysia

■ **FIGURE 5** Denaturant gradient gel electrophoresis (DGGE)

■ **FIGURE 6** Doing FISH analysis to identified microorganism involved during production of biohydrogen

■ **FROM LEFT**
Analysing result using Gas Chromatography at EB Lab, Faculty of Engineering, UPM; Preparing sludge for inoculation

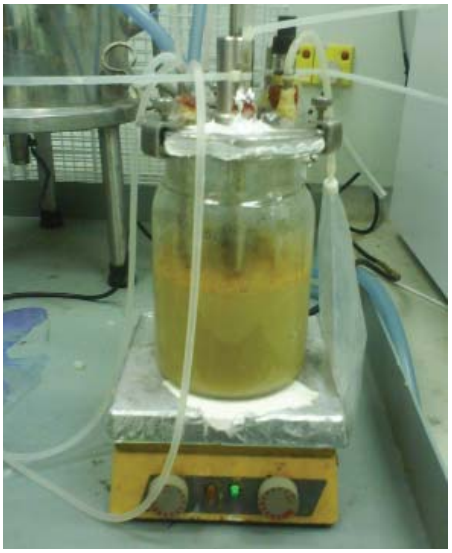
Statistical optimization of biohydrogen production from food waste under thermophilic condition

Fadzillah Ismail

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 affecting biohydrogen production was substrate concentration, temperature, pH and inoculum size. Based on two-level factorial screening, inoculum size shown not significant in biohydrogen production study. 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 second objective study was done by fermentation using 2 L bioreactor at temperature 50°C. Experiment was carried out at initial pH 7.0 until it reach desired pH (5.0, 5.5, 6.0 and 6.5), pH were controlled during fermentation when it reach to the target pH. The effect of volatile fatty acids on biohydrogen production during 24 h incubation was studied. Main volatile fatty acids for biohydrogen production in food waste anaerobic fermentation were lactic acid, butyric acid and acetic acid. Biohydrogen production accompanied with the production of acetic acid ane butyric acid while lactic acid production lead to solventogenesis. These lactic acid present as undissociated acid form which can penetrate through cell membrane and disrupt the production of biohydrogen. ■



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.

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■ **LEFT** 2 L bioreactor used for biohydrogen fermentation

■ **BOTTOM** Bioreactor Engineering Lab, Faculty of Engineering, UPM

Microbial identification from food waste fermentation for biohydrogen production

Nazlina Haiza bt Mohd Yasin

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.

In nature, biohydrogen is produced during acidogenesis where acid forming bacteria produces organic acids compound, hydrogen and carbon dioxide. The anaerobic digestion that converts food waste into hydrogen rich gas was employed by mixed microbial culture readily available in the nature of palm oil mill effluent sludge. Heat treatment should be carried out before start the fermentation to eliminate methanogens, homoacetogens and solvent producing bacteria which considered as HPB inhibitor.

The effects of different initial pH, temperature and substrate composition were studied in 160 mL serum bottle with 100 mL working volume. The optimum initial pH, temperature and substrate composition were 7.0, 55°C and 30% (v/v) respectively. The work was further applied on fermentation processes in 0.5 L bioreactor with 0.25 L working volume at controlled temperature 55°C to study the effect of controlled pH on biohydrogen production.

The identification of microbial profiling and colony presence during fermentation is essential to understand the process in biohydrogen production. 16S rRNA was carried out according to the specific primer to study the present of microbial community in activated POME sludge. Microbial profile was detected using denaturing gradient gel electrophoresis (DGGE). DGGE method has become staple to environmental biotechnology for indicaton of community changes in unknown sample. Fluorescent *in-situ* hybridization (FISH) was used to detect nucleic acid sequences by a fluorescent probe that hybridizes specifically to its complementary target sequence within the intact cell. Microbial quantification based on the specific target probe can be calculated through this technique. Total bacteria, *Clostridium sp.* from cluster I and XI which is main HPB and confirmation of the absence of methanogens was visualized using fluorescent *in-situ* hybridization(FISH). ■

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.

Main supervisor :
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■ **LEFT** Gel cutting under UV light

■ **BOTTOM LEFT** Polyacrylamide gel loading during gel preparation for DGGE

■ **BOTTOM** Identification of aerobic, anaerobic and facultative microbes

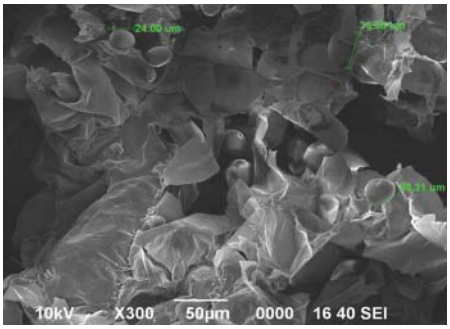
Feasibility of sago hampas as a feedstock for bioethanol production

Dayang Salwani bt Awang Adeni

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. 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 concentration, enzyme dosage, saccharification 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. However, during enzymatic hydrolysis process it was restricted by the physical structures of sago hampas thus it was impossible to get higher glucose concentration. Therefore, the recycle hydrolyzed sago hampas (HSH) solution method was introduced for subsequent saccharification process in order to increase glucose concentration.

Second stage of this study is to further utilize on hydrolyzed sago hampas (HSH) for ethanol fermentation using commercial baker's yeast. Observation on the growth of yeast together with the pregerminate time during inoculum preparation will be conducted initially before ethanol fermentation was carried out. Batch fermentation process is then be carried out using 250 ml shake flask with several parameters are examined such as temperature, pH, substrate concentration, incubation time, agitations rate and inoculum size. The commercial glucose (CG) is used as control substrate throughout this study. The production of ethanol – concentration in fermentation broth, conversion yield and productivity are the main criteria to be observed in ethanol fermentation. The most vital factors that affect the overall fermentation efficiency will be optimized using Response Surface Method (RSM) by Design Expert®, version 7. Another aspect to be considered in this study is to enhance ethanol productivity from HSH. In order to achieve that, some approaches such as scaling up the fermentation process, recycle cell for repeated batch fermentation and introducing fed-batch system will be applied.

High performance liquid chromatography (HPLC) system, equipped with refractive index detector (RID) and BIORAD® Aminex - Fermentation Monitoring column is used as method for analyzing the fermentation broth. Whereas scanning electron microscope (SEM) is used for observing the physical structure of sago hampas. The successful of this study will help to conserve the environment by minimizing the pollution into the waterways as well as beneficial to the sago industries. ■



■ **CLOCKWISE FROM TOP LEFT** Sago hampas hydrolysate and it solid residues; Hydrolyzed sago hampas; Raw sago hampas

RESEARCH OBJECTIVES

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

2010 PUBLICATION

Dayang Salwani Awang Adeni, Suraini Abd-Aziz, Kopli Bujang and Mohd Ali Hassan. (2010). Review: Bioconversion of Sago Residue into Value Added Products. *African Journal of Biotechnology*, 9(14), 2016-2021.
(*See abstract at page 44*)

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Bioethanol and cellulase production from rice straw using local microorganisms

Ahmad Muhaimin bin Roslan

AS ONE OF THE major crops in Malaysia, paddy industries produce a massive amount of rice straw as biomass waste in a seasonal period. Without further use of the rice straw, it's become a usual phenomenon to see burning paddy field and haze in the paddy plantation area after harvesting season. With the application of biotechnology, now there are few potential uses of the rice straw. It can be converted into product such as enzymes, biofuel and etc. Biofuel was a term given to any fuel derived from biomass, for example bioethanol and biobuthanol. It is crucial to have a low production cost of the biofuel since it will determine the final selling price of this kind of fuel.

The main challenge in biofuel production is the cost of commercial enzyme to be used which is very high. It was suggested to produce the enzyme *in situ* to help reduce the production cost. In this study, the rice straw was used as substrate for cellulase and bioethanol production. The rice straw was first pretreated using a milling technique developed by one of the collaborator in this study, AIST. This milling technique was further improved with thermal application to obtain finer product and much more prone to enzymatic hydrolysis. In cellulase production, lignocellulolytic fungi was used to degrade the rice straw while at the same time produce the precious cellulase enzyme. The main target here is to produce high activity cellulase. This is important to ensure high saccharification yield with lower amount of enzyme to be used. Additional biomass (palm oil mill effluent, POME) was also used as additional nutrient to enhance the activity of the cellulase produced. This improves the cellulase produced by several fold. Finally, the fermentation of the sugars was done using yeast which will convert the fermentable sugars into ethanol. All in all, the use of biomass for biofuel production is one of the best ways to mitigate global warming and to help saving mankind from the depletion of fossil fuel. It will be a win-win-win situation where the industries, people and planet will all benefit. ■

RESEARCH OBJECTIVES

1. To improve available pretreatment to obtain higher degree of saccharification.
2. To produce high activity crude cellulase from rice straw using locally isolated fungi through enzyme cocktail approach.
3. To produce high yield of bioethanol from rice straw using crude cellulase cocktail.

Main supervisor :
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■ **TOP LEFT**
Wet disc milling

■ **TOP RIGHT**
Saccarification was carried out on a multistirrer plate in an incubator

■ **LEFT**
HPLC used for sample's sugars determination

Oil palm empty fruit bunch as an alternative cheap substrate for cellulases production

Ahmad Fadhlan bin Hamisan

NOWADAYS utilization of lignocellulosic material from agricultural waste as renewable carbon sources is dependent on the development of economically feasible process technologies for cellulases production. Great amount of lignocellulosic wastes are produced through agricultural activities and industrial processes annually. These wastes generally accumulate in the environment thus causing pollution problem. In Malaysia, several types of lignocellulosic was had been produces annually namely oil palm waste, sugar cane bagasse and rice straw.

Currently, Malaysia is the main exporter in the international market. In the process of oil extraction from the oil palm fruit (OPF, several types of lignocellulosic wastes are produced such as oil palm empty fruit bunch, palm kernel shell and mesocarp fibre. In Malaysia, more than 15 million tons of OPEFB biomass waste is generated annually by palm oil mills. Most of the mills just burned the OPEFB in incinerators and cause pollution problems. So, to raise the value of this biomass, OPEFB can be major source of renewable organic matter. OPEFB can be converted to into valuable product such as biofuels, cheap energy soures, improved animal feed and chemicals.

The first objective of this study was to evaluate the lignin content of OPEFB using kappa number after chemical pretreatment using dilute Sodium Hydroxide (NaOH) as compared with fungal pretreatment by *Phanerochaete chrysosporium* ATCC 32629 as model microorganism using liquid and solid culture techniques. The production of lignin peroxidase for both techniques was compared.

Second objective was optimization of solid state fermentation for cellulases production using pretreated OPEFB by *Aspergillus fumigatus* UPM2. Optimization of cellulase production was done by response surface methodology (RSM). Three factors had been considered for optimization process namely pH, moisture content and substrate. Substrate means the mass of treated OPEFB used during the process. For forming the response surface, fitting the model and predicting the optimum value, central composite design was used. ■

RESEARCH OBJECTIVES

1. Delignification of oil palm empty fruit Bunch (OPEFB) Using Chemical and Microbial Pretreatment Method.
2. Optimization of solid state fermentation for cellulases production using pretreated OPEFB by *Aspergillus fumigatus* UPM2

Main supervisor :
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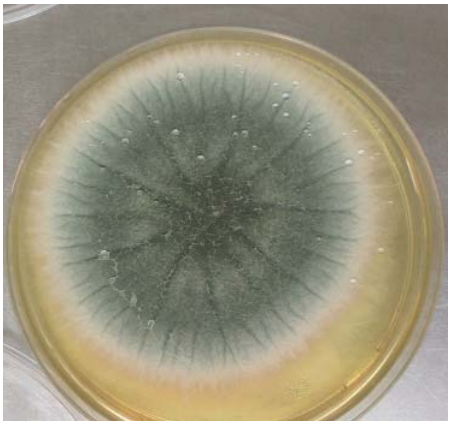

■ **CLOCKWISE FROM RIGHT**
Microbial pretreatment using *Phanerochaete chrysosporium* ATCC 32629 by solid state fermentation;
Production of cellulase using *Aspergillus fumigatus* UPM2 by solid state fermentation;
Culture of *Phanerochaete chrysosporium*;
Culture of *Aspergillus fumigatus* UPM2

Cellulases production from oil palm empty fruit bunch using solid state fermentation

Ezyana binti Kamal Bahrin

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

Bioconversion of value added product using solid state fermentation (SSF) is economical because it requires low energy consumption, low cost equipment, limited water usage and less effluent produced. Moreover, solid state fermentation conditions resemble the real cultivation of fungi in nature. Locally isolated fungus, *Botryosphaeria rhodina* is described as an endophyte which attacks woody host. The ascomycete fungus *Botryosphaeria rhodina* produces a broad range of lignocellulolytic enzymes such as laccases, pectinases, cellulases and xylanases. These complex enzymes play an important role in the degradation process of lignocellulosic materials through a synergistic action. The objective of this research is to study the effects of SSF parameters that influenced cellulase production by *Botryosphaeria rhodina*. *Botryosphaeria rhodina* exhibited its best performance on day 7 of incubation when the initial moisture content was at 20-25 %, initial pH of nutrient was 6 to 7 and with 3-5 g of substrate. Generally, fungi were cultivated at more than 50 % of moisture content in solid state fermentation. However, high cellulase production at low moisture content (20-25 %) is a very rare condition for fungi cultured in solid state fermentation but *Botryosphaeria rhodina* was capable to tolerate this condition. The cellulase produced by *Botryosphaeria rhodina* at the optimal range were as followed: Fpase activity (17.94 U/g) CMCase activity (19.68 U/g) and Beta glucosidase activity (1.13 U/g).

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

RESEARCH OBJECTIVES

1. To optimize cellulase production by *Botryosphaeria rhodina* using RSM
2. To characterize the enzyme kinetic of cellulase by *Botryosphaeria rhodina* in solid state fermentation (SSF)
3. To characterize the substrate fermente by *Botryosphaeria rhodina* in solid state fermentation (SSF)

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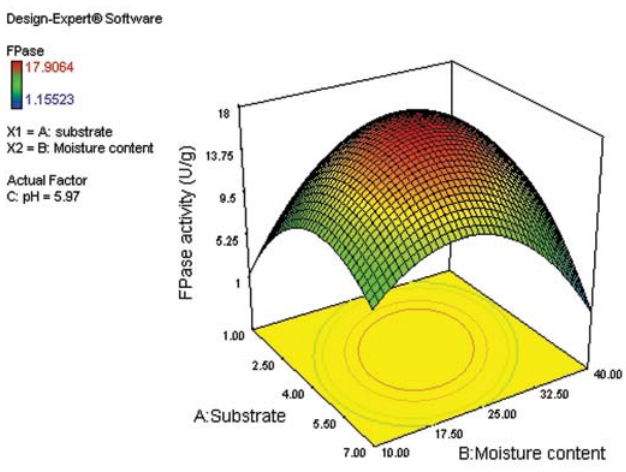
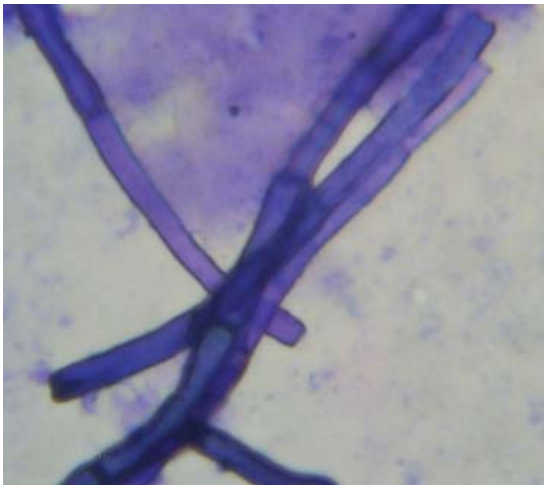


■ **LEFT** Three dimensional response surface plots of independent variables on FPase activity



■ **RIGHT** Ascomycete hyphae cross wall of *Botryosphaeria rhodina* under microscope observation.

Cellulosic bioethanol from steam pretreated oil palm empty fruit bunch

Saleha binti Shamsudin

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

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

Steam pretreatment has been chosen as the most favourable pretreatment of OPEFB. This pretreatment has to be thought to have relatively moderate energy cost production due to the steam is already generated as part of the mill operation for electricity and sterilising the fruit. Besides that, all raw materials to initiate the pretreatment (water, OPEFB, fiber and shells) are available in the mill. This in overall will enhance the sustainability of oil palm plantations. Furthermore steam pretreatment is suitable to be implementing in the palm oil mill as the OPEFB can immediately be processed and saccharified to the biosugars for subsequent bioethanol production.

High pressure steaming is considered one of the most successful options for fractionating wood into its three main components. Heating biomass in the presence of saturated steam of 190oC and 220oC and pressure 1.2 to 4.1 MPa normally is efficient in partially hydrolysed hemicelluloses, modified the lignin, increase in accessible surface area, decrease of the cellulose crystallinity and its degrees of polymerization. Therefore, in this study the high pressure steam of 0.8 to 2.3 MPa will be studied on the effect of enzymatic hydrolysis for biosugars production. The steam pretreatment is believed to convert the pretreated lignocellulosic biomass to more than 80% of sugars yielded from enzymatic saccharification. In this study, a variety of operation temperatures, pressures and residence times to be applied to the OPEFB have to be tested. In general, these parameters however are different depending on the pretreatment strategy as well as on the type and physical of the raw material used to make the pretreatment successfully, effectively and had a positive impact on the overall process. ■

RESEARCH OBJECTIVES

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

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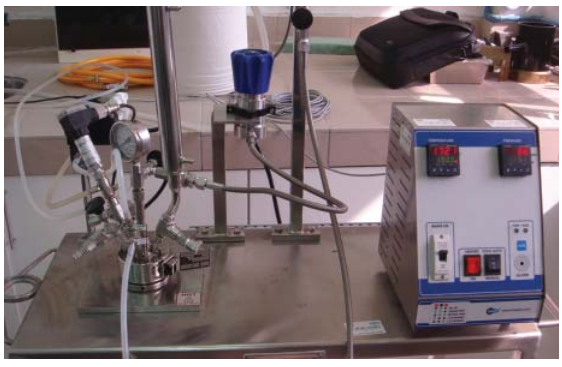

■ **LEFT** High pressure lab autoclave for steam pretreatment of OPEFB

■ **BOTTOM LEFT** The steam pretreated OPEFB and the condensate

■ **BOTTOM** Weighing the ground OPEFB

Bioconversion of oil palm empty fruit bunch into fermentable sugars for bioethanol production

Nurul Kartini Abu Bakar

FROM NATURAL gases to hydro energy, demand of enormous amount of energy every year has initiated a great threat toward current non-renewable energy source such as natural oil and gasses. Currently, world populations are still very much depending on natural sources for energy and fuel generation. Depletion of natural energy sources has initiated many researches on renewable energy sources to meet current demand of energy and fuel. Many had suggested on utilization of biomass wastes for fuel and energy generation.

Current researches had intensified work of utilization biomass such as sugarcane, corn stovers and rice straw for bioethanol, hydrogen and methane production. However, not much was reported on perennial biomass waste such as palm oil biomass. As one of the world largest producer and exporter of palm oil, Malaysia has generated as much as 80 million tones of biomass ranges from empty fruit bunches, fibers, palm kernel cake and palm kernel. Per year, approximately 20 million tones of empty fruit bunches were generated from palm oil factories. Oil palm empty fruit bunches (OPEFB) are obtained from after stripping process of fresh fruit bunches. OPEFB are lignocellulosic biomass which contain mostly cellulose, hemicelluloses and lignin. Cellulose and hemicelluloses are great source of fermentable sugars. Yet, such potential biomass are still under utilization. Current practiced of oil palm plantation has utilized OPEFB as fertilizer and mulching agents which reapplied at plantation area.

As an effort to diversified and improvised utilization OPEFB, many works have focused on conversion of OPEFB biomass to value added products such as bioethanol. This study aimed on conversion of OPEFB into fermentable sugars for bioethanol production using locally isolated fungi cellulase enzyme. Two potential cellulase producer were identified namely *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2. *Trichoderma asperellum* UPM1 produced high activity of β-glucosidase up to 2.90 U/mL and *Aspergillus fumigatus* UPM2 produced high activity of FPase and CMCase up to 0.60 U/mL and 28.19 U/mL respectively. Saccharification of OPEFB from cellulase enzyme produced from the isolated fungi revealed, mixed cellulase enzymes preparation from both isolated fungi cellulase produced highest reducing sugars concentration up to 6.86 g/L correspond to 0.17 g/g yield of reducing sugars per gram of carbohydrate. OPEFB hydrolysate was used for bioethanol fermentation by Baker's yeast. Approximately, 0.59 g/L of ethanol were successfully produced from OPEFB hydrolysate with 36.04 % of ethanol theoretical yield. ■

RESEARCH OBJECTIVES

1. To select appropriate cellulolytic microbial mixed culture for the production of cellulose enzymes
2. To produce OPEFB hydrolysate from OPEFB using crude cellulose enzymes cocktail
3. To produce bioethanol from OPEFB hydrolysate using commercial Baker's Yeast

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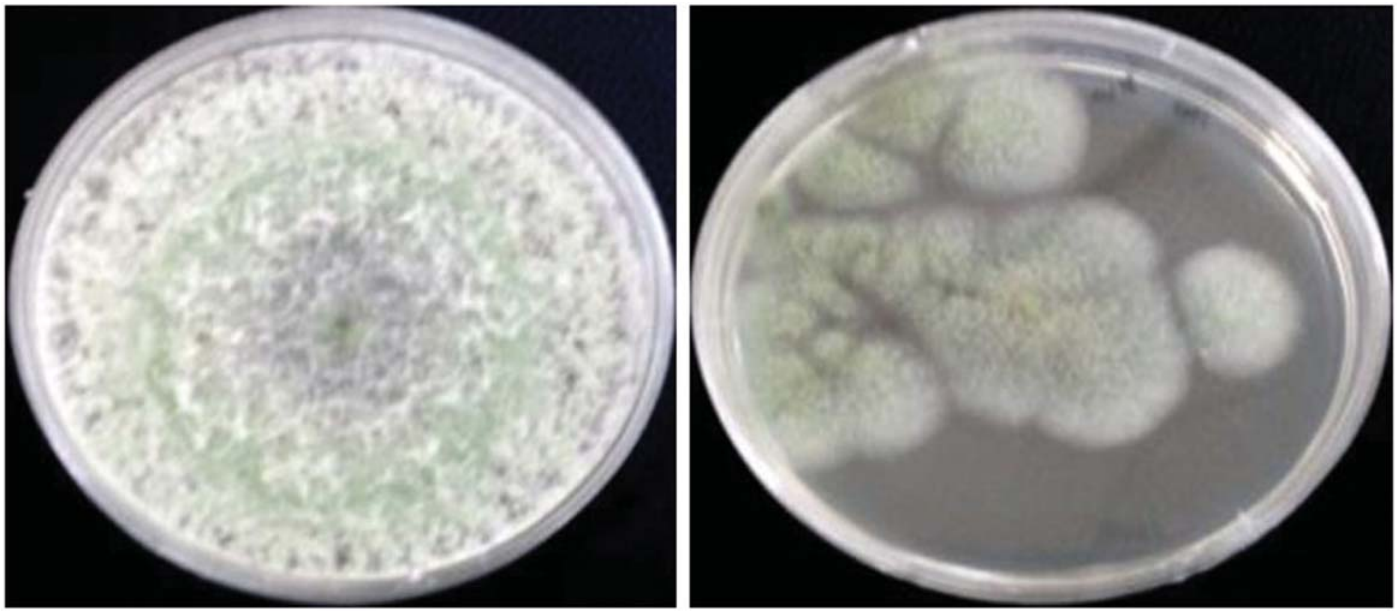
■ *Trichoderma asperellum* UPM1 **LEFT** and *Aspergillus fumigatus* UPM2 **RIGHT**



Production of bioethanol from oil palm empty fruit bunch using crude enzymes cocktail produced by *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2

Zuraidah Zanirun

AS THE WORLD largest producer and exporter of palm oil, Malaysia shared 50% of the palm oil production and 61% of exports. Palm oil industries had contributed crucial income sources for the country. The total planted area of palm oil tree increased by 4.5% to 4.69 million hectares in 2009 (http://econ.mpob.gov.my/economy/Overview_2009.pdf). Thus, more wastes are expected to be generated. Oil palm Empty fruit bunches (OPEFB) is one of the residues remained in a large amount, traditional practice of open burning would be ended with ash which then could be used as good fertiliser. However, it is not recommended as it caused an environmental problem of smoked air. Yet, strategy to address the efficient utilisation or bioconversion into various value added products such as biofuel, animal feed, chemicals and human nutrients should be explored deeply. Recently, much attention has been given to ethanol production from biomass resources as a consequence of a raising oil demand with unstable deliverance prices tempted the search for an alternative biofuel which is renewable and substitutable.

Today, the largest ethanol production mainly located in Brazil and North America produced from sugar cane and corn respectively (IEA,2004). However, the cost of ethanol as an energy source is relatively higher compared with fossil fuel. A dramatic increased in ethanol production using food based technology may not be practical because it will compete for the limited agricultural land needed for food and feed supply (Sun and Cheng, 2002). Thus, lignocellulosic residues of biomass (e.g OPEFB) could be utilise as an alternative sources for the production of low cost ethanol. Brazil's nowadays is the leading country which has a blending of 22-26% of ethanol with gasoline. Due to the growing consumption of oil and availability of oil reserved, as well as the impact of the potentially dwindling supplies and rising prices on the world's economy, ethanol has become the alternative fuel of interest. Utilising and converting them into value added product was crucial to sustain the environment. Cellulose, hemicellulose and lignin are three major components of a biomass material. For bioethanol production, cellulose must be first hydrolysed to glucose before fermented to ethanol and conversion efficiency of cellulose to glucose dependent on the type of pretreatment used from raw materials. Hydrolysis usually catalysed by cellulase enzyme and the fermentation was carried out by yeast or bacteria. Compatibility study of fungi on suitable substrates and culture condition will be carried out to improve enzyme synthesis as well as the targeted enzyme component. Aim of this present study was to achieve the ideal composition ratio of cellulases from either single or mixed culture condition of *T.asperellum* UPM1 and *A.fumigates* UPM2 from potential substrates to be used for the bioethanol production from oil palm empty fruit bunch (OPEFB). The ideal composition ratio of cellulases will expected to produce higher yield of hydrolysed sugars to be converted to ethanol. ■

RESEARCH OBJECTIVES

1. To optimize the production of mixed cellulase enzymes (enzymes cocktail) by strains T. asperrellum KS1 and A. fumigatus KS5 fungi using Response Surface Methodology (RSM) approach
2. To hydrolyse OPEFB to fermentable sugars (polyoses) using the cocktail enzymes produced
3. To separate and convert the polyoses into bioethanol using locally isolated yeasts

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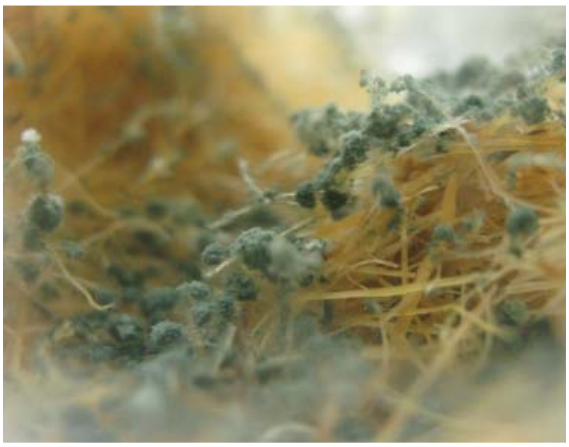

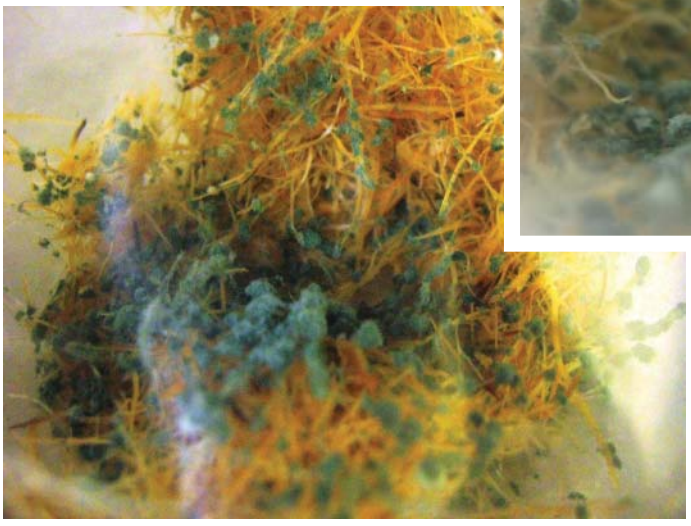

■ The growth of *Trichoderma Asperellum* UPM1 on treated EFB by solid state fermentation

Bioethanol production from glycerine wastes using locally isolated bacteria

Sheril Norliana binti Suhaimi

CURRENT INCREASE of biodiesel production contributes to serious environmental problem. The process of biodiesel production can generate about 10% (w/w) of glycerol as the major byproducts. The abundance of these glycerin wastes will contribute to the environment problem since they cannot be disposed off into environment.

In response to higher availability of this compound in nature, many microorganisms are known to be naturally utilizing glycerol as sole carbon and energy source. Microbial fermentation of glycerol is considered as one of promising applications for the bioconversion of glycerol into valuable compounds. 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. The isolated strain was identified as *Escherichia coli* sp. and able to produce ethanol from glycerol in comparison to several laboratory strains. Understanding on the related metabolic pathways that involved in glycerol fermentation is important for future study to enhance production. The metabolic pathways that are involved in glycerol fermentation will examine through mutation analysis study. 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 effects of these impunities in ethanol production during fermentation of glycerol is examined. Optimization on the fermentation condition is needed to get the optimal condition for microbial conversion of glycerol in order to enhance ethanol production. ■

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)

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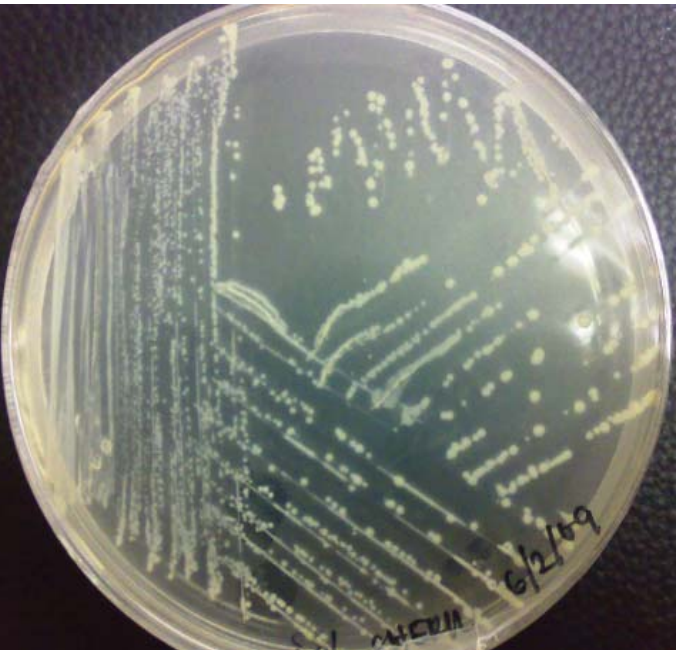

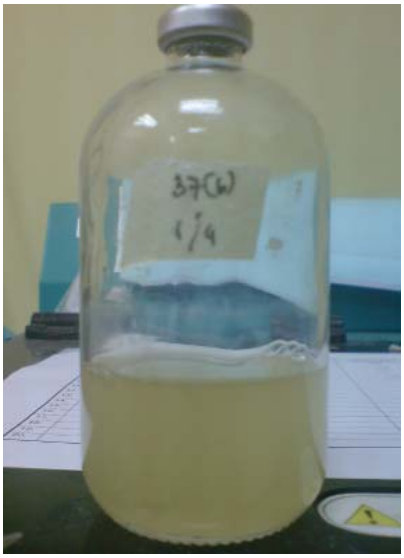

■ **TOP** Flask incubated in shaker at 37°C, 120rpm for ethanol production

■ **FAR LEFT** Isolated bacteria streak on agar plate

■ **LEFT** Serum bottle used for fermentation of glycerol for ethanol production

Production of biobutanol from rice straw using *Clostridium* sp. and *in situ* recovery via gas stripping

Mohamad Firdaus bin Zulkifli

BIOCONVERSION OF agricultural biomass into fuels and chemical feedstock has been increased in research nowadays. Cellulosic biomass such as rice straw is available as substrate for the fermentative derived biofuels as well as ethanol and butanol. Biobutanol currently has potential as attractive fuels due to depletion of fossil fuels. Various recovery techniques for acetone-butanol fermentation were discovered for removal of butanol.Gas stripping is one of the simple and inexpensive recovery technique for low cost recovery of butanol. Since butanol is associated with product inhibition due to the toxicity of butanol on the cell cultures, *in situ* gas stripping recovery is reliable technique for improving the yield and productivity. Biobutanol production from rice straw will be carried out in batch fermentation using *Clostridium* sp. as butanol producing bacterium. Batch fermentation will be performed in 2 L fermenter where Reinforced Clostridial Medium (RCM) will be used as a medium and rice straw as a substrate. Gas stripping will be performed by recycling fermentation gases (CO_2 and H_2) in order to capture ABE and the solvents will be condensed through a condenser, and then collected in a receiver vessel. Cell concentration will be determined by using optical density (OD) and dry cell weight. Glucose contents will be determined by using high performance liquid chromatography (HPLC). The solvents (ABE) concentration will be determined by using gas chromatography (GC). ■

RESEARCH OBJECTIVES

1. To produce biobutanol from batch fermentation of rice straw using *Clostridium* sp.
2. To enhance the yield and productivity of biobutanol from fermentation product (ABE) of rice straw via *in situ* gas stripping recovery technique

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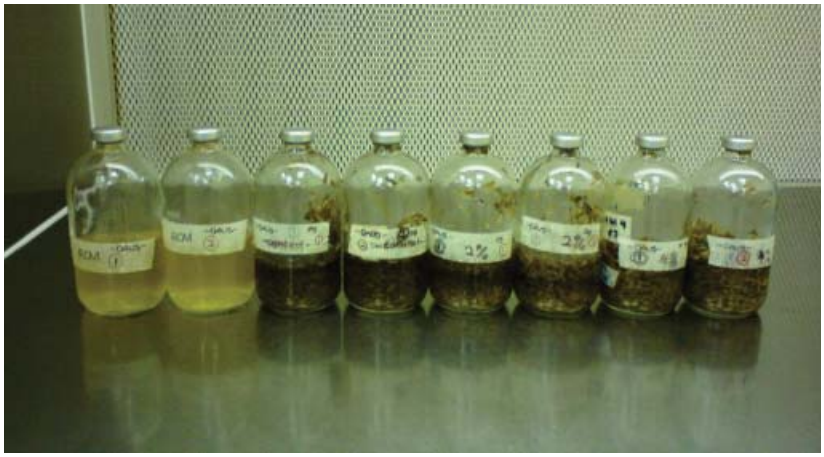
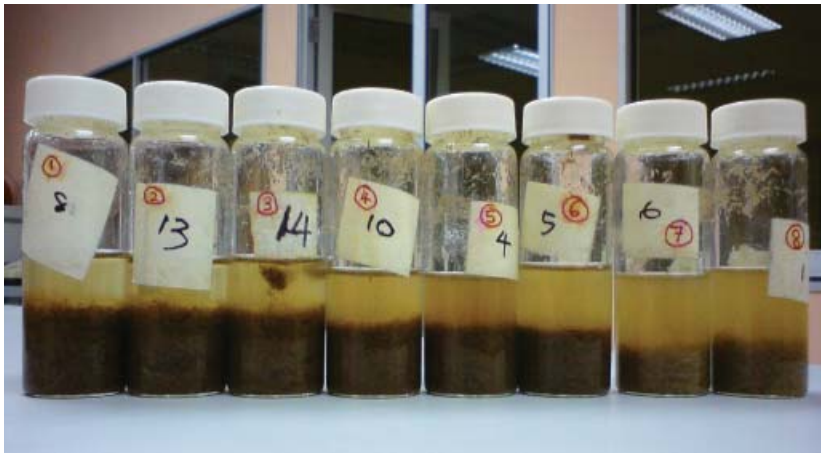
CLOCKWISE FROM RIGHT Fermentation of rice straw in serum bottle; Hydrolysis; Alkaline pretreated rice straw





Biobutanol production from oil palm empty fruit bunches

Mohamad Faizal bin Ibrahim

BIOBUTANOL ($C_4H_{10}O$) or butyl alcohol is an alcohol that can be used as a solvent or fuel produced from biomass by a microbial fermentation. 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.

Malaysia is one of the biggest palm oil producers. As a leading industry in world's oil production, palm oil industry has leaved behind huge amount of biomass from its plantation and milling activities as compared to other type of agriculture biomass. Oil palm empty fruit bunches (OPEFB) is the biggest biomass produced from palm oil industry. The current application of this abundant biomass is through mulching and dumping process. In fact, OPEFB contents high amount of carbon sources in the form of cellulose and hemicelluloses but coated with lignin material. Entirely, this structure was called lignocellulosic material. As we manage to convert the cellulose and hemicelluloses into simple sugar through hydrolysis process, it can be further use for the production of biobutanol. This study is focusing on the production of biobutanol from OPEFB. The OPEFB is converted into fermentable sugar through enzymatic hydrolysis. The OPEFB hydrolysate obtained is further utilized for the production of biobutanol by locally isolated strains mainly from *Clostridium* sp. in anaerobic fermentation. The capability of local microorganism to consume OPEFB hydrolysate and produce biobutanol will be determined based on the amount of biobutanol produced during the fermentation. This study has two main objectives; to obtain biobutanol from OPEFB by locally isolated strains and to optimize the biobutanol production from OPEFB using response surface methodology (RSM) approach. ■

RESEARCH OBJECTIVES

1. To obtain biobutanol from OPEFB hydrolysate
2. To optimize biobutanol production from OPEFB hydrolysate

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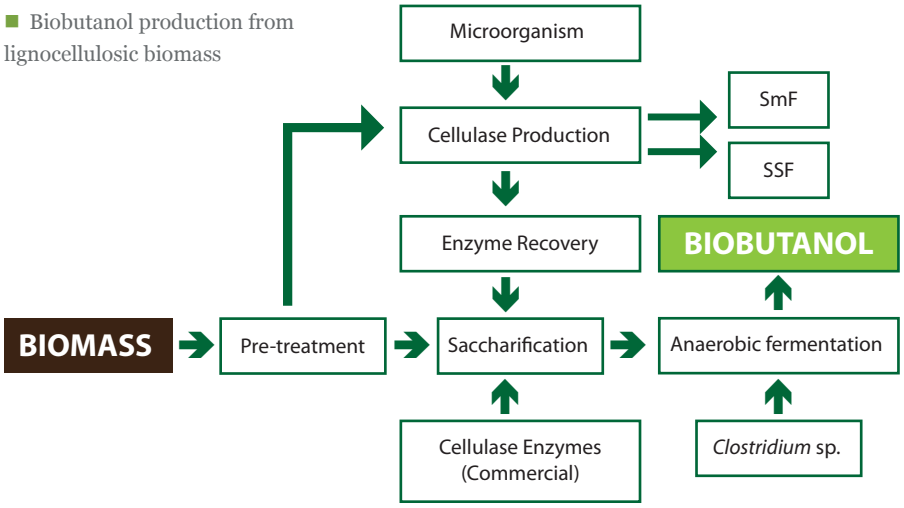
■ Biobutanol production from lignocellulosic biomass



■ Biobutanol production from OPEFB hydrolysate

■ Gas Chromatography (Agilent 6890, US) used for biobutanol analysis

Bioconversion of rice straw to biobutanol by locally isolated bacteria

Nooshin Rahnama

BIOBUTANOL IS A four-carbon alcohol and a potential biofuel .It is a product of microbial anaerobic fermentation. Regarding the constant rise in oil and gasoline prices, the fact that the world supplies are rapidly diminishing and increasing concern for environmental pollution, production of renewable fuels such as bioethanol and biobutanol from cheap and abundant biomass has been a recent issue of interest for many scientists. Thermophilic, anaerobic bacteria such as the solventogenic clostridia naturally possess pathways that allow conversion of sugars into solvents known as acetone- butanol - ethanol (ABE) or solvent fermentation. The typical ratio of ABE is 3:6:1 in which biobutanol is the major product. The clostridia secrete numerous enzymes that facilitate the breakdown of polymeric carbohydrates into monomers.

A very important factor in biobutanol production is fermentation substrate which has the most influence on biobutanol production price. Lignocellulose is the most abundant renewable resource on the planet and has great potential as a substrate for fermentation. Rice, being the main food in many countries including Malaysia, is harvested and rice straw is then burnt causing air pollution and leading to an environmental issue. Therefore, in the current research biobutanol will be produced by fermentation from rice straw. Rice straw is a lignocellulosic material which contains cellulose and hemicellulose that can be hydrolyzed into fermentable sugar (glucose, xylose, ect) by cellulase enzyme. This fermentable sugar will be consumed by clostridia for the production of biobutanol from ABE fermentation. All the samples will be analyzed for biobutanol concentration using gas chromatography (GC), glucose concentration using high performance liquid chromatography (HPLC) and cell concentration using optical density (OD). Production of Biobutanol will then be optimized using statistical approach, Response Surface Methodology (RSM). Fermentation conditions will be optimized for temperature, substrate concentration, pH, inoculum size and biobutanol concentration will be considered as response. An optimized condition needs to be investigated prior to study the production in 2L bioreactor. Pretreatment of rice straw, enzymatic hydrolysis and fermentation are the three processing stages in biobutanol production from rice straw. Unfortunately, biobutanol producing cultures cannot hydrolyze lignocellulosic materials, thus they need to be hydrolyzed prior to fermentation. Pretreatment is the first stage in biobutanol production followed by saccharification. Pretreatment is meant to increase digestibility of biomass and to make cellulose and hemicellulose more accessible to enzymes so that more polysaccharides are converted to monomeric sugars which later will be utilized by bacteria through fermentation process. Pretreatment also breaks down lignin structure that covers cellulose and hemicellulose, therefore increases the accessibility of cellulases. However, the high cost of commercial cellulase makes the bioproduction of biobutanol uneconomical.

Thus, the study is also focusing on cellulase enzyme production from rice straw by fungus and fermentable sugar produced from pretreated rice straw by enzyme cocktail will then be enhanced. Finally in the current study, biobutanol productivity will be enhanced using immobilized cells of the bacteria. The toxicity of biobutanol to the bacteria in the fermentation system will limit the production of biobutanol. By applying adsorption technique, bacterial cells will be immobilized and productivity of biobutanol will be enhanced in a 2 L bioreactor. ■

Anaerobic fermentation for biobutanol production

Biobutanol has several advantages over bioethanol. Biobutanol has higher energy content and lower volatility than ethanol. Biobutanol is less corrosive and less hydroscopic (thus does not pick up water) .Unlike ethanol, biobutanol separates easily from water, making it easier to store under humid conditions. Biobutanol can be used as a direct replacement of gasoline. Being a promising biofuel and playing a major role in the next generation of biofuel, synthesis of biobutanol is the focus of the current study.

RESEARCH OBJECTIVES

1. To enhance fermentable sugar production from treated rice straw using enzyme cocktail.
2. To optimize biobutanol production from rice straw hydrolysate by locally isolated bacteria using RSM approach.
3. To enhance the productivity of biobutanol using immobilized cells of local bacterial isolate.

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Production of biobutanol from pretreated oil palm decanter cake

Mohamad Nafis bin Abdul Razak

OIL PALM DECANTER cake which were produced by 3-phase decanter system for oil recovery was shown high potential biomass resources for the production of cellulases enzymes, polyoses and consequently to biobutanol as sustainable biofuel. Nowadays, cellulases are one of the expensive and precious in the enzyme industries toward the conversion of waste lignocellulosics to liquid fuels. Production of crude cellulases from oil palm decanter cake by locally isolated fungus which are *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2 also studied prior the saccharification process.

Pretreatment of the substrate is the most crucial and tough phase prior to enzyme and polyoses production. Oil palm decanter cake contains high percentage of impurities such as lignin, ash and sand. The effectiveness of various chemical and physical pretreatment (NaOH, HCl and HNO3) to alter lignin content and increase the percentage of cellulose was studied. The effect of pretreatment to the lignocellulose structure was observed by scanning electron microscope (SEM). The treated biomass will proceed to saccharification process by commercial enzyme and crude cellulase.

Polyoses (biosugar) which consist of glucose, xylose, galactose and others sugar are the main intermediate product before the production of biobutanol. Optimization of saccharification process by using response surface methodology approach also will be studied. The parameters are substrate concentration, pH, temperature, agitation and enzyme concentration. The 2-Level Factorial Design in Design-Expert software used to screen the significant parameters for the optimization process. The software also provides central composite design (CCD) which is the most popular response surface method (RSM) design.

Biobutanol has superior fuel properties when compared to bioethanol which become great interest to the world scientist to produce higher amount of biobutanol from biomass. Acetone-Butanol-Ethanol fermentation using *Clostridium* species is the common method for the production of biobutanol. Production of biobutanol by oil palm decanter cake hydrolysate from optimized saccharification process will be studied by using *Clostridium* butyricum (EB6) in anaerobic condition. Gas chromatography used to determine the concentration of butanol produced and high performance liquid chromatography (HPLC) used to determine the concentration of sugar consumption. ■

RESEARCH OBJECTIVES

1. To obtain fermentable sugar from pretreated oil palm decanter cake using enzymes cocktail for biobutanol production
2. To optimize production of polyoses from pretreated oil palm decanter cake by *Trichoderma asperellum* (UPM1) and *Aspergillus fumigatus* (UPM2) crude cellulases

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

■ **RIGHT** *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2 spore suspension

Production of biobutanol from sago pith residue

Siren Anak Linggang

THE AGRICULTURAL industry produces a significant amount of post-processing waste and residue. Particularly in Sarawak, sago starch industry is responsible for the production of a notable amount of residue. Sago pith residue is an abundant lignocellulosic residue that left behind after starch extraction process and contains significant amount of starch, some cellulose, hemicellulose and lignin. This residue has a great potential as economical substrate for the production of biobutanol as an alternative fuel due to their high content of cellulose and hemicellulose. Biobutanol can be produced fermentatively from lignocellulosic residues by solventogenic clostridia. It is very energy efficient and suitable for replacing petrol as fuel in gasoline engines.

Three step applied which are pretreatment of sago pith residue, enzymatic hydrolysis by cellulolytic enzyme that produced by locally isolated fungus and production of biobutanol via acetone-butanol-ethanol (ABE) fermentation in anaerobic condition. In conversion of lignocellulosic biomass to biofuel, the sago pith residue needs to be treated so that the cellulose and hemicellulose in the plant fibers is exposed and more accessible to hydrolyze into simple hexose and pentose sugars. In this study, dried sago pith residue or sago hampas was ground before undergo enzymatic hydrolysis using dextrozyme. The solid part (cellulose) will be separated from hydrolyzed sago hampas solution through filtration. The cellulose obtained will be used as a substrate in this study. This cellulose will be directly converted to fermentable sugars using crude cellulases produced by locally isolated fungus which are *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2. Different parameter such as sago pith residue hydrolysate concentration, pH, temperature and yeast extract concentration will be studied to optimize the production of biobutanol using response surface methodology (RSM) approach. The fermentable sugars obtained will be used as a carbon source for isolted strain A2 in biobutanol fermentation. ■

RESEARCH OBJECTIVES

1. To obtain fermentable sugars from sago pith residue utilizing local microbial enzymes.
2. To obtain biobutanol from sago pith residue hydrolysate by isolated microbe A2.

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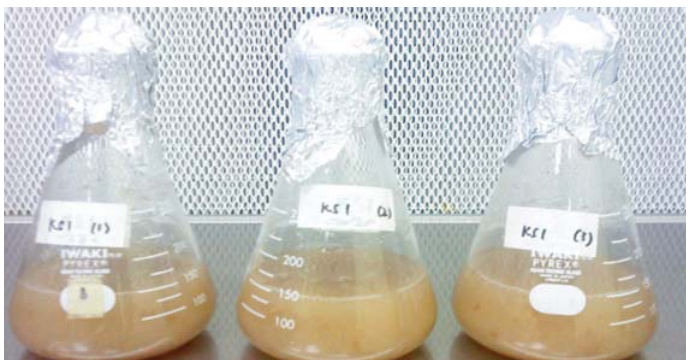
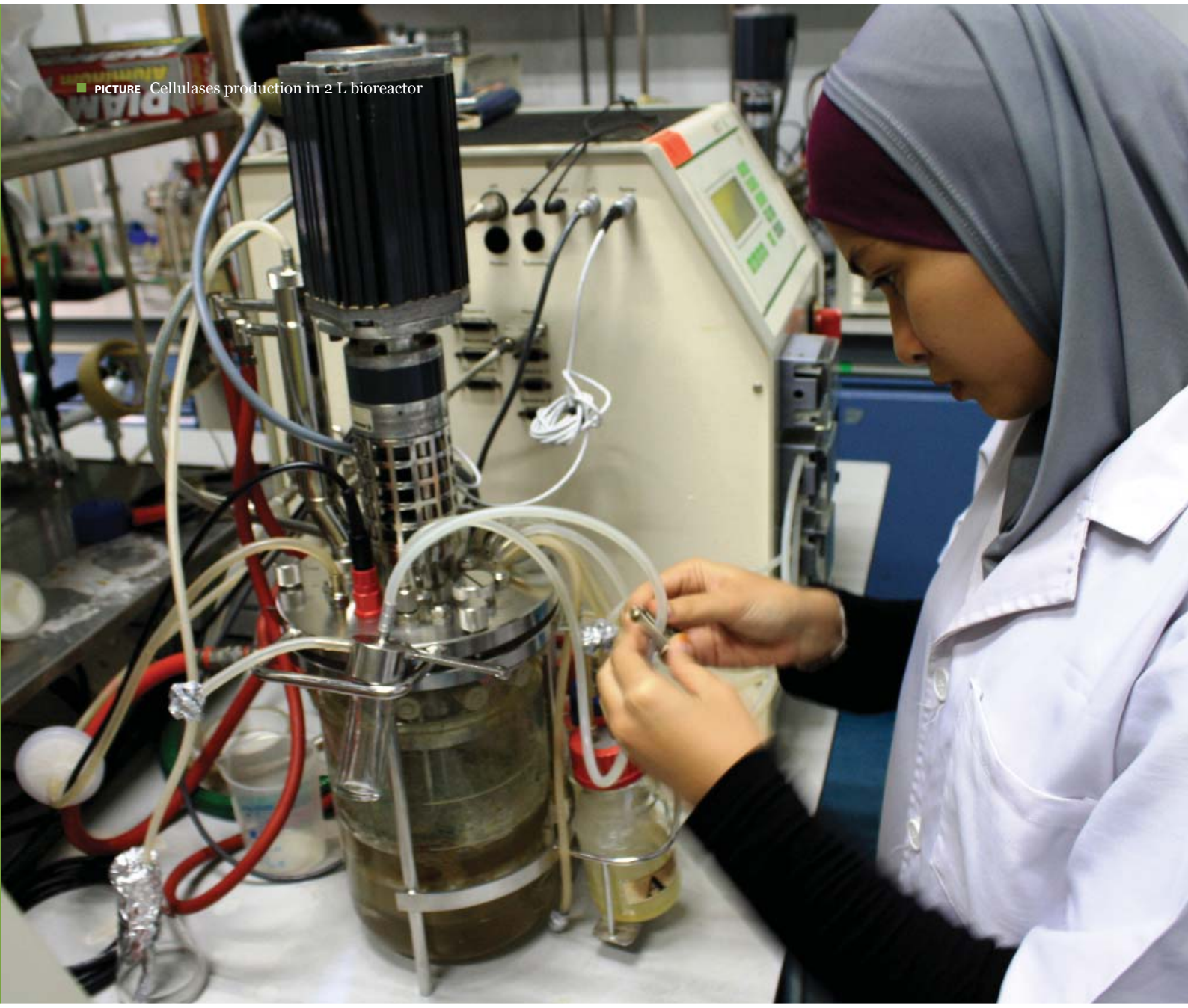


■ **FROM TOP TO RIGHT**
Sago logs; Sago effluent discharge from mill; Wet sago hampas; Enzyme production from sago hampas by KS1



PICTURE Cellulases production in 2 L bioreactor

BIOPLASTIC
RESEARCH GROUP

"Bioconversion of organic wastes into bioacids and bioplastic"

Our aim is to utilize organic wastes for the production of biodegradable plastics. Up to date, we have successfully converted palm oil mill effluent (POME) and kitchen refuse into organic acids, namely acetic, butyric, propionic and lactic acids. These organic acids can be used as the substrate to produce biodegradable plastics, *i.e.* polyhydroxyalkanoates (PHAs). Currently, our bioplastic is being produced by *Comamonas* sp. EB172, a novel acid-tolerant bacterium which was locally isolated. We will continue to explore the potential of other organic wastes / natural resources to be used as substrate for the production of PHAs.

RESEARCH AREAS

At present, we focus on three types of organic waste residues; POME, kitchen waste and oil palm fronds sap.

There are four main activities in our research group:

Pretreatment of wastes

Conversion of organic wastes into bioacids by physical, chemical and biological treatments.

Strain Development

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

Upstream Processing

Upstream processing involves fermentation for the production of PHAs. The fermentation is carried out both in lab scale (shake flask, 2L, 7L and 10L fermenters) and pilot scale (50L and 150L fermenters). Optimization of fermentation parameters is done in order to gain maximum yield and PHA content.

Downstream Processing

The aim of our downstream processing is to recover and purify the PHA produced in bacterial cells using organic solvent-free extraction method. Currently we are proposing two methods, i.e. recovery of PHA using water and sodium hydroxide.
Apart from PHA recovery, our downstream processing also involves chemical recycling of PHA. Chemical recycling is aimed at converting the PHAs into monomers or low molecular weight polymers which can be reused to produce new polymer materials. So far we have tried two methods for the depolymerization of PHA , i.e. pyrolysis and hydrolysis.

PRINCIPAL RESEARCHERS

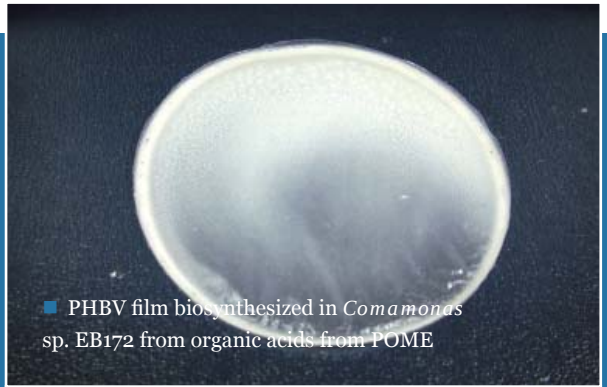
LEFT PROFESSOR DR MOHD ALI HASSAN
RIGHT DR HIDAYAH ARIFFIN

Anaerobic treatment of POME is a 3-in-1 process:

1. to treat the wastewater (POME) from palm oil mill,
2. to produce organic acids, and
3. to generate biogas.

It is thus our dream to have a biorefinery near palm oil mill which integrates the organic acids production, PHA production, PHA recovery and biogas production. Currently, we have a biogas pilot plant in FELDA Serting Hilir palm oil mill meant for producing renewable fuel. Coupling the PHA production with biogas production at the palm oil mill may reduce the PHA production cost and hence, could promote better market for PHA.

There are many applications for PHA, from low-end material products such as plastic packaging to the high-end products like medical suture and artificial heart valve. These applications can be varied by producing PHAs with different characteristics, ranging from brittle and stiff to elastomeric rubber-like materials. This can be done by using various types of microorganism and utilizing other sustainable renewable carbon sources that can produce different types of PHAs with extended characteristics.



PHBV film biosynthesized in *Comamonas* sp. EB172 from organic acids from POME

Concentrated POME and clarified acids

BIG PICTURE BIOPLASTIC

SUBSTRATE

TREATED POME

KITCHEN WASTE

OIL PALM FRONDS

ACID RECOVERY


Nadia


Matun

KITCHEN WASTE


Syaiful Nizam


Mumtaz

TREATED POME

PRODUCTION OF PHA

Comamonas sp.EB172


Rafein


Nadia


Mumtaz

Cupriavidus necator CCUG52238


Mior

STRAIN IMPROVEMENT


Yee

RECYCLING OF PHA


Elmy

SCREENING OF PHA PRODUCERS, MEDIA, CULTURE OPTIMIZATION


Rafein


Azman

PHBV RECOVERY


Mitra


Asma

Anaerobic treatment of palm oil mill effluent and organic acids production in pilot plant scale bioreactor

Syaiful Nizam bin Basri

MALAYSIA IS ONE OF the largest countries that involved in palm oil industry. The total production of palm oil in Malaysia contributed 45% of the palm oil demand. With such a huge production, the palm oil industry generates large amounts of wastewater known as palm oil mill effluent (POME). POME is generated mainly from three major sources, which is sterilizer condensate, hydrocyclone waste and separator sludge. POME is thick brownish liquid which high nutrient content mainly oil and fatty acids. This nutrient content is able to support bacteria growth with the degradation of the waste to reduce its pollution strength. During the biological treatment of POME, by products recovered during anaerobic process of POME are volatile fatty acids that mainly consist of acetic, propionic and butyric acids. Acetic, propionic and butyric acid that produce in anaerobic treatment can be recovered by several methods such as liquid-liquid separation. These acids then will be used as a substrate for polyhydroxylakanoate (PHA) production.

In this research, the recovery process will use rotary evaporation system. With large amount of wastewater generate in palm oil industry each year, the raw material for organic acids production will be continuously exist. Since it was reported that the organic acids generated by the anaerobic fermentation of waste can be used as carbon sources for polyhydroxalkanoate (PHA) production. PHA is a biopolymer and biodegradable thermoplastics that have been produced by various types of bacteria as carbon and energy reserve materials. The high price of PHA has limited the use of these biodegradable plastic for the time being. The production of PHA based on organic acids as carbon sources will be useful to reduce the price and depending on synthetic carbon sources only. Therefore, this study will give significant contribution to new knowledge aside improve efficiency of POME treatment, reducing the cost and enhance the environment. ■

RESEARCH OBJECTIVES

1. To obtained high conversion of organic acids from POME in pilot scale bioreactor.
2. To improve organic acids recovery in pilot scale rotary evaporation system.

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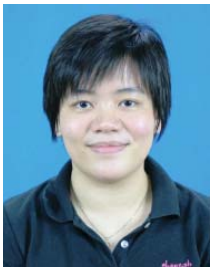
■ **CLOCKWISE FROM RIGHT**
Filter press for solid removal;
Pilot scale rotary evaporator (50 l);
Pilot scale acidogenic bioreactor

Metabolic engineering of *phaZ* and *phaC* genes in *Comamonas* sp. EB172 for polyhydroxyalkanoates production

Yee Lian Ngit



AS WE KNOW MORE than 250 different bacteria have been reported to accumulate PHA as carbon and energy storage compounds. *Comamonas* sp. EB172 is a new local isolate obtained from palm oil mill effluent (POME) sludge. It showed superior growth profile on organic acids. This bacterium was able to synthesize poly(3-hydroxybutyrate-*co*-hydroxyvalerate) (PHBV) by utilizing clarified acids from anaerobically treated POME. The PHA accumulation pathways show that numerous genes have known to encode various enzymes involving in PHAs biosynthesis. Three main genes in microorganism encoding in biosynthesis pathway, named as 3-ketothiolase (*phaA* gene), NADPH-dependent acetoacetyl-CoA reductase (*phaB* gene) and PHA synthase (*phaC* gene) are found to metabolize the carbon sources to final polymer. *phaA* gene condenses two acetyl-CoA molecules to form acetoacetyl-CoA. The next enzyme, NADPH-dependent acetoacetyl-CoA reductase, is catalyzing reduction reaction of acetoacetyl-CoA to (R)-3-hydroxybutyryl-CoA. The last steps for P(3HB) biosynthesis is polymerization of (R)-3-hydroxybutyryl-CoA by PHA synthase.

In order to study the genes involved in the biosynthesis and biodegradation of PHBV, my studies will focus on synthase gene (*phaC*) and depolymerization gene (*phaZ*) of *Comamonas* sp. EB172. Interestingly, *Comamonas* sp. EB172 contained PHA synthase (*phaC*) from class I, acetyl–CoA acetyltransferase (*phaA*), acetoacetyl-CoA reductase (*phaB*) in one operon. Also, the depolymerase gene is between a protein and electron transport complex. With the hyphothesis that genetic modification can increase the PHA productivity or yield, depolymerase gene knock out and over expression of synthase gene will be carried out. Additionally, heterologous expression of *phaAB* genes in *Escherichia coli* to determine the protein produced by the amplified genes is functional or defective. Expresion vector with ampicillin resistant antibiotic will be used for the genes expression in *E. coli*. Through the analysis we will know the PHA biosynthesis is affected by the key enzyme, synthase or the intermediate enzyme such as transferase or reductase. By using the recombinant *Cupriavidus necator* PHB-4 (PHA producer without synthase gene), synthase gene of *Comamonas* sp. EB172 can be expressed and compared the synthase activity with the wild type *C. necator*. Broad-Host-Range plasmid will be used for the expression of synthase gene in *Comamonas* sp. EB172. By insertion of extra synthase gene to over express and increase the PHA production. On the other hand, to avoid the intracellularly accumulated PHA degraded as energy by PHA producer, PHA depolymerase gene can be deactivated by one of the methods such as silencing the depolymerase gene. Depolymerase gene will be active under unfavourable culture condition such as carbon limitation for growth. PHA accumulated tends to be degraded and reutilized as energy sources. Thus, optimum culture medium and conditions are very important for high PHA accumulation.

However, the PHA degradation can be avoided throughout the fermentation process by knocking out the PHA depolymerase gene. Suicide vector will be used as a tool for the deletion of depolymerase gene in *Comamonas* sp. EB172. The accumulated PHA will be maintained or increased by the deleting of depolymerase gene. The recombinant bacteria will use for the PHA production in shake flask. The aim of this study is to improve or maintain the cell yield or PHA content and PHA granules in cell by genetic modification. ■

RESEARCH OBJECTIVES

1. To study heterologous expression of *phaAB* and *phaC* genes in *Escherichia coli* and *Cupriavidus necator* PHB-4.
2. To construct recombinant *Comamonas* sp. EB172 by deleting *phaZ* gene and increasing copy numbers of *phaC* gene.
3. To determine PHA production by using constructed recombinant *Comamonas* sp. EB172.

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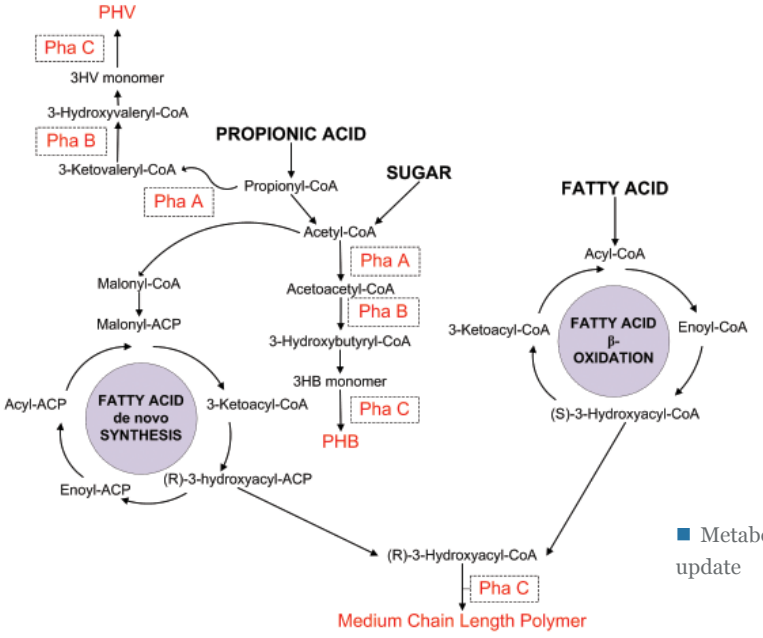

■ Metabolic pathway update

Biosynthesis of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) P(3HB-*co*-3HV)] copolymer from anaerobically treated palm oil mill effluent by locally isolated bacterium

Mohd Rafein Zakaria

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.

The *Comamonas* sp. EB172 cells were rod-shaped, Gram-negative, non-pigmented, non-spore-forming and non-fermentative. Phylogenetic analysis using the 16S rRNA gene sequence showed that the strain clustered with the genus *Comamonas*. Its closest neighbours were the type strains *Comamonas* terrigena (96.8%), *Comamonas* koreensis (93.4%), *Comamonas* composti (92.9%), and *Comamonas* kerstersii (91.1%). The ability of the strain EB172 to produce polyhydroxyalkanoates (PHA) when supplied with organic acids made this bacterium unique among *Comamonas* species. The bacterial strain was clearly distinguished from all of the existing strains by phylogenetic analysis, fatty acid composition and a range of physiological and biochemical characteristics. The G+C content of the genomic DNA was 59.1 mol%. The strain showed good growth in acetic, propionic and n-butyric acids. *Comamonas* sp. EB172 produced 9.8 g/l of cell dry weight and accumulated 59 (wt%) of PHAs when supplemented with mixed organic acids from anaerobically treated palm oil mill effluent. It is evident from the genotypic, phenotypic data and ability to produce PHAs that strain EB172 represents a new strain in the genus *Comamonas* (GeneBank accession no. EU847238).

Based on the shake flask and 2 L fermenter, P(3HB) homopolymer and P(3HB-*co*-3HV) copolymer was produced by *Comamonas* sp. EB172 using single and mixture of carbon sources. Poly(3-hydroxyvalerate) P(3HV) incorporation in the copolymer was obtained when propionic and valeric acid was used as precursors. Incorporation of 3HV fractions in the copolymer varied from 45 to 86 mol% when initial pH of the medium was regulated. In fed-batch cultivation, organic acids derived from anaerobically treated palm oil mill effluent (POME) were shown to be suitable carbon sources for polyhydroxyalkanoate (PHA) production by *Comamonas* sp. EB172. Number average molecular weight (Mn) produced by the strain was in the range of 153 to 412 kDa with polydispersity index (Mw/Mn) in the range of 2.2 to 2.6, respectively. Incorporation of higher 3HV units improved the thermal stability of P(3HB-*co*-3HV) copolymer. Thus the newly isolated bacterium *Comamonas* sp. EB172 is a suitable candidate for PHA production using POME as renewable and alternative cheap raw materials.

As for conclusion, *Comamonas* sp. EB172, demonstrated a suitable bacterial strain for PHA production with ability to consume strong acids produced from anaerobically treated POME. Biopolymer produced resembles the petro-plastic plastics whereas 3HV monomer fraction incorporation in the P(3HB-*co*-3HV) copolymer improves the thermal and mechanical properties. ■

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

2010 PUBLICATION

Mohd Rafein Zakaria, Meisam Tabatabaei, Farinazleen Mohamad Ghazali, Suraini Abd-Aziz, Yoshito Shirai and Mohd Ali Hassan. (2010). Polyhydroxyalkanoate production from anaerobically treated palm oil mill effluent by new bacterial strain *Comamonas* sp. EB172. *World Journal of Microbiology and Biotechnology*, 26(5), 767-774.
(*See abstract at page 37*)

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*, 95(8), 1382-1386.
(*See abstract at page 33*)

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Production of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) by *Comamonas* sp. EB172 from anaerobically treated palm oil mill effluent towards zero emission system

Tabassum Mumtaz

IN THIS PROJECT, pilot scale recovery process was developed for obtaining a clarified solution of mixed organic acids to be used as substrates for PHBV production. The study also aimed at developing a novel fermentation strategy in 2 L scale and up-scaling the fermentation process in 150 L bioreactor based on constant dissolved oxygen tension and constant impeller tip speed.

The PHBV produced were further characterized and compared with the commercially available PHBV to address the market demand. Filtration, evaporation and distillation of treated POME for the recovery and clarification of organic acids was carried out using centrifugation and rotary evaporator in laboratory scale and filter press and rotary evaporator for pilot plant scale studies. When pilot scale set-up was used, the highest concentration obtained was 67.25 g/L upon eight-fold concentration with recovery yield of 83.33% as compared to 85.27% in laboratory scale. The affect of this clarified mixed organic acids on PHBV production by our local isolate, *Comamonas* sp. EB172 were evaluated in 2 L bioreactor.

The time-course and the utilization of carbon and nitrogen throughout the growth cycle of *Comamonas* sp. EB172 was compared using one-step and two-step fermentation methods. *Comamonas* sp. EB172 showed higher tolerance of organic acids in the order of n-butyric acid>acetic acid>propionic acid. The two-step fermentation method was further simplified by eliminating centrifugation step and carrying out growth stage and PHA production stage consecutively in 2 L bioreactor. The fermentation was switched to PHA production stage by introducing nitrogen-free mineral media into broth followed by mixed acids feeding using pH-stat method. By applying this strategy, the maximum biomass obtained in 2 L bioreactor was 10.2 g/L with residual acid around 2-6 g/L and volumetric productivity of 0.1318 g/L/ h. PHA content ranged from 70- 90% (w/w) of the cell with yield of 0.27- 0.4 g PHB/g mixed acid, depending on the concentration of ammonium nitrogen and residual organic acids in the broth, respectively. The size distribution of PHBV granules in *Comamonas* sp. EB172 ranged from 0.11 to 0.67 µm with 5-9 granules in each cell. TEM images of PHBV granules in vivo revealed core-shell structure indicating the formation of block copolymer instead of random copolymer. The extracted polymer had 10-17% of HV content and the chemical structure, thermal and tensile properties of the extracted PHBV were identical to a commercial PHBV. Finally, by applying dual nutrient limitation strategy i.e., dissolved oxygen and nitrogen limitation, scaling up fermentation in 150 L bioreactor resulted in biomass concentration of 5.35 g/L with final PHA content of 72.80% and volumetric productivity of 0.083 g/L/h. The calculated yield was 0.259 g PHBV/g mixed acids. Both yield and PHA content were comparable to that obtained in 2 L scale. The overall results indicated that clarified organic acid mixtures could be suitable substrates for the production of PHA. The fermentation process described herein represents an interesting alternative for the production of polyhydroxyalkanoates from renewable resources. ■

RESEARCH OBJECTIVES

1. To develop process for recovery and clarification of mixed organic acids from anaerobically treated POME in pilot scale.
2. To develop feeding strategy for the production of PHBV by *Comamonas* sp.EB172 in 2 L bioreactor using clarified organic acids obtained from POME and to characterize the PHBV produced.
3. Scale-up production of PHBV by *Comamonas* sp. EB172 in 150L bioreactor.

2010 PUBLICATION

Tabassum Mumtaz, Noor Amalina Yahaya, Suraini Abd-Aziz, Nor'Aini Abdul Rahman, Phang Lai Yee, Yoshihito Shirai, Mohd Ali Hassan. (2010). Turning waste to wealth-biodegradable plastics polyhydroxyalkanoates from palm oil mill effluent – a Malaysian perspective. *Journal of Cleaner Production*, 18(14), 1393-1402.
(*See abstract at page 35*)

Tabassum Mumtaz, Suraini Abd-Aziz, Phang Lai Yee, Wan Md Zin Wan Yunus, Yoshihito Shirai, and Mohd Ali Hassan. (2010). Synthesis, Characterization, and Structural Properties of Intracellular Copolyester Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) Produced by *Comamonas* sp. EB 172 from Renewable Resource. *International Journal of Polymer Analysis and Characterization*, , 329-340.
(*See abstract at page 38*)

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■ **LEFT** Handling 150L Bioreactor at MARDI, Serdang, Malaysia

Production of polyhydroxyalkanoate from oil palm fronds

Mior Ahmad Khushairi Mohd Zahari

POLY(3-HYDROXY-BUTYRATE) (PHB) is a biodegradable thermoplastic polyester accumulated intracellularly by many microorganisms under unfavorable growth conditions. In PHB production, about 40% of the total production cost is for raw material. Thus, the use of a cheaper carbon source is required in order to reduce the high production cost of PHB. Palm oil industry in Malaysia has contributed about 52% of the total world oils and fats export in year 2006. Apart of its contribution to economic growth, palm oil industry also supplies a renewable biomass which can be further utilized to produce other value added product such as bioplastic.

Based on these findings, an attempt has been made to ferment sap from oil palm biomass to produce PHB by using bacteria. This research will be divided into three different stages which are extraction of sugar from oil palm fronds (OPF), followed by, PHB production through fermentation in bioreactor and finally, extraction, purification and characterization of PHB from the cell. Prior to fermentation, juice from OPF will be extracted by using simple physical separation method. At this stage, characterizations of OPF juice will also been carried out. These were including sugar composition, proximate analysis, and so forth. Further pretreatment by centrifugation, membrane filtration and sterilization will be employed to optimize the fermentable sugars production. The next step of this study will be bioplastic fermentation, the stage in conversion of fermentable sugar to PHB by 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 biomass. ■

RESEARCH OBJECTIVES

1. To determine the appropriate sugars extraction and pretreatment method from oil palm biomass.
2. To optimize polyhydroxyalkanoates (PHA) production by using sugars from oil palm biomass in shake flasks.
3. To optimize the production of PHA in 2-L bioreactor.
4. To scale-up the fermentation process from 2L to 50L bioreactor and to optimize the process with respect to the process parameters.

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■ **CLOCKWISE FROM LEFT**
Fresh Oil Palm Fronds (OPF);
Fresh Oil Palm Fronds (OPF) Juice;
Shake flasks study;
2L Bioreactor study

Optimization of polyhydroxyalkanoate production by *Comamonas* sp. EB172

Noor Azman bin Mohd Johar

POLY-B-HYDROXY-BUTYRIC ACID (PHB) is a natural, biodegradable polymer, which is accumulated as an energy reserve material by a large number of bacteria when, nutrients such as nitrogen, oxygen, sulphur, potassium or phosphorous sources are available in limiting concentrations in the presence of excess carbon source.

Nowadays, cost for production of PHB is high compared to the petroleum based plastic. Response surface methodology (RSM) was then used to optimize the composition of culture medium and condition for maximizing the productivity of PHB. A maximum of residual biomass and PHB was obtained using optimized concentrations, representing the validity of the predicted models for residual biomass and PHB production, respectively. In this study, the parameters involved are pH (5 - 9), temperature (25 - 37oC), inoculums size (4 - 10% v/v), acid concentration (5 - 10 g/L) and nitrogen concentration (0 - 1 g/L). All of these parameters were interacted and significantly affected the production of PHB with P value <0.05. Locally isolated bacteria were used in this study, *Comamonas* sp. EB172, which is an acid tolerant bacteria that can consumed acids as their carbon sources. Clarified acids from palm oil mill effluent were used in order waste to wealth principle and can reduce the cost for PHB manufacturing. A significantly higher maximum biomass with PHB content was obtained in a 2 L lab scale bioreactor thus giving a yield of PHB/g carbon source consumed. Batch kinetics can be used for model development, which will facilitate simulation of nutrient limited cultivation(s) for over accumulation of PHB.

RESEARCH OBJECTIVES

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

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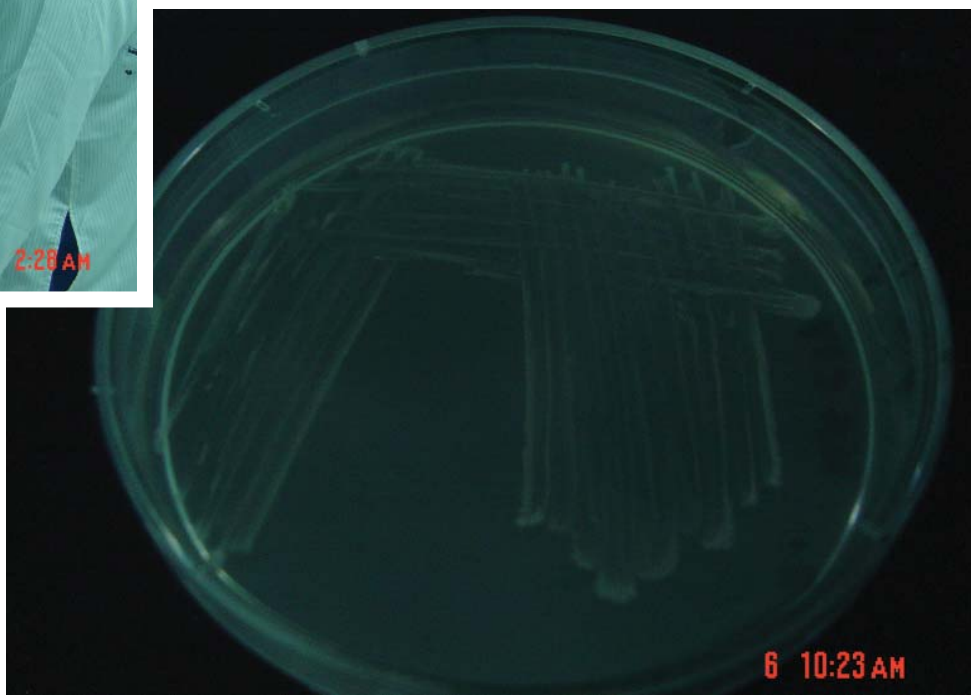
In this work the kinetics of PHAs production and consumption of clarified acids by *Comamonas* sp.EB172 with simultaneous considerations of substrate inhibition, cell growth, and maintenance and product formation were explored. By this kinetics study also can help in scaling up the production PHB in industrial scale. ■



■ **TOP** Training for 2 Liters bioreactor in Universiti Malaysia Pahang (UMP), Malaysia

■ **RIGHT** *Comamonas* sp. EB172 culture

Optimization of organic acids production from kitchen waste using response surface methodology

Halimatun Saadiah Hafid

KITCHEN WASTE consist of uneaten food and food preparation residues from the residential areas, commercial establishments and institutional sources. Kitchen waste is characterized by a high organic content thus its contain carbohydrate, proteins, lipids, and other compounds that are readily biodegradable. As population increased, generation of wastes particularly kitchen waste also increased. Therefore, the best treatment methods to disposed its will be an acute problem due to its characteristics.

In Malaysia, land filling system is a good method but it involves high costs and not suitable for long time purposes due to land scarcity. Thus, studies conducted utilizing kitchen waste for production of value added product is the best alternative. Bioconversion of kitchen waste to intermediates products predominantly organic acids was accomplished by a series of processes in anaerobic digestion catalyzed by a consortium of bacteria. Recently, there are increased in demand from industrial for naturally produced organic acids especially lactic acids for biopolymers industry with application in food industry for packaging, and medical applications.

This study focused on the optimization process of organic acids production utilizing kitchen waste as a substrate. In the optimization process, newly statistical optimizing software so called Response Surface Methodology (RSM) had been introduced to overcome problem of traditional optimization methods "change-one-variable-at-a-time". Previously, the conventional optimization method did not have much information about the parameter studied, but RSM enable information on independent variables involves and the interaction among the variables on the desired product response which describe the behavior of the whole biochemical reaction process. The optimization of organic acids was based on the pH, temperature and inoculums size. The different concentration level of pH, temperature and inoculums size and the interaction among the parameters give a significance effects on organic acids production.

RESEARCH OBJECTIVES

1. To compare organic acids production from kitchen waste and simulated kitchen waste
2. To optimize organic acids production from model kitchen waste using response surface methodology (RSM)

2010 PUBLICATION

Halimatun Saadiah Hafid, Nor'Aini Abdu Rahman, Farah Nadia Omar, Phang Lai Yee, Suraini Abd-Aziz, Mohd Ali Hassan. (2010). A Comparative Study of Organic Acids Production from Kitchen Waste and Simulated Kitchen Waste. *Australian Journal of Basic and Applied Sciences*, 4(4), 639-645.
(*See abstract at page 45*)

Main supervisor :
DR NOR'AINI ABD RAHMAN
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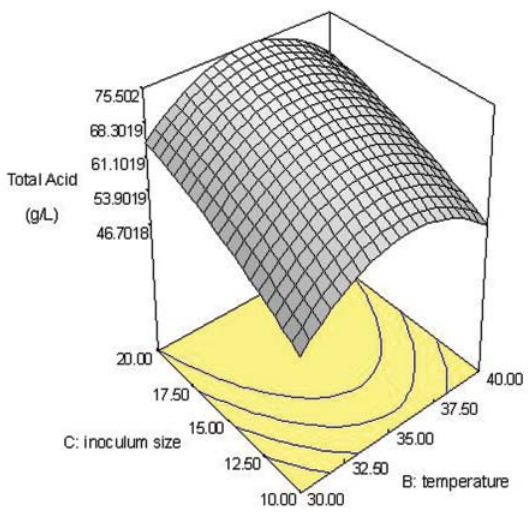
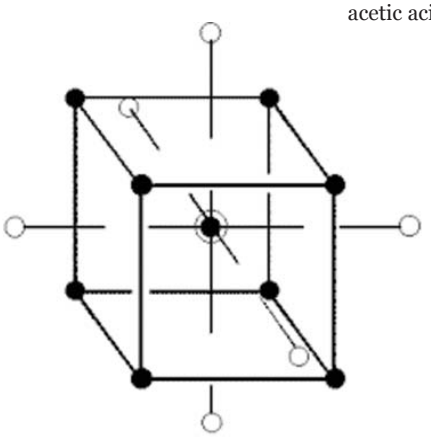
Based on the calculated optimum value that favors the organic acids production has been achieved. During the model validation test, highest organic acids produced which lactic acids is the most dominant followed by acetic acids, propionic acids, and butyric acids. Hence, it has been concluded that RSM is a useful tools for prediction of optimum condition for organic acids production. ■





Response Surface Design
Central Composite

■ **TOP LEFT** Fresh kitchen wastes collected from restaurants

■ **FAR LEFT** Interaction happened between several factors

■ **LEFT** Response surface methodology

Production and recovery of organic acids from fermented kitchen wastes for the production of polyhydroxybutyrate

Farah Nadia Omar

MALAYSIA HAS been experienced a tremendous growth especially in economy and population. These urbanization and industrialization has brought so many changes in Malaysian lifestyle. These include the waste that being generated everyday. Due to its heterogeneous composition, MSW is considered as the most complex solid waste stream.

In Malaysia, debatable issues on MSW have kept going on through out the year. People are complaining, arguing and protesting about the management of MSW everyday. These includes poor management and cleanliness of public places like parks and streets, untidiness of garbage bins and areas surrounding it, inefficient collection of wastes, open dumping of wastes and public health related with MSW problems. Almost 80% of total weight of MSW consisted of kitchen wastes and organics, followed by paper (17%) and plastics (15%). Presently, kitchen waste and other organics have been landfilled together with other wastes. Kitchen waste is the main source of decay, odor and leachate in collection and transportation due to the high volatile solids (85-95%) and moisture content (75-85%). This resulted in various problems like emanating odor, attracting vermin, emitting toxic gasses, contaminating groundwater and wasting landfill capacity. Rather than being degraded in the landfills, all the nutritive organics and intermediate products like organic acids, alcohols and other chemicals, they should be recovered in order to meet the demand of chemicals, bioenergy and biofuels demand of the country. Owning to efficient resource recovery and lessened environmental impact, a lot of researchers around the world opt for anaerobic digestion to treat and utilize the food waste and organics.

Today, researchers around the world have been used kitchen wastes as substrates or medium to produce high value added product like bioplastic, a useful biodegradable plastics which can be used widely in various applications. It has been a good candidate to replace the non-environmental friendly conventional plastics. Bioplastics has few advantages over the conventional plastic where they are thermoplastics, environmental friendly, complete biodegradability and non hazardous. However, bioplastics has not been commercially exploited widely because of its high price as compared to the conventional thermoplastics. Finding less expensive substrate would, therefore, be important for the commercialization of this product. Many researchers have demonstrated that high PHB production cost can be possibly lowered by using unpurified, low cost organic wastes from agricultural and food processing. Since kitchen wastes are nutrition-rich resource, it can be used as a ready-made substrate in fermentation after converting the kitchen wastes to volatile fatty acids such as lactic acid, acetic acid, propionic acid and butyric acid by anaerobic digestion that can be further utilized by PHB-producing bacteria such as recombinant E.coli, *Bacillus* spp., Ralstonia eutropha (currently known as *Cupriavidus necator*), Pseudomonas spp. and others

RESEARCH OBJECTIVES

1. To produce and recover organic acids from kitchen waste
2. To produce PHB from recovered organic acids by using *Cupriavidus necator* CCGUG52238

Main supervisor :
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Education status : PhD, Semester 6
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In this study, kitchen wastes from local restaurants have been used in order to produce organic acids. The organic acids were recovered using an integrated system where it had been centrifuged and filtered in order to obtain purified organic acids. The recovered organic acids were then being used as carbon sources for *Cupriavidus necator* to produce bioplastic. ■

■ **TOP LEFT** Evaporation process for extraction organic acids from food waste

■ **TOP RIGHT** Food waste after filtration

■ **LEFT** Centrifuge the organic acids

Improved recovery of polyhydroxyalkanoate from renewable resources

Nor Asma bt Ab Razak

POLYHYDROXYALKANOATES (PHA) is a complete biodegradable, biocompatible, microbial thermoplastic which has potential to replace petroleum-derived thermoplastics. PHA is an excellent plastic option; a clean energy alternative with no emissions of greenhouse gases, which helps in addressing the challenge of global climate change. PHA are synthesized 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. PHAs are accumulated intracellular and hence their extraction from the cell biomass is a critical step for economic production.

The recovery processes is needed to produce a consistent, usable, clean and purified product while keeping production costs down. PHA production will be conducted using mixed organic acids obtained by anaerobically treated palm oil mill effluent (POME). Organic acids as a carbon and energy sources from waste have been regarded as a promising alternative for the production of PHAs on a large scale and at a low cost. Treated organic acids produced from POME contained acetic, butyrate and propionate which were used to produce a random copolyester of (R)-3-hydroxybutyric acid (3HB) and (R)-3-hydroxyvaleric acid (3HV). This copolyester is more ductile, easier to mold and tougher with wide range of its practical applications.

The objectives of this study are to produce the PHA from organic acid, develop an alternative process recovery of intracellular PHA by using chemical, physical and biological and to develop mass and energy balances for the complete PHA recovery. PHA production in 10 L and 50 L stirred-tank bioreactor using Ralstonia eutropha and *Comamonas* EB172 showed that *Comamonas* EB 172 produce more than 50% of PHA content using organic acids from POME than 30% of PHA content in Ralstonia eutropha. Thus, *Comamonas* EB 172. containing PHA will be recovered by using chloroform, sodium hydroxide and locally isolated enzyme since it can reduce operation cost and also reduce the solvent damage to operator health and environment. In addition to get a higher purity, a purification step could be added to the process. In this study, different initial conditions, effect of drying and washing step of solvent will be done and analysed using statistical software. The properties of PHAs are highly dependent upon their recovery techniques; hence, biodegradable polymer having a wide range of properties. The micrograph, chemical, mechanical and thermal properties are investigated using high pressure chromatography (HPLC), gas chromatography (GC), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), molecular weight (GPC), tensile strength, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). ■

RESEARCH OBJECTIVES

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

Main supervisor :
PROF. DR MOHD ALI BIN HASSAN
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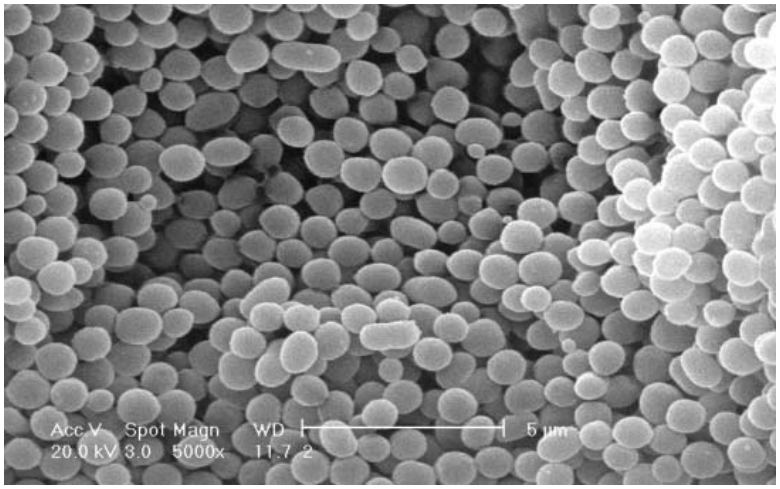
Email: asmaktdi2@yahoo.com



■ **LEFT** PHA in solution

■ **BOTTOM** Morphology of PHA using SEM

Appropriate recovery methods of intracellular polyhydroxyalkanoates from *Comamonas* sp. EB 172

Mitra Mohammadi

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. Polyhydroxyalkanoate (PHA) is energy and carbon storage material synthesized and intracellular accumulated by numerous microorganisms, usually when an essential nutritional element such as nitrogen, phosphorous, oxygen, sulphur, or potassium is limited in the presence of excess carbon source. Due to the similar mechanical properties to synthetic plastics, complete biodegradability, stability and durability, PHA have 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 PHA includes mainly substrate and downstream processing cost. On the other hand, inexpensive and scaleable PHA recovery schemes need to be developed to achieve low-cost production that is competitive with traditional thermoplastics. Obviously, the development of a fermentation strategy that allows high PHA concentration and productivity is most important for reducing the price of PHA recovery. Research has focoused on the search for microorganisms capable of utilizing cheaper carbon substrates, improved frementation strategies, and novel downstream processes to improve yields of polymer, thus reducing the overall cost of the product. Therefore, developing a process that allows a simple, efficient and less polluted recovery of PHA is an attractive proposition. The methods used for PHA recovery concentrate either on its solubilization or on the solubilization of the non-PHA biomass. A variety of solvents can be used for PHA recovery through its solubilization. However, these are costly and also considered as pollutants, and it is obvious that the production of a green bioplastic should not involve such solvents. Sodium hypochlorite, sodium hydroxide, surfactants and enzymes are reported cell solubilizers which generally used with chemical or mechanical supports. However, the procedure is complex and may also degrade the PHA granules which limit their applications.

The application of mechanical methods such as high pressure homogenization for large scale disruption of microbial cells in the PHA recovery process has some weaknesses such as micronization of the cell debris. It avoids following solid–liquid separation by conventional methods such as centrifugation or filtration. 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. Moreover, it was found that addition of water to the system may cause partial depolymerization of PHA which it speeds up the separation process. Cell lysis can be occurred in distilled water during the cell slurry preparation. The reported investigations in the literature reveal important factors affecting microbial cell disruption are microbial strains, their growth and storage history, cell wall structure, distribution time and temperature. Up to date quantitative data to describe comparative assessment of PHA extraction from wild type and genetically modified microorganisms using non-halogenated system are scarce. Therefore, the objective of this research is to study the effect of process parameters as NaOH concentration, incubation time and temperature on the PHA recovery from recombinant *Cupriavidus necator* PHB_4 and *Comamonas* sp. EB172 with different initial PHA content by a simple and reliable method without using any halogenated solvents. ■

RESEARCH OBJECTIVES

1. To study the effect of NaOH treatment on PHA recovery from *Comamonas* sp. EB172 & recombinant Ralstonia sp.
2. To study the effect of water treatment on PHA recovery from *Comamonas* sp. EB172 & recombinant Ralstonia sp.
3. To study the recovery and characterization of PHA from *Comamonas* sp. EB172 & recombinant Ralstonia sp. with different intracellular PHA content

Main supervisor :
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■ Final pellet before **LEFT** and after **RIGHT** recovery process

Controlled degradation of polyhydroxyalkanoates by high pressure steam hydrolysis for chemical recycling

Elmy Nahida binti Othman

POLYHYDROXYALKANOATE (PHA) has unique characteristics of thermoplastic, biodegradable and biocompatible biopolymer which can be produced intracellularly by microorganism and some plant species. This promising biopolymer has been researched since its discovery in 1920s, remarkably starts the green revolution of non-petrochemical aliphatic polyester that is producible via fermentation biosynthesis and has been commercialized as early as in 1962. Dawes (1986) in his monograph emphasized that this compound acts as a reserve material and intermolecular molecule that is highly reduced and able to exert negligible osmotic pressure.

The constituent of PHA, polyhydroxybutyrate acid (PHB) is biocompatible with human as it is also a built compound of blood, made this producible biopolymer able to contribute very significantly in the biomedical applications especially in tissue engineering (Chen & Wu, 2005). It is a good alternative to plastic petroleum which is non-biodegradable and currently depleting. This biodegradable carbon reserve plays important role in the environmental carbon storage. Single use of bioplastics does not support the sustainability of the carbon cycle, therefore chemical recycling of bioplastic is proposed. Chemical recycling is a process to depolymerize polymers to low-molecular-weight materials before repolymerizing it into new polymers. By chemical recycling, cascade utilization of polymers could be introduced before they are finally being released to the environment. Several methods have been used to depolymerize PHA, namely pyrolysis and hydrolysis. Pyrolysis and enzymatic hydrolysis of PHA have been extensively studied; however steam hydrolysis of PHA is yet to be studied. Degradation of PHA in this study, involved with the concept of the material conversion to molecules that built up the original material or lowering of its origin molecular weight (Ariffin *et al.*, 2010) and (Ebdon & Eastmond, 1995). This research is aimed at recovering low-molecular-weight polymers with crotonic acid (CA) and hydroxyalkanoic acid (HA) chain end from polyhydroxyalkanoates (PHA) by high pressure steam (HPS) hydrolysis. These low molecular weight polymers can be used as feedstock for polymerization. Degradation of PHA by HPS hydrolysis will be controlled by several parameters, namely; temperature, pressure and retention time. Two methods of HPS hydrolysis (submerged and solid conditions) will be employed for the degradation of PHA. The experiments will be conducted using autoclave as the reactor. The hydrolysis products will be characterized and the effects of the parameters toward the target product formation will be investigated in details. The analysis of molecular weight will be carried out using gel permeation chromatography (GPC) and the degradation is projected theoretically to follow auto-catalytic hydrolysis mechanism with the identification of critical point, rate constant and activation energy based on relative molecular weight of polystyrene as the standard. The product compounds will be further characterized by 1H and 13C NMR, SEM and DSC. Mass balance for the PHA hydrolysis will also be studied. At the end of this study, it is expected that the degradation mechanisms for PHA hydrolysis can be proposed, with the selective formation of targeted products for chemical recycling. ■

RESEARCH OBJECTIVES

1. To identify the potential of PHA chemical recycling by high pressure steam hydrolysis and to characterize the hydrolysis products.
2. To investigate the effects of high pressure steam hydrolysis parameters on the characteristics of the hydrolysis products.
3. To identify the degradation behavior of PHA steam hydrolysis and to propose the mechanisms involved in steam hydrolysis of PHA.

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BIOFERTILIZER
RESEARCH GROUP

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

RESEARCH FOCUS

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

- Appropriate technology for accelerated composting treatment of oil palm biomass, municipal and organic waste.
- Application of oil palm biomass for increased methane production and POME treatment towards zero discharge solution
- Molecular screening of cellulose and xylanase enzyme from oil palm empty fruit bunch compost
- Zero discharge in palm oil industry
- Composting of oil palm mesocarp fiber and POME anaerobic sludge
- Composting of oil Palm Frond and POME anaerobic sludge
- Composting of continuous sterilizing oil palm empty fruit bunch and raw POME
- Appropriate treatment for palm oil mill final discharged wastewater as recycled water in the mill to replace fresh river water

PRINCIPAL RESEARCHERS

LEFT ASSOC. PROF. DR UMI KALSOM MD SHAH
RIGHT DR NOR'AINI ABDUL RAHMAN

BIG PICTURE BIOFERTILIZER

BIOFERTILIZER

BIOCOMPOST

LARGE SCALE

Dr Azhari Samsu Baharuddin

Mohd Kamahl Kamarudin

SMALL SCALE

Lim Siong Hock

Mohd Najib Ahmad

BIOCHAR

Ahmad Amiruddin Mohd Ali

Mohd Ridzuan Othman

PRODUCTION OF ENZYMES

Mohd Huzairi Mohd Zainudin

Nor Hashimah Abdul Rahman

ECONOMIC ASPECT : ZERO DISCHARGE

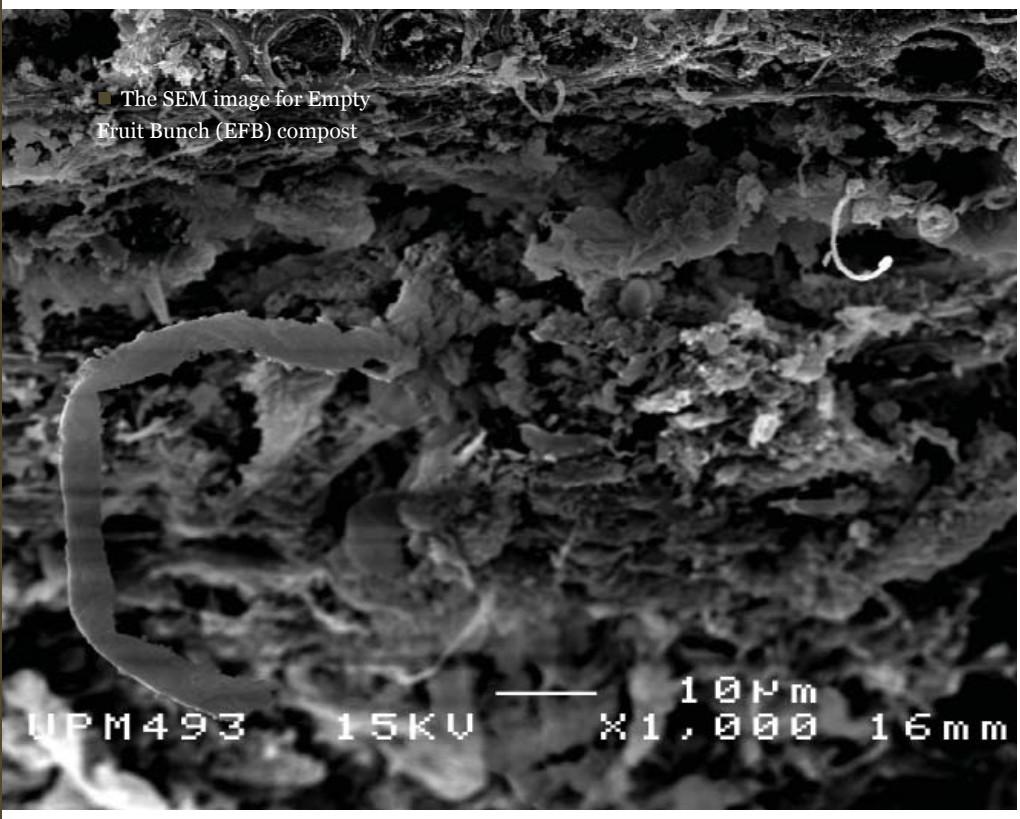
Yoshizaki Tatsuya

Appropriate technology for accelerated composting treatment of oil palm biomass, municipal and organic waste

Dr Azhari Samsu Baharuddin

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 (OPEFB), mesocarp fiber, Oil palm frond (OPF), Palm oil mill effluent (POME) and POME sludge. Currently the solid wastes are 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 biotechnology technique. The composting process utilizes activated POME anaerobic sludge for nitrogen sources 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 biocompost in term of nutrients value and beneficial microbes. Based on EFB biocompost, 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. ■



■ The SEM image for Empty Fruit Bunch (EFB) compost

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 mil 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

2010 PUBLICATION

Azhari Samsu Baharuddin, Mohamad Nafis Ab Razak, Lim Siong Hock, Mohd Najib Ahmad, Suraini Abd-Aziz, Nor'Aini Abdul Rahman, Umi Kalsom Md Shah, Mohd Ali Hassan, Kenji Sakai and Yoshihito Shirai. (2010). Isolation and Characterization of Thermophilic Cellulase-Producing Bacteria from Empty Fruit Bunches-Palm Oil Mill Effluent Compost. *American Journal of Applied Sciences*, 7(1), 56-62.
(*See abstract at page 48*)

Azhari Samsu Baharuddin, Lim Siong Hock, Mohd Zulkhairi Md Yusof, Nor'Aini Abdul Rahman, Umi Kalsom Md Shah, Mohd Ali Hassan, Minato Wakisaka, Kenji Sakai and Yoshihito Shirai. (2010). Effects of palm oil mill effluent (POME) anaerobic sludge from 500 m3 of closed anaerobic methane digested tank on pressed-shredded empty fruit bunch (EFB) composting process. *African Journal of Biotechnology*, 9(16), 2427-2436.
(*See abstract at page 42*)

Main supervisor :
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Application of oil palm biomass for increased methane production and biogas treatment

Ahmad Amiruddin bin Mohd Ali

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

The first part of the study focuses on methods for improving methane production and yield in both batch and continuous anaerobic fermentation systems of POME through utilization of added oil palm biomass. Denaturing gradient gel electrophoresis (DGGE) and Fluorescent *In-Situ* Hybridization (FISH) will be used in tandem to study, visualize and quantify the microbes involved in both types of fermentation systems. The second part of the study focuses on the carbonization of EFB using relatively low cost technology. The end-product is biochar will then be converted to higher grade activated carbon by utilizing excess steam generated from the mill processes. Cheap EFB biochar or activated carbon holds great promise for the industry and environment. It can be used for several applications such as biogas scrubbing or final discharge POME treatment.

This study will involve the former type of application whereby EFB activated carbon will be used to treat biogas generated from the POME anaerobic digestion process. Normal biogas scrubbing systems will be studied in parallel for comparison with the EFB activated carbon system. Purified methane produced could be converted into electricity via gas engines and used in the mill operations. Spent EFB biochar or activated carbon wasted from the biogas scrubbing system will then be analyzed for application as a soil-enrichment agent in the oil palm plantations. This approach could ultimately reduce if not eliminating altogether the need for costly chemical fertilizers. In addition to these potential applications, EFB biochar could also serve as a fuel alternative to replace fossil based fuels and other oil palm biomass resources used in the mill operations. Freed biomass and biochar both possess great commercial value, which could generate significant revenue for the mill and industry. ■

RESEARCH OBJECTIVES

1. To improve methane production and yield from palm oil mill effluent (POME) anaerobic batch and continuous fermentation systems by utilizing added oil palm biomass.
2. To evaluate performance of biogas scrubbing system using mill treated river water and biochar produced from low-cost technology.
3. To produce biochar and activated carbon from oil palm empty fruit bunch (EFB) using low-cost technology and excess steam.

Main supervisor :
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■ Biogas Scrubbing System

■ 50-L Bioreactor Methane Fermentation System

Isolation and characterization of cellulase and xylanase from oil palm empty fruit bunch compost

Mohd Huzairi b. Mohd Zainudin

MALAYSIA HAS become the largest oil palm producer with the production of about 18 million tonnes per year and about 47% of world's supply. Besides producing oil, it has generates abundant of waste such as Palm oil mill effluent (POME), Empty fruit bunch (EFB), Mesocarp fiber and Palm kernel shell. EFB is one the largest waste produce in the mill. Previously, EFB has been dumped for soil mulching in the plantation area. One way to create value added product from these waste are through the compositing using EFB with anaerobic sludge POME.

EFB compost is manageable product which can be use as soil amendment and organic fertilizer. In composting, lignocellulose material breaks down due to the existence of aerobic thermophilic bacteria. The microbial population and microbes capable of producing cellulase and xylanase was investigated. In this study, DNA was extracted and purified from EFB compost by DNA soil extraction kit. The isolated DNA was used for determining the microbial population through microbiota analysis by using culture-independent of 16s rRNA gene amplified directly from the compost. 1500-bp 16S rRNA PCR products were cloned and sequenced. Subsequently, with regard to the results obtained from microbiota analysis, screening and isolation of bacteria producing cellulase and xylanase was done. Several microbes that are able to express cellulase and xylanase have been isolated. In order to identify potential cellulase and xylanase enzyme from nonculturable microbes, metagenomic of EFB compost was done. Metagenome involves the extraction of metagenomic DNA from EFB compost. The DNA is subsequently cloned into vector (fosmid) to construct the metagenomic library. From the metagenomic library, the genes encoding cellulase and xylanase were screened and identified by polymerase chain reaction or activity screening.

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

RESEARCH OBJECTIVES

1. To investigate the microbial community during the oil palm empty fruit bunch compost process
2. To isolate and characterize the expressed cellulase and xylanase from isolated microbes
3. To screen and identify cellulase and xylanase gene through metagenomic approach

Main supervisor :
PROF. DR MOHD ALI HASSAN
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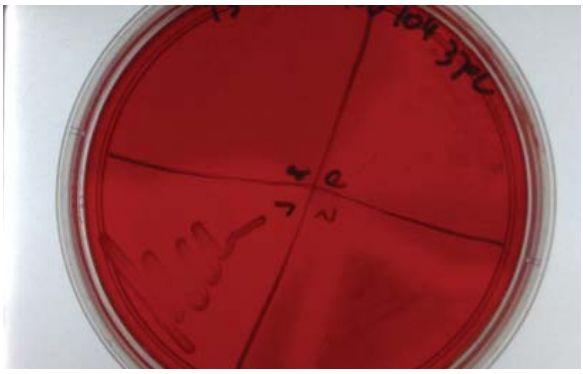
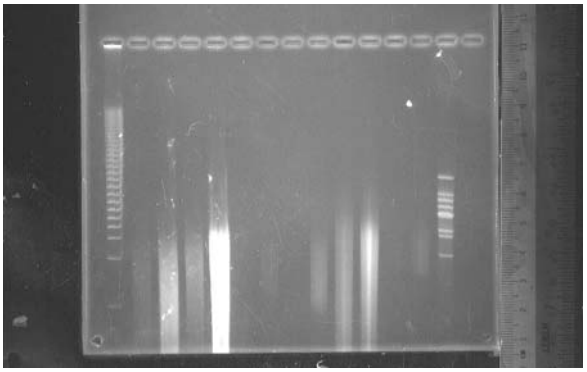
■ Screening of xylanase producing bacteria



■ Screening of cellulase producing Bacteria



■ Pulse Field Gel Electrophoresis of EFB compost

Zero discharge on palm oil industry

Tatsuya Yoshizaki

FOR LONG TIME, palm oil industry has described as a high environment burden industry. By producing crude palm oil (CPO) from Fresh Fruit Bunch (FFB), a big amount of Palm Oil Mill Effluent (POME) which contains high COD/BOD and exhausting methane that is 21 times as effective to Greenhouse effect as CO_2 is discharged. Under status quo, producing CPO and keeping environment in mill are the relationship of Trade-Off. That arranges and optimizes several technologies as a whole mill in order to achieve the balance between economic and keeping environment. The idea for zero discharge is to introduce 2 ways. First one is the POME treatment and biogas capture system. In this POME treatment process, methane is captured, and that is used for electric generating. And the electricity is expected to be used for mill's operation. But this technology is economically-unattractive. Therefore, second way, bio charcoal is introduced. Bio charcoal is made from excess biomass like Empty Fruit Bunch (EFB), shell and fiber. And the biocharcoal is expected not only to make waste water clean-up but also to sell as fertilizer in markets. These two ways should be integrated and support subsidiary each other.

This study is done in a region as well under Bornean Biodiversity and Ecosystems Conservation (BBEC) Program with Japan International Company Agency (JICA) in Sandakan. This project's objective is to improve wet land's pollution at this site and achieve the water quality with Ramsar Conservation. The reason why the wet land is polluted is because of mill's POME discharge located at upstream. Then, this study will contribute to persuade mill's owner to participate the project through making their mill's economy efficiency improve. As a result, that will contribute to environment protection as well. ■

RESEARCH OBJECTIVES

1. To improve methane production and yield from palm oil mill effluent (POME) anaerobic batch and continuous fermentation systems by utilizing added oil palm biomass.
2. To evaluate performance of biogas scrubbing system using mill treated river water and biochar produced from low-cost technology.
3. To produce biochar and activated carbon from oil palm empty fruit bunch (EFB) using low-cost technology and excess steam.

Main supervisor :
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■ **TOP** Biogas capture

■ **RIGHT** Bio charcoal

Composting of oil palm mesocarp fiber by enhancement of palm oil mill effluent anaerobic sludge.

Lim Siong Hock

OIL PALM MESOCARP FIBER (OPMF) is one of the most abundant lignocellulosic wastes produced throughout the year in palm oil industry. The current utilization of OPMF however has created huge environmental pollution to the environment. Due to this reasons, this research was done to utilize OPMF in a better way as composting substrate for biocompost production. POME anaerobic sludge also been used as nitrogen source and microbial seeding for composting process. The addition of POME anaerobic sludge gradually led to prolonged thermophilic condition (50 - 68°C) for about 40 days. This eventually led to feasible composting of OPMF within 50 days with final C/N ratio of 12.6 and considerable amount of nutrients in the final compost. For succession and phylogenetic profile of microbial communities during composting process, Polymerase Chain Reaction–Denaturant Gel Gradient Electrophoresis (PCR-DGGE) analysis has been done. It has been observed that strong hydrolytic microbes have been dominance in thermophillic phase of composting process.

Moreover, beneficial microbes for agricultural purpose also have been observed in the later phase. For structural changes, Scanning Electron Microscopic (SEM) exhibited the structural view of OPMF throughout composting process. The composting process could be accelerated with advanced processing system and the research outcome could improve the degradation as well as nutrient content in compost product. Throughout this study, the production of biocompost from co-composting of OPMF and POME anaerobic sludge in large scale could fully utilize wastes material in the mill as substrates in environmental friendly manner. ■

RESEARCH OBJECTIVES

1. To investigate the feasibility of biocompost production from co-composting of oil palm mesocarp fiber with POME anaerobic sludge in pilot scale.
2. To determine the microbial succession and structural degradation throughout composting process.

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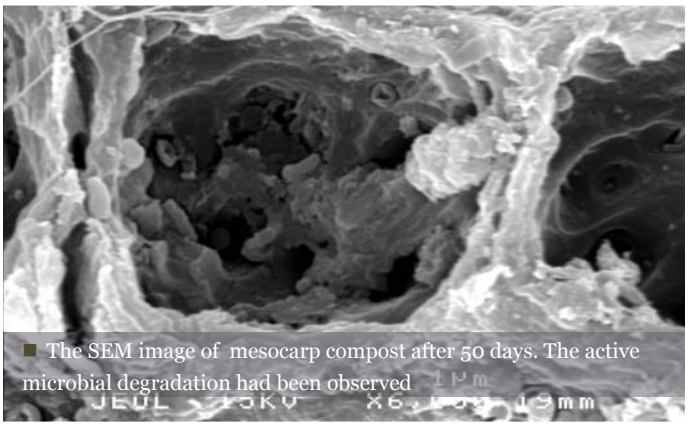
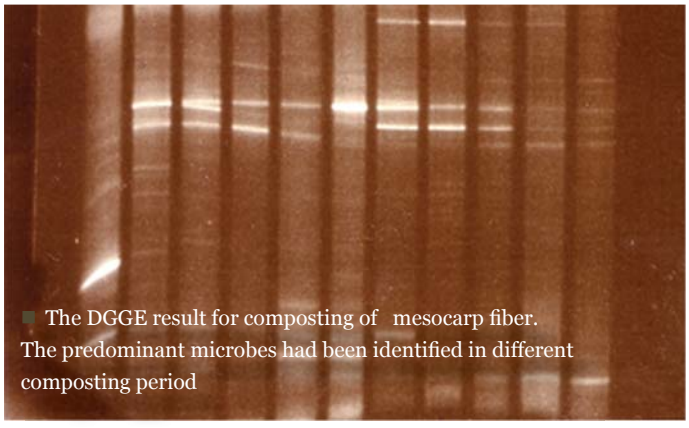


■ The SEM image of mesocarp compost after 50 days. The active microbial degradation had been observed

■ OPMF Biocompost after 50 days of degradation

■ OPMF biocompost after grinding and sieving



■ The DGGE result for composting of mesocarp fiber. The predominant microbes had been identified in different composting period

Appropriate treatment of palm oil mill final discharge wastewater as recycled water for the mill to achieve zero discharge

Mohd Ridzuan bin Othman

IN PALM OIL industry, huge amount of water have been utilized for palm oil sterilization and extraction process. The processing system has been applying widely in Malaysia for year. It has been estimated that around one ton of fresh water was needed for processing every ton of fresh fruit bunch (FFB). As a return, huge amount of wastewater has been generated, treated and discharged to the river every day. Current treatment system applying in oil palm industry is using river water to use for mill. In present study, the effect of coagulant and activated carbon application as appropriate treatment of palm oil mill final discharge wastewater have been evaluated in order to recycled water for the mill to replace fresh river water. Current chemical treatment used at the mill will be used to treat final discharge to achieve zero discharge. Activated carbon is used as absorbent material due to its large number of cavernous pores that provide a large surface area relative to the size of the actual carbon particle and its visible exterior surface. A Jar Test Method is used to stimulate the coagulation and flocculation processes that encourage the removal of COD, color, suspended colloids and organic matter in final discharge wastewater which can lead to turbidity, odor and taste problems. In this research Jar Test is used to determine the optimum operating conditions for final discharge wastewater by optimizing value of pH, dosage of coagulant and activated carbon used and mixing time to improve the performance and/or capacity of existing treatment systems and to reduce capital expenditure on new treatment systems. ■

RESEARCH OBJECTIVES

1. To study an effective dosing of organic and inorganic coagulant usage for the treatment of palm oil mill final discharge wastewater to achieve zero discharge in the mill
2. To develop low cost environmental friendly methods by using organic coagulant to treat palm oil mill final discharge wastewater

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■ **FAR LEFT** Activated carbon from palm oil shell

■ **LEFT** Zeolite for final discharge wastewater treatment

■ **BOTTOM** Final discharge treatment using alum with jar test equipment

Isolation and characterization of ligninolytic bacteria strains from oil palm plantation soils

Nor Hashimah binti Abdul Rahman

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

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

RESEARCH OBJECTIVES

1. To determine bacterial populations from oil palm plantation soils for ligninolytic bacteria strains by using microbiota analysis
2. To isolate and characterize potential ligninolytic bacteria strains for production of lignin-degrading enzymes based on microbiota analysis

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■ **FAR LEFT** DNA extraction from POME sludge

■ **BOTTOM LEFT** Palm Oil Mill Effluent (POME) anaerobic sludge

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

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

Composting of oil palm frond by enhancement of palm oil mill effluent anaerobic sludge

Mohd Najib Ahmad

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

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.

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■ Biocompost Pilot Plant at Taman Pertanian Universiti (TPU), UPM Serdang

Improvement in co-composting process of pressed-shredded empty fruit bunch and raw palm oil mill effluent from continuous sterilizer system

Mohd Kamahl Kamarudin

THE CONTINUOUS sterilizer system is a high performance system in palm oil extraction. In future trend, more and more mills in Malaysia will installing such fresh fruit bunch (FFB) processing system. However, The non-ponding system in continuous sterilization system has generated huge amount of empty fruit bunch (EFB) and raw POME that had create problem to the mills. Therefore, composting of continuous sterilizer EFB with the addition of raw POME was an option to solves wastes accumulation problems in the mills. Currently composting technology on empty fruit bunch (EFB) and raw POME still in the infrant stages. The decomposition of empty fruit bunch (EFB) in acidic condition may inhibit microbial decomposition rate. Therefore, further study regarding microbial decomposition on empty fruit bunch (EFB) was important for utilizating continuous sterilizing empty fruit bunch (EFB). In order to get better understanding on physicochemical in continuous sterilizing empty fruit bunch, detail study on chemical and structural properties has been conducted. The scanning electron microscopy (SEM) and transmission electron microscopy (TEM) give a full picture on structural disruption under sterilization. For composting process, microbial seeding method play an important role in effective composting since raw POME lack of microbes. Hence, effectively utilizing microbial seeding was a key for successful composting process. This study was targeting to deliver a good composting process for raw POME and empty fruit bunch (EFB) for industrial application. ■

RESEARCH OBJECTIVES

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

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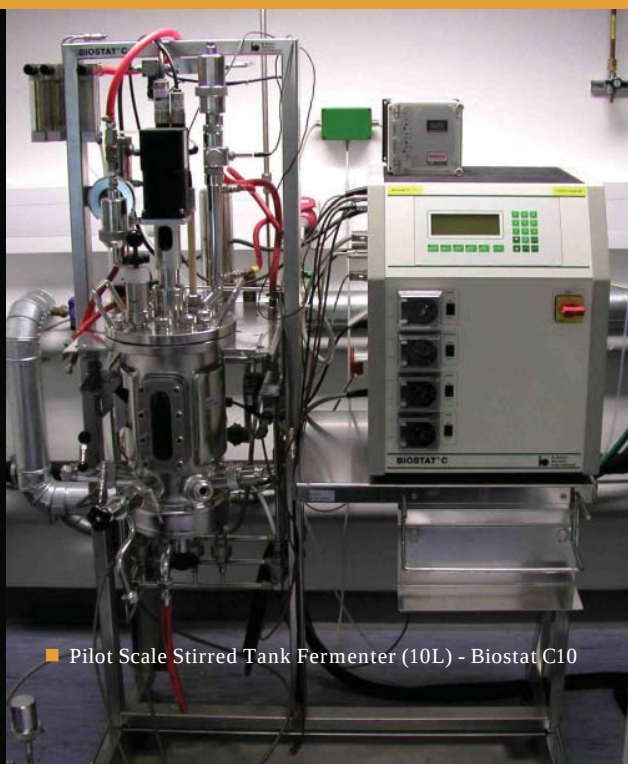
BIOPRODUCT
RESEARCH GROUP

This research group consists of eight different research areas. Our research focus on producing several types of bioproducts using substrates which are shrimp aquaculture waste, sago starch, brown rice, chilli and OPEFB by locally isolated microorganisms. Moreover; downstream processes are also concerned for the bioproducts produced. All projects in this group apply process that utilizes same biological pathways via fermentation of substrates to obtain beneficial enzymes such as chitinase and CGTase; antifungal compound to inhibit anthracnose in chilli; bioadsorption of cyclodextrin; and produce bioflavour compound such as biovanillin and develop system to separate biovanillin.

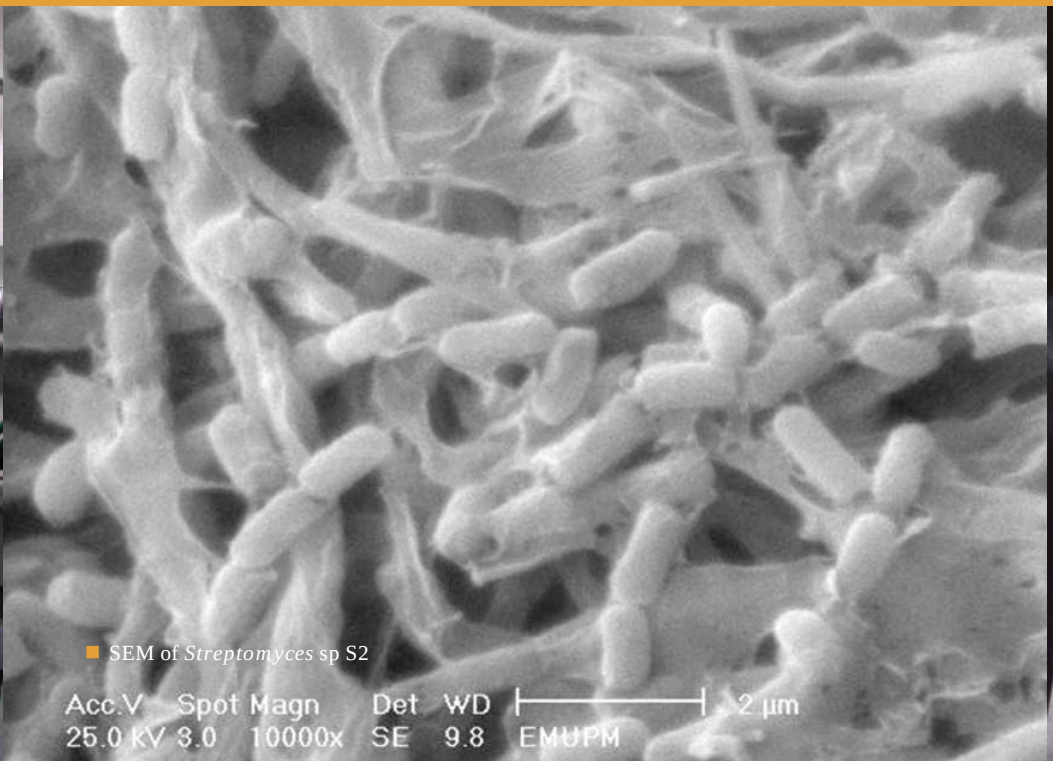
PRINCIPAL RESEARCHERS

LEFT DR HELMI WASOH @ MOHAMAD ISA
RIGHT DR.-ING. MOHD NORIZNAN MOKHTAR

■ Diversity of actinomycetes isolated from Langkawi Island



■ Pilot Scale Stirred Tank Fermenter (10L) - Biostat C10



■ SEM of *Streptomyces* sp S2

■ Force feeding chicken

BIG PICTURE BIOPRODUCT

Nurul Asyifah Mustapha

Enzymatic Improvement of Brown Rice

Anthracnose Disease Biocontrol using Actinomycetes' Antifungal Compounds

Jeffrey Lim Seng Heng

POULTRY FEED

BIOPESTICIDE

BIOPRODUCT

BIOVANILLIN from OPEFB

ENZYMES

Fairouz Jahaan Mohd Aanifah

Microbial Conversion

Chitinase from Shrimp Waste

Sobri Mohd Akhir

Rozaimi Abu Samah

Recovery System Development

CGTase

Molecular cloning approaches

Isolation and Production

Adsorption Of Product

Norhayati Ramli

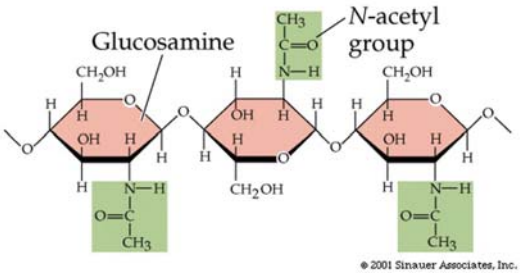
Iman Abdull Halim

Syahinaz Shahrazi

Scaling-up production of chitinase enzyme for shrimp aquaculture waste processing

Sobri Mohd Akhir

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.



■ **MOLECUR STRUCTURE** Chitin

The optimisation of chitinase and N-acetyl glucosamine production by *B. licheniformis* TH-1 was conducted in a 2 L stirred tank fermenter for the maximum yields. Experimental results show the effects of various fermentation conditions such as agitation speed, aeration rate, initial pH, temperature and inoculum size on the cell growth and chitinase production. The maximum chitinase and NAG production were obtained at 1 vvm aeration rate, agitation speed 200 rpm, initial pH of 7.0, temperature 45°C and inoculum size of 10% (v/v). Under this optimised fermentation condition, *B. licheniformis* TH1 produced a total chitinase activity of 2.241 U/mL and NAG of 61.67 mg/L, which is almost 2-fold increase in chitinase activity compared to shake flask fermentation. The increasing of chitinase yield resulted an increment of NAG production and the productivity. The effect of agitation and aeration rates on chitinase production had revealed the intricacies involved in the operation of the chitinase fermentation process. At lower agitation rate, oxygen mass transfer was limited. Whereas, at higher agitation rate, the microbial might suffer strong shear force and cause it to fragment into small piece. 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. This is necessary as it would enable one to minimize production cost and optimize the cost-effectiveness for the overall production process. ■

■ Stirred Tank Fermenter (2L) - Biostat B2

RESEARCH OBJECTIVES

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

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■ Pilot Scale Stirred Tank Fermenter (50L) - Biostat D50

Antifungal compounds produced by a potential indigenous actinomycetes strain for biocontrol of anthracnose in chilli

Jeffrey Lim Seng Heng

ANTHRACNOSE disease on chilli fruits had been known to be a serious problem faced by most of the chilli planters other than Chilli Mosaic Virus (CMV). Once the fruit had been infected by anthracnose the fruit is deem useless and need to be sorted out to reduce further infection on other fruits. Typical symptoms of anthracnose attack on chilli fruits are sunken necrotic tissues, with concentric rings of acervuli. The most widely spread *Colletotrichum* spp. found in the red chilli fruit are the *Colletotrichum capsici* and *Colletotrichum gloeosporioides*.

Actinomycetes a Gram-positive bacteria known for its slow growing organism characteristic with the incubation period of about 7-14 days. The GC content of actinomycetes is between 63-78% and is considered to be the highest GC content for all known bacteria. Actinomycetes produce colonies with powdery or chalky appearance on the media plates which distinguish them from other bacteria during isolation. The earthly smell of produced during the growth of also help to identify the present of these bacteria especially those from the genus of streptomycetes.

Actinomycetes are widely distributed in nature and they thrive in soil where they play an important role in the bioremediation, mineralization and decomposition of organic matter with the production of numerous extracellular enzymes. Actinomycetes act as an important agent in the degradation of organic materials in soil and contribute to the formation of stable humus. As the primary decomposer of tough plant materials (bark, newspapers and woody stems), actinomycetes are effective at attacking the tough raw plant tissues and softening them up for others microorganisms. Actinomycetes bacteria have been known as excellent sources of useful natural products of high commercial value, such as antimicrobial compounds and valuable extra cellular enzymes.

The shift of the trend for actinomycetes from being concentrated on the pharmaceutical industries to the agricultural industries have seen an increased in the isolation of actinomycetes which have the potential to control diseases in selected inmportant food crop around the world. Actinomycetes have given significant results when tested for its activity against fungi such as Alternaria mali, *Colletotrichum gloeosporioides*, Fusarium oxysporum f.sp. cucumerinum, and Rhizoctonia solani were predominant in pepper-field soils. Actinomycetes also have been tested on field for its antagonistic effects on *Colletotrichum* musae on banana fruits. A few of the antifungal compounds which had been produced by actinomycetes are Valinomycin, Bafilomycin B1 and C1 and Gopalamicin. The ability of the actinomycetes to produce useful antifumgal compounds.

In this study, we will be isolating our indigenous actinomycetes from selected local soil area. The isolated actinomycetes would then be tested for the ability to produce antifungal activity against two of the selected *Colletotrichum* sp. (*Colletotrichum capsici* and *Colletotrichum gloeosporioides*) that have been known to cause anthracnose in red chilli fruit. The main objective of this study is to isolate and identify the antifungal compound(s) from the selected actinomycetes and further characterized the compound(s). It is hope that from this study, we would be able to obtain a high activity compound that could be used to control the attack of anhracnose on the chilli. ■

RESEARCH OBJECTIVES

1. To isolate, screen and characterize actinomycetes with antifungal ability.
2. To characterize and identify the potential antifungal compounds produce by actinomycetes.
3. To optimize culture condition for the production of antifungal compounds.

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■ Chilli attacked with antracnose disease

■ Actinomycetes showing antagonistic activity towards *Colletotrichum gloeosporioides*

Over production of cyclodextrin glycosyltransferase through molecular cloning approaches

Norhayati Ramli

CYCLODEXTRIN glycosyltransferase (EC 2.4.1.19) represent one of the most important groups of microbial amylolytic enzymes. This enzyme is a member of alpha-amylase family or glycosidase hydrolase family 13, which forms circular α-(1, 4)-linked oligosaccharide substrates via a covalent intermediate. The non-reducing end of this intermediate is subsequently used as the acceptor that cleaves the covalent enzyme-substrate bond, and a cyclodextrin (CD) is released. CGTase can also use water or the non-reducing end of a free oligosaccharide as an acceptor, which results in hydrolysis or a disproportionation reaction, respectively. Disproportionation can be regarded as the default reaction, and it also catalysed by several other members of the α-amylase family. Major producers of CGTase belong to the *Bacillus* sp. of the genus *Bacillus*. Alkalophilic Bacilli have received the major attention for industrial production of CGTase because of their high activity over a wide range of pH and temperatures. *Bacillus* species constitute the major contributor of industrially important enzymes. The wide range of enzyme application, e.g. in the detergent, pulp and paper industry prompted the isolation of strains from a variety of alkaline environments as a source of enzymes with suitable activities. The cyclization reaction of CGTase from starch and other related α-1,4-glucans are used commercially in producing CD. CD is an important polysaccharide due to its unique hydrophobic interior cavity and hydrophilic surface. CD can encapsulate other hydrophobic organic substances, aiding solubilisation in water. This property is useful in food, pharmaceutical, cosmetic and agricultural applications. The most common available CDs to be synthesized are composed of 6, 7 and 8 glucose units named α-, β- and γ- CD, respectively.

The carbon source is the most important factor determining the rate of the CGTase synthesis and starch is found to be the most common carbon source in production of CGTase enzyme. Various types of starch can be used as substrate for CGTase production, including potato, corn and rice starch. In this study, sago starch will be used as an alternative substrate due to its low production cost and high yields when compared to other starches. The cloning and over expression of CGTase have been carried out by many researches, which hoping could produce the recombinant CGTase-producing bacteria that will enhance enzyme activity and produce less contaminating protein compared to wild type. In this study, the best CGTase producing bacteria; *Bacillus* sp. NR5 UPM has successfully been isolated which produced ß-CD as a main product, followed by γ-CD and α-CD. By using this isolate, the CGTase gene will be isolated using DNA walking strategy and will express into pUC19 expression vector. The over expression of the isolated CGTase gene perhaps will produce the recombinant with higher production of CGTase activity, thus can be used commercially for production of cyclodextrins and maltooligosaccharides. ■

RESEARCH OBJECTIVES

1. To screen, isolate and characterize CGTase producing bacteria, specifically for α-CGTase, ß-CGTase and γ-CGTase production.
2. To isolate CGTase gene by using primer screening approach for construction of CGTase expression system with *Escherichia coli*.
3. To evaluate the production of enzyme by analysing the CGTase activity of the recombinant CGTase produced.

2010 PUBLICATION

Norhayati Ramli, Suraini Abd Aziz, Mohd Ali Hassan, Noorjahan Alitheen, Kamarulzaman Kamaruddin (2010) Potential cyclodextrin glycosyltransferase producer from locally isolated bacteria. *African Journal of Biotechnology*. Vol 9 (43):7317-7321.
(*See abstract at page 43*)

Main supervisor :
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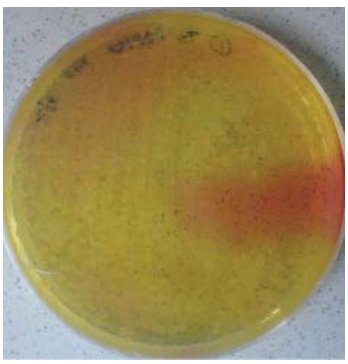
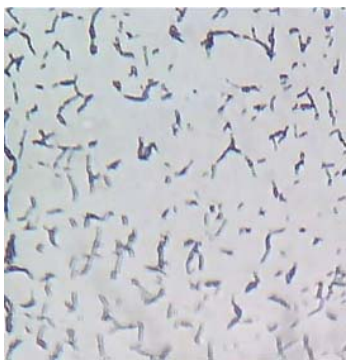
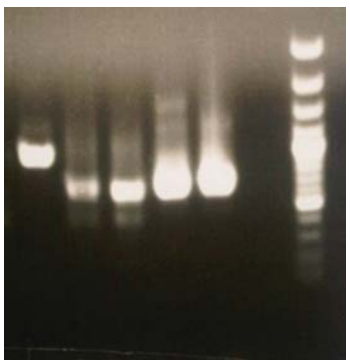
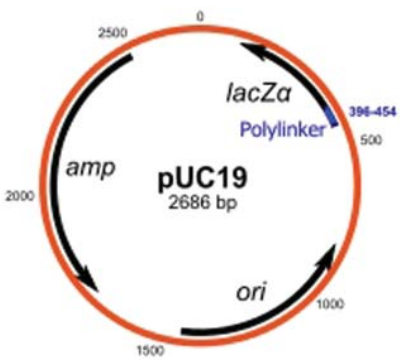
■ **FROM LEFT** Screening of CGTase-producing bacteria with Horikoshi + phenolphthalein; Isolated bacteria (*Bacillus* sp. NR5) UPM; DNA walking results for known sequences of CGTase gene; pUC19 expression system [no '3']





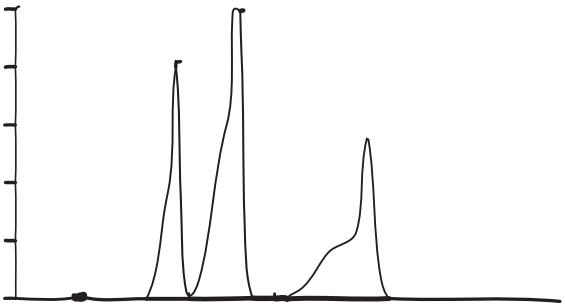




Biovanillin production from alkaline hydrolysate of oil palm empty fruit bunch

Fairouz Jahaan Mohd Aanifah

AGRICULTURAL wastes contain ferulic acid that can be used as a biological precursor to be fermented into vanillin, the active ingredient of the vanilla flavour. Many types of agrowastes; like corn cobs, wheat bran, rice bran, maize bran have been studied for biovanillin production. This research is based on the bioconversion of Malaysia's largest biomass being produced continuously, oil palm empty fruit bunch (OPEFB) to biovanillin.



■ Standard peak of vanillic acid, vanillin and ferulic acid respectively by HPLC

It is known that ferulic acid normally attached to the lignin and hemicellulose of the agricultural residues. Therefore; in this case, as OPEFB is highly lignified, pretreatment to remove lignin is very important, by which the free form of ferulic acid is released. Alkaline hydrolysis was chosen to obtain the ferulic acid in the hydrolysate form. This is because alkaline treatment is well known for its delignification efficiency and breaking up ester bonds attaching ferulic acid to lignins and hemicelluloses.

Basically, this research will be focusing on converting ferulic acid to vanillic acid, the intermediate in this case. *Aspergillus niger* turns ferulic acid into vanillic acid. Later; vanillic acid is fermented into biovanillin by *Phanerochaete chrysosporium*, where resin is used to trap the vanillin and avoid further conversion to alcohols such as vanillyl alcohol. The determination of the these phenolic compounds will be by employing high performance liquid chromatography (HPLC) analysis.

Another vital scope of this study is to optimize both the fermentations' conditions using response surface methodology (RSM). The physical aspects to be optimized comprises of the following parameters; temperature, pH, shaking speed of the incubator, initial inoculum concentration and substrate loading. An optimized condition of shake flask fermentation to produce biovanillin should be achieved at the end of this study. ■

RESEARCH OBJECTIVES

1. To obtain biovanillin from alkaline hydrolysate of oil palm empty fruit bunch containing ferulic acid.
2. To produce biovanillin by optimising ferulic acid bioconversion to biovanillin via two-stage fungal fermentation using Response Surface Methodolgy (RSM).

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■ **CLOCKWISE FROM LEFT**
Aspergillus niger that converts ferulic acid into vanillic acid;
Phanerochaete chrysosporium turns vanillic acid into biovanillin;
Overnight extraction of sample using ethyl acetate;
Sample concentrated using rotary evaporator;
OPEB treated with different concentration of NaOH

Enzymatic improvement of nutritional value of brown rice for poultry

Nurul Asyifah Mustapha

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

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

The addition of enzymes has been widely used to remove the non-starch polysaccharides and to improve the nutritional value of brown rice since poultry cannot produce endogenous enzymes to degrade these NSP. The enzymes addition can supplement or help the endogenous digestive activities of poultry, remove anti-nutritional factors and also render certain nutrients more readily available for absorption and enhance the energy value. 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 optimization using Response Surface Methodology (RSM) for enzymatic hydrolysis of brown rice using commercial enzymes will be done in laboratory scale to produce hydrolyzed brown rice and will be fed to chicken to determine the energy value. ■

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.

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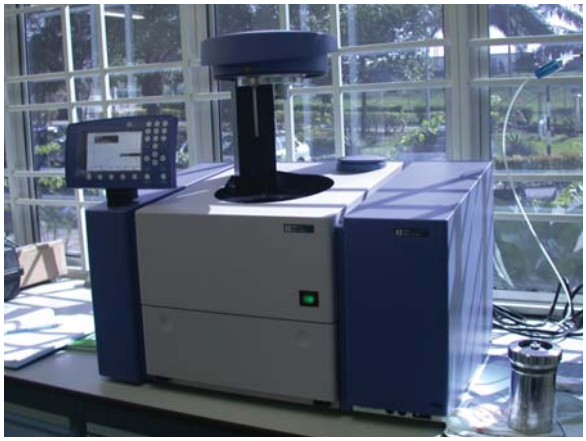

■ **LEFT** Bomb calorimeter for energy determination

■ ANKOM machine for fiber determination

■ Chicken for True Metabolisable Energy (TME) determination

■ Collected faeces for TME

■ Process of collecting faeces

■ Local variety of brown rice MR257

■ Reducing sugar analysis

■ Enzymatic hydrolysis

α-Cyclodextrin glycosyltransferase production from raw sago starch using isolated alkalophilic bacteria

Iman Abdull Halim

SAGO STARCH which is the main carbohydrate source in Malaysia has become an interesting alternative substrate for cyclodextrin glycosyltransferase production because of its low cost of production and high yield when compared to other kinds of starch. Raw starch has a compact crystalline structure, not easily attacked by starch-degrading enzymes. The molecules of sago starch granule are densely packed in a polycrystalline state with inter and intramolecular bonds and are hense insoluble in cold water and often resistant to chemicals and enzymes. Most known cyclodextrin glycosyltrasferase (CGTase) can only convert gelatinized (or physically modified) starches to cyclodextrins with the conventional starch processing insudtry which heated the starch slurry by gelatinization at up to a high temperature of 100°C which increases the energy consumption.

In recent years, a worldwide interest has been focused on the raw starch digesting CGTases which would be of value to simplify the process of starch bioconversion. If an efficient, raw-starch degrading CGTase is available, the energy-intensive gelatinization or physical treatment steps can be avoided, with consequent reduction in cost of production. CD can be synthesized enzymatically by CGTase also known as 1,4-α-D-glucopyranosyl transferase or EC 2.4.1.19. CGTase is produced by various microorganisms, mostly by members of genus *Bacillus*. However, most reported CGTases produce β-CD as main product. Enzymes producing primarily α- and γ-CD are relatively rare. Enzymes capable of predominantly producing a particular type of CD can decrease subsequent purification costs, hence can be valuable. Enzymes from *Klebsiella pneumonia* M 5 al, *Bacillus macerans* IAM 1234, *Klebsiella pneumonia* AS-22 and *Klebsiella oxytoca* 19-1 are the representatives of α-CD producers. Isolated CGTase producer is screened on the types of their CGTase activity, then, the effect of parameters such as concentration of sago starch, inoculum size, initial pH, temperature and agitation speed to production medium with and without pretreatment will be studied on the influence on the enzyme production by the α-CGTase producer. The optimization will be conducted using Response Surface Methodology, thus, produce higher yield of α-CGTase from the optimized production medium. ■

RESEARCH OBJECTIVES

1. To isolate α-, β-, γ-CGTase-producer
2. To optimize the raw sago starch. degrading CGTase production medium by Response Surface Methodology (RSM).

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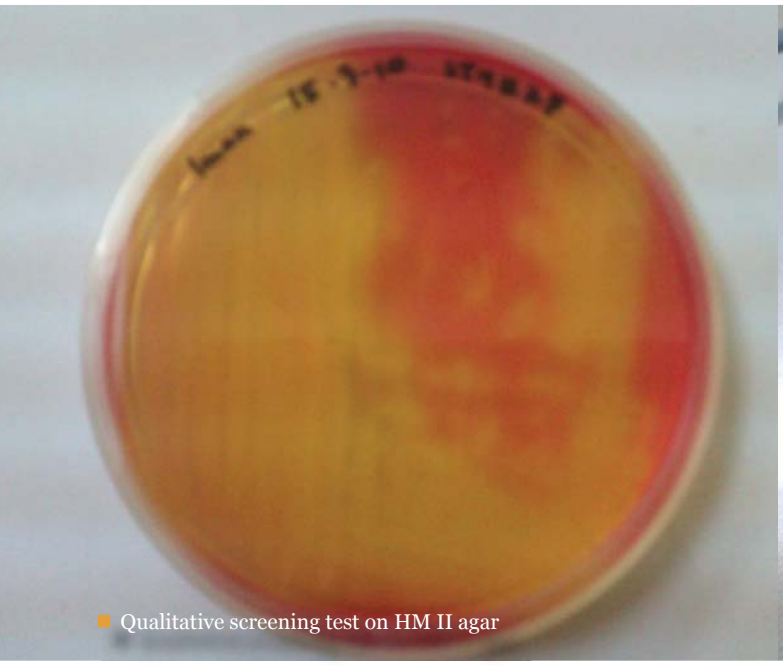
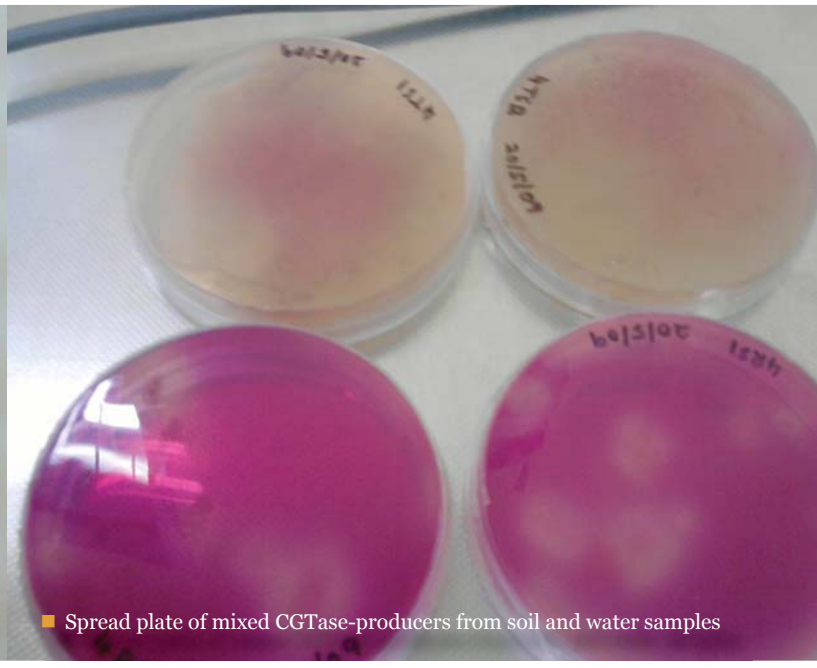


Qualitative screening test on HM II agar



Spread plate of mixed CGTase-producers from soil and water samples

Periodic adsorption-desorption of γ-Cyclodextrin

Syahinaz Shahrazi

THIS STUDY FOCUSES on the purification process of γ-cyclodextrin. This downstream process is the most crucial part in the production of cyclodextrin.

Cyclodextrins are a macrocyclic carbohydrates produced enzymatically from starch using CGTase enzyme with a hydrophilic external surface and hydrophobic interior. There are three different types of well known cyclodextrins according to the number of glucosyl residues known as; α-cyclodextrin (6 glucose units), β-cyclodextrin (7 glucose units) and γ-cyclodextrin (8 glucose units). Compared to α- and β-cyclodextrins, γ-cyclodextrin exhibits more favourable properties in terms of internal cavity size, water solubility and bioavailability, which give wider applications in many fields. However, to date, the most marketed cyclodextrin is β-cyclodextrin and to lesser extend α-cyclodextrin, while the market share of γ-cyclodextrin is considerably small because its low yield. Due to great market demand, the price of γ-cyclodextrin is the highest.

Many attempts have been made to improve the production yield of γ-cyclodextrin. In general, there are two ways to purified γ-cyclodextrin; either using organic solvent to form γ-cyclodextrin precipitate or a non-solvent method which applies physical process. However, organic solvent cannot be used when the product is intended for food or pharmaceutical as it frequently contains traces of the poisonous solvents. The non-solvent method is advantageous since the pure cyclodextrin obtained by the processes have no toxicity, so they can widely be used in the field of food or pharmaceutical. Currently, pure γ-cyclodextrin was achieved via complex and expensive chromatography method. To improve the production of γ-cyclodextrin, adsorption which is an inexpensive non-solvent process was choose to reduce the production cost. This will enable us to fully utilize the benefits especially in food and pharmaceutical area.

Several adsorbent has been identified and proposed by several researchers to have potential in separation γ-cyclodextrin from a mixture of CGTase product. Based on the previous proposed adsorbent, the isotherm between the selected adsorbent and cyclodextrin will be determined using static method. The computational mathematical modelling of the γ-cyclodextrin adsorption-desorption for a single column process have been develop to provide better understanding towards the performance of the process without the building the real adsorbent column. Certain important parameters related to the process are defined and will be used in the mathematical modelling.

gPROMs (general process modelling system), a multipurpose modelling, simulation, optimization and parameter estimation software was used to develop the mathematical modelling. The model is introduced in a specific language which is close to the natural mathematical language. The system interprets this model and links together all the variables and equations to powerful mathematical solvers. The successful single column adsorption-desorption mathematical modelling of γ-cyclodextrin will be applied to develop a periodic adsorption-desorption process to increase the product yield. ■

RESEARCH OBJECTIVES

1. To study adsorption behavior of α-CD, β-CD and γ-CD at different temperatures using selected adsorbents.
2. To develop the semi-continuous separation of γ-CD via periodic adsorption-desorption process using dynamic mathematical modelling and simulation.

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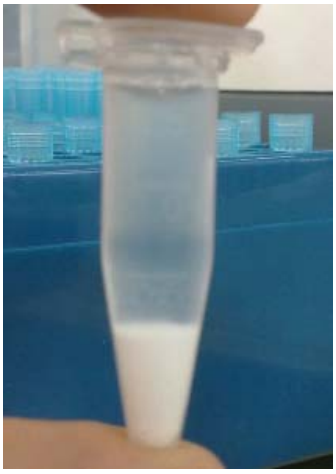
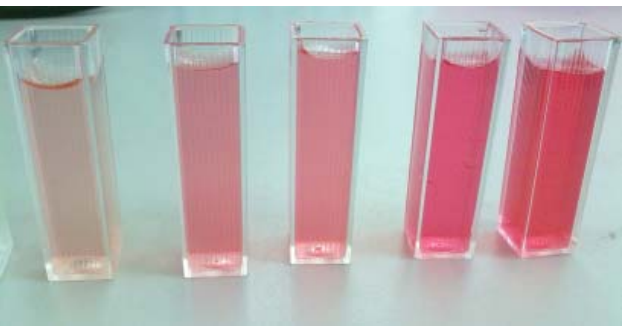
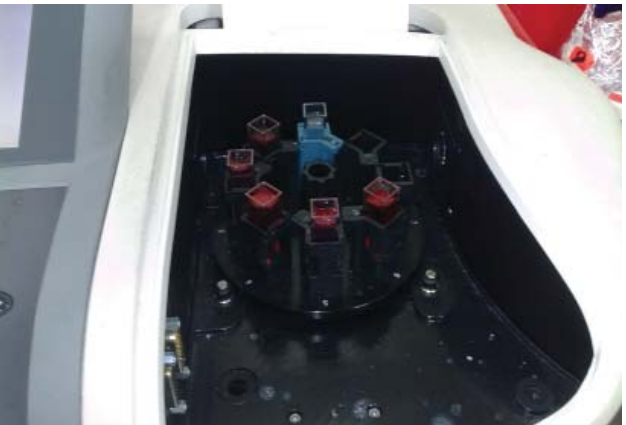


■ Batch method to screen the condition for the adsorption of Cyclodextrins on selected adsorbent.

■ **FIRST BOTTOM** α-Cyclodextrin at different concentration in methyl orange at lower pH. Higher concentration of α-cyclodextrin reduce the color of methyl orange.

■ **SECOND BOTTOM** Determination concentration of cyclodextrin using UV-vis spectrophotometer





Development of a recovery system for biovanillin

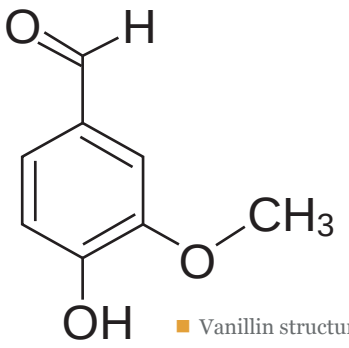
Rozaimi Abu Samah

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

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

This work is proposed to separate vanillin produced via enzymatic reaction, using one of the most abundant by-products in Malaysia, the oil palm empty fruit bunch (OPEFB). Initial study is carried out on the determination of enzymes system involves in the conversion of ferulic acid to vanillic acid by *Aspergillus niger* and the conversion of vanillic acid to vanillin by *Phanerochaete chrysosporium*. Several enzymes are predicted to work synergistically in both of the bioconversion such as arabinase, galactanase, polygalacturonase, xylanase, arabinofuranosidase, galactosidase, rhamnogalacturonase, carboxymethyl cellulose and feruloyl esterase. The production trend of the mentioned enzymes is observed during the bioconversion process.

Consequently, the 'key enzymes' in the cell crude supernatant of both fungi are subjected to shredded and ground OPEFB in order to investigate the ability of the bioconversion to vanillic acid and vanillin via enzymatic route. The shredded and ground OPEFB used is either untreated, heat treated or enzymatic treated with lignin peroxidase, with the aim of aiding the whole bioconversion process.



■ Vanillin structure

The work continues with the determination of the molecular size of the 'key enzymes' in order to prepare a membrane with specific molecular weight cut off. Detailed study on the membrane characteristic is performed. At the same time, an integration of the reaction part with the separation system is also performed. A bench scale membrane system (approximately 1-foot length) is fabricated for the initial study on the parameters involve in the performance of the separation process, such as pressure, feed flow rate, flux and temperature. The pretreatment methods employed on the OPEFB may also be tested using the unit. The data obtained is further analyzed in the determination on the appropriate size of the large scale set up for the recovery of biovanillin. ■

RESEARCH OBJECTIVES

1. To determine the enzymes system involve in the conversion of ferulic acid to vanillic acid using *Aspergillus niger* and of vanillic acid to vanillin using *Phanerochaete chrysosporium*.
2. To compare the performance of crude cell supernatant of Aspergillus niger, *Phanerochaete chrysosporium* and their mixture in the conversion of ferulic acid from OPEFB to biovanillin.
3. To design a separation system for biovanillin using entrapped-enzyme in aqueous solution system.
4. To scale up the biovanillin separation system.

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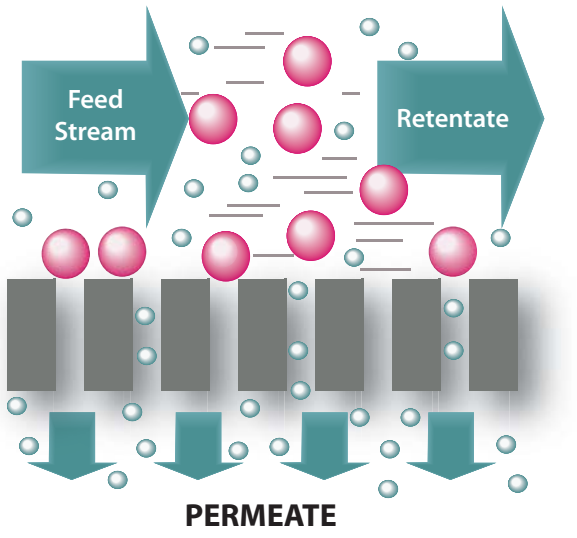
■ Crossflow filtration



PICTURE 2 L Bioreactor located in Biomass Technology Centre

2010 IN PICTURES

■ Minister from MEXT, Japan visiting Biomass Technology Centre,UPM

■ Minister of MOSTI visiting Biogas Plant at FELDA Serting Hilir, Negeri Sembilan

■ Attending World Congress of Industrial Biotechnology IBIO 2010 at Dalian, China

■ Visit Kokura, Kita-Kyushu, Fukuoka, Japan

EU-Malaysia Biomass Stakeholders Forum on 27 April 2010 at Istana Hotel, Kuala Lumpur

JENESYS program at Kyushu Institute of Technology (KIT), Kitakyushu, Japan.

Prof Ali and Prof Shirai at compost pilot plant

Visiting Compost Pilot Plant with Prof Shirai

AFOB Central Office Opening Ceremony on October 2010 at Songdo, Incheon, Republic of Korea

Asian Federation of Biotechnology Executive Board Member Meeting on October 2010 at Songdo, Incheon, Republic of Korea

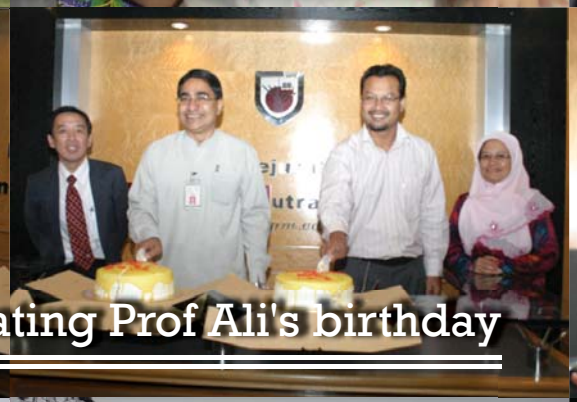
GROUP ACTIVITIES

Badminton games at Putrajaya

Visiting Dr Alawi's newborn, Iman Amani

Bowling tournaments at the MINES

Celebrating Prof Ali's birthday



Dr Alawi and Dr Norjan *soubetsukai*

Volleyball games at Engineering Faculty

Iftar at MAEPS Mardi

FELDA Serting Hilir Biogas Plant

UPM Convocation ceremony

MAHA 2010

UPM Convocation Expo

BioMalaysia 2010

IGEM 2010

PRPI 2010

"Big" Group Meeting

RESEARCH REPORT 2010

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EB GROUP

WASTE TO WEALTH THROUGH BIOTECHNOLOGY

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Welcome to the Environmental Biotechnology Research Group

Waste biomass are a large resource of renewable carbon supplies that have been generated in human daily activities. Waste biomass are coupled to environmental biotechnology and bioprocess engineering through bioprocessing of biomass into higher value bioproducts, designing and modelling processing system, as well as end use of bioproducts in industry, home and transportation in environmental friendly manner.

The Environmental Biotechnology Research Group within the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia undertakes research in core areas of biomass utilization, specifically in production of renewable and valuable green bioproducts. The Group's interests include production of Bioenergy, Biofertilizer, Bioplastics and Bioproducts from oil palm waste biomass. Other potential biomass such as kitchen waste, landfill leachate and sago wastes also been studied.

In the news

VACANCY – Postdoctoral researcher wanted

BIOMALAYSIA 2010

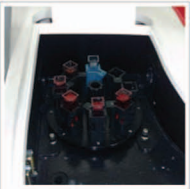
Research Focus

The research work of the group focuses on:

Bioenergy

Biofertilizer

Bioplastic



Bioproduct

Upcoming Events

There are no upcoming EB Group events at this time

Recent Publication

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

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EB GROUP RESEARCH REPORT 2010

WASTE TO WEALTH THROUGH BIOTECHNOLOGY

RESEARCH REPORT 2010

UPM UNIVERSITI PUTRA MALAYSIA BERILMU BERBAKTI

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