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BIO-BASED CROTONIC ACID PRODUCTION ROUTE VIA DIRECT PYROLYSIS OF BACTERIAL POLYHYDROXYBUTYRATE INCLUSIONS

MOHD RAHIMI ZAKARIA @ MAMAT

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MOHD RAHIMI ZAKARIA @ MAMAT

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Chairman : Hidayah Ariffin, PhD
Faculty : Biotechnology and Biomolecular Sciences

Crotonic acid is a short-chain unsaturated carboxylic acid which can be utilized in a wide variety of applications such as resins, polymers, drug-ligand agents, and insecticides. Its current industrial production is from non-renewable petrochemical resource, using hydrocarbon as starting material which is converted into ethylene and several chemical intermediates before obtaining crotonic acid as a product of oxidation of crotonaldehyde. The utilization of hydrocarbon has become a concern due to various environmental and economical issues such as pollution, limited availability and increasing price of the hydrocarbon. Therefore, alternative methods have to be developed to reduce dependence on hydrocarbon and ensure sustainable economy and environment. This study was aimed to develop a simple production method for bio-based crotonic acid via pyrolysis of bacterial polyhydroxybutyrate (PHB) inclusions. Bacterial biomass containing PHB was obtained via fermentation of sugar by PHB producing bacteria, *Cupriavidus necator* NCIMB 11599 at limited phosphate concentration. PHB biomass was subjected to dynamic and isothermal pyrolysis to evaluate thermal degradation behavior and to collect the pyrolyzate produced. The pyrolyzate was analyzed by gas chromatography - mass spectrometry (GC-MS) and proton nuclear magnetic resonance (^{1}H-NMR) to determine its composition. It was found that PHB inclusions with 66±3 % PHB content had different thermo gravimetric (TG) profile compared to pure PHB. PHB inclusions exhibited multi-step degradation which was contributed by presence of PHB, water and bacterial cell components. PHB in the inclusions had temperature degradation range of 270 – 340 °C. Later, when PHB inclusion were pyrolyzed in glass tube oven at its maximum degradation temperature, it was found that the pyrolyzate consisted of 56.8 % monomer, 30.6 % dimer, 9.4 % trimer and 3.2 % impurities. Recovery yield of biocrotonic acid obtained in this study was ~63 %, which is approximately 30 % higher than the conventional petrochemical-based CA. Furthermore, the proposed method also has other advantages such as renewable raw materials with less and simple processing steps. Economic analysis also revealed that crotonic acid price from both proposed bio-based and petrochemical-based was comparable (USD 7.80 - 11.05 and USD 6.75 - 13.50 respectively).

In addition, effects of pyrolysis parameters (temperature and retention time), pretreatment and catalyst were also studied in order to increase biocrotonic acid yield and to reduce the amount of impurities in the pyrolyzate. Results showed that no significant changes of biocrotonic acid yield for temperature range of 300 – 340 °C. Therefore, the temperature of maximum degradation rate of PHB was used for subsequent experiment. Meanwhile, retention time of 20 min showed highest biocrotonic acid recovery yield (66.25 %). Besides that, when the bacterial cells were pretreated by homogenization and combined homogenization with ethanol washing, biocrotonic acid recovery was further improved by 6 % and 8 %, respectively, indicating that improving PHB purity could reduce impurities content and increase the recovery yield. Meanwhile, addition of MgO and $Mg(OH)_2$ as catalysts resulted in significant selective formation of biocrotonic acid. Catalyzed biocrotonic acid production using MgO and $Mg(OH)_2$ yielded 64.07 % and 70.60 % biocrotonic acid with 96.10 % and 95.75% purity respectively. Overall, results obtained from this research showed that biocrotonic acid production from pyrolysis of bacterial inclusions could be a promising method for alternative industrial production of crotonic acid as it does not only provide renewable chemical, but also simpler in production method and yielded higher crotonic acid recovery.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PENGHASILAN ASID KROTONIK BERASASKAN BIO MELALUI PIROLISIS SECARA TERUS BUTIRAN POLIHIDROKSIBUTIRAT DALAM BAKTERIA

Oleh

MOHD RAHIMI ZAKARIA @ MAMAT

Mac 2015

Pengerusi : Hidayah Ariffin, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Asid krotonik adalah asid karboksilik tidak tepu berantai pendek yang boleh digunakan dalam pelbagai aplikasi seperti resin, polimer, ejen ligan dadah dan racun serangga. Penghasilan semasa asid krotonik di peringkat industri adalah dari proses petrokimia dengan menggunakan hidrokarbon sebagai bahan asas yang akan ditukar menjadi etilena dan beberapa bahan kimia perantaraan sebelum menghasilkan asid krotonik sebagai produk daripada pengoksidaan krotonaldehid. Penggunaan hidrokarbon telah menjadi satu kebimbangan disebabkan pelbagai isu-isu alam sekitar dan ekonomi seperti pencemaran, pengeluaran yang terhad dan harga hidrokarbon yang meningkat. Oleh itu, kaedah alternatif perlu dibangunkan untuk mengurangkan kebergantungan kepada hidrokarbon dan memastikan ekonomi dan alam sekitar yang mapan. Kajian ini bertujuan untuk membangunkan satu kaedah pengeluaran asid krotonik berasakan bio yang mudah melalui pirolisis polihidroksibutirat (PHB) dalam bakteria. Biomas bakteria yang mengandungi PHB diperolehi melalui fermentasi gula oleh bakteria yang menghasilkan PHB, *Cupriavidus necator* NCIMB 11599 dengan menghadkan kepekatan fosfat. PHB biomas diuji melalui kaedah pirolisis dinamik dan isoterma untuk menilai tingkahlaku degradasi haba dan untuk mengumpul pirolizat yang dihasilkan. Pirolizat dianalisis dengan gas kromatografi - spektrometri jisim (GC-MS) dan proton nuklear resonans magnet (^{1}H-NMR) untuk menentukan komposisinya. Telah didapati bahawa butiran PHB dengan kandungan PHB sebanyak 66±3 % mempunyai profil termo gravimetrik (TG) yang berbeza berbanding dengan PHB. Butiran PHB mempamerkan degradasi multi-langkah yang telah disumbangkan oleh kehadiran PHB , air dan komponen sel bakteria. PHB dalam butir granul mempunyai suhu degradasi di antara 270 - 340 °C. Kemudian, apabila butir PHB telah di pirolisis di dalam ketuhar tiub kaca pada suhu degradasi maksimum, didapati bahawa pirolizat terdiri daripada 56.8 % monomer, 30.6 % dimer , 9.4 % trimer dan 3.2 % bendasing. Hasil asid krotonik adalah kira-kira 63 %, yang hampir 30 % lebih tinggi daripada pengeluaran konvensional petrokimia. Tambahan pula, kaedah yang dicadangkan juga mempunyai kelebihan lain seperti bahan mentah yang boleh diperbaharui, langkah pemprosesan yang sedikit dan mudah. Analisis ekonomi juga mendedahkan bahawa harga asid krotonik dari kedua-dua kaedah berasaskan bio dan petrokimia adalah setanding (USD 7.80 – 11.05 dan USD 6.75-13.50 masing-masing).

Di samping itu, kesan parameter pirolisis, rawatan awal dan pemangkin juga dikaji untuk meningkatkan hasil asid krotonik-bio dan mengurangkan jumlah bendasing dalam pirolizat itu. Keputusan menunjukkan bahawa tiada perubahan yang besar kepeada hasil asid krotonik-bio untuk lingkungan suhu 300 – 340 °C. Oleh itu, suhu untuk kadar degradasi maksima bagi PHB digunkan untuk eksperimen yang seterusnya. Sementara itu, masa tahanan selama 20 min menunjukkan nilai penghasilan asid krotonik yang paling tinggi (66.25 %). Selain itu, apabila sel-sel bakteria telah dirawat awal oleh penghomogenan dan gabungan penghomogenan dengan basuhan etanol, peningkatan hasil asid krotonik (kira-kira 11 % dan 16 %, masing-masing) diperhatikan, menunjukkan bahawa peningkatkan ketulenan PHB boleh mengurangkan kotoran dan meningkatkan penghasilan. Sementara itu, penambahan MgO dan $Mg(OH)_2$ sebagai pemangkin menyebabkan pembentukan terpilih asid krotonik. Pengeluaran asid krotonik-bio menggunakan MgO dan Mg $(OH)_2$ menghasilkan 64.07 % dan 70.60 % asid krotonik-bio dengan 96.10 % dan 95.75 % ketulenan. Secara keseluruhan, keputusan yang diperolehi daripada kajian ini menunjukkan bahawa pengeluaran asid krotonik-bio daripada pirolisis butiran PHB dalam bakteria boleh menjadi kaedah yang berpotensi untuk pengeluaran alternatif asid krotonik-bio kerana ia bukan sahaja memberi kimia yang boleh diperbaharui , tetapi juga lebih mudah dan memberikan hasil asid krotonik-bio yang lebih tinggi.

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I certify that a Thesis Examination Committee has met on 23 March 2015 to conduct the final examination of Mohd Rahimi Zakaria @ Mamat on his thesis entitled "Bio-based crotonic acid production route via direct pyrolysis of bacterial polyhydroxybutyrate inclusions" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Umi Kalsom binti Md Shah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd. Yunus bin Abd. Shukor, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Nor Azowa binti Ibrahim, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mohamad Suffian bin Mohamad Annuar, PhD
Associate Professor
University of Malaya
Malaysia
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 17 June 2015

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members for the Supervisory Committee were as follows:

Hidayah Ariffin, PhD
Senior Lecturer
Faculty of Biotechology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd Ali Hassan, PhD
Professor
Faculty of Biotechology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
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School of Graduate Studies
Universiti Putra Malaysia

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Signature:	________	Signature:	________
Name of Chairman of Supervisory Committee:	Hidayah Ariffin, PhD	Name of Member of Supervisory Committee:	Mohd Ali Hassan, PhD

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LIST OF ABBREVIATIONS

3HB	3-hydroxybutyrate
4HB	4-hydroxybutyrate
3HHx	3-hydroxyhexanoate
3HV	3-hydroxyvalerate
5HV	5-hydroxyvalerate
AMPS	Acrylamido-2-methyl-1-propanesulfonic acid
CA	Crotonic acid
CoA	Coenzyme A
$CDCl_3$	Chloroform –*d*
CO_2	Carbon dioxide
$CuCl_2$	Copper chloride
DSC	Differential scanning calorimetry
EDTA	Ethylenediaminetetraacetic acid
FAB-MS	Fast atom bombardment mass spectrometry
FID	Flame ionization detector
GC-MS	Gas chromatography mass spectrometry
GTO	Glass tube oven
^{1}H-NMR	Proton nuclear magnetic resonance
H_2O	Water
HV	Hydroxyvalerate
LDL	Low density lipoprotein
MgO	Magnesium oxide
$Mg(OH)_2$	Magnesium hydroxide
MSM	Mineral salt medium
MTBE	Methyl *tert*-butyl ether

NMR	Nuclear magnetic resonance
NPCM	Non-PHA cellular mass
OPFJ	Oil palm frond juice
$PdCl_2$	Palladium chloride
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxy 3-butyrate
PHBHHx	Polyhydroxybutyrate-*co*-hydroxyhexanoate
PHBV	Polyhydroxybutyrate-*co*-hydroxyvalerate
PP	Polypropylene
Py-GC	Pyrolysis-gas chromatography
Py-MS	Pyrolysis-mass spectrometry
SATVA	Subambient thermal volatilization analysis
SDS	Sodium dodecyl suphate
T_d	Degradation temperature
TEM	Transmission electron microscopy
TG	Thermo gravimetry
TGA	Thermo gravimetry anlyzer
TIC	Total ion current
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 Research overview

Fossil feedstocks are primarily used as energy source which cover about 75 % of the world energy demand. Besides that, they are also being utilized to produce variety of products such as polymers and chemicals (Dapsens et al., 2012). However, their usage has been always associated with harmful pollutants which consequently causing global warming and climate changes. In addition, petroleum has faced a drastic increase in price over the last decade due to limited availability and increased demand of the hydrocarbon (Jong et al., 2012). Therefore, efforts have been taken in finding sustainable resources and methods to reduce reliance on the non-renewable hydrocarbons for fuel, energy and chemicals (Armaroli and Balzani, 2007; Pacala and Socolow, 2004). Biomass becomes an interesting alternative for fossil hydrocarbons due to its abundance and ability to be transformed into different products (Dapsens et al., 2012). Furthermore, utilization of biomass can also stimulate development of rural agricultural regions (Jong et al., 2012).

One of the chemicals produced industrially using fossil hydrocarbon is crotonic acid (CA) (Schulz et al., 2003; Arpe, 2010). CA has found its importance in a wide variety of applications. For instance, it can be copolymerized with vinyl acetate, known under different commercial names such as Cevian, Gelva, and Vinac, (Schulz et al., 2003) and be used as a component in hair care and cosmetic products (Campain, 2010). It can also be used in other industries such as coating, absorbent, textile, paint, ceramic, agrochemical and polymer industries (Jasicka-Misiak et al., 2005; Van Walsem et al., 2011). Furthermore, it has great potential as a chemical building block for important chemicals with high market demand such as acrylic acid, butanol, propylene and maleic anhydride (Somleva et al., 2013). However, despite of all its importance, current production method of crotonic acid is from non-renewable petroleum through multiple complicated chemical conversions including steam cracking of heavy hydrocarbon, oxidation of ethylene, aldol condensation of acetaldehyde, dehydration of acetaldol and finally, oxidation of crotonaldehyde into crotonic acid (Arpe, 2010). With growing concern on the impact of hazardous pollutants to the environment, together with limited availability and increasing price of the hydrocarbon, people has started to search for alternative resources and bio-based processes which are safer to both planet and its inhabitant. Recently, there were reports on the use of modified bacteria to produce CA (Koch and Meurer, 2012; Mauch and Schmid, 2008). They manipulated specific bacterial metabolism pathways (2-oxoglutarate and 2-ketoglutarate pathways) which enable the bacterial cells to produce CA which will be secreted into the fermentation broth. However, they did not include the quantitative details and information on recovery methods.

There are many publications on CA as one of the degradation products of polyhydroxybutyrate (PHB) (Morikawa and Marchessault, 1981; Kopinke et al., 1995; Tokiwa and Calabia, 2004; Gonzalez et al., 2005; Ariffin et al., 2008). It has been reported that PHB can be thermally degraded via a process called pyrolysis into its corresponding dehydrated monomers (*cis* and *trans*-CA), dimers, trimers and secondary products such as carbon dioxide (CO_2) and propylene. Degradation products can be influenced by pyrolysis temperature as temperatures below 400 °C produce mainly dehydrated monomer and oligomer of PHB while above 400 °C, secondary products will be observed which resulted from further degradation of primary products (Grassie et al., 1984a; Kopinke et al., 1996; Gonzalez et al., 2005). PHB is thermally degraded via combination of degradation mechanisms including random β-elimination, α-deprotonation, trans-esterification and unzipping β-elimination (Ariffin et al., 2008).

In general, PHB is a bioplastic produced through bacterial fermentation. Nevertheless, due to brittle nature and narrow processing window, PHB can only be used in limited applications. Therefore in this study, PHB biomass produced from bacterial fermentation was used as feedstock for bio-based CA production. Feasibility and sustainability of the process were also evaluated.

1.2 Problem statement

The problem of current industrial production of CA lies with the use of hydrocarbons as raw material. Chemical synthesis of CA uses heavy hydrocarbons such as naphtha to obtain ethylene which can be subsequently converted into CA through multiple and complicated steps. Fossil hydrocarbon, which has high demand in energy and chemical industries, is facing several issues such as non-renewable, decreasing reservoir, elevated price, and more seriously, causing environmental pollutions. Low recovery yield of CA will require another step which is purification step which might influence the production cost and the number of byproducts which needs to be treated.

The second issue that led to this research is an unfavorable narrow processing temperature characteristic of PHB that limits its application as consumer plastics. PHB, in spite of having advantageous in terms of biodegradability and biocompatibility, has limited feasible applications due to brittleness and narrow processing temperatures. PHB is known to melt at approximately 180 °C and degrade at about 250 °C. This has caused difficulties during the molding processing.

Therefore, this research was aimed at finding alternative method for the production of CA, which is renewable and simple through thermal degradation of PHB. The use of PHB for production of CA can broaden its application.

1.3 Objectives of the study

The objectives of this study are:

1) To develop bio-based production of crotonic acid and to compare the proposed method with the chemically-synthesized crotonic acid.
2) To study the effects of selected pyrolysis parameters, pre-treatments and addition of catalyst on the recovery of bio-crotonic acid.

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