UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE) USING COMAMONAS SP. EB172 FROM ORGANIC ACIDS DERIVED FROM ANAEROBIC TREATMENT OF PALM OIL MILL EFFLUEN

TABASSUM MUMTAZ

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By

TABASSUM MUMTAZ

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2010

Dedicated to my father

who would be the happiest and most pleased one in the

world to see me getting PhD degree

10.12.2010

Dedicated to my own "soul out of my soul',

My daughter

Tarannum Tasneem Zaman

23.06.2010

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

PRODUCTION OF POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE) USING *COMAMONAS* SP. EB172 FROM ORGANIC ACIDS DERIVED FROM ANAEROBIC TREATMENT OF PALM OIL MILL EFFLUENT

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Chairman : Professor Mohd. Ali Hassan, PhD

Faculty : Biotechnology and Biomolecular Sciences

Polyhydroxyalkanoates (PHAs) are biodegradable polymers accumulated by certain bacteria as intracellular carbon storage materials in response to inorganic nutrient limitation in the presence of excess carbon. Among the various types of PHAs, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV is the most promising and has great potential as a biodegradable substitute for bulk plastics. However, high production costs associated with substrates have limited the production of these polymers in large scale. In this study, the use of organic waste carbon compounds, in combination with anaerobic treatment, is being evaluated as alternative and appropriate substrates for PHBV production. Acidogenic fermentation of palm oil mill effluent (POME) generates a dilute mixture of organic acids (acetic acid, propionic acid and n-butyric acid) which, has to be recovered before being utilized by microbes for polymer biosynthesis.

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 impeller tip speed. The PHBV produced were further characterized and compared with the commercially available PHBV to address the market demand.

Filtration and evaporation 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% as compared to 85% in laboratory scale. The effect 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 utilization of carbon and nitrogen throughout the growth cycle of *Comamonas* sp. EB172 was compared using batch and fed-batch fermentation methods. *Comamonas* sp. EB172 showed higher tolerance of organic acids in the order of n-butyric acid>acetic acid>propionic acid.

The fed-batch fermentation method was further simplified by eliminating the centrifugation step and operating the growth phase and PHA production phase consecutively in 2 L bioreactor. The fermentation was switched to PHA production phase by introducing nitrogen-free mineral media into the 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 acids around 2-6 g/L and volumetric productivity of 0.1318 g/L/ h. PHBV content ranged from 70- 90% (w/w) of the cell with yield of 0.27- 0.4 g PHBV/g mixed acid. Scaling up fermentation in 150 L bioreactor resulted in biomass concentration of 5.35 g/L with final PHBV 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 PHBV content were comparable to that obtained in 2 L scale. The final HV content in the copolymer ranged from 10-17%.

In order to assess whether the poly (3-hydroxybutyrate-co-3-hydroxyvalerate) produced by *Comamonas* sp. EB172 could be used in practical applications, the physical, thermal and mechanical properties of the polymers were determined. The PHBV with 13% HV content exhibited the mechanical properties of elastic rubber and was less crystalline, having relatively low melting temperature and molecular weight of 130,000 Daltons. The chemical structure of the polymer was also confirmed by NMR and FTIR analyses. 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 overall results indicated that clarified organic acid mixtures derived from the partial anaerobic treatment of POME are suitable substrates for the production of PHBV. Therefore, the bioconversion of POME to PHBV *via* organic acids can be a useful way forward towards the sustainable utilization of POME wastewater.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGHASILAN POLI(3-HIDROKSIBUTIRAT-KO-3-HIDROKSIVALERAT) MENGGUNAKAN *COMAMONAS* SP. EB172 DENGAN MENGEKSTRAK ASID ORGANIK DARIPADA SISA KILANG KEPALA SAWIT TERAWAT ANAEROBIK

Oleh

TABASSUM MUMTAZ

December 2010

Pengerusi : Profesor Mohd Ali Hassan, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Polihidroksialkanoat (PHAs) adalah polimer biourai yang dikumpulkan oleh bakteria sebagai bahan simpanan karbon di dalam sel sebagai tindakbalas terhadap nutrisi tak organik yang terhad dan kehadiran sumber karbon yang berlebihan. Antara pelbagai jenis PHAs, poli (3-hidrosibutirat-ko-3-hidrosivalerat), PHBV menunjukkan potensi yang besar sebagai pengganti plastik biourai. Walaubagaimanapun, kos pengeluaran yang tinggi yang berkaitan dengan substrat telah menghadkan pengeluaran polimer ini dalam skala besar. Dalam kajian ini, penggunaan sisa organik sebatian karbon digabungkan dengan rawatan anaerobik telah dikaji sebagai kaedah alternatif dan substrat yang sesuai untuk penghasilan PHBV. Fermentasi asidogenik dari sisa kilang kelapa sawit (POME) menghasilkan campuran cair asid organik (asid asetik, asid propionik dan asid n-butirat) dan dipekatkan sebelum digunakan oleh mikrob untuk biosintesis polimer.

Dalam projek ini, proses hiliran untuk skala pepandu telah dibangunkan untuk menghasilkan campuran asid organik yang tulen sebagai substrat untuk penghasilan PHBV. Penyelidikan ini juga bertujuan untuk membangunkan strategi suapan substrat dalam skala 2 L dan peningkatan skala untuk proses fermentasi dalam bioreaktor 150 L berdasarkan kelajuan hujung pengaduk yang sama. PHBV yang terhasil telah dikenalpasti dan dibandingkan dengan PHBV yang telah dikomersialkan bagi memenuhi permintaan pasaran.

Penapisan dan pengewapan POME terawat untuk proses hiliran dan pengenalpastian asid organik telah dijalankan melalui kaedah pengemparan dan penyejat berputar dalam skala makmal manakala penapis turas dan penyejat berputar digunakan untuk kajian skala loji pandu. Bagi kajian loji pandu, kepekatan asid organik tertinggi yang diperolehi adalah 67.25 g/L pada kepekatan lapan kali ganda dengan hasil proses hiliran sebanyak 83% berbanding 85% pada skala makmal. Kesan campuran asid organik untuk penghasilan PHBV oleh bakteria tempatan, *Comamonas* sp. EB172 telah dikaji dalam bioreaktor 2 L. Masa tindakbalas dan penggunaan karbon serta nitrogen sepanjang kitaran pertumbuhan *Comamonas* sp. EB172 telah dibandingkan dengan menggunakan kaedah fermentasi sesekelompok dan suapan sesekelompok. *Comamonas* sp. menunjukkan tahap toleransi yang tinggi terhadap asid organik mengikut urutan berikut: asid n-butirik> asid asetik> asid propionik.

Fermentasi suapan sesekelompok telah dipermudahkan lagi dengan mengelakkan proses pengemparan dan operasi fasa pertumbuhan serta fasa penghasilan PHA secara berturut-turut dalam bioreaktor 2 L. Proses fermentasi telah ditukar kepada

fasa pertumbuhan dengan penambahan media mineral bebas nitrogen diikuti dengan suapan asid organik menggunakan kaedah 'pH-stat'. Dengan menggunakan strategi tersebut, biojisim maksimum yang dihasilkan dalam bioreaktor 2 L adalah 10.2 g/L dengan sisa asid tertinggal sebanyak 2-6 g/L dan produktiviti volumetrik 0.1318 g/L/j. Kandungan PHBV dalam lingkungan 70-90% (w/w) dengan penghasilan 0.27-0.4 g PHBV/g campuran asid. Pengingkatan skala fermentasi dalam bioreaktor 150 L menghasilkan kepekatan biojisim 5.35g/L dengan kandungan PHBV akhir sebanyak 72.8%, produktiviti volumetrik 0.083 g/L/h dan penghasilan PHBV yang dikira adalah 0.259 gPHBV/g campuran asid. Nilai penghasilan dan kandungan PHBV tersebut adalah setara dengan keputusan dalam sekala 2 L. Kandungan HV akhir adalah dalam kopolimer adalah dalam julat 10-17%.

Bagi memastikan samada PHBV yang dihasilkan oleh *Comamonas* sp. boleh diaplikasikan secara praktik, ciri-ciri fizikal, terma dan mekanikal telah ditentukan. PHBV dengan kandungan HV 13% menunjukkan ciri mekanikal getah elastik dan kurang kristal. Ia juga mempunyai suhu lebur yang rendah dan berat molekul sebanyak 130,000 D. Struktur kimia polimer juga dipastikan dengan menggunakan analisis NMR dan FTIR. Saiz penyebaran PHBV granul dalam sel *Comamonas* sp. EB 172 adalah dalam lingkungan 0.11-0.67 μm dengan 5-9 granul di setiap sel. Imej TEM bagi granul PHBV *in vivo* menunjukkan struktur selaput teras yang menunjukkan pembentukan blok kopolimer berbanding dengan pembentukan kopolimer secara rawak.

Keputusan keseluruhan menunjukkan campuran asid organik daripada rawatan anaerobik POME boleh menjadi substrat yang sesuai bagi penghasilan PHBV oleh *Comamonas* sp. EB172. Olehitu biopenukaran POME kepada PHBV melalui asid organic adalah satu langkah yang boleh diwajukan kearah pengguraan lestari sisa air POME.

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"Reading maketh a full man, conference a ready man, and writing an exact man."

Francis Bacon

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This list is far from exhaustive; I pray for forgiveness from those I did not mention by name and include them in my heart-felt gratitude.

Finally, I give almighty ALLAH the glory for the wisdom, knowledge and strength to complete this thesis.

Author
December 2010

I certify that an Examination Committee has met on 10/12/2010 to conduct the final examination of Tabassum Mumtaz on her PhD thesis entitled "Production of Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) using *Comamonas* sp. EB172 from Organic Acids Derived from Anaerobic Treatment of Palm Oil Mill Effluent" 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 Doctor of Philosophy.

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

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DECLARATION

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

TABASSUM MUMTAZ

Date: 10 December 2010

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