ABSTRACT

Medium-chain-length polyhydroxyalkanoate (mcl-PHA) is biodegradable polyester that gained serious attention recently, mainly because of its versatility to accomodate a wide range of applications especially in biomedical area. Like other biopolyesters in PHA family, it is synthesized intracellularly by microorganisms under nutrient stress condition i.e abundant carbon sources than other essential nutrients like nitrogen, oxygen, sulphur, etc. It functions as carbon and energy storage to the cells during depletion of carbon sources. A well known group of bacteria able to specifically produce mcl-PHA is fluorescent pseudomonads belonging to rRNA homology group 1.

To make industrial production of mcl-PHA viable, significant efforts have been put into increasing the yield and productivity of the biopolymer inside the bacteria. Equally important is the efficient extraction method to get them. Currently, an extraction method that is both rapid and non-detrimental to the product i.e. mcl-PHA is not available. In this study, a method to achieve the said goal was investigated through the application of ultrasound-assisted process. In addition, a combination of acetone as solvent and heptane as marginal non-solvent was used as the extraction medium. The effects of volumetric energy dissipation, extraction medium ratio and irradiation time on the extraction process were investigated. Frequency of 37 kHz and heptane as marginal nonsolvent facilitated the process. Following optimization, high PHA extraction rate of 74×10^{-3}g PHA g^{-1} dried biomass min^{-1} was observed at ultrasonic energy output, solvent–marginal nonsolvent ratio and irradiation time of 1151 ± 3 J ml^{-1}, 50:50 and 5 min respectively.

The effects of exposure duration and ultrasonic power output on mcl-PHA solution in acetone were also studied. Molecular weight and thermal properties of ultrasound-irradiated mcl-PHA was characterized and compared to control (non-

irradiated mcl-PHA). It was found that at constant volumetric acoustic energy dissipation, prolonged exposure of up to 20 minutes caused slightly significant degradation of mcl-PHA. Under ultrasonic irradiation, degradation mechanism of mcl-PHA was proposed to involve random α-chain scission. It proceeds *via* cleavage of ester linkage at the main chain backbone to form alkenyl terminal.

In a related study, mcl-PHA produced by *Pseudomonas putida* Bet001 was used as a blending component with scl-PHA produced by *Delftia tsuruhatensis* Bet002. The thermal stability and film morphology of the blend between the brittle homopolymer polyhydroxybutyrate (scl-PHA) and flexible heteropolymer polyhydroxyoctanoate (mcl-PHA) were studied. The blends were prepared *via* film casting method and analyzed by differential scanning calorimetry (DSC), Field emmision scanning electron microscopy (FESEM), X-ray diffraction (XRD) and thermogravimetric analysis (TGA). The blend compositions were varied from 0, 25, 50, and 75 % (w/w) of mcl-PHA. The blends showed immiscibility with a morphology that constitutes of crystallite and amorphous phases. Formation of crystallite was observed from the XRD results. Despite the immiscibility, the thermal stability was improved for all blends.

ABSTRAK

Polihidroksialkanoate berantai sederhana panjang (mcl-PHA) adalah poliester terbiodegradasi yang telah mendapat banyak perhatian kerana keserbabolehannya untuk digunakan di dalam pelbagai aplikasi terutama sekali dalam bidang bioperubatan. Seperti biopoliester lain dalam kumpulan PHA, ianya dapat disintesis oleh mikroorganisma di bawah keadaan ketidakstabilan nutrien iaitu semasa sumber karbon adalah lebih tinggi daripada nutrien penting lain seperti nitogen , oksigen, sulfur, dan lain-lain. Fungsinya adalah sebagai penyimpanan tenaga untuk sel bakteria semasa susutan sumber karbon. Contoh bakteria yang dapat menghasilkan secara khususnya polimer ini adalah "fluorescent pseudomonads" dari kumpulan rRNA homologi 1.

Untuk meningkatkan produktiviti penghasilan PHA pada tahap industri, banyak kajian telah dilakukan seperti teknologi kultur baketeria. Walaubagaimanapun, kajian pengekstrakan PHA pada masa yang singkat tanpa merosakan polimer tersebut belum lagi dilakukan. Kajian ini sangat penting kerana ianya dapat mengurangkan kos penghasilan PHA. Sehubungan dengan itu, kajian tentang proses pengekstrakan untuk mencapai matlamat tersebut telah dijalankan dengan menggunakan teknologi gelombang ultrabunyi. Di samping itu, campuran aseton (pelarut) dan heptana (bukan pelarut) telah digunakan sebagai medium pengekstrakan . Kesan pelesapan tenaga, nisbah medium pengekstrakan dan masa penyinaran pada proses pengekstrakan telah disiasat. Kekerapan gelombang pada 37 kHz dan campuran heptana sebagai "marginal non-solvent" memudahkan proses tersebut. Optimumnya, kadar pengekstrakan PHA yang tinggi (74×10^{-3}g PHA g^{-1} dried biomass min^{-1}) diperhatikan apabila tenaga output ultrasonik, nisbah pelarut-bukan pelarut dan masa penyinaran adalah 1151 ± 3 J ml^{-1}, 50:50 dan 5 minit, masing-masing

Kesan jangka masa pendedahan dan tenaga output ultrasonik pada larutan mcl-PHA di dalam aseton juga telah dikaji. Berat molekul dan sifat haba bagi PHA yang didedahkan dengan gelombang bunyi telah dicirikan dan dibanding dengan PHA kawalan (mcl-PHA yang tidak didedahkan). Telah didapati bahawa pendedahan yang berpanjangan sehingga 20 minit boleh menyebabkan degradasi mcl-PHA. Mekanisme pengdegradasian mcl-PHA telah dijangka melibatkan potongan rantai α secara rawak. Ia berlaku melalui proses pemotongan di ikatan ester pada rantai utama untuk menghasilkan hujung rantai yang berakenil .

Di samping itu, mcl-PHA yang dihasilkan oleh *Pseudomonas putida* Bet001 telah digunakan sebagai komponen pengadunan bersama dengan scl-PHA yang dihasilkan oleh *Delftia tsuruhatensis* Bet002. Kestabilan haba dan morfologi filem daripada campuran ini telah dikaji. Campuran ini disediakan dengan cara meruapkan pelarut dari larutan campuran polimer yang pekat dan dianalisis dengan menggunakan DSC, FESEM, XRD dan TGA. Komposisi campuran telah diubah dari 0, 25, 50 , dan 75 % mcl-PHA (w/w). Daripada keputusan FESEM , adunan filem menunjukkan sifat ketidakcampuran dan menunjukkan morfologi yang terdiri daripada gabungan “kristalit” dan fasa “amorphous”. Pembentukan separa kristal telah ditentukan dengan analisis XRD. Walaubagaimanapun, kestabilan haba bagi campuran polimer tersebut bertambah baik.

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May God bless you all

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LIST OF SYMBOLS AND ABBREVIATIONS

mcl-PHA	medium chain length polyhydroxyalkanoate
scl-PHA	short chain length polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHBV	Polyhydroxybutyrate-*co*-valerate
PHBHHx	Polyhydroxybutyrate-*co*-hexanoate
PHO	Polyhydroxyoctanoate
PHHp	Polyhydroxyheptanoate
PHHx	Polyhydroxyhexanoate
PLA	Polylactic acid
DSC	Differential scanning calorimetry
TGA	Thermogravimetric analysis
FESEM	Field emission scanning electron micrography
^{1}H-NMR	Proton nuclear magnetic resonance
FTIR	Fourier transform infrared spectroscopy
XRD	X-ray diffraction
GPC	Gel permeation chromatography
GC	Gas chromatography
PTFE	Polytetrafluoroethylene
TMS	Tetramethylsilane
PS	Polystyrene
ANOVA	Analysis of variance
DF	Degree of freedom
SS	Sum of squares
MS	Mean square

F	F-value
P	P-value
P	Power
cp	Specific heat capacity of water
ΔT	Temperature difference
Δt	Time difference
E	Sonication-dissipitated nergy
V	Volume
M_n	Number-averaged molecular weight
M_w	Weight-averaged molecular weight
PDI	Polydispersity index
q	heating rate (K min^{-1})
k	Boltzman constant ($1.3807x10^{-23}$ J K^{-1})
h	Planck constant ($6.626x10^{-34}$ J s)
E_d	Degradation activation energy (J mol^{-1})
R	General gas constant (8.3143 J K^{-1} mol^{-1})
A	Pre-exponential factor/collision factor (s^{-1})
ΔS	Entropy of activation (J K^{-1} mol^{-1})
T_g	Glass transition temperature
T_c	Crystallization temperature
T_m	Melting temperature
T_p	Peak temperature / most rapid degradation temperature
T_{onset}	Starting temperature of degradation
T_{final}	End temperature of degradation
kPa	kilo Pascal
kDa	kilo Dalton
K	Kelvin

J	Joule
W	watt
min	minute
s	second
ml	mililitre
g	gram
mg	miligram
ppm	part per million
rpm	round per minute
(w/v)	weight per volume
(w/w)	weight per weight
(v/v)	volume per volume

LIST OF APPENDICES