CHARACTERIZATION OF P(3HB-*co*-4HB) COPOLYMER SYNTHESIS CONSISTING OF HIGH 4HB MONOMER USING *Cupriavidus* sp. USMAA1020 TRANSFORMANT STRAINS

MUHAMMAD SYAFIQ BIN ISHAK

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MUHAMMAD SYAFIQ BIN ISHAK

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Main Supervisor : Dr. Kesaven a/l Bhubalan

Co-Supervisor : Prof. Dr. Amirul Al-Ashraf Abdullah

Faculty: School of Marine and Environmental Sciences

Polyhydroxyalkanoates (PHAs) are naturally-occurring biodegradable thermopolyesters accumulated by bacteria. Copolyester of Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)] were noted for its high biocompatibility. This makes it an excellent candidate for medical as well as biopharmaceutical applications. A Gram-negative bacteria, *Cupriavidus* sp. USMAA1020 has the ability to synthesized P(3HB-*co*-4HB) copolymer with various 4HB monomer compositions. Additional copies of PHA synthase gene (*PhaC*) from *Cupriavidus* sp. USMAA1020 ($PhaC_{1020}$) and *Cupriavidus* sp. USMAA2-4 ($PhaC_{2-4}$) the key enzyme in PHA synthesis into *Cupriavidus* sp. USMAA1020 wild-type strain resulted in higher 4HB monomer composition of the copolymer produced. The transformant strains was cultivated with 1,6-hexanediol (0.5175 wt%) and γ-butyrolactone (0.1725 wt%) through one-stage cultivation. 4HB composition up to 93 mol% obtained by *Cupriavidus* sp. USMAA1020$_{phaC1020}$ while 91 mol% 4HB molar fraction was obtained by *Cupriavidus* sp. USMAA1020$_{phaC2-4}$. The 4HB

monomer compositions were in range of 85 - 89 mol% when the copolymer production was scaled-up in 5 L and 30 L bioreactor with a constant oxygen mass transfer rate (K_La). The physical and thermal properties of P(3HB-*co*-4HB) copolymer with 4HB monomer composition of 85 mol% to 93 mol% were characterized. The elongation at break was in range of 310% to 400% and the weight-average molecular weight (M_w) of these copolymers was in the range of 272 kDA to 290 kDA. The melting point (T_m) of the copolymer was decrease in the range of 79 – 50 ˚C with the 4HB molar fraction of the copolymer increase from 85 mol% to 93 mol% while the copolymer crystallinity was increase from 17.2% to 30.7% as the 4HB monomer composition of the P(3HB-*co*-4HB) copolymer increase. The PHA synthase activity for the transformant strain was two-fold higher than wild-type with 171 U/mg and 73 U/mg respectively. The P(3HB-*co*-4HB) copolymer produced in this study has the potential to be used in pharmaceutical and medical applications such as sutures, adjuvant and drug delivery system**.**

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PERINCIAN KOPOLIMER P(3HB-*co*-4HB) DENGAN MONOMER 4HB YANG TINGGI DARIPADA TRANSFORMAN *Cupriavidus* sp. USMAA1020

MUHAMMAD SYAFIQ BIN ISHAK

Ogos 2015

Penyelia Utama : Dr Kesaven a/l Bhubalan

Penyelia Bersama : Prof. Dr. Amirul Al-Ashraf Abdullah

Fakulti: Pusat Pengajian Sains Marin dan Sekitaran

Polihidroksialkanoat (PHAs) adalah termopoliester yang terbiodegradasi dihasilkan oleh bakteria. Kopoliester Poli(3-hidroksibutirat-*ko*-4-hidroksibutirat) [P(3HB-*ko*-4HB)] terbukti mempunyai keserasian yang tinggi dan sesuai di jadikan dijadikan sebagai bahan untuk perubatan dan aplikasi farmaseutikal. Bakteria Gram-negatif, *Cupriavidus* sp. USMAA1020 berkeupayaan menghasilkan kopolimer P(3HB-*ko*-4HB) dengan pelbagai kandungan monomer 4HB. Penambahan gen PHA synthase daripada *Cupriavidus* sp. USMAA1020 (*PhaC*$_{1020}$) dan *Cupriavidus* sp. USMAA2-4 (*PhaC*$_{2-4}$) di dalam bakteria tulen *Cupriavidus* sp. USMAA1020*)* mampu meningkatkan kadar 4HB dengan jumlah monomer yang tinggi. Transforman *Cupriavidus* sp. USMAA1020$_{PhaC1020}$ dan *Cupriavidus* sp. USMAA1020$_{PhaC2-4}$ dibiakkan dengan 1,6-hexanediol (0.5175wt%) dan γ-butirolakton (0.1725wt%) melalui proses pemeliharaan satu peringkat dan masing-masing menghasilkan komposisi 4HB sebanyak 93 mol% dan 91 mol%. Komposisi monomer 4HB berada di dalam julat 85 mol% hingga 89 mol%

apabila penghasilan kopolimer dibiakkan di dalam bioreaktor 5L dan 30L dengan kadar pemindahan jisim oksigen yang malar (K_La). Ciri-ciri fizikal dan termal bagi kopolimer P(3HB-*ko*-4HB) dengan komposisi 85 mol% kepada 93 mol% monomer 4HB telah direkodkan. Pemutusan pemanjangan antara julat 310 % kepada 400 % dan purata berat molekul kopolimer adalah dalam julat 272 kDa hingga 290 kDa. Takat lebur (T_m) kopolimer menurun dalam julat 79 ˚C hingga 50 ˚C dengan pecahan molar polimer 4HB yang meningkat daripada 85 mol% hingga 93 mol%. Kadar kehabluran kopolimer juga meningkat daripada 17.2% kepada 30.7% apabila komposisi P(3HB-*ko*-4HB) meningkat. Aktiviti PHA sintase adalah dua kali ganda daripada bakteria tulen dengan jumlah masing-masing sebanyak 171 U/mg dan 73 U/mg. Dalam kajian ini kopolimer P(3HB-*ko*-4HB) berpotensi untuk digunakan dalam aplikasi farmaseutikal dan perubatan seperti sutur, adjuvan dan sistem penghantaran dadah.