

**BIOSYNTHESIS, FABRICATION AND
ANALYSIS OF BIODEGRADABLE
POLY(3HYDROXYBUTYRATE) AND
ANTIMICROBIAL AGENT BLENDS AS
BACTERIAL CONTROL IN AQUACULTURE**

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Master of Science in the School of Marine and Environmental Sciences
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ABSTRACT

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirement for the degree of Master of Science

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School/Institute/Centre : School of Marine and Environmental Sciences

Bacterial control in aquaculture water system is important because the most prominent problem faced in this system is the high mortality rate caused by uncontrolled bacterial infections. The current measures used to control bacterial infection poses a disadvantage under long term use as it not only affects the environment but humans as well. Poly(3-hydroxybutyrate) [P(3HB)], a bacteria-derived biodegradable polymer was investigated for its potential to act as a matrix for immobilizing antimicrobial agents. The prolonged immobilization of antimicrobial agents on a biodegradable matrix will enable improved inhibition of potentially pathogenic microorganisms in an eco-friendly approach. The P(3HB) antimicrobial film blends will act as a time-releasing system that releases its different types of antimicrobial compounds based on the degradation of the film. Titanium dioxide (TiO₂) is a metal oxide with photocatalytic properties that is able to decompose organic matters through the formation of hydroxyl radicals which makes it known for its antimicrobial properties. Rhamnolipid (RL) are biosurfactant glycolipids that has shown to exhibit antimicrobial actions against bacteria and viruses. Here, P(3HB) and RL was produced via bacterial fermentation using marine *Bacillus megaterium* UMTKB-1 and *Pseudomonas aeruginosa* UMTKB-5 from renewable carbon feedstocks respectively. Blends of P(3HB) and TiO₂ as well as RL was tested with different types of pathogenic bacteria for its effective antimicrobial potential. The P(3HB)-TiO₂ film blends were able to inhibit bacterial growth by reducing the colony-forming-units (CFU) by a

steady decrease on an hourly basis for certain bacteria such as *B. megaterium* UMTKB-1, *E. coli* or *S. aureus*. Antimicrobial test using RL as an antimicrobial agent has produced promising results with an inhibition zone of 12 mm based on disc diffusion test using *B. megaterium* and *Vibrio harveyii*. In general, the inhibition success of the P(3HB)-RL film blends ranges from 60-80%. Environmental test conducted exhibits a significant growth reduction on the *Vibrio* spp. when exposed to RL at approximately 50%. The successful application of P(3HB)-antimicrobial blends as time-releasing microbial inhibition system will serve as an eco-friendly method for pathogenic microorganism control in water treatment or aquaculture systems.

ABSTRAK

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

BIOSINTESIS, FABRIKASI DAN ANALISIS POLI(3-HIDROKSIBUTIRAT) DAN CAMPURAN AGEN ANTIMIKROB SEBAGAI KAWALAN BAKTERIA DALAM AKUAKULTUR

TEH WENG KERN

2018

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Kawalan bakteria dalam sistem air akuakultur adalah penting kerana masalah yang paling menonjol yang dihadapi dalam sistem ini adalah kadar kematian yang tinggi yang disebabkan oleh jangkitan bakteria yang tidak terkawal. Langkah-langkah semasa yang digunakan untuk mengawal jangkitan bakteria menunjukkan kesan-kesan sampingan negatif dari penggunaan jangka panjang kerana ia tidak hanya memberi kesan kepada alam sekitar tetapi juga manusia. Poli (3-hidroksibutirat) [P (3HB)], polimer biodegradasi yang berasal dari bakteria telah dikaji kerana berpotensi untuk bertindak sebagai matriks untuk mengenggam agen antimikrob. Imobilisasi agen antimikrob yang berpanjangan pada matriks biodegradasi mampu meningkatkan potensi perencatan mikroorganisma secara mesra alam. Campuran filem antimikrob P (3HB) akan bertindak sebagai sistem pelepasan masa yang melepaskan sebatian antimikrob yang berbeza berdasarkan degradasi filem. Titanium dioksida (TiO_2) adalah oksida logam dengan sifat fotokatalitik yang dapat menguraikan bahan-bahan organik melalui pembentukan radikal hidroksil yang disifatkan sebagai ciri-ciri antimikrobnya. Rhamnolipid (RL) adalah glikolipid biosurfaktan yang menunjukkan tindak balas antimikrobial terhadap bakteria dan virus. Di sini, P (3HB) dan RL dihasilkan melalui penapaian bakteria menggunakan *Bacillus megaterium* UMTKB-1 dan *Pseudomonas aeruginosa* UMTKB-5 daripada sumber bahan mentah karbon produk sampingan industri. Campuran P (3HB) dan TiO_2 serta RL diuji dengan pelbagai jenis bakteria patogen untuk keberkesanan potensi antimikrobnya. Gabungan

filem P (3HB) -TiO₂ dapat merencat pertumbuhan bakteria dengan mengurangkan unit pembentukan koloni (CFU) dengan penurunan secara tetap setiap jam untuk bakteria tertentu seperti *B. megaterium* UMTKB-1, *E. coli* ataupun *S. aureus*. Ujian antimikrob menggunakan RL sebagai agen antimikrobial menghasilkan keputusan yang memberangsangkan dengan zon perencatan 12 mm berdasarkan ujian penyebaran cakera menggunakan *B. megaterium* dan *Vibrio harveyii*. Secara umumnya, kejayaan perencatan filem P (3HB) – RL terhadap bakteria adalah antara 60-80%. Ujian persekitaran yang dijalankan menunjukkan perencatan pertumbuhan yang ketara terhadap bakteria *Vibrio* spp lebih kurang sebanyak 50% apabila RL digunakan. Penggunaan P (3HB) bersama agen antimikrobial yang berjaya sebagai sistem perencatan mikrobial yang menggunakan system pelepasan masa akan berfungsi sebagai kaedah mesra alam untuk mengawal mikroorganisma patogenik dalam rawatan air atau sistem akuakultur.