PRODUCTION OF POLYHYDROXYALKANOATE (PHA) FROM PALM OIL BY *Pseudomonas oleovorans* AND AN INDIGENOUS BACTERIAL ISOLATE, *Pseudomonas* sp.

BY

ZAZALI ALIAS

A dissertation submitted in partial fulfilment for the Degree of
Master of Biotechnology
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ACKNOWLEDGEMENT

Dengan nama ALLAH yang Maha Pemurah lagi Maha Penyayang

Setinggi-tinggi ribuan terimakasih diucapkan kepada Dr. Irene K.P.Tan yang telah memberi kepercayaan, pendedahan serta tunjuk ajar yang berharga disepanjang waktu kursus. Begitu juga, Profesor K.B. Ramachandran, yang telah membantu memudahkan perjalanan penyelidikan dengan sumbangan pandangan yang membina.

Terima kasih juga diucapkan kepada teman sekerja, Pian, Ho dan Shaza; sokongan moral, buah fikiran serta tunjuk ajar anda semua adalah sangat disanjungi. Begitu juga, En. Karim, yang telah membantu melicinkan penggunaan peralatan makmal.

Terimakasih juga, kepada pihak Jabatan Kimia, makmal Biologi Molekul, Mikologi, N.P.C. dan Alga, diatas sokongan memberi bantuan penggunaan alatan makmal dan analysis. Begitu juga, Molly, Kak Zaini dan Rafiati yang telah membantu mendapatkan bahan-bahan rujukan.

Keluarga tercinta; emak, Ina, kakak, Emy dan terutamanya arwah ayah, anda semua adalah inspirasi untuk menjadi lebih cekal mengejar cita-cita dan kejayaan. Terimakasih yang mendalam diatas segala pengorbanan yang telah diberikan. Begitu juga buat tunang tersayang, Elly, diatas segala sokongan dan galakan.

Buat akhirnya, terimakasih juga, kepada pihak IRPA, UM, yang membantu dari segi kewangan dalam menjayakan projek ini.

Sekian

Zazali Alias

ABSTRACT

In this study, the yield of biomass and polyhydroxyalkanoate (PHA) of *Pseudomonas oleovorans* grown in batch and fed-batch cultivations were compared. When oleic acid (OA) (0.5% w/v) was used as carbon source, a significant increase in biomass and PHA content were observed through fed-batch cultivation. The biomass and PHA content obtained from batch cultivation were 1.45±0.19 g/l and 2.50±0.77 % cell dry weight (CDW) respectively, while in the fed-batch cultivation, the biomass and PHA content increased significantly to 3.35±0.78 g/l and 32.72±0.30 %CDW respectively.

When saponified palm olein (SPO) (0.5% w/v) was used as a carbon source, the batch cultivation yielded 1.54±0.03 g/l biomass and 3.40±0.76 %CDW of PHA. In the fed-batch cultivation, there was no significant increase in biomass yield (1.9±0.66 g/l) but there was a significant increase in PHA content (14.46±1.18 %CDW).

In the batch cultivation, yield of PHA ($Y_{p/s}$), from OA and SPO were 8.05 mg/g and 10.47 mg/g respectively. The polymer obtained when either OA or SPO was used as a carbon source was a medium chain length PHA (MCL_{PHA}) with hydroxyoctanoic acid (C_8) being the major monomer.

Palm oil-mill effluent (POME) was chosen as the source to isolate and screen bacteria capable of utilising palm oil directly for growth and PHA accumulation. Bacterial isolates were screened by using Sudan Black B and Nile Blue A staining.

Out of 45 isolates, none was capable of utilising palm oil directly for growth. Isolate X4.13 was however, able to grow and accumulate PHA from SPO. When isolation was carried out by an enrichment technique, one isolate, FLP1, was found to be able to grow and accumulate PHA directly from crude palm oil, palm olein, palm stearin, palm kernel oil and oleic acid. The superior isolate was identified as *Pseudomonas* sp. The PHA was then analysed to be polyhydroxybutyrate (PHB), a short chain length PHA (SCL_{PHA}).

Thus, palm oil appears to be a suitable carbon source which could be converted to MCL_{PHA} as well as SCL_{PHA} depending on the bacteria metabolising the oil.

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ATP	adenosine triphosphate
CDW	cell dry weight
CFU	colony forming unit
CoASH	free coenzyme A
DO	dissolved oxygen
FAO	fatty acid oxidative enzyme
g/l	gram per litre
GC	gas chromatography
IR	infra red
LCFA	long chain fatty acid
LCL	long chain length
LPS	lipopolysaccharide
MCFA	medium chain fatty acid
MCL	medium chain length
NA	nutrient agar
NADP	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
OA	oleic acid

OD	optical density
P(3HB)	poly-3-hydroxybutyric acid
P(3HV)	poly-3-hydroxyvaleric acid
PHA	polyhydroxyalkanoate
PHB	polyhydroxybutyrate
PKO	palm kernel oil
PO	palm olein
POME	palm oil-mill effluent
r.p.m.	rotation per minute
RF	response factor
SCFA	short chain fatty acid
SCL	short chain length
SD	standard deviation
SPKO	saponifiend palm kernel oil
SPO	saponified palm olein
TCA	tricarboxylic acid
v/v	volume per volume
w/v	weight per volume