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
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at the
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Dengan nama ALLAH yang Maha Pemurah lagi Maha Penyayang

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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 $1.45 \pm 0.19$ g/l and $2.50 \pm 0.77$ % cell dry weight (CDW) respectively, while in the fed-batch cultivation, the biomass and PHA content increased significantly to $3.35 \pm 0.78$ g/l and $32.72 \pm 0.30$ %CDW respectively.

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

Thus, palm oil appears to be a suitable carbon source which could be converted to MCL\textsubscript{PHA} as well as SCL\textsubscript{PHA} depending on the bacteria metabolising the oil.
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>CDW</td>
<td>cell dry weight</td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming unit</td>
</tr>
<tr>
<td>CoASH</td>
<td>free coenzyme A</td>
</tr>
<tr>
<td>DO</td>
<td>dissolved oxygen</td>
</tr>
<tr>
<td>FAO</td>
<td>fatty acid oxidative enzyme</td>
</tr>
<tr>
<td>g/l</td>
<td>gram per litre</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>IR</td>
<td>infra red</td>
</tr>
<tr>
<td>LCFA</td>
<td>long chain fatty acid</td>
</tr>
<tr>
<td>LCL</td>
<td>long chain length</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
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<tr>
<td>MCFA</td>
<td>medium chain fatty acid</td>
</tr>
<tr>
<td>MCL</td>
<td>medium chain length</td>
</tr>
<tr>
<td>NA</td>
<td>nutrient agar</td>
</tr>
<tr>
<td>NADP</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
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<td>OA</td>
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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  saponified palm kernel oil
SPO  saponified palm olein
TCA  tricarboxylic acid
v/v  volume per volume
w/v  weight per volume