EVALUATION OF JATROPHA OIL FOR THE PRODUCTION OF POLYHYDROXYALKANOATES (PHAs) BY Cupriavidus necator H16

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by

NG KO SIN

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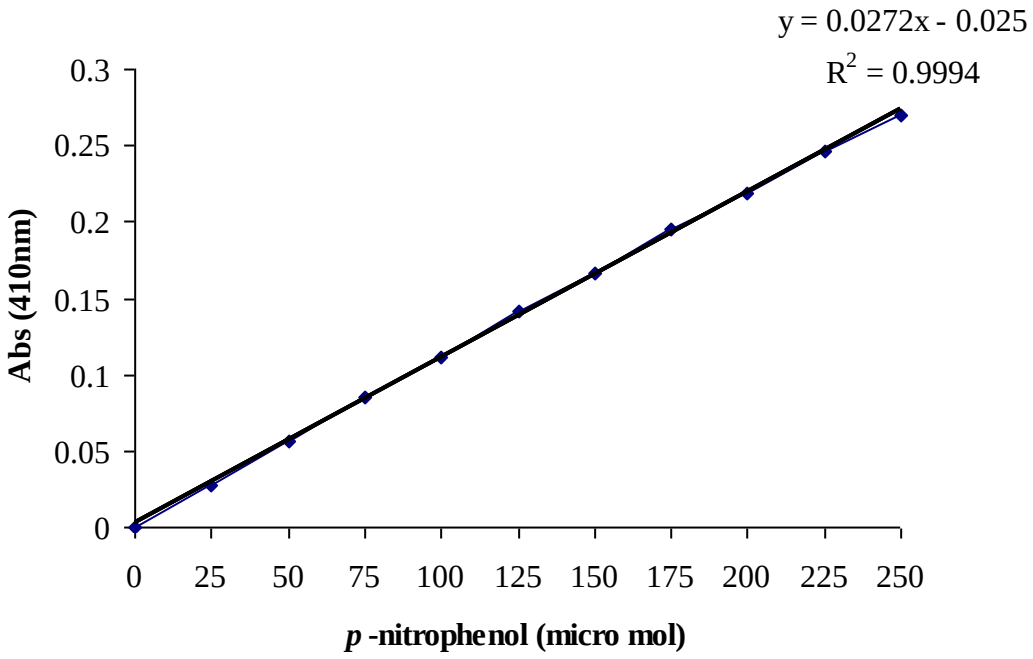
APPENDICES

Appendix A Standard curve of *p*-nitrophenol.



Appendix B Biosynthesis of P(3HB-co-3HV) copolymer by *C. necator* H16 using the mixture of jatropha oil and sodium valerate or sodium propionate[a].

Carbon concentration (g/L)	Total PHA (g/L)	PHA composition (mol%)		Mass concentration (g/L)[b]		Carbon concentration (g/L)		Conversion of precursors into 3HV (%)[e]
		3HB	3HV	3HB	3HV	3HB[c]	3HV[d]	
Sodium valerate								
0.48	8.3	97	3	8.1	0.2	4.51	0.15	31
1.44	5.6	91	9	5.1	0.5	2.85	0.30	21
2.40	5.4	80	20	4.3	1.1	2.42	0.65	27
3.36	4.7	68	32	3.2	1.5	1.79	0.90	27
4.32	5.1	59	41	3.0	2.1	1.69	1.25	29
Sodium propionate								
0.48	6.6	98	2	6.5	0.1	3.62	0.08	17
1.44	5.4	92	8	5.0	0.4	2.78	0.26	18
2.40	4.9	86	14	4.2	0.7	2.36	0.41	17
3.36	3.8	77	23	2.9	0.9	1.64	0.52	16
4.32	4.3	73	27	3.1	1.2	1.76	0.70	16

PHA, polyhydroxyalkanoate; 3HB, 3-hydroxybutyrate; 3HV, 3-hydroxyvalerate

[a] Carbon concentration of oil = Oil utilized (g/L) × 0.76 g carbon/g oil
Assuming that the molar mass of 3HB ($C_4H_6O_2$) and 3HV unit ($C_5H_8O_2$) are 86.11 and 100.13 g/mol respectively (molar mass of butyric acid and valeric acid with two proton less). Molar mass of carbon is 12.01 g/mol.

[b] Mass concentration of 3HB or 3HV (g/L) = Total PHA (g/L) × 3HB or 3HV composition (mol%)

[c] Carbon concentration of 3HB (g/L) = Mass concentration of 3HB × 0.56 g carbon/g 3HB

[d] Carbon concentration of 3HV (g/L) = Mass concentration of 3HV × 0.60 g carbon/g 3HV

e Conversion of precursor into 3HV (%) = Carbon concentration of 3HV (g/L)/ Carbon concentration of precursors (g/L)

Appendix C Conversion percentage of precursors into 3HV monomer.

Strains	Carbon sources	Precursor	Feeding strategy	3HV composition (mol%)	Conversion of precursors into 3HV (%)	Reference
C. necator H16	Sodium acetate	Sodium propionate	Single feeding at 0 h	2 − 44	4 − 9	Doi et al., 1987
C. necator H16	Butyric acid	Propionic acid	Single feeding at 0 h	15 − 85	5 − 13	Doi et al., 1988
C. necator H16	CPKO	Sodium valerate	Single feeding at 0, 12, 24,36,48	4 − 10	7 − 18	Bhubalan, 2010
		Sodium propionate		2 − 11	2 − 15	
C. necator H16	CPKO, Olive oil, Sunflower oil, PKO, Crude palm oil, Cooking oil and Coconut oil	Sodium valerate	Sequential feeding at 48 h and 60 h	3 − 14	2 − 19	Lee et al., 2008
		Sodium propionate		2 − 8	3 − 15	
Cupriavidus sp. JC-64	Cottonseed oil	Valeric acid	Two-stage cultivation. Precursor fed at 0 h of second-stage.	12 − 64	15 − 19	Song et al., 2001
C. necator NCIMB 11599	Glucose	Propionic acid	Two-stage cultivation. Precursor fed at 0 h of second-stage	5 − 9	0.2 – 0.4	Lee et al., 1996

LIST OF PUBLICATION

PEER REVIEWED JOURNAL

Ng, K.-S., Ooi, W.-Y., Goh, L.-K., Shenbagarathai, R. & Sudesh, K. (2010) Evaluation of jatropha oil to produce poly(3-hydroxybutyrate) by *Cupriavidus necator* H16. *Polymer Degradation and Stability,* 95**,** 1365-1369.

Ng, K.-S., Wong, Y.-M., Tsuge, T. & Sudesh, K. (2011) Biosynthesis and characterization of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) copolymers using jatropha oil as the main carbon source. *Process Biochemistry* (In Press)

CONFERENCE PRESENTATION

Ng, K.-S., Ooi, W.-Y., Goh, L.-K., Shenbagarathai, R. & Sudesh, K. (2009) Evaluation of jatropha oil to produce poly(3-hydroxybutyrate) by *Cupriavidus necator* H16. The 2nd International Conference on Bio-based Polymers 2009, Universiti Sains Malaysia. (Poster)

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β	Beta
°C	Degree Celcius
ΔH_m	Enthalpy of fusion
µm	Micrometer
µL	Microliter
%	Percent
±	Plus-minus
× *g*	Times gravity
(*R*)-	*Rectus*-isomer
(*S*)-	*Sinister*-isomer
3HB	3-hydroxybutyrate
3HHx	3-hydroxyhexanoate
3HO	3-hydroxyoctanoate
3HP	3-hydroxypropionate
3HV	3-hydroxyvalerate
4HB	4-hydroxybutyrate
5HV	5-hydroxyvalerate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
ANOVA	Analysis of variance

C	Carbon atom
$C_{12:0}$	Lauric acid
$C_{14:0}$	Myristic acid
C_{16}	Palmitic acid
$C_{16:1}$	Palmitoleic
$C_{18:0}$	Stearic acid
$C_{18:1}$	Oleic acid
$C_{18:2}$	Linoleic acid
$C_{18:3}$	Linolenic acid
$C_{20:0}$	Arachidic acid
$-CH_2$	Methylene group
$-CH_3$	Methyl group
-CH	Methine group
$CaCl_2$	Calcium chloride
$CDCl_3$	Deuterated chloroform
CDW	Cell dry weight
CME	Caprylic acid methyl ester
C/N	Carbon-to-nitrogen
CPKO	Crude palm kernel oil
CoA	-Coenzyme-A
CoASH	Coenzyme-A with sulfhydryl functional group
$CoCl_2 \cdot 6H_2O$	Cobalt (II) chloride hexahydrate
$CrCl_3 \cdot 6H_2O$	Chromium chloride hexahydrate

$CuSO_4 \cdot 5H_2O$	Copper (II) sulphate pentahydrate
Da	Dalton
DMSO	Dimethyl sulfoxide
DSC	Differential scanning calorimetry
EstA	Esterase
FAD	Flavin adenine dinucleotide
$FADH_2$	Reduced flavin adenine dinucleotide
FAO	Facts about Food and Agriculture Organization
$FeCl_3$	Iron (II) chloride
g	Gram
GC	Gas chromatography
GPC	Gel permeation chromatography
h	Hour
ha	Hectare
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
ICI	Imperial Chemical Industries
JaI	Jatropherol-I
kg	Kilogram
KH_2PO_4	Potassium dihydrogen phosphate
kPa	Kilopascal
L	Liter
L.	Linnaeus

m	Meter
mcl-	Medium-chain-length
mcl-PHA	Medium-chain-length PHA
mg	Miligram
$MgSO_4 \cdot 7H_2O$	Magnesium sulphate heptahydrate
MHz	Megahertz
min	Minute
mL	Mililiter
MM	Mineral salts medium
mM	Milimolar
mm^3	Milimeter cube
mol%	Mole percent
M_n	Number-average molecular weight
M_w	Weight-average molecular weight
M_w / M_n	Polydispersity index
N	Normality
N-terminal	Amine terminal
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
Na_2HPO_4	Disodium hydrogen phosphate
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide

NH_4Cl	Ammonium chloride
$NH_4H_2PO_4$	Ammonium dihydrogen phosphate
NH_4NO_3	Ammonium nitrate
$(NH_4)_2SO_4$	Ammonium sulphate
$(NH_2)_2CO$	Urea
$NiCl_2 \cdot 6H_2O$	Nickel chloride hexahydrate
nm	Nanometer
NMR	Nuclear magnetic resonance
NR	Nutrient rich
OD	Optical density
OD_{600}	Optical density at wavelength 600 nm
OD_{410}	Optical density at wavelength 410 nm
P(3HB)	Poly(3-hydroxybutyrate)
P(3HB-*co*-3HV)	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
PAO	Palm acid oil
PKAO	Palm kernel acid oil
PKO	Palm kernel oil
PHA	Polyhydroxyalkanoate
PhaA	*β*-ketothiolase
PhaB	NADPH-dependent acetoacetyl-CoA reductase
PhaC	PHA synthase
PhaP	Phasin
PhaR	PHA-specific regulator protein

PhaZ	PHA depolymerase
PLA	Poly(lactic acid)
*p*NPL	*p*-nitrophenyl laurate
ppm	Parts per million
Psi	Pounds per square inch
PTFE	Polytetrafluoroethylene
PVA	Polyvinyl alcohol
rpm	Rotations per min
Scl-	Short-chain-length
scl-PHA	Short-chain-length PHA
TCA	Tricarboxylic acid
TEM	Transmission electron microscope
TGA	Thermogravimetric analysis
Tukey's HSD	Tukey's Honestly Significant Difference
T_d	Decomposition temperature
T_g	Glass-transition temperature
T_m	Melting temperature
U	Unit
UHMW	Ultra-high-molecular-weight
USD	United States Dollar
v/v	Volume per volume
w/v	Weight per volume
wt%	Dry weight percent

PENILAIAN MINYAK JATROPHA BAGI PENGHASILAN POLIHIDROKSIALKANOAT (PHA) OLEH *CUPRIAVIDUS NECATOR* H16

ABSTRAK

Minyak jatropha ialah sejenis minyak yang tidak boleh dimakan tetapi boleh digunakan sebagai substrat alternatif bagi menggantikan minyak makan dalam penghasilan polihidroksialkanoat (PHA). Minyak jatropha mengandungi asid oleik (42%), asid linoleik (35%) dan asid palmitik (17%) sebagai komponen utama dalam komposisi minyak tersebut. *Cupriavidus necator* H16 menghasilkan sebanyak 87 % (b/b) poli(3-hidroksibutirat) [P(3HB)] daripada 13.1 g/L berat kering sel (BKS) apabila 12.5 g/L minyak jatropha dan 0.54 g/L urea digunakan. Aktiviti lipase dapat dikesan pada peringkat awal penghasilan P(3HB) apabila 1 g/L minyak jatropha ditambah ke dalam medium prakultur. Namun begitu, penambahan minyak dalam prakultur tidak memberi kesan kepada BKS akhir and pengumpulan P(3HB). Kopolimer poli(3-hidroksibutirat-ko-hidroksivalerat) [P(3HB-*co*-3HV)] dengan fraksi molar 3HV yang berlainan (4 – 41 mol%) dapat dihasilkan apabila natrium valerat dan natrium propionat digunakan sebagai prekursor. Jumlah unit 3HV yang dihasilkan dalam polimer adalah berkadar langsung dengan kepekatan prekursor yang dibekalkan. Komposisi 3HV unit (41 mol%) dan BKS (6.7 g/L) yang dihasilkan daripada natrium valerat adalah lebih tinggi berbanding dengan natrium propionat. Sifat-sifat termal homopolimer P(3HB) dan kopolimer P(3HB-*co*-3HV) telah ditentukan dengan menggunakan DSC dan TGA. Selain itu, berat molekul polimer ditentukan dengan menggunakan GPC. Keputusannya menunjukkan PHA yang dihasilkan daripada minyak jatropha pada dasarnya adalah sama dengan polimer yang

dihasilkan daripada sumber karbon yang lain seperti gula dan minyak-minyak tumbuhan lain. Kajian ini telah menunjukkan bahawa minyak jatropha adalah satu sumber karbon yang boleh diperbaharui dan berpotensi bagi penghasilan PHA.

EVALUATION OF JATROPHA OIL FOR THE PRODUCTION OF POLYHYDROXYALKANOATES (PHAs) BY *CUPRIAVIDUS NECATOR* H16

ABSTRACT

Jatropha oil, a non-edible vegetable oil, could serve as an alternative substrate to food grade oils for polyhydroxyalkanoates (PHAs) production. Jatropha oil contains oleic acid (42%), linoleic acid (35%) and palmitic acid (17%) as the major fatty acid constituents. *Cupriavidus necator* H16 accumulated 87 wt% of poly(3-hydroxybutyrate) [P(3HB)] from 13.1 g/L of cell dry weight (CDW) when 12.5 g/L of jatropha oil and 0.54 g/L of urea were used. Lipase activity was detected in the initial stages of P(3HB) production, when 1 g/L of jatropha oil was added to the preculture medium. However, the addition of oil in preculture did not affect final CDW and P(3HB) accumulation. Copolymers of poly(3-hydroxbutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] with different molar fractions of 3HV (4 – 41 mol%) were produced when sodium valerate or sodium propionate were used as precursors. The amount of 3HV units incorporated in the polymer was proportional to the concentration of precursors. However, higher composition of 3HV units (41 mol%) and CDW (6.7 g/L) were produced from sodium valerate compared to sodium propionate. The thermal properties of P(3HB) homopolymer and P(3HB-*co*-3HV) copolymer were determined by Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). The molecular weights of the polymers were determined by Gas Permeation Chromatography (GPC). The results revealed that the PHAs produced from jatropha oil were essentially the same as that produced from other more established carbon sources such as sugars and plant oils. This

study has demonstrated that jatropha oil is a potential renewable carbon source for the production of PHA.

1.0 INTRODUCTION

PHAs are biodegradable plastics that have versatile thermal and mechanical properties which make them potential alternatives to petroleum-based synthetic plastics (Khanna and Srivastava, 2005a, Sudesh and Iwata, 2008). The use of biodegradable plastics will reduce non-biodegradable solid wastes (Shah et al., 2008, Sudesh and Iwata, 2008). PHAs also have great potential in medical applications owing to their *in vivo* and *in vitro* biocompatibility and biodegradability (Chen and Wu, 2005, Martin and Williams, 2003, Ying et al., 2008). To realize this potential, however, the cost of PHAs production must come down several folds. The cost of carbon feedstock can significantly affect the PHAs production cost. Therefore, the identification of alternative cost-effective fermentative substrates for PHAs synthesis has become an important objective for the commercialization of these biopolymers.

Various substrates for PHAs production especially plant oils, e.g. soybean oil (Kahar et al., 2004), palm oil (Lee et al., 2008, Loo et al., 2005), olive oil, sunflower oil and coconut oil (Lee et al., 2008) had been evaluated and found to be excellent carbon sources for PHAs production. P(3HB) yields and the cumulative energy of the production process from plant oils are much higher than that from glucose due to their high carbon content per weight and less usage (Akiyama et al., 2003). However, the use of food grade oils to produce bioplastics may cause imbalance of global food supply and depletion of food sources. It is both wasteful and unethical to convert food sources into bioplastics. Besides, the recent food shortage crisis, limited land availability for food industry and increasing food demand have forced the price of plant oils to increase drastically (Gui et

al., 2008). To overcome these problems, non-edible jatropha oil could be utilized as an alternative substrate for the synthesis of these green materials.

Jatropha oil is a potential renewable resource because jatropha plantations yield large amount of oil, are highly resistant to drought and pests and the oil is relatively cheap and non-edible. Jatropha oil is derived from *Jatropha curcas* seeds. This plant was originally found in the Carribean area but is now widespread throughout Africa, the Americas and much of Asia (Heller, 1996). The plant also is known as "hardy" *Jatropha* due to its resistance to pest and drought, and also its ability to grow on almost anywhere (Openshaw, 2000). The oil yield of this plant is about 1590 kg oil/ha annually which is almost four-fold of soybean, and ten-fold of maize (Fitzgerald, 2006). Recently, jatropha oil has been evaluated as a source of high quality biodiesel production (Patil and Deng, 2009, Rajesh et al., 2008) and the cost is expected to be lower than using rapeseed, soybean, palm oil and waste cooking oil (Lim and Teong, 2010). In Malaysia, *Jatropha* has been put into consideration as supplementary renewable feedstock for biodiesel production in order to balance the renewable energy supply and exportation of biodiesel (Lim and Teong, 2010). The land cultivation will increase to 1 million hectares by the end of 2010. Realizing the increasing potential of *Jatropha* as renewable energy, jatropha oil was evaluated as a feedstock for PHAs production in this study.

The genus *Jatropha* belongs to the *Euphorbiaceae* family which can synthesize several toxic compounds, including carcinogenic phorbol ester, trypsin inhibitor, lectin and saponin (Kumar and Sharma, 2008, Martínez-Herrera et al., 2006). The toxins render the oil non-edible, but should not affect its utility for bioplastics production. Therefore, it

is advantageous to use jatropha oil which is not food grade oil as sole carbon source to produce PHAs.

This study was conducted to evaluate the suitability of jatropha oil for the synthesis of PHA by *C. necator* H16. It is a potential carbon source to substitute food grade oils for large scale production of bioplastics in an economical and sustainable manner.

1.1 The objectives of this study

1. To evaluate the efficacy of jatropha oil as the main carbon source to support the growth and PHAs production by *C. necator* H16.
2. To biosynthesize P(3HB-*co*-3HV) copolymer from jatropha oil with supplementation of precursors.
3. To characterize the physical properties of PHAs produced from jatropha oil.

2.0 LITERATURE REVIEW

2.1 Biobased and biodegradable polymers

Biobased polymers are polymers produced from natural renewable resources and polymerized by chemical and/or biological methods. Biobased polymers are categorized into three groups: bio-chemosynthetic polymers [e.g., poly(lactic acid), poly(butylene succinate), polyvinyl alcohol and polygycolic acid], biosynthetic polymers, bioplastics or naturally occurring polymers (e.g., PHAs) and modified natural polymers (e.g., starch polymers, cellulose derivatives) (Sudesh and Iwata, 2008). For bio-chemosynthetic polymers, the monomers are synthesized biologically and polymerized chemically. An example of bio-chemosynthetic polymer is poly(lactic acid) (PLA). The lactate is synthesized by microbial and chemically polymerized into PLA catalyzed by metal (Jem et al., 2010). Unlike bio-chemosynthetic polymers and biosynthetic polymers, the natural polymers such as starch need to be modified chemically and/or physically to enhance the polymer structure and improve the thermal and mechanical properties (Hoover et al., 2010).

However, not all biobased polymers are biodegradable. Some of the biobased polymers such as crystalline PLA, cellulose derivatives and polythioesters are not biodegradable (Steinbüchel, 2005, Sudesh and Iwata, 2008). Biodegradable polymers can be degraded hydrolytically and/or ezymatically which involves the break down of polymers bonding (Nair and Laurencin, 2007). The natural polymers such as starch and proteins can be ezymatically degraded by various enzymes such as amylases and proteases, while, the polymers possess functional groups such as esters, anhydrides,

carbonates, amides and urea can be hydrolyzed (Flieger et al., 2003, Nair and Laurencin, 2007).

Table 2.1 Some examples of biobased and biodegradable polymers (Flieger et al., 2003, Nair and Laurencin, 2007, Sudesh and Iwata, 2008).

Category	Processes involved	Example	Biodegradability
Bio-chemosynthetic polymers	Biological synthesis of monomers and chemical polymerization	Poly(lactic acid) Poly(butylene succinate) Polyvinyl alcohol Polygycolic acid Polythioesters	Hydrolytically degradable except crystalline poly(lactic acid) and polythioesters
Biosynthetic polymers	Biosynthesis of polymer by microorganisms	Polyhydroxybutyrates	Enzymatically and/or hydrolytically degradable
Modified natural polymers	Chemical modification of natural polymer	Starch polymer Cellulose derivatives Proteins	Enzymatically degradable

Most natural occurring polymers such as PHAs undergo hydrolysis of ester bonds which are catalyzed by intracellular or extracellular depolymerase enzymes (Guérin et al., 2010). These depolymerase enzymes are secreted by various bacteria and fungi during carbon starvation to break down the PHAs into carbon dioxide and water under aerobic conditions or methane under anaerobic conditions for energy utilization. Thin solvent cast PHAs films can be biodegraded in less than two months under tropical conditions with high microbiological activities (Sudesh and Iwata, 2008). PHAs are good candidates for biodegradable plastics applications due to their similar properties to those of conventional plastics but with high biodegradability. The common homopolymer, P(3HB) can be hydrolytically degraded to a normal blood constituent in human's body, thus, it has

potential application as biomaterial (Nair and Laurencin, 2007). However, because of the high crytallinity, this polymer degrades at a slower rate. Another type of PHA, P(3HB-*co*-3HV) with lower crystallinity and higher rate of degradation is suggested as a temporary substrate for tissue engineering such as bone tissue and epithelial tissue (Chen and Wu, 2005, Köse et al., 2003). The PHAs containing 4-hydroxybutyrate (4HB) monomer are probably the best to be used as biomaterials for tissue engineering because it can be hydrolyzed by the lipases of eukaryotes (Sudesh, 2004).

2.2 Polyhydroxyalkanoates (PHAs)

PHAs are biopolymers accumulated in various microorganisms as carbon storage during imbalanced conditions for cell growth (Anderson and Dawes, 1990). PHAs were first discovered as sudanophilic lipid-like inclusion in *Azotobacter chroococcum* and later identified as P(3HB) which was accumulated in *Bacillus megaterium* (Braunegg et al., 1998, Sudesh et al., 2000). Long before that, PHAs were observed as granules in cytoplasm of bacterial cells. To date, various Gram positive and Gram negative bacteria have been identified to biosynthesize PHAs in conditions with excessive carbon and limiting nutrients such as nitrogen, phosphorus, magnesium and phosphorus (Anderson and Dawes, 1990, Doi, 1990, Steinbüchel and Füchtenbusch, 1998).

PHA granules can be observed as refractive inclusions under phase contrast microscope. The lipid-like PHA granules can also be detected easily under fluorescent microscope after they are stained by Nile blue A or its oxazone form, Nile red (Ostle and Holt, 1982, Wu et al., 2003). Both of the fluorescent stains have more specificity and higher affinity toward PHA granules than Sudan black stain. Under transmission electron

microscope (TEM), the ultrastructural cross section of bacteria containing PHA granules can be observed as electron-dense discrete inclusions localized in the cytoplasm (Tian et al., 2005). In some studies, TEM observation was used to study the proliferation of cells and expression of PHA genes on the granules formation and initiation (Jendrossek, 2005, Pötter et al., 2002).

PHAs have been receiving much attraction as a potential partial substitute for conventional plastics. Despite their biodegradability, the material properties of PHAs are very similar to the conventional plastics ranging from polypropylene to synthetic rubber (Lee, 1996b, Sudesh et al., 2000, Verlinden et al., 2007). Unlike conventional plastics, PHAs are produced from various renewable sources such as sugars (Kolibachuk et al., 1999, Lee et al., 1995), fatty acids (Doi et al., 1988), plant oils (Kek et al., 2008, Loo et al., 2005), agricultural by-products (Bormann and Roth, 1999, Gouda et al., 2001) and food industrial wastes (Ma et al., 2005). Until present, approximately 150 different constituents of PHA homopolymers and copolymers have been identified (Steinbüchel and Lütke-Eversloh, 2003). Among these PHAs, P(3HB) and P(3HB-*co*-3HV) are the first and the most studied homopolymer and copolymer.

2.3 Initiation and formation of PHA

PHA granules are insoluble in cytoplasm and surrounded by a layer of phospholipids membrane consisting granule-associated proteins such as PHA synthase (PhaC), PHA depolymerase (PhaZ), phasin (PhaP) and PHA-specific regulator protein (PhaR) (Rehm, 2006, Sudesh et al., 2000), have attracted scientists to investigate the formation and initiation of PHA. The key enzyme for the PHA biosynthesis is PHA

synthases (PhaCs). Basically, during the biosynthesis of PHA, the water-soluble PHA monomers and PhaCs are linked covalently. The continuous growing of polymer chains makes them become more hydrophobic and insoluble in the cytoplasm (Gerngross et al., 1993). The further formation of PHA granules is proposed in two models, micelle model and budding model.

Micelle model (Figure 2.1 A) proposes that the PhaCs and the substrates for the PHA synthases [(*R*)-3-hydroxybutyryl-CoA] are distributed randomly in the cytoplasm (Gerngross et al., 1994). When the polymerization starts, the polymer chains elongates from the PhaCs. The generated hydrophobic polymer chains with one end of hydrophilic PhaCs interact to each other and gather to form micelle like molecules or granules. All the PhaCs stay at the surface of the granules, while the polymer chains continue to grow inwardly and dense. Finally, the dense granules become larger and may fuse with the neighboring granules.

The budding model (Figure 2.1 B) proposes that the PhaCs are attached to the cytoplasmic membrane and the PHA chains which hydrophobically interact with the cytoplasmic membrane are grown into periplasm between the phospholipid bilayer (Tian et al., 2005). The growing PHA granules are budding toward the cytoplasm and surrounded by the membrane. Finally, the PHA granules are detached from the membrane and released into the cytoplasm. Other PHA specific proteins then attach to the surface of the granules. Some studies reported that the PHA granules are localized near the cell poles in *Rhodospirillum rubrum, C. necator H16* and recombinant *Escherichia coli* (Jendrossek, 2005, Peters et al., 2007). However, Tian and co-workers (2005) reported that the formation of granules in wild-type *C. necator* at early stage was

near to the dark-stained mediation elements which were found at the longitudinal centre of cells. A similar observation was reported by Kek and co-workers (Kek et al., 2008). They proposed that the mediations might act as the nucleation sites for the initiation of P(3HB) granules.

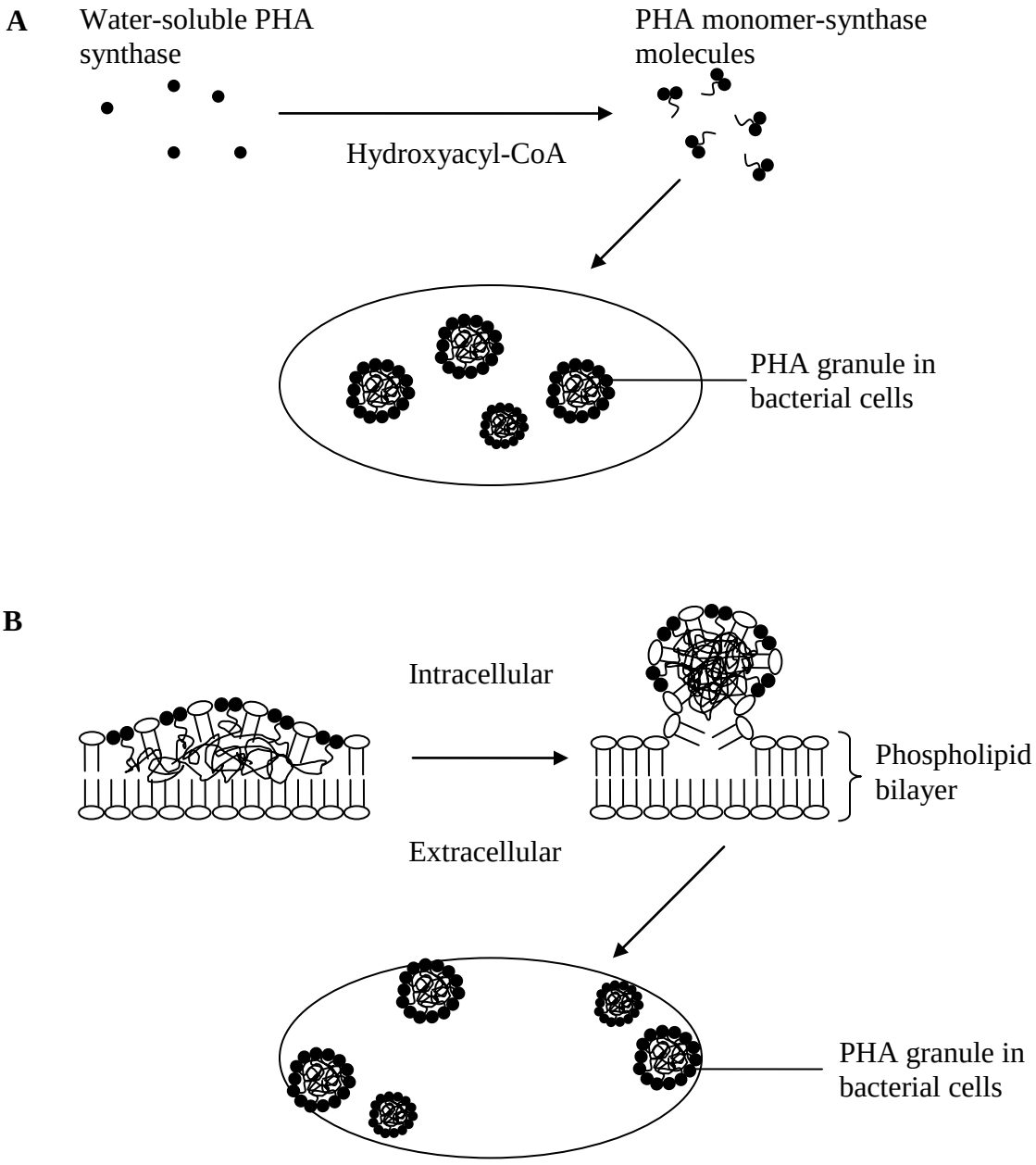
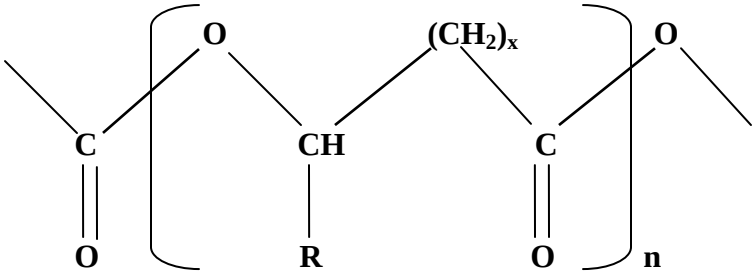


Figure 2.1 Two proposed model of PHA initiation and formation: (A) micelle model and (B) budding model (Rehm, 2006).

2.4 Chemical structure of PHA

The chemical structures of PHAs are constructed based on a basic structure of (*R*)-3-hydroxyalkanoic acid as shown in Figure 2.2. The number of carbon of the side group (R) can vary from methyl (C_1) to tridecyl (C_{13}) (Verlinden et al., 2007) and it is used to classify PHA monomer units. In the biosynthetic mechanisms of bacterial cells, carbon substrates are converted into (*R*)-3-hydroxyacyl-CoA which is then polymerized into a polymer chains catalyzed by PhaC (Doi et al., 1987). Polymerization involves esterification to form ester bonds between carboxyl group and hydroxyl group of monomers. A homopolymer is constructed from the same type of monomer while a copolymer is constructed from different type of monomers. The n refers to the repeating units of monomers and typically in the range of 100 – 30000 (Lee, 1996a). It depends on the type of the monomer produced and the bacterial strain used.



Number of methylene group (x)	Side group (R)	Type of monomer
1	hydrogen	3-hydroxypropionate (3HP)
	methyl	3-hydroxybutyrate (3HB)
	ethyl	3-hydroxyvalerate (3HV)
	propyl	3-hydroxyhexanoate (3HHx)
	pentyl	3-hydroxyoctanoate (3HO)
2	hydrogen	4-hydroxybutyrate (4HB)
3	hydrogen	5-hydroxyvalerate (5HV)

- n refers to number of repeating unit.

Figure 2.2 Classification of monomers based on the chemical structures (Braunegg et al., 1998, Khanna and Srivastava, 2005a).

Based on the number of carbon in the monomers, PHAs are categorized into short-chain-length PHAs (scl-PHAs), medium-chain-length PHAs (mcl-PHAs) and multi-component PHAs (scl-mcl-PHAs) (Braunegg et al., 1998, Khanna and Srivastava, 2005a). Scl-PHA contains 3 to 5 carbon atoms in the monomer while mcl-PHA contains 6 to 14 carbon atoms. A multi component PHA consists of both scl and mcl monomers in the polymer chain. The type of PHAs biosynthesized in bacterial cells depends on the substrate specificity of PhaCs and culture conditions. The *C. necator* can biosynthesize scl-PHAs consisting of monomers with 3-5 carbon atoms whereas *Pseudomonas oleovorans* can biosynthesize only mcl-PHAs (Braunegg et al., 1998, Chan et al., 2006, Sudesh et al., 2000). Only P(3HB) homopolymer is biosynthesized in *C. necator* when carbon sources such as sugars (Doi, 1990), fatty acids (Akiyama et al., 1992) or alcohols (Park and Damodaran, 1994) with even number of carbon atoms are supplied. The P(3HB) homopolymer is a brittle and stiff polymer with high crystallinity. P(3HB-*co*-3HV) copolymer with better properties can be produced in *C. necator* when carbon sources with odd number of carbon atoms are supplemented.

2.5 Poly(3-hydroxybutyrate) [P(3HB)]

P(3HB) is the first and the most studied PHAs. The P(3HB) is a homopolymer consisting of (*R*)-3HB repeating units. P(3HB) is synthesized in bacterial cells as an energy-reserve material during unbalanced conditions for ensuring the survival. Bacteria possess several biosynthetic enzymes which can convert the acetyl-coenzyme A (acetyl-CoA) into P(3HB) via the P(3HB) biosynthetic pathway (Figure 2.3). First, the *β*-ketothiolase (PhaA) condenses two molecules of acetyl-CoA each consisting two carbon

atoms (C_2) into acetoacetyl-CoA (C_4), with four carbon atoms. Then, the acetoacetyl-CoA is reduced to (*R*)-3-hydroxybutyryl-CoA by an NADPH-dependent acetoacetyl-CoA reductase (PhaB). Although NADH-dependent acetoacetyl-CoA reductase are found in *C. necator*, but only the NADPH-dependent acetoacetyl-CoA reductase is involved in P(3HB) biosynthesis (Haywood et al., 1988). Subsequently, the (*R*)-isomer of 3-hydroxybutyryl-CoA as the only accepted isomeric substrate is polymerized into P(3HB) by PHA synthase (PhaC), the key enzyme of P(3HB) biosynthesis (Rehm and Steinbüchel, 1999).

The first enzyme which acts as the regulatory enzyme in the pathway is *β*-ketothiolase (Oeding and Schlegel, 1973). During normal growth conditions, the acetyl-CoA produced is channeled into the tricarboxylic acid (TCA) cycle by citrate synthase and coenzyme A (CoASH) is released. Then, the increasing amount of free CoASH inhibits the *β*-ketothiolase and subsequently the biosynthesis of P(3HB). On the contrary, during imbalanced growth conditions such as limiting nitrogen, the amino acid synthesis pathway would be blocked (Shi et al., 1997). The excessive NADH and NADPH inhibit the citrate synthase and therefore, reduce the carbon-flux into the TCA cycle. The reduced concentration of free CoASH releases the inhibition of *β*-ketothiolase. Thus, the overproduced acetyl-CoA is channeled into the P(3HB) biosynthesis pathway (Anderson and Dawes, 1990, Steinbüchel and Lütke-Eversloh, 2003, Tsuge, 2002, Verlinden et al., 2007).

The P(3HB) accumulated in bacteria is amorphous, while extracted P(3HB) exhibits high crystallinity (Doi, 1990). P(3HB) produced by wild type bacteria normally has weight-average molecular weight (M_w) in the range of $1 \times 10^4 - 3 \times 10^6$ Da with

glass-transition temperature (T_g) at 4 °C and melting temperature (T_m) at 177 °C (Tsuge, 2002). Although the thermal properties and some mechanical properties of P(3HB) is comparable with the polypropylene, P(3HB) is still brittle and stiff (Sudesh et al., 2000). It was suggested that P(3HB) with ultra-high-molecular-weight (UHMW) could improve the properties of polymers. UHMW-P(3HB) (M_w in the range of $3 – 11 \times 10^6$ Da) could be produced by a genetically engineered *Escherichia coli* XL1-Blue harboring PHA biosynthesis genes of *C. necator* H16 (Kusaka et al., 1999). The mechanical properties of P(3HB) also could be improved by the incorporation of other monomers to form copolymers such as P(3HB-*co*-4HB), P(3HB-*co*-3HV) and P(3HB-*co*-3HHx).

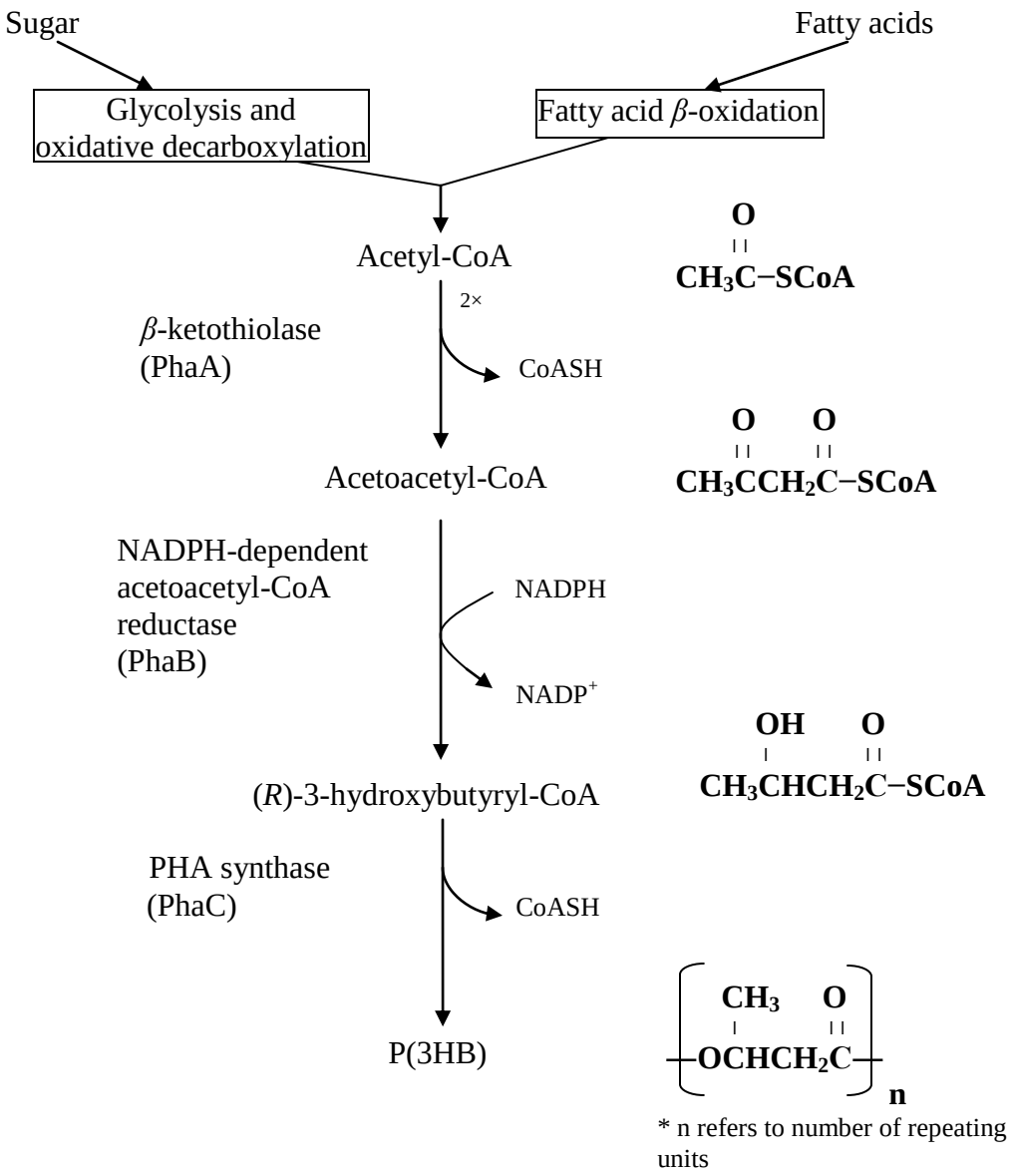


Figure 2.3 Metabolic pathway to biosynthesize P(3HB) (Anderson and Dawes, 1990, Braunegg et al., 1998, Doi, 1990, Steinbüchel and Lütke-Eversloh, 2003).

2.6 Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)]

Incorporation of 3HV monomer into P(3HB) polymer chains has known to have improved the properties of P(3HB) homopolymer. P(3HB-*co*-3HV) has lower crystallinity and melting temperature and greater flexibility and toughness (Luzier, 1992). Similar to other copolymers such as P(3HB-*co*-3HP), P(3HB-*co*-4HB) and P(3HB-*co*-3HHx), P(3HB-*co*-3HV) show isomorphic cocrystallization whereby the 3HB and 3HV units may cocrystallize in a crystalline lattice and the formation of crystal structures depends on the polymeric units containing different compositions of 3HB and 3HV units (Bluhm et al., 1986, Feng et al., 2002, Na et al., 2001). Isomorphic crystallization is a normal phenomenon which can be observed among some natural substances analogous in size and chemical structure such as sodium nitrate, calcium sulfate and barium sulfate. As an isomorphic copolymer, P(3HB-*co*-3HV) with increasing 3HV composition tends to show pseudoeutectic melting behavior allowing a structural transition of P(3HB) lattice to P(3HV) lattice. The structural transition usually occurs in P(3HB-*co*-3HV) with the 3HV content in the range of 30 − 56 mol% (Pseudoeutectic composition) (Bluhm et al., 1986, Mitomo et al., 1993, Mitomo et al., 1995). On one side below the pseudoeutectic point, 3HV units cocrystallize in P(3HB) lattice, while on another side, the 3HB units cocrystallize in P(3HV) lattice. As the 3HV composition increases from 0 – 100 mol%, the melting temperature (T_m) decrease from 177 °C to minimum at about 70 °C at the pseudoeutectic composition, and increases back to 102 – 112 °C at slower rate (Ha and Cho, 2002, Mitomo et al., 1995). Meanwhile, the glass transition temperature (T_g) which depends on the amorphous phase of polymer decreases from 4 °C to about − 16 °C (Ha and Cho, 2002, Shen et al., 2009).

During the crystallization, spherulitic structures which contain stacks of crystalline lamellae grow (Cheng and Sun, 2009, Orts et al., 1995) (Figure 2.4). The crystalline lamellae are formed by the polymers regions containing solely 3HB units or the mixture of 3HB units and 3HV units (Yoshie et al., 2001). In the blends of P(3HB) and P(3HB-*co*-3HV) with low 3HV content, the P(3HB-*co*-3HV) may cocrystallize with P(3HB) due to the similarity in chemical structure and resulted in coexistence of both polymers in a lamella (Conti et al., 2006). The polymer regions with more 3HV units are excluded from the lamellae to form amorphous layers lie in between of crystalline lamellae.

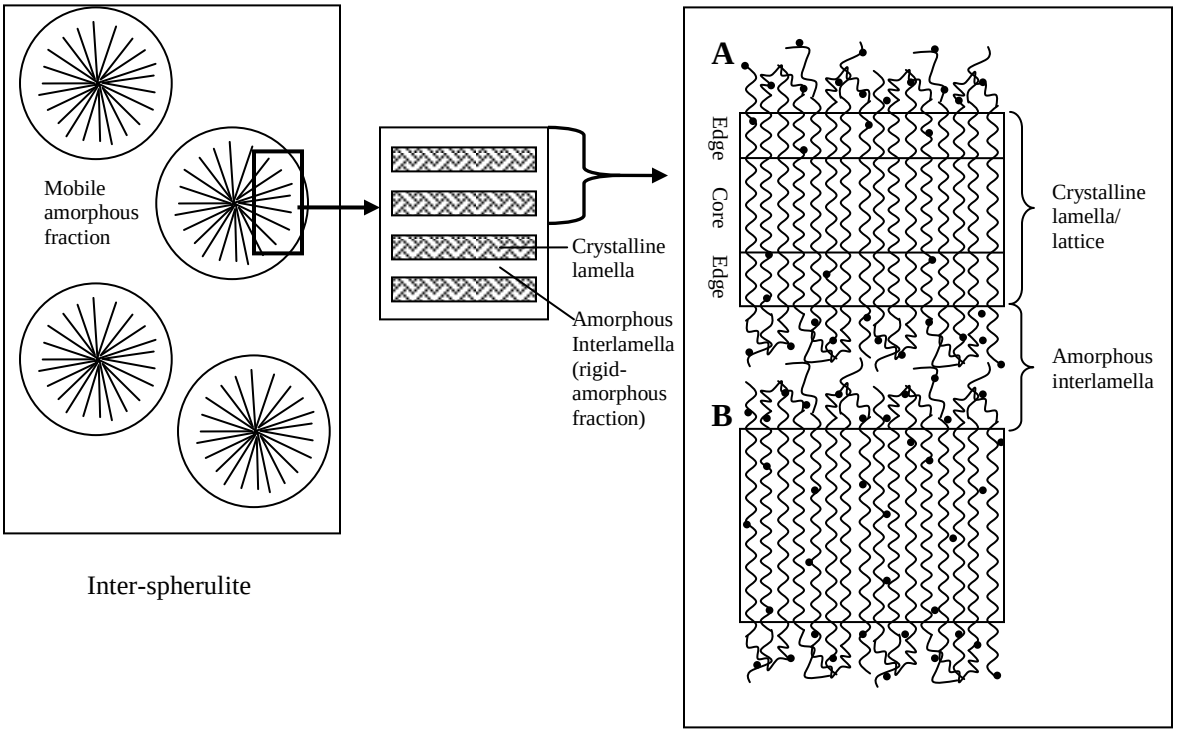


Figure 2.4 The schematic drawings of spherulite containing crystalline lamellae and amorphous region. Two types of lamellae were proposed: (A) sandwich lamella with sole 3HB sequences in the core region and 3HV units exist only in the edge, and (B) lamella with uniform-distributed 3HV units (Cheng and Sun, 2009, Yoshie et al., 2001).

The distribution of 3HV units between the crystalline and amorphous region is affected by crystallization temperature and crystallization rate, which depends on the molecular chain mobility and thermodynamic driving force (Cheng and Sun, 2009). P(3HB) and P(3HB-*co*-3HV) with low 3HV content with fast crystallization rate grow into the lamella and form a P(3HB)-type crystalline lattice, while the chains with more 3HV units are excluded from it (Orts et al., 1995, Yamada et al., 2001). The P(3HB) is more likely to form at the core region (Yoshie et al., 1995). The incorporation of 3HV units in increasing amount will cause more 3HV units distributed into the crystalline lattice. 3HV unit with one more methyl at the side chain than the 3HB unit acts as a defect to expand and loosen the crystalline lattice (Wang et al., 2001). Therefore, incorporation of 3HV into P(3HB) disrupts the crystallinity, lowers the crystallinity and brittleness, but increases the flexibility and toughness (Orts et al., 1995, Yoshie et al., 2001). It also decreases the melting temperature. However, when the 3HV content is more than about 50 mol%, the P(3HV)-type lattice will be formed, and the crystallinity increases gradually (Wang et al., 2001).

C. necator can biosynthesize 3HV monomer with the supplementation of precursors such as alkanoic acids (Du et al., 2001b, Khanna and Srivastava, 2007), alkanoates (Lee et al., 2008, Shang et al., 2004) and alcohols (Park and Damodaran, 1994) with the odd number of carbon atoms. The biosynthesis of 3HV units from propionic acid is similar to the P(3HB) biosynthesis (Figure 2.5). First, the propionic acid is converted into propionyl-CoA. The converted propionyl-CoA intermediate may either condense with one acetyl-CoA generated from the TCA cycle to form 3-ketovaleryl-CoA or eliminate its carbonyl carbon to form acetyl-CoA which condenses with another acetyl-

CoA to generate an acetoacetyl-CoA. The 3-ketovaleryl-CoA and acetoacetyl-CoA are then reduced to (*R*)-3-hydroxyvaleryl-CoA and (*R*)-3-hydroxybutyryl-CoA respectively to be polymerized into P(3HB-*co*-3HV) by PhaC (Braunegg et al., 1998, Doi et al., 1987). The acetyl-CoA which is formed from the propionyl-CoA can also be channeled into TCA cycle for cell metabolism.

On contrary, valeric acid can form valeryl-CoA which is then directly converted to (*S*)-3-hydroxyvaleryl-CoA via *β*-oxidation pathway without being broken down to shorter chain (Doi et al., 1988). Subsequently, the (*S*)-3-hydroxyvaleryl-CoA can be converted into 3-ketovaleryl-CoA which is reduced to (*R*)-3-hydroxyvaleryl-CoA following by the same polymerization. A small amount of converted 3-ketovaleryl-CoA may be degraded into one propionyl-CoA and one acetyl-CoA. Recent report found that a very low epimerase activity in *C. necator* H16 converted the intermediate (*S*)-3-hydroxyvaleryl-CoA directly into (*R*)-3-hydroxyvaleryl-CoA (Mifune et al., 2010), which is normally seen in *R. rubrum* and recombinant *E. coli* expressing the epimerase genes from *Pseudomonas* sp.(Fiedler et al., 2002, Moskowitz and Merrick, 1969, Sato et al., 2007). The epimerization was catalyzed by epimerases including NADH-acetoacetyl-CoA dehydrogenase, (*S*)-hydratase and (*R*)-hydratase.

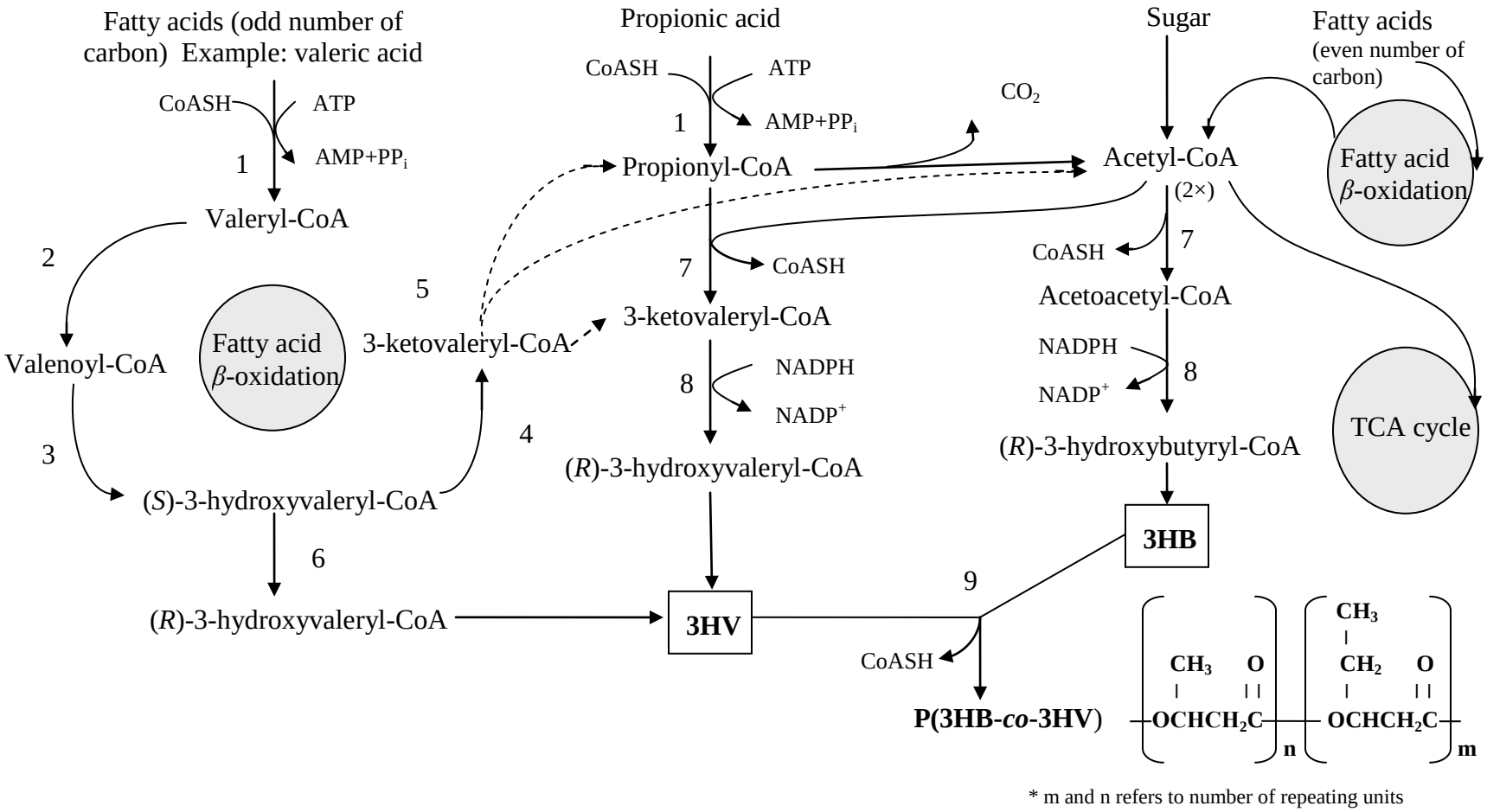


Figure 2.5 Proposed biosynthesis pathway of P(3HB-*co*-3HV) in *C. necator* from the mixture of main carbon sources (sugar or fatty acids with even number of carbon) and precursors (valeric acid or propionic acid). Enzymes: 1. thiokinase; 2. acyl-CoA dehydrogenase; 3. enoyl-CoA hydratase; 4. 3-hydroxyacyl-CoA dehydrogenase; 5. 3-ketoacyl-CoA thiolase; 6. epimerases; 7. *β*-ketothiolase; 8. NADPH-dependent acetoacetyl-CoA reductase; 9. PHA synthase (Bhubalan, 2010, Braunegg et al., 1998, Brigham et al., 2010, Mifune et al., 2010, Steinbüchel and Lütke-Eversloh, 2003, Tsuge, 2002).

2.7 PHAs production from carbon substrates

PHAs are biosynthesized in bacterial cells from various renewable carbon sources. For the large-scale production of PHAs, sugars such as glucose fructose and sucrose have been utilized as the main carbon sources. In 1980s, P(3HB) was produced by *Alcaligenes latus* DSM 1124 in large-scale fermentation using sucrose as the sole carbon source (Hrabak, 1992). At about the same time, the first commercialized PHA copolymer, P(3HB-*co*-3HV) was produced from the mixture of glucose and propionic acid by a mutant strain of *C. necator* and was marketed under the trade name BIOPOL® (Luzier, 1992). However, the high cost of PHAs production and the less competitive price to conventional plastics had led to termination of PHAs production in some companies (Chen, 2010). As the carbon source is the main contributor of the total substrate cost, a low cost carbon source with efficient conversion to PHA is urged to be found (Lee, 1996b, Solaiman et al., 2006).

Plant oils possessing higher carbon content than sugars with lower price, have become feasible alternative substrates for PHAs production. A molecule of fatty acid such as linoleic acid ($C_{18:2}$) can generate nine acetyl-CoA molecules which is 4-fold of sugars, without releasing any carbon dioxide from β-oxidation (Akiyama et al., 2003). The production of PHAs from plant oil was reported to be almost two-fold higher than that from sugar. Besides, PHAs production from oils required less energy and released less carbon dioxide than did the sugar-based production in the life cycle inventory study (Akiyama et al., 2003). Although fatty acids are utilizable for PHAs production, plant oils are preferable substrates compared to the fatty acids because plant oils can be used directly while fatty acids are obtained only after saponification (Solaiman et al., 2006).

Until present, various plant oils such as soybean oil (Kahar et al., 2004, Mifune et al., 2008), olive oil (Chan et al., 2006, Fukui and Doi, 1998), sesame oil (Taniguchi et al., 2003), cottonseed oil (Song et al., 2001), hydrolyzed corn oil (Shang et al., 2008), sunflower oil, coconut oil and palm oil (Lee et al., 2008, Loo et al., 2005) have been studied for the production of scl- and mcl- PHA.

Other carbon sources such as organic waste from agricultural and food industrial also have emerged to be inexpensive feedstock for the production of PHAs. Utilizing the surplus waste materials for PHAs production not only enhances the feedstock conversion into useful products, but also provides an alternative way to solve the disposal issues (Koller et al., 2010). The bioconversion of whey from dairy industry (Koller et al., 2008, Park et al., 2002), molasses from sugar industry (Bengtsson et al., 2010, Gouda et al., 2001), waste lipid and volatile fatty acids from oil production (Bormann and Roth, 1999, Cavalheiro et al., 2009, Dionisi et al., 2005, Gonzalez-Lopez et al., 1996, Kek et al., 2008) and waste water or by-products from industry (Ganzeveld et al., 1999, Yu, 2001) into PHAs were studied. Complex organic wastes may contain nutrients, which are beneficial to PHAs production, but at the same time some organic waste may contain unfavorable compounds or impurities, which may inhibit the cells or cause the processing problematic (Koller et al., 2010). For example, the waste water from olive oil mill contains various volatile fatty acids, which could be used as cheap carbon sources for PHAs production. However, it may also contain toxic phenolic compounds (Ribera et al., 2001). Therefore, the selection of suitable strains incorporating with some pretreatment and feeding strategies are needed for efficient utilization of organic wastes.

2.8 Applications of PHAs

PHAs have potential applications in various fields ranging from packaging to medical. Due to the versatile thermal and mechanical properties which are similar to commodity plastics, PHAs are used as packaging materials and packaging film for bags, containers, paper coating and disposable items (Khanna and Srivastava, 2005a). The water-resistance property of PHAs makes them a suitable biodegradable material for diapers and sanitary towels (Albertsson and Karlsson, 1995). These biodegradable plastics are produced in commercial-scale by several companies such as P&G, Metabolix, Telles (USA), Biomer (Germany), PHB Industrial S/A (Brazil), Mitsubishi Gas, and Kaneka (Japan) (Chen, 2009, Sudesh and Iwata, 2008).

The biocompatibility (Giavaresi et al., 2004) and biodegradability (Foster and Tighe, 1995) of PHAs are desirable properties for medical applications. PHAs such as P(3HB), P(4HB), P(3HO), P(3HB-*co*-3HV), P(3HB-*co*-4HB) and P(3HB-*co*-3HHx) are preferable materials for surgical sutures, cardiovascular patches, tendon repair device, wound dressing and implant scaffolds (Chen and Wu, 2005, Martin and Williams, 2003, Williams et al., 1999). P(3HB) and P(3HB-*co*-3HV) are investigated as materials for bone implant due to their similar mechanical properties to the human bone (Galego et al., 2000). The electrospun scaffolds of P(3HB), P(3HB-*co*-4HB) and P(3HB-*co*-3HHx) have increased the *in vivo* and *in vitro* biocompatibility (Ying et al., 2008). PHAs are suitable to be used as the drug carrier and controlled drug release vector due to their controllable degradability by adjusting the monomer compositions (Juni and Nakano, 1987, Xiong et al., 2010).

2.9 Fatty acid *β*-oxidation

Before entering the fatty acid *β*-oxidation which is also known as fatty acid degradation, oils are broken down into fatty acids by extracellular lipases and are converted to individual acyl-CoA thioesters by thiokinase and CoA-transferase. For example, palmitic acid (C_{16}) is converted to palmityl-CoA. An acyl-CoA is then catabolized via *β*-oxidation involving 4 enzymatic reactions (Figure 2.6) (Braunegg et al., 1998, Steinbüchel and Lütke-Eversloh, 2003). First, the acyl-CoA is oxidized by FAD-dependent acyl-CoA dehydrogenase to *trans*-2-enoyl-CoA, an end product with a double bond between C2 and C3. Then the double bond in *trans*-2-enoyl-CoA is hydrated by enoyl-CoA hydratase and form (*S*)-3-hydroxyacyl-CoA is formed. Following that, the hydroxyl group in (*S*)-isomer of 3-hydroxyacyl-CoA is oxidized to a keto group catalyzed by NAD-dependent 3-hydroxyacyl-CoA dehydrogenase. A 3-ketoacyl-CoA molecule with two keto group is formed and ready to be cleaved by 3-ketoacyl-CoA thiolase to a molecule of acetyl-CoA comprising two carbon atoms and a molecule of acyl-CoA with two carbon atoms shorter than before. The acyl-CoA is oxidized via *β*-oxidation repeatedly to produce a number of acetyl-CoA molecules, which are then channeled into P(3HB) biosynthesis pathway, TCA cycle or other metabolic pathways. A molecule of fatty acid containing an even number of carbon atoms such as palmitic acid, could be oxidized completely to produce 8 molecules of acetyl-CoA. A fatty acid consists of an odd number of carbon atoms such as valeric acid could not be oxidized completely. A propionyl-CoA molecule as a final product is produced.

In the biosynthesis of mcl-PHA, the fatty acids may not undergo a complete *β*-oxidation to generate acetyl-CoA. The intermediate *trans*-2-enoyl-CoA, (*S*)-3-

hydroxyacyl-CoA and 3-ketoacyl-CoA may be converted directly to (*R*)-3-hydroxyacyl-CoA which is the substrate for the PHA polymerization by PhaC. Three types of enzyme activities were investigated in mcl-PHA producers, including the hydratase activities in *Aeromonas caviae*, epimerase and reductase activities in recombinant *E. coli* (Fukui and Doi, 1997, Fukui et al., 1999, Park and Lee, 2003, Taguchi et al., 1999). A very low epimerase activity was also detected in *C. necator* H16 (Mifune et al., 2010).

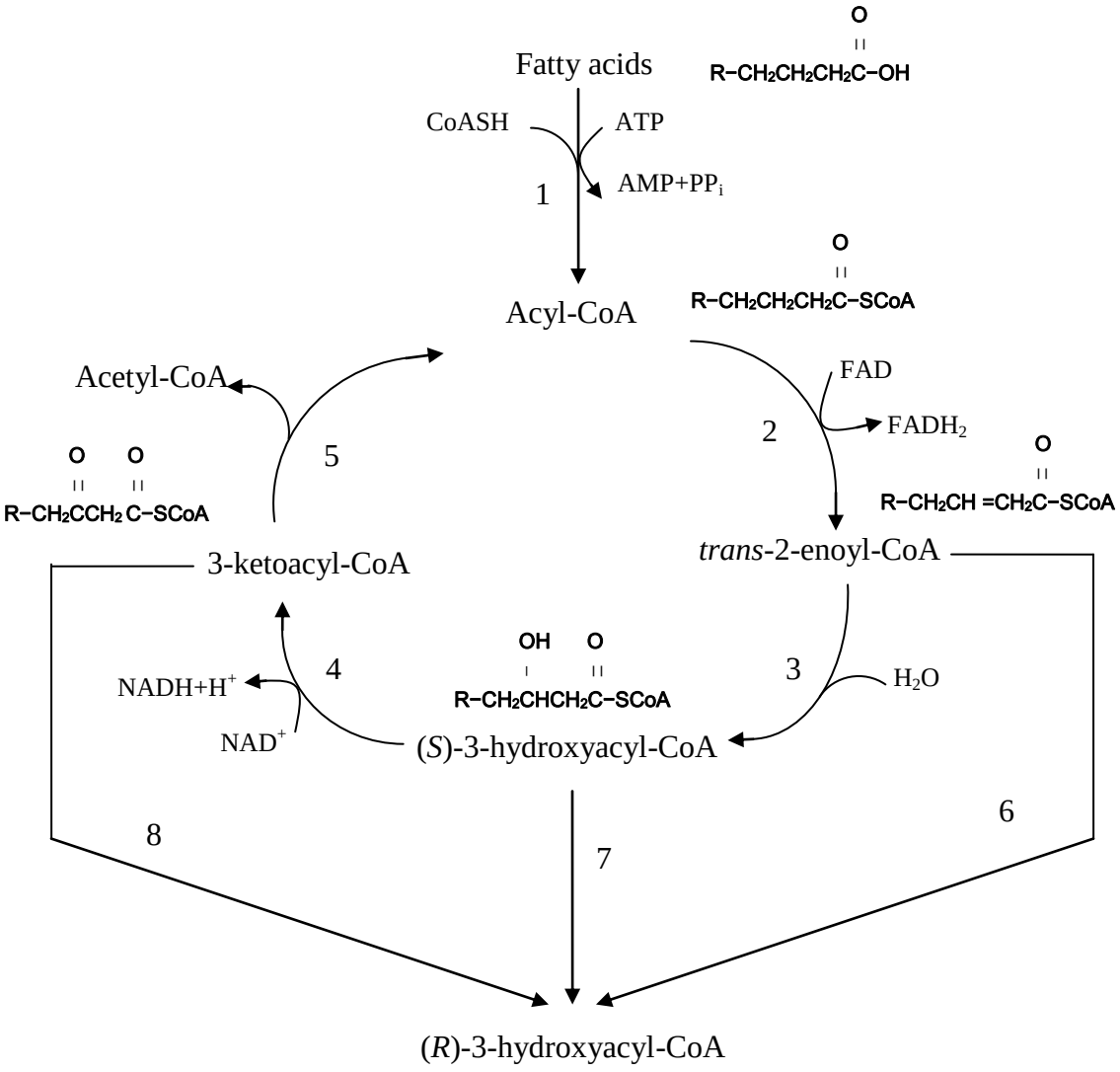


Figure 2.6 Fatty acid *β*-oxidation pathway. Enzymes: 1. thiokinase; 2. FAD dependent acyl-CoA dehydrogenase; 3. trans-2-enoyl-CoA hydratase; 4. 3-hydroxyacyl-CoA dehydrogenase; 5. 3-ketoacyl-CoA thiolase; 6. hydratases; 7. epimerases; 8. reductases (Braunegg et al., 1998, Steinbüchel and Lütke-Eversloh, 2003).

2.10 Lipolytic enzymes

A broad variety of organisms is known to have secreted lipolytic enzymes, mainly for lipid metabolism and signal transduction. Lipolytic enzymes can be classified into different classes, including lipases, esterases and phospholipases (Arpigny and Jaeger, 1999). Esterases are enzymes that hydrolyze ester bonds of soluble or partial soluble molecules, while phospholipases act on phospholipid to release fatty acids and lipophilic molecules. Lipases are hydrolases, which catalyse the hydrolysis of carboxyl ester bonds of triglycerides (triacylglycerol) to release the fatty acids, monoglycerides (monoacylglycerols), diglycerides (diacylglycerols) and glycerols. Lipases are also known as carboxylesterase which catalyze the hydrolysis and synthesis of long-chain glycerides. Although the hydrolysis activity of a lipase does not need any cofactors, the enzyme conformation has to be activated by the interaction with the interfaces of substrate and aqueous solution (Grochulski et al., 1993, Joseph et al., 2008). Lipases have become the potential bio-catalyst in industries of food, detergent, chemical and biomedical due to the wide range of substrate specificities and reactions (Jaeger et al., 1999).

Lipolytic enzymes of a variety of bacteria, including *Pseudomonas* sp. (Dharmsthiti and Kuhasuntisuk, 1998, Ogino et al., 2007, Rosenau and Jaeger, 2000), *Burkholderia* sp (Gupta et al., 2005, Lee and Parkin, 2003) and *Bacillus* sp. (Karpushova et al., 2005, Shi et al., 2010) have been investigated. Recently, the expression of lipase and estrase from *Ralstonia* sp. or *Cupriavidus* sp. in *E. coli* also had been studied (Quyen et al., 2007, Quyen et al., 2005). In a study, the lipase of *Pseudomonas aeruginosa* was reported to act like PHA depolymerase and exhibited hydrolysis activities on linear PHA