



UNIVERSITI PUTRA MALAYSIA

***NON-HALOGENATED RECOVERY METHOD OF INTRACELLULAR
POLYHYDROXYALKANOATES FROM LOCAL ISOLATE
COMAMONAS SP. EB172***

MITRA MOHAMMADI

FBSB 2012 18

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*COMAMONAS SP. EB172***

By

MITRA MOHAMMADI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

February 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Faculty : Faculty of Biotechnology and Biomolecular Sciences

In this study, the use of NaOH treatment and chemical-free aqueous solution for intracellular polyhydroxyalkanoates (PHAs) recovery from local isolate Gram-negative wild type bacteria *Comamonas sp. EB172* at different NaOH concentrations, treatment times, temperatures and agitations is being evaluated as alternative and appropriate methods instead of halogenated solvent extraction system. These methods consist of recovery steps followed by incubation, centrifugation and purification steps using ethanol and water washing. The results of this study were compared with PHA extraction from recombinant *Cupriavidus necator* as a control. The PHA recovered under the most favourable conditions was further characterized.

The chemical-free aqueous solution method as a clean process and low concentration of NaOH treatment were successfully developed for PHA extraction from wild type *Comamonas sp. EB172*. More than 88% purity and 96% recovery yield of PHA were achieved by incubating the wild type bacteria under the mild alkaline treatment using 0.05 M NaOH at 4°C for 1 h. However, up to 96% purity and recovery yield can be obtained by incubation of recombinant *C. necator* with 0.05 M NaOH at 4°C for 3 h. The incubation

of *Comamonas* sp. EB172 in the chemical-free aqueous solution at 30°C for 5 h could efficiently extract the PHA with more than 93% of recovery yield. Besides, shorter incubation time is required for PHA extraction from recombinant strain using the non-chemical method, which resulted in a PHA with 80.6% of purity and recovery yield of 96.1%. Protein determination and transmission electron microscopy images as well as gas chromatography analysis proved that improvement in cell wall permeability and cell membrane breakage are the possible mechanisms of NaOH treatment and chemical-free aqueous solution method on PHA recovery which was accompanied by considerable release of protein after the extraction step. It was found that the effectiveness of chemical and non-chemical treatments depends on the microbial strain. The initial intracellular PHA content also affected the effectiveness of the extraction methods for PHA recovery. Although, the NaOH treatment could recover purer PHA as compared to the chemical-free method, both processes were able to extract the polymer with high yield. The chemical-free aqueous solution method was found to be better than NaOH treatment for PHA extraction in respect of the final polymer molecular weight, which is in fact almost double that of the chloroform-extracted PHA as control.

The overall results in this study indicated that the mild NaOH treatment and the chemical-free aqueous solution methods developed can serve as alternative recovery methods with high potential instead of the conventional halogenated solvent extraction process such as chloroform, since these new methods are environmentally more benign, effective and simple in operation. The recovery of intracellular PHA from *Comamonas* sp. EB172 cells via the recovery methods developed herein can contribute towards the sustainable process of PHA production using organic acids derived from the anaerobic treatment of palm oil mill effluent (POME).

Abstrak tesis kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk Ijazah Doktor Falsafah

**KADEAH PEMULIHAN TANPA HALOGEN BAGI
POLIHIDROKSIALKANOAT INTRASEL DARIPADA
COMAMONAS SP. EB172 PENCILAN TEMPATAN**

Oleh

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Februari 2012

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Di dalam kajian ini, penggunaan rawatan NaOH dan larutan akues tanpa kimia bagi pemulihan polihidroksialkanoat (PHAs) intrasel daripada bakteria Gram-negatif *Comamonas* sp. EB172 pencilan tempatan pada berbagai kepekatan NaOH, masa rawatan, suhu dan pengadukan yang berbeza telah dinilai sebagai kaedah alternatif dan sesuai berbanding kaedah pemulihan menggunakan pelarut berhalogen. Kaedah baharu ini menggunakan langkah pemulihan diikuti pengeraman, pengemparan dan penulinan melalui basuhan etanol dan air. Keputusan kajian ini dibanding dengan kaedah pemulihan PHA daripada *Cupriavidus necator* rekombinan sebagai kawalan. PHA diekstrak pada keadaan yang paling sesuai kemudiannya dicirikan.

Kaedah larutan akues tanpa kimia sebagai proses yang bersih dan penggunaan larutan NaOH pada kepekatan yang rendah telah berjaya dikembangkan untuk pengekstrakan PHA daripada *Comamonas* sp. EB172 pencilan tempatan. Lebih dari 88% ketulinan dan 96% hasilan PHA berjaya didapati melalui pengeraman bacteria berkenaan dalam keadaan rawatan beralkali rendah iaitu 0.05M NaOH pada suhu

4°C selama 1 jam. Untuk *C. necator*, 96% ketulinan dan hasilan didapati selepas penderaman dengan 0.05 M NaOH pada suhu 4°C selama 3 jam. Penderaman *Comamonas* sp. EB172 di dalam larutan akues tanpa kimia pada suhu 30°C selama 5 jam dapat mengekstrak lebih dari 93% hasilan PHA dengan efisien. Disamping itu, masa penderaman yang lebih singkat diperlukan untuk PHA daripada strain rekombinan menggunakan kaedah tanpa kimia dengan menghasilkan 80.6% ketulinan dan 96.1% hasilan. Penentuan protein dan imej transmisi elektron serta analisis kromatografi gas membuktikan pertambahan serapan dinding sel dan pemecahan membran sel sebagai mekanisme rawatan NaOH dan larutan akues tanpa kimia ke atas pemulihan PHA, diiringi oleh rembesan protein selepas langkah pengekstrakan. Tahap kecekapan rawatan didapati bergantung kepada jenis bakteria. Kandungan asal intrasel PHA juga mempengaruhi keberkesanan kaedah pengekstrakan bagi pemulihan PHA. Walaupun rawatan NaOH dapat menghasilkan PHA yang lebih tulen berbanding rawatan tanpa kimia, kedua-dua kaedah didapati berjaya mengekstrak PHA pada tahap yang tinggi. Kaedah pemulihan tanpa kimia didapati lebih baik berbanding kaedah NaOH berdasarkan berat molekul produk polimer PHA, bahkan hampir dua kali ganda berat molekul PHA berbanding kaedah pengekstrakan menggunakan khlorofom sebagai kawalan.

Keputusan keseluruhan di dalam kajian ini menunjukkan kaedah rawatan NaOH dan larutan akues tanpa kimia boleh menjadi kaedah alternatif yang lebih berpotensi berbanding proses pengekstrakan lazim yang menggunakan pelarut berhalogen seperti khlorofom, oleh kerana kaedah baharu ini adalah lebih mesra alam, efektif dan mudah. Pemulihan PHA daripada sel *Comamonas* sp. EB172 melalui kaedah baharu ini juga boleh menjadikan proses penghasilan PHA menggunakan asid organik daripada rawatan efluen kilang sawit lebih lestari dan mesra alam.

ACKNOWLEDGEMENTS

I pray to Almighty God who gave me the thoughts, the will, and guided me to complete this work.

The writing of this dissertation has been a monumental milestone in my academic life. I could not have embarked on this expedition without the passionate and continued support of advisors, colleagues, friends and family.

First and foremost, I would like to express my heartfelt appreciation and gratitude to my supervisor, Professor Dr. Mohd Ali Hassan, for his kind support, patience, motivation, enthusiasm, enlightening lectures, and for many valuable discussions. I would specially like to express my deepest gratitude to my co-supervisor Dr. Phang Lai Yee for her detailed and constructive comments and unconditional support that greatly eased my progress throughout this study. Appreciation is also extended to my co-supervisors Professor Yoshihito Shirai and Dr. Hasfalina Che Man, for their guidance, suggestion and encouragement throughout this project.

My condensed thanks go to Dr. Hidayah Ariffin, UPM, Malaysia, Dr. Yoshito Ando, Kyushu Institute of Technology, Japan and Dr. Kumar Sudesh, USM, Malaysia, for proof-reading and giving helpful suggestions.

I wish to give my sincere gratitude to all the faculty staff at UPM, especially Mr Rosli who helped me with equipments, glasswares and chemicals. I would like to thank Mr Rafie and Ms Aminah at IBS for their assistance with TEM and SEM.

I am very grateful for all the support I received from them over the years.

My special appreciation is also goes to my colleagues Yee Lian Night, Dr. Mei Ling Chong, Dr. Tabassum Mumtaz, Azman and Yik-Kang Kek for their kind help, supportive suggestions and friendly attitude during my study.

This extraordinary voyage would certainly have been less extraordinary and colorful if not for the engaging and heartwarming time spent with friends– Elaheh, Aghdas, Sahar, Ali, Fatemeh, Farzaneh and many more.

I want to thank my friends in Iran Mitra Mehdizadeh, Nafiseh Vahedi, Farzaneh Khatami and Mehdi Mohajerpour for not forgetting me even when I was in abroad, and for keeping contact with words and emails which offered such valuable emotional support that will never be forgotten.

The sacrifices that this work required have been felt most strongly by my parents, husband, sister, brother in-law and brothers. They prevented me from feeling lost on this planet and helped me believing that I was doing the right thing. I would like to express my gratitude for their moral support and encouragements during all these years. In particular, I would like to thank my husband, Ali Mowlavi, for his unconditional support, even when I felt that things were out of proportions. This undertaking would not have been possible without his love.

This list is far from exhaustive; I pray for forgiveness from those I did not mention by name and include them in my heart-felt gratitude.

I certify that an Examination Committee has met on 20/2/2012 to conduct the final examination of Mitra Mohammadi on her PhD thesis entitled “Non-halogenated recovery methods of intracellular polyhydroxyalkanoates from local isolate *Comamonas* sp. EB172” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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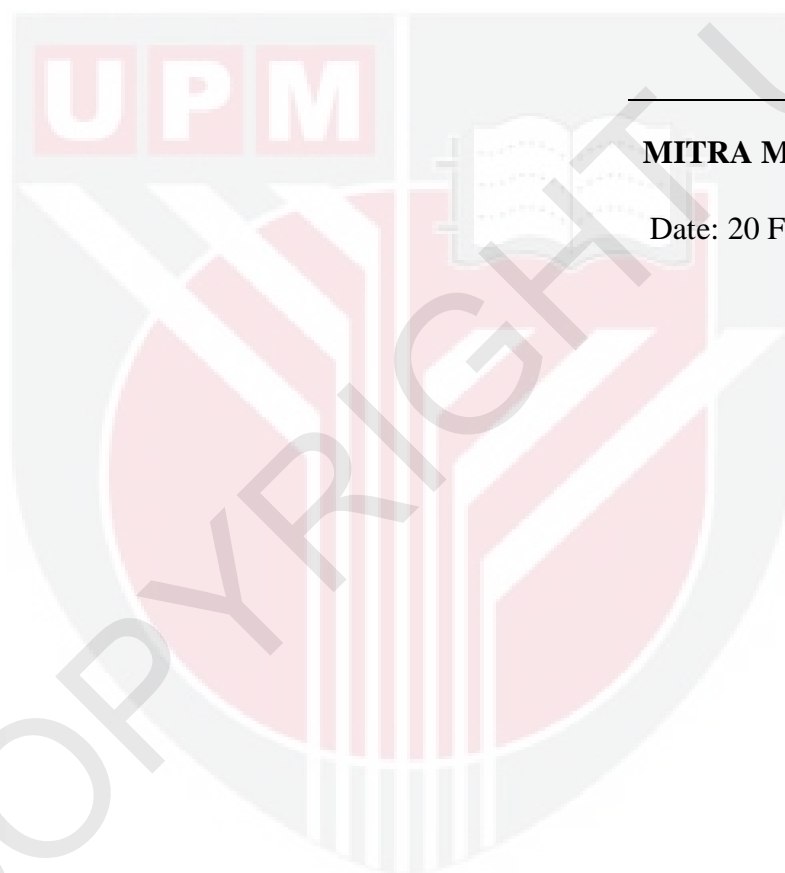
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DECLARATION

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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Date: 20 February 2012.



TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vi
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xiv
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xxi
CHAPTER	
1 INTRODUCTION	1
1.1 Overview of study	1
1.2 Problem statement	6
1.3 Objectives	7
1.4 Thesis plan	7
2 LITERATURE REVIEW	9
2.1 Non-biodegradable plastic	9
2.1.1 Plastic waste management	10
2.2 Polyhydroxyalkanoates	12
2.2.1 Native PHA granules	12
2.2.2 Diversity of PHA	15
2.2.2.1 P(3HB)	17
2.2.2.2 P(3HB-co-3HV)	19
2.2.2.3 P(3HB-co-3HHx)	21
2.3 Production of PHAs	23
2.3.1 Bacterial strains	24
2.3.2 Carbon substrates	29
2.4 Recovery of PHAs	35
2.4.1 Chemical methods	38
2.4.1.1 Solvent extraction	38
2.4.1.2 Chemical digestion	39
2.4.2. Biological method	43
2.4.2.1. Enzymatic digestion	43
2.4.3. Mechanical methods	44
2.4.3.1. Bead mill	44
2.4.3.2. High pressure homogenization	45
2.4.4. Physical methods	46
2.4.4.1. Ultrasonication	46
2.4.4.2. Osmotic shock	47
2.4.4.3. Freezing	48
2.4.5. Other PHA recovery methods	48
2.4.5.1. Supercritical fluid	48
2.4.5.2. Dissolved-air flotation	49
2.4.5.3. Air classification	49

2.4.5.4. Spontaneous liberation	49
2.4.6. Combined methods	50
2.4.7. Sumamry and comparison of PHA recovery method	502
2.5 Applications of PHAs	55
2.5.1 Application of homopolymer	55
2.5.2 Application of copolymer	56
2.5.2.1 Packaging	56
2.5.2.2 Medicine	57
2.5.2.3 Tissue engineering	57
2.5.2.4 Drug delivery	59
2.5.2.5 Blending	59
2.5.2.6 Others	61
3 RECOVERY AND PURIFICATION OF POLYHYDROXYALKANOATES FROM LOCAL ISOLATE <i>COMAMONAS SP. EB172</i> BY SIMPLE DIGESTION WITH SODIUM HYDROXIDE	62
3.1 Introduction	62
3.2 Materials and Methods	65
3.2.1 Chemical reagents	65
3.2.2 PHA-containing biomass	65
3.2.3 PHA recovery using NaOH	67
3.2.4 PHA recovery using chloroform	70
3.2.5 Analytical procedures	72
3.2.5.1 PHA analysis	72
3.2.5.2 Protein determination	72
3.2.5.3 Glycerol determination	73
3.2.6 Data analysis	74
3.3 Results and discussion	74
3.3.1 Single effect of variables on PHA recovery	75
3.3.2 Interaction effect of variables on PHA recovery	833
3.3.3 Proposed mechanisms of PHA recovery using NaOH	877
3.3.4 Mass balance of recovery process	89
3.4 Conclusion	922
4 RECOVERY AND PURIFICATION OF POLYHYDROXYALKANOATES FROM LOCAL ISOLATE <i>COMAMONAS SP. EB172</i> USING CHEMICAL-FREE AQUEOUS SOLUTION METHOD	933
4.1 Introduction	93
4.2 Materials and Methods	95
4.2.1 PHA-containing biomass	95
4.2.2 PHA recovery using chemical-free aqueous solution method	95
4.2.3 Analytical procedures	96
4.2.4 Data analysis	97
4.3 Results and discussion	99
4.3.1 Single effect of variables on PHA recovery	99
4.3.2 Interaction effect of variables on PHA recovery	105
4.3.3 Proposed mechanism of PHA recovery using chemical-free aqueous solution method	109
4.3.4 Mass balance of recovery method	110
4.4 Conclusion	113

5	CHARACTERIZATION OF PHA RECOVERED FROM LOCAL ISOLATE <i>COMAMONAS SP. EB172</i> USING NAOH TREATMENT AND CHEMICAL-FREE AQUEOUS SOLUTION METHOD	114
	5.1 Introduction	114
	5.2 Materials and Methods	117
	5.2.1 Materials	117
	5.2.2 Polymer characterization	118
	5.2.2.1 Transmission electron microscopy (TEM) analysis	118
	5.2.2.2 Scanning electron microscopy (SEM) analysis	119
	5.2.2.3 Nuclear magnetic resonance (NMR) spectroscopy	119
	5.2.2.4 Molecular weight determination	120
	5.2.2.5 Differential scanning calorimetry (DSC) analysis	121
	5.3 Results and discussion	121
	5.3.1 Transmission electron microscopy (TEM) analysis	121
	5.3.1.1 PHA recovered by NaOH treatment	121
	5.3.1.2 PHA recovered by chemical-free aqueous solution method	122
	5.3.2 Scanning electron microscopy (SEM) analysis	124
	5.3.3 Nuclear magnetic resonance (NMR) spectroscopy	127
	5.3.3.1 PHA recovered by NaOH treatment	127
	5.3.3.2 PHA recovered by chemical-free aqueous solution method	132
	5.3.4 Molecular weight determination	135
	5.3.4.1 PHA recovered by NaOH treatment	135
	5.3.4.2 PHA recovered by chemical-free aqueous solution method	137
	5.4 Conclusion	140
6	FACTORS AFFECTING THE PHA RECOVERY PROCESS	142
	6.1 Introduction	142
	6.2 Materials and Methods	144
	6.2.1 PHA-containing biomass	144
	6.2.2 PHA recovery using the NaOH treatment and chemical-free aqueous solution method	144
	6.2.3 Analytical procedures	146
	6.3 Results and discussion	147
	6.3.1 Effect of PHA recovery method	147
	6.3.2 Effect of PHA-producing organism	151
	6.3.3 Effect of initial PHA content	152
	6.4 Conclusion	157
7	SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH	158
	7.1 Summary	158
	7.2 Conclusion	162
	7.3 Recommendations for Future Research	164
	REFERENCES	165
	APPENDICES	180
	BIODATA OF STUDENT	203
	LIST OF PUBLICATIONS	205