PERPUSTAKAAN UNIVERSITI MALAYA

INVC 19/12/00

DEGRADABILITY IN RIVER WATER OF BACTERIAL

POLYHYDROXYALKANOATES (PHA) PRODUCED FROM SAPONIFIED PALM KERNEL OIL (SPKO)



HO YEN HIM

SUBMITTED AS PART OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF TECHNOLOGY (ENVIRONMENTAL MANAGEMENT)



INSTITUTE OF POSTGRADUATE STUDIES AND RESEARCH

1999

UNIVERSITY OF MALAYA 26.12.2001

UNIT RÉPROGRAFI PERPUSTAKAAN UTAMA

ABSTRACT

With the increasing use of plastics, disposal is becoming a major issue. The degree and rate of aerobic biodegradability of a plastic material in the disposal environment determine to what extent and how fast that plastic may be eliminated from the environment. The degradation of polyhydroxyalkanoate (PHA) produced by *Pseudomonas putida* PGA1 from saponified palm kernel oil (SPKO) was studied at 28°C under aerobic conditions in a sterile reactor containing 200-225ml natural water from the river Kayu Ara (Selangor, Malaysia). The time-dependent changes in the weight, surface morphology, monomer compositions and chemical groups of the PHA, CO₂ evolved, dissolved organic carbon, microbial count, pH and released monomers in the test solutions were monitored.

A weight loss of 71.3% of a PHA film was achieved in 86 days. Biodegradation, which was indicated by the amount of CO₂ evolved, contributed 52.4% or more of the total CO₂ from PHA degradation dependent on the incubation period of the film. The rate of monomer hydrolysis was related to the side-chain length of the monomer, and decreased in the following order: C8 > C10 > C12 > C14. Scanning electron micrographs showed that physically, the degradation started with surface lesions and proceeded side ways and inward to form holes and tunnels. By 86 days, the PHA film disintegrated completely into suspended particles in the river water.

ACKNOWLEGEMENTS

I would like to take this opportunity to thank my parents for their patience, understanding and support during my undertaking of this dissertation.

Dr. Irene Tan Kit Ping and Assoc. Prof. Dr. Gan Seng Neon who have been supervising and generously sparing their precious time and energy to guide and assist me throughout the period of this research, without whom this research would not have been completed so smoothly. To them, may I take this opportunity to express my heart-felt gratitude and respect to both of them. May they continue to assist their students and be rewarded with great discoveries that may benefit the society and be returned with joy and satisfaction.

I also wish to thank the Southern Acid (M) Ltd. (Klang, Selangor) for their kind contribution of the palm kernel oil. I'm also grateful to the Ministry of Science, Technology and Environment for their financial support under the funding for Intensification of Research and Priority Areas (IRPA).

Unforgettable is two of my knowledgeable, helpful, humble and jovial colleagues – Suffian and Zazali who have continuously provided their ideas and assistance in the research and make this period an enjoyable and a memorable one. To the staff of IPSP – Mr. Karim, Mr. Osman, Mdm. Vijaya, Mr. Jasmi, Miss Molly Chua and Mdm Zubaidah; to my friends in IPSP, namely Umma, Shazah, Apparna, Mrs. Sen Gupta, Hui Ning, Chee Chong, Ali, Alfred, Kahoru, Hooi Kheng, Pek Yee, Jillian and Joanna, and to Sudesh who visited the lab occasionally, may I express my gratitude for all their assistance and company.

To all whose names are mentioned or not mentioned here, who have helped me in one way or another, may they be blessed with good health, peace in the heart and happiness.

This research has become one year of the history in my life. Life is so precious but it doesn't matter what one is doing, but whether what one chose to do is conducive to one's happiness and peace as well as the happiness and peace of the society. Grasp the challenges as opportunities to develop our mind with the qualities of wisdom, generosity, virtue, right effort, patience, sincerity, determination, kindness and equanimity which is the greatest spiritual power that become an island in a sea of turmoil... May all the merits that I have acquired during this period pave the path to the highest bliss ... of Nibbana.

CONTENTS

	Page
TITLE	i
ABSTRACT	ii
ACKNOWLEGEMENTS	iii
CONTENTS	v
LIST OF TABLES .	ix
LIST OF FIGURES	x
CHAPTER I: INTRODUCTION	1
1.1 The need for biodegradable plastic	1
1.2 Biodegradation	4
1.2.1 The importance of biodegradation	4
1.2.2 Standard definitions & methods to evaluate biodegradability	5
1.2.3 The sequence of biodegradation tests	ç
1.3 Biodegradable plastics in the market	12
1.4 Polyhydroxyalkanoates (PHA)	13
1.4.1 Biodegradation of PHA	15
1.4.2 Factors affecting biodegradation of PHAs	10
1.4.3 Biodegradability tests of PHAs and other related tests	10
1.5 Scope & objective based on biodegradation in environment	nt
management system	1

CHAPTER II: MATERIALS AND METHODS			21
2.1	PHA b	piosynthesis	21
	2.1.1	Bacterial stain	21
	2.1.2	Strain maintenance	21
	2.1.3	Inoculum	21
	2.1.4	Media and growth conditions	21
		2.1.4.1 Palm kernel oil	22
		2.1.4.2 Saponification of PKO	23
	2.1.5	PHA extraction and PHA film casting	23
2.2	Biodeg	gradability test	24
	2.2.1	Sampling river water	24
	2.2.2	Inoculum preparation	24
	2.2.3	Biodegradability experimental design	25
	2.2.4	Initial medium analysis - cell count, pH and DOC	29
		2.2.4.1 Cell count	29
	2.2.5	Test materials	29
	2.2.6	$\ensuremath{\text{CO}_2}$ analysis and final day river water analysis – cell count,	
		pH, DOC and GC	30
		2.2.6.1 DOC measurement for river water	31
		2.2.6.2 Gas chromatography (GC) of river water	31
	2.2.7	PHA sample analysis	33
	2.	.2.7.1 Dry weight	33
	2.	.2.7.2 Scanning electron microscopy (SEM) & phase-contrast	
		microscopy	33
	2.	.2.7.3 Purification of incubated PHA films	33

		2	.2.7.4	Infrared (IR) spectrometry	34
		2	.2.7.5	Molecular weight by gas permeation chromatography	
				(GPC)	34
		2	.2.7.6	Monomer composition by gas chromatography (GC)	34
СНАР	TE	R III:	RESUL	TS AND DISCUSSION	35
3	.1	Prelim	inary stu	dies	35
3	.2	PHA i	nherent b	piodegradability studies	50
		3.2.1	CO ₂ rel	eased	50
		3.2.2	Viable o	cell count and pH	53
		3.2.3	Dissolv	ed organic carbon (DOC) in river water	56
		3.2.4	GC ana	lysis of river water	58
		3.2.5	PHA dr	y weight	60
		3.2.6	Scannir	ng electron micrograph (SEM)	62
		3.2.7	Infrared	d (IR) spectrometry analysis	68
		3.2.8	Molecu	alar weight by gas permeation chromatography (GPC)	73
		3.2.9	Monon	ner composition of PHA by gas chromatography (GC)	73

CHAPTER IV: CONCLUSION

78

APP	PENDIX	82
1	Experimental set up of Sturm test	82
2	Calculation of %CO ₂ -biodegradability	83
3	Standard Curves for methyl ester of 3-hydroxyalkanoic acid	84
4	Calculation of %w/w and %mole of monomer composition in PHA from GC	
	of PHA sample	90
5	Calculation of PHA molecular weight & theoretical carbon dioxide released	
	from 100% degradation of PHA	92
REF	ERENCES	0/1

	LIST OF TABLES	Page
Tab	le	
1.1	Examples of toxic synthetic organic compounds frequently found in plastic	
	industrial waste and their health effects	3
1.2	Standards and practices for testing biodegradability of plastics	7
2.1	Growth and production medium	22
2.2	Test and control reactors set up in terms of the presence of PHA/PHB film and	
	microorganisms, and test period	26
2.3	Mineral salt solution for biodegradability test medium	27
3.1	Number of microorganisms in river water before and after the pretreatment of	
	aeration and storage	36
3.2	Weight loss of PHA & PHB in unstirred water at 28°C	37
3.3	Test reactors and control reactors and the processes that release the CO_2 from	
	the reactor	45
3.4	Time-dependent changes of PHA weight, cumulative CO2 released, pH and	
	number of microorganisms in stagnant river water at 28°C	48
3.5	Monomer composition of PHA in stagnant river water at 28°C	49
3.6	pH and number of microorganisms in stirred river water in the test reactors	
	and control reactors on day 0 and day 86 at 28°C	56
3.7	Monomer compositions of the original PHA film	74
4.1	Weight loss of PHA and PHB for all the runs at their respective full incubation	
	period in river water at 28°C	78
A-1	Calculation of %w/w and %mole of monomer composition in PHA	90
A-2	Calculation of % weight of corbon of a DIJA Film	

A-3 Calculation of theoretical CO₂ released from 100% degradation of a PHA

LIST OF FIGURES

Figure				
1.0	Inter-relationship of degradation modes of degradable plastic materials	5		
1.1	Practical relevance and analytical potential of test methods	10		
1.2	General structure of monomers and PHA polymers	13		
2.1	Biodegradability test set up for Run 2	28		
3.1	Scanning electron micrographs (SEMs) of PHA film surfaces after incubation			
	in unstirred river water at 28°C in trial run for (a) 0 days; (b) 10 days and (c)			
	10 days in sterile condition	39		
3.2	Phase contrast micrographs of PHA film surfaces after incubation in unstirred			
	river water at 28°C for (a) 0 days; (b) 10 days (in sterile river water); (c) 10			
	days; (d) 15 days and (e)-(f) 28 days	40		
3.3	PHA & PHB film surface morphology before and after incubation in unstirred			
	river water at 28°C (Run 2)	41		
3.4	SEMs of PHA film surfaces after incubation in unstirred river at 28°C in Run			
	2 for (a) 0 day; (b) 28 days (in sterile river water); (c) 15 days; (d) 21 days;			
	(e)-(f) 28 days.	42		
3.5	PHA weight change in unstirred river water at 28°C	43		
3.6	pH & number of microorganisms in unstirred river water at 28°C	44		
3.7	Cumulative CO_2 released from degradation of PHA, air supply and $$ microbes			
	in unstirred river water at 28°C	46		

3.8	Monomer composition of PHA in unstirred river water at 28°C	49
3.9	Cumulative CO_2 released and $%CO_2$ -degradability, $%CO_2$ biodegradability	
	and %CO2-physical & chemical degradability of PHA in stirred river water at	
	28°C	51
3.10	Cumulative CO_2 released within 70 days from stirred river water at $28^{\circ}\!\mathrm{C}$	
	which differs in the presence of PHA & microorganisms	52
3.11	Number of microorganisms and pH in stirred river water at 28°C	54
3.12	DOC of stirred river water during the biodegradation of PHA at 28°C	57
3.13	PHA monomer composition in river water within 86 days at 28°C	59
3.14	PHA weight loss in stirred river water at 28°C	61
3.15	SEMs of PHA film surfaces incubated in stirred river water at 28 $^{\circ}\mathrm{C}$ for (a) 0	
	days; (b) 6 days; (c) 41 days; (d) 60 days & (e) 86 days (in sterile river	
	water). Magnification 5.03X10 ² .	65
3.16	SEMs of PHA film surfaces incubated in stirred river water at 28°C for (a) 41	
	days and (b) 86 days (in sterile river water). Magnification 2.22X10 ¹ .	66
3.17	SEMs of PHA film surfaces in stirred river water at 28°C for (a) 0 days; (b) 6	
	days; (c)-(d) 41 days; (e)-(f) 60 days; (g) 86 days (in sterile river water).	
	Magnification 5.00X10 ³ .	67
3.18	(a) IR-absorption spectrum of unincubated PHA	70
3.18	(b) IR-absorption spectrum of PHA incubated in stirred river water for 6 days	70
3.18	(c) IR-absorption spectrum of PHA incubated in stirred river water for 20 days	71
3.18	(d) IR-absorption spectrum of PHA incubated in stirred river water for 41 days	71
3.18	(e) IR-absorption spectrum of PHA incubated in stirred river water for 60 days	72
2 10	(A ID absorption spectrum of DHA incubated in stirred sterile river water for	

	86 days	72
3.19	Monomer composition of PHA film during the 86 days' period of	
	biodegradability test in stirred river water at 28°C	75
A-1	A typical set up of Sturm test for Run 2	82
A-2	A typical set up of sturm test for Run 3	82
A-3	Standard curve for mehyl ester 3-hydroxyoctanoic acid (C8)	84
A-4	Standard curve for mehyl ester 3-hydroxydecanoic acid (C10)	84
A-5	Standard curve for mehyl ester-3-hydroxydodecanoic acid (C12)	84
A-6	Standard curve for mehyl ester 3-hydroxytetradecanoic acid (C14)	85
A-7	Standard curve for mehyl ester 3-hydroxyhexadecanoic acid (C16)	85
A-8	Response factor (RF) of column versus carbon number at various standard	
	concentration of methyl ester 3-hydroxyalkanoic acid	86
A-9	RF of column versus carbon number at various standard concentration of	
	methyl ester 3-hydroxyalkanoic acid	86
A-10	Standard curve for methyl ester 3-hydroxybutyric acid (C4)	8
A-11	Standard curve for methyl ester 3-hydroxyhexanoic acid (C6)	8
A-12	or the state of	
	methyl ester 3-hydroxyalkanoic acid	8

A-13 Standard chromatograph of β-methyl 3-hydroxyalkanoates

88