CHAPTER I

CHAPTER I: INTRODUCTION

1.1 The need for biodegradable plastic

In the last quarter of this century, plastic products have gained universal use, not only in food, clothing and shelter, but also in the transportation, construction, medical and leisure industries (Doi *et al.*, 1996). As a result, plastic production has increased 10% a year for the past three decades (Nebel & Wright, 1996, p.545). It has been predicted that there will be a two to three fold increase in the demand for polymeric materials in the 21st century (Chiellini, 1997). Consequently, plastics contribute a great portion of the total municipal solid waste. In Malaysia, plastic is the third (>5% total waste) major composition of municipal solid waste after organic vegetables (34%) and paper (22%). This amount is comparable to the amount of plastic waste generated by the developed countries such as European Economic Community (EEC) (4.6% by weight), USA (5.0% by weight) and Japan (5.2% by weight) (Agamuthu, 1997). The major challenge faced in the plastic waste treatment is the biodegradability of the plastic. This is an important issue from both the economic and ecosystem perspective.

The major legal treatment of plastic waste in Malaysia is by landfill even though incineration with energy recovery, recycling and the new concept of prevention are other alternatives that coexist for plastic waste treatment in some other countries. An average of 753.4 tonnes per day of municipal plastic waste was generated in Malaysia in 1995. This amount requires about 175 landfills with the size of a football field for complete disposal in a year (Agamuthu, 1997). Not only about RM 13.4 million has to be spent per year (Agamuthu, personal communication, June, 1998), with a 5% annual increment of solid waste from many sectors we also face scarcity of suitable land for disposal. Further more, landfill needs long term management to deal with the potential groundwater pollution from the leachate, air pollution from methane production and land settling due to waste compaction and decomposition. Hence, cost, scarcity of land and incomplete decomposition of plastics depict that landfill may not be a long-term solution to plastic waste management.

Apart from landfill, it is common to find plastics in the roadside litters and rivers. Several hundred thousand tons of plastics are discarded into marine environments via rivers every year in the world. Not only do they impose a negative aestheticism; some are hazardous to the living organisms. It has been estimated by Pruter and Laist that one million animals are killed every year either by choking on floating plastic items or becoming entangled in plastic debris (as cited in Doi *et al.*, 1996). Not only the disposal pose a challenge, but also the production process. The toxic wastes of the synthetic plastic industries are known to cause mutations, cancer, nervous disorders, liver disease and lung disease as shown in Table 1.1 (Grushko, 1992).

Three criteria must be achieved for the disposal of plastic to be considered as environmentally friendly. Firstly, the plastic must be able to degrade rapidly to prevent choking and entanglement of the marine organisms. Secondly, the intermediates of the degradation process must be non-toxic. Thirdly, the degradation process must be complete i.e. the plastic can completely disintegrate into its basic inorganic atoms or molecules to be recycled in the biogeochemical cycle. The imperfection of most of the purported biodegradable plastics is incomplete degradation, forming a fine polymer powder that still resists microbial degradation (Nebel & Wright, 1996). The toxicity of the intermediates is yet to be investigated. Furthermore, some elements in the undegraded polymer powder cannot be returned to the biogeochemical cycle. This may affect the ecosystem equilibrium, which indirectly affect the survival of some of the organisms on earth.

Table 1.1 - Examples of toxic synthetic organic compounds frequently found in plastic industrial waste and their health effects

Chemical	Known health effects[a]							
	Overall toxic effect	Carcino genic	Birth defect	Nervous disorder	ENT irritation	Liver disease	Kidney disease	Lung disease
Chloroethylene	X	X		X	X	X		X
Dicyclopentadiene	X							
β-diethylaminoethyl mercaptan	X			X	X			
Ethylphthalate					X	X	X	
m-Dihydroxybenzene	X				X			
Glycol ether					X		X	X
Acetdimethylamide	X		X	X	X			
N,N-Dimethylamine		X			X			X

Note. [a]Known health effects were determined from volunteers and experimental animals. Adapted from "Handbook of dangerous properties of inorganic and organic substances in industrial wastes" (pp.217, 220-225) by Y. A. M. Grushko, 1992, Boca Raton: CRC Press.

Therefore, when the plastic is destined for disposal, it will be environmentally friendly only if it can completely degrade into its basic inorganic atoms or molecules so that the materials are not toxic, and can be recycled in the environment. This can only be achieved naturally by biodegradation.

1.2 Biodegradation

1.2.1 The importance of biodegradation

When plastics are exposed to a given environment, several outcomes are possible, depending on the nature of the plastic materials. They may remain unchanged in the environment indefinitely and be termed recalcitrant; they may be partially degraded after a long period of time; or they may be degraded completely within reasonably short time and be removed from the environment.

Three modes of degradation may act to degrade a plastic: i.e. photodegradation, chemical degradation and biodegradation as shown in Figure 1.0 (Swift, 1994). It is noteworthy that biodegradation is the only natural pathway for the complete elimination of plastics and fragments from the environment. Hence biodegradability is an important measure of the ultimate degradability of a plastic material.

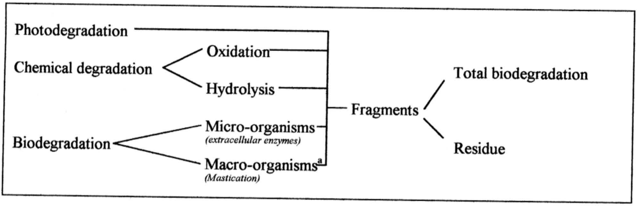


Figure 1.0 - Inter-relationship of.degradation modes of degradable plastic materials.

[a]Macro-organisms are not included in the ASTM definition of biodegradation. From "Expectation for biodegradation testing methods" by Swift, 1994 in *Biodegradable plastic and polymers*, (p.229), Amsterdam: Elsevier.

1.2.2 Standard definitions & methods to evaluate biodegradability

Standard definitions, methods and procedure to evaluate biodegradability under different environmental disposal conditions have been developed or under the development by the American Society of Testing and Materials (ASTM), Organisation for Economic Co-operation and Development (OECD) and International Standard Organisation (ISO) (Narayan, 1997). The standards established by these three organisations are closely parallel with minor variations. In this dissertation, ASTM terminology for definitions will be used as the main reference.

ISO defines degradation as a deleterious change in the chemical structure, physical properties, or appearance of a plastic. A degradable plastic is defined as a plastic which is designed to undergo a significant change in its chemical structure under specific environmental conditions resulting in a loss of some properties that may vary as measured by standard test methods appropriate to the plastic and the application in a period of time that determined its classification. (Note the inclusion of the word "designed", which implies plastics that are intentionally made to be degradable). A biodegradable plastic is defined as a degradable plastic in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi and algae. (Note that the level of biodegradability may be indicated as shown in subordinate definitions for biodegradable plastics; Macroorganisms are not included in this definition). A photodegradable plastic is defined as degradable plastic in which the degradation results from the action of natural sunlight (Wool, 1994).

The ASTM standard test methods and practices that have been developed so far address biodegradability in most environments of global interest. The environments include simulation of compost, landfill, marine, freshwater, soil, activated sludge, aerobic and anaerobic conditions and test of specific organisms (Table 1.2). The tests are based on the chemical balance of the biodegradation process:

i) Aerobic environment:

$$C_p + O_2 \longrightarrow CO_2 + Residue + Biomass$$

ii) Anaerobic environment:

$$C_p \longrightarrow CO_2/CH_4 + Residue + Biomass$$

(C_p denotes the designed biodegradable plastic as the carbon source)

Table 1.2 - Standards and practices for testing biodegradability of plastics

Standard	Test no.	Title	Analytical measurement
ASTM	D5437-93	1. Practice for a simulated marine environment	-
	D5525-4A	2. Practice for a simulated landfill environment	-
	D5509-94 D5512-94	3. Practice for a simulated compost environment	-
	D6002-96	4. Guide to assess compostability	-
	D5209-91	5. Aerobic biodegradation in sewage sludge	CO_2
	D5210-91	6. Anaerobic biodegradation in sewage sludge	CO_2/CH_4
	D5271-92	7. Aerobic biodegradation in activated sludge wastewater	O_2
	D5511-94	8. Anaerobic biodegradation under high-solids anaerobic digestion	-
	D5526-94	9. Anaerobic biodegradation under accelerated landfill	-
	D5338-93	10. Aerobic biodegradation in controlled composting	CO_2
	D5988-96	11. Aerobic biodegradation in soil after composting	-
	D6003-96	12. Aerobic weight loss in solid waste compost	-
	D5247-92	13. Aerobic biodegradability by specific microorganisms	M_w
	D5951-96	14. Practice for preparing residual solids for toxicity testing (Fate & effect testing)	-
	D5152-91	15. Practice for water extraction of residual solids for toxicity testing (Fate & effect testing)	-
	D5510-94	16. Practice for heat ageing	-

Table 1.2 –(Continue) Standards and practices for testing biodegradability of plastics

Standard	Test no.	Title	Analytical measurement
OECD	301A	DOC die-away	DOC[a]
	301B	CO_2 evolution (Sturm test)	CO_2
	301C	MITI	O_2 consumption
	301D	Closed bottle	dissolved O_2
	301E	Modified OECD screening	DOC[a]
	301F	Manometric respirometry	O_2 consumption

[a]DOC = dissolved organic carbon. Adapted from "Standards development for biodegradable plastic" by R. Narayan, 1997, Nov in *UNIDO-ICS International Workshop on Environmentally Degradable Polymers*, (pp.314-316), Pune, India and "Expectation for biodegradation testing methods" by G. Swift, 1994 in Y. Doi and K. Fukuda (Eds.), *Biodegradable plastics and polymers*, (pp.228-236), Amsterdam: Elsevier.

Apart from the standard OECD tests of the respiratory O_2 and CO_2 production (Table 1.2), the physical and chemical changes of the plastic, the soluble and insoluble products, and the biomass can be used as a measure of the biodegradation process. Muller *et al.* (1994) further suggested that a carbon balance should be obtainable to validate the process and the extent of degradation.

The standard definitions, test methods and practices are important as a reference for scientists, marketing agencies, legislators, regulators and consumers. This is to standardise the environmental marketing claim and protect consumer rights. ASTM also expected that modified versions of test methods and practices would be adopted in other countries (Wool, 1994) so that the test results are practical in the local environment to avoid misrepresentation and false claiming (Scott, 1994). As the standard tests are focused at the products to be marketed whereas the bioplastic to be tested in this dissertation is in its preliminary stage of research and development, the theory of the standard test methods and practices will be used as the foundation for designing the biodegradability tests, with suitable modifications based on the local environment and facilities available.

1.2.3 The sequence of biodegradation tests

The sequence in which the biodegradability tests should be done is recommended by OECD as follows (Kitano & Yakabe, 1994):

i) Ready biodegradability tests or screening consist of "stringent tests which provide limited opportunity for biodegradation and acclimatisation to occur. It may be assumed that a chemical giving a positive result in a test of this type will rapidly biodegrade in the environment".

ii) Inherent biodegradability tests consist of "tests which allow prolonged exposure of the microorganisms to the test compound, a more favourable biomass to chemical ratio or other conditions favouring biodegradation. A compound giving a positive result in a test of this type may be classified an "inherently biodegradable", but because of the favourable conditions employed its rapid and reliable biodegradation in the environment may not be assumed".

iii) Simulation of environmental compartments consists of “tests which provide evidence of the rate of biodegradation under some environmentally relevant conditions. Tests of this type may be sub-divided according to the environment they are designed to simulate.

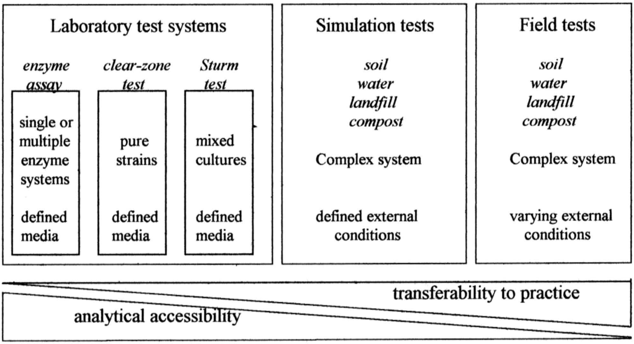


Figure 1.1 – Practical relevance and analytical potential of test methods.

Adapted from “The development and modification of some special test methods and the progress in standardisation of test methods in Germany” by Muller *et al.*, 1994 in Y. Doi and K. Fukuda (Eds.), *Biodegradable plastics and polymers*, (pp.237-249), Amsterdam: Elsevier.

The tests vary in the analytical accessibility and transferability to the actual environment (Figure 1.1). Generally, ready degradability tests in the form of the laboratory test systems are the first choice, followed by inherent test and lastly field test (Kitano & Yakabe, 1994). All the three tests mentioned earlier are important and should be performed as a complete set that support one another. The first two tests enable a well-defined and controlled environment to be set up in the laboratory and hence, the experiment is analytical and repeatable. The third test, is a test of biodegradability in the real world situation, where all mode of degradation, including photodegradation, chemical degradation and biodegradation act in various sequence and proportion. This test may not be well defined and not repeatable due to the dynamic condition of the actual environment but it is the best way to simulate the biodegradability of a compound in the environment. The environments of the disposal facilities should be focused in the tests as these environments are the ultimate disposal sites of the plastics (Scott, 1994).

Another important test is the environmental safety assessment (ESA). The safety of the polymer, degradation products and metabolites in the disposal environment measured in terms of chronic and acute toxicity must be analysed before the product is marketed (Swift, 1994). ASTM has approved two standard practices for preparing the residual solids for toxicity testing (Table 1.2) (Narayan, 1997).

Some other important demands on degradation tests are (Muller *et al.*, 1994) :

- Comparability of different materials.
- Reproducibility.
- Parameters determined must characterise degradation of the polymer matrix.
- Relevance for practical requisition.
- Following analytical measurements (e.g. toxicological tests for intermediate products) must be possible.
- Applicability for commercial products (e.g. no C^{14}-labeling).
- Tests should not exceed economical limits.

1.3 Biodegradable plastics in the market

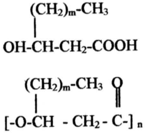
There are three types of biodegradable plastic - synthetic plastics, naturally occurring plastics (bioplastics) and modified natural plastics (artificial bioplastics). The biodegradable synthetic plastics include polylactic acid (PLA), poly(vinyl ester) (PVE), poly(vinyl alcohol) (PVA) and polyesteramides. Starch, cellulose, gelatin, polyhydroxyalkanoate (PHA) are some of the bioplastic products in the market. The currently available biodegradable plastics, the producer, trademark, cost and their applications are summarised by Chiellini (1997).

Even though all the plastics mentioned are biodegradable, there are credits and limitations in each of them. The petrol-chemically-based synthetic plastic may face the limitation of being non-renewable resources, pollution in the production process and incomplete degradation. The artificial bioplastics may face the problem of incomplete degradation as the synthetic part may not be biodegradable. The biopolymers stand out in terms of the resources used are renewable. The natural range of substrates and microbes used for production gives ample structural variety to attain various possible properties for specific applications, and the products stand a high possibility of complete biodegradation. Hence, bioplastic is the most environmentally friendly plastic with lots of potential for development and application in the future. However, much research still has to be done to resolve the problems of high production costs, constraints in processability imposed by inherent structural susceptibility of side reactions, limits of adaptation of existing process technology and machinery, unsatisfactory mechanical properties and lack of standard definitions and test protocols for biodegradable plastics (Chiellini, 1997).

In this dissertation, PHA, a bioplastic produced by *Pseudomonas putida* PGA1 from saponified palm kernel oil, will be tested for its inherent biodegradability in the local river system.

1.4 Polyhydroxylakanoates (PHA)

PHA is a general term for all polyester reserve materials produced by bacteria. It is an intracellular carbon and energy material that is accumulated by a very wide range of bacteria when the carbon supply is adequate but limited in N, P, O and/or S (Byrom, 1994). 90 hydroxyalkanoic acids have been identified as constituents of PHA (Steinbuchel & Valentin, 1995). The monomers consist of 3-hydroxyalkanoic acids as shown in Figure 1.2. The monomeric composition of PHA depends on the bacterial strain and on the carbon source supplied during the accumulation phase. According to the number of carbon atom of the monomers, PHA are classified as short-chain-length PHA (3-5 carbon atoms, PHA_{SCL}), medium-chain-length PHA (6-15 carbon atoms, PHA_{MCL}) and long-chain-length PHA (more than 15 carbon atoms, PHA_{LCL}).



$$\mathrm{OH{-}\overset{(CH_2)_m{-}CH_3}{CH}{-}CH_2{-}COOH}$$

where m = 0 to 12

$$\mathrm{[{-}O{-}\overset{(CH_2)_m{-}CH_3}{CH}{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}]_n}$$

where n = 4000 to 20,000

Figure 1.2 - General structure of monomers and PHA polymers. Modified from "Polyhydroxyalkanoates" by Byrom, 1994 in D. P. Mobley (Ed.), *Plastics from microbes* (p.6), Munich: Hanser Publishers.

The biosynthesis of PHA has been studied using *Rhodospirillum rubrum*, *Alcaligenes ertrophus*, *Azotobacter vinelandii*, *Alcaligenes latus*, *Pseudomonas oleovorans*, *Pseudomonas resinovorans* ATCC, *Pseudomonas putida* KT2442, *Pseudomonas oxalaticus*, *Pseudomonas acidovorans*, *Methylobacterium extorquens* and *Paracoccus denitrificans* etc. Only *Pseudomonas* species has the ability to store polymers containing monomers of 3-hydroxy alkanoate in the carbon chain length C_8 and longer (Byrom, 1994). The PHA copolymers produced by *Pseudomonas putida* PGA1 in this dissertation consists mainly of *n*-alkanoate monomers ranging from C_6 to C_{14}, with C_8 as the predominant component. There is also a small amount of unsaturated monomers, i.e. $C_{12:1}$ and $C_{14:1}$ (Tan *et al.*, 1997).

Poly{*(R)*-3-hydroxybutyrate] (P[*(R)*-3HB]) is the most studied PHA material and is probably the most abundant (Mukai *et al.*, 1994). It can be found not only in microorganisms, but also in plants, animals and humans (Byrom, 1994). Griebel *et al.* isolated PHA from bacteria by various treatments with enzymes (as cited in Mukai, 1994). Lemoigne used chloroform and Williamson and Wikinson used hypochlorite (as cited in Mukai, 1994) to extract PHA from bacteria. At present, the development of PHA as a commercial product is limited to polyhydroxybutyrate (PHB) and its copolymer, PHB/HV (hydroxyvalerate). ICI at Monsanto and Fermentation Institute are the only two producers of PHB copolymers in commercial scale (Chiellini, 1997). There are a few more industries, which are in development stages of PHB/V production. PHB can be used for the production of bottles, film, fibres, non-woven fabric, biomedical devices such as bone plates and screw (Byrom, 1994), fishing net (Cox, 1994) and agriculture mulch film (Scott, 1994). Some other proposed applications include PHA as a carbon substrate for denitrification in the water treatment (Byrom, 1994).

1.4.1 Biodegradation of PHA

PHA can be degraded either intracellularly by intracellular depolymerases of the accumulating strain (Merrick & Doudoroff, 1964) or extracellularly by extracellular depolymerases. The latter are secreted by many bacteria for utilisation of PHA left in the environment after accumulating bacteria have died (Jendrossek *et al.*, 1996). Biodegradation starts when microorganisms begin to grow on the surface of the plastics and secrete extracellular depolymerases that breakdown the polymer into water-soluble oligomers and monomer called hydroxyacids. The hydroxyacids are then transported through the cell wall of microorganisms and utilised as exogenous carbon source (Doi *et al.*, 1994).

Aerobic and anaerobic PHA-degrading bacteria and fungi have been isolated from various ecosystems (Jendrossek, 1996), such as soil (*Pseudomonas lemoignei*, *Acidovorax delafieldii*, *Variovorax paradoxusand comamonas* sp.), laboratory (*Pseudomonas pickettii*), activated sludge (*Alcaligenes faecalis* and *Pseudomonas fluorescence*), sea water (*Comamonas testosteroni*), lake water (*Pseudomonas stutzeri*) and anaerobic sludge (*Ilyobacter delafieldii*). The extracellular PHA depolymerases have been prepared from some microorganisms such as *Pseudomonas strain* P1 , *P. lemoignei*, *A. faecalis* T1, *P. picketti* , *Comamonas* sp. , *P. fluorescens*, *C. testosteroni*, *P. stutzeri* and *Penicillium pinophilium* (Doi *et al.*, 1994). It will be expected that PHA-degrading bacteria and fungi can be found in the river system to be studied in this dissertation.

1.4.2 Factors affecting biodegradation of PHAs

Biodegradation of PHAs is dependent upon the environmental factor and physicochemical properties of PHA. The environmental factors include the type of microorganisms and its activity in the environment concerned, temperature, pH, moisture content, aerobicity, trace nutrient availability and the presence of growth inhibitors in the environment. The physicochemical properties of PHA that influences its biodegradability are (i) the stereoregularity of the PHA: only ester linkages of monomers in the *(R)* configuration are hydrolysed by the depolymerases, (ii) the crystallinity of the PHA: the degradability of a polyester decreases as the overall crystallinity or its crystalline phase perfection increases, (iii) the molecular mass of the PHA: low-molecular-mass polymers are generally degraded more rapidly than high-molecular-mass polyesters, (iv) the monomeric composition of PHA: e.g. the rate of degradation by purified *A. faecalis* PHB depolymerase was slower for PHB than for copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (Doi *et al.*, 1990) (v) the length of side chains and position of hydroxy- group ((i) to (v) are reviewed by Jendrossek, 1996).

1.4.3 Biodegradability tests of PHAs and other related tests

Biodegradability test of PHAs and PHA derivatives have been performed in various environments including fresh water pond (Mergaert *et al.*, 1995), river water (Doi *et al.*, 1996), sea water (Imam *et al.*, 1999), activated sludge, soil (Doi, 1995) and compost heap (Pagga *et al.*, 1995). The parameters used to characterise the biodegradation process are weight loss, change in surface morphology and chemical structure of PHAs, biological oxygen demand (BOD), dissolved organic carbon (DOC) and pH of test solutions, microorganisms count and water soluble products. In one report, PHB and some of its copolymers were found to degrade rapidly in river water of river Arakawa (Saitama, Japan) at 25°C (Doi *et al.*, 1996). The weight-loss biodegradability and BOD-biodegradability were 100% and 80%±5% for 28 days respectively. There was an increase in the microorganisms number in the test river water with time which shows that the PHBV can support growth of microorganisms. There was an *in situ* biodegradability test of PHB and PHBV in the natural waters in Belgium that demonstrated the effective biodegradability of these plastics under real-life conditions (Mergaert *et al.*, 1995).

Apart from biodegradation tests in the environment, many areas of research related to the biodegradation of PHA were also performed:

- Isolation and characterisation of PHA-degrading microorganisms (Doi *et al.*, 1994; Kasuya *et al.*, 1994 and Chang *et al.*, 1994)
- Biochemical properties of PHA depolymerase (Jendrossek *et al.*, 1996)
- Enzymatic model of PHA hydrolysis (Kanesawa *et al.*, 1994; Abe *et al.*, 1995b and Abe *et al.*, 1995a).
- Comparison between enzymatic and non-enzymatic hydrolysis of PHAs (Huang *et al.*,1994).
- Regulation of PHA depolymerase synthesis (Jendrossek *et al.*, 1996).
- Molecular biology of PHA depolymerase (Jendrossek *et al.*, 1996; Fukui & Doi, 1997).
- Influence of the physicochemical properties of PHA on biodegradation (Jendrossek *et al.*, 1996; Abe *et al.*, 1995a; Doi, 1995; Abe *et al.*, 1994; Kanesawa *et al.*, 1994; Marchessault *et al.*, 1994; Ramsay *et al.*, 1994)

- Blending, synthesis and degradation of polyesters related to PHA (Jendrossek *et al.*, 1996; Koyama & Doi, 1995).
- The effects of degraded products on the environment (Chang *et al.*, 1994).
- Methods to enhance degradation (Chang *et al.*, 1994).
- Biotechnology aspects of PHA and PHA depolymerase (Jendrossek *et al.*, 1996).

1.5 Scope & objective based on biodegradation in environment management system

This dissertation reports an integral aspect of bacterial PHA research carried out in the Biotechnology Laboratory, Institution of Postgraduate Studies and Research, University of Malaya, Kuala Lumpur.

In the ISO14000 series, life cycle analysis is one of the assessments of the environmental management standards on industry. It involves looking at all of the environmental impacts associated with a product from 'cradle to grave' (Henry, 1997). In order to comply with the ISO 14000 standard, it is wise that the environmental impact in the process of production, application and disposal of the plastic is minimised. Furthermore, an environmentally sustainable approach should be adopted as far as possible in every process.

In terms of the raw material, it is wise to use renewable resources or recycle the waste as resources. In this dissertation, a renewable substrate, palm kernel oil is used for the synthesis of the PHA as compared to the use of petrol-chemical as the raw material, which is predicted to become limiting in the world by 2010-2020 (Nebel & Wright, 1996). Production of PHAs by fermentation, such as *Pseudomonas putida* PGA1 in the author's laboratory generates less pollution (heat, CO_2) and no hazardous chemicals as compared to chemical production method. This is an environmentally clean technology. As for the disposal of the product, it is better to first minimise the waste based on the 3Rs principle, i.e. reduce, reuse and recycle. However, biodegradability will be the unavoidable factor for the ultimate disposal of plastic to return the elements into biogeochemical cycle of the earth in order to maintain a balanced ecosystem.

The scope of this dissertation is to assess the degradability and biodegradability of PHA synthesised by *P. putida* PGA1 from saponified palm kernel oil (SPKO). The specific objective is to evaluate the inherent biodegradability of PHA in the local river water (Kayu Ara River, Selangor). Therefore, the supportive conditions for biodegradation will be provided in the test. The river water will be used to provide mixed-culture of microorganisms as well as the environment for biodegradation of PHA. Mixed culture will be suitable for inherent biodegradability test because a complete biodegradation may involve various microorganisms working at different stages of the degradation process. Other physical factors such as temperature, aeration, agitation will be fixed at a suitable level.

To meet the objective, the rate of PHA degradation would be measured by looking at the changes of the following factors with time:

i) physicochemical properties:
  a) weight
  b) surface morphology
  c) number (M_n) and weight average (M_w) relative molecular mass
  d) monomer composition
  e) chemical groups

iii) biochemical properties:

- a) CO_2 production
- b) dissolved organic carbon (DOC)
- c) biomass production
- d) pH

Carbon balance of PHA biodegradation (PHA + $O_2 \longrightarrow$ residual PHA + intermediate products + new biomass + CO_2) can also be obtained to verify the extent of PHA biodegradation.