

## *CHAPTER TWO*

### *LITERATURE REVIEW*

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### 2.1 PHA and its importance

All over the world, in the last quarter of this century, the use of plastics became very common and it appears in all aspects of life including food packaging, household materials, shelter, transportation, industrial and also medical use. Due to the expansion of its usage, it is not surprising that tons of plastics are discarded into land, rivers and seas and cause environmental problems. In 1988, packaging material made up 31.6 % of municipal solid waste in the USA (USEPA 1990). This volume of waste will increase with time and thus this problem can only be overcome if disposal routes for plastics are properly planned. The most reasonable route to be taken for waste disposal should be a combination of recycling, land filling and waste-to-energy incineration (Sawada, 1994). Another option for the waste management of polymers is the making of biodegradable plastics.

Earlier work on plastics typically focussed on biodeterioration rather than on biodegradability (Aminabhavi *et al*,1990). A lack of clear, standardized definitions of degradability combined with poorly documented promotional claims left both legislators and the public confused about the merits of these products (Donnelly, 1990). The focus on deterioration usually assessed undesirable changes in appearance and physical properties of

plastics (Yabannavar *et al*, 1993). Therefore, a more clearly definition of biodegradable plastic should be established so that it could be use world wide and applicable to all countries.

## **2.2 Standards development for biodegradable plastics**

There are many different degradation modes in nature combined together to degrade polymer. Degradation of PHA can be induced by many factors including high energy radiation, atmospheric pollutants, mechanical stress, biological action and also hydrolysis. Biodegradation is an event whereby enzymes and/or chemical decomposition associated with living organisms such as bacteria and fungi take place.

American Society for Testing and Materials (ASTM) committee D20.96 is developing standards for terminology and tests for biodegradable plastic materials. It is noteworthy that biodegradation is the only pathway for the complete elimination of plastics and fragments from the environment. Microbial degradation of plastics is initiated by the secretion of enzymes, which cause a chain cleavage of the polymer into oligomers and monomer esters. These water-soluble enzymatic cleavage products are absorbed into the microbial cell where they are metabolized. Under aerobic conditions, the degradation products are carbon dioxide, water and some

organic material. Methane and carbon dioxide are the main degradation products under anaerobic conditions.

The terminology of biodegradable plastic by ASTM D20.96 based on the ISO standard is defined as:

"A degradable plastic in which the degradation results from the action of naturally-occurring microorganisms such as bacteria, fungi and algae".

Test method to measure the biodegradability of plastic materials measures the percent conversions of the carbon from the biodegradable plastic to  $\text{CO}_2$  in an aerobic environment and  $\text{CH}_4$  in addition to  $\text{CO}_2$  in an anaerobic environment. It is noted that the plastic material is the sole carbon source for the microorganism in the experiment.

The equation below is proposed to conduct a carbon mass balance to assess ultimate biodegradation (Swift, 1994):

Aerobic environment :  $\text{C}_p + \text{O}_2 \rightarrow \text{CO}_2 + \text{Residue} + \text{Biomass}$

Anaerobic environment :  $\text{C}_p \rightarrow \text{CO}_2 / \text{CH}_4 + \text{Residue} + \text{Biomass}$

where  $\text{C}_p$  is carbon-containing plastic. Total biodegradation results in complete removal of the carbon from the environment where ;

$$\text{Residue} = 0$$

Incomplete biodegradation may have either  $\text{C}_p$  and /or Residue greater than zero. Currently a carbon mass balance can only be conducted in an aquatic system where the plastic is the sole carbon source. In compost- a

heterogeneous matrix with various carbon sources- the build up of new biomass and the formation of degradation intermediates cannot be determined with sufficient accuracy.

OECD (Organization for Economic Cooperation and Development) is an intergovernmental organization and has objectives similar to ASTM. OECD has developed strategy for biodegradability testing which is based on three levels of testing, namely (Kitano and Yakabe, 1994):

- 1) ready biodegradability or screening
- 2) inherent biodegradability
- 3) simulation of environmental compartments

Ready biodegradability tests are stringent tests which provide limited opportunity for biodegradation and acclimatization to occur. Inherent biodegradability tests are tests which allow prolonged exposure of the microorganisms to the test compound, a more favourable ratio of biomass: chemical or other conditions favouring biodegradation. Simulation tests are tests, which provide evidence of the rate of biodegradation under some environmentally relevant conditions. Tests of this type may be subdivided according to the environment they are designed to simulate.

Ready biodegradability test methods have been carried out on biodegradable plastics and polymers and generally it has been the first choice followed by inherent and simulation tests.

## **2.3 Description of current test methods**

### **2.3.1 Screening tests for ready biodegradability:**

There are 6 test guidelines for determining of ready biodegradability in OECD guidelines. The test protocols described are:

Table 1:Test protocols for determining ready biodegradability in OECD guidelines (with reference to Seal, 1994)

Test	Analytical method
DOC Die-Away or Modified AFNOR test	DOC loss
CO <sub>2</sub> evolution or modified Sturm test	CO <sub>2</sub> evolution
MITI	O <sub>2</sub> consumption
Closed Bottle	O <sub>2</sub> consumption
Modified OECD screening	DOC loss
Manometric Respirometry	O <sub>2</sub> consumption

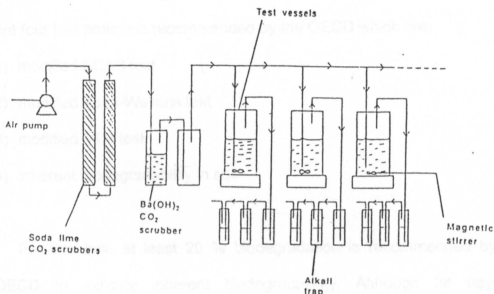
These tests are similar in a number of aspects: they are aquatic and operate under aerobic conditions, the test substance provides the sole

source of carbon, no pre-adaptation with test substance and measurement of ultimate biodegradability.

The modified Sturm test seems to be the preferred technique for polymeric materials. It has been specified by the Italian authorities for assessing biodegradable polymers and has being evaluated by the Biodegradable Plastics Group of the International Biodeterioration Research Group (Seal and Pantke, 1986). A similar ASTM method (D5209) was approved in 1992.

The principle of the Sturm method is as follows; test substance is added to a chemically defined mineral nutrient solution free of organic carbon. An inoculum of sewage microorganisms is added to the solution. The test systems with suitable controls are incubated at ambient temperature with stirring for 28 days. The  $\text{CO}_2$  evolved is trapped in alkali and measured as carbonate by titration or by using carbon analyser. The test arrangement is shown in Figure 1.

**Figure1 : Schematic arrangement of modified Sturm test (with reference to Seal, 1994)**



An experiment was carried out using MITI method (Kitano and Yakabe, 1994) and the biodegradability of the P(3HB-co-3HV) and PCL were dependent on the particle size of the test substance, molecular weight and the activated sludge concentration. It was found that the biodegradation rate was slower with increasing particle size of the plastics. Molecular weight is one of the factors that determine the rate of biodegradation. Polymer with higher molecular weight will be less degraded than the lower molecular weight polymer in 28 days. The increasing of the activated sludge concentration will shorten the half-degradation time of the polymer.



### **2.3.2 Tests for inherent biodegradability**

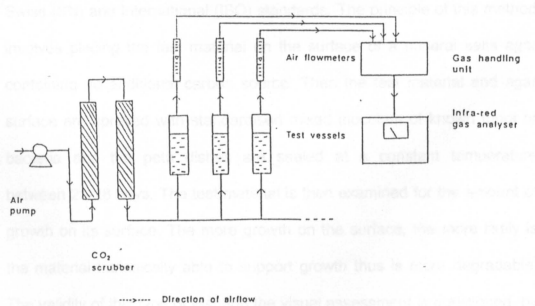
The objective of this test is to test the inherent or intrinsic ability of the test substance to biodegrade and there are no pass or fail criteria. There are four test protocols recommended by the OECD which are:

- 1) modified SCAS test
- 2) modified Zahn-Wellens test
- 3) modified MITI test
- 4) inherent biodegradability in soil

For this test, at least 20 % biodegradation is recommended by the OECD to indicate inherent biodegradability. Although 28 days is recommended, longer exposure of time is permissible and prior acclimatisation procedures is allowed. Sometimes radiolabelled compound is used in this method, particularly where low degradation rates are anticipated and unequivocal measurement of mineralisation is required in the presence of any endogenous respiration. Radiolabelling techniques are valuable particularly for insoluble materials such as polymers where a carbon balance is required to account for the fate of all the carbon in the test system. The test systems vary in design but they measure O<sub>2</sub> consumption, CO<sub>2</sub> evolution or loss in dissolved organic carbon (DOC). For test of inherent biodegradability in soil, the biomass activity and composition is important. Biomass activity is directly proportional to organic

matter levels in soil. As for the screening tests, the best means of applying the polymer is in a powder form. Correct incorporation into the soil is important and preparation of the soils by sieving and standardising on moisture content is a necessary prerequisite to optimising for the biodegradation test. To assess biodegradability, continuous or semi-continuous monitoring of CO<sub>2</sub> using a flow through system and detection by infrared analysis are involved. Unlike the titration method, the test system is never opened to remove and replace the trapping solutions which can affect quality of data. A scheme for a continuous flow system is shown in Figure 2.

Figure 2: Schematic arrangement for assessing biodegradability in soil using infrared detection of CO<sub>2</sub> evolution (with reference to Seal, 1994)



### **2.3.3 Tests for simulation studies**

This test range from laboratory designed equipment, which replicates aerobic sewage treatment and anaerobic sludge digestion. Material is buried in soil or submerged in activated sludge, freshwater or marine environments. The samples will be securely held on some form of racking for aqueous environments. The racks, normally made of stainless steel, are submerged in the liquid medium. The test situation is monitored and the samples are removed periodically.

## **2.4 Other methods involving assessing of polymer biodegradability**

### **2.4.1 Petri dish screen**

This test is used in USA (ASTM), German (DIN), French (AFNOR), Swiss (SN) and International (ISO) standards. The principle of this method involves placing the test material on the surface of a mineral salts agar containing no additional carbon source. Then the test material and agar surface are sprayed with standardised mixed inoculum of known fungi or bacteria and the petri dishes are sealed at a constant temperature between 21-28 days. The test material is then examined for the amount of growth on its surface. The more growth on the surface, the more likely is the material intrinsically able to support growth thus is more degradable. The validity of this type of test and the visual assessment is questioned by

Seal and Pantke (1986). They recommended mechanical assessment instead.

#### **2.4.2 Environmental chamber**

This test requires high humidity (> 90%) to encourage microbial growth. Strips of test material are hung in the chamber sprayed with a standard mixed inoculum of fungi in the absence of additional nutrients and incubated for 28-56 days at constant temperature. A visual assessment is made and rating given based on the amount of growth on the material. This test is particularly stringent and was designed to simulate the effects of high humidity conditions on electronic components and electrical equipment. Growth of fungi across a painted circuit board can result in a gross systems failure in a computer system or military equipment (Seal, 1994). Such a system is valuable in assessing how biodegradable polymers will perform under such conditions.

#### **2.4.3 Soil burial test**

Material is buried in soil beds prepared in the lab using standard sieved soil. The soil beds containing the samples are incubated at a constant temperature for between 28 days to 12 months. The moisture content is set at 20-30%, although it is better calculated as percentage

(40-50 %) of the soil's maximum water holding capacity. Samples are removed for assessment of changes in their properties such as weight loss, mechanical strength changes or a microscopic (light and scanning electron microscopy (SEM)) examination to assess surface damage. Finally, the samples can be used to isolate microorganisms involved in the degradation process.

## **2.5 Choice of environment**

Studies found that fungi (Matavulj *et al*, 1992) and aerobic and anaerobic PHA-degrading bacteria are widely distributed and have been isolated from various ecosystems including soil, compost, aerobic and anaerobic sewage sludge, lake and marine water, estuarine sediment and air (Jendrossek *et al*, 1996). Thus, there are many disposal routes for biodegradable polymer and they can be differentiated into terrestrial and aqueous:

**Table 2 : Disposal routes for biopolymer**

<b>Terrestrial</b>	<b>Aqueous</b>
Landfill	Groundwater
Soil	Waterways
Composting	Marine
	Sewage
	Sediments

PHA – degrading bacteria were isolated from various environments such as soil ( *Pseudomonas lemoigne* ), activated sludge ( *Alcaligenes faecalis* ), laboratory atmosphere ( *Pseudomonas pickettii* ), seawater ( *Comomonas testeroni* ) and lake water ( *Pseudomonas stutzeri* ) ( Doi, 1995).

Some of the test systems stated in Table 2 are well developed while others are still being studied. For example, the aqueous aerobic situation is well served for test protocols whilst testing in sediments and soil require more research and still lack of standardisation. The biodegradability of polymers under anaerobic conditions may be many times less, as oxidative reactions involving oxygenase and molecular oxygen cannot take place in the highly reduced atmosphere. To determine biodegradation, calculation by comparison of the total carbon released as gas with the theoretical maximum derived from the molecular formula has to be made.

Composting environment represents a potential significant route of disposal. Composting is also an ideal environment for rapid degradation of organic matter. Anyway it is not easy to miniaturise or standardise for screening purposes in the laboratory because of the heterogenous nature of composts. This will affect the control experiment of the whole process.

Soil burial is one way to study biodegradation of polymers in nature. Eventhough the study of this type of methods is very scarce, it could be the best way to monitor biodegradation in natural environment.

## **2.6 Degradation of biodegradable polymer through different disposal routes**

### **2.6.1 Composting**

Composting is an ecological and environmentally sound approach to transfer biodegradable waste including the new biodegradable plastics to useful soil amendment product ( Narayan, 1994). Composting represents an increasingly important route of disposal for the organic fraction of municipal solid waste (Starnecker and Menner,1996).Studies on this method are widely conducted. Assessment of biodegradability of plastics under simulated composting conditions in a laboratory test system was carried out by Starnecker and Menner. Biodegradation was monitored by measuring microbial carbon dioxide formation and oxygen consumption. In the heterogenous matrix compost, it is not feasible to assess the completeness of biodegradation due to limited possibilities to analyze degradation intermediates and biomass growth. Carbon dioxide formation may be measured that does not result from microbial degradation of test material. Therefore, a new lab-test was developed by the authors which

replaces the heterogeneous matrix compost by a biologically inert, carbon-free material.

The European Standard WI 261 236 established a strategy that must be followed for every packaging and packaging material intended for composting in accordance with the regulation (Pagga, 1998). In order to avoid hazardous substances accumulating in the environment, no such constituents may be used in packaging materials intended for composting. Such a testing strategy is not only applied for packagings but also for plastics (ISO 15986, 1998) since both have similarities; eg. being composed of biodegradable polymers.

### **2.6.2 Sewage sludge**

Many biodegradation studies were conducted using the sewage sludge since it contains microbes with different species that could increase the rate of biodegradation. The degradation of sheets of poly (2-hydroxybutyrate-co-3-hydroxyvalerate) or also known as BIOPOL by aerobic sewage sludge was carried out (Briese *et al*, 1994). These researchers found out that degradation of the polymer was highly dependent on the pH of the culture medium and was maximal between pH 7 and pH 8.5 where the weight loss of about 60%, 85% and 100% was measured after 4,8 and 12 weeks of incubation. Below pH 6 and above pH



9 the degradation rate was very slow where only 10% of the polymer was degraded after 12 weeks.

### **2.6.3 River water**

River water has borne a major brunt of water pollution due mainly to irresponsible human activities whereby all types of wastes are disposed into the drain and also in the river. This indiscriminate garbage dumping will cause the blockages in drains and streams causing them to overflow during a heavy downpour leading to flash floods and at the same time hazardous to the wildlife especially the fish. As a means to overcome the problem, some scientists studied the degradation of specific wastes in the water, eg. polymers because they constitute a big portion of the wastes. In Japan, the biodegradabilities of 21 samples of biosynthetic and chemosynthetic polyesters were measured at 25°C under aerobic conditions in a temperature-controlled reactor containing 200ml of natural water from river Arakawa (Saitama, Japan) as an inoculum. The degradabilities were measured by monitoring the time-dependent changes in the biochemical oxygen demand (BOD), weight loss (erosion) of polyester film and dissolved organic carbon concentration (DOC) of the test solution (Doi *et al*, 1996). The microbial copolyesters, poly (3-hydroxybutyrate-co-3-hydroxyvalerate), poly (3-hydroxybutyrate-co-4-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxypropionate)

were degraded in the river water at a rapid rate and the weight loss and BOD-biodegradability were 100% and 80% for 28 days respectively. In contrast, the biodegradability of chemosynthetic polyesters were strongly dependent on the chemical structure.

#### **2.6.4 Seawater**

Biodegradability of bacterial copolyesters such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) was studied under marine exposure conditions in sea water (Doi *et al*, 1996) The time-dependent changes in molecular weight and mechanical strength of films, plates and fibers were monitored. The samples were found to be degraded by microorganisms via surface erosion in seawater. From different literature studies researchers found out that in seawater, degradation of PHB did occur and 71% of the initial weight remained after half a year (Mergaert *et al*, 1992). The experiment was carried out in the seawater in the harbour of Zeebrugge. Mukai *et al*, 1994 reported that in aqueous medium, PHA depolymerases were found to be more hydrophobic than those in other environments such as soil and air. High hydrophobicities of PHA depolymerases in aqueous environments may be essential help for efficient adherence of the enzyme to the PHA materials.

### **2.6.5 Freshwater**

Studies were conducted in the freshwater of University Gent, Belgium freshwater pond and the "Schipdonk" canal at Zeebrugge. (Margaert *et al*, 1992). After 186 days, the PHB was found to have lost only 4% of its initial weight. In the canal, after 63 days, 1% weight loss was recorded. This showed that the polymer degrades slowly in freshwater. Studies conducted by Doi *et al*, 1990a found that the polymer chain of a microbial polyester was hydrolyzed in water without enzymes, at a very slow rate. It involved a simple hydrolytic chain scission in the degradation process.

### **2.6.6 Lakewater**

An experiment was carried out in Lake Lugano, Switzerland in order to study the biodegradation of PHA in an aquatic ecosystem under natural conditions (Brandl and Puchner, 1990). Commercially available plastic articles made from PHA, such as shampoo bottles and films were incubated for 250 days in a water depth of 85m by positioning it on the sediment surface by using a buoy. Results demonstrated that in an aquatic ecosystem even under extreme conditions (low temperature, high pressure, no sunlight) plastic articles made from PHA were degraded and the life span of this specific bottle was calculated as 10 years.

### 2.6.7 Soils

The degradation of poly (3-hydroxybutyrate) (PHB) was investigated in 5 different soils in plastic containers (14-22% water content) and incubated at 15°C, 28°C and 40°C in laboratory conditions (Margaert *et al*, 1992). The five different soils were hardwood soil (pH3.9), pinewood soil (pH3.5), sandy soil (pH6.5), clay soil (pH7.1) and loamy soil (pH6.3). After 200 days, all tests pieces lost weight with the highest loss of 26% of initial weight in the pinewood soil, followed by hardwood soil (23%), clay soil (22%), sandy soil (12%) and loamy soil (7%).

Methods for testing biodegradable plastics based on the measurement of gas evolution and residual sample weight in aerobic and anaerobic soils have been investigated (Eya *et al*, 1994). The amounts of CO<sub>2</sub> and CH<sub>4</sub> which evolved from anaerobic Ando soil collected from a farm in Goushicho, Kumamoto, Japan containing powders of Biopol or PCL were significantly less than those from aerobic Ando soil. From the aerobic gas evolution test, the weight loss of samples calculated from CO<sub>2</sub> and CH<sub>4</sub> evolution after 45 days were 14% and the residual weight was 53%. The remaining 33% may be decomposed substrates, cells of microorganisms and others.

2. Field test of plastics biodegradability in soil conducted at 18 locations in Japan and one in US by the Biodegradable Plastic Society, Japan showed that plastics showed different degradability depending on microorganisms in the soil, moisture content of the soil, aerobicity and amendment of organic materials in the soils (Kimura *et al* , 1994).

3. In an experiment conducted, food packaging materials made from highly plasticized polyvinyl chloride (PVC), traditional polyethylene (PE) and polypropylene (PP) were exposed in soil under conditions generally favourable for biodegradation (Yabannavar and Bartha, 1993). During 3 months of exposure, PE and PP films did not undergo measurable deterioration but a group of newly formulated and heavily plasticized PVC films underwent extensive biodeterioration and up to 27.3% of their carbon were converted to CO<sub>2</sub>.

## 2.7 Rationale behind the research of biodegradability of polyhydroxyalkanoates

PHA has been found to be a potential candidate as a biodegradable plastic. Concern over the management of non-renewable resources and the ultimate disposal of plastics has led to the research of this new polymer.

Since PHA is microbially synthesized, therefore it is not surprising that the polymer is readily degraded by a wide range of bacteria, fungi and algae. PHA will be degraded by the microorganisms and ultimate biodegradation will give rise to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  which will be recycled back to the environment. This is what we term as environmental-friendly product. The route of disposal for the petroleum based plastics including incineration and land-filling have not yet come to the ultimate degradation of the polymer. Incineration was even found to emit dangerous gas and residues which will harm the environment and also the living creatures. This is why we should look into something more safe and natural to the nature.

Whatever comes to the world has to be returned back to where it belongs. This is what natural process is and the use of this biodegradable

plastic, PHA can assure that the pollution caused by plastics will be reduced in the next millenium.

## **2.8 Objective of this study**

This study was carried out mainly to test the degradability of PHA produced from saponified palm kernel oil in garden soil. Since our lab is currently doing research on producing medium chain length PHA from local renewable resources which is the palm oil and its derivatives, thus it will be an added advantage to conduct study on the degradability of the polymer in the natural environment such as soil or river. For this degradation project, the garden soil was chosen as the environment since soil has become one of the disposal route for wastes. The test was carried out in the natural and enclosed environment. The enclosed environment study was carried out mainly to monitor the carbon dioxide evolution due to degradation of the test material.