Bioplastics

V C Kalia*, Neena Raizada and V Sonakya
Centre for Biochemical Technology, Mall Road, University Campus, Delhi 110 007, India

Plastics and polymers have become a part of our life today. The annual consumption of plastics in India is 2 kg/person/y. In the other developed countries the per capita consumption of plastics is of the order of 60 kg/y. Some naturally occurring plastic materials have been made use of by man from the earliest times, e.g., Lac and Amber. Today, almost all the plastics are manufactured synthetically and they have much better properties than naturally occurring plastics. The basic raw material for all the modern plastics is crude oil and natural gas. Due to their non-biodegradable nature, environmentalists are campaigning against their production and usage. In India, plastic wastes accounts for 1 to 4 per cent by weight of the total of 80,000 metric tonnes of Municipal solid waste generated daily. In USA, of the 4 lakh tonnes of garbage generated everyday, plastic constitute 30 per cent of its volume. Plastics (polymers) can be degraded by three different processes, either independently or in combination: (i) Light or high energy radiation, (ii) Heat, and (iii) Microbes. In response to increasing public concern over environmental hazard caused by plastic, many countries are conducting various solid waste management programmes including plastic waste reduction by development of biodegradable plastic material. There is an intense research for making the biodegradable plastic material. Some biodegradable plastic materials under development are: (i) Poly hydroxy alkanoates (PHAs), (ii) Poly-lactides, (iii) Aliphatic poly-esters, (iv) Polysaccharides, and (v) Co-polymers and/or blends of above. However, it is possible to produce biodegradable plastics from bio-wastes with the help of a syntrophic population of microbes of diverse origins. This will help to reduce waste management problems and improve environment.

Plastics and polymers have become a part of our life today. The annual consumption of plastics in India is 2 kg/person/y. When compared with the rest of the world this is a very small figure. An average American uses 80 to 90 kg of plastics/y. In other developed countries the per capita consumption of plastics is of order of 60 kg/y and the world average is 15 kg/y. The projected demand of plastics in India is about 25 lakh tonnes/y by the end of this century. There were about 12,500 plastic processing units in India with a total investment of Rs 2,000 crores in 1992. By 2000, we would need about 20,000 processing units which would require an additional investment of Rs 3,000 crores. In the Indian context, it must be borne in mind that the growth of the Indian plastics industry has been phenomenal, where the growth rate is higher than the plastics industry elsewhere in the world. From 1.88 million tonnes (1995-96) the domestic demand for plastics is expected to cross 4 million tonnes by year 2001-2. Furthermore the depletion of already scarce natural resources has established plastics as the material of choice in innumerable applications. For instance, in packaging, plastics are increasingly replacing jute, paper, wood, glass and metal. Even traditional areas, such as agriculture, are also using plastics as part of modern scientific methods for packaging.

Some naturally occurring plastic materials have been made use of by man from the earliest times. Lac was used for molded ornaments and Amber, a yellow coloured fossil polymeric resin formed from trees buried into the ground millions of years ago, was known to the Greeks.

Synthetic Plastics

Today, almost all the available plastics are manufactured synthetically and they have much better properties than naturally occurring plastics. The basic raw material for all the modern plastics is crude oil and natural gas.

Crude oil is recovered from underground oil deposits or from the seabed by offshore drilling. This oil is a mixture of heavy hydrocarbons and is processed in the petroleum refineries to obtain a wide spectrum of industry. Heavy oil molecules when heated under pressure and in the presence of a catalyst breakdown into smaller molecules. This process is called catalytic cracking. The resultant mixture of hydrocarbons is then subjected to fractional distillation. The fractions evaporated at different temperatures can be condensed separately. Naphtha is one such fraction. Naphtha, which closely re-
sembles the petrol used in automobiles, is the main source of chemicals used for making various kinds of plastics. Naphtha is further cracked to obtain still lighter hydrocarbons, which are the raw materials for most of the common plastics used today. Polyethylene and PVC are the two major plastics, manufactured from ethylene gas obtained by cracking naphtha. Natural gas, which also comes out from the oil-fields is a source of yet another important raw material for plastics, called formaldehyde. This chemical which is the raw material for making rigid types of plastics is made from methanol produced from natural gas. There are many other chemicals, which also serve as starting materials for various types of polymers and plastics. Almost all of them are derived from crude oil. Butylene is extensively used for making synthetic rubber.

Plastic vs Pollution
The production and consumption of plastics have increased in geometrical progression in this century. However, due to their non-biodegradable nature, environmentalists are campaigning against their production and usage. In India, plastic wastes accounts for 1 to 4 per cent by weight of the total of 80,000 metric tonnes of Municipal solid waste generated daily. In USA, of the 4 lakh tonnes of garbage is generated everyday, where plastic constitute 30 per cent of its volume.

Every one sees plastic waste dumps daily, but no concerted effort is evident for their disposal. Plastic waste is managed largely by the unorganized section of our society — the rag pickers. These wastes are recycled for producing low quality plastic products. Only 10 per cent of the disposed plastic is recycled through this route. The remaining non-recovered and unused waste takes a very long time to degrade and be reduced to dust. The plastic waste pose a serious problem to landscape, wildlife, marine animals, and become an "environmental eyesore". It is primarily due to the non-biodegradable nature of the plastics. Plastics waste management status in India is given in Table 1.

Plastics have also a costly impact on waste management and municipalities are becoming aware of the significant savings that collection of 'wet' organic wastes in so-called 'biobins' to be composted can provide. For these reasons, reaching the conditions for replacement on non-degradable polymers by degradable plastics, particularly for single-use disposable and packaging applications, is of major interest both to decision-makers and the plastic industry.

<table>
<thead>
<tr>
<th>Table 1 — Plastics waste management status in India</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1995-96</strong></td>
</tr>
<tr>
<td><strong>Consumption of plastics</strong></td>
</tr>
<tr>
<td><strong>Waste available for recycling</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

Anti-plastic Drive
Many Indian states have issued notifications for the use of certain types of plastics, particularly for packing food stuff. Government has issued notification to ban coloured plastic bags. UP government has also planned to ban poly-bags soon. Similarly, Himachal Pradesh government has introduced the HP Non-biodegradable Garbage (Control) Act 1995. It prohibits throwing or depositing plastic articles in public places and facilitates collection through garbage in identifiable and marked garbage receptacles for non-biodegradable. In a step towards eradication of plastics in daily use, Sabarimala, Kerala’s major pilgrim shrine, and the state zoos and museums have banned carrying of plastic bags and containers on their premises.

Ministry of Environment and Forest have issued criteria for labeling "plastic products" as "Environmental Friendly" under its 'Ecomark' scheme in association with the Bureau of Indian Standards. One of the requirements for plastic products, is that the material used for packaging shall be recyclable or biodegradable. Among the various guidelines, a clause reads "no recycled plastic waste shall be used" for critical applications like plastic piping system, water-storage tanks, packaging for food articles.

The Government of India has issued the new guidelines under the National Plastic Waste Management Task Force's (NPWMTF) for plastic industries / manufactures. The salient features of the NPWMTF's report are:

(a) The strategy for effective management of plastics wastes should entail the three Rs: Reduce, Reuse and Recycle;
(b) Manufacture of dirty coloured carry bags with visible contamination and their circulation in the market should be banned;
(c) Thickness of plastic bags to increase from 6 µ to 20 µ for virgin plastic bags and 25 µ for those made out of recycled plastics; and
Table 2 — Polymer degradation pathways

<table>
<thead>
<tr>
<th>Method</th>
<th>Active agent</th>
<th>Heat required</th>
<th>Degradation rate</th>
<th>Eco-friendly</th>
<th>Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photo-degradation</td>
<td>UV or other high energy radiations</td>
<td>No</td>
<td>Slow initiation but fast propagation</td>
<td>No</td>
<td>Yes, expensive</td>
</tr>
<tr>
<td>Thermo-oxidative</td>
<td>Heat + O₂</td>
<td>Very high</td>
<td>Fast</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>degradation</td>
<td>Microbial enzymes</td>
<td>No</td>
<td>Moderate</td>
<td>Yes</td>
<td>Yes, highly acceptable</td>
</tr>
</tbody>
</table>

Figure 1 — Photo and biodegradation of polymers and blends

(d) Virgin bags to be colourless, i.e., translucent, while recycled bags to be in colour.

This never ending waste generation will lead to accumulation of plastic in our environment. There is thus need to develop an eco-friendly strategy for up-gradation of recycling technologies, re-defining guidelines for disposal, formulating specifications and codes of practices (Regulations and Legislation).

Plastic Degradation

Polymers can be degraded by three different processes given in Table 2, either independently or in combination: (i) Light or high energy radiation, (ii) Heat, and (iii) Microbes (Figure 1).

(i) Photo Degradation — It is initiated by the absorption of light energy by reactive groups of the polymer, leading to smaller fragments. The process re-
quires either in-built photo-responsive group or an additive.

(ii) Heat — Degradation of a polymer occurs by heating in the presence of oxygen, i.e., thermo-oxidative degradation. At the primary stage, macro-molecules bonds are broken down with the formation of radical sites. These radical sites react with oxygen to form per-oxo radicals. Thus, long chain polymer molecules are converted into smaller fragments and volatiles. (iii) Biodegradation — For following the biological route, polymers have to be designed in a manner that monomers are susceptible to microbial attack. Alternatively, biodegradable additives or groups have to be blended during polymer formation.

Biological transformation occurs via relatively specific enzymatic processes. Microbes have a significant and dominant role to play. The process is favoured by darkness, high humidity, adequate minerals and other nutrients, temperature, pH, and O₂. Biodegradation is a gradual and slow decomposition of complex materials by living micro-organisms, e.g., fungi, bacteria, and actinomycetes. Aerobic degradation of complex organic material works efficiently till 60 per cent. On the other hand, anaerobic degradation of organic matter to CO₂ and CH₄ occurs at a very high rate of up to 95 per cent. The rest of the matter being inorganic materials easily mixes with soil and increases soil quality. Fungi have been more employed because of their evident invasion properties.

\[
\begin{align*}
\text{Hydrolysis} & \quad \text{Hydroyalted} & \quad \text{Oxidation} \\
\text{POLYMER} & \quad \text{Product} & \quad \text{CO}_2 + \text{H}_2\text{O} \\
\text{(Microbial)} & \quad \text{(Microbial)} \\
\end{align*}
\]

Microbial Biodegradation of Polymers

**Biological Approach for pollution Abatement**

In response to increasing public concern over environmental hazard caused by plastic, many countries are conducting various solid waste management programmes including plastic waste reduction by development of biodegradable plastic material. Thus, there is an intense research going on for making the biodegradable plastic material. Some biodegradable plastic materials under development are: (i) Polyhydroxy alkanoates (PHAs), (ii) Polylactides, (iii) Aliphatic polyesters, (iv) Polysaccharides, and (v) Co-polymers and/or blends of above.

The target can be achieved by either producing plastics containing biological additives (e.g., starch) or produce plastics from biological materials exclusively. The introduction of additives like starch into polyethylene—"Bio-D" bags help it to undergo biodegradation. Starch acts as nutrition for microbes. The major limitation of this type of plastic is the quantity of starch, which can be used as additive, cannot exceed 30 per cent. The degradation rate of this photo-oxidant is quite slow.

A eco-friendly alternative to this "biodegradable polymer" is the production of poly-hydroxy butyrate (PHB). PHB is 100 per cent biodegradable plastic. It can be prepared from renewable sources. Biopol is a PHB which degrades within few months in the soil. However, it is very costly, roughly 25-times costlier than polyethylene, and at present its use is restricted to medical applications only, such as nails for bone consolidation, suture thread, and pharmaceutical capsules. These bio-plastics can potentially replace traditional plastics or petroleum based plastics.

Potential microbial routes to the production of plastics and synthetic fibres can be divided mainly into three categories. These are: (i) The production of bio-polymers processing the properties of plastics, (ii) The use of microorganisms as bio-catalysts to produce specialised chemical intermediates or monomers, and (iii) The release of usable chemicals from renewable carbon sources such as lignin. Microorganisms are being used to produce environmentally-benign plastics.

Polyhydroxy alkanoates (PHAs) are biopolymers accumulated as storage compounds by a large number of different prokaryotes. More than 100 different hydroxy-alkanoic acids have already been detected as constituents of these bacterial polyesters. They are biodegradable thermoplastics and elastomers which have attracted considerable attention from academia and industry for their various technical applications in industry, agriculture, medicine, pharmacology and other areas.

PHA is a biodegradable polymer material that accumulates in numerous microorganisms under unbalanced growth conditions and has been produced on an industrial scale by ZENECAB by using *Alcaligens eutrophus*. PHA is synthesized and accumulated as granules in the cytoplasm of bacterial cells. The native PHA granules that contain lipids and proteins are rapidly hydrolyzed by intracellular de-polymerases under appropriate conditions. High resolution "C NMR spectroscopy of whole cells containing poly-3-hydroxybutyrate (PHB) has revealed that PHB is present predominantly in a higher mobile amorphous state, which seems to allow easy ac-
cess of de-polymerases. Freezing and storage of the PHA granules at low temperature, treatment with acetone or a hypochlorite solution, or even application of strong centrifugal forces to cells containing PHA could trigger irreversible conversion of PHA to the crystalline state. Recently, transmission electron microscope examination of freeze-dried PHA granules revealed that the dry granules have a non-crystalline core and a crystalline shell morphology.

Poly-hydroxy alkanoates (PHAs) are microbial polyester which have received considerable attention as biodegradable alternatives to conventional plastics. Co-polymers of hydroxy-butyrates and hydroxy-valerates including poly (β-hydroxybutyrate-co-β-hydroxy-valerate) (PHBV), from plastics having good mechanical qualities and have been made to incorporate low-value materials into PHBV to reduce their overall cost. Cornstarch is a particularly attractive filler for PHBV-based composites. Moreover, methods for blending starch with several polymers to produce blown film and extruded composites have been developed. Significant biodegradation occurred only after colonization of the plastic, a parameter that was dependent on the resident microbial populations.

Intracellular energy reserves, such as poly-3-hydroxybutyrate (PHB) may also promote environmental persistence. PHB is a homo-polymer of 3-hydroxybutyric acid, which some bacteria accumulate during unbalanced growth to provide an endogenous source of carbon and energy. Indirect evidence for the occurrence of PHB in *Legionella pneumophila* has been provided by Fourier transform infrared spectroscopy of whole cells and by pyrolysis mass spectrometry (MS). *Legionella* species metabolize 3-hydrobutyric acid and provide preliminary chemical evidence for the presence of PHB in chemostat cultures of *Legionella pneumophila*. The PHB was located in electron-dense intracellular inclusions, which fluoresced bright yellow when stained with the lipophilic dye Nile red. A Nile red spectrofluorometric assay provided a more accurate and reliable determination of the PHB content. *L. pneumophila* accumulates significant intracellular reserves of PHB, which promote its long-term survival under conditions of starvation.

PHB has been detected in the cytoplasmic membrane of *Escherichia coli*. *E.coli* cells incorporate PHB into their plasma membranes under growth-limiting conditions and during competence development. Recently, 3 genes responsible for the synthesis of PHA have been cloned from *Alcaligenes eutrophus* and expressed in *E.coli*. PHB was recovered by using a sodium hypochlorite solution or by using dispersions of a sodium hypochlorite solution and chloroform from *A. eutrophus* and a recombinant *E.coli* strain harboring the *A. eutrophus* PHA biosynthesis genes. A recombinant strain of *Escherichia coli* was used to produce poly (4-hydroxybutyric acid), P(4HB), homopolymer by fed-batch culture in M9 mineral salts medium containing glucose and 4-hydroxybutyric acid as carbon sources. The discovery of poly (3-hydroxybutyric acid), poly (3HB), as a storage compound in *Bacillus megaterium* was made in 1926, D(-)-3-hydroxybutyric acid (3HB) remained the only known constituent of bacterial poly-hydroxy-alkanoic acids (PHA) until constituents other than 3HB were reported in the sixties and seventies. However, these reports were either based on preliminary chemical analysis or they failed to attract the deserved attention. In the early eighties, 3-hydroxyvaleric acid (3HV) and 3-hydroxyhexanoic acid (3HHx) were incidently detected as constituents of PHA accumulated by axenic cultures of *Bacillus sps.* or *Alcaligenes eutrophus* and 3-hydroxyoctanoic acid (3HO) was detected as a constituent of PHA accumulated by *Pseudomonas oleovorans*.

Molecular and Biochemical Properties of PHA and PHB

Poly-hydroxy alkanoates (PHAs) are polyesters of various hydroxy-alkanoates. More than 100 different monomer units like 3-OH acyl monomers, have been identified. PHB has lowest molecular weight and is most common in nature. Their molecular weight can be up to 2 million, i.e., 20,000 monomers/polymer molecule. The monomer units of PHA are all in D(-) configuration owing to the stereospecificity of the biosynthetic enzymes.

There are three enzymes present in *A. eutrophus* for PHA biosynthesis. These are – PHA synthase, β-ketothiolase and reductase. Natural producers also have PHA depolymerase that degrades the polymer and uses the breakdown metabolites for cell growth.

Metabolism of PHB occurs as:

\[ \text{PHB} \rightarrow \text{D(-) hydroxybutyric acid} \rightarrow \text{acetoacetate or acetoacetyl CoA} \]

Properties of PHB

1. PHB is water insoluble and relatively resistant to hydrolytic degradation. This differentiates PHB from most other currently available biodegradable
plastics, which are either water soluble or moisture sensitive.

(ii) PHB shows good oxygen permeability.

(iii) PHB has good UV resistance but has poor resistance to acids and bases.

(iv) PHB is soluble in chloroform and other chlorinated hydrocarbons.

(v) PHB is bio-compatible and hence is suitable for medical applications.

(vi) PHB has melting point 175°C and glass transition temperature 15°C.

(vii) PHB has tensile strength of 40 Mpa, which is close to that of polypropylene.

(viii) PHB sinks in water, whereas polypropylene floats. But sinking of PHB facilities its anaerobic biodegradation in sediments.

(ix) PHB is nontoxic.

As many as 91 different HA have been detected as constituents of biosynthetic PHA. That all these HA coenzyme A thioesters are substrates of PHA synthases was concluded in most cases from the occurrence of the corresponding HA in the polyesters and from homologies to known pathways rather than by enzymatic studies. Many more substrates of PHA synthases and therefore new constituents of biosynthetic PPAs could probably be detected by further in vitro studies. Recently, lactyl-CoA was shown to be a poor substrate for the PHA synthases of some bacteria, indicating the incorporation of lactic acid into PHA.

Biosynthesis of PHB

The biosynthesis of PHB in most bacteria is initiated by the condensation of two molecules of acetyl-CoA by 3-ketothiolase to form aceto-acetyl-CoA, which is reduced in an enantiomERICally selective reaction by acetoacetyl-CoA reductase to R-(-)-3 hydroxy butyryl-CoA and is incorporated into PHB by PHB synthase. The acetoacetyl-CoA reductase involved in PHB synthesis is generally considered to be specific for NADPH, although in some bacteria the enzyme has been reported to have activity with NADH as well as NADPH. CC1192 cells retain the capacity for PHB synthesis when they differentiate from free-living cells into the bacteroid form.

Biosynthesis of PHA

Three metabolic phases of the biosynthesis of PHA in bacteria can be distinguished (Figure 2).

**Figure 2** — Three relevant phases of biosynthesis of PHA bacteria.

**Phase I** — A carbon source suitable for biosynthesis of PHA must enter the cell from the environment.

**Phase II** — Anabolic or catabolic reactions — or both — convert the compound into a hydroxyacyl coenzyme A thioester which is a substrate of the PHA synthase.

**Phase III** — PHA synthase, which is the key enzyme of PHA biosynthesis of these thioesters as substrates and catalyzes the formation of the ester bond with the concomitant release of coenzyme A.

The PHA synthase from *T. pfennigii* exhibited the most unusual substrate range among the PHA synthases investigated so far. However, this only became obvious if this PHA synthase was heterologously expressed in a different physiological background.

Most PHA consist of two or more HA. As outlined recently the metabolic reactions occurring in Phase II may result in the formation of various different HA coenzyme A thioesters that are used as substrates by the rather unspecific PHA synthases, thus giving rise to the synthesis of co-polymers.

The potential for the production of new PHA seems to be limited by the availability and costs of chemicals which can be provided as precursor substrates to the bacteria, rather than by the substrate range of PHA synthases. Therefore the chances of obtaining PHA with new HA or with an unusual combination of HA in the future, will depend on the successful screening for bacteria which synthesize these precursor substrates endogenously from simple and cheap carbon sources. Recently, bacteria were isolated which synthesize poly(3HB-co-3HV) or PHA from unrelated substrates.

Despite the excitement of more than 90 different constituents of biosynthetic PHA, it may be pointed out that the commercial exploitation of this variety remains limited. At present, only very few PHA are available in sufficient amounts to allow the evaluation of the physical,
Production of Bio-plastics by Anaerobic Digestion of Biological Wastes

Anaerobic Digestion

Anaerobic digestion process is a multiple stage process (Figure 3). Complex biomolecules are hydrolysed into relatively simpler compounds. A second group of acetogenic and acetoetic bacteria metabolise the hydrolytic products in lower volatile fatty acids, hydrogen and carbon dioxide. In the ultimate stage, methanogens convert these intermediates into methane.

Various process parameters for anaerobic digestion numerous bio-wastes of diverse origins have been established. Municipal market wastes viz. leaves and stalks of radish, cauliflower, cabbage, knol khol and banana, Food Corporation of India waste viz., damaged food grains, National Dairy Development Board Fruit and Vegetable Unit Waste viz. pea-shells, HPMC. Fruit Processing Plant waste viz. Apple Pomace, and Palm Oil Mill Effluent.

Polyhydroxy butyrate (PHB) is the key ingredient for bio-plastic production. The precursor to the formation are lower volatile fatty acids. Poly β-hydroxy butyrate (PHB) accumulates as energy reserve material in many microorganisms like Alcaligenes, Azotobacter, Bacillus, Nocardia, Pseudomonas, and Rhizobium.

PHB has physical properties comparable with polypropylene (PP). PHB comprises repeat units of CH(CH₃)CH₂-CO-O. The difference is that PP shows insignificant degradation while PHB shows complete degradation. PHB sinks while PP floats. Therefore, degradation is easy as sediment. Alcaligenes eutrophus and Azotobacter beijerinckii can accumulate up to 70 per cent of their dry weight of PHB as given in Table 3. These microorganisms can produce the polymer in environment of N and P limitation. At least 40 to 50 per cent of the dry weight of this polymer is required for making the process commercially viable. Extraction of PHB is done by using solvents like halogenated hydrocarbons and purification is done. Molding and extrusion of dried cells is directly possible when PHB contents are high. Lot of work has been done on engineering polymeric properties of PHB. However, PHB is suitable for specialized areas like biomedical use, specially coatings.

Biowastes

From activated sludge from a domestic sewage plant, Wallen and Rohwedder isolated PHA consisting of 3HB, 3HV, and 3HHx, there was also some evidence for the presence of traces of 3-hydroxyheptanoic acid. Analysis of activated sludge from a sewage treatment plant in Veberod (Sweden) revealed PHA, consisting of 3HB, 3HHx, and 3HO.
PHA consisting of 3HB, 3HV, 3-hydroxy-2methylbutyric acid, and 3-hydroxy-2-methyl valeric acid were obtained from activated sludge from a domestic sewage plant in Tokyo, which was withdrawn from the plant and subsequently cultivated in complex medium in a bio-reactor. PHA isolated from an estuarine sediment contained, in addition to 3HB, which contributed to only 30 per cent (w/w) of the constituents, at least five other 3HA.

Biodegradable thermoplastics, poly hydroxy alkanoates (PHAs) were produced from municipal sludge\(^{35}\) and Palm oil Mill effluent\(^{36,37}\). Biodegradable components in municipal sludge and other organic wastes are digested under anaerobic conditions by acidogenic bacteria into volatile fatty acids such as acetic, propionic, butyric acids, and other soluble organic compounds\(^{38,39}\). It has been reported that Alcaligenes eutrophus can utilize individual volatile fatty acid and polymerize the acid into PHAs as its carbon and energy reserve under nitrogen or oxygen limited conditions\(^{39-42,37}\). Many other bacterial strains are also reported to produce PHAs under adverse conditions with different PHA yields\(^{36,43,44}\).

Palm oil mill effluent has been employed as feed material for anaerobic digestion, the intermediate acetic acid was then used as a substrate in the fed-batch production of polyhydroxyalkanoate (PHA) by Alcaligenes eutrophus and by Rhodobacter sphaeroides strain IFO 12203.

PHA has been extracted with hot chloroform from activated municipal sludge and detected in marine sediments\(^{45}\). Biodegradable “enviroplastic” is developed by Planet Technologies, USA. Combinations of various carbon and nitrogen substrates were used to study PHB accumulation and \(H_2\) evolution by Rhodobacter sphaeroides\(^{45}\). PHA was continuously produced by Rhodobacter sphaeroides when sludge particles from anaerobically treated POME were removed\(^{36,37,46}\).

**Biodegradability Tests**

Generally, ASTM recommended microbial cultures are used. Systematic studies, employing wide range of microbes from diverse ecological habitats including hydrocarbon degraders has not been reported. Standard methods for comparison have not so far been established\(^{47}\).

Biodegradability test is dependent upon the objectives, e.g.,

(i) To determine the resistance of polymers from all sources, a mixed inoculum is preferred.

(ii) If the environment where the polymer is expected to be exposed, an inoculum derived from it is an appropriate choice.

(iii) If the end is not known, inocula preferred from soil, humus, domestic sewage or a mixture of known organisms proves effective.

Generally the biodegradability test is carried out by soil burial of the specimen and monitoring various physical, mechanical, and chemical properties of the test specimen as a function of time. Following tests have been in use\(^{47}\):

(i) **Warburg Test** (specification 301) — In this test the quantity of oxygen consumed during biochemical degradation (Biochemical Oxygen Demand, BOD) of a sample is measured as a function of time to the theoretical quantity of oxygen required for complete oxidation of the specimen kept under controlled test conditions. The test samples may be buried under soil or kept outdoors.

(ii) **ASTM D-5338** — The test samples are buried under soil of controlled humidity and composting conditions and loss of physical form, weight, mechanical strength is measured as a function of time. Biodegradation of samples may also be monitored by other test methods viz. a) change of molecular weight due to degradation by gel permeation chromatography, b) viscometry, c) change of carbonyl group content due to degradation by IR Spectrophotometry, d) \(CO_2\) evolution by Liquid Scintillation Counting and e) Colony growth, etc.

(iii) **Microbial Testing** — Pure cultures or enzymes and pure substrates can not be compared to water, sewage and oil environment so as a direct application is highly questionable\(^1\). Degradation pathways occurring in one ecosystem may not be identical with those found in an other.

(iv) **Biodegradation on Solid Medium (Agar plate methods)**\(^{49,49}\) — Most common test for assessing the biodegradability of polymers. It consists of: (1) Selection of suitable specimens, (2) Inoculation with microbes, (3) Incubation, (4) Testing for changes in the specimen. Agar plates as per ASTM standard. High melting agar employed for thermophiles. Plastic sample (2x2cm) was placed on agar plate and inoculated with microbial suspension. Glucose and \(NH_4NO_3\) were used as C and N
source. Microbial growth observed for two weeks (growth indicates non-toxicity of plastic). In controls, C and N were omitted to check if plastic can replace these sources.

(v) Biodegradation on Broth — Physical changes like pH, weight loss (of the sample) and biomass generation were recorded. Fungi is recommended in some of the most important internationally quoted biodegradation tests are given in Table 4. These microbes were initially employed for testing the resistance of cotton textiles. Fungi with an established ability to attack hydrocarbons are the right candidates, along with cellulase utilizers.

Since the number of biological parameters outnumber the individual interspecies differences among most of the soil organisms, adequate proof of a cause and effect relationship can only be provided with the pure cultures, as individual or a consortia.

(vi) Radiocarbon ¹⁴C Studies — The use of ¹⁴C constitutes one means to distinguish CO₂ produced from the polymer and other sources. ¹⁴C polyethylenes 2 per cent/y HDPE film reduced CO₂ evolution on removal of low mol wt components.

(vii) Soil Burial Method — It lacks reproducibility due to climatic factors and lack of control over microbial population Clendining et al., enriched microbes from soil buried polymer. Ennis and Kramer outlined a rapid method of (respirometric) to measure oxygen absorption by microbes and weight loss method.

<table>
<thead>
<tr>
<th>Russian proposal</th>
<th>AFNOR NF X 41-514</th>
<th>ISO 846</th>
<th>ASTM D 1924</th>
</tr>
</thead>
<tbody>
<tr>
<td>USSR-1 1958</td>
<td>Aout 1961</td>
<td>1968</td>
<td>197</td>
</tr>
<tr>
<td>Chaetomium</td>
<td>Chaetomium</td>
<td>Chaetomium</td>
<td></td>
</tr>
<tr>
<td>globosum</td>
<td>globosum</td>
<td>globosum</td>
<td></td>
</tr>
<tr>
<td>Memoniella</td>
<td>echinata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichoderma TI</td>
<td>Trichoderma TI</td>
<td>Trichoderma spp.</td>
<td></td>
</tr>
<tr>
<td>Stachybotrys</td>
<td>Stachybotrys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>atra</td>
<td>atra</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pencillium</td>
<td>Pencillium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bravicompactum</td>
<td>bravicompactum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pencillium</td>
<td>Pencillium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cyclopium</td>
<td>cyclopium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pencillium</td>
<td>Pencillium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>funiculosum</td>
<td>funiculosum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paecilomyces</td>
<td>Paecilomyces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>varioti</td>
<td>varioti</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus</td>
<td>Aspergillus</td>
<td>Aspergillus</td>
<td></td>
</tr>
<tr>
<td>amstlodi</td>
<td>amstlodi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus</td>
<td>Aspergillus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>niger</td>
<td>niger</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paecilomyces</td>
<td>Paecilomyces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>varioti</td>
<td>varioti</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus</td>
<td>Aspergillus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>flavus</td>
<td>flavus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myrothecium</td>
<td>Myrothecium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>verrucaria</td>
<td>verrucaria</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5 — Industrial production of PHA's and other biodegradable plastics

<table>
<thead>
<tr>
<th>Company</th>
<th>Areas of interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berlin Packaging Corp. (USA)</td>
<td>Marketing</td>
</tr>
<tr>
<td>Bioscience Ltd. (Finland)</td>
<td>Medical applications</td>
</tr>
<tr>
<td>Bio ventures Alberta Inc. (Canada)</td>
<td>Production in recombinant E.Coli.</td>
</tr>
<tr>
<td>Metabolix Inc. (USA)</td>
<td>Production in transgenic plants</td>
</tr>
<tr>
<td>Monsanto (USA)</td>
<td>Production from cheap substrates.</td>
</tr>
<tr>
<td>Polyferm INC. (Canada)</td>
<td>Production by A. eutrophus</td>
</tr>
<tr>
<td>Zeneca Bio-products (UK) (former ICI, UK)</td>
<td>Production in transgenic plants</td>
</tr>
<tr>
<td>Zeneca seeds (UK)</td>
<td>Production using A. lactic.</td>
</tr>
<tr>
<td>Petrochemia Danubia (PCD)</td>
<td></td>
</tr>
</tbody>
</table>

Table 6 — Manufactures of biodegradable plastics

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product (Trade name)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Warner- Lambert Company, USA</td>
<td>Noven (Molding grade)</td>
</tr>
<tr>
<td>2 Amke plastics, USA</td>
<td>Polyethylene</td>
</tr>
<tr>
<td>3 Air products and chemicals, USA</td>
<td>Mater-B1 (Molding grade)</td>
</tr>
<tr>
<td>4 Agro Chemical Company, USA</td>
<td>Complast (Master batch)</td>
</tr>
<tr>
<td>5 Novamont, Italy</td>
<td>Poly glycoside</td>
</tr>
<tr>
<td>6 Cabot Europe Ltd, France</td>
<td>poly lactide</td>
</tr>
<tr>
<td>7 Exxon Chemicals, Belgium</td>
<td>and their co-polymers</td>
</tr>
<tr>
<td>8 Phone-Poulenc Che mie, Belgium</td>
<td></td>
</tr>
<tr>
<td>9 Hoechst, Germany</td>
<td></td>
</tr>
<tr>
<td>10 Nova Corporation, Canada</td>
<td></td>
</tr>
<tr>
<td>11 American Cyanide Co. Ltd. USA</td>
<td></td>
</tr>
<tr>
<td>12 ICI, USA</td>
<td></td>
</tr>
<tr>
<td>13 Ethicon Inc.</td>
<td></td>
</tr>
</tbody>
</table>

(viii) **Visual Rating** — It is not a very desired method and is largely a measurement of the effect of the plastic upon the micro-organisms and vice versa. Also, a non-filamentous organism may cause much more severe degradation than a filamentous organism like fungus.

(ix) **Ammonia Production** — Bacteria release ammonia by means of endogenous respiration of their proteins in the absence of an utilizable carbon source. Values of ammonia produced by PVC samples show an excellent inverse correlation to weight loss of the file, and become the basis of a rapid method for assessing biodegradability.

(x) **Non-biological Methods** — These include changes in mechanical, physical and chemical properties like flexibility, elasticity or tensile strength, weight loss has been found to correlate most closely. Loss of transparency and loss of good di-electric properties of films are the basis of a rapid method for assessing biodegradability.

**International Status** — US chemical company Monsanto is already commercially producing (Table 5 and 6) a plastic material called “biopol” by fermentation of the bacterium *Alcaligenes eutrophus*. But the cost of production is high.

**Markets for Biodegradable Polymers**

Man-made polymers have been continuously and systematically improved to make them more stable, mechanically stronger and resistant to chemical and environmental conditions. Rot-resistance and rust-proof char-
acteristics of polymers have increased their demand and popularity. Other features that have made the usage of synthetic polymers in our daily life at an accelerated rate have been their low price, ready availability, wide range of colouration, and ease of fabrication in all the desired shapes. However the disposal of the used product is becoming increasingly difficult. Legislation have been, however, enacted in many developed countries where use of biodegradable plastics in certain applications is mandatory. It is thus estimated that by the end of this century biodegradable plastics will account for about 3 per cent of all plastic waste. Some of the biodegradable polymers are available in market (Table 7).

Present Status of Commercial Biodegradable Plastics

Although, plastics are in use and demand, however the market for degradable plastics is relatively very small. It represents less than 20 per cent of the total demand for plastics in the developed countries like the US or Japan. In India the demand may be less than 5 per cent. The major reason for this is the recycling of plastics. In India, almost every plastic item — films or molded products, domestic or municipal garbage are hand-picked, washed, dried, ground and recycled. Disposal of plastics will pose a real problem only if collection and processing cost of the reclaimed plastics is much more than that of the virgin material. Consequently, markets for degradable plastics will then be bright.

Starch-based plastic products will enjoy the attention of plastic processing industry for biodegradation and cost reduction, since the price of starch is almost 1/5th the cost of poly ethylene. Even partial replacement of the latter by the former by a simple technology appears to be commercially viable. Indian Institute Technology (IIT), Kharagpur has shown that incorporation of starch

<table>
<thead>
<tr>
<th>Field</th>
<th>Product</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Agriculture</td>
<td>(i) Fibre (ii) Film</td>
<td>Netting Mulching controlled release of agro-chemicals and micronutrients, fertilizers.</td>
</tr>
<tr>
<td>2 Horticulture</td>
<td>(i) Film (ii) Moulded item</td>
<td>Nursery bags, Plant cover and plant holders.</td>
</tr>
<tr>
<td>3 Packaging</td>
<td>(i) Film (ii) Blister packaging and bubble sheets</td>
<td>Packaging of perishable foods, dairy products, fruits, Vegetables, hosiery, etc. Fragile goods packaging</td>
</tr>
<tr>
<td>4 Domestic</td>
<td>(i) Films (ii) Moulded products</td>
<td>Shopping bags, composting bags, Diapers, feminine products, hygiene, garbage bags Food containers, vegetable crates, food service items egg-boxes, toys, pens, cutlery and cups, razors, containers for beverages, etc.</td>
</tr>
<tr>
<td>5 Hospital</td>
<td>(i) Moulded products</td>
<td>Disposable needles and syringes, sutures, surgical gloves, gowns, etc.</td>
</tr>
</tbody>
</table>
into LDPE up to 10 per cent only marginally affects the
strength properties of the plastics. The hydrophobicity of
the starch-based plastic products could be controlled by
addition of suitable additives.

It is being realized that the use of long-lasting polymers
for short-lived applications is not entirely justified,
especially when increased concern exists about the
preservation of living systems. The elimination of waste
plastics is therefore of interest in surgery, hygiene, catering,
packaging, agriculture, fishing, and environmental
protection application.

Acceptance of biodegradable polymers is likely to
depend on four unknown:

(i) Customer response to costs that today are generally
2-4 times higher than for conventional polymers,
(ii) possible legislation (particularly concerning water-
soluble polymers),
(iii) The achievement of total biodegradability, and
(iv) The development of an infrastructure to collect,
accept, and process biodegradable polymers as a
generally available option for waste disposal.

Thus the bio-plastics of the future will be produced
from renewable sources, will have a low energy content,
and will display in-use properties similar to those of
conventional plastics.

References

5. Parivesh Newslett (Central Pollution Control Board, Delhi), 2(S) (September 1998) 11.


