Chapter 1

Introduction
1.1 Plastics in demand

Polymers are used in various spheres of human activity. They are synthetic materials, which are well known and widely used, or polymers of biological origin, the so-called biopolymers. Synthetic polymers (nylon, polyethylene, polyurethane) have revolutionized our way of life, but they have also created a number of problems. First, the resources used to produce synthetic polymers are nonrenewable and, second, the application of polymers that cannot decompose in the natural environment and their accumulation lead to environmental pollution, presenting a global problem. Plastics become an essential part of almost every manufacturing industry ranging from automobiles to medicine (Reddy et al., 2003). Today, almost all the available plastics are manufactured synthetically and they have much versatile qualities i.e. strength, lightness, durability and resistance to degradation, structurally manipulated to have a wide range of strengths and shapes than naturally occurring plastics. They have become an important commodity to enhance the comfort and quality of life (Madison and Huisman, 1999; Khanna et al., 2005b). With more and more plastics being employed in human lives and increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastics and biodegradation of plastic wastes has assumed increasing importance in the last few years (Zheng and Yanful, 2005). Outputs of synthetic plastics are huge, increasing annually by about 25 million tons. In developed countries not more than 16-20% of them are recycled and they largely accumulate in landfills. By now, the annual output of polyolefins has reached 150 million tons. While glass containers are, as a rule, involved in the consumer cycle and paper containers degrade in natural conditions, the packaging items made of synthetic polymers can actually persist “eternally”. The main approaches of the plastic refuse policy are now landfilling and utilization. Polymer landfilling is a time bomb, and this problem will have to be solved by the future generations. A more cautious approach is utilization, which can involve several solutions: incineration, pyrolysis and recycling. However, neither incineration nor pyrolysis of plastic can essentially improve the state of the environment. Recycling is some solution, but it involves considerable labour and energy expenditures. Moreover in the recent years, there has been increasing legislative efforts to increase the ban on the disposal of non-biodegradable plastics. It is well understood that synthetic plastics are highly resistant to microbial degradation in landfills. This has become a problem.
for municipalities worldwide because municipal landfills lose capacity because of the accumulation of synthetic plastics.

Specialists argue that a radical solution to the problem of “polymer garbage” is to create and use a wide range of polymers that are, under the appropriate conditions, biodegraded into components innocuous to living and non-living nature. At present there are three main approaches to the development and use of biodegradable plastics: fabrication of plastics based on renewable natural polymers; modification of high molecular-weight synthetic materials, which are currently in wide use, to make them biodegradable; and, finally, synthesis of polyesters of hydroxycarbonic acids. Analysis of the latest literature on the development of biodegradable polymers shows that increasing efforts have been made to synthesize polymers based on hydroxycarbonic acids. Polyesters based on hydroxycarbonic (glycolic, lactic, valeric and butyric) acids are degraded in the environment by exodepolymerases of soil and water microflora, as its growth substrate (Sudesh et al., 2000a).

According to an estimate, more than 100 million tonnes of plastics are produced every year. Forty percent of the 75 billion pounds of plastics produced every year is discarded into landfills. It is well understood that synthetic plastics are highly resistant to microbial degradation in landfills. This has become a problem for municipalities worldwide because municipal landfills lose capacity because of the accumulation of synthetic plastics. Several hundred thousand tonnes of plastics are discarded into marine environments every year and accumulate in oceanic regions.

Recycling can be done but is very tedious. The sorting of the wide variety of discarded plastic material is also a very time-consuming process. Moreover, the presence of a wide variety of additives such as pigments, coatings, fillers, limits the use of the recycled material. Thus significant step towards, replacement of non-degradable polymers by degradable polymers and make environment pollution free is a major interest both to decision-makers and the plastic industry (Anderson and Dawes, 1990; Song et al., 1999).

1.2 Bioplastics: What are they?

Biomaterials include chemically unrelated products that are synthesized by microorganisms or part of them under different environmental conditions (Alias and
Tan, 2005). An important class of biomaterials is bioplastics, which can be defined as polyesters that are widely distributed in nature and accumulate intracellularly in microbes in the form of storage granules. Their physico-chemical properties resemble that of petrochemical (synthetic) plastics. PHAs belong to the polylactides class of naturally occurring biopolymers (Steinbuchel and Hein, 2001). Polyhydroxyalkanoates are completely biodegradable synthesized by bacteria as intracellular storage materials under conditions of stress and act as carbon and energy store. PHAs are accumulated as discrete granules to levels as high as 90% of cell dry weight and are generally believed to play a role as sink for carbon and reducing equivalents (Madison and Huisman, 1999). Polyhydroxyalkanoates are the polyesters of various hydroxyalkanoates which are accumulated as storage material under the conditions of limiting nutritional elements such as Nitrogen, Phosphorus, Sulfur, Oxygen or Magnesium in the presence of excess carbon source. More than 300 different microorganisms are known to synthesize and intracellularly accumulate PHAs (Lee, 1996a). A number of biodegradable plastic materials – mostly biodegradable polyesters, namely polyhydroxyalkanoates (PHAs), polylactides, aliphatic polyesters, polysaccharides, and the copolymer or blends of these, have been developed successfully over the last few years to meet specific demands in various fields and industries (Du and Yu, 2002).

Poly (D-3 hydroxybutyrate) is the most ubiquitous and most intensively studied PHA. The PHA content could be as high as 80% or higher for the sake of efficient recovery and we may call these polymer-filled bacteria as “Plastic Bacteria” (Lee, 1996b). In terms of molecular weight, brittleness, stiffness, melting point and other physical properties PHA is comparable to some of the more common petrochemical-derived thermoplastics. Therefore in certain applications, PHB can directly replace some more traditional, non-biodegradable polymers. Because PHB is resistant to water and UV radiation and is impermeable to oxygen, it is especially suited to use as food packaging (Grothe et al., 1999). Because PHAs are thermoplastics with biocompatible properties, they are being developed as new absorbable materials for implantable medical applications (Du and Yu, 2002). Other applications of PHAs include packaging material, osteosynthetic material in stimulation of bone growth, raw material for production of stereo regular compounds (PHAs being stereospecific), as mulch films in agricultural fields and as hot melt
PHAs can also be depolymerized to optically active bi-functional hydroxy acids. A good example in that context is Merck’s Anti-glaucoma drug “Truspot” which is synthesized from hydrolysis of PHB to R-hydroxybutyric acid and processing thereafter (Reddy et al., 2003).

The best advantage of PHAs is that, on disposal they are completely degraded by microorganisms in various environments such as soil, sea, lake water and sewage. There are two types of PHB polymers: Native and Denatured PHB granules. Native PHB granules containing lipids and proteins are rapidly hydrolyzed by intracellular PHB depolymerase. On the other hand, denatured granules, which are partially crystalline, are hardly hydrolyzed by intracellular depolymerases but can be degraded by extracellular PHB depolymerases into water soluble products. Distribution of PHB- degrading microorganisms, affects the biodegradability of PHA-based plastic. PHB-degraders are found to be widely distributed among the families of Pseudocardiaceae and related genera, Micromonosporaceae, Thermomonosporaceae, Streptosporangiaceae and Streptomycetaceae (Tokiwa and Calabia, 2004).

1.3 Problems and possible solutions

Much effort has been devoted to reduce the price of PHAs by development of bacterial strains, more efficient fermentation and more economical recovery processes (Godbole et al., 2003). High productivity is one of the major factors for economical production of biodegradable polymers (Chen et al., 2001). The carbon source should be inexpensive since it is the major contributor to the total substrate cost (Gao et al., 2002). The isolation and development of bacterial strains that can utilize cheap carbon substrates is pursued intensively. The most common PHA homopolymer, poly(3-hydroxybutyrate) has mechanical properties comparable to those of polypropylene. Unfortunately it is more brittle and very expensive. The brittleness can be diminished by the incorporation of 3-hydroxyvalerate units into the 3-hydroxybutyrate backbone during the fermentation process, resulting in the formation of more flexible poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer (Kim et al., 2000). This blend is commercialized by the name of BIOPOL™. This is about 18 times more expensive than polypropylene.

Recently transgenic plants harbouring *Alcaligenes eutrophus* PHA biosynthetic genes have been developed with the aim of ultimately reducing the price
of PHAs however efficient PHA production in plants needs to be improved to make it competitive with microbial fermentation process.

1.4 Biodegradable plastics and types

1.4.1 Chemically synthesized polymers

Polyglycolic acid, polylactic acid, poly (ε-caprolactone), polyvinyl alcohol, poly (ethylene oxide) fall into this category. These are susceptible to enzymatic or microbial attack. Since they do not match all the properties of plastics, they are not commercially viable as substitute for plastics.

1.4.2 Starch-based biodegradable plastics

In this type, starch is added as filler and cross linking agent to produce a blend of starch and plastic (for example, starch– polyethylene). Soil microorganisms degrade the starch easily, thus breaking down the polymer matrix. This results in significant reduction of degradation time. But such plastics are only partially degradable. The fragments left after starch removal are recalcitrant and remain in the environment for a long time.

1.4.3 Polyhydroxyalkanoates (PHAs)

Polyesters of microbial origin (polymers of hydroxyl fatty acids, or polyhydroxyalkanoates, PHAs) currently occupy a special position among biodegradable polyesters. Interest in PHAs has been growing since the late 190s. This is a new class of natural polyesters, which are not subject to rapid non-biological hydrolysis and whose properties like molecular weight, crystallinity, mechanical strength and degradability can vary substantially. They are promising materials to be applied in food industry (as packaging and antioxidant materials), agriculture (as coating materials for seeds, fertilizers and pesticides, as degradable films and containers for hothouse gardening), and in other spheres, including medicine and pharmacology. They are polyesters of various HAs which are synthesized by numerous microorganisms as energy reserve materials when an essential nutrient such as nitrogen or phosphorus is available only in limiting concentrations in the presence of excess carbon source (Steinbuchel and Hein, 2001). They possess properties similar to various synthetic thermoplastics like polypropylene and hence can be used
in their place. They are also completely degraded to water and carbon dioxide under aerobic conditions and to methane under anaerobic conditions by microorganisms in soil, sea, lake water and sewage.

![Diagram of biodegradation process]

**Figure 1.1: Photo and biodegradation of polymer and their blends**

Polyhydroxybutyrate (PHB) was the first PHA to be discovered and is also the most widely studied and best characterized PHA. PHAs are accumulated as discrete granules to level as high as 90% of cells dry weight (Madison and Huisman, 1999) thus called as polymer filled bacteria as “Plastic Bacteria” (Lee, 1996b). The linear structure of PHA molecules makes them thermoplastic. When heated, molecular chains in a PHA readily shift relative to each other; as a result, material softens and becomes fluid. This is a property of high commercial value: by moulding, extrusion, and other methods, PHAs can be processed into various items and materials. It is noteworthy that processing and molding of numerous currently used synthetic plastics require the addition of various stabilizers, fillers, dyes, etc. This is unnecessary in processing of PHA’s, which can be shaped from solutions and melts. It has mechanical properties very similar to conventional plastics like polypropylene or polyethylene and can be extruded, moulded, spun into fibers, made into films and used to make heteropolymers with other synthetic polymers. PHAs have attracted significant industrial interest because by varying the composition of carbon sources, the chemical and physical properties of PHA can be altered (Nonato et al., 2001). It
also features better gas barrier properties (for oxygen, e.g.) and a better resistance to ultraviolet; it is water- and heat- resistant; and its water vapour permeability is three times lower than that of polypropylene. Plastics produced from PHAs have been reported to be truly, fully biodegradable (Page, 1995).

**Ralstonia eutropha** (formerly *A. eutrophus*) is a bacterial strain that has been the subject of much of the published research because it can accumulate PHAs up to 80% dry weight (Lee, 1996a). Imperial Chemical Industries (ICI), now Zeneca Bio Products, Bellingham, England, produces biodegradable plastics from PHA using *R. eutropha*, and markets them as “BIOPOL” in Europe. BIOPOL was recently produced and sold by Monsanto, a company that acquired the business from Zeneca Bio Products in April 1996 (Braunegg et al., 1998). PHA is of interest because it possesses thermoplastic characteristics. According to Lafferty et al., 1988, PHB has some properties, including tensile strength and flexibility, similar to polyethylene and polystyrene. Plastics produced from PHAs have been reported to be truly biodegradable in both aerobic and anaerobic environments (Page, 1995), unlike many of the “so-called” biodegradable plastics made synthetically. PHAs are composed mainly of poly-beta-hydroxybutyric acid (PHB) and poly-beta-hydroxyvaleric acid (PHV), although other forms are possible. More than 80 different forms of PHAs have been detected in bacteria (Lee, 1996a). Only two forms of PHAs, i.e., PHB homopolymer and 3HB-3HV copolymer are commercially produced by Zeneca. Lafferty et al., 1988 stated that the accumulation of PHA can be stimulated under unbalanced growth conditions, i.e., when nutrients such as nitrogen, phosphorus or sulfate become limiting, when oxygen concentration is low, or when the C:N ratio of the feed substrate is high. Sasikala and Ramana, 1996 summarized nutrient limiting conditions that led to PHA accumulation in different microorganisms. In addition to nitrogen, phosphorus, oxygen, and sulfate limitations, limitations of the following compounds also stimulate the accumulation of PHA: iron, magnesium, manganese, potassium, and sodium.

Although there are presently economic disadvantages and limited uses of bacterial plastics, there is currently a high amount of research devoted to the area of bacterial plastics. It will not be long until science unveils a truly practical and biodegradable plastic. The production of biodegradable plastics on a large scale is limited because of the relative expense of the substrate, low polymer production, and
the cost of maintaining an axenic, i.e., pure, culture. According to Yamane, 1993, higher production costs, especially raw material costs, make it difficult for PHA biodegradable plastics to compete with conventional petroleum based plastics in the commercial marketplace. Lee, 1996b reported that the price of BIOPOL is $16/kg, while the price of synthetic plastics is a lot less expensive, e.g., less than $1/kg for polypropylene. The carbon source should be inexpensive since it is the major contributor to the total substrate cost (Gao et al., 2002). The use of waste as a raw material for PHAs production is a much cheaper carbon source than glucose and propionate used in the traditional Biopol process (Dionisi et al., 2004).

The substitution of petrochemical based plastics by degradable ones like polyhydroxyalkanoates has been one response to the problem. Thus, serious efforts are being made to move PHA production from laboratory to the manufacturing plant. Commercial uses of PHAs have so far been limited to specialty applications where property is more important than the costs. To industrially produce PHA based plastics, the bacterium must be found that can not only produce but also overproduce PHAs. The second criterion for the same bacterium is it must also be able to utilize inexpensive carbon sources. Biodegradable plastics find a broad acceptance in public as natural and environmental-friendly materials. Biodegradable plastics of renewable resources origin also help to preserve the non-renewable resources and contribute to sustainable development (Ren, 2003).

Although biodegradable polymers have been commercial for over 20 years, this niche market is beset with a variety of roadblocks led by high prices and lack of an industrial infrastructure to deal with these materials. The possibility of producing this polymer commercially and at comparable cost has been the main focus in this area (Kadouri et al., 2005a).

1.5 Bioplastics from wastes

Anaerobic digestion has become a key process for both waste reduction and recovery of renewable fuel and other valuable byproducts. The organic compounds are decomposed in a microbial chain to volatile fatty acids (VFAs) in the first phase and then these acids are further metabolized to release byproducts. The microbes involved in the process synergistically as products of phase one become substrate for
phase two (Raizada et al., 2001). The VFAs produced during the acidogenic stage of anaerobic digestion acts as a good process stability indicator.

Considering the fact that biodegradation is the ultimate fate of any material that enters into the environment, it was imperative to change our technologies to be more nature friendly. Recycling and stabilization of waste through anaerobic digestion is better approach (Kalia, 1994; Kalia et al., 2000a,b). During anaerobic digestion, the biodegradable component in municipal sludge and other organic wastes are digested by acidogenic bacteria into VFAs such as acetic acid, propionic acid, butyric acid, valeric acid and other soluble organic compounds. The lower chain fatty acids are then converted to polyhydroxybutyrate (PHB), a key ingredient for bioplastic production by various microbes such as *Ralstonia, Bacillus, Nocardia, Pseudomonas, Rhizobium* etc.

As the first step in pursuit of eventual usage of industrial food wastewater as nutrients for microorganisms to synthesize environmental-friendly bioplastics, the usage of soya wastes and malt wastes from brewery plant have been investigated as a carbon source for production of bioplastics (Alias and Tan, 2005). Edible Oil Mill Effluents (OMEs) were anaerobically fermented to obtain volatile fatty acids (VFAs), which are the most highly used substrate for polyhydroxyalkanotes (PHAs) production (Zazali and Irene, 2005). In recent years, the use of organic wastes (such as swine waste liquor, palm oil mill effluents, and vegetable and fruit wastes) is being studied as an alternative substrate for PHA production (Dionisi et al., 2005).

Conventional methods for searching microbes for a particular characteristics demand high inputs of time and energy. On the other hand, simple bioinformatics searches that integrate multiple sources of data offer faster and more rapid means of identifying new PHA producers (Kalia et al., 2003a,b). These potential PHA producers, in fact have the ability to degrade a wide range of waste materials.

One of the major problems in the commercialization of PHAs is the high production cost. Therefore, much effort has been devoted to the development of efficient recovery processes to lower this cost. The estimated market for biodegradable plastics is about 1.4 billion tons/year, with this demand driven by legislation and by environmental groups’ intent on protecting wildlife and reducing plastic litter (Kadouri et al., 2005b). Biowastes, thus are a major source of organic
matter, which is available in large quantities and can be exploited for producing bioplastics.

Replacement of non-biodegradable by degradable plastic is of major interest both to decision makers and the plastic industry (Song et al., 2001). Biodegradable plastics offer the best solution to the environmental hazard posed by conventional plastics. Nature’s built-in mechanisms and self-regulation ability cannot tackle novel pollutants since these are unfamiliar to it. This has prompted many countries to start developing biodegradable plastic. Biodegradable plastics of renewable resources origin also help to preserve the non-renewable resources and contribute to sustainable development (Ren, 2003).

Wastes of biological origin i.e. municipal market, food-processing industries, and agricultural industries have high organic content and that can be subjected to fermentation (Kalia et al., 1992a,b). Intermediates of fermentation such as volatile fatty acids can be diverted to produce polyhydroxyalkanoates (bioplastics) (Kalia, 1999).

1.6 Objectives of the study

The main objective of this research was to explore ways to improve the economics of manufacturing biodegradable plastics by producing useful by-products (PHAs) from potentially environmentally damaging waste discharges that are expensive to treat. The specific goals of the study include the following objectives:

1. To study microbial diversity in various ecological systems like fermenting biowastes.
2. To characterize microbial isolates for their ability to hydrolyze biowastes.
3. To characterize microbial isolates for their ability to produce polyhydroxyalkanoates.
4. Metabolism of biowastes by hydrolytic and other isolates into PHA.