Studies on biodegradation of modified polypropylene sheets using *Pseudomonas stutzeri* under aerobic conditions

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By

Preetika Rathour

(ROLL NO. 301001018)

UNDER GUIDANCE OF

Dr. Haripada Bhunia

Associate Professor

Department of Chemical Engineering

Department of Biotechnology and Environmental Sciences
Thapar University

Patiala-147004, Punjab

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CERTIFICATE

This is to certify that the thesis entitled **"Studies on biodegradation of modified polypropylene sheets using *Pseudomonas stutzeri* under aerobic conditions"**, is an authentic record of my own work carried out as requirements for the award of degree of Master of Science in Biotechnology from Thapar University, Patiala, under the guidance of Dr. Haripada Bhunia (Associate Professor, ChED) during January to June 2012.

Preetika Rathour

Date: 15 July 2012

Preetika Rathour
(Roll No: 301001018)

It is certified that the above statement made by the student is correct to the best of our knowledge and belief.

Dr. Haripada Bhunia
Associate Professor
Department of Chemical Engineering
Thapar University, Patiala

Countersigned by:

Dr. M. S. Reddy
Professor and Head
Department of Biotech & Env Sciences
Thapar University, Patiala

Dr. S. K. Mohapatra
Dean, Academic Affairs
Thapar University, Patiala

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Place: Thapar University, Patiala Preetika Rathour

ABSTRACT

The ability of bacteria to attack polymers was investigated in pure shake-flask culture studies. The mesophilic bacterium *Pseudomonas stutzeri* (MTCC number 2643) utilized Polypropylene (PP) and its blends with poly-L-lactic acid (PLLA) as the sole carbon and energy sources and degraded them. Incubation of PP and its blends with PLLA with *Pseudomonas stutzeri* for 60 days at 35°C, 120 rpm under aerobic conditions, reduced their weights. As the growth of microbes proportionally increased in synthetic media so it was predicted that the microbes were solely dependent on the polymer samples for its carbon source. Throughout the investigation, all the polymer samples were found to undergo quantitative and qualitative changes by bacteria. Biodegradation was observed in terms of their weight loss. PLLA was degraded maximum by *Peudomonas stutzeri* i.e upto 3.01% weight loss was observed followed by M-*g*-PP 80/6 i.e. upto 2.41%. PP100 had weight loss upto 1.25 %. The above data shows that blend of PP and PLLA blends show effective degradation than PP alone. The degradation results were confirmed by the scanning electron microscopy (SEM) analyses of the polymer samples. SEM micrographs showed the formation of patterns and cracks on the surface of the polymer samples evidencing a profound loss of structure.

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LIST OF ABBREVIATIONS

ASTM	American Society for Testing and Materials
APME	Association of plastic manufacturers in Europe
CFU	Colony forming units
HDPE	High Density Polyethylene
LDPE	Low Density Polyethylene
LLDPE	Linear Low Density Polyethylene
M-*g*-PP	Maleic anhydride grafted Polypropylene
MTCC	Microbial Type Culture Collection
NB	Nutrient Broth
OD	Optical Density
PCL	Polycaprolactone
PE	Polyethylene
PET	Polyethylene teraphthalate
PHA	Polyhydroxyalkanoates
PLA	Polylactic Acid
PLLA	Poly L-Lactic Acid
PP	Polypropylene
PS	Polystyrene
PU	Polyurethane

PVC	Polyvinyl chloride
US EPA	United States Environmental Protection Agency
UV	Ultra-Voilet rays
UV-Vis	Ultra-Voilet Visible Spectroscopy

Chapter-1

INTRODUCTION

Plastics material is one of the most popular materials and indispensable in the present world. The word plastic comes from the Greek word “plastikos”, which means ‘able to be molded into different shapes’ (Joel, 1995). Plastics are manmade long chain polymeric molecules (Scott, 1999). Plastics have become technologically significant since the 1940s and since then they have come to replace glass, wood, masonry and other constructional materials, and even metals in many industrial, domestic, commercial and environmental applications (Cain, 1992).

The attributes like light weight yet stro ng, least energy consumption and minimum emission of pollutants in the air and water during production, inert characteristics, excellent water resistance and barrier properties, excellent insulation and dielectric characteristics, ease of fabrication into variety of shapes and structure – have all made polymers not only a material of choice for an array of applications, use of polymers has become essential in every sphere of our modern life (fig 1.1). The long life of polymer products has added to the convenience (Indian Centre for Plastics in the Environment, 2010).

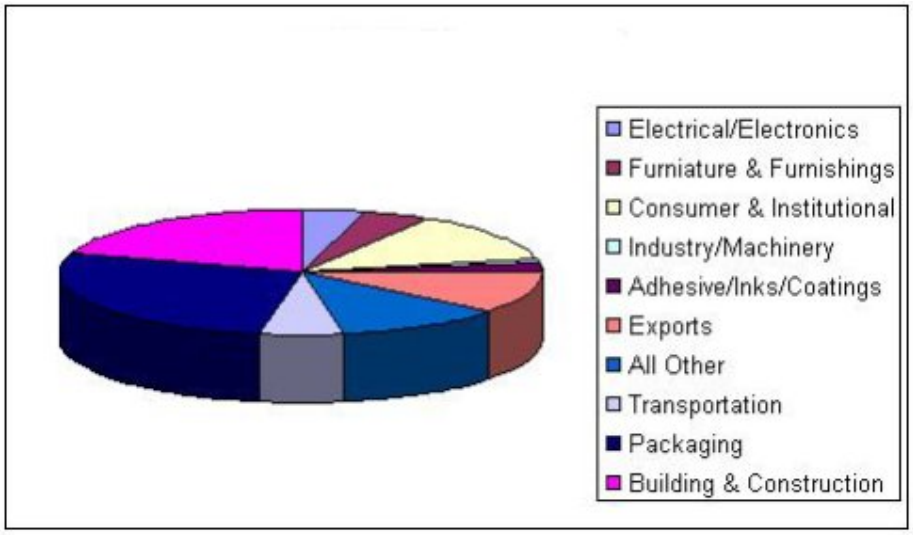


Fig 1.1 Distribution of plastics according to their usage
(Source: Shellie Berkesch Michigan State University)

The plastics we use today are made from inorganic and organic raw materials, such as carbon, silicon, hydrogen, nitrogen, oxygen and chloride. The basic materials used for making plastics are extracted from oil, coal and natural gas (Seymour, 1989). Polymer materials are solid, non-metallic compounds of high molecular weights. They are comprised of repeating macromolecules, and have varying characteristics depending upon their composition. Each macromolecule that comprises a polymeric material is known as a mer unit. A single mer is called a monomer, while repeating mer units are known as polymers (Kolybaba *et al.,* 2003).

Plastics are of two types: synthetic and natural. Synthetic polymers have been developed for durability and resistance to all forms of degradation. These characteristics and others (such as rigidity, permeability and transparency) can be controlled by changing the polymer synthesis process, molecular weight and/or by the use of specific additives (Kyrikou and Briassoulis, 2007).

Synthetic plastics are divided into two groups: water soluble and water insoluble. The former are generally polymers with functional group that affect water solubility such as carboxyl, hydroxyl, amido; the latter are usually non functional polymers commonly referred to as commodity plastics. Commodity plastics are used in packaging, disposable diaper backing, fishing nets and agricultural films. They include polymers such as polyethylene, polypropylene, polystyrene, polyvinyl chloride, poly(ethylene terephthalate), nylon. Some synthetic plastic like polyester polyurethane, polyethylene with starch blends, are biodegradable (Kawai, 1995).

Synthetic plastics are also categorized as: thermoplastics and thermoset plastics (Alauddin *et al.,* 1995). In thermoplastics, the atoms and molecules are joined end-to-end into a series of long, sole carbon chains. These long carbon chains are independent of the others (Allen *et al.,* 1999). This kind of structure in which the backbone is solely built of carbon atoms makes thermoplastics resistant to degradation or hydrolytic cleavage of chemical bonds. Consequently, thermoplastics are considered non-biodegradable plastics. Distinguished from the linear structure of thermoplastics, thermoset plastics have a highly cross-linked structure (Alauddin *et al.,* Scott*,* 1999). Since the main chain of thermoset plastics is made of heteroatoms, it is possible that they are potentially susceptible to be degraded by the hydrolytic cleavage of chemical bonds such as ester bonds or amide bonds (Muller *et al.,* 2001).

Thermoplastics are widely used in packaging and fabrication of bottles and films (Table 1.1). The major types of thermoplastic material include linear, low density polyethylene (LLDPE), high density polyethylene (HDPE), polyvinyl chloride (PVC), low density polyethylene (LDPE), polypropylene (PP), polystyrene (PS) and other resins. Thermoset plastics include a) polyester, one of which is polyethylene terephthalate (PET); and b) polyurethane (PU) (Avella *et al.,* 2001).

TABLE 1.1: Main plastics and their applications

Plastics	Applications
Low density polyethylene (LDPE), linear low density polyethylene (LLDPE), polyvinylchloride (PVC).	Films and Packaging, Bags
Polyethylene terephthalate (PET), PVC, high density polyethylene (HDPE).	Bottles, tubes, pipes, insulation molding
Polystyrene (PS), polypropylene (PP), PVC.	Tanks, jugs, containers, coffee cups, toys
Polyurethane (PU).	Coating, insulation, paints, packing

Natural plastics are products from renewable resources that are totally biodegradable in their natural form. Natural plastics or bioplastics are a special type of biomaterial. They are polyesters, produced by a range of microbes, cultured under different nutrient and environmental conditions (Madison and Huisman, 1999). These polymers, which are usually lipid in nature, are accumulated as storage material (in the form of mobile, amorphous liquid granules), allowing microbial survival under stress conditions (Barnard and Sander, 1989).

The biodegradable plastics are polyesters, namely polyhdroxyalkanoates (PHA), polylactides, aliphatic polyesters, polysaccharides and copolymer or blend of these, and have been developed successfully over the last few years. Polyhydroxyalkanoic acids (PHAs) are most significant due to properties similar to conventional plastics, they may be melted and molded, making them ideal for use in consumer products , and also its complete biodegradability. The most important are poly(3-hydroxbutyrate) and poly(3-hydroxybutyrate-co-3hydroxyvalerate) (Lee, 1996).

With the advances in technology and the increase in the global population, polymers have found wide applications in every aspect of life and industries (Tokiwa *et al*., 2009). This has resulted in single use of polymer products for mass consumption, and consequently a large volume of such products as carry bags, packaging materials, water bottles, dairy containers etc. are being thrown into the garbage (Maiti and Jana, 2005). Polymer accumulates in the environment at the rate of 25 million tons per year throughout the world. Burning of the polymer waste and burying of the polymers releases harmful toxic material which is a major pollutant in the environment. About 3% of polymer material is recycled while remaining remains as litter or landfiller (Kumari *et al*., 2009). It is therefore essential that the risks involved be eliminated or at least reduced to an acceptable level (Poliakoff *et al*., 2002).

Discarded, non-degradable polymers show undesirable environmental problems. These polymers create a threat to diverse animal populations. They have a direct impact on marine ecosystems and are believed to be responsible for the death of a very large number of birds by ingestion or strangulation (Scott and Wiles, 2001). It is estimated that one million tons of polymers are dumped in the sea annually. Entanglement can readily occur in materials with holes or plastic bags. Non-biodegradable polymers also have the capacity to act as disease foci because they persist in the environment for a very long period of time enabling organisms to accumulate (Jayasekara *et al*., 2005).

The dramatic increase in production and lack of biodegradability of commercial polymers, particularly commodity plastics used in packaging (e.g. fast food), industry and agriculture, focused public attention on a potentially huge environmental accumulation and pollution problem that could persist for centuries (Albertsson *et al.,* 1987). The plastic waste is disposed off through landfilling, incineration and recycling. Because of their persistence in our environment, several communities are now more sensitive to the impact of discarded plastic on the environment, including deleterious effects on wildlife and on the aesthetic qualities of cities and forests. Improperly disposed plastic materials are a significant source of environmental pollution, potentially harming life. In addition, the burning of polyvinylchloride (PVC) plastics produces persistent organic pollutants (POPs) known as furans and dioxins (Jayasekara *et al.,* 2005). The estimated figure of plastic waste generation across the Pakistan is 1.32 million tons per annum. This considerable content of plastic in the solid waste generated in Pakistan is of great concern. Plastic waste is released during all stages of production and post consumption every plastic product is a waste (Sabir, 2004).

Polymers can and do degrade by many routes. The polymers can be fragmented through physical forces. Fragmentation often plays an important role in the early stages of degradation and can be brought about by physical forces of mechanical nature. Chemical changes within the polymer can occur and may begin with abiotic degradation (Kyrikou and Briassoulis, 2007). Degradation brought by chemical reactions generally involves chain scission-fragmentation of the polymer chains. Surface erosion can be the result of chain scission resulting from chemical hydrolysis. At some point, some specific polymers may be attacked effectively by microorganisms-the onset of biodegradation. All of these pathways are potential routes for polymer degradation as shown in fig. 1.2 (Arutchelvi *et al*., 2008).

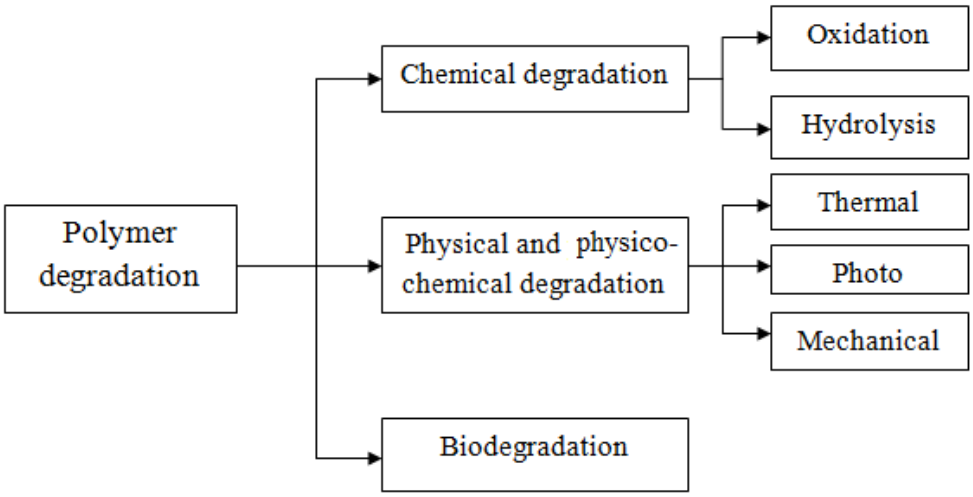


Fig. 1.2 Different pathways for degradation of polymers (Arutchelvi *et al*., 2008)

Chemical degradation is caused by certain chemicals like acids and alkalis, etc. Usage of certain microorganism and enzymes to degrade polymers are classified as biodegradation method of polymers (Premraj and Doble, 2004).

Most plastics tend to absorb high-energy radiation in the ultraviolet portion of the spectrum, which activates their electrons to higher reactivity and causes oxidation, cleavage, and other degradation. Sensitivity of polymers to photodegradation is related to the ability to absorb the harmful part of the tropospheric solar radiation. This includes the UV-B terrestrial radiation (~295– 315 nm) and UV-A radiation (~315–400 nm) responsible for the direct photodegradation (photolysis, initiated photooxidation). Visible part of sunlight (400–760 nm) accelerates polymeric degradation by heating. Infrared radiation (760– 2500 nm) accelerates thermal oxidation (Gugumus, 1990; Pospisil and Nespurek, 1997).

Thermal degradation generally involves changes to the molecular weight (and molecular weight distribution) of the polymer and typical property changes include; reduced ductility and embrittlement, chalking, color changes, cracking and general reduction in most other desirable physical properties (Olayan *et al.,* 1996). Thermal degradation of polymers is 'molecular deterioration as a result of overheating'. At high temperatures the components of the long chain backbone of the polymer can begin to separate (molecular scission) and react with one another to change the properties of the polymer. The chemical reactions involved in thermal degradation lead to physical and optical property changes relative to the initially specified properties.

Oxo-biodegradation process uses two methods to start the biodegradation. These methods are photodegradation (UV) and oxidation. The UV degradation uses UV light to degrade the end product. The oxidation process uses time, and heat to break down the plastic. Both methods reduce the molecular weight of the plastic and allow it to biodegrade.

Biodegradation is the process by which organic substances are broken down by living organisms. The term is often used in relation to ecology, waste management, environmental remediation (bioremediation) and to plastic materials, due to their long life span. Organic material can be degraded aerobically, with oxygen, or anaerobically, without oxygen. A term related to biodegradation is biomineralisation, in which organic matter is converted into minerals (Kyrikou and Briassoulis, 2007).

Biodegradability of the polymer is essentially determined by the following important physical and chemical characteristics: (1) Availability of functional groups that increases hydrophobictiy, (2) Size, molecular weight and density of the polymer, (3) Amount of crystalline and amorphous regions, (4) Structural complexity, (5) Presence of easily breakable bonds such as ester or amide bonds, (6) Molecular composition (blend) and (7) Nature and physical form of the polymer such as whether it is in the form of films, pellets, powder or fibers (Arutchelvi *et al*., 2008).

Plastics are biodegraded aerobically in wild nature, anaerobically in sediments and landfills and partly aerobically and partly anaerobically in composts and soil. Carbon dioxide and water are produced during aerobic biodegradation and carbon dioxide, water and methane are produced during anaerobic biodegradation (Gu *et al.,* 2000). Generally, the breakdown of

large polymers to carbon dioxide (mineralization) requires several different organisms, with one breaking down the polymer into its constituent monomers, one able to use the monomers and excreting simpler waste compounds as byproducts and one able to use the excreted wastes.

A wide variety of organic materials are easily degraded under aerobic conditions. In aerobic metabolism, O_2 is the terminal electron acceptor. When biodegradation follows this pattern, microbial populations quickly adapt and reach high densities. As a result, the rate of biodegradation quickly becomes limited by rate of supply of oxygen or some nutrient, not the inherent microbial capacity to degrade the polymer or other contaminant. Some organic compounds can also be degraded under anaerobic conditions. Under anaerobic conditions, oxygen is absent, nitrate (NO_3^-), sulphate (SO_4^{2-}), ferric iron (Fe^{3+}), manganese (Mn^{3+}, Mn^{4+}), and bicarbonate (HCO_3^-) can serve as terminal electron acceptors, if the microbes have the appropriate enzyme systems. The rate of degradation is usually limited by the inherent reaction rate of the active microorganisms; adaptation is slow, requiring months or years, and metabolic activity results in the formation of incompletely oxidized, simple organic substances, such as organic acids, and by-products such as methane or hydrogen gas. So, aerobic treatment is much safer than anaerobic treatment. Microorganisms adapt easily and early in aerobic conditions; and the danger of release of harmful gases are less. Biodegradation results in complete oxidation of organic substances.

Aerobic biodegradation (Bastioli, 2005):

$$C_{POLYMER} + O_2 \longrightarrow CO_2 + H_2O + C_{RESIDUE} + C_{BIOMASS}$$

Anaerobic biodegradation (Bastioli, 2005):

$$C_{POLYMER} \longrightarrow CO_2 + CH_4 + H_2O + C_{RESIDUE} + C_{BIOMASS}$$

Microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastics (Gu *et al.,* 2000). The biodegradation of plastics proceeds actively under different soil conditions according to their properties, because the microorganisms responsible for the degradation differ from each other and they have their own optimal growth conditions in the soil. Polymers especially plastics are potential substrates for heterotrophic microorganisms (Glass and Swift, 1989).

There is a strong synergism between biodegradation and environmental factors, and biodegradation can, in practice, never be entirely separated from the purely physical and chemical; mainly auto-oxidative progressive ageing, always present as an unavoidable slow but cumulative background effect. Biodegradation is seldom due to a single cause, but a combined effect, including heat, UV light, stress and water. The presence of water is necessity for biodegradation (Albertsson *et al*., 1987).

Biodegradability of the polymer is essentially determined by the following important physical and chemical characteristics: (1) Availability of functional groups that increases hydrophobictiy, (2) Size, molecular weight and density of the polymer, (3) Amount of crystalline and amorphous regions, (4) Structural complexity, (5) Presence of easily breakable bonds such as ester or amide bonds, (6) Molecular composition (blend) and (7) Nature and physical form of the polymer such as whether it is in the form of films, pellets, powder or fibers (Arutchelvi *et al*., 2008).

During degradation the polymer is first converted to its monomers, then these monomers are mineralized. Most polymers are too large to pass through cellular membranes, so they must first be depolymerized to smaller monomers before they can be absorbed and biodegraded within microbial cells. The initial breakdown of a polymer can result from a variety of physical and biological forces (Swift, 1997). Physical forces, such as heating/cooling, freezing/thawing, or wetting/drying, can cause mechanical damage such as the cracking of polymeric materials (Kamal and Huang, 1992). The growth of many fungi can also cause small-scale swelling and bursting, as the fungi penetrate the polymer solids (Griffin, 1980). Synthetic polymers, such as poly (caprolactone) (Toncheva et al., 1996; Jun et al., 1994), are also depolymerized by microbial enzymes, after which the monomers are absorbed into microbial cells and biodegraded (Goldberg, 1995). Abiotic hydrolysis is the most important reaction for initiating the environmental degradation of synthetic polymers (Göpferich, 1997) like polycarboxylates (Winursito and Matsumura, 1996), poly(ethylene terephthalate) (Heidary and Gordon, 1994), polylactic acids and their copolymers (Hiltunen *et al.,* 1997; molecular weights Nakayama et al., 1996), poly(α-glutamic acids) (Fan 1996), and polydimethylsiloxanes, or silicones (Lehmann *et al*., 1995; Xu *et al*., 1998).

Generally, an increase in molecular weight results in a decline of polymer degradability by microorganisms. In contrast, monomers, dimers, and oligomers of a polymer's repeating units

are much easily degraded and mineralized. High result in a sharp decrease in solubility making them unfavorable for microbial attack because bacteria require the substrate to be assimilated through the cellular membrane and then further degraded by cellular enzymes. At least two categories of enzymes are actively involved in biological degradation of polymers: extracellular and intracellular depolymerases (Doi, 1990; Gu *et al.,* 2000).

During degradation, exoenzymes from microorganisms break down complex polymers yielding smaller molecules of short chains, e.g., oligomers, dimers, and monomers, that are smaller enough to pass the semi-permeable outer bacterial membranes, and then to be utilized as carbon and energy sources. The process is called depolymerization. When the end products are CO_2, H_2O, or CH_4, the degradation is called mineralization (Frazer, 1994; Hamilton *et al.,* 1995). It is important to note that biodeterioration and degradation of polymer substrate can rarely reach 100% and the reason is that a small portion of the polymer will be incorporated into microbial biomass, humus and other natural products (Atlas and Bartha, 1997; Narayan, 1993).

Dominant groups of microorganisms and the degradative pathways associated with polymer degradation are often determined by the environmental conditions. When O_2 is available, aerobic microorganisms are mostly responsible for destruction of complex materials, with microbial biomass, CO_2, and H_2O as the final products. In contrast, under anoxic conditions, anaerobic consortia of microorganisms are responsible for polymer deterioration. The primary products will be microbial biomass, CO_2, CH_4 and H_2O under methanogenic (anaerobic) conditions (Barlaz *et al*., 1989) (Fig. 1. 3).

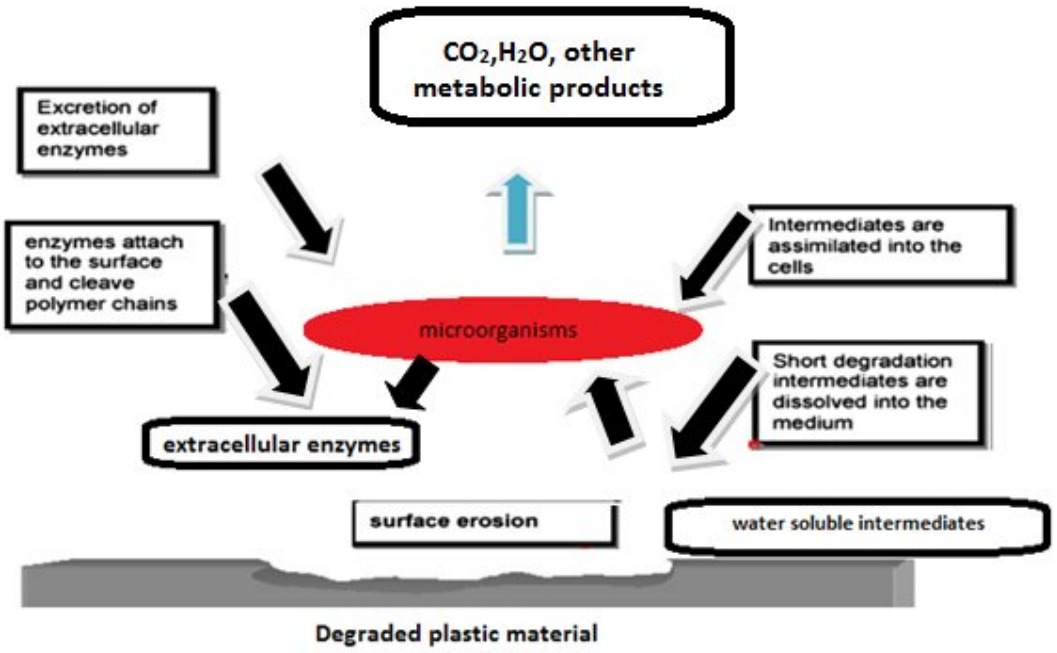


Fig. 1.3 General mechanism of plastic biodegradation under aerobic conditions (Mueller, 2003).

As the polymer usage is unavoidable, ways have to be found to (1) Enhance the biodegradability of the polymers by blending them with biodegradable natural polymers; (2) Mixing with prooxidants so that they are easily degraded and (3) Isolate and improve microorganisms that can efficiently degrade these polymers (Arutchelvi *et al*., 2008). Since most of the polymers are resistant to degradation, research over the past couple of decades has focused on developing biodegradable polymers, which are degraded and catabolized ultimately to CO_2 and water by bacteria, fungi under natural environment (they should not generate any harmful substances) (Premraj and Doble, 2005).

Synthetic polyolefins are inert materials whose backbones consist of only long carbon chains. The characteristic structure makes polyolefins non-susceptible to degradation by microorganisms. However, a comprehensive study of polyolefin biodegradation has shown that some microorganisms could utilize polyolefins with low molecular weight (Yamada-Onodera *et al.,* 2001). The biodegradation always follows photodegradation and chemical degradation.

Polypropylene is one of the synthetic polymers of high hydrophobic level and high molecular weight. In natural form, it is not biodegradable (Kwpp and Jewell, 1992). To make PP biodegradable requires modifying its crystalline level, molecular weight and mechanical properties that are responsible for PP resistance towards degradation (Albertsson *et al.,* 1994) and this can be best achieved by mixing it with synthetic plastics (like poly-L-lactic acid) (Tokiwa *et al.,*1990). These blends are not only have improve mechanical properties but also increase the hydrophilicity of the non biodegradable polymer (Pranamuda *et al.,* 1996).

The successful production and marketing of biodegradable plastics will help alleviate the problem of environmental pollution. In the past 10 years, several biodegradable plastics have been introduced into the market. However, none of them is efficiently biodegradable in landfills. For this reason, none of the products has gained widespread use. Hence, there is an urgent need to develop efficient microorganisms and their products to solve this global issue.

Chapter-2

LITERATURE REVIEW

Plastics are one of the most engineered materials, and have experienced a spectacular growth in both usage and adaptation (APME, 1999). Plastic materials have revolutionized the consumption of durable goods and virtually all major manufacturing industries in the world (Shimao, 2001). Plastics are man-made long chain polymeric molecules (Scott, 1999). They are very widely used, economical materials characterized by excellent all-round properties, easy molding and manufacturing.

Nowadays, a wide variety of petroleum-based synthetic polymers are produced worldwide to the extent of approximately 140 million tons per year and remarkable amounts of these polymers are introduced in the ecosystem as industrial waste products (Shimao, 2001). Traditionally plastics are very stable and not readily degraded in the ambient environment. As a result, environmental pollution from synthetic plastics has been recognized as a major problem. For instance, statistics published by the United States Environment Protection Agency in 2003 indicated that, before recycling, approximately 236 million tons of municipal solid waste was generated in the United States in that year, of which 11.3% was composed of plastics. Only a small fraction of this plastic waste (mostly soft drink and other bottles) was recovered (US EPA, 2005). Their lack of degradability is impacting significantly on rate of depletion of landfill sites and their persistence adds to the growing water and surface litter problem (Kumari *et al*., 2009). It is therefore essential that the risks involved be eliminated or at least reduced to an acceptable level (Poliakoff *et al*., 2002).

In recent years, considerable attention has been focused on biodegradability of polymeric materials. Such materials need to be resistant to degradation both prior to and during use and should be capable of being degraded, if discarded after use, without causing any environmental problems (Upreti and Srivastava, 2003). Two possible approaches to reduce the 'vices of polymeric materials' are (a) to develop biodegradable commodity plastic (Ratajska and Boryniec, 1999), and (b) to identify potential microorganisms and develop protocol to effectively biodegrade the polymeric materials (Kawai, 1995).

Biodegradation is the result of the utilization of the polymer as a carbon source by the microorganisms. This process is facilitated if the microorganism initially forms a biofilm over the polymer surface (Hadad *et al*., 2005). In the case of polymers such as PP, a continuous chain of repetitive methylene units makes it resistant to degradation. The hydrophobic nature of PP hinders the attachment of microorganism on its surface (Arutchelvi *et al*., 2008).

In almost every survey of biological degradation of synthetic polymers, it is fairly clearly stated that polythene is an inert polymer with good resistance to microorganisms. Several reports mention, that fungal growth can occur on the surface of polyethylene. (Dolezel, 1972; Demmer, 1975).

Scott (1999) concluded that attack by microorganisms is a secondary process. This step which determines the rate at which degradable polypropylene is returned to the biological cycle appears to be the rate of the oxidation process which reduces the molecular weight of the molecular weight of the molecule to the value required for biodegradation to occur. It has been shown that biodegradation of polypropylene is affected by preliminary irradiation from UV source by the presence of additives (Albertsson and Ranby, 1979), photodegrative enhancers, by its morphology and surface area, by antioxidants and by its molecular weight (Albertsson and Banhindi, 1980).

These pretreatments either decrease the hydrophobicity of the polymer thereby making it more compatible with the organism or introduces groups such as C=O (carbonyl) or –OH (hydroxyl), which are more prone to degradation. It is reported that UV- treated PP sample is more susceptible to degradation than LDPE (Sameh *et al*., 2006). Biodegradation of polypropylene/starch or polypropylene/cellulose blends has been reported using soil organisms. It is observed that the organisms easily degrade starch or cellulose leaving behind the polymer (Shah *et al.,* 2008, Ramis *et al.,* 2004). These carbohydrates or fillers increase the adhesion of the organisms to the surface of the polymer. Polycaprolactone (PCL) blended PP has also been reported to degrade in the presence of lipase (Weiland *et al*., 1995). PCL is an ester and since lipase is well known to degrade ester linkages, degradation of this polymer is facile. Lipase cannot affect the carbon-carbon present in PP. There are no reports available on the effect of tacticity on the nature and rates of biodegradation as well as on the use of marine organisms to achieve biodegradation. Isotactic polypropylene exposed to bacterial consortia for 175 days had 40% methylene chloride extractable compounds, and this extract was

identified to be a mixture of hydrocarbons (between $C_{10}H_{22}$ and $C_{31}H_{64}$) (Cacciari *et al*., 1993). Thirty to sixty percent growth of *A. niger* was observed on gamma irradiated PP films at the end of 6 weeks, which indicated that the fungus was able to utilize this polymer as its sole carbon source (Alariqi *et al*., 2006).

El-Shafei *et al.* (1998) investigated the ability of fungi and *Streptomyces* species to attack degradable plastics (disposable polyethylene bags containing 6% starch) in pure shake-flask culture studies. Eight different *Streptomyces* strains were isolated and two fungi *Mucor rouxii* NRRL 1835 and *Aspergillus flavus* were used. Ten-day heat treated (70°C) polypropylene films were chemically disinfected and incubated at 30°C, 125 rpm in 0.6% yeast extract medium (pH 7.5) for *Streptomyces* spp. and for the fungi in 3% yeast extract medium (pH 5.5) for 1, 2 and 4 weeks along with an uninoculated control for each treatment. Active enzymes caused changes in the film's mechanical properties and weight.

Most of the examples mentioned above deal with fungi and bacteria based degradation. Based on the literature one could conclude that pretreated polymers degrade more easily than the untreated polymers. Also, degradation is more facile with starch and cellulose blended polymers.

Cell surface hydrophobicity and addition of surfactants showed an important role in biofilm formation, which is prerequisite condition for biodegradation. Degradation leads to decrease in molecular weight, tensile strength and viscosity, formation of new functional groups such as carbonyl, hydroxyl, etc.

Microorganisms capable of degrading other polymers like polystyrene (PS), polycaprolactone (PCL), polyurethane (PU), polyamides (nylons), poly(lactide) (PLA) etc. have also been reported. Polystyrenes have been found to be degraded by fungal cultures like *Trichoderma* sp. and *P. pullulans*; when carbohydrate molecules like glucose, sucrose and lactose were linked to polystyrene-maleic anhydride (Galgali *et al.,* 2004). It was shown in this study that structural features can be incorporated to non-biodegradable polymer to induce biodegradability. *Streptomyces halstedii, Bacillus megaterium, Sphingobacterium spiritivorum, Bacillus cerus* capable of degrading styrene, were isolated from the bed of an experimental biofilter purifying exhaust gases from a cable factory's coil-wire varnishing division (Przybulewska *et al.,* 2006).

In the case of PP, since it is highly hydrophobic with high molecular weight, lacking active functional group, with continuous chain of repetitive methylene units, it shows resistance to biodegradation (Arkatkar *et al*., 2009). To facilitate high degree of degradation PP is blended with different natural plastics like PLLA (Ratajska and Boryniec, 1999). PLA degrading strains phylogenetically belong to *Pseudonocardiaceae* family and related genera, including *Amycolatopsis*, *Lentzea*, *Kibdelosporangium*, *Streptoalloteichus* and *Saccharothrix* (Jarerat *et al.* 2002).

PLA is fully biodegradable when composted in a large-scale operation with temperatures of 60°C and above. The first stage of degradation of PLA (two weeks) is via hydrolysis to water-soluble compounds and lactic acid and rapid metabolisation of these products into CO_2, water and biomass by a variety of microorganisms. There have been reports on the degradation of PLA oligomers (molecular weight ~1000) by *Fusarium moniliforme* and *Penicillium Roquefort* (Chandra and Rustagi., 1998) and the degradation of PLA by *Amycolatopsis sp.* (Pranamuda and Tokiwa, 1999) and by *Bacillus brevis* (Tomita *et al.,* 2000). In addition, enzymatic degradation of low molecular weight PLA (molecular weight ~2000) has been shown using esterase-type enzymes such as *Rhizopus delemer* lipase (Fukuzaki *et al.,* 1989).

Blending PLLA with other polymers can substantially modify the mechanical and thermal properties, degradation rate, and permeability. PLLA/poly (ε-caprolactone) (PCL) blends have been extensively studied. Various compatibilizers such as P (LA-*co*-CL) copolymer were used to improve the miscibility between PLA and PCL. PLLA was also blended with other non biodegradable polymers, including polyethylene, poly (ethylene oxide), poly (ethylene glycol), poly (vinyl acetate), poly (4-vinylphenol), and polyacrylates. Varying degrees of property modifications of PLLA were achieved by blending with these polymers. Many of these blends are immiscible or only partially miscible and may need compatibilizers to increase their compatibility (Singh *et al*., 2011).

Gilmore *et al.* (1993) have carried out degradation study of six types of plastics and plastic blends in municipal wastewater. Samples consist of 6% starch in PP, 12% starch in linear LDPE, 30% Polycaprolactone in LDPE, and poly(-hydroxybutyrate-*co*-hydroxyvalerate) (PHB/V), a microbially produced polyester in activated sludge of 5 months and found no sign of degradation of blended samples except PHB/V, which has showed a

considerable loss of mass and a significant loss of tensile strength in municipal wastewater. Biodegradable polymers can be synthesized by the modification of natural polymers by blending and fermentation.

Composites were prepared by two methods, (i) graft copolymerization (GFC) of isotactic polypropylene (PP) with maliec anhydride, (MAH) followed by esterification with coir fiber and (ii) by direct reactive mixing (DFC) of polypropylene (PP) and ethylene–propylene (EP) copolymers with MAH and peroxide with coir fiber. These composites, after molding in films were examined for susceptibility to biological attack by measuring the percentage weight loss in compost up to 6 months, periodically, and fungal colonization on surface of the samples, when kept as sole carbon source for the growth of *Aspergillus niger* in culture medium up to 40 days. Significant changes were observed depending on the preparation methods during photodegradation and bio-disintegration of composites (Kumar *et al.*, 2006).

Microbe enzymes present in soil accelerate biodegradation of aliphatic polyesters by surface erosion. When the blend films undergo hydrolysis through main chain scission, weight loss of the film will take place, which becomes detectable when water-soluble oligomers produced by microbes diffuse into the surrounding soil (Tsuji *et al.*1998).

Singh *et al.* (2012) studied, the degradability of linear low-density polyethylene (LLDPE) and poly (L-lactic acid) (PLLA) blend films under controlled composting conditions according to modified ASTM D5338 (2003). Differential scanning calorimetry, X-ray diffraction, and Fourier transform infrared spectroscopy were used to determine the thermal and morphological properties of the plastic films. LLDPE 80 (80 wt % LLDPE and 20 wt % PLLA) degraded faster than grafted low-density polyethylene–maleic anhydride (M-g-L) 80/4 (80 wt % LLDPE, 20 wt % PLLA, and 4 phr compatibilizer) and pure LLDPE (LLDPE 100). The tensile strength of LLDPE 100, LLDPE 80 and M-*g*-L 80/4 decreased by 20%, 54%, and. This investigation revealed that introduction of PLLA into LLDPE led to rapid degradation on 35% respectively. The films, as a result of degradation, exhibited a decrease in their mass composting and (LLDPE and PLLA) blend films are more susceptible to biodegradation compared to compatibilizer blend films.

Pseudomonas species degrade polyether, polyesters and polypropylene effectively (Premraj and Mukesh Doble, 2004). Studies show that maximum polypropylene degrading strains belong to genus *Pseudomonas* (Chiellini , 2003). *Pseudomonas stutzeri, Rhodococcus spp. Xanthomonas spp.* and mixed cultures have demonstrated degrading abilities under aerobic conditions (Nawaz *et al.,* 1998). Polypropylene depolymerases are able to degrade all (R) chains, cyclic –(r) oligomers and these enzymes are generally obtained from *Alcaligenes faecalis* and *Pseudomonas stutzeri* (Shimao, 2001).

Reddy *et al.* (2008) produced polyblend fibers from five ratios of polylactic acid/polypropylene (PLA/PP) in an effort to improve the resistance to hydrolysis and biodegradation, and to improve the dyeability of PLA. When made into polyblend fibers, the two polymers, PLA and PP, show partial compatibility and the mechanical properties of the blends are inferior compared to the pure PLA or PP fibers. However, PLA in the blends had substantially better resistance to biodegradation and hydrolysis, and dyeability with disperse dyes, resulting in a polyblend fiber with much better resistance to hydrolysis and similar dyeability to PLA.

Sharma and Sharma (2004) assessed tensile strength, elongation, percentage extension, CFU, BOD & turbidity of plastics to study the extent of degradation of LDP & PP using *Pseudomonas stutzeri* under laboratorytest conditions. Throughout the investigation both the plastic types were found to undergo qualitative and quantitative changes by bacteria but PP was found to be more biodegradable as compared to LDP. Tensile strength was reduced to 6.4% in LDPE and 13.3% in PP as compared to control which shows degradation. *Pseudomonas* was found efficient in degrading polyethylene with its biodegradability of 40.5% (Nanda and Smiti, 2010).

Since *Pseudomonas stutzeri* was reported to degrade polypropylene maximally among all other bacteria therefore we used it to study the biodegradation kinetics of PP and blends of PP and PLLA, under aerobic conditions. The purpose to take polypropylene and its blends is that as its usage is increasing day-by day but less biodegradation studies had been observed on it and because of its blending with biodegradable plastic may lead to its rapid biodegradation.

Chapter-3

MATERIALS & METHODS

3.1 Materials

3.1.1 Polymers

Sheets of Polypropylene (PP) and poly-L-lactic acid (PLLA) were taken. In addition, the sheets of Polypropylene and poly-L-lactic acid blends were used, in which compatibilizer Maleic anhydride was added in different ratios (table 3.1).

Table 3.1 Composition of polymers

Sr. No.	Polymer Blend Code	Polypropylene Wt (%)	poly-L-lactic Acid Wt (%)	Compatibilizer (M-*g*-PP) Wt (%)
1	PP 100	100	0	0
2	PP 90	90	10	0
3	M-*g*-PP 90/4	90	10	4
4	M-*g*-PP 80/6	80	20	6
5	PLLA 100	0	100	0

3.1.2 Bacterial strain

Pseudomonas stutzeri (MTCC number 2643) was procured from Microbial Type Culture & Gene Bank, Institute of Microbial Technology, Chandigarh. This bacterium is a mesophilic, Gram-negative, motile and rod-shaped with a growth optimum at 35°C.

3.1.3 Media for cultivation

Nutrient broth (NB) (g/l containing: 5g peptic digest of animal tissue, 5g sodium chloride, 1.5g beef extract and 1.5g yeast extract) & Nutrient agar (NA) (g/l containing 5g peptic digest of animal tissue, 5g sodium chloride, 1.5g beef extract, 1.5g yeast extract and 15g agar) obtained from HIMEDIA Laboratories Ltd., India, was used to maintain the bacterial culture.

3.1.4 Media for inoculation & degradation

Bacterial strain used, should be capable of utilizing polymer films as the sole source of carbon and energy, hence they were grown on minimal medium containing (g/l of distilled water): 1g NH_4NO_3, 0.7g $MgSO_4.7H_2O$, 1g K_2HPO_4, 1g KH_2PO_4, 0.005g NaCl, and 2mg/l of each of the following micro-elements: $FeSO_4.6H_2O$, $ZnSO_4.7H_2O$ and 1mg/l of $MnSO_4.H_2O$ (Mohdy and Ghanem, 2009). All the chemicals were obtained from HIMEDIA Laboratories Ltd., India.

3.2 Methodology

3.2.1 Aerobic biodegradation

Aerobic biodegradability of degradable polymers by specific microorganisms is determined as per guidelines of ASTM D 5247 standard. For biodegradation studies specific microorganism was used i.e. *Pseudomonas stutzeri* and polymers used were PP and blends of PP and PLLA. Pre-weighed films of the polymers are taken in 250 ml Erlenmeyer flasks containing 150 ml minimal medium inoculated with the culture. The flasks were incubated in shaker incubator (Excella E-24, New Brunswick Scientific, USA) at 120 rpm, at 35°C under aerobic conditions for 60 days. ASTM is currently developing standard practices for exposing degradable plastics to "real systems" environments and reporting the resulting data. The specific microorganisms test method does not represent any real world waste management infrastructure but provides a standard test method to quantify biodegradability using well-defined microbial cultures commonly present in the environment.

3.2.1.1 Weighing of polymer samples

The polymer films were cut into small strips (2cm × 2cm) and disinfected (30 minutes in 70% ethanol) and were allowed to dry overnight at 60°C and then weighed (Goncalves *et al.,* 2009) Ethanol was used to remove any organic matter adhering to the surface.

3.2.1.2 Disinfection of polymer samples

Ethanol was used again to disinfect the polymer pieces. Polymers are dipped in ethanol for 1 hour and then died inside the laminar air flow hood (Thermadyne Pvt. Ltd., Faridabad) on UV treated filter paper so that no microbial community remained adhered to the polymer pieces.

Then the films are added to flasks, each containing 150 ml of synthetic medium which was autoclaved (Equitron, India) at 121°C, 15 psi for 15 minutes.

3.2.1.3 Reviving of bacterial culture

The bacterium was provided in freeze-dried form in a sealed depressurized ampoule. The ampoule was marked near the middle of the cotton wool with a sharp file. The surface around the mark was disinfected with ethanol. A thick cotton wool was wrapped around the ampoule and it was then broken. The pointed top of the ampoule was removed gently (this step had to be performed very carefully as hasty opening may release bacterial spores into the atmosphere).

200 ml of nutrient broth was sterilized by autoclaving at 121°C, 15 psi for 15 minutes. 0.3 to 0.4 ml sterilized nutrient broth was added to the ampoule to make a cell suspension. The cell suspension was added to two Erlenmeyer flasks of 250 ml capacity each containing 100 ml of nutrient broth which were autoclaved at 121°C, 15 psi for 15 minutes. These flasks were incubated for 24 hours at 35°C and 120 rpm in the shaker incubator.

3.2.1.4 Enrichment of *Psudomonas stutzeri*

Enrichment of *Psudomonas stutzeri* was done by using nutrient broth as a medium. Two 250 ml Erlenmeyer flasks each containing 100 ml of nutrient broth were autoclaved at 121°C, 15 psi for 15 minutes. Out of two, one flask was inoculated by bacteria by taking 4 ml inoculums and other was kept blank. Then both the flasks were kept in shaker incubator at 35°C & 120 rpm.

3.3 Analytical Testing Procedures

3.3.1 Growth pattern in Nutrient Broth

Overnight grown culture of *Pseudomonas stutzeri* was inoculated in nutrient broth and other flask with nutrient broth was not inoculated and taken as blank. Both flasks were kept in shaker incubator at 35°C & 120 rpm. Flasks were withdrawn at regular intervals and OD was checked at 620 nm in UV-Vis Spectrophotometer (Model: Lambda 35, Perkin Elmer, USA).

It gives the growth pattern of the *Pseudomonas stutzeri* i.e. lag, log, stationary and death phase of the culture under controlled conditions which can be estimated by measuring the optical density (OD) at regular intervals. OD directly gives the log of number of microorganisms.

3.3.2 Growth kinetics in synthetic media with glucose as carbon source

Overnight grown culture of *Pseudomonas stutzeri* was inoculated in minimal media with glucose (3%) as a sole source of carbon and other flask containing minimal media and glucose was not inoculated and treated as blank. Both the flasks were kept in shaker incubator at 35°C & 120 rpm for the growth of bacteria. Flasks were withdrawn at regular intervals and 2 ml sample was taken in cuvette. OD was checked at 620 nm in UV-Vis Spectrophotometer. This gives the curve representing the behavior of culture different carbon source i.e. glucose.

3.3.3 Growth kinetics in synthetic media with polymers as carbon source

Polymer strips were added in 100 ml of minimal media and 4 ml of inoculums (24 hour old culture in nutrient broth) in 250 ml Erlenmeyer flasks. The flasks were constantly monitored after every 5 days interval to confirm that the *Psudomonas stutzeri* was able to form biofilm on polymer surfaces. 2 ml of media was taken from the flasks and OD was measured at 620 nm to ensure the culture is growing and utilizing the polymers as its carbon source.

3.3.4 Weight loss measurement

Our standard biodegradation assay for PP and its blends with PLLA lasts for 60 days. During this period, the bacteria utilize the carbonyl residues and reduce their concentration. Therefore, it was important to verify that the biodegradability with a reduced level of carbonyls. A simple and quick way to measure the biodegradation of polymers is by determining the weight loss. Loss of polymer integrity leads to weight loss.

To facilitate accurate measurement of the weight of the residual polymer samples (PP and its blends with PLLA), the bacterial biofilm was washed off the film surface with a 2% (v/v) aqueous sodium dodecyl sulphate solution for 4 hr and then with distilled water. The washed polymer samples were placed on a filter paper and dried overnight at 60°C before weighing (Sivan *et al.,* 2006).

3.3.5 Estimation of bacterial biomass colonizing the polymers

As the bacterial cells were strongly attached to the polymer surfaces, it was next to impossible to estimate the population density by standard techniques, such as direct cell counting or plating. Therefore, the population density of the biofilm on the polyethylene surface was estimated by determining the concentration of extractable protein. Colonized polymer samples taken from the bacterial liquid culture was taken and then boiled for 30 min in 5 ml of 0.5 N NaOH. The suspension was centrifuged at 10,000 rpm at 4°C for 15 min; the supernatant was kept aside and the pellet was subjected to the same procedure repeatedly (Sivan and Mor, 2008). The two supernatants were mixed and the protein concentration was determined according to Bradford method, which is a dye binding method using bovine serum albumin (BSA) as a standard (Bradford, 1976). BSA, in crystalline form, was purchased from HIMEDIA Laboratories Ltd., India. 0.1 g of BSA was dissolved in 10 ml of water at room temperature. The stock BSA solution was diluted to obtain the concentrations of 100 – 1,500 μg/ml. 60 μL of each standard was mixed with 940 μL of Bradford reagent. Each sample was incubated at room temperature for 10 minutes. Finally, the absorbance of each standard was measured at 595 nm against a blank which was composed of 60 μL of water and 940 μL of Bradford reagent.

3.3.6 Morphological evaluation using scanning electron microscopy (SEM)

The films were washed in 70 % ethanol solution to remove cell mass from the residual film to the maximum possible and then dried at 60^0C for 24 hrs. These films were used to analyze the surface change and bio-deterioration. Scanning electron micrographs (SEM) of the films were taken with a scanning electron microscope (JEOL, Model JSM 6510 LV). The accelerating voltage was kept at 10 kV. The specimens were coated with 50 μm of thick gold film in an automatic sputter coater (Polaron) to avoid charging under the electron beam prior to SEM studies.

Chapter-4

RESULTS & DISCUSSION

4.1 Aerobic treatment

The biodegradation kinetics of PP and blends of PP and PLLA using *Pseudomonas stutzeri* under aerobic conditions was studied.

4.1.1 Growth pattern of *Pseudomonas stutzeri* in nutrient broth

Growth pattern of *Pseudomonas stutzeri* should be studied before doing further analysis, so as to evaluate the lag, log, stationary and death phases. 1 ml of inoculums (24 hour old culture) was inoculated in 150 ml of nutrient broth taken in 250 ml Erlenmeyer flasks and kept in shaker incubator at 35°C, 120 rpm. Readings in terms of optical density (OD) were taken at fixed intervals i.e. at 620 nm (as shown in table 4.1 and fig. 4.1).

Table 4.1 Growth kinetics of *Pseudomonas stutzeri* in nutrient broth

Time (hours)	Optical Density (OD at 620 nm)
1	0.010 ± 0.001
2	0.012 ± 0.001
3	0.018 ± 0.001
4	0.081 ± 0.008
5	0.116 ± 0.003
6	0.220 ± 0.003
7	0.432 ± 0.004
8	0.670 ± 0.007
9	0.772 ± 0.014
10	0.971 ± 0.008
11	1.059 ± 0.007
12	1.176 ± 0.058
13	1.231 ± 0.007

14	1.269 ± 0.017
15	1.356 ± 0.006
16	1.453 ± 0.022
17	1.554 ± 0.010
18	1.654 ± 0.016
19	1.704 ± 0.003
20	1.729 ± 0.005
21	1.750 ± 0.002
22	1.764 ± 0.005
23	1.770 ± 0.007
24	1.778 ± 0.003

mean ± standard error (n=2)

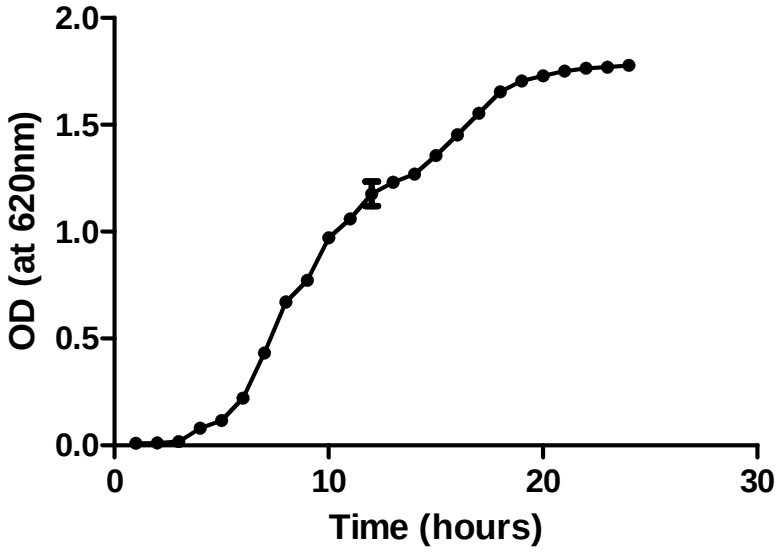


Fig. 4.1 Growth curve of *Pseudomonas stutzeri* in nutrient broth

According to the growth curve obtained (fig. 4.1), it was concluded that lag phase continues for 5-6 hours and after which log or exponential phase goes from 6-17 hours then stationary phase with little or no growth starts, due to lack of nutrients. The purpose of studying growth kinetics of *Pseudomonas stutzeri* was to determine the time period at which exponential phase starts because culture during mid-exponential phase was taken as inoculums for studying biodegradation kinetics.

4.1.2 Growth kinetics in synthetic medium with glucose as carbon source

After studying the growth kinetics of *Pseudomonas stutzeri* in nutrient medium, synthetic medium was taken with the same composition as that of the medium used for polymer degradation, but glucose was added instead of polymer to study its growth pattern. Growth kinetics of *Pseudomonas stutzeri* in synthetic medium, with glucose as a carbon source is shown in table 4.2 and fig. 4.2.

Table 4.2 Growth kinetics in synthetic medium with glucose as carbon source.

Time (hours)	Optical Density (OD at 620 nm)
0	0.014 ± 0.004
1	0.019 ± 0.001
2	0.022 ± 0.001
3	0.052 ± 0.003
4	0.116 ± 0.005
5	0.215 ± 0.10
6	0.289 ± 0.002
7	0.431 ± 0.443
8	0.664 ± 0.016
9	0.872 ± 0.009
10	0.987 ± 0.006
11	1.278 ± 0.064
12	1.422 ± 0.007
13	1.544 ± 0.013
14	1.665 ± 0.024
15	1.724 ± 0.009
16	1.895 ± 0.004
17	1.920 ± 0.008
18	1.929 ± 0.005
19	1.941 ± 0.002
20	1.954 ± 0.002
21	1.957 ± 0.003

22	1.961 ± 0.002
23	1.968 ± 0.003
24	1.973 ± 0.002

mean ± standard error (n=2)

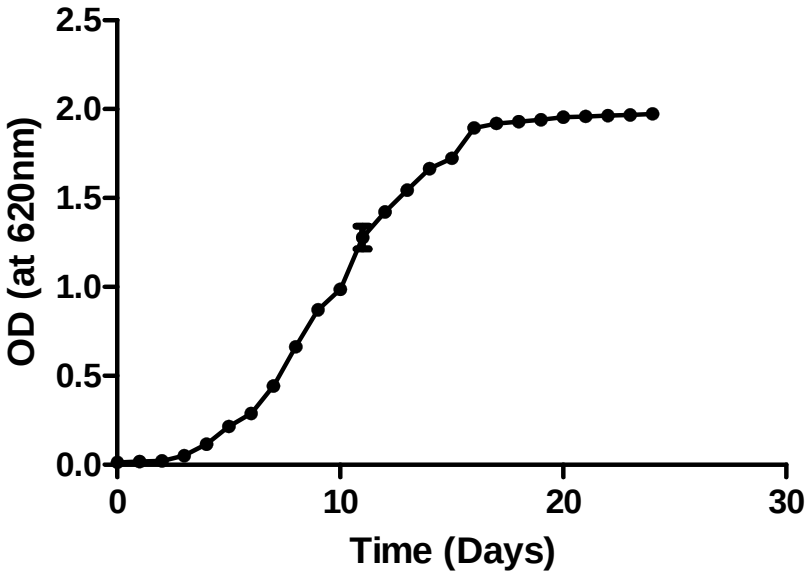


Fig. 4.2 Growth kinetics in synthetic medium (glucose as a carbon source)

From fig. 4.2, we concluded that the growth curve was almost same as that was achieved in nutrient broth. Growth curve of culture represents the increase in number of microorganism with simultaneous decrease in glucose concentration and stationary phase was achieved earlier because of the limited concentration of glucose.

4.1.3 Growth pattern in synthetic media with polymers as carbon source

Table 4.2 and fig. 4.2 represent the growth behavior in media containing same composition but different carbon sources. Readings were taken at 5 day interval to determine the microbial activity. The comparison of growth kinetics in terms of optical density in synthetic media with PP and different blends of PP and PLLA is discussed in table 4.3 and fig 4.3.

Table 4.3 Growth kinetics in synthetic media with plastic samples as carbon source.

Time (days)	Optical Density (OD at 620 nm)				
	PLLA	**M-*g*-PP 80/6**	**M-*g*-PP 90/4**	**PP 90**	**PP 100**
0	0.032 ± 0.004	0.043 ± 0.008	0.038 ± 0.025	0.025 ± 0.005	0.038 ± 0.008
5	0.043 ± 0.005	0.062 ± 0.009	0.052 ± 0.017	0.031 ± 0.009	0.044 ± 0.005
10	0.062± 0.005	0.079 ± 0.005	0.069 ± 0.016	0.034 ± 0.008	0.048 ± 0.006
15	0.081 ± 0.001	0.088 ± 0.007	0.089 ± 0.018	0.043 ± 0.015	0.056 ± 0.010
20	0.095 ± 0.005	0.105 ± 0.001	0.100 ± 0.012	0.048 ± 0.008	0.068 ± 0.13
25	0.121 ± 0.002	0.124 ± 0.005	0.108 ± 0.010	0.063 ± 0.013	0.072 ± 0.016
30	0.140 ± 0.002	0.148 ± 0.003	0.124 ± 0.015	0.070 ± 0.010	0.087 ± 0.008
35	0.164 ± 0.005	0.155 ± 0.005	0.135 ± 0.013	0.078 ± 0.007	0.096 ± 0.012
40	0.173 ± 0.002	0.170 ± 0.10	0.148 ± 0.017	0.094 ± 0.014	0.113 ± 0.019
45	0.201 ± 0.008	0.179 ± 0.009	0.155 ± 0.012	0.116 ± 0.020	0.126 ± 0.009
50	0.264 ± 0.023	0.202 ± 0.019	0.168 ± 0.010	0.137 ± 0.013	0.130 ± 0.013
55	0.280 ± 0.022	0.221 ± 0.020	0.192 ± 0.010	0.153 ± 0.009	0.139 ± 0.014
60	0.341 ± 0.058	0.250 ± 0.140	0.204 ± 0.009	0.161 ± 0.008	0.146 ± 0.014

mean ± standard error (n=2)

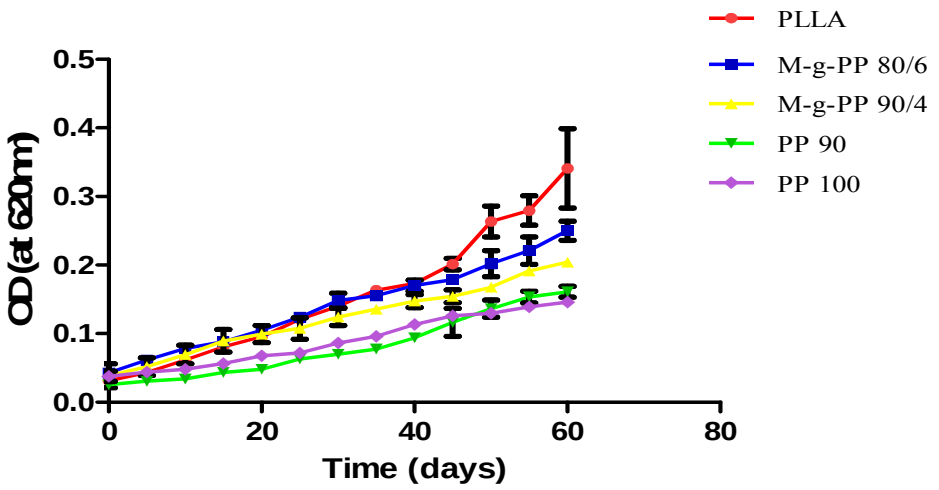


Fig. 4.3 Growth kinetics in synthetic media (with PP and its blends with PPLA as C-source)

When total biodegradation process of any substrate is considered the formation of microbial colonies is critical which can be evaluated by OD measurement (Anjana and Amitabh, 2004).

It can be easily evaluated from fig.4.3 that growth of *Pseudomonas stutzeri* is higher in the synthetic media with PLLA as compared to PP samples. As the surface of PLLA is hydrophilic, therefore more microorganisms attached to its surface and caused its rapid degradation while in case of PP microorganisms took some days to first make the surface hydrophilic initially by the release of certain enzymes particularly esterases and then attached to the surface and used it as its carbon source. In case of blends the growth pattern is nearly same as that of PLLA.

4.1.4 Colony forming units (CFU/ml) measurement

Colony forming units (CFU/ml) of media containing plastic samples were observed at every 5 days incubation period.

Table 4.4 Colony Forming Units/ml (at 10^{-6} Dilution rate) in synthetic media

Time (days)	**Colony Forming Units/ml**				
	PLLA	**M-*g*-PP 80/6**	**M-*g*-PP 90/4**	**PP 90**	**PP 100**
0	6000	7000	4000	3000	5000
5	10000	10000	6000	2000	6000
10	12000	18000	9000	4000	10000
15	18000	29000	16000	8000	12000
20	22000	35000	21000	12000	20000
25	33000	39000	29000	25000	24000
30	51000	68000	33000	29000	29000
35	73000	70000	40000	33000	31000
40	87000	82000	46000	42000	37000
45	93000	101000	51000	46000	42000
50	122000	115000	62000	51000	49000
55	131000	124000	74000	67000	51000
60	149000	130000	98000	72000	58000

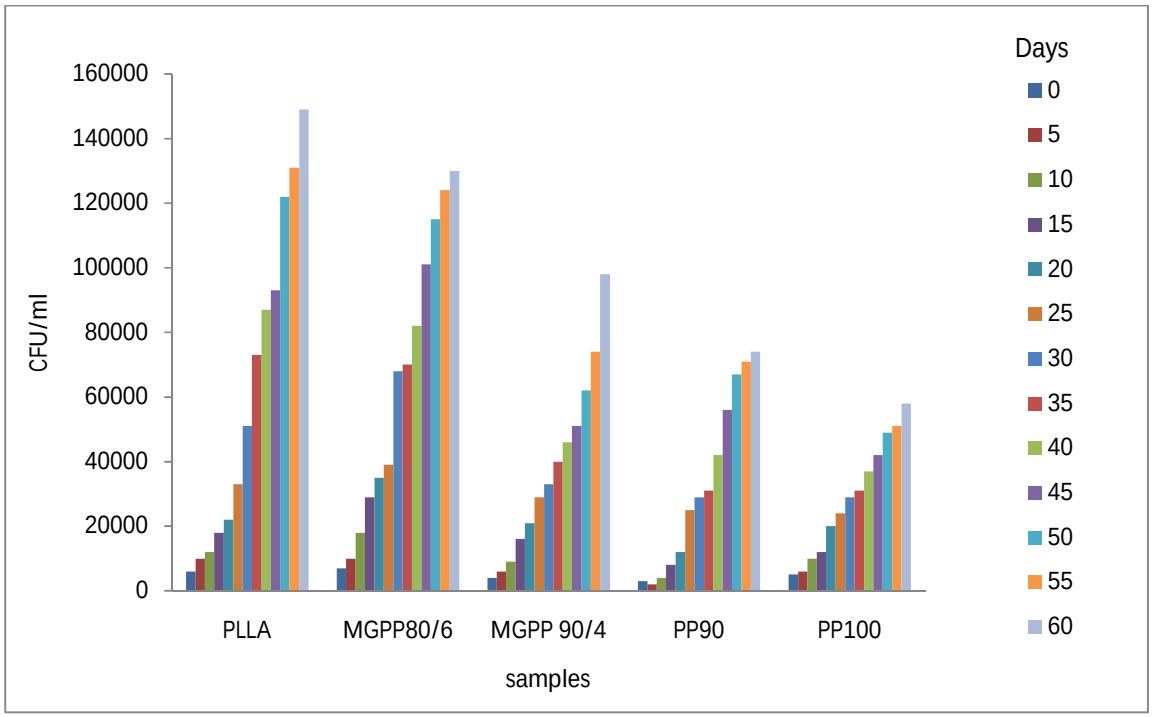


Fig. 4.4 Total viable count (CFU/ml) during degradation of different plastic samples

The increase in CFU/ml with the increase in incubation period indicates that bacteria utilized plastic as its sole source of carbon and thus supporting and enhancing its degradation. Ambika *et al.* (2010) reported that CFU/ml count for *P. stutzeri* was between 10^3 and 10^5 throughout the study period when grown in synthetic media with polypropylene fims. Sharma and Sharma (2004) studied bioegradation of LDP and PP with isolated cultures of *P. stutzeri in synthetic media*, and assessed CFU/ml and found minimum counts 10^1 and maximum 10^3 were recorded. The CFU/ml counts are maximum in case of PLLA i.e. upto the range of 10^5 colonies/ ml, which comply with the maximum OD observed as in the case of liquid media, indicating that microorganisms growing faster in media with PLLA as carbon source, which in turn leads to its degradation, while in case of PP though CFU/ml is increasing but to very lesser extent.

4.1.5 Weight loss measurement

After each incubation period, i.e. at 30, 45 and 60 day interval, the samples were taken out from the flasks containing bacterial culture and synthetic media in aseptic conditions in laminar air flow hood. Polymer samples were then washed, dried and weighed. The total

reduction in weights of the different samples is shown in tables and figures below (tables 4.5-4.9 and fig 4.5-4.9).

Table 4.5 Weight of PP 100 before and after incubation

No. of days	Weight before incubation (mg)	Weight after incubation (mg)	% weight loss
30	135.4	135	0.29
45	208.9	208	0.43
60	160	158	1.25

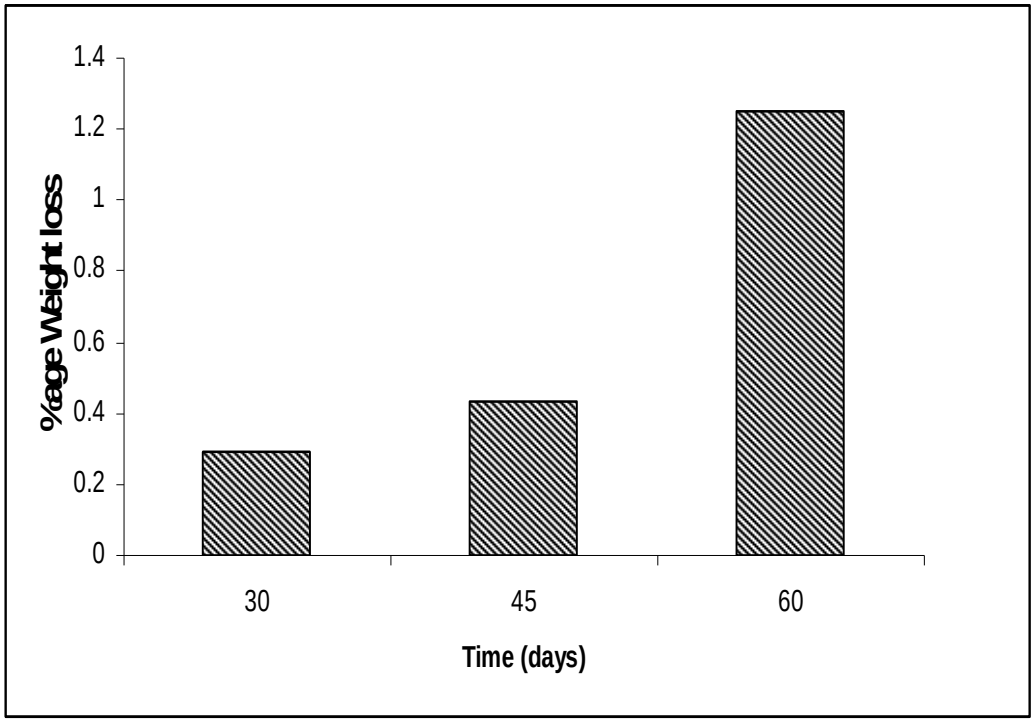


Fig. 4.5 Percentage weight reduction of PP100 after degradation.

Table 4.6 Weight of PP 90 before and after incubation

No. of days	Weight before incubation (mg)	Weight after incubation (mg)	% weight loss
30	115.2	115	0.17
45	87.6	87.1	0.57
60	145.4	144	0.96

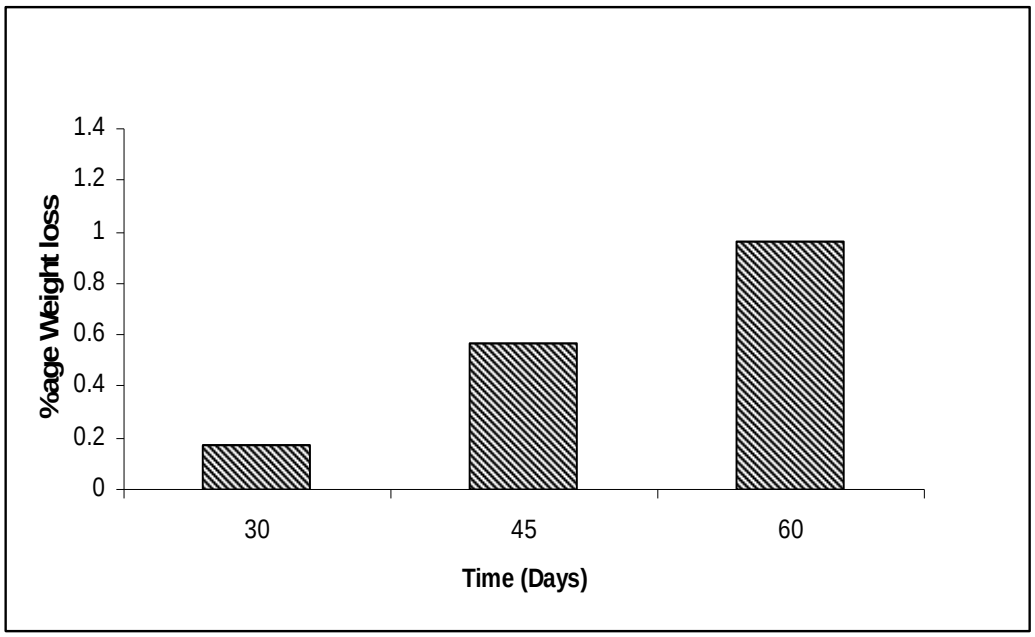


Fig. 4.6 Percentage weight reduction of PP 90 after degradation.

Table 4.7 Weight of PLLA before and after incubation

No. of days	Weight before incubation (mg)	Weight after incubation (mg)	% weight loss
30	155.4	154	0.90
45	208.7	206	1.29
60	195.9	190	3.01

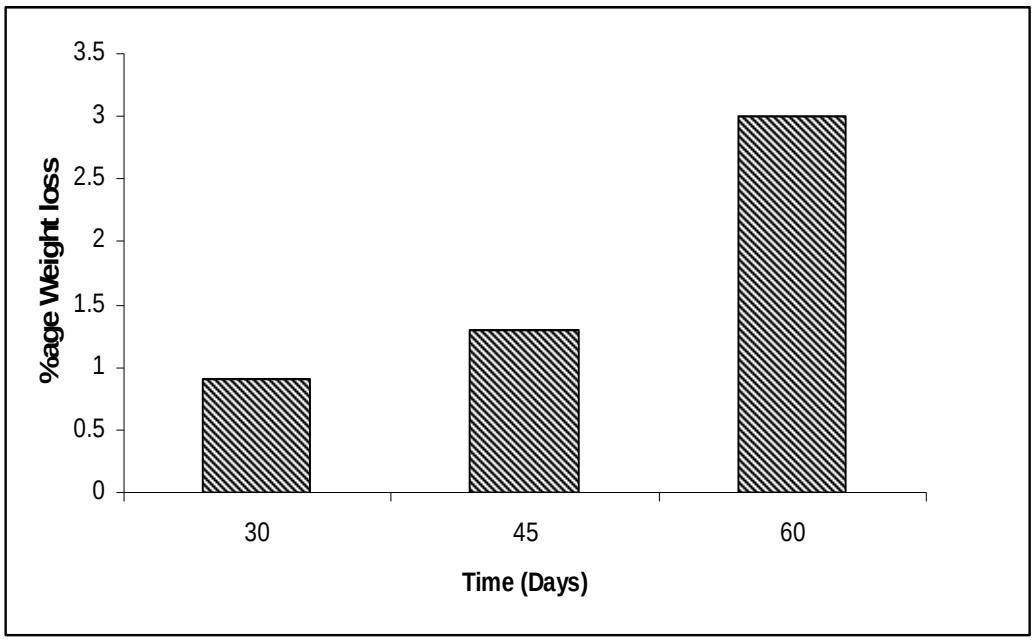


Fig.4.7 Percentage weight reduction of PLLA after degradation.

Table 4.8 Weight of M-*g*-PP 90/4 before and after incubation

No. of days	Weight before incubation (mg)	Weight after incubation (mg)	% weight loss
30	228.5	228.2	0.13
45	199.2	198	0.60
60	173.1	171	1.21

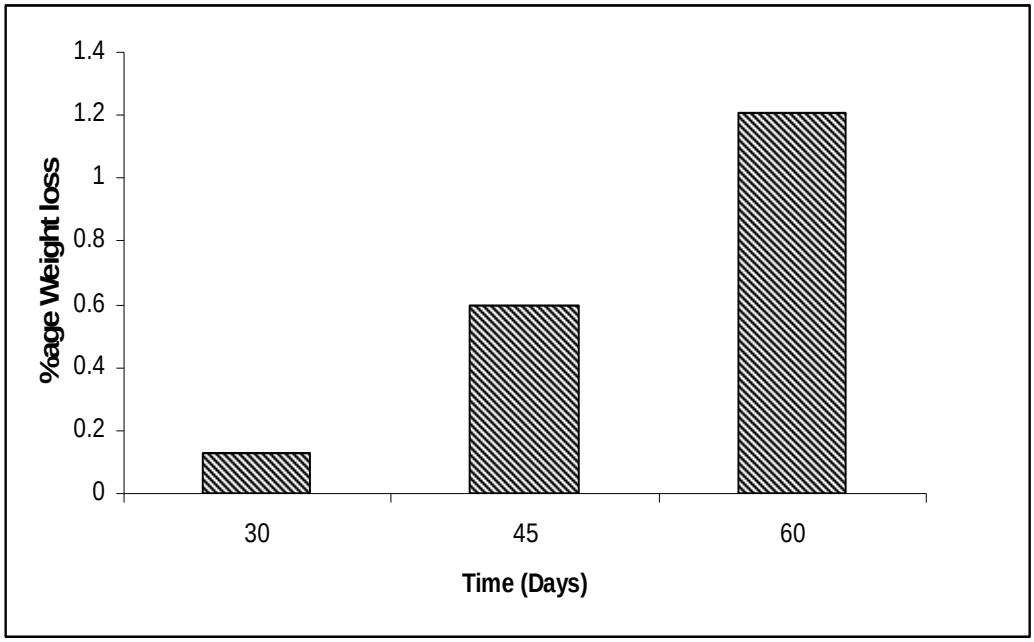


Fig. 4.8 Percentage weight reduction of M-*g*-PP 90/4 after degradation.

Table 4.9 Weight of M-*g*-PP 80/6 before and after incubation

No. of days	Weight before incubation (mg)	Weight after incubation (mg)	% weight loss
30	215.3	215	0.13
45	203.9	203	0.44
60	202.9	198	2.41

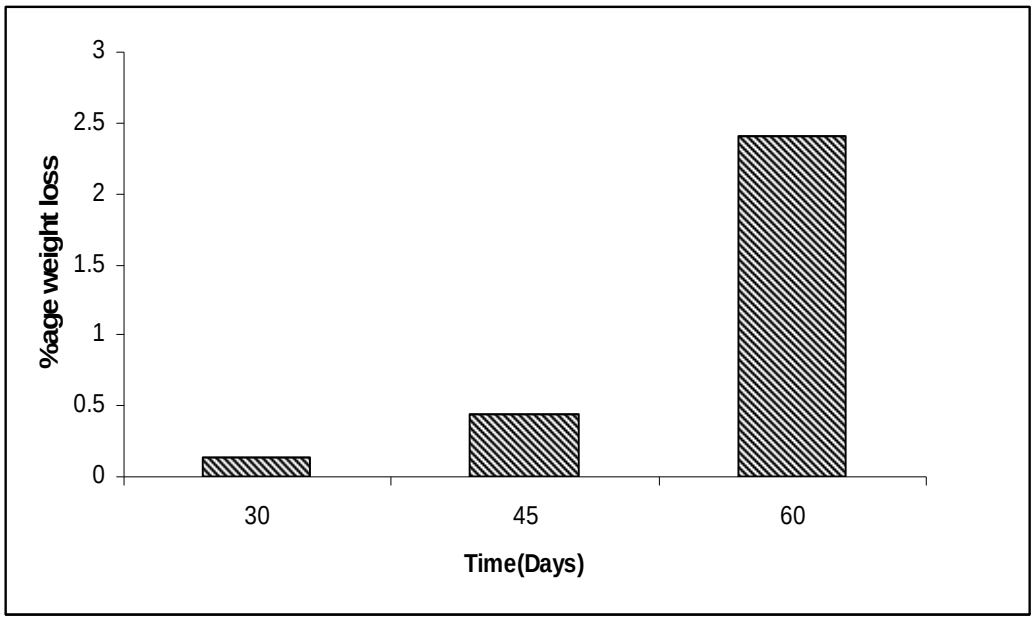


Fig. 4.9 Percentage weight reduction of M-*g*-PP 80/6 after degradation.

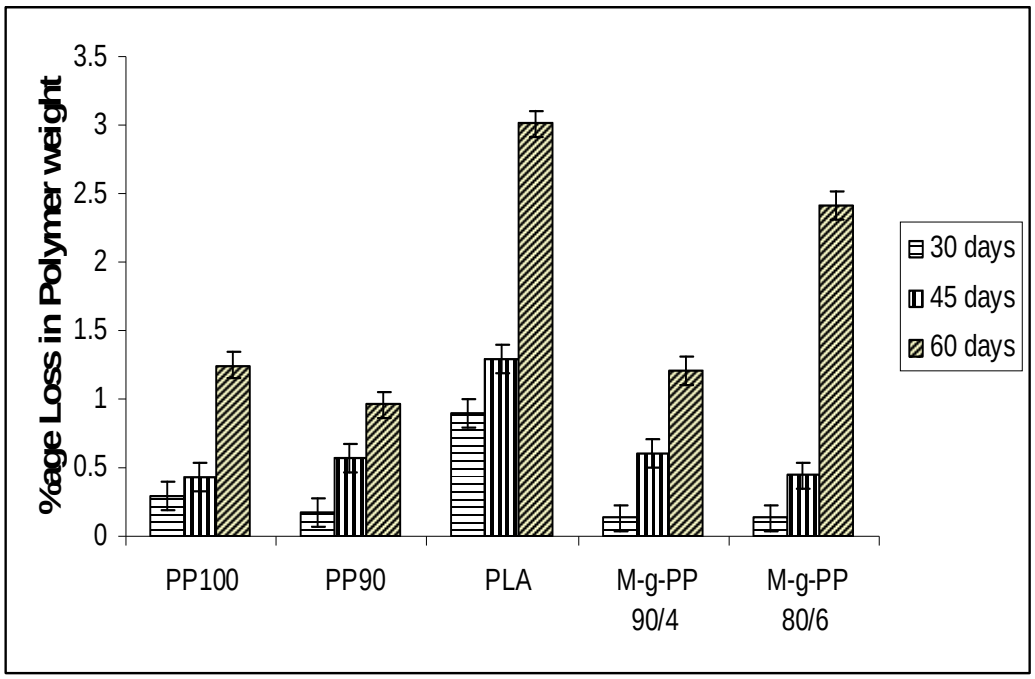


Fig. 4.10 Comparison of percentage weight reduction of different plastic samples.

Kathiresan and Bingham (2001) reported that bacteria caused the biodegradation ranging from 1.19 to 20.54% weight loss for polypropylene and 0.56 to 8.16% for other blended polymers. These microorganisms utilize polymer samples as a sole source of carbon which resulted in their partial degradation. As all the polymers undergo degradation which is observed in terms of their weight loss. PLLA degrade maximum by Peudomonas stutzeri i.e upto 3.01% followed by M-g-PP 80/6, which is degraded upto 2.41%. PP100 degraded upto 1.25 %. The above data shows that blend of PP and PLLA show effective degradation than PP alone.

4.1.6 Protein estimation

Growth of microorganisms (i.e. biofilm) on the surface of the polymer was monitored by quantifying the total proteins extracted from the biofilm. The readings were taken after the interval of 16, 30 and 60 days at 660 nm. The OD was compared with the OD of the known sample i.e. bovine serum albumin (BSA) was taken as standard in this case. The standard curve of BSA was obtained (fig. 4.10) using known concentrations of BSA as summarized in table 4.10.

Table 4.10 Standard curve of BSA

BSA µg/ml	Volume (µL) of 10 mg/ml BSA Stock	Volume (µL) of Distilled water	Absorbance at 595nm
100	5	495	0.10 ± 0.02
200	10	490	0.18 ± 0.03
400	20	480	0.44 ± 0.03
600	30	470	0.62 ± 0.03
900	45	455	0.82 ± 0.02
1,200	60	440	1.16 ± 0.06
1,500	75	425	1.27 ± 0.07

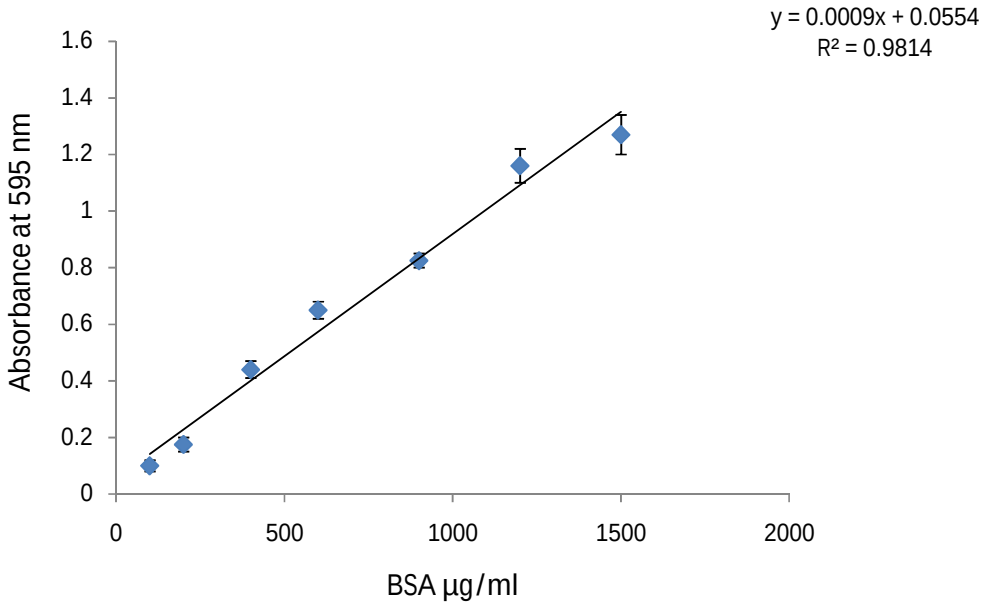


Fig. 4.11 Standard curve of BSA

Table 4.11 OD measurement of suspension of plastic samples

Time (days)	Optical Density (OD at 595 nm)				
	PLLA	**M-*g*-PP 80/6**	**M-*g*-PP 90/4**	**PP 90**	**PP 100**
30	0.28	0.17	0.11	0.10	0.09
45	0.59	0.31	0.18	0.21	0.10
60	0.67	0.39	0.21	0.32	0.15

Table 4.12 Estimation of protein Concentration

Time (days)	Protein concentration (mg/ml)				
	PLLA	**M-*g*-PP 80/6**	**M-*g*-PP 90/4**	**PP 90**	**PP 100**
30	0.28	0.14	0.07	0.05	0.04
45	0.65	0.32	0.15	0.19	0.05
60	0.77	0.42	0.20	0.33	0.12

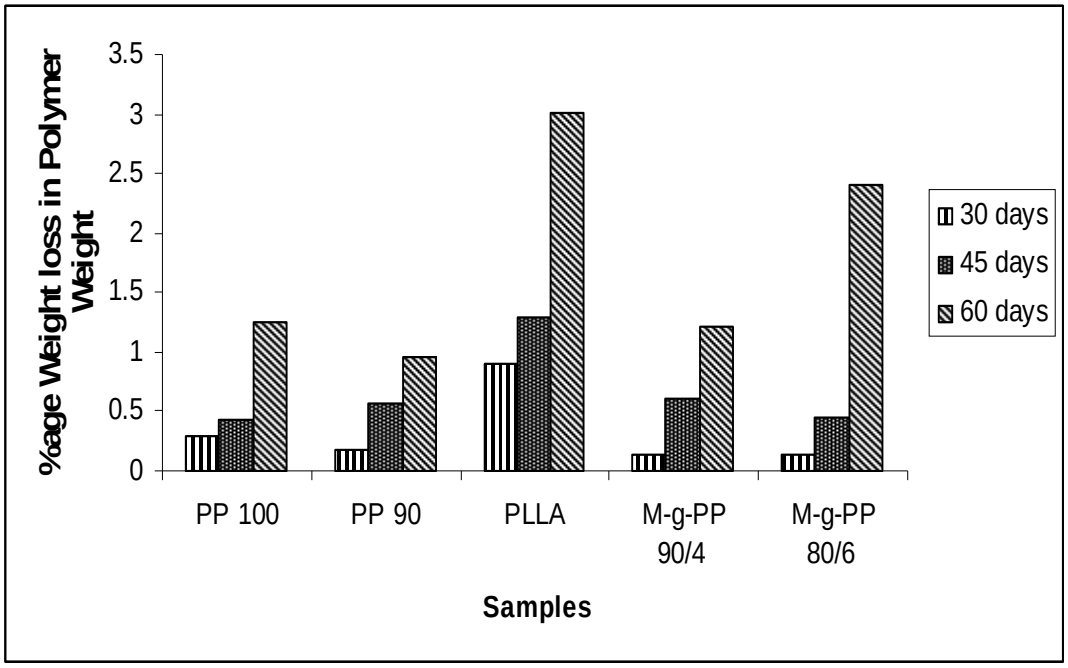


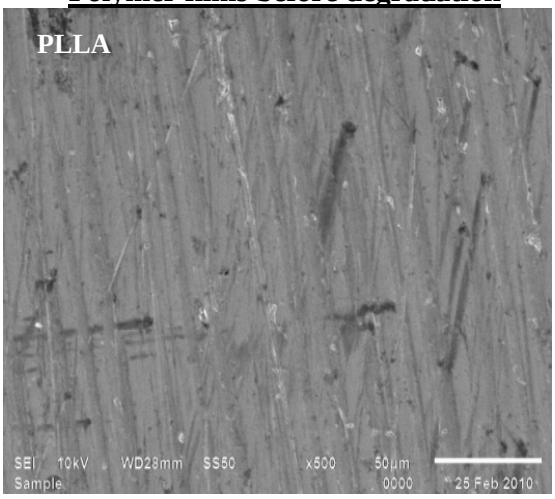
Fig. 4.12 Comparison of protein content of microbial biofilm on the surface of different plastic samples

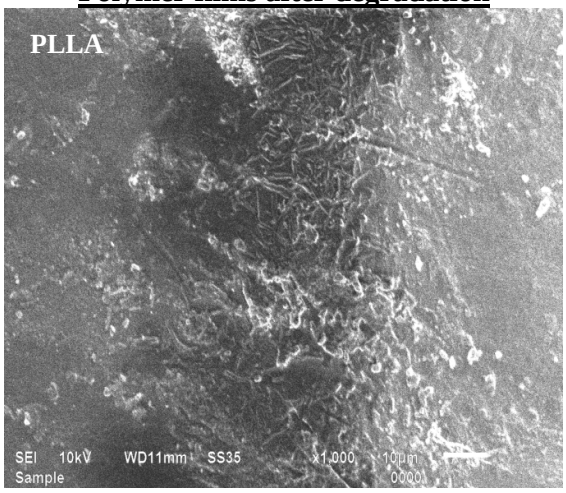
The fig. 4.12 represents the changes in protein concentration of the biofilm which was formed on the polymer surfaces. There observed an increase in protein concentration consistently which reflects an increase in the biomass. The figure also depicts the comparison between the concentrations of proteins extracted from different plastic samples.

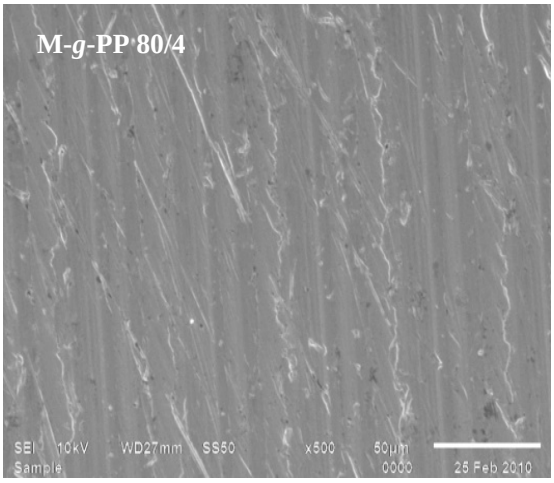
4.1.7 Morphological evaluation using scanning electron microscopy (SEM)

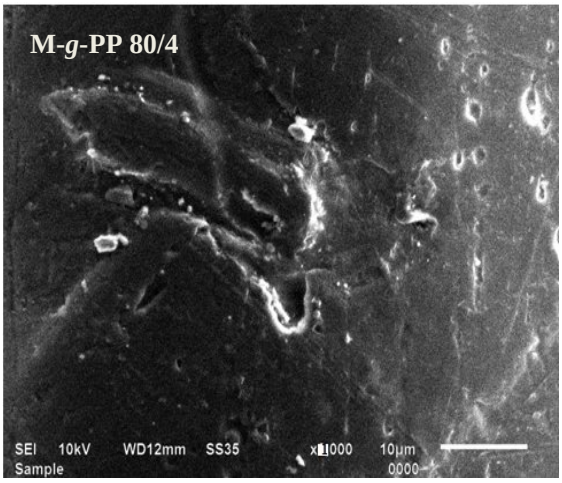
For evaluation of the surface changes and bio-deterioration in polymer films scanning electron micrographs of the films after 60 days of degradation studies with *Pseudomonas stutzeri* were taken with a scanning electron microscope (JEOL, Model JSM 6510 LV).

Polymer films before degradation | **Polymer films after degradation**









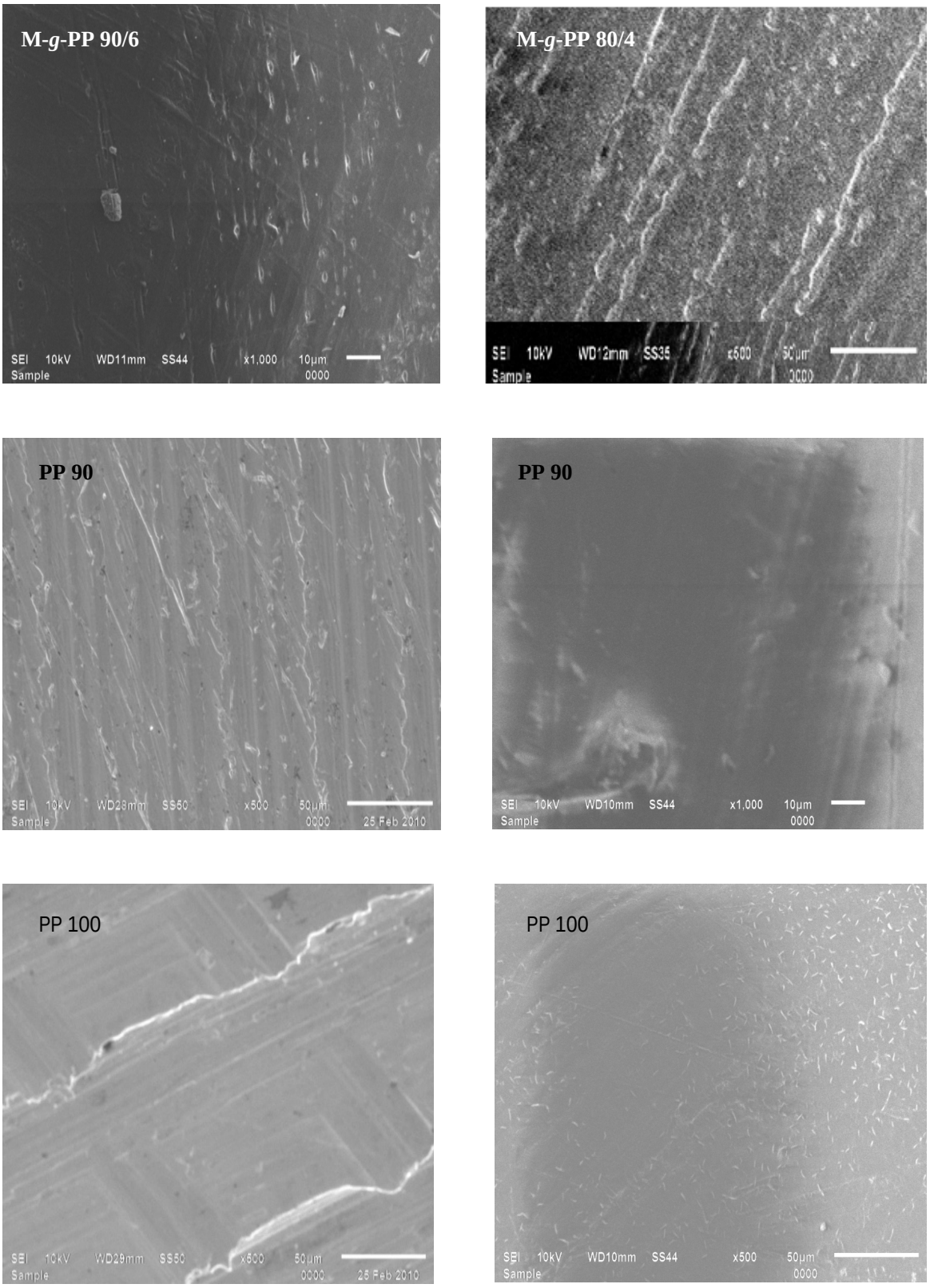


Fig 4.13 SEM micrographs of different polymer samples before and after degradation

The structural changes in the form of pits and erosions observed through scanning electron microscopy indicated damages on the surface of different polymer samples, incubated with *Pseudomonas stutzeri*. Surface of PLLA was observed to be rough with well-defined numerous shallow pits. As the surface of PLLA is hydrophobic and microorganisms easily adhere to the surface and after the removal of microorganisms, the surface became physically pitted and eroded (Otake *et al.,* 1995). The microbial attack on the blends of PP and PLLA caused rearrangement of the polymer chains and changes in the PP amorphous phase and interphases of the different polymers (Goncalves *et al.,* 2009) and thus leads to the surface erosion. There were several irregular cracks and pits of variable sizes on the surface M-*g*-PP 90/4 and M-*g*-PP 80/6. Surface of PP 90 was smooth with very few deformities. Polypropylene is resistant to decomposition by microorganisms (Arcana *et al.,* 2005), although can occur the appearance of cracks caused by contraction of the surface layers as one of the consequences of 'chemi-crystallization' (Craig *et al.,* 2005). Surface of the PP 100 was observed rough as compared to the sample before degradation but no marked holes and pits were noticed.

Chapter-5

CONCLUSIONS & FUTURE PROSPECTS

5.1 Conclusions

One of the key advantages of a pure culture biodegradation assay is that we can identify the part of the biodegradation which is due to chemical degradation. This study reveals that the selected polymer and its blends with appropriate additives can be biodegraded provided the right microbial strain is used under appropriate conditions. Culture enrichment methods were found effective for enhancing the capabilities of a bacterium in utilizing the polypropylene (PP) and its blends with poly-L-Lactic acid (PLLA) as the sole carbon and energy source. Maximum biodegradation was observed in case of PLLA followed by M-*g*-PP 80/6 and then M-*g*-PP 90/4. Although biodegradation of PP was very minute, the culture was able to survive in media containing PP and thus, the polymer showed a little degradation. Biodegradation leads to decrease in molecular weight. It is clear from the study that the bacterium *Pseudomonas stutzeri* is able to degrade the CH_2 backbone of the polymer under consideration and its blends with PLLA with/without traces of compatibilizer. PLLA increases the surface hydrophobicity, which is prerequisite for biodegradation. Hence degradation is more facile in blends of PP and PLLA. Biodegradation by *Pseudomonas stutzeri* is also supported by the SEM micrographs, which showed drastic surface change in the polymer samples.

5.2 Future prospects

1. Modify the polymer for microbial utility by (i) increasing the percentage of natural/biodegradable polymers; (ii) using pro-oxidants as additive; and (iii) pretreatment (e.g. photodegradation / UV radiation) of the polymer(s).
2. Modify the microbes to utilize the polymer by (i) varying and optimizing the medium composition and thus enhancing the utilization of polymer; and (ii) using genetic engineering, thus enabling the microorganism to utilize the polymer more.
3. Over-expressing the gene to increase the enzyme production, which is responsible for biodegradation, and purify the same to re-utilize for the purpose.

REFERENCES

Abd El-Mohdy H.L. and Ghanem S., Biodegradability, antimicrobial activity and properties of PVA/PVP hydrogels prepare by gamma irradiation, *Journal of Polymer Research,* 2009; 16: 1-10.

Albertsson A.C., Andersson S.O. and Karlsson S., The mechanism of biodegradation of polyethylene, *Polymer Degradation and Stability*, 1987; 18: 73-87.

Albertsson A.C. and Ranby B., Effects of increased solar ultraviolet radiation on materials, *Journal of Photochemical and Photobiology*, 1979; 46: 96–103.

Albertsson A.C. and Banhidi Z.G., Environmental biodegradation of polyethylene. Journal of *Polymer Degradation and Stab*. 1980; 81: 441–452.

Alariqi S.A.S., Pratheep Kumar A., Rao B.S.M. and Singh R.P., Biodegradation of γ-sterilised biomedical polyolefins under composting and fungal culture environments, *Journal of Polymer Degradation and Stability*, 2006; 91: 1105–1116.

Alauddin M., Choudkury I.A., Baradie M.A., Hashmi M.S.J., Plastics and their machining: a review*, Journal of Materials and Processing Technology,* 1995; 54: 40–46.

Albertsson A.C., Andersson S.O. and Karlsson S., The mechanism of biodegradation of polyethylene, *Polymer Degradation and Stability*, 1987; 18: 73-87.

Allen A.B, Hilliard N.P., and Howard G.T., Purification and characterization of a soluble polyurethane degrading enzyme from *Comamonas acidovorans*, *International Journal Of Biodeterioration and Biodegradation,*1999; 43: 37–41.

Ambika A., Asha A.J., Sumit B., Parasu V.U., Mukesh D., Growth of *Pseudomonas and Bacillus* biofilms on pretreated polypropylene surface, *International Journal Of Biodeterioration and Biodegradation,* 2010; 64: 530-536.

APME (Association of plastic manufacturers in Europe) An ananlysis of plastic consumption and recovery in Western Europe, 1999.

Anjana S. and Amitabh S., Degradation assessment of low density polyethylene (LDP) and polyethene (PP) by an indigenous isolate of *Pseudomonas stutzeri, Journal of Scientific And Industrial Research,* 2004; 63: 293-296.

Arcana I.M., Bundjali B., Yudistira I., Jariah B. and Sukria L, Biodegradation of starch polyethylene films in soil and by microbial cultures*, Journal of Polymers*, 2007; 39:13-37.

Arkatkar A., Arutchelvi J., Bhaduri S., Uppara P.V. and Doble M., Degradation of unpretreated and thermally pretreated polypropylene by soil consortia, *International Biodeterioration & Biodegradation*. 2009; 63: 106-111.

Arutchelvi J., Sudhakar M., Arkatkar A., Doble M., Bhaduri S. and Uppara P.V., Biodegradation of polyethylene and polypropylene, *Indian Journal of Biotechnology*, Jan 2008; 7: 9-22.

Atlas R.M., and Bartha R., *Microbial Ecology: Fundamentals and Applications. 4th Edition*, Pub: Benjamin & Cummings Menlo Park, 1997.

Avella M., Bonadies E., Martuscelli E. and Rimedio R., European current standardization for plastic packaging recoverable through composting and biodegradation, *Journal of Polymer Testing,* 2001; 20: 517–521.

Barlaz M.A., Ham R.K. and Schaefer D.M., Mass-balance analysis of anaerobically decomposed refuse, *Journal of Environmental Engineering,* 1989; 115: 1088–102.

Barnard G.N. and J.K. Sander, The poly-beta-hydroxybutyrate granule *in vivo.* A new in sight based on NMR spectroscopy of whole cell, *Journal of Biology and Chemistry,* 1989; 264: 3286-3291.

Bastioli C., *Handbook of biodegradable polymers*, Rapra Technology Limited, United Kingdom, 2005.

Bradford M.M., Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein- dye binding, *Journal of Analytical Biochemistry,* 1976; 72: 248-254.

Cain, Microbial degradation of synthetic polymers, *Journal of Microbial control of pollution*. Forty-eighth symposium of the society for general microbiology held at the University of Cardiff, 1992; 293-338.

Cacciari I., Quatrini P., Zirletta G., Mincione E., Vinciguerra V., Lupattelli P. and Sermanni G.G., Isotactic polypropylene biodegradation by a microbial community: physicochemical characterization of metabolites produced, *Journal of Applied and Environmental Microbiology*, 1993; 59: 3695–3700.

Chandra R. and Rustgi R., Biodegradable polymers, *Progressive Polymer Science*, 1998; 23: 1273-1335.

Craig I.H., White J.R. and Kin P.C., Degradation of poly(β-hydroxyalkanoates) and polyolefin blends in a municipal wastewater treatment facility, *Journal of Polymers*, 2005; 46: 505.

Demmer F., Environmental biodegradation of polyethylene. *Polymer Degradation and Stab*ility, 1975; 81: 441–452.

Doi Y., Effect of starvation on the adhesive properties of xenobiotic degrading bacteria, *Process Biochemistry*, 1990; 38: 909-914.

Dolezel B., Biodegradable polymers in the environment, *British Plastics*, 1972; 49: 105

El-Shafei H.A., El-Nasser N.H.A., Kansoh A.L. and Ali A.M., Biodegradation of disposable polyethylene by fungi and *Streptomyces* species, *Polymer Degradation and Stability*, 1998; 62: 361-365.

Fan K., Gonzales D. and Sevoian M., Hydrolytic and enzymatic degradation of poly (g-glutamic acid) hydrogels and their application in slow-release systems for proteins, *Journal of Environment and Polymer Degradation,* 1996; 4: 253–60.

Fukuzaki H.M., Yoshida M.A. and Kumakuna M, synthesis of copolyesters (D,L-Lactic acid) with relatively low molecular weight *& in vivo* degradation, *Journal of Environment and Polymer Degradation,*, 1989; 25: 1019-1026.

Frazer A.C., O-methylation and other transformations of aromatic compounds by acetogenic bacteria, *Analyst*, 1994; 121: 1451–1456.

Galgali P., Puntambekar U.S., Gokhale D.V. and Varma A.J., Fungal degradation of carbohydrate-linked polystyrenes, *Carbohydrate Polymers*, 2004; 55: 393-399.

Gilmore D.F., Antoun S., Lenz R.W. and Fuller R.C., Degradation of poly(β-hydroxyalkanoates) and polyolefin blends in a municipal wastewater treatment facility, *Journal of Polymers and Environment* 1993; 1: 269–274.

Glass J.E., Swift G., Agricultural and Synthetic Polymers*, Biodegradation and Utilization*, ACS Symposium Series, 433. Washington DC, American Chemical Society, 1989; 9–64.

Goldberg D, A review of the biodegradability and utility of poly (caprolactone), *Journal of Environment and Polymer Degradation*, 1995; 3: 61–8.

Goncalves S.P.C., Cinaglia D.L. and Martins S.M., Biodegradation of the films of PP, PHBV and its blends in soil, *Journal of Polymers and Environment,* 2009; 17: 280-285.

Göpferich A., Mechanisms of polymer degradation and elimination, *Handbook of Biodegradable Polymers,* Amsterdam, Harwood Academic, 1997; 451–71.

Gugumus F., Photooxidation of polymers and its inhibition, *Oxidation inhibition in organic materials,* 1990: 29–162.

Gu J.D., Ford T.E., Mitton D.B. and Mitchell R., Microbial corrosion of metals, *The Uhlig Corrosion Handbook. 2nd Edition.* Pub: New York: Wiley, 2000; 915–27.

Gurusewak S., Navleen K., Haripada B., Pramod K.B., and Uttam K.M., Degradation behaviours of linear low-density polyethylene and poly(L-lactic acid) blends, Wiley Online Library, 2011.

Hadad D., Geresh S. and Sivan A., Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*, *Journal of Applied Microbiology*, 2005; 98: 1093-1100.

Hamilton J.D., Reinert K.H., Hogan J.V. and Lord W.V., Polymers as solid waste in municipal landfills, *Journal of Air Waste Management,* 1995; 43: 247–51.

Heidary S. and Gordon B., Hydrolyzable poly(ethylene terephthalate), *Journal of Environment and Polymer Degradation,* 1994; 2: 19–26.

Jarerat A., Paranamuda H. and Tokiwa Y., Poly(L-lactide)-Degrading activity in various actinomycetes, Journal of Macromolecular Bioscience, 2002; 2: 420-428.

Jayasekara R., Harding I., Bowater I. and Lornergan G., Biodegradability of selected range of polymers and polymer blends and standard methods for assessment of biodegradation, *Journal of Polymers and the Environment*, 2005; 13: 231-251.

Joel F.R., *Polymer Science & Technology: Introduction to polymer science*, Pub: Prentice Hall PTR Inc., Upper Saddle River, New Jersey 07458, 1995: 4-9.

Jun H.S., Kim B.O., Kim Y.C., Chang H.N. and Woo S.I., Synthesis of copolyesters containing poly(ethylene terephthalate) and poly(e-caprolactone) units and their susceptibility to *Pseudomonas sp.* Lipase, *Journal of Environment and Polymer Degradation,* 1994; 2: 9–18.

Kamal M.R., Huang B. Natural and artificial weathering of polymers, *Handbook of Polymer Degradation*, 1992; 127–68.

Kawai F., Bacterial degradation of glycol ethers, *Applied Microbiology and Biotechnology*, 1995; 44: 532-538.

Kathiresan K. and Bingham B.L., Biology of mangroves and mangrove ecosystems, *Journal of Advances in Marine Biology,* 2001; 40: 81-251.

Kolybaba M., Tabil L.G., Panigrahi S., Crerar W.J., Powell T. and Wang B., *Biodegradable polymers: past, present, and future*, CSAE/ ASAE Annual Intersectional Meeting, 2003.

Kumar A.P., Pandey J.K., Kumar B. and Singh R.P., Photo-/Bio-degradability of Agro Waste and Ethylene–Propylene Copolymers Composites Under Abiotic and Biotic Environments, *Journal of Polymers and Environment*, 2006; 14: 203–212.

Kumari K., Anand R.C. and Narula N., Microbial degradation of polyethylene (PE), *The South Pacific Journal of Natural Science*, 2009; 27: 66-70.

Kwpp L.R. and Jewell W.J., Biodegradability of modified plastic films in controlled biological environments, *Journal of Environmental Technology,* 1992; 26: 193-198.

Kyrikou I. and Briassoulis D., Biodegradation of agricultural plastic films: A critical review, *Journal of Polymers and the Environment*, 2007; 15: 125-150.

Lee S., Bacterial polyhydroxyalkonoates, *Journal of Biotechnology and Bioengineering,* 1996; 49: 1-14

Madison M. and G.W. Huisman., Metabolic engineering of poly(3-hydroxyalkanoates): from DNA to plastic. *Journal of Microbiology and Molecular Biology*., 1999; 63: 21-53.

Maiti S. and Jana T., *Biodegradable polymers: Polymer recycle and waste management*, Anusandhan Prakashan, Midnapore (India), 2005.

Muller R.J., Kleeberg I., and Deckwer W.D., Biodegradation of polyesters containing aromatic constituents*, Journal of Biotechnology*, 2001; 86: 87–95.

Nanda S. and Sahu S.S., Biodegradability of polyethylene by Brevibacillus, Pseudomonas, and Rhodococcus spp., *New York Science Journal*, 2010; 3(7): 95-98.

Nawaz M.S., Billaedeau S.M. and Cerniglia C.E., Microbial degradation of hydrocarbons in the environment, *Microbiological Reviews*, 1990; 54: 305-315.

Olayan H.B, Hamid H.S. and Owen E.D., Photochemical and thermal crosslinking of Polymers, *Journal of Macromolecule Sciences*, 1996; 36: 671–719.

Otake Y., Kobayashi H., Murakami N. and Ono K., Biodegradation of low density polyethylene,polystyrene, polypropylene and urea-formaldehyde resin buried under soil for 32 years, *Journal of Applied Polymer Sciences,* 1995; 56: 1789-1796.

Pranamuda H. and Tokiwa Y., Degradation of poly(L-lactide) by the strains belonging to the genus *Amycolatopsis*, Biotechnology Letters, 1999; 21: 901-905.

Pranamuda H., Tsuchii A. and Tokiwa Y., Poly-L-lactide degrading enzyme produced by *Amycolatopsis* sp., *Journal of Macromolecule Biosciences,* 2001; 1: 25-29.

Poliakoff M., Fitzpatrick J.M., Farren T.R. and Anastas P.T., Green chemistry: Science and politics of change, *Science*, 2002; 4: 297.

Pospisil J. and Nespurek S., Highlights in chemistry and physics of polymer stabilization. *Macromolecule Symposium*, 1997; 115: 143–63.

Premraj R. and Doble M., Biodegradation of polymers, *Indian Journal of Biotechnology*, 2005; 4: 186-193.

Przybulewska K., Wieczorek A. and Nowak A, Isolation of microorganisms capable of styrene degradation, Polish Journal of Environment Studies, 2006; 15(5): 777-783.

Ramis X., Cadenato A., Salla J.M., Morancho J.M., Valles A *et al,* Thermal degradation of polypropylene/ starch based materials with enhanced biodegradability, *Journal of Polymer Degradation and Stability*, 2004; 86: 483-491.

Ratajska M. and Boryniec S., Biodegradation of some natural polymers in blend with polyolefines, *Polymers Advanced Technologies*, 1999; 10(10): 625-633.

Reddy N., Nama D. and Yang Y., Polylactic Acid/Polypropylene polyblend fibers for better resistance to degradation, *Journal of Polymer Degradation and Stability*. 2008; 93: 233-241.

Sabir, I., Plastic Industry in Pakistan. http://www.jang.com.pk/thenews/investors/nov/index.html , 2004.

Sameh A.S., Alariqi, Kumar A.P., Rao B.S.M. & Singh R.P., Biodegradation of γ- sterilized biomedical polyolefins under composting and fungal culture environments, *Journal of Polymer Degradation and Stability*, 2006; 91: 1105-1116.

Scott G., 'Green' polymers, *Polymer Degradation and Stability*, 2000; 68: 1-7.

Scott G., Environmental biodegradation of hydrocarbon polymers: initiation and control, *Journal of biodegradable Plastics and Polymers,* 1999: 79-91.

Scott G. and Wiles D.M., Programmed-life plastics from polyolefins: a new look at sustainability, *Biomacromolecules*, 2001; 2(3): 615-622.

Seymour R.B., Polymer science before & after 1899: notable developments during the lifetime of Maurtis Dekker, *Journal of Macromolecule Science and Chemistry,* 1989; 26: 1023–32.

Shah A.A., Hasan F., Hameed A. and Ahmed S., Biological degradation of plastics: a comprehensive review, *Journal of Biotechnology Advances*, 2008; 26: 246-265.

Shimao M., Biodegradation of plastics, *Journal of Current Opinion in Biotechnology*, 2001; 12: 242-47.

Shimura M., Kimbara K., Nagato H. and Hatta T., Isolation and characterization of a thermophilic *Bacillus* sp. JF8 capable of degrading polychlorinated biphenyls and naphthalene, *FEMS Microbiology Letters*, Sept.1999; 178(1): 87–93.

Singh G., Bhunia H., Rajor A., and Choudhary V., Thermal properties and degradation characteristics of polylactide, linear low density polyethylene, and their blends, *Polymer Bulletin*, 2011; 66: 939-953.

Singh G., Kaur N., Bhunia H., Bajpai P.K., Mandal U.K., Degradation Behaviors of Linear Low-Density Polyethylene and Poly(L-lactic acid) Blends, *Journal of Applied Polymer Science*, 2012; 124: 1993-1998.

Sivan A. and Mor R., Biofilm Formation and partial biodegradation of polystyrene by the actinomycete *Rhodococcus ruber, Journal of Biodegradation,* 2008; 19: 851-858.

Sivan A., Szanto M ., Pavlov V., Biofilm development of the polyethylene degrading bacteria *Rhodococcus ruber, Journal of Applied Microbiology and Biotechnology,* 2006; 72: 346-352.

Sonil N. and Samiti S.S., Biodegradability of polyethylene by *Brevibacillus, Pseudomonas, and Rhodococcus* spp., *New York Science Journal*, 2010; 3(7): 95-98.

Swift G., Non-medical biodegradable polymers: environmentally degradable polymers. *Handbook of Biodegradable Polymers*, Amsterdam, Harwood Academic, 1997; 473–511.

Tokiwa Y. and Calabia B.P., Biological production of functional chemicals from renewable resources, *Canadian Journal of Chemistry*, 2008; 86: 548-555.

Tokiwa Y., Calabia B.P., Ugwu C.U. and Aiba S., Review: Biodegradability of plastics, *International Journal of Molecular Sciences,* 2009; 10: 3722-3742.

Tomita K., Kuroki Y., Hayashi N. and Komukai Y., Isolation of a thermophile degradation of Poly(Butylene Succinate-co-Butylene adipate), *Journal of Biosciences and Bioengineering,* 2000; 90: 350-352.

Toncheva V., Bulcke A., Schacht E., Mergaert J., and Swings J., Synthesis and environmental degradation of polyesters based on poly (ε-caprolactone), *Journal of Environment and Polymer Degradation,* 1996; 4: 71–83.

Upreti M.C. and Srivastava R.B., A potential *Aspergillus* species for biodegradation of polymeric material, *Current Science*, 2003; 84(11): 1399-1402.

US EPA, United States Environmental Protection Agency, Municipal solid waste management, 2005.

Weiland M., Daro A. & David C., Biodegradation of thermally oxidised polyethylene, *Journal of Polymer Degradation and Stability,* 1995; 48: 275-289.

Winursito I., and Matsumura S., Biodegradability, hydrolytic degradability, and builder performance in detergent formulations of partially dicarboxylated alginic acid *Canadian Journal of Chemistry,* 1996; 4: 113–21.

Xu S., Lehmann R.G., Miller J.R. and Chandra G., Degradation of silicone polymer as influenced by clay minerals, *Journal of Environental Science and Technology,* 1998; 32: 1199–206.

Yamada-Onodera K, Mukumoto H, Katsuyaya Y, Saiganji A, and Tani Y, Degradation of polyethylene by a fungus, *Penicillium simplicissimum* YK, *Journal of Polymer Degradation and stabilization,* 2001; 72: 323-327.