Biodegradation of Plastics: Polyethylene Terephthalate (PET), Polystyrene (PS) and Polyethylene (PE)

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DEDICATION

Every work that is challenging needs not only our efforts but also the guidance and motivation of people that play a major role in our life and are much close to us. We dedicate our efforts to our **parents** and to those whose affection, love and attention made our journey successful.

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Rida Zainab

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TABLE OF CONTENTS

Contents **page #**

LIST OF FIGURES

Figure # **Page #**

LIST OF TABLES

Table # **page #**

LIST OF ABBREVIATIONS

MMT	Million metric tons
PP	Polypropylene
PUR	Polyurethane
PA	Polyamide
PS	Polystyrene
PET	Polyethylene terephthalate
PVC	Polyvinyl chloride
LDPE	Low Density Polyethylene
HDPE	High Density Polyethylene
PTFE	Polytetraflourethylene
AMU	Atomic mass unit
PHA	Polyhydroxy alkanoic acid
DDE	Dichlorodiphenyldicholoroethylene
PCB	Polychlorinated Biphenyls
BPA	Bisphenol A
US EPA	United States Environmental Protection Agency
TBBPA	Tetrabromobisphenol A
PBDES	Polybrominated diphenyl ethers
Atm	Atmospheric Pressure
UV	Ultravoilet
PMMA	Polymethylmethacrylate
PVAL	Polyvinyl alcohol
TGA	Thermal Gravimetric Analysis
AFM	Atomic Force Microscopy

SEM	Scanning Electron Microscopy
CFCs	Chlorofluorocarbons
NMR	Nuclear Magnetic Resonance
FTIR	Fourier-transformed infrared spectroscopy
XRD	X-Ray diffraction
GPC	Gel Permeation Chromatography
HPLC	High Pressure Liquid Chromatography
GC-M	Gas Chromatography-Mass Spectroscopy
MSM	Minimal Salt media
GCF	Gross Calorific Value
PLA	Polylactic Acid

ABSTRACT

With the increase in human population, there has been increase in plastic production and its usage. There is wide range of applications of Polyethylene (PE), Polystyrene (PS) and Polyethylene terephthalate (PET). These plastic types contain many hazardous chemicals like Phthalates, Bisphenol A as additives which can leach into environment and cause various health hazards to humans. Microorganisms use plastics as carbon source through process of degradation. The aim of the present project is the isolation of bacterial strains having the ability to degrade plastics, PE, PS and PET. The bacterial and fungal strains were isolated from soil samples of plastic dump. Bacterial isolates were inoculated in liquid Mineral salt media with sterilized plastic pieces and incubated in shaking incubator. The degradation ability was to test with growth of microbes and then testing degradation by different analytical techniques like FTIR, NMR, ATM etc.

As there is accumulation of millions of tons of plastic waste every year, so this results in significant decay of environment. Recent clean-up strategies tend to reduce the negative effects caused by plastic pollution but increase in production of plastics makes it difficult. The current project also focused on the social aspect of the plastic pollution. Awareness campaign was launched with posters and banners of harmful effects of plastic pollution. Further a questionnaire was developed of 25 questions to know about plastic usage, its disposal and hazards and 300 responses were obtained from people of different age group. The results showed that more than 64 % people use different types of plastics in their daily life and majority prefer plastic bags due to durability and cheapness. About 53.6 % people do not have knowledge about biodegradable plastics. When use of alternative to plastic was questioned, 97.7% people think cloth bags should be used and 83% people of in-favor of recycling of plastics bags. About 97 % people were in favor of ban on non-biodegradable plastic bags and enforcing law. Survey results indicates that 48 % people think burying plastic waste is best disposable method while 33 % were in favor of burning. More than 60 % people through plastic bags and disposable cups etc in the dustbin. People do have knowledge about the hazards of plastics (97%) while only 67 % have specific knowledge of carcinogens released from plastics and majority do not have knowledge about the micro plastics.

So it is concluded that there should be more research needed for plastic degradation, awareness about hazards of plastics and effective strategies should be applied to reduce plastic pollution. There is need of extensive campaign on social media about the hazards of plastics. Social media, universities, and schools should play their part in awareness of plastic hazards among people.

CHAPTER 1

INTRODUCTION

Plastic is a versatile and most commonly usable materials of twentieth century. In 1909, Baekeland coined the word plastic. The word plastic came from Greek word “Plastikos” which means molding. Plastics are semisynthetic or synthetic substances which are obtained from the petrochemical substances. Plastics show similarities to the natural plant and trees resins. Due to its versatile nature it is used instead of metals, glass, and woods. Modern plastic development started somewhat 59 years ago. Plastic is used in construction, food, transportation, shelter, clothing, sports, medical and many other aspects. Due to its vast characteristics like stability, light weight, low cost, unbreakable nature and durability it is used in many aspects of daily life. Polymers are synthesized from monomers through polymerization by the process of condensation and addition.

Since 1950, the global production of plastics was 1.5 Mt. About 6.3 BT. of plastic wastes has been generated in 2015. The global production of plastics increases 9 % i.e. from 348 to 359 Mt. from 2017-2018. The worldwide production of synthetic polymers is 350-400 MMT. (million metric tons) annually.

Plastic have many types, its classification is based upon chemical composition, structure and durability. The most important types of plastics are as polypropylene (PP), polyurethane (PUR), polyamide (PA), polystyrene (PS), polyethylene (PE), polyethylene terephthalate (PET) and polyvinyl chloride (PVC). Polymers have versatile nature which resulted in the production of large amount of wastes. Polymers are used in packaging, agriculture sector, fishing nets and food industries. After using such large amount of plastics are then discarded into landfills which are most dangerous environmental threat, because of non-biodegradable nature of synthetic polymers. There is an important threat to world life and environment because of accumulation of synthetic polymers. Human activities like industrial and household wastes lead to the pollution of soil. Many animals die by eating and being stuck in the plastic traps. Some plastic particles mimic human hormones that resulted in human health problems. Furthermore, the polymers show resistance to degradation because of materials used in production. Non biodegradability and resistivity properties of polymers make them a critical waste.

Polymers wastes have now started world attention because of the different health and environmental hazards. As these polymers are marine and terrestrial life hazards. It is studied that almost 5 to 13 MMT (million metric tons) of polymers entered the oceans annually, having negative effects on different ecosystems and on animals and human health. Due to plastic burning dioxins compounds are releases to the environment which are carcinogenic.

Consequently, it is needed to make biodegradable plastics that can be degraded by microorganisms. Such polymers have carbon chain which act as an energy source for microorganisms like fungi and bacteria. Many microorganisms are investigated and studied that have potential to produce plastic degrading enzymes which results in the degradation of polymers, having nonpoisonous by products. It is considered as one of the safest methods used for the degradation of polymers. Thus, this thesis is presented to focus on the role of microorganisms in plastic degradation.

In 2017, PE accounts for 33% global plastic wastes and is most widely used plastic Most Low-Density Polyethylene produced by America almost 820000 metric tons. And most High-Density Polyethylene is produced by China almost 4.16 MMT (million metric tons) by the year 2019. Many polyethylene products in our daily life is most common like pharmaceutical and food packaging film, pipe, cable and wire insulation and most consumed polyethylene are High Density Polyethylene, Linear Low-Density Polyethylene and Low-Density Polyethylene. Many bacteria like *Rhodococcus, Bacillus, Streptococcus, Staphylococcus* and *Klebsiella, Pseudomonas, Acenitobacter, Ralstonia* etc. have ability to form biofilm on the surface and hence cause the breakdown of the surface. Many fungal species like *Penicillium, Fusarium, Aspergillus, Cladosporium* etc. are involved in the degradation of polymers.

Polystyrene is an important plastic that can be used in various things of everyday life including household appliances, construction, electronics, sports and leisure, toys and packaging of food. Global production of EPS and PS was 6.6 million Mt and14.7 million Mt per annum in 2016. From total waste production in last 10 years, PS accounts for about 10 % wt. of total plastic. The production and capacity will reach 10.9 and 15.2 million tons in 2024. WWF-Pakistan is one of the largest conservational organizations that are working against environmental degradation and climate change in country. According to WWF-Pakistan, approximately 250 million tons of garbage includes pet bottles, plastic bags and food scraps of polystyrene.

These forms of polystyrene i.e. Expanded Polystyrene (EPS) and High Impact Polystyrene (HIPS) provide the excellent facility of insulation and mechanical properties. It is used in construction, arts and crafts, electrical appliances, food packaging etc. It is also used for construction purposes to insulate walls, ceilings and floors. It also provide sound proof specialty to walls. PS is also used in laboratory items like petri dishes, test tubes, trays etc.(Willis et al.,2016)

PET almost accounts for half of the production of synthetic polymer production in the world due to its applications in food packaging and synthetic fiber. However, some of the components of plastics also possess threat to the environment especially to aquatic life (Devi et al., 2015). The high stability and durability of PET make it highly resistant to degradation in the natural environment thus damaging aquatic life badly. The inappropriate methods of disposal also made PE, PS and PET to affect the environment, as these methods result in release of several toxic compounds or pollutants that result in air pollution. This also led to the production of plastic pollution as the current methods of disposal of these plastics including landfilling, incineration and recycling has failed to be effective methods to dispose these wastes. So, there is a need of efficient and effective alternative methods that are environment friendly and not hazardous to human health as well.

Biodegradation is an effective alternative to dispose PE, PS and PET waste. However, its commercial application is not yet known. These materials are biodegraded with the help of certain set of enzymes both intracellular and extracellular that are secreted by different bacteria and fungi present in the soil samples. These enzymes convert the polymers of plastics into monomers that were further taken up by the cell as a carbon source (Le et al., 2019).

Aims and Objectives:

1. Research work aims are about:

- Biodegradation of plastics for effective remediation of environmental plastic pollution.
- To aware people about plastic pollution.

Objectives:

- To isolate microorganisms for having ability to degrade plastics from contaminated sites (PE, PS and PET).
- Biodegradability of isolated bacterial and fungal colonies in shake flask.
- To analyze possible alternatives to plastics and strategies to reduce plastic pollution.

The objectives of survey include:

- To assess the level of awareness, behaviors and attitudes regarding plastic usage in everyday life.
- To know about strategy of waste disposal by people.
- To know about awareness of plastic hazards on human beings and environment among people.

CHAPTER 2

LITERATURE REVIEW

2.1 History of plastics:

Since, the discovery of bands, balls and figures from natural rubber in 1600BC the human get benefits of the polymers. In Great International Exhibition in 1862, a first human made plastic was presented by Alexander Parkes in Landon. He then called it "Parkesine" but now it is known as 'celluloid'. L.H. Baekeland a chemist in 1907 was working to produce synthetic varnish but he accidentally produces formula for the synthetic polymer which comes from the coal tar. He then named it 'Bakelite'. Then in 1909 the chemist Baekeland gave the name plastic for these completely new materials.

In 1914 a first patent was registered for polyvinyl chloride, which is used in water pipes and vinyl sidings. During the first and second world war plastic served as a substitute for metal, wood and glass. New different kinds of plastic like polycarbonate, polyurethane, polystyrene, polyester, polyvinyl chloride, polypropylene, polymethyl Methacrylate and silicones were discovered after the Second World War. Plastic is now considered as common in the consumer society (Abrusci et al., 2011).

More than hundred years back the history of synthetic polymer started, though plastic in comparison with other materials are considered as modern. The modern plastic development started in the first half of 20th century with fifteen different new kinds of plastic synthesis. Plastic have unique characteristics as they are resistant to light and chemicals and show resistivity to different temperatures.

Synthetic polymer is somewhat similar to the natural resins which are found in the plants and trees. Because of versatile nature of plastic, a great increase in its production and use occur in the past thirty years, and it is used in every respect of life. Plastics are strong, cheap, light weight and durable and these characteristics make it used for the manufacturing of wide variety of products.

Plastic took years to degrade and cause pollution. Although many types of plastic materials are available in the market, only some of these are used as a commodity because of more volume and low price. Polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC), high-density polyethylene (HDPE), low density polyethylene (LDPE) fulfill approximately total demand of 90 %. The leading producer of plastic is China which accounts for 23.9 % production of plastic. The second world most producer of plastic is Europe which produce almost 21.5% lagging after China (Devi et al., 2015).

2.2 Classification of plastic:

Plastic is classified into different types, classification based on chemical structure and properties are as follows:

2.2.1 Classification based on Thermal properties:

Plastic is classified into two different kinds based on thermal properties.

1. Thermoplastic polymers
2. Thermosetting polymers

2.2.1.1 Thermoplastics:

On heating, its chemical composition doesn't change and due to this reason, it undergoes multiple time molding. Thermoplastics are of different types including polyvinyl chloride, polyethylene, polystyrene, polytetrafluorethylene (PTFE) and polypropylene (PP). Thermoplastics are also called as common plastics, its molecular weight ranges from 20,000-500,000 AMU. It is consisting of variable number of repeating units that comes from monomers (Alshehrei, 2017).

2.2.1.2 Thermosetting Polymers:

Thermosetting polymers cannot be modified and melted but remain solid. These plastic structures have high cross-linked bonds due to this reason they are irreversible and are not recyclable, while the thermoplastic have linear structure (Raziyafathima et al., 2015). Example of thermosetting polymers is polyurethanes, epoxy resins and phenol-formaldehyde etc.

2.2.2 Classification Based on Designed Properties:

This kind of plastic classification is based on the manufacturing design and process. Differences are based on the different kind of parameters used in plastics like durability, electrical conductivity, tensile strength, thermal stability and degradability.

2.2.3 Classification Based on Degradability Properties:

This is the kind of classification in which either the plastic is degradable or non-degradable is based on its chemical properties (Ghosh et al., 2013). Synthetic polymers which are usually non-biodegradable come from petrochemicals. In synthetic polymers many repetitions of monomers occur which lead to production of high molecular weight polymer.

In contrast, degradable polymers are composed of material that is renewable that can be degraded easily and completely when exist in natural form like material of living algae, animals and plants as starch, cellulose, proteins source etc. Many microorganisms can also produce these materials. Degradable plastic degradation occurs with water, UV, change in pH and enzymes. Degradable plastic is classified into four types (Arikan & Ozsoy, 2014).

2.2.3.1 Biodegradable Bio-plastic:

This kind of plastic is completely degradable by microorganisms, without production of any kind of toxic material. The word “biodegradable” means that a material which is degraded by living sources like microorganisms which lead to production of biomass and biogas i.e.; water and carbon dioxide.

One of the significant types of biodegradable plastic is polyhydroxyalkaanoate (PHA). They have properties like conventional plastic. It is completely degradable thus it is ideal for consuming purpose. An example of important PHAs is polyhydroxy butyrate (Raziyafathima et al., 2015).

2.2.3.2 Compostable Plastic:

Compostable plastic is the one in which decomposition occurs by microorganism in a composting process, in which the decomposition rate is equal to the decomposition of other material. The clear visible toxicity is not detected but by special standard test toxicity can be measured and determined.

Table 1 Types of synthetic plastic (Okunola et al., 2019**;** Eyerer et al., 2010**;** Yogalakshmi & Singh, 2020)

Plastic type	Chemical composition	Properties	Applications	Human health Hazards
Polyethylene terephthalate	Condensed polymer Formed by esterification of ethylene glycol with terephthalic acid or dimethyl terephthalate and antimony trioxide is used as a catalyst	Smooth, transparent and thin, soften at 80ºC, Anti-Air prevent entrance of O_2, solvent resistance, manufactured for single use.	Used in production of soft drinks, disposable salad dressing and water bottles.	Leaching of some additives (acetaldehyde, antimony and phthalates) at higher temperatures, Antimony is toxic.
High density polyethylene (HDPE)	A macromolecule (chain like structure) formed by polymerization of ethylene.	Opaque, Vary from hard to semi flexible, heat and chemical resistant, at 75ºC get soft	Refrigerators, shampoo, milk and ice-cream containers, detergent bottles, grocery bags	Generally Safe for drinks and food, long exposure to sunlight may make it harmful.
Polyvinyl chloride (PVC)	A polymer formed by polymerization of vinyl chloride. Have chemical constituents like phthalates, BHA and dioxin.	Hard, strong, tough and sort of clear, softens at 80°C, can be solvent cementing.	Used in packaging fruit juice, cooking oil, plumbing pipes and fittings, wall cladding, electrical material roof sheets and blood bags	Highly toxic due to presence of phthalates, cause different diseases i.e. chronic bronchitis, birth defects, genetic changes etc.
Low density polyethylene (LDPE)	Contains many short and long side-chain branches with 4,000-40,000 carbon and formed via free radical polymerization at high temperature.	Soft, fragile and rigid, heat resistant ,softens at 70°C	Cling film, refuse or garbage bags, Frozen food bags, squeezable bottles, flexible container lid.	Not hazardous to human body.
Polypropylene (PP)	Polymer made from propylene monomers.	Hard and strong, semitransparent, soften at 140°C.	Bottle caps, drinking straws, car batteries, syringes, packaging medicine and beverages, garden furniture and kettles.	No harmful substances, considered safe for humans.
Polystyrene (PS)	A petroleum-based, aromatic hydrocarbon made up of styrene subunits.	Clear, hard and brittle, Alkali resistant, taste and odor free, soften at 95°C	laboratory ware, polystyrene cups, disposable plates, trays, and cutlery, brittle toys and food insulation	Hazardous to health, human carcinogen, can be neurotoxic causing cytogenetic, hematological effects
Others (polycarbonate)	A Thermoplastic polymer with carbonate as a backbone	tough, amorphous and transparent	Beverage and baby milk bottles, shatterproof windows, electronics and automobiles.	PC Contain BPA, longer exposure to heat leach out BPA.

2.2.3.3 Photodegradable Bio Plastic:

Photodegradable bio plastic is the plastic having sensitive group to light. Long toxic exposure to ultraviolet light can lead to the degradation of structure of plastic, which further makes it easily available for the microbial degradation of plastic. Such plastic remains non-degraded in landfill because of having no light (Arikan & Ozsoy, 2014).

2.2.3.3 Bio-based bioplastics:

Bio-based bioplastics are those plastics that are derived from the carbon source of some renewable forestry and agriculture source that is soya bean proteins, corn starch and cellulose.

2.2.4. Classification Based on Chemical Structure:

This classification is based on the type of reactions occurred for its formation. Addition polymers are referred to it if all the monomers atoms combined to form plastic. Condensation polymers are referred to those synthetic polymers in which water molecules resulted from the combination of polymers. Some monomers formed double bond with each other between carbon atoms are called olefins and polyolefin are referred to as commercial polymers. This include different synthetic polymers like polyethylene terephthalate, polyethylene, polyvinyl chloride, polystyrene and polyurethane (Shah et al., 2008).

2.2.5 Classification Based on Uses and Manufacturing:

This class of classification is based on manufacturing process and its uses, as some plastics are low cost, and some are relatively high, and durable products. This includes the usage of plastic products for different purposes. In case of PS, General Purpose Polystyrene is used in disposable transparent cups and toys while HIPS is used in Formation of electrical wires (Raziyafathima et al., 2015).

2.2 Plastic Hazards:

Approximately 6.3 billion tons of plastic have been produced worldwide since 1950 – 2018 and among it approximately 9 to 12% of plastic has been incinerated and recycled. The great input of plastic into environment is due to insufficient circular plastic economy as well as low recycling and reusing rate, which leads to environmental and human health. As plastics are present in different sizes, so their exceeding amount have different negative effects. More the smaller size of plastic it will have more surfaces to volume ratio and will harm more. Smaller size also increases the toxicological potencies of plastic.

When there is plastic accumulation in environment then by the process of UV radiation, slow decomposition, biodegradation and weathering that results into formation of smaller plastics called mesoplastics, microplastics and nano plastics. They all are classified in different sizes as well as in shapes for Example spheres, fibers and fragments depending upon their functions. Mesoplastics are those having size of plastics from 5-40 mm. Microplastics are small particles having the size of greater than 100 nm and less than 5 mm. nano plastics are having size of less than 100nm.Nano plastics are most dangerous of all because of having smaller size.

2.2.1 Sources:

Microplastics can be classified into Primary and Secondary plastics depending upon the source from which they have originated.

1. Primary Microplastics are produced through manufacturing process by tearing and abrasion of large products like electronic waste, wheels, tyres and boards. These either intentionally or accidently directly unleash into environment in as microfibers, microbeads and micropellets.
2. Secondary Microplastics are obtained from break down of large or macroplastics by the help of biological, physical and chemical forces. The forces that undergo breakdown are Mechanical forces, UV degradation, oxidation and thermal degradation etc.

2.2.2 Effect of Plastic on Environment:

Due to its vast application and usage, plastic unfortunately has become part of every environment including both terrestrial and aquatic environment. During the manufacturing of plastics, it is treated with different additives, stabilizers, colorant moieties, heavy metals etc. so during disposal these components (phthalates, acetaldehyde and antimony) will leach into the soil and contaminate soil and water reservoirs. incineration of PE, PS and PET also leads to evolution of harmful gases also termed as green-house gases and results in global warming and affecting ecosystem adversely e.g. CO_2 and methane produces by breakdown of nylon act as a powerful greenhouse gases (Proshad et al., 2017). Plastic waste also effect the aquatic life, almost 80% of plastic waste is disposed of to the rivers and oceans untreated. The microorganisms present in water also tend to degrade the plastic waste, but this leads to the accumulation of toxic compounds that will affect the aquatic life present in water. Thus, the microorganisms present on surface water get colonized by different non-native species. Moreover the plastic debris present in marine and terrestrial environment get utilized by animals and fish leading to obstruction and physical damage to animal digestive system results in malnutrition, starvation and ultimately death of organisms. It also limits the ability of organisms to reproduce thus destroying the niche of the area (Ilyas et al., 2018).

2.2.3 Effect on Animals Health:

Due to the hazardous effects of plastic and plastic products wastes on animals, it adversely affects the food supplies for the consumption of human. There are several reports that threat us about the survival of marine life due to the plastic and its product's waste. Entanglement and ingestion is the major route through which animals are exposed to plastic wastes. Mostly ingestion is more dominant than entanglement. Many times, the animals that are present in water mistakenly ingest plastic waste as food.

Furthermore, the entanglement of marine animals by plastic and its products can harm them and even death can also occur. Studies show that above 260 species of invertebrates and vertebrates of marine animals are entangled by plastics or ingest plastics, which leads to above 4,000,000 death of these animals. Mostly those marine animals are subjected to plastic wastes which use jellyfish for food, as it is being confused with the plastic by certain marine animals; most specifically turtles get more confused. Similarly, seabirds confuse in microplastics for fishes and cattle fish. Thus, ingestion of plastic polymer can cause damage to digestive tract which causes malnutrition, starvation and eventually death. The entanglement of animals by plastic makes them unavailable for predator because they are unable to untangle themselves. Nets of plastic and other plastic waste also damage coral reefs. Fishing net that is also known as "Ghost net" is discarded in the water and trap marine animals, which in return leads to starvation and death (Hammer et al., 2012).

2.2.4 Effect on Public Health:

Generally, it is believed that plastic has no or less effect on human health. but different kinds of monomers and additives of plastic retained in the environment are highly risky and hazardous to human health. Many additives of plastic wastes are high disruptor of endocrine and hence are carcinogenic. The main route of these additives to human health is inhalation, ingestion and skin contact. The major problem is the ingestion of microplastics by aquatic and marine life which further effect public health by consumption of these marine animals and it is a major threat to public health. Studies shows that plastic contamination persist in human bodies from environmental contamination

2.2.5 Effect of Plastic Additives on Public Health:

In plastic production, various kinds of different additives are added to make the plastic which cause very dangerous effect to human health. The following are various types of additives used in plastic making

2.2.5.1 Bisphenol A:

The main and hazardous monomer present in polycarbonate plastics and PVC is bisphenol A. It is present in polycarbonate water bottles. These plastic release BPA into environment when discarded. They are most commonly found in leachate of landfills where plastic waste is dumped. They get released into environment because during polymerization, some of monomers are not bound together and hence can easily release into environment. It causes serious hazards to human health. It acts as potential hazard to endocrine system thus causing disruption to normal hormonal functioning. Various other health hazards caused by BPA include heart attacks, angina, cardiovascular disease and male sexual dysfunction

2.2.5.2 Phthalates:

Phthalates are composed of diesters of phthalic acid. They are added in PVC to provide it flexibility and elasticity. Plastic manufacturing include more than 25 different phthalate esters. They do not bind covalently to polymer hence making an ease to release into environment. One of additive includes Di (2-ethylhexyl) phthalate (DEHP). It is associated with various health concerns to human beings. DEHP is converted to MEHP on entering body. It gets converted by lipases and is absorbed by body. It causes asthma, cancer development, hormone disruption, genital malformations and reproductive problems.

2.2.5.3 Benzene:

Benzene has high hazard level that is used as raw material in different monomers like ε-caprolactam, styrene and adipic acid. Butadiene is used as raw material in monomers like lauryl lactam, sebacic acid and hexamethylenediamine. These both are considered as carcinogenic as well as germ line mutagenic. They also damage fertility.

2.2.5.4 Vinyl chloride:

It is a monomer that is used to make plastic called Vinyl chloride. This monomer is considered as most health hazardous material. It also causes infertility. The raw materials used to make this monomer are acetylene and ethylene that is less hazardous than monomer itself.

2.2.6 Hazards of PS on Human Health:

When inhaled, it causes irritation to skin, eyes and gastrointestinal tract. High exposure in humans causes effects on Central Nervous System like weakness, fatigue, depression, headache and dysfunction. It also causes effects on blood and kidney enzymes functions. When animals such as Rats are exposed to high concentration of styrene, they show problems like kidney damage, liver, sperm, hearing loss, changes in lining of nose, nasal and eye irritations. Disturbance of nervous system causing tiredness, color vision, feeling drunk, balance problems and also lung tumors

2.2.7 Hazards of PET on Human Health:

Terephthalic acid cause unclear vision, pain in eyes and slight irritation, when a person gets exposed with it. It also causes irritation of mucosal surface when inhaled. According to a study based on animal diseases, prolonged or high oral exposure may cause changes in presence of blood in urine, kidneys or formation of gall stones in bladder. Concentrations of equal to or greater than 0.1% of TPA is listed by OSHA and NTP as a carcinogen. To keep employee from its exposure sufficient ventilation must be provided. TPA must be kept in air tight containers. Severe burns are caused when molten polymer adhere to skins. Unclear vision and eye pain is caused if PET particles get exposed to eye. It is observed that no skin sensitizations and irritation is caused by TPA. However, decomposition of PET generate products that cause respiratory, eye and skin irritations. NTP and OSHA has not mentioned PET as carcinogen if its concentration lies in range of 0.1%. Local ventilation must be used to control fumes during hot processing of PET (Okunola et al., 2019).

2.3 Types of Plastic Degradation:

Change in polymer properties because of chemical, physical and biological reactions which results into breaking of bond between polymers and successive transformations chemically called as polymer degradation. As a result of degradation, there are many changes which can be observed in properties such as mechanical, optical and electrical characteristics in cracking, erosion, change in color and separation of phase. There are different classification of polymers based on their nature of causative agent which includes the following types of photo-oxidative degradation, ozone-induced degradation, thermal degradation, biodegradation, mechanochemical degradation and catalytic degradation.

2.3.1 Photooxidative Degradation:

The process of decomposition of material through the action of light is called photodegradation. The decomposition by light is considered as primary sources to damage polymer at normal conditions in environment. Usually, visible and UV light are the main components to initiate degradation in many polymers. The UV radiations of length (290-400nm) in sunlight usually determine the life span of polymeric substance in outdoor environment. Ether parts present in soft parts of polymer, undergo degradation through photo irradiation. It generates different groups such as propyl, formaldehyde, aldehyde and ester etc. UV radiations have enough energy to cause breakage of C-C bonds in polymer. Different types of plastics have different wavelengths of UV to cause degradation activity. For example: For polyethylene, the wavelength is usually 300nm and its 370nm for PP. Physical and optical characteristics of plastics are changed due to photodegradation. Some of the important degradation effects include yellowing of plastics, loss of mechanical properties such as tensile strength of polymer as well as changes in molecular weight and mass of polymer. PP and PE films after exposure to UV light lose their mechanical integrity, strength and extensibility. In PS there is excessive chain scission through photodegradation. By the addition of flame retardants in PS, the exposure of UV to PS polymer prevents the risk of fire and hence can be used as construction of building materials.

The rate of weathering of polymer increases as conditions for moisture content and temperature becomes favorable, with an increase in UV flux. Plastic such as PP upon tensile stress in photooxidative and thermooxidative environment causes increase in brittleness of polymers. In one case Tensile stress of having value 0-6.86MN/m^2 was applied to observe the unstabilized and stabilized PP type. The conditions include photooxidative aging at 45°C, thermooxidative aging at 80-130°C and relative humidity of 65%. According to kinetic evaluation of unstabilized PP during thermooxidative aging, it was observed that weight losses of tension stressed and unstressed plastics have obeyed first order kinetic equations. Photochemical degradation has also been increased by introduction of carbonyl groups on polymer backbone. PS undergo ring opening reactions by photochemical reactions which leads to cleavage of backbone.

2.3.2 Thermo-oxidative Degradation:

The principle of photodegradation and thermo degradation are same as they do oxidation for degradation. Thermal degradation process is initiated by UV light that depolymerizes the polymer by forming weak links instead of acting at the terminal ends of the macromolecule. At this high elevated temperature, a large number of polymer additions are formed. The reactions occurring in the degradation widely depends upon factors i.e. rate of heating, pressure, reaction medium, and geometry of reactor. Thermodegradation of PET and other polyesters yields variety of products i.e. formaldehyde, acetaldehyde, formic acid, acetic acid and CO_2 and H_2O. (Le et al., 2019).

2.3.3 Catalytic Degradation:

Waste polymers conversion into hydrocarbons through catalytic transformation having high commercial value is of great interest. Polyolefins are catalytically or thermally degraded into oils and gases. The major part of industry and domestic waste include PP, PE and PS and interest has been direct for their breakdown. The addition of catalyst is advantageous in a way that the products that are obtained by pyrolysis of plastic waste, their quality is being improved by addition of catalyst. It also causes lowering of temperature of decomposition. It causes the formation of final product by giving selectivity to them. Hydrogen transfer reactions are being favored by addition of solid catalysts which include zeolites, because of presence of many acidic sides on them. The access of molecules to catalyst takes place under two conditions. First include pore size of catalyst as well as end products growth inside pores. Zeolites catalysts therefore are being used for shape and molecular sieving selectivity. A study shows that presence of catalyst causes decrease in activation energy of reaction. Different types of catalysts have been used for plastic degradation like TEMPO, PetCo which is supported over SiO_2. Non-Zeolite and Zeolite catalysts, Catalysts of transition metals like (Co, Mo, Ni, Cr and Fe). They are being supported on Al_2O_3 and SiO_2 and zirconium hydride.

2.3.4 Mechanochemical Degradation:

It includes degradation of polymers under strong ultrasonic irradiations and mechanical stress. Mechanochemical degradation include breakdown of molecular chains of polymers under mechanical force, which is being aided by chemical reaction. For example, there is development of plasticity of rubber by breakage of chain where mastication of rubber takes place. In presence of Oxygen, mastication of rubber takes place and it degrades rapidly while in presence of nitrogen it does not undergo mastication. This is because of reason that in presence of oxygen, there are formation of oxygen radicals that act as scavenger. By addition of polybutadiene with cis-polyisoprene or addition of butyl rubbers while mechanical mixing of polymers leads to changes in properties of polymers. During Mechanodegradation of polymers, there is decrease in width of molecular function of polymer as well as rupture of double bonds and cross links between plastics. Due to reaction of side chain radicals, there is an increase inside chain branching. PMMA mechanochemical degradation occurs in presence of nitroxides, which acts as chain terminating agents and creates macro-radicals. These radicals are used in free radical polymerization reaction. PVC mechanochemical dichlorination occurs in presence of various oxides like Fe_2O_3, CaO, Al_2O_3 and SiO_2.In air, it causes reduction of MW of PVC.

2.3.5 Ozone-induced Degradation:

Ozone-induced degradation is basically a slow process and polymer tend to retain its properties for long time. Only small amount of ozone can accelerates degradation of polymer. Due to this, there is impairment of electrical and mechanical properties of specimens as well as its molecular weight reduction. This process cause the formation of oxygen containing compounds in saturated polymers, When there is exposure to ozone, then there is formation of carbonyl groups on surface of polymers which are based on ketones, aliphatic esters, lactones and carbonyl associated with styrene. With change of time and concentration, there is formation of hydroxyl, ether and terminal vinyl groups. These ozone based reactions causes breakage of C-C bonds. There is formation of intermediate during reaction such as per oxy radicals, which causes decomposition of molecules. In poly(vinyl alcohol) , there is oxidation of alcoholic groups in chain, with formation of ketone groups, which causes keto enol tautomerism and causes further ozone attack. The interaction energy of PVAL is 47.3kJ/mol. It forms strong hydrogen bond with ozone. FTIR study indicates presence of ketone groups and carboxylic acid end groups in polymer backbone. The attack of ozone on PS is slow as compared to other polymers. The yield of products obtained from this is 18% ketone, 35% peroxide and 47% acid. Ozone effect PE and polyamide films differently. It causes increase in C-N chain in polyamide and PE films degradation and causing increase in oxygen containing functional groups. The oxides of Sulphur, Nitrogen and ozone cause UV induced oxidative damage of plastics especially in rubber products. Ozone cause stretching in rubber forming cracks in it. Properties of LDPE, nylon and PP are changed when exposed to ozone. Increase in surface oxidation of PP increases after exposure to ozone.

2.3.6 Biodegradation:

A biological activity that leads to certain chemical or physical change in any matter is referred as biodegradation. Many microorganisms are associated with the natural as well as artificial plastic's degradation that usually includes actinomycetes, bacteria and many fungal species. Plastic are known to be degraded biologically in nature either aerobically (in the presence of oxygen) in certain landfills and sediments. It also degrades the plastic anaerobically (without oxygen) or partially in soil and compost. Certain end products are produced as a result of aerobic and anaerobic degradation such as, production of water and CO_2 during aerobic process takes place. On the other hand, CO_2, methane and water are produced during the anaerobic process of biodegradation (Alshehrei, 2017). Its details are as follows:

2.3.6.1 Aerobic Biodegradation:

Aerobic respiration is also known for aerobic biodegradation. The removal of various hazardous pollutants from different sites containing wastes is done by the help of aerobic degradation. During this process, the conversion of organic compounds into smaller ones takes place in the presence of O_2 as an electron acceptor by the aerobic microbes. The end products in this aerobic process includes water and CO_2 as given in figure below (Priyanka & Archana, 2011).

2.3.6.2 Anaerobic Biodegradation:

In contrast to aerobic biodegradation, the breakdown of complex organic pollutants in anaerobic biodegradation is done in the absence of O_2 with the help of anaerobic microorganisms. It is also as much as important and active in removal of harmful pollutants at the sites containing wastes. For the generation of simpler compounds by the breakdown of complex ones, anaerobic microbes use sulphate, manganese, iron, CO_2 and nitrate as an electron acceptor, as given in the figure 1.

Microorganisms are able to undergo the depolymerization of polymers efficiently outside the cell by the use of certain enzymes that they secrete extracellularly to use these polymers as a source of energy and carbon. As we know that these molecules of polymers are insoluble in water and long, they are not taken up by the microbes directly from their membranes outside to the biochemical machinery inside the cell. For this purpose, certain extracellular enzymes are released. For the process of polymer's degradation, both extracellular as well as intracellular enzymes play a major role in this conversion. However, this demand of microbes for carbon and energy source is fulfilled by the release of enzymes i.e. majorly exoenzymes that results in production of small weight molecules and short chains that usually monomeric, dimeric and oligomeric etc. This low molecular weight, small products are easily passed through the outer membrane of bacterial cell by providing them energy.

The process of degradation in which polymers are broken down initially is termed as Depolymerization. However, Mineralization is the production of inorganic specie(s)/substances from organic matter as a product during degradation such as CH_4, CO_2, H_2O and many others.

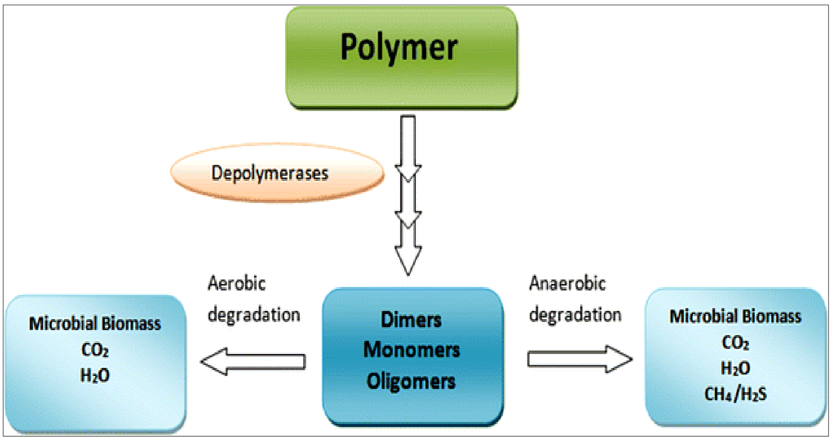


Figure 1: End Products of Aerobic and Anaerobic Degradation

2.3.6.3 Mechanism of Biodegradation:

Fungi and bacteria produce various extracellular enzymes like depolymerases and laccases to degrade various types of plastics. Bacteria and fungi undergo enzymatic and metabolic mechanisms to degrade these polymers. The end product include H_2O and CO_2. Every strain of fungi and bacteria contains different kinds of enzymes having different nature and catalytic activity that vary from specie to specie. Enzymes are known to degrade different polymers because of having specificity. The example includes *Brevibacillus* spp. And some strains of *Bacillus* that produces protease enzymes that cause breakdown of polymers. Lignin degradation takes place by laccases that are secreted by various strains of fungi. They have ability to catalyze non-aromatic and aromatic compounds through oxidation process. Microorganisms and enzymes tend to attach to both non-degradable and degradable plastics like PHB, PET, PHA, PLA, PBS and PCL etc. There is efficient degradation of polymers in environment in presence of these enzymes. The degradation of plastic start with the adherence of microbe with the polymer surface leading to the formation of bio-film through surface colonization. Hydrolysis of plastics undergo in two steps: In the first step, there is adherence of enzymes to the surface of polymer substrate and causes hydrolytic division which causes the breakdown of polymer into small oligomers, dimers and monomers. The next step causes the mineralization of monomers to produce H_2O and CO_2. In aerobic degradation, oxygen is being used up by bacteria as terminal electron acceptor. This causes synthesis of small organic compounds. End products involve the production of H_2O and CO_2.. In anaerobic conditions, other gases apart from oxygen play their role as terminal electron acceptor. Like iron, nitrate, sulphate, manganese and carbon dioxide. New pathways need to be explored in this field to efficiently degrade polymer. Fossil based polymers which are also called non-degradable polymer are called so because microbes are unable to degrade them for many years. Their degradation process is very slow. They can be completely biodegradable if new strains are being found and that explain process in detail.

2.4 Polyethylene terephthalate:

Polyethylene terephthalate, a widely used synthetic polymer developed by DuPont in the mid-1940s, first patented in 1973 (Le et al., 2019). A high molecular weight thermoplastic belongs to polyester family, composed of terephthalic acid and ethyl glycol monomers. these polyester family polymers are semi crystalline, hygroscopic and colorless in nature with increase thermostability and outstanding water, gas, and moisture barring properties. PET have excellent physical and chemical properties due to which it has wide application in textile industry as a fibers and clothing but its widely used as a packaging material in different industries(Kawai et al., 2019). Among polyesters PET are produced widely about 30.3×10^6 tons globally, while 120,000 metric tons of bottle grade material in Pakistan annually (Danso & Zimmermann, 2018).

2.4.1 PET Composition and Production:

PET is composed of polymeric units of ethylene terephthalate with ($C_{10}H_8O_4$) repeating units and molecular weight of 30,000 to 80,000 $gmol^{-1}$. These repeating units are synthesized of two monomers i.e. ethylene glycol and terephthalic acids that are polymerized to form a stiff, semi-crystalline structure. The process of pet manufacturing occurs in two steps (Taniguchi et al., 2019).

2.4.1.1 First Step: Esterification or Transesterification:

a) Initially ethyl glycol and zinc ions are mixed as a catalyst prior to be placed in a reactor. after this esterification reaction takes place where ethylene glycol reacts with terephthalic acid (TPA) and water is formed as a bi product. At a temperature range of 240°C and 260°C and pressure of 300-500 kPa leads to formation of bis(hydroxyethyl) terephthalate (BHET) (Koshti et al., 2018).

b) Transesterification: this process occurs if we use dimethyl terephthalate (DMT) instead of TPA. DMT reacts with (EG) at pressure 100 kPa and temperature of 140°C to 220°C, forming BHET as a product and methanol as a bi product (Gomes et al., 2019).

2.4.1.2 Second Step: Polycondensation:

At this stage two or three polycondensation or polymerization tests are performed. Transesterification reaction will again take place that displaces the EG from BHET at 250 to 280°C and pressure of 2 to 3 kilo Pascal. There will be further polycondensation of reacting molecules at 270 to 280°C and 50-100 kPa to form PET resins. At this stage these polymers are ready to be used for different applications and will be processed into different forms by extrusion or blow molding process. After extrusion process of PET, they are cooled quickly and are being cut into small pieces or pellets. These pellets are again brought up to heat and melted to a molten liquid form. This form is easily can be molded or extruded into any shape to form any product of use (Godwin, 2011).

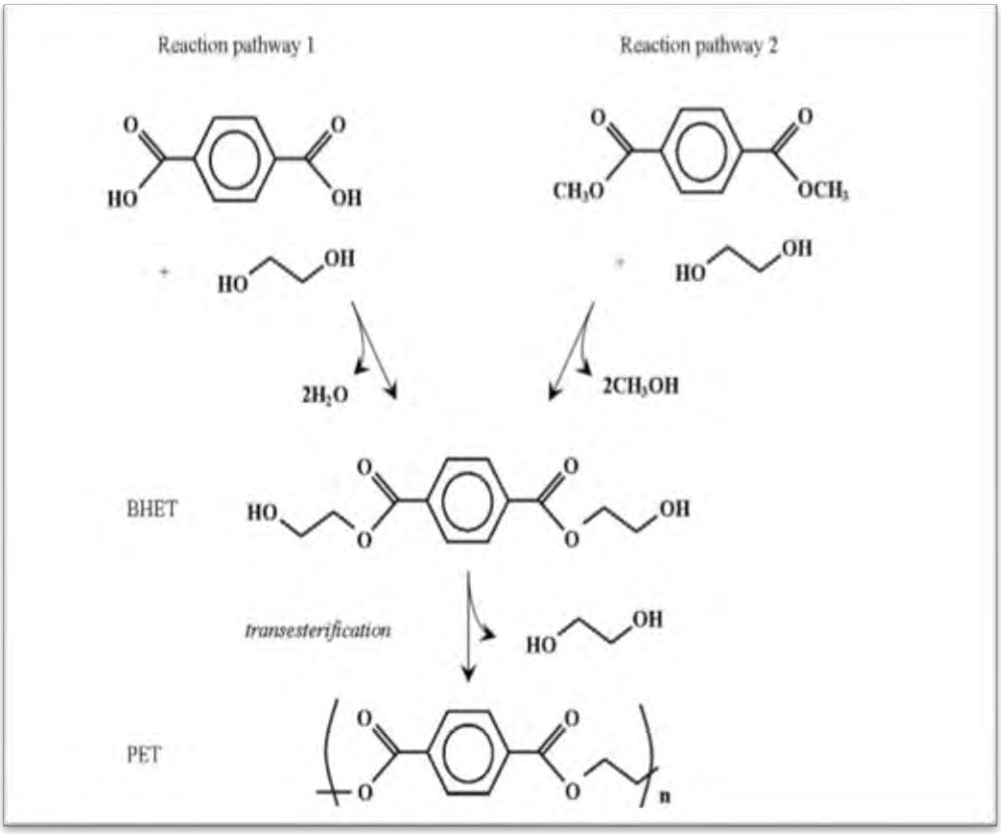


Figure 1: Manufacturing process of PET

2.4.2 Applications of PET:

PET due to its good physical and chemical properties has wide range of applications in packaging of food and beverages more specifically for soft drinks, juices and water. It is also used for packaging of salad dressings, peanut butter, cooking oils and different other liquids i.e. mouthwash, shampoo, liquid hand soaps and sanitizers, window cleaner and even used for packing of toys and sports products like tennis balls. For special carry-home food containers and food trays different grade type of PET materials are used that can also be used in oven and microwave and has no harmful effect. PET can also be used to create the PET films that have further applications to be used as a photo, video or packaging film.

2.4.3 Global safety Approval of PET:

Food and drug administration (FDA) and health-safety agencies all over the world approved PET safe for packaging material of food and beverages. The extensive studies, regulatory approvals and testing methods have shown the safety of polyethylene terephthalate for food, beverages, pharmaceutical and medical applications. This wide acceptance is due to the fact that it do not contain any additives like bisphenol-A or phthalates, besides that it is also inert and strong and don’t react with the food and any other material packaged in it. Because of these unique properties, PET is becoming the world's preferred packaging material for foods and beverages.

2.4.4 Microbial Degradation of PET:

Polyethylene terephthalate is a highly thermostable, crystalline polymer among polyesters and is considered recalcitrant to biodegradation thus lying in category of non-biodegradable plastic. However there are certain set of enzymes (extracellular and intracellular enzymes) produced by different bacteria and fungi i.e. PET hydrolases or polyester hydrolases that actively hydrolyze these polymers(Kawai et al., 2019).

2.4.5. Enzymes and Microorganisms Involved in PET Biodegradation:

Only few bacterial and fungal species are studied currently that are involved in partial degradation of PET molecules(Kawai et al., 2019). The bacterial strain that are involved in degradation of PET are mostly related to Gram positive phylum *Acitinobacteria*, however their turn over is quiet low. The best characterized examples from these phyla belong to two genre i.e. *Thermobifidia* and *Thermomonospora*. The enzymes produced from *Ideonella sakaiensis* are the best studied model and termed as a potent PET degrader. The enzymes produced from these microorganisms that hydrolyze PET are usually serine hydrolases that include esterase, lipases, tannases, Cutinases etc. These enzymes have two components: A catalytic triad; that is composed of histidine, serine and aspartate residues and an Ab hydrolase fold. Thermal stability and specific binding to PET molecules is maintained by several disulfide bonds, created by cysteine residues (Danso et al., 2019). Some of the enzymes produces by different PET degrading microorganism are discussed below.

2.4.5.1 Esterases:

During the degradation of synthetic polymer like PET; the polymer usually have ester bonding, that can be disrupted by different enzyme like esterases that are found ubiquitously in living organisms. Esterase modifies the PET polymers surfaces and enhances its properties i.e. surface hydrophilicity, cation dye binding ability, and wettability. The action of esterases are usually observed in initial steps of degradation of PET by many species i.e. *Nocardia*, *Thermomonospora fusca* KW3b and some *Bacillus* species. *Roseateles depolymerans* TB-87 strain is considered to have some novel set of esterase that have wide substrate specificity and activity towards aliphatic co-polyester (Moog et al., 2019).

2.4.5.1 Lipases:

Lipases involved in biodegradation are thought to be involved in layer by layer degradation approach where lipases act on the ester bond of the PET molecule. They are involved in biodegradation of both aliphatic and aromatic polyesters. Lipases show greater rate of degradation towards aliphatic substances rather than aromatic ones. Because they act more effective on hydrophobic substances as the concentration of enzyme increases. The microorganisms involved in lipase production are *Candida cylindracea*, *Pseudomonas* species, *Aspergillus oryzae* and some Egyptian bacillus strains (Koshti et al., 2018).

2.4.5.2 Cutinases:

These enzymes also belongs to family of lipases but are obtained from plant pathogenic fungi that hydrolyze cutin. In genus *Tricoderma*, it was observed that addition of hydrophobins results in modification of PET and effects the activity of cutinase enzyme in *Humicola insolens* and ultimately resulting in PET degradation. Another thermophilic bacterium i.e. *T. fusca* degrades the co-polyesters of aromatic and aliphatic compounds via cutinase activity, during the compositing process.

2.4.5.3 METase:

METase are the enzymes that hydrolyze the catalysis of product mono (2hydroxyethylene) terephthalic acid (MHET) to TPA and EG. Different PETase produce MHET an intermediate product during degradation process that is further internalized by the cells and inside cell it is hydrolyzed by METase. The MHET from the *Ideonella sakaiensis* has shown that MHETase is a ligand free with non-hydrolysable MHET analogue bound. They possess a lid domain and 4 MHET moieties get bound to one end. Three MHET to subsite 1 and one MHET to subsite 2 and the ester bond cleavage is between these two subsites located next to catalytic serine residue. These enzymes are considered to be regulatory part of the PET degradation pathway

2.4.6 Factors Affecting PET Degradation:

Several factors and characteristics of PET i.e. minimal reactive functional groups in the backbone, chain mobility, surface hydrophobicity and crystallinity cause hindrance in degradation process. The backbone of PET contains different highly stable C-C bonds and is not accessed by hydrolyzing enzymes, thus not susceptible to degradation. Therefore, certain abiotic endotype activities i.e. UV irradiation and oxidation are made prior to enzymatic degradation. These thermo-oxidative and photodegradative activities will expose functional groups and improve the hydrophilicity of the polymer leading to formation of carbonyl groups in PET that will aid the degradation process (Wei & Zimmermann, 2017). On the other hand the ester bond in backbone are more susceptible to degradation as they have high ratios of aromatic terephthalates that have reduced chain mobility and ultimately results in low hydrolysability of the ester linkage. Another factor that makes PET more prone to enzymatic degradation is its semi-crystalline nature having both amorphous and crystalline domains. PETase have an ability to adhere to the amorphous domain and cause depolymerization process (Dutt & Soni, 2013).

2.4.7 Biodegradation of PET:

The process of biological degradation of polymer is a multi stage process. It initiates with the photodegradation that is followed by thermo-oxidative degradation and results in the reduction of the molecular weight of a polymer to sufficient number so that microorganisms are able to grow on to the polymer surfaces. Because the hydrophobic nature and other physical properties don't let bacteria to grow onto their surfaces so that is why abiotic degradation is sometime necessary prior to degradation. Microorganisms will attach on the surface of polymer and colonize the exposed surface of plastic. These microorganisms mineralize the monomers by the process known as bio mineralization. Now these mineralized monomers can easily get through the semi permeable microbial membrane and taken up as a carbon and energy source ultimately result into degradation (Taniguchi et al., 2019).

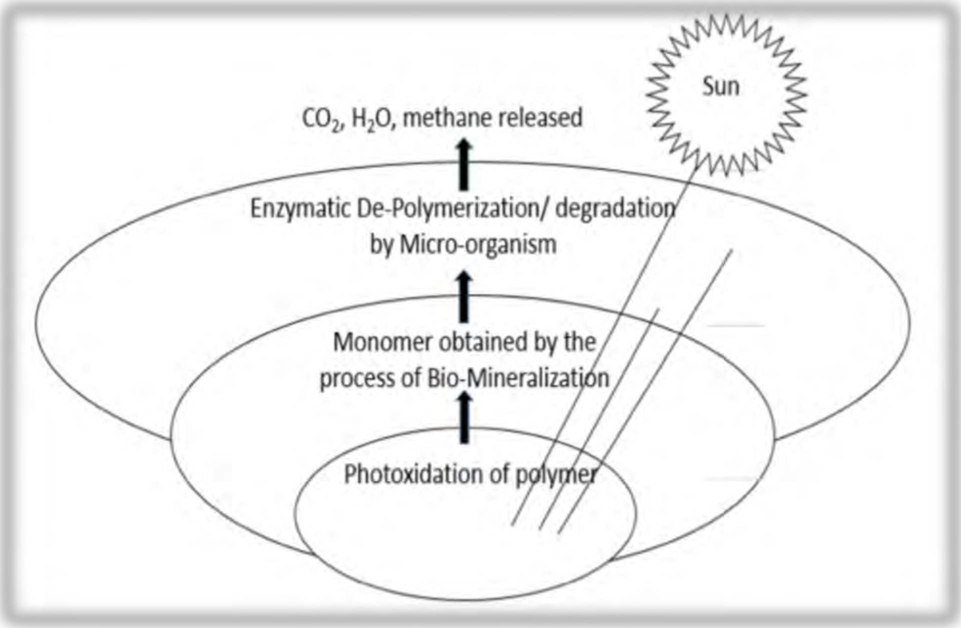


Figure 2 : Biodegradation process of PET in natural environment

2.4.7.1 Degradation Pathway:

The process of enzymatic hydrolysis starts with the action of PETases, that hydrolyses the PET into terephthalic acid (TPA) and ethylene glycol (EG). Usually PETases act on the polymer and convert it to intermediately products mono-(2-hydroxyethyl) terephthalate (MHET) and bis-(2-hydroxyethyl) terephthalate (BHET). MHETase catalyze the conversion of mono(2hydroxyethylene) terephthalic acid

(MHET) to TPA and EG. The membranes of microbes consist of TPA transporters that will bring the TPA molecule inside the cell. TPA molecule inside the cell undergoes series of reaction and gets converted into protocatechuic acid (PCA) and gets integrated into metabolism via tricarboxylic acid (TCA) cycle. Before generating TCA cycle intermediates, protocatechuate undergo the ring cleavage through aromatic catabolism pathway. Whereas EG get its way to TCA cycle by converting into glyoxylate and utilized in different biosynthetic pathways (Wilkes & Aristilde, 2017).

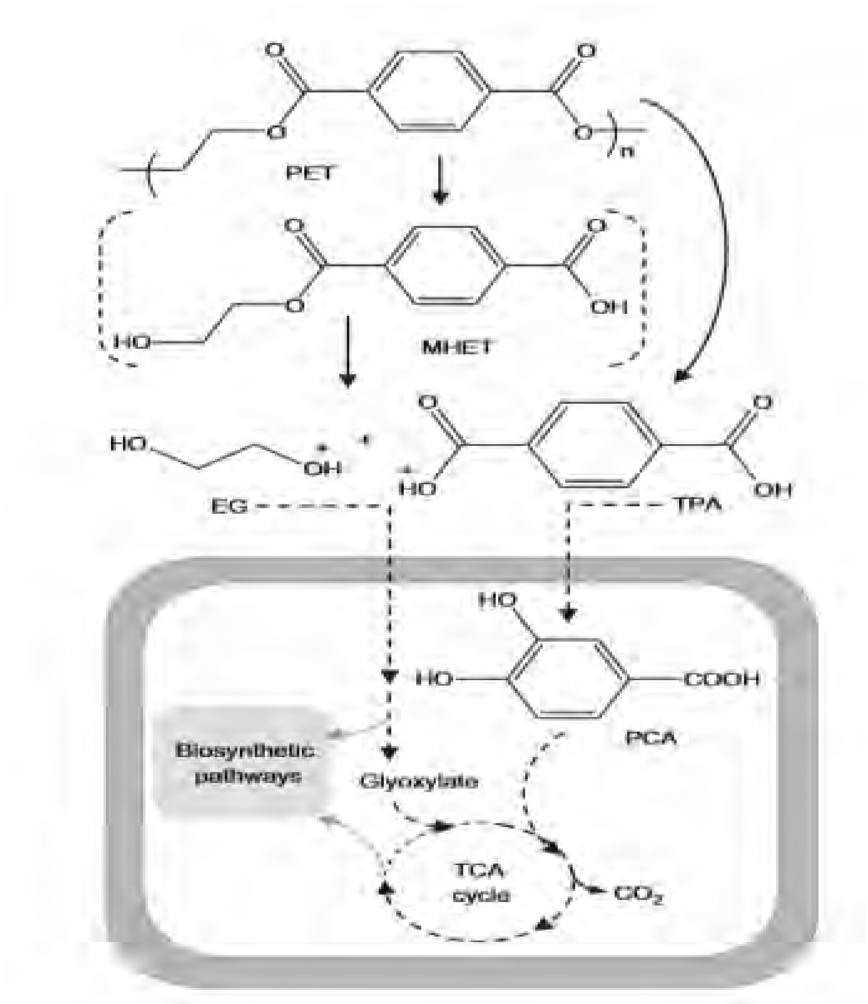


Figure 3: The enzymatic process of PET degradation

2.5 Polystyrene:

Polystyrene acts as poor barrier for water vapors and oxygen. It is inexpensive and amorphous plastic. It is chemically inert having linear molecules. It is chemically inert, so alkalis, acids, oxidizing and reducing agents have little effect on it. It has high tensile strength and provides excellent transparency. It exists in various forms like coextruded with other plastics like polyethylene, monoextruded or it can be molded in various shapes to give particular product. As polystyrene is chemically stable polymer, it is called white pollution known as accumulation of plastics on earth. Additives are chemicals that are added in plastic to increase its stability and provide durability. In polystyrene the main additives added are: plasticizers, antioxidants, flame retardants, acid scavengers, heat and light stabilizers, pigments, lubricants as well as thermal stabilizers, Each of them has a distinct role in enhancing the properties of polystyrene plastic. Addition of pigments provides colors to plastics, Nucleators reduce processing time and catalyst deactivators are added for neutralization of catalyst residues.

2.5.1 Forms of Polystyrene:

Depending upon structure, PS is divided into three forms:

1. Isotactic polystyrene is one containing all phenyl groups on one side .
2. Atactic polystyrene is one containing phenyl groups randomly.
3. Syndiotactic polystyrene is one containing phenyl groups on alternating sides of chain.

2.5.2 History of Polystyrene:

Polystyrene polymer was discovered accidently in 1839 in Germany by Edward Simon. He obtained oily substance from resin *Liquidambar orientalis* It is a Turkish sweet gum tree. When this oily substance exposed to air it transformed into jelly like material, which he named as styrol oxide. Marcelin Berthelot identifies a polymerization process for conversion of styrol to metastyrol.

2.5.3 Synthesis of Polystyrene:

The synthesis of polystyrene begins by heating the crude oil or natural gas in a process called “cracking”. The styrene monomer is made from alkylation of benzene and ethylene. At 850℃ the yield of ethylene is 30%, which is dependent upon cracking temperature. The reaction takes place in presence of Aluminium trichloride that acts as catalyst. Ethylene is passed through benzene in presence of catalyst that results in production of ethyl-benzene. The next step involves the passing of ethyl benzene over a catalyst called magnesium oxide or iron oxide. Ethyl-benzene dehydrogenation forms styrene, which at high temperature is polymerized to give polystyrene (Naima et al., 2011).

Depending upon Applications of usage of polystyrene, its products are made by extrusion, injection molding, vacuum molding and blow molding. For hard, clear and brittle type of general polystyrene products, injection molding and extrusion is performed. For construction industry, insulation sheets are produced which are obtained from extruded polystyrene. Expanded polystyrene foam products are made from thermoforming.

2.5.3.1 Additives and Their Properties:

Additives present in Polystyrene may be divided into following categories:

2.5.3.1.1 UV Stabilizer:

In outdoor environment, Polystyrene is exposed to photons generated by sunlight having range of 100nm-1mm. It includes visible, ultraviolet and infrared radiation. It leads to photooxidation of plastic. This oxidation of PS is prevented by addition of UV stabilizer in Polystyrene during its formation. By adding this, PS will be prevented from oxidation hence making it longer time to degrade. The UV stabilizers that are added in PS are Tinuvin P, Tinuvin 770 that acts as UV absorber.

2.5.3.1.2 Antioxidants:

When Polystyrene is exposed to environment, it causes formation of Reactive Oxygen Species (ROS) due to oxidation. It starts deteriorating the structure and colour of PS. It also affects its mechanical properties. It is mainly induced by UV light or either through thermal process. This process is called autooxidation because the radical chain mechanism initiate by itself. The antioxidants added in PS are Alicyclic Bromine, Irganox 1076 and Wytox (John, et al., 2018).

2.5.3.1.3 Flame Retardants:

When there is presence of Oxygen and Heat, then a process known as Combustion initiates. Polymers also tend to burn like other organic substances. When PS is exposed to heat, than hydrocarbons present in PS break down into smaller subunits. The smaller molecules that are formed include Sulphur, nitrogen, oxygen, fluorine that acts as potential hazard to health and environment. These smaller molecules are released into atmosphere because they have volatile nature. Therefore, to prevent this and maintain the structure of PS, Flame retardants are added to it. Flame retardants that are added to PS include Decabromodiphenyl oxide, Penta-bromochlorocyclohexane etc.

2.5.3.1.4 Processing Lubricants:

Zinc stearate, mineral oil and stearic acid acts as processing lubricants.

2.5.4.1.5 Colorants:

The pigments or ozocolorants are added in polystyrene for colour development. They are added in polystyrene in range of 0.25-5 %w/w.

2.5.4.1.6 Functional Additives:

Additives are added in polystyrene to enhance its functional capability and performance during plastic formation. These are added during injection moulding, during shaping of polymer, blow moulding, extrusion and vacuum moulding.

2.5.4.1.7 Composites:

Composites are materials that are formed by the combination of two or more constituents, having different chemical properties and are insoluble in each other. Filler materials are added in plastic such as silicon carbide, magnesium hydroxide, zinc oxide and aluminium oxide. Mica and barium sulphate, caolin, calcium carbonate. By adding these materials plastic tend to become tougher, stiff, and hard. It also improves its heat distortion temperature. It also reduces cost of components. Filler materials are added in up to 50% (Al-Malaika, et al., 2017)

2.5.5 Types of Polystyrene:

Depending upon production and usage, Following are types of polystyrene:

2.5.5.1 General Purpose Polystyrene:

The most simple and cost efficient type of Polystyrene. It is brittle and has low impact strength. It is clear, glass like appearance and has its applications in food packaging. It can easily be molded into different shapes. It is also used to make disposable glass. It is used to make CD cases and boxes etc.

2.5.5.2 High Impact Polystyrene:

Pure PS is in brittle in nature. This brittle nature of PS is decreased by adding rubber to Polystyrene which increases its strength, resistance and durability. It provides resistance when there is chance of shock loads. The PS is mixed with cis-1,4-polybutadiene which is a synthetic rubber. Double bonds present in polybutadiene causes its polymerization with styrene. It will add only slight improvement in strength of PS. It is less transparent as compared to pure PS. Before molding into specific shapes, pigments are added.

2.5.5.3 Extended Polystyrene:

Expanded polystyrene is also known as Styrofoam. Thermoplastic foam that contains polystyrene beads. The styrene undergoes polymerization to give EPS. Hexane and pentane are added as a blowing agent in EPS. These blowing agents will expand polymeric chains in order to get low density of polystyrene. The amount of blowing agent added is 5-10 %. The material is heated at 100-110°C. The rising temperature causes the polystyrene chain to become softer. They are used worldwide in construction because of its favorable properties and facile manufacturing process such as high strength, low density, impact resistance, sound proofing and low thermal conductivity.

2.5.6 Stages:

The conversion of Polystyrene to EPS undergoes 3 stages:

2.5.6.1 Pre-Expansion:

In first stage, steam having temperature between 80-100°C is provided to raw materials in pre-expanders. This process creates uniform cellular structures in form of small closed cells. These small cells contain air in them. During pre-expansion, beads are kept apart from fusion due to presence of agitator.

2.5.6.2 Intermediate Maturing and Stabilization:

During intermediate maturing and stabilization, the internal gas of beads experience volume expansion. As a result, air can easily penetrate cellular structures of beads. This process is carried out in aerated silos.

2.5.6.3 Expansion and Final Molding:

Through expansion process, the steam is provided in order to binds beads together. This will provide a molded shape like blocks (Hafizah et al., 2019).

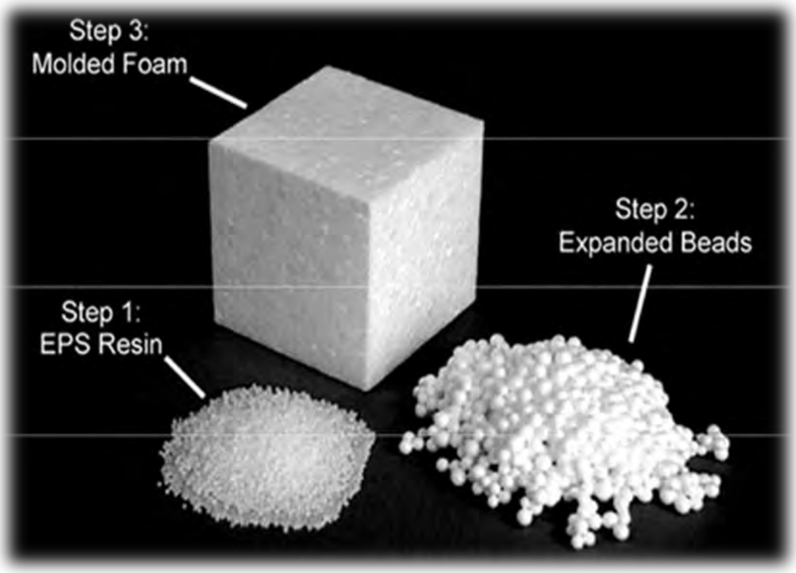


Figure 4: Expanded Polystyrene

2.5.7 Microbial Degradation:

The ability to use synthetic polymer as sole carbon source at least by one microorganism is known as biodegradation. Several bacterial and fungal species have been isolated that can degrade polymers. Synthetic polymers are more difficult to degrade as compared to composite mixtures of starch and polystyrene. Photo or thermal oxidation facilitates degradation of polystyrene. They create carbonyl groups at surface of polymer that facilitate degradation when microbes attach and forms biofilm on them.(Yutaka, Charles et al.,2009)

2.5.7.1 Fungal Degradation:

Fungal isolates causing degradation of Polystyrene are:

- ^{14}C labelled PS films having 29kDa and MW of 15 after subjected to ozonation pre-treatment is degraded by Fungus *Penicillium variabile* CCF3219 after 16 weeks incubation in a liquid medium with pH 7.5 having no carbon source.
- Brown Rot fungi strains of *Gloeophyllum* including *G. trabeum* DSM 1398 and *G.striatum* DSM 9592. About 50% reduction in PS is observed after 20 days of incubation.
- White Rot fungi isolates named *Pleurotus ostreatus, Phanerochaete crysosporium, Trametes versicolor.* These fungi include ligninolytic enzymes

including laccases, lignin peroxidase and manganese peroxidase.(Dominik, Jennifer et al.,2019)

2.5.7.2 Bacterial Degradation:

Bacterial isolates causing degradation of PS are:

- PS flakes degradation by actinomycetes *Rhodococcus ruber* isolates C208. Incubation in shaker flask causes 0.8% weight loss after 8 weeks at 28 °C.
- Styrofoam PS films that are degraded by *Exiguobacterium sp.*strain YT2 that is isolated from *Tenebrio molitor* Linnaeus larvae's gut. Degradation is observed after 60 days of incubation. It decreases the PS weight by 11%.
- Recent study shows biodegradation of predegraded General purpose and High impact PS flakes by bacterial strains *Cupriavidus necator* H16 in presence of oxidized Co-stearate and Mn-myristicate and utilized predegraded PS as carbon source and produce PHAs (Ho et al., 2017)
- Pseudomonas sp. Causes degradation of HIPS that is polymer of polybutadiene and Polystyrene. *Pseudomonas aeruginosa,* were capable of degrading plastic having PS and PLA.
- The gut of Super worms named Zophobas atratus were capable of degrading PS. The study showed that the larvae contain bacterium named *Pseudomonas sp*. DSM 50071. The activity was checked when the super worms were fed by antibiotic gentamicin containing bran. These results in suppression of gut bacteria and have decreased PS activity. It shows the presence of bacteria in gut of super worm.

2.5.7.2.3 SEM Analysis:

The growing colonies of *Pseudomonas sp.* were observed under SEM. Pseudomonas sp. Used PS as C-source. When white colonies were washed with SDS buffer, the surface of plastic was observed under SEM. In comparison to Control, the result shows that the rough edges of PS were converted to smooth form as a result of degradation. There was formation of holes in plastic.

2.5.7.2.3 Analysis of C and O Atoms:

Carbon and Oxygen atom composition were observed at edges where *Pseudomonas sp.* were grown in comparison to Control. There was no carbon change but there were more concentration of Oxygen atom that shows that PS degrading bacteria has released enzymes that promote oxidation during degradation. The biofilm formation causes increase in contact area between plastic and bacteria. As oxidation causes degradation, functional groups such as carbonyl and hydroxyl groups are formed by Β-oxidation. Bacteria use Β-oxidation process in TCA cycle for energy metabolism. After degradation the contact angle between water droplets and PS increases due to oxidation. As a result, the structure of PS changes from hydrophobic to hydrophilic. As compared to control, in FTIR analysis a peak was observed at $1715cm^{-1}$ where there is formation of C=O bond. Weak absorptions were also observed at $3600cm^{-1}$ which shows the presence of O-H bonds. This indicates that alcohols are also generated at intermediate stage.. There were also secretion of enzymes like lipases, esterases, Cutinases and hydrolases by bacteria.

2.5.7.3 Enzymatic Degradation:

There are two types of enzymes that cause degradation of polymer.

1. Extracellular
2. Intracellular

2.5.7.3.1 Extracellular Enzymes:

Extracellular enzymes cause exo attack. In this there is formation of small monomers, oligomers when hydrolytic cleavage takes place at chain terminus. Extracellular enzymes include depolymerases and hydrolases that act on different types of polymers. A brominated HIPS was effectively degradable by extracellular depolymerase produced by *Pseudomonas sp*. Esterases also causes degradation of HIPS. Degradation of Vinyl chloride by Alkene monooxygenase secreted by *Pseudomonas putida* is reported. Hydroquinone peroxidase secreted from lignin decolorizing *Azotobacter beijrinkii* HM121 are able to degrade PS and it gives CO_2 and H_2O. It undergoes degradation in two phases: In first phase there is formation of dichloromethane and water and in Organic phase it is converted to small products that are soluble. This reaction takes place at 30 for 5 min.

2.5.7.3.2 Intracellular Enzymes:

Intracellular enzymes are those that cause endo attack. They cause hydrolytic cleavage at any point other than terminus. It causes reduction in MW of polymer (Wilkes, et al., 2017).

2.5.7.4 Mechanism of Degradation:

Microorganisms degrade plastic in multi-stage process. This process mainly depends upon both biotic and abiotic factors. Microbes first attach to the surface of plastic and form biofilm. This took place according to structure of polymer like hydrophobic nature, electrostatic interactions and roughness. The biofilm development also depends upon temperature, limitation of light, Oxygen and salinity. Because of Hydrophobic nature of microbial membranes, and large size of these molecules microbes are unable to use polymer as carbon source. For this microbes release extracellular enzymes like depolymerases that degrade these polymers into oligomers, dimers and monomers

Steps involved in degradation of polymers are as follows:

1. First microbes attach on the surface of polymer and colonize the exposed surface of plastic. Enzymes first adhere to surface and then break polymer by hydrolysis.
2. Microbes then start utilizing polymer as carbon source and hence their growth on polymers starts.
3. Microbes start assimilating the monomers and oligomers (Fasil et al., 2019).

2.5.7.5 Degradation Pathways:

The degradation of polystyrene undergoes in two processes. There is no evidence of degradation of styrene in environment. But one of the important route observed for degradation of styrene is in microorganisms. The microbiological activity is also important for degradation of industrially released styrene and similar compounds. These metabolic pathways are also important because this process contains enzymes that are involved in formation of valuable compounds. Both processes involve enzymes and cofactors. These two processes take place in presence of oxygen. Two types of pathways include:

1. Side Chain Oxidation
2. Direct Ring Cleavage

2.5.7.5.1 Side Chain Oxidation:

Substituted phenylacetic acid is generated from styrene by co-metabolic transformation. The pathways and enzymes involved in this pathway produce products like phenylacetic acid and styrene oxides. In this pathway, vinyl-side chain of styrene is being attacked. In first step, Styrene with help of enzyme called styrene monooxygenase to convert vinyl side chain to an epoxide by using molecular oxygen. SMO consists of two components called StyA which is called oxygenase and other component called StyB known as reductase. In second step, the enzyme called styrene oxide isomerase known as StyC, styrene oxide undergoes isomerization to phenylacetaldehyde. In the last step a enzyme named phenylacetaldehyde dehydrogenase undergo catalysis and leads to formation of phenylacetic acid which is central metabolite of reaction.

2.5.7.5.2 Importance of Pathway:

The chemicals obtained through these pathways are of great importance with respect to pharmaceuticals, agriculture and in industries. These also play role in giving flavor to food. Styrene Oxides are used in pharmaceuticals and synthesis of enantiomers substances. Phenylacetaldehydes are used in perfume industry as fragrances. They are also used in different pharmaceuticals, herbicides, fungicides and insecticides. Phenylacetic acid are used as precursors for drugs and pharmaceutical products. For example, they are used in production of penicillin. Other example include 4-isobutyl-a-methylphenylacetic acid also known as ibuprofen. Phenylacetic acid play important role for food industry and cosmetics. Sometimes modifications are done to side-chain oxygenation. This ensures enlarge product and substrate spectrum. This produces 2-phenylethanols that are valuable products because they play important role as precursor, disinfectant and flavor.

2.5.7.5.3 Direct Ring Cleavage:

Direct ring cleavage starts with dihydroxylation of aromatic ring initially. This reaction is catalyzed by enzyme 2,3-dioxygenase. This enzyme is followed by enzyme 2,3-dihydrodiol dehydrogenase. As a result of these steps, there is formation of styrene *cis*-glycol by oxidation of styrene. This step is followed by formation of compound known as 3-vinylcatechol. Further metabolites can be made by this by cleavage at ortho-or meta- position. This will lead to formation of central intermediates like acetaldehyde, acrylic acid and pyruvate. This pathway is also important because of degradation of various aromatic compounds like phenol, toluene as well as ethylbenzene (Juliane et al., 2018).

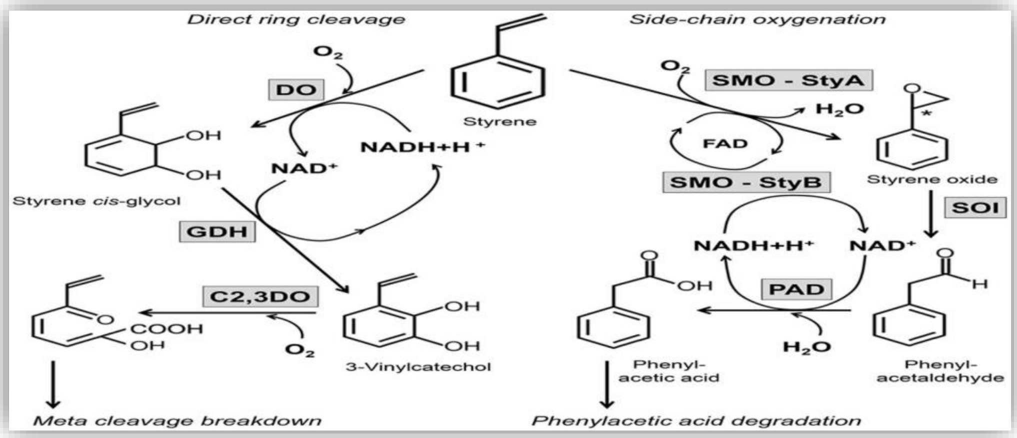


Figure 5 : Polystyrene Degradation Pathways

2.6 Polyethylene:

Products of polyethylene in our daily life are most common like pharmaceutical food packaging film, pipe, cable and wire insulation. As polyethylene polymer is the most usable plastic in our daily life, so its production occurs in a huge amount. Many products can be manufactured from polyethylene which includes plastic film, plastic bags, injections, milk barrels and other various kinds of plastics. Furthermore, it can also be used for sheets, profile, pipe, different containers and cable cladding. Among world top five production polyethylene is one of them. The main varieties and most consumed polyethylene are High Density Polyethylene, Linear Low-Density Polyethylene and Low-Density Polyethylene. Polyethylene plastic is very sensitive to mechanical and chemical stress and heat aging capacity is poor. With density of structure, the properties of polyethylene also change. Different production methods can be used to make different density plastics i.e. **0.88- 0.96 g/cm^3**. Polyethylene polymer can be synthesized by thermoplastic general molding techniques. Polyethylene plastic is used in the manufacturing of containers, mono filaments, thin films, pipes, cables, wires and other necessities also used in different insulating materials, radar and in TV. The polyethylene production highly increase the development of petrochemical industries . Polyethylene also have role in the astrophysics, nuclear physics as a diffusing agent and is also useful in the reactor operation to count the number of reactions in nuclear physics (Ghatge et al., 2020).

2.6.1 Types of Polyethylene:

Polyethylene plastics have density range from 0.88 to 0.96 g/cm^3 with different molecular weight and with different branching. According to American Society for Testing and Material have classified polyethylene into five different categories. (Malpass, 2010)

2.6.1.1 Low-Density Polyethylene:

LDPE is made by free radical polymerization, as these results in the processing of long and short chain polyethylene branches. From these mixtures of long and short chain polyethylene, it is not easy to make crystalline structure. The tensile stress of LDPE is lower as compared to other but having more desirable properties for formation of making plastic wraps and films which are further used in the plastic wraps and shopping bags. Furthermore, it is also used in the medical films and packaging.

2.6.1.2 Linear Low-Density Polyethylene:

This LLDP is more linear than Low Density Polyethylene and due to this reason; it has more tensile strength and is having short branches. LLDP is mostly used in many products like pipes, toys, bubble wrap and containers. (Malpass, 2010)

2.6.1.3 Medium Density Polyethylene:

MDPE is produced from catalysts like metallocene, Ziegler Natta and silica chromium. It is equivalent to High Density Polyethylene because of best stress resistance and low density. It is more likely used in medical application, piping and plumbing.

2.6.1.4 Crossed Linked Polyethylene:

It is an analogue of polyethylene. It cannot be defined by its molecular weight specific range. It is equivalent to cross linked HDPE or MDPE with strength intermediate in between of both. This cross-linked polyethylene can be obtained by multiple methods that is peroxide mixed into HDPE prior to removal at maximum temperature to create bonds in between the atoms of carbon. Another method of electron beam is used to cross link the atoms. This plastic is thermoset, unlike thermoplastics and due to this reason; it has more stable dielectric properties and chemical resistance in comparison to these plastics which are not crossed. This plastic also has various uses like as plumbing material and is used as an insulating material for high voltage cables.

2.6.1.5 High Density Polyethylene:

It is most frequently available type of plastic. It is cheap as compared to other types of plastic. Optimized processing method is available for large scale application, high quality and low cost. HDPE has high density and hence high tensile strength. It gets melted at high temperature and pressure. It is used for making materials which are used and is autoclaved and sterilized, mostly for medical purpose.

Furthermore, Ultra High Molecular Weight Polyethylene is also available in this category, has long polymer chain and are highly resistant of high temperature and pressure. This long chain backbone polymer helps it to become more stable. It is also highly resistant to corrosive chemicals. It is used in suspended cable and lines, in Armor of vehicles and fibers. (McKeen, 2014)

2.6.2 Structure of Polyethylene:

Polyethylene structure is very simple. It is the most usable material. Its composition is **$–CH_2$-** repeating units. Thus, polyethylene polymer is formed by the polymerization of monomeric ethylene that is **[$CH_2{=}CH_2$].** The characteristics of polyethylene polymer depends on the polymerization. For High Density Polyethylene synthesis catalyst like Organic compound and polymerization of Ziegler Natta is carried at medium pressure that is 15 to 30 atm. Under such conditions the polyethylene synthesized is having large chain up to several thousands of linear geometries. Low Density Polyethylene is synthesized by keeping the pressure 100-300 MPa and temperature 190-210 C in the presence of catalyst like peroxide which results in the free radical polymerization. Also, the structure of LDPE is branched (Malpass, 2010).

2.6.3 Polyethylene Usage:

Polyethylene is unique synthetic polymer because of its characteristics and is having great chemical and physical properties. Polyethylene has best dielectric characteristics and has good mechanical characteristics. The production cost is low, and the molding process is easy. It has much importance because of the following uses:

2.6.3.1 Electrical Insulation:

Because of its great dielectric characteristics, high stability, moisture resistance and many other properties it is used as an electrical insulator.

2.6.3.2 Resistant to Chemicals:

Because of its nature, it is resistant to chemicals that are why it is used in pipes and as an anti-corrosion lining.

2.6.3.3 Packaging Materials:

Polyethylene plastic is of low cost, has soft nature, low density, water resistant, chemical resistant and more tear strength having all these properties are important in the packaging materials, thus in the market it has high value and thus it replaces celluloids.

2.4.3.4 After the Radiation Treatment:

a. Will not crack due to the environmental stress.

b. Difficult to deform.

c. Resistant to high temperature.

d. Good insulating material.

Thus, after the radiation treatment, its uses increase because of its wide range of characteristics. Example includes transformers and capacitors in which it is used as an insulating material and is used in aircrafts because of its high temperature resistant nature. During the radiation treatment occurrence of crosslinking reactions make difficulties in the processing of the polymer.

Furthermore, there are other many uses of polyethylene like uses in medical equipment, wood, spraying of metal, fabrics etc. and as rubber reinforcing material in HDPE is used (Malpass, 2010).

2.6.4 Properties of Polyethylene:

Polyethylene has several chemical and physical properties which are as follows:

Ethylene monomer alone is transparent molecule but when it combines to form polymer it becomes opaque because most scattering of light occurs. The branches of polyethylene affect the polymerization. It is more difficult if there is more branching in it. The temperature at which the polyethylene melts also depends upon the branches of polyethylene, the more the branches; the more will be the melting temperature. It comes in the range of **90-130°C.** Polyethylene polymers are generally waxy with translucent materials that are tough, soft, lighter, good dielectric material and non-toxic. It burns even after the fire that shows that it has flammable characteristics. It has low water permeability. As the crystallinity increases, the transparency decreases. In some cases, with the increase in crystallinity and molecular weight the transparency also increases.

The melting point of LDPE is 112°C and HDPE is 132 to 135°C. Normally, polyethylene is not soluble in any of the solvent at room temperature, but if we rise temperature to 70°C it starts its dissolution in amyl acetate, trichloroethylene, toluene and some other solvents. PE is non-toxic, odorless, waxy, and excellent in chemical stability, good low temperature performance. PE absorption of water is low but can be dissolved in certain organic chemicals solvent because of its linear structure. Polyethylene has greater insulating ability and cannot swell up. Moreover, environmental stress can change polyethylene structure and make it badly resistive to heat. Polyethylene properties depend upon the structure and density of polymer.

2.6.4.2 Chemical Properties:

Polyethylene is highly chemically stable. PE is stable to highly basic and acidic solution like sodium hydroxide, potassium hydroxide, hydrochloric acid, phosphoric acid and amines at 25° C. Moreover, Sulfuric acid and nitric acid shows destructive effect towards polyethylene. PE is also susceptible to thermal oxidation, ozone decomposition and photooxidation. In the presence of Ultraviolet light, it also shows degradation. Exposure to irradiation results in broken chain, unsaturated groups and cross-linking. It is resistant to organic solvent and different alkali and acid corrosions but show sensitivity towards antioxidants like nitric acid. Polyethylene gets oxidized in the oxidizing environment. Thermoplastic resins polymerization results in the ethylene. Ethylene copolymers like alpha-olefins are also added in industry in small amount.

The properties of polyethylene changes with changes in the density and structure of polyethylene. Different kind of polyethylene product is achieved through various methods. Thermoplastic molding process is used to manufacture polyethylene. Polyethylene is used as wire, films, pipe, container, radar, television and in other different uses. In developing countries, the production of polyethylene increases because of petrochemical industries (Malpass, 2010).

2.6.5 Applications of Polyethylene:

Polyethylene has a wide range of requirement because of its uses as it is used in hollow products, thin films and in sundry goods. The characteristic of polyethylene changes with the addition of different additives and these additives are added to the polyethylene to make it stable, some additives like ultraviolet light absorbent like o-hydroxybenzophenone. Some of the applications of the polyethylene are as follow:

2.6.5.1 Film:

Half of the film is produced by blowing low density polyethylene. The film produced have more tensile stress and is transparent. Its uses include as packaging material, agriculture film, used in different clothing, foods, fertilizer, medicine and in industrial packaging .It can also be used for packaging of heavy material objects. High Density Polyethylene production was started in 1975. It has low temperature, high strength, machinability and moisture. Linear Low-Density Polyethylene is also used in the synthesis of films. The toughness and strength of Linear Low-Density Polyethylene are better than LDPE. It is rigid and resistant to puncture. It is not highly transparent but are given good quality than HDPE. Polyethylene can be used in the manufacturing of aluminum foil, paper and other coating materials.

2.6.5.2 Hollow Products:

To produce hollow products mostly High-Density Polyethylene is used. Such products can be made by the blow molding barrels, tanks, bottles, cans and can also be made casting procedure in large containers and other tanks.

2.6.5.3 Tube Sheet:

Tubing of polyethylene can be produced by the extrusion method. The pipes made from the High-Density Polyethylene have more strength and due to this reason, it is used in the underground. Secondary processing is also done to the extruded sheets of plastics. Through foaming the HDPE can be used for foam, building materials and foaming materials.

2.6.5.4 Fiber:

It is referred as ethylene fiber by China. It is made using low-pressure polyethylene. Different materials like ropes, flasks and fishing net are synthesized from polyethylene polymer. In industries, polyethylene can also be used for the alkali and acidic fabrics. The polyethylene having ultra-high strength can be used in the making of bullet proof automobiles, vest and offshore operations.

Sundry goods can be prepared by injection of molding. Various kinds of sundry goods are artificial flowers, small containers, tractor parts, groceries, turn over boxes and bicycle (Zhong et al., 2018).

2.6.6 Biodegradation of PE:

The biodegradation of polyethylene PE has been studied by many scientists. But still the exact mechanism of biodegradation is not fully known. However, it is recommended that some biotic and abiotic factors play an important role in the biodegradation of PE in the environment.

Biodegradation of PE is performed either by using pure cultures that can degrade the PE or using the complex microbial consortia from different habitats such as marine or terrestrial habitats. However, it was thought that biodegradation using complex microbial consortia facilitates the degradation of PE. Furthermore, waxworms are used for the better biodegradation of PE (Abrusci et al., 2011).

2.6.6.1 Bacterial Biodegradation of PE:

Different types of polyethylene can be degraded by different bacterial genera. More than 20 bacterial genera are known for the degradation purposes. These genera include Gram-positive and Gram-negative species such as *Rhodococcus, Bacillus, Streptococcus, Staphylococcus* and *Klebsiella, Pseudomonas, Acenitobacter, Ralstonia* etc. Most of these bacterial species have ability to form biofilm on the surface and hence cause the breakdown of the surface.

Various studies have been conducted on the bacterial genera *Pseudomonas* to check the degrading abilities of synthetic polymers and the by-products. *Pseudomonas* species have the extracellular hydrolytic and oxidative enzymes and their activities that are helpful in the uptake and degradation of polymeric fragments and involves in controlling the interaction between polymer surface and biofilms. A species of *Pseudomonas* i.e. *P. fluorescens* is involved in the complete degradation in water of PE. It is basically a surfactant and a biosurfactant and is involved in polymer degradation and oxidation.

Rhodococcus ruber (C208) strain degrades the PE at the rate of 0.86% per week. The presence of mycolic acid layer i.e. a hydrophobic cell surface plays an important role in biofilm formation on the surface of PE. Similarly, *Brevibacillus borstenlensis* that use carbon and energy source reduces the 30% molecular weight of PE film during 30 days of incubation. *Klebsiella pneumoniae* after thermal treatment degraded HDPE. This bacterial specie attaches strongly to the surface and form biofilm of more thickness during 60 days of incubation (Ghatge et al., 2020).

Table 2: PE Degrading Bacterial Strain

Bacterial strain	Substrate
Streptomyces badius, S. setonii,	starch-PE
Arthrobacter paraffineus	LDPE, HDPE
Brevibacillus borstelensis	LDPE
Nocardia asteroids	LDPE, HDPE
Rhodococcus rhodochrous	LDPE, HDPE
Bacillus halodenitrificans	LDPE
Bacillus sphericus	LDPE, HDPE
Arthrobacter sp.	HDPE
Pseudomonas sp.	HDPE
Staphylococcus epidermidis	LDPE
Rhodococcus rhodochrous	LDPE, HDPE, LLDPE
Arthrobacter viscosus, Acinetobacter baumannii *Bacillus amyloliquefaciens, B. cereus B. circulans, B. mycoides, B. pumilus, B. thuringienesis, M. luteous, M. lylae*	LDPE
Pseudomonas fluorescens, Paenibacillus macerans, Rahnella aquatilis	LDPE
Staphylococcus cohnii	LDPE
Staphylococcus xylosus	LDPE
Microbacterium paraoxydans	LDPE
Pseudomonas aeruginosa	LDPE
Rhodococcus ruber C208	LDPE
Pseudomonas sp. AKS2	LDPE
Klebsiella pneumonia	HDPE

2.6.6.2 Fungal degradation of PE:

Along with bacteria, different fungal genera are also involved in the degradation of PE. These genera include *Penicillium, Fusarium, Aspergillus, Cladosporium* etc. Fungi have ability to attach to the charged surfaces of polymers, produce extracellular enzymes that target the insoluble fibers and withstand adverse growth conditions. That is why fungi are considered better and more than bacteria during the PE degradation.

Commonly used method for the analysis of biodegradation of PE is weight loss measurement. For instance, *A. japonicans* and *A. niger* are thought to be involved in the biodegradation of LDPE and it decreases the dry weight by 11.1% and 5.8%. Studies conducted by Das and Kumar on the biodegradation of LDPE using *Aspergillus* and *Fusarium* species in which 5% and 8-9% weight reduction was observed after the incubation of 60 days. *P. chrysogenum* NS10 and *P. oxalicum* NS4 are also involved in the biodegradation of HDPE and LDPE using response surface methodology for the optimization of growth media for increasing the weight of mycelium. The SEM and AFM can be done for the rectification of biodegradation of PE by fungal strains leads to biofilm formation and morphological changes on the surface such as cracks and pits (Ghatge et al., 2020).

Table 3: PE Degrading Fungal Strains

Fungal strain	Substrate
Fusarium redolens	LDPE C14 labelled; UV treated
Verticillium lecanii	LDPE
Phanerochaete chrysosporium	LDPE/starch
Penicillum simplicissimum	HDPE UV treated
Aspergillus niger	Thermal treated LDPE
Penicillum pinophilum	Powdered LDPE
Cladosporium cladosporioides	Degradable polyethylene green film
Gliocladium virens	Thermal treated LDPE
Aspergillus flavus	LDPE and HDPE film

2.6.6.3 Enzymatic Degradation of Polyethylene:

In the complex process of polyethylene biodegradation different biotic and abiotic factors are involved. The combine effect of biotic and abiotic factors result in the degradation of the polymer and make the surface available for the biodegradation of polymer. More frequent fragmentation of the polymer is done by the extracellular enzymes. In the degradation of polyethylene thermoplastic many lignin like degrading enzymes also take part. Oligomers after cleavage of polymer are transported to cell to further break into monomers. In the backbone of polyethylene polymer, absence of hydrolysable group causes the restriction of the biodegradation of polyethylene. Biodegradation is further accelerated by the pretreatment of the polymer with the oxidizing agent or UV irradiation. Substrate like peroxidation is used while studying the biodegradation of polyethylene. Polyethylene biodegradation involved enzymes that can degrade polymer having C-C oxidizable bonds in lignin, such enzymes include laccases (EC 1.10.3.2.), lignin peroxidase (EC 1.11.1.14) and manganese peroxidase EC 1.11.1.13). Pretreated polyethylene with ultraviolet can be degraded by copper required laccase obtained from *R. ruber*. Degradation mediated by laccase of polyethylene resulted in the availability of carbonyl group for the biodegradation and loss in weight is significantly observed. Weight loss can be significantly observed in the biodegradation of polyethylene by treatment of PE with laccase that is obtained from the *Trametes versicolor* along with mediator like 1-hydroxybenzotriazole. *P. chrysosporium* isolate ME-446, IZU-154 and enzymes like manganese peroxidase (EC 1.11.1.13) obtained from white rot fungus play important role in the biodegradation of High Density Polyethylene degradation is stimulated in the presence of different surfactant and partially purified manganese peroxidase (EC 1.11.1.13). In the degradation of nylon-66 the manganese peroxidase (EC 1.11.1.13) obtained from strain IZU-154 can play active role by oxidize the 2,6-dimethoxyphenol. An increase secretion of manganese peroxidase (EC 1.11.1.13) and laccases (EC 1.10.3.2.) by the *B. cereus* was noted when the polyethylene polymer was prior exposed to ultraviolet irradiation.

Furthermore, A negligible weight loss was observed when polyethylene was treated with the partially purified manganese peroxidase (EC 1.11.1.13) and laccases (EC 1.10.3.2.) obtained from the *P. simplicissimum.* The lignin peroxidase (EC 1.11.1.14) play important role in the biodegradation of heated polyethylene in the presence of concentrated lignocellulose supernatant *Streptomyces* species. Almost 70% of the high molecular weight polyethylene degradation occurs by treating PE with *P. chrysosporium,* MTCC_787 strain. Peroxidases play an important role in the polyethylene biodegradation (Ghatge et al., 2020).

2.7 Factors Affecting Biodegradation of Plastics:

Many factors that can affect the process of biodegradation include exposure condition, enzymatic characteristics and properties of polymers. Some of the factors that affect the degradation of plastics are as follows:

2.7.1. Exposure Conditions:

2.7.1.1 Moisture:

The requirement for energy and multiplication of microorganisms, water play an important role in the growth of microorganisms. As the microorganisms growth increases the chances of the biodegradation of the plastic also increases. Thus, the biodegradation of polymers increases as the growth of microorganisms increases with the moisture. Hence, the moisture content in the environment enhances the hydrolysis reactions thus generate more chain reactions (Ahmed et al., 2018).

2.7.1.2 pH and Temperature:

The hydrolysis reaction can be modified by the basic and acidic condition of the pH . Example includes the PLA capsule hydrolysis occurs more at optimal condition, at the pH 5. The microbial growth and the degradation process affect with the production of the degrading product, which further affect the PH.

In the same way, the degradation ability of the enzymes is also affected by the temperature. Biodegradation of polymers having high melting point is less possible. The increase in the temperature causes decrease in the potential of enzyme degradation . Hydrolysis of PCL polyester increases with the *Rhizopus delemar* purified lipase show low melting point (Tokiwa et al., 2009).

2.7.1.3 Enzyme Characteristics:

Various enzymes have unique active site and have the potential to degrade different kinds of plastics. Enzymes that is obtained from the fungal species like *A. niger* and *A. flavus* quickly degrade the polyester having straight chain and is of di acid monomers consist of 6-12 C-atoms in comparison with other kind of monomers. It is studied that extra cellular enzymes are involved in the PHB depolymerization. Due to 3D structure and hydrophobicity the polymer derived from petrochemicals cannot be easily degraded.

Moreover, because of the polyethylene hydrophobic nature it presents the microbial biofilm formation thus it plays role in the reduction of biodegradation (Hadad et al., 2005).

2.7.2. Polymer Characteristics:

2.7.2.1 Molecular Weight:

In defining the properties of polymer, the molecular weight plays important role with respect to degradability. With the increase in the molecular weight the degradability gets decreased. *R. delemar* strain produce lipase which shows maximum degradability towards low molecular weight polymer. It is easier for microorganisms to biodegrade polymer having low molecular weight.

2.7.2.2 Shape and Size:

Polymers properties like size and shape also play an important role in the degradation of the plastic. Large surface area polymers can be quickly and easily degraded in comparison with the short or small surface area. For the degradation of various kinds of plastics there are standard criteria of size and shape.

2.7.2.3 Additives:

Contaminants like non polymeric filler and dyes have effects on the degrading ability. It is observed that the thermal stability of the polymer gets reduced with the increase in the ligno cellulosic fillers. Interfacial adhesion and the dispersal between the thermoplastic polymer and the lignoo cellulosic filler are the force behind thermal stability in composite system. In the same way, metals can also act as an excellent pro oxidant in the manufacturing of polyethylene which are sensitive to the thermo oxidative biodegradation.

2.7.2.4 Biosurfactants:

Biosurfactants compounds are produced on the surface of the living organisms and are amphophilic in nature. Due to high degradability and low toxic nature it is added to the polymers to improve the degradation process. Specific functional groups present in the biosurfactants which help in the biodegradation process and have the potential to undergo the degradation under extreme pH, salinity and temperature.

2.8 Methods of Disposal:

The large application of plastic almost in every sector has led to plastic accumulation in every environment leading to devasting environmental and health issues. Some of the major end-of-life options for plastics include:

- Landfill
- Incineration
- Reprocessing and recycling
- Biological waste treatments (anaerobic digestion and composting)

2.8.1. Landfills:

Landfilling is considered as one of the easiest and widely used method of disposing all kinds of municipal solid waste. About 60% of plastic waste produced is dumped in landfills every year. Landfill is designed in such a way that it will separate out the dumped trash from the surrounding environments. It will be kept dry under the covering of soil (Adamcová & Vaverková, 2014). Landfilling Is not an effective method of waste disposal because of following disadvantages: the degradation rate inside the landfills are quite slow, these waste even persist inside landfills for more than 20 years. The low degradability is because of limiting oxygen and anaerobic conditions inside the landfill. This will result in occupation of that particular space for longer time and no more be utilized for productive means i.e. agricultural and irrigation purposes (Sharon, 2012). Another limitation to this process is production of different secondary pollutants that are either released as gases or remain in leachate these include benzene, toluene, ethyl and trimethyl benzenes etc.

2.8.2. Incineration:

It includes a thermal treatment of plastic in presence of oxygen, usually at higher temperatures. This method has been used frequently for the disposal of waste. It generates a large number of gases and heat that can be used as an energy source. Incineration of PET produces high energy content that is equivalent to soft coal. At least 23 MJ/kg amount of energy is being produced alone from constituents of municipal solid waste. This process is also advantageous over landfill that it doesn't require significant space for longer time. However incineration of PET produces many harmful gases that act as a greenhouse gases and affects the atmosphere adversely. (Dutt & Soni, 2013).

2.8.3 Recycling:

A process in which a product or material is brought back to the position where it can be used again. Recycling is advantageous in a way that it partially depolymerize the polymers so that it get innovated to different new products. Most of the synthetic plastics formed have a limited ability to be recycled this is due to their mechanical strength and thermostability. Recycling of plastic can be achieved by two processes: mechanical and chemical recycling (Koshti et al., 2018).

2.8.3.1 Mechanical or Secondary Recycling:

This process is important only for thermoplastic polymers such as polystyrene, PE and PET etc. because in this process high temperature is provided to re-melt plastics and to mold them into various shapes and hence there is formation of new products. This process includes physical processes like shredding, cutting, washing into granulates or solvent. It also involves pellet of appropriate quality that is used for manufacturing by re-melting. The end result includes the decrease in plastic waste.

2.8.3.2 Chemical Recycling:

Chemical processing of plastic polymer can be performed by depolymerization of polymers that can be carried out by hydrolysis, methanolysis, glycolysis or amino lysis (Raheem et al., 2019). After chemo lysis, different monomers are formed that can be used as a raw material to produce new plastic. This process is not as much preferred though it is cost effective (Gomes et al., 2019).

2.8.4 Biological Waste Treatments; Anaerobic Digestion or Composting:

Composting process can biologically transfer and convert the biodegradable wastes and biodegradable plastics into products that are useful and valuable for soil amendment under suitable composting conditions. In Composting process microbial population degrade the heterogeneous organic matter under controlled aerobic and moist environmental conditions. such as composting processes as well as other Aerobic waste management systems facilitates the addition of carbon and other sufficient nutrients to the soil. However, composting facilities in the Europe are limited.

Some of the composting systems facilities deals only with garden waste and these composting facilities are not adapted for processing packaging materials in the composting waste. Infrared detection technology made it easy to separate conventional plastic from biodegradable and compostable plastics (Rujnić-Sokele & Pilipović, 2017).

2.9 Reduction and Control of Plastic Waste:

Due to such vast hazards of plastic to environment and human health, the need of moment is to reduce and control the production of plastic waste by reducing the manufacturing of plastic and plastic products, prohibiting the excessive packaging. Implementing and promoting the use of some alternative products that are environment friendly and safe. Some of the strategies are mentioned below.

2.9.1 Policy Making; Laws and Legislation:

To combat the environmental pollution of plastic, we must have some laws and policies to be followed and enforced. These policies must mandate plastic producers to list the ingredients and composition of plastic products related to the plastic constituents. If that plastic product contains some harmful chemical then that plastic must be banned. In 2004 a precedent including harmful effects of Chlorofluorocarbons was enacted. This causes 200 countries to completely stop production of CFCs. Government should have to implement the laws and regulation that will checkup the production, consumption, usage and disposal methods of plastics (Farah, 2019). The plastic to be packaged must be employed to all these stages of 3Rs: Reduce, Recycle and Reuse to prevent from indiscriminate methods of disposal.

2.9.2 Education and Public Awareness:

There is a need to educate the public about the potential environment pollution health effects made by the plastic waste. Most of the common and general population don't know about the basic potential hazards of plastics. Proper awareness campaign must be run to aware people about the toxic chemical constituents of plastic, the way to dispose, reuse and reduce the use of plastic in their daily life. Beside that educational schools and institutions must include the way of plastic pollution reduction and the proper methods of disposing it to their curriculum to educate the students at different levels, public have knowledge that plastic is synthetic, biodegradable, or made up of recycled material (Proshad et al., 2017).

2.9.3 Bio-upcycling of Plastic Waste:

A mixture of variety of plastic waste is added. Theses mixtures are mechanically grinded first into different plastic fragments. These plastic fragments are than biologically depolymerized by enzymes called depolymerases that depolymerizes the plastic fragments for recycling. The products that obtained are separated from rest of products. Then through bio fermentation, there is process of depolymerized products. It is utilized as feedstocks to produce certain bio-degradable chemicals like succinic acids, PHA and biosurfactant. (Huo et al., 2020).

2.9.4 Implementation OF 4R's:

There was a well understood concept of the three R's: Reduce, recycle, the 4^{th} R refers to the remediate. There all help in a same way to cut down the amount of waste that we throw away.

2.9.4.1 1^{ST} R; Reduce:

The best way to manage plastic pollution is to stop the production of plastics and switch to the alternative products. But as plastics are the part of our lives so we can reduce the use of plastic by source reduction. This can be done through the personal awareness as one must have to buy in bulk instead of separately and use less packaging, Buy durable goods to avoid the plastics etc.

2.9.4.2 2^{nd} R; Recycle:

Among other methods, recycling is the most easiest and convenient way. As a consumer of plastic product we can also play our roles in recycling of product by putting plastic waste in a right bins for disposal , this will prevent plastics to be land filled rather it will get recycled with other products of same kind. Recycling is done in a series of steps that take up the plastics and process them to remanufacturing the other new products.

2.9.4.3 3^{rd} R; Reuse:

Reuse of plastic material in one or either way. Either reuse of the plastic in same form i.e. reuse of PET bottles instead of buying new or by reuse them or by modifying them through creativity by converting into other products i.e. use coffee can to pack a lunch, use PET bottles to make pots, pencil box etc.

2.9.4.4. 4^{th} R; Remediate :

The other and newest technique to treat the plastic waste is to remediate the plastic waste. Remediation sorts the plastic waste in order to get rid of toxic substances i.e. plasticizers and additives that are effecting human and environment. One way of remediation is to treat the plastic with the fungi and bacteria that will hasten the degradation process thus depolymerizing the plastic polymers in a natural way that is safe for environment with no side effects. Example of remediation includes Bioremediation both in-situ and ex-situ remediation of plastic products.

2.9.5 Alternatives:

Following are the alternatives that can be used instead of plastic to reduce pollution.

2.9.5.1 Biodegradable Plastics:

Biodegradable plastics are those that can easily be degraded under natural conditions. Its properties remain same during storage period and fulfill our requirements. It is degraded by microbes (algae, fungi, bacteria) and gives H_2O, CO_2, CH_4 and biomass. An ideal BP exist as part of carbon cycle in environment. These plastics are made from renewable raw materials including cellulose, lignin, starch and bioethanol. In market the natural biodegradable polymers include polyhydroxyalkaanoate (PHA), polylactic acid (PLA), polyhydroxy butyrate (PHV). It also cause harm to environment in for of micro and nano plastics but less than conventional plastics (Maocai et al., 2020).

Although BPs production and usage is still under development, but there are substitution effects of these plastics in various fields. It includes Disposable plastic products of everyday life like Disposable lunch boxes, garbage bags, plastic bags and product packaging bags. They are being used in Agriculture mulch film which is made of PP which is non-degradable. It takes hundreds of years to degrade causing poor aeration and decrease in crop yield. To solve problem Agricultural degradable mulch films are used. It also has applications in High-end Market including 3D printing materials, Medical supplies. PHA, PLA, PCL and other BPs are used because of having good biocompatibility.

2.9.5.1.2 Advantages of Bio-degradable Plastics;

- It prevents the animals from injuries that are caused by plastic pollution.
- They help in soil enrichment by returning to soil through organic wastes and composting.
- Their degradation does not require labor and labor cost, because they are naturally degraded in environment.

2.9.5.2 Hurdles of Using BPs:

Firstly, Out of 335 million tons of plastic, It accounts for only 0.5% of production. It is expected to increase to 2.62% by 2023. The key reason of using petrochemical based plastics is because it is cheap; the process of production is too mature. The price of producing BPs is 3-10 times more than that of conventional plastics. Some BPs is not reliable as conventional plastics like PHB that is used in packaging in food because it acts as barrier to oxygen but has low plasticity and strength. BPs needs certain environmental conditions for degradation. BPs lack a favorable management system of sanitary landfill or incineration .Change of human behavior regarding usage of BPs is needed. People need to aware of this and should take plastic pollution seriously.

2.9.5.3 Alternatives to Plastic Bags:

The eco-friendly alternatives to plastic bags include paper bags, jute bags, cloth bags, reusable bags and bio-degradable bags. Jute bags are made of fiber named jute, which mostly contains cellulose. It is used as packaging material such as salt, grain sugar etc. They are also used for grocery purpose. Jute bags are also known as hydrocarbon free bags because during manufacturing they are treated with vegetable oil to destroy effects of hydrocarbons. They are able to turn to plant manure. Paper bags can also be used instead of plastic bags. The natural fibers used in paper bags are recyclable. But they are not long lasting. The best alternative to this is fully degradable plastic bag. The reusable bags include cane woven baskets, cloth bags that may act as reusable alternatives.

2.9.5.4 Alternatives to Plastic Toys:

Toys are made of different types of plastics like polystyrene, polyethylene, Chemicals called BPA is added and plasticizers called phthalates are also present. These are toxic substances and children are continuously exposed to it. So eco-friendly toys should be made. A choice based conjoint analysis was performed among parents in 2014. Results showed that parents preferred to choose toys that are made of non-toxic material. An alternative for this may be toys made of completely biodegradable plastics like PHA etc. other alternative may be sand based toys that can be degraded (Agnes et al., 2017).

2.9.5.5 Alternatives to Plastic Microbeads:

Microbeads are solid spherical beads having size of 1mm. They are made of non-biodegradable plastics. They are used in cosmetics, tooth paste, body wash, scrub and face wash etc. These microbeads pollute the drinking water as well as other water bodies like lakes and rivers. They are ingested by fish and other small creatures in water and clog their lungs. Alternatives to these oil based microplastics include chitin or cellulose based microplastics that can be easily degraded. Other alternative include formation of polyhydroxyalkaanoate (PHA) beads that can be used to protect environment (Jalil, et al., 2013).

CHAPTER 3

METHODOLOGY

3.1 Sample Collection:

For the isolation of particular microorganisms having desired characteristic we isolate our sample from the place where is more chances of occurrence of that particular microorganisms. For example, if we require protein degrading or lipid degrading bacteria, we will isolate our sample from area that is rich in protein and lipid contents. Similarly, for the isolation of PE, PS and PET degrading microorganisms we will collect our samples that are abundant in these sources. Usually sampling is done from the open dumps that have all these kind of plastic as a waste because there is more probability of occurrence of plastic degrading microorganisms at these sites. During our experiment the soil sample was taken from a 20 years old open dump site that is in Peshawar city. The samples were collected from the top layer of soil about 1 inch deep in a sterile bag and were used after 1 week of sampling.

3.2 Media Preparation:

The media that is used for enrichment or growth of degrading microorganisms is Minimal salt media (MSM). It contains only inorganic salts with no carbon source in it, plastic polymers will act as a carbon source, only those microorganisms will survive that will take up plastic as a carbon source. The pH of media will be adjusted at 7 and media was autoclaved at 12˚C for approximately 20-15 mins. The composition of MSM is described in table below.

Table 4: Composition of Minimal Salt Media

no	Composition	Quantity (g/l)	No	Composition	Quantity (g/l)
1	$K_2\ HPO_4$	1.0	**7**	$MgSO_4.\ 7H_2O$	0.5
2	NaCl	1.0	**8**	$CuSO_4.\ 5H_2O$	0.001
3	KH_2PO_4	0.2	**9**	$ZnSO_4.\ H_2O$	0.001
4	$CaCl_2.\ 2H_2O$	0.002	**10**	$MnSO_4.\ H_2O$	0.001
5	Boric acid	0.005	**11**	$Fe^2\ (SO_4).\ 7H_2O$	0.01
6	$NH_4(SO_4)^2$	1.0	**12**	Distilled water	1L

3.3 Isolation of Microorganisms from Sample:

3.3.1 Serial Dilution:

A method used to reduce the number of cells present inside the dense sample having uncountable number of microorganisms in it. Each dilution will reduce the concentration of microorganisms by specific amount. 9ml of diluent i.e. normal saline or distilled water is drawn into a 10 test tubes. 1g of soil sample is mixed with the 1ml of distilled water and added to test tube 1 with help of micropipette. Making it total volume of 10 ml with initial dilution of 10^{-1}. Now 1 ml of mixture is drawn from the 1^{st} test tube of 10^{-1} dilution and emptied into the second tube. Same process is applied to rest of tubes.

3.3.2 Inoculation by Spread Plate Method:

The dilutions 10^{-4}, 10^{-5} and 10^{-6} were further inoculated on a nutrient medium for the growth of microorganisms for fungal growth. We inoculated the sample from each dilution to Sabouraud Dextrose Agar and incubated, While for the bacterial growth the diluted sample is spread on a nutrient agar. 0.1ml of sample from each dilution is drawn through the pipette and placed at the center of agar plates. With the help of sterile L-shaped glass spreader it is evenly spread over the surface of agar medium and incubated at 37°C for 24 hours for bacterial growth and 20-25°C for 2 to 3 days for fungal growth. After incubation we have got almost 30 separate colonies of bacteria and 15 colonies of fungi.

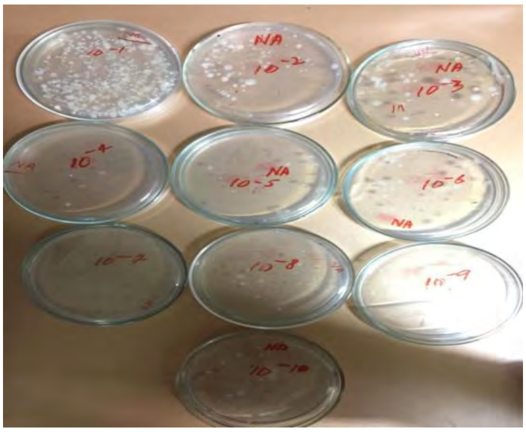


Figure 6*:* Bacterial colonies obtained from spread plate method

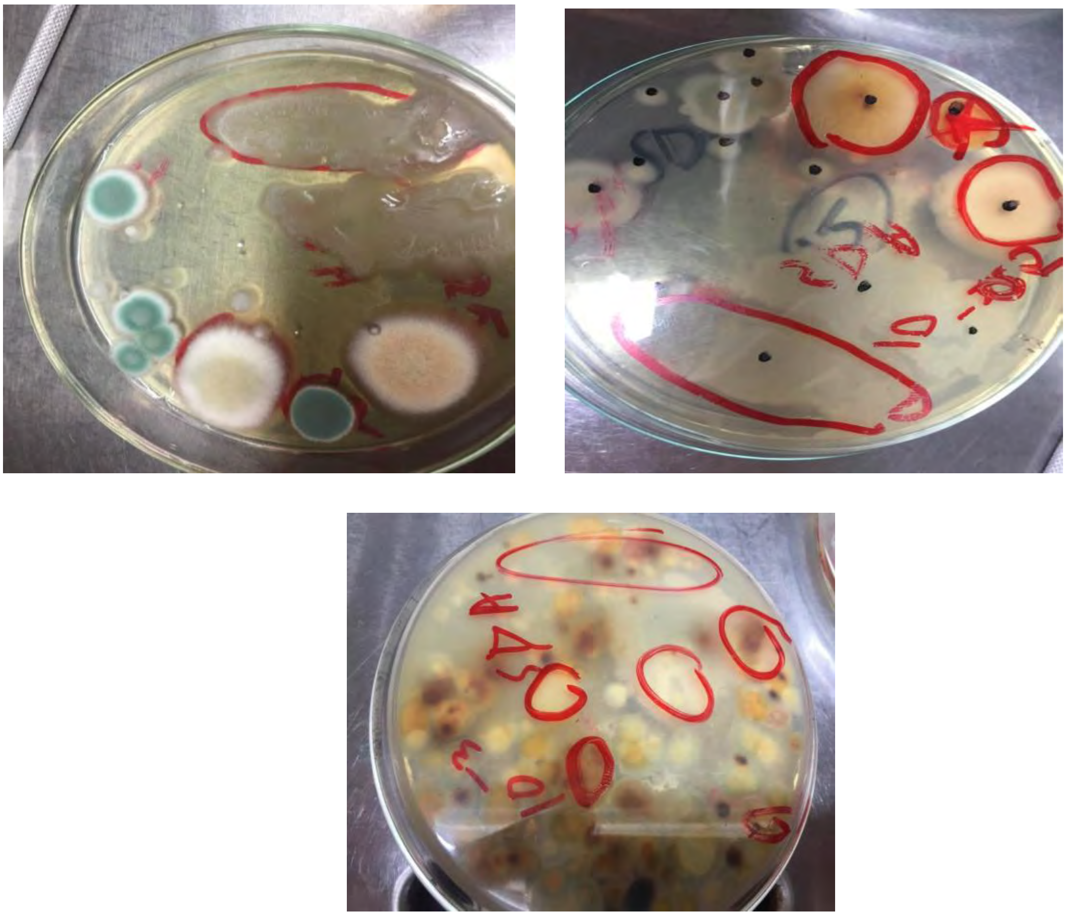


Figure 7*:* Fungal Colonies Isolated from Soil Sample

3.3.3 Sub-Culturing:

Sub culturing for the isolated colonies were done by two ways. For the sub culturing of bacterial colonies, we subculture the number of colonies obtained from the culturing to a separate plate by streaking method. Almost 30 bacterial colonies were obtained that were further streaked out. In case of fungi almost 15 isolated colonies were sub cultured by point inoculation method. All the sub cultured plates were incubated at 37°C for 24 hours and 20-25°C for 2 to 3 days respectively.

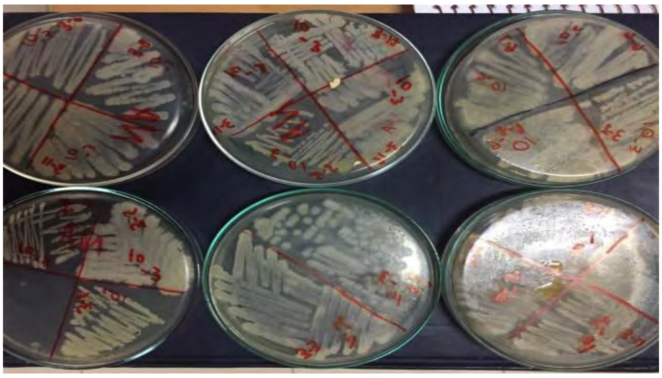


Figure 8 :Pure colonies obtained via streak plate method.

3.4 Screening of Plastic Degrading Microorganisms:

3.4.1 Pre-treatment of Plastic:

All three plastics were cut down into the small strips of equal size and weight and are being transferred to a fresh solution of 70 ml Tween 80, 10 ml bleach, and 983 ml distilled water for approximately 30 to 60 minutes. After this these plastic strips were transferred onto another beaker having distilled water and stirred for 1 hour. At last, they were aseptically placed for 30 mins in the 70% ethanol solution. Sometimes plastic strips were also placed under UV for 15 minutes as a final treatment process.

Figure 9: Weighed and Equal Size Strips of PE, PS, PET used for degradation

3.4.2 Degradation of Pretreated Plastic:

This process was carried out in form of triplicate. Four set were prepared for all three types of plastic types i.e. PS, PE, PET with one control. Total 12 conical flasks were prepared having 300 ml of MSM so that only those bacteria will able grow that take up plastic as a sole carbon source. At first, we add the bacterial and fungal colonies isolated from the soil sample in each flask with help of loop except for the control that is a microbial free medium. After then initially weighed and pre-treated strips were aseptically transferred to the conical flasks and kept in a shaker for 10, 20, 30 and 40 days respectively. Flasks were analyzed after each 10 days of incubation and then re-cultured. Plastic strips were collected, washed thoroughly by distilled water, dried and finally analyzed to check the degradation.

3.5 Analysis of Degradation:

The analysis of biodegradation can either be done by the visual observation of strips including roughness on the surface of plastic, crack or the hole formation, color change or formation of biofilm on surfaces of polymers. We can also analyze the degradation process by monitoring the gas formation or by measuring the weight loss that is calculated by formula: **Initial weight-Final weight *100**. Different analytical techniques can also be used for the analysis of degradation i.e. FTIR, NMR, XRD, HPLC etc. these techniques will tell about the complete insight of polymers that are bought up by bacteria on their surfaces.

3.5.1 Standard Testing Methods:

3.5.1.1 Radiolabeling:

In this method, the explanation and calculations are done easily in the presence of ^{14}C test material. A^{14}C marker is placed in environment having microbes by placing in any material. The amount of $^{14}CO_2$ that is produced is approximated. This method usually shows precision and is known to be much accurate in detecting of the polymer degradation by microorganisms. The release of simple (CO2) and radioactive carbon dioxide ($^{14}CO2$) is estimated simply without any hindrance. However, the problem associated with disposal as well as its uneasy availability makes it difficult to deal sometimes.

3.5.1.2 Visual Information:

The changes that a plastic can undergo can be evaluated with the help of many tests. These tests hence do not tell about the biodegradation directly but the visible changes in the plastic gives the indication that some microbial activity is being going. The visual changes that takes place includes formation of biofilms, defragmentation, cracks, color change etc. The interpretations about microbial degradations can be made with the help of either Atomic Force Microscopy or Scanning microscopy. The slow degradation process can be explained by the release of small crystalline substances, that is the result of degradation of polymers. The other techniques by which the degradation of complex polymer can be studied includes Nuclear Magnetic Resonance Spectroscopy (NMR), X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and many others.

3.5.1.3 Molar Mass and Mechanical Properties Changes:

Out of some events, that indicates the degradation of polymers, a small change in the mass shows changes in mechanical properties of the polymer. As a result of change in mass, the property of tensile strength is affected. However, the degradation process in abiotic conditions results in no change in mass, but some changes in mechanical properties are observed. It is used in abiotic condition to detect degradation. Similarly, the changes in the polymer materials by the action of enzymes also results in no or some mass loss. However, because of the enzymatic action major changes in mechanical properties takes place if more loss of mass takes place. In this depolymerization process, the inner material remains safe without any changes, but surface material is changed due to enzymatic actions and hence helps in the detection.

3.5.1.4 Controlled Composting Test:

Some active methods against the recycling of organic material in waste as well as treating it properly is done with the help of composting in a controlled way. With the help of this facility, the biodegradable waste can be recovered hence increasing the efficiency of landfills. However, it should be focused that the material that is not in compatibility with the composting should not be considered in recycling because the material only compatible with the composting will be recycled and the presence of incompatible materials will decrease the efficiency in return. This test method evaluates based on the release of CO_2 gas. Moreover, it should be kept in consideration that the release of toxic substances towards compost by the packaging material should be avoided, otherwise it may affect the plant growth, human and animals living in a habitat.

3.5.1.5 Enzymatic Degradation:

A two-step hydrolytic process includes the disintegration of complex polymers that includes at first, the binding of the enzyme with its polymer's substrate and after that the cleavage of the material takes place. For example, Both the extracellular and intracellular enzymes (depolymerases) are thus involved in the proper degradation of polyhydroxy butyrate by the fungi and bacteria specific to them. Degradation that takes place extracellularly is done by the hydrolytic reaction, either in the presence or absence of microbes. However, the intracellular hydrolysis of PHB is done by the bacteria. Hence the phenomena of depolymerization takes place in which the microorganisms convert the complex polymers to simple short fragments, taken easily by microbes as energy source thus releasing the enzymes. These short molecules are then subjected to mineralization i.e. formation of CH4, CO2 etc. takes place.

3.5.1.6 Clear Zone Formation:

This specialized test is used to determine the potentially active microorganisms against the polymer degradation. It based on the agar plate medium on which the dispersion of the complex polymer is done in the medium having agar and the polymer is added in the form of fine small particles to be acted by microorganisms efficiently. After the addition of microorganisms, the formation of clear zone after their action indicates the active microbes against the polymer degradation. Also clear zones formation tells about the microbial action related to their quantity.

3.5.1.7 Determination of Residual Polymer by Weight Loss:

By the degradation step, it is thus referred that the mass loss has taken place and hence degradation is done in the presence of microbes. However, the proof for the proper activity of biodegradation is not achieved by the test. Sometimes, due to the natural tendency of the polymer to undergo degradation during treatment, some material disintegrates thus allowing changes in the polymer even before detection of degradation. To avoid this, certain recovery methods can be followed such as the method of extraction, in which the polymer if obtained in powder form can be determined for its degradation that either some changes has taken place or not. By the comparison of the degraded polymer and the residual polymer, the results can be interpreted easily based on the weight of the polymers.

3.5.1.8 O_2 Consumption/ CO_2 evolution:

Usually, besides some traditional or conventional methods of detection of gases, this method involves the use of indicators or detectors that are active in determining the amount of O_2 or CO_2 involved in the process of degradation. We know that the microbes that are dependent on the oxygen concentration undergo aerobic degradation hence producing CO_2 as a major end product. Normally the indication about the presence of polymer degrading microbes in the environment is made by the amount of consumed oxygen in the environment and the evolution of CO_2 by these microbes as result of degradation. The more O_2 present in the environment, it will be utilized by the degrading microbes. The modern analytical techniques used for the detection of gases have many uses, but beside its uses it also has some disadvantages regarding the rate of the degradation processes taking place i.e. it should take place with continuous rate without getting slow to give appropriate indication of O2 and CO_2. Also, one of the accurate methods for the determination includes the small bottles closed tightly acting as a chamber for degradation in which CO_2 production or O_2 utilization can be analyzed. This method is more appropriate for the determination of polymers degradation in aqueous systems. For the determination of solid degradation, the method of Composting test is used and is much useful. However, the release of CO_2 from other sources such as soil that interrupts the CO_2 measurement can be avoided by the use of the combination of microbial population (Mixed), inert and porous carbon free film to avoid excessive CO_2 production. (Shah et al., 2008)

3.5.2 Analytical Techniques in Biodegradation Studies:

Various analytical techniques are used to determine the interaction of the polymer with microorganisms and hence the resultant changes that takes place. Through these analytical techniques, the generation of small organic groups as a result of degradation as well as change in structure of certain polymers can be analyzed.

3.5.2.1 Fourier-transform Infrared Spectroscopy (FTIR):

FTIR is utilized in the studies of polymer's degradation, so that the changes that have undergone in the presence of microbes can be detected. These techniques are much helpful in explaining the different characteristics of polymers that includes Hydrogen bonding, degradation reactions, detection of end groups and the configuration of polymers in solid and liquid forms.

3.5.2.2 Nuclear Magnetic Resonance Spectroscopy (NMR):

NMR is considered as the most multifaceted technique as it is used as an important tool in many studies related to degradation. NMR is used to determine those fractions that are soluble, gives indirect results of the changes in molecular weight, to get the idea about changes in the polymer's chemistry.

3.5.2.3 Gel Permease Chromatography (GPS):

GPC that is also known as size exclusion technique is a traditional method that determines and are known to undergo the separation of particles or molecules on the basis of its respective sizes. This technique is applied and helpful after biodegradation in detecting the changes in the molecular weight of polymer used.

3.5.2.4 High Pressure Liquid Chromatography (HPLC):

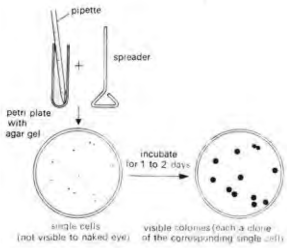
By this widely used technique, the separation of the molecules on the basis of their specific sizes is done efficiently. In this technique, basically the molecules that are smaller in size will more slowly due to less density as compared to the molecules that are larger in size are will move faster and elude first because of greater density, weight and size. The products that are produced as a result of degradation taking place metabolically are examined by the HPLC. Similarly, by the application of Reverse Phase Liquid Chromatography, bioconversion products of various chemical compounds such as phenylacetaldehyde, 2-phenylethanol, epoxy styrene and styrene are identified.

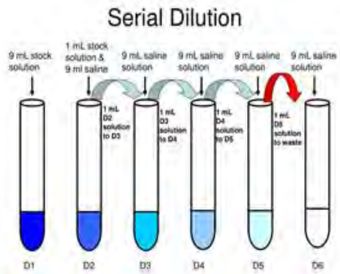
3.5.2.5 Gas Chromatography-Mass Spectrometry (GC-MS):

One of the common techniques that is applied to isolate the various organic and inorganic compounds from each other on the basis of the difference in the nature of mobile and stationary phase is (GC-MS). For those compounds that are difficult to isolate, Mass spectroscopy is also accessible, thus evaluating the substances both quantitatively and qualitatively. Similarly, many other detectors are present for the purpose of specialized detecting that includes Atomic Emission (AE), Flame Ionization (FI) and many others. This method of analysis is much useful in the quantification and detection of impurities or volatile species, for trace pollutants determination, polymer having additives and also in detection of certain coatings and resins with monomeric residues.

3.5.2.6 Thermo Gravimetric Analysis (TGA):

This technique tells about the effect of heat and its resultant changes in the sample that are provided to the sample under controlled environment. Energy of activation, Maximum decomposition rate and its related temperature and the rate at which decomposition is taking place can be studied easily by thermo gravimetric technique. Also, during the degradation of the sample thermally, the release of different gases can also be detected by this (Mahalakshmi, 2014)





Sampling from 20 year old open dump , located in Peshawar

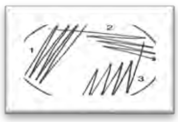
10x **serial dilution** of sample

Inoculation of diluted sample onto nutrient agar and SDA for bacterial and fungal growth respectively by **spread plate method**

Sub culturing to get isolated colonies

Figure 10: Overview of Biodegradation Procedure

point inoculation for fungal sub culturing on SDA



Streaking on nutrient agar for sub culturing of bacterial colonies

Isolation of plastic degrading microorganisms from sample

1. Preparation of MSM
2. pre-treatment of plastics
3. inoculate colonies in 100ml of flask containing MSM

Put the flasks in shake flask incubator at 37 C for 40 days and during that re- culture after every 10 days

Analysis of biodegradation

1. Weight loss measurement
2. Microscopy
3. By analytical techniques.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Expected Results:

In natural environment, microorganisms play a vital role in degradation of the materials including both synthetic and natural polymers. Usually in biological degradation process, microorganisms depolymerizes the polymer by excreting certain set of enzymes i.e. intracellular and extracellular hydrolases and depolymerases. Different extracellular enzymes hydrolyze the polymer by converting it to oligomers, dimers and ultimately to monomeric form so that they are actively utilized by the Microorganisms as a carbon and energy source.

In a current study PE, PS and PET were used for determining the degradation activity that is done by the different bacteria and fungi.. The source of these plastics were plastic bags which contains LDPE, plastic cups and plastic bottles. The degradation rate of all these plastics in environment is very slow because they are Petro -based plastics. Their degradation rate may take several of years. They have hydrocarbon backbone that is difficult to degrade. Bacteria and fungi also need proper environment conditions to degrade them. Total 30 bacterial and 15 fungal species were isolated by secondary screening the soil sample, obtained from the 20-year open dump site of Peshawar. All of these colonies were inoculated to Minimal Salt Medium along with PE, PS and PET to check the degradation ability of these isolated colonies. Some microbes degrade aerobically while some degrade anaerobically. The size of these plastics were 3cm X 3cm.It is expected that out of these isolated microorganisms some PE, PS and PET degrading microorganisms are also present.

The microorganisms involved in PET degradation includes *Alteromonas* belongs to class γ-proteobacteria, Gram positive phylum *Acitinobacteria, Thermobifidia* and *Thermomonospora,* consortium no 46, *Ideonella sakaiensis* and some species of pseudomonas and bacillus. These species has ability to degrade plastic as sole carbon source.

The microbes that were able to degrade PS may include some species of Actinomycetes *Rhodococcusruber*, *Exiquobacterium spp*, *Cuproidus nector*, *Zophobus atratus*, *Shingobacterium*, *Serratia marcescens* and species of *Pseudomonas* and *Bacillus*. Whereas fungi including *Penecillium variabile*, *Geophyllum trabeum*, *Pleurotus ostreatus*, *Phenerochaete crysosporium c*onsuming plastic as sole carbon source.

The microorganisms that cause degradation of PE may include *Pseudomonas spp*, *Bacillus spp*, *Staphylococcus*, *Rhodococcus ruber*, *Microbacterium paraoxydans* and fungal species of *Fusarium redolens*, *Verticillium lecanni*, *Aspergillus niger, Glioclodium vitens* and *Aspergilus flavus*. *Bacillus spp*. has ability to grow on minimal salt media. Microbes use plastics as a C-source. They also have shown the zone of clearance around the colonies when grown o the agar plate, this is because of secretion of extracellular enzymes. Some species i.e. *Klebsiella*, *Staphylococcus* and *Pseudomonas* also degrade PE by changing mechanical properties like tensile strength, optical changes and decolorization. Staphylococcus has expected to show the weight loss of 52%, while *Psuedomonas* show the 11% degradation by weight loss. The degradability of these species onto the polystyrene PE and PET can be measured by techniques including weight loss, clear zone formation, gas production via different analytical techniques that includes FTIR, HPLC, NMR, SEM etc. Actinomycetes i.e. *Rhodococcus ruber* has shown the degradability by 0.8% weight loss after 8 weeks of incubation, while Exquobacterium spp has shown 11% decrease in weight in 60 days and the fungal strain i.e. *Geophyllum trabeum* DSM, it has shown the 50% reduction in weight when incubated for 20 days. In IR spectroscopy, the oxidation of some carbonyl and hydroxyl groups were observed by the presence of absorbance peaks or oxidation peaks at 1740 cm^{-1} and 3400 cm^{-1} which highlights the oxidation process during the biodegradation by different fungal species. In HPLC, biodegradation products were measured that were phenyl-1,2 ethane diol 2- phenyl ethanol, produced by the *Penecillium chrysosporium* hence showing the degradation of polystyrene.

The physical changes after the degradation by microorganisms were visualized by visual observation revealing the brownish white colouration, with brittle crumpled places on the PET strip. The degradation and hydrolysis of aromatic rings results in the foul smelling of the PET sheet. SEM micrographs has also shown the crystal of the polymer and presence of microbes onto the sheets i.e. colonies of branched mycelium of *Actinomycetes* with width of approximately 0.1µ to 0.25µ. Some bacillus species were also observed that degrades dimethyl phthalate by the action of esterases. *Bacillus* sp. has shown about 8% of decrease in weight over the period of 20 days. Enzyme production was observed by the formation of clear zones around the colonies of following species *Nocardia* and *Bacillus subtilis*. Moreover *Comamonas acidovorans* TB-35 also degrading polyester polyurethane by the action of esterases.

4.2 Survey:

4.2.1 Background:

Plastics cheapness, durability and versatility have earned them a place in every aspect of life. In 2016, the amount of manufactured plastics reached 335 million tons. There are different types of plastics that are used in different forms in everyday life. The types include It includes Polyethylene, Polypropylene, polystyrene etc. They are used in different types of everyday life like plastic bags, toys, disposable cups, pipes, plastic bins and plastic bottles etc. Plastic bags and food packaging materials are most abundantly used in everyday life. 64% of their usage that is being discarded after short interval. Because of lack of manageability of plastic waste there is increase in plastic pollution day by day. Due to its non-degradable nature, it is causing as hazard to human and environment. Chlorinated plastic release harmful chemicals in environment.

Plastics also release various toxic chemicals like Polychlorinated biphenyls and Bisphenol A that causes serious health effects including cancer causing mutations and endocrine disruptions. The disposal of plastic waste in landfilling and oceans had led to a global issue unfortunately. The most visible effect of plastic pollution on environment is harming and killing of marine organisms through ingestion and entanglement. Recycling of plastics must be done in order to reduce waste and to produce products that have same quality as of original plastic products. Alternatives of plastics are present called ‘biodegradable’ plastics that are made from naturally occurring sources of plants, animals and microorganisms. They are easily degradable without causing plastic pollution. These plastics should be produced abundantly so that they can replace conventional plastics in daily life. In this study we examined society’s point of view regarding plastic usage, its disposal and hazards.

4.2.2 Methodology:

4.2.2.1 Research Design:

An online and physically distributed survey of closed ended questions were asked randomly from people of different age groups in order to get knowledge and perceptions of people about plastic usage, its disposal and hazards. Participants were given the option of selecting the answers for them. The questionnaire contains 5 pages. A total sample size of about 300 responses was collected from people of different age groups. After collection of responses, analyses were done about the answers given by people.

Out of 300 responses, 76.1% were done by females and 23.9% by males. The age of participants who attended survey and submitted response ranges from 18-44. The maximum responses were filled by people of age factor 22. Participants were also asked if they were engaged in any pro-environmental activity like recycling of water bottles or plastic bags or non-environmental behaviors like purchasing new water bottle after every usage and buying new plastic bag instead of using old. Participants were asked 25 questions in total that measured their knowledge and awareness about plastic usage, its disposal and hazards. The questionnaire was divided into three. Sections First section contains demographic information of participants including Age, Gender, Education and Occupation. Second section contains questions regarding plastic usage having 13 questions, while Section three contains questions related to Plastic disposal and its hazards having 12 questions.

4.2.2.2 Data Collection:

The survey was randomly distributed through online website to people of different age groups belonging to different fields. Data collection began on 4th July 2020 and continued until 20th July 2020. The duration of Data collection was about two weeks.

4.2.2.3 Data Analysis:

Approximately 300 Responses of survey were collected online through Google. docs. It provides summary as well as individual answers to overall questions. Results are obtained through graphical representation in form of Pie-charts having percentage of people responding to a particular question. In results below, both Pie-chart and Bar graphs are drawn to add statistics related to answers given by people of all questions. The statement in figure below every Pie-chart represent question whose answer are represented in form of Pie-chart. Pie-chart contains Data related to single question while Bar graphs shows representation to more than one question. The number in start of each statement below the explanation of Bar graph represents the question number.

Table 5: Frequencies of Age Groups

Age Group	Frequency
18-21	70
22-25	120
26-29	48
30-33	20
34-37	16
38-41	16
42-45	10

4.2.3 Results:

4.2.3.1 Section II:

This section includes questions related to “Plastic usage”

4.2.3.1.1 Representation through Pie-Chart:

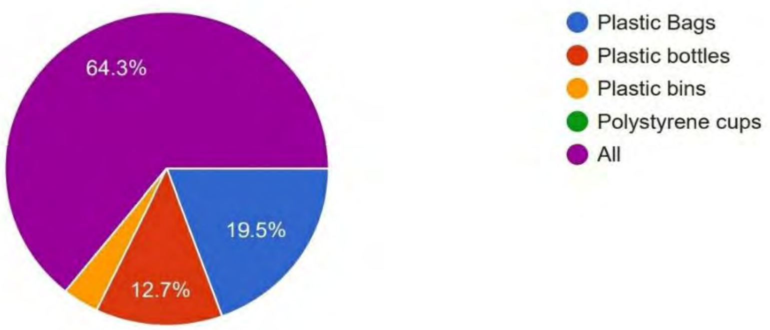


Figure 11 : Usage of Plastic Products in Daily Life

According to survey, the statistics of using all four types of plastics by people in everyday life is 64.3%. Over 19.5% prefer to use plastic bags in daily life, while 12.7% people said they only use plastic bottles in daily life. Only about 3.5% people used plastic bins. Hence it is evident that there is vast usage of plastic among people

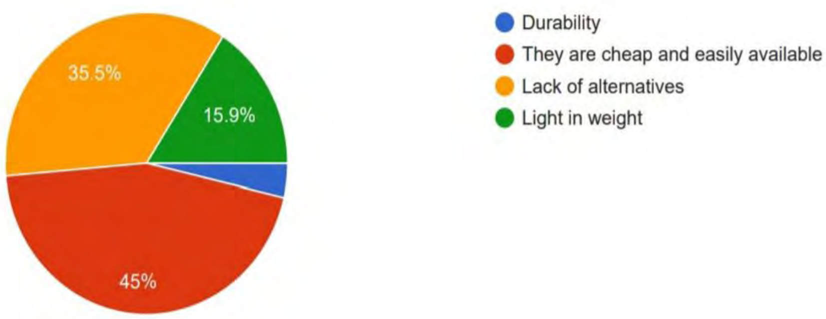


Figure 12: Preference of Plastic Bags and Cups Usage

The statistics obtained from survey shows that about 45% people said that they use plastic bags and cups because they are cheap and easily available. While, 35.5% people said because of lack of alternatives to plastic bags and cups, they use them. However, about 15.9% use them because of having lighter weight. Only 3.6% used them because of having durability.

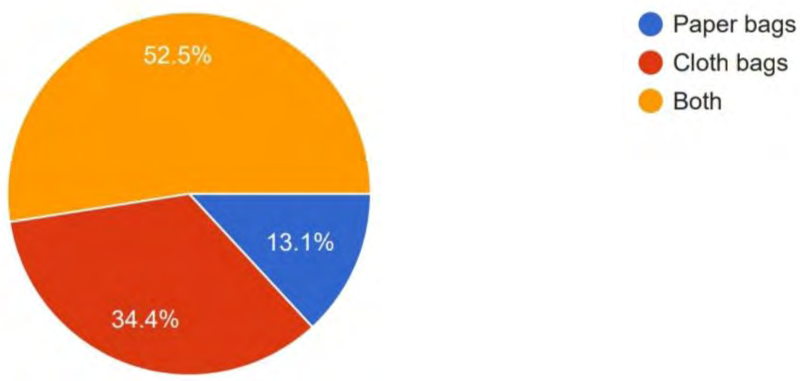


Figure 13: Usage of Alternatives to Plastic Bags

The statistics shows that about 52.5% people used both paper bags and cloth bags. About 34.4% people prefer to use cloth bags while only 13.1% people use paper bags.

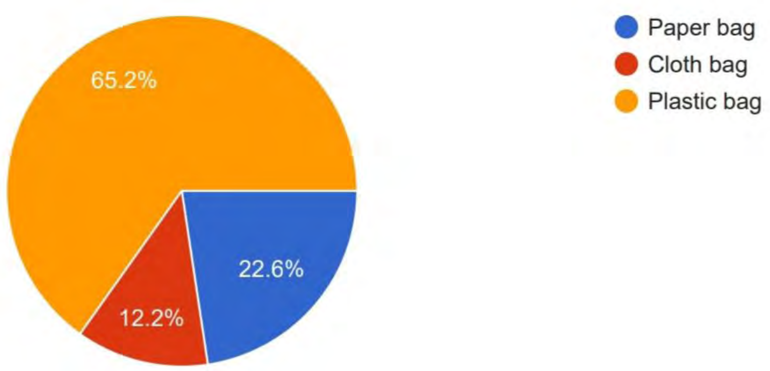


Figure 14: Cheapness of Plastic or Cloth Bag

Statistics from pie chart shows that 65.2% people thinks that plastic bag is cheaper as compared to cloth and paper bags. While 22.6% people thinks paper, bag is cheaper. Only 12.2% people thinks that cloth bag is cheaper.

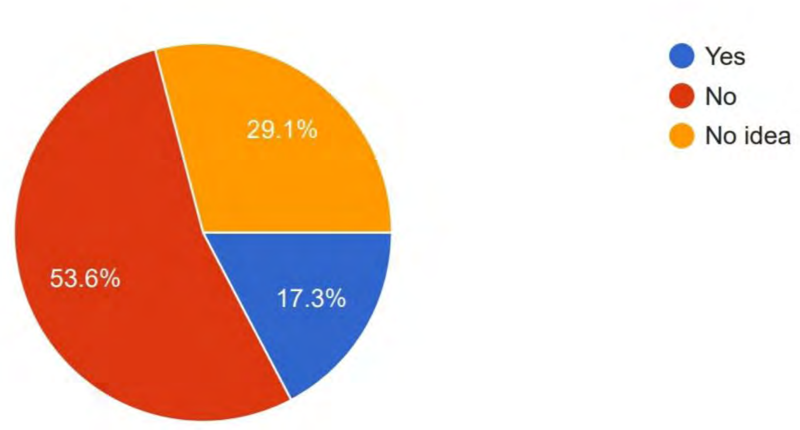


Figure 15 : Knowledge of Using Bio-degradable Plastic

Pie-chart shows that only 17.3% people make sure that plastic they are using is biodegradable before usage. While results show that 53.6%. However, statistics shows that 29.1% people have no idea about usage of biodegradable plastics.

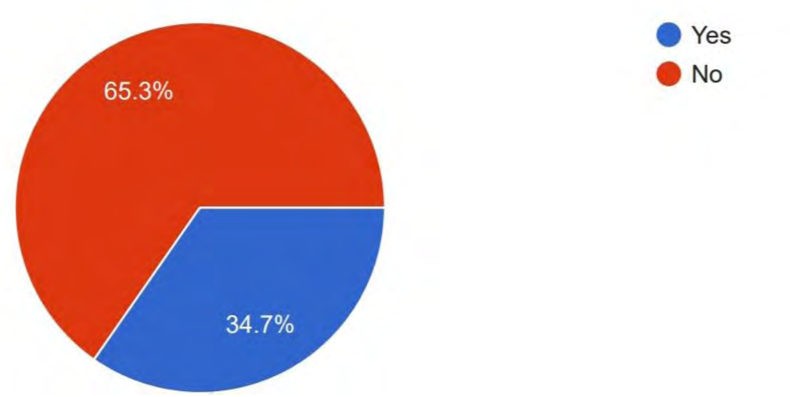


Figure 16 : Knowledge of symbols of Plastic

Results of pie-chart shows that about 34.7% of people consider these symbols while using plastics while 65.3% people don't consider these symbols.

4.2.3.1.2 Representation Through Bar Graphs:

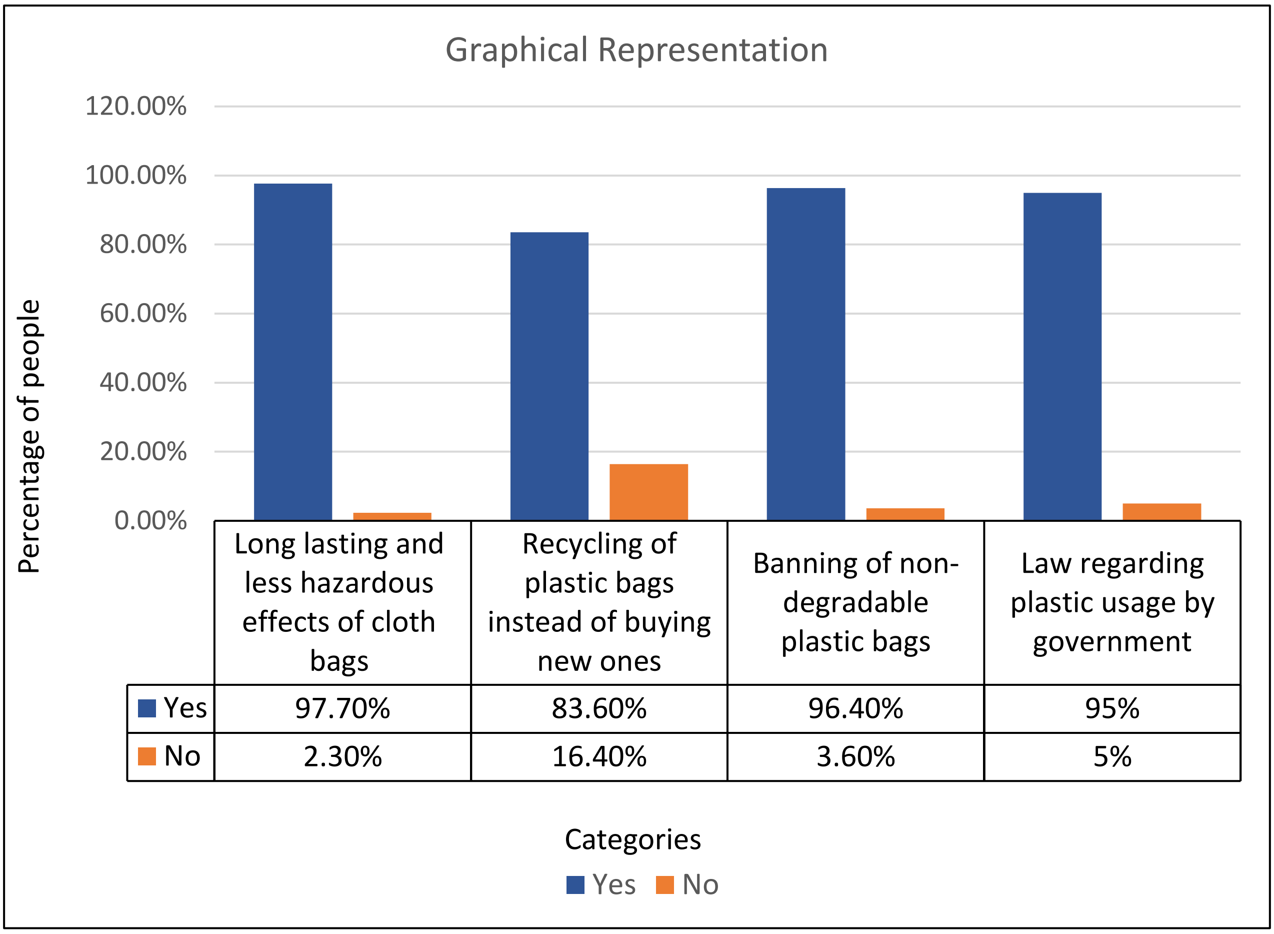


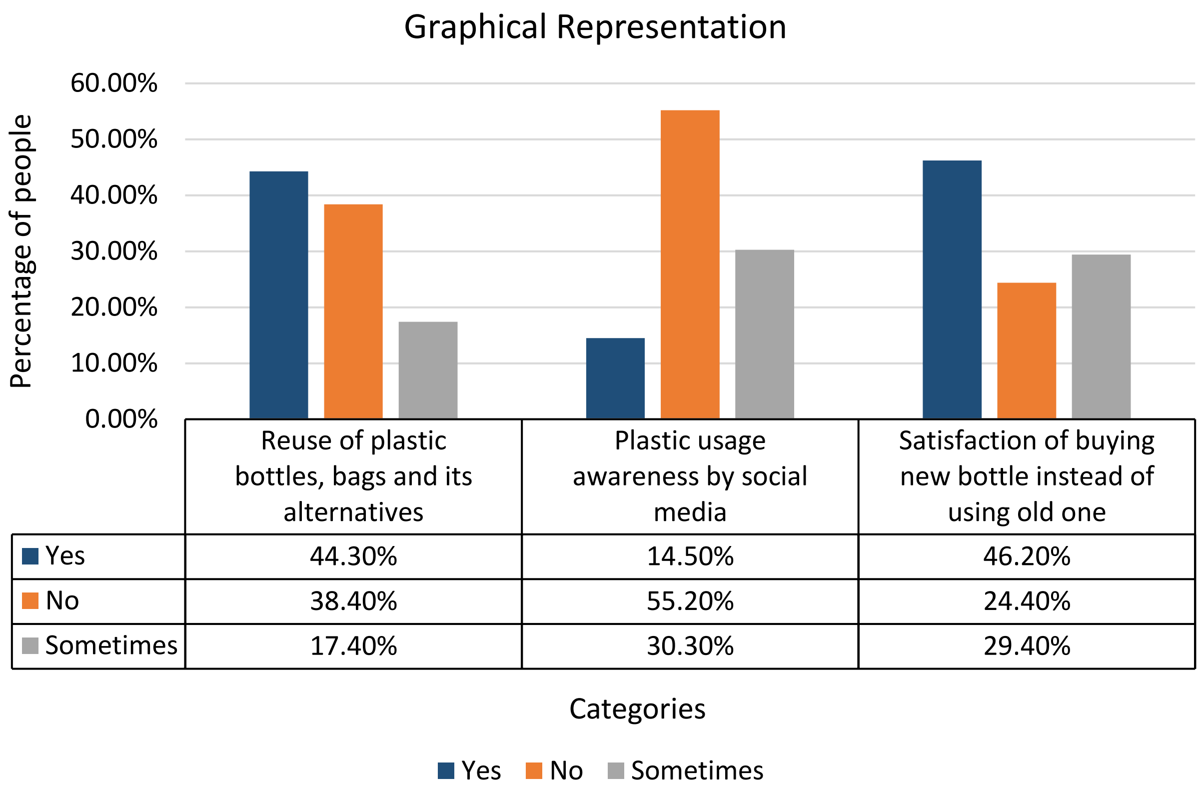
Figure 17 :Bar Graphs Showing Data Related to Plastic Usage

7. Bar chart shows that about 97.7% people thinks that cloth bags are longer lasting and less hazardous as compared to plastic bags. While about 2.30% thinks that cloth bags are not less hazardous.

8. According to survey about 83.60% people were in favor of recycling of plastic bags while about 16.40% of people were in favor of buying new bags instead of recycling.

9.About 96.40% people were in favor of Non-degradable plastic ban while small percentage of people about 3.60% were not in favor of non-degradable plastic ban.

11.Bar chart shows that about 95% people were in favor of enforcing law about plastic usage by government while only 5% people thinks no law enforcement about plastic ban by government.



	Reuse of plastic bottles, bags and its alternatives	Plastic usage awareness by social media	Satisfaction of buying new bottle instead of using old one
Yes	44.30%	14.50%	46.20%
No	38.40%	55.20%	24.40%
Sometimes	17.40%	30.30%	29.40%

Figure 18: Bar Graphs Showing Data Related to Plastic Usage

4. According to statistics obtained, about 44.30% people were in favor of reuse of plastic bottles, bags and its alternatives, while 38.40% people think that there should be no reuse of plastic bottles, bags and its alternatives. Only 17.40% of population reuse their plastic bottles, bags and its alternatives.

10. According to survey, about 14.50% people thinks that social media provide enough awareness regarding plastic usage. 55.20% people were not in favor of it while 30.30% people thinks that sometimes media provide enough awareness.

12. Statistically, about 46.20% people feel satisfaction about buying new water bottle instead of reusing old one. 24.40% people think that buying new water bottle doesn't provide any satisfaction to them. However, statistics of people that sometimes feel satisfaction of buying new water bottle is 29.40%.

4.2.3.2 Section III:

This section includes questions related to "Plastic disposal and Hazards"

4.2.3.2.1 Representation through Pie-Charts:

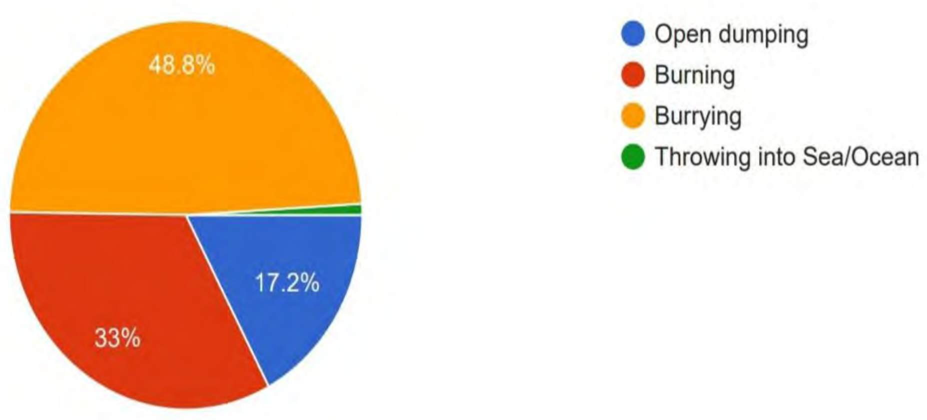


Figure 19: Best way of Disposal of Plastic

Above data shows that about 48.8% of people think that burying is best method of disposal. 33% of people think that burning is the best method. According to 17.2% people, Open dumping is best method of all. However, only 1% people think that best method is throwing into Sea/Ocean.

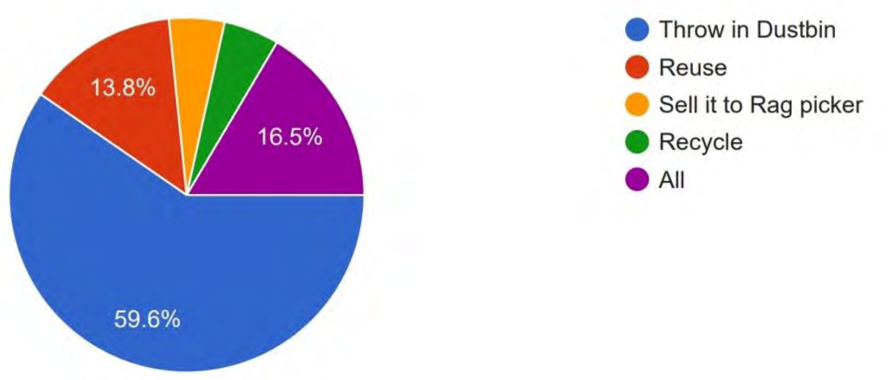


Figure 20: Behavior of people after Using Plastic

According to above statistics, about 16.5% people use all methods after finishing using plastic. Almost 59.6% people throw plastic in dustbin after its usage. About 13.8% people reuse that plastic. 5.1% people sell it to rag picker after its usage and 5% people recycle it.

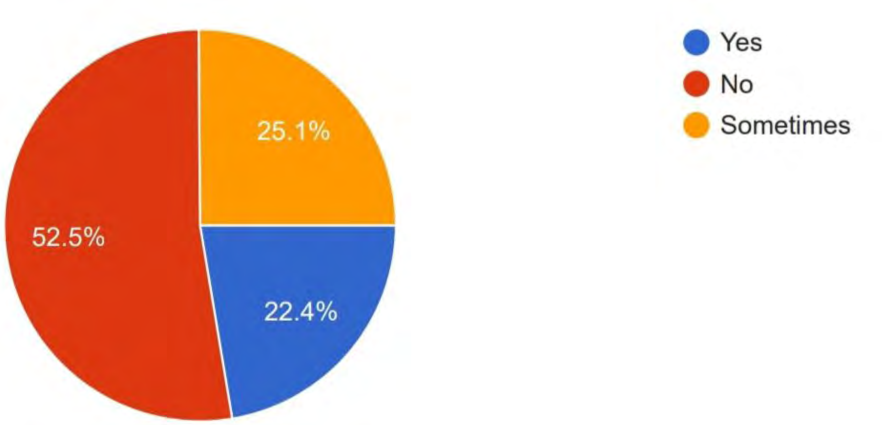


Figure 21: Heating of Food in Plastic or Polystyrene

Statistical analysis shows that about 22.40% people heat food in plastic or polystyrene material at home, while 52.50% people don't heat food in plastic or polystyrene material at home. About 25.10% people heat food material in plastic sometimes.

4.2.3.2.2 Representation through Bar graphs:

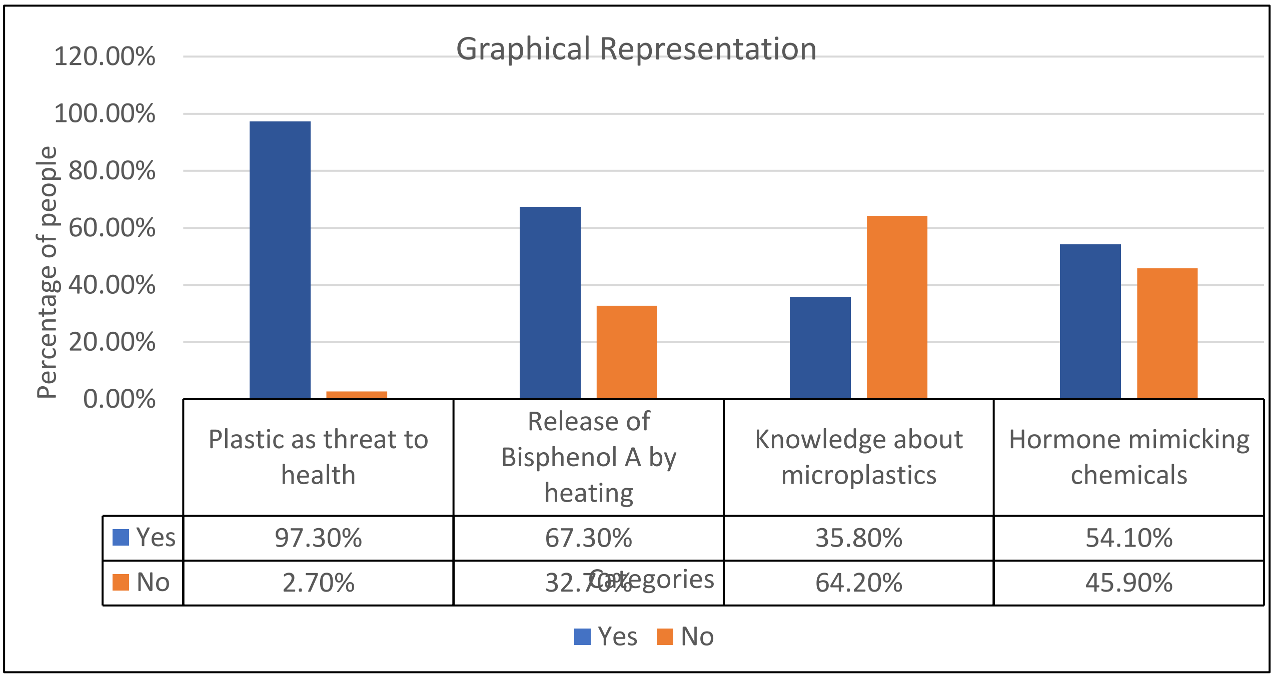


Figure 22*:* Bar Graph Showing Data Related to Plastic Hazards

16. Results from above bar graph shows that about 97.30% people have knowledge about plastic as threat to health, while only 2.70% don't consider plastic as threat.

20. From above results second bar graph shows that about 67.30% of people have knowledge regarding release of Bisphenol A from plastic after heating that cause brain damage, while 32.70% percent people have no knowledge about it.

22. Third bar graph shows that about 35.80% people have knowledge about microplastics while 64.20% people have no knowledge about it.

24. The last bar graph shows that about 54.10% people have knowledge about release of hormone mimicking chemicals by heating, while 45.90% people have no idea about it.

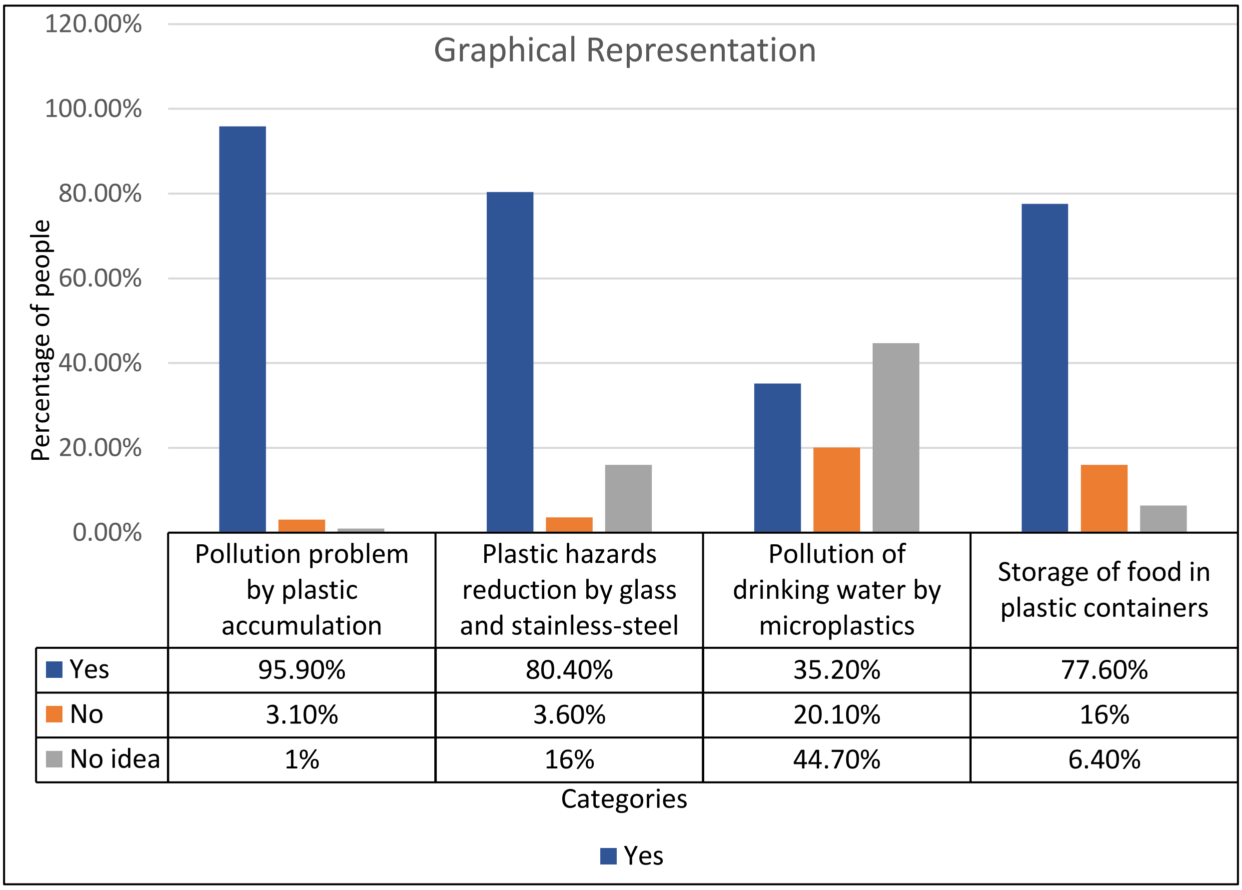


Figure 23: Bar Graph Showing Data Related to Plastic Hazards

19. First bar graph shows that about 95.90% people think that plastic accumulation is causing pollution problem. 3.10% people think that plastic accumulation does not cause pollution problem according to above data. Only small fraction of people about 1% have no idea about this.

21. From above data about 80.40% people thinks that plastic hazards in humans can be reduced by using glass or stainless-steel containers, while about 3.60% think that using glass and stainless-steel will not reduce plastic hazards. 16% people have no idea about this.

23. Third bar graph shows that about 35.20% people have knowledge of microplastics causing health hazards to human by drinking water.20.1% people have no knowledge about this while approximately 44.7% people have no idea about this information.

25. According to statistics, about 77.60% people think that storing food in plastic containers effect health. while 16% people think that string food in plastic container do not affect human health. However, 6.40% people have no idea about it.

Conclusion:

Polyethylene, Polystyrene and PET play an important role in our everyday life because of having durability and cheapness. These plastics are used in form of plastic bags, plastic cups, plastic bottles and toys as well as for other purposes. Plastic accumulation in an environment is leading a great threat to environmental and marine life. There are many microorganisms that have capability to degrade PE, PS and PET. They have slow rate of degradation process. The rate of degradation is modulated by UV or Oxidation. Microbial degradation of plastics depends upon degree of oxidation crystallinity and most important is molecular weight of plastic. After degradation, changes in physic-chemical properties of plastics can be analysing by SEM, FTIR and AFM. The purpose was to isolate that particular microbe that grow on PE, PS and PET. A survey was conducted to know about usage, Disposal and hazards awareness of people related to plastics. More research regarding microbial degradation of polyethylene is still in process.

Appendix:

Plastic: Its usage, Disposal and Hazard

This survey aims to access the use of plastic in daily life, to aware people about its adverse effects on environment and human beings. Every respondent should fill the form voluntarily.

Age: **Gender:**

Level of Education: **Occupation:**

Plastic Usage

1. What kind of plastic products do you use in your daily life?

   a. Plastic bags b. Plastic bottles c. Plastic bins

   d. Polystyrene cups e. All

2. Why you prefer to choose plastic bags and plastic cups?

   a. Durability b. They are cheap and easily available

   c. Lack of alternatives d. Light in weight

3. What kind of alternatives you prefer to use instead of plastic bags?

   a. Paper bags b. Cloth bags c. Both

4. Do you reuse plastic bottles, bags and its alternatives?

   a. Yes b. No c. Sometimes

5. Which of the following is cheaper in your opinion?

   a. Paper bag b. Cloth bag c. Plastic bag

6. Do you make sure that plastic you are using is biodegradable?
   a. Yes b. No c. No idea

7. Do you think cloth bags are longer lasting and have less hazardous effects as compared to plastic bags?
   a. Yes b. No

8. Do you think we should recycle our plastic bags instead of buying new ones?
   a. Yes b. No

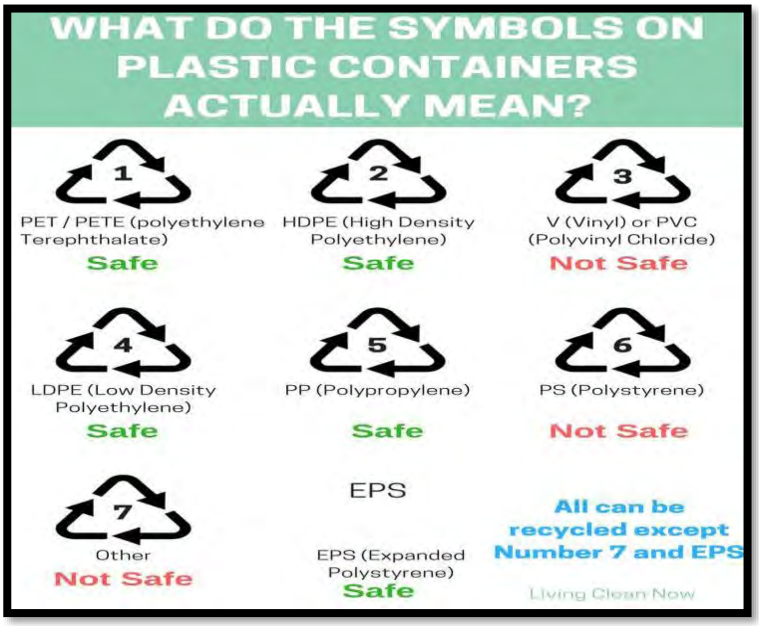
9. Do you think non-degradable plastic should be completely banned?
   a. Yes b. No

10. Do you think social media provide enough awareness regarding plastic usage?
    a. Yes b. No c. Sometimes

11. Do you think Government should make any law regarding plastic usage?
    a. Yes b No

12. Do you think that buying new mineral water bottle gives you satisfaction instead of using old one?
    a. Yes b. No c. Sometimes

13. Do you consider these symbols while using plastic?
    a. Yes b. No



Plastic Disposal and Hazards:

14. Which of the following is best way to dispose plastic?

a. Open dumping b. Burning c. Burrying

d. Throwing into Sea/Ocean

15. When you have finished using plastic, how you treat it?

a. Throw in dustbin b. Reuse c. Sell it to Rag picker

d. Recycle e. All

17. Do you think plastic is a threat to your health?

a. Yes b. No

18. Do you heat food or drinks in plastic or polystyrene material at home?

a. Yes b. No c. Sometimes

19. Do you think that using hot food in plastic containers cause cancer?

a. Yes b. No c. No idea

20. Do you think plastic accumulation lead to pollution problem?

a. Yes b. No c. No idea

21. Do you know that heating plastics release certain chemicals called Bis phenolA that cause brain damage?

a. Yes b. No

22. Do you think that glass or stainless-steel containers can reduce plastic hazards in humans?

a. Yes b. No c. No idea

23. Do you have any idea about Microplastics?

a. Yes b. No

24. Do you know that micro plastics (<5 mm) pollute drinking water causing hazards to Human health?

a. Yes b. No c. No idea

25. Do you know that heating plastic release hormone mimicking chemicals?

a. Yes b. No

26. Do you think stored food in plastic containers also effect health?

a. Yes b. No c. No idea

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