“Synthesis and Biodegradation of HDPE Microplastics using Bacteria Isolated from Polluted Riverside Soil”

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT
FOR REQUIREMENT OF THE DEGREE OF
MASTER OF TECHNOLOGY
IN
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Submitted by
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(2K19/IBT/05)

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CANDIDATE'S DECLARATION

I, **Megha, 2K19/IBT/05**, student of **M.Tech (Industrial Biotechnology)**, hereby declare that the project dissertation titled **"Synthesis and Biodegradation of HDPE Microplastics using Bacteria Isolated from Polluted Riverside Soil"** is submitted to Department of Biotechnology, Delhi Technological University, Delhi, by me in partial fulfillment for the award of degree of **Master of Technology (Industrial Biotechnology)**. This thesis is original work done by me and contains information with proper citation. This project work has not formed the basis for award of any degree, fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify the project dissertation entitled **"Synthesis and Biodegradation of HDPE Microplastics using Bacteria Isolated from Polluted Riverside Soil"** submitted by **Megha, 2K19/IBT/05**, Department of Biotechnology, Delhi Technological University, Delhi, in partial fulfillment for the award of degree of **Master of Technology (Industrial Biotechnology)**, is a project done by the student under my guidance and supervision. To best of my knowledge and belief, the work has not formed basis for any degree or other work to this university or elsewhere.

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ABSTRACT

Plastic wastes in environment pose an ever-increasing ecological threat towards the environment. Biodegradable plastics can be considered environment friendly, have a range of potential applications. However, the persistent use of petroleum-based plastic products has created a menace to the environment and its sustainability. In this project, degradation of high density polyethylene using microorganisms was analyzed for around 40 days of incubation in liquid culture method. Two bacterial strains, A and B, were isolated from a landfill site to mitigate the degradation of HDPE microplastics. These bacterial strains were allowed to grow on a mineral salts medium (MSM) without a carbon source. A shake flask liquid culture was maintained to observe the growth of bacterial cells when infused with HDPE microplastics. The biodegradation extent of HDPE microplastics was analyzed by recording the reduction in weight of HDPE microplastics before and after bacterial incubation. The morphological and structural changes of HDPE microplastics were observed by scanning electron microscopy, Fourier Transform Infrared microscopy, transmission electron microscopy, whereas thermo gravimetric analysis could provide degradation rates of microplastic particles with respect to temperature. Therefore, this study is helpful in assessing biodegradation of HDPE microplastics to remediate the environment and provide sustainability.

Key words: Biodegradation, Bacterial Isolates, HDPE Microplastics, Plastic Pollution, Sustainability

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LIST OF ABBREVIATIONS AND SYMBOLS

º - Degree Celsius

HDPE- High-Density Polyethylene

LDPE- Low-density Polyethylene

TGA- Thermo Gravimetric Analysis

UV- Ultraviolet

BPA- Bisphenol A

MSM- Mineral salt media

PCB- Polychlorinated Biphenyls

CHAPTER 1 - INTRODUCTION

Plastic pollution symbolizes one of the most serious risks to ecosystem and human health. Many of over-all mess unrestricted in the normal atmospheres fit in to the minor size plastic-debris i.e., (microplastics and nanoplastics) which come after an extensive range of causes, comprising attire, cosmetics, trawling, & manufacturing methods (Alimi et al., 2018). The ingestion of tiny microplastic by aquatic species like turtles, mammals, cetaceans, fish, seabirds, is producing worry within the methodical society, administrators, procedure creators or the common civic (Andrady, 2011). Micro-plastics can also affect the marine plants while causing harm to the physical characteristics and soil biota (Li et al., 2015). But the effect of microplastics on marine organisms is more harmful compared to aquatic plants. As microplastics already exist in a variety of seafood matters, there is powerful encouragement for the allocation of microplastic elements to the human beings (Zhu et al., 2018). When human consumes plastic debris, it can cause direct or indirect health problems on human and water bodies. Different sectors contribute to large amounts of plastic waste as depicted in figure 1.1 (Geyer et al., 2017). Especially because of microplastics small size, particles could be easily consumed or collected in the brain, or nerves, and also in the circulatory system of the creatures which causes many adverse effects (Bouwmeester et al., 2015).

HDPE MICROPLASTICS

In recent years, research studies focusing on fate, source, allotment and migration of microplastics have gained significant importance because of the widespread pollution caused by small plastic fragments (Avio et al., 2017). Different type of microplastics are present in environment including low-density and high-density polyethylene. Of these, HDPE is a synthetic polymer having hydrophobic nature and high molecular weight (Balasubramanian et al., 2014). Different abiotic factors including temperature, pH, and light influence the decomposition of synthetic polyethylene with application of microorganisms. Several effects of polyethylene exist in the natural ecosystem causing detrimental impact on environment. For various years, researchers have made significant contributions in improving the stability and degradability of polyethylene. The disposal of HDPE microplastics in ecosystem causes many problems and threat to biological ecosystem (Balasubramanian et al., 2014). But effective polyethylene degrading microorganisms are needed to remove these microplastics (Chowdhary et al., 2020a).

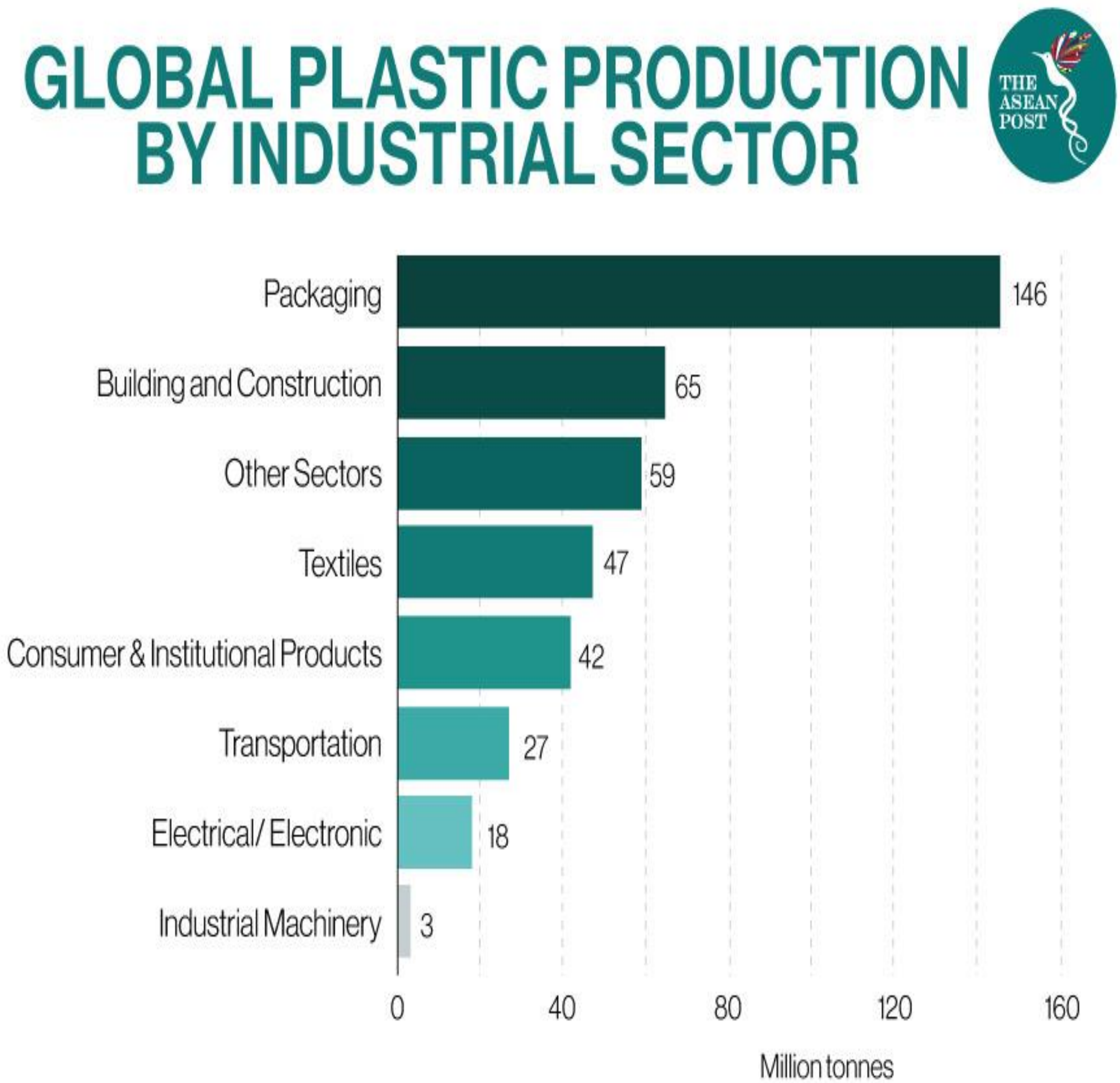


Figure 1.1: Production of plastic from different commercial units (Adapted from Geyer et al., 2017)

Elimination of plastic materials using physical or chemical approaches is expensive and produces organic pollutants including persistent organic pollutants, volatile organic pollutants and heavy metals, reported to be toxic pollutants causing alterations in soil structure and stability, depletion of ground water sources and dangerous to human and animals (Ojha et al., 2017). However, decomposition of large synthetic polymers using microorganisms produces carbon dioxide, water and methane.

BIODEGRADATION OF HDPE MICROPLASTICS

Huge amounts of plastic products are disposed each year from packaging materials and other commercial products thereby polluting the land and water bodies. These plastic particles are degraded by light called photo-degradation, by utilizing heat energy, or using microorganisms known as biodegradation (Ahmed et al., 2018). Although biodegradable plastic products are being used in today's scenario, their utilization is not feasible or accessible to a large section of the population (Lambert and Wagner, 2017). Mineralization of large synthetic polymers to carbon dioxide requires various microorganisms that help in breakdown of polymer into its monomers and by-products with excreted wastes (Ojha et al., 2017). So the eco-friendly process of biodegradation can help to remove plastic waste. During the process of biodegradation, microorganisms utilize oxygen present in the air to survive. These microorganisms utilize the oxygen to secrete polymer degrading enzymes that facilitate the breakdown of synthetic polymers into its by-products along with carbon dioxide and water (Albertsson et al., 1998). Many enzymes are used to degrade plastics including esterases, lipases, and cutinases that have potential to breakdown polymers (Liebminger et al., 2007). Different microorganisms have been studied to report the degradation of plastics, and hydrophobic nature of HDPE microplastics makes it adverse to biodegradation (Yoshida et al., 2016). Hence it is necessitated to solve the problem of HDPE microplastics and restore the natural ecosystem through biodegradation process.

OBJECTIVE OF THE STUDY

Therefore, this project is done to determine biological breakdown of HDPE. Degradation was preceded after inoculation of microplastics culture with bacteria isolated from a polluted riverside soil. To study characteristic attributes of HDPE microplastics after treatment with microorganisms could facilitate research on plastic degradation in the environment employing microbes.

The objectives of the study are:

a) Synthesis of HDPE microplastics

b) Biodegradation of microplastics by bacteria

c) Analysis of degradation of microplastics by bacteria

Various analytical techniques were utilized in a view to determine extent of degradation of plastic sheets (HDPE). Different analytical method employed in this study includes:

a. Transmission electron microscopy (TEM),

b. Scanning electron microscope (SEM),

c. Fourier-transform infrared (FTIR) spectroscopy,

d. Growth pattern using Spectrophotometer, and

e. Thermo Gravimetric analysis (TGA)

These methods could help in assessing the degradation rate of HDPE microplastics after bacterial inoculation.

CHAPTER 2 – REVIEW OF LITERATURE

Plastic Pollution

Pollution due to plastic accumulation is identified as the most prominent contaminant of concern because of its properties of flexibility, durability, low cost, corrosion resistance and easy handling (Botterell et al., 2019). The worldwide increase in production and mass consumption has caused ubiquitous accumulation of plastics in oceans and soils (Wang et al., 2019). Moreover, traditionally-employed plastics usually petroleum-hydrocarbon fossil fuels, are major drivers of diverse environmental harms including climate alteration and biodiversity failure, thereby requiring to be removed completely from environment. Furthermore, plastics that are biodegradable in laboratory conditions as well as in waste management and natural conditions merely exist (Briassoulis and Innocenti, 2017). Large plastic fragments interact with marine taxa by various processes including ingestion and entanglement. However, these large particles in marine environment are worn-out into minor pieces by UV degradation, physical abrasion and wave action, eventually forming microplastics (de Sá et al., 2018). Microplastics are small synthetic fragments formed by breakdown of large macro plastic particles utilizing different mechanical and physical processes. Approximately, 90% of plastic waste in oceans is microplastics owing to their very small size of less than 5mm (Auta et al., 2017). Abundance of microplastics in aquatic ecosystem poses potential threat to aquatic flora and fauna with significant adverse impacts on oceans, rivers, coastal areas, and seas.

Source of microplastics in ecosystem

Prevalence and accumulation of microplastic particles in environment can be attributed to various sources. The major sources of microplastic can be attributed as primary microplastic that results from direct release of pellets or powders and microbeads from cosmetic formulations in addition to secondary microplastics arising from fragmentation of large plastic particles (Thompson, 2015). Table 2.1 provides a brief summary of all the sources of microplastics from different sectors through which they pollute the environment.

Table 2.1: Source of microplastics in environment

Category	Industry source	Microplastic contaminant	References
Waste management industry	Solid waste and wastewater	Microbeads, fragments and fibres	(Verma et al., 2016)
Producers	Plastic producers, recyclers	Pellets and fragmented plastic materials	(Bai et al., 2018)
Consumers	Shipping or offshore industry	Paints, pipes and clothes	(Jambeck et al., 2015)
	Aquaculture	PVC pipes and lines, nets	(Thompson, 2015)
	Agriculture	Nutrient pills, pots and greenhouse sheets	(Alimi et al., 2018)
	Sports	Turfs	(Lynch, 2018)
	Fisheries	Fishing gear and packages	(Andrade et al., 2019)
	Construction	Packaging and polymer cement materials	(Kawecki and Nowack, 2019)
	Textile industry	Fibres and clothing	(Mishra et al., 2019)
	Tourism industry	Consumer goods,	(Garcés-Ordóñez

		cosmetics, textile fibres	et al., 2020)
	Terrestrial transportation	Pellets and tyres	(Emmerik and Schwarz, 2020)
Individual	Cosmetics	Microbeads and packaging, Containers	(Zhao et al., 2019)
	Food and drinks	Containers, plastic bags and bottles, caps, cups, plates	(Bauer-Civiello et al., 2019)

Impacts of microplastics in environmental systems

Emergence of microplastic in marine ecosystem results in either accumulation of plastic on water surface or benthic zone of water bodies (Au, n.d.). Microplastics are potentially available to wide range of organisms that consider small size of microplastics to be food source (Galloway et al., 2017). Different microplastic fragments have been ingested by aquatic organism including zooplanktons (Botterell et al., 2019), crustaceans, molluscs, sea birds and corals (Hall et al., 2015). The ingestion of microplastics have various detrimental effects on organisms such as restricted growth, reproduction, reduced feeding and physical injury (Bellasi et al., 2020) (figure 2.1). Microplastics offer more surface area-to-volume ratio for accumulation of various contaminants including toxic metals and polychlorinated biphenyls (PCB) (Ozcan et al., 2013). The major threat caused by surface microplastic is on duckweed that causes abaxial leaf shallow of the duckweed (Egbeocha et al., 2018). Solid microplastic with harsh ends can directly disturb the end length of the duckweed and also cause influence on the end tissues. The chemicals can bioaccumulate in biological tissue and cause adverse effects in aquatic food chains. Moreover, added chemicals and additives in plastic manufacturing and organic pollutants are real threats to marine organisms (Hong et al., 2018).

So, when microplastic enters the marine ecology and connects with the floras, they quickly get ingested and also result in formation of biofilms (Gong et al., 2019). The immediate outcomes of ingestion and entanglement could occur in the marine or seaside biotic organisms that may get damaged lethally. Sub-deadly impacts include damage of the sensitivity, impairing reproduction capability, damage of mobility, reduced growth and body condition, lack of ability to escape from the predators (Zhu et al., 2018). The consumed particles frequently contain the micro debris sized particles that are capable to enter easily into the gut without harming or affecting the beings. These microplastic particles can stick inside in the throat, stomach, or gastro- intestinal region and can originate harm in the body.

Fishes, sea-birds, sea-turtles, and aquatic organisms can become knotted in or ingest plastic particles, which results in causing suffocation, food shortage, and dying (Ozdilek et al., 2006). Many birds in the marine ecosystem also encounter with microplastics in water bodies in search for food, and thereby meet plastic debris. Fishing lines and six-pack rings are the supreme reasons of tangling by sea-birds (Savoca et al., 2016). Many researchers have found occurrence of microplastic in the intestine of numerous fishes. Microplastic consumption through the marine lobster *Nephrops norvegicus* was experimentally observed as their learning proved that 83% of trial mammals had confirmed test positive, while examining on the similar species found 262 micro-plastic molecules from the 103 individuals gathered from the field (Murray and Cowie, 2011). Marine mammals like dolphins and whales are known to ingest majority amount of microplastic debris (Frasier, 2020). Many scientists have examined microplastic consumption by microbiota like zooplankton (Botterell et al., 2019), marine isopod—*Idotea emarginata, Calanus helgolandicus; Daphnia magna*; Amphipod *Orchestoidea tuberculate* (Cole et al., 2015). A number of other species like birds and turtles were found to consume the marine debris. This consumption of microplastic can diversely affect the respiratory system and can cause damage in different organism, also harm to the stomach lining of digestive system.

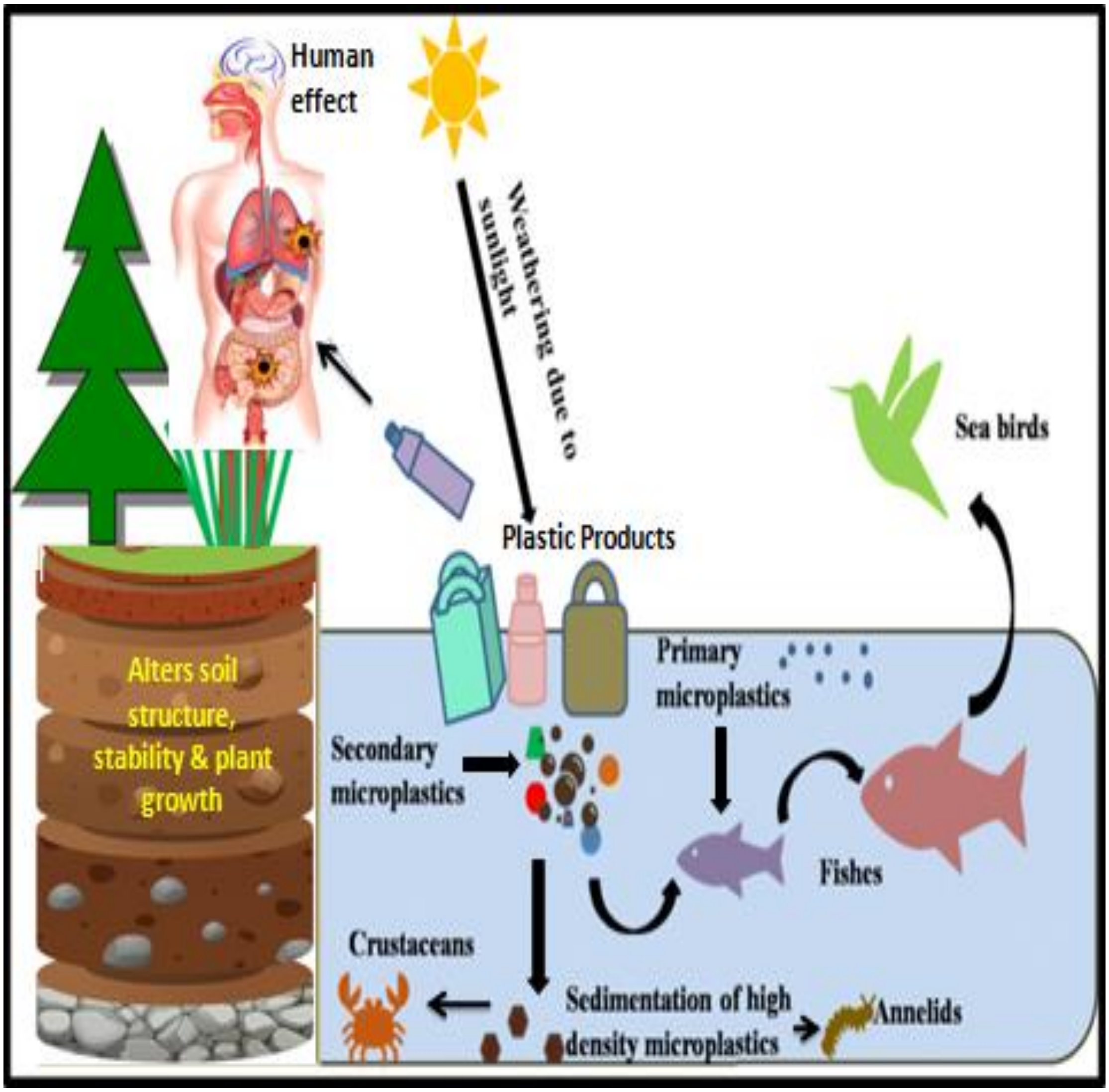


Figure 2.1: Microplastics effect to aquatic organisms, soil, plants and human

The effect of different microplastics were determined in spring onion (*Allium fistulosum*) (de Souza Machado et al., 2018b). The structural property of soil was affected by all type of plastics. Significant decrease in soil bulk density was observed in soils treated with PE, PP, PET whereas increase in soil bulk density in rhizosphere was observed in all the plastics. Addition of plastics in soil can affect nitrogen cycling and soil organic carbon. Critical limits for plastic contamination are rarely defined in researches which make it harder to evaluate the bearing capacity of agricultural ecosystems (Ruimin et al., 2019). The continuous use of plastic films has left residual plastic film particles in farm soil affecting infiltration of water. Residual film in farms affects soil porosity as it is expected to occupy soil pores and also affects water infiltration by blocking the soil pores (Wang et al., 2020). Microplastics taken up by soil organisms accumulate in their system. The accumulation of plastics can affect the feeding behavior and growth of the organisms. An experiment by Cao et al., 2017 observed that higher concentrations of microplastics i.e. 1% and 2% (w/w) in mixed soil caused 27.6% and 29.8% decrease in the weight of earthworms affecting their growth and results in lethality. An additional experiment through Lwanga et al., 2017 showed lower concentration of microplastic in soil leads to higher concentration of plastic in casts, chicken gizzard and chicken faeces (Lwanga et al., 2017).

Plastics have harmful chemicals like Bisphenol A (BPA), phthalates, and poly- fluorinated chemicals that affect human and environment (Hahladakis et al., 2018). The toxic compounds in plastics cause problems like vision failure, eye irritation, difficulty in breathing, respiratory problems, liver and lung problems, cancers, skin disorders, dizziness and headache, birth defects, gastrointestinal, cardiovascular, genotoxic problems etc. (Proshad et al., 2017). Ingestion of plastic debris by individuals can cause complete intake of earthly and marine foodstuffs. Even though seafood is an accepted cause of threat to the human regimen, existence of plastic remains in aquatic seafood still requires more research. Marine food is a vital component for individual regimen; besides presence of microplastics in aquatic food lead to a severe risk to individual (Bouwmeester et al., 2015). Marine food might be polluted by the microplastics from the consumption of normal prey, observance of the organisms or throughout handling and marketing period. Several studies have confirmed the existence of plastics debris in the eatable fishes, prawns, and by consuming them; microplastics enter in humans as an outcome of the bio-magnifications (Prata, 2018). Foodstuffs are not only the cause of microplastic accumulation in human. The plastic exposure can also arise from breathing of the air or breathing treatments/tablets. It can straightly inhale and can accumulate in the breathing system of the human body (Gasperi et al., 2018). Adversative health effects can decrease country's efficiency and waged proficiency with harmful influences on public and economic features of the exaggerated zone. Although many studies have focused on microplastics in intestinal tracts of aquatic organisms, most research have focused on identifying impacts of microplastics using scientific concentrations that are outside range defined by natural levels present in oceans (Critchell and Hoogenboom, 2018). Moreover, various bio solids leached from wastewater treatment plants also contribute substantial proportion of microplastics fragments. The microbeads in cosmetic products and fibres also cause pollution of microplastics in aquatic ecosystem (Mason et al., 2016). Microplastics bioaccumulation in marine environment increases with decreasing size and serve as a surface for proliferation of bacterial pathogens (Michielssen et al., 2016). Thus, it is essential for determining the risks linked with ingestion of microplastic particles to identify potential response and behaviour of microplastics in aquatic ecosystem.

HDPE Microplastics

The two forms of polyethylene (HDPE and LDPE) are common types of polymer used in various products including carrier bags, plastic bottles, cosmetics, pharmaceuticals and other (Thompson, 2015). Weathering process results in decomposition of around 80% polyethylene forming microplastics (Grause et al., 2020). HDPE is a thermoplastic with a linear structure and having no degree of branching (figure 2.2). The temperature required for its manufacturing is low (70-300 ºC) and pressure of (10-80bar). It is mostly derived from modified natural gas (methane, ethane, propane) or catalytic breakdown of crude oil into gasoline (“Polyethylene (PE) Plastic,” n.d.).

HDPE is flexible, weather resistant, and displays toughness at very low temperatures. The various properties of HDPE are:

i. Melting point: 120-140 ºC

$-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-$

Figure 2.2: Structure of HDPE

ii. Density: 0.93 to 0.97 g/cm^3

iii. High tensile strength

iv. Low cost polymer

v. Low temperature resistance capability

vi. Excellent electrical insulator

vii. Low water absorption

viii. Resistance to alcohols, solvents, acids and alkalis

ix. Poor resistance to hydrocarbons

x. Resistance to UV light poor

Biodegradation of Microplastics

Different properties of microplastics including their hydrophobicity and lack of metabolic activity to polymerize the plastics, makes it difficult to undergo biodegradation (Chowdhary et al., 2020b). However, biodegradation is possible by formation of microbial biofilms on surface of microplastic fragments (Rummel et al., 2017). These biofilms allow for growth of bacteria and other organisms that could potentially help in degradation of plastic (Lobelle and Cunliffe, 2011). Also the weight of various plastic polymers can be reduced by incubating different microbial strains (Harshvardhan and Jha, 2013). Additionally, surface of microplastics containing pits could also be an indicator for bacterial species to degrade the polymers (Zettler et al., 2013). The plastic fragments formed after the chemical (abiotic) degradation are buried deep in marine environment and takes years for degradation (Fotopoulou and Karapanagioti, 2019). The microbial biofilms attached to the surface of polymer allows the formation of various enzymes that induce breakdown of plastic by hydrolysis (Ho et al., 2018) (figure 2.3).

Various microorganisms have potential to produce enzymes that result in degradation of polymers; for example, *Thermobifida fusca* produces an enzyme, hydrolase capable of degrading PET (Barth et al., 2016; Jabloune et al., 2020). Strains of *Bacillus cereus* and *Bacillus sphericus* produce peroxidase that helps to degrade PE (Yuan et al., 2020). Similarly, degradation of polyethylene (PE) by alkane hydroxylases obtained from *Pseudomonas* sp. E4 act as important contributors in LDPE degradation (MoonGyung et al., 2012). Also, fungal and bacterial laccases help in oxidation of PE and heme peroxidases act as fungal degraders of PE (Gómez-Méndez et al., 2018). Thermophilic consortium including *Brevibacillus* sp. and *Aneurinibacillus* sp. could also enhance degradation of polyethylene and polypropylene (Skariyachan et al., 2018). Enzymatic degradation of PET using hydrolases, esterases, proteases and cutinases has shown to hydrolyze PET surfaces. Modification in enzymes can improve the specificity and efficiency of PET degradation. For example, recent studies have shown that microbial species, *Ideonella sakaiensis,* has the capability to degrade PET by action of enzymes (Glaser, 2019). A hydrolytic reaction of PET under the enzyme, PETase, has efficiency to produce ethylene glycol and terephthalate that are required for microbial growth (Vandermaesen et al., 2016). Similarly, other enzymes are employed for degradation of microplastics PU, and 6-aminohexanoate oligomers (PA) (Wei and Zimmermann, 2017). This is the most effective method for overcoming the problem of plastic degradation and water contamination.

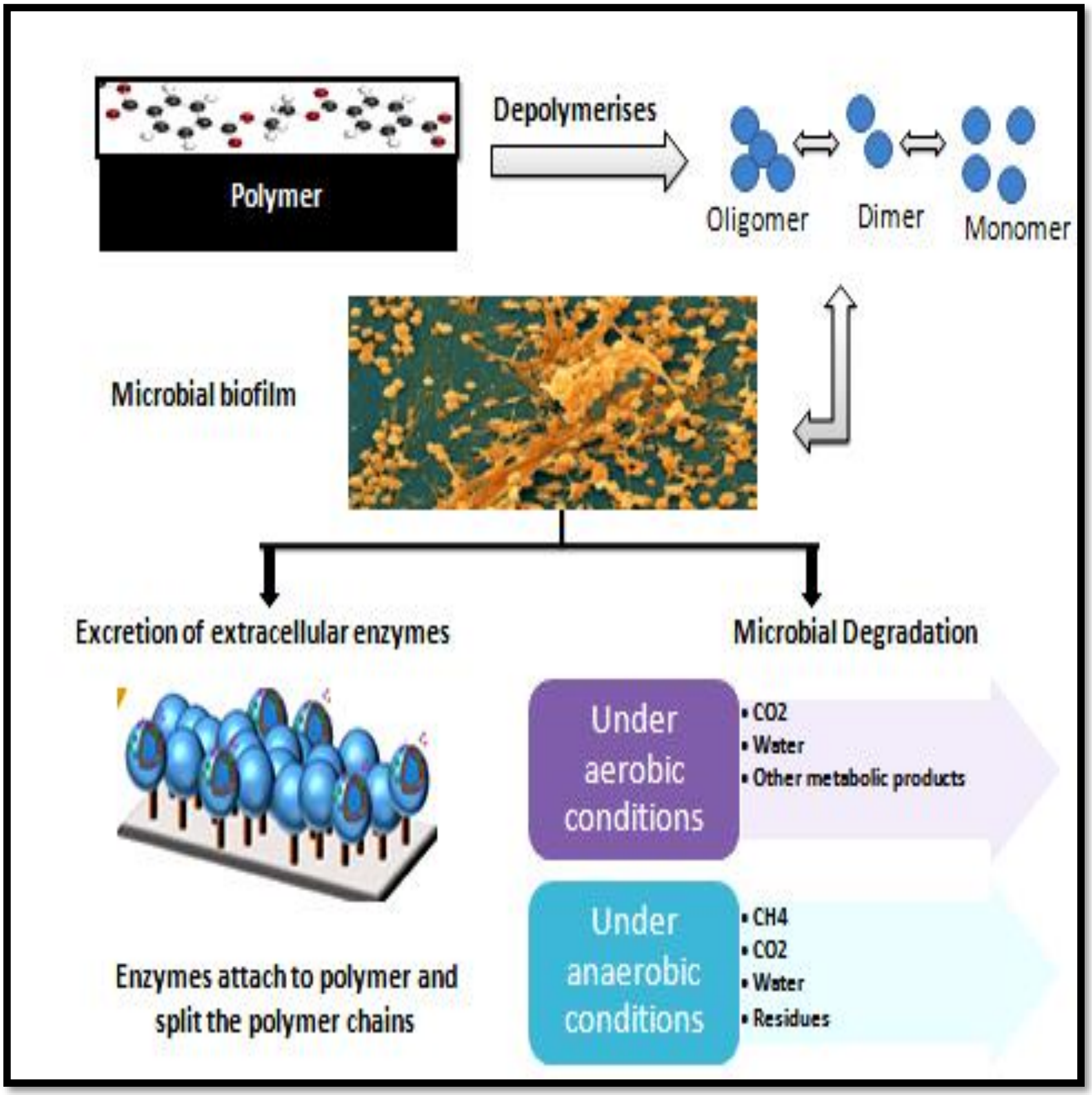


Figure 2.3: Mechanism demonstrating Biodegradation of polymer

Microbial degradation of synthetic polymers i.e. high-density polyethylene (HDPE) has been tremendously studied to investigate the capability of microbes (bacteria and fungi) from natural soil and water environment that facilitate degradation. Various species such as *Bacillus sp*. (Auta et al., 2018), *Rhodococcus sp*. (Auta et al., 2018), *Zalerion maritimum* (Paço et al., 2017), *and Pseudomonas sp.*, can help in reducing the weight of polymer materials inducing physicochemical changes and surface morphological structures and chemical morphology (Ahmed et al., 2018). The primary procedure for biodegradation of plastic is initiated by sticking of microbes on polymer surface and their proliferation (Kawai et al., 2019). These microbes help in excretion of extracellular enzymes that result in breakdown of plastics (Alshehrei, 2017). The enzymatic hydrolysis occurs in two ways: first is oligomers, dimers and monomers release degradation products that are converted to carbon-dioxide and water when enzyme attaches to polymer and hydrolytic division occurs (Roohi et al., 2017). Secondly, polymers are degraded by microbes in absence of air and new enzymes are needed to degrade the plastic in anaerobic conditions (Pathak and Navneet, 2017). Thus biodegradation of polymers results in production of microbial biomass, carbon-dioxide and water that can be used by aquatic flora and fauna.

This project work is done to analyze potential of bacterial strains in degrading HDPE microplastics. It is helpful in demonstrating the effects of biodegradation of HDPE microplastics by analyzing various parameters when exposed to a bacterial consortium isolated from polluted riverside soil. The results were analyzed and compared with control to address significant changes observed after incorporation of microplastics with microbes and determine their degradation potential.

CHAPTER 3 – MATERIALS AND METHODS

HDPE microplastic pellets and all chemicals used throughout this study are analytical and gradient grade obtained from standard manufacturers.

I. Collection of sample and Bacteria Isolation

The soil samples were collected from Hindon riverside soil at depths of 15 to 30 cm. Serial dilution followed by plating on nutrient agar (NA) plates kept at 25 ºC helped in identification of bacterial cultures. Morphologically differentiated bacterial colony be identified and then sub cultured for three generations in mineral salts medium (MSM) (Deionized water: 1litre; Dipotassium hydrogen phosphate: 2.27g; Potassium dihydrogen phosphate: 0.95g; Ammonium sulphate: 0.67g; $Na_2EDTA.2H_2O$:6.37g; $ZnSO_4.7H_2O$:1.0g; $CaCl_2.2H_2O$:0.5g; $FeSO_4.7H_2O$:2.5g; $NaMoO_4.2H_2O$:0.1g; $CuSO_4.5H_2O$:0.1g; $CoCl_2.6H_2O$:0.2g; $MnSO_4.H_2O$:0.52g; $MgSO_4.7H_2O$:60g).

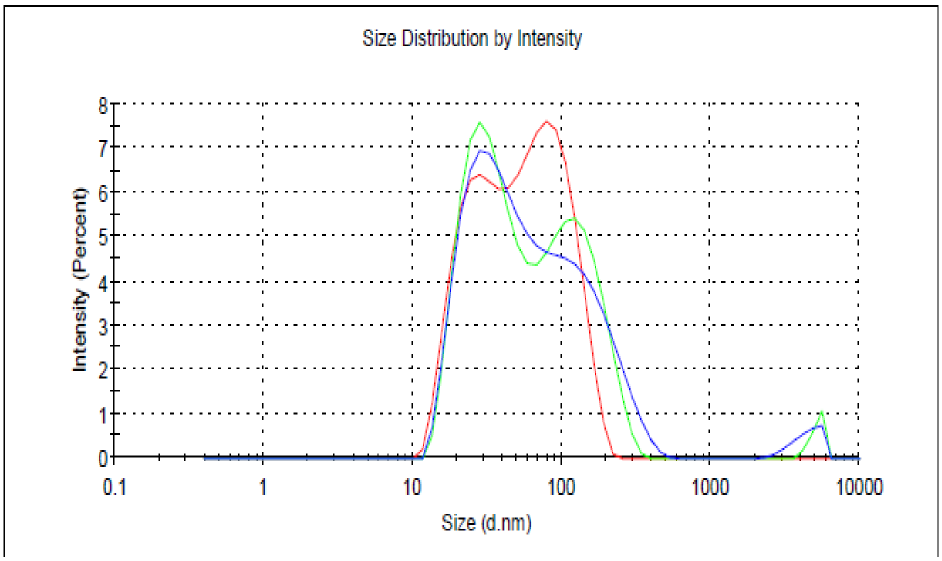
The mineral salts media contain all the essential nutrients excluding the carbon resource. Every bacterial isolate was grown in MSM containing 0.5g of HDPE microplastics. The media was observed for growth by comparing it with a control set (containing media but no polymer) maintained simultaneously.

II. HDPE Microplastic piece measurement

To compare size reduction of HDPE microplastics after biodegradation, ascertaining their size prior to experimental study is necessary. Size of HDPE microplastics was determined by means of Zetasizer Nano ZS (Malvern Instrument, UK) at an angle of 173° at 25 °C as shown in graph 3.1. To execute HDPE microplastic particle size, these particles were vortexed and then sonicated for 15 minutes. The resulted solution was subjected to fragment size laboratory analysis. Unit size was analyzed using refractive index of HDPE (1.49979) and (0).

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	48.09	Peak 1:	81.49	62.9	35.73
PdI:	0.382	Peak 2:	26.17	37.1	7.353
Intercept:	0.959	Peak 3:	0.000	0.0	0.000
Result quality	Good				



Graph 3.1: Size distribution of HDPE microplastics

III. Synthesis of HDPE microplastics

HDPE microplastics were synthesized using a method described by (Crespy et al., 2007) with some modifications. A solution containing 1g of the HDPE and 20 ml of xylene was stirred on magnetic stirrer for 1 hour until completely dissolved. Then in a 100 ml deionised water the HDPE solution was added slowly while keeping the sonication at optimum amplitude range of 70% (Branson sonifier W450 Digital) for 30s in cool condition. The final solution is then centrifuged, washed with water and ethanol, and finally air dried. Further the characterization of its size and thermal properties was done.

Figure 3.2: Branson sonifier W450 Digital

IV. Bacterial inoculums preparation and assessment of HDPE degradation

Bacterial culture obtained from serial dilution of samples collected from polluted soil was revived on nutrient agar plates. The bacteria culture was inoculated into 100 ml of MSM broth with and without containing 0.5 g of HDPE microplastics. The bacteria were allowed to grow in nutrient broth flasks kept in rotating shaker at 30 ºC at 120 rpm. Third generation culture is utilized for the biodegradation study, using log phase with an absorbance of 0.8 at 600 nm. The cultures in log phase with an absorbance of 0.8 at 600 nm were used in the degradation experiments. The bacterial growth of the culture was monitored by determining its absorbance at 600 nm using Eppendorf UV-Vis Spectrophotometer, (Bio Spectrometer basic model). The different parameters such as pH and Optical density (OD) were observed at every 7 days for 35 days.

Figure 3.3: Eppendorf UV-Vis Spectrophotometer for analysis of Optical Density

V. Determining residual reduction in weight of microplastic particles

Proceeding 35 days of incubation, the HDPE microplastics were recovered by process of filtration. Plastic particles were washed with 70% solution of ethanol followed by drying at 50 ºC in oven for the night. Left over weight of microplastics polymer were observed towards determining degradation of microplastics (Mohan et al., 2016). The weights before experimental study were obtained similar to methodology explained in above sections. Plastic polymer disintegration was ascertained in terms of percentage weight loss by following formula:

Weight loss of polymer in terms of percentage

$$= \frac{\text{Initial weight of polymer - Final weight of polymer}}{\text{Initial weight of polymer}} \times 100$$

VI. Scanning Electron Microscope (SEM)

The bacterial culture recognized as potential microplastic degraders were observed by scanning electron microscopy to determine their structural morphology (Zeiss Sigma VP Scanning Electron Microscope).

Figure 3.4: Zeiss Sigma VP Scanning Electron Microscope

VII. Fourier-transform infrared (FTIR) spectroscopy of HDPE microplastic

Changes occurred before and after the degradation of microplastic polymers were analyzed by FTIR spectroscopy (Perkin-Elmer 400 FT-IR/FT-FIR) at around 4000-450 cm^{-1} range of frequency.

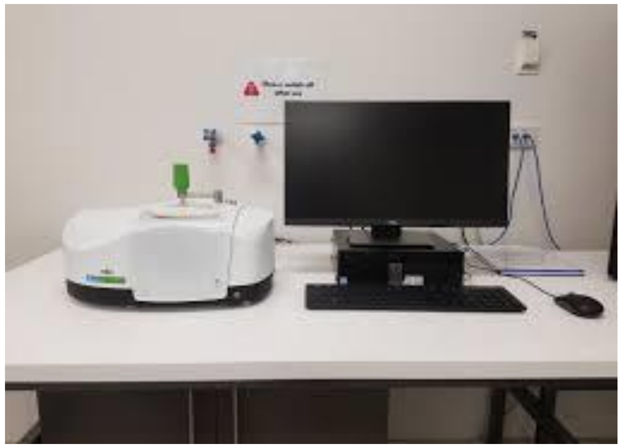


Figure 3.5: Perkin Elmer Spectrum 2 FTIR system

VIII. Thermo gravimetric analysis (TGA) of HDPE microplastics

The TGA of the treated and untreated HDPE microplastics was performed using Perkin Elmer thermo gravimetric analyzer TGA 4000.

Figure 3.6: Perkin Elmer thermo gravimetric analyzer TGA 4000

IX. Transmission electron microscopy (TEM) of HDPE microplastics

The ultra structure of HDPE before and after treatment with bacterial culture was determined by TEM (Tecnai G2 200 KV HRTEM SEI HOLLAND).

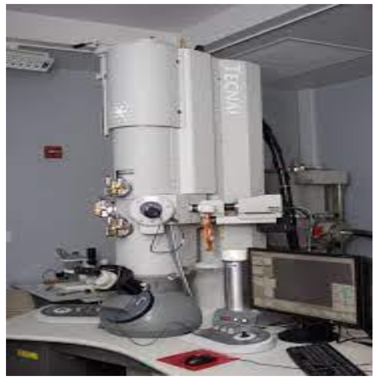
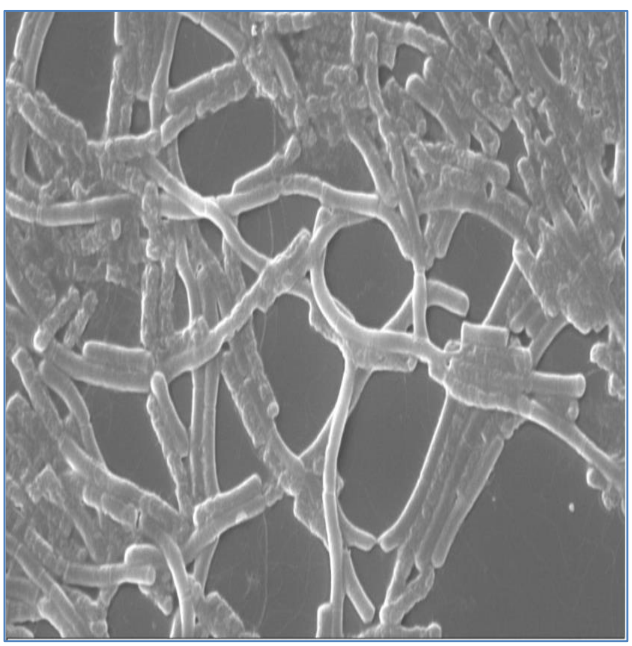


Figure 3.7: Tecnai G2 200 KV HRTEM SEI HOLLAND for TEM analysis

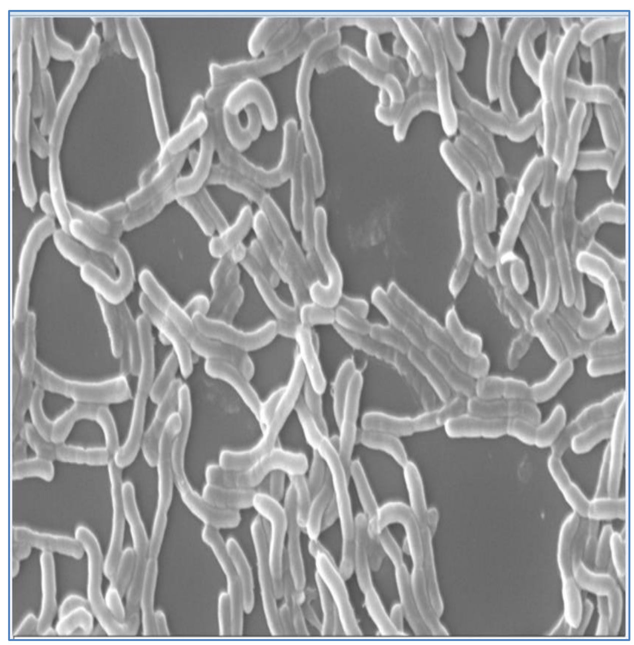
CHAPTER 4 – RESULTS AND DICUSSION

I. Bacterial screening of isolates

Landfill sites are considered as habitats with high microbial counts and distribution. These microbes are environmental ecosystem for large diversity of bacteria (Stamps et al., 2016). A global necessity in today's scenario is to combat plastic contamination, particularly microplastics. So, possible solution to remediate environment from microplastics are utilization of microbes that help in degradation of plastic pollutants. From the present study, two bacterial isolates, bacteria A and bacteria B were isolated and capable of growing on microplastic-infused media. These bacterial isolates had the ability to degrade microplastics due to their possibility of having enzymatic components that helped in degradation. Two bacteria identified were determined to be gram-positive with rod-shaped and variable rod structural morphology as observed by SEM.



Bacteria A (Rod-shaped)



Bacteria B (Variable-rods)

Figure 4.1: SEM images of bacterial isolates

II. Determination of weight loss in HDPE microplastics by bacterial isolates

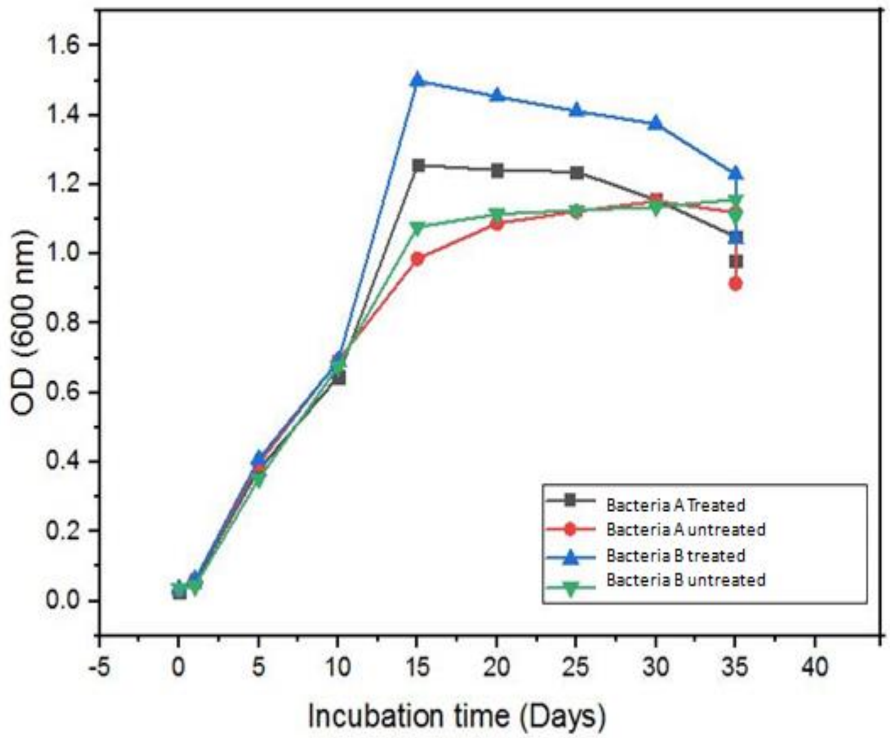
The action of bacterial isolates on HDPE microplastics resulted in a weight loss of microplastic particles. The reduction in weight was observed to be around 58% and 73% after 35-40 days of incubation with bacterial strains A and B. This finding has implied capability of bacterial strains containing enzymes to act on HDPE microplastics and subsequently cause their degradation. Control flasks showed no bacterial strains.

Treatment with Bacteria A= $\frac{.5\ g - .2119\ g}{.5g} \times 100 = 58\%$

Treatment with Bacteria B = $\frac{.5\ g - .1354\ g}{.5g} \times 100 = 73\%$

III. Growth pattern of bacteria A and B on microplastic exposure

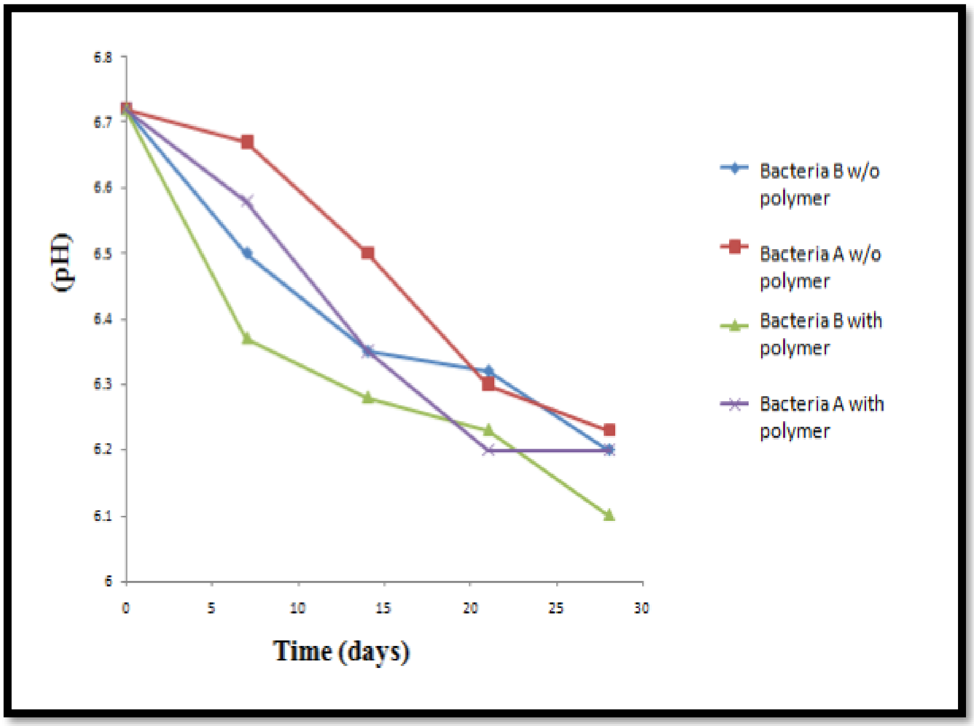
The growth patterns of bacterial isolates are presented in graph 4.2. Both the bacterial strains demonstrated significant growth on exposure to HDPE microplastics. The increase in growth pattern could be attributed to interaction between cell membrane of bacteria and microplastics allowing for metabolism. The highest OD for bacteria A was observed after 15 days of inoculation at 1.25nm and for bacteria B after 15 days incubation at 1.5nm. Gradual decrease in trend was observed after an exposure of around 35 days due to less bacterial counts. Increase in microbial biomass because of substrate utilization by bacteria caused the microplastics degradation. The decline in growth of bacterial cell occurred due to lyses of cell, depletion of nutrients and inhibition products. The decreasing trend was also due to inability of bacteria to adapt culture conditions and also degradation products of microplastics could also render the culture media unfavorable for growth and proliferation of bacterial isolates. Thus a declining trend was observed after 20-25 days.



Graph 4.2: Growth curve before and after treatment

IV. Change in pH during degradation

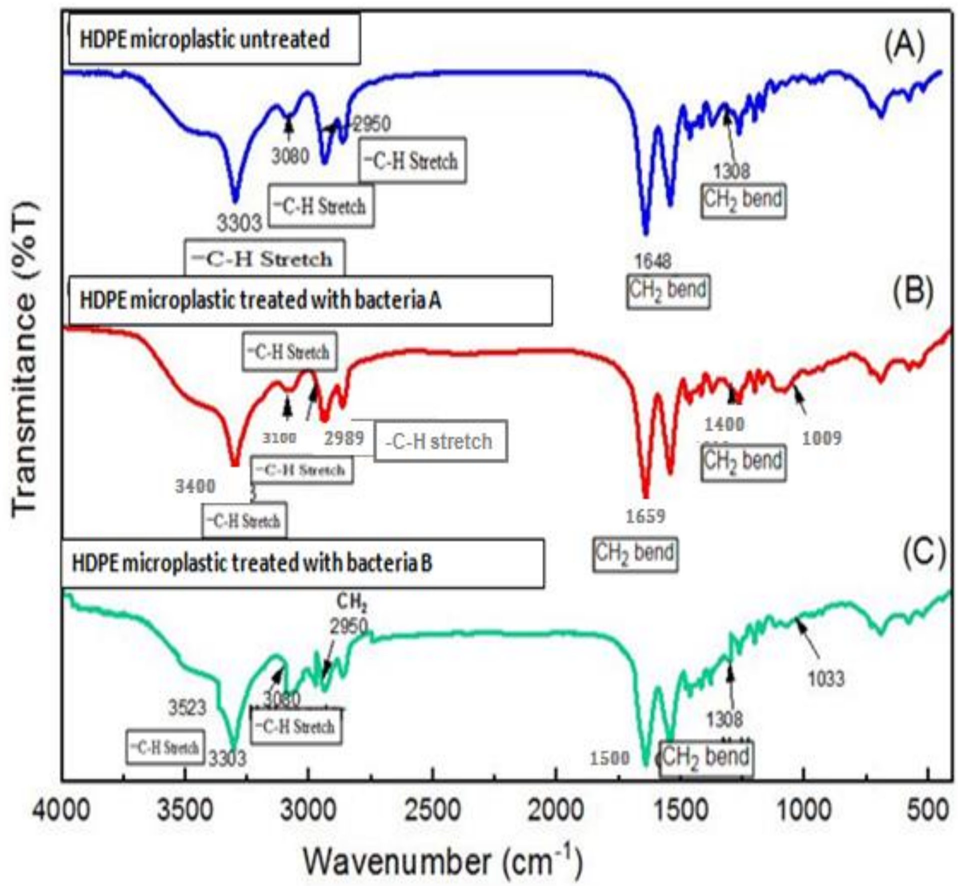
The survival and activity of microorganisms is analyzed using pH which determines its bacterial population, enzyme activity and degradation rates. Graph 4.3 demonstrates the change in pH of bacterial isolates upon exposure to microplastics cultured mineral media. The degradation of microplastics reduced pH of aqueous media towards acidity. Similar pH observations could be seen in both the bacterial strains A and B. The pH values determined the optimal rate of growth of bacteria and decreasing pH trend could be attributed to the production of metabolites in degradation of microplastics. This study suggested facilitating pH-modulating metabolic products formed by bacterial strains. Hence, degradation of HDPE microplastics changed the structure of polymer. This changing trend in pH values is indicative of decomposition potential of bacteria A and B for HDPE microplastics respectively.



Graph 4.3: Change in pH of bacterial isolates upon exposure to microplastics

V. FTIR spectra of HDPE microplastic

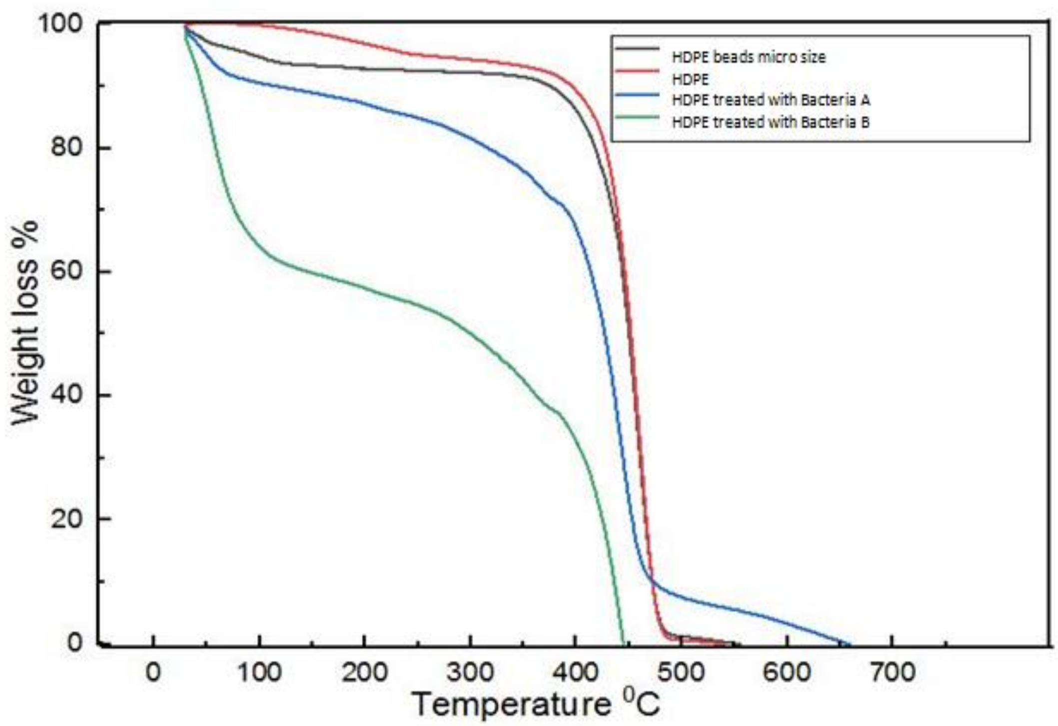
Structural changes in HDPE microplastics after incubation through bacterial strains were analyzed by means of FTIR spectroscopy. Graph 4.4 (a), (b), and (c); demonstrates the FTIR spectra of treated HDPE microplastics incubated with bacteria A and B respectively for a period of around 40 days. This can help in determining chemical bonds present within samples. In HDPE microplastics not incubated with bacterial strains (a), the absorption peaks with intense bands were observed at 1308 nm and 1648 nm whereas strong bands could be seen at 2950 nm, 3080 nm and 3303 nm due to -C-H stretch. In HDPE microplastics treated with bacteria A (b), the absorption peaks with intense bands were observed at 1009 nm, 1400 nm and 1659 nm due to CH_2 blend, and strong bands at 2989 nm, 3100 nm and 3400 nm due to -C-H stretch. In HDPE microplastics treated with bacteria B (c), the absorption peaks with intense bands were observed at 1033nm, 1308 nm and at 1500 nm due to CH_2 bond, and strong bands at 2950 nm, 3080 nm, 3303 nm and 3523 nm due to -C-H stretch.



Graph 4.4: FTIR spectra of HDPE microplastics

VI. Thermo gravimetric analysis (TGA) analysis of HDPE microplastics

Thermo gravimetric analysis data on various conditions of HDPE microplastics under study were depicted in graph 4.5. In case of HDPE micro sized beads and HDPE, there is not much weight loss observed which depicts the resistance of the plastic to the temperature based on their size. Whereas when subjected to microbial degradation, the amount of weight loss was more in bacteria B (80 %) as compared to bacteria A (50%). The shift in the temperature observed in Bacteria A demonstrates that weight loss starts at about 120 ºC and in Bacteria B at 100 ºC which depicts the change in thermal properties of the microplastics.



Graph 4.5: TGA analysis of HDPE microplastics before and after treatment

VII. Transmission Electron Microscopy (TEM) analysis of HDPE microplastics

The morphological characteristics of HDPE microplastics with and without treatment with bacteria A and B could be observed by TEM. Figure 4.5 depicts the morphology of HDPE microplastics after incubation with bacteria demonstrating the degradation of microplastics debris by bacteria and formation of cracks and holes in the microplastics depicts their degradation.

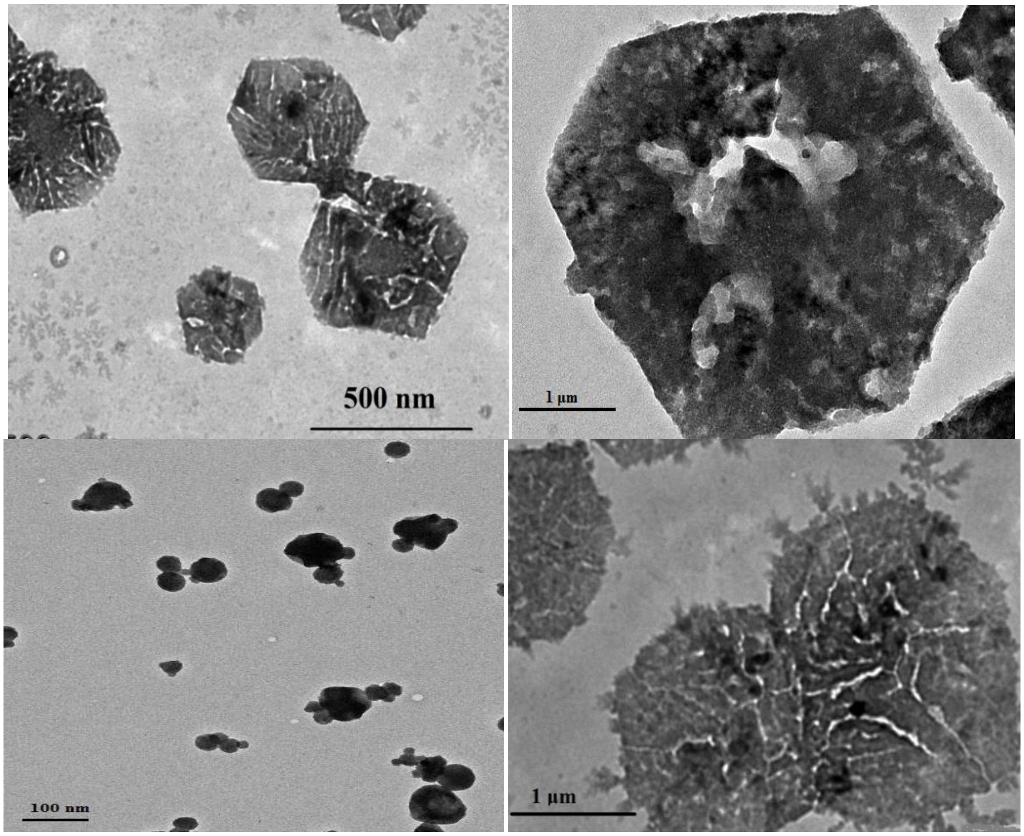


Figure 4.5: TEM analysis of HDPE microplastics

CHAPTER 5 – CONCLUSION

Plastic pollution is creating a menace to the environment with potential threats to marine, land and humans. It is essential to combat plastic waste and remediate the ecosystem to enhance the biodiversity. Biodegradable microorganisms act as eco-friendly sources to remediate plastic contaminants by degrading the plastic particles. This study demonstrated the potential of bacterial strains isolated from a polluted riverside soil in degrading HDPE microplastic particles. The in vitro biodegradation assay of HDPE microplastics demonstrated the capability of two bacteria, A and B, respectively, to degrade the microplastics. Growth patterns of the two bacteria when infused with microplastics could be observed to determine the potential of bacterial isolates in degrading microplastics. Also the reduction in absorption peaks of microplastics could be analyzed by FTIR analysis, and subsequent structural and morphological changes by SEM, TEM confirmed the biodegradation efficacy. Thermo gravimetric analysis further facilitated the degradation potential of bacterial isolates. Therefore, this study is considerably important in identifying microbes helpful in degradation of plastic contaminants and facilitating efficiency of various microorganisms in degradation. The utilization of microorganisms provides a new strategy for enhancing the degradation of microplastic pollutants and providing sustainable environment.

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Plastic Pollution by COVID-19 Pandemic: An Urge for Sustainable Approaches to Protect the Environment

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Abstract

COVID-19 pandemic has created prolonged impact globally and destructed the life all over the world. The necessary use of personal protective equipments, masks, gloves and other plastic products has to some extent reduced transmission of virus. However, impact of plastic waste generated worldwide due to the pandemic has affected the environment globally. The corona virus disease (COVID-19) has destructed and altered every part of life and environment globally. Potential impacts on environment are seen due to the transmission of virus as well as slowdown in economic activities as lockdown prevails. Increased biomedical waste, improper usage and disposal of surgical masks, disinfectants, gloves, and increasing plastics wastes from domestic households continuously endangers environment. Not only it has impact on environment, but also deteriorates human health in the future. Global environmental sustainability is necessitated to overcome plastic pollution problem and facilitate strategies to recycle and reuse plastics products. This review highlights the influence of COVID-19 on wastes generated by plastic products along with environmental challenges and repercussions. Also measures to combat plastic pollution problem have to be implemented for future protection and safety of the environment.

Keywords: COVID-19, Deteriorates, Environmental sustainability, Plastic Pollution, Strategies

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Plastic pollution by COVID-19 pandemic: An urge for sustainable approaches to protect the environment

Highlights

Starting the occurrence of COVID-19 virus, several organizations and agencies have recommended the use of prompt plastic products and packaging materials to control transmission of virus. However, scientific community is worried on monitoring and combating plastic waste residues generated by COVID-19 pandemic. Most of plastic residues are produced from RT-PCR tests of which approximating 97% of total plastic waste is incinerated to reduce hazardous chemicals from leaching in environment[18].

According to the reports by [18], around 15439 tonnes of plastic wastes have been generated until August 2020. Globally, the amount of plastic residues from different continents is Asia (9600 tonnes)), Europe (2200 tonnes), South America (560 tonnes), North America (2500 tonnes), Africa (270 tonnes) and Oceania (200 tonnes). The countries with most amounts of plastic wastes include China (38%), Russia (7%), United States (15%), India (6.4%), Germany (1.9%), Italy (1.6%), United Kingdom (3%), Spain (1.6%) and Turkey (1.2%) [18].

Different plastic sources identified from COVID-19 tests include plastic swab, falcon tubes, plastic tip, plastic pipettes, buffer plastic bottles, aerosol plastic barrier tips, 96-well PCR plastic plate, and optical plastic plate and eppendorf plastic tubes. Therefore, each test estimates to around 37g of plastic residue that is left out in the environment [18].

In today's scenario, disposal of plastic wastes generated from COVID-19 depends largely on its nature, whether it is classified as biohazardous or non-biohazardous. Biohazardous waste is mostly incinerated emitting various toxic chemicals that pollute the environment [19] whereas non-biohazardous waste usually ends up in landfill sites thereby being exposed to wild animals and birds. The burning of biohazardous waste also causes air pollution thereby increasing particulate matter in air and increasing the chance of COVID-19 infection and other respiratory disorders [8].

The pollution caused by these plastic residues has many different biological influences in the assemblage specific levels of ecosystem. Plastic residues disintegrating into microplastics affect the marine plants while causing harm to the physical characteristics and soil biota [20]. Especially due to microplastics small size, they can be easily consumed or collected in the brain, or nerves, and also in the circulatory system of the creatures which causes many adverse effects [21]. Sub-deadly impacts include damage of the sensitivity, impairing reproduction capability, damage of mobility, reduced growth and body condition, lack of ability to escape from the predators [22]. Many scientists have examined microplastic consumption by microbiota like zooplankton [24], marine isopod—*Idotea emarginata, Calanus helgolandicus; Daphnia magna*; Amphipod *Orchestoidea tuberculate* [25]. Adversative health effects can decrease country's efficiency and waged proficiency with harmful influence on public and economic characteristics. Rethinking and redesigning of PPE kits and plastic products is necessitated to overcome the plastic waste pollution. Improvements in recycling procedures to ensure sustainable use and improve air and water quality are recommended.

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