"Role of *Corynebacterium striatum* in the degradation of nylon-6,6 microplastics"

A DISSERTATION

SUBMITTED IN PARTIAL
FULFILLMENT FOR REQUIREMENT
OF THE DEGREE OF
MASTER OF
TECHNOLOGY IN

INDUSTRIAL BIOTECHNOLOGY

Submitted by
URJA SHARMA
(2K20/IBT/11)

Under the supervision of
Prof. Jai Gopal Sharma

DEPARTMENT OF
BIOTECHNOLOGY
DELHI TECHNOLOGICAL
UNIVERSITY
Bawana road, Delhi – 110042

JUNE – 2022

CONTENTS

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
Bawana road, Delhi-110042

CANDIDATE'S DECLARATION

I, Urja Sharma, 2K20/IBT/11, student of M.Tech (Industrial Biotechnology), hereby declare that the project dissertation titled "Role of *Corynebacterium striatum* in the degradation of nylon-6,6 microplastics" is submitted to the Department of Biotechnology, Delhi Technological University, Delhi, by me in partial fulfillment of requirement for the award of degree of Master of Technology (Industrial Biotechnology). This thesis is original work done by me and not obtained from any source without proper citation. This project work has not previously formed the basis for award of any degree, diploma, fellowship or other similar title or recognition.

Place: Delhi **Urja Sharma**

Date : 30-05-2022

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
Bawana road, Delhi-110042

CERTIFICATE

I hereby certify that project dissertation titled "Role of *Corynebacterium striatum* in the degradation of nylon-6,6 microplastics" submitted by Urja Sharma, 2K20/IBT/11, Department of Biotechnology, Delhi Technological University, Delhi, in partial fulfillment of requirement for the award of degree of Master of Technology (Industrial Biotechnology), is a project done and carried out by the student under my supervision. To best of my knowledge and belief, this work has not been submitted in part or full for any degree or diploma to this university or elsewhere.

Prof. Pravir Kumar
Head of Department
Department of Biotechnology
Delhi Technological University
Delhi - 110042

Prof. Jai Gopal Sharma
Supervisor
Department of Biotechnology
Delhi Technological University
Delhi - 110042

ACKNOWLEDGEMENT

I express my deep gratitude and indebtedness to **Prof. Jai Gopal Sharma**, Professor, Department of Biotechnology, DTU, Delhi, for his guidance, and valuable feedback throughout this project work. His able knowledge and supervision with unswerving patience fathered my project work at every stage, for without his encouragement, the fulfilment of task would have been impossible and difficult.

I wish to express my gratitude towards our Head of Department, **Prof. Pravir Kumar**, Department of Biotechnology, DTU, Delhi, for showing interest and providing help throughout the period of my project work.

I express my heartfelt thanks to PhD scholar, **Ms. Neha Tiwari** for her constant support, motivation, active cooperation, sincere help and guidance throughout the whole project.

I am genuinely appreciative of all my Friends for their support and suggestions during my work.

Lastly, I would like to thank the Almighty GOD and my parents, whose committed and untiring efforts towards me have brought me at this stage of my life.

Urja Sharma
(2K20/IBT/11)
Date: 30-05-2022

ABSTRACT

Plastic is one of the most important materials that is used in our quotidian lives. The application of plastic spreads from textile to cosmetic industries, construction to electronic industries as well as from transportation to aerospace industries. The reason for such extensive use of plastic is its durability, light-weight, cost and malleability. However, leakage of this plastic into the environment has proven harmful and has various adverse effects on humans and flora and fauna. Smaller plastic fragments, also known as microplastic (upto 5mm), have gained ground lately due to their widespread occurrence in the oceans, rivers, land and their toxicological effects. In this study, the role of *Corynebacterium striatum* in the degradation of nylon-6,6 microplastics has been studied. The bacterial strain were grown on triple sugar agar and liquid culture was maintained in order to study the bacterial cell's growth. Further, the bacterial culture was grown in MSM medium with minimal glucose concentration as control and in the presence of nylon-6,6 microplastic as treatment. This study focuses on the role of enzymes like laccase and peroxidase in the biodegradation of microplastic. The degradation of nylon-6,6 was analyzed by measuring the reduction in the nylon-6,6 microplastic weight. To study the structural parameters, scanning electron microscopy and to study the degree of bio-decomposition, soluble oxygen demand analysis was done. To confirm the presence of adipic acid monomer, HPLC was performd. Therefore, this study helps in understanding and assessing the role of enzymes in the biodegradation of nylon-6,6 microplastics in order to provide a solution remediate the environment.

Key words : Microplastics, Biodegradation, Soluble Oxygen Demand Analysis, High Performance Liquid Chromatography, Scanning Electron Microscopy, Laccase, Peroxidase

LIST OF FIGURES

LIST OF TABLES

LIST OF GRAPHS

LIST OF ABBREVIATIONS AND SYMBOLS

º - Degree

µ- Micro

HDPE- High-Density Polyethylene

LDPE- Low-density Polyethylene

SEM - Scanning Electron Microscopy

HPLC - High Performance Liquid Chromatography

PE- Polyethylene

PET- Polyethylene Terephthalate

PP- Polypropylene

EVA – Ethylene-vinyl-acetate

PS- Polystyrene

PVC – Polyvinyl Chloride

FTIR – Fourier Transform Infrared Spectroscopy

MSM- Mineral Salt Media

MP – Microplastic

OD – Optical Density

FDA - Fluorescein Diacetate

ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

SCOD - Soluble Oxygen Demand

TCA – Trichloroacetic Acid

CHAPTER 1 : INTRODUCTION

Plastics, also known as synthetic polymers, are known to cause serious and enduring environmental effects. The fact that justifies the extensive use of plastic is its high durability, inert nature, low cost and stability. Lack of safe disposal methods causes the environmental accumulation of plastic that pose ecological risk to the flora and fauna [1] . The uncontrolled use of plastic is being done for purposes like packaging, transportation and in various industries. Most of the plastics are non-degradable in nature and persist in the environment for a prolonged period of time. The broken fragments of plastic have gained great consideration owing to their toxic effects. Microplastics can be defined as almost 0.1μm-5mm long fragments of plastic. In a study, it was discovered that the contribution of microplastic debris by particular geographical areas were as follows: Northern part of America (17.2%), the Middle Eastern region (8.7%) and Africa and Southeast Asia (15.9%) [2]. The distribution of global plastic production as of 2021 is mentioned in Table 1. Only 21% of the total plastic that is produced is being successfully handled and tackled. The composition of plastic includes carbon, silicon, hydrogen, nitrogen, oxygen and chloride. Poly (ethylene terephthalate) (PET), nylons, polybutylene terephthalate (PBT), polypropylene (PP), polystyrene (PS), polyethylene (LDPE, LLDPE , HDPE and MDPE), polyurethane and polyvinyl chloride (PVC) are included in the category of plastics.

Various factors that affect plastic degradation include presence or absence of sunlight, temperature, pH, humidity and salinity. One of the major parameters that determine the biodegradation of plastic is its molecular weight. Low molecular weight plastics are easier to get biodegraded. Melting point is another important parameter that has an effect on enzymatic degradability ; higher the melting point, lower would be its biodegradability. Crystallinity as well as modulus of elasticity are also known to affect the polymer degradability. Addition of antioxidants and stabilizers cause toxicity to microorganisms and thus, slow down the process of degradation. The physical form, structural and molecular composition of polymers may also affect the biodegradation rate [3].

Table 1. Distribution of Global Plastic Production as of 2021

COUNTRY/REGION	PERCENTAGE
CHINA	32 %
NAFTA*	19%
REST OF ASIA	17%
EUROPE	15%
MIDDLE EAST, AFRICA	7%
LATIN AMERICA	4%
JAPAN	3%
CIS**	3%

*North American Free Trade Agreement
**Commonwealth of Independent States
Source : Plastics Europe Market Research Group (PEMRG) / Conversio Market & Strategy GmbH.
Estimated data.

A ubiquitous polymer, nylon, has various applications in our day to day lives. A few applications include reservoirs, fish nets, bristles of brushes, slide, gear, electrical connectors, fabric, bearings, sports and recreational equipments [4]. Nylons are long chain synthetic polymers of amide (polyamide) and have amide groups as the major and integral part of the polymer chain. Adipic acid and hexamethylenediamine are the two monomers that make up nylon-6,6 polymer. These show commendable chemical, electrical as well as thermal resistances [5]. Out of the various types of nylon, nylon-6,6 shows greater strength due to the effective and increased level of H-bonding. This causes an increase in the melting temperature as well as the crystallinity. With the increase in crystallinity, density, stiffness, tensile stress, yield stress, chemical resistance also increase and provide much better dimensional properties [4].

One of the most recent method for plastic degradation involves combination of UV-light along with mechanical disruption that allows the breakdown of plastic into micro and nano sizes. These nano and micro-sized plastics are known to pass into the food chain and thus, turn out to be in the intestine. The most attractive method for plastic management is the enzymatic degradation. The degradation of polyethylene occurs in two steps in case of microbial enzymes. First, the enzyme adheres to PE which is the substrate and further, cleavage of hydrolytic bonds is catalyzed. Disintegration into shorter chains of oligo-,di- and mono-mers of complex polymer allows passage of these simpler forms through bacterial membranes and act as energy and carbon sources. The above stated procedure is termed as de-polymerization. The process of mineralization involves the degradation that leads to the production of carbon dioxide, methane and water. In simple words, biodegradation occurs when microorganisms utilize polymers as their substrate and secrete extracellular enzymes that adhere to its surface and cause cleavage of the polymer and thus, biodegradation then takes places, producing methane, carbon dioxide, and water as end products [3].

Various fungal as well as bacterial strains are known to produce enzymes that aid in the degradation of plastics. *Aspergillus flavus*, *Aspergillus niger*, *Pestalotiopsis microspore* and *Phanerochaete chrysosporium* are a few fungal strains that utilise plastic as substrates by secreting enzymes like glucosidase, catalase, protease, serine hydrolase and manganese peroxidase respectively. Examples of a few bacterial strains that cause biodegradation of plastic by secreting enzymes like lipase, serine hydrolase and a few others include *Penicillium*, *Rhizopus arrizus*, *Pseudomonas stutzeri*, *Rhizopus delemar* etc.[1] Natural consortia of *Brevibacillus agri* btDSCE02, *Aneurinibacillus aneurinilyticus* btDSCE01 and *Brevibacillus brevis* btDSCE04 has also shown better degradation of PP, LDPE and HDPE compared to a single population [6].

In this study, *Corynebacterium striatum* a gram positive, aerobic bacterium is being studied for its potential to degrade nylon-6,6 microplastics. The study focuses on the enzymatic degradation of microplastic and the bio-degaradtion is recorded by measuring the reduction in the dry weight of the

microplastic, both before and after the bacterial incubation. Surface morphology was analysed by using scanning electron microscope and to confirm the presence of adipic acid and hexamethylenediamine monomers, high performance liquid chromatography was conducted.

CHAPTER 2 : REVIEW OF LITERATURE

2.1 CHEMICAL COMPOSITION OF MICROPLASTICS

The polymeric raw substances, such as monomers, and chemical additives, are the main components of MPs. Monomers, the fundamental components of plastic polymers, generate a biochemically inert structure. Nylon, PET, PP, PVC, HD/LD-PE and PS are some of the most often utilized monomers. Chemical additives, such as retardants used for flames, plasticizers, anti-oxidants, fillers, colours, UV and heat stabilizers, and lubricants, are also included in MPs. Plasticizers are chemical compounds with features such as low vapour pressure, chemical stability, and inability to dissolve in liquids. They are used to improve plastic's workability, distensibility, and mobility. Inert fillers are used to adjust the working, strength, shrinkage, and flow qualities of the resin, whereas reinforcing fillers are preferable to the base resin because of their strength. Soluble or insoluble dyes are compounds that contain organic or inorganic material in the form of tiny powders and are employed to give the polymer the desired colour. To prevent the plastics from degradation caused by light, heat, or UV radiation, UV and heat stabilizers are added. Lubricants aid in the convenience of plastic processing by increasing the flow properties of the plastic material. In their investigation, Lithner et al. (2011) rated the polymers according to their hazardous qualities and discovered that styrene is a probable carcinogenic or mutagenic carrier and one of the most dangerous polymers [7].

2.2 SOURCES OF MICROPLASTIC

Generally, there are two forms in which microplastics enter into the environment; primary and secondary [2]. Primary microplastics are polymers that are created in tiny sizes [8]. Primary microplastics are generally utilized in commercial uses (cosmetics, microfibers from nets, clothing, textiles, air-blasting media, drug vectors etc [9,8,10]. Due to the continual fragmentation of bigger plastics, secondary microplastics now dominate marine habitats. However, some debate and confusion regarding the sources of 1^0 microplastics, especially in terms of the amounts released in

each category still exists.. 2^0 microplastics are microplastics made from larger plastic fragments that have been broken down into tiny particles by physio-chemical, or biological processes like break down of water bottles etc [9]. As a result, secondary microplastics come from a variety of places. The decomposition rate of macroplastics (> 5 mm) is affected by environmental elements such as sunlight and temperature along with the qualities of plastic materials (e.g., density and size) [11].

Plastic breakdown is often caused by weathering. Another key process is photodegradation which is basically a degradation caused due to sunshine, which can cause link cleavage, resulting in plastic breakdown and oxidation [12,8, 13,14]. Mechanical forces such as fluctuation, abrasion and turbulence can also cause plastic particles to break. Furthermore, with the changes in the external environment, like that in case of low oxygen content at very deep ocean depths or in the benthic zone where the marine environment is of low energy, the pace of microplastic disintegration slows substantially [15,16]. MPs are frequently employed in sectors like as textiles, autos, electronics, and paints, and can then be discharged straight into the water, affecting the aquatic ecosystem. Plastic fibres used in the textile industry are another source of MPs, as many clothing are comprised of these fibres, which can release ultrafine particles into the environment when washed. Other well-known sources include agricultural plastic film, tyres, paint particles, polymeric materials used for medicine delivery, debris generated by ships on the water, and plastic food packaging items, among others. Industrial emissions, breakdown of bigger plastics materials, particles produced from traffic, garbage dumped in landfills, and resuspension of road dusts are also sources of MPs in the air [17, 18].

To pinpoint the specific source of secondary microplastics, it is important to initially determine the macroplastics (> 5 mm) sources and related degradation processes. However, it is hard to find out the exact sources because both micro sized and macro sized plastics as the process of their breakdown is dynamic in nature.

2.3 COMPARING MICROPLASTICS FROM SOIL WITH THE AQUATIC SYSTEM

The majority of the plastic garbage turns up in the ocean or is thrown on land [19,20]. Microplastic pollution is prevalent in the marine, freshwater, as well as in the terrestrial environments [21]. The aquatic environment is a hub for microplastics from many sources, while the terrestrial area also plays a role in microplastic storage. The comparison of microplastic characteristics in several environmental matrices may provide some information about microplastic source and dispersion.

According to previous research, the most common microplastics in aquatic environments are fibrous and fragmental ones that are made of PE and PP with small particle sizes [22,23]. Microplastics that generally occur in soil have a physiological morphology and chemical composition that is comparable to that of microplastics found in aquatic settings. The most common morphologies of soil-microplastics are fragments and fibres, and they are chemically composed of PP and PE. Also, less than 1 mm sized microplastics are present and identified in soils more often, and the amount of microplastic decreases as the particle size increases. Microplastics originating from aquatic environment and soil come in a wide range of colours.

The constancy of microplastic features and composition in the soil as well as in the aquatic environment tend to indicate a few inter-connections between each other and their pathways of source.[23]. The fundamental grounds of the relationship between microplastic components existing in the soil and aquatic ecosystems is that they share or have similar pollution sources. Apart from atmospheric transmission, widely regarded common sources in soil and aquatic habitats include sewage and sludge, as well as garbage that has been poorly or insufficiently processed [24,25]. Irrigation water sources such as rivers, reservoirs, and groundwater can carry microplastics onto agriculture. However, further information about the linkages or source (pathways) of microplastics found in the soil and aquatic ecosystem is lacking and also, the quantitative processing and simulation of interaction data is lacking.

2.4 TOXICOLOGICAL IMPACTS OF MICROPLASTIC

The building blocks of plastics are long chain organic polymers. Because of their huge molecular size, which renders them biochemically inactive, these polymers are not considered to be especially dangerous to the environment. Throughout the life cycle of plastics, many hazardous compounds such as monomers, chemical additives, and their degradation product or by product may be released. PUR, PVC, and PAN are the most dangerous polymers, whereas EVA, PP, PVAc and PE are the least harmful. Vinyl chloride and styrene monomers, for example, have both been demonstrated to be mutagenic and carcinogenic in humans, whilst BPA monomer affects endocrine function. Monomers like ethylene and propylene, on the other hand, are thought to be the least toxic to people [26]. Air pollution can be generated by the volatilization of compounds released from polymers such as styrene, formaldehyde, and vinyl chloride, according to one study [22] . PET, polycarbonate, and high-density polyethylene are all extensively used polymers in the food and beverage industry. According to several research, BPA produced from the polycarbonate polymer is linked to a number of human health issues, including type 2 diabetes, obesity, impaired sperm production, cardiovascular disease, and an increased risk of breast and prostate cancer [27]. PET uptake by humans, on the other hand, can cause health problems such as persistent pneumonia, allergies, asthma, gastrointestinal blockage, and so on [28]. To make PVC flexible, phthalates are employed, which are dangerous to human health since they can cause skin disease, ulcers, vision loss, genetic abnormalities, birth defects, and other problems [27].

During the manufacturing process, additives are added to plastics to increase qualities such as strength, workability, UV or heat resistance, and so on. Because these chemicals are not necessarily attached to the polymer of the plastics, they have a variety of toxicological consequences [18] . Because MPs have a bigger ratio of surface area to volume, this release of additives is more effective. Furthermore, many chemicals, such as PBDEs (heat resistant) and nonylphenol (antioxidant), are extremely hazardous [11]. In addition, additives are released more frequently in places with greater temperatures [18]. Phthalates (plasticizers) can affect the development of amphipods and crustaceans,

animal reproductive, and genetic abnormalities due to their capacity to modify the endocrine function of the body [29]. Plasticizers are also highly hazardous to plants; for example, the heat-resistant thermoplastic tetra chlorophenol is directly toxic to phytoplankton. The impacts of toxicology on human health are still in their early stages. Human intake of fish containing MPs has been linked to health problems such as inflammation, cell necrosis, and other issues [30] . In addition, ingesting MP-contaminated food can create major difficulties for humans as well as the organisms at the topmost level of the food-chain.

2.5 ENVIRONMENTAL FATE OF MICROPLASTICS

Microplastics carry dangerous compounds for two reasons: (a) to improve tensile strength, substances are intentionally added to plastics during the process of production and (b) adsorption of various substances on the plastic surface in nature [31]. By using acid digestion to investigate the plastic and metal relationship, Vedolin et al. (2017) discovered the existence of numerous heavy metals that have been adsorbed on the plastic surface, implying that micro-sized plastics can act as aquatic contaminants as well [32]. Microplastics have the potential to transport heavy metals into the food-chain. Heavy metals leak into the water column due to sorption with a variety of other substances such as additives and antioxidizers [33].

2.5.1 Interaction with biotic pollutants

Microorganisms like as bacteria, algae, and viruses can live in MPs. These microbes can attach themselves to the MPs' outer surface and then populate the food-chain, causing danger to other organisms. The adhesion along with the growth of microorganisms on MPs can be owed to the fact that MPs provide a better environment for the growth of immobile microorganisms than planktonic life, and the inorganic and organic nutrients or substances adsorbed onto the MPs' surface provide a suitable support for the adhered microorganisms' growth [34]. When microorganisms enter the aquatic environment, they quickly cover the surface of the MPs and establish a stable biofilm in around 7 days. Other zooplanktons may be attracted to the surface of the MPs by these biofilms [35].

When compared to MPs alone, MP breakdown after microbe attachment will be more difficult and have greater detrimental effects on other species.

2.5.2 Interaction with abiotic pollutants

MPs can adsorb a variety of chemical pollutants found in the environment, including polycyclic aromatic hydrocarbons, organochlorine insecticides, antibiotics, heavy metals and polychlorinated biphenyls owing to their chemical and physical features. Due to its high sorption capacity, previous investigations have revealed that microplastics contain higher concentrations of contaminants on them than the surrounding environment [36]. Because of their enormous surface area and hydrophobicity, they absorb more contaminants than organic pollutants. Because plastics have diverse physical and chemical properties, the adsorption of contaminants on them varies depending on the kind of plastic. In comparison to polyethylene and polystyrene, polyethylene is better at adsorbing organic pollutants with various hydro-phobicities, whereas polyamide has a strong affinity for trimethoprim [37]. In addition, the size of the microplastic has an impact on its adsorption rate and capacity. Because of their higher surface area, nano- and micro-sized plastics can absorb more contaminants than millimetre-sized plastics [38].

2.6 ENZYMATIC DEGRADATION OF MICROPLASTIC

Enzyme technology can be used to produce, isolate, purify, and provide enzymes for the purpose of destrupting the plastics, which is interesting. These enzymes are biodegradable and innocuous. A set of enzymes has been degrading a few polymer polymers chains (PS, PE, PVC and PP) throughout the previous decade. Esterases, protease, cutinase, and laccase are examples of such enzymes. Enzymatic breakdown is the most appealing way to deal with plastic trash. There are two phases in the breakdown of plastic by microbial enzymes. First, the enzymes bind to the plastic substrate before catalyzing the cleavage of hydrolytic bonds. Fungi and bacteria breakdown plastic through intracellular and extracellular depolymerases. Intracellular degradation hydrolyzes endogenous carbon content produced by growing bacteria, whereas extracellular degradation occurs when

external carbon sources are used but not necessarily by accumulating bacteria [39]. Complex polymers break down into oligo-, di- and mono- mers which can pass through bacterial membranes and provide carbon and energy. Depolymerization is the name for this process. Water (H_2O), methane (CH_4) or carbon dioxide (CO_2) are the end products of the process of mineralization [1]. Physical elements such as temperature, pressure, and moisture mechanically degrade polymers, causing biological forces such as bacterial enzymes and metabolites to initiate the process. Various studies have highlighted the degradation of microplastic by enzymes produced by microorganisms. The bacterium *Ideonella sakaiensis* can use PET as its major and most important source of energy and carbon requirements. Terephthalic acid and ethylene glycol (monomers of PET) are produced by the conversion of PET into its monomers by two active enzymes in this bacterium; MHETase and PETase [40]. Protease produced by *Brevibacillus spp.* is also known to degrade low molecular weight PLA. *Rhodococcus ruber* C208 produced laccase and *Penicillium simplicissimum* produced manganese peroxidase have also shown the degradation of polyethylene [41,42].

CHAPTER 3 : METHODOLOGIES

3.1 Preparation of microbial culture for the biodegradation study

The bacterial strain were grown on triple sugar agar and liquid culture was maintained in order to study the bacterial cell's growth. The microplastic degrading bacterium were inoculated into fresh minimal salt media with minimal concentration of glucose as control while 100ml of minimal salt media and 0.4g of sterilized nylon-6,6 microplastics containing flasks were inoculated by 500µl of the bacterial strain as treatment and cultured in a shaker incubator at 30°C until their population density reached log phase, which was defined at an absorbance of 1.00 ± 0.05 at 600 nm. All the experiments were done in duplicates. The flasks were then placed in a shaker incubator for 21 days at 30°C and 120 rpm. For a period of 40 days, the pH, optical density (OD), total enzyme activity, and laccase activity of each flask were measured every 5 days. OD was collected at 600 nm to ensure the growth of microorganisms in the experimental flasks.

3.2 Biodegradation Experiments (*in vitro*)

3.2.1 Laccase Enzyme Activity

ABTS (substrate) was used to determine the activity of laccase from the culture media of *C. striatum*. Two hundred microliters of culture media were mixed with 200 µl of 20 mM ABTS in 1000 µl of 50 mM sodium acetate buffer (pH 5.5) and further incubated for 5 min at 30 °C. The oxidation of ABTS leads to the formation of green colour and it was measured spectrophotometrically at A420 (ε = 36000°L·mol-1·cm-1). One unit of activity of laccase was defined as the amount of enzyme required to oxidize 1 µmol of ABTS per minute at 30 °C.

3.2.2 Peroxidase Enzyme Activity

Methylene Blue assay for peroxidase activity was done. The assay mixture contained 2.2 ml of supernatant, 0.1 ml of methylene blue (1.3 mM), while the buffer system was substituted by 0.6 ml

of Tris-HCl buffer (0.5 M, pH 8.0). The reaction was started by the addition of 0.1 ml of 2.7 mM H_2O_2. The change of the dye to Azure C was monitored by the measurement of the decrease in absorbance at 664 mn. The results were expressed as the change of absorbance per minute (mM methylene Blue).

3.2.3 Determination of Dry Weight

The nylon-6,6 microplastics in the samples were filtered out using a 0.46m cellulose acetate membrane filter once the incubation periods were completed. This filtrate can further be used for performing SCOD analysis. The filters were then washed for 4 hours in a 2 percent (w/v) sodium dodecyl sulphate (SDS) solution to remove the bacterial films that had colonised the surface of the microplastic particles, and then dried overnight in a 50°C oven. Using an analytical balance, the amount of microplastic degraded was calculated by comparing the dried polymer weight before and after the test. Furthermore, the percentage of weight loss due to degradation of nylon-6,6 microplastic was calculated using the following equation:

$$\%\text{weight loss} = ((W_0 - W) / W_0) \times 100\ \%$$

where,

W_0 = initial weight of the nylon-6,6 microplastic in grams

W = residual weight of the nylon-6,6 microplastics after degradation period in grams.

3.2.4 High Performance Liquid Chromatography of nylon 6,6

Treated metabolite along with appropriate control samples were also analysed for adipic acid using Dionex model HPLC (Thermo Fisher make) equipped with a PDA detector at wavelength 425 nm. The Thermo fisher C18 column (3 µm, 150 x 4.6 mm) act as the stationary phase. The mobile phase was a mixture of acetonitrile with 0.1% formic acid and water (50:50 v/v) for hexamethylenediamine whereas a ratio of 0.1% formic acid and acetonitrile (60:40 v/v) for adipic acid. Separation was performed at a flow rate of 1 ml/min at 30 ºC temperature.

3.2.5 Fourier transform infrared (FTIR) analysis of nylon-6,6 microplastic

The changes that occurred after the degradation of nylon-6,6 microplastic polymers were analysed using FTIR spectroscopy (Perkin-Elmer 400 FT-IR/FT-FIR) in the 2000-450 cm^{-1} frequency range with appropriate control samples.

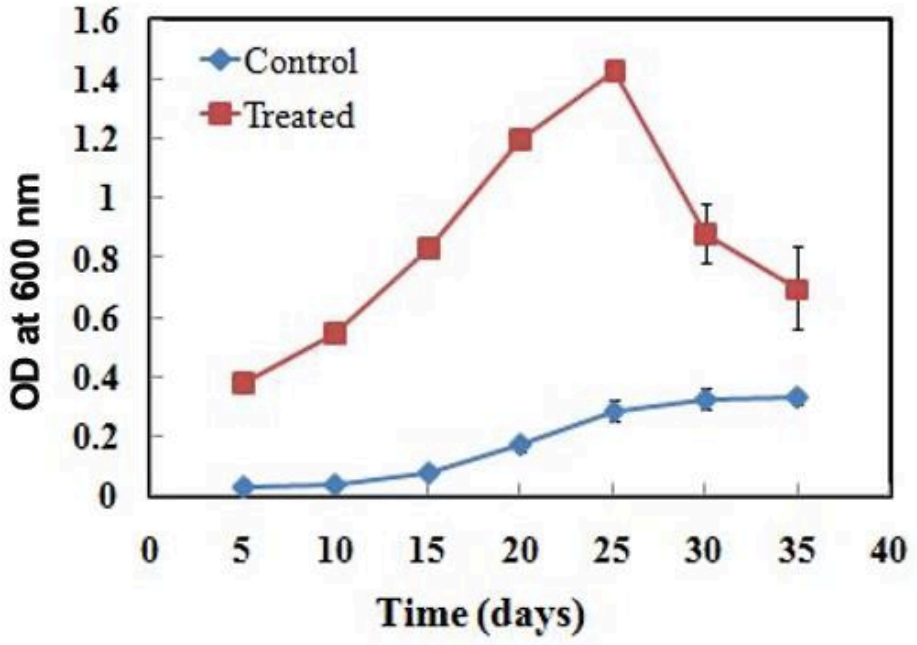
3.2.6 Scanning Electron Microscope (SEM) Analysis

The microplastic after degradation along with the control were coated (sputter) with a layer of gold at 26 mA under Ar atmosphere at 0.5 MPa and was then examined under microscope subsequently.

CHAPTER 4 : RESULTS AND DISCUSSIONS

4.1 Growth pattern of control and treated sample

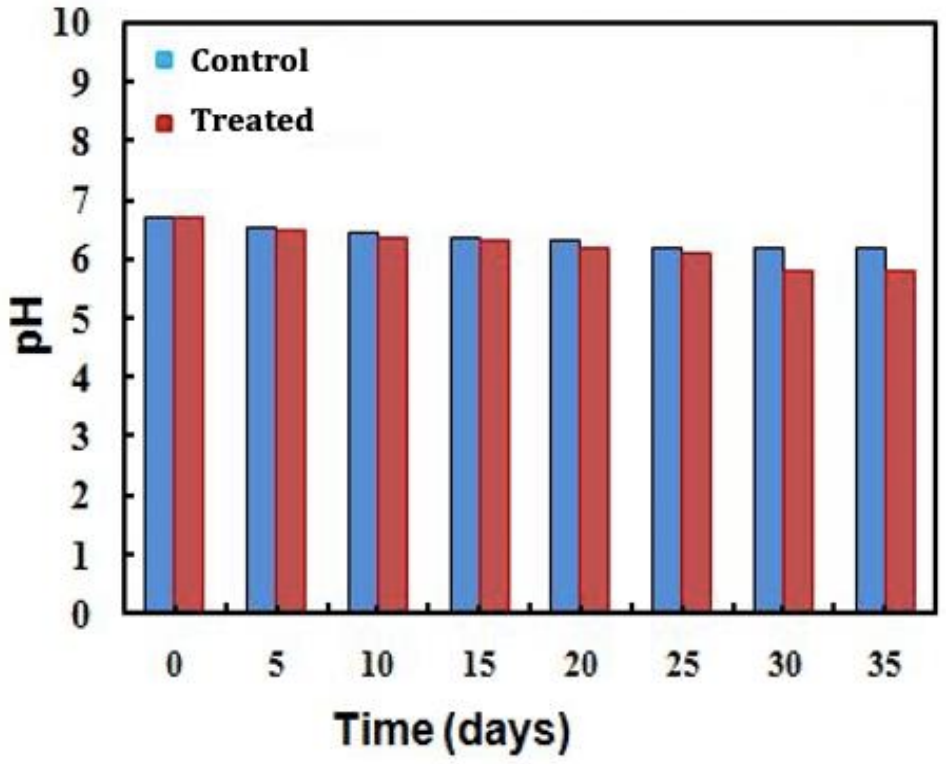
The pattern of bacterial growth for both the control and treated sample are presented in graph 1. The increase in the bacterial growth of the treated sample can be attributed to the cell membrane and microplastics interaction, allowing metabolism. The maximum OD for the treated sample was observed around 25 days of inoculation at ~1.5 nm while the maximum OD for the control was observed on day 35 at 0.3 nm. After 25 days, a gradual decrease in the pattern was observed in treated sample because of the less bacterial counts. Increase in the microbial biomass due to the utilization of substrate by the bacteria caused the degradation of microplastic. Cell lysis, inhibition products and nutrients depletion can be the reasons for the declination in the growth of the bacterial cells. Also, unfavorable growth and proliferation conditions of culture media caused by the degradation products of microplastic might be another reason for this declining trend observed after 25 days.



Graph 1. Growth curve of control and treated sample

4.2 Change in pH

Graph 2 demonstrates the changes in pH of both the control and treated samples. In order to determine the survival and the activity of microorganisms, pH analysis is done which helps in determining the enzymatic activity, bacterial population and the degradation rates. The decrease in pH towards acidity observed in the case of treated samples is indicative of microplastic degradation. Production of metabolites during microplastic degradation caused the pH to decrease. The degradation of nylon-6,6 microplastic changed the polymer structure and the negative trend in the pH values is suggestive of the bacterium's decomposition potential for nylon-6,6 microplastic.



Graph 2. pH changes in control and treated sample

4.3 SEM Analysis

To confirm the degradation of microplastic and to study the surface morphology, scanning electron microscopy was done before and after the bacterial treatment. Figure 4.3 a) indicates the surface morphology of nylon-6,6 microplastic before the bacterial treatment. Prominent changes after the bacterial treatment like the presence of holes and thinning of microplastic from the sides can be seen in figure 4.3 b). Changes in the surface morphology are indicative of the bacterium's ability to degrade nylon-6,6 microplastic.

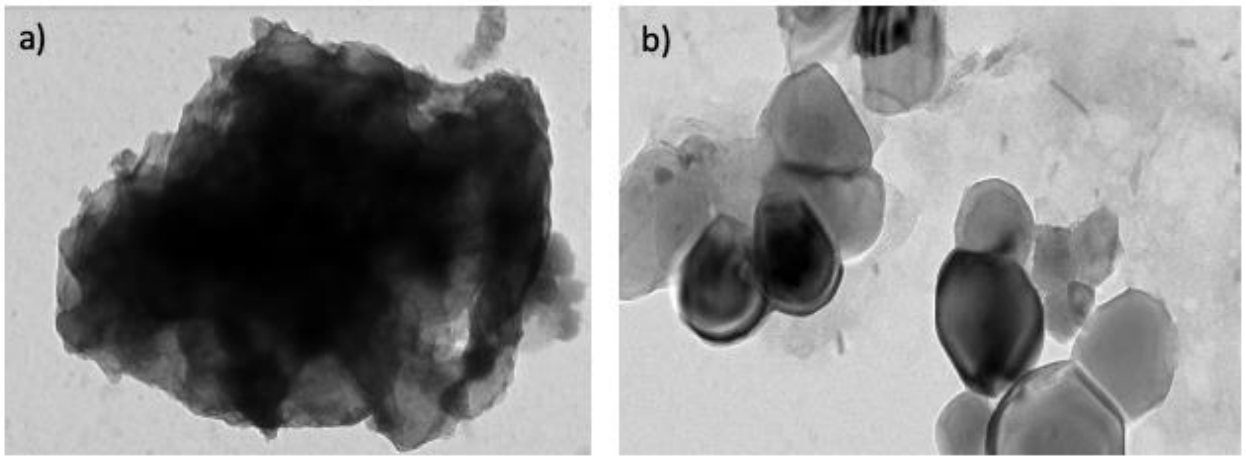


Figure 1. SEM of a) MP before bacterial treatment and b) MP after bacterial treatment

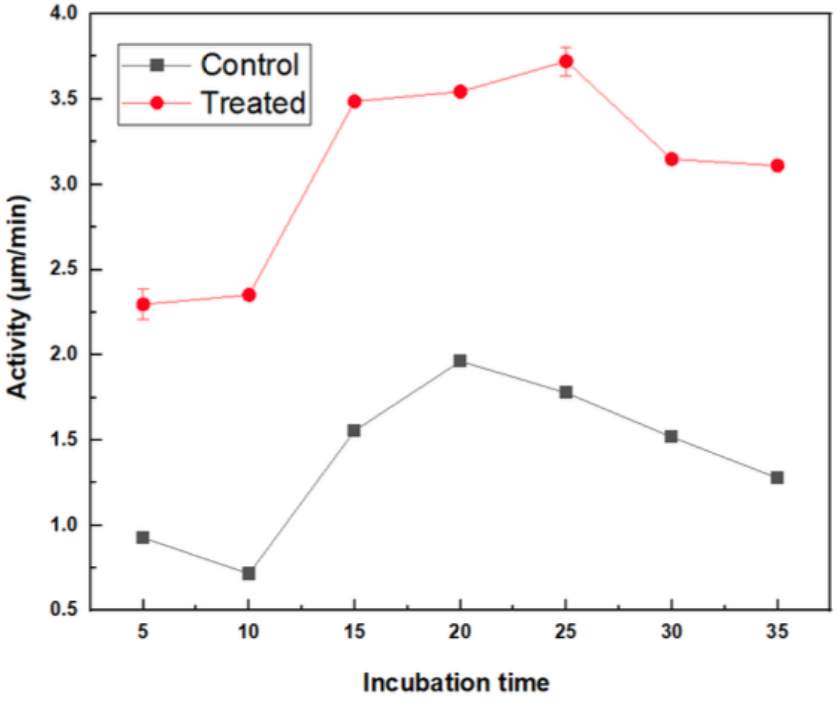
4.4 Determination of weight loss in nylon-6,6 microplastic by bacterial isolate

The action of *Corynebacterium striatum* on nylon-6,6 microplastic led to a weight loss of 15% after 40 days of incubation with bacterial strain. This study is indicative of the ability of the bacterial strain and the enzymes to act on nylon-6,6 microplastic and cause its degradation via various metabolic reactions that led to adsorption and breakdown of the microplastic.

Treated sample : ((0.4 g - 0.34 g)/0.4 g) x 100 = 15%

4.5 Laccase enzyme activity

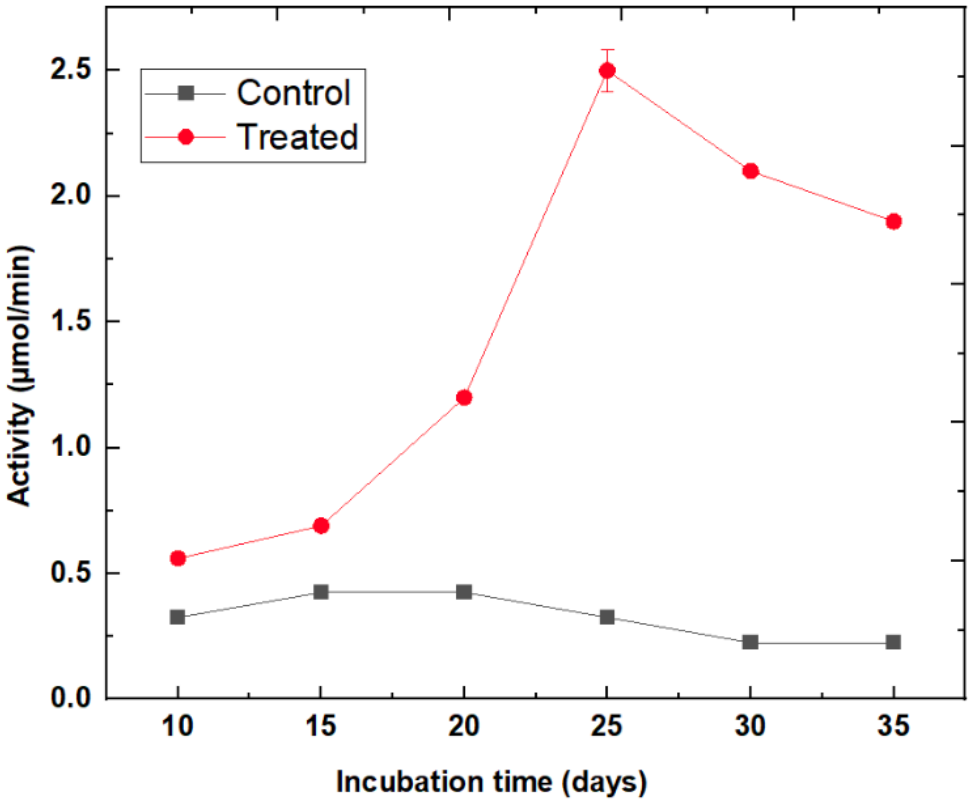
Increase in the activity of laccase in the presence of nylon 6, 6 microplastics than the absence of microplastics was observed and can be seen in graph 3. These findings confirm the presence of laccase required for the nylon 6, 6 degradations. Upon the interaction with microplastic, the levels of secretion of *C.striatum* tend to increase and at day 25 (3.5 µmol/min in 5 min reaction time), maximum activity was shown. After 25 days, decrease in the activity was observed. It can be concluded that the presence of laccase enzyme activity is needed in order to biodegrade nylon-6,6.



Graph 3. Laccase enzyme activity

4.6 Peroxidase enzyme activity

Graph 4 represents increase in the activity of peroxidase in the presence of nylon 6, 6 microplastics than the absence of microplastics. These findings confirm the presence of peroxidase required for the nylon 6, 6 degradations. It was observed that peroxidase enzyme secretion of *C.striatum* increased with interaction and showed maximum activity at day 25th (2.5 µmol/min in 5 min reaction time) and decreased thereafter. Thereafter, the conclusion is the presence of peroxidase enzyme is required in the biodegradation of nylon 6, 6.



Graph 4. Peroxidase enzyme activity

4.7 HPLC

Interestingly, the existence of adipic acid in the treated metabolite was evidenced by HPLC examination after 40 days of treatment and reported as 1.356 mg/l ± 0.006 respectively.

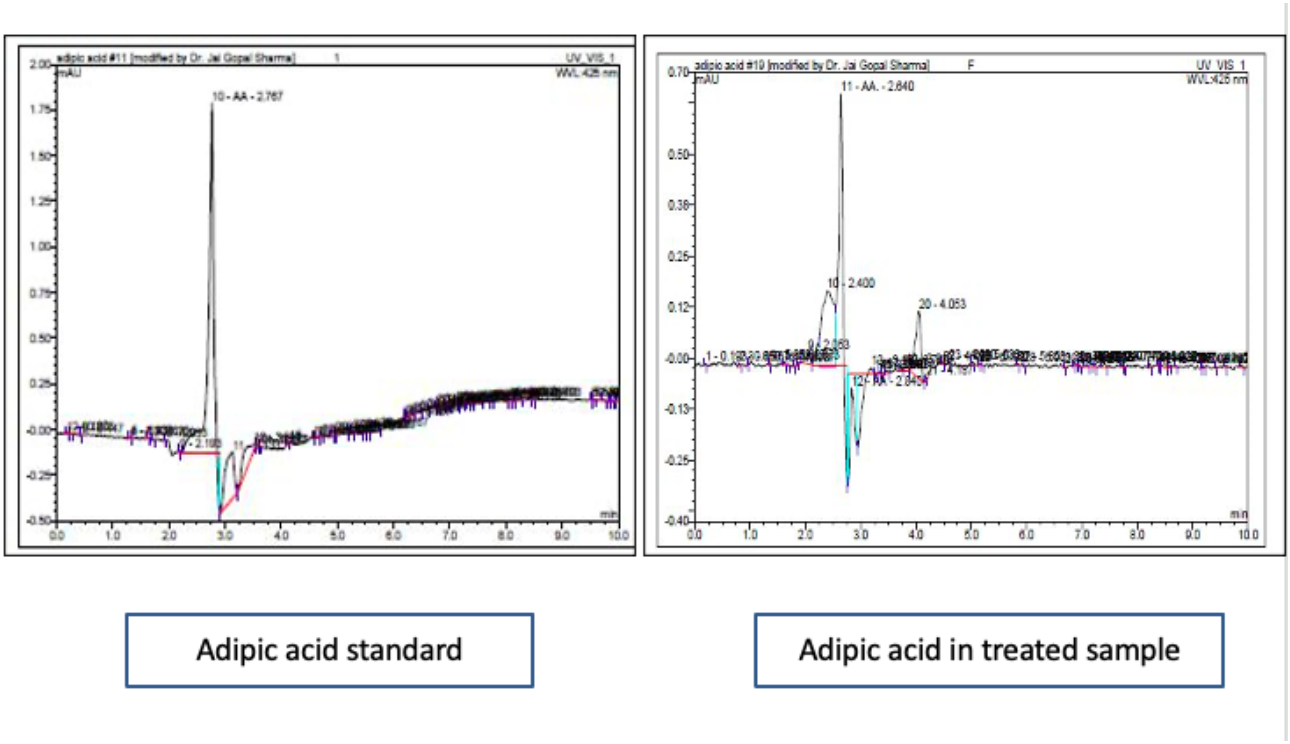


Figure 2. High Performance Liquid Chromatography of nylon-6,6

4.8 FTIR

The presence of amide groups is well reflected as a strong band at 1637 cm^{-1} due to the –C=O stretching vibrations (amide I). The band at 1544 cm^{-1} is assigned to $–NH_2$ deformation (amide II) of polyamide chain. The –C-H stretching vibrations of $–CH_2$ axial groups close to carboxyl groups of the polymer chain and –C-N stretching of the amide III band appeared at 1273, and 1204 cm^{-1}, respectively. The observed alterations in the chemical structure of nylon 6, 6 particularly in amide (-CONH-) functional groups after microbial interaction, confirm breaking of intermolecular cohesive forces between nylon molecules degradation.

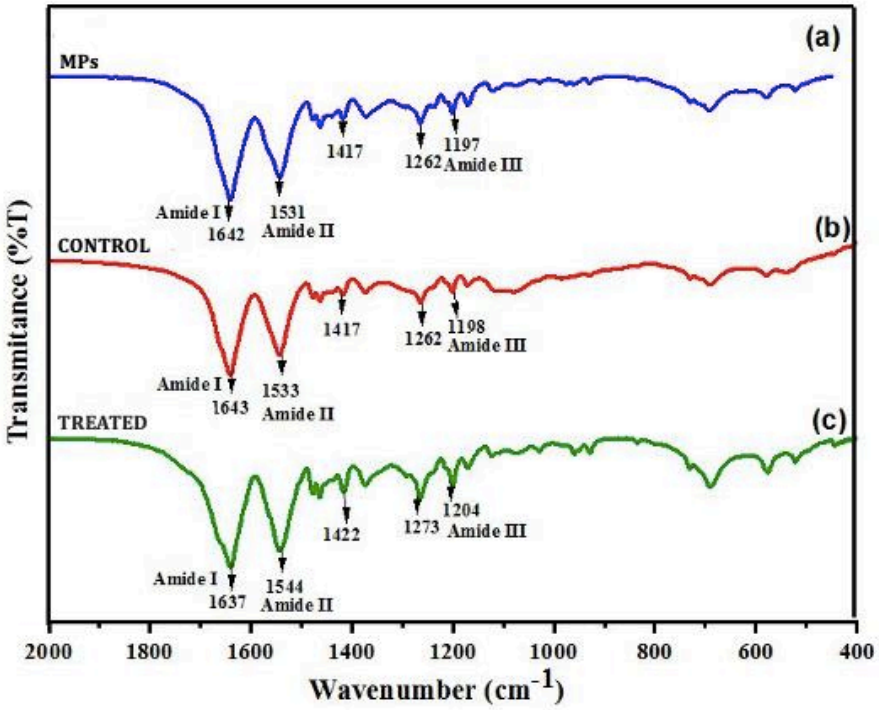


Figure 3. Fourier transform infrared (FTIR) analysis of nylon-6,6 microplastic

CHAPTER 5 : CONCLUSION

The presence of microplastics and their harmful effects on the ecosystem is a known fact. Plastic is extensively used in various industries and its smaller fragments are known to cause toxic effects not only to the environment but also cause severe health related issues in humans. This necessitates the need to degrade microplastics that are present in the environment and by utilizing microorganisms for microplastic remediation, a new and efficient strategy can be established . This study focused on the potential of *Corynebacterium striatum* in the degradation of nylon-6,6 microplastic. The growth pattern of the bacteria is indicative of the interaction between the cell membrane and the microplastic. Reduction in the pH level of the treated sample indicates the degradation of nylon-6,6. The SEM images also confirm the degradation where thinning of microplastic as well as presence of holes can be seen. Also, a 15% reduction in the dry weight of microplastic was observed. The increase in the laccase and peroxidase enzyme activity is also indicative of their role in the degradation process of nylon-6,6. Presence of adipic acid which was confirmed by HPLC is suggestive of the breakdown of nylon-6,6 into its monomer (adipic acid). Alterations in the chemical structure of nylon-6,6 after the microbial interaction confirms the breakdown of intermolecular cohesive forces. Therefore, this study confirmed the possibility of degradation of nylon-6,6 by the aid of *Corynebacterium striatum*.

REFERENCES

1. Bhardwaj H, Gupta R, Tiwari A. Communities of Microbial Enzymes Associated with Biodegradation of Plastics. J Polym Environ [Internet]. 2013 Jun;21(2):575–9.

2. Boucher J, Friot D. Primary microplastics in the oceans: A global evaluation of sources [Internet]. IUCN International Union for Conservation of Nature; 2017.

3. Kale SK, Deshmukh AG, Dudhare MS, Patil VB. Microbial degradation of plastic: a review. 2015;10.

4. Vagholkar P. Nylon (Chemistry, Properties and Uses). Int J Sci Res. 2016 Sep 1;5:349–51.

5. Kiron MI. Nylon 66 Fiber: Preparation, Properties and Applications [Internet]. Textile Learner. 2013.

6. Jaiswal S, Sharma B, Shukla P. Integrated approaches in microbial degradation of plastics. Environ Technol Innov [Internet]. 2020 Feb;17:100567.

7. Lithner D, Larsson A, Dave G. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci Total Environ. 2011 Aug 15;409(18):3309–24.

8. Cole M, Lindeque P, Halsband C, Galloway TS. Microplastics as contaminants in the marine environment: A review. Mar Pollut Bull [Internet]. 2011 Dec;62(12):2588–97.

9. Auta HS, Emenike CU, Fauziah SH. Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. Environ Int [Internet]. 2017 May 1;102:165–76.

10. Patel MM, Goyal BR, Bhadada SV, Bhatt JS, Amin AF. Getting into the brain: approaches to enhance brain drug delivery. CNS Drugs. 2009;23(1):35–58.

11. Horton AA, Walton A, Spurgeon DJ, Lahive E, Svendsen C. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci Total Environ [Internet]. 2017 May;586:127–41.

12. Andrady AL. Microplastics in the marine environment. Mar Pollut Bull [Internet]. 2011 Aug ;62(8):1596–605.

13. Li WC, Tse HF, Fok L. Plastic waste in the marine environment: A review of sources, occurrence and effects. Sci Total Environ [Internet]. 2016 Oct;566–567:333–49.

14. Wagner M, Scherer C, Alvarez-Muñoz D, Brennholt N, Bourrain X, Buchinger S, et al. Microplastics in freshwater ecosystems: what we know and what we need to know. Environ Sci Eur [Internet]. 2014 Jul 9;26(1):12.

15. Sources, fate and effects of microplastics in the marine environment (Part 1) [Internet]. GESAMP.

16. Wang J, Zheng L, Li J. A critical review on the sources and instruments of marine microplastics and prospects on the relevant management in China. Waste Manag Res J Int Solid Wastes Public Clean Assoc ISWA. 2018 Oct;36(10):898–911.

17. Tiwari N, Santhiya D, Sharma JG. Microbial remediation of micro-nano plastics: Current knowledge and future trends. Environ Pollut [Internet]. 2020 Oct 1;265:115044.

18. Gong J, Xie P. Research progress in sources, analytical methods, eco-environmental effects, and control measures of microplastics. Chemosphere [Internet]. 2020 Sep;254:126790.

19. Liu Y, Zhang J, Tang Y, He Y, Li Y, You J, et al. Effects of anthropogenic discharge and hydraulic deposition on the distribution and accumulation of microplastics in surface sediments of a typical seagoing river: The Haihe River. J Hazard Mater [Internet]. 2021 Feb;404:124180.

20. Akdogan Z, Guven B. Microplastics in the environment: A critical review of current understanding and identification of future research needs. Environ Pollut [Internet]. 2019 Nov ;254:113011.

21. Yang L, Li K, Cui S, Kang Y, An L, Lei K. Removal of microplastics in municipal sewage from China's largest water reclamation plant. Water Res [Internet]. 2019 May;155:175–81.

22. Hale RC, Seeley ME, La Guardia MJ, Mai L, Zeng EY. A Global Perspective on Microplastics. J Geophys Res Oceans [Internet]. 2020 Jan;125(1).

23. Yang L, Zhang Y, Kang S, Wang Z, Wu C. Microplastics in soil: A review on methods, occurrence, sources, and potential risk. Sci Total Environ [Internet]. 2021 Aug;780:146546.

24. Allen S, Allen D, Phoenix VR, Le Roux G, Durántez Jiménez P, Simonneau A, et al. Atmospheric transport and deposition of microplastics in a remote mountain catchment. Nat Geosci [Internet]. 2019 May;12(5):339–44.

25. Dris R, Gasperi J, Mirande C, Mandin C, Guerrouache M, Langlois V, et al. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environ Pollut [Internet]. 2017 Feb;221:453–8.

26. Wright SL, Kelly FJ. Plastic and Human Health: A Micro Issue? Environ Sci Technol [Internet]. 2017 Jun 20 [cited 2022 May 23];51(12):6634–47.

27. Proshad R, Kormoker T, Islam MdS, Haque MA, Rahman MdM, Mithu MdMR. Toxic effects of plastic on human health and environment : A consequences of health risk assessment in Bangladesh. Int J Health [Internet]. 2017 Dec 18;6(1):1.

28. Dhaka V, Singh S, Anil AG, Sunil Kumar Naik TS, Garg S, Samuel J, et al. Occurrence, toxicity and remediation of polyethylene terephthalate plastics. A review. Environ Chem Lett [Internet]. 2022 Jun 1;20(3):1777–800. Available from: https://doi.org/10.1007/s10311-021-01384-8

29. Halden RU. Plastics and Health Risks. Annu Rev Public Health [Internet]. 2010 Mar 1];31(1):179–94.

30. Rochman CM, Hoh E, Kurobe T, Teh SJ. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci Rep [Internet]. 2013 Dec;3(1):3263.

31. Ajith N, Arumugam S, Parthasarathy S, Manupoori S, Janakiraman S. Global distribution of microplastics and its impact on marine environment—a review. Environ Sci Pollut Res [Internet]. 2020 Jul;27(21):25970–86.

32. Vedolin MC, Teophilo CYS, Turra A, Figueira RCL. Spatial variability in the concentrations of metals in beached microplastics. Mar Pollut Bull [Internet]. 2018 Apr;129(2):487–93.

33. Kang J, Zhou L, Duan X, Sun H, Ao Z, Wang S. Degradation of Cosmetic Microplastics via Functionalized Carbon Nanosprings. Matter [Internet]. 2019 Sep;1(3):745–58.

34. Bakir A, Rowland SJ, Thompson RC. Transport of persistent organic pollutants by microplastics in estuarine conditions. Estuar Coast Shelf Sci. 2014 Mar 1;140:14–21.

35. Lobelle D, Cunliffe M. Early microbial biofilm formation on marine plastic debris. Mar Pollut Bull [Internet]. 2011 Jan;62(1):197–200.

36. Yu Q, Hu X, Yang B, Zhang G, Wang J, Ling W. Distribution, abundance and risks of microplastics in the environment. Chemosphere [Internet]. 2020 Jun;249:126059.

37. Li J, Zhang K, Zhang H. Adsorption of antibiotics on microplastics. Environ Pollut [Internet]. 2018 Jun;237:460–7.

38. Koelmans AA, Besseling E, Wegner A, Foekema EM. Plastic as a Carrier of POPs to Aquatic Organisms: A Model Analysis. Environ Sci Technol [Internet]. 2013 Jul 16;47(14):7812–20.

39. Tokiwa Y, Calabia BP. Review Degradation of microbial polyesters. Biotechnol Lett [Internet]. 2004 Aug;26(15):1181–9.

40. Palm GJ, Reisky L, Böttcher D, Müller H, Michels EAP, Walczak MC, et al. Structure of the plastic-degrading Ideonella sakaiensis MHETase bound to a substrate. Nat Commun [Internet]. 2019 Dec;10(1):1717.

41. Santo M, Weitsman R, Sivan A. The role of the copper-binding enzyme – laccase – in the biodegradation of polyethylene by the actinomycete Rhodococcus ruber. Int Biodeterior Biodegrad [Internet]. 2013 Oct;84:204–10.

42. Sowmya HV, Ramalingappa, Krishnappa M, Thippeswamy B. Degradation of polyethylene by Penicillium simplicissimum isolated from local dumpsite of Shivamogga district. Environ Dev Sustain [Internet]. 2015 Aug;17(4):731–45.

PAPER NAME

Thesis_Urja_M.Tech.docx

WORD COUNT

6086 Words

CHARACTER COUNT

34327 Characters

PAGE COUNT

30 Pages

FILE SIZE

8.9MB

SUBMISSION DATE

May 25, 2022 5:40 PM GMT+5:30

REPORT DATE

May 25, 2022 5:42 PM GMT+5:30

7% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

- 5% Internet database
- 5% Publications database
- Crossref database
- Crossref Posted Content database
- 3% Submitted Works database

Excluded from Similarity Report

- Bibliographic material
- Small Matches (Less then 8 words)

April 16, 2022

Dear Urja Sharma

I am pleased to inform you that your manuscript titled "Nanotechnology for the bioremediation of heavy metals and metalloids" (Manuscript Number: JABB-2022-01-034) is accepted for publication in the Journal of Applied Biology & Biotechnology.

You are advised to add the published article to your research gate, google scholar, and ORCID profile to enhance the discoverability of the published content.

The journal encourages the authors to host the published version of the manuscript to their institutional repositories.

You are required to make the payment of article processing charges. The payment details will be sent in a separate email.

I would like to remind you that you could send your future manuscripts to Journal of Applied Biology & Biotechnology.

Sincerely yours,

Editor
Journal of Applied Biology & Biotechnology
email: editor@jabonline.in