SCREENING AND IDENTIFICATION OF SOIL MICROORGANISMS WITH POTENTIAL OF PLASTIC DEGRADATION ABILITY.

A DISSERTATION

SUBMITTED TO THE

SCHOOL OF BIOENGINEERING & BIOSCIENCES

IN PARTIAL FULFILMENT FOR THE REQUIREMENT OF THE DEGREE

OF MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by: Rajdeep Kaur (11201169)

Under the guidance of:

Dr. Rattandeep Singh

MAY, 2017

LOVELY PROFESSIONAL UNIVERSITY

PHAGWARA (PUNJAB)-144411

DECLARATION

I hereby declare that the project work entitled as "Screening and identification of soil microorganism with potential of plastic degradation ability" submitted to Lovely Professional University, is a record of an authentic work done by me, under the guidance of Dr. Rattandeep Singh, Lovely Professional University, in order to fulfill the requirement of project. This work has not be copied from any source and whatever decoration and connection made in the circuit is a total dedicated work of mine.

Rajdeep Kaur(11201169)

CERTIFICATE

This is to certify that the Dissertation entitled "**Screening and identification of soil microorganism with potential of plastic degradation ability.**" submitted by Rajdeep Kaur (11201169) in partial fulfilment of the requirement for the award of degree of M.sc in Biotechnology to Lovely Professional University, Phagwara Punjab. It is a record of the candidates own work carried out by her under my supervision. The matter embodied in this thesis is original and has not been submitted for the award of any other degree.

Approved as to style and content by:

Dr. Rattandeep Singh,
Assistant Professor
School of Bioengineering and Biosciences

ACKNOWLEDGEMENT

It is my honour to present my project on "Screening and identification of soil microorganism with potential of plastic degradation ability".

I wish to express my sincere gratitude to my project supervisor **Dr. Rattandeep Singh**, **Assistant Professor**, who gave me the motivation to take the project and inspired me each point of time. I deeply thank him for his dedication and patience provided for my project work. I owe a deep sense of gratitude **to Dr. Umesh Gautam, Head of Department of Biotechnology** for his continuous encouragement and guidance whenever needed.

Also, I sincerely thank all the faculty members who helped me at various stages of our project. My thanks also go out to **Er. Chirag Chopra, Head of Laboratory of Biotechnology** for encouraging and supporting me during my project.

Last but not the least I would like to thanks my parents who always supported and encouraged me, that always helped me to move forward. I would like to thank all my friends for their kind support and stimulating thoughtful discussions that helped me so much in my project.

Rajdeep Kaur (11201169)

Abbreviations

ABS	Acrylonitrile butadiene styrene
PVC	Poly vinyl chloride
PHB	Poly hydroxyl butyrate
PHV	Polyhydroxyvulerate
PHA	Polyhydroxyalkanoate
PET	Polyethylene terephthalate
HDPE	High density polyethylene
LDPE	Low density polyethylene
PP	Polypropylene
PS	Polystyrene
SPI	Society of plastic industry
BPA	Bisphenol A
YPD	Yeast Potato Dextrose Agar Media

Table of Contents

List of figures:

List of tables:

Abstract:

Plastic is of great use, but at same time has lots of disadvantages as well, pollution of which is one of the major cause including both soil and air pollution. Burning plastic releases lots of toxic chemicals that are really harmful to health, so to resolve this issue microorganisms can be used. The enzymes secreted by microbes are of great use and thus play an important role in degradation of plastics. With this motive, the aim of the study was to isolate microorganism with capability to degrade the plastic. 5 different fungi were isolated form soil and were further used in the plastic degradation studies. 2 different plastics, that is, LDPE and polystyrene were used in the study for the degradation. Out of 5 isolated fungi one showed maximum LDPE as well as polystyrene degradation. Upon partial identification of the fungi, it was identified a species belonging to *Rhizopus* genus. This species showed 20.17% of degradation of LDPE film in 50 days whereas 16.34% degradation of the polystyrene film in 30 days upon determination of weight loss before and after treatment with the fungi. Also, FTIR analysis of the films was done so as to confirm the change in the surface properties of the plastic. Thus, using microbes as an alternative solution for degradation of plastic can be of great use with minimal harm to mankind and offers a ecofriendly process.

Chapter 1

Introduction:

From the past few years, materials of plastics have gained a great attention due to their use in food, shelter, transportation, cloth, construction and various other industries. The word plastic comes from the Greek word plastikos, which means 'able to be molded into varied shapes' [1]. Plastic, in simple language can be defined as a polymer which on heating becomes mobile and thus accordingly can be cast into the moulds [2]. Polyethene constitutes of at least 64% of total constituent of a plastic. It is a linear polymer that comprises monomeric units of ethylene (C_2H_4) [3]. Increasing demand of plastics in day to day life has also increased the risk of pollution and other health related hazards.

Plastic, becoming as the most adaptable synthetic and manmade substance is being used for various purposes. Having lot of advantages, degradation of plastic has become a major issue as it has number of harmful affects due to its accumulation in the soil and thus leading to soil pollution [4].Plastics that are used by us are generally made from, some of the organic and inorganic raw material including carbon, nitrogen, silicon, chloride , oxygen and hydrogen. The material required for making the plastic are obtained from oil, coal and natural gas [2]. The most conventionally used plastics include polystyrene, polypropylene, polyvinyl chloride, and polyethylene terephthalate and polyethylene [5]. Plastic accumulation in the terrestrial habitats has led to its ingestion by the endangered California condor, *Gymnogyps californianus* and caused a further decline of its population [6]. Polythene can even lead to blockage in the intestine of fish, marine mammals and birds [7].

Plastic can be classified into two major types; Thermoplastics and Thermosetting plastics. Thermoplastic is a type of plastic which upon heating softens and upon cooling gets hard. When heated again and again thermoplastics can be molded into any shape and also they do not even undergo any change in the chemical composition. Example of thermosetting process includes Rubber Vulcanization. There are three types of thermoplastics including, crystalline, amorphous and semi-crystalline type [8, 9]. On the other hand, thermosetting plastics are those, which upon heating becomes insoluble and infusible materials. These polymers have permanent irreversible

polymerization. The first step in this type of polymerization includes polymer formation having linear chains whereas the second step includes cross linking between the structures formed. The end product formed can be either rigid or it may be flexible [10]. Examples of thermoplastics are ABS (acrylonitrile butadiene styrene), polyester and PMMA (acrylic). Polystyrene can be either thermoplastic or thermoset.

Other types of plastics include: Natural and Synthetic plastics.

Natural plastics are those, which are obtained naturally and thus can be extracted from plants, algae etc. Natural plastics are generally biodegradable. Whereas, Synthetic plastics are those which are formed as a result of a number of chemical reactions which thus leads to polymerization of the monomers, thus formation of plastic occurs [11]. Plastic contains additives such as plasticizer which can cause higher extent of toxicity. Plasticizers are those substances includes either low-melting solids or high-boiling organic liquids. Upon their addition to hard plastics, the flexibility and durability of the plastics get improved. It has been reported that plant death has been caused by the use of a plasticizer known as di butyl phthalate [12]. A number of experiments has also been to identify the potential toxicity of plasticizers especially phthalate plasticizers on human health. The results of several experiments showed a number of severe implications such as hepatic peroxisome proliferation, hormonal disorders, inducing allergic symptoms in children, reproductive toxicity, and carcinogenicity [13]. The three main ways to handle the plastics after its use include incineration, land filling, and recycling each of which has its own drawback. Land fill occupies wide space of field that can be efficiently used for other means like agriculture [14]. This method also consumes large amount of time, due to low oxygen concentrations during landfill thus leading to anaerobic conditions [15]. Incineration requires use of heat to bury the plastic. This process leads to accumulation of toxic materials in the environment therefore enhancing the chances of health related problems [14]. Chemical and mechanical processing of PET (polyethylene terephthalate) helps in PET recycling [16]. Chemolysis of PET results in depolymerization of plastic. Hydrolysis, methanolysis, glycolysis or aminolysis can lead to depolymerization reaction. Mechanolysis includes various properties to be changed like Flake size Melting temperature ,Viscosity (η), Water content ,Dye content, PVC content, Polyolefin content, Metal content, Yellowing index [16, 17].

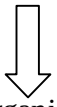
Other type of plastic is the biodegradable plastic, that include starch based and bacteria based plastics. Starch based plastics are generally derived for sources like wheat, potatoes, corn and rice. Starch is transformed into an altered polymer so as to solve the issue of starch deformation. The process results in conversion of lactic acid monomer to a polymer known as polylacitide (PLA). PLA and PGA are example of starch based polymers. Bacteria based plastics include PHA (poly hydroxyl acetone) that is polymer of PHB (poly hydroxyl butyrate) and PHV (poly hydroxyvulerate). Polyhydroxyalkanoates (PHA), a family of diverse bio-polyesters, used as biodegradable plastics [18].

Any physical or chemical change in a polymer occurs due to environmental factors such as light, heat, moisture, chemical conditions and biological activity. This change leads to loss of properties in the polymer and is referred as degradation. Photo degradation of plastic happens in presence of light. Thermal degradation happens due to heat whereas Biodegradation of plastics means degradation of plastic using micro organism when provided appropriate conditions [19].There are various factors that influence the biodegradability of the plastics. Environmental factors such as humidity, temperature, pH, salinity, water, stress the presence or absence of oxygen, sunlight and culture conditions not only affect the polymer degradation, but also have a great influence on the microbial population and enzyme activity [20].

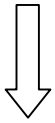
To overcome the problem of soil pollution, biodegradation of plastic can be achieved using consortium of microorganism that widely include filamentous fungi and bacteria. Microbial act on plastic can be either directly or indirectly that consequently leads to deterioration and discoloration of the plastic. Plastic itself serve as carbon or nitrogen source for the effective growth of the microorganisms. Fungi and bacteria have been widely used for degradation of plastics. Degradation of plastics by microorganism is achieved with help of enzymes secreted by the microbe. These enzymes tend to cleave the chains of polymer into monomeric units, thus leading to effective degradation of the plastic. The increasing rate at which the plastic is accumulating in the environment has lead to increase in pollution and therefore is major concern to the scientists [21].

1.1 GENERAL MECHANISM OF BIODEGRADATION:

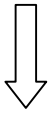
Specific microorganism.



Adherence of micro organism to the surface of plastic.



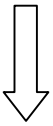
Plastic now serves as the sole carbon source for the microorganism, thus biofilm formed on the surface of the plastic and colonization start to happen.



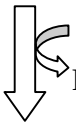
Release of extracellular enzyme, and its attachment to the plastic surface.

.

Cleavage of the polymer chains and its change to monomer units by the specific action of the enzyme.



Assimilation, that is, integration of the various molecules transported to cytoplasm of microbe during microbial metabolism.



Release of carbon dioxide, water etc.

Complete degradation of plastic and excretion of various metabolites.

The purpose of the study is to isolate micro organism from soil and screen it for the potential to secrete various enzymes such that it is capable to degrade the plastics.

Chapter 2

REVIEW OF LITERATURE:

Plastics are long chain of monomer units, thus resulting in formation of a polymeric chain. From the last few years there is uncontrolled use of plastics for various purposes including packaging, transport, building and construction, transport, agriculture, furniture, toys and sports etc. Plastics are widely used not only due to the reason that they have good mechanical and thermal properties but also due to their high stability and durability [22]. While no authentic figures are available on total accumulation of plastic waste in India, it is evaluated to be approximately 5.6 million tonnes per annum (TPA), which means it is about 15,342 tonnes per day (TPD).

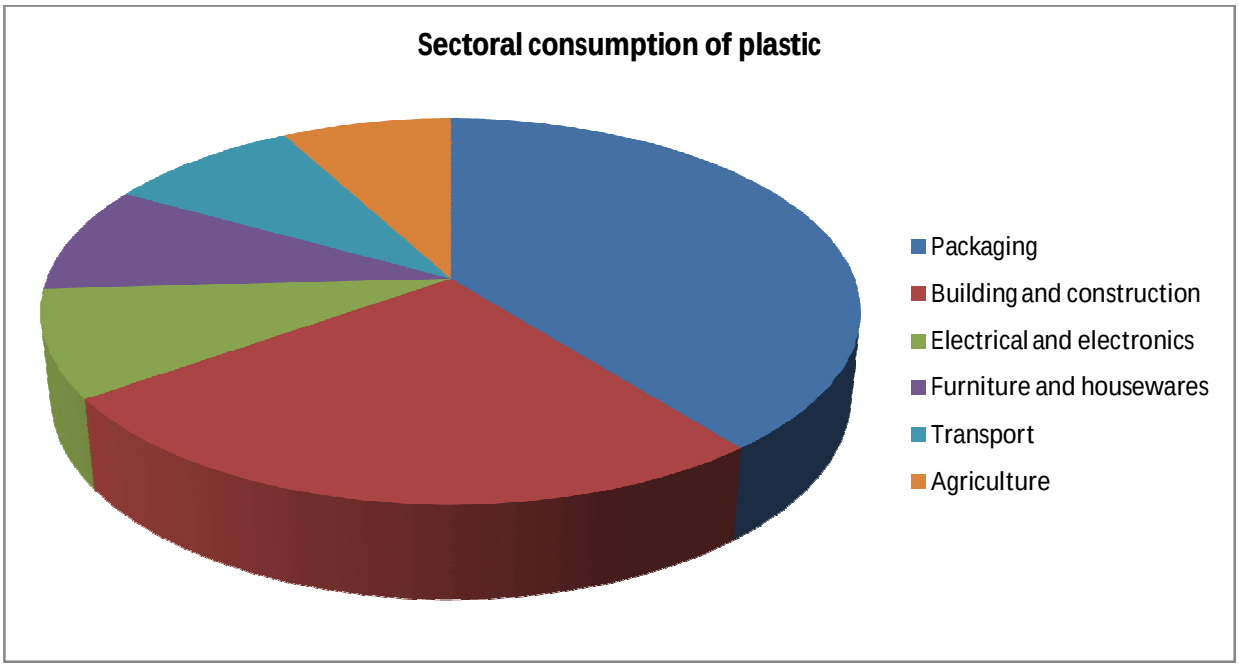


Figure no.1 Sectoral consumption of plastic

80 percent of the total plastic waste generated in India is contributed by thermoplastics or recyclable plastics, while remaining 20 per cent is accounted by the thermoset plastics or non-recyclable plastics. The food packaging industry comprises mostly use polyethylene terephthalate (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC), low-density polyethylene (LDPE), polypropylene (PP), and polystyrene (PS) as the main components of common food packaging plastics

Table no. 1 **The Society of the Plastics Industry (SPI) established a plastic classification system in for identification of plastics by consumers.**

Serial number	Type of plastic	Uses
1.	Polyethylene Terephthalate (PET)	Beverage, medicine jars, rope, clothing, bottles, and carpet fiber.
2.	High Density Polyethylene (HDPE)	Containers of milk, motor oil, soap bottles, detergents, shampoos and conditioners, and bleaches
3.	Poly Vinyl Chloride (PVC)	Used for all kinds of pipes and tiles.
4.	Low Density Polyethylene (LDPE)	Cling-film, squeezable bottles, sandwich bags and plastic grocery bags.
5.	Polypropylene (PP)	Lunch boxes, margarine containers, yogurt pots, syrup bottles, prescription bottles.
6.	Polystyrene (PS)	Disposable coffee cups, plastic food boxes, plastic cutlery and packing foam
7.	Polycarbonate and Polylactide	Baby bottles, compact discs, and medical storage containers

Table no. 2 Type of plastics, examples and their code designations.

Type of plastic	Code designations
	Code 1 (PET) Eg. Bottles
	Code 2 (HDPE) Eg. Milk containers
	Code 3 (PVC) Eg. Plumber pipes
	Code 4 (LDPE) Eg. Plastic bags
	Code 5 (PP) Eg. Lunch box
	Code 6 (PS) Eg. Coffee cups
	Code7 (Miscellaneous types) Eg. CDs

The Society of the Plastics Industry (SPI) established a plastic classification system in for identification of the plastics by the consumers. These include;

High plastic accumulation has occurred in the natural environment and in landfills. Around 10% by weight of the city waste is plastic. Plastic that is discarded as such contaminates many natural terrestrial, freshwater and marine habitats [23]. A portion of the polymers have higher level of lightness(46%, US EPA 2006), and subsequent things of plastic trash, for example, plasticizers like adipates and phthalates which are added most usually to weal the plastic cases of which incorporate polyvinyl chloride, in order to make the item sufficiently bendable. Hints of these mixes can exit out of the item. This intensifies that filter from polystyrene nourishment compartments have been anticipated to meddle with hormone works and should have cancer causing impact [24].

The plastic debris has a deleterious effect on the aquatic life. The polymers despite of having buoyant nature get folded with the marine life and this cause them to sink at the seabed [25]. Accumulation of plastic debris also causes major issues related to aesthetic problems, and eventually represents a hazard to maritime activities such as fishing and tourism [26]. Use of huge amount of different chemicals for the manufacture of plastics is reported to be toxic.

Bisphenol A (BPA) may leech into the contents/liquids that the plastic container is holding. BPA acts as basic monomer of polycarbonate plastics, some of those that are used includes for bottled water, food packaging and other items. It has also been reported as an endocrine disrupting chemical which interferes with normal hormonal function [27, 28, 29].

PVC is used in various products in the home including water pipe, may contain phthalates, BPA, flame retardants such as PBDEs or TBBPA, cadmium, lead and organotins, all of which have been shown in animal studies to result in obesity. Large amount of phthalates and BPA, as well as other plastic additives, are present in the human population [28].

Phthalates are that class of chemicals which are used for softening of plastics, for example, PVC (Polyvinyl Chloride), it tie the aromas in items, and go about as solvents or fixatives, like the event of nail shines. People get presented to it in various courses, like, Inhalation i.e., taking in exhaust or scents from the solvents and fixatives, Ingestion that happens during biting on a plastic toy subsequently makes little openings in the plastic, giving a space to draining of chemicals from the toy into the baby's mouth, Absorption that happens because of utilization of

salve, scents, and antiperspirants. Antagonistic wellbeing impacts, for example hormone interruption, formative and regenerative issues, asthma, low sperm count, undescended testicles, genital distortions, untimely puberty, and advancement of few tumors {29].Inappropriate discarding of plastic play a major role in causing harm to life by increasing rate of environmental pollution. Also, burning of plastics such as polyvinylchloride (PVC) release persistent organic pollutants (POPs) called furans and dioxin [30]. Having almost similar material properties as compared with conventional plastics, polyhydroxyalkanoates (PHA), polycaprolactone, polylactides, polysaccharides and copolymer like biodegradable plastics such as polyesters or blend of these have been rapidly developed over the last few years.

Different types of plastics get degraded in a different way depending on the type of polymer it is. Plastic can be either thermoset or thermoplastic [9], or it can be either natural or synthetic. Thus degradability is also affected by type of polymer. Various micro organisms have been associated with plastic degradation, which happens as a result of secretion of specific enzymes that act upon the polymeric plastic thus leading to it degradation. Plastic biodegradation begins actively under different soil conditions according to their physical and chemical properties, as the degrading source that is the microorganism differ from each other and also they have their own optimal growth conditions in the soil. Plastics generally act as potential substrates for microorganisms that are heterotrophic in nature that is they can use plastic as both its carbon and energy source [31].

Both fungi and bacteria have been reported for the purpose of microbial degradation of plastics. Examples of these include, *Sreptomyces, Aspergillus niger, Pseudomonas, Aspergillus flavus, Aspergillus japonicas, Pestalotiopis microspora, Pseudomonans putida, Penicillium simplicissium, Gloephyllum trabeum, Micrococcus luteus, Masoniella* species, *Bacillus mycoids, Bacillus subtilis, etc.* Micro organisms living in the gut of bacteria also leads to plastic degradation as reported recently. All of these are associated with plastic degradation and that too with the specific type of plastic.

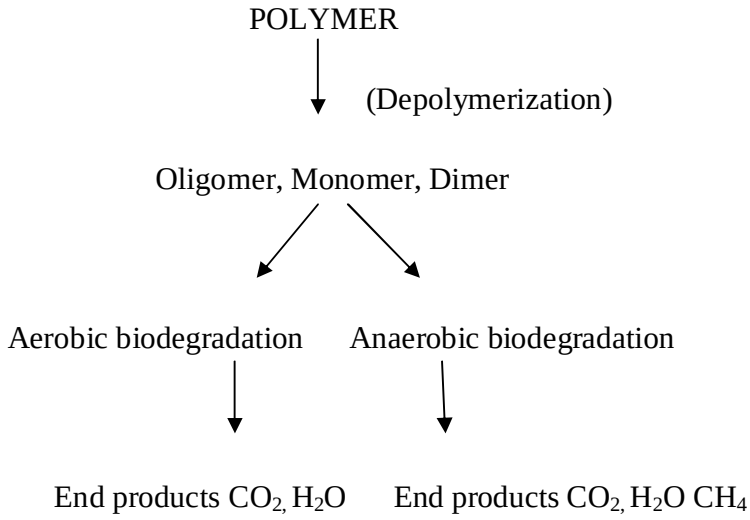
Due to diverse forms of enzymes secreted by the fungi it has been dominating over bacteria in the bioremediation processes. One of such fungi reported is *Phanerochaete chrysosporium*, which is also known as white rot fungus is able produce wide range of enzymes of great importance in concern to plastic biodegradation.

Table no. 3 Some of the literature reports conducted, on Biodegradation of plastic using various microorganisms including bacteria and fungi.

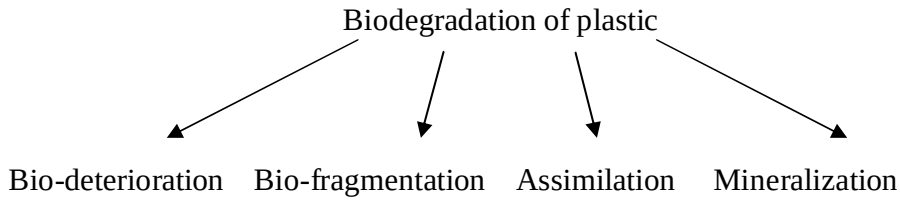
Sr. No.	Micro organism used	Plastic used	Year	Reference number
1.	*Aspergillus niger*, *Aspergillus japonicas*	LDPE (Low density polyethylene)	2012	[32]
2.	*Pseudomonas*, *Streptomyces* species	Polythene, Plastic	2011	[33]
3.	*Pestalotiopis microspora*	PUR(Poly urethane)	2011	[34]
4.	*Pseudomonas , Aspergillus glaucus*	Polythene, Plastic	2002	[35]
5.	*Gloephyllum trabeum*	Polystyrene sulfonate	2015	[36]
6.	*Phanerochaete chrysosporium*	Starch based plastic polymer	2003	[37]
7.	*Bacillus mycoids, Bacillus subtilis*	LDPE and HDPE	2013	[38]
8.	*Penicillium*	Polyethylene	2000	[39]
9.	Micrococcus Luteus, Masoniella Sp	Plastic cups	2014	[40]
10.	*Pseudomonans putida*	Polythene bags, plastic cups & bags, milk cover	2014	[41]
11.	*Pleurotus ostreatus*	Oxo-Degradable-Polyethylene and Polylactic Acid Films	2014	[42]

Sr. No.	Micro organism used	Plastic used	Year	Reference number
12.	*Sreptomyces, Aspergillus niger, Pseudomonas, Aspergillus flavus*	Low density polyethylene (LDPE)	2015	[43]
13.	*Pseudomonas, Aspergillus, Bacillus, Penicillium* species	Polyethylene	2012	[44]
14.	Mealworms gut- bacteria	Polystyrene	2015	[45]
15.	*Pseudomonas*	Synthetic Polyethylene	2010	[46]
16.	*Aspergillus and Fusarium* species	LDPE	2014	[04]
17.	*Trichoderma harzianum*	Polyethylene	2014	[47]
18.	*Bacillus amyloliquefaciens*	LDPE	2015	[48]
19.	*Aspergillus clavatus* JASK 1	LDPE	2016	[49]
20.	*Bacillus weihenstephanensis, Burkholderia cepacia, Escherichia coli Aspergillus niger*	Plastic carry bag	2014	[50]

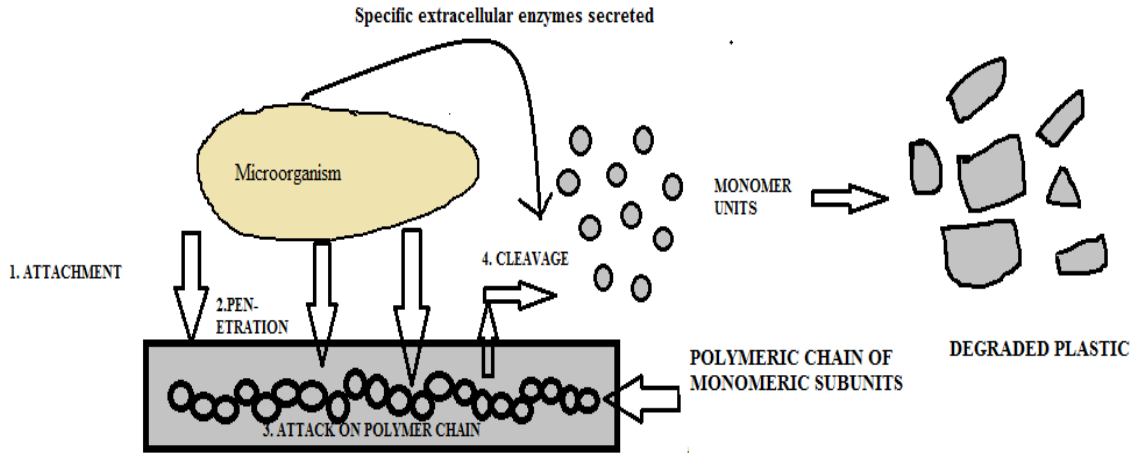
Biodegradation reactions of plastic follow two types of pathways, aerobic reaction and anaerobic reaction. Both of this leads to a release different end products depending on the pathway the process follows. In presence of oxygen aerobic biodegradation occurs and production end product like carbon dioxide takes place. On the other hand anaerobic biodegradation involves absence of oxygen and release of end products like carbon dioxide and methane. [51]



Due to plastic accumulation at a very high rate, the degradation of this plastic waste has become issue of concern. Degradation of plastic can be achieved with help of energetically, chemically, and biologically. Energetically it can be either thermal or radiant type of energy. Radiant energy types include UV rays, gamma rays, or ion beams. Chemical degradation is done with help of various chemicals such as acids or alkali. Micro organism can be used to degrade biodegradable plastics, which serves as biological means of degradation [51].



The main steps that are involved in the biodegradation of plastic includes; Bio-deterioration (involves attack of microbial consortia on the plastic which leads in physical and chemical changes in plastic), Bio-fragmentation (leads to the breakdown of oligomer units to dimer and monomer units), Assimilation (transportation of integrated molecules to the cytoplasm), Mineralization (complete degradation and release of end products) [51, 52].



<u>Figure no.2.</u> Action of microorganism on polymeric chain of plastic and its breakdown into monomer subunits with the specific action of enzymes secreted by microorganism.

Degradable plastics are those that can undergo chemical changes under specific environmental conditions (as described by the ASTM, American Society of Testing Materials and ISO, Indian standard Organization).

Plastic degradation occurs only when appropriate conditions are provides. The process of biodegradation generally happens as a result of release of enzyme by the microorganism and their direct action on the plastic thus leading to its breakdown.

Enzymes are biological catalysts that enhance the rate of chemical reaction that is, the conversion of particular substrates into specific products by providing favorable conditions that further decrease the activation energy of the chemical reaction. Enzyme may be either a protein or a glycoprotein. Enzymes have active sites; these sites are directly involved in the reaction. An enzyme may also have one or more extra groups that are covalently or non-covalently bonded to the active sites are essential for catalytic activity; the protein or glycoprotein moiety in such type of enzyme is known as apoenzyme, while the nonprotein moiety is known as the prosthetic group. The combination of the apoenzyme with the prosthetic group results into holoenzyme [53].

Microbial enzymes are generally involved in the process of biodegradation. These enzymes include laccase, cutinase, hydrolase, esterase, protease and urease. Laccases are polyphenol oxidases. These enzymes are involved in the catalysis and the oxidation of phenolic and aromatic compounds), with reduction of oxygen to water. Laccase enzymes are found among various plants, fungi and bacteria, and are involved in many biological functions, such as breakdown of complex polymers (lignin, humic acid) into simple monomers, lignifications reactions, detoxification, morphogenesis, sporulation, etc. [53, 54]. Proteases enzymes are those that lead to the breakdown of protein molecule by hydrolyzing the peptide bond. Proteases are majorly divided into two categories , exopeptidases and endopeptidases [53].

Peroxidases are those enzymes that catalyze the lignin oxidation and other phenolic compounds. Peroxidases are extensively classifies into lignin peroxidase (LiP), manganese-dependant peroxidase (MnP), and versatile peroxidase (VP) and have been studied frequently due to their ability to degrade toxic wastes in environment [53, 54]. Ureases are those enzymes that hydrolyze urea and librate ammonia. Esterases are those group of enzyme that catalyze cleavage and ester bond formation. Esterases are involved in hydrolysis of carboxyl ester. Another group of enzymes lipases are also involved in plastic degradation and are highly involved in hydrocarbon elimination from soil. Lipases catalyze the hydrolysis of triacylglycerols to glycerol and free-fatty acids [55].

There are various factors that directly are involved in the biodegradation of the polymers. The characteristic properties of plastics influence the rate of biodegradability. Chemical and physical properties of plastic affect the process of degradation, some of these properties include, surface conditions plastic such as surface area, hydrophilicity and hydrophobicity of the plastic, the first order structures of plastic which include chemical structure and molecular weight of the plastic and the high order structures involving glass transition temperature, melting temperature, elasticity and crystallinity of plastics [56].

Crystallinity of a polymer affects drastically the rate of degradation. Microbial enzymes generally attack on the amorphous regions of polymers. Therefore, more region of crystallinity will directly affect the rate of degradation leading to decrease in degradation. Smaller plastic debris is degradable at higher rate as compared to larger plastic particles. Biodegradation is also enhanced by abiotic hydrolysis, photo-oxidation and physical disintegration. These processes

increase the surface area of the polymer and thus reduce the overall molecular weight of the polymer; leading to microbial degradation. Temperature, pH, oxygen availability and plastic concentration also influence the plastic degradation rates [5, 57].

Chapter 3

SCOPE OF STUDY

- This experimental study is related to degradation of plastic with the beneficial use of microorganisms.
- The chemical methods available for degradation of plastic generally release toxic fumes and gases in the atmosphere, therefore degradation by the use of microorganisms will minimize this risk.
- The present research work can isolate some microbial species that has the ability to degrade plastic and is of industrial applicability.

Chapter 4

OBJECTIVES OF STUDY

- Isolation of microbial species from plastic disposed soil.
- Partial identification of the isolated microbial species with potential of plastic degradation.
- Physical and Chemical analysis of microbial degradation of plastic and change in various characteristics after degradation.

Chapter 5

Materials and Methods:

6.1 Collection of the sample

Soil sample were collected in lock zip seal plastic bags from the village Pharala, near Behram, from 2 different sites with maximum plastic disposed garbage and 1 soil sample was collected from Ludhiana as well. 1 soil sample was specifically collected from the soil where plastic bags and as well as polystyrene plates were dumped in soil nearly 4-5 cm in depth.

Isolation of microorganisms was done from the soil sample collected from the sites where maximum amount of plastic was disposed off. Soil samples were collected from different sites. The collected soil sample was subjected to serial dilution, and then dilutions were made 1:10 (10^{-1}), 1:100 (10^{-2}), 1:1000 ($10^{-3)}$ and so on. 0.85% NaCl was used for making dilutions. From each dilution, 100 µl of sample was taken and spreading was performed in order to isolate the microbial species. Yeast Potato Dextrose Agar (pH 5.4) was used was used for isolation of fungal species.

Incubation of fungal plates were done at 25°C ± 2°C for 5-7 days and then growth was observed and further steps were performed.

6.2 Sub culturing of fungal species for purification of single type of species.

Fungal species were obtained after 5-7 days of spreading on YPD agar. In order to obtain a pure culture the fungal mycelia was further inoculated onto YPD agar plates.

6.3 Pretreatment of plastic and polystyrene.

Both plastic bag as well as polystyrene plates was cut into very small pieces and were then were grinded to get a fine powder with help of grinding machine separately [48].

6.4 Screening of the purified isolates, with potential to degrade plastic and polystyrene.

The purified isolates were further screened for their ability to degrade plastic. For screening the purified fungi were inoculated on 2% agar containing 1% powdered plastic and some low concentration of salts including, $(NH_4)_2SO_4$ (0.6g/l), KH_2PO_4 (1.3g/l), (Na $_3$PO $_4$)2H $_2$O (0.12g/l), MgSO $_4$.7H $_2$O (0.3g/l), KCl (0.3g/l).

The polystyrene and plastic powder was exposed to UV light for 20 minutes and then mixed with autoclaved 2% agar, finally poured to plates and inoculated with the fungal cultures. These plates were then incubated at 25℃ ± 2°C for 5-7 days.

Further screening of the isolates to degrade plastic film was carried out on YPD agar plate containing the plastic film inoculated with the purified fungal culture. Similarly plates were then incubated at 25°C ± 2℃ for 37 days so as to check the weight loss in plastic film after degradation [35, 41]. Also the fungus that was showing fastest growth was inoculated in YPD broth along with plastic film in 4 different flasks and incubated at 25°C ± 2°C. With certain intervals the plastic was weighed after 12, 22, 36 and 50 days respectively to check if loss in plastic film has taken place.

Polystyrene films similarly were cut and put in YPD broth along with different purified cultures. These flasks were also then incubated at 25℃ ± 2℃ for 30 days. As described earlier weight was checked later.

6.5 Washing of the plastic and polystyrene films after the incubation period along with the fungus.

The plastic and polystyrene films were carefully taken out with forceps in laminar air flow from the plates after their respective incubations along with the fungus. These films were then washed with 70% ethanol and then incubated in shaker in 70% ethanol at room temperature for 2 hours. After 2 hours the films were then washed with distilled water and put on filter paper and dried overnight [48]. Next day each plastic film was weighed carefully and the films were further stored in zip lock plastic bag for further analysis each of which were labeled respectively.

6.6 Analysis of the change in properties of the plastic surface.

The chemical changes in the surface of the plastic were further determined by the FTIR technique that tells us about the change in surface properties of plastic. These changes may include change in functional groups of a polymer, or a stretch in the carbon-carbon double bond.

Chapter 6

RESULTS AND DISCUSSION:

Isolation of the microorganisms was done from then soil with maximum disposed plastic. After 6-7 days of incubation fungal colonies were isolated and each dilution had different colonies, these colonies were then purified, to obtain a single type of fungus.

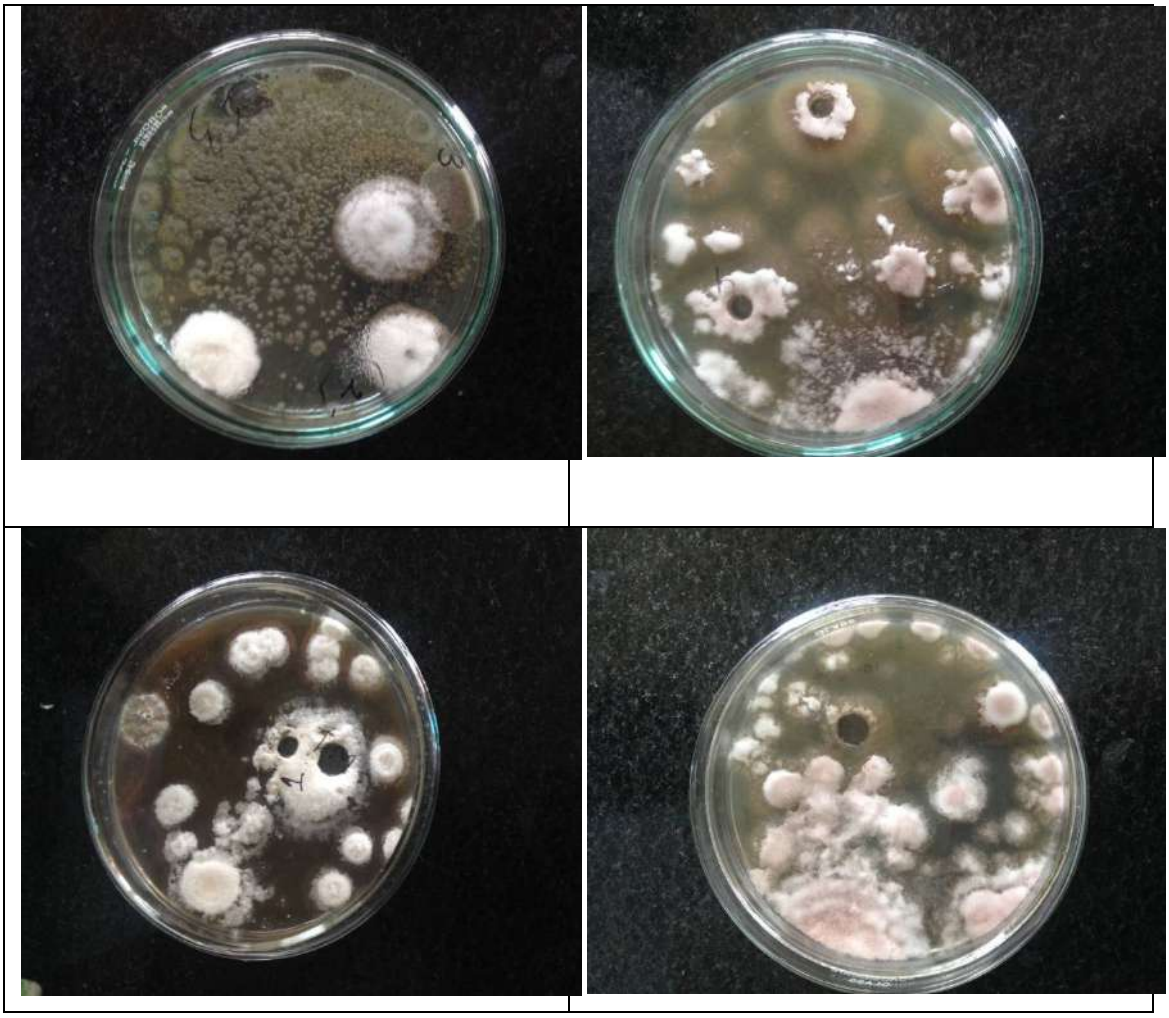


Figure. 3.a. Isolated fungal colonies after spreading of the serially diluted sample from soil sample 1. (From top left dilution 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}).

Figure.3.b. Isolated fungal colony from soil sample 2 (dilution 10^{-2}, rest all the plates were bacteria contaminated).

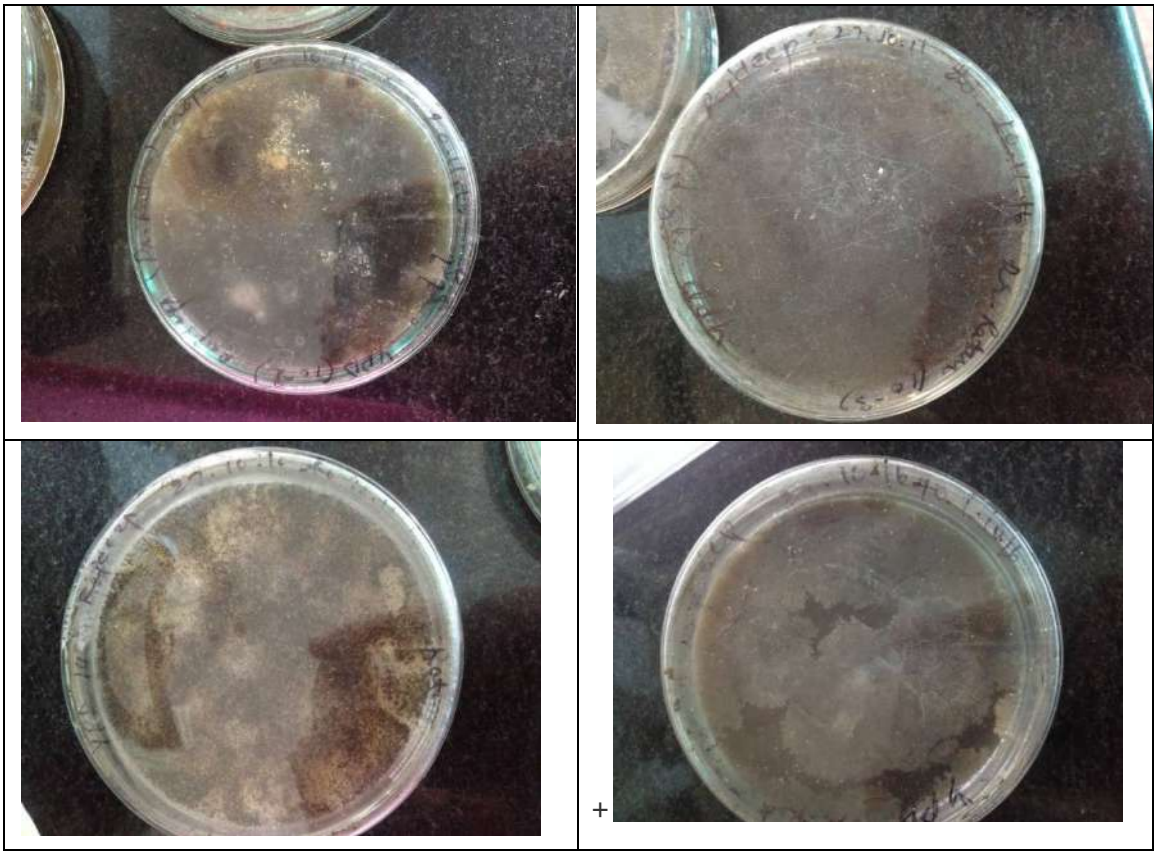


Figure.3c. Isolated fungal colony from soil sample 3(dilution 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}).

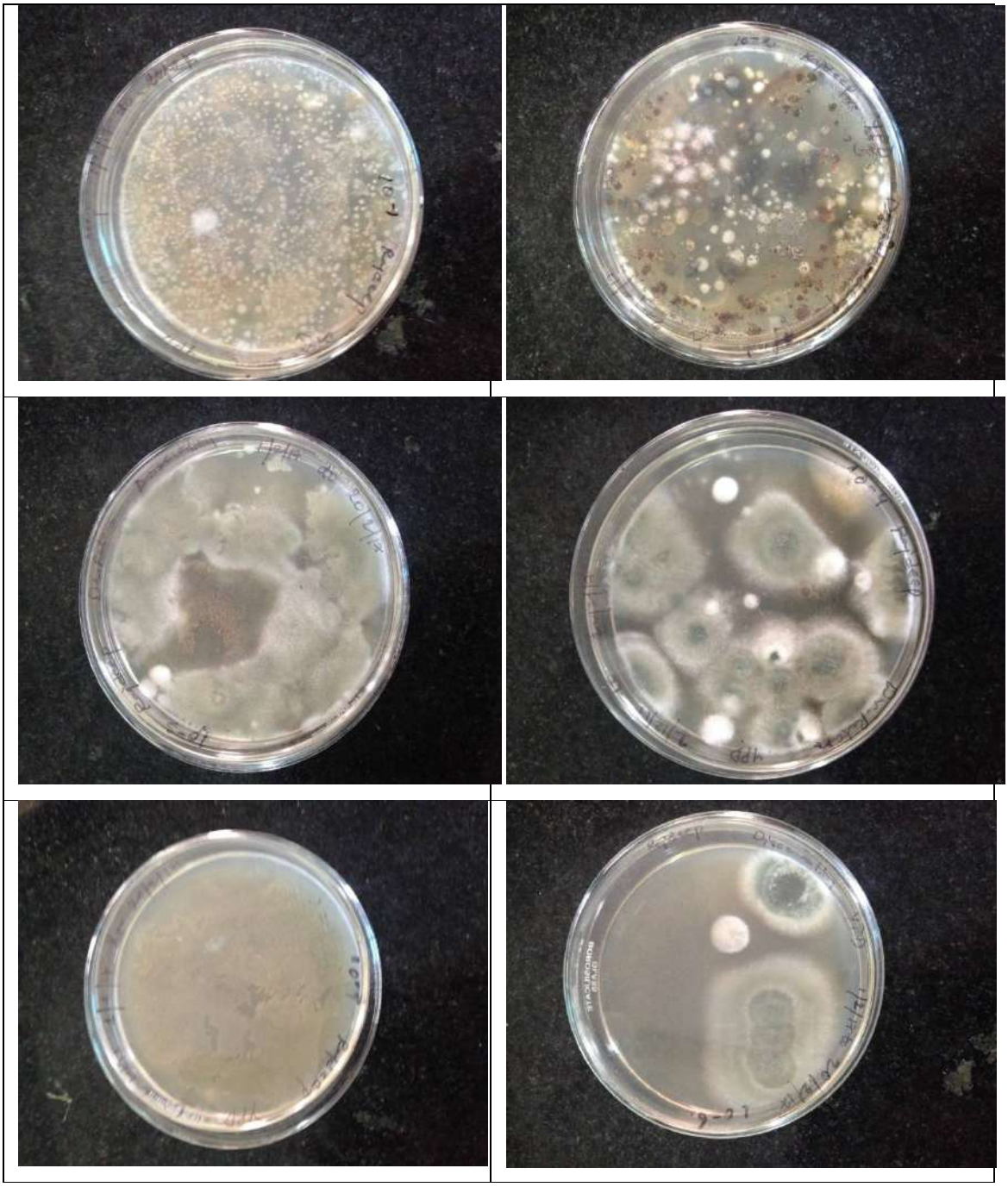


Figure.3d. Isolated fungal colony from soil sample 4 (dilution $10^{-}2$, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}).

Table no. 4. Morphological characteristics (Colony morphology of the fungal isolates)

Serial number	Soil Sample	Color of colony	Colony Characteristics
1.	Sample 1	White colored colony	Raised , Cottony
2.	Sample 1	Whitish - brown colored colony	Flat, Cottony
3.	Sample 1	Green colored colony	Powdered, Flat
4.	Sample 2	White colored colony	Raised, Cottony
5.	Sample 3	Black colored colony	Powdered, Flat
6.	Sample 4	Green colored colony	White margin
7.	Sample 4	White colored colony	Raised , sticky texture

From the soil sample 1, 2, 3 and 4 different fungal colonies were obtained. From sample 1, three colonies, whereas form sample 2 and sample 3 only single type of fungal colony was obtained. From soil sample 4 that was isolated specifically from plastic and polystyrene dumped soil, 2 colonies were obtained. Morphological characteristics of these colonies were carefully observed, like, the texture of colony, the elevation of colony, the color of colony etc.

<u>Figure no.4.</u> Purified fungal colonies obtained from the 4 different soil samples, which were used in the plastic degradation study (RR, WG, ASN, GG, and WW).

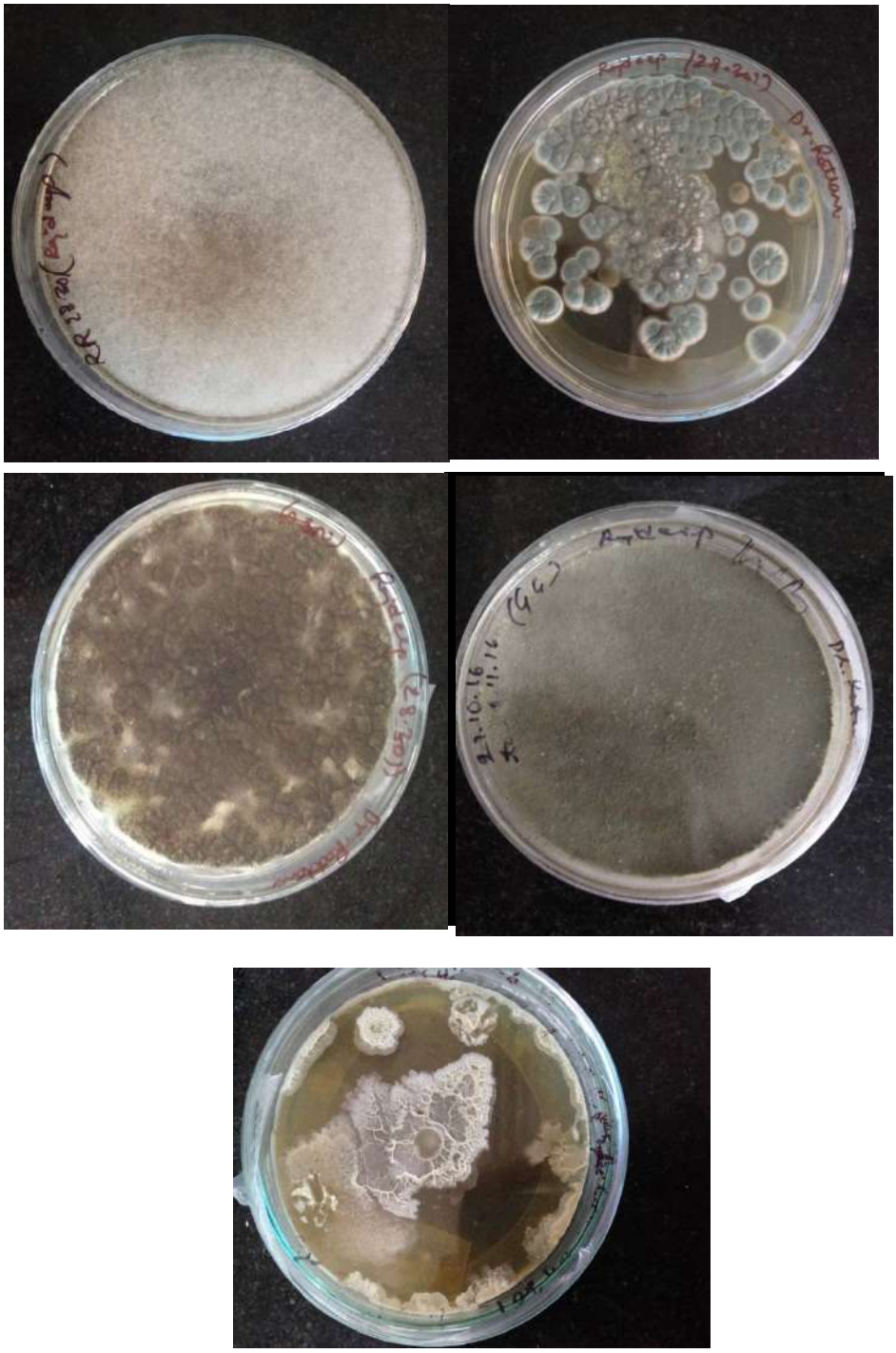
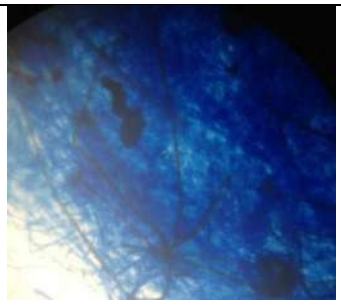
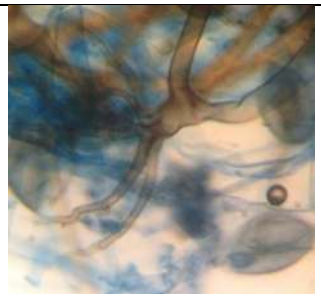
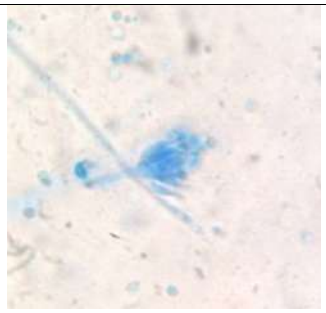
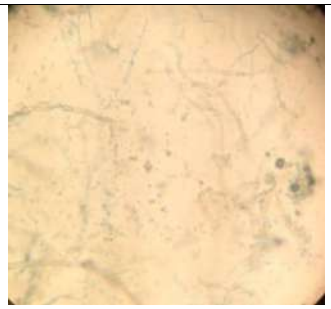
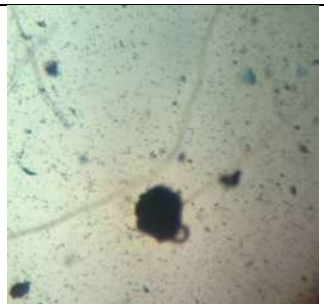
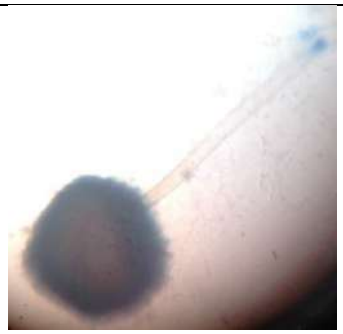
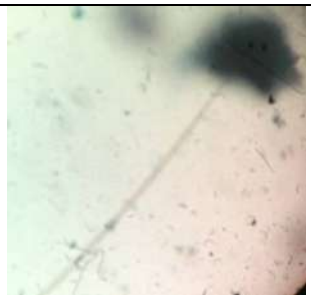
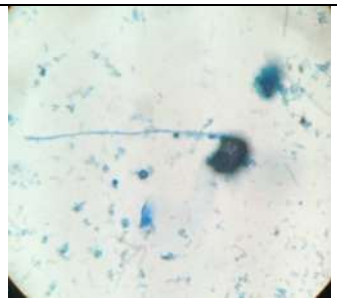


Table no.5 Microscopic identification of the fungus (LPCB staining)

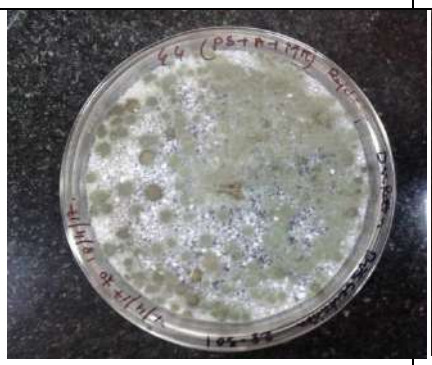
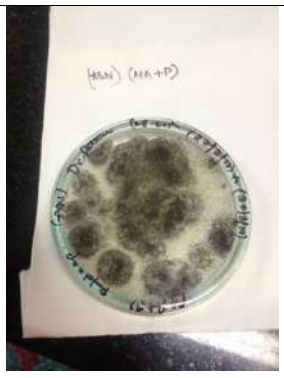
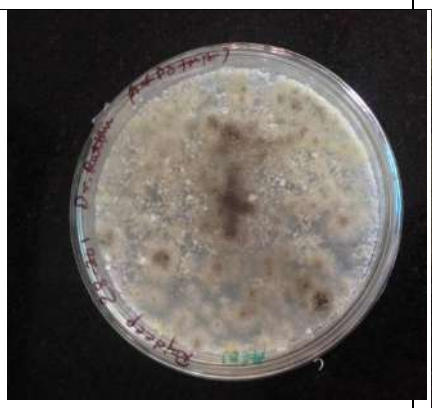
RR (Presence of rhizoids)		
GW (Presence of brush-like structure and septate hyphae)		
ASN (Presence of hyphae and spores)		
GG (Presence of spores and hyphae)		
WW		

	
Polystyrene powder	Plastic (LDPE) powder

Figure no.5. Both polystyrene and plastic (LDPE) were powdered using grinder for primary plastic degradation studies.

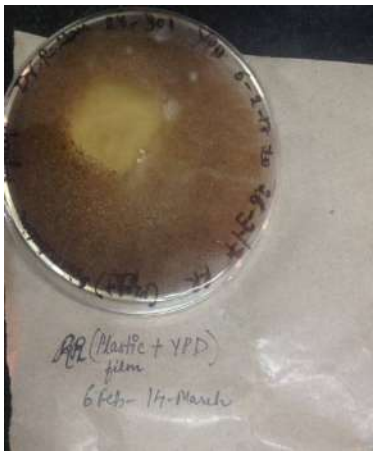
Inoculation of the 5 purified cultures in 2% agar containing some salts in very low concentration such that the salt acts as the nitrogen source and the plastic powder acting as the only carbon source. This formulated media acted as a media that contained both nitrogen (use of salts) as well as carbon source (plastic powder and polystyrene powder). The control plates that contained only 2% agar and fungus were also used such that growth pattern between the plates for a single species can be observed. In the plates containing plastic powder, low concentration of salts and 2% agar dense growth was observed as compared to plates containing only 2% agar and low concentration of salts. From this observations it was interpreted that plastic was acting as carbon source in the plates were the plastic powder along with some salts was prepared, thus this acts as a media for the fungus which will allow the fungus to degrade the plastic by utilizing it.

<u>Table no. 6</u>: Comparison of growth for 5 different species inoculated in 2% agar containing 1% plastic powder and polystyrene powder (acting as carbon source) and low concentration of salts (acting as nitrogen source), 2% agar, and the control plates containing only 2% agar and low concentration of salts.

Fungus	(AGAR + LDPE Powder + FUNGUS)	(AGAR + POLYSTYRENE Powder + FUNGUS)	(AGAR + FUNGUS)
RR			
GG			
ASN			

Fungus	(AGAR + LDPE Powder + FUNGUS)	(AGAR + POLYSTYRENE Powder + FUNGUS)	(AGAR + FUNGUS)
WW			
GW			

Dense growth was observed in those plates where both carbon and nitrogen source was used as compared to the plates containing only nitrogen source. Polystyrene and plastic powder were acting as carbon source, whereas low concentration of salts were acting as nitrogen source. Thus indicating that the plastic and polystyrene along with the nitrogen source were utilized by the fungi.



WW (YPD agar+ Plastic film) RR (YPD agar + Plastic film) GW (YPD agar + Plastic film)

ASN (YPD agar+ Plastic film) GG (YPD agar+ Plastic film)

<u>Figure no.6.</u> The next step was to check the degradation of the plastic films inoculated in YPD agar medium and weight reduction after the treatment of plastic with fungus for 37 days.

Figure no.7. **Plastic films from the YPD agar plates were taken out with help of forceps and dipped in 50ml of 70% ethanol in different flasks and incubated in shaking incubator for 2 hours.**

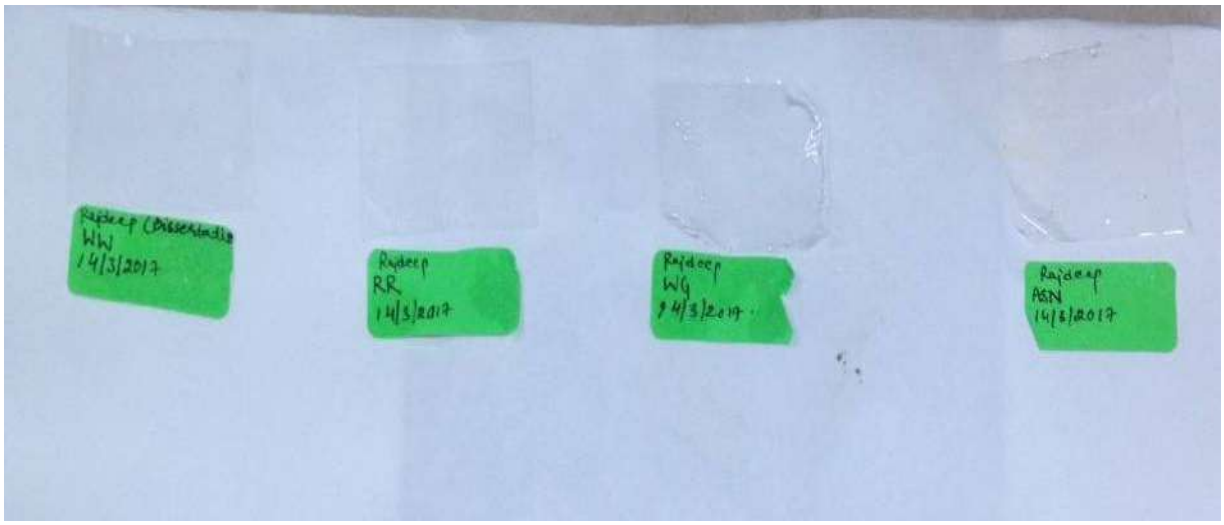


Figure no.8. **Plastic films allowed to dry overnight on filter paper and then were weighed and compared with the weight of untreated plastic.**

Table no. 7 Degradation of plastic using fungal species:

Serial number	Plastic sample	Weight of plastic (in grams)
1.	Control (Untreated, UT)	0.0570
2.	**Treated plastic films**	
2a.	GG, from soil sample 1 (Treated)	0.0479
2b.	RR, from soil sample 2(Treated)	0.0477
2c.	ASN, from soil sample 3(Treated)	0.0511
2d.	WG, from soil sample 4 (Treated)	0.0505
2e.	WW3, from soil sample 4 (Treated)	0.0513

Percentage weight loss in plastic film

Formula

$$\frac{\text{Initial weight of plastic film (I)} - \text{Final weight of plastic film after degradation (F)}}{\text{Initial weight of plastic film (I)}} \times 100$$

Table no. 8 Calculation of percentage weight loss after degradation studies of plastic by fungal species.

Fungus	Formula for calculation	Percentage weight loss
GG	0.0570 – 0.0479/ 0.0570*100	15.96%
RR	0.0570 – 0.0477/0.0570*100	16.31%
ASN	0.0570 – 0.0511/0.0570*100	10.35%
WG	0.0570 – 0.0505/0.0570*100	11.40%
WW3	0.0570 – 0.0513/0.0570*100	10%

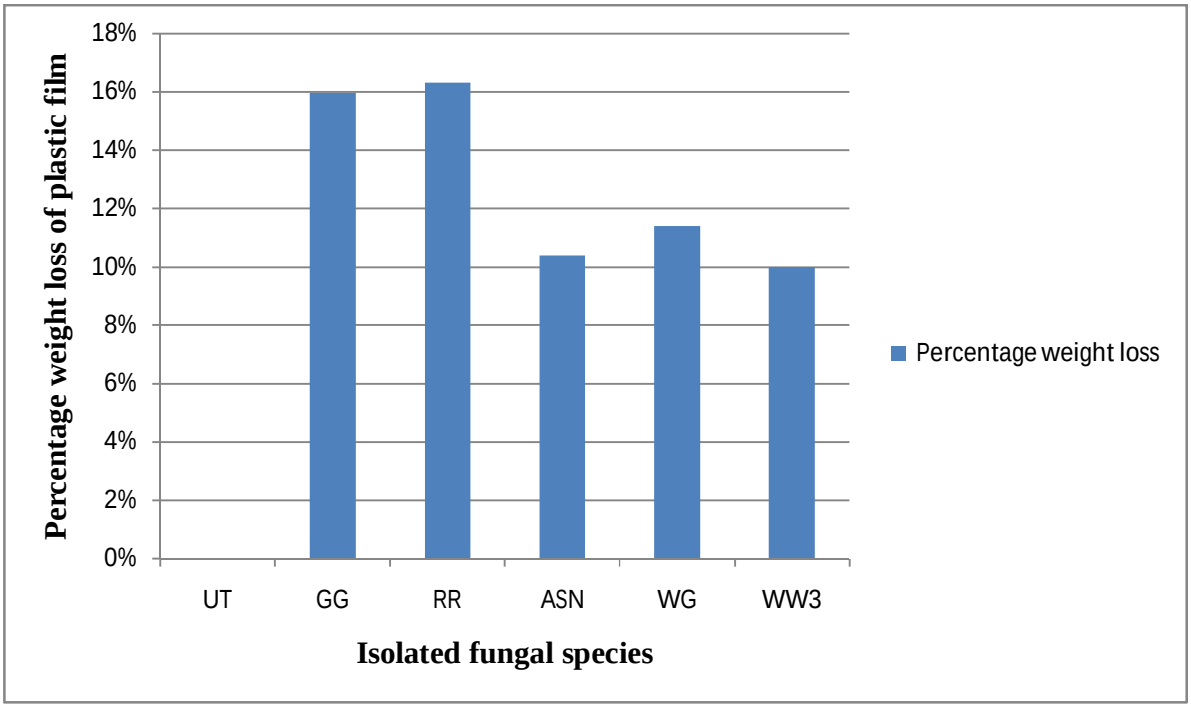


Figure no.9. Percentage weight loss in plastic film by 5 different fungal species

Plastic film degradation by RR fungi after 12, 22 and 36 days.

Table no. 9 Degradation of plastic film for RR fungi for 50 days

Time period	Total number of days	Weight of plastic film (in grams)
6 Feb – 18 Feb	12 days	0.0525g
6 Feb – 28 Feb	22 days	0.0506g
6 Feb – 14 March	36 days	0.0473g
6 Feb – 28 March	50 days	0.0455g

Table no.10 Calculation of percentage weight loss of plastic film by RR fungi in 50 days.

Fungus RR (Total days)	Calculation	Percentage weight loss
12days	0.0570 – 0.0525/0.0570*100	7.89%
22 days	0.0570 – 0.0506/0.0570*100	11.22%
36 days	0.0570 – 0.0473/0.0570*100	17.01%
50 days	0.0570 – 0.0455/0.0570*100	20.17%

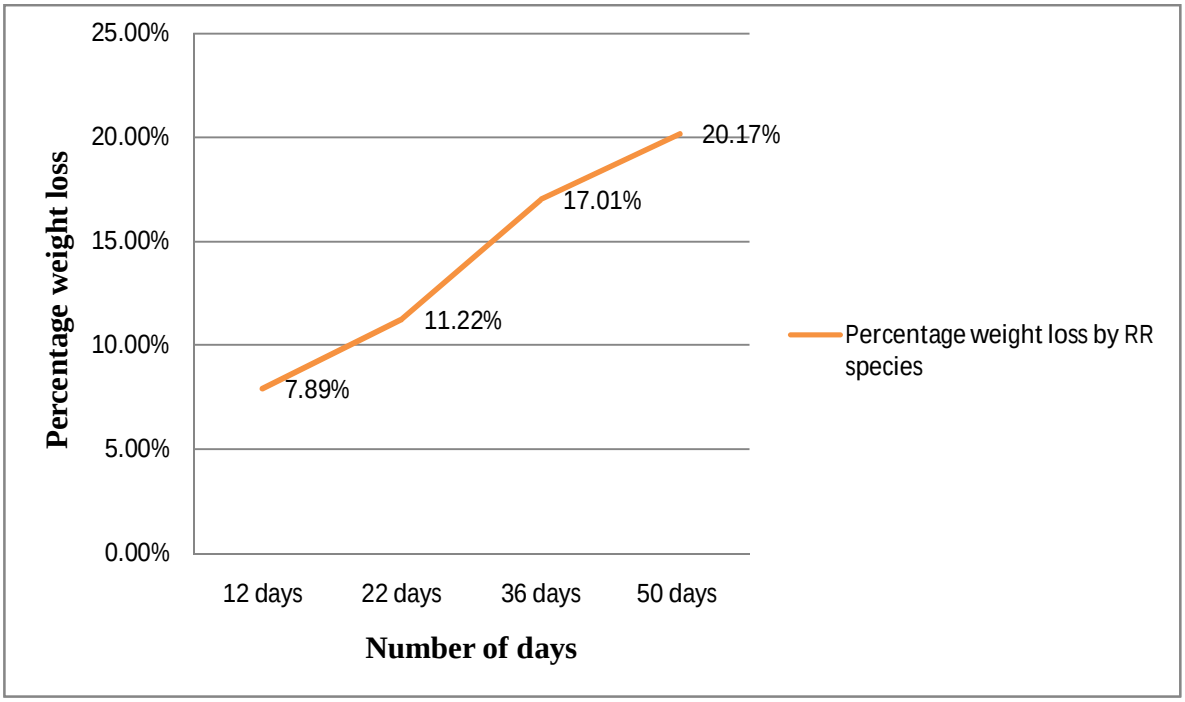


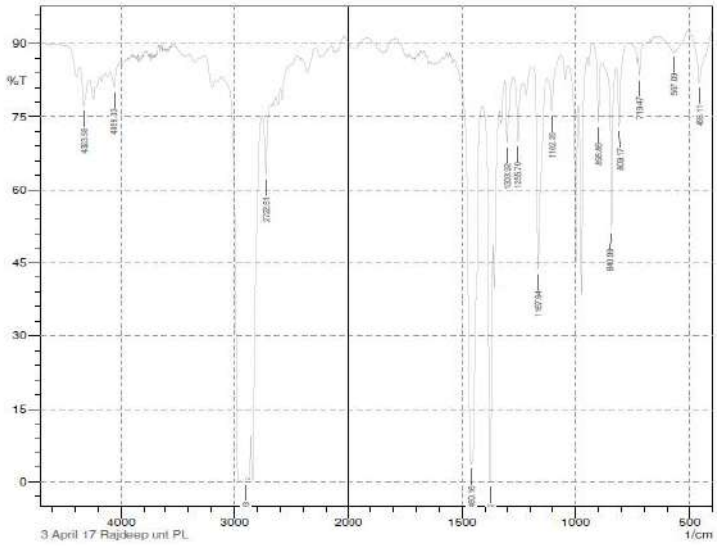
Figure no.10. Percentage weight loss of plastic by RR fungi in 50 days.

FTIR Analysis of Plastic film (LDPE):

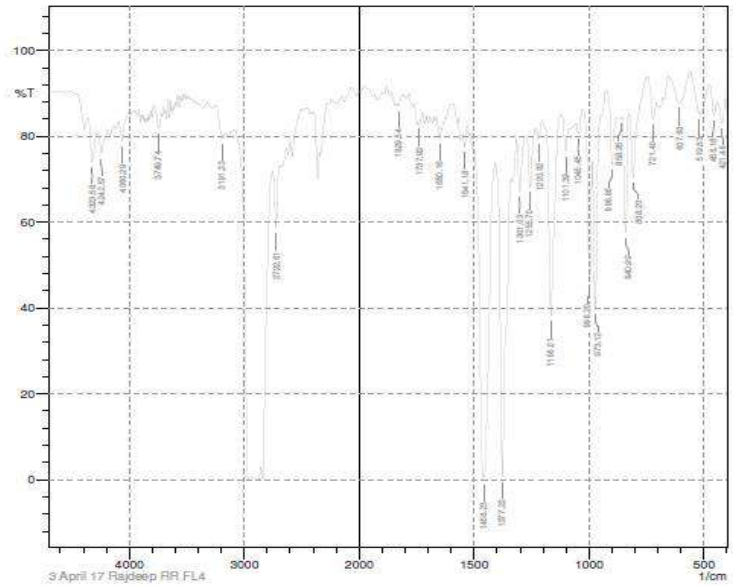
The spectral data of original LDPE molecule was compared with that degraded by fungus after 50 days by the RR species, revealed a visible change in molecule. In untreated plastic apart from larger peaks there were less peaks in the range of 1500-2000 cm^{-1}, whereas in the treated plastic small peaks of 1829.54, 1737.92,1650.16 and 1541.8 cm^{-1} appeared, which shows a drastic change in the polymeric molecule to the monomeric unit or less molecular weight compounds.

Studies shown by Ojha *et al*, also showed similar kind of peaks and change in the peaks after treatment with the fungi *Penicillium*. This was done for LDPE film as well as HDPE film in the study performed by them [58].

FTIR analysis of the plastic film (LDPE):



<u>Figure no.11.</u> FTIR analysis of untreated plastic film (LDPE).



<u>Figure no.12</u> FTIR analysis of treated plastic film (LDPE) by RR fungi in 50 days.

ASN (PS+ YPD broth) **GW(PS+ YPD broth)** **GG (PS+ YPD broth)**

RR (PS+ YPD broth) **WW (PS+ YPD broth)**

<u>Figure no.13.</u> Degradation of polystyrene films by fungal species for 30 days.

Table no. 11 **Degradation of polystyrene films for 30 days in duplicates by 5 species.**

Serial no.	Polystyrene sample	Weight of Polystyrene film (in grams)	
1.	**Untreated**	0.0220 gm	
2.	**Treated polystyrene samples**	**D1**	**D2**
2a.	GG, from soil sample 1 (Treated)	0.0211	0.0214
2b.	RR, from soil sample 2(Treated)	0.0175	0.0193
2c.	ASN, from soil sample 3(Treated)	0.0206	0.0213
2d.	WG, from soil sample 4 (Treated)	0.0212	0.0210
2e.	WW3, from soil sample 4 (Treated)	0.0218	0.0219

Table no. 12 **Calculation of percentage weight loss of polystyrene film by the fungal species.**

Fungus	Calculation	Percentage weight loss
GG	0.0220 – 0.0212/0.0220 * 100	3.4%
RR	0.0220 – 0.0184/0.0220 * 100	16.34%
ASN	0.0220 – 0.0209/0.0220 * 100	5%
WG	0.0220 – 0.0211/0.0220 * 100	4.09%
WW3	0.0220 – 0.0218/0.0220 * 100	0.60%

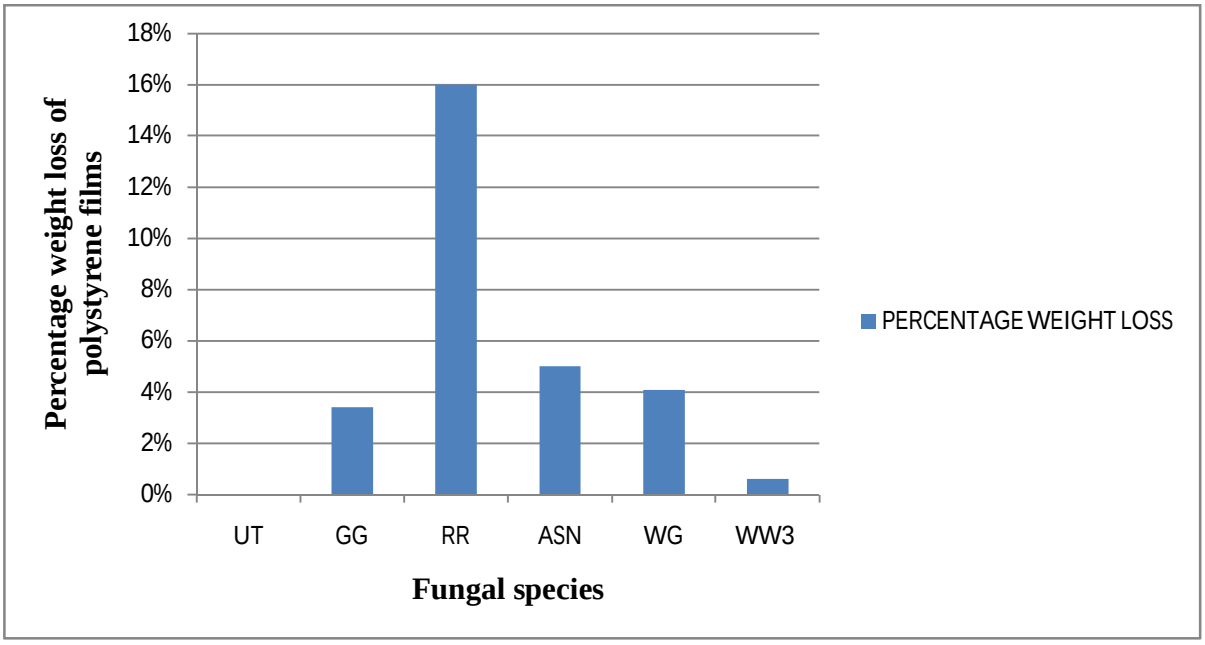


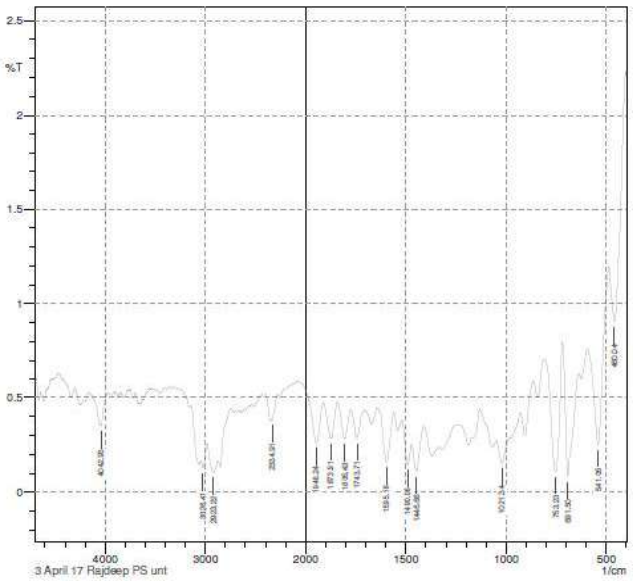
Figure no.14. Percentage weight loss of polystyrene film by 5 fungal species.

FTIR analysis of polystyrene film:

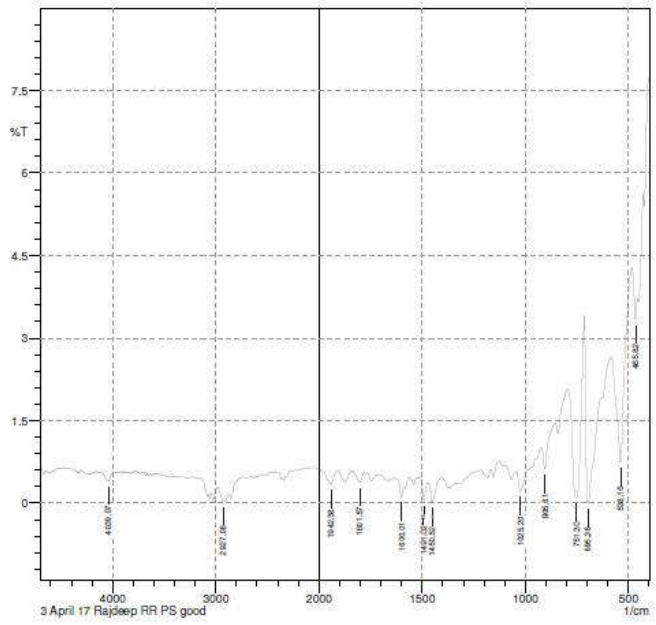
The spectral data of original Polystyrene molecule was compared with that degraded by fungus after 50days reveals a visible change in molecule. In the treated polystyrene as compared to the untreated, the peaks of 1743.71 cm^{-1} were missing, similarly another peak of 905.61 cm^{-1} is found in the treated polystyrene. Also in range of 2000-1500, the peaks observed in untreated plastic were then found missing in the treated plastic.

Studies have also shown that photoxidative degradation of polystyrene shows a FTIR spectra that shows a change in the peak before and after treatment at different time intervals. These studies also showed stretch in the bond after treatment with UV [59].

FTIR analysis of polystyrene film:



<u>Figure no.15.</u> FTIR analysis of untreated polystyrene film.



<u>Figure no.16.</u> FTIR analysis of treated polystyrene film for 30 days by RR.

Discussion:

Fungal species showed enormous potential to degrade the plastic and polystyrene films. Species belonging to *Rhizopus* genus out of all the 5 different fungi showed maximal degradation of both plastic and polystyrene film. The analysis for weight loss was done by determining the weight loss of plastic film before and after treatment. Out of the 5 species 3 of these were identified partially on the basis of their macroscopic and microscopic characteristics. These 3 were belonging to genus *Rhizopus, Aspergillus, Penicillium.* In comparison to other studies done by various researchers, *Rhizopus* species showed 20.17% degradation for plastic films in 50 days whereas 16.34% degradation for polystyrene films in 30 days as compared to plastic degradation shown by *Aspergillus glacus* and *Aspergillus niger*, being 7.26% and 5.54% in 30days [35]. Studies shown by Raaman et al, concluded that *Aspergillus niger and Aspergillus japonicus* show degradation of plastic as 5.8% and 11.11% in 1 month [32].

On the other hand not much work has been done on degradation of polystyrene so as the use of polystyrene plates increases day by day for daily needs, degradation of these types of plastic is becoming greater issue. Thus, fungus belonging to *Rhizopus* genus shows a higher potential to be used for the degradation purposes being an ecofriendly step towards degradation.

Chapter 7

SUMMARY AND CONCLUSION:

Plastic disposal is becoming the great issue nowadays and is of great concern as it is the major cause to increase the pollution. Not only human beings get affected by this but animals even get affected by this. Therefore to deal with this problem, some of the conventional methods include land filling, incineration and recycling. Out of these, land filling and incineration both lead to soil pollution, if not disposed properly, as well as air pollution hence causing a number of health related issues. Recycling is one of the best method and leading to minimizing various risks and health related issues.

Microorganisms are the most effective ones to deal with this problem. Due to huge diversity of microorganisms they have been use widely in bioremediation specifically fungi. Similarly, for plastic degradation even microorganism can be used and hence being a ecofriendly process this will surely reduce the problem of plastic disposal and hence proper disposal and degrading of plastic.

Therefore, making proper procedures for plastic disposal and use of various fungal consortia can really solve these major problems. Although this may take a little larger time but can be very promising for future prospective if proper plans are made.

REFERENCES:

1. Fried. J. R, *Polymer science and technology, Introduction to polymer science*. 3rd edn. 1995, Prentice Hall, Upper Saddle River, pp 4–9

2. Seymour. R. B, *Polymer science before & after 1899: notable developments during the lifetime of Maurtis Dekker*. 1989, J Macromol Sci Chem , pp.1023-1032

3. Sangale. M. K, Shahnawaz. M & Ade. A. B, *A Review on Biodegradation of Polythene: The Microbial Approach.* 2012, J Bioremed Biodeg, pp. 1-9.

4. Singh. J & Gupta. K.C, *Screening and Identification of Low density Polyethylene (LDPE) Degrading Soil Fungi Isolated from Polythene Polluted Sites around Gwalior city (M.P.)*. 2014, Int.J.Curr.Microbiol.App.Sci, pp. 443-448.

5. Tokiwa.Y, Calabia. B. P, Ugwu. C. U & Aiba. S, *Biodegradability of Plastics.* 2009, Int J Mol Sci, pp. 3722-3742.

6. Mee. A, Rideout. A. B , Hamber. A. J, Todd. J. N, Austin.G, Clark. M & Wallace. M. P, *Junk ingestion and nestling mortality in a reintroduced population of California Condors Gymnogyps californianus*. 2007, Bird Conservation International, pp. 119-130.

7. Spear, L.B., Ainley. D. G & Ribic. C. A, *Incidence of plastic in seabirds from the tropical pacific, 1984–1991: Relation with distribution of species, sex, age, season, year and body weight.* 1995, Marine Environmental Research, pp. 123-146.

8. Amin. S & Amin. M, *Thermoplastic elastomeric materials and their use in outdoor electrical insulation*. 2011, Rev. Adv. Mater. Sci, pp. 15-30

9. Legge. N. R, Holden. G & Schroeder. H. E, *Thermoplastic elastomers: a Comprehensive Review.* Munich : Carl Hanser Verlag, 1987.

10.*Plastic & thermoplastic elastomer materials,online technical report* (Minnesota Rubber and QMR Plastics, section 5), 2003.

11. http://www.cmu.edu/gelfand/k12-teachers/polymers/natural-synthetic-polymers/.

12. Fyfield. T. P, Hardwick R.C & Cole. R. A, *Plant damage and death caused by plastics containing the plasticizer dibutylphthalate.* 1984, Short communication, pp. 39-41.

13. Reddy. J. K, *Peroxisome Proliferators and Peroxisome Proliferator-Activated Receptor α.* 2004, Am. J. Pathol, pp. 2305-2321.

14. Denoncourt. J. M, Wallace. S. J, Solla. S. R & Langlois. V.S, *Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species.* 2015, General and Comparitive Endocrinology, pp. 74-88.

15. Hopewell. J, Dvorak. R & Kosior. E, *Plastics recycling: challenges and opportunities.* 2009, Philos Trans R Soc Lond B Biol Sci , pp. 2115-2126.

16. Awaja. F & Pavel. D, *Recycling of PET.* 2005, European Polymer Journal, pp. 1453-1477.

17. Sinha. V. K, Patel, Patel. M. R & Patel. J.V, *PET waste management by chemical recycling: A review.* 2010, Journal of Polymers and the Environment, pp. 8-25.

18. Berkesch. S, Biodegradable Polymers: A Rebirth of Plastic. 2005, Michigan State University, pp. 1-14.

19. Andrady. A. L, *Microplastics in the marine environment.* 2011, Marine Pollution Bulletin, pp. 1596-1605.

20. Sihag. S, Pathak. H, Jaroli. D. P, *Factors Affecting the Rate of Biodegradation of Polyaromatic Hydrocarbons.* 2014, International Journal of Pure and Applied Bioscience, pp. 185-202.

21. Premraj. R & Dobley. M, *Biodegradation of Polymers* . 2005, Indian Journal of Biotechnology, pp. 186-193.

22. Andrady A. L., Neal M. A, Applications and societal benefits of plastics. 2009, Phil. Trans. R. Soc. B 364, pp. 1977-1984.

23. Barnes D. K. A, *Remote islands reveal rapid rise of Southern Hemisphere sea debris.* 2005, The Scientific World JOURNAL, pp. 915-921.

24. Webb. H. K, Arnott. J, Crawford. R. J & Ivanova. E. P, *Plastic Degradation and Its Environmental Implications with Special Reference to Poly(ethylene terephthalate).* 2013, Polymers, pp. 1-18.

25. Laist D. W, *Impacts of marine debris: entanglement of marine life in marine debris including a comprehensive list of species with entanglement and ingestion records.* 1997, Marine Entanglement Research, pp. 99-413.

26. Abdulla. A, *Maritime traffic effects on biodiversity in the mediterranean seas.* Malaga, Spain : IUCN, Gland, Switzerland and Malaga, Spain. 2008.pp. 104-106

27. Vogel. S. A, *The Politics of Plastics: The Making and Unmaking of Bisphenol A "Safety"*. 2009, Am J Public Health, pp. 559-566.

28. Thompson. R. C, Moore. C. J, Saal. F. S, Swan. S. H, *Plastics, the environment and human health: current consensus and future trends*. 2009, Philos Trans R Soc Lond B Biol Sci , pp. 2153-2166.

29. Meeker. J. D, Sathyanarayana. S & Swan. S. H, *Phthalates and other additives in plastics: human exposure and associated health outcomes*. 2009, Phil. Trans. R. Soc. B, pp. 2097-2113.

30. Shibamoto. T, Yasuhara. A & Katami. T, *Dioxin formation from waste incineration*. 2007, Rev Environ Contam Toxicol., pp. 1-41.

31. Glass. J. E & Swift. G, *Agricultural and Synthetic Polymers, Biodegradation and Utilization*. 1989, American Chemical Society, Washington DC, ACS Symposium series, pp. 9-64,

32. Raaman. N, Rajitha. N, Jayshree. A & Jegadeesh. R, *Biodegradation of plastic by Aspergillus species isolated from polyethene polluted sites around Chennai.* 2012, J. Acad. Indus. Res, pp. 313-316.

33. Usha. R, Sangeetha. T & Palaniswamy. M, *Screening of Polyethylene Degrading Microorganisms from Garbage Soil*. 2011, Libyan Agriculture Research Center Journal International, pp. 200-204.

34. Russell. J. R, Huang. J, Anand. P, Kucera. K, Sandoval. A. G, Dantzler. K. W, Hickman. D, Jee. J, Kimovec. F. M, Koppstein. D, Marks. D. H, Mittermiller. P. A, Núñez. S. J, Santiago. M, Townes. M. A, Vishnevetsky. M, Williams. N. E, Vargas. M. P. N, Boulanger. L. A, Slack. C. B, Strobel. S. A, *Biodegradation of polyester polyurethane by endophytic fungi*. 2011, Appl Environ Microbiol pp. 6076–6084

35. Kathiresan. K, *Polythene and Plastics-degrading microbes from the mangrove soil.* 2003, Rev. Biol. Trop., pp. 629-634.

36. Krueger. M. C, Hofmann. U, Moeder. M & Schlosser. D, *Potential of Wood-Rotting Fungi to Attack Polystyrene Sulfonate and Its Depolymerisation by Gloeophyllum trabeum via Hydroquinone-Driven Fenton Chemistry*. 2015, PLoS One.

37. Roldán-Carrillo T, Rodríguez-Vázquez R , Díaz-Cervantes D , Vázquez-Torres H , Manzur-Guzmán A & Torres-Domínguez A, *Starch-based plastic polymer degradation by the white rot fungus Phanerochaete chrysosporium grown on sugarcane bagasse pith: enzyme production*. 2003, Bioresource Technology, pp. 1-5.

38. Ibiene. A. A, Stanley. H. O & Immanuel. O. M, *Biodegradation of Polyethylene by Bacillus sp. Indigenous to the Niger Delta Mangrove Swamp.* 2013, Nig J. Biotech, pp. 68-79.

39. Onodera. K. Y, Mukumoto. H, Katsuyaya. Y, Saiganji. A, Tani. Y, *Degradation of polyethylene by a fungus Penicillium simplicissimum YK*. 2000, Polymer degradation and stability , pp. 323-327.

40. Sivasankari. S & Vinotha. T, *In Vitro Degradation of Plastics (Plastic Cup) Using Micrococcus Luteus and Masoniella Species*. 2014, Scholars Academic Journal of Biosciences, pp. 85-89.

41. Saminathan. P, Sripriya. A, Saminathan. P, Sripriya. A, Nalini. K, Sivakumar. T, Thangapandian. V, *Biodegradation of Plastics by Pseudomonas putida isolated from Garden Soil Samples.* 2014, Journal of advanced botany and zoology, pp. 1-4.

42. Luz J. M. R, Paes. S. A, Nunes. M. D, Silva. M. C. S & Kasuya. M .C. S, *Degradation of Oxo-Biodegradable Plastic by Pleurotus ostreatus*. 2013, PLoS ONE .

43. Deepika. S & Jaya. M, *Biodegradation of low density polyethylene by micro-organisms from garbage soil.* 2015, Journal of Experimental Biology and Agricultural Sciences, pp. 15-21.

44. Mahalakshmi. V, Siddiq. A & Andrew. S. N, *Analysis of Polyethylene Degrading Potentials of Microorganisms Isolated From Compost Soil.* 2012, International Journal of Pharmaceutical & Biological Archives , pp. 1190-1196.

45. Yang. Y, Yang. J, Wu. W. M, Zhao. J, Song. Y, Gao .L, Yang. R & Jiang. L, *Biodegradation and Mineralization of Polystyrene by Plastic-Eating Mealworms*. 2015, Environ Sci Technol. , pp. 12087-12093.

46. Nanda. S, Sahu. S.S, Abraham. J, *The biodegradation of natural and synthetic polyethylene by Pseudomonas Species*. 2010, J. Appl. Sci. Environ. Manage., pp. 57-60.

47. Sowmya. H. V, Ramalingappa & Krishnappa. M & Thippeswamy. B, *Degradation of polyethylene by Trichoderma harzianum--SEM, FTIR, and NMR analyses.* 2014, Environ Monit Assess. , pp. 6577-6586.

48. *Das. M. P & Kumar. S, An approach to low-density polyethylene biodegradation by Bacillus amyloliquefaciens.* 2015, 3. Biotech., pp. 81-86.

49. Gajendiran. A, Krishnamoorthy. S & Abraham. J, *Microbial degradation of low-density polyethylene (LDPE) by Aspergillus clavatus strain JASK1 isolated from landfill soil.* 2016, 3. Biotech, pp. 52

50. Mukherjee. S & Chatterjee. S, *A comparative study of commercially available plastic carry bag biodegradation by microorganisms isolated from hydrocarbon effluent enriched soil.* 2014, Int.J.Curr.Microbiol.App.Sci, pp. 318-325

51. Starnecker. A & Menner. M, *Assessment of biodegradability of plastics under stimulated composting conditions in a laboratory test system.* 1996, Int Biodeterior Biodegrad, pp. 85-92.

52. Gu. J. D, Ford. T. E, Mitton. D. B & Mitchell. R, *Microbial corrosion of metals.* New York, U.S.A : Whiley, 2000, pp. 915-927.

53. Karigar. C. S & Rao. S. S, *Role of Microbial Enzymes in the Bioremediation of Pollutants: A Review.* 2011, Enzyme Research, pp. 1-11.

54. Viswanath. B, Rajesh. B, Janardhan. A, Kumar. A.P & Narasimha. G, *Fungal Laccases and Their Applications in Bioremediation.* 2014, Enzyme research, pp. 1-21.

55. Dashtban. M, Schraft. H, Syed. T. A & Qin. W, *Fungal biodegradation and enzymatic modification of lignin.* 2010, International journal of biochemistry and molecular biology, pp. 36-50.

56. Tokiwa. Y, Ando. T & Suzuki. T, *Degradation of polycaprolactone by a fungus.* 1976, J. Ferm. Technol. , pp. 603-608.

57. Iwata. T, Doi. Y, *Morphology and enzymatic degradation of poly(L-lactic acid) single crystals.* 1998, Macromolecules, pp. 2461- 2467.

58.Ojha. N, Pradhan. N, Singh. S, Barla. A, Shrivastava. A, Khatua. A, Rai. V, Bose.S, *Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimization.* 2017, Nature, pp.

59.Yousif.E, Haddad. R, *Photodegradation* and *photostabilization of polymers, especially polystyrene: review.* 2013, Springer Plus, pp. 2-32

.