

**CLEAVAGE OF POLYMERS INTO MONOMER BY
THE BIODEGRADABLE BACTERIA**

**S. PRIYA
(17PZOO11)**

**Thesis Submitted to
Avinashilingam Institute for Home Science and Higher Education for
Women
Coimbatore - 641 043**

In partial fulfilment of the requirements for the
Degree of Master of Science in Zoology

APRIL 2019

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Signature of the
Head Of the Department

Signature of the
Supervisor

Acknowledgement

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who have contributed towards shaping this thesis”*

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I. INTRODUCTION

Matter is destroyed and created all the time. But, if there is anything that goes against this rule, it can create havoc to the entire universe. One such matter is plastic (Gnanavel *et al.*, 2012). Development in science and technology, especially over the last 2 decades, have led to the production of a number of synthetic polymers worldwide. The polymers are chains of monomers linked together by chemical bonds (Shah *et al.*, 2008). It becomes mobile upon heating and can be casted into moulds. They are non-metallic compounds and plastic derived materials can be pushed into shapes (Sharma *et al.*, 2014). Polymers such as lignin, starch, chitin, etc., are present in the environment naturally (Dey *et al.*, 2012). As plastic packages are light – weight and easier to handle the adaption of these products is growing. Manufactures are also preferring the use of plastic packaging solutions, due to lower costs of production. There are a wide variety of innovative, cost – effective and sustainable packaging solution emerging in the market (Halaye, 2019).

Nowadays, synthetic polymers are used in several industries, of which packaging application covers 30% of plastic use throughout the world (Kumar *et al.*, 2011). In the nineteenth and twentieth centuries, plastic played a revolutionary role in the packaging industries. Thereafter, approaches to transportation were changed with the introduction of carrying bags made of polyethylene (Nerland *et al.*, 2014). Plastic bags, straws, stirrers, water bottles, and most food packaging are some of the most common single-use plastics, used throughout the world. Since plastic takes more than 500 years to decompose, as per the experts, it is one of the biggest threats to the well-being of the environment today (Upadhyay, 2019). Unpredicted use of synthetic polymers is leading towards the accumulation of increased solid waste in the natural environment. This affects the natural system and creates various environmental hazards. Now such types of synthetic compounds have become a nuisance affecting natural resources like water quality and soil fertility by contaminating them (Bhatnagar and Kumari, 2013; Ojo, 2007; Arutchelvi *et al.*, 2008).

In the 1990s, plastic waste was found to have tripled and is continuously increasing in the marine environment (Moore, 2008). The level of debris materials increased markedly from 1990 to 1995 on Bird Island of South Georgia; similarly, the garbage amount doubled in the coastline area of the UK during 1994–1998 (Walker *et al.*, 1997; Barnes, 2002). The use of this polymer is growing at a rate of 12% per year and about 140 million tons of synthetic polymers are produced worldwide each year (Sharma *et al.*, 2014). The total demand for plastic was 107 million tons in 1993, which increased to 146 million tons in 2000. The growth rate of the plastic industry in Pakistan is 15% per annum (Shah *et al.*, 2008).

Plastic waste is being generated rapidly worldwide. The UK, China and India contribute 1 million tons, 4.5 million tons and 16 million tons, respectively (Kumar *et al.*, 2011). The annual production of plastic was estimated as 57 million tons in Europe in 2012 (Nerland *et al.*, 2014).

Plastic materials have become versatile, competitive and reliable substitutes for traditionally used metal, leather and wood materials in past 5 decades because of their toughness, flexibility and physical properties (Sivan, 2011). Plastic waste recycling has largely unsuccessful outcomes; Of the over 1 trillion plastic bags dumped per annum in US, only 5% are recycled (Shah *et al.*, 2008). Low density polyethylene (LDPE) is one of the major sources of environmental pollution (Sharma *et al.*, 2014).

Plastic bag packing for hot edible items causes migration of harmful chemicals to food items. These include Styrene which is carcinogenic, Phthalates and Bisphenol A, which causes diabetes and diseases of the heart and liver (Sung, 2010). Usage of plastic also gives several environmental risks, including leaching chemicals and toxins into surrounding ecosystems, threatening the health of local animal populations and releasing greenhouse gases into the air.

Bioremediation is one of the way to reduce the adverse effects and can serve as a potential tool (Ali *et al.*, 2014).

Bacterial and fungal species are the most abundant biological agents found in nature and have distinct degradation abilities for natural and synthetic polymers. Among the huge microbial population associated with polymer degradation, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Streptomyces badius*, *Streptomyces setonii*, *Rhodococcus ruber*, *Comamonas acidovorans*, *Clostridium thermocellum* and *Butyrivibrio fibrisolvens* are the dominant bacterial species. Similarly, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium lini*, *Pycnoporus cinnabarinus* and *Mucor rouxii* are prevalent fungal species (Pathak *et al.*, 2017).

Microbes cause cleavage of the polymer chain using certain enzymes and convert them into monomers and oligomers. Currently enzymatic degradation is the mostly widely used methods for plastic waste treatment. This method of biodegradation by microbial enzymes increases the rate of degradation of plastic without causing any harm to the environment (Bhardwaj *et al.*, 2012). There are four mechanisms by which plastics degrade in the environment namely, Photodegradation, thermo- oxidative degradation, hydrolytic degradation and biodegradation by micro-organisms (Andrady, 2011).

A recent research article has suggested that the presence of groups such as, esterases, lipases and cutinases are responsible for degrading different forms of plastics (Hadad *et al.*, 2005).

It is necessary to degrade polyethylene from atmosphere. Therefore, an attempt has been made in this dissertation to isolate those micro-organisms that degrades the polythene. Considering all facts, the present study was considered with the following objectives.

- To isolate the biodegradable bacteria from the soil sample.
- To conduct the biochemical parameters of the isolated biodegradable bacteria and to assess its polyethylene degrading efficiency.
- To recommend the biodegradable bacteria for the decomposition of polyethylene material to the Governmental and non- governmental organization.

II. REVIEW OF LITERATURE

Synthetic polymers are widely used both in food or commercial industry. Plastic, one of its products is known to be expanding with changing lifestyles and increasing population. However, it causes deleterious effects on the environment due to its non-degrading nature. Burning of this plastic waste and burying of the plastics releases harmful toxic material which is a major pollutant in environment. Also, there is an undesirable influence on the environment and it is known to be cause problems with waste deposition and utilization. The most commonly used non-degradable solid waste is polythene which is a linear hydrocarbon polymers consisting of long chains of the ethylene monomers. Thus, there is a tendency to substitute such polymers with polymers that undergo biodegradable processes. Biodegradable plastics are environment friendly; they have an expanding range of potential application and are driven by the growing use of plastics in packaging.

This review describes biodegradation processes and its mechanisms, the microorganisms which involved the reactions of importance in the biodegradation of plastics.

II. 1. PLASTICS

II. 1.1. Plastics: An Introduction

Plastics are polymers derived from petrochemicals which are further synthetically made from monomers by some chemical processes to produce these long chain polymers (Shimao, 2001).

Plastics are light weight, low cost, highly durable and are of high strength. In our daily life the plastics are available in various forms such as nylon, polycarbonate, polyethylene-terephthalate, polyvinylidene chloride, urea formaldehyde, polyamides, polyethylene, polypropylene, polystyrene, polytetraflouro ethylene, polyurethane and polyvinyl chloride. The annual production of plastics has doubled over the past 15 years to 245 million tonnes. Production of plastic has increased from 204 million tonnes in 2002 million tonnes in 2013, representing a 46.6 % increase. During the past three decades, plastic

materials are widely used in transportation, food, clothing, shelter construction, medical and recreation industries, fishing nets, packaging, food industry and agricultural field (Vatseldutt *et al.*, 2014).

Under the natural condition degradable or non-degradable organic materials are considered as the major environmental problem, *e.g.* plastics. The accumulation of these plastic wastes created serious threat to environment and wild life (Chua *et al.*, 2003). The environmental concerns include air, water and soil pollution. The dispersal of urban and industrial wastes contaminates the soil. The soil contaminations are mainly made by human activities (Ghosh *et al.*, 2005). Environmental pollution is caused by synthetic polymers, such as wastes of plastic and water-soluble synthetic polymers in wastewater (Premraj *et al.*, 2005).

Many animals die of waste plastics either by being caught in the waste plastic traps or by swallowing the waste plastic debris to exert ruinous effects on the ecosystem (Usha *et al.*, 2011). Some of the plastic products cause human health problems because they mimic human hormone. Vinyl chloride is classified by the International Agency for the Research on Cancer (IARC) as carcinogenic to humans (Ruthann *et al.*, 2007). It has also shown to be a mammary carcinogen in animals. PVC is used in numerous consumer products, including adhesives, detergents, lubricating oils, solvents, automotive plastics, plastic clothing, personal care products (such as soap, shampoo, deodorants, fragrances, hair spray, nail polish) as well as toys and building materials.

Styrene is classified by IARC as possibly carcinogenic to humans and is shown to cause mammary gland tumours in animal studies. It also acts as an endocrine disrupter (Gray *et al.*, 2009). BPA has been linked with premature birth, intrauterine growth retardation, preeclampsia and still birth (Benachour *et al.*, 2009). It has also been noted that prolonged exposure to BPA shows a significant effect on the sex hormones (progesterone) in females (Hao *et al.*, 2011).

Burning plastics usually produce some noxious gases like furans and dioxins which are dangerous greenhouse gases and play an important role in ozone layer depletion (Pilz *et al.*, 2010). In fact, dioxins cause serious problems in the human endocrine hormone activity, thus becoming a major concern for the human

health. Dioxins also cause very serious soil pollution, causing a great concern for scientific community worldwide. Phthalates and Bisphenol A are closely related in thyroid causing dysfunction in humans.

II. 2. ORIGIN OF PLASTICS – AN INTERESTING STORY

The first man-made plastic was created by Alexander Parkes who publicly demonstrated it at the 1862 Great International Exhibition in London. The material, called Parkesine, was an organic material derived from cellulose that once heated could be molded and retained its shape when cooled.

Celluloid

Celluloid is derived from cellulose and alcoholized camphor. John Wesley Hyatt invented celluloid as a substitute for the ivory in billiard balls in 1868. He first tried using a natural substance called collodion after spilling a bottle of it and discovering that the material dried into a tough and flexible film. However, the material was not strong enough to be used as a billiard ball, not until the addition of camphor, a derivative of the laurel tree. The new celluloid could now be molded with heat and pressure into a durable shape.

Besides billiard balls, celluloid became famous as the first flexible photographic film used for still photography and motion pictures. Hyatt created celluloid in a strip format for movie film. By 1900, movie film was an exploding market for celluloid.

Formaldehyde Resins – Bakelite

After cellulose nitrate, formaldehyde was the next product to advance the technology of plastic. Around 1897, efforts to manufacture white chalkboards led to casein plastics (milk protein mixed with formaldehyde) Galalith and Erinoid are two early tradename examples.

In 1899, Arthur Smith received British Patent 16,275, for "phenol-formaldehyde resins for use as an ebonite substitute in electrical insulation," the first patent for processing a formaldehyde resin.

However, in 1907, Leo Hendrik Baekeland improved phenol-formaldehyde reaction techniques and invented the first fully synthetic resin to become commercially successful with the trade name Bakelite.

Timeline - Precursors

- 1839 - Natural Rubber - Method of processing invented by Charles Goodyear
- 1843 - Vulcanite - Invented by Thomas Hancock
- 1843 - Gutta-Percha - Invented by William Montgomerie
- 1856 - Shellac - Invented by Alfred Critchlow and Samuel Peck
- 1856 - Bois Durci - Invented by Francois Charles Lepage

Timeline - Beginning of the Plastic Era with Semi-Synthetics

- 1839 - Polystyrene or PS discovered by Eduard Simon
- 1862 - Parkesine - Invented by Alexander Parkes
- 1863 - Cellulose Nitrate or Celluloid - Invented by John Wesley Hyatt
- 1872 - Polyvinyl Chloride or PVC - First created by Eugen Baumann
- 1894 - Viscose Rayon - Invented by Charles Frederick Cross and Edward John Bevan

Timeline - Thermosetting Plastics and Thermoplastics

- 1908 - Cellophane - Invented by Jacques E. Brandenberger
- 1909 - First true plastic Phenol-Formaldehyde trade name Bakelite - Invented by Leo Hendrik Baekeland
- 1926 - Vinyl or PVC - Walter Semon invented a plasticized PVC.
- 1933 - Polyvinylidene chloride or Saran also called PVDC - Accidentally discovered by Ralph Wiley, a Dow Chemical lab worker.
- 1935 - Low-density polyethylene or LDPE - Invented by Reginald Gibson and Eric Fawcett
- 1936 - Acrylic or Polymethyl Methacrylate

- 1937 - Polyurethanes tradenamed Igamid for plastics materials and Perlon for fibers. - Otto Bayer and co-workers discovered and patented the chemistry of polyurethanes
- 1938 - Polystyrene made practical
- 1938 - Polytetrafluoroethylene or PTFE tradenamed Teflon - Invented by Roy Plunkett
- 1939 - Nylon and Neoprene - Considered a replacement for silk and a synthetic rubber respectively by Wallace Hume Carothers
- 1941 - Polyethylene Terephthalate or Pet - Invented by Whinfield and Dickson
- 1942 - Low-Density Polyethylene
- 1942 - Unsaturated Polyester also called PET patented by John Rex Whinfield and James Tennant Dickson
- 1951 - High-density polyethylene or HDPE tradenamed Marlex - Invented by Paul Hogan and Robert Banks
- 1951 - Polypropylene or PP - Invented by Paul Hogan and Robert Banks
- 1953 - Saran Wrap introduced by Dow Chemicals.
- 1954 - Styrofoam a type of foamed polystyrene foam was invented by Ray McIntire for Dow Chemicals
- 1964 – Polyimide
- 1970 - Thermoplastic Polyester this includes trademarked Dacron, Mylar, Melinex, Teijin, and Tetoron
- 1978 - Linear Low-Density Polyethylene
- 1985 - Liquid Crystal Polymers

II. 2.2. CLASSIFICATION OF PLASTICS

Polymers are made up from non-renewable as well as renewable feedstock. These polymers are well known for their diverse applications in industries, domestic appliances, transportation, construction, shelters, storage and packaging practices.

II. 2.2.1. Natural Polymers

Natural polymers are found abundantly in nature in the forms of biopolymers and dry material of plants (Leschine, 1995). The constitution of the plant cell wall differs with the composition of the lignocellulosic biomass (cellulose, hemicellulose and lignin), which provides strength (Premraj and Doble, 2005).

Lignocelluloses play a critical role in developing plant biomass, in which cellulose, hemicellulose and lignin are the major building blocks of the natural polymer (Perez *et al.*, 2002).

II. 2.2.2. Synthetic Polymers

Plastics are manmade compounds that consist of a long chain of polymeric molecules and unusual bonds, with excessive molecular mass and halogen substitutions. Nowadays plastic manufacturing involves different inorganic and organic materials, including carbon, hydrogen, chloride, oxygen, nitrogen, coal and natural gases (Shah *et al.*, 2008).

The most widely used polymers contributing to plastic waste are Low - Density Poly Ethylene (LDPE), High-Density Poly ethylene (HDPE), polyvinyl chloride, polystyrene and polypropylene with 23, 17.3, 10.7, 12.3 and 18.5%, respectively, and the remaining 9.7% of other types of polymer (Puri *et al.*, 2013). The polymer production in 2012 was estimated as polyethylene 30% (LLDPE and LDPE 18%, HDPE 12%), polypropylene 19%, polyvinyl chloride 11%, polystyrene 7%, polyethylene terephthalate 7% and polyurethanes 7% worldwide (Nerland *et al.*, 2014).

Different types of plastics and its applications – Table 1

S. No	Types	Applications	References
1	Polyethylene terephthalate	drink bottles, peanut butter jars, plastic films, microwavable packaging	Vona <i>et al.</i> , 1965
2	Polylactic acid	A biodegradable thermoplastic that can be converted into a variety of aliphatic polyesters derived from lactic acid	Noopur <i>et al.</i> , 2015
3	Polyetherimide	A high temperature, chemically stable polymer that does not crystalline	Noopur <i>et al.</i> , 2015
4	Phenolics	Used for insulating parts in electrical fixtures, paper laminated product and thermally insulation foams	Noopur <i>et al.</i> , 2015
5	Polyvinyl chloride	Plumbing pipes and guttering, shower curtains, window frames, flooring	Vona <i>et al.</i> , 1965
6	Polyethylene	Wide range of inexpensive uses including supermarket bags, plastic bottles	Vona <i>et al.</i> , 1965
7	Polyvinylidene chloride	Food packaging	Noopur <i>et al.</i> , 2015

8	Low density polyethylene	Outdoor furniture, sliding, floor tiles, shower curtains, packaging	Noopur <i>et al.</i> , 2015
9	Polyamides	Fibers, tooth brush bristles, fishing line, under the hood car engine mouldings	Noopur <i>et al.</i> , 2015
10	Polypropylene	Bottle caps, drinking straws, yogurt containers, appliances, car fenders	Vona <i>et al.</i> , 1965
11	Polycarbonate	A blend of PC and ABS that creates stronger plastic. Used in car interior and exterior parts and mobile phones	Vona <i>et al.</i> , 1965
12	Polystyrene	Packaging foam, food containers, plastic tableware, disposable cups, plates, cutlery, CD and cassette boxes	Vona <i>et al.</i> , 1965
13	Melamine formaldehyde	One of the aminoplasts used as a multi colorable alternative to phenolics, biodegradable and heat resistant thermoplastic composed of modified corn starch	Noopur <i>et al.</i> , 2015
14	Acrylonitrile butadiene	Electronic equipment cases (e.g., computer styrene monitors, printers, keyboards) drainage pipe (ABS)	Noopur <i>et al.</i> , 2015

15	High impact polystyrene (HIPS)	Refrigerator liners, food packaging, vending cups	Noopur <i>et al.</i> , 2015
16	Polyester (PES)	Fibers, textiles	Noopur <i>et al.</i> , 2015
17	Nylon	Small bearings, speedometer gears, windshield wipers, water hose nozzles, football helmets, race horse shoes, cell phone.	Noopur <i>et al.</i> , 2015
18	Polytetrafluoro ethylene (PTFE)	Electronics bearing, nonstick kitchen utensils.	Mukherjee <i>et al.</i> , 2014
19	Polymethyl methacrylate (PMMA)	Contact lenses, glazing, aglets, florescent light diffusers, rear light covers for vehicles.	Ghosh <i>et al.</i> , 2013
20	Urea formaldehyde	Wood adhesive and electrical switch housings	Ghosh <i>et al.</i> , 2013
21	Poly etheretherketone(PEEK)	Strong chemical and heat resistant thermoplastic biocompatibility allows for use in implant application and aerospace moulding.	Ghosh <i>et al.</i> , 2013

II.3. PLASTICS AND ENVIRONMENTAL HEALTH

II. 3.1. The Good: Medical and Public Health Applications of Plastics

In medicine alone, the diversity of plastics uses is incredible. Prosthetics, engineered tissues, and microneedle patches for drug delivery are all possible with polymers. In many sectors of society, plastics have replaced glass, wood, fibers, and metal in various products, including dishware, clothing, food packaging, personal care products, and others; these uses have already been explored elsewhere (Andrady *et al.*, 2009).

Disposable products in particular have been a major application for plastics within the last century because plastics are both inexpensive and lightweight (Holmgren, 1974).

Syringes are a good example of how plastics have benefited public health through single-use applications and later, through reusable products. Healthcare workers have long cited convenience for choosing disposable products (Battersby, 1999). Disposable plastic items such as latex gloves, intravenous (IV) bags and dialysis tubes are inexpensive and allow for patient safety as well as time savings, due to eliminating the need to sterilize used equipment (Steinglass *et al.*, 1999).

Disposable syringes in particular were in focus during the early 1980s as a way to reduce the risk of transmitting blood-borne infections such as Human Immunodeficiency Virus (HIV) and hepatitis B through injections from used needles. With reusable syringes, there is a risk of needle-sticks while capping and re-sterilizing the syringe and improper sterilization techniques can cause transmission of communicable diseases (Battersby, 1999). However, against the explicit recommendations, disposable syringes can and still were being reused; therefore, efforts were made to develop a plastic auto-disable syringe that locks after single use (Drain *et al.*, 2003).

Although, these are more expensive than conventional disposable syringes, they prevent reuse and possible infection (Drain *et al.*, 2003). Reusable syringes are experiencing a comeback in developing countries where, single-use syringes still pose a health risk from improper disposal, and sterilizable syringes can always

be at hand to administer critically important vaccinations (Drain *et al.*, 2003). However, the problems raised by opponents of sterilizable syringes – such as easy breakage and the difficulty of cleaning syringes made of metal and glass – have been solved by designing a sterilizable syringe completely made of plastic (Battersby, 1999). Today, plastics are thus facilitating the manufacture of both throwaway and reusable medical syringes.

The development of plastics has led to other disposable items similarly ubiquitous to hospitals, such as Intra Venous (IV) bags and tubing. These are used for immediate drug-delivery, to treat dehydrated patients through fluid replacement, to transfuse blood and to correct electrolyte imbalances as quickly as possible. The IV injection of fluids and medicines into the bloodstream is by far the fastest method of delivering remedies into the body. The importance of IV bags and tubing in caring for hospital patients is clear in the fact that they constitute 20–25% of hospital waste (Lee *et al.*, 2002).

Polymers have also been utilized in the development of innovative materials and methods of healing patients. Absorbable sutures are made of polymers that can be designed to biodegrade over differing time periods depending on patient needs (Pillai *et al.*, 2010). Since they do not require surgical removal following implantation, they also reduce the number of procedures a patient must undergo. Polymers have demonstrated their value in pharmaceuticals, as controlled drug delivery systems used by ten millions of people each year, and in orthopedics: the polymer polymethylmethacrylate is used as bone cement in total hip replacements. Tissue engineering has more recently been significantly advanced by polymers, as polymer scaffolds can be designed to biodegrade and can have a variety of different structures (Liu *et al.*, 2012).

II. 3.2. The Bad: Health Effects of Plastics

However, the widespread use of plastics facilitates continuous contact of these materials with the human body and with it daily exposure to ingredients in plastics. Although plastics components do not have significant bioaccumulation potential (except when accidentally ingested and becoming entrapped in the gastrointestinal system), biomonitoring studies have demonstrated the presence of

steady-state concentration of plastics components in the human body, thereby reflecting the ongoing balance of constant exposure, metabolism and excretion of these compounds (Calafat *et al.*, 2005). This situation implies that in today's plastics-enabled society there are no control groups to be found to analyze the effects on human health from low-level, environmental exposures to plastic constituents. Everybody is being exposed to some degree at any given time from gestation through death. Detectable levels of bisphenol A have been found in the urine of 95% of the adult population of the United States (vandenburg *et al.*, 2007).

In recent years, there have been several epidemiological studies and controlled animal experiments performed concerning the health effects of plastic components such as BPA and DEHP. Associations were found between exposure to these compounds and destructive effects on health and reproduction, such as early sexual maturation, decreased male fertility, aggressive behavior, and others (Kandarakis *et al.*, 2009).

At present, BPA has been one of the first plastic materials to be recognized for its potential harm. Evidence of potential harm has been deemed sufficient by the U.S. Food and Drug Administration (FDA) to issue a statement that "recent studies provide reason for some concern about the potential effects of BPA". In response to such concerns, BPA also recently has been banned in the U.S. from use in infant bottles and spill-proof cups for toddlers in an effort to protect a particularly vulnerable population. In Canada and the European Union, polycarbonate plastics made from BPA have also been banned from use in baby bottles.

II. 4. DIVERSITY OF POLYMER DEDRADATION

Living organisms are involved in the breakdown of plastic material and consequently the recycled form reverses back to the environment. Anaerobic microbial degradation releases greenhouse gas (methane) in landfills, which increases global warming. Aerobic conditions are essential for fungal degradation while bacterial degradation proceeds in aerobic as well as anaerobic conditions (Kumar *et al.*, 2011; Chandra *et al.*, 1998).

Plastic can be reduced in an eco-friendly manner with the help of soil bacteria and proper water availability. Decomposition of the polymer depends on its chemical composition, which supports the growth of microorganisms in the form of nutrient sources. The starch-based polymer is favorable for microbial attack, and hydrolytic enzymes act on the polymer matrix to reduce their weight. Polymer made from starch or flax fiber shows greater biodegradability as compared to other synthetic polymers. Microorganisms also play an important role in the degradation of petroleum-based polymers. Petroleum-based polymers such as polyolefin are degraded through photo-degradation (Kumar *et al.*, 2011; Sen and Raut, 2015).

Emerging technology is continuously involved in improving the processing of biopolymers by using an additive (benzophenone) during their construction. Additives play a significant role in the chemical process during photo-degradation. Such amendments affect their thermal sensitivity and UV-absorbing capacities. Chemically sensitive polymers have a better biodegradability rate compared to other polymers. Similarly, thermal exposure is also involved in the breakdown of a polymer into simpler forms that increase the availability of micro-organisms. Nodax is alkaline in nature and generally involves in the structural change of the polymer (Kumar *et al.* 2011; Augusta *et al.*, 1993).

Renewable resources are also used in the formation of biodegradable materials. Animal and plant originated compounds are susceptible to microbial degradation (Schink *et al.*, 1992).

Development of bio-based materials is beneficial for our environment's sustainability, maintenance of greenhouse gas emissions, etc. (Song *et al.*, 2009). This type of material's manufacturing practice plays a significant role in the environment by reducing the amount of dumped polymer waste (Leja and Lewandowicz, 2010).

Many synthetic polymers are degraded under exposure to solar ultraviolet (UV) radiation, photo-oxidative, thermo-oxidative and photolytic reactions (Singh and Sharma, 2008).

In the natural environment, hydrolytic properties of seawater, oxidative properties of the atmosphere and sunlight radiation (UVB) make the polymers fragile and eventually break them into smaller pieces (Moore, 2008).

The American Society for Testing and Materials (ASTM) and International Standards Organization (ISO) provided the analytical protocol for plastic degradation on the basis of alteration in chemical structure and loss of physical properties of plastic (Kumar *et al.*, 2011).

II. 5. INVOLVEMENT OF MICROORGANISMS FOR DEGRADATION OF PLASTICS

Microorganisms such as bacteria, fungi and actinomycetes degrades both natural and synthetic plastics (Gu *et al.*, 2000). The richness of microbes able to degrade polythene is so far limited to 17 genera of bacteria and 9 genera of fungi (Hugenholtz *et al.*, 1998). Microbial degradation of plastics is caused by oxidation or hydrolysis using microbial enzymes that leads to chain cleavage of the large compound polymer into small molecular monomer by the metabolic process (Kumar *et al.*, 2013). The microbial species associated with the degrading materials were identified as bacteria, fungi, actinomycetes sp. and *Saccharomonospora* genus (Chee *et al.*, 2010).

The microorganism's growth is influenced by several factors including the availability of water, redox potential, temperature carbon and energy source (Sand, 2003). Microorganisms secreted by both exoenzymes and endoenzymes that are attached to the surface of large molecular substrate and cleave in to smaller segments (Albinas *et al.*, 2003). Recently reported, degrading enzymes are produced by several microorganisms (Gnanavel *et al.*, 2003). Microorganisms recognize polymers as a source of the organic compounds (Premraj *et al.*, 2005).

II. 5.1. Bacteria

Microorganisms such as *Bacillus megatserium*, *Pseudomonas* sp., *Azotobacter* sp., *Ralstonia eutropha*, *Halomonas* sp., etc are used to degrade plastics (Chee *et al.*, 2010). In addition, *Bacillus brevis* (Tomita *et al.*, 1999), *Acidovorax delafieldii* BS-3, *Paenibacillus amylocticus* TB-13 (Teeraphatpornchai

et al., 2003), *Bacillus pumilus* 1-A (Hayase *et al.*, 2004), *Bordetella petrii* PLA-3 (Kim *et al.*, 2010) *Pseudomonas aeruginosa* PBSA-2 (Lee *et al.*, 2010), *Shewanella* sp. CT01 are examples of bioplastic degrading bacteria (Sekiguchi *et al.*, 2010). In addition to these strains, a thermophilic bacterium, *Bacillus brevis*, with PLA-degrading properties has been isolated from soil.

In past years polyethylene degrading bacteria has been reported such as, *Acinetobacter baumannii*, *Arthrobacter* spp, *Viscosus* spp, *Pseudomonas* spp, *Arthrobacter viscosus*, *Bacillus amyloliquefaciens*, *Thuringiensis*, *Mycoides*, *Cereus pumilus*, *Staphylococcus cohnii*, *Xylopus* spp, *Pseudomonas fluorescens*, *Rahnella aquatilis*, *Micrococcus luteus*, *Lylae*, *Paenibacillus macerans*, *Flavobacterium* spp, *Delftia acidovorans*, *Ralstonia* spp *Rhodococcus erythropolis*, *Pseudomonas aeruginosa* (Koutny *et al.*, 2009) and *Bacillus brevis* (Watanabe *et al.*, 2009).

The microbial species that identified from the sample polythene bags tested were *Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Diplococcus* sp., *Micrococcus* sp., *Pseudomonas* sp. and *Moraxella* sp. The microbial species are associated with the degrading materials were identified as bacterial genus like (*Pseudomonas*, *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Moraxella*) (Shift *et al.*, 1998).

II. 5.2. Fungi

The growth of many fungi can also cause small-scale swelling and bursting, as the fungi penetrate the polymer solids. In recent years fungal strains have been reported for plastic degradation such as *Aspergillus versicolor* (Pramila *et al.*, 2011), *Aspergillus flavus* (Ramesh *et al.*, 2011), *Chaetomium* spp (Sowmya *et al.*, 2012), *Mucor circinellodites* species etc.

The polythene bags were degraded by some fungal species identified such as, *Aspergillus niger*, *A. ornatus*, *A. nidulans*, *A. cremeus*, *A. flavus*, *A. candidus* and *A. glaucus* were the predominant species. The microbial species are associated with the degrading materials were identified fungi (*Aspergillus niger*, *Aspergillus glaucus*. *Aspergillus* sp., is effective in biodegradation as plastics studies.

Many studies on fungal degradation of the bioplastic have also been performed including *Paecilomyces lilacinus* D218 (Oda *et al.*, 1995), *Fusarium moniliforme* Fmm (Torres *et al.*, 1996), *Aspergillus flavus* ATCC9643 (Benedict *et al.*, 1983), *Thermoascus aurantiacus* IFO31910 (Sanchez *et al.*, 2000), *Tritirachium album* ATCC22563, *Paecilomyces verrucosum* (Jarerat *et al.*, 2001), and *Aspergillus sp.*XH0501-a (Li *et al.*, 2011).

On the other hand, polylactic acid (PLA) is subjected to degradation by only two genera of fungi (*Penicillium roqueforti* and *Tritirachium album*) reported that *Aspergillus niger* van Tieghem F-1119 had the ability to degrade PVC. PHB and polyesters are degraded by many fungal genera such as *Acremonium*, *Cladosporium*, *Debaryomyces*, *Emericellopsis*, *Eupenicillium*, *Fusarium*, *Mucor*, *Paecilomyces*, *Penicillium*, *Pullularia*, *Rhodosporidium*, and *Verticillium* (Nitskii *et al.*, 1987). Similarly, PCL is degraded by *Aspergillus*, *Aureobasidium*, *Chaetomium*, *Cryptococcus*, *Fusarium*, *Rhizopus*, *Penicillium*, and *Thermoascus*.

PEA is degraded by *Aspergillus*, *Aureobasidium*, *Penicillium*, *Pullularia*. Fungus like *Alternaria solani*, *Spicaria sp.*, *Aspergillus terreus*, *Aspergillus fumigates*, *Aspergillus flavus* were isolated from soil where plastic have been dumped. These caused significant weight loss in the PS PUR blocks in the shaken cultures, reaching up to 100% in case of the isolate *Fusarium solani* (Ibrahim *et al.*, 2013).

II. 5.3. Actinomycetes

PLA-degrading actinomycetes, and *Amycolatopsis sp.* Strain isolated from the sample, reduced 100 mg of PLA film by ~60% after 14 days in liquid culture at 30°C. There are many species of microorganisms which can degrade PLA, PCL and PBS such as actinomycetes.

Several actinomycetes including *Amycolatopsis sp.* 3118 (Ikura *et al.*, 1999), *Amycolatopsis sp.* HT-6 (Pranamuda *et al.*, 1999), *Saccharothrix* JMC9114, *Kibdelosporangium aridum* JMC7912, *Actinomadura keratinilytica* T16-1 (Sukkhum *et al.*, 2011), *Amycolatopsis thailandensis* PLA07 (Chomchoei *et al.*, 2011), *Streptomyces bangladeshensis* 77T-4 (Hsu *et al.*, 2012), *Streptomyces*

thermoviolaceus subsp. *thermoviolaceus*76T-2 were reported as bioplastic degraders (Chua *et al.*, 2013). *Cryptococcus* sp. S-2 (Masaki *et al.*, 2005) and *Pseudozyma antarctica* JCM10317 (Shinozaki *et al.*, 2013) were reported to be bioplastic-degrading yeasts.

II. 6. FACTORS AFFECTING THE BIODEGRADATION OF PLASTICS BY MICROBES

There are many factors that determine the biodegradability of plastics by microorganisms. The chemical and physical properties of plastics mainly influence the biodegradation capacity. Other than that physicochemical nature like surface area, hydrophilic, and hydrophobicity, molecular weight, chemical structure, melting temperature, crystallinity etc play important roles in the biodegradation processes (Tokiwa *et al.*, 2009).

In general, polyesters with side chains are less assimilated than those without side chains. The molecular weight is also important for the biodegradability because it determines many physical properties of the polymer. Increasing the molecular weight of the polymer decreased its degradability. Morphological characters of polymers greatly affect their rates of biodegradation. The degree of crystallinity is a crucial factor affecting biodegradability, since enzymes mainly attack the amorphous domains of a polymer. The crystalline part of the polymers is more resistant than the amorphous region. The rate of degradation of Plastics decreases with an increase in crystallinity of the polymer (Iwata and Doi, 1998; Tsuji *et al.*, 2002).

II. 7. BIODEGRADATION MODE

Microorganisms being a part of delicate biological system are highly adaptive to environment and secrete both endoenzymes and exoenzymes that attack the substrate and cleave the molecular chains into segments (Albinas *et al.*, 2003; Huang *et al.*, 1990).

The secreted enzymes are proteins of complicated chemical structure with high molecular weights possessing hydrophilic groups such as -COOH, --OH, and -NH₂ (Potts, 1978) which can attack and eventually destroy almost anything. Several factors including the availability of water, temperature, oxygen usage, minerals, pH, redox potential, and carbon and energy source influence the growth

of microorganisms (Holmes, 1988; Sand, 2003). The degradative action of fungi and bacteria on the polymeric material is a result of enzyme production and resultant breakdowns to the non-living substrate in order to supply nutrient materials.

When biodegradable plastics decompose biologically, the resulting natural components do not affect the environment in any non-ecofriendly way. Even though the ordinary, non-biodegradable plastics do not release harmful by-products into the environment, they are relatively dangerous to ecosystem by causing unnecessary logging and dumping. With biodegradable plastics, which decompose more quickly, these negative effects are not altogether removed, that means the biodegradable plastics must not be dumped in the natural environment. However, if they do they will definitely cause less damage than non-biodegradable plastics. The advantages of biodegradable plastics over other types of plastics are of value only if proper handling of biodegradable plastics is applied; that is, if after they have been used, the plastics are disposed of under such conditions that enable their biological decomposition and the entering of the products into natural cycles (Sharma *et al.*, 2015).

II. 8. MECHANISM OF POLYMER BIODEGRADATION

Microorganisms break down the compounds into a simpler form through biochemical transformation. Bio-degradation of polymer is described as any alteration of the polymer properties such as digestion by microbial enzymes, reduction in molecular weight, and loss of mechanical strength and surface properties, in other words, the breakdown of material into fragments via microbial digestion. Degraded particles are redistributed and probably non-toxic to the environment. In nature, microorganisms form catalytic enzymes for biodegradation (Hadad *et al.*, 2005)

The above approach is proficient for environmental waste management, and microorganisms involved in this process for oxidation serve as a tangible alternative mode to maintain the healthy environment (Singh and Sharma, 2008). The degradation process is accomplished by microorganisms via different enzymatic activities and bond cleavage. This degradation occurs in sequential steps,

bio-deterioration (altering the chemical and physical properties of the polymer), bio-fragmentation (polymer breakdown in a simpler form via enzymatic cleavage) and assimilation (uptake of molecules by microorganisms) and mineralization (production of oxidized metabolites (CO₂, CH₄, H₂O) after degradation), Mineralization of polymers takes place in both aerobic and anaerobic conditions. In the aerobic condition, CO₂ and H₂O are formed, while under anaerobic conditions, CH₄, CO₂ and H₂O are produced (Singh and Sharma, 2008).

The biodegradation procedure of a few polymers is known (Shimao, 2001). Most of the microbial communities are able to utilize polyester and polyurethane at a slower rate (Dey *et al.*, 2012; Schink *et al.*, 1992). Starch- or cellulose-based plastics are biodegradable; they degrade easily through composting, which can reduce landfilling and solve the waste management problem. Biodegradation with the help of microorganisms is an approachable way to clean up such plastic waste. Microorganisms are able to utilize synthetic polymers, but the composition of the polymer and manufacturing process need to be defined for the biological activity on the polymer material (Sivan, 2011; Song *et al.*, 2009; Leja and Lewandowicz, 2010, Kumar *et al.*, 2011).

Biodegradability of synthetic polymers with chemical groups that are susceptible to microbial attack can be carried out with polycaprolactone, poly- β -hydroxyalkanoates and oil-based polymers (Leja and Lewandowicz 2010, Song *et al.*, 2009). Enzymes of microbial origin are employed to control pollution and contribute to developing an eco-friendly environment. Diverse forms of microflora are known to utilize them through the mineralization process.

III. MATERIALS AND METHODS

The methodology adopted for the present study “Cleavage of polymers into monomer by the biodegradable bacteria” are discussed under the following headings:

III. 1. SAMPLE COLLECTION

The soil sample was collected from a chronically dumped site located in Chennai (Corporation dump yard), Tamilnadu. Soil was collected from a depth of 3-5cm, and sealed in plastic bags immediately after sampling, then transported in an ice chest and used for the isolation of bacteria.

III. 2. COLLECTION OF POLYETHYLENE FILM

The LDPE films used for this study were obtained from local market where it is sold as 20 micron thick carry bags. LDPE films were cut into (3X3 cm) strips and then washed with 70% ethanol for 30 min, washed with distilled water, and air dried for 15 minutes in Laminar air flow chamber and was added to the medium.

III. 3. PREPARATION OF LDPE POWDER (LOW DENSITY POLYETHYLENE)

The washed LDPE sheets were cut into bits and were immersed in 20ml of xylene. The bits were boiled for 15 minutes using xylene which dissolves the LDPE film. The residue were crushed when it was warmed by using hand gloves. The resulting LDPE powder were washed with ethanol to remove residual xylene and were allowed to evaporate to remove ethanol. The powder were dried in hot air oven at 60°C overnight.

III. 4. ISOLATION OF MICROORGANISMS

The plastic degrading micro-organisms were isolated using Mineral Salt Medium (MSM). Mineral salt medium agar were prepared by using the following constituents in 100 ml distilled water. The composition of MSM were as follows.

1. K_2HPO_4 – 1g,
2. KH_2PO_4 – 0.2 g,

3. NaCl-1g,
4. CaCl₂.2H₂O- 0.002g,
5. MgSO₄.7H₂O-0.5g,
6. CuSO₄.5H₂O-0.001g,
7. ZnSO₄.7H₂O-0.001g,
8. MnSO₄. H₂O- 0.001g,
9. FeSO₄.7H₂O-0.01g.

III. 4.1. CULTURING OF MICROORGANISMS

To study the characteristics of a polyethylene degrading bacteria, it must be separated from all other species. To obtain a pure culture, streak plate technique was proceeded.

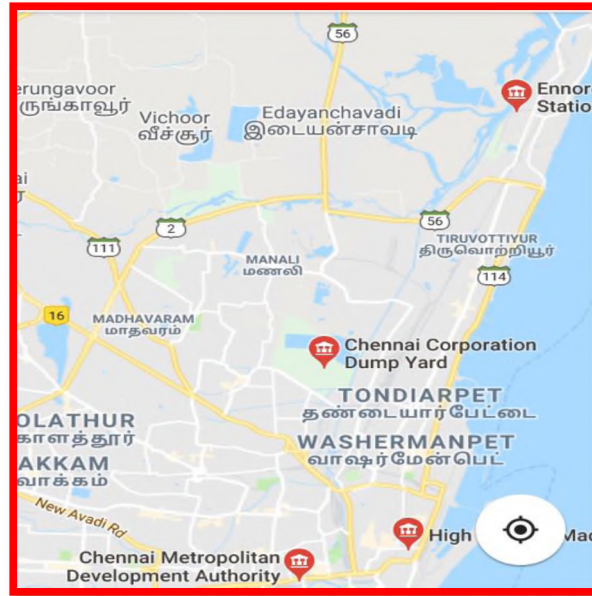
III. 4.2. SPREAD PLATE TECHNIQUE

Mineral Salt Medium was prepared and sterilized. The sterilized medium were poured into the sterilized petri plate and allowed to solidify. The soil sample was serially diluted from 1 to 10. From each dilution, 0.1 ml of sample was transferred into the petri plate with the help of sterile micropipette. The 0.1 ml of the sample present on the surface of agar was spread evenly by using L-rod. Plates were incubated at 37°C for 24 hrs.

III. 4.3. STREAK PLATE TECHNIQUE

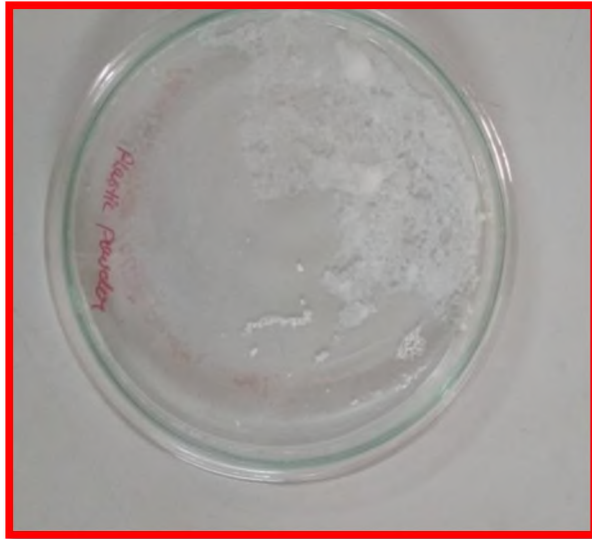
The microbial mixture were poured into the freshly prepared Mineral Salt Medium with the help of the inoculation loop and then streaked over the surface. This streak thin out the colony and separated from each other. The plates were incubated at 37°C for 24 hours.

PLATE - I



LOCATION OF SAMPLE COLLECTION

PLATE - II



PREPARATION OF LDPE POWDER

PLATE - III



ISOLATION OF MICROORGANISMS

III. 5. SCREENING OF POLYETHYLENE DEGRADING MICROBES

Opacity method

This method is to screen the polyethylene degrading microorganisms. The substrates like 1% of starch, 1% of gelatin and 1% of tween-80 were added individually to MSM agar medium along with the polymer sample. The bacterial sample were loaded. The plates were incubated at 37⁰C for 3-4 hours. After incubation, the formation of clear hallow indicates the first step of bacterial degradation.

III. 6. IDENTIFICATION TEST FOR MICROORGANISMS

III. 6.1. GRAMS STAINING

The Grams staining method is one of the widely used differential staining methods in bacteriology. The bacteria was stained by basic primary stain, followed by the addition of a mordent (Gram's iodine), and then decolorized by ethyl alcohol. Some of the bacteria remain their strain colour in certain conditions, while some are decolorized. Therefore the bacteria can be classified into two groups, the former is Grams positive bacteria and the latter is grams negative bacteria. For clear observations a final step of counter staining with safranin or basic fushin is applied after the decolourization. The colour of Gram positive bacteria remains purple while the Gram negative bacteria were stained to appear red.

Heat fixes the sample to the slide by carefully passing the slide with a drop or small piece of sample on it through a Bunsen burner. Add the primary stain (Crystal violet) to the sample/slide and incubate for 1 minute. Rinse slide with a gentle stream of water for a maximum of 5seconds to remove the unbound crystal violet. Add Gram's iodine for 1min this is a mordant or an agent that fixes the crystal violet to the bacterial cell wall. Gentle stream of water. Add the safranin to the slide and incubate for 1 minute. And then it was viewed under microscope.

III. 6.2. MOTILITY TEST

Vaseline or Petroleum jelly were spreaded on the four corner of a clean cover slip by using toothpick. A small drop of the bacterial suspension were placed

in the center of a cover slip by using the inoculated loop. The contact were made between the cover slip and the slide with the help of the Vaseline. The drop were examined by first locating its edge under low power and followed by high-dry objective (40 x).

III. 6.3. BIOCHEMICAL TEST

The selected organisms were identified using VITEK 2 Compact system at Bioline Laboratory, R.S Puram. It is a computer based technique for the identification of species. It also monitors the growth and the activity of the organisms. The reagent cards have 64 wells and each well contains different test substrates. Substrates assess various metabolic activities such as alkalisation, acidification, enzyme hydrolysis. It has several advantages like rapid identification, a simple methodology, high level of automation and taxonomically updated databases.

III. 7. MICROBIAL GROWTH STUDIES

The microbial growth were monitored by using HB and L URO QUATTRO at Bioline Laboratory, RS Puram. The number of colonies formed were plotted in graph.

III. 8. LABORATORY MICROBIAL DEGRADATION ASSAY OF LOW DENSITY POLY ETHYLENE (LDPE)

Determination of weight loss

To determine the quantity of plastic degraded by the selected bacterial isolates, the percentage weight loss method have been followed. Pre- weighed LDPE (0.5g) were transferred to the conical flask containing 50ml of culture broth medium, inoculated with bacterial species. Control was maintained with LDPE in the microbe-free medium. Different flasks were maintained for each treatment and left in a shaker. After one month of shaking, the LDPE were collected, washed thoroughly using distilled water and ethanol, shade dried and then weighed for final weight.

weight loss of the LDPE was calculated,

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

III. 9. FOURIER TRANSFORM INFRARED (FT-IR) ANALYSIS

To determine the formation of new or disappearance of functional groups was carried out on plastic sample Fourier Transform Infrared Spectroscopy have been carried out in the frequency range 3600- 600 cm^{-1} .

III. 10. SCANNING ELECTRON MICROSCOPY OF POLYETHYLENE

The changes in the morphology of the LDPE were analyzed by using SEM (Scanning Electron Microscopy).

IV. RESULTS AND DISCUSSION

The present study aimed to degrade the plastic film using microbes isolated from dumped soil sample. Two different bacterial strains have been isolated from chronically contaminated soil sample. Bacterial strains were chosen by screening from opacity method and they were identified based on their morphological and biochemical characteristics. Further, the isolated bacterial strains were used for the biodegradation of plastic wastes. The result has been discussed under the following headings.

IV. 1. ISOLATION OF MICROBES FROM CORPORATION DUMP YARD

The bacterial strains responsible for plastic degradation have been isolated from chronically contaminated dumped soil by using Mineral Salt Medium (MSM). The isolated bacterial strains were found to be colorless in MSM medium. To conform whether the isolated bacterial strains possess plastic degrading ability, the opacity method was followed.

IV. 2. SCREENING OF POLYETHYLENE DEGRADING MICROBES

To conform whether the isolated bacterial strain possess plastic degrading ability, the opacity method was followed. The isolated bacterial strains were translucent in Mineral Salt Medium. Hence, opacity formed cannot be able to measure.

Gelatin and Tween 80 are the derivative of petroleum product, it is used for screening potent microbes. The bacteria utilizes the substrates as a sole carbon source and forms opacity (Vignesh *et al.*, 2016).

IV. 3. IDENTIFICATION OF BACTERIAL STRAINS

IV. 3.1. COLONY COLOUR

The obtained bacterial colonies were translucent in Mineral Salt medium, Whereas the same bacterial strains were observed to be pink and green color in Mac Conkey (Isolate – 1) and Blood agar (Isolate – 2) medium respectively.

IV. 3.2. SIMPLE STAINING

The simple staining method was used to found out the morphology of the isolated bacterial strains .

A simple stain consists of a solution of single dye. Basic solution such as Acetylene Blue, Grams Safranin, Crystal violet is useful for staining most bacteria. These stains will readily give up a hydroxide ion or accept a hydrogen ion which leaves the stain positively charged. Since the surface of the most bacterial cells is negatively charged. These positively charged stains adhere readily to the cell surface (Kambe *et al.*, 1995).

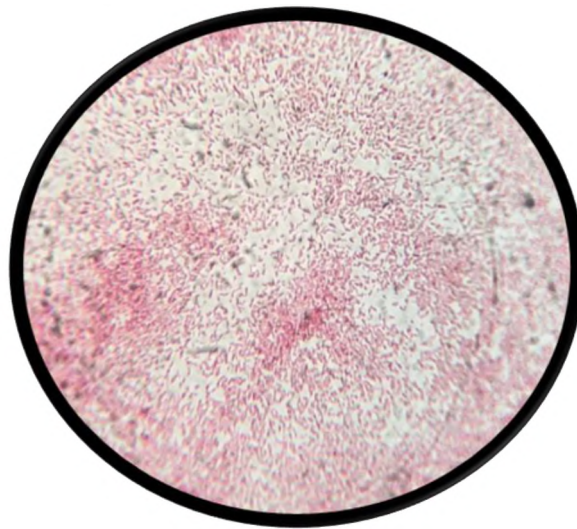
IV. 3.3. GRAMS STAINING

For further clarification of the isolated bacterial strains, Grams staining have been followed. The results revealed that the bacterial strains were gram negative bacilli.

IV. 4. BIOCHEMICAL PROPERTIES OF THE BACTERIAL ISOLATES

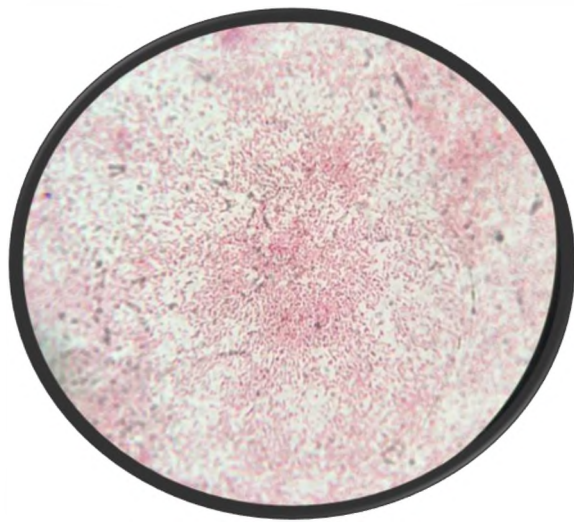
The results of the table 2 and 3 revealed that presence or absence of 64 substrates from the bacterial isolates 1 and 2. Through this VITEK 2 COMPACT, it was conformed that the selected bacterial isolates were *pseudomonas aeruginosa* and *klebsiella pneumoniae*.

PLATE – IV



GRAMS STAINING FOR ISOLATE – 1

PLATE – V



GRAMS STAINING FOR ISOLATE – 2

BIOCHEMICAL PROPERTIES FOR ISOLATE 1 – TABLE 2

WELL NO	NAME OF THE SUBTRATE	PRESENCE/ ABSENCE
2	Ala - Phe - Pro- ARYLAMIDASE	-
3	ADONITOL	-
4	L – Pyrrolydonyl – ARYLAMIDASE	-
5	L – ARABITOL	-
7	D – CELLOBIOSE	-
9	BETA – GALACTOSIDASE	-
10	H2S PRODUCTION	-
11	BETA – N – ACETYL – GLUCOSAMINIDASE	-
12	Glutamyl Arylamidase pNA	+
13	D – GLUCOSE	+
14	GAMMA – GLUTAMYL – TRANSFERASE	+
15	FERMENTATION GLUCOSE	-
17	BETA – GLUCOSIDASE	-
18	D – MALTOSE	-
19	D – MANNITOL	-
20	D – MANNOSE	-
21	BETA – XYLOSIDASE	-
22	BETA – Alanine arylamidase pNA	+
23	L – Proline ARYLAMIDASE	+
26	LIPASE	-
27	PALATINOSE	-
29	Tyrosine ARYLAMIDASE	-
31	UREASE	-
32	D – SORBITOL	-
33	SACCHAROSE/SUCROSE	-
34	D - TAGATOSE	-
35	D – TREHALOSE	-
36	CITRATE (SODIUM)	+

37	MALONATE	+
39	5 – KETO – D – GLUCONATE	–
40	L – LACTATE alkalisation	+
41	ALPHA – GLUCOSIDASE	–
42	SUCCINATE alkalisation	+
43	Beta – N- ACETYL- GALACTOSAMINIDASE	–
44	ALPHA – GALACTOSIDASE	–
45	PHOSPHATASE	–
46	Glycine ARYLAMIDASE	–
47	OENITHINE DECARBOXYLASE	–
48	LYSINE DECARBOXYLASE	–
53	L – HISTIDINE assimilation	–
56	COUMARATE	–
57	BETA – GLUCORONIDASE	–
58	O/129 RESISTANCE	–
59	Glu – Gly – Arg –ARYLAMIDASE	–
61	L – MALATE	+
62	ELLMAN	–
64	L – LACTATE assimilation	+

BIOCHEMICAL PROPERTIES FOR ISOLATE 2 – TABLE 3

WELL NO	NAME OF THE SUBTRATE	PRESENCE/ ABSENCE
2	Ala - Phe - Pro- ARYLAMIDASE	–
3	ADONITOL	+
4	L – Pyrrolydonyl – ARYLAMIDASE	–
5	L – ARABITOL	–
7	D – CELLOBIOSE	+
9	BETA – GALACTOSIDASE	+
10	H ₂ S PRODUCTION	–
11	BETA – N – ACETYL – GLUCOSAMINIDASE	–
12	Glutamyl Arylamidase pNA	–
13	D – GLUCOSE	+
14	GAMMA – GLUTAMYL – TRANSFERASE	+
15	FERMENTATION GLUCOSE	+
17	BETA – GLUCOSIDASE	+
18	D – MALTOSE	+
19	D – MANNITOL	+
20	D – MANNOSE	+
21	BETA – XYLOSIDASE	+
22	BETA – Alanine arylamidase pNA	–
23	L – Proline ARYLAMIDASE	–
26	LIPASE	–
27	PALATINOSE	+
29	Tyrosine ARYLAMIDASE	–
31	UREASE	+
32	D – SORBITOL	+

33	SACCHAROSE/SUCROSE	+
34	D - TAGATOSE	-
35	D – TREHALOSE	+
36	CITRATE (SODIUM)	+
37	MALONATE	+
39	5 – KETO – D – GLUCONATE	-
40	L – LACTATE alkalisation	+
41	ALPHA – GLUCOSIDASE	-
42	SUCCINATE alkalisation	-
43	Beta – N- ACETYL- GALACTOSAMINIDASE	-
44	ALPHA – GALACTOSIDASE	+
45	PHOSPHATASE	+
46	Glycine ARYLAMIDASE	-
47	OENITHINE DECARBOXYLASE	-
48	LYSINE DECARBOXYLASE	+
53	L – HISTIDINE assimilation	-
56	COUMARATE	-
57	BETA – GLUCORONIDASE	-
58	O/129 RESISTANCE	+
59	Glu – Gly – Arg –ARYLAMIDASE	-
61	L – MALATE	-
62	ELLMAN	-
64	L – LACTATE assimilation	-

IV. 5. MICROBIAL GROWTH STUDIES

The HB and L UROQUATTRO monitored the growth phase of the bacterial isolates and quantitative bacterial count in the form of Colony Forming Unit/ ml. The figure 1 shows the growth phase of the bacterial isolates and quantitative bacterial count of 10,000,000 CFU/ml from *Pseudomonas aeruginosa* (Isolate – 1). Similar count have also been observed for *Klebsiella pneumoniae* (Isolate – 2).

IV. 6. LABORATORY MICROBIAL DEGRADATION ASSAY OF LDPE

The % weight loss method have been followed to estimate the quantity of plastic degradation by the two isolated bacterial strains.

Determination of weight loss

% of weight loss,

$$= \frac{0.50-0.42}{0.50} \times 100$$

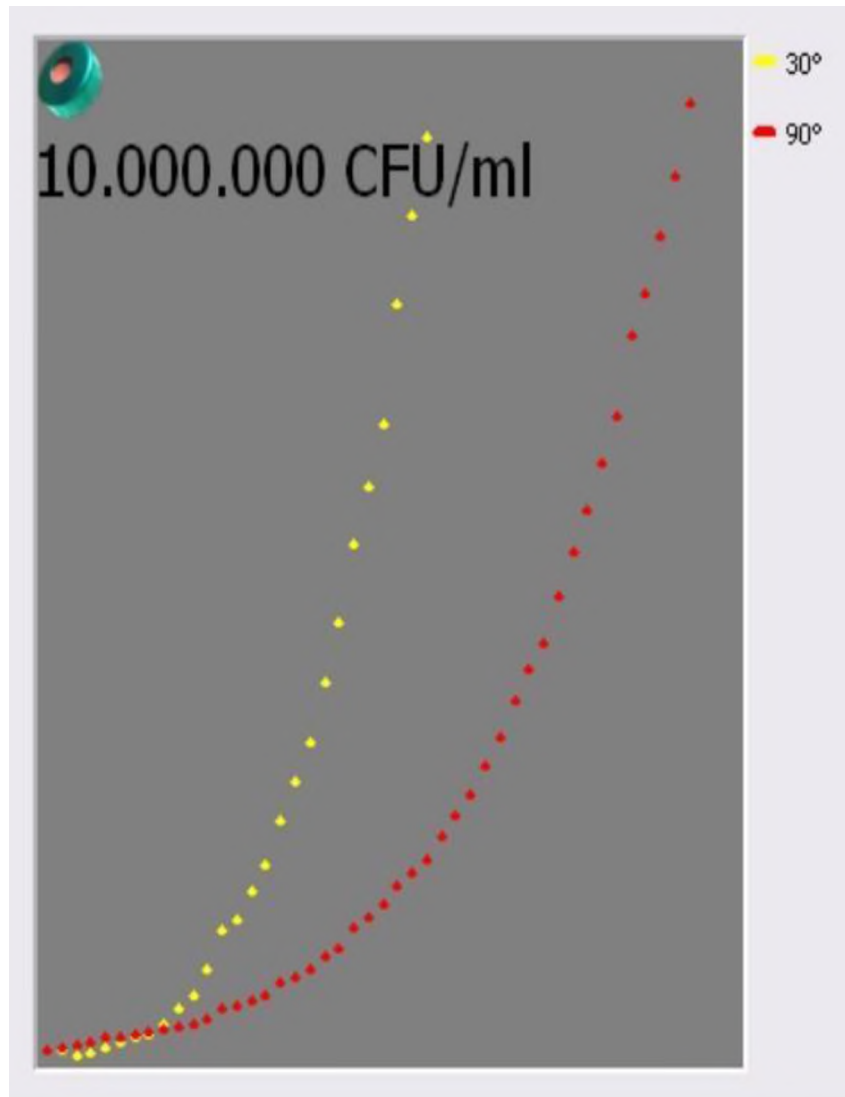
The percentage of weight loss of 0.50 g of plastic in 1 month is 16 %.

IV. 7. FOURIER TRANSFORM INFRARED ANALYSIS (FT – IR)

Infrared spectroscopy gives information on the vibrational and rotational modes of a molecule and hence an important technique for identification and characterization of a substance (Lalitha *et al.*, 2013).

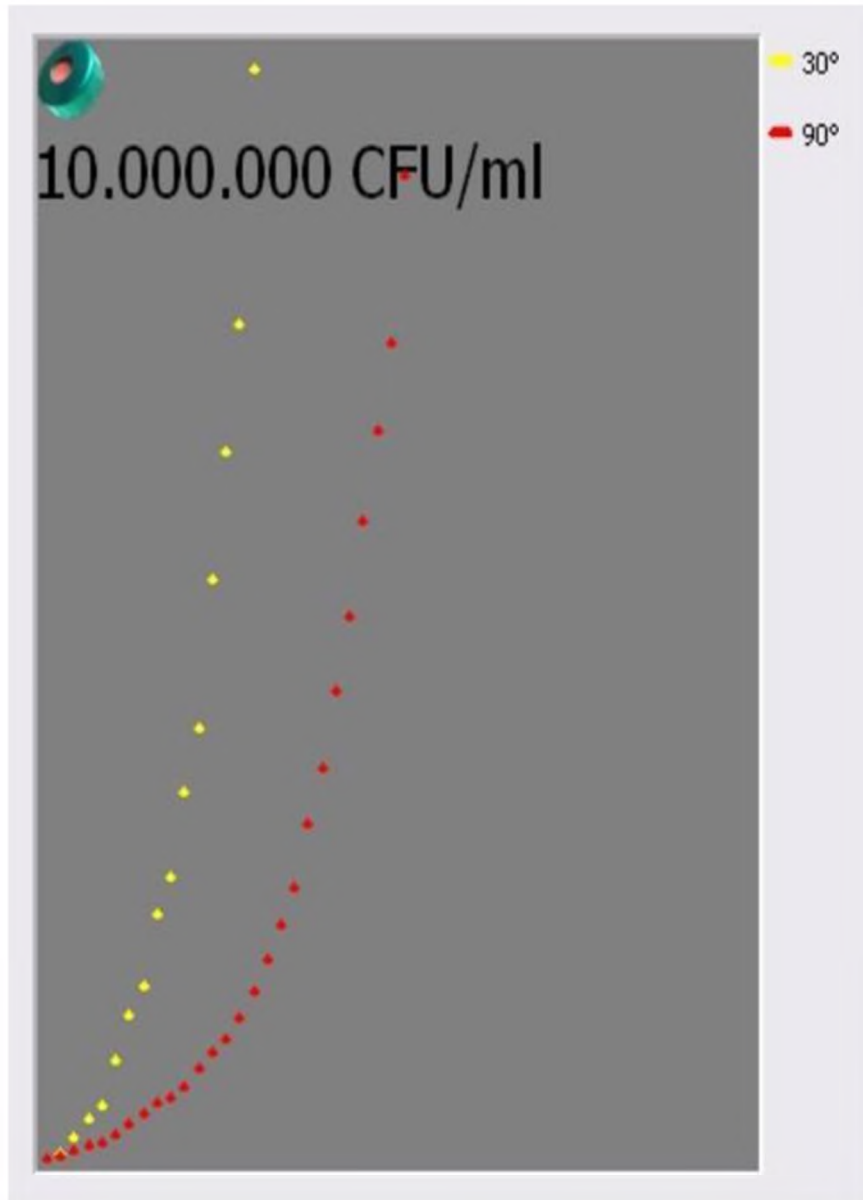
The FTIR spectroscopy analysis identified the changes in the functional groups that responsible for the reduction of polymer into monomer which were shown in the figure 3 and 4. This figure revealed strong peaks at 2916, 2353, 2021, 1651, 1203. The strong band at 2916 cm^{-1} are assigned to the OH stretching vibration. The peak at 1203 cm^{-1} attributed to the stretching vibration of alcohols, carboxylic acids, esters and ethers. The peak at 2353 cm^{-1} corresponds to the stretching vibration of alkenes. The smallest peak at 2021 cm^{-1} were identified as stretching vibration of COOH groups. The peak at 1651 cm^{-1} were identified as stretching vibration of aromatic protons. All these changes in functional groups confirmed the degradation occurs in LDPE film.

FIGURE – 1



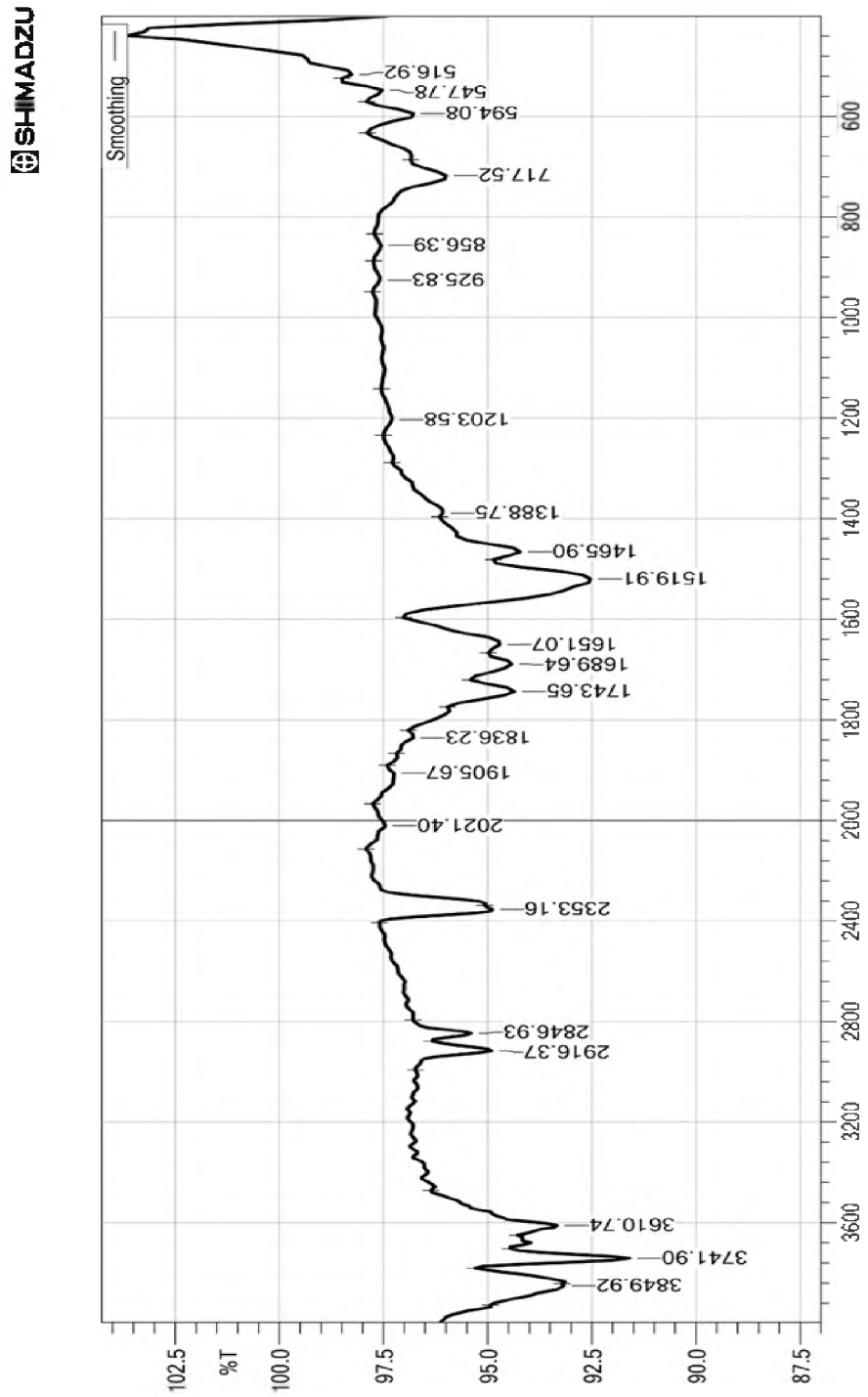
GROWTH PHASE OF PSEUDOMONAS AERUGINOSA

FIGURE - II



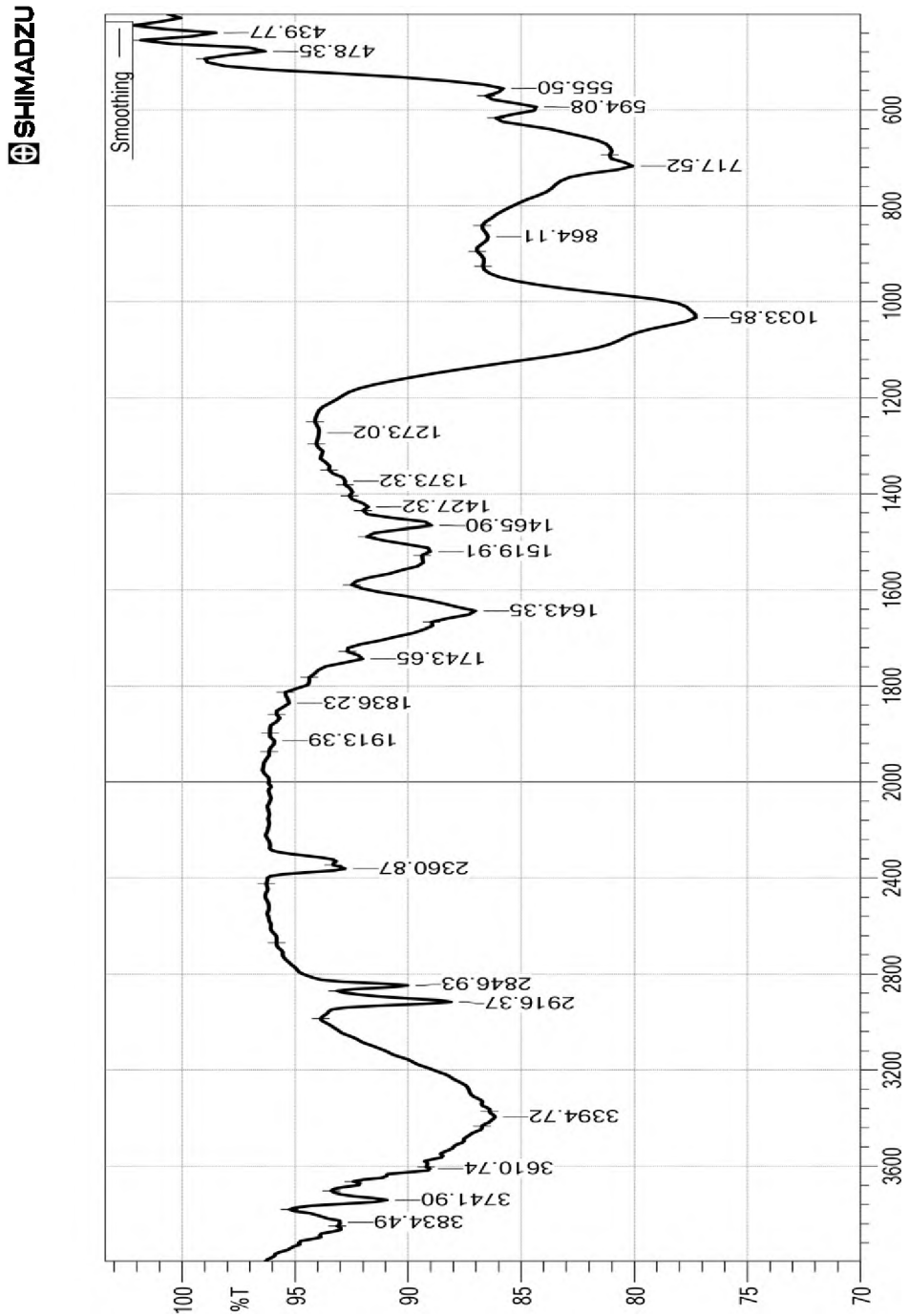
GROWTH PHASE OF KLEBSIELLA PNEUMONIAE

FIGURE – III



FT – IR SPECTRUM ANALYSIS – BEFORE DEGRADATION

FIGURE – IV

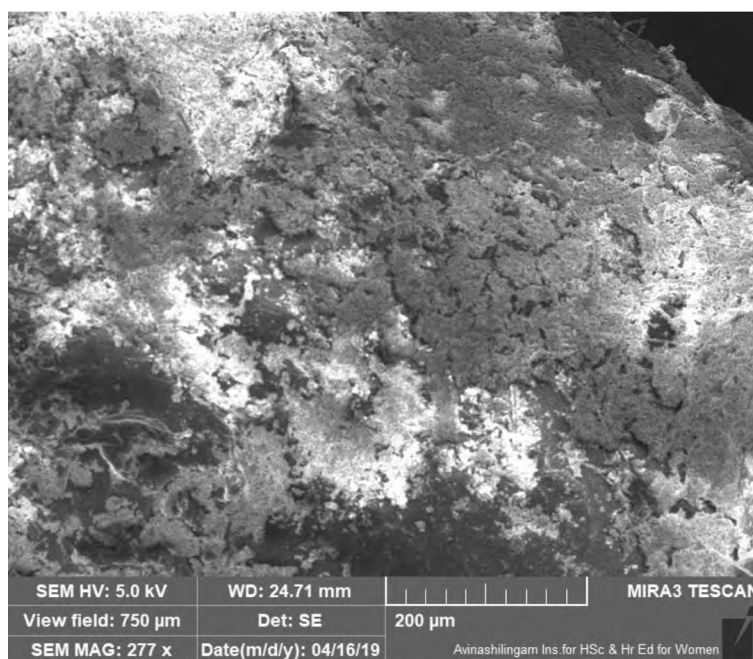


FT – IR SPECTRUM ANALYSIS - AFTER DEGRADATION

IV. SCANNING ELECTRON MICROSCOPY OF POLYETHYLENE

The surface morphology of the treated LDPE was analyzed through Scanning Electron Microscopy to check for any structural changes on the Low Density Poly Ethylene. Figure 5 shows the micrographs of the Scanning Electron Microscopy of LDPE powder after incubation with bacterial isolates. Erosions of the LPDE powder were observed . cavities are also observed on the polyethylene powder.

FIGURE - V



SCANNING ELECTRON MICROSCOPY

V. SUMMARY AND CONCLUSION

Industrialization as well as social development is playing a significant role in the generation of solid waste. Microorganisms play a significant role in biological decomposition of materials, including synthetic polymers in natural environment. High Density Polyethylene (HDPE) and Low Density Polyethylene (LDPE) are the most commonly used Synthetic plastics and they are slow in degradation and causing serious environmental problems. Nowadays, there are few biodegradable water soluble polymers available commercially, in the spite of the obvious and pressing need for environmental protection.

- ✦ The present study deals with the isolation, identification and degradation ability of plastic (LDPE) degrading microorganisms from soil.
- ✦ From the soil sample, the plastic degrading bacterial strains were isolated using Mineral Salt Medium.
- ✦ To obtain a pure culture of bacterial strains, streak plate method has been followed.
- ✦ Since, the isolated bacterial strains were translucent in MSM medium, the opacity formed cannot be measured.
- ✦ To identify the bacterial isolates from the chronically contaminated soil sample, various staining and biochemical methods have been followed.
- ✦ The staining procedure revealed that bacterial isolates were gram negative rod shaped bacillus.
- ✦ Motility test performed for the bacterial isolates indicated that the isolate 1 showed motility and isolate 2 is non – motile in nature.
- ✦ The biochemical parameters were analyzed by using VITEK 2 COMPACT, at Bioline Laboratory.
- ✦ The results showed that the presence of 2 bacterial strains namely *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.
- ✦ After identifying the plastic degradable bacterial strains from the contaminated soil, the growth studies have been performed using HB and L URO QUATTRO.
- ✦ The results registered that the growth curve of bacterial isolates and quantitative count in the form of Colony Forming Unit/ ml.

- ✚ FT – IR results indicated the obvious changes in functional groups occurred between pre and post LDPE powder.
- ✚ The Scanning Electron Microscopy results indicated that the erosions and cavities were observed on LDPE powder.
- ✚ The overall investigation can be concluded that *Pseudomonas sp.*, and *Klebsiella sp.*, exhibited significant role in polyethylene degradation ability. Various polyethylene degradation methods are available in literature but the cheapest, eco-friendly and adequate method is degradation using microbes.

VI. REFERENCES

- Ali, M.I., Ahmed, S., Robson, G., Javed, I., Ali, N., Atiq, N. and Hameed, A., 2014. Isolation and molecular characterization of polyvinyl chloride (PVC) plastic degrading fungal isolates. *Journal of basic microbiology*, 54(1), pp.18-27.
- Andrady, A.L., 2011. Microplastics in the marine environment. *Marine pollution bulletin*, 62(8), pp.1596-1605.
- Andrady, A.L. and Neal, M.A., 2009. Applications and societal benefits of plastics. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1526), pp.1977-1984.
- Arutchelvi, J., Sudhakar, M., Arkatkar, A., Doble, M., Bhaduri, S. and Uppara, P.V., 2008. Biodegradation of polyethylene and polypropylene.
- Augusta, J., Müller, R.J. and Widdecke, H., 1993. A rapid evaluation plate-test for the biodegradability of plastics. *Applied microbiology and biotechnology*, 39(4-5), pp.673-678.
- Barnes, D.K., 2002. Biodiversity: invasions by marine life on plastic debris. *Nature*, 416(6883), p.808.
- Battersby, A., Feilden, R. and Nelson, C., 1999. Sterilizable syringes: excessive risk or cost-effective option?. *Bulletin of the World Health Organization*, 77(10), p.812.
- Benachour, N. and Aris, A., 2009. Toxic effects of low doses of Bisphenol-A on human placental cells. *Toxicology and applied pharmacology*, 241(3), pp.322-328.
- Benedict, C.V., Cook, W.J., Jarrett, P., Cameron, J.A., Huang, S.J. and Bell, J.P., 1983. Fungal degradation of polycaprolactones. *Journal of Applied Polymer Science*, 28(1), pp.327-334.
- Bhatnagar, S. and Kumari, R., 2013. Bioremediation: a sustainable tool for environmental management—a review. *Annual Research & Review in Biology*, pp.974-993.
- Bhardwaj, H., Gupta, R. and Tiwari, A., 2012. Microbial population associated with plastic degradation. *Open Access Sci Rep* 1: 272.

- Calafat, A.M., Kuklennyik, Z., Reidy, J.A., Caudill, S.P., Ekong, J. and Needham, L.L., 2004. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environmental health perspectives*, 113(4), pp.391-395.
- Chandra, R.U.S.T.G.I., 1998. Biodegradable polymers. *Progress in polymer science*, 23, pp.1273-1335.
- Chee, J.Y., Yoga, S.S., Lau, N.S., Ling, S.C., Abed, R.M. and Sudesh, K., 2010. Bacterially produced polyhydroxyalkanoate (PHA): converting renewable resources into bioplastics. *Current research, technology and education topics in Applied Microbiology and Microbial Biotechnology*, 2, pp.1395-1404.
- Chomchoei, A., Pathom-Aree, W., Yokota, A., Kanongnuch, C. and Lumyong, S., 2011. *Amycolatopsis thailandensis* sp. nov., a poly (l-lactic acid)-degrading actinomycete, isolated from soil. *International journal of systematic and evolutionary microbiology*, 61(4), pp.839-843.
- Chua, A.S., Takabatake, H., Satoh, H. and Mino, T., 2003. Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent. *Water Research*, 37(15), pp.3602-3611.
- Chua, T.K., Tseng, M. and Yang, M.K., 2013. Degradation of Poly (ϵ -caprolactone) by thermophilic *Streptomyces thermoviolaceus* subsp. *thermoviolaceus* 76T-2. *AMB Express*, 3(1), p.8.
- Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T. and Gore, A.C., 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocrine reviews*, 30(4), pp.293-342.
- Dey, U., Mondal, N.K., Das, K. and Dutta, S., 2012. An approach to polymer degradation through microbes. *IOSR J Pharm*, 2(3), pp.385-388.
- Drain, P.K., Ralaivao, J.S., Rakotonandrasana, A. and Carnell, M.A., 2003. Introducing auto-disable syringes to the national immunization programme in Madagascar. *Bulletin of the World Health Organization*, 81, pp.553-560

- Ghosh, S.K., Pal, S. and Ray, S., 2013. Study of microbes having potentiality for biodegradation of plastics. *Environmental Science and Pollution Research*, 20(7), pp.4339-4355.
- Ghosh, M. and Singh, S.P., 2005. A review on phytoremediation of heavy metals and utilization of it's by products. *Asian J Energy Environ*, 6(4), p.18.
- Gnanavel, G., JayaValli, M.V.P., Thirumarimurugan, M. and Kannadasan, T., 2012. Degradation of plastics using microorganisms. *International Journal of Pharmaceutical and Chemical Sciences*, 1, pp.691-694.
- Gray, J., Evans, N., Taylor, B., Rizzo, J. and Walker, M., 2009. State of the evidence: the connection between breast cancer and the environment. *International journal of occupational and environmental health*, 15(1), pp.43-78.
- Gu, J., Ford, T.E. and Mitchell, R., 2000. Microbiological corrosion of metals. *Uhlig's corrosion handbook (2nd edition)*.
- Hadad, D., Geresh, S. and Sivan, A., 2005. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *Journal of applied microbiology*, 98(5), pp.1093-1100.
- Hao, J., Wang, J., Zhao, W., Ding, L., Gao, E. and Yuan, W., 2011. Effect of bisphenol A exposure on sex hormone level in occupational women. *Wei sheng yan jiu = Journal of hygiene research*, 40(3), pp.312-4.
- Hayale T, (2019) – Market Research News.
<https://themarketresearchnews.com/2019/04/16/plastic-bottles-market-analysis-status-and-business-outlook-2019-to-2023/>
- Hayase, N., Yano, H., Kudoh, E., Tsutsumi, C., Ushio, K., Miyahara, Y., Tanaka, S. and Nakagawa, K., 2004. Isolation and characterization of poly (butylene succinate-co-butylene adipate)-degrading microorganism. *Journal of Bioscience and Bioengineering*, 97(2), pp.131-133.
- Holmes PA (1988). In: *Developments in Crystalline Polymers*, edited by Basset DC (Applied science) UK.
- Holmgren, J.H., 1974. Advantages of disposables: safety, cost, convenience. *Modern hospital*, 122(1), pp.35-35.

- Hsu, K.J., Tseng, M., Don, T.M. and Yang, M.K., 2012. Biodegradation of Poly (β -hydroxybutyrate) by a novel isolate of *Streptomyces bangladeshensis* 77T-4. *Botanical Studies*, 53(3).
- Huang, J.C., Shetty, A.S. and Wang, M.S., 1990. Biodegradable plastics: a review. *Advances in Polymer Technology*, 10(1), pp.23-30.
- Ibrahim, D., Bankole, O.C., Ma'aji, S.A., Ohize, E.J. and Abdul, B.K., 2013. Assessment of the strength properties of polystyrene material used in building construction in Mbora district of Abuja, Nigeria. *International Journal of Engineering Research and Development*, 6(12), pp.80-84.
- Ikura, Y. and Kudo, T., 1999. Isolation of a microorganism capable of degrading poly-(L-lactide). *The Journal of general and applied microbiology*, 45(5), pp.247-251.
- Iwata, T. and Doi, Y., 1998. Morphology and enzymatic degradation of poly (L-lactic acid) single crystals. *Macromolecules*, 31(8), pp.2461-2467.
- Jarerat, A. and Tokiwa, Y., 2001. Degradation of Poly (L-lactide) by a Fungus. *Macromolecular Bioscience*, 1(4), pp.136-140.
- Kim, M.N. and Park, S.T., 2010. Degradation of poly (L-lactide) by a mesophilic bacterium. *Journal of applied polymer science*, 117(1), pp.67-74.
- Koutny, M., Amato, P., Muchova, M., Ruzicka, J. and Delort, A.M., 2009. Soil bacterial strains able to grow on the surface of oxidized polyethylene film containing prooxidant additives. *International Biodeterioration & Biodegradation*, 63(3), pp.354-357.
- Kumar, S., Das, P.M., Rebecca, L.J. and Sharmila, S., 2013. Isolation and identification of LDPE degrading fungi from municipal solid waste. *Journal of chemical and pharmaceutical research*, 5(3), pp.78-81.
- Kumar, A.A., Karthick, K. and Arumugam, K.P., 2011. Biodegradable polymers and its applications. *International Journal of Bioscience, Biochemistry and Bioinformatics*, 1(3), p.173.
- Kumar, A.A., Karthick, K. and Arumugam, K.P., 2011. Biodegradable polymers and its applications. *International Journal of Bioscience, Biochemistry and Bioinformatics*, 1(3), p.173.

- Lee, B.K., Ellenbecker, M.J. and Moure-Eraso, R., 2002. Analyses of the recycling potential of medical plastic wastes. *Waste management*, 22(5), pp.461-470.
- Leja, K. and Lewandowicz, G., 2010. Polymer Biodegradation and Biodegradable Polymers-a Review. *Polish Journal of Environmental Studies*, 19(2).
- Leschine, S.B., 1995. Cellulose degradation in anaerobic environments. *Annual review of microbiology*, 49(1), pp.399-426.
- Li, F., Hu, X., Guo, Z., Wang, Z., Wang, Y., Liu, D., Xia, H. and Chen, S., 2011. Purification and characterization of a novel poly (butylene succinate)-degrading enzyme from *Aspergillus* sp. XH0501-a. *World Journal of Microbiology and Biotechnology*, 27(11), pp.2591-2596.
- Liu, X., Holzwarth, J.M. and Ma, P.X., 2012. Functionalized synthetic biodegradable polymer scaffolds for tissue engineering. *Macromolecular bioscience*, 12(7), pp.911-919.
- Lugauskas, A., Levinskait, L. and Pečiulyt, D., 2003. Micromycetes as deterioration agents of polymeric materials. *International biodeterioration & biodegradation*, 52(4), pp.233-242.
- Masaki, K., Kamini, N.R., Ikeda, H. and Iefuji, H., 2005. Cutinase-like enzyme from the yeast *Cryptococcus* sp. strain S-2 hydrolyzes polylactic acid and other biodegradable plastics. *Appl. Environ. Microbiol.*, 71(11), pp.7548-7550.
- Mogil'nitskii, G.M., Sagatelyan, R.T., Kutishcheva, T.N., Zhukova, S.V., Kerimov, S.I. and Parfenova, T.B., 1987. Disruption of the protective properties of the polyvinyl chloride coating under the effect of microorganisms. *Prot. Met. (Engl. Transl.); (United States)*, 23(1).
- Moore, C.J., 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. *Environmental research*, 108(2), pp.131-139.
- Muthur Noopur, Sharma Sakshi, Nupur Singh Snnuradha. Toxicity and biodegradation of plastics: A review. *J Environ Dev* 2015; 9(3): 906-913.
- Nerland, I.L., Halsband, C., Allan, I. and Thomas, K.V., 2014. Microplastics in marine environments: Occurrence, distribution and effects.

- Oda, Y., Asari, H., Urakami, T. and Tonomura, K., 1995. Microbial degradation of poly (3-hydroxybutyrate) and polycaprolactone by filamentous fungi. *Journal of fermentation and bioengineering*, 80(3), pp.265-269.
- Ojo, O.A., 2007. Molecular strategies of microbia adaptation to xenobiotics in natural environment. *Biotechnology and Molecular Biology Reviews*, 2(1), pp.1-13.
- Pathak, V.M., 2017. Review on the current status of polymer degradation: a microbial approach. *Bioresources and Bioprocessing*, 4(1), p.15.
- Pérez, J., Munoz-Dorado, J., De la Rubia, T.D.L.R. and Martinez, J., 2002. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *International microbiology*, 5(2), pp.53-63.
- Pillai, C.K.S. and Sharma, C.P., 2010. Absorbable polymeric surgical sutures: chemistry, production, properties, biodegradability, and performance. *Journal of biomaterials applications*, 25(4), pp.291-366.
- Pilz, H., Brandt, B. and Fehringer, R., 2010. The impact of plastics on life cycle energy consumption and greenhouse gas emissions in Europe. *Summary report June*.
- Pramila R, Ramesh KV. Biodegradation of LDPE by fungi isolated from municipal landfill area, J. Microbiol. Biotechnol Res 2011; 1(2): 131-136.
- Pranamuda, H. and Tokiwa, Y., 1999. Degradation of poly (L-lactide) by strains belonging to genus Amycolatopsis. *Biotechnology Letters*, 21(10), pp.901-905.
- Premraj R, Mukesh CD. (2005). Biodegradation of polymer. *Indian Journal of Biotechnology*, 4: 186-193.
- Puri N, Kumar B, Tyagi H (2013). Utilization of recycled wastes as ingredients in concrete mix. *International Journal of Innovative Technology and Exploring Engineering* 2(2):74–78.
- Rudel, R.A., Attfield, K.R., Schifano, J.N. and Brody, J.G., 2007. Chemicals causing mammary gland tumors in animals signal new directions for epidemiology, chemicals testing, and risk assessment for breast cancer prevention. *Cancer: Interdisciplinary International Journal of the American Cancer Society*, 109, pp.2635-2666.

- Sanchez, J.G., Tsuchii, A. and Tokiwa, Y., 2000. Degradation of polycaprolactone at 50° C by a thermotolerant *Aspergillus* sp. *Biotechnology Letters*, 22(10), pp.849-853.
- Sand, W., 2003. Microbial life in geothermal waters. *Geothermics*, 32(4-6), pp.655-667.
- Schink, B., Janssen, P.H. and Frings, J., 1992. Microbial degradation of natural and of new synthetic polymers. *FEMS microbiology reviews*, 9(2-4), pp.311-316.
- Sekiguchi, T., Sato, T., Enoki, M., Kanehiro, H., Uematsu, K. and Kato, C., 2011. Isolation and characterization of biodegradable plastic degrading bacteria from deep-sea environments. *JAMSTEC Report of Research and Development*, 11, pp.33-41.
- Shah, A.A., Hasan, F., Hameed, A. and Ahmed, S., 2008. Biological degradation of plastics: a comprehensive review. *Biotechnology advances*, 26(3), pp.246-265.
- Sharma, J., Gurung, T., Upadhyay, A., Nandy, K., Agnihotri, P. and Mitra, A.K., 2015. Isolation and characterization of plastic degrading bacteria from soil collected from the dumping grounds of an industrial area. *Int J Adv Innov Res*, 3, pp.2278-7844.
- Shima, M., 2001. Biodegradation of plastics. *Current opinion in biotechnology*, 12(3), pp.242-247.
- Shinozaki, Y., Kikkawa, Y., Sato, S., Fukuoka, T., Watanabe, T., Yoshida, S., Nakajima-Kambe, T. and Kitamoto, H.K., 2013. Enzymatic degradation of polyester films by a cutinase-like enzyme from *Pseudozyma antarctica*: surface plasmon resonance and atomic force microscopy study. *Applied microbiology and biotechnology*, 97(19), pp.8591-8598.
- Singh B, Sharma N (2008) Mechanistic implications of plastic degradation. *Polym Degrad Stab* 93:561–584.
- Sivan, A., 2011. New perspectives in plastic biodegradation. *Current opinion in biotechnology*, 22(3), pp.422-426.
- Song, J.H., Murphy, R.J., Narayan, R. and Davies, G.B.H., 2009. Biodegradable and compostable alternatives to conventional plastics. *Philosophical*

transactions of the royal society B: Biological sciences, 364(1526), pp.2127-2139.

- Sowmya, H.V., Ramalingappa, M. and Krishnappa, M., 2012. Degradation of polyethylene by *Chaetomium* sp. and *Aspergillus flavus*. *Int. J. Recent Sci. Res*, 3(513), p.e517.
- Steinglass, R., Boyd, D., Grabowsky, M., Laghari, A.G., Khan, M.A., Qavi, A. and Evans, P., 1995. Safety, effectiveness and ease of use of a non-reusable syringe in a developing country immunization programme. *Bulletin of the World Health Organization*, 73(1), p.57.
- Sukkhum, S., Tokuyama, S., Kongsaree, P., Tamura, T., Ishida, Y. and Kitpreechavanich, V., 2011. A novel poly (L-lactide) degrading thermophilic actinomycetes, *Actinomadura keratinilytica* strain T16-1 and plasmid sequencing. *African Journal of Microbiology Research*, 5(18), pp.2575-2582.
- Suman, M. and Shamba, C., 2014. A comparative study of commercially available plastic carry bag biodegradation by microorganisms isolated from hydrocarbon effluent enriched soil. *International Journal of Current Microbiology and Applied Sciences*, 3(5), pp.318-325.
- Sung, G.B., 2010. *Ban on Plastic Bags Usage-Is It a Right Move? An Empirical Study On Consumer Perception And Practice*. Research Report in partial fulfillment of the requirements for the degree of Master of Business Administration
- Swift G. Handbook of biodegradable polymers. CRC Press; 1998.
- Szumigaj, J., Żakowska, Z., Klimek, L., Rosicka-Kaczmarek, A. and Bartkowiak, A., 2008. Assessment of polylactide foil degradation as a result of filamentous fungi activity. *Polish Journal of Environmental Studies*, 17(3), pp.335-341.
- Teeraphatpornchai, T., Nakajima-Kambe, T., Shigeno-Akutsu, Y., Nakayama, M., Nomura, N., Nakahara, T. and Uchiyama, H., 2003. Isolation and characterization of a bacterium that degrades various polyester-based biodegradable plastics. *Biotechnology letters*, 25(1), pp.23-28..
- Tokiwa, Y., Suzuki, T. and Ando, T., 1976. Degradation of polycaprolactone by a fungus. *Journal of Fermentation Technology*.

- Tomita, K., Kuroki, Y. and Nagai, K., 1999. Isolation of thermophiles degrading poly (L-lactic acid). *Journal of bioscience and bioengineering*, 87(6), pp.752-755.
- Torres, A., Li, S.M., Roussos, S. and Vert, M., 1996. Screening of microorganisms for biodegradation of poly (lactic-acid) and lactic acid-containing polymers. *Appl. Environ. Microbiol.*, 62(7), pp.2393-2397.
- Tsuji, H. and Suzuyoshi, K., 2002. Environmental degradation of biodegradable polyesters 1. Poly (ϵ -caprolactone), poly [(R)-3-hydroxybutyrate], and poly (L-lactide) films in controlled static seawater. *Polymer Degradation and Stability*, 75(2), pp.347-355.
- Upadhyay, NDTV NEWS (2019).
<https://swachhindia.ndtv.com/goa-bans-single-use-plastic-september-2019-33440/>
- Usha, R., Sangeetha, T. and Palaniswamy, M., 2011. Screening of polyethylene degrading microorganisms from garbage soil. *Libyan agriculture research center journal international*, 2(4), pp.200-204.
- Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N. and Welshons, W.V., 2007. Human exposure to bisphenol A (BPA). *Reproductive toxicology*, 24(2), pp.139-177.
- Vatseldutt, S.A., 2004. Isolation and characterization of polythene degrading bacteria from polythene dumped garbage. *Biotechnol Lett*, 53, pp.1181-1189.
- Vignesh, R., Deepika, R.C., Manigandan, P. and Janani, R., 2016. Screening of plastic degrading microbes from various dumped soil samples. *Int Res J Eng Tech*, 3(4), pp.2493-2498.
- Walker, T.R., Reid, K., Arnould, J.P. and Croxall, J.P., 1997. Marine debris surveys at Bird Island, South Georgia 1990–1995. *Marine pollution bulletin*, 34(1), pp.61-65.
- Watanabe, T., Ohtake, Y., Asabe, H., Murakami, N. and Furukawa, M., 2009. Biodegradability and degrading microbes of low-density polyethylene. *Journal of applied polymer science*, 111(1), pp.551-559.