BIODEGRADATION OF HDPE PLASTICS BY BACTERIA ISOLATED FROM PETROCHEMICAL OIL CONTAMINATED SITES

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(20PBT002)

Under the guidance of

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Professor

A Thesis submitted to Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore – 641043

In Partial Fulfilment of the Requirement for the Degree of

Master of Science in Biotechnology

May 2022

Certificate

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Signature of the Guide

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Signature of the Head of the Department

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Introduction

1.0 INTRODUCTION

Plastics are low-cost, long-lasting organic polymers that are manufactured artificially. These materials are frequently employed in modern civilization and have become crucial. Polypropylene, polystyrene, and polyethylene are examples of plastics. polyethylene is the most widely used plastic on the planet. Every year, 140 million tonnes are manufactured. Because of its versatility and efficacy, low-density polythene (LDPE) has been widely employed. Globally, between 500 billion and 1 trillion plastic bags are consumed each year. These packaging materials are thrown in landfills after use, causing pollution because they are non-biodegradable in natural environments. The hydrophobic carbon backbone and large molecular weight of LDPE were thought to contribute to the polymer's resistance against biological attack. As a result, the rapid biodegradation of plastic has become a topic of attention in the waste management problem throughout time (Kyaw *et al.*, 2020).

Plastic is the most versatile 'manmade' synthetic substance made from fossil fuel resources. allow for the majority of industrial and technological advancements. Revolutions of the nineteenth and twentieth centuries Plastic materials have become widely used in the previous 25 years since they are increasingly employed in food, clothes, and other products Housing, Transit, Building, Medical care, and Recreation. Plastics are made out of petroleum-based materials. Resins such as polythene and polyester materials polypropylene are resistant to biodegradation. Plastics that are resistant to this are disposed of in landfills and are preserved in their original state perpetuity. Plastics have a lot of advantages over other materials. alternative materials are lightweight, low-cost, and environmentally friendly. In the last 30 years, the use of plastics has increased dramatically (Chowdhurcy *et al.,* 2020).

Low-density polyethylene is one of the major sources of environmental pollution. Polyethylene is a polymer made of long chains of ethylene monomers. The use of polyethylene growing worldwide at a rate of 12% per year and about 140 million tons of synthetic polymers are produced worldwide each year. With such a large amount of polyethylene gets accumulated in the environment, generating plastic waste ecological problems are needed thousands of years to efficiently degradation. Microorganisms can degrade the plastic in over 90 genera, from bacteria among them; *Bacillus megaterium, Pseudomonas sp., Azotobacter, Ralstoniaeutropha Halomona ssp.* Plastic degradation by microbes due to the activity of certain enzymes that cause cleavage of the polymer chains into monomers and oligomers. Plastic that has been enzymatically broken down is further absorbed by the microbial cells to be metabolized.

Aerobic metabolism produces carbon dioxide and water. Instead, anaerobic metabolism produces carbon dioxide, water, and methane as end products. This study aims to isolate, screen, and identification the bacteria from contaminated soil with plastic waste that can degrade low-density polyethylene (LDPE) (Amal *et al.,* 2015).

High-density polyethylene is one type of polyethylene. Because of its longevity, HDPE is commonly found in the environment. It can, however, be bioaccumulated into living creatures or the environment after being broken down into minute particles by external causes. Sustainable and cost-effective HDPE treatment solutions are required to decrease HDPE-induced bio risks (Kang *et al*., 2019).

HDPE is widely used in commercial applications and is used in large quantities in common industrial processes like extrusion, injection molding, and blow molding. The ultimate optical, mechanical, and thermal properties of HDPE are influenced by the crystallization process. change the physical properties of HDPE by influencing crystallization from the melt stage. Clarity, cycle speed, and modulus are all improved by specific. Because of its slower crystallization rate polypropylene (PP) is more widely developed than HDPE, allowing for better control in attaining property improvements. While some are efficient at increasing HDPE physical characteristics, further control over the crystallization processed results in even more improvements in specific attributes. Though progress has been made in identifying viable for HDPE, the severity of the problem remains (Karl, 2021).

Many countries prohibit municipal garbage incineration due to the presence of plastics in these wastes. Instead, plastics are burned and landfilled in an open, unregulated manner. The open burning of these wastes, which releases toxins into the air, can cause a variety of health concerns. Furthermore, burning plastics produces persistent organic pollutants known as furans and dioxins. burning polyethylene, polyurethane, polyvinyl chloride, and polystyrene produces toxic irritant products that cause immune disorders and lung diseases, and are classified as possible human carcinogens. Plastic can degrade in a variety of ways, including chemical, thermal, photooxidation, and biodegradation, all of which take a very long period to complete depending on the type of plastic (Moszczynski *et al.*, 2021).

Synthetic polymers were first manufactured more than half a century ago and have since become a viable alternative to natural materials in practically every sector. They become an integral part of our daily lives. Oil, coal, and natural gas are the primary raw materials used to make plastics. In the last four decades, the development and use of synthetic plastics have transformed the character of garbage. Plastics have now superseded natural materials in various facets of human life and have become an indispensable part of society. Although polymers' endurance is one of their advantageous features, it is also one of their significant environmental issues. The plastic deposition has expanded dramatically during the previous two decades, and plastics have become ubiquitous. The biological approach to bioremediation is biodegradation. To eliminate harmful contaminants, microorganisms are used. Some limitations of conventional approaches are not present in biological degrading methods. Polyethylene is an important component of essential structure (Ferronato and Torretta, 2019).

Microorganisms are used in the majority of plastic biodegradation experiments. The majority of organic wastes are degraded by microbes and contribute to biological productivity either directly or indirectly. Microorganisms are capable of digesting most organic and inorganic materials; hence the microbial breakdown of plastic and polythene waste material is of great interest (Ramman *et al*., 2018).

The gradual destruction of the natural environment that has occurred over the last few decades as a result of the systematically increasing manufacture of synthetic polymer materials has prompted the hunt for technological advancements that will provide environmentally benign materials. In addition, the growing importance of sustainability encourages the development of bio-based and biodegradable polymers, which are sometimes mislabelled as "bioplastics." Incapability to deteriorate. The problem of synthetic polymer compounds persisting in the environment for hundreds of years has led the manufacture of polymer materials with the addition of components that may hasten their deterioration in recent years has become increasingly essential. Furthermore, as the public's awareness of environmental issues grows, so does the demand for novel materials. This will not have a substantial impact on the environment (Mierzwa *et al*., 2019).

Direct breakdown of HDPE by microorganisms using only the polymer as a carbon source is one of the most common ways of facilitating disintegration and subsequent degradation. polythene bags manufactured of HDPE in both natural and artificial environments. *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, have previously been shown to biodegrade synthetic plastics (Norman *et al*., 2012).

The present study was focused on the objectives

- To isolate and identify the HDPE degrading bacteria from petrochemical oil-contaminated sites.
- To study the role of the biodegradation process of isolated bacteria.

Review of Literature

2.0 REVIEW OF LITERATURE

2.1 Introduction

The term plastic was derived from the Greek word ‘Plastikos’ meaning – that can be easily molded or shaped. In the year 1846, Plastic was first discovered by the famous German chemist name Christian Schonbein. Plastics are a wide range of synthetic or semi-synthetic materials that has polymers as the main constituent. Plastic as polymers of long carbon chains (Nelson, 2011). The plastics include different varieties. They are discussed below in detail.

2.2 Types of plastic

Acrylic or Polymethyl Methacrylate (PMMA), Polycarbonate (PC), Polyethylene (PE), Polypropylene (PP), Polyethylene Terephthalate (PETE or PET), Polyvinyl Chloride (PVC), Acrylonitrile-Butadiene-Styrene (ABS)(Carlos *et al.,* 2021).

2.2.1 Acrylic or polymethyl methacrylate

Acrylic is a transparent thermoplastic used as a lightweight, shatter-resistant alternative to glass. Acrylic is typically used in sheets to form create products such as acrylic mirrors and acrylic plexiglass. The transparent plastic can be made coloured and fluorescent, abrasion-resistant, bullet-resistant, UV-tolerant, non-glare, anti-static, and many more. In addition to being than glass and polycarbonate sheeting, acrylic is seventeen times more impact resistant than glass, easier to handle and process, and has endless applications (Carlos *et al.*, 2021).

2.2.2 Polycarbonate (PC)

Polycarbonate is an excellent engineering plastic that is as clear as glass and two hundred and fifty times stronger. Tough, stable, and transparent thirty times stronger than acrylic, clear polycarbonate sheets are also easily worked, molded, and thermoformed or cold-formed. Although extremely strong and impact-resistant, polycarbonate plastic possesses inherent design flexibility. Unlike glass or acrylic, plastic sheets can be cut or cold-formed on-site without pre-forming and fabrication. Polycarbonate plastic is in a wide variety of products including greenhouses, DVDs, sunglasses, police riot gear, and more (Bai *et al*., 2020).

2.2.3 Polyethylene (PE)

The most common plastic on earth, polyethylene can be manufactured in varying densities. Each different density of polyethylene gives the final plastic unique physical properties. As a result, polyethylene is in a wide variety of products. The four common polyethylene derivatives are LDPE, MDPE, HDPE and UHMWU (Wang *et al.*, 2015).

2.2.3.1 Low -density polyethene (LDPE)

This density of polyethylene is ductile and used to make products like shopping bags, plastic bags, clear food containers and disposable packaging (Wang *et al.*, 2015).

2.2.3.2 Medium-Density Polyethylene (MDPE)

Possess more polymer chains and, thus, greater density, MDPE is typically in gas pipes, shrink film, carrier bags, screw closures, and more (Wang *et al.*, 2015).

2.2.3.3 High-Density Polyethylene (HDPE)

More are rigid than both LDPE and MDPE, HDPE plastic sheeting is in products such as plastic bottles, piping for water and sewer, snowboards, boats, and folding chairs (Wang *et al.*, 2015).

2.2.3.4 Polyethylene (Ultra High Molecular Weight UHMWPE)

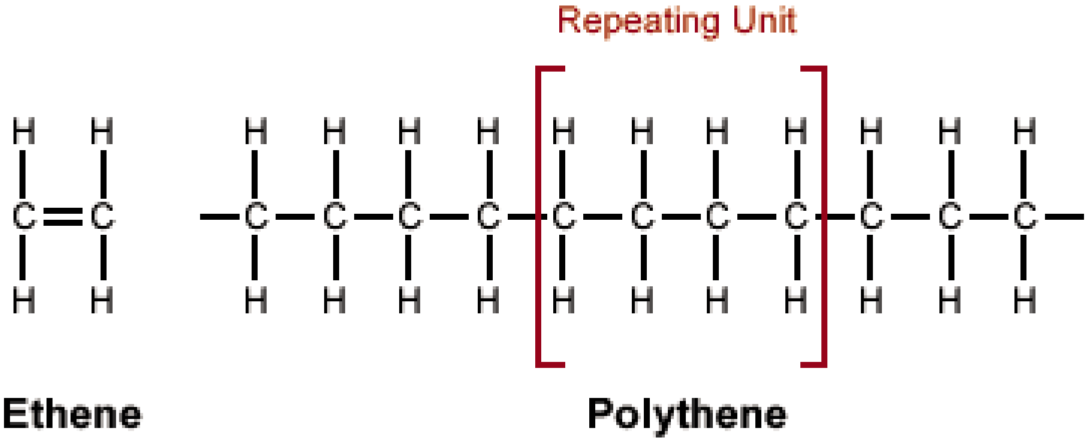
Ultra-high molecular weight is not much denser than HDPE. Compared to HDPE, this polyethylene plastic is much more abrasion resistant due to the extreme length of its polymer chains. Possessing high density and low friction properties, ultra-high molecular weight is in military body armor, hydraulic seals and bearings, biomaterial for hip, knee, and spine implants, and artificial ice-skating rinks (Wang *et al.*, 2015).

2.3.1 Polyethene structure

Polyethylene is a hydrocarbon polyolefin made of long chains of $\mathbf{(C_2\,H_4)_n}$ molecules, where n describes the number of units of ethylene ($C_2\,H_4$) that are joined together in the chain; n ranges from thousands to hundreds of thousands of units. An olefin (alkene) is a hydrocarbon with a C=C double bond (Robinson *et al.*, 2021).

2.3.2 Preparation of Polyethylene

Polyethylene, a word meaning many ethylenes, is prepared in one of two ways: either by putting ethylene gas under enormous pressure with radical activators, or by mixing ethylene in a liquid with catalysts. In each case, the process quickly and efficiently joins the ethylene units into a chain of polyethylene. After that, polyethylene is made into different forms: extruded into thread and tubes, rolled into films, or injection-moulding (Robinson *et al.*, 2021).



2.3.3 Polymerization of Ethylene

This method of joining many units (or "-mers") together chemically, like beads in a necklace, is called polymerization. The monomer, ethylene, comes from natural gas or from processed petroleum, and end with PE polymer (David *et al.*, 2015).

Chain reaction

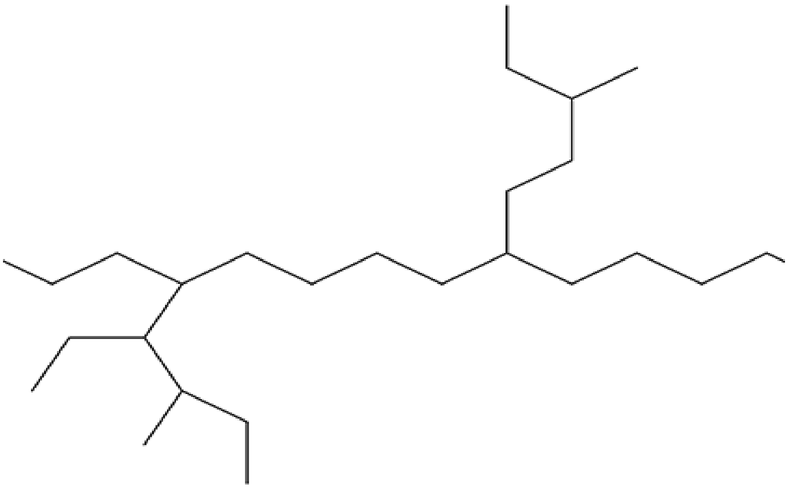
Ethylene has a double bond between two carbons. This double bond makes ethylene unstable and ready to react. With enough pressure and heat and the addition of radicals or catalysts, the double-bond of the monomer breaks and connects with another monomer, growing the PE polymer in a chain reaction (David *et al.*, 2015).

2.3.4 Classification of polyethylene

LDPE

Low- density polyethylene is producing under very high pressure in oldest polyethylene polymerization process developed in Britain in 1930**.**

Figure 1: Branched-chain of LDPE



The branched chains of LDPE stick out and prevent the polymer backbone from lining up well. This creates a loose, amorphous (disorganized) structure, like a pile of newly cut fir trees with tangled branches. This molecular structure is important, as it provides the elasticity, resistance to tears, and strength in all directions that is valuable to consumers (David *et al.*, 2015).

2.3.5 LDPE preparation

40 percent of all PE polymer is LDPE. The high-pressure polymerization requires radical initiators. The free radicals, which are oxygen-based substances with an extra 2electron, grow the chain and make bushy side branches in random places. LDPE is made in hot steel tubes called autoclaves under the intense pressures of thousands of atmospheres. The process converts ethylene to PE polymer in about a minute (Shi *et al.*, 2019).

2.3.6 LDPE Applications

Low-cost, lightweight LDPE is used in waterproof packaging, plastic bags, bubble wrap, disposable bottles, FDA-compliant pharmaceutical equipment, and electrical insulation (Shi *et al.*, 2019).

2.3.7 High-density polyethylene (HDPE)

High-density polyethylene (HDPE) is made **of** linear, relatively unbranched, chains. HDPE can be rolled into a thin film with a crisp, crinkly feel that is strong in one direction. It can also be extruded into durable containers such as bottles and pipes (Shi *et al.*, 2019).

Figure 2: L**inear polymer chains of HDPE**

The linear polymer chains in HDPE arrange closely like a neat pile of uncut logs in what is called a crystalline structure. Molecules in the HDPE structure are so close that short-distance intermolecular forces (between molecules) hold them together, similar to two pieces of static fabric that cling. This linear structure makes HDPE stronger, denser, and more fibrous than LDPE while being less flexible (Sharma *et al.*, 2020).

2.3.8 HDPE preparation

HDPE is produced in a different polymerization process than LDPE, using catalysts under less extreme pressure. This is less expensive than LDPE production. The catalysts add ethylene to the ends of a growing polymer chain without attacking the backbone in the middle. Therefore, this process forms long, relatively unbranched chains that pack together with high density. The commonest process is Zeigler-Natta polymerization which uses titanium catalysts. The process was developed in the 1960s in the USA, Germany, and Italy and was an important step in reducing the cost of production of plastics. Chromium oxide catalysts may also be used. These catalytic processes account for 60% of polyethylene production (Aoshima and Yata, 2018).

2.3.9 HDPE Applications

HDPE has higher tensile (pulling) strength than LDPE, and is long-lasting and solvent resistant. It is primarily used for tough, flexible containers such as garbage bins, jerry cans, automobile gas tanks, water and sewage pipes. It is a major component of toys, fishing nets, and telecommunications cables (Dawn *et al.,* 2021).

2.4. Polypropylene (PP)

Polypropylene material is a thermoplastic polymer and the world’s second-most widely produced synthetic plastic. Its widespread use and popularity are undoubted because polypropylene is one of the most flexible thermoplastics on the planet. Although PP is stronger than PE, it still retains flexibility. It will not crack under repeated stress. Durable, flexible, heat resistant, acid resistance and cheap, polypropylene sheets are used to make laboratory equipment, automotive parts, medical devices and food containers (Liu *et al.*, 2019).

2.5 Polyethylene terephthalate (PETE or PET)

Polyethylene is the most common thermoplastic resin of the polyester family; PET is the fourth-most produced synthetic plastic. Polyethylene Terephthalate hasan excellent chemical resistance to organic materials and water and is easily recyclable. It is practically shatterproof and possesses an impressive high strength to weight ratio. This plastic material is in fibers for clothing, containers for foods and liquid, glass fiber for engineering resins, carbon nanotubes, and many other products that are used on a daily basis (Xuemin *et al.*, 2019).

2.6 Polyvinyl Chloride (PVC)

Polyvinyl chloride is the third-most produced synthetic plastic polymer, the PVC can be manufactured to possess rigid or flexible properties. It is well-known for its ability to blend with other materials. For example, expanded PVC sheets are a foamed polyvinyl chloride material that is ideal for products like kiosks, store displays, and exhibits. The rigid form of PVC is commonly used in construction materials, doors, windows, bottles, non-food packaging, and more. With the addition of plasticizers such as phthalates, the softer and more flexible form of PVC is in plumbing products, electrical cable insulation, clothing, medical tubing and other similar products (Raewyn *et al.*, 2020).

2.7 Acrylonitrile-Butadiene-Styrene (ABS)

Acrylonitrile- Butadiene- styrene Created by polymerizing styrene and acrylonitrile in the presence of polybutadiene, ABS is robust, flexible, glossy, and highly processable and impact resistant. It can be manufactured in a range of thicknesses from 200 microns to 5mm with a maximum width of 1600mm. With a relatively low manufacturing cost, ABS plastic sheeting is typically used in the automotive and refrigeration industries but is also in products such as boxes, gauges, protective headgear, luggage, and children’s toys (Alessandro *et al.*, 2016).

2.8 Status of Polythene Pollution

The use of plastic, especially polythene is growing day by day. Every year 25 million tons of synthetic plastics are being accumulated in the sea coasts and terrestrial environment. Polythene constitutes 64 percentage of the total synthetic plastic as it is being used in huge quantity for the manufacture of bottles, carry bags, disposable articles, garbage containers, margarine tubs, milk jugs, and water pipes. Similarly, in the marine environment alone, out of total marine waste, plastic shares about 60-80 percentage by mass. All the polythene waste along with other plastic wastes generated by the human activity finally enters into marine water through rivers, canals/channels and municipal drainages. Therefore, the beaches were reported to be the excellent depository sites for the polythene (plastic) wastes. At dumping sites, polythene waste degraded with both chemical and mechanical weathering but it takes long time for mineralization and may remain in the microscopic form for long time. Annually 500 billion to 1 trillion polythene bags are being used routinely all over the world (Avinash *et al.*, 2020).

Polythene is strong and highly durable and takes up to 1000 years for natural degradation in the environment. Furthermore, plastic degrades by sunlight into smaller toxic parts contaminating soil and water where they can be accidentally ingested by animals and thereby enter the food chain especially in the marine biota. To the marine life polythene waste is recognized as a major threat. Sometimes, it could cause intestinal blockage in the fishes, birds and marine mammals. As per report due to plastic pollution in the marine environment minimum 267 species are being affected which includes all mammals, sea turtles (86%) and seabirds (44%). The death of terrestrial animals such as cow was reported due to consumption of polythene carry bags. The polythene leads to blockage of their digestive tract. It is also found that the polythene remains undigested in the stomach of the animals, after the death of the animals the polythene is again being eaten by some other animal and the cycle continues. The undigested polythene was found to be responsible for various problems in the animals such as during the digestion the fermentation process and mixi ng of the other contents were hampered due to ingested polythene and leads to indigestion. The ingested polythene blocks the opening between omasum and reticulum which leads to death of the animal if the polythene will not be removed, impaction: due to accumulation of large quantity of polythene bags in the rumen becomes impact which leads to ruementory .due to blockage of the reticulum and omasum with polythene, accumulation of gases takes place in rumen, which leads to death of the animal if not removed properly. In the digestive track around the polythene deposition of salt takes place that leads to formation of stone like structure which hampers the food passages and leads to pain and inflammation of rumen. The accumulation of polythene in the stomach of the animals (cow) leads to increased sensitivity to infections such as haemorrhagic septicemia. The widely used packaging plastic (mainly polythene) constitutes about 10 percentage of the total municipal waste generated around the globe. As per literature, every year hundred thousand tons of plastics have been degraded in the marine environment resulting death. The use of polythene is increasing every day and its degradation is becoming a great challenge. In the year 2021 about 57 million tons of plastic waste was generated around the world annually. Only a fraction of this polythene waste is recycled whereas most of the wastes enter into the landfills and take hundreds of years to degrade (Avinash *et al.*, 2020).

2.9 Cost-Effective Methods of Polythene Degradation

The process which leads to any physical or chemical change in polymer properties as a result of environmental factors (such as light, heat and moisture, etc.), chemical conditions or biological activity is said to be polymer degradation. Based on the factors responsible for the degradation of the polymers, three types of polymer degradation methods are cited in the literature such as photodegradation, thermos-oxidative degradation, and biodegradation. Biodegradation is a natural process of degrading materials through microbes such as bacteria, fungi and algae. The biodegradation involves microbial agents and does not require heat. Organic material can be degraded in two ways either aerobically or anaerobically. In landfills and sediments, plastics are degraded anaerobically while in composite and soil, aerobic biodegradation takes place. Aerobic biodegradation leads to the production of water and CO2 and anaerobic biodegradation results in the formation of water, CO2, and methane as end products. Generally, the conversion of the long-chain polymer into CO2 and water is a complex process. In this process, various different types of microorganisms are needed, with one leading to the breakdown of the polymer into smaller constituents, one utilizing the monomers and excreting simple waste compounds as by-products, and one using the excreted waste. The efficiency of this method is moderate but is environmentally friendly. This method is cheap and widely accepted. Depending upon the formulation of the biodegradable polythene carry bags, three types along with one standard polythene were studied for their degradation potential in the marine water. It was reported that after 40 weeks of exposure period the surfaces of the biodegradable polythene carry bags degraded less than 2% whereas the degradation of standard polythene was negligible (Sangale *et al.,* 2012).

2.10 Polythene Biodegradation

The degradation of polythene begins with the attachment of microbes to its surface. Various bacteria (*Streptomyces viridosporus T7A, Streptomyces badius 252,* and *Streptomyces setonii 75Vi2)* and wood degrading fungi produced some extracellular enzymes which leads of degradation of polythene. In wood degrading fungi, the extracellular enzymatic complex (ligninolytic system) contains peroxidases, laccases and oxidases which leads to the production of extracellular hydrogen peroxide. Depending upon the type of the organism or strain and culture condition, the characteristics of this enzyme system varies. For degradation of lignin, three enzymes such as lignin peroxidase, manganese peroxidase, and phenoloxidase containing copper also known as laccase. Based on the capabilities of these lingolytic enzymes, they are being used in various industries such as agricultural, chemical, cosmetic, food, fuel, paper, textile and more interesting point is that they are also reported to be involved in the degradation of xenobiotic compounds and dyes. During lignin degradation, phenolic compounds are being oxidized in the presence of H2O2 and manganese by manganese peroxidase (MnP). MnP oxidizes Mn-II to Mn-III and monomeric phenols. phenolic lignin dimmers and synthetic lignin are in turn oxidized by Mn-III via the formation of phenoxy radicals. There is no such report in case of polythene degradation but a similar trend is predicted. The byproducts of the polythene varied depending upon the conditions of degradation. Under aerobic conditions, CO2, water and microbial biomass are the final degradation products whereas in case of anaerobic/ methanogenic condition CO2, water, methane and microbial biomass are the end products and under sulfidogenic condition H2S, CO2 and H2O and microbial biomass are reported to be the end products (Zhang *et al.*, 2020).

2.11 Determination of Polythene Degradation

The level of polythene degradation can be determined by the various methods as well as analytical techniques. At topographical level, the Scanning Electron Microscopy (SEM) are being used to see the level of scission and attachment of the microbes on the surface of the polythene before and after the microbial attack. The micro destruction of the small samples is widely analyzed by an important tool such as Fourier Transform Infrared spectroscopy (FT-IR), and due to the recent upgradation of this instrument the map of the identified compounds on the surface of the sample can be documented via collection of large number of FT-IR spectra. To measure the physical changes of the polythene after the microbial attack various parameters are usually used to determine the weight loss, percentage of elongation and change in tensile strength (Sivan *et al.*, 2011).

Materials and Methods

3.0 EXPERIMENTAL PROCEDURE

3.1 INTRODUCTION

The present study focused mainly to the isolate, identify and characterize the bacteria from the soil samples of petrochemical oil contaminated sites and their role in biodegradation of HDPE plastics. In recent years there have been numerous negative reports on plastic bags and their devastating impact on environment, sparking public interest in finding a solution ratio the problem of plastic trash. Polyethylene a chemically inert polymer made up of carbon and hydrogen is used to make the majority of shopping bags. Municipal solid trash management has been hampered by the littering of plastic carry bags and pouches. The high-density polyethylene plastics in this investigation purchased from a local store in Coimbatore (commercially available as 50-micron plastic carry bags).

3.2 SOIL SAMPLE COLLECTION

Soil samples were collected from petrochemical oil contaminated site in two selected sites such as Kovai pudhur and Narasimmanaickenpalayam area of Coimbatore, Tamil Nadu, India.They were designated as KP1 and NP1. They were collected from the ground surface to a depth of 5cm and promptly transferred to the laboratory in sterile zip lock bags.

PLATE 1: SOIL SAMPLE FROM SELECTED AREA

KP1

NP1

3.3 ISOLATION OF BACTERIA

The soil samples were utilized to isolate polyethylene degrading bacteria by enrichment technique in synthetic medium (SM) containing polyethylene as a carbon source. One gram of the soil sample was added as inoculum source to the flask containing 50ml of the SM. HDPE films were cut into small pieces (about 3×3 cm each), weighed at a concentration of 300mg/100ml, disinfected in 70 percent ethanol and air dried in laminar air flow chamber and was added to the enrichment medium which was then incubated at 30°C for 7 days in a shaker incubator (150 rpm) (Gilan *et al.*, 2004). After incubation, 10ml of the incubated culture was transferred to a freshly prepared 90ml of SM, containing HDPE film as sole carbon source and incubated for another week and the same cycle was repeated. Once for every 7 days the subcultures were made up to 35 days. After the sub culturing, the enriched culture broth was spread on the nutrient agar plates. Morphologically distinct colonies were selected, purified and maintained on slant nutrient agar for further studies. The samples were kept at 4°C and used without being pre-treated.

3.4 IDENTIFICATION OF POLYETHYLENE DEGRADING BACTERIA

The polyethylene degrading bacteria were identified according to the morphological, cultural and biochemical characteristics by following Bergey's Manual of Systematic Bacteriology (Sneath, 1986).

3.4.1 MORPHOLOGICAL CHARACTERIZATION

3.4.1.1 GRAM'S STAINING (Gram,1884)

Gram's staining is used to differentiate gram positive and gram negative organisms. Gram's iodine act as a mordant that causes the crystal violet to penetrate and adhere to the gram-positive organisms. A little drop of the isolate's bacterial suspension was obtained and smears were formed on a glass slide by heat fixation. The crystal violet staining reagent was flooded onto the slide and incubated for 1 minute. The smear was gently cleaned for 2 seconds with a direct stream of tap water. After that, the slide was filled with iodine mordant and leave lit for 1 minute. The smear was blotted with absorbent paper after being rinsed with the decolorizing agent ethanol. For 2 minutes the slide was soaked in counter stain. The slide was wiped with absorbent paper after being rinsed with water. It was inspected a microscope at (100X magnification).

3.5 BIOCHEMICAL CHARACTERIZATION

Bergey's Manual of Systemic Bacteriology was used to identify the isolated strains using conventional biochemical testing.

3.5.1 CATALASE TEST

Catalase enzyme is found in most bacteria. It catalyzes the breakdown of hydrogen peroxide with the release of free oxygen. The catalase enzyme produced by bacteria that respire using oxygen shows a positive result for the catalase test bubbling appears as a result of catalase enzyme production followed by the breakdown of hydrogen peroxide with release of elemental oxygen. The isolated strain was inoculated on a nutrient agar slant and incubated for 24 hours at 37ºC. After the incubation period poured 1ml of 3% hydrogen peroxide down the slant and looked for gas bubbles. The appearance of gas bubbles as a result of hydrogen peroxide being broken down into oxygen and water was considered a positive catalase test result.

3.5.2 STARCH HYDROLYSIS TEST

The test for Starch hydrolysis is done to identify bacteria that can hydrolyse starch using the enzymes alpha-amylase and oligo-1,6-glucosidase. A clear zone around the line of growth after the addition of iodine solution indicates a positive result. A negative result indicates a blue, purple or black coloration of the medium. The isolate was created by inverting a single streak on a starch agar plate for 72-96 hours at 37º C. For 30 seconds the plates were soaked with Gram’s iodine solution. The plates were checked for starch hydrolysis surrounding each isolate’s growth line (i, e.,) for a change in the medium’s color. Positive starch hydrolysis is characterized by a clear zone enclosing the microbial colonies.

3.5.3 GELATIN LIQUEFACTION TEST

Liquefaction of gelatin being the most common proteolytic property is used as an index to determine the proteolytic activity of the organism. Gelatin is liquefied by the microbes capable of producing proteolytic exoenzyme known as gelatinase acts on gelatin present in the medium. This can be detected by observing liquefaction at a very low temperature. Positive results indicate liquefaction. The isolate was inoculated for 4-7 days at 37ºC on gelatin agar deep tubes. Following incubation, the tubes were placed in the refrigerator for 15 minutes at 4ºC, to check gelatin liquefaction.

3.5.4 INDOLE TEST

Tryptophan is oxidized with tryptophanase resulting in the formation of indole, pyruvic acid and ammonia. The development of cherry red later indicates positive test. No cherry red layer formation confirmation for the negative result of this test. The peptone broth was made sterilized in an autoclave for 15 minutes at 121 pounds. These tubes were infected with the isolated strain and incubated for 48 hours at 37ºC. The broth that had not been inoculated was maintained as a control sample. After 48 hours incubation period,1ml of Kovac’s reagent was applied to the test sample and control tube. At intervals of roughly 10-15 minutes the tubes were gently shook. To allow reagent to rise to the top, the tubes were allowed to stand. The appearance of the cherry red color showed a positive reaction.

3.5.5 METHYL RED TEST

Bacteria belonging to *Enterobacteriaceae* ferment glucose to produce acetic acid, lactic acid, succinic acid, formic acid, ethanol, carbon dioxide and hydrogen. Due to the abundant acid production, pH of the medium drops from 10 to 4.5 which can be detected using the methyl red indicator. The positive results indicate the colour changes from yellow into red colour. The negative result indicates no colour changes. The MR-VP broth was produced and sterilized for 15 minutes at 121 pounds. The tubes were infected with the isolates strain and incubated for 24 hours at 37ºC. The control sample was retained uninoculated broth. After incubation, each tube received 5 drops of methyl red indicator. The formation of the red colour suggested a positive reaction.

3.5.6 VOGES-PROSKAUER TEST

The Voges – Proskauer test is used to determine if an organism produces acetyl methyl carbinol from glucose fermentation. Positive result is indicated by the development of pink color in 2-5 minutes. Negative results indicate no color development. For 48 hours the isolated culture was inoculated in an MR-VP medium at 37ºC. After 48 hours of the incubation period, added 1ml of 40% sodium hydroxide and 35 percent alpha naphthol. The development of pink color 2-5 minutes, which turned scarlet in 30 minutes showed a good reaction.

3.5.7 CITRATE UTILIZATION TEST

The citrate test is used to determine the ability of the bacteria to use sodium citrate as the only source of carbon and inorganic ammonium hydrogen phosphate as a source of nitrogen. Positive results show the growth with color change from green to intense blue along the slant. Negative results indicate no color change, slant remains green in color. The isolated organism was inoculated on agar slopes and incubated at 37ºC for 96 hours. The only carbon source available to the bacteria in this medium is citrate. Only when the bacterium develops by consuming citrate and the media turns a vivid blue colour as a result of the increased media P^H was a favourable outcome noticed.

3.6 EVALUATION OF BACTERIAL CELL SURFACE HYDROPHOBICITY

In biodegradation investigations, hydrophobicity is an important surface feature because the relationship between surface and microbe hydrophobicity determines the extent of colonization on the polymer substrate (Restrepo florez *et al.*, 2014). The bacterial surface must also be by hydrophobic in order to form a biofilm on polyethylene (Haded *et al.*, 2005). The bacterial adhesion to hydrocarbon (BATH) experiment was used to measure the hydrophobicity of the bacterial cell surface (Rosenberg *et al.*, 1980). Bacteria were cultivated in nutrient broth medium until they reached mild-logarithmic phase, then centrifuged and washed with phosphate urea magnesium sulphate (PUM) buffer, which contained 17Gk$_2$HPO$_{4,}$7.26g KH2PO4, 1.8g urea and 0.2g MGSO$_{4.}$7H$_2$O per litre. The bacterial cells were resuspended in PUM buffer at an optical density of 1.0-1.2 at 400 nm after being washed. Aliquots of this suspension (1.2ml each) were transferred to a set of test tubes, to which increasing quantities (0-0.2) of toluene were added. To aid phase separation the test tubes were agitated for 10 minutes and then allowed to rest for 2 minutes. The aqueous suspension's OD 400nm was determined. The blank was made up of cell-free buffer. The studies were done in triplicates with the findings given as the mean ± SE of the triplicates.

3.7 SCREENING FOR POLYETHYLENE DEGRADATION BY BACTERIAL ISOLATES

3.7.1 *IN VITRO* BIODEGRADATION ASSAY

The isolated bacterial strains were individually evaluated for HDPE degrading efficiency using an *in vitro* biodegradation assay. The bacteria were isolated and inoculated for 20 days in synthetic medium (SM) containing polyethylene as the sole carbon source. The growth of the bacterial isolates was observed during the incubation period. After the biodegradation assay's incubation period the dry weight of the leftover HDPE film was determined and bacterial biomass in biofilm colonizing the polyethylene surface was evaluated by measuring the protein concentration. Scanning Electron Microscopy (SEM) was used to examine morphological changes, while Fourier Transform Infrared Spectroscopy (FTIR) was used to examine chemical property changes.

The following paragraphs describes the experimental approaches done to test the polyethylene broken down by the bacterial isolates.

GROWTH CURVE OF THE BACTERIAL BIOMASS

Each isolated bacteria was cultured in an HDPE film containing SM and compared to one another in terms of growth. Bacterial isolates were cultured in nutrient broth overnight until they reached log phase (absorbance of 0.6 at 600nm). 10 percent of this log phase culture was inoculated in 250ml of Erlenmeyer flask containing 50 ml synthetic media and polyethylene sheets and cultured at 30ºC for 20 days on a shaker incubator (150rpm). The test was carried out in three different ways. The control was kept under the same circumstances as the non-inoculated minimum broth supplemented with untreated HDPE films. 20 days of incubation cell growth was measured at intervals in each of the triplicates. Periodic sampling (day 0,3,7,9,14,17 and 19) from SM was used to determine cell density using optical density at 600 nm.

3.7.2 DETERMINATION OF DRY WEIGHT OF RESIDUAL HDPE

After 20 days of incubation in SM with the bacterial isolates, HDPE films were removed and the weight loss was measured. The bacterial population coating the polyethylene surface was removed by treating it with a 2%(v/v) aqueous sodium dodecyl sulfate (SDS) solution for 4 hours and then washing it with distilled water to permit accurate measurements of residual HDPE dry weight (Haded *et al.,* 2005). The HDPE films samples were then weighed after being dried overnight at 60ºC. A digital scale that could accurately measure up to 0.01 was used to record the drop in weight of HDPE films.

3.7.3 QUANTIFICATION OF BACTERIAL BIOMASS (Lowry *et al.,* 1951)

The method developed by (Gilan *et al.,* 2004) was used to calculate the quantitative amount of bacterial biomass on treated HDPE. Standard approaches such as direct cell counting or plating were unable to assess the population density because the bacterial biofilm was tightly adherent to the polyethylene surface. As a result, protein concentration was used to determine the population density of the biofilm on the polyethylene surface. Pieces of HDPE film colonized with SM were rinsed quickly in water, then agitated overnight. The supernatant was preserved and the pellet went through the same process once more. The two supernatants were combined and the protein concentration in each supernatant was determined according to Lowry *et al.* (1951) with the results combined. The studies were done in triplicates with the findings given as the mean ± SE of the triplicates.

3.7.5 SCANNING ELECTRON MICROSCOPY (McMullan, 1948)

Scanning electron microscopy was used to investigate the changes in the surface morphology of the HDPE films before and after exposure to the bacterial isolates. After 20 days of incubation the LDPE films were removed and fixed for 3 hours with 5% glutaraldehyde (0.1M), 0.1M phosphate buffer saline (pH 7.4). After completely rinsing the HDPE films in 0.1M PBS they were post fixed using 2 percent glutaraldehyde, 3 percent formaldehyde solution (v/v) in 0.1M PBS. The films were then carefully cleaned in 0.1 M PBS before being dehydrated in 70, 90 and 100 percent ethanol. Before imaging the films were dried overnight and sputter coated with gold. The biofilm layer generated on the surface of the HDPE films treated with bacteria was washed off with 2 percent SDS before SEM observation to analyze the surface changes (Kyaw *et al.*, 2012).

3.7.6 FOURIER TRANSFORM INFRARED SPECTROSCOPY (Michelson, 1907)

The polyethylene films were investigated by FTIR to detect the development of new functional groups or changes in the amount of existing functional groups in the bacterial isolate treated polyethylene. FTIR analysis was used to determine the changes in polymer bonds caused by biodegradation.

Results and Discussion

4.0 RESULTS AND DISCUSSION

The present study aimed mainly on the isolation of bacteria from soil samples collected at the HDPE plastic dumped near, petrochemical oil contaminated sites in order to identify the potent soil bacteria with unique characteristics and their role in the biodegradation of HDPE plastics and the results obtained are presented in this chapter.

4.1 ISOLATION OF BACTERIA

Soil samples from petrochemical oil contaminated area Kovaipudhur and Narasimmanaickenpalayam were collected and designated as KP1 and NP1. Bacteria which are able to utilize HDPE as the carbon source were isolated and identified from the soil samples by enrichment technique. The synthetic medium (SM) was used for the untreated HDPE plastic as the sole carbon source for the enrichment culture procedure.

4.2 IDENTIFICATION AND CHARACTERIZATION OF BACTERIAL ISOLATES

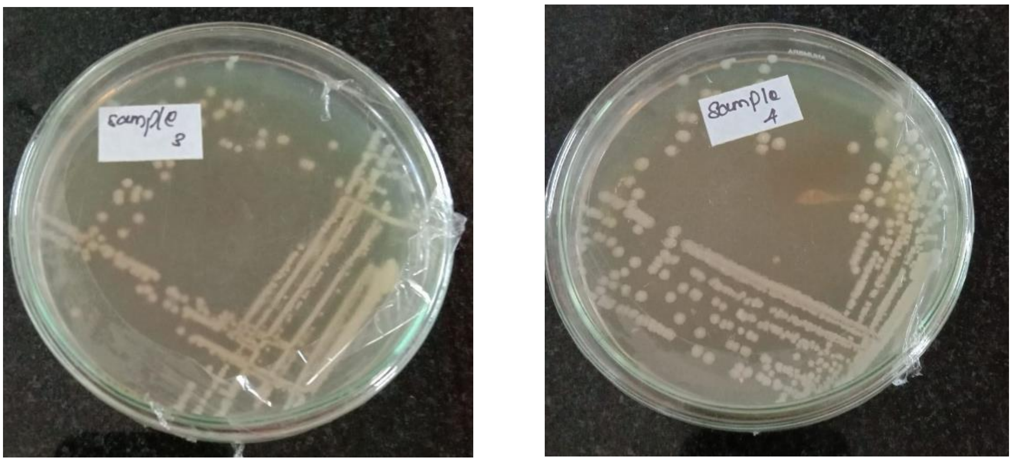
The isolated bacterial strains were subjected to a set of morphological and biochemical analysis for the purpose of identification. Microscopic examination such as gram's staining and biochemical characterization tests such as catalase test, gelatin liquefaction test, starch hydrolysis test, citrate test, methyl red test, Voges prokasuer test and indole test were used for (perform as the preliminary tests for the) identification of the bacterial isolates. The results of the microscopic observation and biochemical tests were analysed according to Bergey's Manual of systematic Bacteriology. The morphological examination and biochemical characterization test revealed that the two bacterial isolates such as, KP1 and NP1 probably belong to the *Bacillus spp* according to the Bergey's Manual Systemic Bacteriology.

4.2.1 MORPHOLOGICAL CHARACTERIZATION

The isolated bacterial strains were subjected to morphological characterization for the purpose of identification. Preliminary identification of these bacterial isolates was carried out by microscopic and biochemical characterization. The results of microscopic observation and biochemical tests were analyzed according to Bergey's Manual of systematic Bacteriology. Plate 2 and Table 1 represents the results of morphological characterization of bacterial isolates of KP1 and NP1.

PLATE 2

MORPHOLOGICAL CHARACTERIZATION OF KP1 AND NP1



KP1 **NP1**

TABLE 1

MORPHOLOGICAL CHARACTERIZATION OF KP1 AND NP1

Morphology	KP1	NP1
Colony morphology (Nutrient agar)	Opaque, cream coloured and also slightly convex with irregular margins	Concave, smooth and milk white, whitish creamy

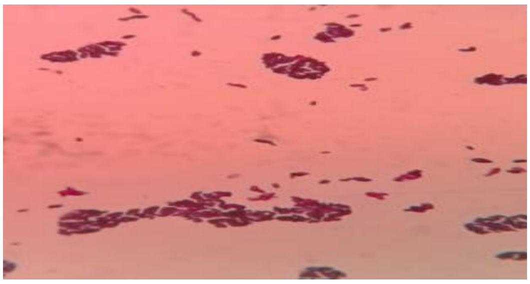
From the above Plate 2 and Table 1 represents the colony morphology of bacterial isolates of KP1 and NP1. The isolated bacterial strain KP1 shows Opaque, cream coloured and also slightly convex with irregular margins. The bacterial strain NP1 shows concave, smooth, and milk-white, whitish creamy. The results of morphological characterization of the isolated bacterial strains revealed that the bacterial isolates KP1 and NP1 might belong to *Bacillus spp.,*

4.2.1.1 GRAM'S STAINING

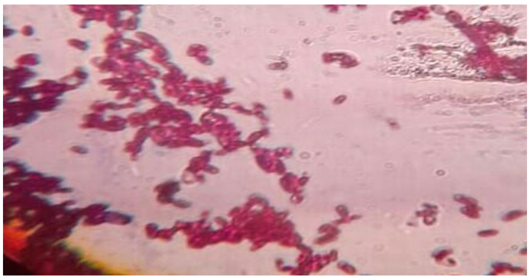
Gram's iodine is used in Gram's staining procedure to differentiate Gram-positive and Gram-negative bacteria. Gram's iodine acts as a mordant that causes the crystal violet to penetrate and adhere to the organism. Plate 3 and Table 2 represent the results for two bacterial isolates KP1 and NP1

PLATE 3

MICROSCOPIC EXAMINATION OF BACTERIAL ISOLATES



KP1



NP1

TABLE 2: MICROSCOPIC EXAMINATION OF BACTERIAL ISOLATES

DESCRIPTION	KP1	NP1
Shape	Rod	Rod
Gram’s staining	Positive	Positive

From the above Plate 3 and Table 2, it is clear that the isolated two bacterial strains KP1 and NP1 shows purple colour rod-shaped gram-positive bacteria under the microscopic examination.

Wang *et al.* *(*2015) reported that the Colonies are round and pale yellow. The bacteria were Gram-positive, rod-shaped and terminal circular, with single or short chain arrangement. Williams *et al.* (2018) reported the isolated bacteria SNS5 was carried out to morphological, biochemical and genetic criteria for identification and was gram-positive bacteria.

4.2.2 BIOCHEMICAL CHARACTERIZATION

The isolated bacterial strains were identified by conventional biochemical tests in accordance with Bergey’s Manual of Systemic Bacteriology.

4.2.2.1 CHARACTERIZATION OF BACTERIAL ISOLATES BY CATALASE ASSAY

Catalase enzyme is found in most of the bacteria. It catalyzes the breakdown of hydrogen peroxide with the release of free oxygen. The catalase enzyme produced by bacteria that respire using oxygen shows the positive result for catalase test, bubbling appears as a result of catalase enzyme followed by the breakdown of hydrogen peroxide with release of elemental oxygen. plate represents the results for catalase test of isolated two bacterial strains KP1 and NP1.

PLATE 4

CHARACTERIZATION OF BACTERIAL ISOLATE BY CATALASE ASSAY

KP1 **NP1**

From the above Plate 4, it is clear that the isolated two bacterial strains (KP1 and NP1) shows a bubble, because catalase enzyme produced by bacteria that respire using oxygen indicates the positive result for catalase test.

Reiner. (2010) reported *Micrococcaceae* shows the positive result for catalase test and catalase negative *Streptococcaceae.* Bacterial isolates KP1 and NP1 shows positive for catalase. Hence, isolated bacterial strains KP1 and NP1 may belongs to *Bacillus spp.,* because it shows positive results for catalase test.

4.2.2.2 CHARACTERIZATION OF BACTERIAL ISOLATES BY GELATIN LIQUEFACTION ASSAY

Liquefaction of gelatin being the most common proteolytic property is used as an index to determine the proteolytic activity of the organism. Gelatin liquefied by the microbes capable of producing proteolytic exoenzyme known as gelatinase, which acts on gelatin present in the medium. This can be detected by observing liquefaction at a very low temperature. Positive results indicate the liquefaction. Plate 5 represents the result of two isolated bacterial strains KP1 and NP1.

PLATE 5

CHARACTERIZATION OF BACTERIAL ISOLATES BY GELATIN LIQUEFACTION

KP1

NP1

The above Plate 5, explains that the isolated two bacterial strains KP1 and NP1 shows a liquefaction of gelatin medium and therefore indicates the positive result for gelatin liquefaction test.

Edison *et al.* (2012) reported the ability of microorganisms to create gelatinases is detected by the gelatin hydrolysis test. *Serratia, Pseudomonas, Flavobacterium,* and *Clostridium* are all identified using this test. It differentiate pathogenic *Staphylococcus* gelatinase negative and gelatinase positive bacterium *Staphylococcus epidermidis, Bacillus subtills, B.anthracis, , B. subtilis, Clostridium perfringens* and *Clostridium*. The isolated bacterial strains KP1 and NP1 shows positive for gelatin liquefaction test and therefore may belongs to bacillus spp.

4.2.2.3 CHARACTERIZATION OF BACTERIAL ISOLATES BY INDOLE ASSAY

Tryptophan is oxidized with tryptophanase resulting in the formation of indole, pyruvic acid and ammonia. Positive results indicates the cherry red layer formation and negative test indicates the no cherry red layer formation. Plate 6, represents the result of two isolated bacterial strains KP1 and NP1.

PLATE 6

CHARACTERIZATION OF BACTERIAL ISOLATES BY INDOLE

KP1 **NP1**

The above Plate 6, it is clear that the isolated two bacterial strains KP1 and NP1 shows no cherry red layer. It indicates the negative result for indole test.

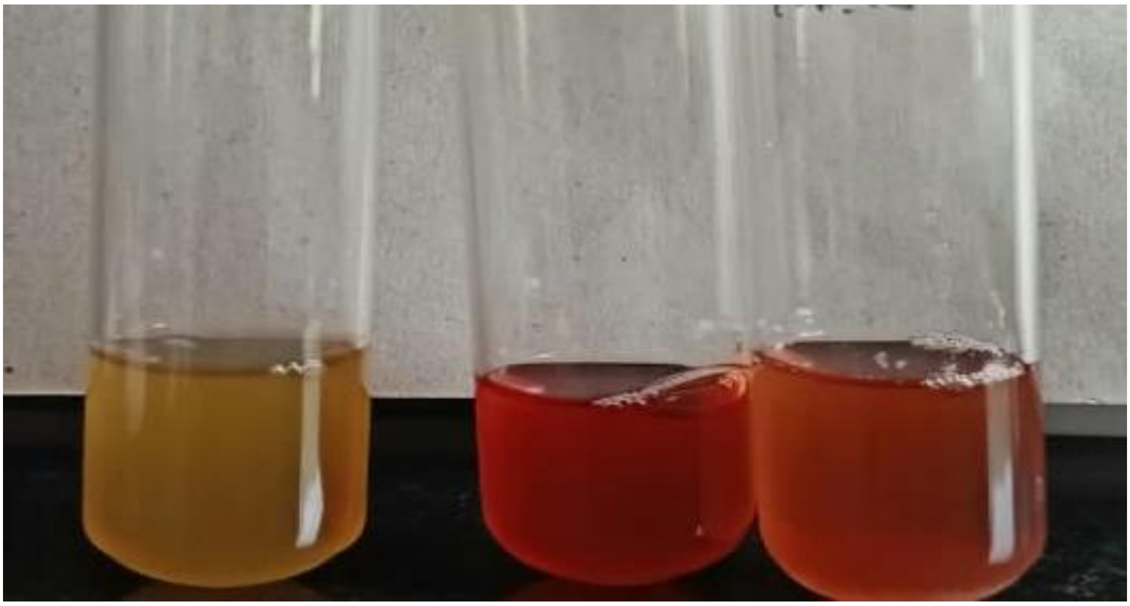
Macwilliams (2009) reported the presence of indole test is indicated by the formation of a brown-red to purple-red colour (benzaldehyde reagents) or a blue colour (cinnamaldehyde reagent) within 20 seconds. A negative test is either colourless or slightly yellow in appearance for this test. The isolated bacterial strains KP1 and NP1 shows negative result for indole test

4.2.2.4 CHARACTERIZATION OF BACTERIAL ISOLATES BY METHYL RED ASSAY

Bacteria belonging to *Enterobacteriaceae* ferment glucose to produce acetic acid, lactic acid, succinic acid, formic acid, ethanol, carbon dioxide and hydrogen. Due to the abundant acid production, pH of the medium drops from 10 to 4.5 which can be detected using methyl red indicator. The positive results indicate the colour changes from yellow into red colour and negative results indicates no colour development. Plate 7 represents the isolated of two bacterial strains KP1 and NP1 results for methyl red test.

PLATE 7

CHARACTERIZATION OF BACTERIAL ISOLATES BY METHYL RED



KP1 and NP1

The above Plate 7, explains that the isolated two bacterial strains (KP1 and NP1) shows colour changes from yellow into red colour. It indicates the positive result for methyl red test.

Tankeshwar *et al*. (2019) reported a positive test when the surface of the medium develops a stable red colour, indicating adequate acid production to drop the pH. Other organisms producing smaller amounts of acid from the test substrate may create an intermediate orange colour between yellow. The isolated bacterial strains shows positive for methyl red test, which is similar to the study done by Tankeswar *et al.* (2019)

4.2.2.5 CHARACTERIZATION OF BACTERIAL ISOLATES BY VOGES-PROSKAUER ASSAY

The Voges – Proskauer test is used to determine if an organism produces acetyl methyl carbinol from glucose fermentation. Positive result is indicated by the development of pink colour in 2-5 minutes. Negative results indicates the no colour development. Plate 8, isolated represents result f two bacterial strains shows the results of Voges-Proskauer test.

PLATE 8

CHARACTERIZATION OF BACTERIAL ISOLATES BY VOGES PROSKAUER ASSAY

KP1 **NP1**

The above plate 8, it is clear that the isolated two bacterial strains KP1 and NP1 shows no colour development or no development of pink colour, it indicates the negative result for Voges-Proskauer test.

4.2.2.6 CHARACTERIZATION OF BACTERIAL ISOLATES BY CITRATE UTILIZATION

The citrate test is used to determine the ability of the bacteria to use sodium citrate as the only source of carbon and inorganic ammonium hydrogen phosphate as a source of nitrogen. Positive results shows the growth with colour change from green to intense blue along the slant. Negative results indicates no colour change, slant remains green in colour. Plate 9, represents the results of citrate utilization test for the bacterial isolates KP1 and NP1

PLATE 9

CHARACTERIZATION OF BCATERIAL ISOLATES BY CITRATE UTILIZATION

KP1

NP1

The above Plate 9, indicated the results for the isolated two bacterial strains(KP1 and PN1) where shows, colour change from green to intense blue along the slant, it indicates the positive result for citrate utilization test.

Allaf *et al*.(2011) reported biochemical characteristic performed from *bacillus spp*, it was isolated from different sample the citrate utilization test is positive for *bacillus sp*.The isolated bacterial strains (KP1 and NP1) shows positivity for citrate utilization test, which is similar to the study done by Al-Alaf *et al.* (2011). Hence our bacterial isolates may belong to *Bacillus spp.*,

TABLE 3

BIOCHEMICAL CHARACTERIZATION- KP1 AND NP1

Biochemical test	**KP1**	**NP1**
Catalase test	Positive	Positive
Gelatin liquefaction test	Positive	Positive
Indole test	Negative	Negative
Methyl red test	Positive	Positive
Voges- Proskauer test	Negative	Negative
Citrate utilization test	Positive	Positive

4.3 BACTERIAL CELL SURFACE HYDROPHOBICITY

Bacterial cell surface hydrophobicity is an important factor that influences bacterial adhesion. In this study bacterial cell surface hydrophobicity was assessed by BATH assay using hydrocarbon such as toluene in various concentrations. Figures 3 and 4 shows the results of hydrophobicity of bacterial isolates.

FIGURE 3

HYDROPHOBICITY OF BACTERIAL ISOLATES BY BATH ASSAY

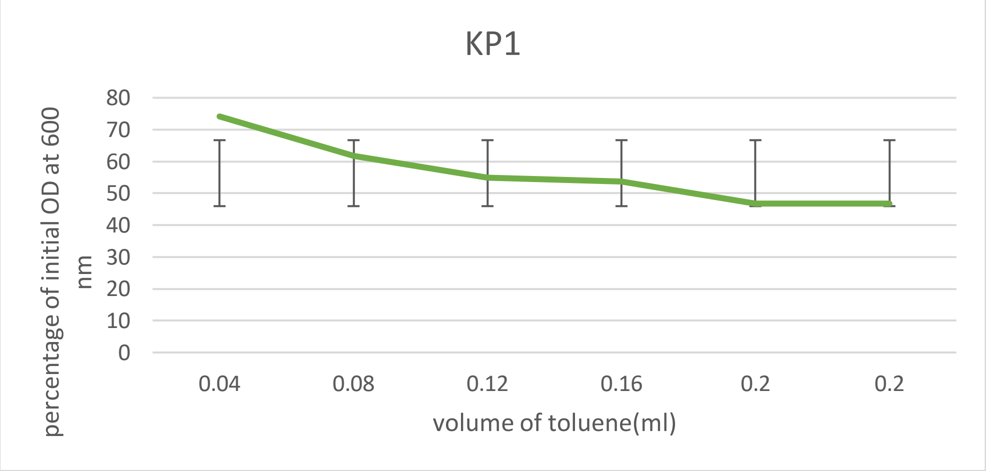
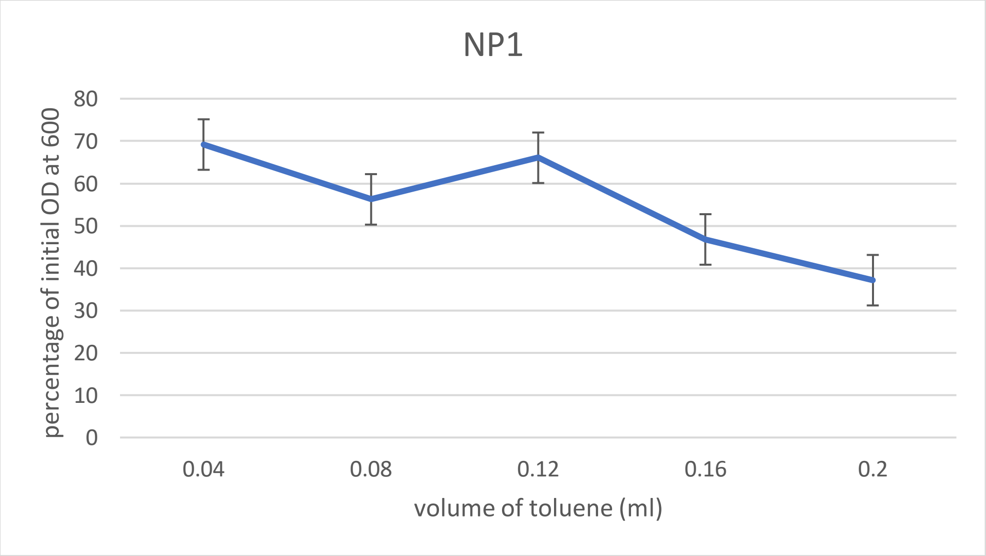


FIGURE 4: NP1



From Figure 3 and 4 it can be inferred that the turbidity of bacterial suspensions namely, KP1 and NP1 decreased from the initial level at all concentrations of toluene studied. KP1 bacterial isolate showed a maximum reduction in turbidity (74.13 %,61.65%,54.95%,54.70%,46.71%) respectively at 0.04 ml, 0.08 ml, 0.12 ml, 0.16 ml and 0.2 ml of toluene) when compared to NP1. 54.95 % decrease in the concentration of turbidity, KP1 shows more hydrophobic than NP1.

Popovici *et al.* (2010) bacterial adherence to hydrocarbons, or BATH, is a technique for assessing bacterial cells' hydrophobic surface properties. The affinity of a strain for water is determined by completely mixing a culture with a hydrocarbon mixture and then measuring the decrease in the culture phase's optical density. The strain's hydrophobicity is correlated with a decrease in optical density. The goal of this study is to use the BATH test to identify the hydrophobic surface properties of possible drinking water pathogens. Two *Vibrio cholerae* strains were discovered to be the most hydrophobic. Two *Escherichia coli* wild type strains were determined to be the least hydrophobic**.**

High cell surface hydrophobicity in all the test samples further confirmed the biofilm formation, as the hydrophobicity of any organism has a direct correlation with its potential to bind to non-polar hydrocarbons such as polyethylene (Sarker *et al.,* 2020).

4.4 SCREENING FOR HDPE DEGRADING BACTERIA

4.4.1 *In vitro* BIODEGRADATION ASSAY

Biodegradation assay is important for the screening for HDPE degrading bacteria. In this assay bacterial strains were inoculated with synthetic medium, it contains HDPE plastics bacterial strains use HDPE plastics as sole carbon source. During 20 days of incubation the synthetic medium contains HDPE plastics it becomes turbidity, it indicates the growth of isolated bacterial strains KP1 and NP1.

4.4.2 GROWTH CURVE OF BACTERIAL ISOLATES

Growth curve of bacterial isolates done for measurement of bacterial isolates in various phases. During 20 days of incubation of synthetic media the growth curve of bacterial isolates was measured at the days (3,7,9,14,17 and 19) at 600nm.

PLATE 10

SYNTHETIC MEDIA INOCULATED WITH HDPE FILMS

KP1 **NP1**

FIGURE 5

GROWTH CURVE OF BACTERIAL ISOLATES

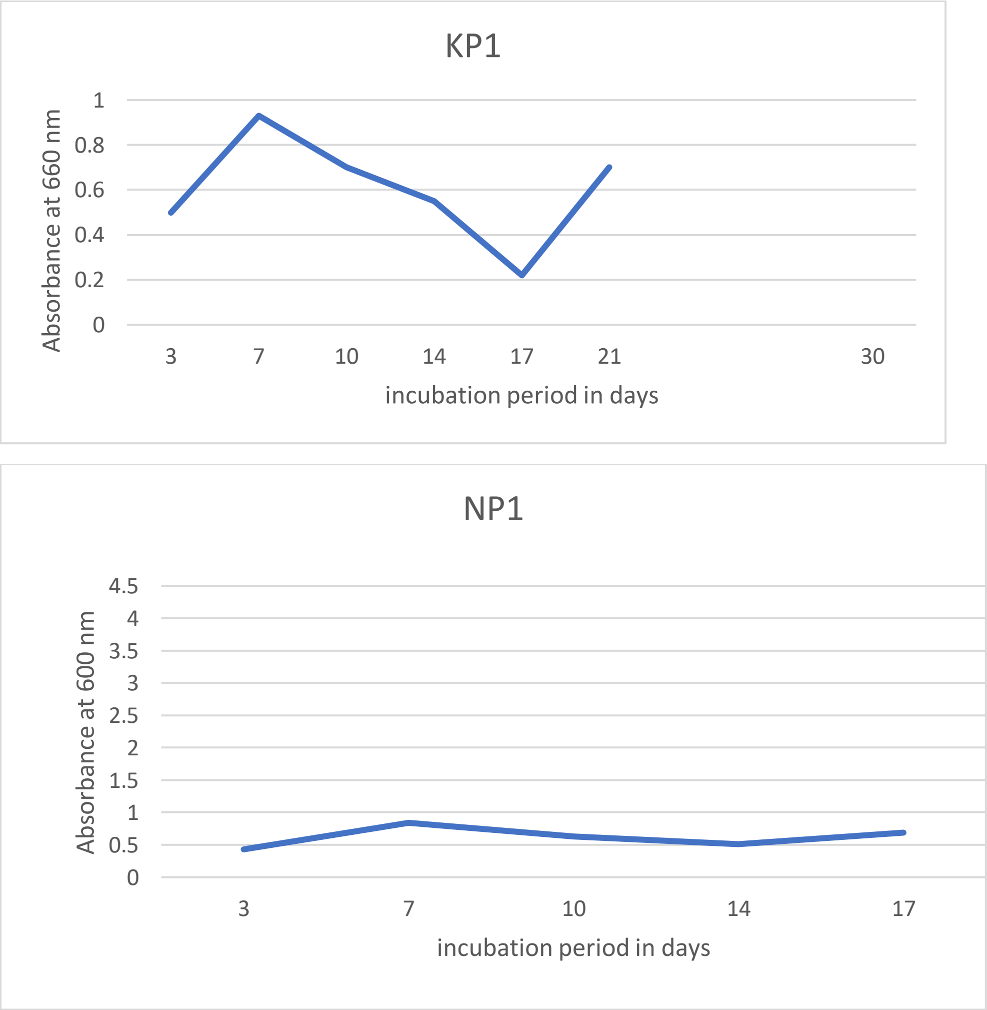
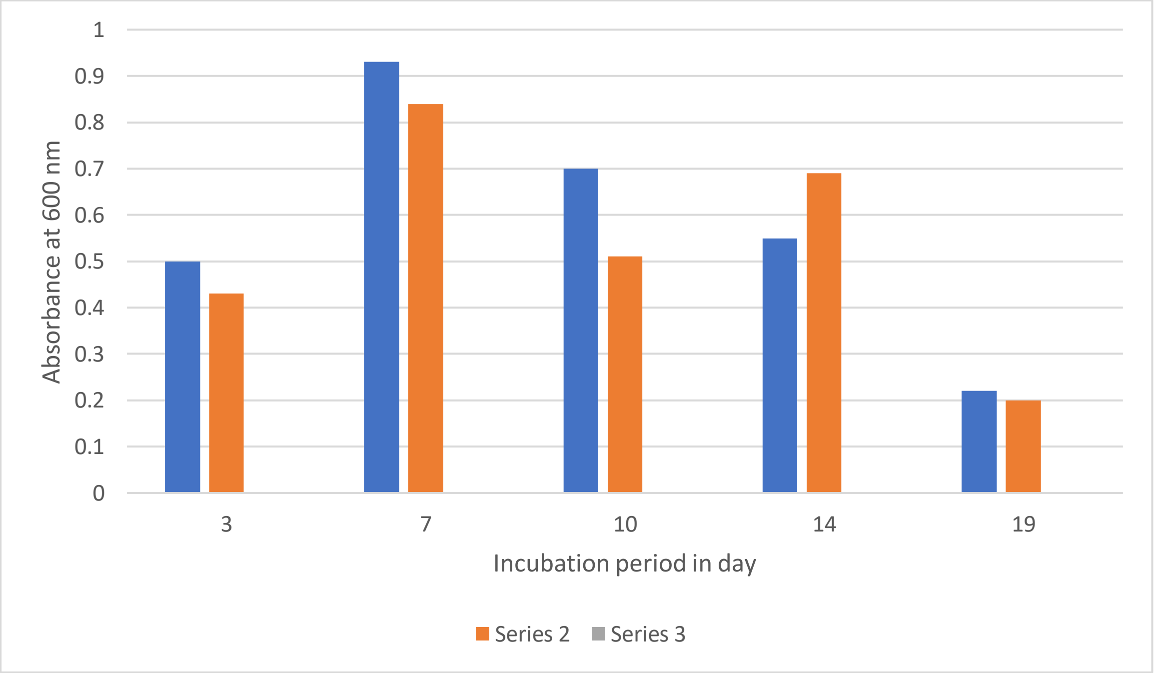


FIGURE 6

COMPARISION OF GROWTH CURVE OF THE BACTERIAL ISOLATES AT DIFFERENT TIME POINTS



During the sampling period, the growth rates of KP1 and NP1 were nearly identical. The organism used as HDPE as sole carbon source. During incubation period medium shows turbidity, it indicates the organisms growth.

(Zahari and Esa, 2020) reported the results showed that the growth curves of *C. tropicalis* on both LDPE and SBP were slightly increase from week 1 to 7 along the biodegradation study. For *B. subtilis* the growth was increase from week 1 to 5 and slowly decrease along the incubation period. The highest growth curve of B. subtilis can be observed in SBP at week 5 with 9.1 x 108 CFU/ml. While for *C. tropicalis*, the highest growth rate can be shown at week 4 in SBP with 9.6 x 108 CFU/ml. The formation of biofilms by both plastics within 7 days had reduced the hydrophobicity of both polymers and yet it improved the degradation rate. This can be observed after a week of incubation whereas the microbial growth of both strains were slightly increased. This indicates that the biodegradation were rapidly progressed which means it has make an easier movement for microbial attack to degrade the plastic. In addition, the presence of plastics as sole source of carbon may favor the growing of both strains, especially for petroleum-based microbes. In this study, there were no other nutrients supplied to the cultures such as glucose and the changes of microbial growth can be seen through the presence of LDPE and SBP.

4.4.3 DETERMINATION OF DRY WEIGHT OF RESIDUAL POLYETHYLENE

In the present study degradation of HDPE of the bacteial isolates was measured by weight loss of the HDPE film after 20 days of incubation. Figure 7 and Table 4 represents the reduction of HDPE after 20 days of incubation with the bacterial isolates.

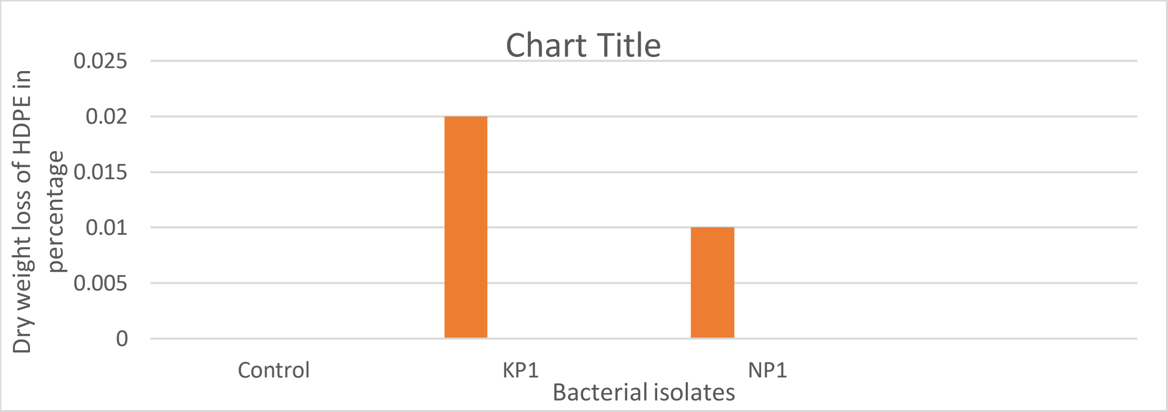
TABLE 4

REDUCTION OF HDPE AFTER 20 DAYS OF INCUBATION WITH THE BACTERIAL STRAINS

S.no	Bacterial isolates	Weight loss of HDPE
1	KP1	0.015±0.040
2	NP1	0.025±0.012

FIGURE 7

REDUCTION OF HDPE AFTER 20 DAYS OF INCUBATION WITH THE BACTERIAL STRAINS



The weight loss of HDPE films of bacterial isolates KP1 and NP1 was found to be in 0.025±0.040 and 0.015±0.012 ,respectivelyKP1, shows significant weight loss when compared to NP1.

For reduction of dry weight of HDPE mean weight changes were computed and used to compare the effectiveness biodegradation between fungi and bacteria as well as between the two sets of polyethylene sheets (30 and 40 microns). The highest degradation activity for bacteria was a mean of 35.72± 4.01% and 20.28± 2.30% attributed to *Bacillus cereus* strain A5, a (MG645264) and *Brevibacillus borstelensis* strain B2,2 (MG645267) respectively. Genus *Aspergillus*, *Bacillus*, and *Brevibacillus* were confirmed to be good candidates for Low-Density Poly Ethene bio-degradation (Muhonja, 2019).

This study clearly indicates that our bacterial isolates were also good candidate in biodegradation of HDPE.

4.5 QUANTIFICATION OF BACTERIAL BIOMASS

The protein estimation was done to know the protein content attached in the bacterial biofilms.The bacterial biomass tightly attached to the HDPE plastics. The formation of biofilm indicates the biodegradtion of HDPE. Plate 11, represents the biofilm protein content of baterial isolates.

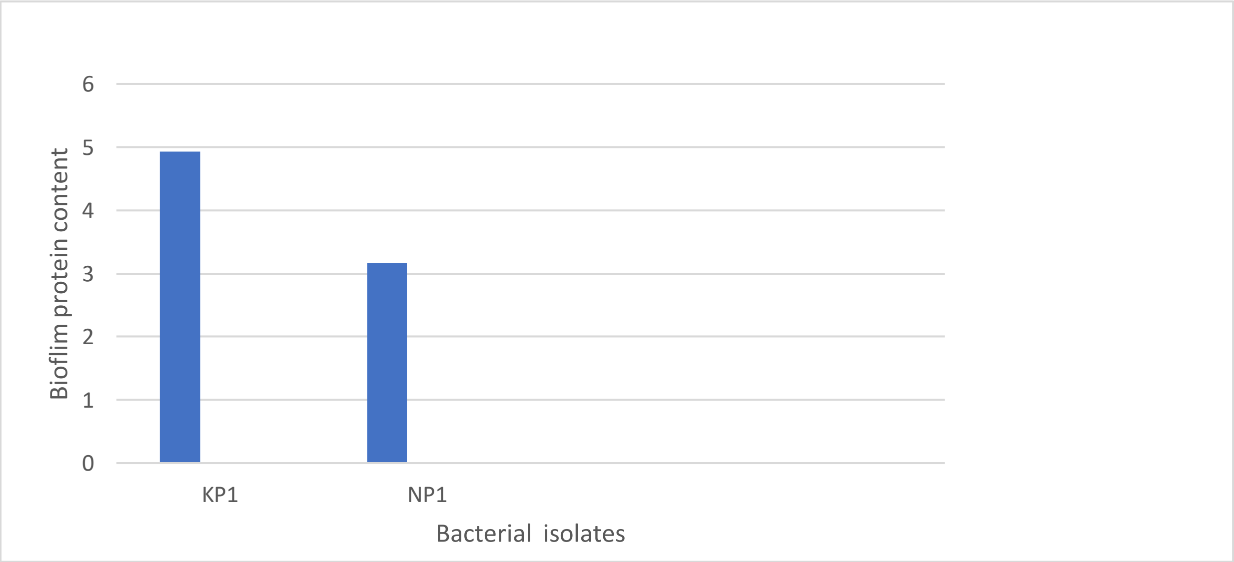
PLATE 11

BIOFILM WITH NaOH SOLUTION

KP1 **NP1**

FIGURE 8

PROTEIN CONTENT OF BIOFILM BACTERIAL ISOLATES



Biomass attached to HDPE films indicates that the protein content. The biofilm protein content observed as 4.93±1.234 µg mg^{-1} and 3.17±0.006µg mg^{-1} polyethylene (HDPE) for the bacterial isolates SN1 and PN1 respectively. When compared KP1 and NP1 the highest protein content (9.06 ±0,006) was observed KN1.

Santhoshkumar (2013) reported that the high hydrophobic nature of BSM-2 helped it for maximum biofilm formation on the LDPE and thus showed highest capability of colonization. It is evident that the bacterial isolate *Bacillus amyloliquefaciens* BSM-2 have significant colonization than BSM-1 after 60 days of incubation, resulting formation of dense biofilm. As large numbers of bacterial cell of BSM-2 were attached on the LDPE, the level of extracted protein concentration from the film was high (101µg/ml) and facilitated efficient biodegradation as compared to strain BSM-1 prototype.

4.6 FOURIER TRANSFORM INFRARED SPECTROSCOPY

FTIR analysis is used for the formation and disappearance of chemical functional groups. It indicates the changes in the HDPE film. Table 5. at different wavenumbers of the FTIR spectra, it delivers information on the bond and the related functional groups.

TABLE 5

CHARACTERIZATION PEAK IN FTIR

S.no	Wave number	Bond	Functional group
1	300-2850	-C-H Strech	Alkanes
2	2850-2695	H-C=O: C-H Strech	Aldehydes
3	710-1665	-C=O Strech	Ketones, aldehydes
4	1470-1450	-C-H Bend	Alkanes
5	1320-1000	-C-O Strech	Alcohols, carboxylic acid, esters, ethers.
6	1000-650	=C-H Bend	Alkenes
7	3600-3200	O-H Strech, H bonded	Alcohol

The peaks obtained in the region of 2916 cm^{-1}, 2846 cm^{-1}, 1465 cm^{-1} and 725 cm^{-1} were assigned for CH_2 asymmetrical stretching, CH_2 symmetrical stretching, bending deformation and rocking deformation respectively in the results of FTIR analysis.

4.6.1 FTIR ANALYSIS OF UNTREATED HDPE WITH BACTERIAL ISOLATES

The structural changes in HDPE film were investigated using FTIR spectroscopy in this work. The plastic film is placed on top of synthetic media that has been infected with a variety of microbial species KP1 and NP1. The FTIR spectra of untreated HDPE, treated HDPE such as KP1, and treated HDPE such as NP1 are shown in Figures 8, 9 and 10.

The FTIR spectra of untreated HDPE revealed a multitude of peaks, indicating the HDPE complexity. The spectrum of untreated HDPE revealed distinctive absorption bands at 2916.37 cm^{-1}, 2846.93 cm^{-1}, and 1018.41 cm^{-1}, which correspond to CH2 asymmetrical stretching, CH2 asymmetrical stretching, and bending deformation, respectively.

FIGURE 9

FTIR SPECTRA OF UNTREATED HDPE

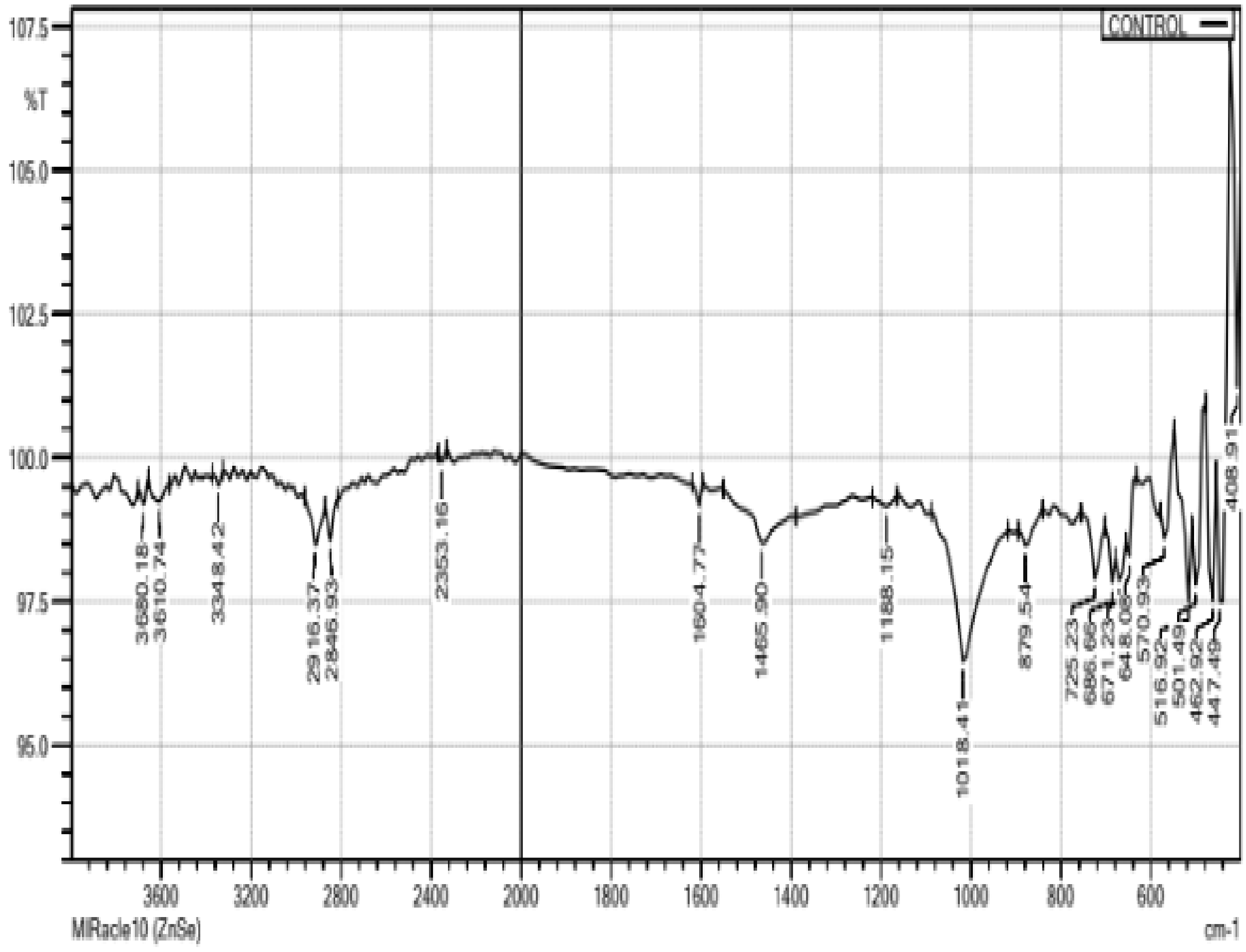


FIGURE 10

FTIR SPECTRA OF KP1 TREATED HDPE

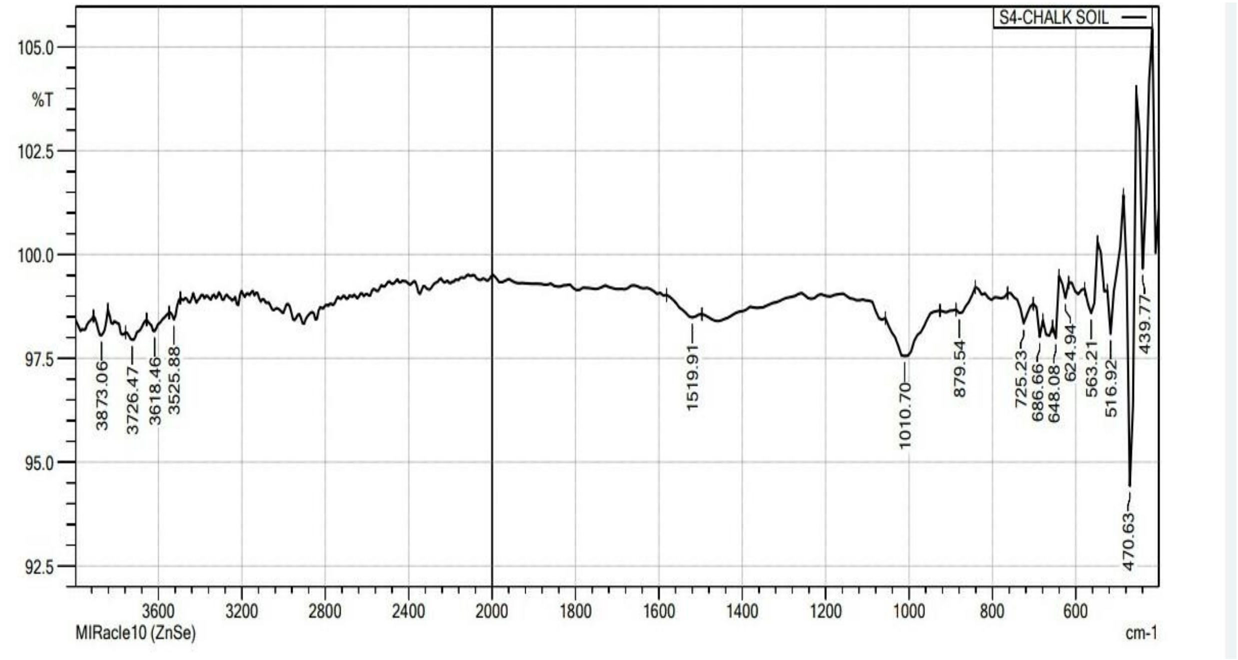
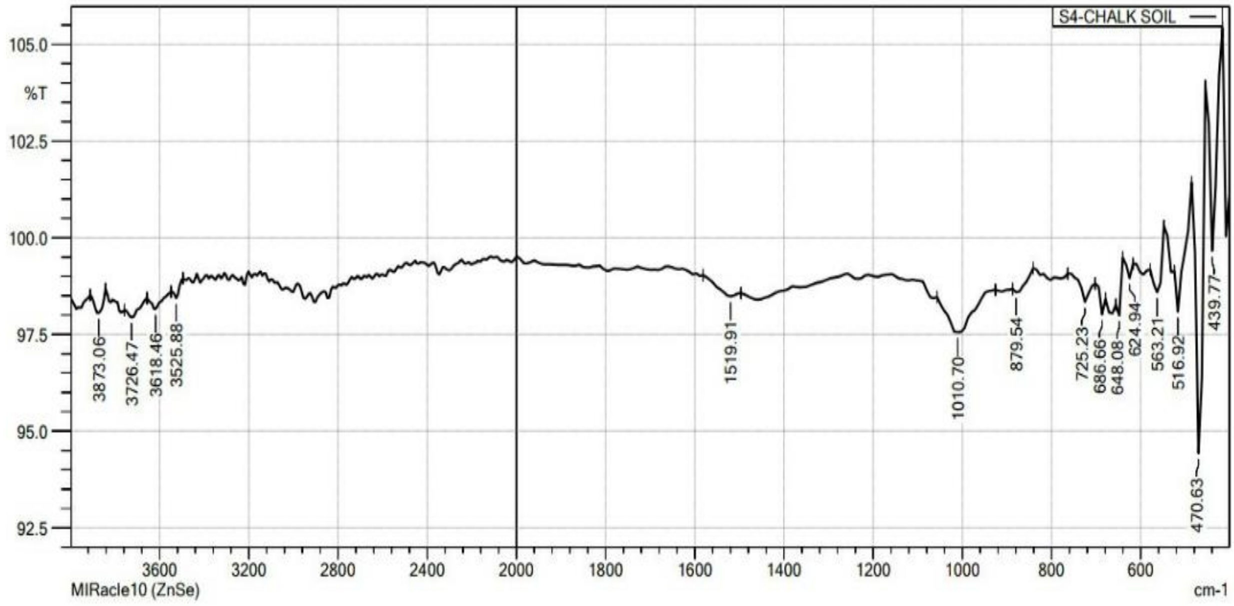


FIGURE 11

FTIR SPECTRA OF NP1 TREATED HDPE



The FTIR spectra of untreated HDPE displayed a number of peaks reflecting the complex nature of HDPE. The spectrum of untreated HDPE showed characteristics absorption bands at 2916.37 cm^{-1} corresponds to CH_2 asymmetrical stretching, 2846.93 cm^{-1} corresponding to CH_2 asymmetrical stretching and 1018.41 cm^{-1} corresponding to bending deformation

The creation of absorption peaks at 3873.06 cm-1, 725.23 cm^{-1}, and 686.66cm^{-1} correlates to C=o bends in the FTIR spectra of KP1 treated HDPE. The natural peaks at 2908.65 cm^{-1} and 2846.93 cm^{-1} did not show any changes. In the treated HDPE plastics, the band that was observed for untreated HDPE at 879.54 cm^{-1} and 516.92cm^{-1} decreased to 725.23cm^{-1} and 532.9323cm^{-1} respectively and peaks seen at 3610.74 cm^{-1}, 3348.42 cm^{-1}, 2916.37 cm^{-1}, 2553.16 cm^{-1}, 1604.77 cm^{-1},1465.90 cm^{-1}, and 1188.15 cm^{-1}disappeared. Peak at 1018.41 cm^{-1}, there was a deformation of peak.

Peaks at 3873.06 cm^{-1} and 3525.88 cm^{-1} in the carbonyl region were seen in the FTIR spectra of HDPE treated with NP1. The bands that were 671 cm^{-1} and 570 cm^{-1} for untreated HDPE have been reduced to 624.94cm^{-1} and 563.21 cm^{-1}, respectively. Peaks at 3873.06cm^{-1}, 3726.47 cm^{-1}, 3618.46 cm^{-1}, 3218.46 cm^{-1}, 1519.91 cm^{-1}, 1010.70 cm^{-1} ,and 879.54 cm^{-1}, also vanished. At 1018.41 cm^{-1}, a peak deformation was detected. Peaks of 501.49 cm^{-1} and 462.92 cm^{-1} for untreated HDPE were enhanced to 563.21 cm^{-1} and 516.92 cm^{-1}, respectively.

The treated HDPE film with bacterial strains showed negligible modifications in already existing peaks of untreated HDPE but gave peaks with strong intensity in the region of 1018.41 cm^{-1}, which corresponds to -C-O stretching, suggesting that these modifications could be due to bacterial isolates' action. The formation of absorption peaks in the spectra at 3873.06 cm^{-1} and 3618.46 cm^{-1} suggests that the bacterial isolates are biodegrading the HDPE.

Muhonja *et al.* (2018) reported that the emergence of new peaks between 1700 and 1650 is indicated by analysis of the polyethylene spectral data. New peaks can also be observed between 1000 and 1100 meters. The new peaks around 1700 and 1650 indicate the formation of aldehydes and ketones, respectively are polyethylene biodegradation intermediate products. The area with a higher peak. The correlation between absorbance and new peaks in the 1000-1200 cm^{-1} region of the FTIR spectrum and Alcohols, both primary and secondary. The major bands of the LDPE sheets under investigation are made up of a band and a band. As an asymmetric stretching, a band approximately 2900 cm^{-1} can be assigned to CH2.

(Rajandas *et al.*, 2012) explans thatMicroorganisms have been found to have an adaptive ability to break down oxidised polymers efficiently. In this study, Fourier Transform Infrared coupled Attenuated Total Reflectance (FTIR-ATR) spectroscopy was used to establish a fast and sensitive analytical technique for measuring low-density polyethylene (LDPE) biodegradation by microorganisms. To show the efficacy of this technology, two bacterial strains, *Microbacterium paraoxydans* and *Pseudomonas aeruginosa*, were used to biodegrade LDPE. These bacterial cultures were acclimatised to nitric acid prepared LDPE for two months prior to measurement. For further two months, the acclimatised strains were put through an *in vitro* biodegradation experiment with prepared LDPE as the only carbon source. Following that, biodegradation was measured using FTIR-ATR spectroscopy with different amounts of LDPE standards for comparison.

4.7 SCANNING ELECTRON MICROSCOPIC ANALYSIS OF HDPE TREATED WITH BACTERIAL ISOLATES.

SEM analysis shows the surface changes in the HDPE films. Figure 11 depicts the SEM images of the untreated HDPE (control). The figure illustrated a smooth morphology. The images KP1 and NP1 SEM images shows alternation in the surface morphology of HDPE that treated with bacterial isolates. The results are depicted in Figure 12 and 13.

FIGURE 12

SCANNING ELECTRON MICROGRAPHS OF UNTREATED HDPE FILMS

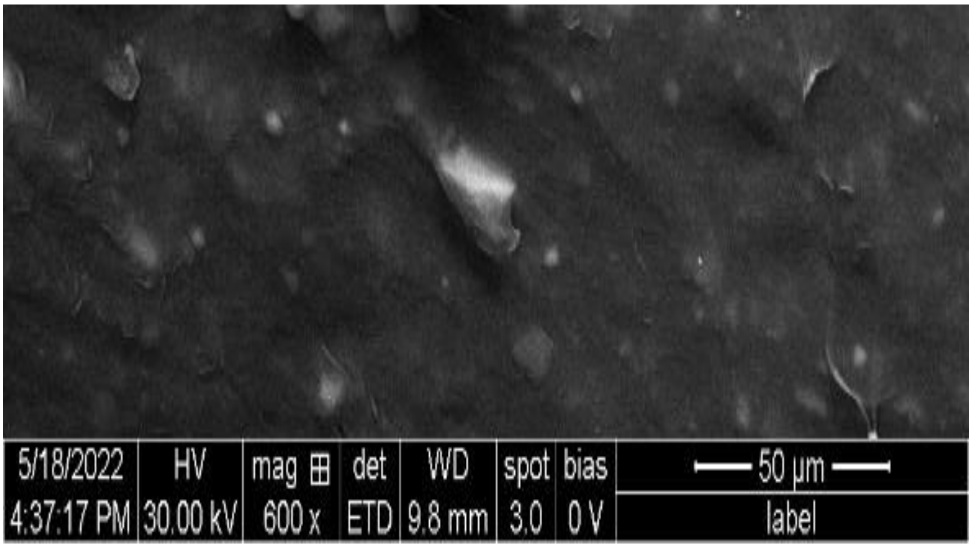


FIGURE 13

SCANNING ELECTRON MICROGRAPHS OF HDPE FILM TREATED WITH KP1

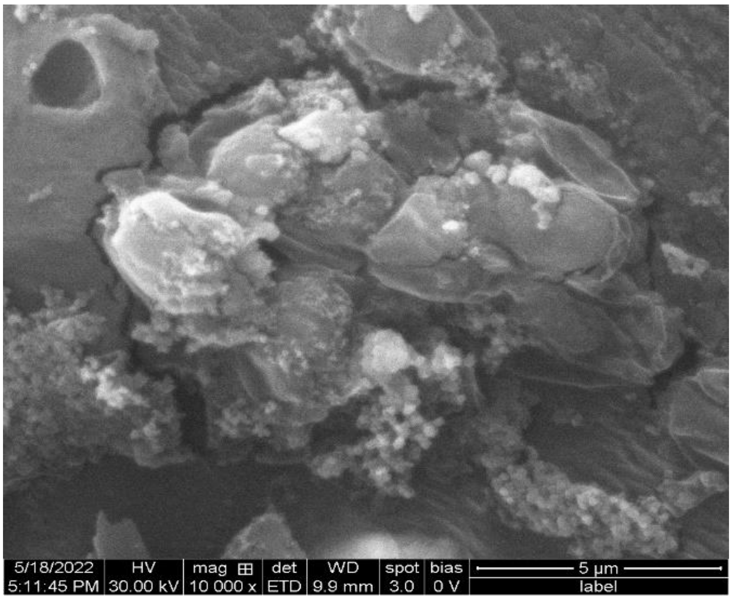
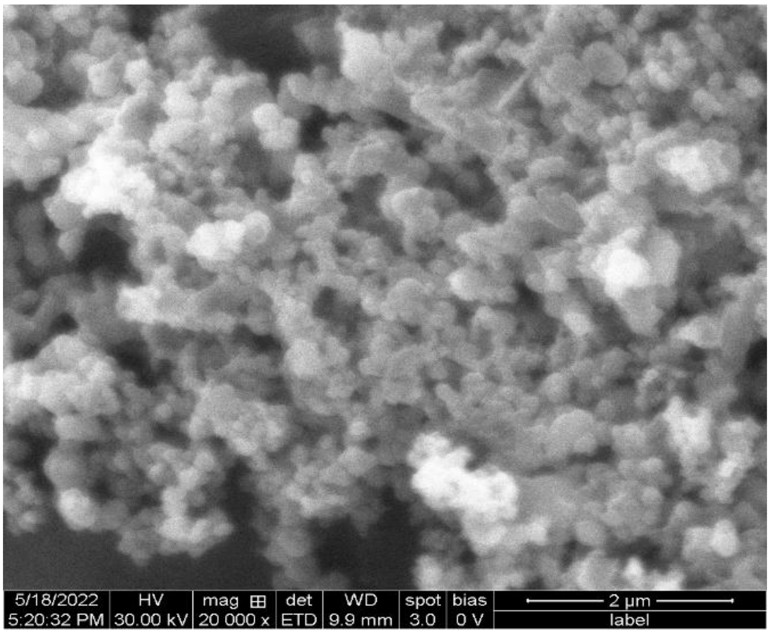


FIGURE 14

SCANNING ELECTRON MICROGRAPHS OF HDPE FILMS TREATED WITH NP1



Scanning Electron Micrographs of the treated HDPE films revealed non-uniformly spread whitened areas, erosion zones, wound-out areas and clamp development in the current investigation. After 20 days of incubation of HDPE films recovered from synthetic media and allowed to some washing procedure. Results of HDPE indicates the surface morphology changes in the HDPE surface, it results in biodegradation of plastic. Bacterial isolates KP1 and NP1 both shows surface changes and biofilm formation in the surface of the film.

SEM data acquired after biodegradation of a sample film of HDPE by Farzil *et al.* (2017) indicated good digestion on the film surface, but not for complete HDPE destruction.

Summary and conclusion

5.0 SUMMARY AND CONCLUSION

Plastic and polyethylene waste accumulating in the environment are posing an ever-increasing ecological threat. Biodegradable plastics are environment friendly; they have an expanding range of potential application and are driven by the growing use of plastics in packaging. In recent years, many researchers have studied biodegradable plastic production for use in packaging applications. Biodegradable plastics do not deleteriously affect the environment and are naturally broken down by enzymes endemic to local microorganisms, producing water, carbon dioxide, methane, and biomass as by-products of degradation. Plastics are resistant to microbial attack since during their short time of presence in natural evolution could not design new enzyme structures capable of degrading synthetic polymers.

The present study aimed mainly at the isolation of bacteria from soil samples collected at the petrochemical oil-contaminated sites in order to identify the potent soil bacteria with unique characteristics and their role in the biodegradation of HDPE plastics.

The soil samples were collected from HDPE plastic waste disposal sites in two selected sites Kovaipudhur and Narasimmanaickenpalayam areas of Coimbatore, Tamil Nadu, India. Next, the isolation of bacteria was done and isolated bacterial strains named as KP1 and NP11, this bacterial strains may belongs to *Bacillus spp.,* For identification of HDPE degrading bacteria morphological characterization such as Gram's staining and biochemical characterization such as catalase test, gelatin liquefaction test, methyl red test, indole test, citrate utilization test, Voges- Proskauer test was done. Results of Gram's staining showed purple colour rod-shaped gram-positive bacteria, it may belong to *Bacillus spp.,* for bacterial isolates KP1 and NP1.

Hydrophobicity of bacterial isolates KP1 and NP1 was determined by BATH assay. Results for this assay showed reduction of 53.95 turbidity in KP1 when compared to NP1.The isolated bacterial strains were screened individually for the HDPE degrading efficiency by the *in-vitro* degradation assay, in this assay bacterial isolates KP11 and NP1 inoculated with synthetic medium containing HDPE as sole carbon source. During incubation period of 20 days the bacterial growth curve measurement was done periodically, it shows growth curve of bacterial isolates KP1 and NP1 at different phases and determination of dry weight loss of HDPE was done, it showed results in KP1 was found to be 0.025± 0.040 and foe NP1 was found to be 0.015±0.012, when compared between two bacterial strains, weight loss observed in KP1.

For quantification of bacterial biomass, protein estimation was done, it showed the results of protein content present in the bacterial biofilm on the HDPE surface. The bacterial isolates KP1 and PN1 showed protein content present in the HDPE surface, such as 9.06±1.23 µg mg^{-1} and 3.17±0.006µg mg^{-1} respectively. When comparing KP1 and NP1, the highest protein content present was found to be in the KP1.

Fourier Transform Infrared Spectroscopy analysis was done for the HDPE films, it showed the formation and deformation of new functional groups. For both KP1 and NP1 new peaks were obtained, also disappeared peaks were obtained. Scanning electron microscopy (SEM) showed surface morphology changes in the HDPE films, it shows the bacterial biofilm formation occurs on the surface of HDPE and it indicates the process of biodegradation.

Conclusion

To conclude this study, indicate that the isolated bacterial strains KP1 and NP11 may belong to *Bacillus spp.*, and the bacterial biofilm formation occurs in the bacterial strain KP1, it showed that *Bacillus spp.*, have the capacity to form biofilm formation in the surface of HDPE. Hence it showed the process of biodegradation.

Future studies

- Sequencing of the isolated bacterial strains *Bacilus spp.*,
- It is suggested to collect more soil samples from various plastic waste disposal site for comparison of those soil samples for biodegradation process.

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Bibliography

6. BIBLIOGRAPHY

- Al-Allaf, M. A. (2011). Isolation of Bacillus spp. from some sources and study of its proteolytic activity. Tikrit Journal of Pure Science, *16*(4), 1813-1662.
- Alessandro,.,Wellberg, E. A., Lewis, A. S., Terrell, K., Abel, E. D., Muller, W. A., ... and Anderson, S. M. (2016). Abstract PR03: GLUT1 is required for induction of mammary tumorigenesis by activated ErbB2/HER2/Neu.Lewis, A. S., Terrell, K., Abel, E. D., Muller, W. A., ... and Anderson, S. M. (2016). Abstract PR03: GLUT1 is required for induction of mammary tumorigenesis by activated ErbB2/HER2/Neu.
- Amal A .,Hussein, A. A., Al-Mayaly, I. K., and Khudeir, S. H. (2015). Isolation, Screening and Identification of Low Density Polyethylene (LDPE) degrading bacteria from contaminated soil with plastic wastes. Mesopotamia Environ. *J*, *1*(4), 1-14.
- Aoshima, M., and Yata, K. (2018). Two-sample tests for high-dimension, strongly spiked eigenvalue models. Statistica Sinica, 43-62.
- Avinash, R. D. K. (2020). Fish Diversity of Sai River flowing through Raebareli District of Uttar Pradesh (India).
- Bai, B., Liu, Y., Meng, X., Liu, C., Zhang, H., Zhang, W., and Jin, H. (2020). Experimental investigation on gasification characteristics of polycarbonate (PC) microplastics in supercritical water. Journal of the Energy Institute, *93*(2), 624-633.
- Carlos-Alberola, M., Gallardo Izquierdo, A., Colomer-Mendoza, F. J., and Barreda-Albert, E. (2021). Design of a municipal solid waste collection system in situations with a lack of resources: Nikki (Benin), a case in Africa. Sustainabilit*y*, *13*(4), 1785.
- Chowdhury, G. W., Koldewey, H. J., Duncan, E., Napper, I. E., Niloy, M. N. H., Nelms, S. E., and Nishat, B. (2021). Plastic pollution in aquatic systems in Bangladesh: A review of current knowledge. Science of the Total Environment, *761*, 143285.
- David, L., Bhandavat, R., Barrera, U., and Singh, G. (2015). Polymer-derived ceramic functionalized MoS2 composite paper as a stable lithium-ion battery electrode. Scientific reports, *5*(1), 1-7.
- Dawn, S., Chatterjee, S., Chakrabarty, S., Mufti, S., Bakshi, A. K., and Sapra, B. K. (2021). Cosmic ray neutron spectrometry and dosimetry at High Altitude Research Laboratory, Gulmarg, Kashmir, India. Radiation Physics and Chemistry, *185*, 109523.
- Gram, C. (1884). Ueber die isolirte Farbung der Schizomyceten in Schnitt-und Trockenpraparaten. *Fortschritte der Medicin*, *2*, 185-189.

- Edison, T. J. I., and Sethuraman, M. G. (2012). Instant green synthesis of silver nanoparticles using Terminalia chebula fruit extract and evaluation of their catalytic activity on reduction of methylene blue. Process Biochemistry, *47*(9), 1351-1357
- Fazil, M.,Rehman, S., Nabi, B., Khan, S., Bari, N. K., Singh, R., ... and Ali, J. (2017). Role of P-glycoprotein inhibitors in the bioavailability enhancement of solid dispersion of darunavir. BioMed research international, *2017*.
- Ferronato, N., and Torretta, V. (2019). Waste mismanagement in developing countries: A review of global issues. International journal of environmental research and public health, 16(6), 1060.
- Gillan, S. L. (2004). Recent developments in corporate governance: An overview. Journal of corporate finance, *12*(3), 381-402.
- Haded, M., Sabbouh, M., and Sharaf, A. N. S. (2005). Evaluation of heritability and genetic advance for yield and fiber traits among cotton f2 hybirds. *Damascus* university journal of the agricultural sciences *(syria)*.
- Kang, B. R., Kim, S. B., Song, H. A., and Lee, T. K. (2019). Accelerating the biodegradation of high-density polyethylene (HDPE) using Bjerkandera adusta TBB-03 and lignocellulose substrates. Microorganisms, 7(9), 304.
- Kar, K. K. (2021). Ramdas V. Mangore1, Sandeep S. Ahankari2, Kapil Dev Verma1, and. *Handbook of Fly Ash*, 301.
- Kyaw, B. M., Saxena, N., Posadzki, P., Vseteckova, J., Nikolaou, C. K., George, P. P., and Car, L. T. (2020). Virtual reality for health professions education: systematic review and meta-analysis by the digital health education collaboration. *Journal of medical Internet research*, *21*(1), e12959.
- Liu, X., He, P., Chen, W., and Gao, J. (2019). Improving multi-task deep neural networks via knowledge distillation for natural language understanding. arXiv preprint arXiv*:1904.09482*.
- Lowry Classics, O., Rosebrough, N., Farr, A., & Randall, R. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, *193*(1), 265-275.
- MacWilliams, M. (2009). Indole Test Protocol. American Society for Microbiology.
- Mierzwa-Hersztek, M., Gondek, K., and Kopeć, M. (2019). Degradation of polyethylene and biocomponent-derived polymer materials: an overview. Journal of Polymers and the Environment, *27*(3), 600-611

- Moszczynski, M., Green, D., Asbah, S., Morgan, C., Klyn, B., Foutry, G., ... and Smith, T. (2021). Using mobile phone data for epidemic response in low resource settings—A case study of Malawi. *Data* and *Policy*.
- Muhonja, W. L. (2008). Process evaluation of adolescent and youth sexual reproductive health (AYSRH) programme on utilisation of sexual and reproductive health services: Case study of Kinondoni Municipality (Doctoral dissertation, Mzumbe University).
- Nelson, J. W. (2011). Measuring caring-The next frontier in understanding workforce performance and patient outcomes. Nursing Economics, *29*(4), 215.
- Norman, R. E., Byambaa, M., De, R., Butchart, A., Scott, J., and Vos, T. (2018). The long-term health consequences of child physical abuse, emotional abuse, and neglect: a systematic review and meta-analysis. PLoS medicine, *9*(11), e1001349.
- Popovici, J., White, C. P., Hoelle, J., Kinkle, B. K., and Lytle, D. A. (2014). Characterization of the cell surface properties of drinking water pathogens by microbial adhesion to hydrocarbon and electrophoretic mobility measurements. Colloids and Surfaces B: Biointerfaces, 118, 126-132.
- Raaman, N., Rajitha, N., Jayshree, A., and Jegadeesh, R. (2008). Biodegradation of plastic by Aspergillus spp. isolated from polythene polluted sites around Chennai. *J* Acad Indus Res, *1*(6), 313-316.
- Raewyn .k .,Piccardo, M., Renzi, M., and Terlizzi, A. (2020). Nanoplastics in the oceans: Theory, experimental evidenceand real world. Marine Pollution Bulletin, 157, 111317.
- Rajandas, H., Parimannan, S., Sathasivam, K., Ravichandran, M., and Yin, L. S. (2012). A novel FTIR-ATR spectroscopy based technique for the estimation of low-density polyethylene biodegradation. Polymer Testing, *31*(8), 1094-1099.
- Reiner, K. (2010). Catalase test protocol. American society for microbiology, 1-6.
- Robinson, A. J., Prunet, G., Pawula, F., Fleury, G., Cloutet, E., Hadziioannou, G., and Pakdel, A. (2021). A review on conductive polymers and their hybrids for flexible and wearable thermoelectric applications. *Materials Today Physics*, *18*, 100402.
- Rosenberg, M., Gutnick, D., and Rosenberg, E. (1980). Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS* microbiology letters, *9*(1), 29-33.

- Michelson, P. F., Leahy, D. A., Hall, T. A., Finley, J. P., Cominsky, L. R., & Srinivasan, R. (2001). Phase-dependent spectral variability in 4U 1907+ 09. *The Astrophysical Journal*, *555*(2), 967.
- Sangale, M. K., Shahnawaz, M., and Ade, A. B. (2012). A review on biodegradation of polythene: the microbial approach. *J* Bioremed Biodeg, *3*(10), 1-9.
- Sathoskumar, C. and Anjan (2013). Ecological Concern in Ruskin Bond's Short Stories. *Veda's: Journal of English* Laguage and Literature *(JOELL)*, *4*(4), 287-290.
- Sharma, V., Bose, S., Kundu, B., Bodhak, S., Mitun, D., Balla, V. K., and Basu, B. (2020). Probing the influence of γ-sterilization on the oxidation, crystallization, sliding wear resistance, and cytocompatibility of chemically modified graphene-oxide-reinforced HDPE/UHMWPE nanocomposites and wear debris. *ACS* Biomaterials Science and Engineering, *6*(3), 1462-1475.
- Shi, Y ,Wang, D., Yang, B., Chen, Q. T., Chen, J., Su, L. F., Chen, P., ... and (2019). A facile evaluation on melt crystallization kinetics and thermal properties of low-density polyethylene (LDPE)/Recycled polyethylene terephthalate (RPET) blends. Advanced Industrial and Engineering Polymer Research, *2*(3), 126-135.
- Sivan, A. (2011). New perspectives in plastic biodegradation. Current opinion in biotechnology, 22(3), 422-426.
- Sneath, P. H. (1986). Endospore-forming Gram-positive rods and cocci. Bergey's manual of systematic bacteriology, 2, 1104-1207
- Tankeshwar, K., Kumar, V., Kaur, H., and Deep, A. (2019). Computational analysis to study the effect of infusion of Tetracyanoquinodimethane in zinc based metal-**organic framework.** ***Materials Research Express***, *7*(1), 015001
- Wang, Y. Y., Chen, Y. R., and Sarkanen, S. (2015). Path to plastics composed of ligninsulphonates (lignosulfonates). Green Chemistry, *17*(11), 5069-5078.
- Williams, B., Mancia, G., Spiering, W., Agabiti Rosei, E., Azizi, M., Burnier, M., ... and Desormais, I. (2018). 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH). European heart journal, *39*(33), 3021-3104.
- Xuemin, L. I. U., Mingyuan, L. I., Qiqing, C. H. E. N., and Huahong, S. H. I. (2019). Research progress on sorption of organic pollutants by microplastics. *Environmental* Chemistry, *41*(4), 1101-1113. Piccardo, M., Renzi, M., and Terlizzi, A. (2020)

Nanoplastics in the oceans: Theory, experimental evidence and real world. Marine Pollution Bulletin, 157, 111317.

- Zahari, A. R., and Esa, E. (2020). A longitudinal study of corporate social responsibility activities and brand equity in Malaysia. Global Business and Management Research, 12(4).
- Zhang, J., Gao, D., Li, Q., Zhao, Y., Li, L., Lin, H., and Zhao, Y. (2020) Biodegradation of polyethylene microplastic particles by the fungus *Aspergillus flavus* from the guts of wax moth Galleria mellonella. Science of the Total Environment, 704, 135931.

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