ISOLATION, IDENTIFICATION AND SCREENING OF BACTERIA WITH PLASTIC DEGRADATION POTENTIAL FROM THE SAMPLES COLLECTED FROM NJARACKAL , ERNAKULAM

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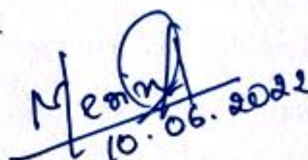
CERTIFICATE

This is to certify that the dissertation titled," **ISOLATION, IDENTIFICATION AND SCREENING OF BACTERIA WITH PLASTIC DEGRADATION POTENTIAL FROM THE SAMPLES COLLECTED FROM NJARACKAL , ERNAKULAM** " is an authentic record of work carried out by **EMILY SANDRA FRANCIS** under the supervision and guidance of Ms. MERIN ALICE GEORGE, Assistant Professor, Department of Botany & Centre for Research, St. Teresas's College (Autonomous), Ernakulam in partial fulfilment of the requirement for the Master's Degree of Science in Botany.



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INDRODUCTION

I see a future where getting to work or to school or to the store does not have to cause pollution.

- Bernie Sanders

The systematic pollution of our environment is one of the biggest hazards that humanity faces today. People are becoming increasingly aware of the threat posed by pollution and governments are enacting legislations aimed at protecting the environment.During the last few decades, the global environment has gone through serious challenges and changes. Population pressure has escaped rapidly consequently resources have dwindled.Pollution is an undesirable change in the physical and biological operations of our air, land and water. They may be or will be harmful to human life, species our industries process; living condition and culture assets or deteriorate our material resources.

Human being can be exposed to pollutant in many ways through the air they breathe, the water they drink, the food they eat and the cosmetics, drugs and other products they use. The continuing discovery of previously unsuspected hazards from various chemicals and other substances underscore the point. The environmental and human health effect of even those substances identified for priority consideration.Scientific developments have been a growing concern about the links between the health and environment and worldwide industrial, land and resources management practices. Today there is a growing concern for global scale environmental degradation brought by combinations of all people on earth**.**

From different hydrocarbons and petroleum derivatives high molecular weight organic polymers are obtained. These polymers are known as plastic (Ahmed *et al*., 2018). The word‘‘plastic”derived from the Greek word ‘‘Plastikos”, that means which can be molded into different shapes. Plastics stated as the polymers which start moving on heating so can be casted into moulds (Kale *et al*., 2015). Generally, plastic materials are derived from petrochemicals except biodegradable bioplastic (Akmal *et al*., 2015; Getachew and Woldesenbet, 2016). Plastic consists of chloride, oxygen, hydrogen, carbon, silicon and nitrogen. Polyethylene consists of 64% of total plastic and its general formula is CnH2n (Kale *et al*., 2015).

For packaging and many other purposes like agricultural films formation, diaper packaging and fishing nets plastics are used.Plastics play an important part in every sector of

economy all over the world. In highly growing areas i.e. agriculture, building and construction, health and consumer goods, plastics use ensures that they are in high demand and without plastics no one can do work.Plastics, the backbone of many industries, are used in manufacturing of various products that are used in our daily life i.e. defense materials, sanitary wares, tiles, plastic bottles, artificial leather and different other household items. Plastics are also used in packaging of food items, pharmaceuticals, detergents and cosmetics (Thakur, 2012; Piergiovanni and Limbo, 2016).

One of the rapidly growing fields in global industry is the production of synthetic plastics. Plastics are more superior than other materials due to their unique properties. These properties have been led to increase the plastic production scale to 20 folds since 1964 (Ellen MacArthur Foundation 2016), and production scale exceeds 300 million tons/year (Plastics Europe 2015) in 2015 it reached to 335 million tons (Plastics Europe 2017) (Urbanek *et al.*, 2018). There are advantages and disadvantages of plastics. Plastics are strong, durable, and light weight. On the other hand, they are harmful to the natural environment, resistant to degradation and leading to environmental pollution. On our planet, plastics pose a serious threat by accumulating in large quantities (Ahmed *et al.*, 2018; Yang et al., 2020; Al-Thawadi, 2020).

Plastics can be differentiated into degradable and nondegradable polymers on the basis of their chemical properties (Ghosh *et al.*, 2013). Plastics that are obtained from renewable resources are biodegradable plastics. These are naturally degradable, as a source of cellulose, starch and algal material, an important component in plants, animals and algae. These polymers are also produced by microorganisms. Non-degradable plastics, typically known as synthetic plastics, are derived from petrochemicalsand are higher in molecular weight due to the repetitions of small monomer units (Imre and Pukánszky, 2013).

The development and use of synthetic plastic has changed the nature of waste in last 3–4 decades (Sheavly, 2005). Over this period, it has replaced natural material in various aspects of human life and become an indispensable part of our society. Although the durability of plastic is ones of its most beneficial qualities, this same property is a major problem for our environment (Sivan, 2011). Plastics are chemically synthesized long-chain polymers (Scott, 1999) and are globally produced on a substantial scale. As per a recent estimate of the Central Pollution Control Board, New Delhi, India, 8 million tons of plastic products are consumed every year in

India alone. A study on plastic waste generation in 60 major Indian cities revealed that approximately 15,340 tons per day ofplastic waste is generated in the country (Central Pollution ControlBoard (CPCB) New Delhi, India, 2013). Low-density polyethylene (mainly used as carry bags) constitutes the major portion of thiswaste problem.

In the last two decades, the rate of plastic deposition has increased tremendously, and plastic has intruded into the marine environment. Plastic is found floating in oceans everywhere from the polar regions to the equator and has become one of the mostcommon and persistent pollutants of seas and beaches worldwide (Frias *et al*., 2010; Moore, 2008; Teuten *et al*., 2009). Plastic debris is one of the largest contaminants of the marine environment. Polyethylene is the most commonly found non-degradable solid waste and has recently been recognized as a major threat to marine life. There are reports that suggest that polyethylene causes blockages in the intestines of fish, birds and marine mammals. In addition, entanglement in or ingestion of this waste has endangered hundreds of different species (Teuten *et al*., 2009; Secchiand Zarzur, 1999; Spear *et al*., 1995).

Polyethylene represents up to 64% of the synthetic plastics thatare discarded within a short period after use (Byuntae *et al*., 1991).It is highly resistant to acids, alcohols, bases and esters. It is also biologically inactive and considered a recalcitrant material. It sinertness is due to the high molecular weight, hydrophobicity and lack of functional groups recognized by microbial enzymaticsystems (Hamid, 2000). Polyethylene is a concern for waste management due to its accumulation in landfills and natural habitats (Thompson *et al*., 2009). Hence, a suitable method for disposal thatis eco-friendly must be found. Recycling of polyethylene was considered a solution but has failed to provide safe disposal of thesematerials (Sivan, 2011); in this regard, microbial degradation isone of the best options. Some reports on the biodegradation of plastics indicate that it could be a viable proposition when suitable microorganisms are utilized (Singh and Sharma, 2008; Shah *et.al.,* 2008). Studies on polyethylene biodegradation (Albertson, 1980; Albertsson *et. al.,* 1987), including the biotic environment (Shah *et. al*., 2008), have been reported.

The purpose of this study was to isolate bacteria from Plastic and sediment collected from polluted Perumpilly ketu of Njarackal panchayat of Ernakulam district and screening of the potential Plastic degrading bacteria and identifying the highest potential bacteria that degrade

the plastics. If plastics can be degraded by microbes, it will reduce solid waste very naturally which causes environmental issues.

Perumpilly ketu of Njarackal panchayat of Ernakulam district was selected as the area for the study. High industrial pollution,dumped plastic waste from household as well as industry,many sewage connection ,minimal waste clearence are some of the reasons to select this area.

Sediment and plastics sample were collected prior to experiment commencement from selected study areas were the plastic accumulation was observed high. Sediments were collected in aseptic bottles along with plastic sample.

AIM AND OBJECTIVE

- Collection of plastic and sediment samples from the area of study.
- Microbial analysis of collected plastic and sediment samples.
- Identification and characterization of heteromorphic bacteria showing plastic biodegradation potential using weight loss method.
- Biochemical analysis of heteromorphic bacteria which showed highest plastic degradation potential.
- Molecular level identification of the screened bacterial isolates that showed highest plastic degradation potential.

REVIEW OF LITERATURE

Plastics are man-made long chain polymeric units (Scott, 1999). Synthetic polymers started to substitute natural materials in almost every area more than half a century ago and nowadays plastics have become an indispensable part of our life. With time, the stability and durability of plastics have been improved continuously, and hence these groups of materials are considered as a synonym for materials that are resistant to many environmental influences. The word 'plastic' is derived from the Greek word "plastikos", that means 'able to be molded into various shapes and sizes' (Joel, 1995). The plastics are made from inorganic and organic raw materials, used today, such as carbon, silicon, hydrogen, nitrogen, and oxygen. The basic materials are extracted from oil, coal and natural gas used for making plastics (Seymour, 1989).

Plastics are resistant to microbial attack, because their short time of presence in nature evolution could not design new enzyme structures capable of degrading synthetic polymers (Mueller, 2006) .Nowadays, a wide variety of petroleum-based synthetic polymers are produced worldwide to the extent of approximately 140 million tons per year and remarkable amounts of these polymers are introduced in the ecosystem as industrial waste products (Shimao, 2001).

The synthetic plastics are used in packaging of products like food, medicines, cosmetics, detergents and chemicals. Approximately 30% of the plastics are used worldwide for packaging applications. The utilization is still expanding at a high rate of 12% per annum. They have replaced paper and other cellulose-based products for packaging because of they have better physical and chemical properties, such as strength, lightness, resistance to water and most waterborne microorganisms. The plastics used in packaging are polyethylene (LDPE, MDPE, HDPE and LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), polyethylene terephthalate) (PET), poly(butylene terephthalate) (PBT), nylons are widely used. The widespread applications of plastics are not only due to their favorable mechanical and thermal properties but also mainly due to the stability and durability (Rivard et al., 1995). Plastics (polymers) have attracted more public and media attention than any other component of the solid waste stream because of their durability and visibility in litter. The

total world demand for plastics was over 107 million tones in 1993 and it was estimated about 146 million tonnes in 2000.

The dramatic increase in production and lack of biodegradability of commercial polymers, mainly commodity plastics used in packaging (e.g. fast food), industry and agriculture, has focused public attention on a potentially huge environmental accumulation and pollution problem that could persist for centuries (Albertsson *et al*., 1987). Plastic waste is disposed off through the process such as landfilling, incineration and recycling. Several communities are now more sensitive to the impact of discarded plastic on the environment because of their persistence in our environment, including deleterious effects on wildlife and on the aesthetic qualities of cities and forests. Improperly disposed plastic plays significant role in potentially harming life by causing environmental pollution. In addition to this, the burning of polyvinylchloride (PVC) plastics produces persistent organic pollutants (POPs) known as furans and dioxins (Jayasekara *et al*., 2005).

Synthetic plastics like polyester polyurethane, polyethylene with starch blend, can biodegrade, although most commodity plastics used now are either non-biodegradable or take decades to degrade. This growing concern about degradable polymers has raised and promoted research activity world wide to either modify current products to promote degradability or to develop new alternatives that are degradable by any or all of the following mechanisms: biodegradation, photodegradation, thermal degradation and environmental erosion (Kawai, 1995).

In 1980′s, scientists started to look if plastics could be designed to become susceptible against microbial attack, making them allowed to degrade in a microbial active environment. Biodegradable plastics has opened the way for new considerations of waste management strategies since these materials are designed to degrade under environmental conditions or in municipal and industrial biological waste treatment facilities (Augusta *et al*., 1992 and Witt *et al*., 1997).

Several biodegradable plastics have been introduced into the market in the past 10 years and none of them is found efficiently biodegradable in landfills. For this reason, none of the plastic products has gained widespread use (Anonymous, 1999). At present, biodegradable

plastic represents just a tiny market as compared with the conventional petrochemical materials. The bioplastics will comparatively prove cheaper when oil prices will continue to hike up. The plastic shopping bags could be made from Polylactic acid (PLA) a biodegradable polymer derived from lactic acid although not in use today. This could be said as one form of vegetable-based bioplastic which biodegrades quickly under composting conditions without leaving toxic residue. But, bioplastic can have its own environmental impacts, depending on the way it is produced .There is an urgent need to develop efficient microorganisms and their products to solve this global issue (Kathiresan, 2003). This paper reviews the current research on the degradation of the biodegradable plastics

Plastic pollution accumulating in an area of the environment is considered “poorly reversible” if natural mineralization processes occurring there are slow and engineered remediation solutions are improbable. Should negative outcomes in these areas arise as a consequence of plastic pollution, they will be practically irreversible. Potential impacts from poorly reversible plastic pollution include changes to carbon and nutrient cycles; habitat changes within soils, sediments, and aquatic ecosystems; co-occurring biological impacts on endangered or keystone species; ecotoxicity; and related societal impacts. The rational response to the global threat posed by accumulating and poorly reversible plastic pollution is to rapidly reduce plastic emissions through reductions in consumption of virgin plastic materials, along with internationally coordinated strategies for waste management.

Plastic pollution is found globally from deserts to farms, from mountaintops to the deep ocean, in tropical landfills and in Arctic snow. Reports of plastic debris in the marine environment date back half a century (Geyer *et.al.*,2017) with continuing accumulation on the ocean surface over the past 60 year. Emissions of plastic are increasing and will continue to do so even in some of the most optimistic future scenarios of plastic waste reduction (Wikipedia 2018 thermoplastics). Estimates of global emissions of plastic waste to rivers, lakes, and the ocean range from 9 to 23 million metric tons per year, with a similar amount emitted into the terrestrial environment, from 13 to 25 million metric tons per year as of 2016 (Fenichell,1996). Following business-as-usual scenarios, these estimated 2016 emission rates will be approximately doubled by 2025. Scenarios that include concerted, joint global action—such as

implementing the Basel convention to prevent transport of plastic waste to countries with poor management systems, or the European Union target to recycle more plastic as part of the transition to a circular economy—still forecast continuous yearly increases in plastic emissions (Fenichell, 1996).

Accumulation of plastic in the environment occurs when the rate at which plastic pollution enters an area exceeds the rate of natural removal processes or cleanup actions. Plastic is persistent in the environment, with rates of natural removal on the scale of decades to centuries (UK Patent office, 1865). Cleanup actions are not feasible in many areas of the global environment where plastic accumulates, particularly in remote locations. Plastic therefore fits the profile of a "poorly reversible pollutant," both because emissions cannot be curtailed and because it resides in the environment for a long time (Vitale, 1898). A central concern about poorly reversible pollution is that if it accumulates to levels that exceed effect thresholds, this transgression will trigger negative impacts that themselves cannot be readily reversed because it will not be possible to rapidly reduce pollution levels below the threshold (Bauman,1872 and Von Pechmann, 1898).

It has been reckoned that, the total mass of virgin plastics ever made amounts to 8.3 billion tonnes (Geyer *et.al.*,2017), mainly derived from natural gas and crude oil, used as chemical feedstocks and fuel sources. Between 1950 and 2015, a total of 6.3 billion tonnes of primary and secondary (recycled) plastic waste was generated, of which around 9% has been recycled, and 12% incinerated, with the remaining 79% either being stored in landfills or having been released directly into the natural environment. In the 'New Plastics Economy – Rethinking the Future of Plastics' report, the fate of plastic packaging waste is further quantified, concluding that approximately 40% of plastic packaging goes to landfill, while 32% leaks out of the collection system: that is, either it is not collected at all, or it is collected but then illegally dumped or mismanaged, and ends up directly in the environment. The statistic has been given that at present rates of production and pollution, by 2050, there will be more plastic than fish in the sea (by mass) (Ellen Mac Arthur Foundation, 2017). Only 28% of 'plastic packaging' was collected, of which half was incinerated to provide energy, while the other half was recycled

Plastics are typically cheap to manufacture, and hence are used on a very large scale for many essential purposes of modern civilisation .These materials are typically also chemically resistant, meaning that they degrade only slowly and hence billions of tonnes of plastics have accumulated in the environment. Land, waterways and oceans can become polluted by plastics, and living organisms, particularly those in ocean environments, can be harmed, for example by becoming entangled by plastic materials from packaging or discarded fishing lines, or they can ingest plastic waste. The latter may cause various health problems, either by direct physical action of the plastic items or particles, or potentially from the release of chemicals contained within the plastics that interfere with physiological processes: for example, by acting as endocrine disruptors, which disturb various hormonal mechanisms, both in animals and humans.

Plastics tend to be exceptionally stable and durable, which is why they have gained their popularity and wide application in society; however, these same qualities render them persistent in the environment, and resistant to decomposition when it is desired to dispose of them (Webb *et.al*.,2013). Under environmental conditions, plastics may undergo degradation by four principal mechanisms: photodegradation, thermooxidative degradation, hydrolysis and biodegradation by microbes50. Photodegradation by sunlight is generally the initial event, which primes the material for subsequent thermo-oxidative degradation,(Raquez *et.al*., 2011)As a result of these processes, the plastic becomes brittle and steadily dissociates into increasingly smaller fragments: finally, down to the molecular level, such that they can be metabolized by microbes(Zheng *et.al*.,2005) which either incorporate the carbon atoms from the polymer chains into biomolecules, or oxidise them to CO2 (Yamanda-Onodera *et.al*., 2001). The overall process of decomposition is very slow, however; an estimated 50 years for a foam plastic cup, 400 years for a plastic drinking cup, 450 years for a disposable nappy, and 600 years for a fishing line (Le Guern, 2018). The persistence of plastic in the oceans is enhanced by the limited availability of oxygen, and by the cooling effect of the water; also, rates of hydrolysis are too low to provide an effective route for the decomposition of most polymer components of plastic debris (Andrady, 2011)

Any physical or chemical change in polymer as a result of several environmental factors, such as light, temperature, moisture, chemical conditions or biological activity. Processes

that induce changes in polymer properties due to physical,chemical or biological reactions resulting in subsequent chemical transformations (formation of structural in homogeneities) are categorized as polymer degradation. Degradations are reflected as changes in properties of material (mechanical, optical or electrical characteristics), in cracking, erosion, discoloration, phase separation and delamination. The changes include chemical transformation and formation of new functional groups (Pospisil *et.al.*, 1997). The degradation will be photo, thermal or biological.

Sensitivity of polymers to photodegradation is related to the ability to absorb the harmful part of the tropospheric solar radiation. And this includes the UV-B terrestrial radiation (~ 295–315 nm) and UV-A radiation (~ 315–400 nm) responsible for the direct photodegradation (photolysis, initiated photooxidation). Visible part of sunlight (400–760 nm) accelerates polymeric degradation by heating. Infrared radiation (760–2500 nm) accelerates thermal oxidation (Gugumus, 1990 and Pospisil *et.al.*, 1997). The absorbance of high-energy radiation in the ultraviolet portion of the spectrum by most plastics, results in activation of their electrons to higher reactivity and that causes oxidation, cleavage, and finally process of degradation. Thermal degradation of polymers is 'molecular deterioration as a result of overheating'. The components of the long chain backbone of the polymer can begin to separate (molecular scission) at high temperatures and react with one another to change the properties of the polymer. Various chemical reactions involved in thermal degradation lead to physical and optical property changes relative to the initially specified properties. Thermal degradation of plastics generally involve changes to the molecular weight (and molecular weight distribution) of the polymer and typical property changes include; reduced ductility and embrittlement, chalking, color change, and general reduction in most other desirable physical properties (Olayan *et al*., 1996).

Biodegradation is the process by which organic substances are broken down by living organisms. This term is often used in relation to ecology, waste management, bioremediation and to the plastic materials, because of their long life span. (Gu *et al*., 2000a).

Microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastics (Gu *et al*., 2000a). The biodegradation of plastics proceeds actively under different soil conditions according to their properties, because the microorganisms that are responsible for the process of degradation differ from each other and they have their own optimal

growth conditions in the soil. Plastics are potential substrates for heterotrophic microorganisms (Glass *et.al*., 1989).

Biodegradation is governed by different factors that include characteristics of polymer, type of organism, and nature of pretreatment. The characteristics of polymer such as mobility, crystallinity, molecular weight, functional groups and substituents present in its structure, and plasticizers or additives when added to the polymer all play a significant role in its degradation (Artham *et.al*., 2008; Gu *et al*., 2000)

The polymer is first converted to its monomers during degradation, after which these monomers are mineralized. Most polymers are too large that they can pass through cellular membranes, so for this they must first be depolymerized to smaller monomers before they can be absorbed and biodegraded within the microbial cells. The initial breaking down of polymers can result from a variety of physical and biological forces (Swift, 1997). Any of the physical forces, such as heating, cooling, freezing, thawing, wetting or drying, can cause damage to the mechanical property such as the cracking of polymers (Kamal *et.al*., 1992). The growth of many fungi on polymers can also cause small-scale swelling and bursting, as the fungi get penetrated in the polymer solids (Griffin, 1980). Synthetic polymers, such as poly(caprolactone) (Toncheva *et al*., 1996; Jun *et al*., 1994), can also be depolymerized by microbial enzymes, then the monomers are absorbed into microbial cells and biodegraded (Goldberg, 1995). The most important reaction for initiating the environmental degradation of synthetic polymers is the abiotic hydrolysis (Göpferich, 1997) like polycarboxylates (Winursito and Matsumura, 1996), polyethylene terephthalate ,poly (α-glutamic acids) (Fan *et al*., 1996), and polydimethylsiloxanes, or silicones.

Generally, an increase in molecular weight results in a decline of polymer degradability by microorganisms. In contrast, a polymer's repeating units are monomers, dimers, and oligomers are much easily degraded and mineralized. There could be a sharp decrease in solubility due to high molecular weights of plastics making them unfavorable for microbial attack because bacteria require the substrate to be assimilated through the cellular membrane and then further degraded by cellular enzymes. There are at least two categories of enzymes that are actively involved in biological degradation of polymers: extracellular and intracellular depolymerases (Doi, 1990; Gu *et al*., 2000b). Exoenzymes from microorganisms break down complex polymers during degradation yielding smaller molecules of short chains, that are small

enough to pass the semi-permeable outer bacterial membranes, and then utilized as carbon and energy sources. This process is called depolymerization. The degradation is called mineralization when the end products are Carbon dioxide, water, or methane (Frazer, 1994; Hamilton *et al*., 1995). It is important to note that the biodeterioration and degradation of a polymer substrate can rarely reach 100% and the reason is that a small portion of the polymer will be incorporated into microbial biomass, humus and other natural products (Atlas *et.al*., 1997 and Narayan, 1993). the dominant groups of microorganisms and the degradative pathways associated with polymer degradation are often determined by the environmental conditions. When Oxygen is available, aerobic microorganisms are mostly responsible for the destruction of complex materials, yielding microbial biomass, Carbon dioxide, and water as the final products. Anaerobic consortia of microorganisms are responsible for polymer deterioration under anoxic conditions. The microbial biomass, Carbon dioxide, methane and water are the primary products under methanogenic (anaerobic) conditions (Barlaz *et al*., 1989) (e.g. landfills/compost)

Plastic is the most used material for food wrapping and is basically made of PE material (Agustien *et al*., 2016). Shopping bags are PE bags. These bags are composed of PE and 10% of the municipal waste, all over the world, is due to the excessive use of plastic material which is mainly PE (Begum *et al*., 2015). Usage of PE bags all around the globe is about 500 billion to one trillion annually. The plastic accumulation in the terrestrial environment or in the sea coast is about 25 million tons every year

PE is chemically inert and hydrophobic in nature and microorganisms have no appropriate mechanism to digest these synthetic plastics (Yoon *et al*., 2012). PE polymers are used by microorganisms as a substrate for their growth. Erosion, discoloration, cracking and phase separation are the indicators of PE degradation (Trivedi *et a*l., 2016, Agustien *et al*., 2016)

PE degradation is further classified into two classes: abiotic and biotic. In abiotic degradation all natural factors like temperature, ultraviolet rays cause degradation of PE while in case of biotic degradation microorganisms are involved that consume the plastics by changing their properties (Sen *et.al*., 2015). As PE is safe, cheap, harmless and stable in the environment, and is easy to proceed, it is one of the polymers that are mostly seen all over the world. The two possible ways by which PE usefulness is maintained in nature are to use microbes in order to

degrade polymers or PE. The second is to make polymers artificially that are prone to degradation by microorganism.

Polyolefins, low density PE, are unreactive in their chemical nature. For a shorter period 95 °C is used while for a longer time it may be used at 80 °C (Billmeyer, 1984). Due to incomplete crystallinity that ranges in 50–60%, there are several properties of low-density polyethylene (LDPE) such as rigidity, tensile strength, flexibility and tear strength. The carbonyl group, generated in polyethylene oxidation, is used by microorganisms for its degradation (Cornell *et al*., 1984; Awasthi *et al*., 2017).

The oxidative degradation mechanism, used for non-hydrolysable polymers e.g. polyethylene and polypropylene, leads to loss in molecular weight of polymers. Several oxidative enzymes are involved in oxidation of ethylenic groups; these enzymes are monooxygenase, peroxidase, manganese, peroxidase, dehydrogenase and oxidase. By the action of extracellular and intracellular enzymes, polymers convert into oligomers and monomers that are utilized by microorganisms for a source of energy (Arkatkar *et al*., 2009). β-oxidation of fatty acids that occurs in animals and humans shows similarities with β-oxidation of polyethylene.

Microorganisms that are capable of degrading polymers have been investigated and isolated from the natural environment. Polymer materials that are used for microbial degradation e.g., polyethylene and polypropylene (Park *et.al*., 2019). Polymer degrading microbial species that are associated with degradation were identified as Streptococcus, Klebsiella, Micrococcus Staphylococcus, Pseudomonas (Das *et.al.,* 2015). Biodegradability of polyethylene is enhanced by blending polyethylene with different additives, by adding these additives auto-oxidation of polyethylene enhances, by which molecular weight of polymer reduces and microorganisms then easily degrade these low molecular weight polymers.

MATERIALS AND METHODOLOGY

I. SAMPLE COLLECTION

Sediment and plastics sample were collected Prior to experiment commencement from selected study areas were the plastic accumulation was observed high.Perumpilly ketu of Njarackal panchayat of Ernakulam district was selected as the area for the study .Sediments were collected in aseptic bottles along with Plastic sample and stored at 4°C.

II. GLASSWARE

Test tubes, culture tubes, Petri dishes and Erlenmeyer flasks are the most important types of glassware in a microbiological lab. The apparatus are washed with soap and water. Placed in hot air oven (dry heat) at 180°C for 2-3 hrs. After sterilization, apparatus kept in for holding time to slowly cool.

III. AUTOCLAVING/ STERILIZATION

An autoclave that uses steam under pressure to kill harmful bacteria, viruses, fungi, and spores on items that are placed inside a pressure vesse was used.. The items are heated to an appropriate sterilization temperature for a given amount of time. It was used to kill spores and ells of microorganisms. Agar filled culture vessels are placed for about 20-30 mins in 15lbs under 120°C.

IV. INCUBATION

The cultured bacterial cells were kept in an incubator which is used to grow and maintain microbiological cultures or cell cultures. It maintains optimal temperature, humidity and other conditions such as the CO2 and oxygen content of the atmosphere inside.

V. REFRIGERATION

Working bacterial stocks can be streaked onto agar plates and stored at 4°C for daily or weekly usein a refrigerator. Culture dishes should be wrapped with laboratory sealing film (plastic or paraffin) and stored upside down (agar side up) to minimize contamination and to keep both the culture and agar properly hydrated.

VI. MICROBIOLOGICAL ANALYSIS

a) Isolation of Bacterial Strains

Serial Dilution

***Principle**:*

It is a method that involves the dilution of specimen in successive stages. It is a simple procedure for the isolation of bacteria. The specimen is serially diluted in a sterile liquid.

***Materials**:*

Conical flask, test tubes, distilled water, petri dishes, nutrient agar medium, autoclave, cotton, pipette, soil samples

***Procedure**:*

Sample or the culture is taken in a test tube and five test tubes, each with 9ml of sterile diluents, which can either be distilled water or 0.9% saline, are taken. A sterile pipette is taken. 1ml of properly mixed sample/ culture is drawn into the pipette. The sample is then added to the first tube to make the total volume of 10ml. This provides an initial dilution of 10^{-1}. The dilution is thoroughly mixed by emptying and filling the pipette several times . The pipette tip is discarded and anew pipette tip is attached to the pipette. Now 1ml of mixture is taken from the 10^{-1} dilution and is emptied into the second tube. The second tube now has a total dilution factor of 10^{-2}. The same process is repeated for the remaining tubes, taking 1 ml from the previous tubes and adding into the next 9ml diluents. As five tubes are used, the final dilution for the bacteria or the cell will be 10^{-5} (1 in 1,00,000)

i. Spread plate

Principle:

Spread plate method is used for isolation of pure culture from mixed colonies. In this technique, the microorganisms are spread over solidified agar plates with the help of a L-rod (L-shaped glass tube), when petri plate is spinning on a turn table.

Materials:

Bacterial culture, nutrient agar plates, measuring cylinder, L- rod, spirit lamp, absolute alcohol, sterile pipette.

Procedure:

Nutrient agar medium preparation

To 100 ml of distilled water taken in a conical flask, add 2g of nutrient agar and 1.3 g of nutrient broth. Mix well and heat it in the oven till it just start to boil. Pour the mixture into sterile petri plates. Keep the plates undisturbed till it solidifies (setting time)

1. A loop full of bacterial culture was suspended in sterile saline in a test tube and mixed thoroughly to get a homogenous suspension.
2. 5ml of this suspension was inoculated into the surface of agar plate using a sterile pipette.
3. The plates were placed on a turn table.
4. The L-rod was sterilized by dipping it first in absolute alcohol and then flaming on Bunsen burner. It was allowed to cool.
5. The lid of the plate was removed and kept on a turn table.
6. The L-shaped glass rod was placed on the agar containing the inoculum and moved back and forth gently to spread the culture evenly on agar surface.
7. When the turn table stops spinning, the lid was put over the petri plate.
8. The L-rod was sterilized.
9. The petri plate was incubated at 30°C for 24 hrs.
10. The plates were observed after incubation.

ii. Quadrant streak

Principle:

The quadrant streaking method enables us to work with an individual or well-isolated colonies. It is a rapid isolation method. It sequentially dilutes the population size of the microorganism in the original sample.

Materials:

Nutrient agar medium, distilled water, autoclave, heater, sterile petri plates, glass rod, inoculating loop, beaker, cotton, aluminium foil or paper, Bunsen burner, bacterial culture tube.

Procedure:

Nutrient agar medium preparation

To 100 ml of distilled water taken in a conical flask, add 2g of nutrient agar and 1.3 g of nutrient broth. Mix well and heat it in the oven till it just start to boil. Pour the mixture into sterile petri plates. Keep the plates undisturbed till it solidifies (setting time)

1. Flame the loop and wire and streak a loopful of broth as at A in the diagram.
2. Re-flame the loop and cool it.
3. Streak as at B to spread the original inoculum over more of the agar.
4. Re-flame the loop and cool it.
5. Streak as at C
6. Re-flame the loop and cool it.
7. Streak as at D.
8. Label the plate and incubate it inverted at 30°C for 24hrs.

iii. Slant Agar Preparation of Bacterial Culture

Materials:

Nutrient agar medium, distilled water, autoclave, heater, culture tubes, glass rod, measuring cylinder, pH meter, test tube stand, cultured bacteria

Procedure:

A. <u>Preparation of agar medium</u>

Weigh 2g of nutrient agar medium and transfer them into 100ml beaker. Add distilled water to make the volume of 100 ml. Gently heat the contents with slight agitation to dissolve the ingredients. Measure the pH of the medium using a ph meter and adjust to 7.0 by adding a drop of either HCl or NaOH solution.

B. <u>Preparation of Agar Slants and inoculation</u>

Prepared agar medium is dispensed into test tube. Dispense 5 ml of molten agar into each test tubes using pipettes Prepare cotton plugs and place them loosely on to the mouth of the tubes containing hot agar. The amount of medium maybe increased or decreased according to the volume of the culture tubes. Sterilize the tubes at 121°C for 15 minutes with plugs loosely on. While the medium is still hot, tilt the rack onto a thick book or other solid surface so that the medium in the tube is slanted. Allow the medium to harden in this position. When the medium is cooled tighten the plugs. After cooling of agar slants, the bacteria is inoculated one by one in each of the slant accordingly using inoculation loop. After inoculation, incubate the tubes for 24 hrs. After 24hrs, take out the slants from the incubator and store in the refrigerator.

VII. SCREENING OF BACTERIA:-

a) Weight-Loss Technique

The method is carried out to test the polythene degrading bacteria from the isolates obtained.

- ***Materials*:**

Sterile 100ml conical flasks, 1000 ml conical flask, glass rod, measuring cylinder, sterile nutrient broth (50ml each), distilled water, cotton plugs, pre-weighed polyethylene pieces, incubator

- ***Methods*:**

Weigh 7.15g of nutrient broth is added to conical flask containing 550 ml of distilled water and autoclaved. It is then transferred to 100ml conical flask and plugged with cotton loosely. After the broth is cooled, a loop full of the purified bacterial isolates are inoculated into each culture vessel and labelled uniquely. The plastic pieces were pre-weighed around 95.6 milligrams and were transferred to each conical flask aseptically. It is incubated at 37°C for 28 days. The vessels were shaken at regular intervals initially for even distribution in the medium. After 28 days, the polythene pieces were removed, washed with distilled water, air dried and weighed to note the final weight.

VIII. BIOCHEMICAL ANALYSIS

a) Grams Staining

Principle:

This method s primarily one to differentiate Gram positive and Gram negative bacteria

Materials:

Bacterial culture, Crystal violet, Gram's iodine, 95% ethanol, safranin, distilled water, glass slide, inoculating loop, spirit lamp, microscope

Procedure:

Prepare a smear of bacteria using inoculating loop on a clean glass slide and heat fix it. Flood the slide with crystal violet staining reagent for 1 minute. Rinse the reagent off gently using distilled water. Flood the slide with Gram's iodine for 1 min. Wash with a gentle indirect stream of distilled water for 2 seconds. Flood the smear with ethanol for 5 seconds. Blot dry the smear and counter stain with safranin for one minute. Rinse off the stain, air dry and keep it undisturbed.

b) IMViC Tests

i. Indole test

Principle:

Indole is generated by reductive deamination from tryptophan via the intermediate molecule indolepyruvic acid

Materials:

Culture tubes, cotton plugs,

(a) Composition of medium per litre –

Tryptone	1.5 g
NaCl	20g
pH	7.2+/- 0.3

(b) Kovac's reagent

Paradimethylaminobenzaldehyde	5g
Amyl or isoamyl alcohol	75ml
Concentrated HCl	25ml

Procedure:

Dispense the medium in 5ml aliquot into tubes and autoclaved for 15 mins at 15lbs. Inoculate the tube of tryptone water with a loop full of 24hrs culture growth .Inoculation for 48-96 hrs at 37°C. After inoculation, 0.5ml Kovac's reagent is added to each tube without mixing and hold for 2-5mins. Appearance of dark red ring indicates positive result.

ii. Methyl Red (MR) Test

Principle:

The acid so produced decreases the pH to 4.5 or below, which is indicated by a change in the colour of methyl red from yellow to red.

Materials:

Culture tubes, cotton plugs,

(a) glucose-phosphate peptone water medium

Glucose	5g
K_2HPO_4	5g
Peptone	5g

(b) methyl red indicator

Dissolve 0.1g methyl red in 300ml 95% ethylalcohol in standard flask dilute to 500ml with distilled water.

***Procedure*:**

Inoculate the test organism in liquid medium (glucose-phosphate broth) incubate at 37°C for 2-5 days. Add 5 drops of 0.04 solution of methyl red along the sides of the test tube . Read the results immediately.

iii. Voges- Proskauer (VP) Test

***Principle*:**

The Voges-Proskauer (VP) test is used to determine if an organism produces acetylmethyl carbinol from glucose fermentation.

***Materials*:**

Barrit's reagent A and B

***Procedure*:**

Inoculate test organisms in glucose-phosphate broth and incubate at 37°C or 30°C for 48 hrs only. Barrit's reagent A and B in equal amounts is added.

iv. Citrate Test

***Principle*:**

This test detects the ability of an organism to utilize citrate as the sole source of carbon and energy.

***Materials*:**

(a) Simmon's citrate agar medium

Sodium citrate	2.0g

$MgSO_4$	0.2g
$(NH_4)H_2PO_4$	1.0g
K_2PO_4	1.0g
NaCl	5.0g
Bromothymol blue	0.08g
Agar	15.0g
Distilled water	1L
pH	7.0

(b) Bacterial culture
(c) Inoculation needle
(d) Culture tubes
(e) Incubator

***Procedure*:**

Prepare a few slants of Simmon's citrate agar medium. Inoculate the slants by stabbing to the base of the slant. Thereafter, streak the surface. Keep the tubes at 37°C for 48 hrs for incubation. Examine the tubes for result. If the culture grows, it causes blue color of the medium due to change in pH. (Alkaline indicated by citrate utilization)

OBSERVATION AND RESULTS

AREA UNDER STUDY

Perumpilly ketu of Njarackal panchayat of Ernakulam district was selected as the area for the study. The following figure shows the images of the area under study from where the samples where collected.

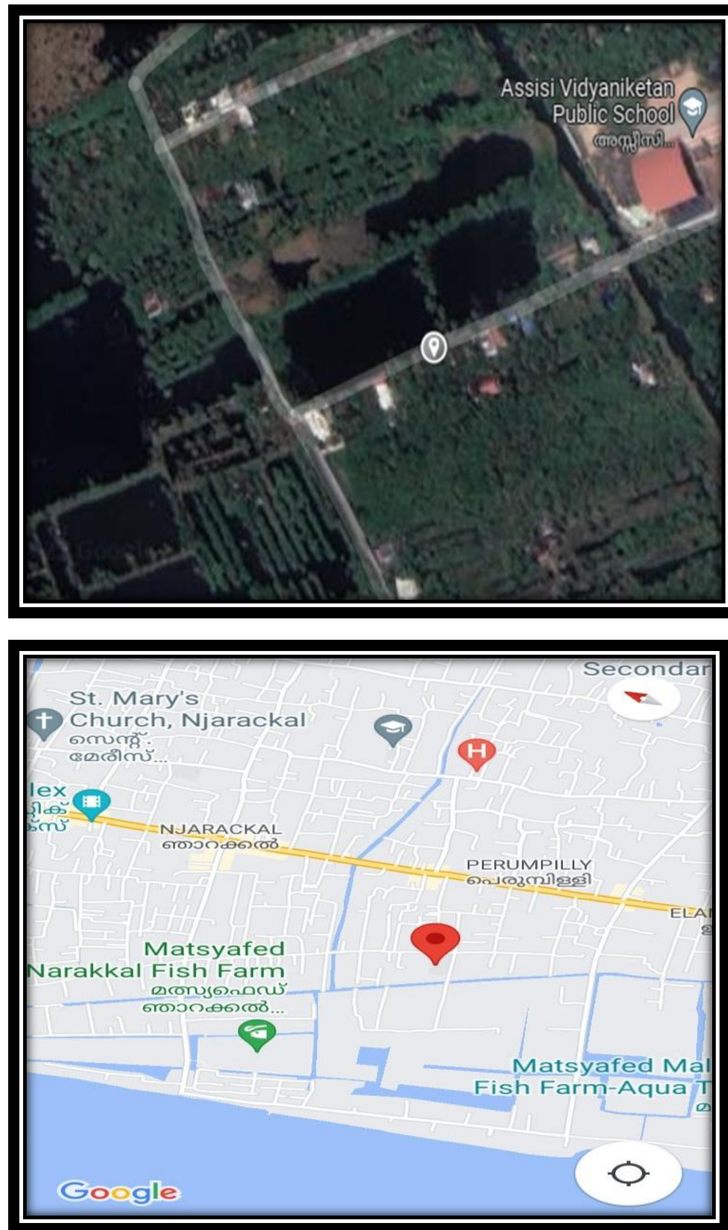


Figure 1: Google Map Showing Study Area

Figure 2 : Polluted Area Of Perumpilly Ketu

Figure 3 : Area From Which Sample Were Collected

a) Isolation of Bacterial Strains

The bacteria were distinguished based on the morphology of the colonies formed on the spread plate cultures as shown below

i. Spread Plate

***Sediment Sample* :**

Figure 4: spread plate of sediment sample

***Plastic Sample* :**

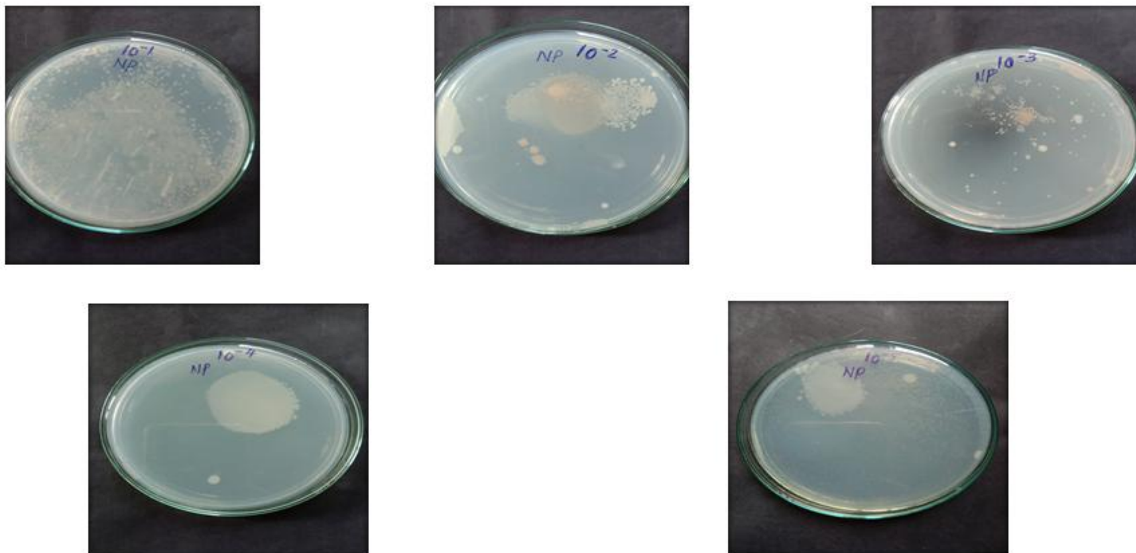


Figure 5 : spread plate of plastic sample

ii. Streak Plate

Bacterial colonies so noted were individually streaked into solid nutrient cultures to obtain purified isolates as well as for the ease of storing them.

Sediment Sample **:**

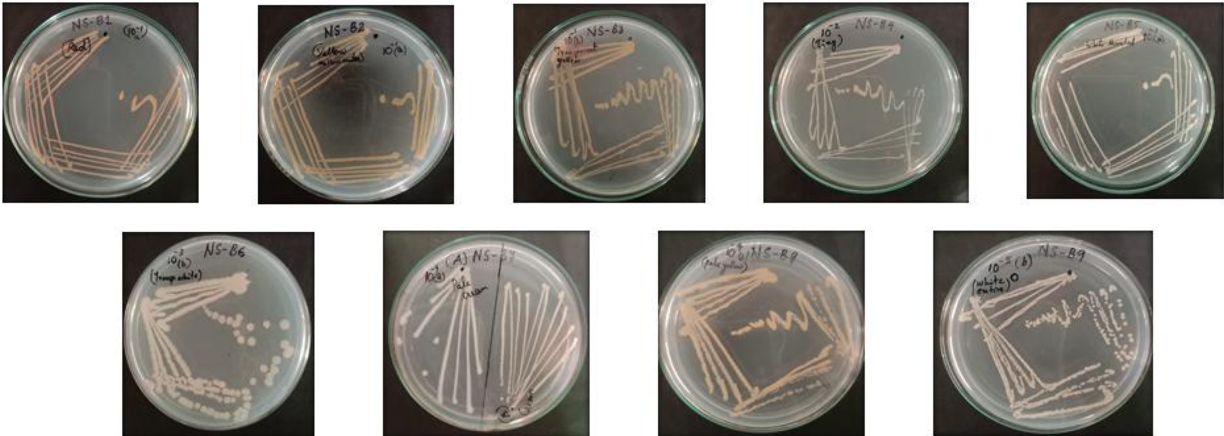


Figure 6 : streak plates of sediment sample

Plastic Sample **:**

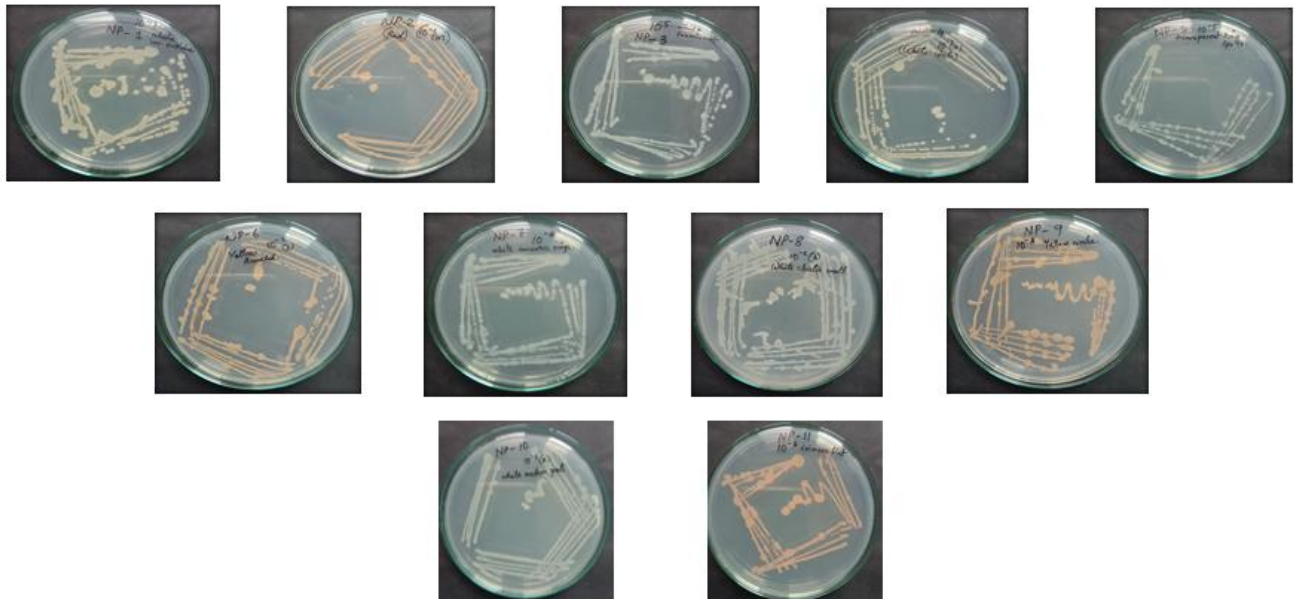


Figure 7 : streak plate of plastic sample

iii. Slant Preparation

Bacterial colonies so obtained from the streak plates were individually inoculated into a culture tube containing the slant agar medium to obtain purified isolates as well as for the ease of storing them.

***Sediment Sample*:**

Figure 8 : Agar Slants Containing Bacterial Isolates From Sediment Sample

***Plastic Sample* :**

Figure 9 : Agar Slants Containing Bacterial Isolates From Plastic Sample

VII) SCREENING OF BACTERIA :

The following picture shows the nature of the cultures during incubation for screening the bacteria of its Degradation potential.

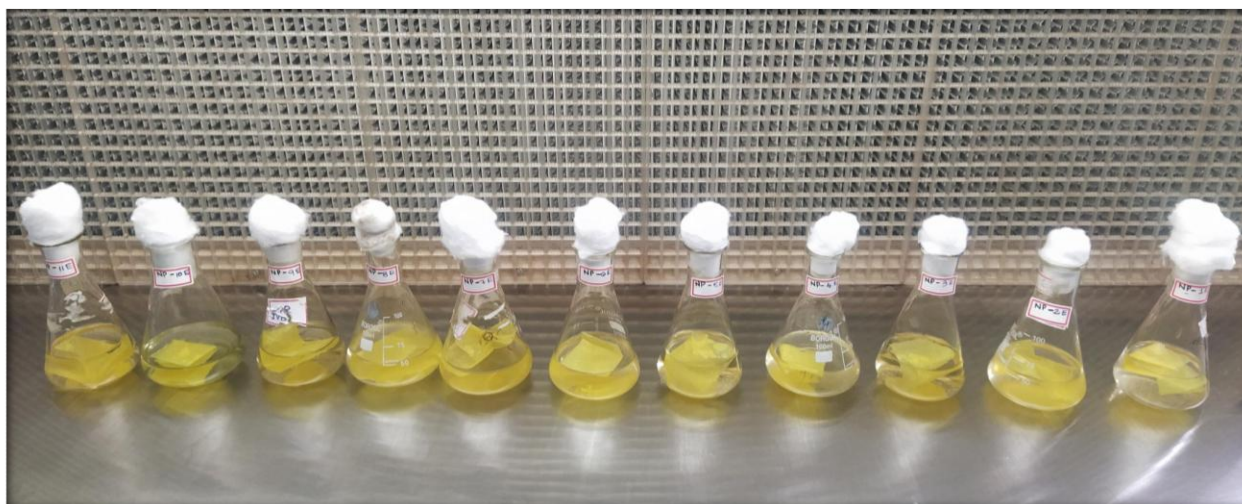


Figure 10 : Images Of Weight Loss Method For The Determination Of Plastic Biodegradation Capacity By Bacterial Isolates.

VIII) BIOCHEMICAL TEST :

a) GRAM'S STAINING

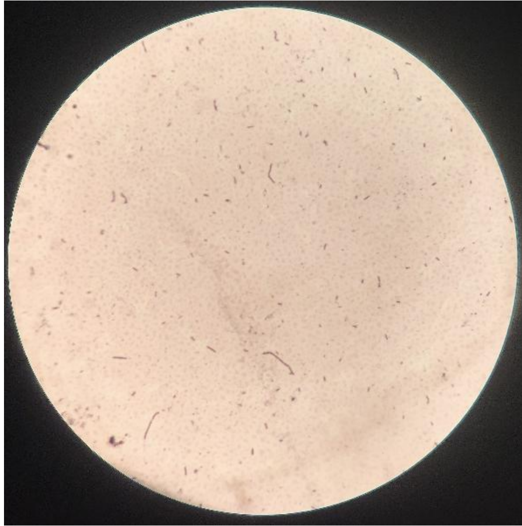


Figure 11 : Isolate NP 01

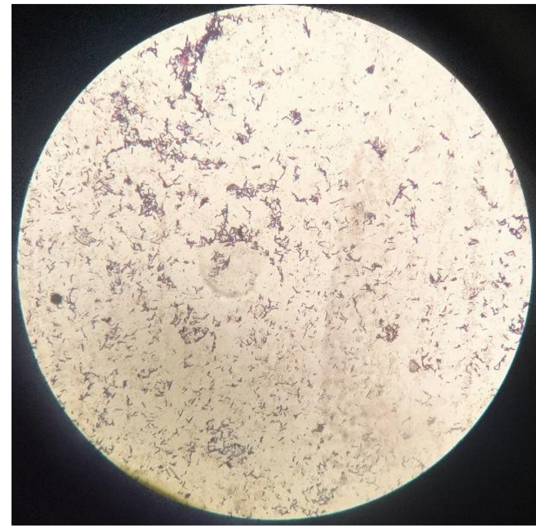


Figure 12 : Isolate NP 07

b) IMViC TEST

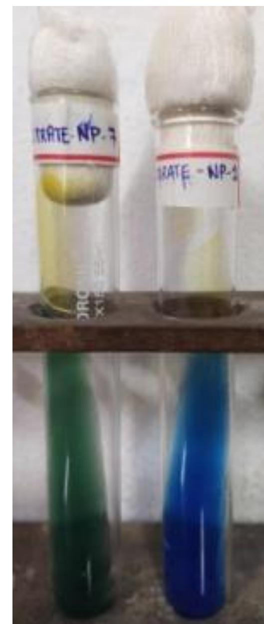


Figure 13 : Result of IMViC test

RESULT

➢ MORPHOLOGY OF COLONY

The bacteria were isolated from samples by serial dilution and observed for peculiar morphological characters in their colony which are shown below:

COLONY MORPHOLOGY – SEDIMENT SAMPLE

DILUTION No.	Sl. No.	COLONY MORPHOLOGY	CODE
10^{-1}	1.	**Small irregular round**	NS1
	2.	**Large yellow hollow centered**	NS2
	3.	**Small transparent yellow**	NS3
10^{-2}	1.	**Tiny transparent spots**	NS4
10^{-3}	1.	**Large branched white**	NS5
	2.	**Large transparent white**	NS6
10^{-4}	1.	**Medium pale cream**	NS7
	2.	**Small pale yellow**	NS8
10^{-5}	1.	**Medium round white**	NS9

COLONY MORPHOLOGY – PLASTIC SAMPLE

DILUTION NO.	SL. No.	COLONY MORPHOLOGY	CODE
10^{-1}	1.	**Small irregular white**	NP1
10^{-2}	1.	**Small irregular red**	NP2
	2.	**Small circular white**	NP3
	3.	**Large branching yellow**	NP4
	4.	**Small irregular white clusters**	NP5
10^{-3}	1.	**Flat circular round crimson**	NP6
	2.	**Medium round white**	NP7
	3.	**Small round yellow**	NP8
10^{-4}	1.	**Concentric circles white**	NP9
10^{-5}	1.	**Tiny transparent spots**	NP10
	2.	**Oval translucent white**	NP11

➢ COLONY COUNT :

Sediment sample

DILUTION CONCENTRATION NO.	NO. OF COLONIES
10^{-1}	162
$10-^{2}$	112
$10-^{3}$	323
$10-^{4}$	2082
$10-^{5}$	2145

Plastic sample

DILUTION CONCENTRATION NO.	NO. OF COLONIES
10^{--1}	198
$10-^{2}$	224
$10-^{-3}$	445
$10-^{4}$	1025
$10-^{5}$	2113

VII) SCREENING OF BACTERIA FROM SEDIMENT SAMPLE :

a) Polyethylene degradation capacity by weight loss method

The following table depicts the amount of weight lost in the polyethylene when subjected to bacterial degradation in synthetic media. Certain isolates showed significant biodegrading potential out of which two isolates were selectively screened for biochemical analysis.

BACTERIAL ISOLATE FROM PLASTIC	INITIAL PLASTIC WEIGHT (g)	FINAL PLASTIC WEIGHT (g)	WEIGHT LOSS (g)
Isolate 1	0.0956	0.0936	0.002
Isolate 2	0.0959	0.0935	0.0024
Isolate 3	0.0934	0.0928	0.006
Isolate 4	0.0953	0.0938	0.0015
Isolate 5	0.0933	0.0922	0.0011
Isolate 6	0.0952	0.0934	0.0018
Isolate 7	0.0947	0.0922	0.0025
Isolate 8	0.0958	0.0936	0.0022
Isolate 9	0.0959	0.0936	0.0023

VII) SCREENING OF BACTERIA FROM PLASTIC SAMPLE :

a) Polyethylene degradation capacity by weight loss method

The following table depicts the amount of weight lost in the polyethylene when subjected to bacterial degradation in synthetic media. Certain isolates showed significant biodegrading potential out of which two isolates were selectively screened for biochemical analysis

BACTERIAL ISOLATE FROM SEDIMENT	INITIAL PLASTIC WEIGHT (g)	FINAL PLASTIC WEIGHT (g)	WEIGHT LOSS (g)
Isolate 1	**0.0951**	**0.0920**	**0.0031**
Isolate 2	0.0958	0.0928	0.0030
Isolate 3	0.0956	0.0932	0.0024
Isolate 4	0.0959	0.0935	0.0024
Isolate 5	0.0959	0.0930	0.0029
Isolate 6	0.0956	0.0926	0.0030
Isolate 7	**0.0956**	**0.0907**	**0.0046**
Isolate 8	0.0947	0.0932	0.0015
Isolate 9	0.0931	0.0929	0.0002
Isolate 10	0.0956	0.0935	0.0021
Isolate 11	0.0945	0.0931	0.0014

VIII) BIOCHEMICAL ANALYSIS :

a) Gram's Staining and Motility Test:

SL NO.	TEST	Isolate 1	Isolate 7
1.	**Gram's staining**	POSITIVE (+)	POSITIVE (+)
2.	**Motility test**	NON MOTILE	NON MOTILE

b) IMViC Test :

SL NO.	TEST	Isolate 1	Isolate 7
i.	**Indole Test**	Positive(+)	Positive(+)
ii.	**Methyl red Test**	Negative (-)	Negative (-)
iii.	**Voges Proskauer Test**	Negative (-)	Negative (-)
iv.	**Citrate Test**	Positive (+)	Negative (-)

IX) <u>MOLECULAR IDENTIFICATION</u> :

Following the biochemical characterisation, the bacterial strains where screened for biodegradation potential among which two strains coded NP01 and NP07 showed the highest degradation capacity .These isolates where subjected for DNA isolation and PCR amplification followed by sequencing of the DNA so obtained and finally the strain coded NP01 was identified as ***Priestia magaterium*** and isolate strain coded NP07 was identified as ***Bacillus cerus***.

a) DNA EXTRACTION

Genomic DNA of the selected isolate strains showing hightest degradation capacity was isolated using nucleospin Tissue kit (Macherey- Nagel) following manufacturer's instructions.

b) AGAROSE GEL ELECTROPHORESIS OF PCR PRODUCT

Purified DNA product was used foe amplification by using specific primers and PCR product was loaded on 1.2% Agarose Gel Electrophoresis and bands were observed under gel documentation system.

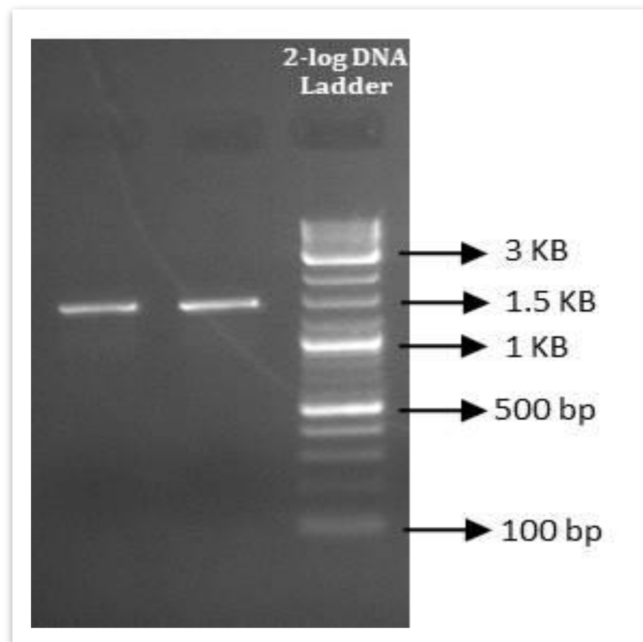
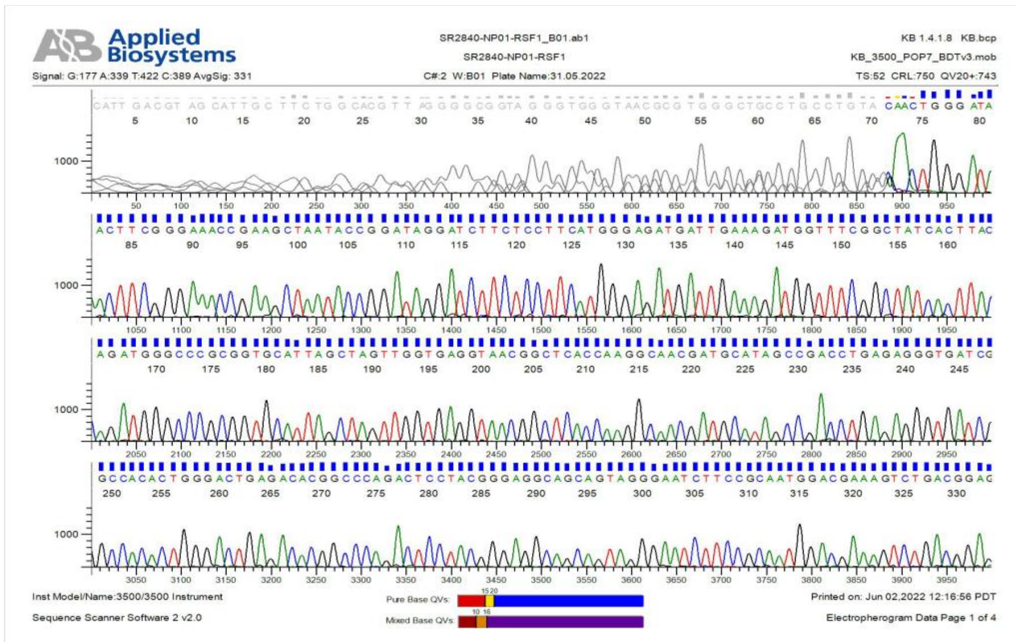


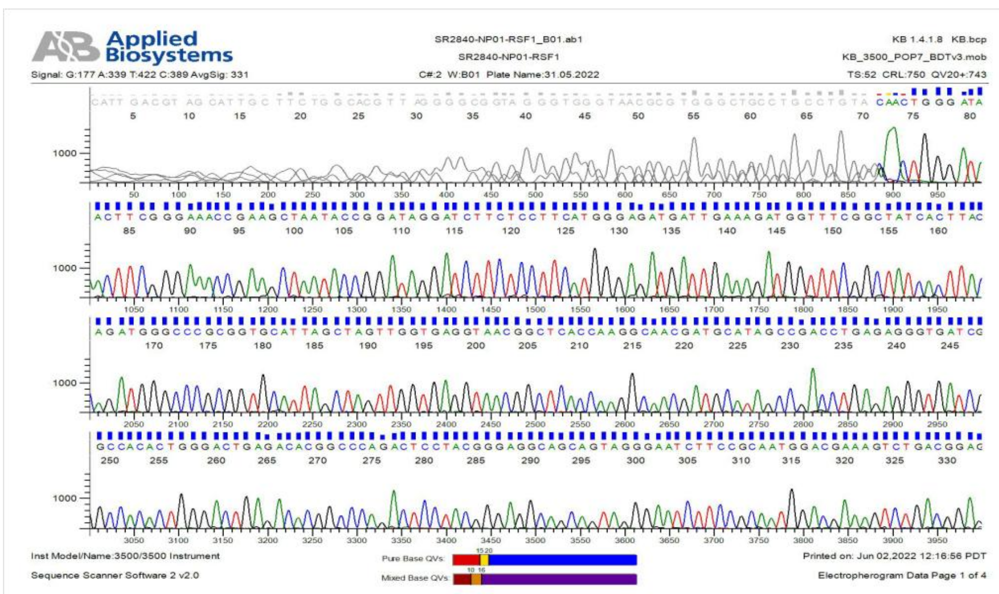
Figure: Band Observed on Gel Documentation

c) SEQUENCE ANALYSIS:

Nucleotide sequence determination PCR products of the 16S rDNA of strains were analysed for nucleotide sequences. The homology of partial sequences obtained were compared with the sequences from the DNA database and similarity showing above 95% were retrieved by Nucleotide Basic Local Alignment Tool (BLAST) program at national centre for biotechnology information server. Sequence obtained were compared against the sequences available in NCBI, database using BLAST.

Figure : sequence analysis of isolate code **NP 01**





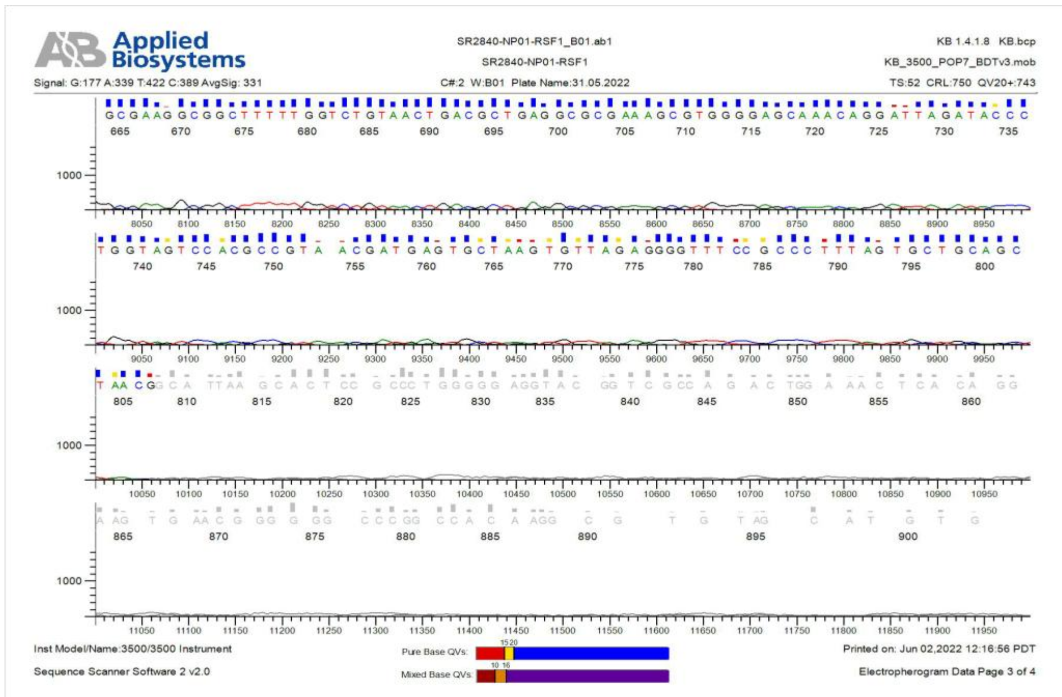


Figure : sequence analysis of isolate code **NP 01**

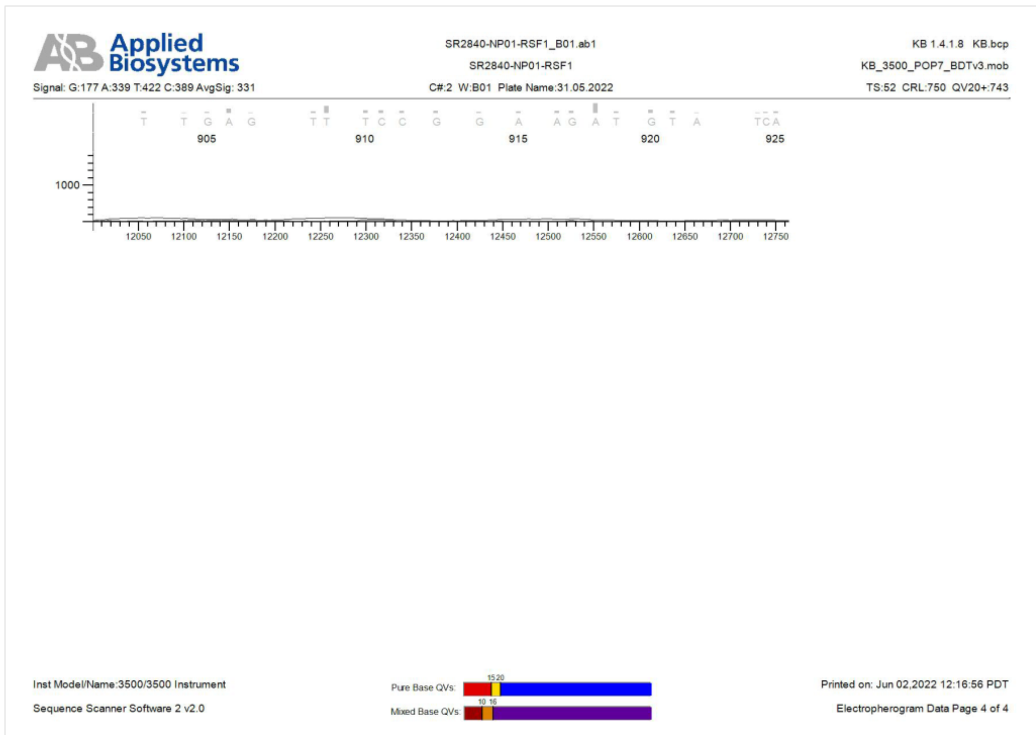


Figure : sequence analysis of isolate code **NP 01**

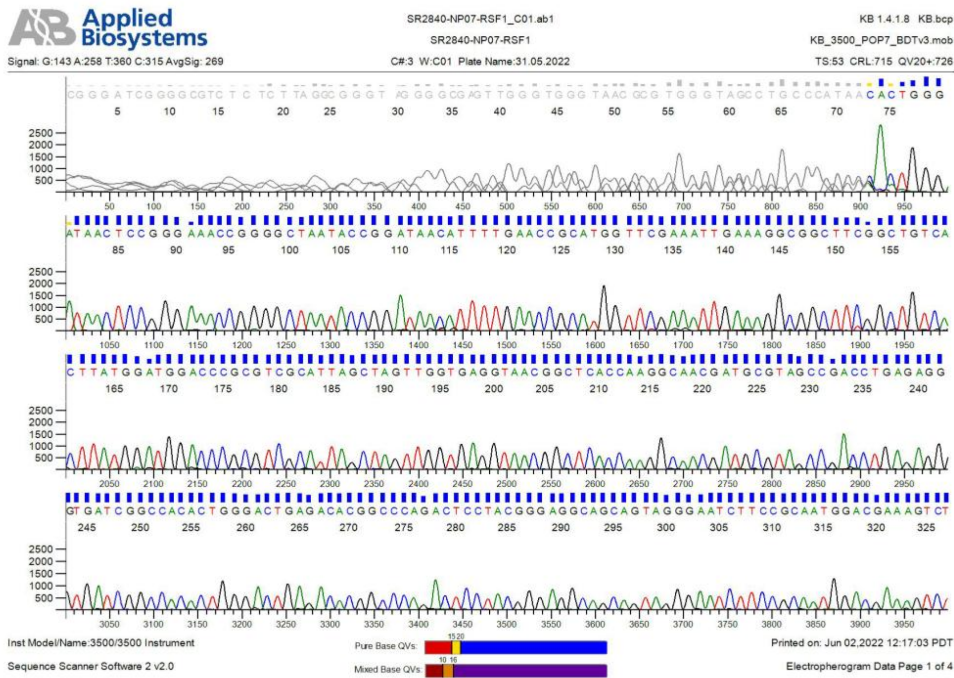


Figure : sequence analysis of isolate code **NP 07**

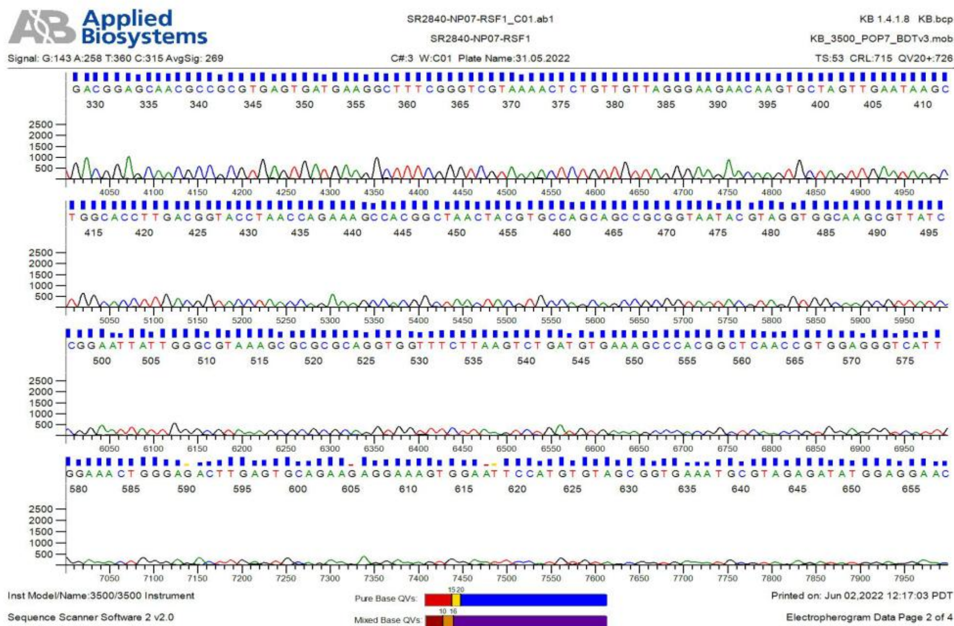


Figure : sequence analysis of isolate code **NP 07**

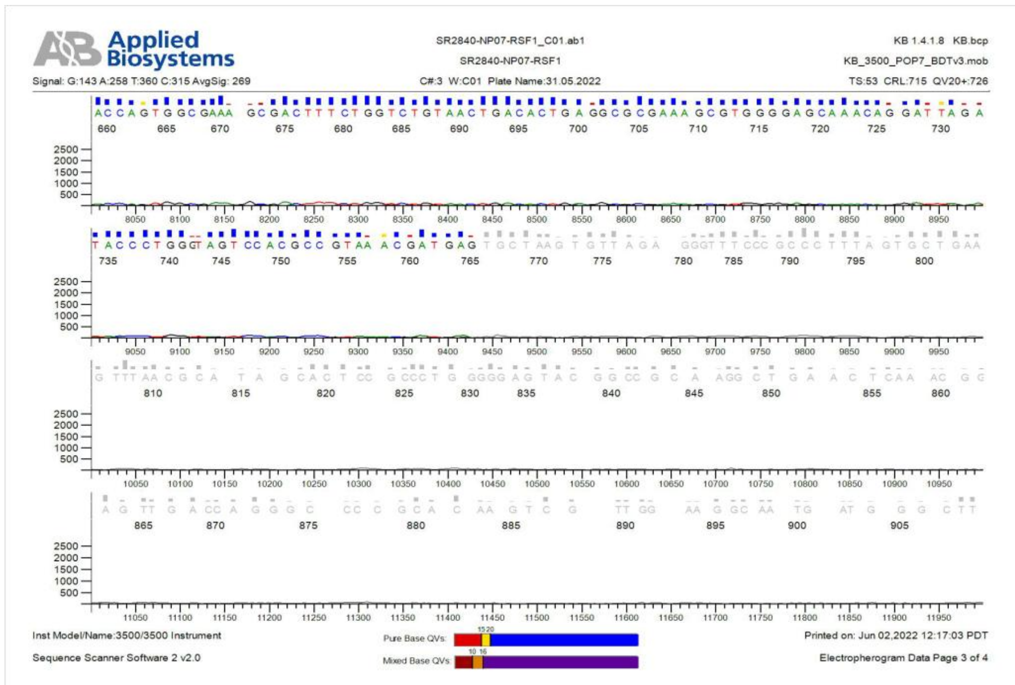


Figure : sequence analysis of isolate code **NP 07**

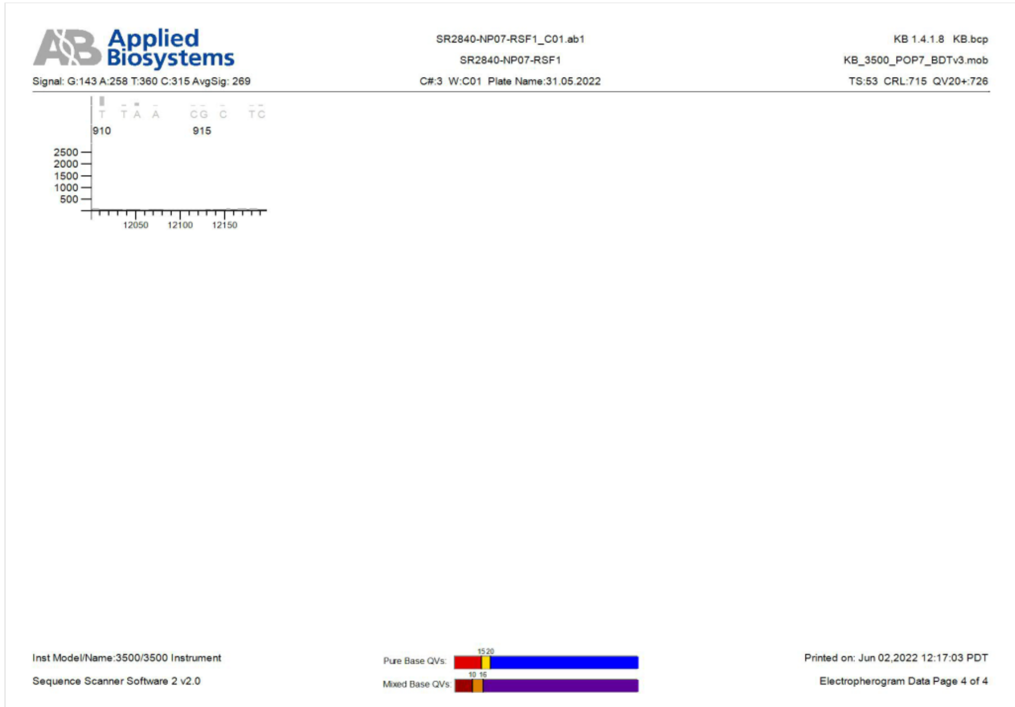


Figure : sequence analysis of isolate code **NP 07**

d) DNA SEQUENCES :

DNA Sequence Of Isolate NP 01 ***: Priestia magaterium***

GCCGAACTGAGAATGGTTTTATGGGATTGGCTTGACCTCGCGGTCTTGCAGCCCTTT
GTACCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGAC
GTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTAA
ATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCA
CGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGTCCCCCGAAGGGGAA
CGCTCTATCTCTAGAGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTG
CTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAG
TTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCAC
TAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAG
GGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCA
AAAAGCCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACAC
GTGGAATTCCGCTTTTCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCAC
GGTTGAG

DNA Sequence of Isolate NP 07: ***Bacillus cerus***

CACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCAT
GGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATT
AGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAG
GGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAG
TAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATG
AAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAA
GCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGC
CGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCG
CAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTG
GAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGA
AATGCGTAGAGATATGGAGGAACACCAGTGGCGAAAGCGACTTTCTGGTCTGTAAC
TGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGGTAGTC
CACGCCGTAAACGATGAG

DISCUSSION

The word ‘‘plastic’’derived from the Greek word ‘‘Plastikos”, that means which can be molded into different shapes. Plastics stated as the polymers which start moving on heating so can be casted into moulds. Generally, plastic materials are derived from petrochemicals except biodegradable bioplastic. Plastic consists of chloride, oxygen, hydrogen, carbon, silicon and nitrogen. Polyethylene consists of 64% of total plastic and its general formula is CnH2n. One of the rapidly growing fields in global industry is the production of synthetic plastics. Plastics are more superior than other materials due to their unique properties and due to their overuse and rapid accumulation they impart a high threats to ecosystem. Hence measure needed to be taken for eradicating them. In this regard, microbial degradation is one of the best options. Some reports on the biodegradation of plastics indicate that it could be a viable proposition when suitable microorganisms are utilized

This study has covered the major concerns about the natural and synthetic polymers, their types, uses and degradability also it has looked at the disposal methods and the standards used in assessing polymer degradation. Another area examined has been the biodegradation of plastics by the liquid culture method. It is clear that most recalcitrant polymers can be degraded to some extent in the appropriate environment at the right concentration.

Microorganisms that are capable of degrading polymers have been investigated and isolated from the natural environment. Polymer materials that are used for microbial degradation e.g., polyethylene and polypropylene (Park and Kim, 2019) similarly in this study bacterial colony were isolated by means of of streak and slant isolation method and were found to have different growth rate , colour and pattern of growth. The isolated bacterial strains were then examined for their plastic biodegradation capacity by means of weight loss method and was found that certain stain clearly showed the degradation capacity

Similar Microbial degradation of a solid polymer like polyethylene that requires the formation of a biofilm on the polymer surface to enable the microbes to efficiently utilize the non-soluble substrates by enzymatic degradation activities were also found in the culture tube with the inoculum and plastic kept for the analyze. Development of multicellular microbial

communities known as biofilm, attached to the surface of synthetic wastes confers that they are the powerful degrading agents in nature.

The results obtained were in agreement with the studies that the bacterial colonies isolated from the plastic showed a clear extent of biodegradation of that plastic and was able to found out by means of weight loss method. Each microbe has different characteristic, so degradation ability possessed will be variated between one microbe with another. Microbe different characteristic includes type of enzyme produced for biodegradation process that helped in polymer degradation. Exoenzymes from microorganisms break down complex polymers during degradation yielding smaller molecules of short chains, that are small enough to pass the semi-permeable outer bacterial membranes, and then utilized as carbon and energy sources.

Result of present study agrees with the fact that microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastics (Gu et al., 2000a).From the study we could conclude that there are potential bacterias in nature that has high plastic biodegradation capacity and in such a way plastic waste that accumulate in our ecosystem can be naturally eradicated by means of such natural microbes with such potential to degrade plastic.

SUMMARY AND CONCLUSION

Biodegradation is the process by which organic substances are broken down by living organisms. This term is often used in relation to ecology, waste management, bioremediation and to the plastic materials, because of their long life span.

This study has covered the major concerns about the natural and synthetic polymers, their types, uses and degradability also it has looked at the disposal methods and the standards used in assessing polymer degradation. Another area examined has been the biodegradation of plastics by the liquid culture method also called as weight loss method. It is clear that most recalcitrant polymers can be degraded to some extent in the appropriate environment at the right concentration.

The present study deals with the isolation, identification and degradative ability of plastic degrading microorganisms from plastic and sediment samples collected from polluted Perumpilly ketu of Njarackal Panchayat of Ernakulam district . Sediment and plastics sample were collected prior to experiment commencement from selected study areas were the plastic accumulation was observed high. This plastic was used to study their biodegradation by microorganisms isolated from them.

Microbial degradation of a solid polymer like polyethylene requires the formation of a biofilm on the polymer surface to enable the microbes to efficiently utilize the non-soluble substrates by enzymatic degradation activities. Development of multicellular microbial communities known as biofilm, attached to the surface of synthetic wastes have been found to be powerful degrading agents in nature. When the total biodegradation process of any organic substrate is considered the formation of microbial colony is critical to the initiation of biodegradation. Thus, the duration of the microbial colonization is an important factor that effects total degradation period. The bacterial isolates were screened to identify the one with highest potential by using weight loss method. The screened microbial species that showed greater degrading capacity for plastic were identified as both Gram positive . In the present study pieces of plastics with equivalent weight were inoculated in the liquid culture medium inoculated with bacterial isolates and kept for 28 days to observe the percentage of weight loss by bacteria.

About 11 different bacterial strains were individually isolated from the plastic sample collected and were given a code starting with NP following the numbers from 1 to 11 respectively. Similarly 9 different strains were also isolated from the sediment sample collected The result shows the degradative ability of the microorganisms after 28 days of incubation. The percentage of weight loss due to degradation was found more by strains coded NP01 and NP 07 . This shows it has the greater potential of degradation compared to other bacteria.

Later the DNA isolation ,PCR amplification, Gel electrophoresis of PCR products and futher sequencing of the DNA so obtained were done to identify the bacterial species. Sequence so obtained were run on BLAST (Basic local alignment tool) to identify the species which produced a result of two bacteria named ***Priestia magatarium*** and ***Bacillus cerus***. This clearly showed that these two species were having the highest potential for plastic biodegradation compared to others. Among these two bacterial isolates *Bacillus cerus* showed more degradation capacity than *Priestia magatarium*

From the study we could conclude that there are potential bacterias in nature that has high plastic biodegradation capacity and in such a way plastic waste that accumulate in our ecosystem can be naturally eradicated by means of such natural microbes with such potential to degrade plastic. In the present work the bacterial strains that showed high plastic biodegradation capacity were identified as *Priestia magatarium* and *Bacillus cerus*. Among them isolate coded NP07 that was identified as *Bacillus cerus* showed greatest degrading ability than all other strains. The isolated microbes were native to the site of polyethylene disposal and shown some degradability in natural conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media.

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