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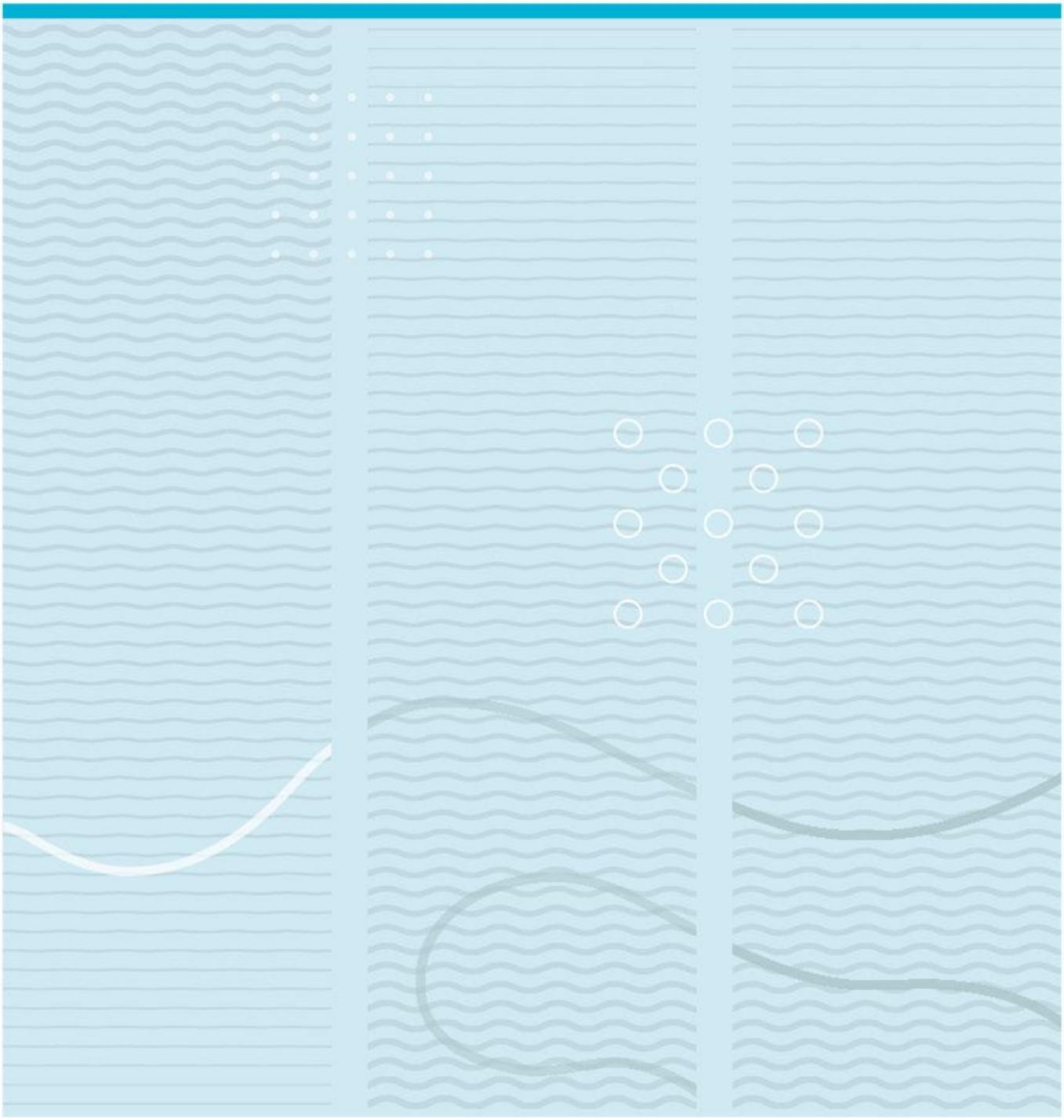
Isolation and growth of plastic-degrading microbes



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Abstract

Plastic waste is a significant threat to wildlife and marine life, presenting a potential for a biotechnological based on biodegradation using bacteria that break down polymers in plastic into monomers or oligomers, allowing them to be disposed of more quickly (Khan and Majeed 2019). This work aims to isolate and grow microorganisms that can degrade low-density polyethylene (LDPE) and use it as a carbon source. Low-density polyethylene (LDPE) was used as a substrate, which was industrially produced without any additives, and a piece of old plastic was used as a source of microorganisms. Microorganisms were cultured in a growth medium to which (LDPE) was added to obtain only LDPE degradation isolates. The following isolates were obtained (AH1, AH2, AH3, AH4, AH5, AH1a, AH1b, AH3b1, AH4b1, AH4b2, AH4b3, AH3a, AH4a, and AH1a). Each of the isolates (AH1, AH2, AH3, AH4, AH5, AH1a, AH1b, AH4b3, AH4b2) was marked by staining and some tests were performed to characterize them. The isolates were identified by 16s RNA is sequencing. Some analytical tests such as the growth test, the CO2 produced test, and a weight loss test was also performed to monitor biodeterioration. Only AH2 showed growth in the first stage of growth measurement of isolates (AH1, AH2, AH3, AH4, and AH5), and only AH4b2 reported growth in the second stage of growth measurement of isolates (AH4b2, AH4b3, AH1a, and AH1b). The isolates (AH4b2 and AH2) both showed negative growth during the growth confirmation stage. When measuring the amount of carbon dioxide produced and calculating the weight loss for the isolates (AH4b2 and AH2), negative results were obtained, which indicates the unreliability of biodegradation and it requires more investigation.

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List of Abbreviations

PE	Polyethylene
PP	Polypropylene
PU	Polyurethanes
PS	Polystyrene
PVC	Polyvinyl Chloride
PET	Polyethylene Terephthalate
PTFE	Polytetrafluoroethylene
PHAs	Polyhydroxyalkanoates
PHB	Polyhydroxy Butyrate
PLA	Polylactic acid
HDPE	High-density polyethylene
MDPE	Medium density polyethylene
LDPE	Low-density polyethylene

LLDPE	Linear low-density polyethylene
VLDPE	Very-low-density polyethylene
LDPE	Ultra-low-density polyethylene
PMMA	Polymethyl methacrylate
PVAc	Poly (vinyl acetate)
PCL	(poly[caprolactone])
PLA	Polylactide
PC	Polycarbonate
LMWPE	Low molecular weight polyethylene
UV	Ultraviolet
V	Vanadium
Co	Cobalt
Ti	Titanium
TGA	Thermogravimetric analysis
FTIR	Spectroscopy
VMR	Nuclear Magnetic Resonance
GPC	Gel permeation chromatography
HPCL	High-Performance Liquid Chromatography Systems
SEM	Scanning electron microscope
POPs	Persistent organic pollutants
BFRs	Brominated flame retardants
FTIR	Fourier transform infrared analysis
SEM	Scanning electron microscopy
XRD	X-ray diffraction analysis
NBT	Nanobarium titanate

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Abdulaziz Hajyousef

1 Introduction

Plastic was not popular, but in the past six decades it has become a multi-use and essential product in daily life that cannot be dispensed with, and this is due to its properties, chemical composition, and applications (Alabi, Ologbonjaye et al. 2019)**.** Plastic has become a necessary need in our daily lives, and the word "plastic" is a Greek word that means something that can be shaped into different shapes (Shah, Hasan et al. 2008). Plastic is a synthetic polymer that contains hydrogen, silicon, oxygen, nitrogen, and chloride. Plastic is manufactured from different derivatives such as oil, coal, and natural gas derivatives and has different types such as polyethylene (PE), Polyethylene Terephthalate (PET), nylons, Polypropylene (PP), Polystyrene (PS), Polyvinyl Chloride (PVC), and Polyurethane (PUR), Which is the most widely used type of plastic in the packaging process, accounting for 90% of the volume of plastic used in packaging (Alavi, Thomas et al. 2014), (Alshehrei 2017). And the impact of packaging materials is not limited to the environment only, there may be a transfer of chemicals added to packaging materials to improve their properties to food and their interaction with it, causing a decrease in food quality at a certain concentration (Bhunia, Sablani et al. 2013). Plastic is a synthetic material that has been widely spread due to its good advantages such as lightweight, low cost, and durability. It is involved in many industries such as food, medical, transportation, and clothing industries. Plastics consist of petroleum materials such as polythene and polypropylene, which are resistant to biodegradation (MICROBES and AMOAH 2016). Most of the plastic that is used is inert due to poor waste management and a lack of oversight. Plastic is produced unevenly from one country to another, as China ranks first in the world in the production of plastics with a waste rate of 23.9% and Europe is in second place with a rate of 21% of the global production of plastics. We can estimate the annual use of plastic bags at 500 billion to a trillion bags (Khan and Majeed 2019). In terms of consumption, India recorded an increase in consumption, reaching 400 tons/year in 1992 and then increasing to 5 million tons /and the increase reached 8 million tons/year in 2008, and this increase in consumption continues from year to year due to the increasing demand for plastic (Singh and Ruj 2015). It has been pointed out that the rate of plastic production will double by 2025 and triple by 2050, compared to increased growth rates (Lusher, Hollman et al. 2017). Plastics are stable and slow to decompose, which is a problem for the environment. In 2003, according to statistics published by the US Environmental Protection Agency, 236 million tons of solid waste were generated before being recycled, 11% of which consisted of plastic (mostly from soft drinks and other bottles) (Zheng, Yanful et al. 2005). The dangers of plastic come from entering the environment, accumulating, and then transporting in the form of microplastics through food chains (Hermabessiere, Dehaut et al. 2017). The term microplastics first appeared in the year 2004, which refers to the fragmentation of

plastic into fragments with a diameter of less than 5 mm, which arises from the production of plastic pellets within the microsize range and the degradation of plastic debris of larger size, as after decomposing into fragments it becomes less subject to physical and optical degradation and this It causes the accumulation of microplastics and has sustainable effects on the environment, and it may enter the human body through the fresh and saltwater environment because of the accumulation in the bodies of animals that humans feed on (Li, Zhu et al. 2020). And because of the additives that plastic enjoys and the increase in consumption in the past years, plastic production doubled from 1.5 million tons in 1950 to 360 million tons in 2018 fig1. Concerns about plastics were only aesthetically polluting the environment and then soon became an environmental problem of global concern due to the formation of microplastics smaller than 5 mm, their transport and accumulation in the food chains, and the formation of new environments for pathogens (Matjašič, Simčič et al. 2021). Billions of tons of plastic are produced annually, but only about 21% is dealt with. It is disposed of either on land in various ways, such as burning or throwing into the oceans, causing environmental pollution. On land, burning leads to chemical pollution. In the oceans, plastic kills millions of species, including endangered ones, through ingestion or entanglement and impeding movement (Kaushal, Khatri et al. 2021). Indonesia comes after China in the production of plastic waste at a rate of 3.2 million tons annually, which is approximately 10% of the global total of plastic waste, and statistics indicate that 15% of daily waste is plastic (Asiandu, Wahyudi et al. 2021).The process of burning plastics will lead to the release of organic pollutants into the environment, which are persistent, for example, when burning polyvinyl chloride in waste incinerators, furans and dioxins will be released, which cause lung and immune system problems and are classified as carcinogens (Alshehrei 2017). This huge production of plastic in the world is multi-use, as 30% is used in food packaging, clothing, detergents, cosmetics, etc. and there is an annual increase of 12%. Plastic has been relied on more than paper or replacement because of its tensile strength, air resistance, and biodegradation (Muhonja, Makonde et al. 2018). Various physical and chemical methods are relied upon to get rid of plastic waste. All these methods are characterized as being environmentally useless by causing harm to the environment and humans, and costly economically. There are many of these methods, including burning and the pollutants left behind in the environment, followed by backfilling, which takes a great deal of time and because of anaerobic conditions as well. Pollutants such as dioxins, which have a harmful effect, are released. Also, of these methods used is recycling, which is a method that does not achieve the required efficiency in addition to the high economic costs. Efforts had to be made to search for a feasible and effective way to get rid of plastic waste and achieve environmental and economic feasibility through biodegradation, as this method depends on the ability of microorganisms to degrade plastic through extracellular enzymatic secretion and convert polymers into simpler

components (Gupta and Devi 2017, Chen, Dai et al. 2020). Research work on the biodegradation approach of plastics has been started in 1970, as many types of plastic materials did not comply with the microbial attacks, such as polyethylene, Polypropylene, Polystyrene, and Polyvinyl Chloride, and many microorganisms were identified, such as fungi and bacteria, which had an amazing ability to biodegradation for plastic (Iram, Riaz et al. 2019). Also, some manufactured plastics such as polyester and polyurethane can degrade by mixing them with starch, but most of the manufactured plastics used today are not biodegradable or take hundreds of years to decompose, and due to the presence of biodegradable polymers, this matter called for thinking about modifying the current products until, they are biodegradable or try to find new biodegradable polymers through a combination of mechanisms (biodegradation, photolysis, thermal degradation and environmental erosion (Shah, Hasan et al. 2008). In general, the biodegradation of plastic by microorganisms is a very slow process, and some microorganisms are ineffective in degrading some types of plastic (Khan and Majeed 2019).

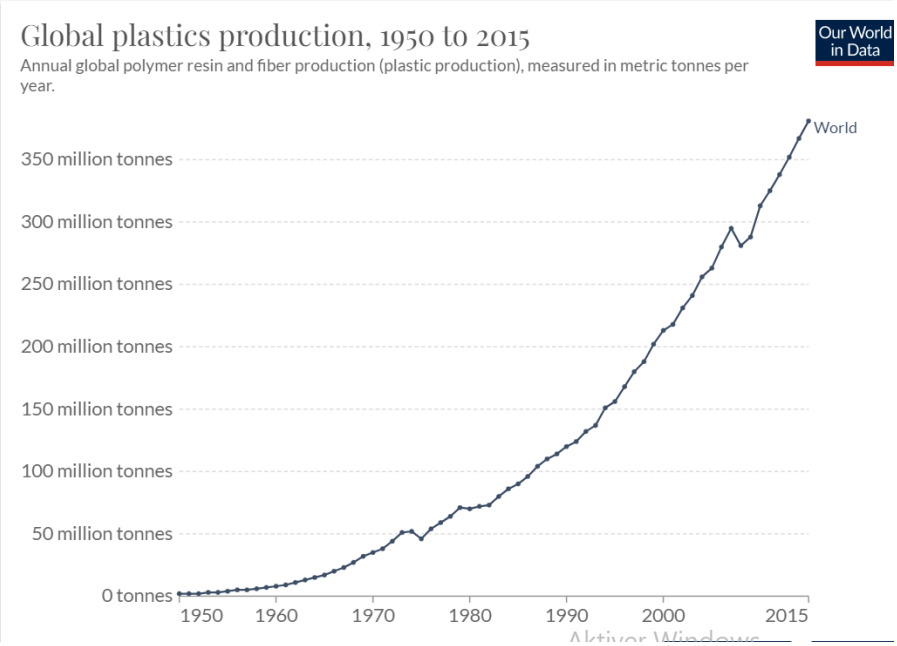


Figure1. Global plastic production from 1950 to 2015 (Ritchie and Roser 2018).

1.1 Aims and objectives of the study

Isolate microbes can use polyethylene as a sole carbon source.

Investigate their identity and growth characteristics.

1.2 Hypotheses

H0: There is the reliability of biodegradation

HA: There is the unreliability of biodegradation

1.3 Plastics

Plastic is an umbrella term that includes a wide range of materials made from polymers and moldable additives (Clunies-Ross 2019). Plastics are made from any material that contains carbon and hydrogen, but at present fossil fuels (oil and gas) are the leading material in the plastics industry. The global plastic production process requires approximately 4% of raw materials from oil, gas, and similar energy (Andrady and Neal 2009). Life is not without polymers, which play an important role in our daily lives, whether they are synthetic polymers such as plastics and adhesive waste, or natural biological ones such as cellulose, proteins, and nucleic acids. **Polymer** is defined as a two-syllable word, poly, meaning multiple, and mer, meaning part or segment. Therefore, Polymers are made up of smaller units called monomers. An example of polymers is polyethylene, which is the most widely used polymer and consists of a group of ethylene monomersfig2. (Nambiar, Padma et al. 2018). **Natural polymers** are condensation polymers resulting from the condensation reaction in nature. Water and methanol form their by-products. They are found in milk, cellulose, plants, and insects. **Synthetic polymers** are derived from petroleum oil after cracking and refining petroleum derivatives. Nylon, Teflon, polyethylene, bakelite, elastomer, and polyvinyl chloride are examples of synthetic polymers. Celluloid is an industrial plastic discovered by the American industrialist Jhon Wesley Hyatt and for which he obtained a patent (Nambiar, Padma et al. 2018). The production of synthetic polymers is increasing because of replacing natural fibers such as wool, silk, and cotton with synthetic fibers, which is another source that represents a large part of the production of synthetic polymers, as the production of synthetic fibers reached 61 million tons in 2015 (Lusher, Hollman et al. 2017). In terms of structure, polymers can be divided into linear, branched, and crosslinked polymers. Linear polymers are polymers in which monomer molecules are joined together one length and continue to form a polymer molecule. Branched polymers are characterized by the presence of

side branches for each molecule of linked monomer molecules that protrude from central branching points of different lengths along the main polymer chain in the form of a comb with long branches (A), short branches (B), or a tree structure (C). The importance of branching comes from changing the properties of the polymer (crystallization), whereby increased branching leads to a decrease in crystallization and thus the polymer is more susceptible to degradation. To produce polymers in which polymer molecules are attached at points other than their ends, we call these crosslinked polymers fig3 (Odian 2004).

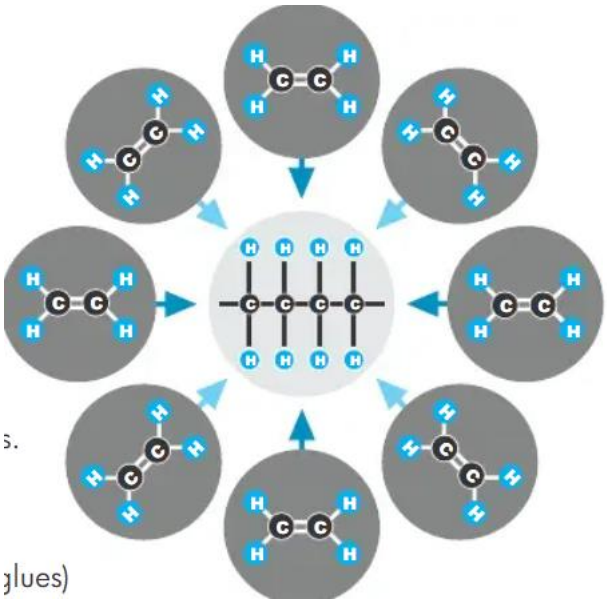


Figure2. Ethylene (monomer) molecules combine to former a polyethylene polymer

(Nambiar, Padma et al. 2018).

Plastic is characterized by a chemically inert nature, which makes it highly durable and gives it an undesirable property, which causes creating environmental challenges through the inability to dispose of plastic waste or recycle it properly, as it accumulates as garbage and poses a serious threat to the environment. The duration of plastic life in nature has not been properly evaluated due to insufficient time, but most types of plastic can accumulate in the environment for up to 5 decades. Also, 60-80% of the garbage in the world is in the form of plastic (Nerland, Halsband et al. 2014).

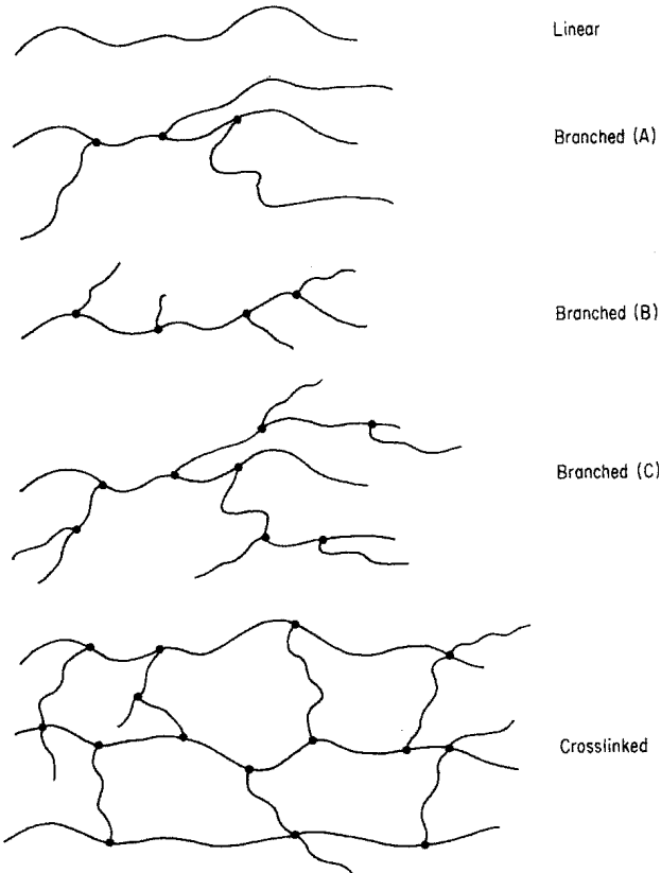


Figure3. Structure of polymers (linear, branched, and cross-linked)

(A) long, (B)short, (C) tree (branched) (Odian 2004).

1.4 The important and use of plastic

The use of plastic includes all aspects of life in society, from the production of clothes, shoes, and products used in health and food. More than 40 million tons of plastic from nylon, polyester, and acrylic are used in the garment industry by converting them into textifibersres. Plastic also forms a large part of the shoe industry, where polyurethane is used in the manufacture of outsoles and footbeds, or by using another flexible material. Other industrial polymers are also used in the manufacture of upper parts of shoes. From a health point of view, plastic facilitates the provision of clean drinking water through transportation and reduces food waste through packaging. Due to the lightweight of plastic, it reduces transportation costs, and this reduces carbon dioxide emissions into the atmosphere, as plastic enters the manufacture of vehicles by up to 20% in each of the wheels, doors, electrical, and electronics. Plastic is also used in the manufacture of aircraft, such as the Boeing Dreamliner, with a percentage of up to 50% (Andrady and Neal 2009). In the field of agriculture and horticulture, plastic is used in many agricultural applications such as sheets and films to protect crops

from damage, in transporting and storing products in the form of containers, and in the process of supporting irrigation and preserving wastewater. Plastic also enters many applications in the construction sector such as floors, walls, upholstery, windows, insulation, and hoses, which improve performance and reduce high costs (Clunies-Ross 2019). Thus, plastic achieves high efficiency in use at all levels and in all sectors, due to its diversity of design with a high degree of strength, durability, rigidity, corrosion resistance, ductility, electrical and thermal insulation, vital inertness, durabiliqualityity, and noncityicity fig4 (Andrady and Neal 2009).

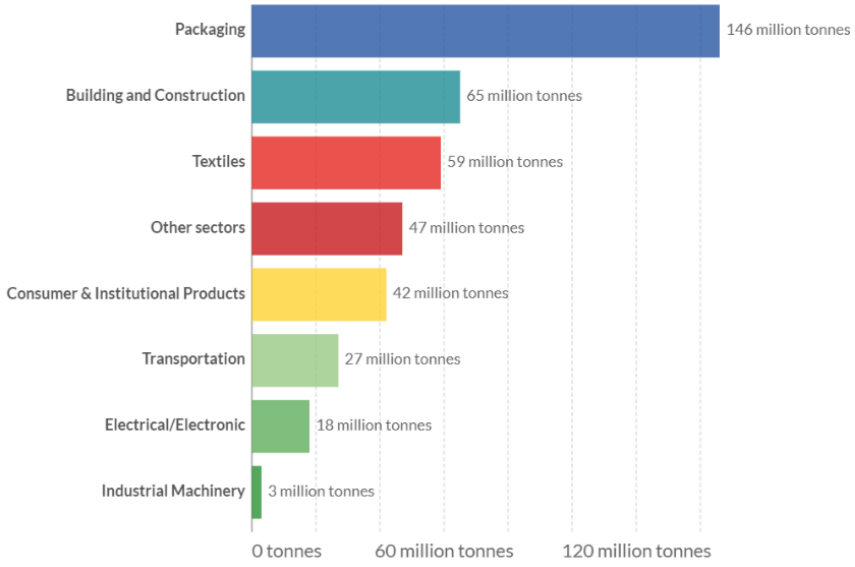


Figure4. Plastic production by sector in 2015, with packaging having the largest share, with production reaching 146 million tonnes (Ritchie and Roser 2018).

1.5 Type of plastics

Categories and Classification of Plastics

1.5.1 Thermal Properties

Plastics are known as industrial polymers and their manufacture is through two processes, the first process is by breaking the double bond in the original olefin through additional polymerization to form new carbon-carbon bonds, which are all carbon-chain said polymers, whereas polyolefins such as polyethylene and polypropylene are manufactured through this reaction. Or the second process of manufacturing polymers depends on the condensation or elimination of water between the carboxylic acid and the alcohol or amine to form a polyester or polyamide. We also get polyurethane through this reaction. Plastics are divided into two groups depending on their thermal properties **thermoplastic** polymers and **thermosetting** polymer. Thermoplastic polymers are plastic materials

that when heated do not undergo any chemical change in their composition and can be formed repeatedly resulting from the first type of reaction. It consists of chains with repeating subunits derived from monomers. Each polymer chain has thousands of repeating units. Thermosetting polymers are characterized by their resistance to hydrolysis or hydrolysis of chemical bonds (Zheng, Yanful et al. 2005). Polyethylene (PE), Polypropylene(PP), polystyrene(PS), polyvinyl chloride(PVC), and polytetrafluoroethylene(PTFE) are examples of thermoplastics (Raziyafathima, Praseetha et al. 2016). The chains of polymers of this type are cohesive without cross-links between the chains, with weak attractive forces, which make them soft upon heating fig5(right). Thermosetting polymers are the product of the second type of reaction mentioned above, after being converted to a liquid state by heat, subject to freezing in an irreversible process. They are characterized by having a highly bonded structure such as ester bonds or amide bonds, which distinguishes them from thermoplastics such as the vulcanization of rubber (Zheng, Yanful et al. 2005). Formaldehyde polyurethanes, Phenol are examples of thermoset plastics (Raziyafathima, Praseetha et al. 2016). The polymer chains here are interconnected through strong covalent bonding which makes them unbreakable upon heating and inelastic fig5(leftt).

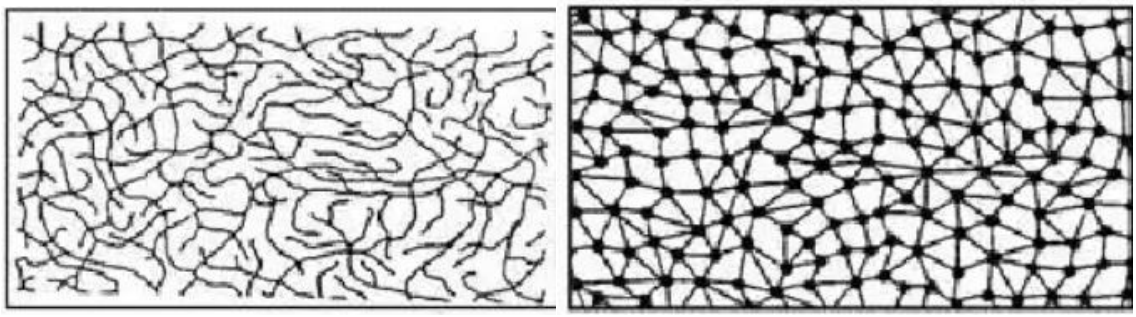


Figure5. Polymeric chains: On the left are the polymeric chains of thermoplastics and on the right are the polymeric chains of thermosetting plastics (Chaudhari 2014).

1.5.2 Design Properties

There is a classification of plastics based on their manufacturing and design processes, which include electrical conductivity, toughness, tensile strength, degradability, and thermal stability (Alshehrei 2017).

1.5.3 Degradability Properties

Depends on chemical properties to classify plastics as pre-degradable or non-biodegradable. Non-biodegradable plastics include synthetic elastomers, which contain a high frequency of micro-monomer units resulting in a high molecular weight. Biodegradable plastics are made from natural

materials that completely decompose, such as components of live plants and algae as a source of proteins, starch, and algal materials. Or by a group of microorganisms. Biodegradable plastics break down upon exposure to ultraviolet (UV) rays, water, and enzymes and gradually change the ph. Biodegradable plastics are divided into four groups Photodegradable bioplastics, compostable bioplastics, bio-based bioplastics, and biodegradable bioplastics (Reddy, Ghai et al. 2003, Alshehrei 2017).

1.6 Type of plastic degradation

There is a group of mechanical, light, thermal, and chemical transformations that plastic undergoes in nature as a result of exposure to external conditions, weather, burial, and aging before the start of biological decomposition (Lucas, Bienaime et al. 2008). Plastics deteriorate in nature because of physical or chemical changes in the polymer structure because of environmental factors such as heat, light, humidity, chemical conditions, or biological activity. These processes which cause a change in the structure of the polymer due to the physical, chemical and biological reactions which subsequently cause a change are called polymer decomposition (Shah, Hasan et al. 2008). Depending on the nature of the factors involved in polymer degradation, polymer degradation has been categorized as thermal degradation, photo-oxidative degradation, mechanochemical degradation, catalytic degradation, ozone-induced degradation, and biodegradation (Singh and Sharma 2008).

1.6.1 Thermal degradation

Thermal and photochemical degradations are classified as oxidative degradation, where they are similar under normal conditions with a difference in the sequence of initial steps that lead to the spontaneous oxidation cycle and also differ in the place of occurrence where the thermal reactions occur in almost all parts of the polymer and the photochemical reactions occur within the surface (Singh and Sharma 2008). Thermal degradation is a process of partial deterioration of plastic due to high temperatures. At high temperatures, the components of the long chains of the polymer separate, followed by the interaction of the chains with each other, which in turn leads to a change in the properties of the polymer (Shah, Hasan et al. 2008). Polystyrene (PS) is degraded by heat to give organic compounds such as phenol, quinine, naphthalene, and diphenylamine at experimental temperatures of 350-450 °C. Also, the reactions that occur during the thermal degradation process depend on several factors such as pressure, heating rate, reaction medium, and reactor geometry. Polymers also have a high viscosity, which makes the process complicated by impeding heat transfer (Singh and Sharma 2008). There are chemical reactions involved in thermal deterioration that ultimately led to a change in the physical and optical properties of the polymer. Changes in the

physical properties include the molecular weight distribution of the polymer, while changes in the optical properties include color change, cracking, chalking, embrittlement, and a decrease in ductility(Shah, Hasan et al. 2008). It was confirmed too that the thermoplastics of low-density polyethylene(LDPE) showed susceptibility to thermal-oxidative radiation through the addition of auxiliary oxidants such as (Zn, Cu, Ag, Co, Ni, Fe, Mn, Cr, and V) (Lucas, Bienaime et al. 2008).

1.6.2 Mechanochemical degradation

This type of chemical-mechanical degradation of polymers occurs under the influence of mechanical stress through strong ultrasonic radiation. When mechanical shear or stress has applied the breakdown of the molecular chains occurs through a chemical reaction known as mechanochemical degradation. High molecular weight polystyrene (PS) deteriorates through turbulent flow and lower drag reduction efficiency with time is due to the mechanical degradation of polymer molecules. One study indicated that when mechanical mixing of binary mixtures of cis-polyisoprene with stereo-regular poly(butadiene), and with butadiene methyl, styrene, or butyl rubbers leads, significant changes occurred in the molecular properties of the components due to the mechanical deterioration of polymers during the mechanical mixing process. Also, the mechanochemical reaction to remove chlorine from polyvinyl chloride (PVC) using powders of different oxides, such as Cao, Fe2o3, Si,o2, and Alo3, in the air, leads to a reduction in the molecular weight polyvinyl chloride (PVC) (Singh and Sharma 2008).

1.6.3 Photo-oxidative degradation

The process by which high-intensity photon particles are bombarded to degrade long-chain polymers into single units (Iram, Riaz et al. 2019). Oxo-biodegradation in this process uses two methods to initiate biodegradation: Photolysis (UV) and oxidation, in photolysis, the final product is reduced by (UV) rays, and oxidation breaks down the plastic through time and heat. Both methods reduce the molecular weight of the plastic and thus allow the plastic to degrade (Lucas, Bienaime et al. 2008). Naturally, this process occurs through light, as most synthetic polymers are subjected to degradation caused by ultraviolet and visible light, where the life of the polymers is determined by the near near-ultraviolet in sunlight within the range 290of -400 nm (Singh and Sharma 2008). Ultraviolet rays have enough energy to crack the carbon-carbon bond. The wavelength of ultraviolet rays that causes the most damage to plastic depends on the available bonds, and therefore there is a difference in deterioration according to different wavelengths of rays and different types of plastic, as it is 300 nm for polyethylene(PE) and 370 nm for polypropylene (PP) (Singh and Sharma 2008). To increase the effectiveness of the physical and chemical procedures for polymer degradation, synthetic oxo-

degradable synthetic polymers were introduced, which consist of oxidation-supporting units, which often consist of a mineral base such as Mn, Co, Fe, or Ti, and when subjected to photo or thermal degradation, they give a partial formation (Iram, Riaz et al. 2019).

1.6.4 Catalytic degradation

This type of waste catalytic conversion of polymer to high-value hydrocarbons is important in this field. Polyolefins polystyrene, polyethylene, and polypropylene, which make up a large part of household waste, are of interest in the field of catalytic conversion as they degrade into oils and gases. An investigation was carried out for the catalytic degradation of polyolefins, where thermogravimetric analysis (TGA) was used as a method to screen the catalysts, and it was concluded that the presence of the catalyst led to a decrease in the energy required for activation (Garforth et al). A range of different stimuli such as Pt–Mo supported over Sio2 and Pt-Co, zeolite catalysts and non-zeolite catalysts, transition metal catalysts (Cr, Ni, Mo, Co, Fe) on the support (Al2o3, Sio2), zirconium hydride and zeolite was used in polymer degradation. The process of adding a catalyst not only improves the quality of the thermal transformation products for plastics but also includes reducing decomposition temperatures and achieving selectivity for a specific product (Singh and Sharma 2008). The thermal or catalytic deterioration of plastic waste into fuel represents a great potential to be a successful commercial move in the future because plastic waste is a cheap source of raw materials in periods of depletion of natural resources (Akpanudoh, Gobin et al. 2005).

1.6.5 Ozone-induced degradation

It is caused by atmospheric ozone where polymers deteriorate under normal conditions when aging oxidation processes are slow and polymer maintains its properties for a longer period. The ozone in the air accelerates the aging period of polymers even at very low ozone concentrations. This process of saturated polymers is accompanied by an intense formation of compounds that contain oxygen, a change in the molecular weight, and the weakening of the mechanical and electrical properties of the samples. The polymers when exposed to ozone result in the formation of a variety of unsaturated carbonyl and carbonyl products, followed by the formation of graded groups of aliphatic esters, ketones, and lactones as well as aromatic carbonyl associated with the styrene phase. Ozen et al. reported an attack on polystyrene, one of the unsaturated polymers, where the yield from this attack was 35% peroxide, 18% ketone, and 47% acid, similar to the products obtained from oxidation of the free radical chain (Singh and Sharma 2008).

1.6.6 Biodegradation

Biodegradation may occur through one of the above-mentioned mechanisms (thermal degradation or mechanochemical degradation or photo-oxidative degradation or ozone-induced degradation or catalytic degradation) individually or a combination of mechanisms (Singh and Sharma 2008)**.** Biodegradation has been defined in different ways (Singh and Sharma 2008). Biodegradation is a process in which microorganisms are relied upon to break down organic matter into simpler substances under aerobic and anaerobic conditions. This term is used in waste management, environmental treatment, and plastics due to its longevity (lifespan of deterioration). Where the products of cracking are converted into metals within the so-called metallurgical process (Shah, Hasan et al. 2008). Within this concept, there is a need for different types of organisms, some of which break down long-chained polymers into simple shapes, while the other section can use simple monomers and liberate some simple wastes, and another section can destroy these wastes (Iram, Riaz et al. 2019). Biodegradation on the other hand is defined as a process by which substances are degraded into carbon dioxide, methane, water, inorganic compounds, or biomass where the enzymatic action of microorganisms is the dominant mechanism. Biodegradation is also defined as the tendency of a substance to break down into the molecules that make up it within the natural processes, which are often microbial digestion, where the receptors resulting from the degradation are non-toxic to the environment and are redistributed through the sulfur, nitrogen, and carbon cycles. Biodegradation of polymers is enhanced by increasing their microbial colonizing surface area or by decreasing their molecular weight through abiotic hydrolysis degradation, physical disintegration, and photo-oxidation of polymers (Singh and Sharma 2008). Biodegradation can be considered as a chemical sourced from microorganisms that attack the polymer. Enzymes are chemicals that come from microorganisms and are catalytic to attack the polymer, and the attack depends on the availability and specificity of the enzymes, providing sites for the enzymes to attack the polymer and providing a coenzyme when needed. Both enzymatic and hydrolytic processes may contribute to degradation through different levels during the different stages of degradation. The onset of degradation may be through hydrolysis and with progression, the polymer breaks down and there is an increase in surface area and accessibility resulting in enzymatic hydrolysis may be prevalent. Thus, biological degradation is comprehensive of all types of degradation that occur in the body, regardless of whether they are the result of metabolism or hydrolysis. Biodegradation is also defined as the conversion of substances into less complex intermediate substances or final products through simple hydrolysis or dissolution or the action of biologically constituent entities such as enzymes and other organism products. Degradation of polymer molecules may occur, but not necessarily to produce fragments, but the integrity of the material decreases within this process (Singh and Sharma 2008). It is a process

that occurs in the environment by microorganisms (bacteria and fungi) through the events of changing or transforming any enzymatic decomposition in the structure of chemicals that are foreign to the environment to metabolites such as Co2, H2O, and CH4, for example when plastic waste is thrown into the aquatic environment. This plastic waste goes through several stages, the first of which are processes the physical process is through fractionation first and the formation of smaller particles (microplastics), and therefore the decomposition of plastic waste is affected by biotic and abiotic factors. So, the ability of microorganisms to adhere to the surface of the plastic and form films does not depend only on microbes but is related to other factors such as the characteristics of water and the nature of the surface that is targeted by amicroorganisms (Urbanek, Rymowicz et al. 2018). This change of substance in which microorganisms participate occurs under both aerobic and anaerobic conditions. Biodegradation under aerobic conditions results in CO2, H2O, and, under anaerobic conditions, CO2, H2O in addition to CH4 (Alshehrei 2017). The ability of microorganisms to degrade the polymer decreases when the molecular weight of the polymer is increased as the increase in the molecular weight leads to a significant decrease in solubility and becomes unsuitable for microbial attack (Shah, Hasan et al. 2008).

1.7 Steps of plastic degradation by microorganisms

It includes many steps, namely:

1.7.1 Bio-deterioration

The surface deteriorathat causes a change in the physical, chemical, and mechanical surface properties of the plastic. It is caused by microbes and other organisms that cause physical and chemical degradation. It is through the weakening of the polymer structure by abiotic factors wthatabiotic factors act as a synergistic factor or as a contributor to the initiation of biodegradation with microbial activity. Biodegradation takes place through the formation of a microscopic membrane on the surface or inside the polymer fig6 (Iram, Riaz et al. 2019) (Dussud and Ghiglione 2014).

1.7.2 Bio-fragmentation

Catalytic procedures, work to cleave polymer plastics into an oligomer or monomer by enzymes or free radicals that are secreted outside the cell by bacteria. Polymers are characterized by high molecular weight and do not cross the cell wall, but microorganisms secrete extracellular enzymes that catalyze reactions at the polymer boundary. To perform biodegradation, there must be an imbalance in the charge, as biodegradation seeks to stabilize the polymer, where bacteria work to dislodge the balanced charge of the long chains of the polymer by secreting enzymes such as

oxygenase, which add oxygen to the long carbon chain, thus forming groups with less resistance to degradation biological (Dussud and Ghiglione 2014). This stage requires energy to cleave the bonds and obtain oligomers or monomers capable of crossing the cell membrane, which comes from various mechanical, thermal, chemical, or biological sources fig6 (Lucas, Bienaime et al. 2008).

1.7.3 Assimilation

Inclusion in the cytoplasm of transported molecules in microbial metabolism. The Assimilation indicates the integration of the atoms inside the microbial cell, but the monomers may not be completely degraded, this leads to the formation of secondary metabolites, and because of the lack of the metabolic ability to convert them, they are transported outside the microbial cell and there is no need to metabolize or store them. These secondary receptors can be used from a neighboring cell to achieve further deterioration fig6 (Dussud and Ghiglione 2014).

1.7.4 Mineralization.

Oxidative receptor secretion (CO2 ، N2 ، CH4 ، H2O), refers to the complete breakdown of molecules fig6 (Dussud and Ghiglione 2014).

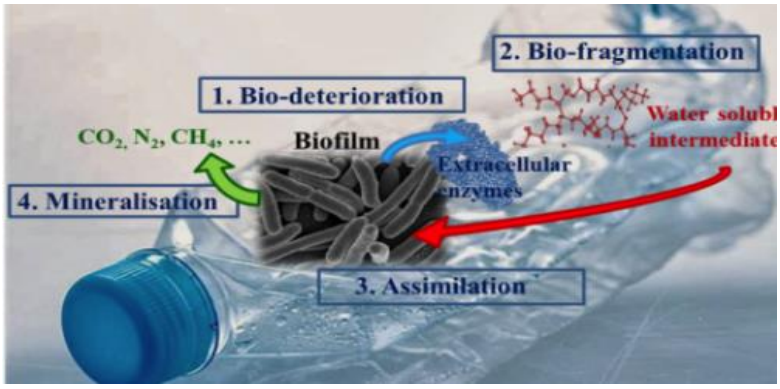


Figure6. The steps of plastic biodegradation by a microorganisms

(Dussud and Ghiglione 2014).

1.8 Aerobic and anaerobic biodegradation

1.8.1 Aerobic biodegradation

It is also called aerobic respiration, which is an important part of natural attenuation, which includes decontamination of waste disposal sites. Aerobic microbes work to break down organic matter into smaller or shorter-chained compounds, and the by-product is CO2 and H2O, depending on oxygen. C plastic + O2 → CO2 + H2O + C residual +Biomass fig7 (Alshehrei 2017).

1.8.2 Anaerobic biodegradation

It is also important to reduce pollution in waste sites under anaerobic conditions where oxygen is not available. Sulfur, nitrate, and iron are used as a source to break down organic matter into smaller long-chained compounds that microorganisms cannot utilize and transport into the cell. To solve this problem, the presence of organic compounds with long chains, microorganisms developed a strategy by secreting enzymes outside the cell that convert the compounds into short chains that are used as a source of carbon and energy by microorganisms and are known as depolymerization. The by-product of this process is (CO2, H2O, and CH4), which known as the mineralization process

C plastic → CH4 + CO2 + H2O + C residual +Biomass fig7 (Alshehrei 2017).

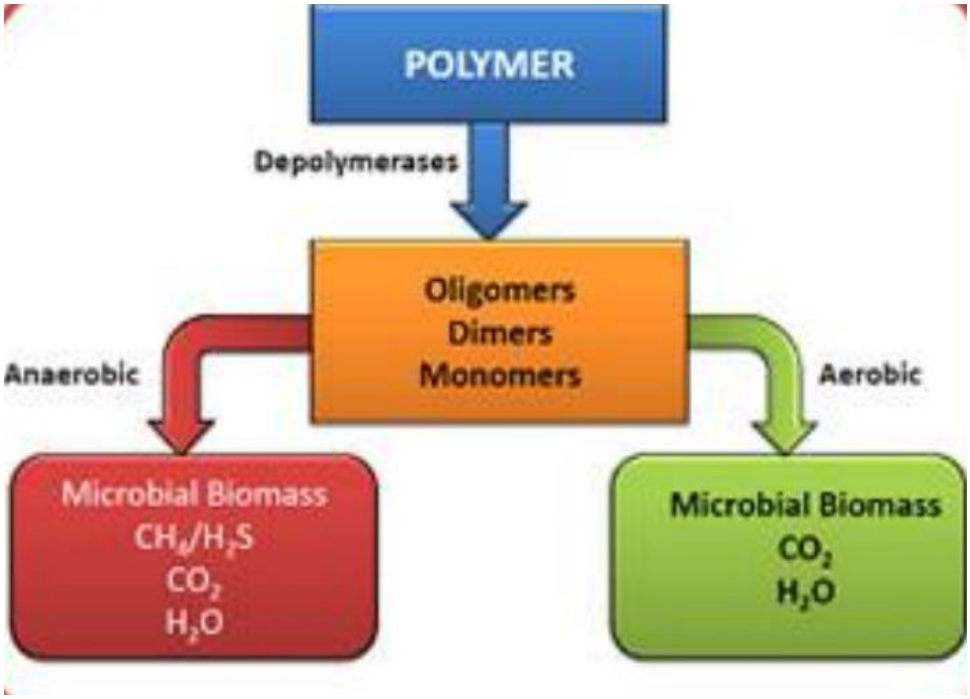


Figure7. Degradation of polymers under aerobic and anaerobic conditions (Mohan 2011).

1.9 Biodegradation of natural plastics

Interesting because most of them are biodegradable and are synthesized by microorganisms. It enters under the name of biomaterials, which is a polyester that is widely distributed in nature and is collected inside the cells of microorganisms in the form of granules with properties like those of petrochemical plastics. These polymers are constructed from hydroxy acyl derivatives through a metabolic pathway. Bioplastics differ in their monomer composition, macromolecular structure, and physical properties (Luengo, García et al. 2003) (Chandra and Rustgi 1998). In addition to being manufactured by microorganisms, it can also be synthesized by resources available in nature such as components of live plants and algae which are used as a source of cellulose, starch, protein, and algal

materials. Bioplastics degrade upon exposure to UV rays, water, and enzymes, and the pH changes gradually. Bioplastics are divided into four groups: photodegradable bioplastics, biodegradable bioplastics, bioplastics, and biodegradable bioplastics. Most biodegradable polymers are characterized by the presence of water-degradable bonds along the backbone of the polymer chain: polyester, polyamide, polyurea, polyurethane, polyanhydride, and polyphosphate. Polysaccharides such as starch are among the most commercially used polymers table1 (CATIA BASTIOLI 2021).

Class of bioplastic	Consist of	Degrading
polyhydroxyalkanoates (PHAs)	biodegradable polyester	*Burkholderia, Bacillus, Cupriavidusand Nocardiopsis*, a new enzyme depolymerase
Polyhydroxy Butyrate (PHB)	is made by microorganisms in response to physiological stress	105 species of fungi that were isolated from natural habitats and lichens proved 41. Of them the ability to degrade PHA
polylactic acid (PLA)	is made from corn starch, sugar cane, or tapioca root.	*B. licheniformis and Amycolatopsis sp* in soil

*Table*1. *Some types of bioplastics, their components, and the way they are degraded (Khan and Majeed 2019).*

1.10 Biodegradation of synthetic plastics

The advantages that industrial plastic enjoys in terms of low cost, weight, and durability make it a basic material in many industries such as the automobile industry, medical equipment, children's toys, and food packaging. Many polymers and additional materials such as plasticizers, metallic materials, and UV stabilizers are included in the plastic composition, whereas industrial polymers are manufactured from fossil materials such as crude oil, coal, or natural gas (Biopolymers). Synthetic plastics are slow to degrade in nature, where several environmental factors participate in their decomposition, then these factors are followed by the role and impact of microorganisms (biodegradation) through hydrolysis and oxidation. Where microorganisms attack the surface of the plastic and secrete enzymes that break the polymers of high molecular weight and turn them into polymers of low molecular weight that dissolve in water and make the properties of the surface

of the plastic more susceptible to attack by microorganisms (Shah, Hasan et al. 2008, Haben Fesseha 2019). By relying on the degradation pathways, synthetic plastics are divided into two groups: plastics with carbon backbone chains (Polyethylene**,** Polypropylene**,** Polystyrene**,** and Polyvinyl Chloride) and plastics with heterogeneous atoms in the main chain **(**Polyethylene Terephthalate**,** and Polyurethanes) fig8 (Mohanan, Montazer et al. 2020).

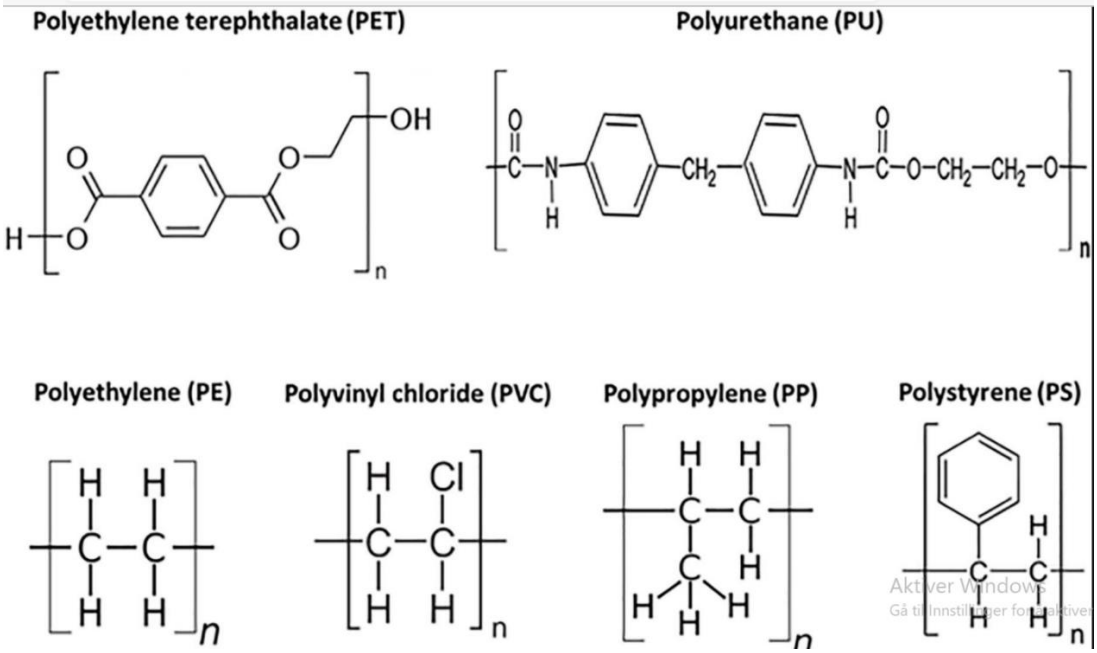


Figure8. Structures of synthetic common polymers (Mohanan, Montazer et al. 2020).

1.10.1Plastic with carbon spine chains.

Polyethylene**,** Polypropylene**,** Polystyrene**,** Polyvinyl Chloride

The backbone of the chains of this group contains only carbon atoms. These polymers mainly contribute to packaging materials. In carbon-carbon backbone polymers, photooxidation (UV and oxygen) is the abiotic initiating factor responsible for polymer degradation as photooxidation leads to chain separation. For polypropylene, polyethylene, and polystyrene photooxidation reduces the molecular weight and forms carboxylic groups as final groups and then UV light starts to dechlorinate polyvinyl chloride. Thus, within this group, abiotic degradation is first, then biological degradation (Mohanan, Montazer et al. 2020).

1.10.1.1 Polyethylene (PE)

It belongs to the group of polyolefins and is characterized as inert materials consisting of long chains of carbon that are not degradable by microorganisms (Shah, Hasan et al. 2008) Polyethylene is a high molecular weight synthetic polymer that is highly resistant to hydrolysis and does not degrade in its natural form and takes

different densities and different physical structures depending on the different manufacturing processes of the linear chains (Alavi, Thomas et al. 2014)Table2. It poses a danger to the environment due to its use in the packaging(Shah, Hasan et al. 2008). Some studies have demonstrated the ability of microorganisms to degrade low molecular weight polyethylene oligomers (MW=600_800) by *Actinobacteria spp* (Alshehrei 2017, Haben Fesseha 2019). However, high molecular weight polyethylene cannot be degraded by microorganisms. Efforts were made not only on polyethylene which biodegrades but also on polyethylene, which does not biodegrade due to extensive use as a packaging material (Shah, Hasan et al. 2008). Biodegradation by microorganisms of polyethylene is preceded by photolysis and chemical decomposition. Biodegradation of polyethylene occurs through hydrolysis and oxo-photolysis where UV light activates an inert material such as polyethylene at the start of the reaction (Haben Fesseha 2019). Another method used to stimulate the inert material is polyethylene, before the reaction begins, adding nitric acid to act as a catalyst and activator in preparation for the start of the role of microorganisms fig9 (Haben Fesseha 2019). For polyethylene to become biodegradable, an adjustment must be made in its crystalline level, molecular weight, and mechanical properties, which play an important role in the resistance of polyethylene to degradation, to be available to microorganisms for biodegradation (Shah, Hasan et al. 2008). It was also found that after exposure of polyethylene (PE) to ultraviolet rays or heat treatment, it becomes subject to depolymerization by some types of bacteria, such as *Bacillus spp, Rhodococcus ssp,* and *Pseudomonas ssp,* and some types of fungi, such as *Aspergillus* and *Fusarium* where the carbon chains of the polymer become sensitive to biodegradation. The ability of thermophilic bacteria *Brevibacillus borstelensis* strain 707 was shown that at a temperature of 50 ° C for 30 days, the weight loss of polyethylene material was achieved at an average of 11%, where the rate of weight loss can be increased to three times when using the photo-oxidized polymer (Wierckx, Narancic et al. 2018). Also, through research, the ability to degrade polyethylene (PE) was demonstrated by bacteria that live in the intestines of the wax worm (*Galleria melonella*) (Mohanan, Montazer et al. 2020) it was a *Bacillus ssp* strain that was isolated from waxworms and incubated for 60 days with PE film, it caused a loss of 10% of the weight of polyethylene (PE) (Wierckx, Narancic et al. 2018). And the isolation of microorganisms capable of hydrolyzing polyethylene(PE) in soil, water, and activated sludge (Mohanan, Montazer et al. 2020).

Types of polyethylene	Density (g/cm^3)
High-density polyethylene (HDPE)	0.94–0.96
Medium-density polyethylene (MDPE)	0.93–0.94
Low-density polyethylene (LDPE)	0.91–0.93
linear low-density polyethylene (LLDPE)	0.91–0.93
Very low-density polyethylene (VLDPE)	0.89–0.91
Ultra-low-density polyethylene (ULDPE	< 0.89

Table2. Types of polyethylene (Alavi, Thomas et al. 2014).

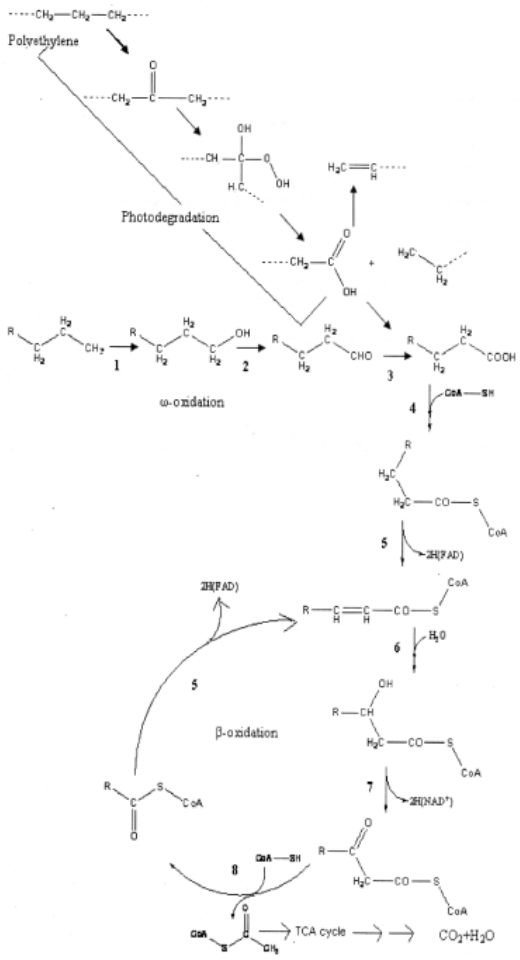


Figure9. Mechanism of biodegradation of polyethylene

Photooxidation pathway supporting biodegradability

(Arutchelvi, Sudhakar et al. 2008).

1.10.1.2 Polypropylene (PP)

It is a thermoplastic synthetic that has wide uses in daily life in plastic molds, stationery, folders, diapers, and non-absorbable threads. Studies have been conducted to prove the biodegradation of polypropylene by microorganisms, through which the contribution of microbial groups, fungi, and bacteria such as *Aspergillus Niger, Pseudomonas,* and *Vibrio bacteria*, to the degradation of polypropylene (PP), has been demonstrated, as the viscosity decreased and new groups are formed, which are carbonyl and carboxyl during the deterioration process (Alshehrei 2017, Haben Fesseha 2019). It was also observed that the weight of PP decreased by an average of 2.5% of its total weight after one year of incubation with *Bacillus* and *Pseudomonas* strains where polypropylene (PP) was treated with high temperatures compared to another group that was not exposed to high temperature and therefore no significant change in its weight was observed

(Wierckx, Narancic et al. 2018). Some studies have attempted to increase the biodegradability of PP by facilitating the formation of biofilm microbes on the surface of polypropylene (PP) by adding natural biodegradable polymers such as natural fibers or starch. However, the biodegradation efficacy was limited (Jeon and Kim 2016).

1.10.1.3 Polystyrene (PS)

It is a high molecular weight synthetic polymer that is recyclable but not biodegradable. It has been reported to biodegrade at room temperature but in very low amounts. It has the advantage that it expands upon heating above the glass transition temperature but returns to the solid-state upon cooling. It is transparent and is used as cutlery and cups, as well as for packaging and insulation (Tokiwa, Calabia et al. 2009). It is manufactured from a styrene monomer and may be in a solid or foamy state, while a styrene monomer is liquid and has a relatively low melting point (Ho**,** Roberts et al. 2018**).** Reports revealed that mealworm gut bacteria were able to metabolize half the number of PS used as carbon source within 16 days using pure bacterial cultures of *Exiguobacterium sp.* The ability of the YT2 strain isolated from the gut of mealworm to degrade polystyrene (PS) was reduced to 7% within 60 days of incubation (Wierckx, Narancic et al. 2018). Bacterial decomposition of PS and release of styrene, benzene, acrolein, and toluene when exposed to heat and chemicals have also been reported (Shah, Hasan et al. 2008, Alavi, Thomas et al. 2014). Biodegradation of polystyrene (PS) by use of pure microbial strains was studied as Shimbe et al reported biodegradation of modified PS by pure strains of *Pseudomonas areuginosa*. Another approach was also taken by placing polystyrene (PS) in different conditions such as marine, sludge, compost, or soil to demonstrate the ability to biodegrade or find microbes capable of degrading polystyrene (PS). Through this approach, Nikolic et al. monitored biodegradation through mass reduction, where samples of mixed polymer PS-graft-starch were used within earthen containers containing different types of soil at a depth of 6 cm, where the soil-grown with patience achieved the highest level of biodegradation(Ho**,** Roberts et al. 2018**).** Polystyrene PS may be degraded by microorganisms but tfew studies haveindicated or reported on enzymes or microorganisms that are used in biodegradation (Ho**,** Roberts et al. 2018**).**

1.10.1.4 Polyvinyl Chloride (PVC)

It is ranked third in the world in terms of production after polyethylene and polypropylene. It is widely spread due to its low cost and excellent physical and chemical properties (Wang, Wang et al. 2020). It is used in the construction industry in pipes, insulation fittings, electrical wires, leather products, and building floor coverings (Shah, Hasan et al. 2008). No complete or successful biodegradation of polyvinyl chloride (PVC) has been reported so far as it showed high resistance when buried in soil for 32 years and no meaningful biodegradation was observed (Wierckx, Narancic et al. 2018). According to Karpas et al. low molecular weight polyvinyl chloride (PVC) can be biodegraded by the black mold fungus. There are numerous studies on the acoustic and thermal degradation of polyvinyl chloride (Alshehrei 2017).

1.10.2 Plastic with heterogeneous bonds

(Polyethylene Terephthalate**,** Polyurethanes)

Plastics of this group contain heteroatoms in their main chain and their polymers are subject to hydrolysis as ester or amide bonds. The plastics in this group can deteriorate through photo-oxidation, hydrolysis, and biodegradation. The degradation results in smaller fragments and final carboxylic groups (Mohanan, Montazer et al. 2020).

1.10.2.1 Polyethylene Terephthalate (PET)

The molecular weight of this group is from 30,000 to 80,000. It has different properties. It is a polymer with a semi-crystalline structure and is chemically thermally stable. Microbial degradation affects the crystal structure as the microbes inside the polyethylene terephthalate (PET) were viewed through an electron microscope. The studies also showed that chemical changes occurred to the polymeric chains through X-rays (Alshehrei 2017). The importance of pyrolysis (biotechnological conversion) in converting polyethylene terephthalate (PET) to polyhydroxyalkanoate by different species of *Pseudomonas* has been pointed out. Polyethylene terephthalate (PET) was converted through pyrolysis to terephthalate which was used as feedstock for P. putida Go16. The degradation of polyethylene terephthalate (PET) films depends on a variety of factors such as the degree of crystallinity, orientation of polymer chains, and purity. Degradation of commercially available (pure and amorphous) polyethylene terephthalate (PET) film has been demonstrated at temperatures of 50°C (approximately 5%) and at temperatures rising to 55,60,65°C the degradation has increased to more than 50% (Ho**,** Roberts et al. 2018**).**

1.10.2.2 Polyurethanes (PU)

It is used in industries such as pillows, rubber goods, leather, adhesives, plastic foams, and paints, and most of its commercial products are derived from polymer diol such as PCL-diol. There are two types of polyurethane, the ester type, and the ether type. It was reported by Darby and Kaplan that polyester polyurethane (ES-PU) was more susceptible to fungal attacks than ether polyurethane (ET-PU). Studies have shown that no microbe can completely degrade polyurethane, and this leads to it being difficult to determine what is the fate of the residues after degradation by microorganisms and enzymes for ES-PU and whether ET-PU has been degraded to a large degree by microbes (Tokiwa, Calabia et al. 2009).

1.11 Enzymes involved in the hydrolysis of Petro-polymers (synthetic polymers)

Enzymes are defined as catalysts that increase the rate of chemical reactions in environments that are not suitable for chemical reactions. Through the binding of enzymes on the surface, they form active sites in which the reaction between the substrate and the enzyme takes place leading to a chemical reaction and the formation of a specific product. Some enzymes are specific to a specific substrate and others attack a series of substrates. To achieve the integrated activity, the enzymes must bind with cofactors of organic origin, such as metal ions, or of inorganic origins, such as ATP. Enzymes follow different mechanisms of catalysis either by changing the substrate by adding free islands or by chemical alternative methods. As a result, the behaviour of different enzymes (complementary or synergistically) makes it impossible to identify enzymes so some examples of enzymes that degrade polymers will be presented. Enzymes follow different mechanisms of catalysis, either by changing the substrate, by free radicals, or by chemical methods **(**CATIA BASTIOLI 2021**).**

1.11.1Enzymic hydrolysis

Several different enzymes such as proteases, esterase, and glucoside hydrolysates are involved in hydrolysis to separate glycoside bonds, peptide bonds, most ester bonds in proteins, nucleic acids, polysaccharides, and polyhydroxy alkanoic acids. The type of enzyme used also depends on the type of bond to be broken down.

1.11.1.1 Proteases

These proteolytic enzymes catalyse the hydrolysis of amide and sometimes ester-bound hydrolysis. By its mechanism of action, it is divided into four groups (a) the serine proteases, (b) the cysteine proteases, (c) the metal-containing proteases, and(d) the aspartic proteases.

1.11.1.2 Esterase (EC 3.1)

Most tissues contain a large group of these enzymes with esterase activity. You add water as a second additional component to split ester bonds. They are divided into groups depending on the acid involved in the ester substrate. (a) carboxylic ester hydrolases (EC3.1.1), (b) thiol ester hydrolases (EC 3.1.2), (c) phosphoric monoester hydrolases (EC 3.1.3), (d) phosphoric diester hydrolases (EC 3.1.4) and (e) sulfuric ester hydrolases (EC 3.1.6)

1.11.1.3 Glycosidases

These enzymes cleave the bond in each of the sugars such as starch, cellulose, inulin, and their derivatives. And its types

(a)the amylases (EC 3.2.1.1and EC 3.2.1.2) hydrolyze the α -1,4 and/or α -1,6 glucosidase linkages, and (b) cellulase (EC 3.2.1.4) that act on β -1,4 glucose linkages in cellulose and derived polymers

1.11.2Enzymic Oxidation

They are called oxidoreductases and are a large group of enzymes by which biological oxidation is induced. Oxidation of the substrate occurs in several ways as it is characterized depending on the electron acceptors (B, O2, or H2O2) and the formed product, which are explained in the table3. reactions (1): It contains the largest number of oxidoreductases. In these reactions, enzymes are catalysed to oxidize the substrate by removing hydrogen or an electron by sharing a receptor such NAD +, NADP +, ferricytochrome, and son (2 and 3) reactions**:** Oxygen is present or involved in these reactions and therefore does not occur except in aerobic conditions. The catalyst is carried out with the participation of a catalyst, which appears when the cycle is completed, and the catalyst does not appear in the equation as in the first type of reaction. These reactions are characterized by the fact that they are one-way and are not renewed except by using another enzyme. Reactions (4) to (7), in these reactions, the substrate is oxidized by incorporating one or more oxygen atoms into the substrate. The enzymes that carry out the fusion process are called oxygenases because these are like the reactions that occur by chemical and photochemical processes. The catalyst is done by mono-oxygen by introducing one oxygen atom to the substrate as a hydroxy group, or by Dioxygenases catalysing the introduction of an entire oxygen molecule in the substrate as a carboxyl group.

Reaction formula			
$AH_2 + B$	$\rightarrow A + BH_2$		(1)
$AH_2 + O_2$	$\rightarrow A + H_2O_2$	(H_2O_2-forming oxidases)	(2)
$AH_2 + ½\ O_2$	$\rightarrow A + H_2O$	(H_2O-forming oxidases)	(3)
$A + H_2O + B$	$\rightarrow AO + BH_2$		(4)
$A + H_2O_2$	$\rightarrow AO + H_2O$		(5)
$A + O_2 + BH_2$	$\rightarrow AO + B + H_2O$	(monooxygenases)	(6)
$A + O_2$	$\rightarrow AO_2$	(dioxygenases)	(7)

Table3. Oxidative enzymes in biological systems ***(****CATIA BASTIOLI 2021****).***

Esterases, hydrolysis, cutinises, lipase, binding units, polyesterase, laccases.

Petrochemical products are characterized by high stability and resistance to biodegradation in nature, but with this stability, some microorganisms can degrade these materials (synthetic plastics) or what is known as Petro-polymer. Microorganisms are living bioreactors that break down polymer chains through the enzymatic secretion of microorganisms and then absorb and metabolize the products of enzymatic hydrolysis (hydrolysis). Speaking of synthetic plastics such as polypropylene and polyethylene, the enzymes that break them down are not known, but despite that, some types of enzymes that break down the Petro-polymer have been identified (Mohanan, Montazer et al. 2020). In terms of enzymatic decomposition, petroleum polymers can be divided into two groups, the first group of petroleum polymers that hydrolyze (polyethylene terephthalate**,** polyurethanes) and the second group of petroleum polymers that do not hydrolyze (Polyethylene**,** polypropylene**,** polystyrene, and polyvinyl Chloride) and each group follows a biodegradation path significantly different from the second**. Esterases** are known as the enzymes that cleave ester bonds, where ester bonds in petroleum plastic polymers are the same as ester bonds in the polyester polymer. Different types of esters differ in terms of protein, biological functions, and substrate properties. Both (polyethylene terephthalate and polyurethanes) have ester bonds in their backbones, and this makes them more available for biodegradation compared to other polymers with a basic carbon chain (Polyethylene**,** polypropylene**,** polystyrene, and polyvinyl chloride). Ester-based enzymes have been identified and identified their activities that hydrolyze high molecular weight polymers (Polyethylene**,** polypropylene**,** polystyrene, and Polyvinyl Chloride), but to date, no enzymes with the ability to effectively biodegrade have been identified. Some of these enzymes secreted by microorganisms can degrade petroleum polymers and synthetic polyesters fig10. **Cutinises**: It represents one of the classes of esters and its importance comes from its ability to analyze polyesters that have a high molar mass, which are hydrolysates α or carboxylic ester hydrolysates that are extracted from fungi that cause plant diseases. A group of chitinases was purified and distinguished from bacterial strains of thermophilic actinomycetes such as *Thermomonospora fusca, Thermobifida fusca, Thermobifida alba, Thermobifida cellulosilytica, and Thermomonospora curvata*. Cutinases that degrade aliphatic polyesters such as PCL and aromatic aliphatic polyesters such as polyethylene terephthalate have also been reported. Polyethylene terephthalate hydrolysis can occur through two mechanisms, either by enzymatic modification of the surface of the polyester and this is done through surface modification enzymes such as lipase, kinases, proteases, and carboxylesterase, or enzymatic polymerization. A limited number of cutinizes that can be considered polyethylene terephthalate hydrolases that can break down the intrinsic mass of polyethylene terephthalate by 10% have been known since the first discovery of polyethylene terephthalate hydrolase. Studies have been able to test several hydrolases for the surface hydrolysis

of polyethylene terephthalate fibers such as **lipase** from *Candida Antarctica, Triticum aestivum, Thermomyces lanuginosus, and Burkholderia spp,* and fungal Cutinises from *Fusarium solani, Penicillium citrinum,* and *Aspergillus oryzae, Humicola insolens* and those from *actinomycetes, Saccharomonospora viridi, T. fusca, Thermobifida cellulosilytica, Thermobifida alba, carboxylesterases* and Esterases (*Thermobifida halotolerans*) (Mohanan, Montazer et al. 2020).

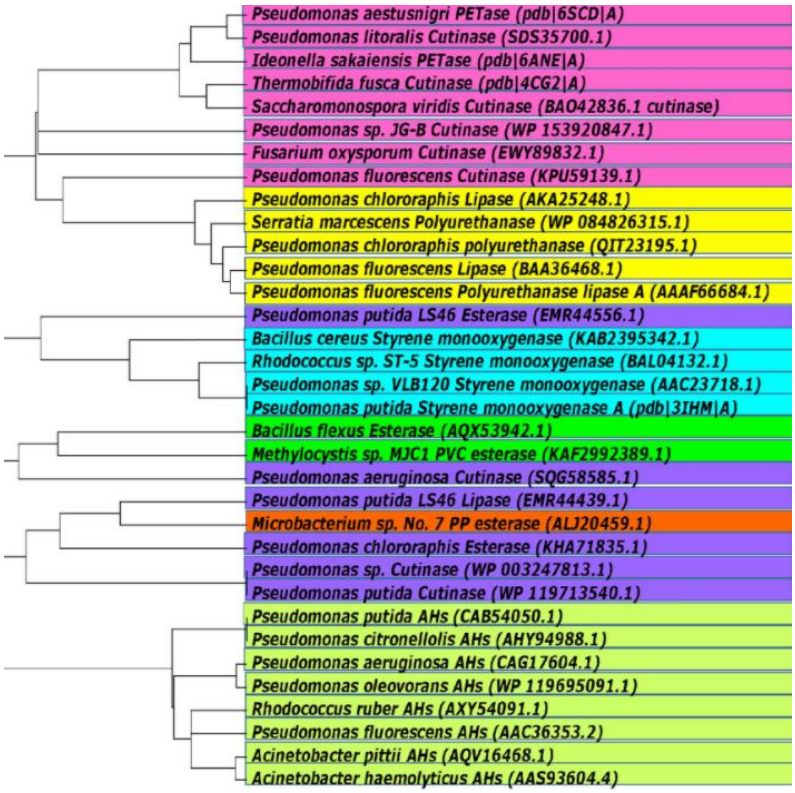


Figure10. The genetic tree that was established using molecular evolutionary genetics analysis, which shows the evolutionary relationship between bacteria based on the symmetry of the amino acid sequences of the dominant enzymes of the Petro polymer (Mohanan, Montazer et al. 2020).

In 2005 it was reported that depolymerization using 19 PET hydrolysis enzymes (PHES) was derived from esters lipase and kinases 4, 11, 13 of the bacteria *Idonella sakaiensis* Where these enzymes are active at high temperatures and weakly active at moderate temperatures, this constitutes an obstacle to degradation at the waste site. work continued with the enzyme and improving its stability and durability by constructing the famous Fast-PETase engineering variants ThermoPETase17 and DuraPETase22 through logical protein engineering and re-design where the thermal stability and catalytic activity of these two mutants were improved in certain conditions with a continued decrease in activity at moderate temperatures with the emergence of activity Excellent for Fast-PETase at moderate temperatures and moderate pH suitable for PET degradation. Fast-PETase is effective in the degradation (depolymerization) of thermoformed polycarbonate (pc-PET) products. Fifty-one samples of plastic products were taken after being used in food packaging, medicine, office supplies,

and cosmetics, and treated with Fast PET use enzyme at a temperature of 50 ° C, which led to complete deterioration of the perforated samples of PET in one week (Lu, Diaz et al. 2022). It is also possible to increase the hydrolysis of the polymers through the **binding units** such as the binding unit (PBM) as in the polyurethane PU, where the fusion between the PA polyamide and the PBM binding unit leads to a fourfold improvement in the hydrolysis of the polyurethane polymers compared to the original enzyme (Mohanan, Montazer et al. 2020). It was also demonstrated that the E4 strain plays an important role in the biodegradation of non-oxidative low molecular weight polyethylene (LMWPE), as low molecular weight polyethylene (LMWPE) has a molecular weight that is much higher than the upper limit that can penetrate microbial membranes. Jeon and Kim have reported on the alkane one-oxygen enzyme (AlKB1, AlKb2) that participates in the biodegradation of low molecular weight polyethylene (LMWPE) from *Pseudomonas* aeruginosa and its functional properties (Mohanan, Montazer et al. 2020). It was found that the mechanism of these regulatory enzymes was different and that AlKb2 was more effective in degrading than AlKB1 (Mohanan, Montazer et al. 2020). There is a possibility that there is a group of polyesterases that still need to be studied and discovered, especially in bacteria, which are a major source of chitinases. This was explained by Danson and others based on the Markov model. The marine strain of *Pseudomonas* has been considered a source of hydrolytic enzymes, which include several types which are halophilic and can tolerate heavy metals, and biotic and hydrocarbon proteins. In Austria at the Biotechnological Centre of Industry polyesterase activity was also detected in *Pseudomonas.* The presence of polyesters genes was also confirmed in *Pseudomonas* Proteinogenic using sequential research. In addition, it has been reported that several strains of *Pseudomonas* are effective in degrading many compounds and plastic materials such as (Polyethylene**,** polypropylene**,** polystyrene, and polyvinyl Chloride) (Mohanan, Montazer et al. 2020). The role of laccases (manganese peroxides and ligand peroxides) isolated from Tramets has been indicated in the degradation of high molecular weight (PE-HMW) polyethylene membrane with the presence of hydroxy benzotriazole-1 as a mediator in the oxidation of non-phenolic substrates by the enzyme (Wierckx, Narancic et al. 2018). It should be noted the role of natural polymers such as starch in increasing the degradability of plastic (Petro-polymer) when mixing the two, and this is due to the enzymatic hydrolysis of starch, which leads to the polymer (plastic) becoming porous and more biodegradable and non-biotic. Karimi and Beria reported on a mixture of starch and low-density polyethylene by using alpha-amylase as an aqueous solution, which in turn analyzed the starch as a first step and then polyethylene (Mohanan, Montazer et al. 2020).

1.12 Mechanism of biodegradation of plastic by microorganisms

Plastic degradation occurs microbially through the secretion of extracellular enzymes, which is known as the process of hydrolysis under aerobic and non-aerobic conditions. The biological decomposition of plastic takes place through several stages, the first of which begins with the agglomeration and gathering of microbes on the surface of the plastic, where it works to change the physical and chemical properties of the plastic and then convert it into water-soluble monomers through the secretion of various enzymes such as proteases that stimulate ureases or esters that make different polymers. The microbes eventually mineralize the polymer decomposition product to CO2, H2O, and CH4 where they absorb the final degradation product So the biodegradation of plastic is done through two processes: first hydrolysis and then oxidation within microorganisms(Khan and Majeed 2019). Several physical and biological forces contribute to polymer cracking such as heating, posting, drying, hydration, freezing, and thawing. Also, the fungi when it grows on the surface of the plastic cause swelling and explosion within small dimensions causing the penetration of the polymer and converting it into smaller absorbable units. Bacterial enzymes also play an important role in depolymerization, then the monomers are absorbed into the microbial cells and biodegrade(Shah, Hasan et al. 2008). Four different mechanisms are involved in the occurrence of biodegradation, namely dissolution, charge formation, dissolution, hydrolysis, and enzyme catalytic degradation (Singh and Sharma 2008).

1.12.1Solubility

The hydration of the polymer depends on the hydrophilic ability of the polymer. The hydration is caused by a disruption of the stable secondary and tertiary structure through hydrogen bonds and van der Waals forces. The polymer chains may be soluble in water during or after the hydration process, or cleavage of the polymer backbone may occur through enzyme-catalyzed hydrolysis or chemical hydrolysis resulting in a loss of polymer strength. In non-swellable polymer systems, the reduction in the molecular weight of the polymer may cause a loss of cohesion between the polymer chains (Singh and Sharma 2008).

1.12.2 Ionization

Through the methods of ionization or protonation of a suspension group, the initially insoluble polymers become soluble in water. As with polyacids, they become soluble and hydrophilic at higher PH. At a pH greater than 6, allulose acetate phthalate becomes water-soluble, but at lower pH, poly

(vinyl acetate phthalate) and hydroxypropyl methylcellulose phthalate are ionized (Singh and Sharma 2008).

1.12.3 Hydrolysis

Water-insoluble polymers containing ester or suspended anhydride groups are converted to water-soluble polymers if the anhydrides or esters are hydrolyzed to form ionized acids on the polymer chain. An example of this is poly(methacrylate) and poly (methyl methacrylate), which are esters derived from poly (acrylic acid) and poly (methacrylic acid) that are insoluble in water, but upon hydrolysis of the suspended esters or subsequent ionization of the carboxylic group, they become soluble in water. Natural polymers degrade through hydrolysis, but synthetic polymers are insoluble in water as they tend to be more crystalline making them insoluble in water. For the hydrolysis of synthetic polymers to occur, they must possess hydrolytically unstable bonds that are sufficiently hydrophilic to reach the water. Biodegradable polymers include both esters and ester-derivative polymers (Singh and Sharma 2008).

1.12.4 Enzyme catalytic degradation.

Enzymes are proteins that act as catalysts for a specific reaction or group of reactions such as hydrolysis, esterification, oxidation, reduction, molecular conversions, and synthesis. PE, PS, PP, and PMMA are more stable polymers due to their hydrophobicity and no hydrolyzable bonds.
Some natural polymers undergo enzymatic degradation followed by decompression or degradation at the end of the chain, an example is a degradation of starch am-amylase to maltose starting at the end of the chain. The degradation of poly (vinyl acetate) (PVAc) catalyzed lipase was studied by Chattopadhyay and Madras, and it was observed that the ester bonds in the side chains were broken to produce oligomers with acidic groups and alcohols. There are many side branches in PVAc because chain transfer to the methyl groups of the acetate groups of PVAc occurs frequently during PVAc polymerization. In one study, enzymatic degradation of poly(ε-caprolactone) in supercritical carbon dioxide (scCO2) was successfully performed. *Candida Antarctica* lipase has smoothly catalyzed the hydrolytic degradation in scCO2 to give oligo (ε-caprolactone) (Singh and Sharma 2008).

1.12.5 Microbial degradations

This degradation is caused by naturally occurring microorganisms such as bacteria, fungi, algae, etc. The components resulting from the action of microorganisms are invisible and there is no need for sifting after composting. The production of degradable plastic is an important global step because of its complete decomposition in nature and its being environmentally friendly and important in

reducing the burden of pollution in a landfill. To increase the biodegradability of synthetic plastics, natural polymers are added that increase the effectiveness of microbial degradation of synthetic plastics Microbial degradation is enhanced by the addition of natural polymers to synthetic plastics to form a biodegradable plastic, forming a biodegradable plastic as occurs in synthetic polyolefins, making them susceptible to microbial degradation. These additions serve to separate the continuity of the C-C bond in the chain of polyolefin. Some additives also contain hydrophilic groups that make synthetic plastics hydrophilic and subject to photo and chemical degradation. Through studies, some strains of bacteria such as *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and fungi *Penicillium simplicissimum* have been reported as the most organisms that degrade plastics. Haddad et al. isolated thermophilic bacterial strains of *Brevibacillus borstelensis* for the degradation of low-density polyethylene (LDPE) degradation and studied the effect of ultraviolet photooxidation on the biodegradation efficacy of polyethylene which enhanced the biodegradation (Singh and Sharma 2008).

1.13 Measuring Biodegradation of Polymers

When studying biodegradation, the role and impact of the environment cannot be neglected. The polymer chemistry is insufficient. Therefore, biodegradation depends on the chemistry of the polymer and a set of environmental factors, namely, the presence of microorganisms, the abundance of oxygen, the amount of available water, temperature, and the chemical environment. To facilitate the study, the biodegradation zones are divided into aerobic decomposition zones characterized by the presence of oxygen and anaerobic decomposition zones with no oxygen, and they are also divided into two parts: solid environments and liquid environments. The table14 shows different environments in which biodegradation can occur. For example, in landfills, the solids are in high quantities and are suitable for measuring the biodegradation of the polymer. On the other hand, there is a necessity for aquatic biodegradation for fishing nets or exposure to undesirable substances. As a result of the difference in approach and definition of biodegradation, many methods for measuring biodegradation have been developed and described which in turn do not provide a complete definition and characterization due to the incubation of the polymer substrate with microorganisms or enzymes, where such information is not available. Four approaches are available to study biodegradation in detail monitoring bacterial growth, monitoring substrate depletion, monitoring reaction products, and monitoring changes in substrate properties. Several methods are adopted for estimating biodegradation as enzyme Assays, plate tests, respiration tests, gas (CO2 or CH 4) evolution tests, radioactively Labelled Polymers, laboratory-scale simulated accelerating environments, and natural environments – field trials all these methods are based on the four bases they mentioned. We will

carry out some of these laboratory experiments to quantify the biodegradation of Low-density polyethylene in this study.

	aquatic	*high solids*
aerobic	• aerobic waste water treatment plants • surface waters, e.g., lakes and rivers • marine environments	• surface soils • organic waste composting plants • littering
anaerobic	• anaerobic waste water treatment plants • rumen of herbivores	• deep sea sediments • anaerobic sludge • anaerobic digestion/ biogasification • landfill

Table4. Classification of polymer biodegradation environments

(CATIA BASTIOLI 2021)

1.13.1Weight loss

Weight loss is calculated by the difference between the weight of the polymer before exposure to the microbes and the weight of the polymer after exposure to the microbes. Weight loss depends on the type and properties of the polymer used, the pre-treatment of the plastic, the type of bacteria that degrade the plastic, and conditions during the experiment such as study duration, pH, and temperature. 65.2% of studies reported a weight loss of up to 10% or 10 to 20%. While some studies have not observed any change in weight due to the use of untreated polymer as a control (Matjašič, Simčič et al. 2021). Weight loss may occur because of volatile and soluble impurities and therefore measuring weight loss is not an effective method for measuring the biodegradability of samples. Also, this method is concerned with the first stages of biodegradation and does not give any information on mineralization (Ho, Roberts et al. 2018).

1.13.2(Mechanical changes) Tensile strength

It is measured in megapascals and is measured by a tensile testing machine as a percentage of the losses in tensile strength at the breaking point or the final tensile strength (the amount of pressure the material can withstand during expansion to the breaking point). 17.4 of studies have indicated the use of tensile strength as evidence of biodegradation. A Significant decrease in tensile strength was reported when performing biodegradation experiments where one experiment indicated a decrease in tensile strength of 50% for heat-treated high-density polyethylene (HDPE) after incubation for 60 days. It was also indicated that the tensile strength decreased by more than 50% when investigating the

biodegradation of PE by bacterial strains (Bacillus sp) extracted from the guts of the plastic-eating waxworms (Matjašič, Simčič et al. 2021).

1.13.3 Visual changes (Surface changes)

The visual changes in the before and after images from SEM were relied upon to evaluate the surface changes of the plastic, as 60% of the studies indicated the presence of changes on the surface of the plastic in the shape of holes, cracks, and pores. SEM images of HDPE were provided by Kiao et al. The images indicated superficial changes after three months of incubation with selected Pseudomonas strains. Superficial changes also occurred when PP was exposed to different Bacillus strains for an incubation period of 12 months. SEM experiments also revealed the degradation of PET, and PS by different strains under different incubation times (Matjašič, Simčič et al. 2021). In addition to advanced observations with SEM, optical microscopy, electron microscopy, polarization microscopy, force microscopy, and atomic force microscopy are used. These methods used are characterized by being cheap and fast, but they are qualitative, due to the use of additives with polymer, and the differences may be caused by chemical or physical deterioration and not f by biodegradation (Ho, Roberts et al. 2018).

1.13.4 Oxygen consumption

The amount of oxygen consumed during biodegradation is an indicator or evidence of this deterioration. The amount of oxygen consumed is measured by comparing biological oxygen demand with chemical oxygen demand, where a spirometer is used to measure oxygen consumption. The MITI (Ministry of International Trade and Industry, Japan) test is used as a method for determining oxygen consumption (Ho, Roberts et al. 2018).

1.13.5 Radiolabeling

This technique is non-destructive to biodegradation and is used to examine the susceptibility of polymer to biodegradation. In this technique, the carbon in the polymer is determined through radiation, using iso-tope14C carbon and exposing it to a microbial environment, and a comparison is made between the amount of 14CO2 or the amount of 14CH4 radioactive with the radioactive product to determine the duration of exposure. In this technique, a flash counter is used to measure the amount of 14CO2 released. This method does not interfere with materials that are added to the polymer or impurities that are subject to deterioration. This method is blamed for the high costs of selecting and preparing polymers that have a radioactive mark, as it requires special equipment, trained technicians,

and special laboratories, as well as the presence of radioactive waste and how to dispose of it (Ho, Roberts et al. 2018).

1.13.6Chemical changes

Infrared Fourier transform spectroscopy was used to evaluate chemical changes in the treated polymer (60% of studies used this method). The changes are depicted as FTIR spectra and explained as carbonyl band intensity changes. For example, changes in the carbonyl index from 0.196 to 0.143 have been reported for LDPE treated with Arthrobacter paraffin and changes in the double bond index from 0.275 to 0.250. While when measuring the absorption of FTIR spectroscopy for PP that underwent treatment at 1377 cm, there was a clear decrease when PP was exposed to bacteria, where the methyl group index showed a decrease from the value of 1.0 to the value. 0.85 after six months of incubation, 0.75 after 9 months, and 0.70 after a year. TGA was also followed to evaluate changes in the thermal profiles. This was shown by Bhatia et al in virgin LDPE which had a steep deterioration curve between 450 and 500°C, while degraded LDPE showed a three-stage weight loss of 22%, 33%, and 46% under the temperature of 50°C, 100°C, and 175 degrees Celsius, respectively. It was concluded that bacterial cultures due to direct enzymatic fission and internalization of low molecular weight chains can accelerate the degradation. Also, in addition to FTIR and TGA spectroscopy, NMR and HPLC were used to evaluate the degradation of high-impact PS with the help of microbes. They used dissolved chloroform before and after NMR analysis of high-impact PS samples. The absence of atoms at peaks of 3 ppm and 4 ppm that corresponded to -CH2-Br was shown to be due to bacteria, in which the bromination process was released from high-impact PS to form methyl bromine. Also, the presence of phenyl ethanol, an intermediate product in the biodegradation of PE was detected by analysis of culture medium treated with *Bacillus spp* and *Pseudomonas spp*. GPC was used to determine the molecular weight (Mw) and average molecular mass (Mn) of biodegradable plastics and their polydispersity index (Mw/Mn). Novotny et al. used GPC (gel permeation chromatography) with FTIR to evaluate the degradation of LLDPE previously treated with the bacteria *Bacillus amyloliquefaciens*. Neither the properties of the pristine LLDPE nor the abiotic control was changed after 60 days of incubation at 28°C. The pre-treated LLDPE when exposed to bacteria had an increase in manganese and an increase in the molecular weight, while the dispersion index decreased from 16.9 to 9.4. It was concluded that the removal of oligomers occurred in LLDPE by microbial action (Matjašič, Simčič et al. 2021).

1.13.7Biogas evolution (CO_2, CH_4)

To determine the ultimate biodegradability or activity of a polymer, analytical parameters (carbon dioxide or methane) developed from biodegradation are used. Where the mechanism is the consumption of oxygen by microbes to oxidize carbon compounds and obtain carbon dioxide, which is one of the main metabolic products. With the onset and continuation of biodegradation, carbon dioxide production develops progressively, which serves as a measure of biodegradation. The incremental evolution of carbon dioxide is expressed as a percentage of the theoretically expected value of the conversion of total carbon to carbon dioxide. The evolution of carbon dioxide, which results from oxidation, is the most widely used method for measuring or determining biodegradation. A set of tests have been approved and standardized to evaluate the biodegradation of polymers under aerobic conditions such as the fertilization test, and the modified Strum test. As for the determination of the effectiveness and ability of biodegradation under anaerobic conditions, it is by determining and measuring the increase in volume or pressure because of the evolution of methane gas. Standardized tests to determine the biodegradability of polymers under anaerobic conditions include the anaerobic digestion test and the anaerobic sludge test (Ho, Roberts et al. 2018).

1.14 Factors affecting biodegradation of plastics

The biodegradation of plastics is influenced by a variety of factors including polymer properties, type of organism, and environmental conditions table5. It has been clarified through studies that the important role of the environment in biodegradation in terms of participation in biodegradation and its amount. And how polymer chemistry controls the physical and chemical properties of the material and its interaction with the environment (biological reaction). It has been proven that the process of linking the polymer structure to the amount of biodegradation is a challenge because sometimes there is an overlap between different factors, and thus, it is difficult to determine the initial effects and the association. The process of accessing the enzyme systems is the first and most important step for the polymer because the biodegradation is outside the cell through the enzyme breakdown of the polymer (CATIA BASTIOLI 2021). The polymer properties that are most important in determining the degradability of the polymer include the functional groups that are added when the hydrophilic groups increase the degradation increases. Molecular weight and density the higher the molecular weight, the slower the degradation (Haben Fesseha 2019, Khan and Majeed 2019). Studies showed that PCL was slowly degraded due to its high molecular weight greater than 4000 by lipase enzyme of *R.*

Delmar strain compared with low molecular weight polymer (Bahl, Dolma et al. 2021). The form of the polymer is amorphous or amorphous since the amorphous has the fastest deterioration. Structural complexity Polymer causes slow degradation. Hardness The higher the hardness, the slower the deterioration. Nature and physical form of the polymer (Haben Fesseha 2019, Khan and Majeed 2019). It was found that polymers with large surface areas deteriorate faster than polymers with small surface areas (Bahl, Dolma et al. 2021). When a comparison is made between low-density polyethylene (LDPE) and high-density polyethylene (HDPE) in terms of crystallinity, branches, and, molar mass, (LDPE) is characterized by being amorphous, with one branch or many branches, and consisting of one or more comonomers, and therefore this branching makes polyethylene chains Low-density ethylene (LDPE) is more degradable and the triple carbon atoms at the branch sites are more vulnerable to attack and because HDPE's high molar mass makes it difficult to access the polymer chains by microorganisms and their enzymes. Also, a study was conducted to compare the biodegradability between different thickness polyethylene types which were pre-treated LDPE, HDPE, and Linear Low-Density Polyethylene LLDPE by *Rhodococcus rhodochrous*, which expresses one of the most effective types of bacteria in PE degradation. It was the structural differences in polyethylene polymers such as the carbonyl groups, the unsaturated carbon-carbon double bond, and the hydroxide group formed during polymerization and subsequent treatment that was first consumed by the bacteri,a and thus a rapid growth occurred (Mohanan, Montazer et al. 2020). The abundance, growth, and diversity of microorganisms depend on a range of factors such as environmental conditions, pH, temperature, soil moisture content, and other factors as soil moisture content has an important role in the growth of soil microbes. As the moisture content increases, the hydrophilic division of microbes will increase. Higher temperatures also reduce the ability of enzymes to degrade, and thus there is an inverse relationship between polymers with high melting point and degradation. The change in the pH will affect the amount of the degradation reaction due to the effect on the growth of the microbe’s table (Bahl, Dolma et al. 2021).

Factors affecting the process of biodegradation of plastics by a microorganisms	
Exposure condition	Polymer characteristics
PH	Flexibility
Temperature	Function groups
Moisture	Morphology
Bio-surfactant	Cross-linking
Enzyme	Additives
Microbial strain	Blend
	Co-polymers
	Molecular weight

Table5. Factors affecting the process of biodegradation of plastics by microorganisms (Khan and Majeed 2019).

Recent studies have indicated that pollutants in the atmosphere may be a source of nutrients needed by some microorganisms. Precipitation of sulfur dioxide and aromatic and aliphatic hydrocarbons from urban air has been reported on many plastics (Michel and Joe). These absorbed contaminants may prefer to be colonized by other microbial species (Lucas, Bienaime et al. 2008). Also, surfactant-reducing substances when added facilitate biodegradation because they have specific functional groups that allow activity to occur under critical conditions such as pH, salinity, and temperature extremes. Thus, plastics of petrochemical origin cannot easily degrade in the environment due to their three-dimensional structure and resistance to hydrolysis. The hydrophobic nature of polyethylene leads to the formation of a biofilm of microorganisms that reduces biodegradation (Bahl, Dolma et al. 2021).

1.15 Modification of polymers and incentivizing bacteria to enable biodegradation

Due to the presence of a carbon-carbon bond in both oligomers and polymers (with high molecular weight) they are unable or slow degraded by the enzyme. The molecular weight is converted to low molecular weight polymers which are then consumed by microorganisms via biodegradation. There are two approaches to converting a high molecular weight polymer into a low molecular weight polymer: The first approach is by introducing functional groups such as ester into the main chain which are cleaved through chemical decomposition. The second approach is by inserting a functional group that is in the main chain that undergoes photochemical cleavage reactions and is a typical carbonyl group (Chandra and Rustgi 1998). This is with regard to the polymer and with regard to bacteria, the use of nanoparticles that are incorporated as an enhancer for bacterial degradation has been indicated, many of which serve to enhance thermal, mechanical, and stability besides biological degradation. For synthetic plastics, some nanoparticles enhance the heat, mechanical, and stability with biodegradation. The nanoparticles are used as nanoparticles by forming nanocomposites with a large surface area known as clay nanocomposites. The addition of nanobarium titanate (NBT) was indicated to influence the growth of the cycle of bacterial consortia that degrade LDPE. NBT reduces the time of the delay phase and increases the time of the fixed exponential phase for the accelerated growth of the bacterial consortia, thus aiding in the biodegradation of waste plastics (Bhatia, Girdhar et al. 2013).

1.16 Plastic biodegradation bacteria (*Proteobacteria, Firmicutes, Actinobacteria*)

Of the 145 studies, 138 reported biodegradations of one type of polymer, and of the 138 studies, 103 reported 246 bacterial strains that biodegrade plastic Table. Most of the bacterial strains that were isolated belonged to phyla *Proteobacteria* (48%), *Firmicutes* (37.4%), and *Actinobacteria* (9.8%) (Matjašič, Simčič et al. 2021).The genus *Pseudomonas* is one of the most common in soil and water and contains 216 species active in biodegradation. Jacquin et al. reported the efficacy of *Pseudomonas* strains in biodegradation (Matjašič, Simčič et al. 2021). Common strains of the genera *Bacillus* and *Brevibacillus* have also been included. One study in deep-sea waters in Toyama Bay also reported the isolation of two types of bacteria that degrade PCL. The isolated strains belonged to the genus Pseudomonas, where PCL was degraded at 4°C. At depths of 5000-7000m, bacteria belonging to the genus *Shewanella, Moritella, Psychrobacter,* and *Pseudomonas genera* were isolated from sediment

samples, where six isolated strains showed their ability to degrade degradable PCL (Urbanek, Rymowicz et al. 2018). One of the most important features of bacteria that degrade plastics is the ability to use different organic compounds as a source of carbon and energy. Some species can use more than 100 different compounds, including biomaterials of unknown origin, making them important tools in bioremediation (Matjašič, Simčič et al. 2021). As for the family *Firmicutes*, members of which are Gram-positive bacteria with a primary presence in the soil and the ability to form endospores. Several of its strains can produce extracellular hydrolytic enzymes that break down complex polymers such as lipids, which are a carbon source and electron donor. Many baskets of this family also produce insecticides and antibiotics. The genus *Bacillus* constitutes the most organisms in this family with 282 species, which can form cylindrical or oval endospores and can act as aerobes or facultative aerobes (Matjašič, Simčič et al. 2021). Actinobacteria are aerobic Gram-positive, and their strains are rod-shaped to filamentous, found mainly in soil and plants. In commercial terms, it is used to produce insecticides and antibiotics (Matjašič, Simčič et al. 2021). *Marine Actinobacteria* are also a treasure trove of secondary receptors, each of which is believed to be able to produce 15-25 secondary independents (Puttaswamygowda, Olakkaran et al. 2019). Studies have been made to effective bacteria in the start of vital degradation of artificial plastic. Where a 21% signal. From studies to *Pseudomonas* and 17% signal to *Bacillus* and 17% signal to a mixture of *pseudomonas* and *bacillus* (Matjašič, Simčič et al. 2021).

Polymer	**Bacteria/sample origin**	**Phylum**	**Identified bacteria capable of plastic biodegradation**	**References**
HDPE	Contaminated site	Actinobacteria	*Arthrobacter* sp. GMB5, *Leucobacter* sp., *Micrococcus* sp.	*(Kunlere, Fagade et al. 2019)* *(Devi, Ramya et al. 2019)*
HDPE	Contaminated site	Firmicutes	*Bacillusspp., B. amyloliquefaciens, B. aryabhattai, B. cereus, B. licheniformis,B. pumilus, B. subtilis, Staphylococcus sp.*	*(Kunlere, Fagade et al. 2019)* *(Devi, Ramya et al. 2019)*

HDPE	Mix	Firmicutes	*Brevibacillus borstelensis* KY4948 6	*(Mohanrasu, Premnath et al. 2018)*
HDPE	Other	Firmicutes	*Bacillus* spp., *B. cere us, B. sphericus, Pae nibacillus* spp.	*(Kumari, Chaudhary et al. 2019)* *(Sudhakar, Doble et al. 2008)* *(Skariyachan, Setlur et al. 2017)*
HDPE	Contaminated site	Proteobacteria	*Achromobacter xylosoxidans* PE-1, *Acinetobacter sp., Klebsiella pneumoniae* CH001, *Pseudomonas* sp. GMB7, *P. aeruginos a*	*(Awasthi, Kumar et al. 2017)* *(Balasubramanian, Natarajan et al. 2010)* *(Kowalczyk, Chyc et al. 2016)* *(Kunlere, Fagade et al. 2019)* *(Devi, Ramya et al. 2019)*
HDPE	Other	Proteobacteria	*Pseudomonas* spp., *P. fluorescens, Serra tia marcescens, Stenotr ophomonas* spp.	*(Baculi, Melegrito et al. 2017)* *(Skariyachan, Setlur et al. 2017)*
LDPE	Banks/SM	Acidobacteria, Firmicutes & Proteobacteria	Consortium of: Pseudomonas otitidis SPT1, Bacillus aerius SPT2, Acanthopleuribacter pedis SPT3, Bacillus cereus SPK1	(Anwar, Negi et al. 2013)
LDPE	NA	Acinetobacteri a,	Consortium of: Actinobacteria,	(Zhang, Zhang et al. 2020)

		Gemmatimonadetes & unknowns	Gemmatimonadaceae and some unknowns	
LDPE	Banks/SM	Actinobacteria	*Arthrobacter oxydans, A. globiformis, Microbacterium paraoxydans* (GenBank ID: HQ185284)	*(Carol, Karpagam et al. 2012) (Rajandas, Parimannan et al. 2012)*
LDPE	Contaminated site	Actinobacteria	*Cellulosimicrobium funkei, Micrococcus luteus*	*(Montazer, Habibi-Najafi et al. 2018) (Muhonja, Makonde et al. 2018)*
LDPE	NA	Actinobacteria	*Arthobacter paraffineus*	*(Albertsson, Erlandsson et al. 1998)*
LDPE	Contaminated site	Bacteriodetes	*Spingobacterium multivorum*	*(Montazer, Habibi-Najafi et al. 2018)*
LDPE	Contaminated site	Cyanobacteria	*Oscillatoria subbrevis, Phormidium lucidum*	*(Sarmah and Rout 2018)*
LDPE	Banks/SM	Firmicutes	BP/SU1 of *Staphylococcal epidermis*	(Chatterjee, Roy et al. 2010)
LDPE	Contaminated site	Firmicutes	*Bacillus cereus, B. niacini, B. pseudomycoides, B. safensis, Bacillus* sp., *Bacillus* sp. *ISJ55, B. subtilis, B. thuringiensis, B. toyonensis, Brevibacillus borstelensis,*	*(Anbuselvi and Pandey 2015) (Bardají, Furlan et al. 2019) (Deepa 2019) (Muhonja, Makonde et al. 2018) (Muthumani and Anbuselvi 2015)*

			B. parabrevis, Lysinibacillus macroides, Paenibacillus sp. (*GenBank MK053775*), *Staphylococcus* sp., *Streptococcus, Streptococcus/Staphylococcus**	
LDPE	Mix	Firmicutes	*Bacillus subtilis* MTCC 9447	*(Skariyachan, Manjunatha et al. 2016)*
LDPE	Other	Firmicutes	*Bacillus* sp. YP2, *Bacillus* sp. *B. sphericus*, *B. cereus*, *Bacillus* spp., *Paenibacillus* spp.	*(Kumari, Chaudhary et al. 2019)* *(Skariyachan, Setlur et al. 2017)* *(Sudhakar, Doble et al. 2008)* *(Yang, Yang et al. 2014)*
LDPE	Banks/SM	Proteobacteria	*Pseudomonas aeruginosa* PAO1 ATCC 15729, *P. aeruginosa* ATCC 15692, *P. aeruginosa* (GenBank ID: HQ185285), *P. putida* KT2440 ATCC 47054, *P. syringae* DC 3000 ATCC 10862	*(Kyaw, Champakalakshmi et al. 2012)* *(Rajandas, Parimannan et al. 2012)*

LDPE	Contaminated site	Proteobacteria	*Acinetobacter pitti, Alcanivorax borkumensis, Citrobacter amalonaticus, Delftia tsuruhatensis, Escherichia coli, Klebsiella* sp., *Ochrobactrum intermedium, O. oryzae, O. pseudintermedium, Pseudomonas aeruginosa, P. aeruginosa SKN1* (*ID*: *9702593*), *P. citronellolis* EMBS027 KF361478, *P. putida*, *Pseudomonas* spp., *Stenotrophomonas humi, S. maltophilia, S. pavanii* CC18, *P. stutzeri*	*(Anbuselvi and Pandey 2015)* *(Anbuselvi and Pandey 2015)* *(Bhatia, Girdhar et al. 2014)* *(Deepika and Jaya 2015)* *(Delacuvellerie, Cyriaque et al. 2019)* *(Mehmood, Qazi et al. 2016)* *(Montazer, Habibi-Najafi et al. 2018)* *(Muhonja, Makonde et al. 2018)* *(Muthumani and Anbuselvi 2015)* *(Nourollahi, Sedighi-Khavidak et al. 2019)* *(Sharma and Sharma 2004)* *(Skariyachan, Megha et al. 2015)*
LDPE	Mix	Proteobacteria	*Enterobacter* spp., *Pantoea* spp., *Proteus* spp., *Pseudomonas putida* MTCC 2445, *Pseudomonas* spp., *P. stutzeri* MTCC 2643	*(Skariyachan, Manjunatha et al. 2016)*

LDPE	Other	Proteobacteria	*Enterobacter asburiae*, *Enterobacter cloacae AKS7*, *Pseudomonas* spp., *Stenotrophomonas* s pp.	*(Sarker, Chakraborty et al. 2020)* *(Skariyachan, Setlur et al. 2017)* *(Yang, Yang et al. 2014)*
LDPE	Other	Firmicutes & Proteobacteria	Consortium of: *Serratia* sp. KC1-MRL, *Bacillus licheniformis* KC2-MRL, *B.* sp. KC3-MRL and *Stenotrophomonas* sp. KCMRL	(Jamil, Zada et al. 2017)
LLDPE	Banks/M	Firmicutes & Proteobacteria	Consortium of: *Pseudomonas aeruginosa & Brevibacterium sp*.	(Matjašič, Simčič et al. 2021)
LLDPE	Banks/M	Proteobacteria	*Microbulbifer hydrolytic IRE-31* (*ATCC 700072*)	*(Li, Wei et al. 2020)*
LLDPE	Other	Firmicutes	*Bacillus amyloliquefaciens* (GenBank accession no. KT185076)	*(Novotný, Malachová et al. 2018)*
LLDPE	Other	Proteobacteria	*Serratia marcescens marcescens*	*(Azeko, Etuk-Udo et al. 2015)*
LMWPE	Contaminated site	Proteobacteria	*Stenotrophomonas panacihumi* PA3-2.	*(Jeon and Kim 2016)*
PE	Banks/M	Actinobacteria	*Rhodococcus ruber* strain C208	*(Santo, Weitsman et al. 2013)*
PE	Contaminated site	Actinobacteria	Arthrobacter spp., consortium	(Jin and Kim 2017)

			of: *Arthrobacter*, *Curtobacterium*, *Gordonia* and *Rhodococcus*	(Puglisi, Romaniello et al. 2019)
PE	Other	Actinobacteria	*Micrococcus* sp., *Brevibacterium* sp., *Streptomyces albogriseolus* LBX-2	*(Brandon, Gao et al. 2018)* *(Kathiresan 2003)*
PE	Banks/SM	Firmicutes	*Lysinibacillus fusiformis*	*(Mukherjee, RoyChaudhuri et al. 2017)* *(Shao, Chen et al. 2019)*
PE	Contaminated site	Firmicutes	*Bacillus aquimaris*, *B. boroniphilus*, *B. drentensis*, *B. firmus*, *B. idriensis*, *B. luciferensis*, *B. marisflavi*, *B. megaterium*, *B. muralis*, *B. mycoides*, *B. pumilus*, *B. simplex*, *B. subtilis*, *B. sp.*, *Paenibacillus woosongensis*	*(Puglisi, Romaniello et al. 2019)*
PE	Mix	Firmicutes	*Bacillus cereus* VASB1/TS, *Lysinibacillus fusiformis* VASB-14/WL, *Staphylococci*	*(Rani and Rao 2012)* *(Shahnawaz, Sangale et al. 2016)*
PE	Other	Firmicutes	*Bacillus* sp., *B. gottheilii*, *B. cereus*, *Staphylococcus* sp., *Streptococcus* sp.	*(Kathiresan 2003)* *(Auta, Emenike et al. 2017)*

PE	Other	Fusobacteria	*Sebaldella termitidis*	*(Brandon, Gao et al. 2018)*
PE	Contaminated site	Proteobacteria	16 isolates from three genera: *Comamonas*, *Delftia*, and *Stenotrophomonas*, recombinant strains by *Escherichia coli* BL21 and *P. aeruginosa* E 7, *Acinetobacter johnsonii*, *Comamonas testosteroni*, *Pseudomonas* sp., *P. aeruginosa*, *P. alcaligenes*, *P. plecoglossicida*, *P. thivervalensis*, *Stenotrophomonas maltophilia*	(Jeon and Kim 2016) (Peixoto, Silva et al. 2017) (Puglisi, Romaniello et al. 2019) (Satyalakshmi 2016) (Shahreza, Sepahy et al. 2019) (Skariyachan, Megha et al. 2015)
PE	Mix	Proteobacteria	*Pseudomonas putida*	*(Rani and Rao 2012)*
PE	Other	Proteobacteria	*Citrobacter* sp., *Diplococcus* sp., *Enterobacter* sp., *Kosakonia* sp., *Moraxella* sp., *Pseudomonas aeruginosa*, *Pseudomonas* sp.	*(Brandon, Gao et al. 2018)* *(Li, Wei et al. 2020)* *(Kathiresan 2003)* *(Nanda, Sahu et al. 2010)* *(Ren, Men et al. 2019)*

Table6. The table shows a list of bacteria that can degrade polymers, which were taken from different sites. Contaminated sites Bacterial samples were taken from contaminated sites such as landfills, contaminated soil, activated sludge, and dumpsites. Banks/SM bacterial samples from banks of

strain and/or of their synthetic mixtures of SM. A mixture is a mixture of bacteria samples taken from different contaminated and non-contaminated environments. Others refer to bacterial samples taken from different natural environments such as soil, sediments, freshwater, animal intestines, marine environments, plant nodes, and mangroves (Matjašič, Simčič et al. 2021).

2 Issue

2.1 The effect of plastic debris

From the poles to the equator, plastic debris accumulates in natural habitats and is an obvious component of the marine environment. Plastic debris spreads and pollutes marine habitats, from distant shores to crowded coasts to deep, hard-to-reach seas. Several reports indicated that this spread of plastic debris everywhere leads to ingestion by living organisms because slow or non-degradation (persistence in the environment) can cause weakness in the movement of living organisms and then death. Other reports have also shown evidence of plastic fragmentation, making it available for ingestion by large numbers of living organisms. Also, there have been predictions for more than 30 years that ingestion of plastic debris could transfer toxic chemicals to wildlife This effect is not limited to debris, ingestion and chemical transfer, but also public health problems caused by additives during manufacturing that can be transmitted to humans directly (Thompson, Swan et al. 2009).

2.1.1 Microplastics MP, Nano plastics

Primary and secondary (microplastics, nano plastics)

Scientists use microplastics and nano plastics(MPs) as a guide to express the contemporary period, which is the era of plasticine, but despite its distribution and its abundant presence in the environment, there is a complexity in fully understanding its effects due to its different physical and chemical properties, which give it multifaceted pressures (Campanale, Massarelli et al. 2020) Plastic particles with a diameter of less than 5 mm were first discovered in 1972 in the Sago Sea, where the term microplastics was not known until 2004, when many studies appeared that attached great importance to microplastics (Nerland, Halsband et al. 2014). Microplastics are defined as plastic particles with sizes less than 5 mm, microplastics originate through two sources, a primary source,

and a secondary source. The primary source of PM is directly produced at a size of one mm including plastic pellets, those used in cosmetics or clothing fibers that are found in wastewater treatment plants (primary microplastics) fig 11(Left). Secondary sources arise from the breakdown of large plastic pieces due to friction or mechanical corrosion and light oxidation in the environment (secondary microplastics)fig12(Right) (Hermabessiere, Dehaut et al. 2017)(Dioses-Salinas, Pérez-Baca et al. 2019). The primary microplastics that are produced industrially and used in raw materials enter the environment through losses during transportation and distribution processes or through surface runoff from treatment plants because of their smallness and inability to retain them. Secondary microplastics have multiple pathways to enter the environment, first through the residual water from washing or drying textiles, and then through wastewater entering the environment, secondly through the weathering processes of plastics that are used in agricultural applications, where they enter the soil through surface runoff, and thirdly, entry to the environment through The products of tire erosion, which results in fine particles that settle in the environment through air and runoff Fourth through weather factors by ultraviolet rays within the landfills where a fragmentation process occurs and thus the possibility of microplastic particles entering the atmosphere and rivers by wind and runoff Fifthly in coastal areas On beaches, plastic garbage weathers as it stays in sediments or is transported further from the shore (Lusher, Hollman et al. 2017). Nano plastics is defined as a material with a range ranging from 1 nm to 100 nm that may be primary or secondary and arise from weathering or fragmentation of microplastic debris and enter the aquatic environment through wastewater. There is insufficient information about nano plastics in terms of their occurrence and distribution in the environment, due to the lack of sufficient methods to detect them (Lusher, Hollman et al. 2017) were confirmed by the World Health Organization on the presence of microplastics in the environment and the effect of nano and microplastic particles on human health, and through one of the studies that were conducted, 0.44 MPs/g microplastic and nanoparticles were found in sugar and 0.11 MPs/g MP in salt and MP0 .03 MPs/g in alcohol and 0.09 MPs/g in canned water and 80g daily basis of microplastics found in vegetables and fruits that are eaten on a daily basis as the plant absorbs it from the soil(Campanale, Massarelli et al. 2020). These two forms of plastics settle in the environment and pose a threat to all forms of life (Silva, Bastos et al. 2018).

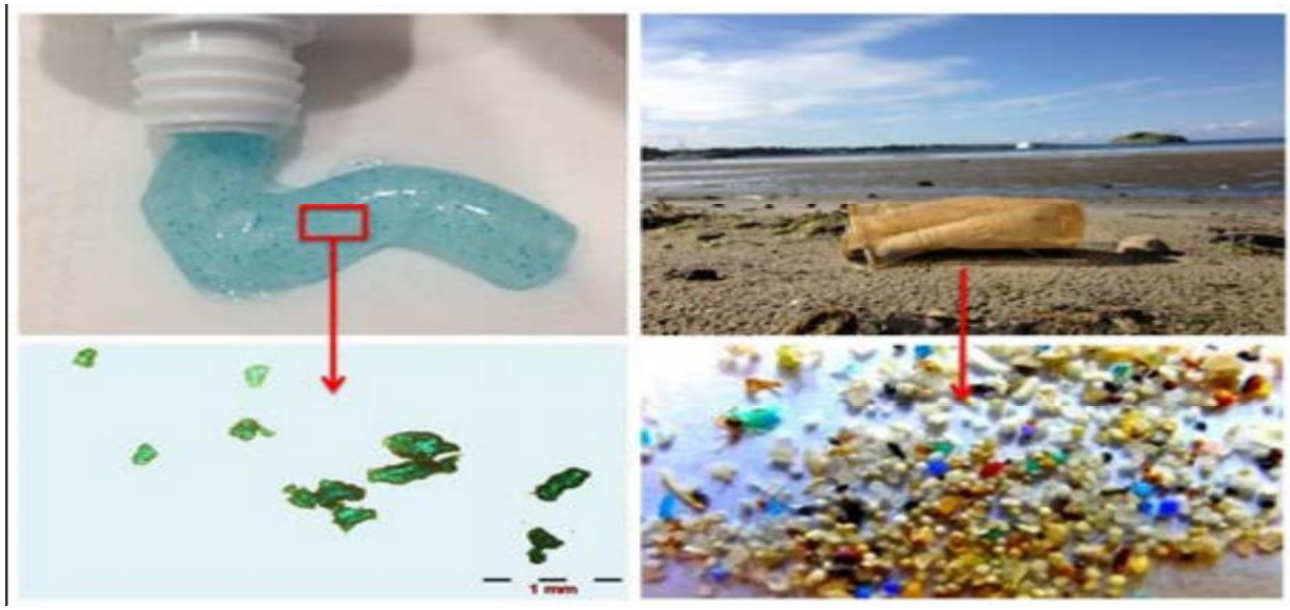


Figure11. Examples of microplastics and their sources on the left are the microplastics from the main source (cosmetics) and on the right are the microplastics from the secondary source (the breakdown of large plastic pieces) (Nerland, Halsband et al. 2014).

2.1.1.1 Microplastic in Ocean

Attention has been paid to the concentration and properties of plastics since the first report was published indicating the spread of plastic at a rate of 3,500 pieces in the marine basins of the North Atlantic Ocean. Plastic waste enters the marine environment through many sources, the most important of which are coastal waste, fishing and marine industries, and the resulting waste from the plastic industry, which finds its final downstream seawater. Plastic waste follows many paths before entering marine waters, from homes through cosmetics, through wastewater to rivers, and from there to seas and oceans. Mechanical factors and ultraviolet rays contribute to this fragmentation of large pieces and their transportation over long distances. Then the accumulation of plastic on the surface of the water and then diving into the water towards the bottom due to the loading of dissolved vital and abiotic compounds. Thus, the plastic will reach the sediment and take a longer time to decompose due to the absence of light and lower temperatures and the energy input is low compared to surface waters that are characterized by abundant oxygen therefore the oxidation processes will be effective for polymers in bright water (Urbanek, Rymowicz et al. 2018).

2.1.1.1.1 Impact of microplastics in ocean

2.1.1.1.1.1 Ingestion.

The turtles can make a hole in the esophagus or intestines and thus die when eating plastic, even in small quantities, as when a turtle was rid of an intestinal blockage on the beaches of Florida, the animal defecates 74 foreign bodies during a whole month, four of which were latex balloons and different types of hard plastic and a piece of material It resembles carpet plus two balls of tar of 2-4 mm. Also, ingestion of plastic leads to a feeling of satiety in the animal and an enemy of the ability to eat, and thus a decrease in growth and death of the animal in the end (Wabnitz and Nichols 2010). A study proved in the year 2003-2007 the presence of large amounts of plastic in the North Sea in Norway, where it was found that 95% of the birds of this region have plastic materials in their intestines, with an average of 35 plastic particles per bird (Nerland, Halsband et al. 2014). Fish that accumulate plastic in their intestines get hungry and malnourished, which leads to mortality and decreasing numbers, between 1 and 7.2 plastic particles were detected in the digestive system of wild- caught fish (Nerland, Halsband et al. 2014). The marine arrowworm *Parasagittal setosa* was also indicated after feeding on blue plastic fibers of approximately 3 mm in length fig12(Right) (Clunies-Ross 2019). The digestive system of Atlantic mackerel was also reported to contain orange fibers during a sport fishing expedition in Steingrund in the vicinity of Helgoland Island in the summer of 2013, Where the effects of plastic ingestion were not mentioned, the focus was only on the detection of plastic in the digestive system fig12(Left) (Rummel, Löder et al. 2016).

Figure12. On the left is the mackerel that ingested the orange PE fibres(Rummel, Löder et al. 2016). On the right is the marine arrowworm after eating blue plastic fibres, 3 mm long (Clunies-Ross 2019)

2.1.1.1.1.2 Entanglement

The effect of the ropes is by preventing or hindering turtles from diving to search for food, or by obstructing the surface for breathing, or cutting the limbs, and this leads to preventing or limiting the movement of the animal fig13. Through studies, 92 dead turtles were mentioned on the beaches of Rio Grande do Sul State in Brazil, due to the wreck of human origin. This wreck contains plastic bags and ropes that caused blockage and injuries to the digestive system (Wabnitz and Nichols 2010)**.** The

impact of plastic is not limited to large animals in the marine water environment but includes small organisms such as mussels and zooplankton through ingestion of microplastics and thus accumulate in the food chain due to feeding on them by other organisms (Urbanek, Rymowicz et al. 2018).

Figure13. An entanglement caused by a fishing net in the Southern Ocean in remote Bouvet Øyan the Southern Ocean (Jepsen and de Bruyn 2019).

2.1.1.1.1.3 Interaction.

Marine wildlife is at risk due to fishing gear that is deliberately discarded, lost, abandoned due to the drift, interception, and interaction of this equipment with living organisms. Globally, the quantities of lost fishing gear in the oceans amounted to 6.4 million tons annually (Wilcox, Heathcote et al. 2015). The interaction includes collision and erosion. The impact is, for example, of fishing gear on the reef which, by colliding with the reef, causes the corals to erode and destroy their ecosystem also by affecting the penetration of light and the exchange of oxygen by the plastic ball resulting from the interaction of the substrate with the plastic (Panda, Singh et al. 2010). One study reported in 2010 an annual accumulation of abandoned fishing gear at a rate of over 52 tons in the northwester Hawaiian Islands and once this equipment is lost in the water, it drifts and causes entanglement and impedes movement, and wildlife becomes threatened by the entanglement from days to years, and the entanglement results in the drowning of animals, exposure to wounds and drag during the swimming process, and they are more vulnerable to predation (Wilcox, Heathcote et al. 2015).

2.1.1.2 Microplastics in freshwater ecosystems

There is less information available on plastic pollution in freshwater systems compared to plastic pollution in marine systems, indicating a constant need for more knowledge about plastic pollution in freshwater systems. When comparing research studies, it was found that 87% of the studies dealt with plastic pollution in marine environments, and only 13% of them dealt with fresh water. Studying plastic pollution in these systems is important to make estimates of the amount of plastic entering the seas from rivers. Despite the scarcity of information regarding plastic pollution, evidence has been indicated for the presence of plastic within these ecosystems (Williams and Simmons, 1996), including pristine and remote sites (Horton et al., 2014). Chinese rivers contribute two-thirds of the plastic that is dumped into the oceans, with 7out of 20 indicated to be polluted with plastic (Blettler, Abrial et al. 2018). One study confirmed the presence of microplastics in the two largest lakes in Norway, Mjøsa, and Femunden, in sediment cores, where microplastics were found larger than 36 µm. It was also indicated that every nine of the 12 samples contained microplastic ranging from 0 to 14 plastic particles for each sample, and thus this indicates microplastics inputs from the 1970s in lake Miøse (Lusher, Buenaventura et al. 2018).

2.1.1.3 Microplastics in the soil

2.1.1.3.1 Sources of plastic in the soil

Plastic particles enter the soil through the accumulation of particles in the sludge resulting from wastewater treatment. It has been reported that microplastics are present in treated wastewater sludge at a rate of 15,385 particles $kg-1$. Therefore, the use of sludge in agricultural lands will lead to the arrival of many particles The problem of the source of soil pollution with these particles. Reports in both North America and Europe indicate that about 44,000-300,000 and 63,000-430000 tons of microplastics are released annually using sludge on agricultural land. Organic fertilizers may act as carriers through which microplastics enter the soil. The amount of microplastics in the fertilizer has been indicated, may reach 1200 mg/kg in Germany each year, where approximately 0.035 to 2.2 trillion tons of microplastics were entered in soil because of the composting process. The soil content of fine particles was indicated because of using plastic covering for crops, which amounted to 18,760 pieces per kilogram, where the size of the plastics was less than 1 mm. urban because of atmospheric precipitation. Playground grass is also a source of soil pollution as up to 2,630 tons of plastic particles can be dumped each year. Microplastic pollution in the terrestrial system is more dangerous than in the aquatic environment**,** but few studies are available about it in terrestrial systems (Ya, Jiang et al. 2021)

2.1.1.3.2 Plastic impact on soil animals and soil plants

2.1.1.3.2.1 Soil animals

Soil animals are indirectly affected by microplastics changing their surrounding environment. The earthworm is a typical species in the soil environment, and therefore the effect of microplastics on these worms is more studied, as its effect is related to the type and concentration of plastics, causing damage to the immune system, and inhibiting the growth rate. The effect of exposure to microplastics on spermatozoa invertebrates were also reported for the first time. Also, microplastics can cause changes in the intestinal flora of soil animals. In snails, feeding on sheets bearing nano plastics can alter the behavior and vitality of the intestinal microbiota fig 14 (Ya, Jiang et al. 2021).

2.1.1.3.2.2 Soil plants

The influence of soil plants depends on the type of microplastics, as microplastics can cause clogging of the pores of the cress seeds and prevent water absorption, and thus there will be a delay in the plant and the growth of the roots of the cress plant. Also, the negative effects of microplastics on growth and antioxidative defense systems have been reported in lettuce plants. As for the soil to which sludge containing microplastics was added, an increase in tomato plant growth was observed, and on the other hand, it negatively affected the fruit yield as production decreased. The nano plastics can also accumulate in the leaves of the mung plant and cause root growth inhibition. The experiments also proved a relationship between the type of effect on plants and the size of microplastics, as it was found that plastics with 100.nm have an inhibitory effect on growth rates in the *Vicia faba* plant, and oxidative damage and toxicity increased more than five micrometers of microplastics. As for the indirect effects of microplastics, it is represented by a change in the physical and chemical properties of the soil and thus changing the growing conditions and ways of supplying plants with nutrients. If a lot of information is missing about the effect of microplastics on plants, such as the accumulation and transfer of microplastics in plant tissues, toxic effects, and stress responses in plants fig14 (Ya, Jiang et al. 2021).

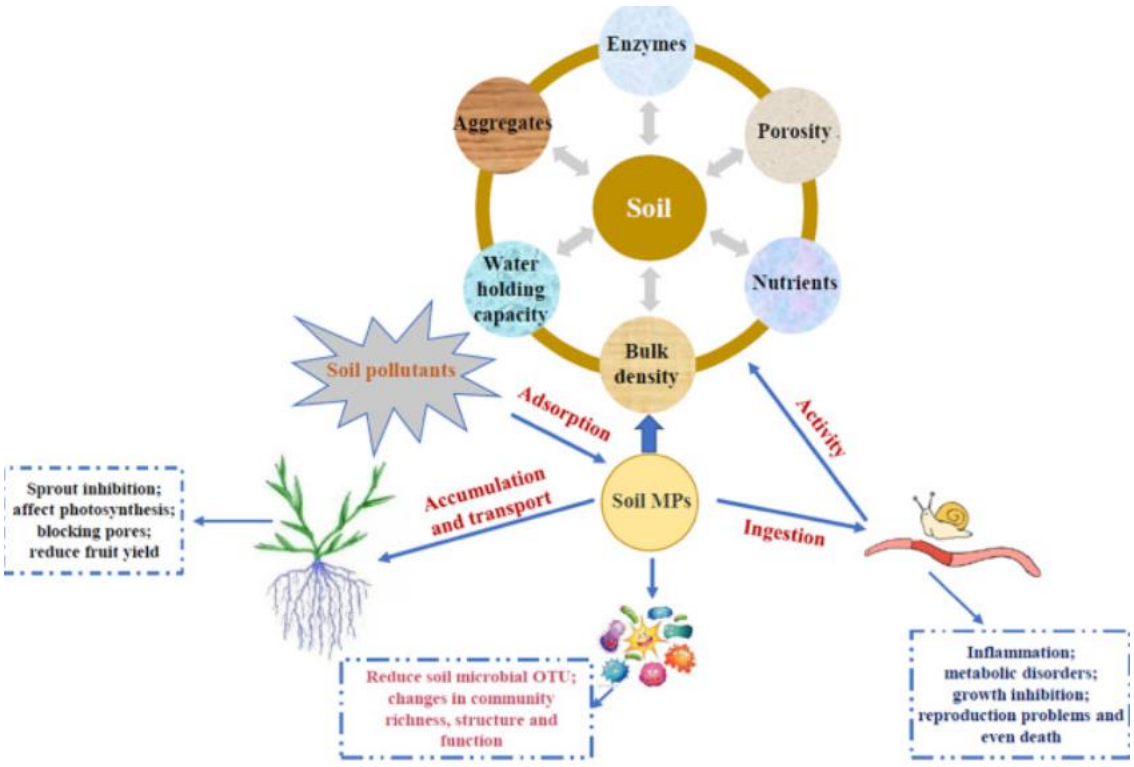


Figure14. The impact of microplastics on soil and it,s function, as well as the interaction with soil animals, plants, and microorganisms (Ya, Jiang et al. 2021).

2.1.1.4 Microplastics in food

Experiments with commercially farmed bivalves in both *Mytilus edulis* and *Crassostrea Gigas* demonstrated the presence of microplastics on average of 0.36 ± 0.07 particles/g in (*Mytilus edulis*) and 0.47 ± 0.16 particles/g in (*Crassostrea Gigas*), indicating that consumers of shellfish could ingest 11,000 microplastics annually. It was also found through the research that 80% of culture oysters on the coast of China contain microplastic particles at a rate of 0.62 particles/g. An investigation was also conducted on the presence of microplastics in the species found in the markets and supermarkets in China, where nine bivalves were used, which were proven to inhale microplastics at a concentration of 2.1 to 10.5 particles/gr In terrestrial systems, microplastics have been confirmed in terrestrial edible snails (De-la-Torre 2020). Through studies on fish, it was found that fish swallow microplastic particles and store them in the intestine. The intestine is an inedible part for humans, but it is used as food by other organisms or used to feed other organisms, and therefore the harm will return to humans through return and accumulation at higher levels. In the chain, as for mussels, the entire digestive system is eaten, which is the part used to store microplastic particles, and thus transfer them to higher levels in the food chain (Rainieri and Barranco 2019) .Microplastics are now ubiquitous around us in

drinking water, in food, in sugar, beer, honey, tap water, table salt, and in low concentrations, but chronic exposure to them threatens human health (De-la-Torre 2020)

2.1.2 Plastic ball

Microorganisms attack the plastic that constitutes one of their habitats. Because of this attack and colonization by microorganisms, these organisms form biofilms on the surface of the plastic known as the plastic ball (Kirstein, Wichels et al. 2019). This plastic ball plays an important role in its impact on the fate of plastic and its impact on the marine environment through the production of polymeric materials produced by extracellular microorganisms that contribute to the joint assembly of microorganisms and microplastics, which causes an increase or decrease in the deposition rates of algal blooms. With the impact on the function of the ecosystem, microorganisms can be a host for pathogens, which can travel long distances through the sea environment by dispersal of plastics, thus effectively achieving the spread of infectious diseases. Also, the production process of biomaterials causes a change in the physical properties of plastics. The chemical will increase the chances of colonization by metazoan larvae. This system of the plastic ball is also characterized by the growth of vital cracks on the surface of the plastic and between its voids, which causes the plastic to lose its physical integrity, which is known as biodegradation, which contributes to the decomposition of plastic debris into microplastics compared to bioanalysis (Cheng, Jacquin et al. 2021). Plastic debris (Plastic debris consists of PE on the sea surface and then PP followed by PS) affects marine organisms through ingestion, entanglement, and impeding movement. There are also concerns that some types of plastic debris absorb chemicals such as persistent organic pollutants (FCB) or act as carriers of them and thus amplify them in the food chain (Bryant, Clemente et al. 2016), in addition to the absorption of toxic metals and their inflation as well, of which mercury is one of the most important of these metals where mercury receives great attention as it is one of the global pollutants and is characterized by high toxicity to animals and humans, as it accumulates in a several of living organisms and some of its organic forms, especially methyl mercury (Barboza, Vethaak et al. 2018). Through studies, the rapid microbial colonization of plastic debris has been shown and there is a classification difference between the microbes that colonize plastic in the Atlantic Ocean and that colonize plastic in the ocean water column. The colonization of plastic by microbes was first demonstrated in 1972 (Bryant, Clemente et al. 2016). Also, it was proved that there are differences between bacteria that live on plastic and bacteria that live freely or in the surrounding seawater on organic molecules (Jacquin, Cheng et al. 2019). One study, by examining plastic particles with a diameter greater than 5 mm collected by scanning electron microscopy (SEM) from the North Pacific Ocean, demonstrated that the colonization process of the samples was largely caused by the

encapsulation of bryozoans. The colonizing organisms varied between algae, microbial biofilms (idioms), organoids, spiral and rod-shaped cells as well. It was also observed that bacterial cells possess prosthetic limbs and long filaments on the surfaces of bryozoans fig15 (Bryant, Clemente et al. 2016)

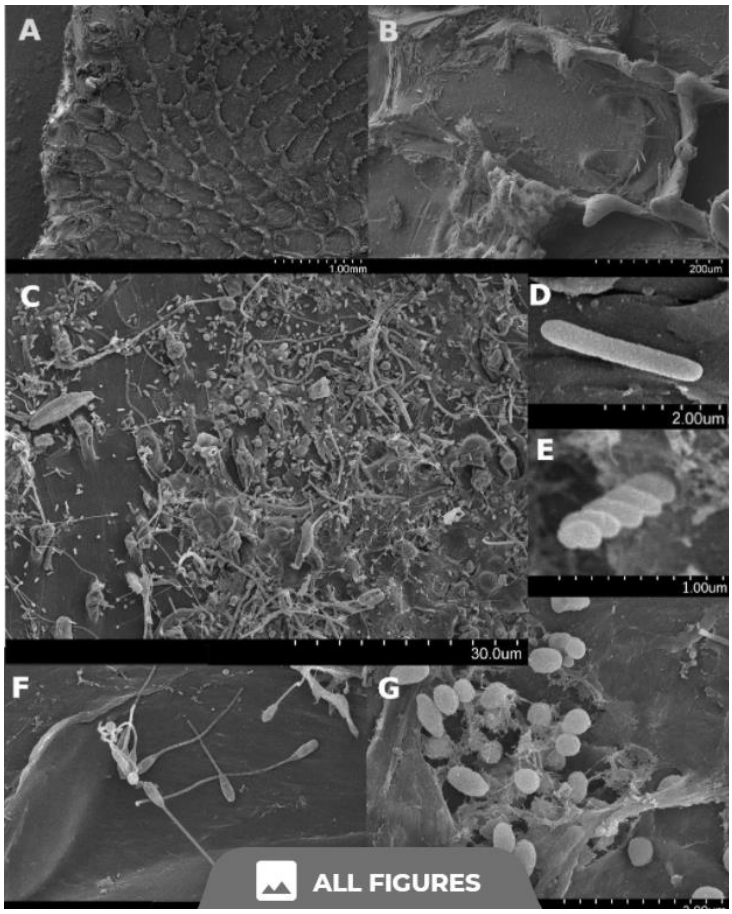


Figure15. SEM scanning electron image of microorganisms on the surfaces of microplastics. A. bryozoan colony on the surface of a plastic particle. B. An individual bryozoan contains diatom-shaped organisms. c. Anterior membrane area. D to G. Cells on the surfaces of plastic particles (Bryant, Clemente et al. 2016)

2.2 The duration of life of plastic wastes/The service life

The increase in the consumption of plastic leads to a greater accumulation of waste, which leads to the difficulty of disposal, due to the duration of life of plastic waste, which is very small, as 40% of it has a lifespan of less than a month. Through the areas of application, the average service life of plastic ranges from 1 to 35 years, as it varies from country to country. In India, the average service

life of plastic is 8 years, which is less than the average service life in Germany, which is estimated at 14 years. This difference in service life reflects that a high percentage of plastic is used in short-lived products (the share of plastic packaging is 40% in India, compared to 27% in Germany.) (Panda, Singh et al. 2010).

2.3 Plastic additives

Plastic additives are defined as chemical compounds that are added to plastics to give them certain properties or are combined to facilitate the manufacturing process (Alabi, Ologbonjaye et al. 2019), where it can reach 4% and may be organic and inorganic (Pattanayak 2018). Some of these additives are used as monomers such as bisphenol A, which is a polycarbonate (PC) monomer and is also a stabilizing agent in other polymers. The additives are used to give plastic flexibility, as flame retardants, or to resist pigment and oxidation. Brominated Flame, Retardants (BFR), Phthalates, Nonylphenol, and Bisphenol A (BPA), are the most additives to plastics that enter the marine environment through industrial and municipal wastewater, surface runoff and atmospheric sedimentation, and through agricultural sewage through rivers table7. The accumulation and deterioration of plastic debris can be a major entry for plastic compounds into the oceans due to the leaching of these materials through large and small plastics. The harmful effects of plastic additives on humans have been indicated, as the table shows the different types of plastic additives produced, their effects and functions table7 (Hermabessiere, Dehaut et al. 2017).

Additives	Function	Effects
Brominated Flame	Reduce flammability in plastic	Potential endocrine disruptors
Retardants (BFR)	Reduce flammability in plastic	Potential endocrine disruptors
Phthalates	Plastic softening	Endocrine disruptors
Nonylphenol	Antioxidant and plasticizer	Endocrine disruptors
Bisphenol A (BPA)	Monomer in polycarbonate and epoxy resins	Endocrine disruptors
Irganox®	antioxidant	Estrogen mimic

Table7. List of the most popular plastic additives, functions, and effects of their use (Hermabessiere, Dehaut et al. 2017).

It has been proved that polystyrene microplastics can absorb triazole fungicides, which may cause by hydrophobicity and electrostatic interaction, which is one of the main reasons for absorption (Ya, Jiang et al. 2021). As for heavy metals, a laboratory study was conducted on the absorption behavior of chromium in the human digestive system, taking into account the types of microplastics that are non-degradable (PP, PE, PVC, PS) and microplastics degradable Polylactide (PLA), where the results showed in the digestive phase of the stomach, stimulated by acids, the ability to release Cr of microplastic particles (Campanale, Massarelli et al. 2020).

2.4 Migration of chemical compounds from packaging plastic to food

The term migration is defined as the occurrence of the spread of substances from areas of high concentration to areas of low concentration (Guerreiro, de Oliveira et al. 2018) from packaging materials to the surface of the food, where this migration is linked to a several of properties such as contact duration, contract area, contact temperature, and materials that enter into the composition of the packaging material in addition to the type of food item. It was found that with the increase in temperature, there was an increase in the migration and spread of monomers, oligomers, and other compounds, and the migration and waiting rate was 6-7 times when the food was subjected to extreme temperatures (from freezing temperature to cooking temperature) (Bhunia, Sablani et al. 2013).There is a transfer of monomers, oligomers, and contaminants from packaging materials to foods, in addition to materials or compounds known as additives that are added to packaging materials to improve their manufacturing quality. Additives include plasticizers, antioxidants, light stabilizers, thermal stabilizers, lubricants, antistatic agents, slip materials, antistatic agents, as well as migratory solvents such as ethyl acetate, toluene, hexane, and dyes, which are also a source of nuisance and concern (Arvanitoyannis and Bosnea 2004). Several studies confirmed the migration of the styrene monomer present in PS to food, where styrene is the second most common monomer used in the production of packaging materials and styrene is a toxic substance that causes irritation in the throat, nose, and eyes when exposed to its vapors and it has a toxic effect on the liver and causes neurological impairment as it acts as a depressing agent for the nervous system, the daily exposure to this substance is estimated at 18.2-55.2 micrograms/person and an annual exposure of 6.7- 20.2 mg/person(Arvanitoyannis and Bosnea 2004, Bhunia, Sablani et al. 2013). Isocyanates are used in polyurethane polymers and adhesives, which are considered toxic, as their health effects have been documented, and their residual amount in plastic intended for use should not exceed 1.0 mg/kg and

use of small amounts of nylon oligomer, caprolactam residues, and nylon monomer give a bitter taste for food, therefore, do not use in packaging food prepared for cooking (Arvanitoyannis and Bosnea 2004).One study showed the presence of five contaminating compounds in pieces of beef that were in direct contact with vacuum plastic packages. Three of these compounds enter the manufacture of polymers, namely phthalic anhydride, which is used in the process of manufacturing organic polymers, adding stability to the final product and the second compound is polycarbonate. Ethylene glycol is added with other polymers to achieve hardness and ductility, and the third compound is dioctyl phthalate, which is used as a plasticizer, which proves that these compounds came from plastic packages (Guerreiro, de Oliveira et al. 2018).

2.5 Manufacturing

The process of plastic production, use, and transportation requires large quantities of mineral oil, and therefore the continuation and increase in the plastics industry make this industry occupy 20% of the total global oil consumption by the year 2050. The manufacturing process also reduces the storage of hydrocarbon fuels at high rates and the production of dangerous gases, these dangerous gases can also be produced during the production of forms of plastic that can be used through primary plastic materials. For example, when polyurethane is formed, it consumes 11% of total chlorine and 85% of total phosgene, which is considered globally hazardous. Plastic production is a source of danger to workers and a source of pollution to the environment and causes the death of a large number of people, animals, and plants, and this is due to the accidental leakage and occurrence of by-products and the toxicity of plastic materials themselves (Pattanayak 2018).

2.6 Human exposure to microplastics and the effect on human health.

Human exposure to microplastics through feeding on seafood is the main pathway for microplastics to the digestive system Fig16 (De-la-Torre 2020, Usman, Abdull Razis et al. 2020). Seafood is not the only source of human exposure to microplastics, but also through inhalation, skin contact, and ingestion, through dust generated by human activities, industrial textiles, and powdered synthetic rubber tires (Usman, Abdull Razis et al. 2020). Although the skin contributes to preventing the entry of microplastics into the body, microplastics can enter in other ways, such as wounds in the skin, through the thyroid gland, or through hair follicles, where these sources explain the pathways of entry of microplastics into the body, the most dangerous of which is seafood, which causes greater danger due to weathering, residual monomers, leaching of plastic additives, and interaction with toxic substances and microorganisms that cause diseases (Usman, Abdull Razis et al. 2020). Microplastics

affect human health indirectly through changes in the aquatic microbial community where they transfer genes or through their role in increasing the spread and resistance of microbes, Where which was referred to as the transmission of Bisphenol A (ARB) antibiotic-resistant bacteria from aquatic environments to soil and then to humans, and this is due to the presence of microplastics, work as a vehicle to microbes fig16 (Usman, Abdull Razis et al. 2020). The accumulation of microplastics in the cells and tissues of organisms that live in water exposes humans to dangers due to the ingestion of microplastics, as chromosomes change, causing cancer, obesity, and sterility (Usman, Abdull Razis et al. 2020). Regarding the amount of microplastic particles in men through the digestive system, microplastic particles were detected at a rate of 20 plastic particles, mostly polyethylene and polypropylene, with a size ranging from 5 to 500 mm per 10 g, although the digestive system is responsible for excreting 90% of micro and nano plastics ingested (Campanale, Massarelli et al. 2020). The genotoxic effects on lung epithelial cells and macrophages have been reported due to polystyrene particles with a size of 50 nm, this is due to the fact that the human lung has a wide surface lumen with a thin tissue barrier of fewer than 1 μm that allows the nanoparticles to penetrate the bloodstream and throughout the body (Campanale, Massarelli et al. 2020).

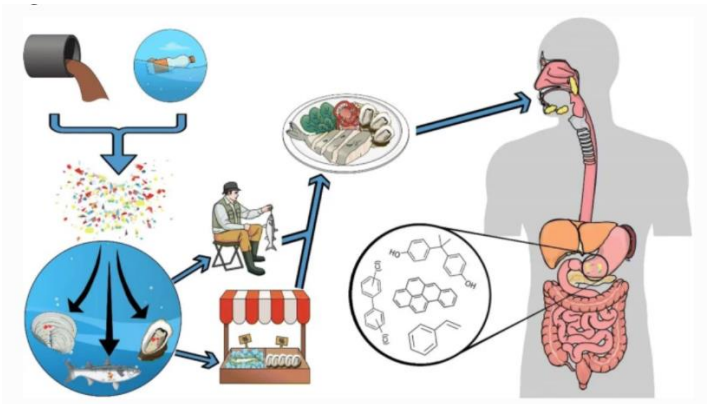


Figure16. The figure shows the path of entry of microplastics to the food web, then to our food, and from there to the body and organs(De-la-Torre 2020).

2.7 Disposal of plastic waste.

Plastic waste and municipal waste are a source of bothering to the environment and there is an ongoing search until now for ways to properly use and dispose of plastic waste. Because of the difficulty of deteriorating plastic waste and its accumulation, makes the disposal of municipal waste very difficult (Vasudevan, Sekar et al. 2012). Globally, plastic that is disposed of as waste is sent to landfills, incineration, or recycling. Until 2015, 8.3 billion tons of plastic were produced, which is equivalent to more than a ton per person. 6.3 billion tons were disposed of, of which 76% were

wasted. Between 1950 and 2015, 9% of plastic waste was recycled and 12% and 76% were incinerated. Accumulated in landfills and the environment. The amount of waste generated by an individual varies greatly from country to country fig17 (Clunies-Ross 2019). In the Kingdom of Saudi Arabia, approximately 12 million tons of municipal solid waste are produced each year, and plastic constitutes 15-20% of this waste, 40% is organic waste, and 20% is paper waste. According to statistics issued by the Makkah Municipality, 82,933 municipal waste was generated, of which 26% was plastic (Alshehrei 2017).

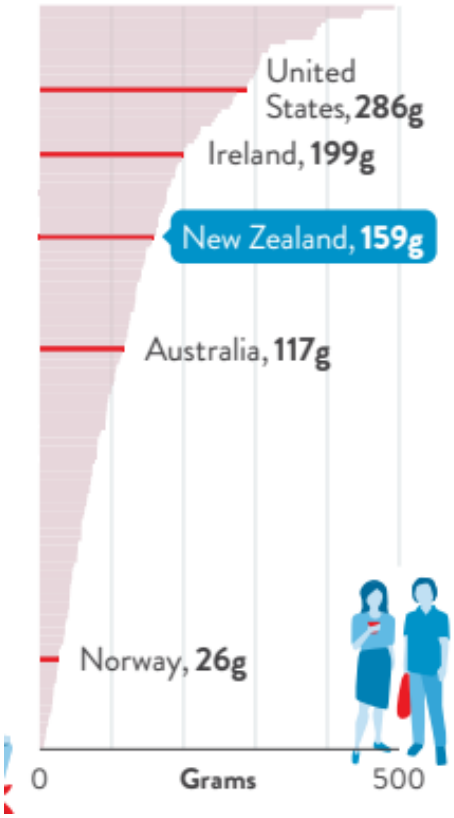


Figure17. The amount of plastic waste generated by the individual by country in 2016 (Clunies-Ross 2019).

2.7.1 Landfill

In the developed countries of the world, solid waste is disposed of in a special place known as a landfill, where it is compressed, reduced in size, and then buried. Solid waste remains in the landfill for many years because of slow or no biodegradation and the scarcity of both oxygen and ultraviolet rays (the requirements of biodegradation). The type of plastic and environmental conditions plays a role in determining the duration of the microbial degradation process (Clunies-Ross 2019). Recent studies have shown solid waste components in landfills, where combustible materials constitute 20-

30%, plastic is the main component of combustible materials, 50-60% are soil materials, and the remaining 10% are inorganic materials (Zhou, Fang et al. 2014). Unmanaged and well-organized landfills can be considered as the source of waste entering the environment through torrents and floods (Clunies-Ross 2019), The process of dumping plastic waste in the landfill also causes damage to the soil and animals, as the soil is exposed to decay and increases the risks of animals consuming plastic (Rajmohan, Ramya et al. 2019). An open landfill is also a suitable place for the reproduction of rodents and insects, which in turn transmit dangerous pathogens, and the fullness of the landfill increases the cost due to the difficulty of finding new burial sites, and this requires larger tunnels to dispose of solid waste (Abebe 2018). At the moment, plastic landfills are less preferred in the UK due to public health concerns, the plethora of toxic substances, and their potential for infiltration into soil and groundwater (Alabi, Ologbonjaye et al. 2019). Although there are many disadvantages to this method, it is the most widely used because of the low cost it achieves compared to other methods. The cost per ton that is deposited in the landfill is $62.50, while recycling costs $108.50 per ton, and Incineration is the most expensive at an average of $175. per ton and fertilization cost 115 dollars per ton.

2.7.2 Incineration

Plastic waste can also be disposed of through the incineration process to obtain energy used in heating and electricity generation. The burning of plastic waste leads to the release of fumes and toxic compounds such as dioxins and persistent organic pollutants. These emissions can be mitigated by adjusting the optimal conditions (incinerator temperature) (Alshehrei 2017, Clunies-Ross 2019). The incineration process takes place directly in the air at a temperature of 850 degrees Celsius, so the liquid waste is converted into carbon dioxide, water, and fuel, and the waste incineration process does not require burial of waste, and this leads to the production of energy, which can benefit for use in other fields (Rajmohan, Ramya et al. 2019). The incineration process is very popular in Denmark, Sweden and Japan and is also used in some Asian countries such as China and Japan, but given global warming, researchers have proven that burning plastic waste packaging is dangerous to the environment. In India, energy production decreases when burning, due to the high percentage of moisture in waste, which ranges between 60%-65%, and therefore it is considered a method that does not achieve success (Singh and Ruj 2015). A change in the hydrogen ace may occur due to the chemical interaction between the combustion products of plastic and water, and this leads to a change in the functions of the water systems, as well as the deposition of ash and other products resulting from combustion on the soil and plants and the occurrence of its migration during rain to groundwater,

polluting it or absorbed by the plant and thus moving within the food chains (Alabi, Ologbonjaye et al. 2019).

2.7.3 Recycling

This method reduces the plastic that is disposed of in the environment and is not economically viable due to higher recycling costs compared to plastic that is made from fossil fuels. 14-18% of the world's plastic waste is recycled (Clunies-Ross 2019), in China, 6 million tons of plastic waste were recycled in 2005, and this amount is very small compared to plastic production, and the percentage of recycling constitutes one-fifth of consumption (Zhang, Zhu et al. 2007). While in developed countries the rate of recycling has increased since the 1980s, the recycling rate is reported in both the United States and Europe, in Norway more than 40% is recycled, Sweden 12.54% is recycled, the United States 32% and France 23% of plastic waste table8. Some countries such as New Zealand do not have the possibility of recycling and send recyclable plastic waste to other countries such as China, where China imported more than 50% of recyclable plastic waste from several countries, and import ban laws were issued, causing the accumulation of plastic waste in the source countries. The recycling process requires the disposal of contaminants such as food residues and compounds that consist of difficult materials, and this requires the dismantling of each type of plastic (Clunies-Ross 2019). There are challenges in reusing recycled or recovered plastic materials due to some concerns related to safety standards, for example, the food packaging process requires a layer of the modern plastic coating due to contact with food, which prevents the use of recovered plastic and this is due to additives that may cause an increase in the toxicity of recovered plastic also the cost of collecting, sorting and the required size that hinders the recycling process and makes many plastic materials not achieve the economic feasibility of recycling Where transparent or opaque PET and colorless HDPE have a high recycling value (Clunies-Ross 2019). Also, electronic plastic waste constitutes at least 20% of plastic waste and its recycling is complicated by the presence of brominated flame retardants and persistent organic pollutants, Which can be recovered when recycled (Sahajwalla and Gaikwad 2018) and used in new products such as children's toys, personal care and hair supplies (Clunies-Ross 2019). And this is not in accordance with Stockholm law on recovery of POPs and BFRs and this requires more efficient technologies for recycling electronic plastic waste (Sahajwalla and Gaikwad 2018).

COUNTRY RECYCLING	RECYCLING %	INCINERATION %	LANDFILL %
Germany	38,6	60,6	0,8
Spain	36,5	17,1	46,6
United Kingdom	31,1	38,3	29,6
Italy	29	33,8	37,2
Poland	26,8	29,1	44,1
France	22,8	44,2	32,5
United states	9,1	15,5	75,4

Table8. Percentage of methods used to treat plastics from municipal waste in Europe 2015 and the United states 2016 (Clunies-Ross 2019).

3 Methods

The protocols were obtained and applied in laboratory experiments by Professor Andrew Jenkins.

3.1 Material

Material

Erlenmeyer flasks, Petri-dishes, Test tubes, Beakers, Pipette, Micropipettes, Filter paper, Distilled water, Sterile water, water, Tube holder, magnetic, glass slides, aluminum foil, Tweezers, inoculating loop, syringe, syringe needles, dräger pump, dräger CO2 measuring tubes, self-sealing rubber caps, Cellulose stoppers, filter funnel, coffee filters.

Equipment

Electronic weighing scale, Refrigerator, Autoclave, Oven, Hotplate magnetic stirrers, Microscope, Stove, Bio photometer, Agitator microtiter.

Chemicals

½ TSA, Agar (soy agar), M9 minimal salts (growth media), Trace element, Cu/Fe, PE (substrate) Low-density polyethylene (LDPE) was used without any additives, Safranin, Crystal violet, Lugols, Glycerol, Ethanol, Hydrogen peroxide H2o2, N, N, N, N -tetramethyl-1,4phenylen-diammonumdichlorid (Oxidase reagent), SDS.

3.2 Method

3.2.1 Sample collection

The microbial samples (isolates) that were isolated and studied were obtained from an old piece of plastic taken from the Klyve area in the municipality of Skien, in the Vestfold and Telemark country.

3.2.2 Sterilization

Sterilize both liquid and solid growth media, flasks, and bottles used in isolation tests and tests to determine the biodegradation activity of low-density polyethylene, in the autoclave at a temperature of 121 ° C for 40 minutes to ensure that there is no microbial growth.

3.2.3 Preparation of the growth media (Liquid media)

Preparation of growth medium for the growth of bacteria that will be cultured and isolated to study its effectiveness in the biodegradation of low-density polyethylene (LDPE). The components have 5.6 g M9 salts, distilled water 500 ml, and 500 µl Trace elements. The components are placed in a bottle with an airtight seal and placed in an autoclave for sterilization. Temperature 120°C for 40 minutes to ensure that there is no bacterial growth.

Components of growth medium Liquid media	
5,9 gr	M9 Salts
500 ml	Distilled water
500 µl	Trac element

3.2.4 Preparation of sold media (1/2 TSA+Agar)

The importance of agar comes from forming a medium for the growth of bacteria by providing elements necessary for growth. We will rely on the agar that is poured into the dishes to isolate and grow the bacteria that contribute to the degradation of plastic (LDPE). We take an airtight bottle and put 10 gr (1/2 TSA media) in it, then add 5 gr of agar, then add 500 ml of distilled water. We put the bottle in the autoclave at 121 ° C for 40 minutes to sterilize and obtain a homogeneous liquid. The agar is characterized by its solidification at room temperature, so after taking it out of the autoclave, it is placed in an oven to maintain the liquid consistency to pour into dishes when needed.

Components of growth medium Solid media	
10 gr	½ TSA media
500 ml	Distilled water
5 gr	Agar

3.2.5 Microbial analysis

The microbial analysis included cultivation, isolation, purification, characterization, identification, and determination of the ability of microbes to degrade plastics.

3.2.6 Cultivation of plastic degrading microorganisms

We take two Erlenmeyer flasks with a capacity of 150 ml each and put them in the autoclave for sterilization at 121 ° C for 40 minutes. After sterilizing the bottles, we put in each flask 150 ml of sterilized media, we add 100 µl of copper and iron mixture, and we also add 1 g of low-density polyethylene, in addition to placing a small piece of plastic in each beaker that was brought from the forest. The flasks are wrapped in aluminum foil to block out the light, so we don't have algae. The flasks are placed on the vibrating machine for 6 weeks at a rate of 100 rpm.

150 ml	Media
100 µl	Cu/Fe
1 gr	LDPE
1 cm	small piece of plastic

3.2.7 Isolation and Purification of plastic-degrading microbes

After six weeks of incubating the low-density polyethylene in the media, we removed the aluminum that was wrapped in the beaker and took a magnet. We sterilized it and placed it in the beaker. Then the beaker was placed on the spinning device so that the plastic flakes floated on the surface of the solution. Then, using a pipette, we took a portion of the solution 3 ml. We prepared Petri dishes and poured the agar and after solidification, we spread the solution on Petri dishes more than once to get pure colonies fig19 and fig20.

3.2.8 Samples preservation

The 20% glycerol was prepared with Nutrient broth and placed in small 3ml tubes. A portion of AH1 culture was placed in the first tube, then a portion of AH2 culture was placed in the second tube, and so on for all cultures that were isolated and kept in a freezer at _70°C. This step is intended to retain isolatess for use when needed.

Preservation materials	
Nutrient broth	80%
Glycerol	20%

3.2.9 Characterization of isolates

3.2.9.1 *Morphology characterization*

_Gram staining

We will color cultures that we got (AH1, AH2, AH3, AH4, AH5, AH1a, AH1b, AH4b2, AH4b3). Gram stain is used to distinguish between microbes, and which group they belong to by farging the cell wall. They are either Gram-negative with red colour or Gram-positive with blue color.

Bacterial preparation and fixation.

We take a slide with a drop of water in the middle and by inoculation loop we take 100 µl of bacterial culture and transfer it to a drop of water, then we spread it over the slide and leave it to dry. After it dries, we pass it over a blue flame.

1. Flood the slide with crystal violet for 1 minute.
2. Rinse with Lugols iodine for a minute.
3. Rinse with water for five seconds.
4. Rinse with ethanol until no color.
5. Rinse with water five seconds.
6. Flood the slide with safranin for a minute
7. Rinse with water for a minute

We wait for the slide to dry and check with the microscope figure21.

3.2.9.2 Biochemical test

_Oxidase test

This test is either negative or positive to determine the ability of microorganisms to secrete the enzyme cytochrome oxidase, which is a factor in aerobic respiration that transfers electrons to oxygen and forms water. After we prepared the reagent by taking 0.05 gr of oxidase reagent with 3ml of sterile water, we took a filter paper and put it in a Petri dish, and by inoculating loop we took part of the microbial culture from the available isolates (AH1, AH2, AH3, AH4, AH5, AH1a, AH1b, AH4b2,

AH4b3) and put it on the filter paper and then added a drop of the reagent to each portion extracted from the isolates, some isolates were colored in purple, which indicates that they are oxidase-positive, and some of them are colorless, which indicates that they are oxidase-negative (KOMAL 2019) table9.

0.005g	Oxidase reagent
3 ml	Sterile water

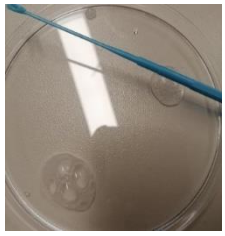
Oxidase test

_Catalase test

This test is used to detect catalase enzyme in aerobic and anaerobic bacteria, where anaerobic bacteria lack this enzyme, that is, they are catalase-negative (Reiner 2010). This indicates that microbes produce the enzyme catalase that they use to protect themselves from the oxidative damage of hydrogen peroxide(H_2O_2).

$2H_2O_2 \rightarrow 2H_2O + O_2$

1 ml of hydrogen peroxide was taken in a tube, and we added 9 ml of sterile water to it, then we took a drop of this solution and put it in a petri dish to test the microbial cultures from (AH1, AH2, AH3, AH4, AH5, AH1a, AH1b, AH4b2, AH4b3). The appearance of bubbles indicates that the microbial isolates are catalase-positive and catalase-negative when no bubbles appear table9.



1 ml	H2o2
9 ml	Sterile water

3.2.10 Identification of plastics degrading strains

The bacteria were identified after performing sequencing analysis of 16S rRNA genes with bacteria grown in nutrient broth by polymerase chain reaction (PCR) by Daniel Abiriga a Ph.D. student at USN University. After obtaining the sequences, I performed sequence editing through BLAST search and identification of microbial samples table10.

3.2.11 Analytical tests to monitor biodegradation of LDPE

3.2.11.1 Assessing microbial growth on plastic (LDPE)

Dilution method for counting the number of bacterial colonies

Before dilution (isolates preparation)

Two groups were prepared for each sample of microbial isolates, a control group, and a test group to measure the growth using the dilution method and count the number of colonies after spreading on the agar. We took two Erlenmeyer flasks with 250 ml capacity for each isolate with tampons and added to each flask 50 ml of growth medium and then sterilized using an autoclave. After sterilization, a plastic tube was taken and we added to it a sterile growth medium, then using an inoculating loop, part of the bacterial culture was transferred to the tube and then a shaking process was performed to obtain homogeneity, that is, to achieve the spread of bacteria within the growth medium. Then we added to the first flask (test group) 0.5 ml of the bacterial culture from tube and 2 g of low-density polyethylene (substrate). In the second flask (control group) we added only 0.5 ml of the bacterial culture which was also diluted without substrate. We repeated the previous steps for all isolates (AH1, AH2, AH3, AH4, AH5, AH1a, AH1b, AH4b2, AH4b3).

Test group	Control group
2-gram LDPE	Without LDPE
50 ml medium	50 ml medium
0.5 ml bacterial culture	0.5 ml bacteria culture

Dilution method to count colonies

Calculation of the dilution factor

Dilution= (volume of culture)/ (the volume of culture +volume of diluent)

= 0,5/ (0,5+4,5)

= 0,1

dilution factor= 10 per plate

CUF/ml= Number of colonies. dilution factor

(Colony-forming units)

We take five or six tubes of 10 ml, and we number them from 0.1, 0.01, 0.001 to 0,000001. We place in each tube 4.5 ml sterile growth medium. We take 0.5 ml from the control group bottle and add it to the first tube. Then 0.5 ml is transferred from the first tube to the second to the third to the fourth and so on in succession. We repeat the same steps for the control group. Repeat these steps for all samples. We take six Petri dishes for the six-tube set (test set) that were prepared in the previous paragraph. We number the dishes from - 1 to - 6 in a row. We take 0.5 ml from the first tube to the first plate and then from the second tube to the second plate until the sixth plate in a row. We repeat the steps for the control group. These steps are also repeated for all samples. After transferring the diluted solution to the dishes, we pour the agar into the dishes and wait for two days until we get clear, countable bacterial colonies. We repeat these steps for a period of 20 to 25 days, where every two to three days we count the bacterial colonies. with an increase in the number of colonies, we get an increase in growth fig22.

3.2.11.2 Measuring CO2 production (AH4 b2, AH2)

A part of a colony that can utilize low-density polyethylene (substrate) as a carbon source is transplanted into an airtight growth medium. The carbon dioxide content is measured after an adequate incubation period, and a comparison is done with a negative control containing only the substrate within the growth media and another containing only a bacterial culture inside the growth medium. The purpose of the test is to measure the susceptibility of organic compounds to biodegradation by microorganisms (Strotmann, Reuschenbach et al. 2004). Measurement of CO2 is slightly different from a weight loss experiment in principle. The microbial may break the substrate (PE) into soluble components causing weight loss without the need for further metabolism.

Metabolism of PE occurs according to the following equation

$2\ CH_2 + \ 3\ O_2 \rightarrow 2\ CO_2 + 2\ H_2O$

Procedure:

Six Erlenmeyer flasks of 250 ml capacity with self-closing stoppers were sterilized by autoclaving (three flasks per culture AH4b2 and AH2).

Each flask was filled with 50 ml of sterile growth medium.

A portion of the fresh bacterial culture was taken for both isolates AH4b2 and AH2 and the suspension of A540=0.5 was made by Bio photometer by dilution by growth medium until we got A540=0.5.

Three flasks were prepared for each bacterial culture as follows.

a. Test. 1 ml of culture and 1 g of the substrate (LDPE).

b. No substrate control. 1 ml of culture.

c. Sterile control. 1 g of substrate (LDPE).

Covered and incubated for six weeks.

For carbon dioxide measurement.

Figure 18 shows how we put the device together.

Pump up the pressure, then let it go.

The small round indication glass on the top of the pump should be noted. When the pump is compressed, this changes, and when the correct amount of air is extracted, it returns to its former appearance.

To prevent air from entering the flask and diluting the headspace gas, keep the 50 ml syringe topped up with water.

Examine the tube and compare the position of the purple fig34 sector's edge to the tube's scale.

Take note of this number table11.

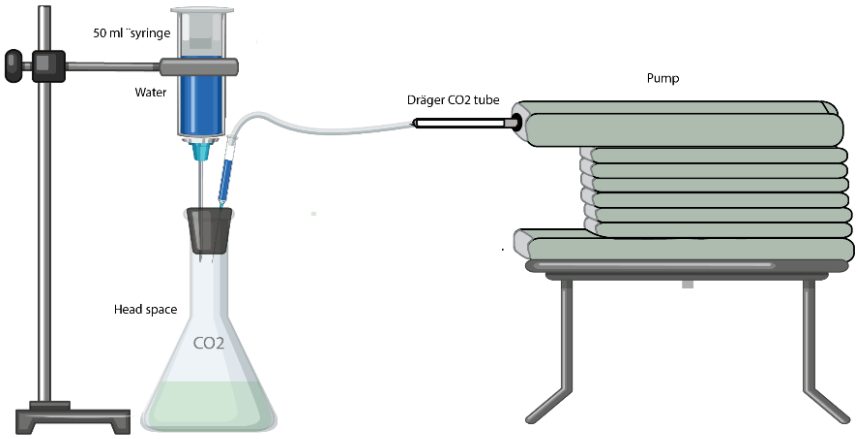


Figure18. The method used to measure CO2 produced (Zolanvari 2021).

3.2.11.3 Weight-loss experiments (AH4b2, AH2)

This method is based on calculating the weight difference before and after biodegradation. Where the substrate low-density polyethylene is incubated with a bacterial culture within a growth medium for a certain period. The substrate (LDPE) is then filtered through a pre-weighed filter, washed, dried, weighed and determined for weight loss.

Weight loss = (initial weight of substrate) – (final weight of substrate).

Not only is the degradation of plastics measured by bacterial culture, but the degradation of insoluble materials is also measured.

Procedure:

Six Erlenmeyer flasks of 250 ml capacity with cellulose stoppers for each isolate were sterilized in an autoclave. 50 ml sterile growth medium was placed in each flask along with 1 g(LDPE) (substrate). A homogeneous suspension of the bacterial culture) (AH4b2, AH3) was made by dilution with a

Biophotometer until we got A540 = 0.5. We added 1 ml of the suspension to three flasks and the other three are a negative control without bacterial culture.

Incubation was carried out with shaking for six weeks.

Six weeks later

Weigh six coffee filters per sample accurately.

Using a coffee filter, filter each flask.

Wash each flask with 1 percent SDS to remove any remnants of the substrate.

500 mL distilled water to clean the filter

Clean the filter (substrate with filter).

After the filter has dried, weigh it.

After 24 hours, repeat the drying process.

Continue drying if there is a weight difference.

Calculate the rate of loss in the substrate using the table12.

4 Results

4.1 Isolation

After six weeks of cultivation and taking part in the growing medium and spreading it to the agar, we obtained a different set of colonies fig19.

Figure19. Different types of colonies after cultivation and isolation.

4.2 Purification

We got a different group of microorganisms (impure colonies) fig19. The agar was poured into the dishes and the colonies were spread on the agar more than once and we obtained pure samples fig20.

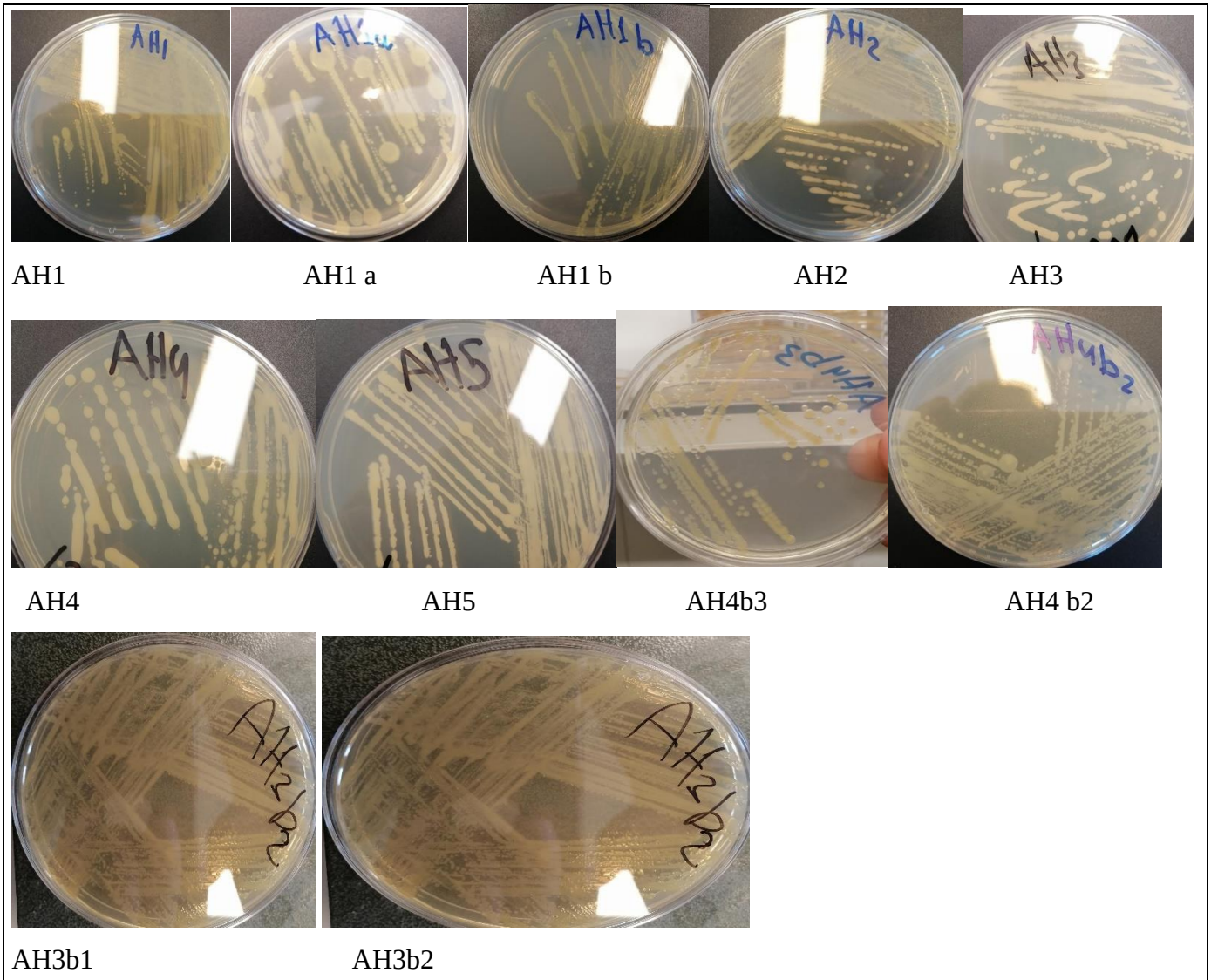
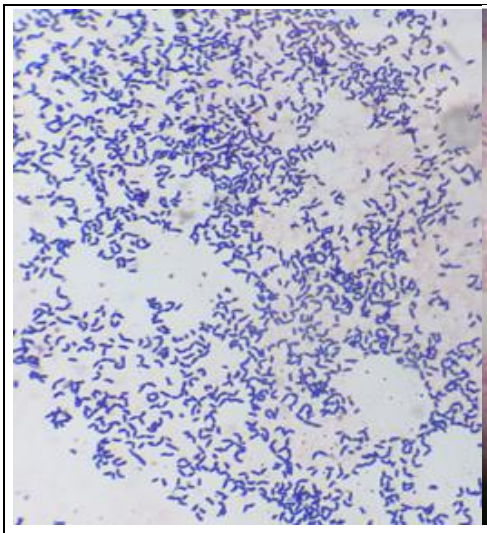


Figure20. Isolates after purification, the isolates showed slightly different colors and different forms of colonies.

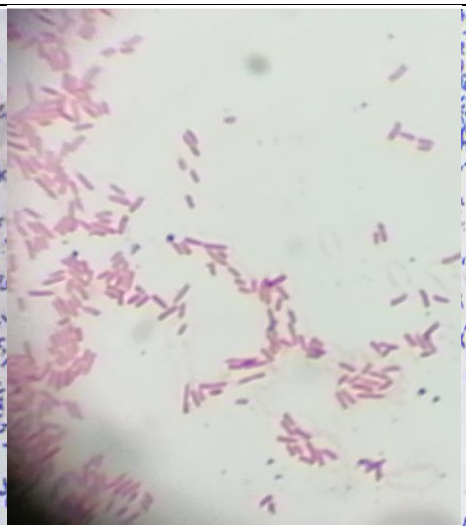
4.3 Characterization of strains

4.3.1 Morphology characterization and biochemical test

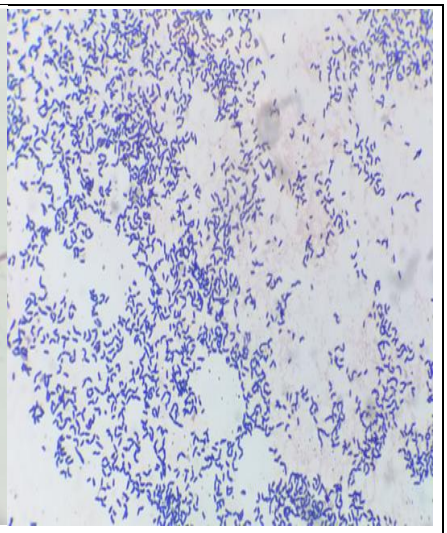
Determination of morphological characteristics, color, and shape of strains under the microscope with a magnification of X100, after staining fig21 and biochemical test table9.



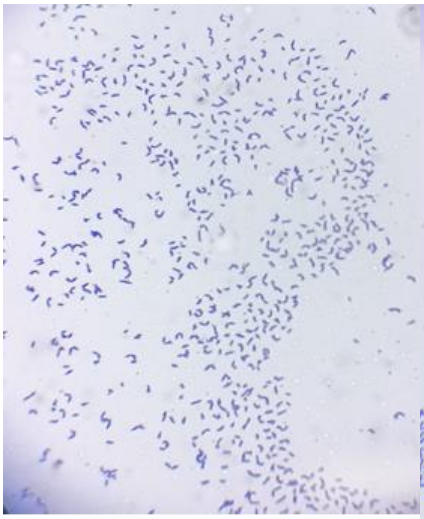
AH1Gram positive rod



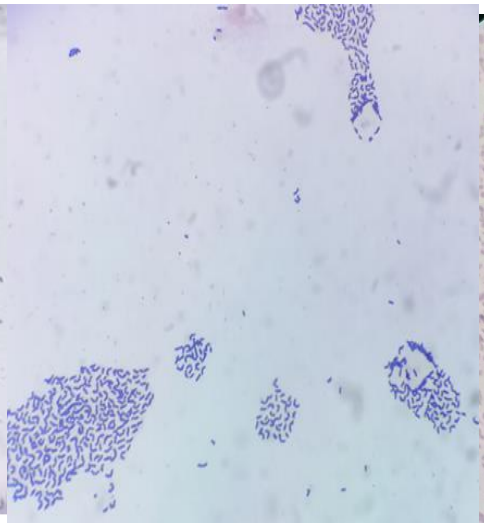
AH2 Gram negative rod



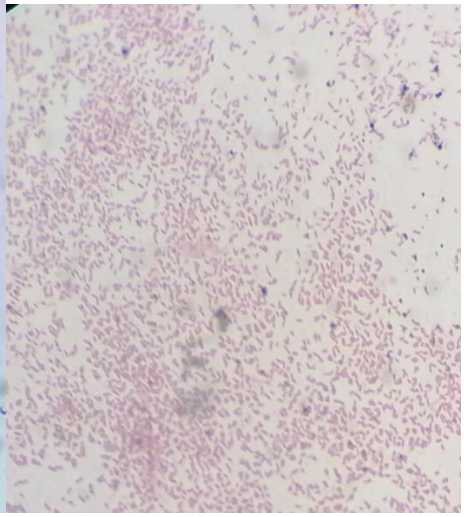
AH3 Gram positive rod



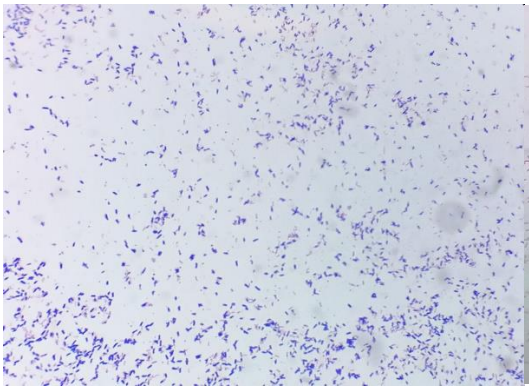
AH4 Gram -positive rod



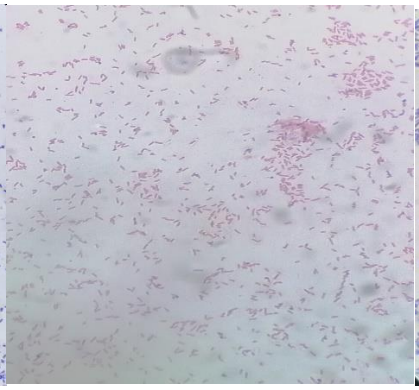
AH5 Gram- positive rod



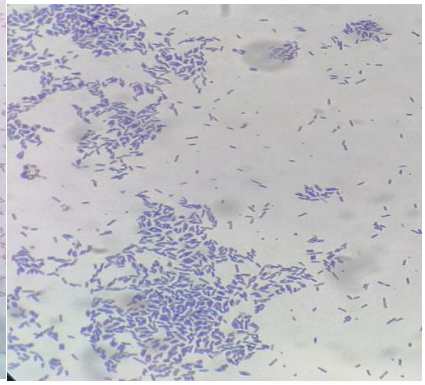
AH1a Gram-negative rod



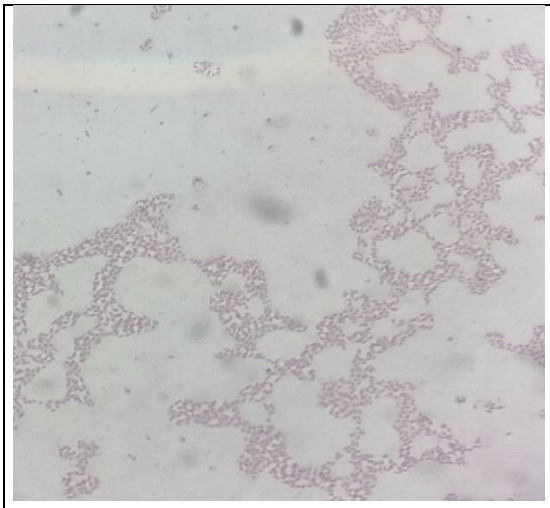
AH1 b Gram -positive- short rod



AH4 b2 Gram-negative spherical



AH4 b3 Gram- positive rod



AH3b1, AH3b2 Gram-negative rod

Figure21. Isolates after staining and microscopy, AH1Gra- positive rod, AH2 Gram-negative rod, AH3 Gram-positive rod, AH4 Gram-positive rod, AH5 Gram-positive rod, AH1 a Gram-negative spherical, AH1 b Gram-positive- short rod, AH4 b2 Gram-negative spherical, AH4 b3 Gram-positive rod, AH3b1Gram-negative rod

_Isolates after staining and biochemical test (Gram, Oxidase test, Catalase test)

Strain	Gram	Oxidase test	Catalase test
AH1	Positive	Negative	Positive
AH1 a	Negative	Positive	Negative
AH1 b	Positive	Negative	Positive
AH2	Negative	Positive	Negative
AH3	Positive	Positive	Positive
AH3 b1	Negative	Positive	Positive
AH3 b2	Negative	Positive	Positive
AH4	Negative	Positive	Negative
AH4 b2	Negative	Positive	Negative
AH4 b3	Positive	Negative	Positive
AH5	Positive	Negative	Positive

Table9. The results of both staining, oxidase, and catalase testing of microbial isolates.

4.4 Identification of plastics degrading strains

Sequences were entered, a blast search was performed, and samples with similarity close to 100% were selected table10.

Isolates	species	Match
AH1a	*Stenotrophomonas pavanii*	98,22%
AH3b2	*Stenotrophomonas maltophilia*	98,22%
AH1b	*Microbacterium saperdae*	100%
	Microbacterium hydrocarbonoxydans	100%
	Microbacterium phyllosphaerae	100%
AH2	*Pseudomonas helmanticensis99,62*	99,62%
AH4 b2	*Pseudomonas migulae 99,43*	99,43%
AH3 b1	*Pseudomonas canadensis*	99,81%
	Pseudomonas salomonii	99,81%
AH4a	*Rhodococcus erythropolis*	100%
AH4 b1	*Rhodococcus qingshengii JCM 15477*	100%
	Nocardia coeliaca	100%
AH3a		
AH5		
AH4 b3	*Stenotrophomonas rhizophila 99,25*	99,25%
	Stenotrophomonas bentonitica 99,6	99,6%

Tabl110. The table shows the identity of microbial samples by sequencing and blast search.

4.5 Analytical tests to monitor biodegradation of LDPE

4.5.1 Assessing microbial growth on plastic (LDPE)

We counted the colonies of the samples (AH1, AH2, AH3, AH4, AH5, AH1a, AH1b, AH4b2, AH4b3) fig22, recorded them in Excel, and represented them graphically. We noticed an increase in growth for both sample AH4b2 and AH2, and to verify the growth, we measured the growth again, counted the colonies, recorded data, and represented it graphically for each of AH4b2 and AH2, and negative results were obtained, meaning no growth occurred figures from 23 to 33I.

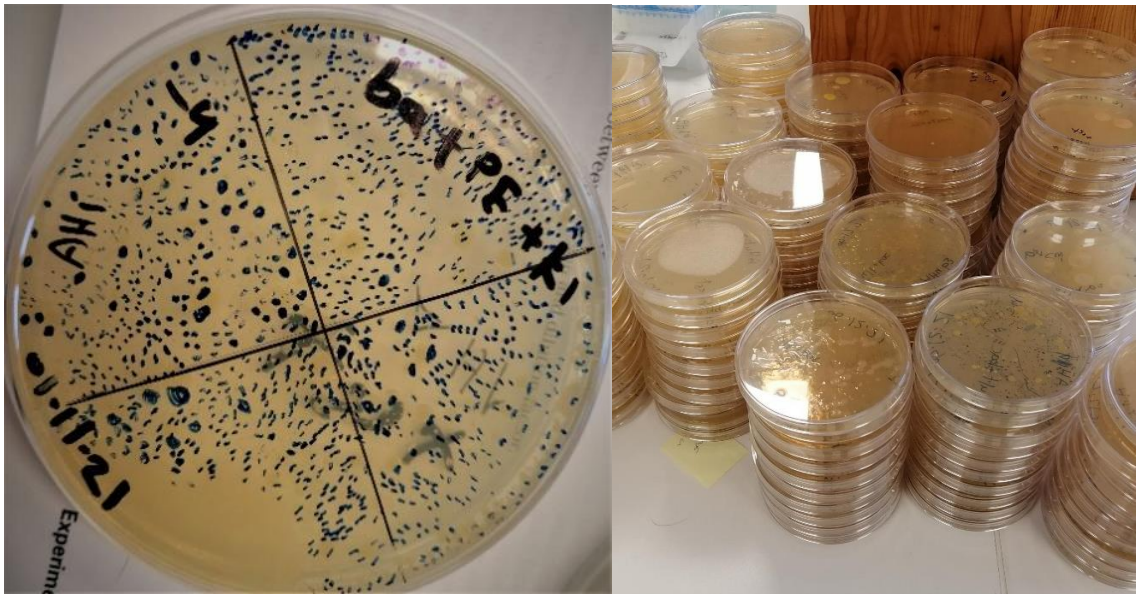


Figure22.Counting bacterial colonies after their appearance on the surface of the agar.

The first stage of growth measurement

The growth of samples and colony counting for 37 days was measured for each of the test group and the control group for samples (AH, AH2, AH3, AH4, AH5), using different dilutions fig 23, 23I, 24, 24I, 25, 25I, 26, 26I, 27, 27I.

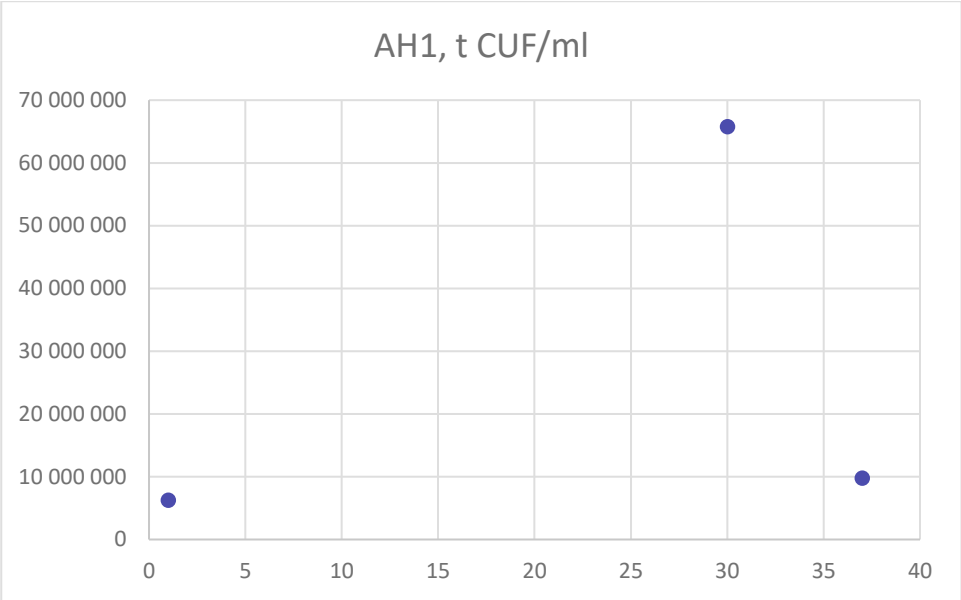


Figure 23. CUF/ml the number of bacteria cells per ml (AH1. test group)

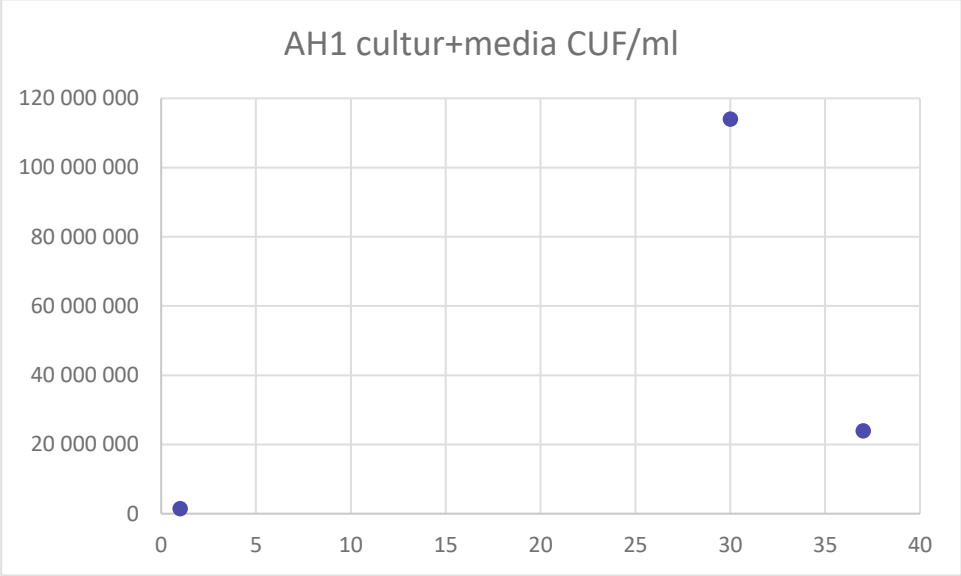


Figure23I. CUF/ml the number of bacteria cells per ml (AH1.control group)

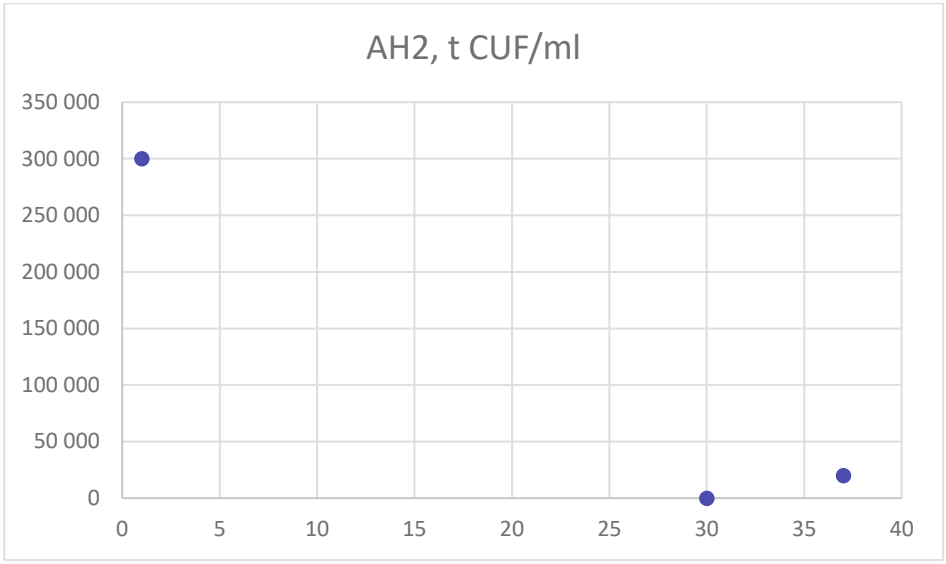


Figure24. CUF/ml the number of bacteria cells per ml (AH2.test group)

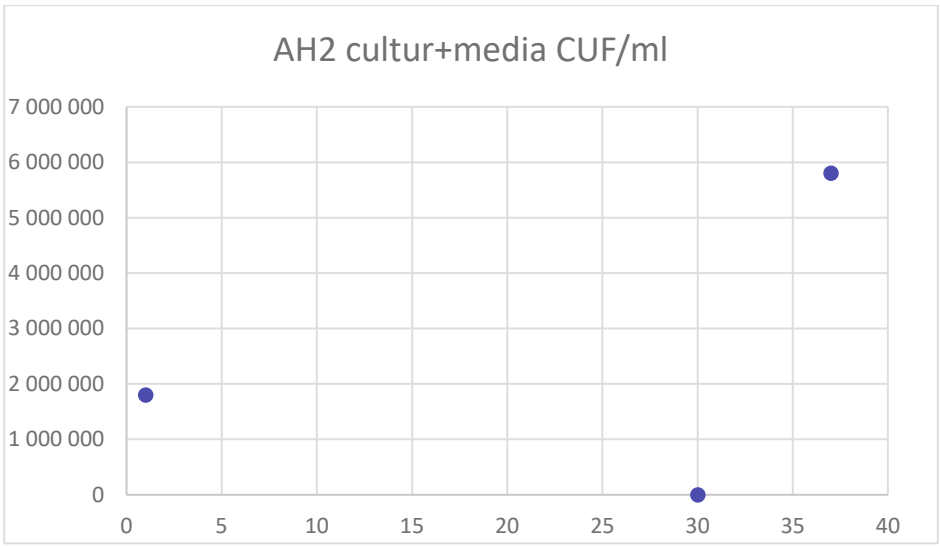


Figure24I. CUF/ml the number of bacteria cells per ml (AH2.control group)

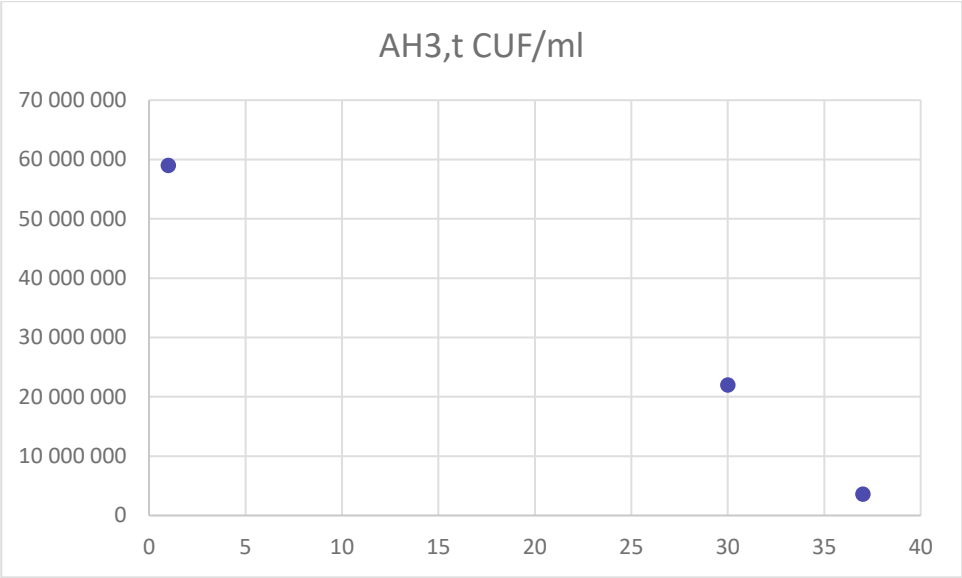


Figure25. CUF/ml the number of bacteria cells per ml (AH3.test group)

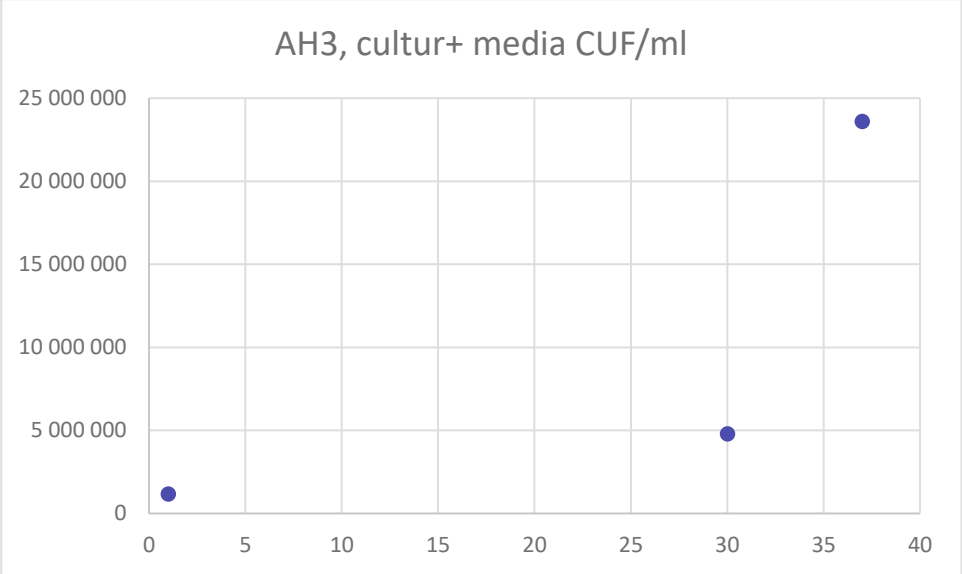


Figure25 I. CUF/ml the number of bacteria cells per ml (AH3.control group)

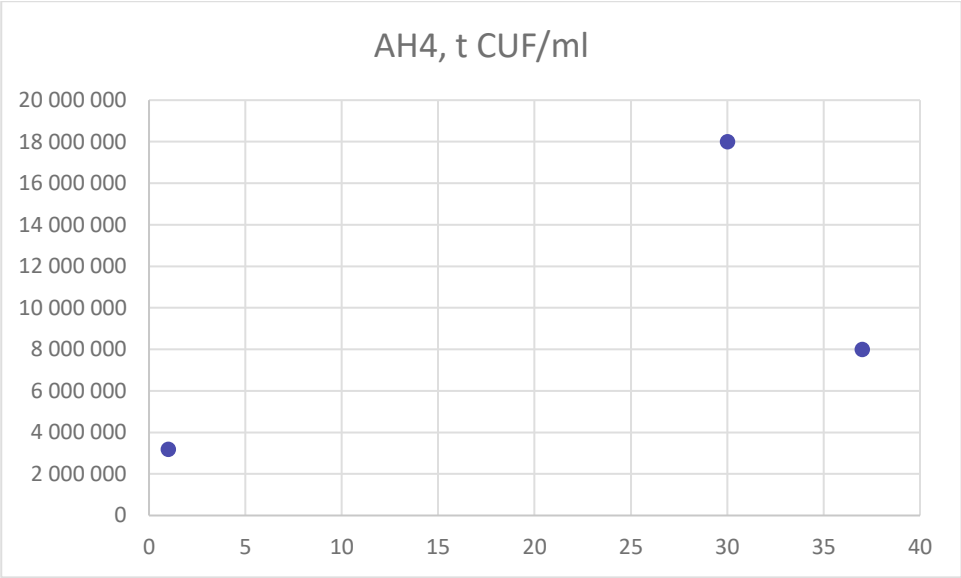


Figure26. CUF/ml the number of bacteria cells per ml (AH4.test group)

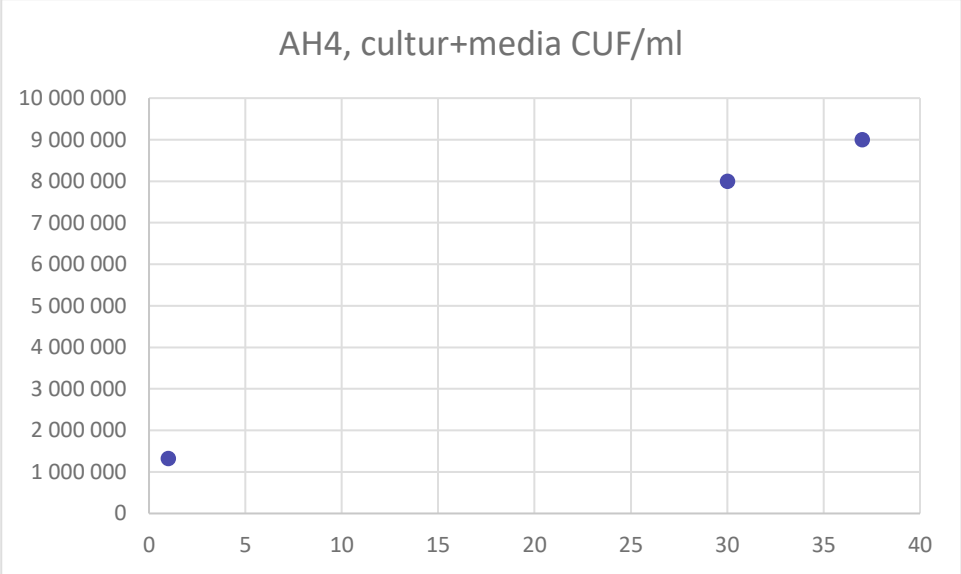


Figure26 I. CUF/ml the number of bacteria cells per ml (AH4.control group)

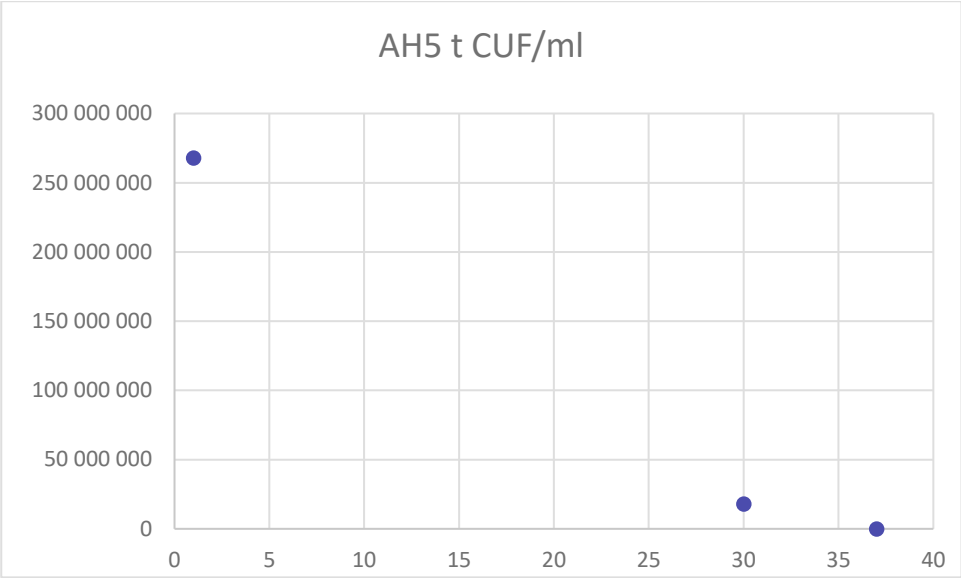


Figure27. CUF/ml the number of bacteria cells per ml (AH5. test group)

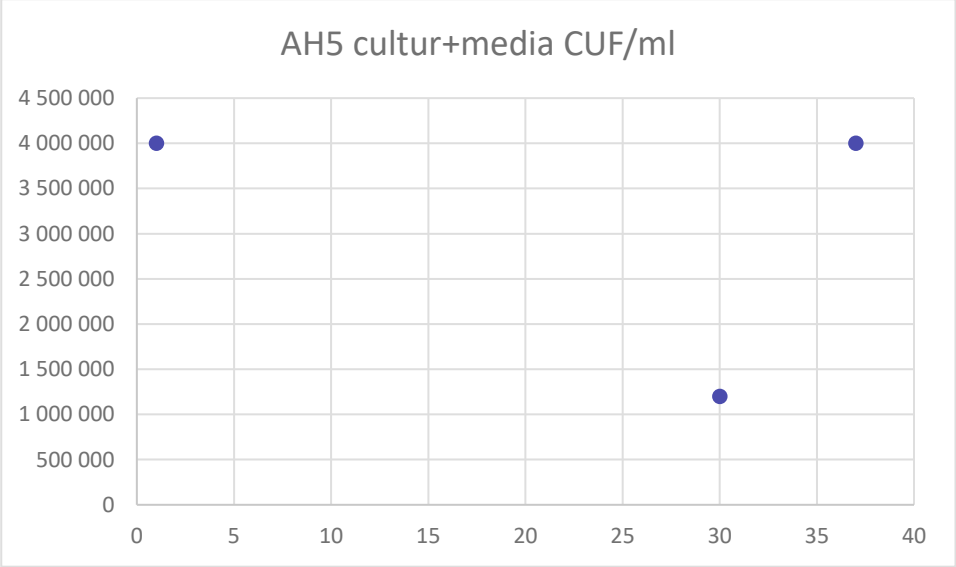


Figure27 I. CUF/ml the number of bacteria cells per ml (AH5. control group)

The second stage of growth measurement

The growth of samples for 37 days was measured for each of the test group and the control group for samples (A4b2, AH4b3, AH1a, AH1b), using different dilutions fig 28, 28I, 29, 29I, 30, 30I, 31, 31I.

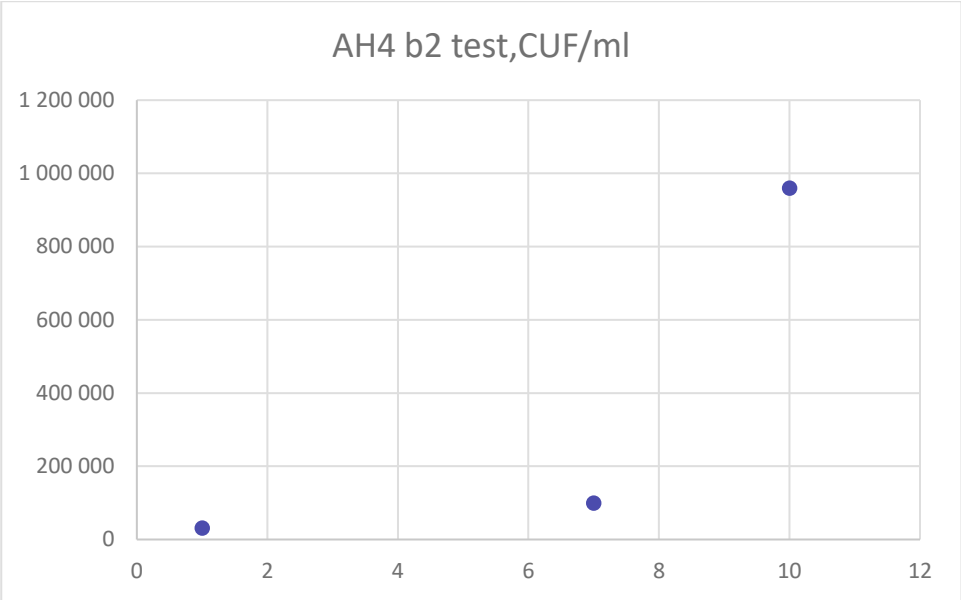


Figure28. CUF/ml the number of bacteria cells per ml (AH4b2. test group)

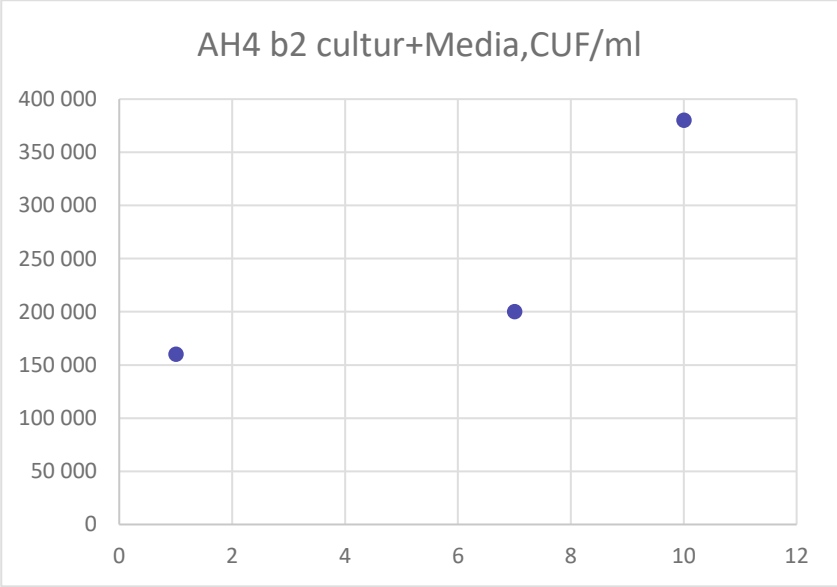


Figure28I. CUF/ml the number of bacteria cells per ml (AH4b2. control group)

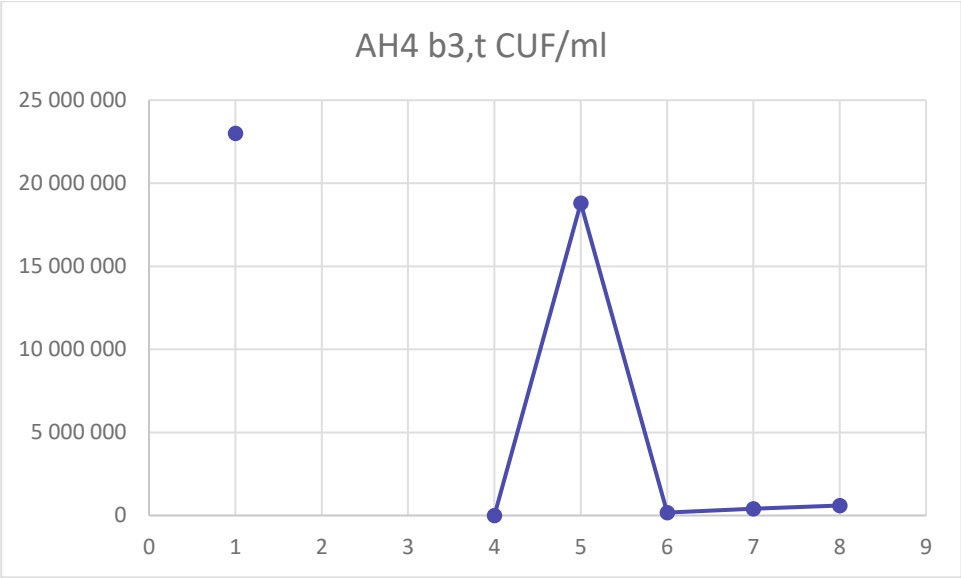


Figure29. CUF/ml the number of bacteria cells per ml (AH4b3. test group)

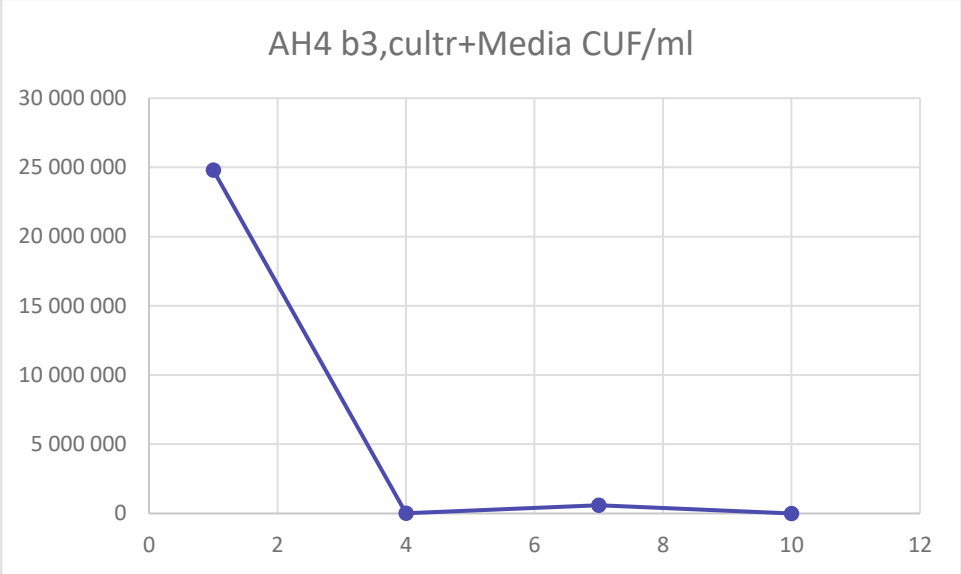


Figure29 I. CUF/ml the number of bacteria cells per ml (AH4b3. control group)

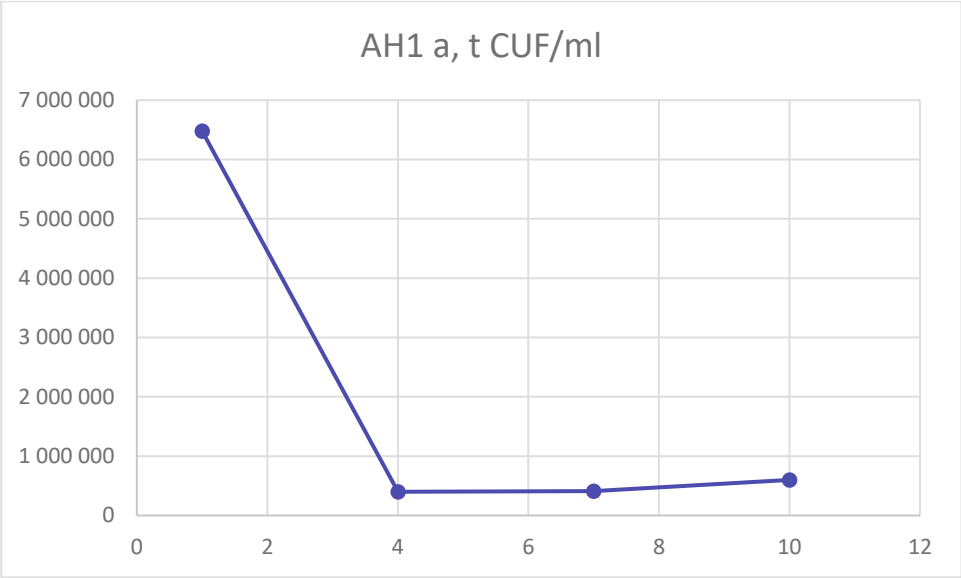


Figure30. CUF/ml the number of bacteria cells per ml (AH1 a. test group)

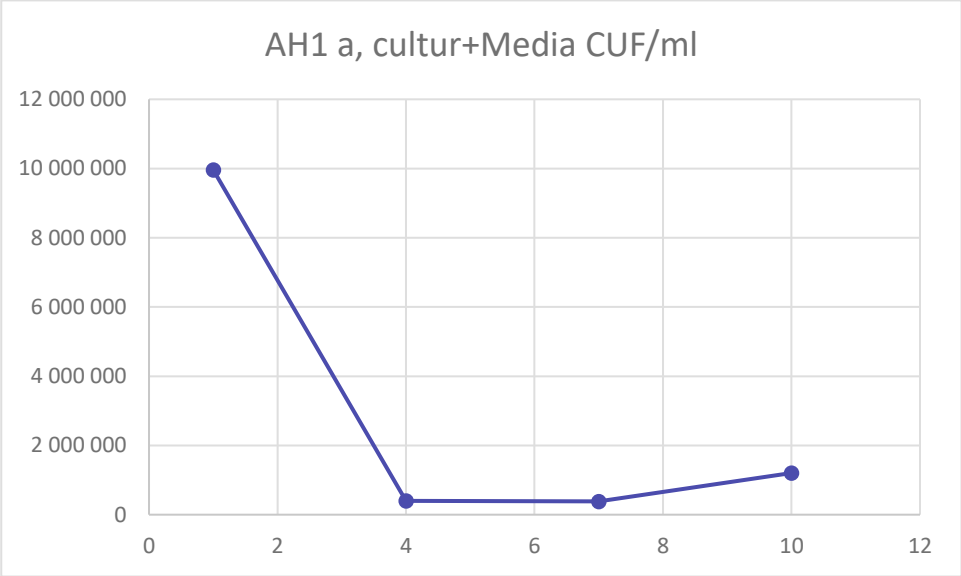


Figure30 I. CUF/ml the number of bacteria cells per ml (AH1a. control group).

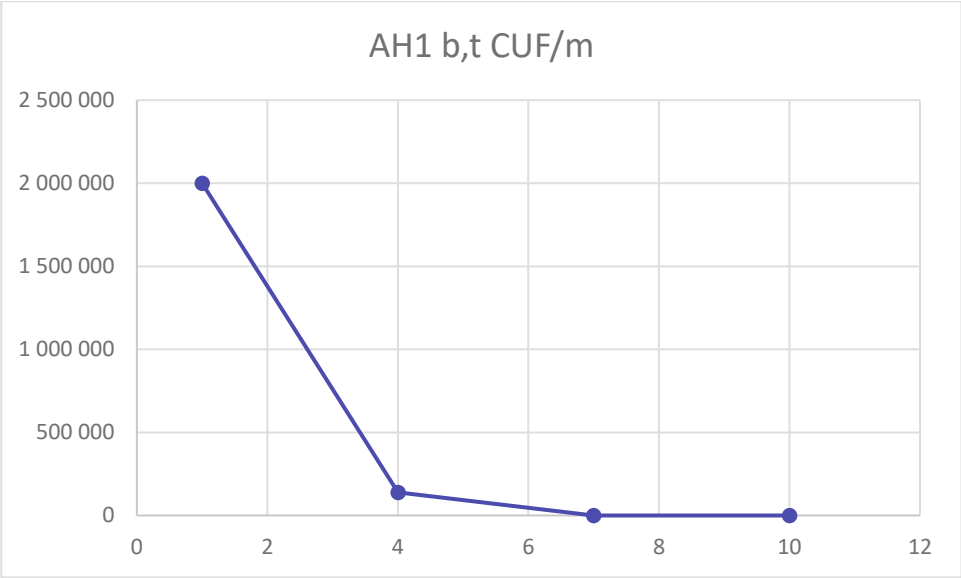


Figure31. CUF/ml the number of bacteria cells per ml (AH1b. test group)

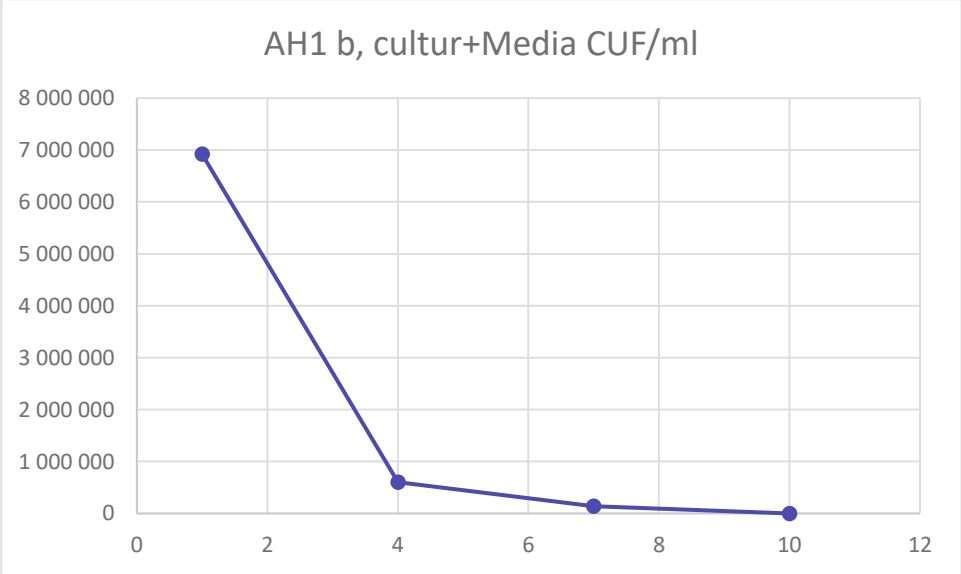


Figure31 I. CUF/ml the number of bacteria cells per ml (AH1b. control group)

Growth measurement for both (AH4b2, AH2)

Measure the growth for samples (AH4b2, AH2) again to confirm and prove the presence of growth for 16 days, using different dilutions fig32, 32I, 33, 33I.

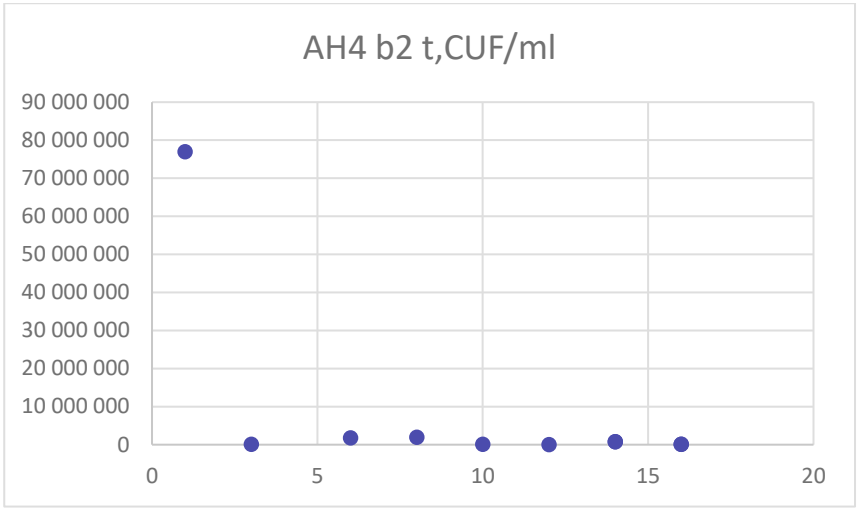


Figure32. CUF/ml the number of bacteria cells per ml (AH4b2. test group)

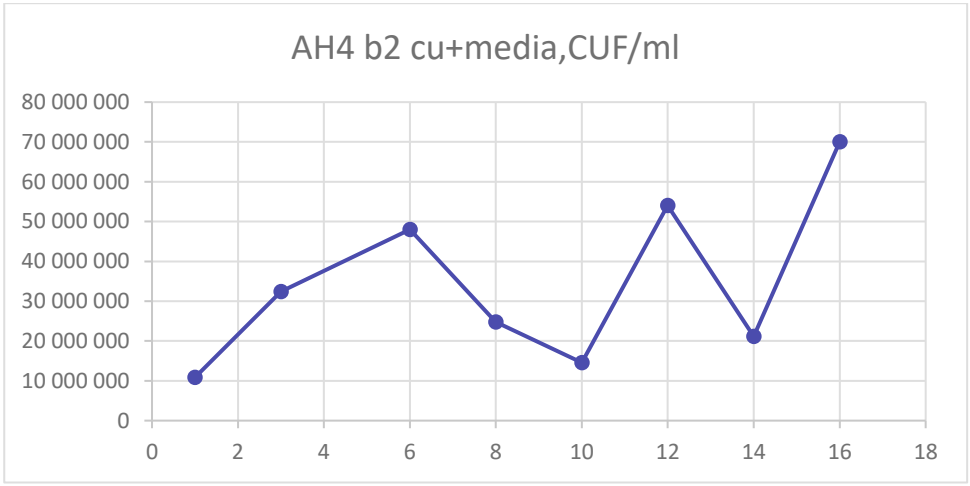


Figure32 I. CUF/ml the number of bacteria cells per ml (AH4b2.control group)

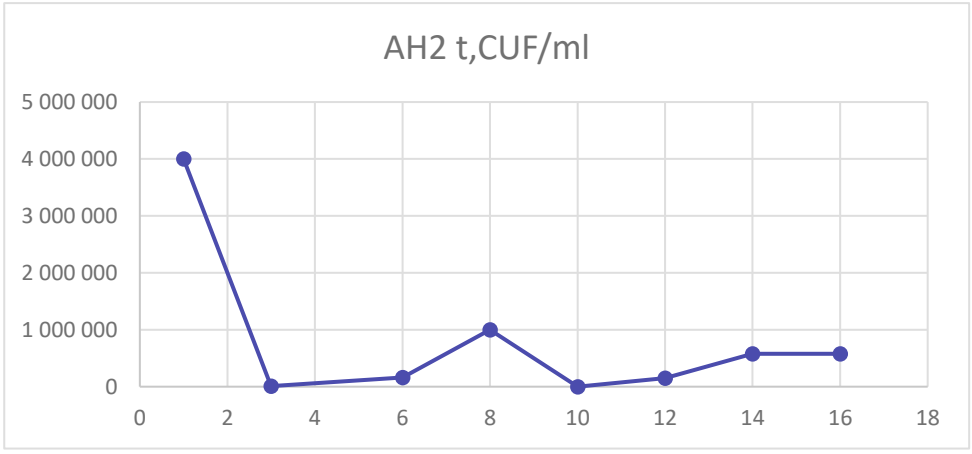


Figure33. CUF/ml the number of bacteria cells per ml (AH2. test group)

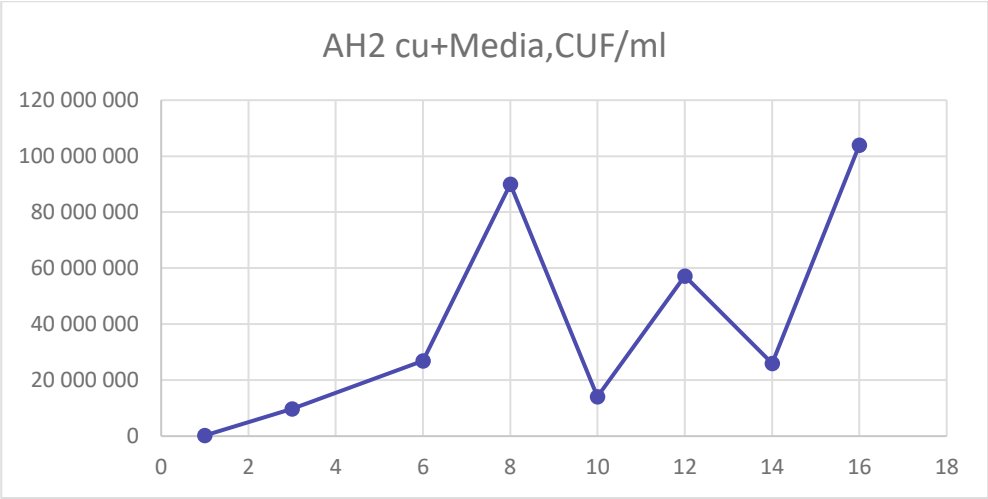


Figure33 I. CUF/ml the number of bacteria cells per ml (AH2. control group)

4.5.2 Measuring CO2 production (AH4 b2, AH2)

After preparing three groups (test, control, sterile control) for each of the samples (AH4b2, AH2), and after six weeks of incubation, we measured the CO2 produced using the device in the fig18. We obtained a purple color representing CO2 production, but less than 50 ppm in trace amounts table11.

Strain		CO2 (ppm)	
AH4b2	Test	<50	Negative
	Control	<50	Negative
	Sterile control	<50	Negative
AH2	Test	50	Negative
	Sterile control	<50	Negative

Table11. CO2 product value for Isolates AH4b2 and AH2.

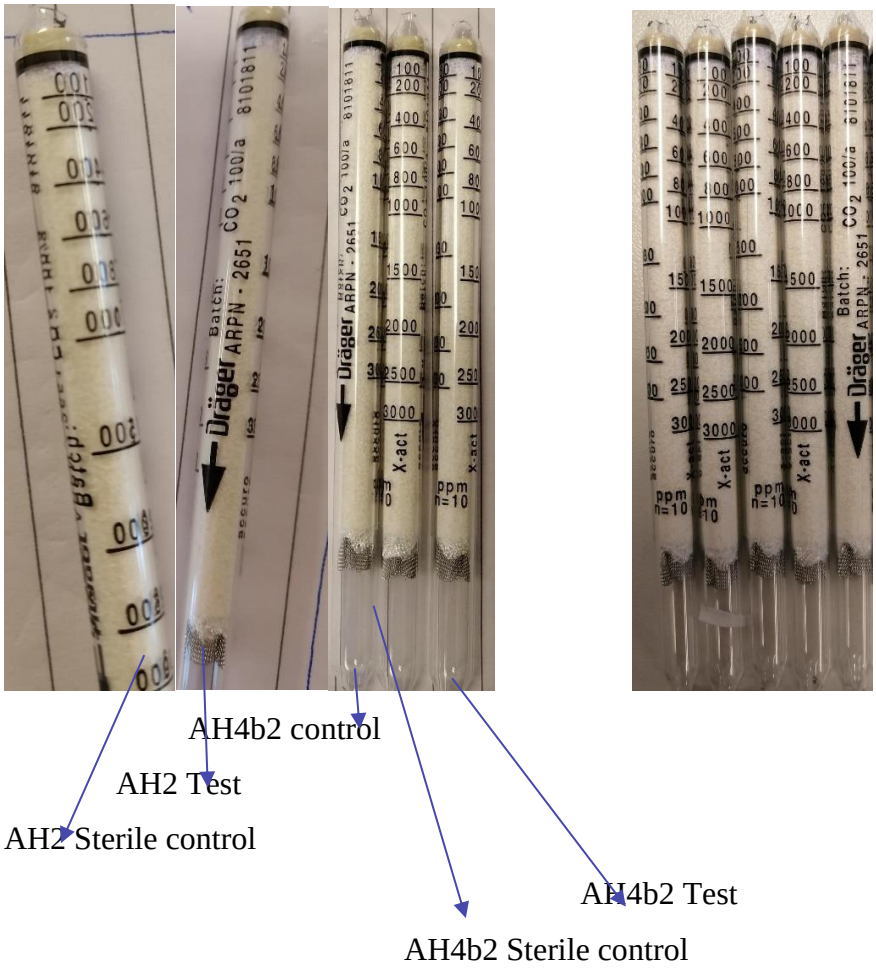


Figure34. Measure the CO2 produced for each of the isolates AH4b2 and AH2 where the blue colour indicates the presence of CO2 production.

4.5.3 Weight-loss experiment (AH4b2, AH2)

After 6 weeks of incubation for 12 flasks 6 for each isolate (three test I.II, III and three control I, II, III), filtration and washing were carried out using SDS and distilled water, then drying and readings (weight) were taken more than once until the weight was stable and the weight loss was calculated table12.

Isolates	Filter weight	Dish Weigh t	Dish+filter+ substrate (PE) Weight	Dish+filter+ substrate (PE) Weight Fourth day	Dish+filter + substrate (PE) Weight Fifth day	substrate (PE) Weight	Initial weight substra te	Weight loss 1- substrat e (PE)

			Third day					Weight
AH4b2 Test								
I	1,58	7,07	9,67	9,64	9,61	0,96	1	0,04
II	1,57+1,57	7,07	11,28	11,22	11,19	0,98	1	0,02
III	1,58	7,07	9,72	9,69	9,68	1.03	1	-0,03
AH4b2 control								
I	1,58+1,56	7,07	11	10,95	10,90	0,69	1	0,31
II	1,58+1,58 + 1,58	7,07	12,81	12,71	12,68	0,87	1	0,13
III	1,59	7,07	9,72	9,69	9,67	1,01	1	-0,01
AH2 Test								
I	1,57+1,56	7,07	11,04	10,98	10,94	0,74	1	0,26
II	1,59+1,56	7,07	11,20	11,12	11,06	0,84	1	0.16
III	1,58+1,59	7,07	11,23	11,18	11,15	0,91	1	0,09
AH2 control								
I	1,57+1,57	7,07	11,31	11,27	11,22	1,01	1-	-0,01
II	1,56+1,51	7,07	11,20	11,17	11,13	0,99	1-	0,01
III	1,57+1,57	7,07	11,20	11,15	11,12	0,91	1-	0,09

Table12. The table indicates the weight loss after complete drying of AH4b2 and AH2 for the test and control groups.

5 Discussion

This study included the isolation and growth of microorganisms and their study to determine their ability to biodegrade plastics (LDPE). Plastic waste is spread everywhere due to industrialization, modernity, and lack of awareness. Soil is characterized as a haven for microorganisms such as bacteria and fungi, which attack waste and use it as a source of nutrients. Studies indicate the presence of large numbers of microorganisms that have been isolated from soil contaminated with plastic waste with a high ability to degrade synthetic plastics (Gupta, Devi et al. 2016). Studies conducted in the past fifty years have been able to identify the strains that react with LDPE causing biodegradation using different LDPE types. Species capable of degrading LDPE were estimated to be 19 bacterial and 12 fungal species with the potential to increase these numbers due to RNA-based isolation and characterization methods (Sen and Raut 2015). Also indicated in the table6 are the most important strains that participate in the deterioration of polyethylene and some of its types and the areas or the isolation environment. Both (AH1, AH2, AH3, AH4, AH5, AH1a, AH1b, AH4b2, AH4b3) microorganisms were isolated and purified. Both AH4b2 and AH2 showed growth when measuring growth, the first stage of measurement, and then showed negative growth when measuring growth, the second stage of measurement. The production of CO2 was examined after planting for 6 weeks for both H4b2 and AH2, the production was very low when the measurement was made. Also, AH4b2 and AH2 showed negative results when conducting the weight loss test, after drying the samples and calculating the weight loss, the numbers were large and did not match the measured CO2 production.

5.1 Isolation, purification, and characterization of plastic degrading microorganisms

Microorganisms were grown in saline (M9) medium containing nutrients needed for growth and low-density polyethylene as the sole carbon source where only microorganisms that attack LDPE and use it as a carbon source grow. Six weeks later, the microorganisms were isolated by spreading on agar plates. By isolating, we obtained a different group of microorganisms that formed colonies of different sizes and colors fig19. The spreading process was repeated on agar plates to obtain pure colonies representing only one type of microorganism as microorganisms were obtained (AH1, AH1a, AH2, AH3 AH4, AH4a, AH5, AH1a, AH1b, AH4b2, AH4b3, AH3b1, AH3b2) fig20. The

obtained isolates were all bacterial isolates, no fungal isolates were obtained. The morphological and chemical characteristics of the samples were determined, some were gram-negative, some were gram-positive, some were spherical, some were rod-shaped fig21, some were oxidase-negative, and some were oxidase- and catalase-negative table9. Zahra Zolanvari used the same method to isolate and determine the characteristics of the isolates, as it indicated thirteen bacterial isolates, nine of which are gram-negative in the shape of a rod and one negative in the shape of a cocci or spherical, while three isolates were gram-positive, two in the shape of a rod and one in the shape of a cocci or spherical in addition to a fungal isolate one (Zolanvari 2021). Also referred to by Nida Khan are bacterial strains sourced from groundwater from Revdalen in Norway that were isolated by Daniel Abiriga. Bacteria and fungi were also isolated from soil at five different sites and were identified based on morphological and chemical characteristics and selection between microbial species, according to their ability to damage polythene and plastic (Priyanka and Archana 2011).

5.2 Identification of plastics degrading strains

The sequences were obtained by Daniel Abiriga, a Ph.D. student at USN University, then we edited the sequences through BLAST search and identified them by taking the sample with a similarity percentage close to 100%. The samples were as follows: AH1a ***(****Stenotrophomonas pavanii)****,*** AH3b2 ***(****Stenotrophomonas maltophilia)* one report referred to the genus *Stenotrophomonas*, which contains seven rod-shaped gram-negative species, which matched with microscopic examination of AH1a and AH3b2 (Ramos, Van Trappen et al. 2011). AH1b (*Microbacterium saperdae, Microbacterium hydrocarbonoxydans, and Microbacterium phyllosphaerae).* For *Microbacterium*, it matches our description in terms of shape and gram staining, as it was described as Gram-positive rod-shaped, which includes forty species isolated from different environments, including soil, insects, clinical samples, marine environment, and dairy (Mounier, Coton et al. 2017). AH2, AH4b2 (*Pseudomonas helmanticensis99,62,* and *Pseudomonas migulae 99,43),* Information about *Pseudomonas helmanticensis* is gram-negative and positive for oxidase and catalase. This information matches our results in terms of gram staining and oxidase, but not in terms of catalase(Ramírez-Bahena, Cuesta et al. 2014). AH3b1 (*Pseudomonas canadensis, Pseudomonas salomonii).* The genus Pseudomonas is the largest Gram-negative genera, and this corresponds to the results of staining, as this genus contains a wide genetic diversity. Pseudomonas subspecies represent a small part of this genus (Girard, Lood et al. 2021). AH4a, AH4b1, AH3a, AH5 (*Rhodococcus erythropolis, Rhodococcus qingshengii JCM 15477, Nocardia coeliaca),* Members of the genus *Rhodococcus* are generally surviving in nature and are commonly Gram-positive(Xu, He et al. 2007) . AH4b3 (*Stenotrophomonas rhizophila 99,25, Stenotrophomonas bentonitica99,6).* Priyanka and

Archa at (2011) collected different soil samples from different sources and conducted a comparison analysis between the biodegradation of polythene and plastic. There were different types of fungi and bacteria as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus lactis*, *A. niger*, *Aspergillus nidulance*, Aspergillus flavus, *Aspergillus glaucus*, *Micrococcus, Penicillium, Pseudomonas, Proteus vulgaris*, Where these species recorded a high efficiency in the degradation of both polythene and plastics (Gajendiran, Krishnamoorthy et al. 2016). Through one study, JAPE1 and AJ1 strains were identified based on the genome sequences of 18SrRNA and 16SrRNA, where searches for similar sequences by BLAST search showed that the strains JAPE1 and AJ1 showed a similarity of 99% with both *Aspergillus nomius* and *Streptomyces sp* in a row (Delacuvellerie, Cyriaque et al. 2019). Johan Comm (USN), in her paper, referred to the use of six microbial strains provided by Andrew Jerkins and Ph.D. student Daniel Abiriga, R4-2(g), *Pseudomonas silesiensis.* R1-1(a) *Rhodococcus degradans.* R4-3(d), *Pseudoarthrobacter phenanthrenivorans.* R4-2h, *Microbacterium hydrocarbonoxydans.* R1-4(b), *Pseudoarthrobacter sulfonivorans.* R4-2(c), *Paraburkholderia xenovorans. Lysinibacillus sp* was isolated and identified as a new strain for the degradation of low-density polyethylene and polypropylene by culturing it in a microbial culture medium without any treatment (Jeon, Park et al. 2021).

5.3 Assessing microbial growth on plastic (LDPE)

Growth was assessed by counting bacterial colonies by dilution method and pouring agar over the diluted liquid. Bacterial colonies were counted for each of the samples AH1, AH2, AH3, AH4, and AH5 for each of the test samples, and the control samples and the graphic representation was done through excel. for the first stage, the growth of each of AH1, AH2, AH3, AH4, and AH5 was evaluated, and the colonies were counted three times during thirty-seven days through dilutions of different fig 23, 23I, 24, 24I, 25, 25I, 26, 26I, 27, 27I. The results were negative with a significant decrease in the value for all samples except for AH2. The second stage, the growth was evaluated for each of AH4b2, Ah4b3, AH1a, AH1b, and AH2, the colonies were counted, and the values were recorded four times for ten days. Also, there was a decrease in the values, only an increase in growth was recorded by increasing the number of colonies for each AH4b2 and AH2 Fig 28, 28I, 29, 29I, 30, 30I, 31, 31I. It was suggested by Andrew Jerkins to reassess the growth of both AH4b2 and AH2, where the bacterial colonies of AH4b2 and AH2 were counted eight times during sixteen days and the results were negative, meaning that no growth occurred fig32, 32I, 33, 33I**.** Jhon comm signal when examining the growth of isolates through ATP meter that the ATP values of isolates R1-1(a) and R4-2(g) showed metabolic activity on cultures containing low-density polyethylene. Therefore, the increase in ATP measurement indicates that these isolates can Plastic is used as a carbon buffer

by forming biofilms on the surface of the polymer (plastic), while the isolates R4-2(c), R4-2(h), R1-4(b), and R4-3(d) showed low values, and therefore there was no continuity in the investigation of these isolates. It indicated the existence of a growth curve gradient between high and low for isolates R1-1(a) and R4-2(g) due to adding a functional group on the carbon atoms, becoming more resistant to microbial attack and this is consistent with the biodegradation of the polymer that was suggested by (Arutchelvi, Sudhakar et al. 2008), where the strains bind to the hydrophilic polymer and deplete the hydrophilic carbon leading to a decrease in growth due to the polymer's transformation into a hydrophobic polymer. Zahra Zolanvari also indicated that the method of measuring ATP is a reliable method for estimating and measuring bacterial growth, as its results agree with (Koutny, Sancelme et al. 2006) (Zolanvari 2021), Which indicated that the presence of polyethylene as a single carbon buffer at the beginning of the incubation led to a rapid growth that may result from the consumption of extracted compounds with low molecular weight resulting from the biodegradation and oxidation of polyethylene, which ends with the carboxyl group, and then a decrease in metabolic activity occurs after this rapid growth. According to Zakaria, using the approach to determine the optical density (OD) at 540 nm using a UV spectrophotometer, the kinetics of the growth of the bacterial isolate *Bacillus tropicus* (MK318648) were determined. And that is by determining the dry mass (DCW) at 70 degrees Celsius and showing its relationship with OD through linear regression, where the biomass or the amount of growth was calculated, and the specific growth rate of bacteria was estimated at 0.246 g L−1 h−1 or 1.138 h−1 and indicating the log phase or the exponential phase there is a sharp change in the biomass of bacteria with the corresponding time in the growth curve (Samanta, Datta et al. 2020). The decrease in growth may be explained by the attack on the amorphous areas by microorganisms and their consumption due to their ease. This consumption leads to an increase in the rate of crystallization as small crystals are consumed and an increase in the proportion of large crystals, but there are no adequate studies indicating what happens after this consumption (Sen and Raut 2015). It has been pointed out that the polymer chains are cut into smaller pieces through enzymes secreted by microorganisms, and the low molecular weight of the polymer occurs as the first stage of degradation, but there is no change in elongation, causing growth stunted, as this agrees with a study by Lee et al. Lee et al also indicated that exposing the polymer to ultraviolet rays caused a 95% decrease in the elongation and, consequently, an increase in the rate of biodegradation (Esmaeili, Pourbabaee et al. 2013). Structural changes in LDPE and OPP were indicated by FTIR and XRD and correlated with pretreatment whereby the pretreatment induces a change in polymer structural structure, surface roughness, and effective biofilm formation. FTIR spectra of LDPE incubated with mixed culture and LYSINIBACILLUS SP JJY0216 showed lower density compared to Controlling for the region of peak uptake where the differences were not so great also overall changes of OPP

intensity with mixed culture and LYSINIBACILLUS SP JJY0216 were like LDPE. By performing XRD powder analysis to determine the degree of crystallinity for both LDPE and OPP and whether it occurs before or after biodegradation, the results showed prominent peaks at 21.4 and 23.5 from meridional position 2, which indicates the crystal structure of LDPE and prominent peaks for OPP at 15.6 and 21.3 at angular position 2 This also indicates the crystal structure of OPP. By calculating the crystallinity of both LDPE and OPP using a comparison ratio of 46% for the control, LDPE biodegradable by mixed culture and *Lysinibacillus sp* JJY0216, and the crystallization increase was 52% and 49%, respectively, as there are no significant differences in crystallinity between the control and degraded OPP (Jeon, Park et al. 2021). Microorganisms live within a certain pH range and are therefore affected by its changes as bacteria prefer a somewhat neutral alkaline pH (CATIA BASTIOLI 2021) . It was necessary to link the stop or decrease of growth to the pH balance, but when measuring the pH was 7.140 for the growth medium after sterilization without any additions and it was slightly low 6.852 for the test groups (culture, growth media, substrate) and the control groups (culture growth media) After several days of incubation, there are no significant differences in pH.

5.4 Co2 evolution test (AH4 b2, AH2)

The carbon that the microorganisms use when attacking the LDPE is supposed to be converted to carbon dioxide as a product of the respiration process and thus can be used as an indirect measure of the amount of LDPE the microorganisms have consumed. The ability to continuously monitor Co2 evolution outside the system enables the determination of not only polymer consumption but also the rate of biological degradation (Sen and Raut 2015). The Co2 produced was measured after six weeks of incubation for samples AH2 and AH4b2 fig18. It was noticed that the purple color indicates Co2 produced fig34. The amount of Co2 produced was very little and the samples showed negative results table11. The reason may be the death of microorganisms that were used in the incubation and the presence of other organisms because of using a non-sterile substrate in all stages of the study. This study agreed with some of what Zahra Zolanvari referred to, as the same method was used to measure the amount of Co2 produced for isolates. The isolates ZZ-1, ZZ-5, ZZ-8, and ZZ-9 showed values less than 50 ppm, thus indicating a lower confidence level for biodegradation. Isolates ZZ-3, ZZ-7, ZZ-11, and ZZ-12-2 showed the best results after 14 days of incubation without any additives, while ZZ-13, ZZ-3, and ZZ-2 showed the best results after 21 days of incubation at Addition of a mixed component of FeCl2 and CuSo4. The amount of carbon dioxide in the atmosphere at 420 ppm was indicated and linked to the negative results, as there were no errors regarding the interpretation of the

results, and all isolates indicated a lower confidence level for biodegradation (Zolanvari 2021). When studying the measurement of carbon dioxide evolution, the determination of both volatile compounds, biomass, and the proportions of both dissolved and insoluble parts of the polymer must be considered, but the determination of biomass and insoluble residues pose some obstacles and requires further investigation. The Co2 evolution test is subject to some limitations such as leakage into the complex system and consequently, a decrease in the amount of Co2 measured. Microorganisms can utilize impurities attached to the surface of polyethylene or embedded in the polyethylene chain as a source of energy, affecting the amount of carbon dioxide produced (Itävaara and Vikman 1996).

5.5 Weight-loss experiments (AH4b2, AH2)

The method of determining the percentage of lost weight is one of the effective and primary methods that are used to estimate or determine the rate of biological degradation of any polymeric film. The percentage of weight loss depends on the surface area of the polymer as biodegradation is triggered on the surface accessible from the membranes using single carbon atoms (Samanta, Datta et al. 2020). When assessing growth, we obtained negative results for samples including AH2 and AH4b2. Andrew Jerkins proposed to continue and measure the rate of weight loss of LDPE by incubating with samples AH4b2 and AH2. A test group and a control group were prepared for the samples AH2 and AH4b2 and incubated for six weeks with low-density polyethylene as a substrate, and after six weeks washing was performed using SDS, filtering, drying, and calculating the weight loss. The results were significantly no match for the CO2 product table12. Jhon Comm indicated in her study the percentage change in weight after 60 days of incubation for samples R1-1a, R4-2 g, and R1-1a/R4-2 g. The percentage change in weight was 6.5%, 3.5%, and 21.5% for each of R1-1(a), R4-2 (g), and R1-1(a)/R4-2 (g)., respectively, where the same method was used by washing with SDS, filtering and drying. One study indicated significant differences in the final weight compared to the initial weight. Whereas the chemically treated polyethylene bags showed twice the percentage of weight loss compared to the untreated bags. The weight loss of the polyethylene films is due to the breakdown of the carbon backbone which results from the enzymatic hydrolysis of the experimental bacteria (Bardají, Furlan et al. 2019). It was indicated that LDPE that was incubated in the soil for ten years showed a higher degradation rate that ranged from 3.5 to 8.4 compared to previous studies indicating a lower degradation rate of LDPE where LDPE was subjected to oxidation to increase biodegradation (Abraham, Ghosh et al. 2017). Measuring weight loss is not

an effective method for measuring biodegradability due to volatile and soluble impurities that may cause weight loss. Also, this method is concerned only with the early stages of degradation and does not give information on mineralization (Ho, Roberts et al. 2018).

6 Conclusion, recommendations, and possible future alternatives.

This study was conducted based on the widespread and increasing use of plastic in all sectors and aspects of life, as plastic waste accumulates in the environment and causes many problems for all forms of life. The problem is also exacerbated by the inefficiency and high cost of the methods used to dispose of plastic waste. Together, these factors prompted us to think of the biodegradation of plastic using microorganisms, due to the positive effects on the environment. This study included the isolation of more than eleven microorganisms in the laboratory that could use low-density polyethylene (LDPE) as a carbon source, and thus this indicates the presence of these microorganisms in nature. There was an agreement between these studies and many studies, including those that were conducted at USN, in terms of isolates' presence, characterization, and identification. But they did not agree on determining the effectiveness of biodegradation by measuring growth and determining the amount of CO2 produced and weight loss, which showed unreliability for biodegradation and reject the null hypothesis. This study did not refer to external biodegradation conditions, as the experiments were carried out at laboratory temperatures in the range of 20-25 °C. Thus, in situ studies are the most important in providing us with information on biological degradation (Matjašič, Simčič et al. 2021). In the introduction, it was pointed out the role of these factors (abiotic factors) in accelerating the rate of biological degradation of the polymer, such as ultraviolet rays, as many research papers indicated that exposure of the polymer to ultraviolet rays led to an increase in the rate of biological degradation and an increase in the percentage of weight loss in addition to the effect of other abiotic factors So there is a need to study on-site (Esmaeili, Pourbabaee et al. 2013). The biodegradation of LDPE is complex and difficult to understand the process, so the method of using pure strains was followed in this study to elucidate the mechanisms of LDPE biodegradation. This approach is suitable for evaluating environmental conditions and their effect on LDPE degradation and for investigating metabolic reactions. However, this approach is criticized for ignoring the possibility that LDPE biodegradation is the result of joint action between different species of microorganisms (Sen

and Raut 2015). The microorganisms that were used were isolated from the forest. It should be noted that the landfill sites represent rich sites that contain a large diversity of microorganisms, which can be used to obtain isolates that give more comprehensive information on biological degradation. Also, more standards must be used to examine changes in the physical properties of the polymer such as strength, crystallization, and comparison of spectroscopy (SEM, XRD, and FTIR) data (Sen and Raut 2015).

Recommendations, and possible future alternatives.

_The biodegradation process requires further study to verify the ability of microorganisms to attack and degrade polymeric materials and further discovery regarding the enzymatic system.

_Molecular engineering techniques: By designing a microbial community specialized in this field, it works to make biodegradation more efficient through genome fusion techniques, thus focusing on proteins and genomics.

Recommendations, and possible future alternatives.

_Plastic-eating insects: The activity of these insects must be considered and an attempt to exploit them in industrial applications and scientific research

_Since the biodegradation of algae does not require a pre-treatment or carbon source, and therefore the biodegradation of algae as an upward method may be better than bacterial or fungal biodegradation. Also, about algae, there is a need to confirm the role of free radical-producing algae in increasing the effectiveness of biodegradation of plastic polymers, as free radicals increase the polarity of the polymer due to their oxidative stress and the polymer is ready for biodegradation.

_Nanotechnology: The application of nanotechnology in the manufacture of plastics can overcome the defects of biodegradation and control the rates of biodegradation (Ali, Elsamahy et al. 2021).

_Achieving sustainable environmental safety by urging future generations to use biodegradable plastics(Bahl, Dolma et al. 2021), And that is by replacing part of the traditional plastic materials that depend on petroleum in their manufacture with bioplastics, which achieves savings in energy consumption and reduces heat emissions (Piemonte 2011).

_Improving the performance of polymers for longer use in different sectors such as construction, aviation equipment, and containers, considering the preservation of these materials in their immutability and their derivation from materials from renewable sources (wood fibers in composite materials or vegetable oil in a refractory substance).

_Producing polymers with short lifespans and intending to rapid biodegradation, as in single-use packages, planting pots, and others (Lucas, Bienaime et al. 2008).

_There must be knowledge and understanding of the place and mechanism of polymer degradation when adopting the use of biodegradable polymer and knowledge of the environmental impacts on the

life cycle and trying to reduce them in addition to achieving real commercial gains (Singh and Sharma 2008).

_We must enhance and increase the effectiveness of biodegradation by mixing synthetic polymers with biodegradable natural polymers such as starch or cellulose. Also, by mixing industrial polymers with prooxidants so that they are degraded easily (Arutchelvi, Sudhakar et al. 2008).

_Plastic waste: About plastic waste, its entry into water can be reduced (seas, rivers stations) through good waste management, as plastic waste leaks due to poor waste management and this occurs especially in low- and middle-income countries. Or by adopting an approach to remove them from beaches and seas, due to the inadequacy of a good waste management approach, as they must be recovered (Ritchie and Roser 2018).

_There must be internationally comparable measurement methods for determining biodegradation efficacy and this indicates that there is a need for all details to be standardized to operate in the same ways by all researchers (Sen and Raut 2015).

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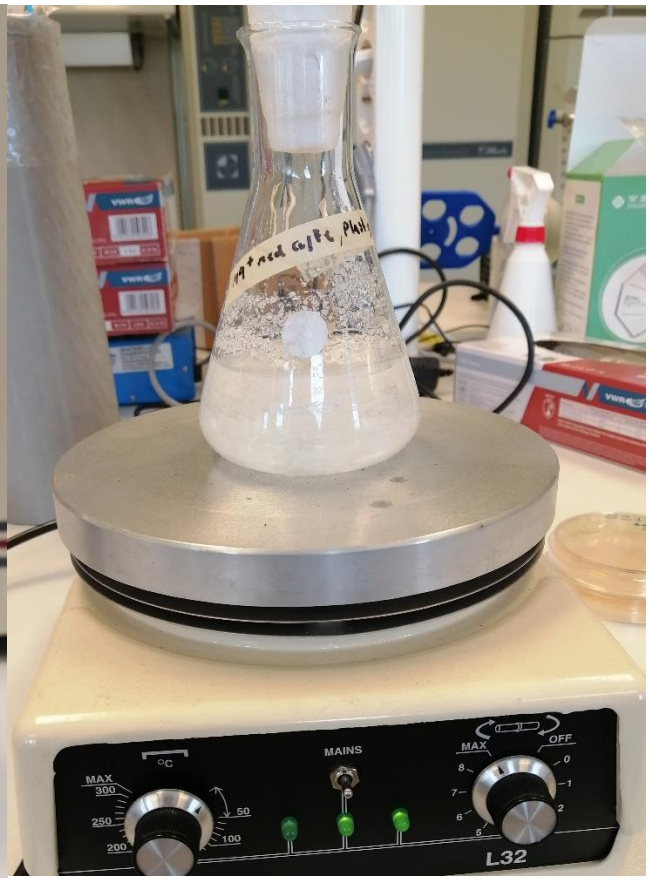
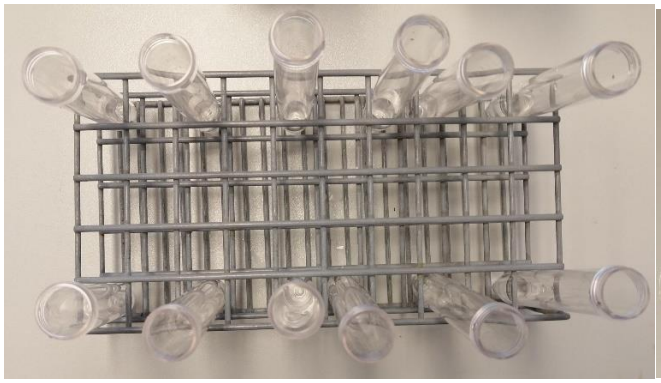
8 Annexes

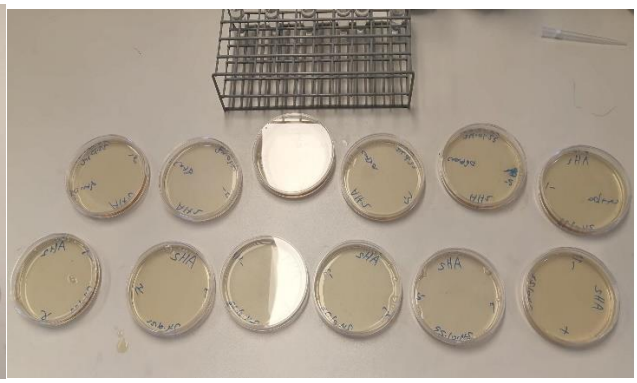
8.1 Annex1: source of microorganisms

8.2 Annex2: Isolation and cultivation of microorganisms that can degrade low-density polyethylene.



8.3 Annex4: Cultivation of isolates and growth measurement (dilution method).





8.4 Annex5: Filter, drying, and weight loss calculation.

8.5 Annex6: Cultivation of isolates and measurement of carbon dioxide produced.