ISOLATION AND SELECTION OF POLYETHENE PLASTIC-DEGRADING MICROBIAL STRAINS

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Jimma, Ethiopia

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ABSTRACT

Plastic bags or "festals" are made from polyethene. Due to their molecular stability, plastics do not easily breakdown into simpler components; therefore, they are not considered biodegradable. Worldwide 500 billion to 1 trillion plastic bags are being produced each year and it persists in the environment between 20 and 1000 years before they decompose. The majority of these synthetic plastics do not degrade in the environment, and incineration of plastics generates CO2 and the highly poisonous dioxins. There is yet no report on microbial polyethene and plastic degradation in Ethiopia. Advances in making polymers in more environmentally friendly and sustainable manner can have significant beneficial consequences. The aim of this study was to isolate and screen polyethene degrading microbes from garbage dumps and soils covered with vegetation in and around Jimma town, South Western Ethiopia. Screening of isolates was based on their ability to degrade low and high density polyethene.

Microbes were isolated using *Streptomyces* and *Sphingomonas* selective media as well as enrichment broth procedure using polyethene as a sole carbon and energy source. Preliminary qualitative and quantitative screening based on color clearing and weight loss of plastic respectively by pure shake flask culture assays were used to screen plastic-degrading microbes. These microbes were tested for degradation of heat pre-treated (at 70^{o}C) and untreated polyethene plastic separately and in combination. Degradation was measured in terms of weight loss after incubation for six weeks on a shaker. Among the tested microbes *Sphingomonas* strain LBG-5 demonstrated the highest degradation of both heat-treated and untreated low density and high density polyethene plastics. This strain degraded about 20% and 8% weight of heat-treated low density and high density polyethene sheets respectively within a period of six weeks. Garbage dumps are found out to be good source of plastic-degrading microbes that degrade low density polyethene better than high density. Microbes degrade polyethene better when they are combined. Heat pre-treated polyethene sheets showed greater degradability than untreated ones. Degradation of polyethene is influenced by physical, chemical and biological factors such as: heat exposure, the type of plastic and the kind of microbial strain to which it is exposed.

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ABBREVIATIONS

PE	Polyethene
LDPE	Low Density Polyethene
HDPE	High Density Polyethene
SPE	Society of Plastic Engineers
ICI	Imperial Chemical Industries
UK	United Kingdoms
WYE	Water-yeast Extract Agar
FEC	Final enrichment cultures
rpm	rounds per minute

CHAPTER ONE

BACKGROUND OF THE STUDY

1.1 INTRODUCTION

Interest in environmental issues is still growing and there is increchapasing demand to develop materials which do not burden the environment significantly. Awareness of the waste problem and its impact on the environment has awakened new interest in the area of degradable polymers. Biodegradability becomes more and more important for the so-called plastics, because many of them can not be recycled (due to low thermal resistance) [1]. Incineration of plastics generates highly toxic compounds [2] and are environmental pollutants, such as vinyl chloride, acrylonirrile, CO_2 and dioxin) [3], remaining in the environment.

The whole world seems to be wrapped in plastic. Almost every product we buy including foods we eat and many of the liquids we drink come encased in plastic. According to the report of Society of Plastics Engineers (SPE), over 200 million tons of plastic are manufactured annually around the world [4]. The scientific knowledge and the technological advances in the field of polymers have enabled the development of several applications of these materials. In recent times, the excessive consumption of synthetic plastics derived from petroleum has had an adverse impact on the environment because the majority of these synthetic plastics do not degrade in the environment and incineration of plastics generates CO_2 and dioxin. These molecules increase the warming of the earth and environmental pollution [2]. The accelerated consumption of polymers, mainly in the applications of disposables, such as packaging, has roused strong concerns due to the generation of residues from the polymeric materials [3].

Plastic bags or “festals” are made from polyethylene (PE), a polymer consisting of long chains of the ethylene monomer (C_2H_4). Plastic bags are very popular in everyday life but have a negative impact on the environment. Each year, an estimated 500 billion to 1 trillion

plastic bags are consumed worldwide. This comes out to be over one million per minute. They are very durable, and may persist in the environment from 20 to 1,000 years before they decompose [5].

Many approaches have been proposed for solving the worldwide problem of plastic wastes, such as recycling and using biodegradable materials. Biodegradable plastics have several excellent properties and may serve as alternatives to non-degradable ones. First, biodegradable plastics are degraded by microorganisms in the natural environment. Second, they can be composted, and burn with a lower calorific value than that of non-biodegradable plastic materials [2]. However, these plastics are less durable for having low impact/shock and thermal resistance and costly so that their adoption in the area of consumer durable goods, particularly electronic products, remains limited [1].

Polyethylene is not considered biodegradable as it takes several centuries until it is efficiently degraded. However, it is oxo-biodegradable plastic, meaning it degrades more quickly after exposure to physical factors like heat as it produces free radicals on the long polyethylene chain, causing the material to lose some of its physical properties, to become oxidized, and, possibly, to become more accessible to microbial biodegradation [6]. Exposure to such factors as sunlight, heat, and mechanical stress ultimately reduces oxo-biodegradables to a mix of water, CO_2, and biomass, making them easy and safe to compost [6].

In recent time,s efforts are being made to make plastics more environmental-friendly; therefore, in order to recover plastic hydrolysates, research have been done to develop a cost-effective biodegradable plastic recycling system using different microbes particularly fungi and bacteria.

In this study, bacteria mainly *Streptomyces* spp. and *Sphingomonas* spp. and other microbes that use polyethene as a sole source of carbon and energy were isolated and screened for degradation of polyethene plastic. Plastic biodegradation was evaluated by weight loss. Chemical degradation of the plastic was initiated by a 70°C heat pretreatment of the plastic.

Microbial transformation of the pretreated plastic material after inoculation of degrading microbe and incubation under aerobic conditions was demonstrated.

1.2 STATEMENT OF THE PROBLEM

The wide use of polyethylene makes it an important environmental issue. Though they can be recycled, most of the commercial polyethylene ends up in landfills and in the oceans notably the Great Pacific Garbage Patch [7]. In Ethiopia, plastic bags are being disposed to road sides, water ways, agricultural fields and other inappropriate places. Polyethylene is not considered biodegradable, as it takes several centuries until it is efficiently degraded [7]. Furthermore, plastic photo-degrade into smaller toxic parts due to uncontrolled oxidation by UV generates toxic substances such as vinyl chloride, acrylonirrile and dioxin [3]. These products contaminate the soil and waterways [8], where they can be accidentally ingested by animals and thereby enter the food chain [5].

Polyethylene chokes the drains, the water bodies, pollutes the land and poisons us slowly but surely [9]. The majority of these synthetic plastics do not degrade in the environment, and incineration of plastics generates CO_2 and the highly poisonous dioxins. These molecules increase the warming of the Earth and environmental pollution [2].

In recent times studies are being done on plastic bioremediation. However, little is known about the degradation process including the mechanism of degradation and the enzymes involved in the degradation process. Several bacterial and fungal organisms have been found to degrade polyethylene plastics [16,19-22,36]. Although studies have been done in the industrialized world, this field of research is untouched in Africa. The existing plastic-degrading microbes are slow acting; they degrade polyethylene plastics only slowly since many of the existing plastic – degrading strains decompose the plastics through co-metabolism which explains their low efficiency level. Co-metabolism is a process by which a microbe degrades the pollutant but does not derive energy from the activity [10]. This is due to the enzymes of low specificity that also act upon the pollutant when a metabolizeable nutrient starch, cellulose or lignin is available along with the pollutant. The task at hand is to come up with bacterial strains that can degrade plastics as a source of carbon and energy which endow with better efficiency. Such strains may be more efficient than those that act co-metabolically.

1.3 SCOPE OF THE STUDY

This investigation dealt only in the isolation and selection of the most efficient plastic-degrading strains of mainly *Streptomyces* and *Sphingomonas* bacteria and others that use polyethene as a sole source of carbon and energy from the environment, particularly from soil. A number of microbes were found to degrade polyethene [16,19-22,36] and among these microbes Streptomyces and Sphingomonas were found to cause better degradation. The selection of the most efficient among these microbial strains was on the basis of their ability to degrade heat-treated and untreated LDPE as well as HDPE plastics. The selected strains were also tested for their combined ability to degrade both types of polyethene plastic. The analysis of other environmental, physical and biological factors that may influence degradation of plastic by these microbes will be the subject of future studies.

1.4 LIMITATION OF THE STUDY

The researcher faced the following problems:

1. Unsteady power and water supply which might delayed the performance of some laboratory experiments; and
2. Because of disposal of six weeks old labeled experimental treatments by the janitors it was difficult to finish the experiment during the time allotted.

CHAPTER TWO

REVIEW OF RELEVANT LITRATURES

2.1 Brief History of Plastics and Polyethylene

Polyethylene was first synthesized by the German chemist Hans von Pechmann who prepared it by accident in 1898 while heating diazomethane. When his colleagues Eugen Bamberger and Friedrich Tschirner characterized the white, waxy, substance that he had created they recognized that it contained long -CH_2- chains and termed it polymethylene [7].

The first industrially practical polyethylene synthesis was discovered again by accident in 1933 by Eric Fawcett and Reginald Gibson at the Imperial Chemical Industries (ICI) works in Northwich, England. Upon applying extremely high pressure several hundred atmospheres to a mixture of ethylene and benzaldehyde they again produced a white, waxy, material. Because the reaction had been initiated by trace oxygen contamination in their apparatus the experiment was, at first, difficult to reproduce. It was not until 1935 that another Imperial Chemical Industries (ICI) chemist, Michael Perrin, developed this accident into a reproducible high-pressure synthesis for polyethylene that became the basis for industrial production beginning in 1939 [7].

By 1936, American, British, and German companies were producing "polymethyl methacrylate" PMMA, better known as "acrylic." Although acrylics are now well-known for the use in paints and synthetic fibers, such as "fake furs," in their bulk form they are actually very hard and more transparent than glass, and are sold as glass replacements under trade names such as "plexiglas" and "lucite." Plexiglas was used to build aircraft canopies during the Second World War and it is now used as a marble replacement for countertops [11].

Subsequent landmarks in polyethylene synthesis have revolved around the development of several types of catalyst that promote ethylene polymerization at more mild temperatures and pressures. The first of these was a chromium trioxide-based catalyst discovered in 1951

by Robert Banks and J. Paul Hogan at Phillips Petroleum. In 1953 the German chemist Karl Ziegler developed a catalytic system based on titanium halides and organo-aluminium compounds that worked at even milder conditions than the Phillips catalyst. The Phillips catalyst is less expensive and easier to work with, however, and both methods are used in industrial practice [7].

By the end of the 1950s both the Phillips- and Ziegler-type catalysts were being used for HDPE production. Phillips initially had difficulties producing a HDPE product of uniform quality and filled warehouses with off-specification plastics. However, financial ruin was unexpectedly averted in 1957 when the hula hoop, a toy consisting of a circular polyethylene tube, became a fad among the youth in the United States [7].

A third type of catalytic system, one based on metallocenes, was discovered in 1976 in Germany by Walter Kaminsky and Hansjörg Sinn. The Ziegler and metallocene catalyst families have since proven to be very flexible at copolymerizing ethylene with other olefins and have become the basis for the wide range of polyethylene resins available today, including very low-density polyethylene and linear low-density polyethylene. Such resins, in the form of fibers like Dyneema, have, as of 2005 begun to replace aramids in many high-strength applications [7].

Dyneema is a superstrong polyethylene fiber that offers maximum strength combined with minimum weight. It is up to 15 times stronger than quality steel and up to 40% stronger than aramid fibers, both on weight for weight basis. Dyneema floats on water and is extremely durable and resistant to moisture, UV light and chemicals. The applications are therefore more or less unlimited. Dyneema is an important component in ropes, cables and nets in the fishing, shipping and offshore industries. Dyneema is also used in safety gloves for the metalworking industry and in fine yarns for applications in sporting goods and the medical sector. In addition, Dyneema is also used in bullet resistant armor and clothing for police and military personnel [12].

Aramids are a family of nylons, including Nomex® and Kevlar®. Kevlar® is used to make things like bullet proof vests and puncture resistant bicycle tires [13]. Aramid fibers are a

class of heat-resistant and strong synthetic fibers. They are used in aerospace and military applications, for ballistic rated body armor fabric, and as an asbestos substitute. The name is a shortened form of "aromatic polyamide". They are fibers in which the chain molecules are highly oriented along the fiber axis, so the strength of the chemical bond can be exploited [14].

Until recently the metallocenes [a compound with the general formula of $C_5H_{52}M$ consisting of two cyclopentadienyl anions Cp, which is $C_5H_5^-$ bound to a metal center M in the oxidation state II.] [15,35] were the most active single-site catalysts for ethylene polymerisation known—new catalysts are typically compared to zirconocene dichloride. Much effort is currently being exerted on developing new, single-site so-called post-metallocene catalysts that may allow greater tuning of the polymer structure than is possible with metallocenes. Recently, the work of Fujita at the Mitsui Corporation , amongst others, has demonstrated that certain salicylaldimine complexes of Group 4 metals show substantially higher activity than the metallocenes [7].

Polyethylene is cheap, flexible, durable, and chemically resistant. LDPE is used to make films and packaging materials, including plastic bags, while HDPE is used more often to produce containers, plumbing, and automotive fittings. While PE has low resistance to chemical attack, it was found later that a PE container could be made much more robust by exposing it to fluorine gas, which modified the surface layer of the container into the much tougher "polyfluoroethylene" [11].

2.2 General Description of Polyethenes

Polyethylene IUPAC name polyethene or polymethylene is a polymer consisting of long chains of the monomer ethylene IUPAC name ethene. The recommended scientific name polyethene is systematically derived from the scientific name of the monomer. In certain circumstances it is useful to use a structure-based nomenclature; in such cases IUPAC recommends polymethylene*;* [poly(methanediyl) is an non-preferred alternative]. The

difference in names between the two systems is due to the opening up of the monomer's double bond upon polymerization [7].

In the polymer industry, the name is sometimes shortened to PE in a manner similar to that by which other polymers like polypropylene and polystyrene are shortened to PP and PS respectively. In the United Kingdom the polymer is commonly called polyethene, although this is not recognized scientifically [7].

The ethene molecule known almost universally by its common name ethylene, C_2H_4 or $CH_2=CH_2$, two CH_2 groups connected by a double bond. Thus the structure:

Figure 2.1 Chemical structure of ethylene (monomer of polyethene)

Polyethylene is the most popular plastic in the world. It is a thermoplastic commodity heavily used in consumer products. Thermoplastics can be repeatedly softened by heating and hardened by cooling. Thermosetting plastics, on the other hand, harden permanently after being heated once [16,36]. Over 60 million tons of the material is produced worldwide every year [7]. This is the polymer that makes grocery bags, shampoo bottles, children's toys, and even bullet proof vests. For such a versatile material, it has a very simple structure (see figure 1), the simplest of all commercial polymers. A molecule of polyethylene is nothing more than a long chain of carbon atoms, with two hydrogen atoms attached to each carbon atom (Figure 1) [17]. This material evolved into two forms, low density polyethylene (LDPE) and high density polyethylene (HDPE) (see below).

A **B**

Figure 2.2 Chemical structures of high density polyethylene HDPE A and low density polyethylene LDPE B.

In some polyethylene, the carbons, instead of having hydrogens, have long chains of polyethylene attached to them (Figure1B). This is called branched, or low-density polyethylene, or LDPE. When there is no branching, it is called linear polyethylene, or HDPE. Linear polyethylene is much stronger than branched polyethylene [17]. This is because LDPE contains a greater degree of long chain branching so that the matrix is less dense and does not offer as much tensile strength as the HDPE. Since this is the case, LDPE is less expensive to produce and process than similar polymers. On the other hand, HDPE is a more durable grade of polyethylene due to a higher density and linear crystallization. The polyethylene properties of this material make it suitable for use in producing many types of stress-resistant plastics intended for consumer and commercial use. For example, HDPE is used to make gallon-sized milk containers, as well as plumbing fixtures [18].

2.3 Microbial degradation of polyethylene

The disposal of non-degradable synthetic polymers has become a worldwide environmental problem, and there is a need for the development of biodegradable polymers.

Biodegradable polymers are recently developed materials in the field of polymers. Their main characteristic is that they are biodegradable through the action of microorganisms in appropriate environmental conditions. When in contact with the biodegradable polymer, the microorganisms produce enzymes that break down the material in progressively smaller segments; that is to say, they reduce its average molecular mass, favoring its degradation in the environment [16,36].

Studies on biodegradation of synthetic polymers and oligomers have shown close relationships between biodegradability and chemical structure. The synthetic polymers that biodegrade tend to have structures similar to those found in naturally occurring polymers, suggesting that microbial populations produce enzymes that do not discriminate between

polymers of similar structure. Hydrolysis and oxidation are the primary processes involved in polymer degradation [19].

There are a number of microbes that are known to be responsible for Polyethene degradation. Bacteria studied for plastic-degrading activities were *Moraxella, Pseudomonas, Staphyloccoccus, Micrococcus* and *Streptococcus* and two fungal species, *Aspergillus niger* and *A. glaucus*. These microbes were separately allowed to degrade the polyethylene and plastics under shaker cultures for a month. Among the *bacteria, Pseudomonas* and *Moraxella* sp. were found most active in degrading 20.54% of polyethylene, and 8.16 % of plastics in one month period. Among the fungal species, *Aspergillus glaucus* was more active than *A. niger* in degrading 28.8% of polyethylene and 7.26% of plastics within a month [20].

Polyethylene was also degraded by bacteria such as *Streptomyces viridosporus, S. badius,* and *S. setonii*. *S. viridosporus* is the overall best, with an average reduction among treatments of 21% (range, 11.8 to 67.8%), although, there was no significant difference among bacterial treatments [21].

Recently Daniel Burd [22], a 16 year old Canadian, won the Canada-Wide Science Fair in Ottawa after discovering that *Sphingomonas*, a type of *bacterium*, can degrade plastic bags. He was able to degrade 43% of some plastic within six weeks. Burd says this should be easy on an industrial scale: all that's needed is a fermenter, a growth medium and plastic, and the bacteria themselves provide most of the energy by producing heat as they metabolize. The only waste is water and a bit of carbon dioxide [22].

2.4 Physical factors influencing biodegradation

UV light is a known initiator of polyethylene oxidation and this photo-oxidant activity is enhanced by the addition of transition metals such as cobalt, manganese, nickel, and zinc, which are also used as pro-oxidant catalysts. To enhance the degradation of polyethylene, chemical or photo initiators or both are added to the degradable plastic films. For polyethylene films containing photo- and pro-oxidants, the initiators of oxidation are light and temperature, respectively. Both the pro-oxidant and the photo-oxidant produce free radicals on the long polyethylene chain, causing the material to lose some of its physical properties and become easily oxidized. The resulting molecular change makes the polymer more accessible to microbial degradation. UV-treated films showed the greatest biodegradation by bacteria [21].

Oxo-biodegradability is triggered by exposure to oxygen, heat and UV light and therefore shortens the shelf life of plastic products. Plastic bottles will oxo-biodegrade in landfills, ditches, rivers and oceans [23]. Other studies showed that heat and UV treatments both generate very different residual oxidized polyethylene products, which had direct effects on the biodegradability of the polymer. Generally, the UV-treated films were more recalcitrant than heat-treated films, suggesting that there are differences between the two residual polyethylenes [21].

SIGNIFICANCE OF THE STUDY

Plastics have become a significant environmental problem. The problem of plastic pollution in Ethiopia is apparent by their presence in streets, water ways, garbage dump sites and agricultural lands.

A number of approaches have been proposed to tackle this problem mainly bio-plastic synthesis and plastic degradation. Plastic degradation could have a real impact on the amount of garbage in landfills or of litter that ends up in our oceans, waterways, farm lands and on our streets; and also in preventing toxicity and pollution problems associated with plastics.

There is yet no report on microbial polyethene and plastic degradation in Ethiopia. This study may contribute to the scientific knowledge in the area of plastic degradation and provide base line information for future improvement of degradation efficacy of plastic degrading microbes in the Ethiopian context. This study may also provide the inputs to develop bioremediation as a means of eliminating solid pollutants in the environment and the associated public health problems. The effective isolates may be employed to degrade plastic materials in garbage dumps and landfills there by reducing the volume of solid wastes.

CHAPTER THREE

OBJECTIVES AND HYPOTHESIS

3.1 General objective:

The general objective of the study was to obtain microbial strains with high polyethene plastic-degrading ability.

3.2 Specific objectives:

a. To isolate *Streptomyces* spp. and *Sphingomonas* spp. *bacterium* and other polyethylene-degrading microbes from environmental samples

b. To screen these microbial isolates for the most efficient ones based on their efficiency of degrading polyethylene plastic.

c. To assess the influence of pre-heat treatment and pro-oxidants in the degradation of polyethylene.

3.3 Hypothesis

If polyethene-degrading microorganisms do exist in Ethiopia, then it will be possible to isolate, screen and select among them the most efficient ones based on degradation efficacy, and use them for degradation of plastic bags.

CHAPTER FOUR

METHODS AND MATERIALS

4.1 Polymer:

A polyethylene of low density (LDPE) and polyethylene of high density (HDPE) were the polymers used. These are commercially produced: HDPE by Exxon Mobil Chemical company and LDPE by Sabic Chemicals company and were supplied by ABC Plastic Factory PLC. For the qualitative colorimetric screening in method 1 (i.e. based on color change to narrow down the number of isolates for the subsequent test (see below)), PE that can be autoclaved was bought and brought from Philippines.

Preparation of PE powder for the enrichment isolation procedure

Shredded PE film and table salt (NaCl) were ground for several minutes using medicinal plant grinder blender. The mixture of ground PE film and NaCl was transferred into cylinder (1 L capacity) containing distilled water and washed. The floating layer of PE particles was collected on filter paper, washed three times with distilled water and dried in an oven at 50°C overnight. Dried PE powder was then passed through a sieve [22].

4.2 Soil sample collection and processing

A total of 20 soil samples were collected at a depth of 3-5 cm from garbage dumps and grounds covered by vegetation in and around Jimma town, about 345 km, south west of the capital Addis Ababa, Ethiopia. Each sample of 20g was transferred to sterile sample bottles and transported to the laboratory and stored at 5^{o}C. Each soil samples (10g wet weight) were transferred separately into 250 ml flask containing 90 ml of buffered phosphate solution (pH 7.0), shaken vigorously for 2 min and left to stand for 10 min to let the soil particles to settle. The resulting supernatant was serially diluted to 10^{-5} to 10^{-7} and 0.1 ml of each dilution was surface plated in duplicates on selective media (*Sphingomonas* and *Streptomyces*) and incubated at 37 ^{o}C for 4-10 days. The soil samples were collected at

waste dumps around airport, mercato, mentina, koche, uorael church and inside Jimma university campus.

4.3 Cultures and Growth Conditions

4.3.1Isolation of Polyethylene Degrading Microorganisms

Two methods were used to isolate polyethene-degrading microorganisms: 1) using enrichment medium and 2) using selective medium.

4.3.1.1. Isolation of PE degrading microorganisms by enrichment procedure

The fundamental reason behind the enrichment procedure was to create strong selective conditions using an organic pollutant as the only source of carbon as described by Kleeberg *et al*. [24]. The enrichment medium consists of 0.1% $(NH4)_2SO_4$, 0.1% $NaNO_3$, 0.1% K_2HPO_4, 0.1% KCl, 0.02% $MgSO_4$, 0.01 % yeast extract and 0.2 % polyethene powder, using tap water as solvent.

Soil samples two replicates per sampling site were mixed thoroughly and used as a source of potential PE degrading microorganisms. One gram of the soil was added to 100 ml of enrichment medium in a flask and incubated at 37^oC for 2 weeks with shaking at 100 rpm. Then, 10ml of the broth was taken from the enrichment flask with visible growth, re-inoculated into 100ml of fresh enrichment medium and incubated under the same conditions for 2 weeks. The same procedure was repeated a third time. The final enrichment cultures (FEC) were aseptically filtered through filter paper to remove any remaining polyethylene powder.

Figure 4.1 Isolation of plastic-degrading microorganisms using polyethene. Left, incubation with shaking of the enrichment medium; Right, close-up of medium showing PE plastic as carbon source.

A 0.1ml aliquot from each filtrate was surface plated on pre-dried sterile solid enrichment medium and incubated at 37°C until visible growth is observed. Bacterial colonies with different morphologies were picked and purified by repeated streaking on nutrient agar plates and transferred to slants for maintenance. Fungal isolates were purified and maintained on Sabourauds Dextrose agar.

4.3.1.2. Isolation of plastic-degrading microbes using selective medium

A. Isolation of *Streptomyces* by selective medium:

Soil samples (two replicates per sampling site) was serially diluted to 10^{-5} to 10^{-7} and 0.1 ml of each dilution was surface plated in duplicates on water-yeast extract (WYE) agar as described in Vinhas *et al*. [3]. The composition of the WYE medium is as follows (per liter): 0.5 g of K_2HPO_4, 0.25 g of yeast extract and 18g of agar. The pH was adjusted to pH 7.0 by addition of NaOH. The inoculated plates were incubated at 37 ^{o}C for 4-10 days. Distinct colonies were streak plated on nutrient agar until pure culture was obtained.

B. Isolation of *Sphingomonas* by selective medium:

Soil samples (two replicates per sampling site) was serially diluted to 10^{-5} to 10^{-7} and 0.1 ml of each dilution was surface plated in duplicates on mineral medium containing glucose at 15g/L as sole carbon source and 200 µg/ml of the aminoglycoside antibiotic streptomycin [25]. The composition of mineral medium is (per liter): 4.60 g of KH_2PO_4, 11.60 g of

$NaHPO_4 \cdot 12H_2O$, 1.00 g of NH_4Cl, 0.50 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $CaCl_2 \cdot 2H_2O$, and 0.01 g of $FeCl_3$. The pH was adjusted to pH 7.0 by addition of NaOH. The inoculated agar plates were incubated at 37 °C for 4 – 10 days. According to Karolien *et al*. [25]**,** bacteria growing under these conditions and with yellow pigmentation (colony morphology) are strains of *Sphingomonas* which can tolerate the presence of 200 μg/ml of streptomycin. Distinct colonies were streak plated until pure culture was obtained.

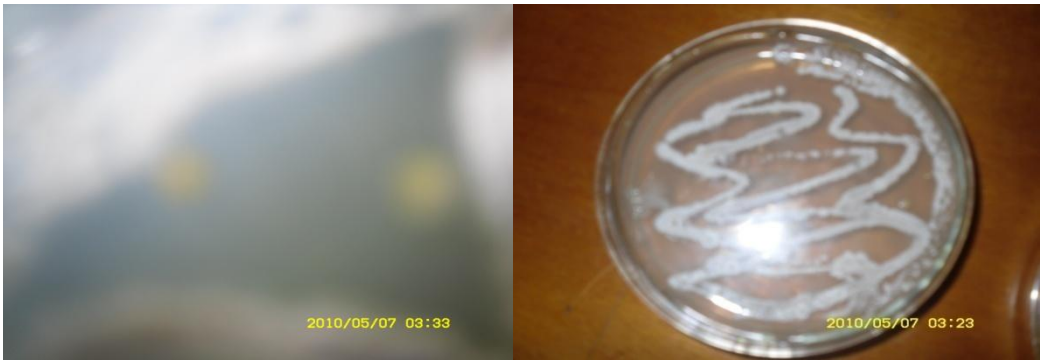

Figure 4.2 Streak-plated microbial isolates on purifying plates. Left, Sphingomonas; right, Streptomyces

4.3.2 Maintenance of cultures:

The strains of bacteria and fungus were maintained on slants of agar medium respectively overlaid with sterile mineral oil. The slants were stored at 5ºC.

4.4 Screening of isolates:

i. ***Method 1:*** Qualitative method using colored LDPE sheets (to narrow down the number of strains for quantitative screening). A rapid screening test was made using colored LDPE as developed by Cook [26]; LDPE plastic sheets were cut into discs to fit the diameter of the petridish. The microbial strains from 0.1 ml of overnight broth cultures maintenance media were streak-plated on mineral medium (MinB agar) as described by Kleeberg *et al*. [24] **(**four isolates per petridish and each isolate was plated in triplicate). Mineral media (Min B agar) is composed of 1g of NH_4NO_3, 0.5 g of $MgSO_4 \bullet 7H_2O$, 0.5 g of NaCl, 0.01 g of $FeSO_4 \bullet 7H_2O$ 1 g of $K2HPO_4$ and 20 g agar per liter of distilled water. Plastic sheets were placed on the top of the streaked media and incubated at 37

^{O}C for 15 days. The selection of isolates was based on color change upon removal of the dye on the surface of these plastic sheets. The cultures with clearing surrounded by original color of the plastic sheet were chosen for further tests using Method 2. In the case of *Streptomyces*, nutrient agar was used in addition to the plastic substrate since *Streptomyces* were unable to grow when only PE was used.

ii. ***Method 2*:** Quantitative method of screening procedure is similar to those described by Kathiresan [20]. To assess microbial degradation of polyethene plastic, 20 pre-weighed 1×0.5 inch LDPE strips were sterilized by soaking in 95% ethanol, washed with sterile distilled water and aseptically transferred to conical flasks containing 50 ml of MinB broth medium with the exception of *Streptomyces* inoculated alone, in this case nutrient broth was used. Three replicates of the medium were inoculated with 0.1 ml of overnight nutrient broth culture per strain and incubated for six weeks with shaking at 100 rpm. Control medium was not inoculated with strains. Then, the medium was filtered to retain the discs and the discs were washed to remove the remaining *bacterium*. The strips were air-dried and weighed. High percentage reduction in mean weight of the plastic indicates efficient plastic degradation. In the case of *Streptomyces*, glucose was added in addition to the plastic substrate since *Streptomyces* were unable to grow when only PE was used.

Figure 4.3 Screening of plastic-degrading strains using method 2. Left, incubation with shaking of medium containing plastic (blue) as carbon and energy source; Right, close-up of medium showing the plastic.

4.5 Selection of the Efficient Isolates:

Based on their degradation efficacy using Method 2, three most efficient strains were chosen for the subsequent two further tests:

i. Test of select efficient strains for degrading ability on HDPE individually. In this test, Method 2 was used except that HDPE strips were used as carbon source. Efficiency of degradation was measured in terms of mean weight reduction.
ii. Test of efficacy of combination of strains for degradation of LDPE and HDPE. In this test, Method 2 was employed with LDPE and HDPE strips as carbon and energy source and the media separately inoculated with different combinations of isolates. Assessment of efficiency was the same as above.

4.6 Assessment of Influence of Heat in Biodegradation

In this test, Method 2 was employed. There were four treatments replicated three times, and pro-oxidants shown below were added to all treatments.

The treatments were as follows:

1. Plastic + *Bacterium* + Heat
2. Plastic + *Bacterium*
3. Plastic + Heat
4. Plastic (negative control)

4.7 Degradable plastic pretreatment

Plastic pretreatment was based on the methodology used by Lee *et al* [21]:

i. Addition of Pro-oxidants- Pro-oxidants are mixtures of transition metals and lipids that induce oxidative stress, either through creating reactive oxygen species (ROSs) or inhibiting antioxidant systems. In this study iron in the form of $FeSO_4$ (0.01g/l) and two drop/L soy bean oil were added.

ii. Heat treatment: To accelerate the pro-oxidant activity, strips of LDPE and HDPE degradable plastics were placed in a 60°C forced-air oven for 14 days in order to stimulate the thermophilic phase of the process.

iii. Chemical disinfection: The disinfection procedure used with each pretreated film consisted of placing the plastic into a covered beaker, adding a fresh solution of universal disinfectant containing 7 ml of Tween 80, 10 ml of bleach, and 983 ml of sterile water, and stirred for 30 min [21]. The mixture was filtered through filter paper in order to remove the liquid and the plastic was washed with sterile water, placed into a covered beaker of sterile water, and stirred for 60 min at room temperature. The plastic was then filtered and aseptically transferred into a standing 70% (vol/vol) ethanol solution and left for 30 min. The plastic was then placed into a pre-weighed sterile petridish. The dishes with plastic were placed into an incubator at 45 to 50°C to dry overnight, allowing to equilibrate to room temperature, and were weighed to +0.1-mg accuracy; the weight of the plastic film was then determined.

4.8 Test used to evaluate changes in degradable plastics

Weight loss: Biodegradation was followed by weight loss. It was determined by the difference in the weight of plastic between before and after incubation.

4.9 Data quality management

The whole experiment was performed based on standard methodology described above and standardized laboratory procedures, and the necessary precautions were made. Instruments were checked and calibrated before use.

4.10 Dissemination plan

Three copy of the report will be submitted to college of public health and medical sciences of Jimma University and other relevant authorities. The report will also be submitted for publication.

CHAPTER FIVE

RESULTS

5.1 Result of isolation

After sample soils were collected from garbage dumps and grounds covered with vegetation and isolated using selective media with subsequent purification by streaking, tentatively identified four isolates of *Streptomyces* and four isolates of *Sphingomonas* strains were obtained (Table 5.1).

Table 5.1 Plastic-degrading microbial strains isolated from the environment

Isolate Number Code	Tentative Identification	Source
LBG 1	*Streptomyces*	Garbage dump
LBG 2	*Streptomyces*	Garbage dump
LBG 3	*Streptomyces*	Vegetation covered ground
LBG 4	*Streptomyces*	Vegetation covered ground
LBG 5	*Sphingomonas*	Garbage dump
LBG 6	*Sphingomonas*	Garbage dump
LBG 7	*Sphingomonas*	Vegetation covered ground
LBG 8	*Sphingomonas*	Garbage dump
LBG 9	*Sphingomonas*	Vegetation covered ground
LBG 10	Fungus	Garbage dump
LBG 11	Fungus	Garbage dump
LBG 12	Fungus	Vegetation covered ground
LBG 13	Bacterium	Garbage dump
LBG 14	Bacterium	Vegetation covered ground
LBG 15	Bacterium	Garbage dump
LBG 16	Bacterium	Vegetation covered ground
LBG 17	Bacterium	Garbage dump
LBG 18	Bacterium	Vegetation covered ground

The study also employed shaken enrichment broth procedure that use polyethene as the sole source of carbon and energy in order to create a selective condition. After isolation on solid enrichment media, colonies with different morphologies were picked and purified by streaking on agar plates; as a result six unidentified bacteria, three unidentified fungal isolates and tentatively identified *Sphingomonas* strain were obtained. The results of this isolation are given in Table 5.1.

From the above two isolation techniques *Sphingomonas* and *Streptomyces* selective media and enrichment broth methods, a total of eighteen microbial isolates were found (four *Streptomyces*, five *Sphingomonas*, six unidentified *bacterium* and three unidentified fungal strains). The microbial isolates were cultured on slants and overlaid with sterile mineral oil for maintenance at 5^{o}C.

With the exception of test on combination of isolates, no weight loss and even no visible growth was observed on treatments inoculated with *Streptomyces* sp. alone and either LDPE or HDPE polyethene were used as a sole source of carbon and energy. Therefore, nutrient agar was used and glucose was added in addition to the plastic substrate in qualitative and quantitative screening respectively.

5.2 Qualitative screening of isolates

If these microbes can degrade colored polyethene, the dyes of these bags get removed causing a color change. Therefore, to narrow down the number of strains for the subsequent quantitative screening a rapid screening was used. The cultures with clearing surrounded by original color of the plastic sheet were chosen for further tests using Method 2.

All microbial strains that are found after both methods of isolation and from both soil sample sources (i.e. garbage dumps and grounds covered with vegetation), were tested in the laboratory for their ability to degrade the polyethene sheets through use of rapid color-clearing technique. As a result six isolates: LBG-1, LBG-2, LBG-5, LBG-13 (gram-negative and rod shaped *bacterium*), LBG-10 and LBG-11, isolated from garbage dumps, were found to cause a better (visible) color change (Figure 5.1). However, all microbes isolated from soil samples taken from grounds covered with vegetation were unable to cause visible color change on the polyethene sheets during the period of incubation (i.e. 37 ^{O}C for 15 days).

Table 5.2 Result of qualitative screening of isolates

Isolate Number Code	Tentative Generic Identification	Color-clearing
LBG 1	*Streptomyces*	+
LBG 2	*Streptomyces*	+
LBG 3	*Streptomyces*	-
LBG 4	*Streptomyces*	-
LBG 5	*Sphingomonas*	+++
LBG 6	*Sphingomonas*	-
LBG 7	*Sphingomonas*	-
LBG 8	*Sphingomonas*	-
LBG 9	*Sphingomonas*	-
LBG 10	Fungus	+++
LBG 11	Fungus	++
LBG 12	Fungus	-
LBG 13	*Bacterium*	++
LBG 14	*Bacterium*	-
LBG 15	*Bacterium*	-
LBG 16	*Bacterium*	-
LBG 17	*Bacterium*	-
LBG 18	*Bacterium*	-

Legend: -, no clearing; +, slight clearing; ++, moderate clearing; +++, high clearing of dye.

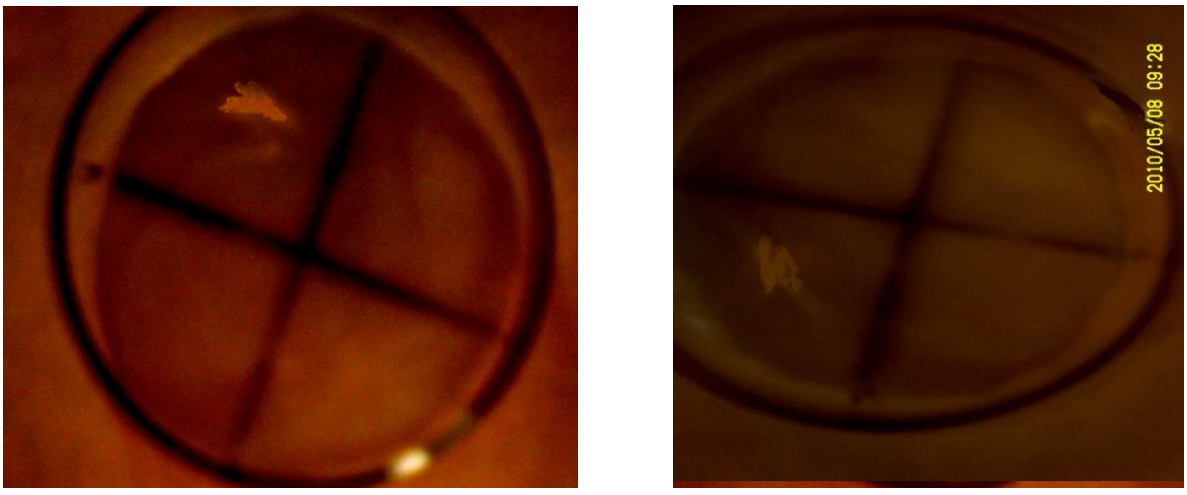

Figure 5.1 Qualitative screening result of Sphingomonas strain LBG-5 (left) and fungal strain LBG-10 (right). The clearing of the color of the plastic disk indicates degradation of the material by microbial action.

5.3 Quantitative screening results

After six weeks of incubation on shacked flask, weight loss data was recorded for each strain in each treatment. The numbers represent an average percent weight loss of duplicate.

5.3.1 Polyethene degradation by individual microbial strains

5.3.1.1 LDPE degradation

Six microbial species, isolated from soil samples taken from garbage dumps, selected in the qualitative colorimetric screening, were tested for their ability of degrading LDPE plastic. The species tested were: two *Streptomyces* sp. (LBG-1 and LBG-2), one *Sphingomonas* sp. LBG-5, two fungal isolate (LBG-10 and LBG-11) and one rod shaped gram-negative *bacteria*l strain (LBG-13). These microbes were separately allowed to degrade LDPE under shaken flask cultures for six weeks. The results are shown in Table 5.3.

Table 5.3 Heat-treated and untreated LDPE degradation (%) by individual strains

Strain	**Genera (Group)**	**Average heat untreated LDPE weight loss (%)**	**Standard error of the mean**	**Average heat-treated LDPE weight loss (%)**	**Standard error of the mean**
LBG-1	*Streptomyces*	10.31 ± 0.13	0.075	15.18 ± 0.68	0.393
LBG-2	*Streptomyces*	6.12 ± 0.09	0.052	7.80 ± 0.03	0.017
LBG-5	*Sphingomonas*	12.73 ± 0.43	0.248	19.74 ± 2.12	1.224
LBG-13	Bacterium	3.77 ± 0.13	0.075	5.95 ± 0.35	0.202
LBG-10	Fungus	7.11 ± 0.01	0.006	9.31 ± 1.78	1.028
LBG-11	Fungus	4.91 ± 0.11	0.064	6.16 ± 0.66	0.381
Control		0	0	1.13 ± 0.03	0.017

As indicated in Table 5.3, among the six strains tested, *Sphingomonas* strain LBG-5 demonstrated greater degrading ability in both heat-treated and untreated LDPE strips with an average percent weight loss of 19.74 and 12.7,3 respectively. *Streptomyces* sp. LBG-1 caused 10.31% of weight reduction on untreated and 15.18% on heat-treated LDPE films. The fungal isolate LBG-10 conceded the third reduction in percent weight loss on both treatments while the unidentified bacteria caused the least weight reduction of all. However all the strains tested showed a much better degradation as compared to the heat untreated (0% weight loss) and the heat-treated control (1.13 %).

In all the treatments heat-treated LDPE stripes showed much better degradation compared to untreated ones. The heat-treated but un-inoculated LDPE treatments demonstrated a slight degradation in terms of average % weight loss.

As mentioned in the methodology part of this report, on the basis of their efficacy to degrade LDPE three strains namely *Sphingomonas* LBG-5, *Streptomyces* LBG-1 and fungus strain LBG-10 were chosen for test on HDPE degradation and combination of strains.

5.3.1.2 HDPE degradation

The best three strains selected based on their efficacy of degrading LDPE, were tested for their ability to degrade HDPE plastic. The species tested were: *Sphingomonas* strain LBG-5, *Streptomyces* strain LBG-1 and fungus strain LBG-10 in decreasing order of degrading low density PE. These microbes were separately allowed to degrade HDPE under shaker flask cultures for six weeks.

Similar to the result of degradation on LDPE, *Streptomyces* strain LBG-1 caused 5.53 and 4.10 average percent weight reduction on heat-treated and untreated HDPE strips respectively, next to *Sphingomonas* strain LBG-5 with mean weight loss of 8.01% for heat-treated and 5.59 % of untreated ones. The results are shown in Table 5.4.

Table 5.4 Weight loss (%) of heat-treated and untreated HDPE due to degradation by individual strains.

Strain	**LBG-5 (*Sphingomonas*)**	**LBG-1 (*Streptomyces*)**	**LBG-10** (Fungus)	**Control**
Average heat-treated HDPE weight loss	8.01 ± 0.32	5.53 ± 0.05	4.12 ± 2.01	0.76 ± 0.01
Standard error of the mean	0.185	0.029	1.160	0.006
Average untreated HDPE weight loss	5.59 ± 0.42	4.10 ± 0.11	3.75 ± 0.22	0
Standard error of the mean	0.242	0.064	0.127	

The fungal isolate LBG-10 caused the lowest weight reduction of all the strains in both the heat-treated and untreated HDPE while the control showed no change in weight. Similar to the result of LDPE test, the heat-treated HDPE sheets again showed greater degradation compared to the untreated sheets. The heat-treated but un-inoculated HDPE treatments demonstrated a slight degradation in terms of average weight loss.

Prooxidant transition metal combinations demonstrated the greatest effect on the degradation of heat-treated LDPE and HDPE films inoculated with all the three strains. Upon activation by heat in the presence of oxygen, prooxidants produce free radicals on the polyethylene chain, which result in oxidation and a change in physical properties of the polyethene sheets.

5.3.2 Polyethene degradation by combination of strains

The three most efficient strains tested on HDPE were also combined to see the effect of combination of strains on the degradation of LDPE and HDPE plastic sheets. Combinations of these strains were allowed to degrade LDPE or HDPE under shaken flask cultures for six weeks. The results are shown in Table 5.5. The numbers presented here are mean percent weight loss caused by the combination of isolates on non-heat-treated PE strips.

Among the three combinations, LBG-5 + LBG-10 *Sphingomonas* and fungal combination demonstrated the highest degradation in terms of mean weight loss conceding 14.01% reduction on LDPE followed by LBG-5 + LBG-1 *Sphingomonas* and *Streptomyces* that resulted 13.33% average weight loss. However, LBG-1 + LBG-10 caused the least degradation of all the three combinations. (Table 5.5)

Table 5.5 Weight loss (%) of LDPE and HDPE due to degradation by combination of strains.

Strains	**LBG-5+ LBG-1** *(Sphingomonas+ Streptomyces)*	**LBG-5 + LBG-10** *(Sphingomonas+* Fungus)	**LBG-1 + LBG-10** *(Streptomyces+* Fungus)	**Control**
Average LDPE weight loss %	13.33 ± 0.71	14.01 ± 0.15	10.97 ± 0.56	0
Standard error of the mean	0.410	0.087	0.323	0
Average HDPE weight loss %	7.31 ± 1.13	6.34 ± 0.25	5.17 ± 0. 03	0
Standard error of the mean	0.652	0.144	0.017	0

Unlike the result on LDPE strips, LBG-5 + LBG-10 (*Sphingomonas* and fungi) combination resulted in lesser degradation on HDPE (6.34% mean weight reduction) compared to that of *Sphingomonas* and *Streptomyces* combination that showed the highest degradation (7.13% weight reduction) of all the combinations. The least mean weight loss among the combinations on HDPE sheets was demonstrated by LBG-1 + LBG-10 (*Streptomyces* and Fungi) combined.

CHAPTER SIX

DISCUSSION

The fundamental reason behind the enrichment broth procedure is to create strong selective conditions using an organic pollutant i.e. polyethene as the only source of carbon and energy as described by Kleeberg *et al*. [24]; So that, only microbes that can metabolize polyethene as a primary metabolite can be isolated.

Soil samples were collected from garbage dumps and grounds covered with vegetation in and around Jimma town. This is to enable the comparison of degradation ability between the isolates from the two sources of soil samples and among isolates. This was done in view of the fact that previous researchers [27] claim that adaptation, in addition to other factors, also plays a major role in determining biodegradation rates.

The results of qualitative screening demonstrated that microbes, isolated from soil samples taken from grounds covered with vegetation, were unable to cause color change on the polyethene sheets during the period of incubation (i.e., at 37 ^{O}C for 15 days). These results corroborate the findings of previous researchers [27]. Polyethene plastic wastes are found in garbage dumps and microbes at these sites adapt themselves to polyethene environment and acclimatize their metabolism enabling them to metabolize polyethene better than those that are from vegetation-covered soil.

Six microbial isolates, selected based on their efficacy on qualitative screening, were separately tested for degradation of heat-treated and untreated LDPE under shaken flask culture for six weeks. All the tested microbes were able to degrade both the heat-treated and untreated low density polyethene sheets compared to the corresponding controls. Among the strains tested, *Sphingomonas* strain LBG-5 demonstrated the greatest degrading ability with an average percent weight loss of 19.74 (heat- treated) and 12.73 (untreated) LDPE followed by *Streptomyces* strain LBG-1. The unidentified gram-negative rod-shaped *bacterium*l strain

LBG-13 caused the least weight reduction. In all microbial treatments the heat-treated film degradation was greater compared to the untreated ones.

The three microbial strains that were able to cause better degradation on LDPE plastic were chosen to test their ability to degrade HDPE individually and in combination. The microbes chosen were *Sphingomonas* strain LBG-5, *Streptomyces* strain LBG-1 and fungus strain LBG-10.

When tested individually for their ability to degrade the heat-treated and untreated high density polyethene plastic, *Sphingomonas* strain LBG-5 was able to degrade HDPE strips better than *Streptomyces* strain LBG-1 and fungus strain LBG-10, with mean percentage weight loss of 8.01 for heat-treated and 5.59 % for untreated films. *Streptomyces* strain LBG-1 ranked the second in degradation ability and fungus strain LBG-10 caused the least degradation of both heat-treated and untreated PE films.

Similar to the result of degradation on LDPE, the heat-treated HDPE sheets showed greater degradation compared to the untreated sheets. However, HDPE sheet degradation by all the three strains was lower compared to LDPE sheets in both heat-treated and untreated ones. The biodegradation of polymers is a complex process depending on various factors relating to the polymer or the media or the environment where degradation takes place [28].

Studies on biodegradation of synthetic polymers and oligomers have shown close relationships between biodegradability and chemical structure [19,28,29]. HDPE has a closely packed structure (i.e. linear crystallization) [28] which makes it difficult for enzymes of microorganisms to access the C-bonds and degrade it [7,13,30]. In addition, as a result of this chemical structure property, HDPE has high tensile strength, very low moisture absorption, better chemical resistance and is stronger and harder than LDPE. More flexible polymer chains fit more easily to the active site of the enzyme and this promotes biodegradability [28]. Thus, the ability of microbes to cause weight loss is greater on LDPE compared to HDPE as density, crystallization and structural complexity such as linearity of the substrate are among the factors that affect biodegradability [28,29].

However, in some previous studies that used powdered polyethene [27] and starch blended polyethene [31], HDPE showed greater degradation than that of the LDPE; this could be due to the fact that the availability of moisture [28] (i.e. in this case: moisture absorbed by starch) is a prerequisite for biological decay [29]. Also, substrate size is one of the factors that affect biodegradability [28,32]. Therefore, much better moisture absorption by the starch and size reduction through powdering might contributed to the greater HDPE degradation despite characteristic low moisture absorption by HDPE compared to LDPE [31].

Of all the strains tested *Sphingomonas* strain LBG-5 was able to degrade both low density and high density polyethene plastic better than the other plastic-degrading microbes tested.

As can be understood from the result of the rapid qualitative screening and as observed in the first few weeks of incubation in heat-treated and untreated plastics inoculated with the microbes, discoloration of the plastic strips could be due to the first step of biodegradation of polymers (i.e. attachment of microorganism to the surface of the polymer) [29].

In both LDPE and HDPE plastic degradation tests by individual microbial strains, the heat-treated but un-inoculated treatments demonstrated a slight degradation in terms of weight loss. This indicates that, hence polyethene is oxo-degradable [6], upon exposure to heat the plastic undergoes chemical degradation as explained by Arutchelvi [29]. The higher weight loss in heat-treated films than untreated films is because polyethene is oxo-degradable meaning it degrades more quickly after exposure to physical factors like heat [6]. A variety of environmental factors such as oxygen, temperature, sunlight, water, stress, living organisms and pollutants may affect the degradation of polymer [33]. Exposure to heat facilitates polymers to decompose into smaller parts (monomers) [6,8,23]. Also, physical and chemical degradation facilitates microbial degradation and complete mineralization of the polymer happens due to biodegradation, which is generally the final step [29].

The combined degradative activities of the isolates had additive effects. The three best microbes were used in various combinations to degrade LDPE as well as HDPE sheets. In all combinations tests, an increased degradation of both LDPE and HDPE was observed

compared to degradation by individual strains. Combinations of strains enabled faster degradation of low density than of high density polyethene [22].

Pro-oxidants produce free radicals on the long polyethylene chain, causing the material to lose some of its physical properties, to become oxidized, and, possibly, to become more accessible to microbial biodegradation [19]. Pro-oxidants were added to all treatments because as reported earlier [21,29], biodegradation of plastics can be initiated and facilitated by the addition of pro-oxidants. The results of this study showed polyethene plastic degradability and as well greater degradability compared to previous studies that do not employ the use of pro-oxidants [33].

There was no visible growth and weight loss by *Streptomyces* sp. when polyethene was used as sole source of energy/carbon; strengthening the scientific fact that *Streptomyces* have a characteristic secondary metabolism/co metabolism [34].

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

Due to their molecular stability, plastics do not easily break down into simpler components; therefore they are not considered biodegradable. Though polyethene is synthetic polymer and persist in the environment, microbes were able to degrade it in controlled environmental conditions. Garbage dumps are found out to be good source of plastic-degrading microbes.

Soil microorganisms are able to degrade polyethene as a sole source of carbon and energy or co-metabolically. Among the tested microbes, *Sphingomonas* strain LBG-5 can degrade polyethene plastic as a sole source of carbon and energy and better than the other microbes *Streptomyces* strain LBG-1 also has the potential to degrade it. Microbes degrade polyethen better when they are combined.

Degradation of polyethene is influenced by physical, chemical and biological factors such as: heat exposure, the type of plastic and the kind of microbial strain to which the polyethene is exposed. Low density polyethene is more suseptible to microbial degradation than the high density polyethene. Pre-treatment of heat facilitates and enhances polyethene degradation. Hence, polyethene have only CH_2 groups, meaning that its surfaces are hydrophobic and microorganisms can attach to the surface if the polymer is hydrophilic; heat treatment of the plastic can lead to insertion of hydrophilic groups on the polymer surface making it more hydrophilic.

7.2 RECOMMENDATIONS

The degradability of synthetic plastics particularly polyethene is influenced by a number of factors. A variety of environmental factors such as oxygen, temperature, sunlight, water, stress, living organisms and pollutants may affect the degradation of polymer. it is important from the viewpoint of bio-remediation to determine the biodegradability of plastics and microorganisms responsible for it. Understanding of these factors will have significant contribution in solving the pollution problems associated with these plastics and in making them environmentally friendly. In previous and this study, it was possible to assess some factors. However, this research was very basic.

More investigation should be done in order to get better understanding on the biodegradation of polyethene; such as: physico-chemical, biological and environmental factors. Future studies can also be done on understanding of the degradation process and also on different property changes of polyethene in degradation. For example, in this research the degradation has been found based on the mean weight loss. Other properties such as the thickness, tensile strength, percentage elongation, molecular weight change and visual observation can also be used to find the degradation rate of the polymer.

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