
# BACTERIAL REMEDIATION OF POLYETHYLENE

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**AZEKO, SALIFU TAHIRU**

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## DECLARATION

I hereby declare that this thesis work entitled 'Bacterial Remediation of polyethylene' is the result of investigation carried out by me, Azeko Salifu Tahiru under the supervisions of Professor Wole Soboyejo at the African university of Science and Technology, Abuja-Nigeria in the Department of Materials Science and Engineering and Doctor Shola Odusanya at the Sheda Science and Technology complex, Abuja-Nigeria in the Biotechnology Advanced laboratory and that no previous submission for a degree or diploma of this university or elsewhere has been made. Related work by others which served as source of knowledge has been duly acknowledged and referenced.

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Azeko, Salifu Tahiru Date

## ACKNOWLEDGEMENT

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The second half of my acknowledgement is dedicated to every single person who mentioned, nagged, bugged and even pressured me into finishing my thesis work. Your words probably went unappreciated at that time but please know that they really did help.

My parents have been the most significant people in my life so far and this part of my life was no exception. Without your support, persistence and love I would not be where I am today. Finally, I would like to express my sincere thanks to my brother Ali for being there when I needed him. Your words and actions are inspiring to me. Thank you!

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## ABSTRACT

Improper disposal of commodity plastics e.g. polyethylene (PE) in the environment causes land pollution, soil infertility, is unsightly and poses dangers to plant and animal life. The current effort describes the bacteria mediated biodegradation of commodity plastics by *Serratia marcescens marcescens* without first subjecting the plastics to thermo-oxidative ageing. It further elaborates on the mechanism and breakdown that are involved in the biodegradation of PE. 90 ml of carbonless medium containing essential minerals and vitamins (minus carbon source) was measured into seven conical flasks and 2 g of powdered PE was poured into each flask. 5 ml of three overnight cultures of *Serratia marcescens marcescens* was measured into six flasks. The other flask served as the control. The samples were incubated at $30^{o}$ C, 141 revolutions per minute (rpm) in a rotary shaker for four weeks to observe the degradation incidence. After 4 weeks, 5 ml of cell-free supernatant from *Serratia marcescens marcescens* culture was measured into a sterile conical flask to which 2 g of sterile PE was added. This was incubated at $30^{o}$C & 141 rpm in a shaker for four weeks. It was discovered that, the supernatants from *Serratia marcescens marcescens* degrade PE faster than the bacteria with a percent of degradation of 37.5 in a month. The SEM micrographs revealed that the biodegradation of polyethylene occurs due to the presence of voids and pits, which indicates the bacteria feeding on the PE.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background

The world has an abundance of land and resources [1]. However, due to human carelessness and negligence, we have contaminated the environment. Industrial activities generated from the past have resulted in polluted lands and landfills that are potentially harmful to the health and wellbeing of people [1]. There is, therefore, a need for improved methods of waste disposal that could limit the long term effects on human wellbeing.

Plastics are artificially made long chain "polymeric molecules" and come from the Greek word "Plastikos", indicating that they are able to form different shapes [2]. Nowadays, plastics are composed of organic and inorganic raw materials comprising Hydrogen, Carbon, Oxygen, Silicon, Nitrogen and chloride. They are often obtained from oil, coal and natural gas [3].

In most cases, plastics cannot be degraded easily by enzymes and microbes [4]. There are therefore environmental concerns and about the 30% of plastics that are used worldwide in the packaging of food, detergents, chemicals etc. occurs at an annual rate of 12% [5]. These include plastics such as; polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET) among others are used in packaging products.

Plastics have been used increasingly due to their attractive combination of stability as well as their balance of thermal and mechanical properties [6]. However, the pile-up of non-biodegradable plastics has been a source of increasing environmental concerns [7]. Furthermore,

since plastics are typically disposed of in landfills, there are serious concerns about the pile-up of plastic wastes that are non-biodegradable. There are also environmental concerns about the toxic fumes [8].

Polyethylene (PE) is a synthetic polymer that comprises repeating units of smaller molecules (monomers) of ethylene in a long chain. The annual production of synthetic polymers worldwide is approximately 140 million of tones with a utility rate of 12% [9]. Thus makes it difficult to develop an efficient way of disposing of it. The resulting landfills pose a serious threat to the environment [10].

However, PE can be degraded by chemical, thermal, photo and biodegradation. The two mechanisms that aid in the biodegradation of polyethylene are hydro-biodegradation and oxo-biodegardation. Hydro biodegradation is the reaction that involves the degradation of plastics by breaking down water molecules into protons and hydroxide anions. While Oxo-biodegradation involves the reaction of plastic with oxygen to molecular fragments and these smaller molecules are then biodegraded by microorganisms and converted into carbon dioxide, water and biomass [11].

Recent work [12, 13, and 14] has shown that some microbes such as *rhodococcus spp*, *Penicillium simplicissimum,* Brevibacillus and *pseudomonas* spp among others are able to biodegrade PE and use it as a source of carbon and energy.

The studies have revealed that microorganisms found it difficult to degrade PE with large molecular weight and vice versa. PE with small molecular weights are preferred in biodegradation since microorganisms easily degrade and use them as sources of carbon and energy a process called mineralization [15, 16].

The crystalline structure, molecular weight and mechanical properties of PE need to be altered for biodegradation, since these properties serves as opposition towards its degradation [17]. The hydrophobic level and molecular weight/polymer chain length of PE need to be reduced to enable easy access to microbial degradation [18].

Recent studies [9, 14] explain the various microorganisms that can be used in the biodegradation of PE and also how to alter its properties to enable easy degradation. However, the studies fail to elucidate the mechanism of degradation and the kinetics of breakdown of PE molecules in our current scientific understanding.

### 1.2 Problem of Plastic Waste

Figure 1.0: Plastic waste dumped in Sheda community, Abuja-Nigeria

The production of plastics from industries enters the ecosystem in large amounts which pollutes the environment. The presence of plastics such as polyethylene (PE) in the environment causes soil infertility and poses danger to animal life. Also, the existence of plastics in nature leads to water pollution and reduces landfills. Hence, there is the need to find appropriate ways of disposing of plastic waste.

### 1.3 Approaches to Plastic Remediation

They are quite a number of methods used in plastic remediation. Some of these include: Thermal degradation, Chemical degradation, Photo-degradation and Thermo-oxidative degradation.

These processes eliminate plastic waste in the environment. However, they are costly and environmentally unfriendly. Hence the need to find an alternative approach that is cost effective and environmentally acceptable.

Biodegradation is an alternative approach that can be used in disposing of plastic waste.

### 1.4 Unresolved Research Question(S)

Although a lot of researches have demonstrated on the use of microbes in the breakdown of polyethylene (PE) molecules, the mechanism and kinetics of the breakdown are yet to be elucidated. They also fail to use culture enrichment technique in culturing the microbes. There is, therefore, the need to determine the mechanism and the rate of degradation of PE to enable a potential for scale-up.

## 1.5 Scope of Work

The primary aim of this research was to explore the potential for degrading polyethylene (PE) by exposure to micro-organisms (bacteria) under well controlled conditions. The scope of the research is summarized below.

- Culturing of bacteria using an enrichment technique: Soil samples were collected at places where PE was not dumped, diluted with water and exposed to fresh powdered PE in an incubator shaker to determine which microorganism survives and this microorganism cultured in the lab.
- Pre-treatment and powdering of PE: This was done to remove dirt and any organic matter adhering to the surface, and also to obtain the PE in powdered form.
- Treatment of PE: The isolated bacteria was inoculated with the powdered PE and carbonless media and incubated for ten weeks to determine the rate of biodegradation.
- Characterization of PE: This was used to determine the changes in PE that occur during biodegradation. The degradation products were characterized as a function of time to determine the interaction between the bacteria and the PE.
- Modeling of PE Student T- Distribution.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Introduction

The release of highly toxic substances into the environment due to industrial and agricultural activities has increased drastically in recent years [19]. Soil contamination by pesticides, nitrogen and polycyclic aromatic hydrocarbons amongst others and heavy metals are major environmental problems that need to be address worldwide especially in Africa. This is attributed to the fact that, they cause soil infertility, pose danger to animal life and leads to water pollution and reduction in landfills [20].

Intrinsic biodegradation, a natural process is gaining more attention and may occur at the contaminated soil and microbial organisms which transform most of the polluted chemicals/substances into harmless form can contribute to its remediation [21, 22, and 23]. The environment and the chemical may however, undergo biodegradation depending on numerous factors in relation to their physical and chemical properties in which they are located. Incineration and volatilization which are conventional treatments of waste moves pollutant from one place to another and thus creating new waste and failing to solve the problem.

Several studies [24] have been carried to show that microorganisms are capable of biodegrading organic pollutants. Yet more contaminated sites are still found, indicating the availability of pollutants in the fields and hence the need to remedy and restore the damage environment to its original state.

Bioremediation, an alternative approach is usually preferred to the traditional method, since it is nontoxic and cleaner technique used in eliminating pollutants from the environment. Plants,

microorganisms, enzymes and plant-microorganisms population are the main agents used in bioremediation process [25].

This chapter will focus on polymer degradation mechanism, corrosion of polymers, materials characterization and the degradation models such as kinetic and stochastic models that can be used in the present study.

## 2.2 Degradation of Polymers

Polymer degradation involves the physical or chemical change that occurs due to heat, light, moisture or biological activities. The above factors cause the polymer to alter its properties and this aid in its degradation. Mechanical, optical and electrical features in cracking and phase separation occur as a result of changes in material properties including scission, chemical transformation and creation of new functional groups [26].

The degradation of polymers can occur due to the effects of photons, heat and biological organisms. Photosensitivity is due to the ability of polymers to absorb photons. This can induce photolysis, i.e. the breakdown of polymer chains. The factors responsible for photolysis and photo-oxidation include the UV-B terrestrial radiation and UV-A radiation with wavelengths of (~295–315 nm) and (~315–400 nm) respectively. The sunlight that reaches the earth with wavelength of 400-760 nm can be used to speed up the degradation of polymers by heating. Thermal oxidation can also be increased by infrared radiation (760-2500 nm) [27].

Majority of polymers are able to absorb radiation with high energy in the ultraviolet part of the electromagnetic spectrum. This triggers their electrons to higher reactivity and leads to oxidation,

cleavage and other degradation mechanisms [28]. Degradation of polymers by thermal effects is due to overheating. The constituents of the long chain strength of the polymer starts to detached at greater temperatures and react with each other to alter the polymer properties. Thermal degradation deals with changes in molecular weight and distribution of the polymer. These changes involves the reduction of ductility and "embrittlement", variations of color, "chalking", "cracking" and the lessening of other required physical properties [29].

The two methods used in Oxo-biodegradation to initiate biodegradation include photo-degradation (U V) and oxidation. The photo-degradation uses ultra violet light which aid in the degradation of the final product. Time and heat is used in the oxidation process for easy breakage of the plastic molecules. The two methods described above decreases the weight of the plastic molecules and permits biodegradation [30]. Biodegradation involves the breaking down of organic substances by bacteria. It is used mostly in ecology, waste management and bioremediation. Biodegradation also applies to plastics due to their long existence in the environment. Organic materials can be degraded with or without oxygen and also organic matter is changed into minerals by a process called biomineralization [31].

Biodegradation of plastics with oxygen (aerobic) occurs in harsh environment and in sediments and landfills plastics are biodegraded without oxygen (anaerobic) and partially aerobic and anaerobic in soil and composts. The by-products of aerobic biodegradation involve Carbon dioxide and water whilst that for anaerobic biodegradation consists of water and methane [32]. Mostly, bulk polymers can be broken down to carbon dioxide which needs numerous different organisms. A group of organism breakdown the polymer into its various components, while

others utilize the monomers and release waste compounds as by-products and also uses the expelled waste [33].

### 2.3 Corrosion of Polymers

Corrosion of polymers is a loosely term that is used for its degradation. They are usually difficult to dissolve due to their high molecular weight especially when mixed with other substance [34]. Corrosion of polymers can be related to a glass which is govern by diffusion-controlled leaching (ion exchange) and depends strongly on pH of contacting solution [35]. The rate of ion exchange is inversely proportional to pH as $10^{-0.5\,pH}$ and an increase in pH leads to a decrease in the rate of ion exchange.

The corrosion rate of a polymer can be determined using the normalized corrosion rate of an element (NCR) expressed as: NCR= $\frac{\boldsymbol{M_i}}{\boldsymbol{Sw_i t}}$, where $\boldsymbol{M_i}$ is the total amount of released species in the media (g), S is the media-contacting surface area ($cm^2$), t is the time of contact (days) and $\boldsymbol{w_i}$ is the weight fraction content of the element in the polymer (d).

The diffusion controlled normalized leaching rate of cations from polymers (DNLR) in $g/cm^2$ d can be expressed as: DNLR= $\boldsymbol{2\rho\sqrt{\frac{D_i}{\pi t}}}$ , where $\rho$ is the density of the polymer, t is the time (days) and $D_i$ is the i-th cation effective diffusion coefficient ($cm^2$/d) which depends on pH of contacting media as $D_i = D_{i0} \times 10^{-pH}$ [34].

## 2.4 Characterization Techniques

### 2.4.1 Differential Scanning Calorimetry (DSC)

DSC is a technique used to determine the heat flow between a sample and a reference especially when the material is heated or cooled or under isothermal [36]. DSC is used in the analysis of semi-crystalline plastics such as polyolefin [37]. DSC can be used to distinguish the different types of polyethylene (PE) from their melting points and endotherms as shown in figure 2.0.

Even though DSC is not very sensitive, its thermogram can be used to identify plastics from glass transition as illustrated in figure 2.1 [38]. In DSC application, temperature difference is converted into energy measurement per unit mass that is related to the transition in which the temperature difference occurred [36]. At concentration less than 5%, DSC may not be able to detect transitions within materials.

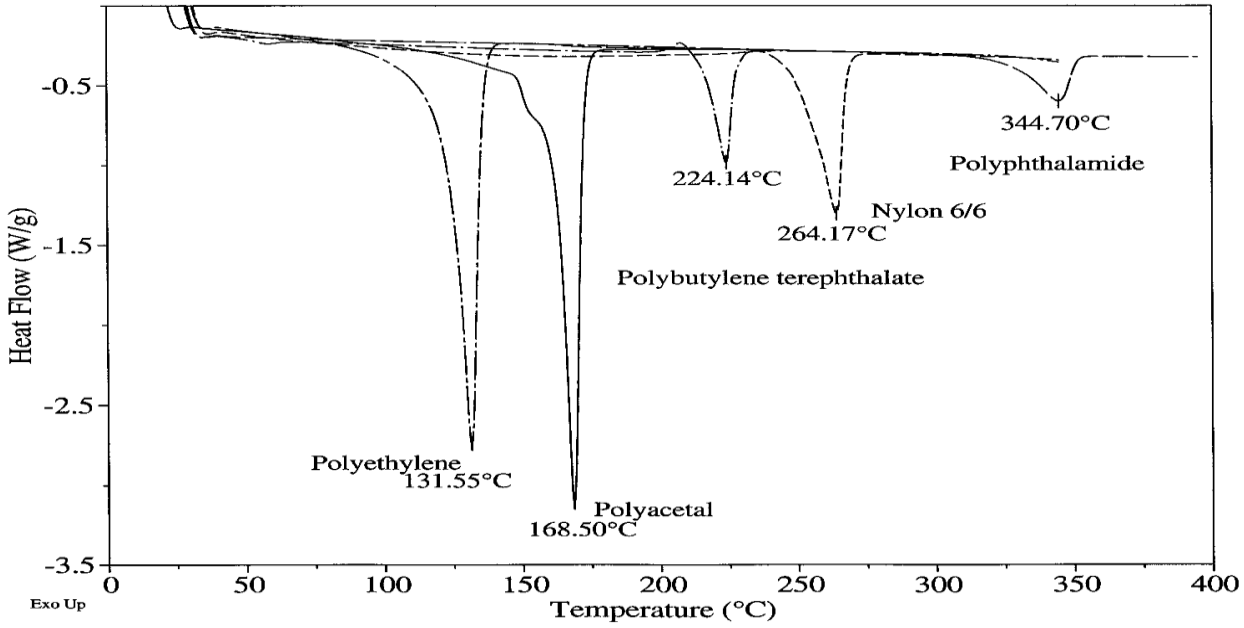


Figure 2.0: Differential Scanning Calorimetry used to identify polymeric materials by determination of their melting point

(Adapted from Jeffrey A. Jansen. Characterization of Plastics in Failure Analysis)

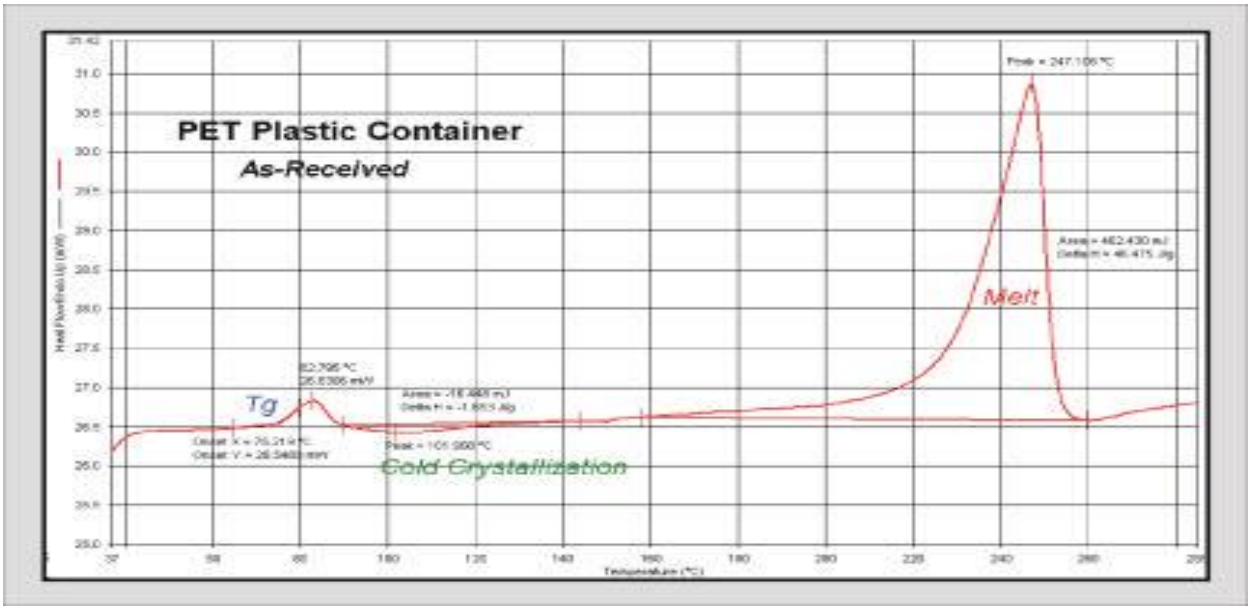


Figure 2.1: Differential Scanning Calorimetry used to identify polymeric materials by determination of their melting point

**(**Adapted from W.J. Sichina. DSC as Problem Solving Tool)

### 2.4.2 Thermogravimetric Analysis (TGA)

TGA is a technique that measures the quantity and the materials weight change with respect to temperature or time in an atmosphere which is well controlled [36]. Volatilization or decomposition can reduce the weight of the material. Also, gas absorption or reactions that involve chemicals can increase the weight content of the material. In polymeric materials, TGA gives important information concerning the composition and thermal stability as illustrated in figure 2.2. The data obtained comprises volatiles and inorganic filler contents, thermal decomposition onset and antioxidants [39].

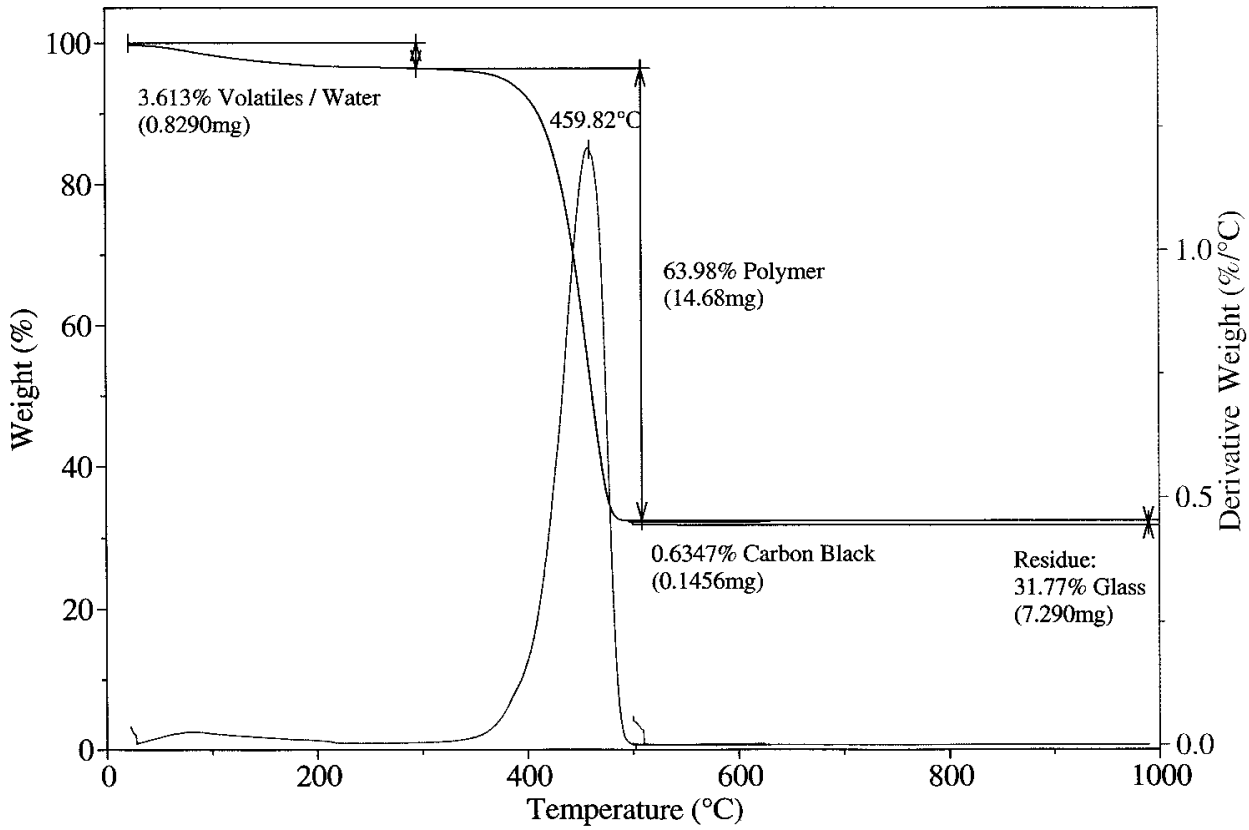


Figure 2.2: Thermogravimetric analysis thermogram showing the weight-loss profile for a typical plastic resin

(Adapted from Jeffrey A. Jansen. Characterization of Plastics in Failure Analysis)

### 2.4.3 Gel Permeation Chromatography (GPC)

GPC is a technique used in determining the molecular weight distribution of polymers. It is sometimes called the size exclusion chromatography and uses packed column to separate various constituents of the polymer [36]. Detectors are used in identifying the changes in molecular weight and the results obtained correspond to the actual molecular weight and molecular weight distribution of the polymer. The polymer produces molecular weight distribution in a histogram and the average molecular weight can be obtained. GPC uses small sample size of about 30 to 120 $\mu$g for complete analysis of plastics [40]. However, this technique requires sophisticated instrumentation and usually difficult to interpret results.

## 2.5 Degradation Models

### 2.5.1 Kinetic Models

Thermal degradation of polymers is the initial step in developing a model used in plastic pyrolysis in complete scale systems [41]. This polymer is described in relation to its intrinsic kinetics, implying that, heat and mass transfers are not limited. Lumped-parameter kinetic models do not take into consideration the rigorous and exhaustive description of the chemistry of thermal degradation of polymers. Thermal degradation of poly-olefins is usually described by single step degradation [42, 43] in the Arrhenius equations rate of nth order. Another approach [44] fitted their experimental data consisting of multiple independent parallel reaction kinetics. This model made an assumption that, volatilization products comes from a large number of independent parallel reactions of first-order.

The activation energy probability density function for polyethylene (PE) pyrolysis when calculated yielded very small best-fit values [41]. This indicates there is very little degradation pathways to apparent kinetics and also similar activation energy would be produced if many parallel reactions are involved. Activation energies and pre-exponential factors from literature range from 45 to 73 kcal/mol and $10^{11}$ and $10^{19}$ $S^{-1}$ respectively [45, 46].

The description of the kinetics of thermal degradation reactions involves numerous equations but taking into consideration the role they play in their mechanisms [47]. The following equation describes the reaction rate based on the degree of conversion.

$\alpha = \frac{m_o - m_i}{m_o - m_f}$, where $m_0$, $m_f$ and $m_i$ denote the initial, final and current sample mass at the moment *t*, respectively.

The kinetic equation of process in general can be written as follows;

$\frac{d\alpha}{dt}$= K (t) $f(\alpha)$, where $f(\alpha)$ and *k* (*T*) are the conversion and temperature functions respectively

The Arrhenius equation below describes the rate constant temperature dependence.

K = $A \exp\left(-\frac{E}{RT}\right)$, where *A* is the pre-exponential factor, *T* is the absolute temperature, *R* is the universal gas constant, and *E* is the apparent activation energy of the process [47].

Upon substitution, the following equation is obtained.

$\frac{d\alpha}{dt}$=$A \exp\left(-\frac{E}{RT}\right)) f(\alpha)$.

For a solid-state reaction, $f(\alpha)$depends on the mechanism reaction which is obtained below.

$f(\alpha) = \alpha^m(1-\alpha)^n[-\ln(1-\alpha)]^p$, where *m*, *n* and *p* are empirically obtained exponent factors, one of them always being zero [48, 49].

### 2.5.2 Stochastic Models

Most of the studies in polymer science currently apply deterministic models based on exact chemical reaction or molecular weight distribution equations [50]. Some studies [51] applied Markov chain model in the stochastic analysis of stepwise enzymatic cellulose degradation. The model predicted the change in the number or concentration distribution of cellulose chains based

on their lengths as gotten from hydrolysis at different times. Some Markov chain models [52, 53, 54] have been established to investigate mixing and attrition or breakage of particles.

With respect to modeling principle, the degradation of molecules is similar to the breakage of particles. The splitting of molecules and particle breakage is a stochastic model since their precise structures and molecular weight distribution is not known in detailed [50].

The molecular weight (MW) of the polymer is considered to be between the maximal MW in the original polymer and the lowest in the degradation product. This MW is then arbitrary divided into smaller intervals from the highest to the lowest one [55]. Modeling can start at any stage of degradation since the Markov chain is a homogenous one. The law of large numbers, ${}_{n}p_i$ gives the expectable proportion of molecules that will split in the interval $M_i$ during $\Delta t$ time step.

The development of a stochastic model is very complex and there is the need to choose limited MW intervals. For example, 5-10 MW intervals may be enough to use in the model [55].

${}_{n}p_i$ represents the split probability of molecules in the interval $M_i$ during $\Delta t$ time step. The reaction mixture consist of large molecules and from the law of large numbers, the spilt probability represents the expected molecules splitting in $M_i$ interval in $\Delta t$ time step.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Introduction

The potential use of S*erratia. marcescens marcescens* and/or its enzymes will be explored in this chapter, in which the experimental procedures will be presented. The underlying of causes of the biodegradation will be elucidated via experiments that will determine whether the bacteria or the supernatant extracts from the bacteria are responsible for the biodegradation of PE.

The null hypothesis ($H_o$) of this research states that: "mean of the supernatant extracts from *Serratia. marcescens marcescens* are responsible for the biodegradation of PE". The alternative hypothesis ($H_a$) states that: "mean of the supernatants extracts from *Serratia marcescens marcescens* are not responsible for biodegradation of PE". A student t- distribution will then be employed to test the null hypothesis at 95% confidence interval and to either reject or fail to reject the above mentioned claim.

This chapter presents the procedures that were followed in the bacteria and supernatant experiments. The degradation mechanisms were studied by characterizing the PE after different amounts of exposure to S*erratia. marcescens marcescens* and its supernatants.

### 3.2 Experimental Procedures

#### 3.2.1 Powdering of PE

Pure water bags made of PE were obtained and washed manually with absolute ethanol using cotton wool and rinsed severally with distilled water to remove dirt and other organic particles adhering to the surface and then allow to air dry for 30 minutes. A solvent was kept in a

container and heated to a temperature of 140$^{o}$C in a fume cubic hood. The PE bags were then dropped one after the other into the container containing the solvent and continuously stirring for dissolution until complete saturation. Filtration was performed using a white cloth to obtain powdered PE and then rinsed severally with acetone to remove solvent. The acetone was allowed to evaporate and the PE was air dried and obtained in powder form.

### 3.2.2 Bacteria Experiments

#### 3.2.2.1 Culturing of *Serratia marcescens marcescens*

Figure 3.0: *Serratia marcescens marcescens* cultured in peptone glycerol agar for 72 hours

Soil sample was obtained from Sheda community in the Federal Capital Territory- Abuja, Nigeria. 100 g of the soil sample was diluted with 100 ml of water and stirred for uniformity. 50 ml of the sample was exposed to 5 g of powdered PE for four months to determine which

microorganism survives using the culture enrichment technique. The surviving microorganism, when cultured in peptone glycerol agar, produces a red pigment called prodigiosin which gives an indication of *Serratia. marcescens marcescens* as indicated in fig 3.0 above. This bacterium was then cultured in 100 ml of nutrient broth for 48 hrs.

### 3.2.2.2 Carbonless Media Preparation and Inoculation

The carbonless media was prepared in 1000 ml conical flask with various compositions of nutrients. Distilled water was kept in a beaker and stirred to produce a uniform mixture. A 50 ml of macro stock was measured into the distilled water and stirred for uniformity. A 5 ml of micro stock was also measured into the media and mixed into the solution. Also, 5 ml of both vitamin and iron stock were also measured and kept separately in the media until complete mixing. The mixture was continuously stirred until a carbonless media was obtained. Seven empty conical flasks, in addition to the carbonless media, all covered with cotton wool and Aluminum foil, were autoclaved to remove any contaminants and to produce a sterile environment. After autoclaving, the samples were moved to a lamina flow hood. 2 g of powdered PE were then measured in six different beakers and placed in a lamina flow hood.

Subsequently, 90 ml of carbonless media was measured into each flask and the 2 g of powdered PE was poured into each flask. 5 ml of the nutrient broth that contains S*erratia marcescens marcescens* were measured into six flasks and corked tightly with cotton wool and Aluminum foil. The other flask served as the control. After inoculating the bacteria with the PE, the samples were incubated in a shaker at $30^{o}$C, with 141 revolutions per minute (RPM), for ten weeks to study the degradation of PE.

### 3.2.2.3 Mass Loss Experiment

Figure 3.1: Experimental Setup for Mass loss

Pure water bags made of PE were washed manually with absolute ethanol using cotton wool and rinsed severally with distilled water to remove dirt and other organic particles adhering to the surface and allow to air dry for 30 minutes. The PE was cut into spherical parts with the aid of a perforator for uniformity of surface area. Two empty conical flaks were autoclaved to produce a sterile environment and moved to the lamina flow hood. 1 g of spherical PE were each measured into two different beakers and placed in the lamina flow hood.

Consequently, 90 ml of carbonless media were measured into each flask and the 1 g of spherical PE was then poured into both flasks. 5 ml of nutrient broth that contains the bacteria was

measured into one flask and corked tightly. The other flask serves as the control and was corked tightly to prevent air from entering in. Two hypodermic syringes were each pierced into the cork of the two flasks in a way not to touch the media as illustrated in fig 3.1. The samples were incubated in a shaker at 30$^{o}$C with 141 RPM for four weeks to determine the mass loss and diffusivity of species in the media.

### 3.2.3 Supernatant Experiment

This experiment was carried out to determine if the supernatant of *Serratia marcescens marcescens* are responsible for the biodegradation of PE. Five empty conical flasks were autoclaved and moved to the lamina flow hood for inoculation.

The biodegraded PE that were exposed to the bacteria for a period of four weeks was poured into a 0.22 $\mu m$ filter to separate the bacteria from its supernatants as shown in fig 3.2. A vacuum pump of about 34474-62053 Pa (5-9 Pounds/square inch, psig) was applied to filter the supernatants, leaving the PE and the bacteria on top.

40 ml of the supernatant were each measured into four conical flasks and the remaining flask serves as the control with 40 ml of carbonless media. 2 g of PE was then poured into each flask and corked tightly with cotton wool and Aluminum foil and incubated in a shaker at 30$^{o}$C with 141 RPM for four weeks.

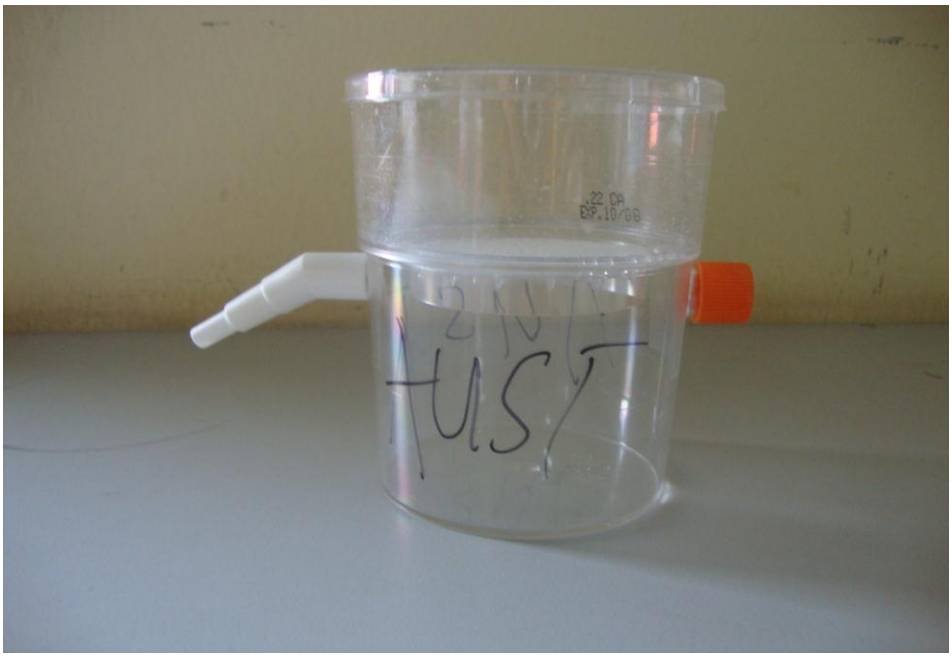


Figure 3.2: A sample of 0.22 μm filter

### 3.2.4 Material Characterization

#### 3.2.4.1 Scanning Electron Microscopy (SEM)/ Energy Dispersive Spectroscopy (EDX)

The SEM machine was vented and changed to the variable pressure (VP) aperture. A conducting double sided carbon tape was placed on a multi-stub sample holder and the various samples of the PE put on it as illustrated in fig 3.3. The sample holder was then mounted into the specimen chamber for analysis. A vacuum was created and the electron beam switched on. The machine settings were adjusted to enable proper alignment of the samples on the beam and the variable pressure aperture and accelerating voltage set to 50 Pa and 20 kV respectively. The samples were analyzed and SEM images taken. The machine was changed to the EDX mode using INCA software analyzer to determine the number of elements present in the samples.

Figure 3.3: Multi-stub sample holder of SEM

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Bacteria Results

After incubating the samples in the conical flasks for ten weeks, the flasks were removed from the incubator shaker and tested for the presence of the bacteria or any possible contamination. The result showed the presence of S*erratia marcescens marcescens* in the medium devoid of contamination.

It was found that the initial weight of 2 g of PE in the medium containing *Serratia marcescens marcescens* decreased with number of days. This is shown in Table 4.0. This is attributable to the bacteria using PE as its energy and carbon source [12, 13 and 14]. The weight of the control (which was not exposed to bacteria) remained unchanged as expected.

Also, it was observed that, the degradation products increased as the bacteria were exposed to longer time periods as illustrated in table 4.1**.** Furthermore, the average percent of biodegradation rate showed a level of significance of 36.0 for 70 days incubation indicating that the bacteria uses PE as source of nutrients, as shown in table 4.2 and figure 4.2**.**

Table 4.0: The mass of PE after weeks of incubation with *Serratia marcescens marcescens*

| Mass (g) | Time (days) |
|---|---|
| 2.00± 0.27 | 0 |
| 1.93± 0.20 | 14 |
| 1.84±0.11 | 28 |
| 1.76±0.03 | 42 |
| 1.55±0.18 | 56 |
| 1.28±0.45 | 70 |

Table 4.1: The biodegradation Products of *Serratia marcescens marcescens* after eight weeks of incubation

| Biodegradation products( in grams) | Time (in days) |
|---|---|
| 0.00±0.27 | 0 |
| 0.07±0.20 | 14 |
| 0.16±0.11 | 28 |
| 0.24±0.03 | 42 |
| 0.45±0.18 | 56 |
| 0.72±0.45 | 70 |

Table 4.2: The Percentage of biodegradation after PE exposure to *Serratia marcescens marcescens*

| Percent of Biodegradation | Time (days) |
|---|---|
| 0.00±13.67 | 0 |
| 3.50±10.17 | 14 |
| 8.00±5.67 | 28 |
| 12.00±1.67 | 42 |
| 22.50±8.67 | 56 |
| 36.00±22.33 | 70 |

From the graph of biodegradation products with respect to time as illustrated in figure 4.1, it was noticed that for the first two weeks of incubation, the biodegradation was rate was slow. However, average rate of biodegradation increases tremendously after the second week. This may be attributable to a lag phase of growth where the micro-organism mobilizes enzymes to effect the biodegradation. The increases seen in the second week may be explained as an exponential phase of growth after the microbe has mobilized enzymatic breakdown of plastics [14].

From figure 4.0, it was observed that the average mass of biodegradation decreases as a function of time.

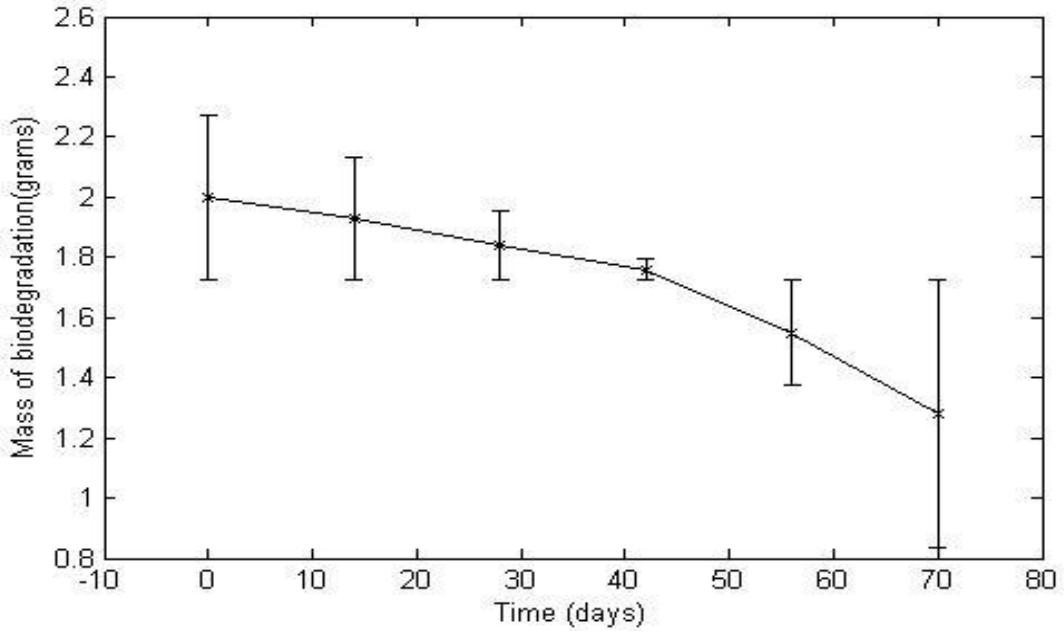


Figure 4.0: Trend showing mass of biodegradation of PE after Ten weeks of incubation with *Serratia marcescens marcescens*

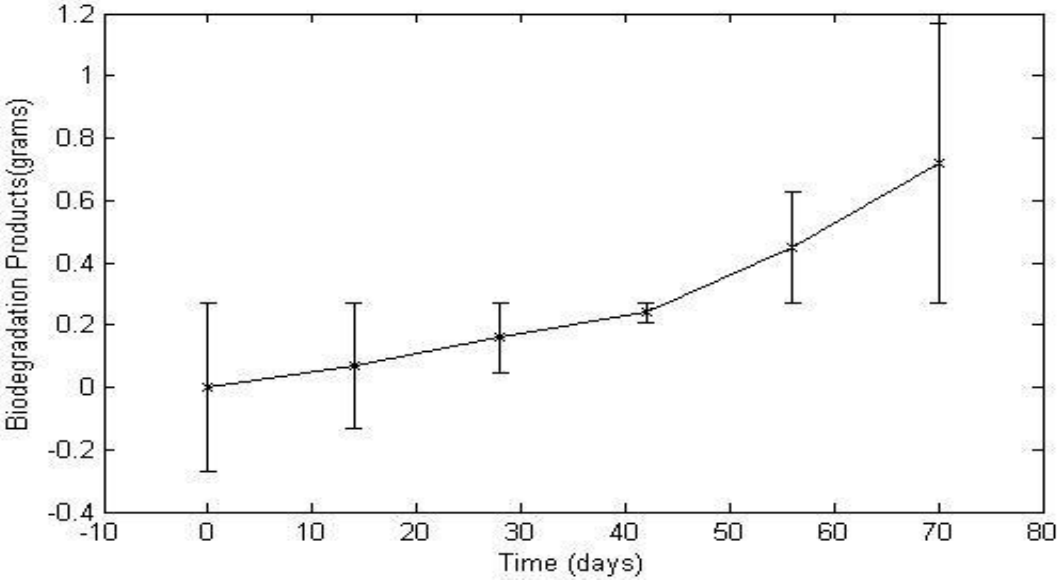


Figure 4.1: Trend showing biodegradation products of PE after Ten weeks of incubation with *Serratia marcescens marcescens*

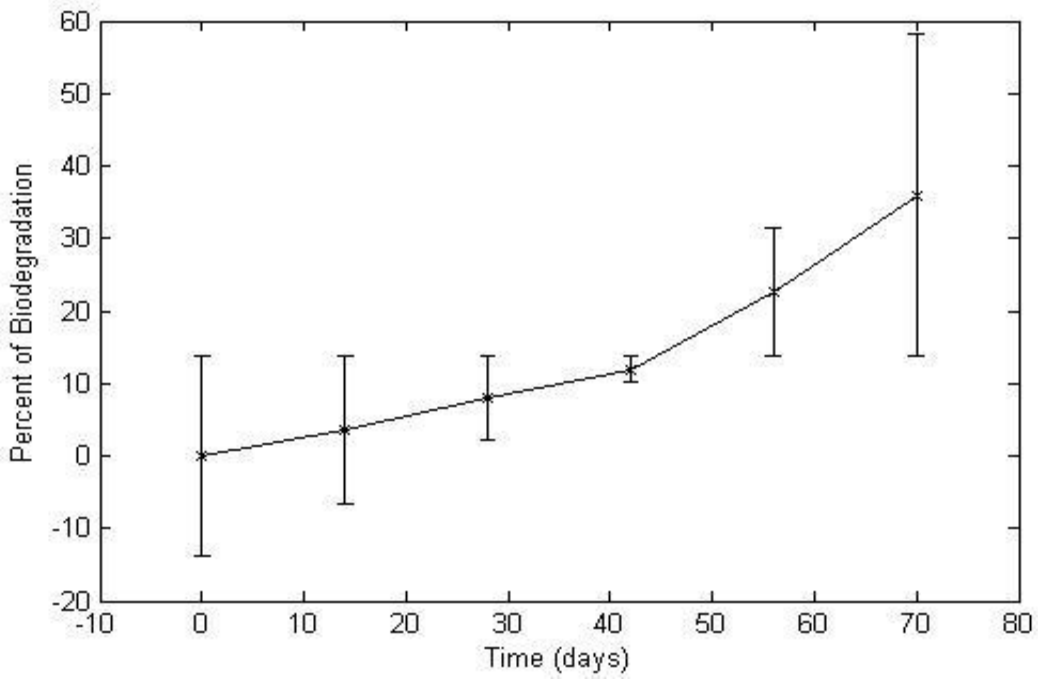

Figure 4.2: Trend showing percent of biodegradation of PE after eight weeks of incubation with *Serratia marcescens marcescens*

**4.2 Supernatant Cultures**

A similar method was employed to ensure that the supernatant extract from *Serratia marcescens marcescens* was not contaminated after four weeks of incubation as applied in the bacteria experiment. The result when tested shows the absence of *Serratia marcescens marcescens* and only its supernatant was present.

From table 4.3, it was realized that the initial mass of 2 g of PE decreased tremendously to 1.25 g after four weeks of incubation with supernatant extract from the bacteria. This explains that, the supernatant effectively breaks down long PE molecules and aids in faster degradation than the bacteria itself. Also, with respect to figure 4.3, it was observed that the mass of PE decreases as a function of time.

Table 1.3: The mass of PE after four weeks of incubation with supernatant extract

| Mass (g) | Time (days) |
|---|---|
| 2.00±0.34 | 0 |
| 1.87±0.21 | 7 |
| 1.69±0.03 | 14 |
| 1.47±0.19 | 21 |
| 1.25±0.41 | 28 |

The average biodegradation products were drastically increased from 0 to 0.75 g in 28 days which constitute about 37.50 % of biodegradation as illustrated in table 4.4 and table 4.5 respectively. This explains the ability of the supernatant to degrade PE molecules effectively. However, this is a necessary but not sufficient condition to conclude that, the supernatant extracts from *Serratia marcescens marcescens* are responsible for PE biodegradation.

Table 4.4: The Biodegradation Products of supernatant extract after four weeks of incubation

| Biodegradation Products (grams) | Time (days) |
|---|---|
| 0.00±0.34 | 0 |
| 0.13±0.21 | 7 |
| 0.31±0.03 | 14 |
| 0.53±0.19 | 21 |
| 0.75±0.4060 | 28 |

Table 4.5: The percent of biodegradation after PE exposure to supernatant extract

| Percent of Biodegradation | Time (days) |
|---|---|
| 0.00±17.0 | 0 |
| 6.50±10.5 | 7 |
| 15.50±1.5 | 14 |
| 26.50±9.5 | 21 |
| 37.50±19.5 | 28 |

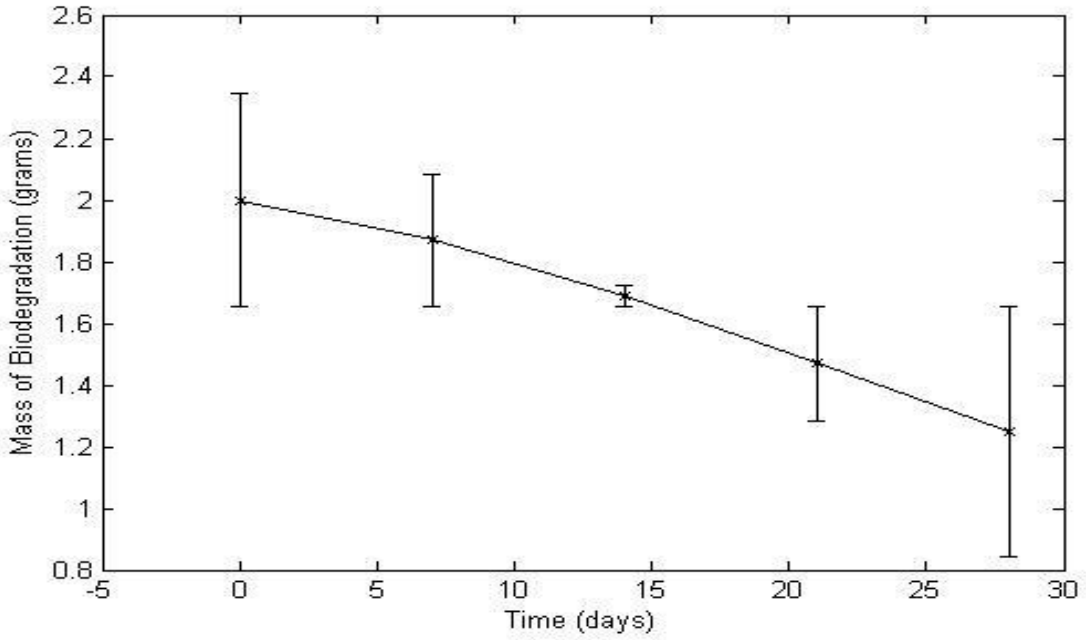


Figure 4.3: Trend showing the mass of biodegradation of PE after four weeks of exposure by supernatant extract

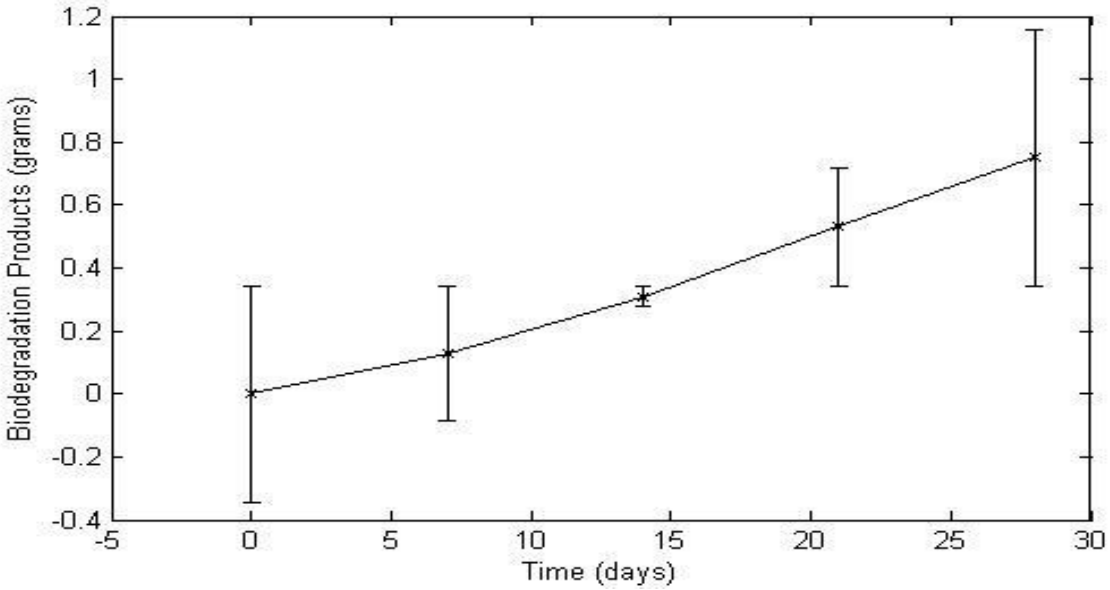


Figure 4.4: Trend showing the biodegradation products of PE after four weeks of exposure by supernatant extract

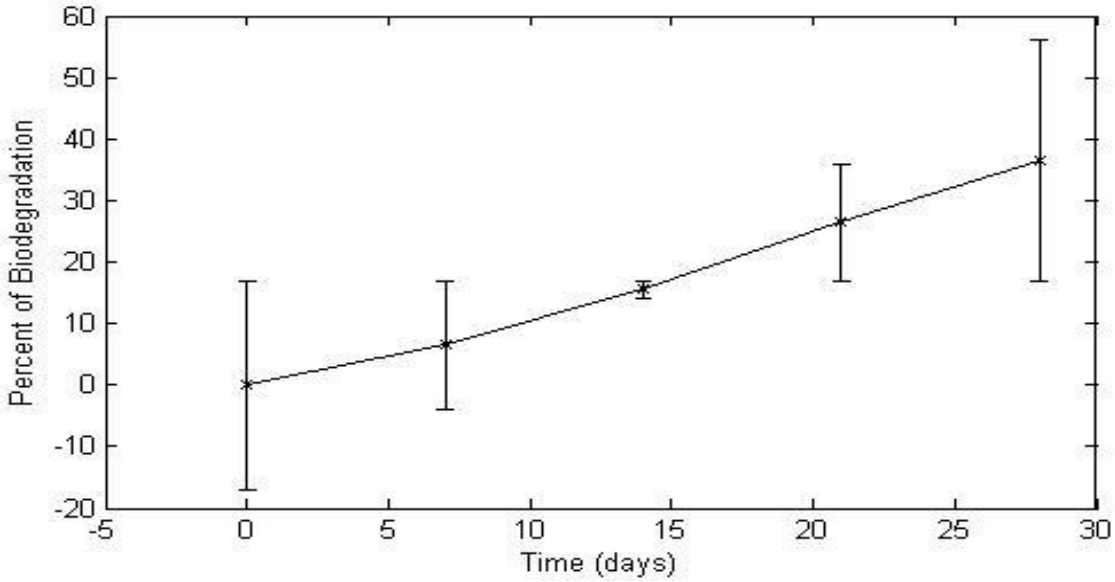

Figure 3: Trend in the percent of biodegradation of supernatant extract after four weeks of incubation

With reference to figures 4.4 and 4.5, it was noticed that the average biodegradation products increases tremendously for 28 days of incubation giving an average percent of biodegradation of 37.5. This behavior explains that the supernatant extract can biodegrade most of the PE molecules within three months.

However, it was realized that the average mass of biodegradation of PE exposed to supernatant decreased tremendously than those exposed to the bacteria as illustrated in figures 4.3 and 4.0 respectively. Also, the average biodegradation products of the supernatants increases at a faster rate than those obtained from the bacteria as shown in figures 4.4 and 4.1 respectively.

### 4.3 Modeling of Polyethylene biodegradation using Student T-Distribution

Based on the results obtained in the supernatant experiment discussed above, there is the need to actually test whether if the supernatant extract from *Serratia marcescens marcescens* are responsible for the biodegradation of PE or is it the bacteria. The null hypothesis ($H_o$) of this research states that, "mean of the supernatant extract from *Serratia marcescens marcescens* are responsible for the biodegradation of PE". The alternative hypothesis ($H_a$) states that: "mean of the supernatant extract from *Serratia marcescens marcescens* are not responsible for biodegradation of PE". These hypotheses will be tested at a significance level of 0.05.

Using Minitab software with one sample T and 95% confidence interval, we obtain the following results as illustrated in table 4.6.

Table 4.6: One-Sample T: Mass (g), Time (days)

Test of mu = 2 vs not = 2

| Variable | N | Mean | StDev | SE Mean | 95% CI | T | P |
|---|---|---|---|---|---|---|---|
| Mass (g) | 5 | 1.656 | 0.302 | 0.135 | (1.281, 2.031) | -2.55 | 0.063 |

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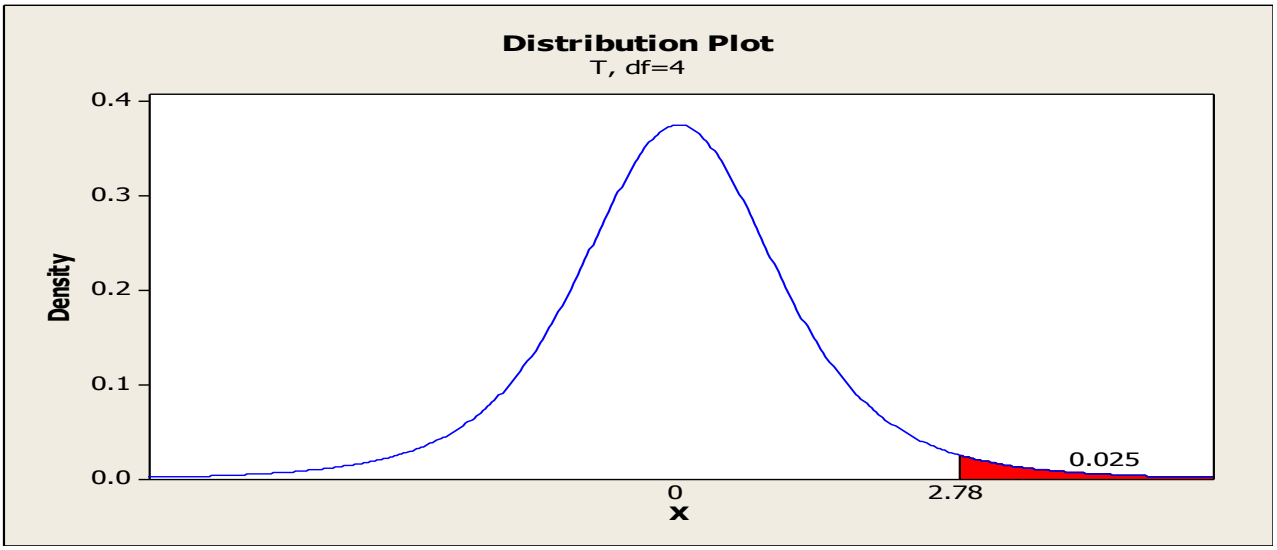


Figure 4.6: One tail probability distribution plot at $\alpha/2 = 0.025$

From the one sample T as illustrated in table 4.6, it was noticed that the p-value of 0.067 for mass is greater than the significance level of 0.05 and hence we fail to reject the null hypothesis. Also, the T-value of -2.55 is less than the critical value of 2.78 as shown in the distribution plot in figure 4.6, hence we fail to reject the null hypothesis at 95% confidence interval (CI) since it lies within the critical region and conclude that there is no evidence to reject the null hypothesis and the mean of the supernatant extract from *S. marcescens marcescens* are responsible for biodegradation of PE.

## 4.4 Material Characterization

### 4.4.1 Scanning Electron Microscopy (SEM)

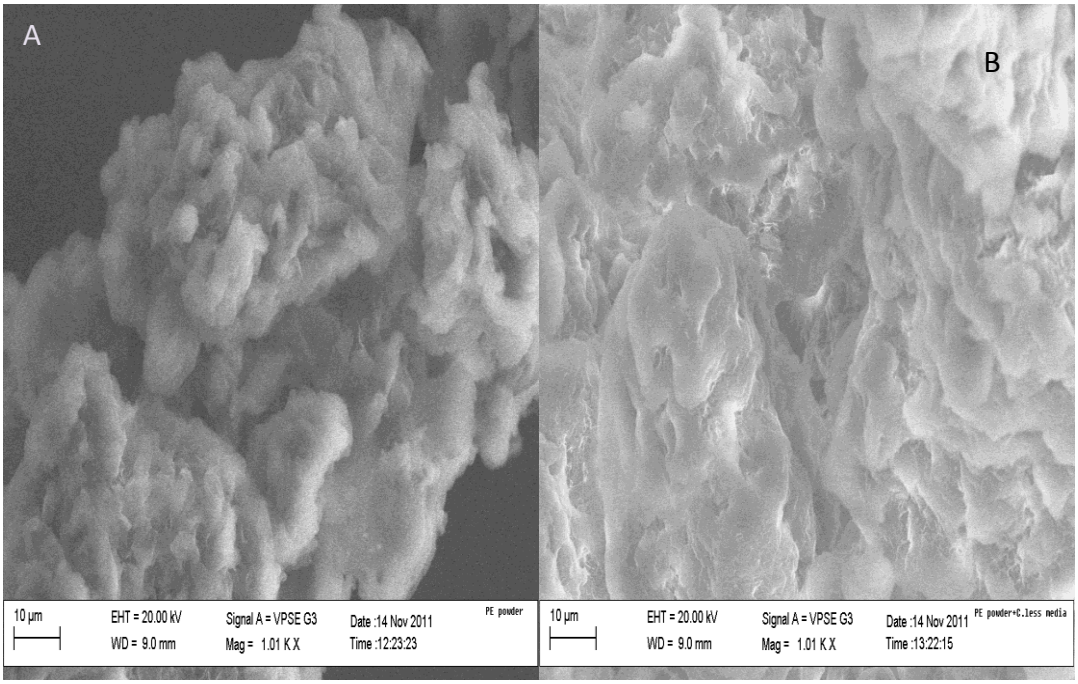


Figure 4.7: SEM micrographs of A) Untreated powdered PE, B) Untreated powdered PE with carbonless media

The PE samples both treated and untreated were viewed with the Scanning Electron Microscope to determine the changes that took place. Micrograph A in figure 4.7 produced an SEM image similar to the one exposed to carbonless media in micrograph B of figure 4.7. This implies that, the media did not have any effect on the PE. However, it was noticed that the PE exposed to *Serratia marcescens marcescens* for one month produces an image with voids and pits. This may mean that the more vulnerable portions of the PE are being eroded by the bacteria as illustrated in figure 4.8 of micrograph C.

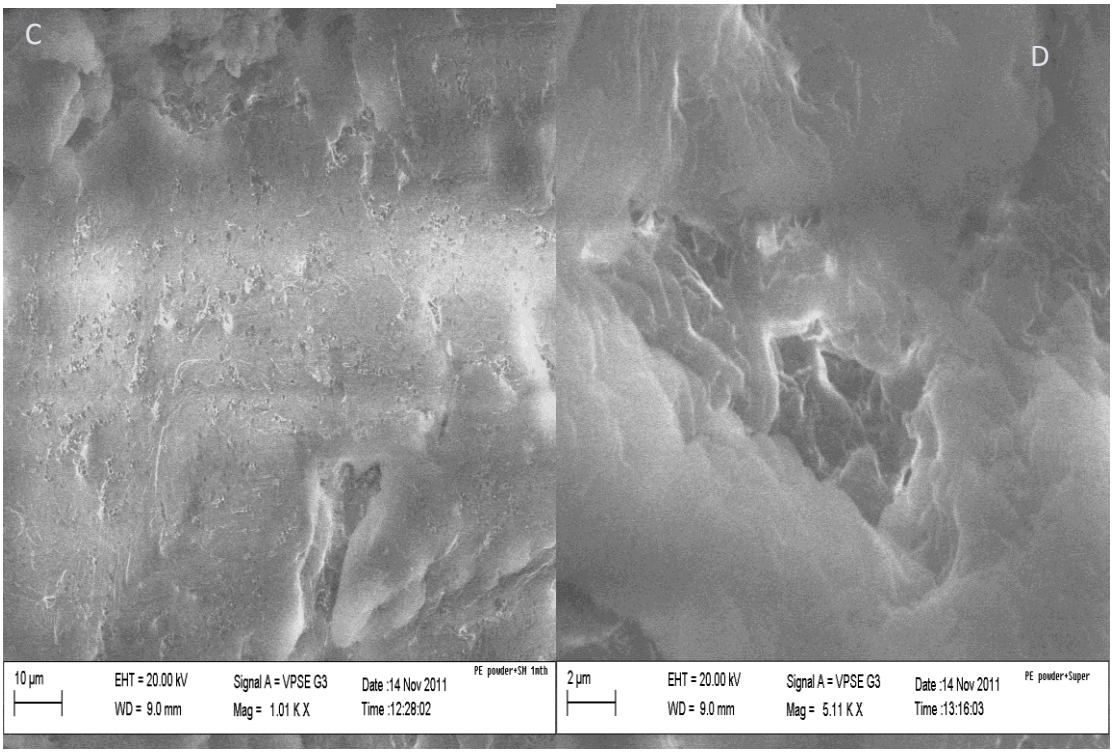


Figure 4.8: SEM micrograph of powdered PE C) exposed to *Serratia marcescens marcescens*, D) exposed to supernatant extract

Furthermore, the image produced by exposing PE to supernatant were found to contain more voids and pits than those with the bacteria as shown in figure 4.8 of micrographs D and C respectively. This suggests the ability of the supernatant to degrade PE faster than the bacteria. The amorphous region of the PE are expected to be loosely packed thus enzymes from the bacteria may easily access these portions of PE compared with the more tightly packed crystalline portions [4, 5, and 6]. Besides, when the bacteria were exposed to PE film for a month and washed, big voids were found as illustrated in figure 4.9 of micrograph E. However, the virgin PE film contains no voids as in figure 4.9 of micrograph F.

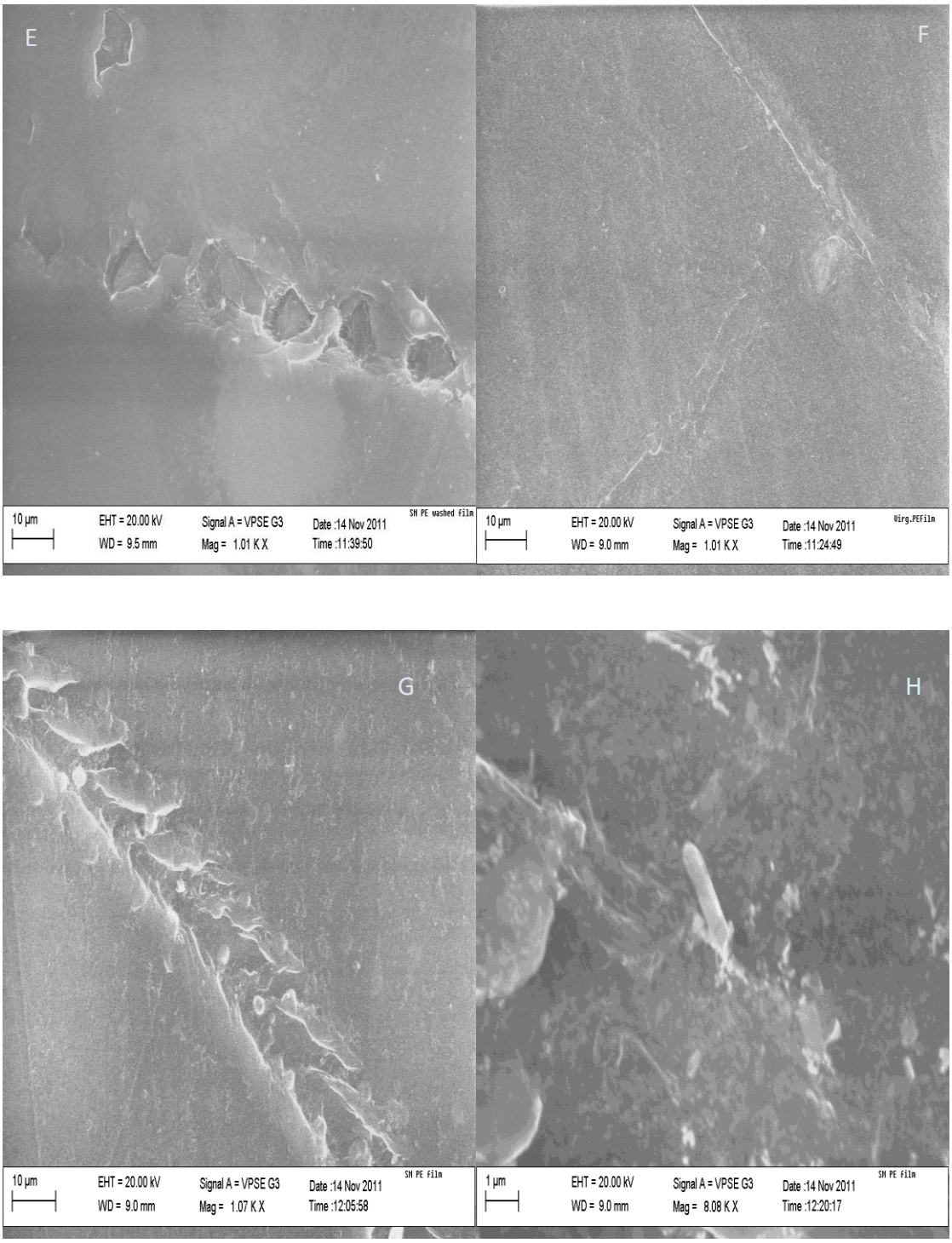


Figure 4.9: SEM micrograph of E) PE film exposed to *Serratia marcescens marcescens* and washed, F) Virgin PE film, G) PE film exposed to *Serratia marcescens marcescens*

Also, when the bacteria were exposed to PE film for month without washing, large deformations or discontinuities are observed at low magnification as illustrated in figure 4.9 of micrograph G. When view at high magnification, rod-like shapes started appearing, suggesting the presence of *Serratia marcescens marcescens* bacteria on the sample as illustrated in figure 4.9 of micrograph H.

### 4.4.2. Energy Dispersive Spectroscopy (EDX) Results

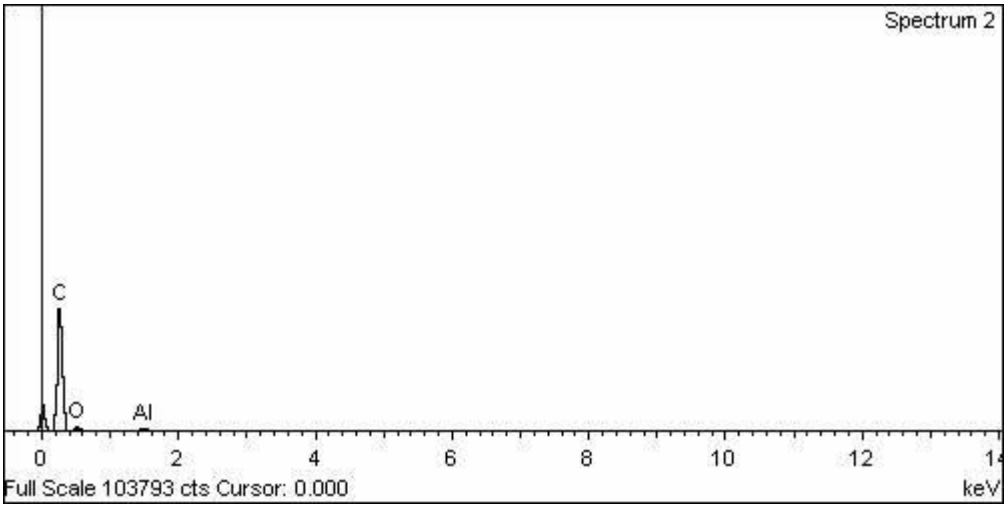


Figure 4.10: Composition of elements in PE without Carbonless media

Table 4.7: Percentage of elements in PE without Carbonless media

| Element | Weight% | Atomic% |
|---|---|---|
| C K | 84.86 | 88.33 |
| O K | 14.61 | 11.42 |
| Al K | 0.53 | 0.25 |
| Totals | 100.00 | |

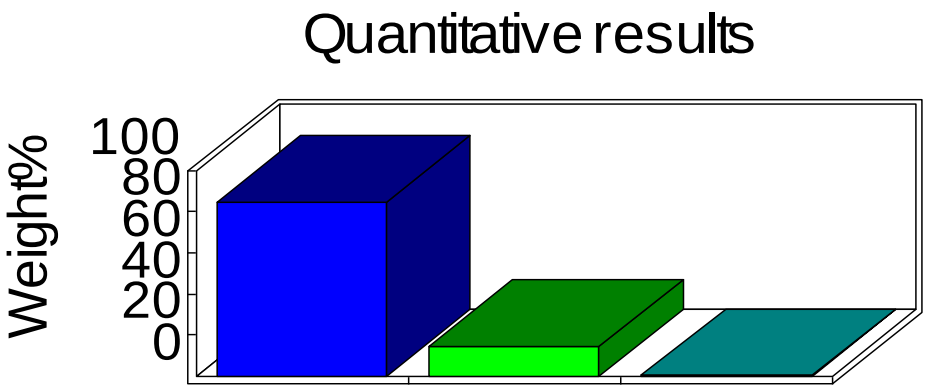


Figure 4.11: A graph showing weight percent of elements in PE without Carbonless media

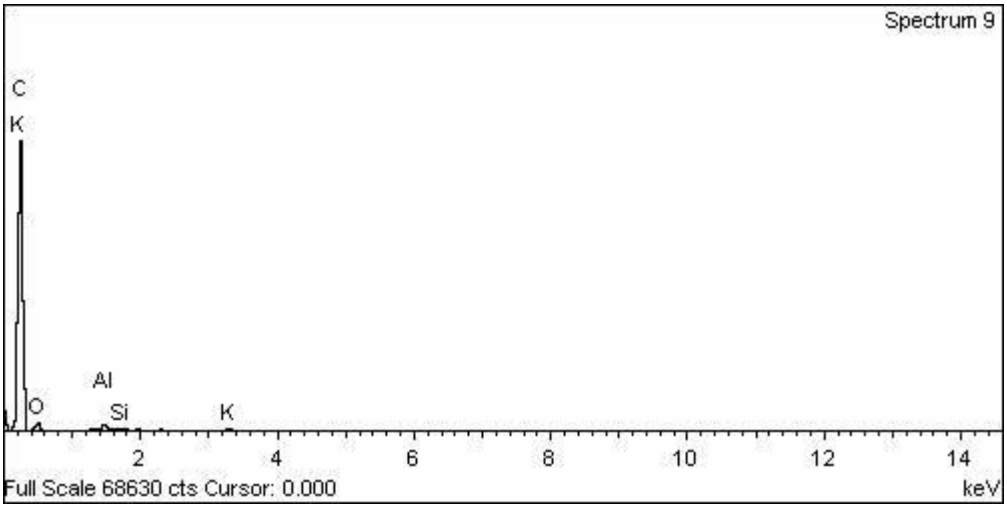


Figure 4.12: Composition of elements in PE with Carbonless media

**Table 4.8**: Percentage of elements in PE with Carbonless media

| Element | Weight% | Atomic% |
|---|---|---|
| C K | 85.65 | 89.20 |
| O K | 13.24 | 10.35 |
| Al K | 0.57 | 0.27 |
| Si K | 0.18 | 0.08 |
| K K | 0.21 | 0.07 |
| Fe K | 0.15 | 0.03 |
| Totals | 100.00 | |

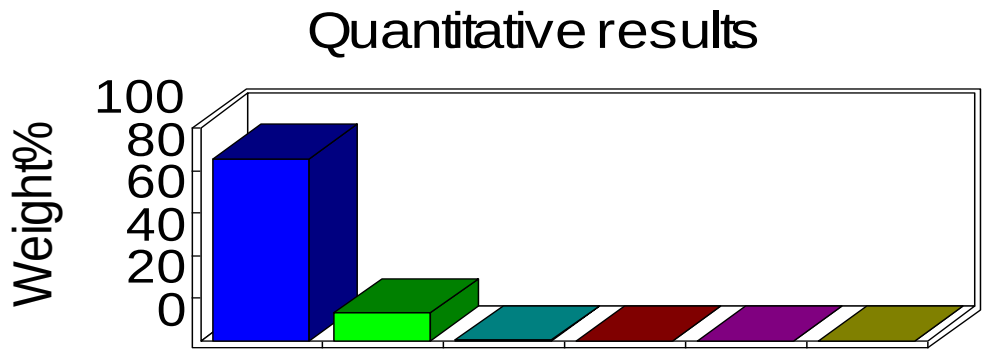


Figure 4.13: A graph showing weight of elements in PE with Carbonless media

From Table 4.7 and Figure 4.11, it was noticed that the elements present were Carbon (C), Oxygen (O) and Aluminum (Al) whilst in Table 4.8 and Figure 4.13 elements such as Silicon Potassium and Iron were included to the existing elements in Table 4.7 and Figure 4.11. This tells us that the inclusion of the media added some elements in the PE. It was also noticed that, the weight percent of C increases slightly whilst there is a minute decrease in O upon the addition of media as compared to the virgin powdered PE as illustrated in figures 4.10 and 4.12 and tables 4.7 and 4.8 respectively.

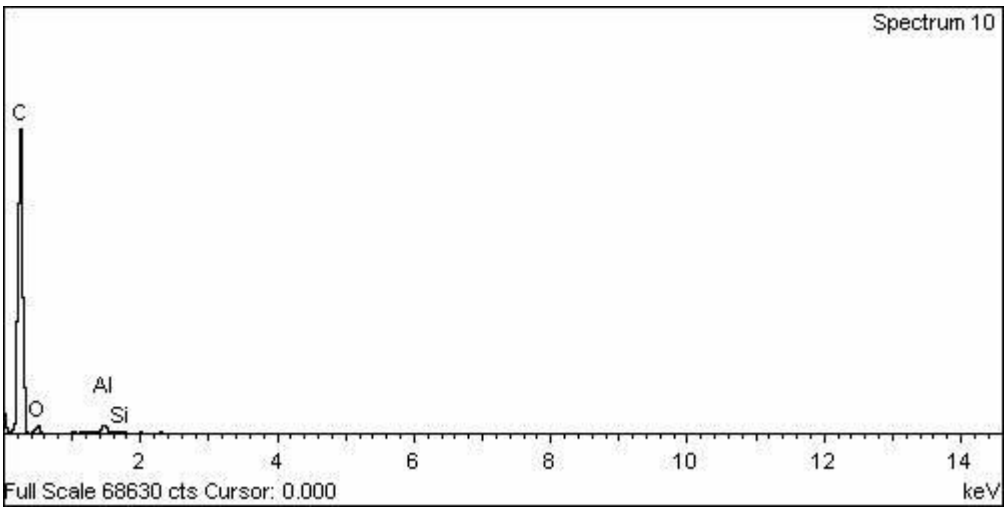


Figure 4.14: Composition of elements PE with *Serratia marcescens marcescens*

Table 4.9: Percentage of elements in Polyethylene in the presence of *Serratia marcescens marcescens*

| Element | Weight% | Atomic% |
|---|---|---|
| C K | 88.41 | 91.40 |
| O K | 10.44 | 8.10 |
| Al K | 0.88 | 0.41 |
| Si K | 0.08 | 0.04 |
| S K | 0.07 | 0.03 |
| Fe K | 0.12 | 0.03 |
| Totals | 100.00 | |

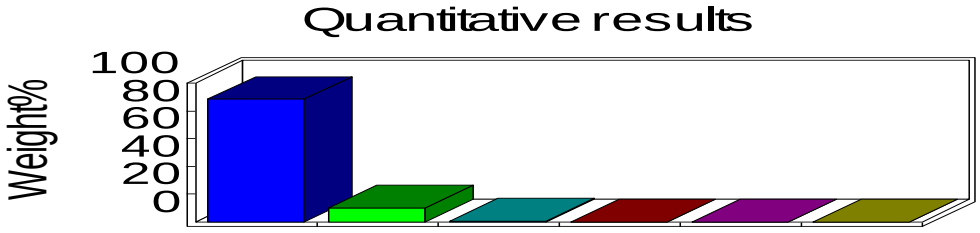


Figure 4.15: Figure 4.15: A graph showing weight of elements in PE with *Serratia marcescens marcescens*

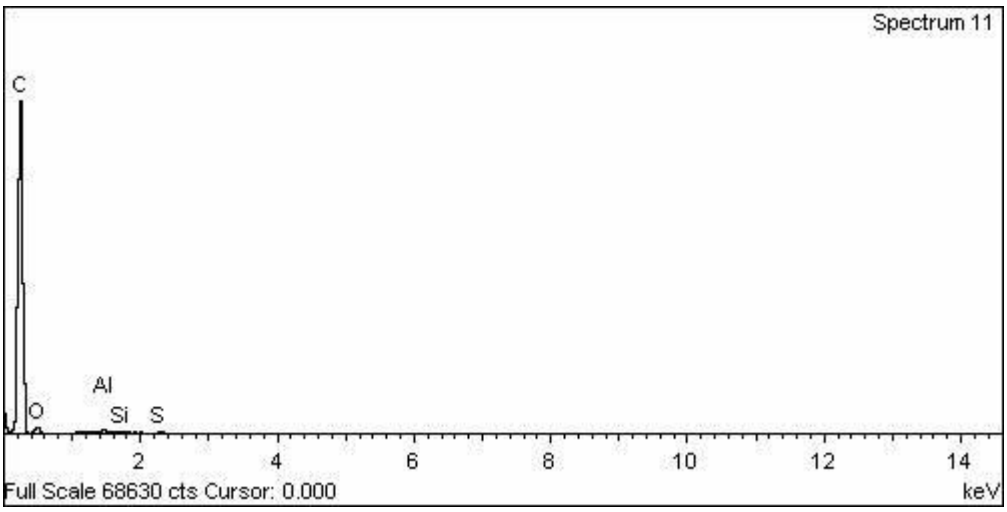


Figure 4.16: Composition of elements in PE with supernatant extract

Table 4.10: Percentage of elements in PE with supernatant extract

| Element | Weight% | Atomic% |
|---|---|---|
| C K | 90.66 | 92.94 |
| O K | 8.95 | 6.89 |
| Al K | 0.22 | 0.10 |
| Si K | 0.10 | 0.04 |
| S K | 0.07 | 0.02 |
| Totals | 100.00 | |

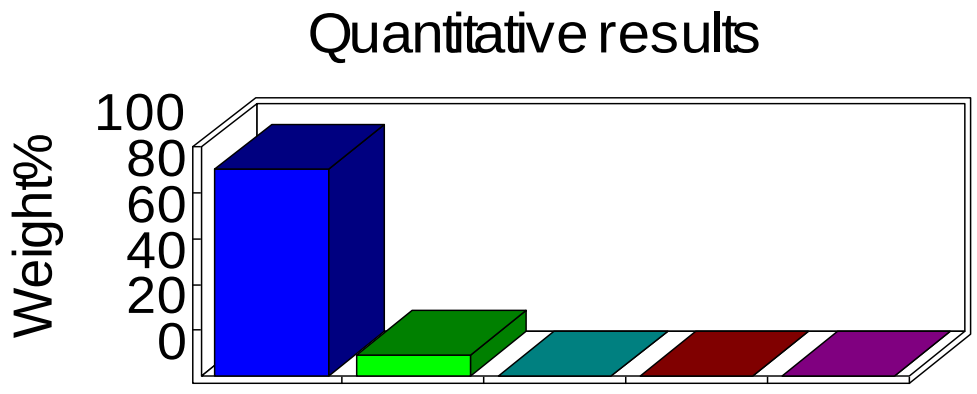


Figure 4.17: A graph showing weight percent of elements in PE with supernatant extract

From Table 4.9 and Figure 4.14, it was realized that the carbon content increases while the Oxygen content decreases upon exposure to *Serratia marcescens marcescens* as compared to Tables 4.7 and 4.8 and Figures 4.10 and 4.12. Furthermore, from Table 4.10 and Figure 4.17 it can be seen that there were inconsistencies in the content of elements as compared with Figure 4.15.

In summary, EDX analysis is a semi-quantitative approach and may not necessary give the true representation of the elemental content of the samples under consideration. However, it can give a scenario of what is happening in the sample.

### 4.5 Implication of Results

The implications of the current research work are quite enormous. First and foremost, the use of the culture enrichment technique is a useful tool in optimizing the microbial population of microbes capable of biodegradation in the presence of a recalcitrant substrate. These microorganisms will then be used in bioremediation of plastic waste on the environment. This could be a breakthrough in environmental waste management since the technique has the potential of producing other microorganism that could be used in degrading plastics.

Also, *Serratia marcescens marcescens* a gram negative bacterium was able to biodegrade polyethylene (PE). The supernatant of this bacterium also exhibited biodegradation potentials.

# CHAPTER FIVE

## 5. 0 CONCLUSIONS AND FUTURE WORK

### 5.1 Conclusion

Based on the deductions drawn from the experimental results of this thesis, the following conclusions could be made;

- A method for powdering of plastics was demonstrated using a proprietary solvent. This approach will increase the surface area of the plastics exposed to the environment.
- The culture enrichment technique should be encouraged as a method of choice in increasing the microbial population of cells in the presence of recalcitrant substrates. This technique could be a breakthrough since it may be used to identify micro-organisms with unique biodegradative tendencies.
- *Serratia marcescens marcescens* a gram negative bacterium demonstrated capacity to use polyethylene plastic as its sole carbon source. The supernatant of the bacteria was also demonstrated as capable of degrading plastics indicating that it is an enzyme mediated process in the extracellular matrix.
- The Scanning Electron Microscopy revealed the degradation of polyethylene due to presence of voids and pits.

### 5.2 Recommendations for Future Work

Further research should be conducted to determine the twin effect of pH and Temperature on the biodegradation rate, thus providing insights on the most critical parameter controlling the biodegradation of plastics by this bacterium. A kinetic model that uses Arrhenius equation

considerations may be appropriate to fully decipher the mechanistic aspects of the biodegradation of PE by bacteria. The data generated could be fitted into various theoretical models depicting a rate dependent biodegradation process.

In addition, more research should be carried out to determine the diffusion and reaction mechanisms between the bacteria and the polyethylene.

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## APPENDIX A (CHAPTER THREE)

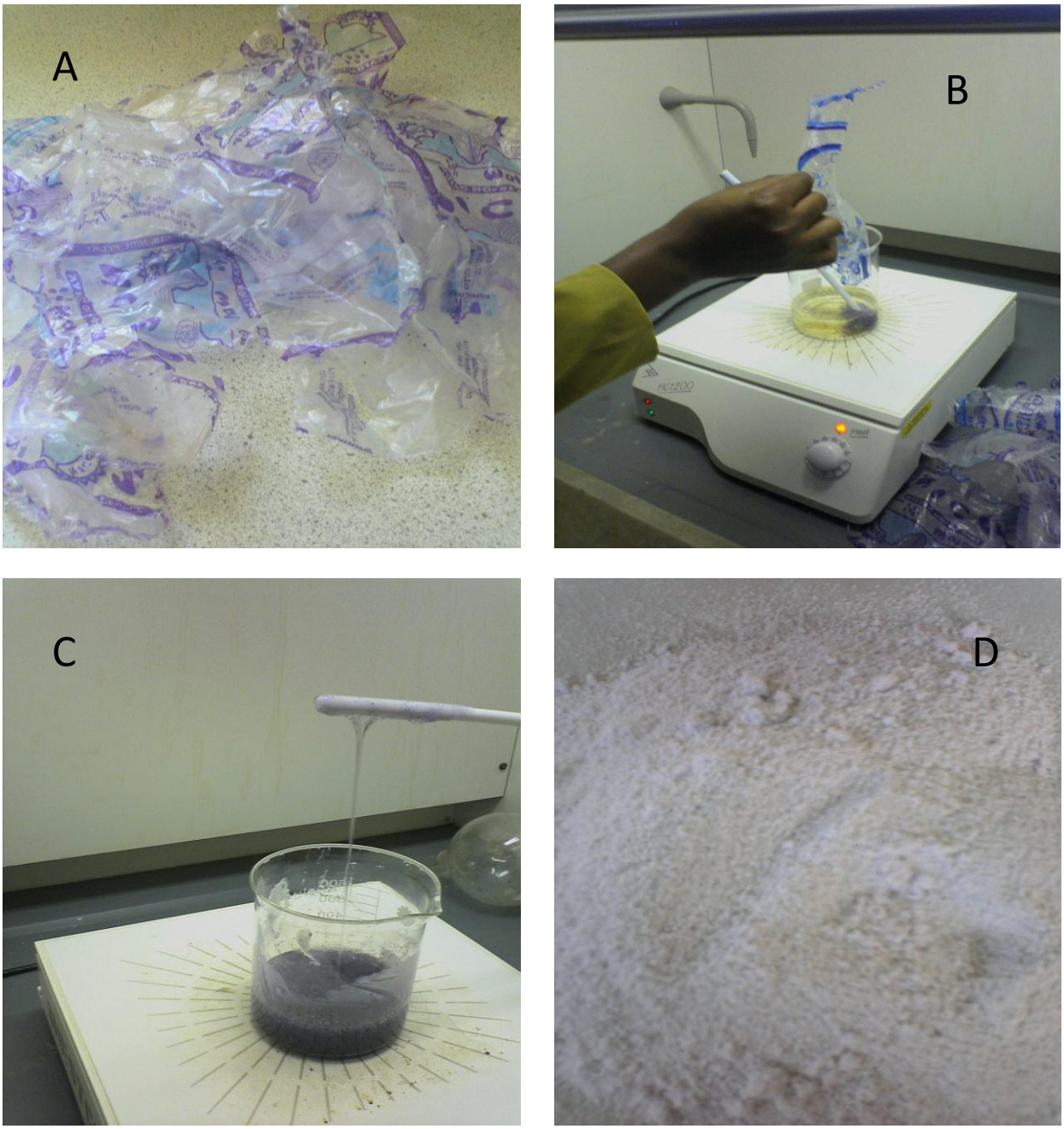


Figure A.1: Stages involved in powdering of polyethylene (PE)


Figure A.2: Process of inoculating *Serratia marcescens marcescens* and its supernatant with powdered polyethylene

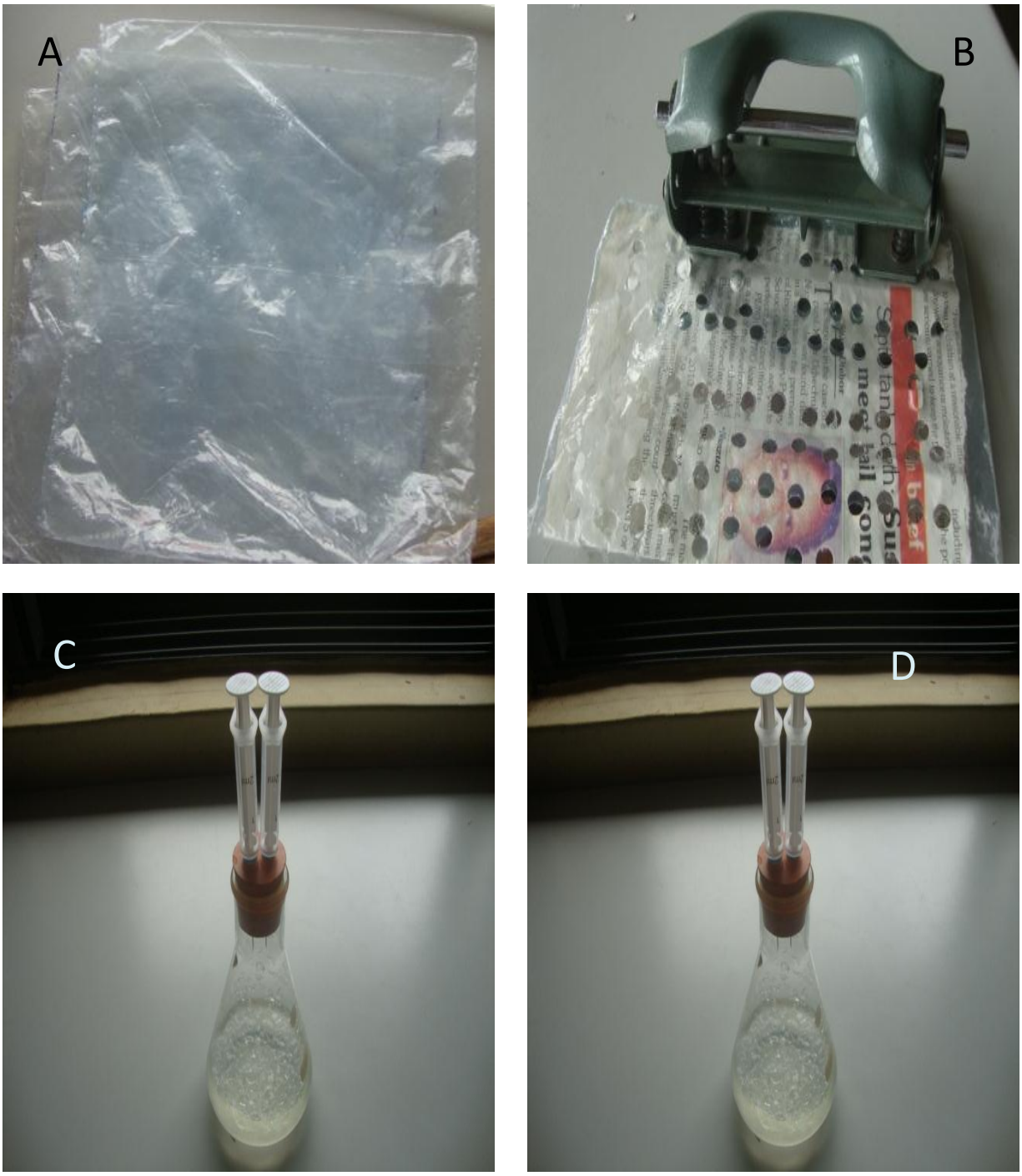


Figure A.3: Stages in Mass Loss Experiment

## APPENDIX B (CHAPTER FOUR)

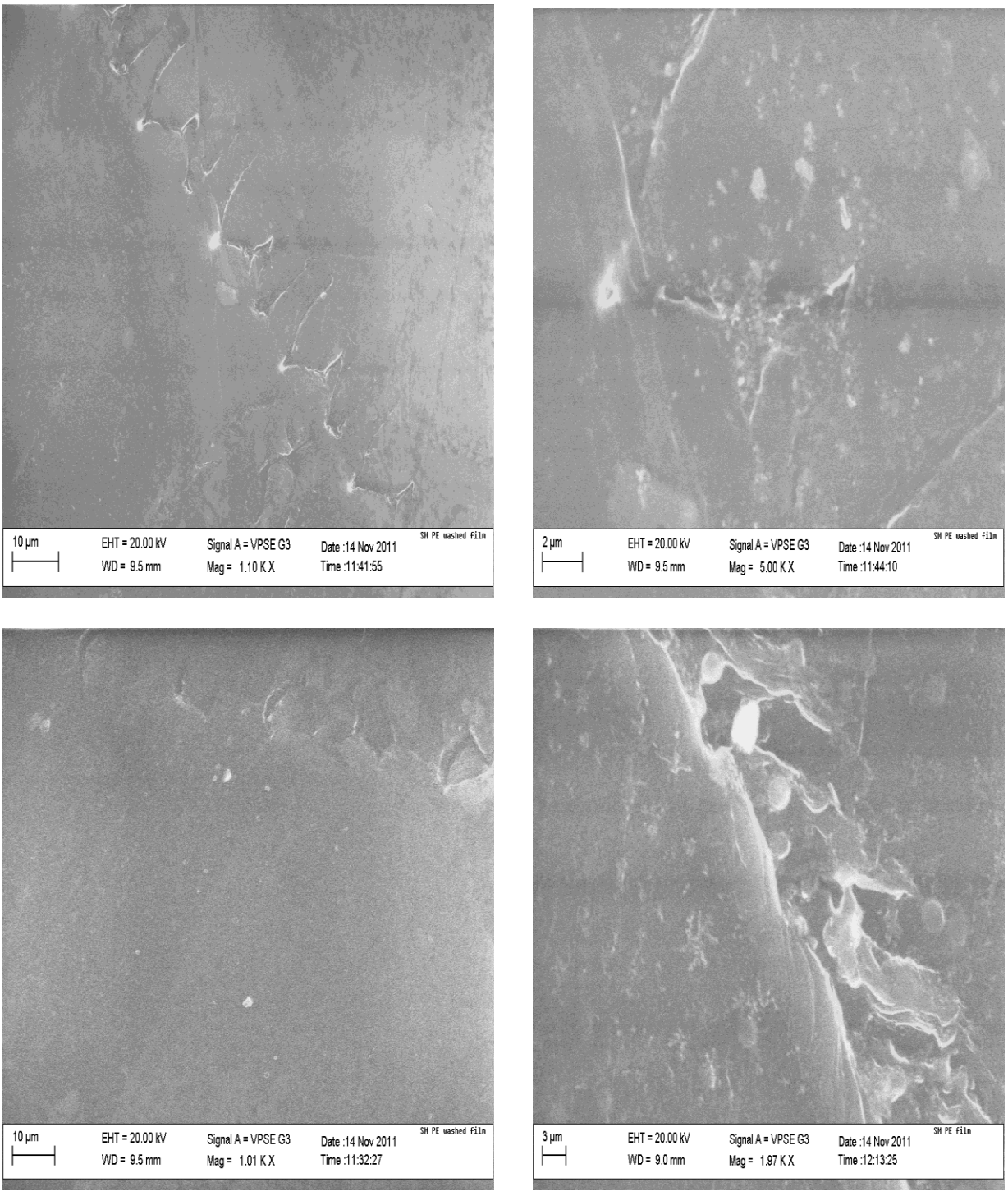


Figure B.1: Signs of *Serratia marcescens marcescens* feeding on polyethylene