TALLINN UNIVERSITY OF TECHNOLOGY
SCHOOL OF ENGINEERING
Department of Civil Engineering and Architecture

ANALYSIS OF THE INFLUENCE OF DIFFERENT CATALYSTS ON (PLA)-BASED PLASTIC AEROBIC BIODEGRADABILITY

ERINEVATE KATALÜSAATORITE MÕJU ANALÜÜS (PLA)-PÕHISELE PLASTILE AEROOBSEL BIOLAGUNDAMISEL

MASTER THESIS

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Tallinn 2023

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Department of Civil Engineering and Architecture

THESIS TASK

Student: Florence Olamide Alabi, 215121EABM

Study programme: Environmental engineering and management, EABM03/18

main speciality: Environmental engineering and management

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Thesis topic:

(in English) *ANALYSIS OF THE INFLUENCE OF DIFFERENT CATALYSTS ON (PLA)-BASED PLASTIC AEROBIC BIODEGRADABILITY*

(in Estonian) *ERINEVATE KATALÜSAATORITE MÕJU ANALÜÜS (PLA)-PÕHISELE PLASTILE AEROOBSEL BIOLAGUNDAMISEL*…

Thesis main objectives:

1. To prepare raw materials and test materials.
2. To carry out tests that will help to determine level of biodegradation.
3. Calculation of amount of carbon dioxide and percentage of biodegradation.
4. Comparison of results.

Thesis tasks and time schedule:

No	Task description	Deadline
1.	Preparation of raw material	04.12.2022
2.	Experimentation	17.03.2023
3.	Introduction and methodology	11.02.2023
4.	Final submission of report	05.05.2023

Language: English

Deadline for submission of thesis: "...."..05..2023

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PREFACE

I would like to thank almighty God for making all these possible, He made completing my thesis a reality.

I would like to show my heartfelt gratitude to my supervisor, Pavlo Lyshtva for guiding me, for being supportive all through the process of my thesis and for the opportunity that was given to me to do this research work, I am very grateful.

I would also like to thank my loving family, my mum Mrs L.A Fatoki, my aunties Mrs Adebunmi Agboola and Justice Abiola Adewemimo and my wonderful siblings Oluwayimika and Eniami Fatoki. Thank you for your support and your love, it kept me all through my stay at Tallinn University of Technology.

To the love of my life, my husband Mr Oluwashola Alabi, thank you so much for being my support system, for your constant prayers and for constantly being my helpmate.

This study is about the Aerobic Biodegradation of plastics using enzymes and compost activator. The aim of this study is to investigate the reaction of enzymes and compost activator in the degradation of plastic which will help in creating a sustainable environment.

List of abbreviations and symbols

C	Carbon
$CaCl_2$	Calcium chloride
CO_2	Carbondioxide/ carbon (II) oxide
CH_4	Methane
$FeCl_3$	Iron (III) chloride
$FeSO_4$	Iron (II)sulphate heptahydrate
H_2O	Water
H_3BO_3	Boric acid
HDPE	High density polyethylene
ISO	International standard organization
KH_2PO_4	Potassium dihydrogen phosphate
KI	Potassium iodide
LDPE	Low density polyehylene
M	Mole per litre
Mg	Milligram
$MgSO_4$	Magnesium sulphate heptahydrate
$MnSO_4$	Manganese (II) sulphate
M-O	Methyl-orange indicator
NaCl	Sodium chloride
Na_2CO_3	Sodium carbonate
$(NH_4)_6Mo_7O_{24}$	Ammonium molydate tetrahydrate
PE	Polyethylene
PET	Polyethylene terephthalate
PETE	Polyethylene terephthalate
PHAs	Polyhydroxyalkanoic acids
PLA	Polylactic acid
PP	Polypropylene
P-P	Phenolphthalein indicator
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride

1. INTRODUCTION

Plastic is the group name given to organic polymers with high molecular weight. Plastics are usually derived from hydrocarbons and products of petroleum. They are made up of chloride, hydrogen, nitrogen, silicon, carbon, and oxygen [1].

Plastics have several properties that make it widely used; they include being lightweight, durable, flexible, transparency, low thermal conductivity and plastics are good insulator [2]. Although plastics are widely being produced and used, it also has adverse effect on the environment.

"Plastics live longer than humans", this statement means that plastics take a long time to decompose depending on environmental factors like sunlight and the composition of the plastic. Decomposition takes between 20 to 500years [3]. While still in the environment, plastics release toxins into the ground and surrounding water. This can cause harm to animals, plants, and human [4].

Recently, research have shown effective result from enzymes and microbes degrading plastics. This thesis has to do with the determination of ultimate aerobic biodegradability of plastic materials under controlled composting conditions. This is done by analysing the carbon dioxide given off by our mixtures. The general method used was based on ISO 14855-1:2012 standard.

The aim of this study is to investigate the reaction of enzymes and compost activator in the entire process of plastic degradation. PLA – (Polylactic acid) based plastic was used. To attain this goal, there are certain objectives that were achieved which includes:

- To source and prepare all the necessary materials required for the test. This also involves preparing all necessary tools and equipment.
- To carry out tests that will help to determine mass of carbon dioxide, theoretical amount of carbon dioxide and percentage of biodegradation.
- To compare the results of each category of sample that were tested.

2. LITERATURE REVIEW

Plastics are organic polymer items comprising of an extensive variety of engineered or semi-manufactured natural and inorganic mixtures [5]. Plastics are composed of long carbon chains back-bone formed through the polymerization [6]. Plastics comprises of majorly hydrogen, carbon, sulphur and other inorganic and organic compounds gotten from fossil fuels.

2.1 Categories and classifications of plastics

2.1.1 Classification of plastics based on manufacturing and uses

There are 7 different types of plastics as shown in Figure 2.1. Being able to identify the different types of plastic helps to better understand the health risks and how best to recycle them. The 7 types are:

1. PET: is the same as PETE which stands for Polyethylene Terephthalate. PET is often utilized in polyester fabrics and food packaging because it is transparent, light, and strong [7].
2. HDPE: refers to High Density Polyethylene. It is applied in construction materials such as pipes, it is also utilized in containers and cartons due to its strength and moisture resistance [7].
3. PVC: means Polyvinyl Chloride. This is the most dangerous plastic to human health because it leaches dangerous toxins throughout its life span. PVCs are applied in construction because of their rigidity and hardness. PVC is impermeable to germs and is easy to disinfect therefore it is used for medical purposes [7].
4. LDPE: represents Low Density Polyehylene. LDPE is a soft, flexible, and clear version of HDPE. It is usually used to line the inside of beverage cartons. It is also applied in coating materials of surfaces prone to corrosion [7].
5. PP: stands for Polypropylene. It is a type of plastic that is flexible and is heat resistance which makes it suitable for packaging and storing hot food [7].
6. PS or Styrofoam: means Polystyrene. It is a type of rigid plastic that insulates and is cheap. It is utilized in food packaging and building. Polystyrene is a dangerous type of

plastic because like PVC, it leaches dangerous toxins that can be consumed by humans through food [7].

7. Others: This represents all other plastics that do not belong to the previously mentioned types of plastics [7].

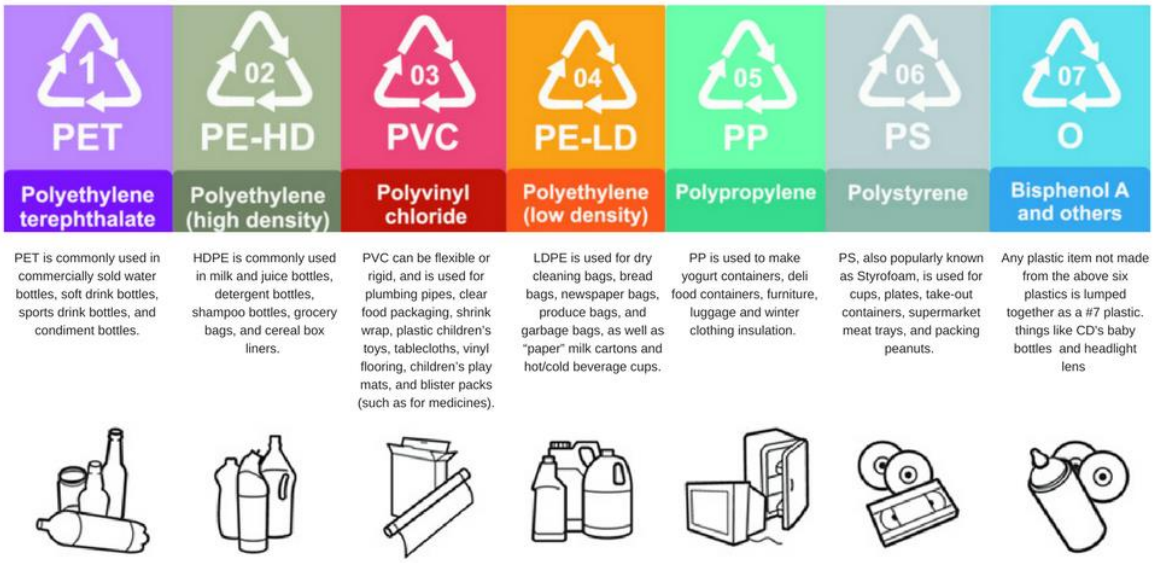

Figure 2.1 Types of plastics based on manufacturing and use *[7]*

2.1.2 Classification based on thermal properties

There are majorly 2 categories of plastics namely:

1. Thermoplastics: are long chain macromolecules where the molecules and atoms are joined into a progressively long, sole carbon chains. Thermoplastics don't change their compound arrangement when heated. These incorporates unique sorts of polymer plastic which are PTFE (polytetrafluoroethylene), PVC (polyvinyl chloride), PP (polypropylene), PS (polystyrene) and PE (Polyethylene). The other name given to this class is Normal Plastics, ranging from 20,000 to 500,000 AMU in atomic weight; thermoplastics have unique quantities of rehashing units gotten from a straightforward monomer unit [8].
2. Thermosetting Polymers: are a type of polymer that is formed through a process called step-growth polymerization. Unlike thermoplastic polymers, thermoset plastics cannot be melted or reshaped after they have been formed. This is because the chemical

change that occurs during the creation of thermoset plastics is irreversible. Additionally, the highly cross-linked structure of thermoset plastics makes them non-recyclable. Examples of thermoset plastics include phenol-formaldehyde, polyurethanes, and many others [9].

2.1.3 Classification based on design properties

Plastics can be classified according to their properties and how they are used in manufacturing and design. These properties can include electrical conductivity, durability, tensile strength, degradability, and thermal stability. Plastics with specific combinations of these properties can be chosen for specific applications based on the requirements of the product being made [9].

2.1.4 Classification based on degradability properties

The degradation properties of plastics can be used to differentiate between degradable and non-degradable polymers. Degradable plastics break down into smaller molecules over time through natural processes such as exposure to sunlight or water. Non-degradable plastics, on the other hand, do not break down and remain as they are indefinitely. The chemical properties of a polymer determine whether it is degradable or non-degradable [10].

Non-biodegradable plastics, also known as synthetic plastics, are made from petrochemicals and have a high molecular weight due to the repetition of small monomer units. In contrast, biodegradable plastics are made from renewable resources such as plant, animal, and algal materials. They can also be produced by microorganisms and are able to break down into their natural forms through the process of biodegradation. Biodegradable plastics are made from sources such as cellulose, starches, protein, and algal materials [11].

Biodegradable plastics can break down over time when exposed to UV light, water, enzymes, and changes in pH. The main types of degradable plastics are:

1. Photodegradable bioplastics: are broken down when exposed to UV light the way compostable bioplastics are broken down when exposed to microorganisms in a composting environment and bio-based bioplastics made from renewable resources and broken down through natural processes. Biodegradable bioplastics are broken down through a combination of these processes [12].

2. Bio-based bioplastics: are derived from renewable resources, such as corn starch, soy protein, and cellulose, are referred to as bio-based. These materials are made entirely from carbon sources that come from agricultural and forestry industries [9].
3. Compostable bioplastics: These decompose through biological processes at a similar rate to other compostable materials. Compostable materials do not leave behind any visible toxic residue during the composting process. To be classified as bio-compostable, a plastic must first pass standardized tests that evaluate its total biodegradability, degree of disintegration, and the potential ecological toxicity of its degraded materials [9].
4. Biodegradable bioplastics: can fully break down into biogases and biomass (mostly carbon dioxide and water) through the action of microorganisms, without leaving any visible toxic residue. This process of decomposition occurs naturally when the material is exposed to a microbial environment and humidity. The term "biodegradable" refers to the ability of a material to disintegrate or break down in this way [13]. Polyhydroxyalkanoic acids (PHAs) are a type of biodegradable plastics that have properties similar to traditional plastics. They can fully break down through natural processes and do not leave any toxic residue. PHAs can be melted and moulded, making them useful for consumer products. Figure 2.2 shows the typical PHA structure, as well as the structures of two commonly used PHAs: poly (3-hydroxybutyrate) and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) [14].

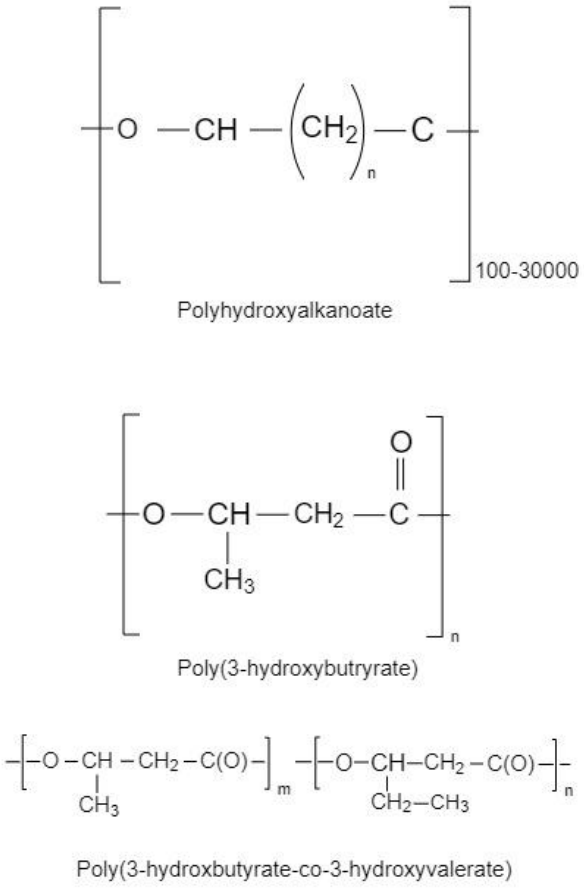

Figure 2.2 Structure of biodegradable plastic polyhydroxyalkanoates (PHA) and its derivatives poly(3-hydroxybutyrate) PHB and poly (3-hydroxybutryrate – co-3-hydroxyvalerate*) [9]*

2.1.5 Classification based on chemical structure

Synthetic plastics are divided into two categories based on the type of chemical reaction that creates them: addition polymers and condensation polymers [15]. Addition polymers are created when all atoms in the monomers are incorporated into the polymer chain. In contrast, condensation polymers are formed when some atoms in the monomers are released as small molecules, such as water, during the polymerization process. Most addition polymers are made from monomers with double bonds between carbon atoms, known as olefins. These include polyolefins like polyethylene, polypropylene, and polystyrene. Condensation polymers, on the other hand, are made from monomers with different groups of atoms that can bond together through ester or amide links. Examples include polyvinyl chloride,

polypropylene, polystyrene, polyurethane, and polyethylene terephthalate as illustrated in Figure 2.3 [15].

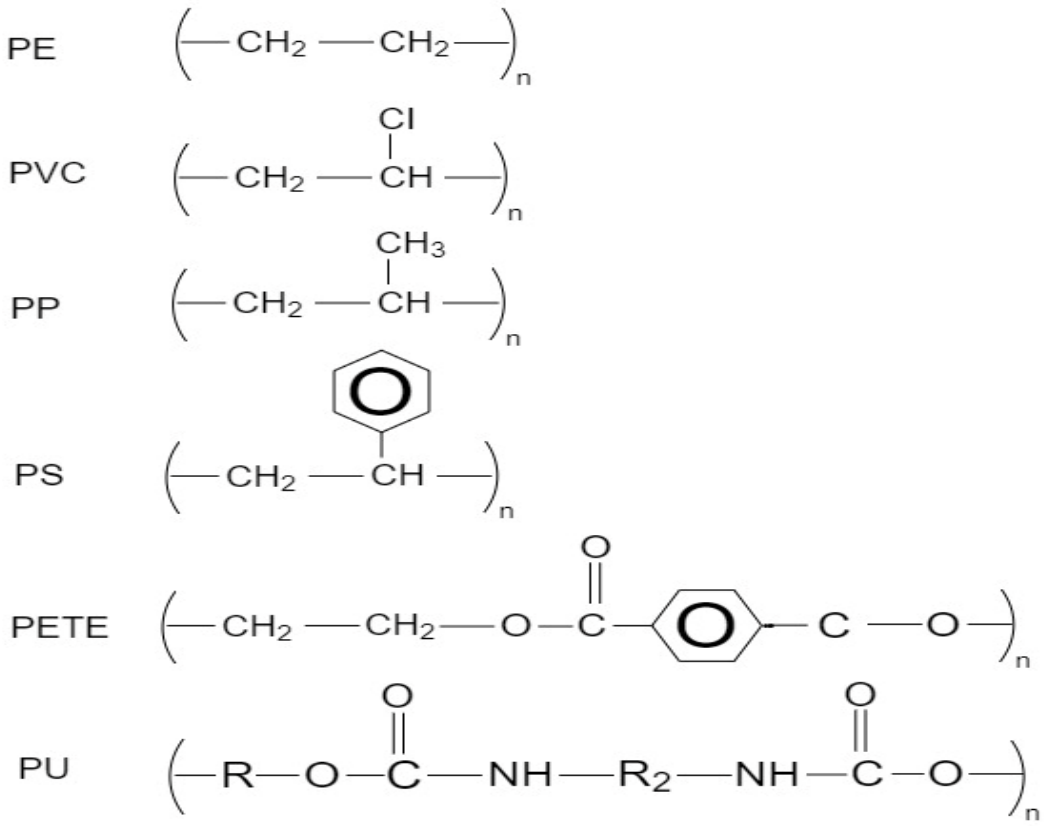

Figure 2.3 Chemical structures of petrochemical plastics Polyethylene (PE), Polyvinyl chloride (PVC), Polypropylene (PP), Polystyrene (PS), Polyethylene Terephthalate (PET) and Polyurethane (PU) *[9]*

2.2 Biodegradation

Biodegradation is defined as a transformation or alteration of chemicals introduced into the environment through the actions of microbial organisms, either through metabolism or the use of enzymes [16].

When biodegradation involves the use of enzymes, the enzymes break down substances in a laboratory setting or within an organism. Biodegradation can be characterized as follows:

a. The primary alteration of the chemical structure of the substance, leading to the loss of a specific property.
b. Biodegradation that removes undesirable properties of the compound and is considered environmentally acceptable. This is often related to primary biodegradation, but it depends on the conditions in which the products are released into the environment.
c. The ultimate complete breakdown of the compound into simple molecules that are either fully oxidized or reduced (such as carbon dioxide/methane, nitrate/ammonium, and water). It's important to note that the products of biodegradation can sometimes be more harmful than the substance that was degraded [17].

2.2.1 Biodegradation of plastics

The microorganisms in biodegradation can break down both natural and synthetic plastics. Biodegradation of plastics typically occurs under different conditions, such as in the presence of oxygen (aerobic biodegradation), without oxygen (anaerobic biodegradation), or partially in the presence of oxygen (as in compost or soil). During aerobic biodegradation, carbon dioxide and water are produced, while anaerobic biodegradation produces carbon dioxide, water, and methane. These reactions are shown in Figure 2.4 [9].

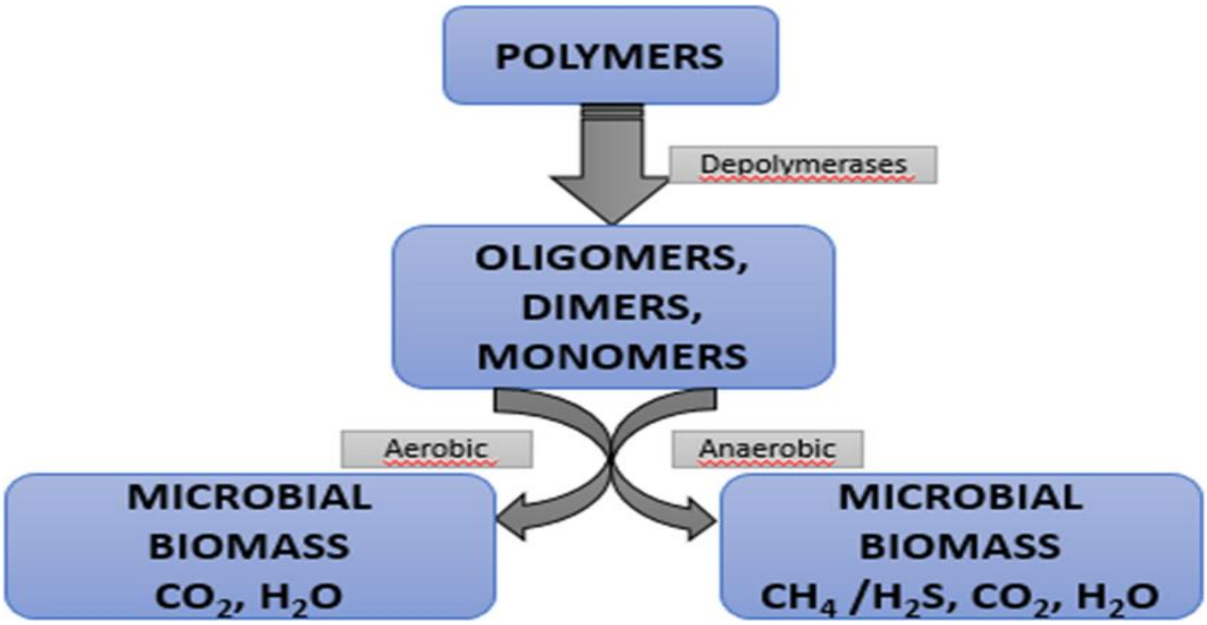

Figure 2.4 Reaction pathways during degradation of polymers *[18]*

There are 4 phases of general plastics biodegradation namely:

1. Aerobic Phase: During the first few days, aerobic microbes become established as moisture accumulates in the environment. Biodegradable plastics begin to swell and weaken as their molecular structure is altered to create space for microbial growth and moisture. Oxygen is replaced with carbon dioxide. [19]
2. Anaerobic Phase: After about two weeks to six months, the concentration of oxygen decreases, and an anaerobic process begins. During this initial phase, microbes break down large polymers into smaller monomers through an enzymatic process, releasing monomers that mix with organic plastic additives and cause further swelling and opening of the polymer chains. Acidogens convert the monomers into fatty acids and produce carbon dioxide. [19]
3. Anaerobic methanogenic, unsteady phase: Between 6-18 months, the colonies of microbes continue to grow, consuming the polymer chain and creating larger molecular spaces. Acetogenesis occurs, converting fatty acids into acetic acid, carbon dioxide, and hydrogen. The rate of carbon dioxide production gradually decreases, and hydrogen production eventually stops. [19]
4. Anaerobic methanogenic, steady phase: Between 1-5 years, the final stage of decomposition occurs. This involves methanogenesis, where colonies of microbes continue to consume the remaining surface of the polymer and convert acetates into methane and carbon dioxide, while consuming hydrogen. This methane can be converted into energy through biotechnology. [19]

2.2.2 Aerobic biodegradation

Aerobic biodegradation, also known as aerobic respiration, is a process that involves the use of oxygen by microbes to break down organic chemicals into smaller compounds as represented in equation 1.1. This type of biodegradation plays a significant role in the natural reduction of contaminants at hazardous waste sites. During aerobic biodegradation, the microbes utilize oxygen as an electron acceptor, releasing carbon dioxide and water as by-products. The process of aerobic biodegradation can be represented as follows:

$$\text{C plastic} + O_2 \rightarrow CO_2 + H_2O + \text{C residual} + \text{Biomass} \qquad \text{equation (1.1)}$$

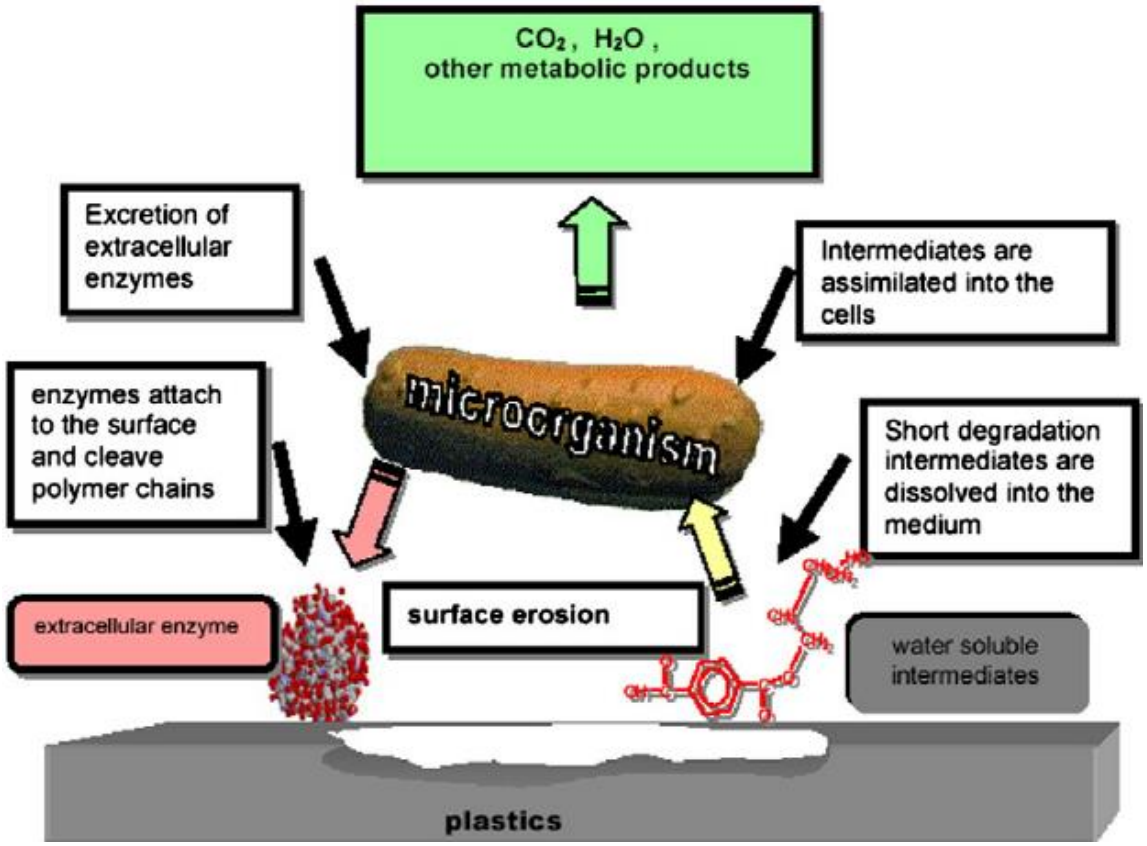

Figure 2.5 General mechanism of plastic biodegradation under aerobic conditions *[20]*

2.2.3 Anaerobic biodegradation

The breakdown of organic contaminants by microorganisms in the absence of oxygen is known as anaerobic biodegradation. It plays a crucial role in the natural attenuation of contaminants at hazardous waste sites. Some anaerobic bacteria rely on electron acceptors such as nitrate, sulphate, iron, manganese, and carbon dioxide to break down organic chemicals into smaller compounds [21]. The process of anaerobic biodegradation can be represented in equation 1.2 below:

$$\text{C plastic} \rightarrow CH_4 + CO_2 + H_2O + \text{C residual} + \text{Biomass} \qquad \text{equation (1.2)}$$

It has several uses in many different disciplines, such as environmental science, microbiology, and renewable energy [9].

Mechanisms of Anaerobic Degradation

The process of anaerobic degradation involves the collaboration of various microorganisms, including methanogens, acetogens, and sulphate-reducing bacteria. Methanogens play a vital role in anaerobic degradation, as they produce methane as a by-product of their metabolism. Acetogens convert organic compounds into acetate, which can then be used by methanogens to produce methane. Sulphate-reducing bacteria use sulphate as an alternative electron acceptor to oxygen [9].

There are different types of anaerobic degradation, including acetogenesis, syntrophy, and anaerobic respiration. Acetogenesis is the process of converting organic compounds into acetate, while syntrophy involves the cooperation of multiple microbial species in the breakdown of organic compounds. Finally, anaerobic respiration involves the use of alternative electron acceptors, such as sulphate or nitrate, instead of oxygen [9].

The efficiency of anaerobic degradation is affected by several factors, including temperature, pH, nutrient availability, and the type of organic matter being degraded. Methanogens, for instance, require a specific range of pH and temperature to function optimally. Additionally, the type of organic matter being degraded can affect the rate and efficiency of the process, with some types of organic matter being more easily degraded than others [9].

Applications of anaerobic degradation

Anaerobic degradation has numerous applications, including wastewater treatment, biogas production, and the treatment of organic waste. In wastewater treatment, anaerobic digestion is often used to reduce the organic content of sewage and produce biogas. Biogas can be used as a source of renewable energy, either for electricity generation or as a replacement for fossil fuels. Additionally, anaerobic digestion can be used to treat organic waste, such as food waste or agricultural waste, reducing the amount of waste that needs to be disposed of in landfills [9].

2.3 Enzymes

An enzyme is a protein that acts as a biological catalyst, meaning that it increases the rate of a specific chemical reaction within a cell. Enzymes are not consumed or destroyed during the reaction and can be used repeatedly. Cells contain a diverse array of enzyme molecules, each of which is specialized for a particular chemical reaction [22].

Microbes, including bacteria and fungi, produce enzymes that can break down toxic natural compounds. These enzymes, which are essential for bioremediation, are a practical and environmentally friendly biotechnology. In recent years, there has been an increase in research and development in this field, highlighting the need for further progress in this area. The microbial enzymes involved in bioremediation play a crucial role in reducing toxic pollutants and produce valuable by-products. These microbes use biochemical reactions to extract energy and break down bonds, resulting in the production of harmless compounds. The mechanisms of microbial enzymes responsible for bioremediation, such as laccases and hydrolases, have been extensively studied. [22].

2.3.1 Plastic degradation by enzymes

Degradation of plastics using enzymes is a way of depolarizing waste petro-plastics into monomers before recycling or a way of mineralizing plastics into new biomass, carbon dioxide and water with associated production of valuable bioproducts [23].

Microbes that can produce and keep PHA below nutrient-limited condition can metabolize and degrade the same when constraint is removed. The ability to store PHA does not automatically mean the ability to degrade [24].

Single polymers are too large to be moved directly through the bacterial wall, therefore bacteria will need to secrete extracellular hydrolases able to change the polymers into corresponding hydroxyl acid monomers. The result of PHB hydrolysis is R-3-hydroxybutyric acid, at the same time the extracellular degradation of PHBV results to 3-hydroxybutyrate and 3-hydroxyvalerate. The monomers are water soluble and small enough to pass through the cell wall producing water and carbon dioxide. Resulting water and carbon dioxide are a result of being metabolized by β-oxidation and tricarboxylic acid cycle (TCA) under aerobic condition while methane is likewise produced under anaerobic condition [24].

The enzymatic degradation of polymers through hydrolysis is a two-step process, wherein the enzyme binds to the polymer substrate, after which it finally catalyses Intracellular and Extracellular depolymerases in PHB/PHBV-degrading microorganism. Intracellular degradation is the hydrolysis of an endogenous carbon reservoir by gathering bacteria. In the same way extracellular degradation involves the use of exogenous carbon supply but not compulsorily by accumulating microbes. Polyhydroxyalkanoate-degrading PHA microbes produce PHA depolymerases that hydrolyse polymers extracellularly to water-soluble merchandise and make use of the hydrolysis products as carbon and energy sources for growth [24].

Biodegradation of plastics involves microorganisms excreting extracellular enzymes. These enzymes attach themselves to the surface of the plastic and hydrolysis to short polymer intermediates, which are ultimately assimilated by microbial cells as carbon source to release CO_2 [25].

There are some examples of enzymes that are involved in biodegradation of plastics, they include:

1. Cutinases: These enzymes identify themselves by their ability to hydrolyze polyesters with high molar masses as a subclass of esterase enzymes. Cutinases are products of penicillium citrinum, aspergillus oryzae, humicola insolens, pichia pastoris and fusarium solani. It was found that Fusarium solani pisi cutinase (FsC) and Humicola insolens cutinase (HiC) are capable of degrading low crystallinity PET films with 97% weight loss within 96 hours [26].
2. Lipases: Lipase enzyme is a naturally occurring enzyme found in the stomach and pancreatic juice. Its function is to digest fats and lipids, helping to maintain correct gallbladder function. Lipase is the one such widely used and versatile enzyme. These enzymes are obtained from animals, plants and as well as from several microorganisms and are sufficiently stable. However, microbial lipases are the only catalysts that are used significantly in the commercial world [27].

   Lipases are enzymes that catalyze the hydrolysis of lipids. They are also the subclass of esterases enzyme. It has been shown that some fungal species produce lipases and are involved in plastic degradation, including Rhizopus delemer, Candida antarctica, Termomyces lanuginosus and Candida rugosa, which degrade poly (butylene succinate-cohexamethylene succinate) copolymer [28].

3. Proteases: Proteases can be obtained from animal, plant (or vegetable), and microbial sources. Animal proteases include pancreatic trypsin, chymotrypsin, pepsin, and renin. These are produced in small quantities because their production depends on the availability of livestock for sacrifice so that these proteases can be extracted from their organs or tissues. In the group of proteases of plant origin, papain and bromelain are illustrative examples; the first is removed from the shell of the fruit *Carica papaya*, and the second from the trunk and juice of the pineapple. However, the use of plants as a source of proteases is strongly influenced by factors of viability, cultivation, climatic conditions, and lengthy extraction processes. For the reasons above, most of the commercially relevant enzymes are produced from a limited number of microorganisms. Additionally, microbial enzymes are preferred over those of others because they can usually be obtained in abundant quantities, on a regular basis and of uniform quality. Microbial enzymes are generally more stable than their animal and vegetable counterparts. In addition, production processes are faster due to their short duplication period and relatively simple nutritional requirements. It is possible to manipulate microbes genetically and environmentally to obtain the desired characteristics, such as increasing the activity and yield of the enzyme of interest [29].
4. Esterases: It is a group of hydrolases that has a wide substrate tolerance and can catalyse a wide variety of reactions even in organic solvents [30]. Esterases are enzymes with the ability to selectively react at a specific level and produce a specific stereochemistry. They are hydrolase enzymes that can break down ester bonds (into alcohol and acids with addition of water molecules) and synthesize them without additional substances. This makes esterases highly valuable in biocatalytic applications, such as the production of chiral drugs and agricultural chemicals [31]. These enzymes produced fungi and bacteria take part in degradation of plastic.
5. Laccase: Laccases are found in plants, insects, bacteria, and fungi. Laccases are found in plants like turnip, potatoes, cabbages, and other vegetables. They are involved in catalysing the oxidation process of phenolic compounds where they utilize molecular oxygen as co-substrate. The end products are water and some by-products [32]. Laccases have the special ability to degrade lignin by oxidizing them [33]. These enzymes are generally found in fungi and plants but recent research have shown that they are also found in bacteria like Marinomonas mediterranea, S.lavendulae and S.cyaneus [34].

6. Peroxidases: These enzymes have the ability to reduce hydrogen peroxide and some hydroperoxides to water, oxidizing substrates. Peroxidases are classified according to the interaction with peroxide into heme and non-heme [34].

2.3.2 Factors affecting enzymic biodegradation

Biodegradation of plastics can be aided by the following factors:

1. Molecular weight of polymers.
2. Density of polymers.
3. Presence of easily breakable bond e.g. amide bonds, ester bonds.
4. Rate of hydrophobicity.
5. Amount of crystalline and amorphous region – crystalline degrades slower than amorphous.
6. Structural complexity of polymers.
7. Composition of molecules.
8. Physical form of polymers – fibers, pallets, films and powder.
9. Hardness of polymers [35]

2.4 Compost activators

Composting is a valuable process that manages organic waste by breaking them down into nutrient-rich materials called compost. The process involves the activity of various microorganisms that decompose organic matter into simpler compounds. However, the process can be slow, and the quality of the final product can vary depending on some factors such as material composition, environmental conditions, and the presence of inhibitory factors. Compost activators are additives that are added to the composting material to quicken the decomposition process and improve the quality of the by-product [36].

2.4.1 Types of compost activators based on additives

There are 2 major types of compost activators namely: natural and synthetic additives.

1. Natural activators: This type of activators comprises of animal manure, plant-based materials, and microbial inoculants. Animal manure is a common activator because it contains high levels of nitrogen and other nutrients that stimulate microbial activity. Plant-based materials, such as alfalfa meal, blood meal, and bone meal, are also used as activators, as they provide a source of nitrogen, phosphorus, and other micronutrients. Microbial inoculants involve the addition of microorganisms to the composting material to enhance the activity of microorganisms already present [37].
2. Synthetic activators: These are mainly chemical fertilizers, enzymes, and other additives. Chemical fertilizers, such as ammonium nitrate and urea, provide a source of nitrogen and other nutrients. Enzymes, such as cellulases and ligninases, break down complex organic molecules, such as cellulose and lignin, into simpler compounds. Other additives, such as surfactants and pH adjusters, enhance microbial activity and optimize the conditions for composting [38].

2.4.2 Types of Compost Activators based on reaction

Compost activators can be broadly categorized based in reaction into three types: chemical, biological, and physical.

1. Chemical compost activators: This includes materials such as urea, ammonium sulphate, and phosphate. These activators work by providing the necessary nutrients for microbial growth and activity. Biological compost activators include microorganisms such as bacteria and fungi that are added to the compost to enhance the microbial community and accelerate the composting process. Physical compost activators include materials such as air, water, and mechanical agitation, which improve the physical conditions of the compost, such as aeration and moisture content. One limitation of chemical compost activators is that they can be expensive and difficult to obtain. Many chemical compost activators are industrial by-products that are not readily available to the average gardener or composter. Additionally, excessive use of nitrogen-rich chemical activators can lead to nitrate pollution in soil and groundwater,

which can be harmful to human health and aquatic life. A study by the University of Missouri Extension found that excessive use of ammonium nitrate in compost led to high levels of nitrate in the soil, which can be toxic to plants and inhibit their growth. Therefore, the use of chemical compost activators must be carefully monitored to avoid harmful effects on the environment [39].

2. Biological compost activators: This include bacteria and fungi which can also have limitations. The microbial community in the compost is already unique and by introducing new microorganism, may have significant impact on the composting process. Additionally, some microorganisms may be more effective in certain conditions like temperature, than others. Therefore, it can be challenging to identify and introduce the right strain of microorganisms for a particular composting situation. A study by the University of California found that the addition of microbial inoculants did not significantly affect the rate of decomposition or the quality of the product. However, the study did note that the addition of microbial inoculants may have benefits for specific composting situations, such as composting animal waste or improving the quality of compost for commercial purpose [39].

   Another limitation of biological compost activators is that they can introduce pathogens into the compost. While most microorganisms in compost are beneficial, some can be harmful to human health, such as Salmonella and E. coli. Therefore, the use of biological compost activators must be carefully controlled to prevent the introduction of harmful pathogens into the compost. A study by the University of Minnesota found that adding high levels of E. coli to compost resulted in increased levels of E. coli in the final product, which could be harmful to human health. The study recommends that the use of biological compost activators should be carefully monitored to prevent the introduction of harmful pathogens into the compost [39].
3. Physical compost activators, such as air and water, can also have limitations. One limitation is that they may not be sufficient to improve the composting process. For example, if the compost is too dense, adding air and water may not be enough to stimulate the microbial activity [39].

2.4.3 Mechanisms of Compost Activation

The effectiveness of compost activators depends on their mechanisms of action. Natural activators, such as animal manure and plant-based materials, provide a source of nutrients

that stimulate microbial activity. Microbial inoculants introduce new microorganisms to the composting material, enhancing the diversity and activity of the microbial community. Synthetic activators, such as chemical fertilizers and enzymes, provide specific nutrients or enzymes that can promote the breakdown of specific compounds [36].

The activity of microorganisms in composting is influenced by temperature, moisture content, pH, and aeration. Compost activators can influence these factors by providing nutrients, adjusting pH, or improving aeration. For example, adding animal manure to the composting material can increase the temperature of the pile, accelerating microbial activity. Enzymes break down complex organic molecules into simpler compounds, which can be used as a source of energy and nutrients by microorganisms [36].

2.4.4 Effectiveness of Compost Activators

The effectiveness of compost activators depends on various factors, including the type of feedstock, the composition of the activator, and the environmental conditions. Animal manure is a popular activator, providing a source of nitrogen, phosphorus, and other nutrients that stimulate microbial activity. The quality of the manure can vary depending on the type of animal, diet, and management practices. Plant-based materials, such as alfalfa meal and blood meal, are effective activators, providing a source of nitrogen and other nutrients but they can be expensive and challenging to transport and store.

Microbial inoculants are another effective activator, introducing new microorganisms to the composting material, enhancing the diversity and activity of the microbial community. Synthetic activators, such as chemical fertilizers and enzymes, can provide specific nutrients or enzymes that promote the breakdown of specific compounds. Overuse of synthetic activators can harm the microbial community and lead to the accumulation of harmful compounds in the final product [36].

3. METHODOLOGY

This chapter focuses on the methodology of this thesis. The general method used in this work is based on ISO 14855-1:2012 standard which focuses on determination of the ultimate aerobic biodegradability of plastics based on organic compounds under controlled composting conditions. Enzymes were added and the amount of carbon dioxide given off and the degree of disintegration of the plastic at the end of the test were measured and compared. This test method is designed to yield percentage conversion of the carbon in the test material to the evolved carbon dioxide and the rate of conversion.

3.1 Apparatus and materials

3.1.1 Apparatus

The following apparatus were used during this thesis:

- Composting vessels- glass bottles
- Air pump
- Pipes
- Volumetric pipette
- Burette
- Gas tight tubes
- Measuring scale
- Water bath

3.1.2 Materials

The following materials were used:

- Boric acid - H_3BO_3
- Potassium iodide - KI
- Iron (III) chloride – $FeCl_3$
- Manganese (II) sulphate – $MnSO_4$

- Ammonium molydate tetrahydrate – $(NH_4)_6Mo_7O_{24}$
- Iron (II)sulphate heptahydrate - $FeSO_4$
- Potassium dihydrogen phosphate – KH_2PO_4
- Magnesium sulphate heptahydrate – $MgSO_4$
- Calcium chloride – $CaCl_2$
- Sodium chloride – NaCl
- Sodium hydroxide - NaOH
- Broth
- Urea
- Corn starch
- Cellulose
- Compost
- Water
- NaOH
- Enzymes
- Vermiculite
- Polylactic acid – PLA
- Compost activator

3.2 Procedure

3.2.1 Preparation of vermiculite

Vermiculite was activated by inoculating it with a solution of compost, organic and inorganic nutrients. The inoculum and vermiculite were mixed in the ratio 3:1.

To prepare inoculum, 2 other mixtures were prepared as follows:

1. Preparation of 1litre of trace-element solution with the chemicals and amounts illustrated in Table 1 is based on the standard of 100% pure chemical.

Table 3.0.1 Actual composition of 1 litre of trace-element solution

Chemical	H_3BO_3	KI	$FeCl_3$	$MnSO_4$	$(NH_4)_6Mo_7O_{24}$	$FeSO_4$
Amount	0.4946g	0.1010g	0.2014g	0.2239g	0.2124g	0.7322g

Each substance in Table 1 was measured based on level of purity and the standard quantity. The higher the purity, the lower the weight.

2. Preparation of 1 litre of mineral solution as illustrated in Table 2.

Table 3.0.2 Actual composition of 1 litre of mineral solution

Chemical	KH_2PO_4	$MgSO_4$	$CaCl_2$ (10% solution)	NaCl (10% solution)	Trace-element solution from Table 2
Amount	0.9970g	1.0238g	1ml	1ml	1ml

This also like the previous mixture was calculated based on the level of purity and the standard quantity.

3. Inoculum mixture is as follows:

Table 3.0.3 Actual composition of 1 litre of inoculum solution

Chemical	Mineral Solution from Table 4	Suitable nutrient broth	Urea	Corn starch	Cellulose	Compost extract
Amount	500ml	13g	5.8058g	20g	20g	500ml

For Table 3, only the equivalent weight of 100% pure urea was calculated as the urea used was 99.9% pure.

Compost preparation

Compost was prepared by mixing with water in the ratio 20:80. 600grams of compost was used, it was then mixed with 2400g of water. The water and compost were mixed for 40mins and then filtered through two sieves of different mesh sizes. The sizes are 1.0cm and 0.5cm.

3.2.2 Preparation of materials

1. Vermiculite: 210grams of vermiculite were measured into all 15 bottles.

Figure 3.1 Activated vermiculite sample

2. PLA: Polylactic acid granules as illustrated in Figure 3.2 was used and measured into 9 compost vessels. 13.85g of PLA was measured into each bottle.

Figure 3.2 PLA-based samples used

3. Enzymes: About 2.2385g of crushed enzymes illustrated in Figure 3.3 was used where the percentage of lipases is 60.79%, amylases is 36.47% and proteases is 2.74%. These enzymes were used as a catalyst for Category C (three bottles of vermiculite+ PLA-base plastic + crushed lipases + amylase + protease enzymes).

Figure 3.3 Enzyme used

4. Compost activator: Two different compost activators illustrated in Figure 3.4 and 3.5 were used for the Category D (three bottles of vermiculite + PLA-based plastic + brown compost activator) and E (three bottles of vermiculite + PLA-based plastic + white compost activator). They are white and brown compost activators. 2.2385g of compost activator were used for both categories i.e 2.2385g of brown compost activator for Category D and 2.2385g of white compost activator for Category E.

Figure 3.4 Brown compost activator

Figure 3.5 White compost activator

3.2.3 The setup

The materials used and compared were in 5 different categories and they were setup in the order:

(A) three bottles of vermiculite

(B) three bottles of vermiculite + cellulose

(C) three bottles of vermiculite + PLA-base plastic + crushed lipases + amylase + protease enzymes

(D) three bottles of vermiculite + PLA-based plastic + brown compost activator

(E) three bottles of vermiculite + PLA-based plastic + white compost activator

The entire setup is illustrated in Figure 3.6 and 3.7. Compressed air at a constantly low pressure was supplied to the system, passing through a solution of sodium hydroxide absorption system thereby removing the carbon dioxide which shows that biodegradation has taken place.

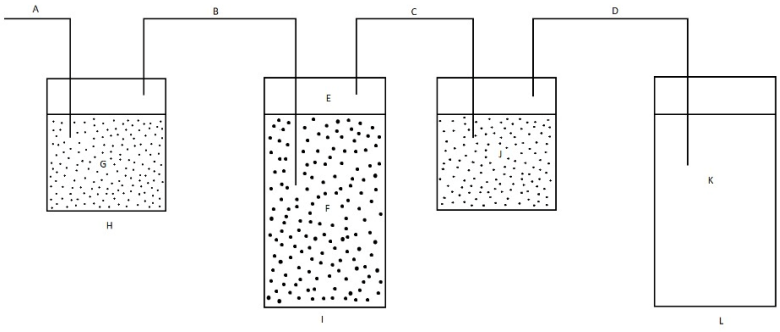

Figure 3.6 Layout of the system

Where

A - air
B – CO_2
C – water removal system
D – exhaust air
E – head space
F – test mixture
G – NaOH solution
H – CO_2-removal system
I – composting vessel
J – HCl solution
K -NaOH solution
L – CO_2 determination system

NaOH was also measured out based on 2 concentrations, 0.5M and 0.75M. 98% pure NaOH was used in this study, so the amount of NaOH that was used equivalent to the concentrations was 20.4082grams/litre and 30.6122grams/litre respectively.

Figure 3.7 Actual layout of the system

3.2.4 Incubation period

Incubation period was 104 days, but sampling was done between 7-12 days. 20ml samples from each CO_2 determination system (NaOH solution) were taken and titrated to determine the amount of carbon dioxide generated. The NaOH solution in each bottle was then replaced after each titration with fresh 1000ml mixture of 0.5M/0.75M of NaOH depending on CO_2 evolution.

Titration

Titration of the NaOH was done with 0.5M concentration of hydrochloric acid in two stages. The first stage was with phenolphthalein indicator (P-P). 3 drops of P-P indicator were added to 20ml sample of NaOH solution to turn it from pink to clear white colour. The second stage

involved titrating the colourless solution from the first stage with the same 0.5M concentration of hydrochloric acid after 3 drops of methyl-orange indicator (M-O) was added. This changed the solution from colourless into pink. The first stage showed the midpoint while the second stage showed the endpoint.

3.3 Precautions

In carrying out the experiment, the following precautions were taken:

- Ensured the bottles were properly sealed to avoid leakage.
- Ensured that the water bath was constantly filled with water.
- Avoided parallax error in reading the pipette and burette.
- Avoided gas leakage through tubes by ensuring they were tight.

3.4 Equations for calculations

Determination of carbon dioxide

The amount of carbon dioxide was determined by titration following ISO 19679:2020. The CO_2 given off reacts with NaOH as follows:

$$2\ NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O \qquad \text{equation (3.1)}$$

NaOH solution was used as CO_2 absorbing agent and had unreacted NaOH and Na_2CO_3 as shown in equation 3.1 but during titration, these two unreacted compounds react with HCl to give the following reactions:

$$NaOH + HCl \rightarrow NaCl + H_2O, \text{ at pH 7} \qquad \text{equation (3.2)}$$

$$Na_2CO_3 + HCl \rightarrow NaHCO_3 + NaCl, \text{ at pH 8.5} \qquad \text{equation (3.3)}$$

Phenolphthalein indicator (P-P) was used to identify the single end point of pH between the range 7 and 8. A further titration of $NaHCO_3$ with HCL and methyl-orange indicator (M-O) to give equation 3.4.

$Na_2CO_3 + HCl \rightarrow NaHCO_3 + NaCl$, at pH 4 equation (3.4)

The result gotten from the titration will help to determine the mass of CO_2 given in equation (3.5)

Mass of CO_2 = volume of titrant x molarity of acid equation (3.5)

x molecular weight of CO_2

Determination of theoretical amount of carbon dioxide

The theoretical amount of carbon dioxide represented by $ThCO_2$ is calculated as grams per vessel is given as:

$ThCO_2 = M_{TOT} \text{ X } C_{TOT} \text{ X } \frac{44}{12}$ equation (3.6)

Where,

M_{TOT} - total dry solids, in grams

C_{TOT} – proportion of total organic carbon in the total dry solids, in grams

44 and 12 – molecular mass of carbon dioxide and the atomic mass of carbon respectively

Determination of percentage biodegradation

From the theoretical and cumulative amount of carbon dioxide, the percentage of biodegradation can be calculated as shown in equation (3.7).

$D_t = \frac{(CO_2)_T - (CO_2)_B}{ThCO_2} \times 100$ equation (3.7)

Where,

$(CO_2)_T$ – cumulative amount of CO_2 evolved in each composting vessel with test material, grams per vessel.

$(CO_2)_B$ – mean cumulative amount of CO_2 evolved in blank vessels, grams per vessel

$ThCO_2$ – theoretical amount of CO_2 that can be produced by the test material, grams per vessel.

4. RESULTS ANALYSIS

The entire process took 145 days but incubation and titration lasted for 107 days. Titration was done between 10 – 12 days and the data were measured and interpreted.

4.1 Amount of CO_2 produced

Altogether there were 15 bottles, and each bottle was titrated. The released CO_2 from each bottle was calculated from the titration result and cumulative amount of carbon dioxide per bottle is illustrated in Table 4.1 while the cumulative amount of CO_2 per category is illustrated in Table 4.2.

Table 4.1 Cumulative amount of carbon dioxide per bottle

CUMULATIVE AMOUNT OF CARBON DIOXIDE (g)															
DAYS	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**	**B12**	**B13**	**B14**	**B15**
7	1.14	0.48	0.88	1.87	2.31	2.42	2.86	4.62	4.07	1.50	1.47	1.43	1.14	1.21	1.21
18	1.94	1.36	1.43	5.39	6.12	10.97	5.65	9.32	8.18	2.46	2.42	2.27	1.87	1.98	2.09
30	3.30	3.08	2.71	10.86	11.04	15.51	9.06	12.14	11.11	4.99	4.88	4.55	4.47	4.95	5.28
41	7.78	6.20	5.65	14.67	14.27	18.15	11.77	14.67	15.51	8.00	7.26	7.41	7.30	8.25	7.48
51	9.76	7.79	7.13	18.91	17.57	19.25	12.98	18.58	19.69	11.02	10.56	11.26	10.60	11.55	10.18
62	10.41	8.45	7.74	23.68	21.13	21.78	15.01	21.26	23.75	15.19	14.24	12.96	15.86	13.47	12.26
73	11.18	8.73	8.01	25.54	21.79	22.65	16.27	24.55	26.71	18.86	16.92	13.84	18.93	14.29	14.08
83	11.62	9.05	8.39	27.18	23.37	23.47	19.39	27.45	28.79	21.43	18.23	15.15	20.25	15.39	15.50
93	12.27	9.44	9.76	29.26	25.23	24.57	21.68	29.69	30.65	24.82	20.09	17.50	22.98	17.74	17.25
104	12.49	9.82	10.20	30.68	26.16	25.44	22.99	30.62	31.85	26.68	21.02	19.36	25.16	21.23	19.27

Table 4.2 Cumulative amount of carbon dioxide by category

CUMMULATIVE AMOUNT OF CARBON DIOXIDE BY CATEGORY (g)										
CATEGORY	**7**	**18**	**30**	**41**	**51**	**62**	**73**	**83**	**93**	**104**
A	0.83	1.58	3.03	6.54	8.23	8.87	9.31	9.69	10.49	10.84
B	2.20	7.49	12.47	15.70	18.58	22.20	23.33	24.68	26.35	27.43
C	3.85	7.71	10.77	13.99	17.08	20.01	22.51	25.21	27.34	28.49
D	1.47	2.38	4.80	7.56	10.95	14.13	16.54	18.27	20.80	22.35
E	1.19	1.98	4.90	7.68	10.78	13.87	15.77	17.04	19.32	21.89

A graph showing the cumulative amount of CO_2 is given below in Figure 4.1.

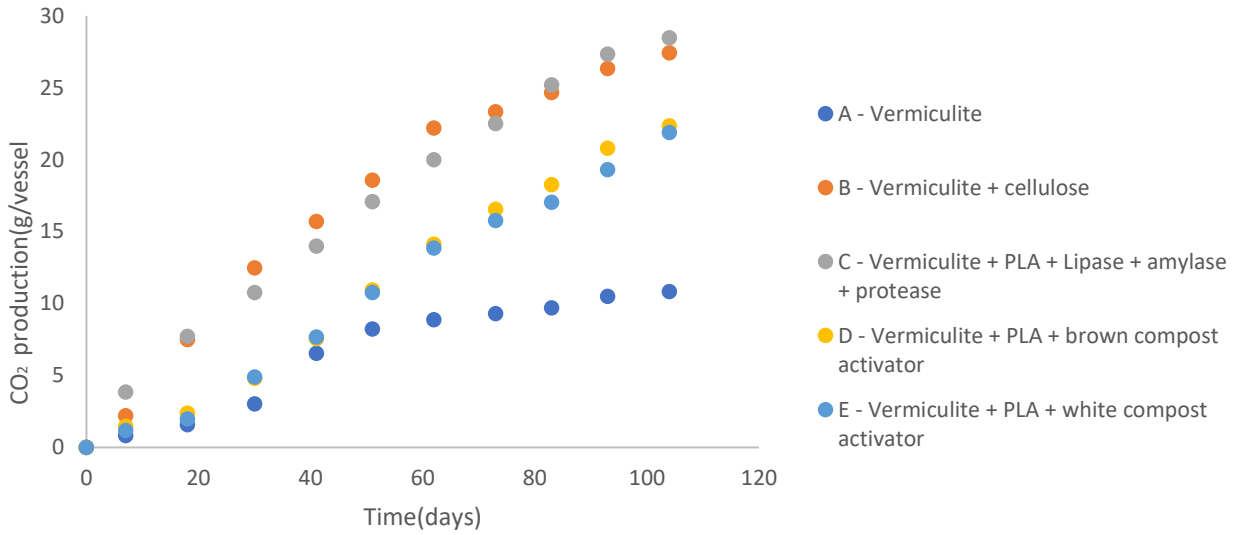

Figure 4.1 CO_2 evolution curve of Categories A, B, C,D and E between 104 days

From day one, the results show that category A(vermiculite-blank) released the lowest amount of carbon dioxide with value 0.83g while category C(vermiculite + plastic + enzymes) released the highest amount with value 3.85g. This trend also was the same at the end of the 104th day. The values of category D and E were relatively close, but category D had the highest value of the two.

4.2 Theoretical amount of carbon dioxide

To calculate the percentage of biodegradation, the theoretical amount of CO_2 was first calculated using equation 3.6 to give 22.8205g for PLA-based as shown in equation 4.1 material and 23.3449g for cellulose material as shown in equation 4.2.

$ThCO_2 = 13.83061 \text{ X } 0.45 \text{ X } \frac{44}{12} = 22.8205g$ equation (4.1)

$ThCO_2 = 14.47 \text{ X } 0.44 \text{ X } \frac{44}{12} = 23.3449g$ equation (4.2)

4.3 Percentage of biodegradation

Table 4.2 shows the percentage of biodegradation and Figure 4.2 shows the biodegradation curve.

Table 4.3 Percentage of degradation

PERCENTAGE OF BIODEGRADATION (%)										
CATEGORY	**7**	**18**	**30**	**41**	**51**	**62**	**73**	**83**	**93**	**104**
B	5.87	25.35	40.43	39.22	44.33	57.09	60.07	64.20	67.95	71.07
C	13.23	26.89	33.91	32.62	38.81	48.82	57.88	68.01	73.84	77.35
D	2.79	3.54	7.77	4.45	11.92	23.05	31.70	37.61	45.19	50.46
E	1.55	1.77	8.20	4.98	11.17	21.90	28.32	32.23	38.69	48.43

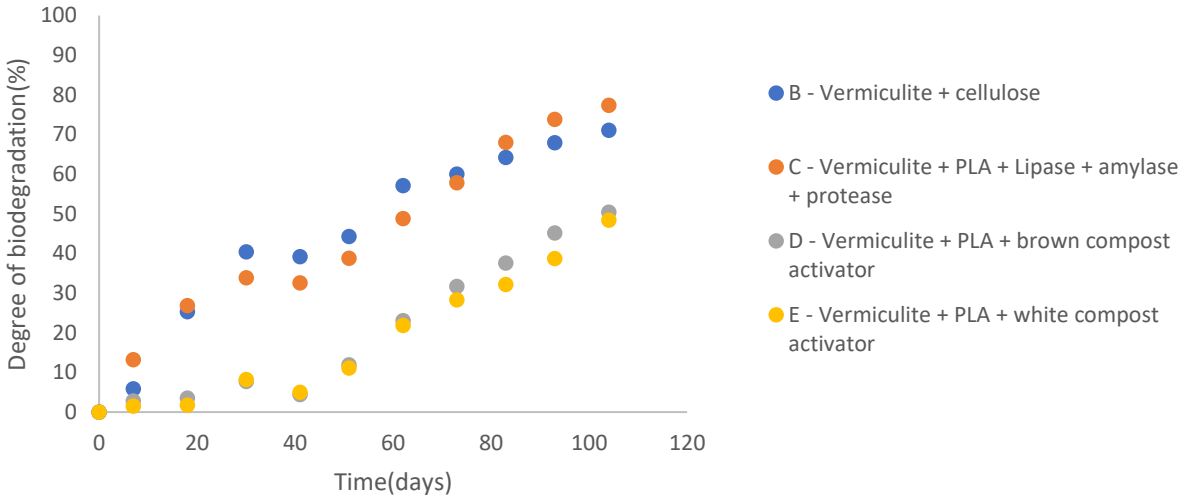

Figure 4.2 Biodegradation curve of Categories A, B, C, D and E between 104 days

The biodegradation curve in Figure 4.2 shows the different trends for category B, C, D, and E as category A is the blank sample. From day 1, category B (vermiculite + cellulose) had the lowest percentage of degradation of 6%. The result shows that category C had the highest degree of degradation followed by category D and then category E.

4.4 Comparison of influence of the catalyst

Out of the three categories which are vermiculite+ PLA-based plastic + crushed lipases + amylase + protease enzymes, vermiculite + PLA-based plastic + brown compost activator and vermiculite + PLA-based plastic + white compost activator, the category with enzymes showed the highest value all round.

In terms of amount of carbon dioxide produced, Category C with vermiculite + PLA-based plastic + crushed lipases + amylase + protease enzymes gave off more CO_2 than the categories with vermiculite + PLA-based plastic + brown compost activator and vermiculite + PLA-based plastic + white compost activator because at the end of the 104 days Category C produced 28.4g of CO_2 while Category D and E produced 22.35g and 21.89g of CO_2 respectively.

The amount of carbon dioxide evolved has effect on the level of degradation. In the same way as the amount of carbon dioxide given off, the category with enzymes had the highest degree of degradation compared to that of the category with compost activator.

Category D and E had close figures in term of amount of carbon dioxide and degree of degradation all through the experiment but there were some abnormalities. From day 7, Category D - vermiculite + PLA-based plastic + brown compost activator had higher value of 1.47g for amount of carbon dioxide evolved and 2.79% degree of degradation compared to that of category E - vermiculite + PLA-based plastic + white compost activator which had 1.19g of CO_2 produced and 1.55% degree of degradation. Category D and E continued the same trend except on the 30^{th} and 41^{st} day, where Category E had higher values of carbon dioxide produced than Category D which were of 4.90g and 7.68g. The same exemption applies to the degree of degradation.

Category B - three bottles of vermiculite + cellulose which is the reference material helps to determine a good material for degradation. Category B in comparison to Category C shows a significant result because at the beginning and the end of the experiment, Category C had higher values of 13.23% and 77.35% in terms of degradation. This result shows that enzymes are indeed a good agent of degradation.

Overall, Category C with the mixture of enzymes lipases, amylase and protease showed higher potential in degrading the sample PLA-based plastic than Category D with brown compost activator than Category E with white compost activator.

Table 4.4 represents the summary of all the results for the amount of materials, total dry solids, cumulative amount of carbon dioxide and percentage of biodegradation.

Table 4.4 Comparison of final result

	CATEGORY B	CATEGORY C	CATEGORY D	CATEGORY E
AMOUNT OF MATERIAL (g)	14.47	13.85	13.85	13.85
TDS (%)	44.00	45.00	45.00	45.00
CUMMULATIVE AMOUNT OF CARBON DIOXIDE (g)	27.43	28.49	22.35	21.89
PERCENTAGE OF BIODEGRADATION (%)	71.07	77.35	50.46	48.43

4.5 Comparison with other studies

This chapter presents a review and comparative analysis of studies that investigate the potential of catalysts in promoting the aerobic biodegradation of plastics. By analysing the methodologies, catalyst types, outcomes, and limitations of these studies, we can gain valuable insights into the current state of research in this field.

A study conducted by Yufang Wu et al.[40], focused on the degradation of polyethylene terephthalate (PET) using a metal-based catalyst in a laboratory-scale experiment. The study was performed under controlled experimental conditions and the degradation process was monitored over a period with had a total yield of 98%.

Seon Yeong Park and Chang Gyun Kim carried out a study on degradation of plastic using bacterial colony [41]. They carried out an experiment where Bacillus and Paenibacillus was introduced to PE microplastics, and the result showed reduction in dry weight of particles by 14.7% after 60days.

Another study by Qian Ying Lee and Hong Li on photocatalytic degradation of plastic shows that sunlight is a natural energy source that can also help in degrading plastics [42]. The research also shows that even though photocatalytic degradation of plastic is possible, it degrades at a slow rate.

All these studies show the potential of catalysts in enhancing degradation rates and inducing structural changes in plastics. It is also important to note that these studies were carried out in small scale laboratory facilities and may not represent surrounding environment.

5 CONCLUSION AND RECOMMENDATION

From the results and calculations, category C showed a more upward trend compared to category D and E. The sample of a mixture of vermiculite, plastic and enzymes produced more CO_2 compared to the other categories with the highest value of 28.49g at the end of the experiment. This same category C also had the highest degradation percentage of approximately 77% and showed more potential in degrading plastic compared to category E and D that had approximate degree of degradation as 50% and 48% respectively. This means that enzymes are a better degrading agent compared to the brown and white compost activator.

There were some irregularities in the result which is due to some of the following reasons:

- Difference in the concentration of the acid used in titration; on the 55^{th} and 66^{th} day - 0.4984M acid was used, on the 76^{th}, 86^{th} and 97^{th} day – 0.4966M and 0.5M was used for the remaining days.
- Parallax error during titration.
- Gas leakage during the experiment.

My recommendation is that more research should be carried out on the quantity of enzymes needed and time required for degradation of plastics. Even though the brown and white compost activators did not show as much efficiency as enzymes, studies should also be carried out on them as they show potential and can serve as an alternative to enzymes.

SUMMARY

Plastics are in high demand because of several reasons such as durability, strength, increasing population etc. This has led to the mass production of plastic which is a huge problem has these plastics have adverse effect on the environment and humans. More sustainable means need to be devised to help manage and curb the situation such introducing the use eco-friendlier products and finding the best means to decompose them. This study aims to combat the later, to investigate the reaction of enzymes and compost activator in the entire process of plastic degradation.

PLA – (Polylactic acid) based plastic was used and the degree of degradation was analysed using different catalysts. There were five different categories namely:

(A) three bottles of vermiculite

(B) three bottles of vermiculite + cellulose

(C) three bottles of vermiculite+ PLA-base plastic + crushed lipases + amylase + protease enzymes

(D) three bottles of vermiculite + PLA-based plastic + brown compost activator

(E) three bottles of vermiculite + PLA-based plastic + white compost activator

Cellulose category was the reference material and plain vermiculite bottles were the blank sample used for calculation. ISO 14855-1:2012 was the basis of the analysis. The titration assessment lasted for 97 days, and the data was recorded and shown in this thesis report.

From all the results and calculations, enzymes show to be a better agent of degradation compared to the other catalysts used i.e., brown and white compost activator.

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APPENDICES

TITRATION RESULT 1									
BOTTLE	RESULT 1A(ml)	RESULT 2A(ml)	RESULT 1B(ml)	RESULT 2B(ml)	RESULT 1C(ml)	RESULT 2C(ml)	AV.1(ml)	AV.2(ml)	AV.2 - AV.1(ml)
1	19.40	20.50	19.30	20.50	19.70	20.50	19.47	20.50	1.03
2	19.90	20.30	19.90	20.30	19.80	20.30	19.87	20.30	0.43
3	19.50	20.20	19.30	20.10	19.30	20.20	19.37	20.17	0.80
4	18.50	20.30	17.30	19.20	18.90	20.30	18.23	19.93	1.70
5	18.30	20.20	18.20	20.40	18.00	20.20	18.17	20.27	2.10
6	17.80	20.40	17.90	20.40	18.80	20.30	18.17	20.37	2.20
7	18.50	20.50	17.50	20.40	17.40	20.30	17.80	20.40	2.60
8	15.80	19.70	15.50	19.90	15.40	19.70	15.57	19.77	4.20
9	16.80	20.50	16.70	20.40	16.90	20.60	16.80	20.50	3.70
10	19.00	20.30	19.20	20.50	19.00	20.50	19.07	20.43	1.37
11	19.10	20.40	18.90	20.20	19.10	20.50	19.03	20.37	1.33
12	19.00	20.30	19.10	20.40	19.10	20.40	19.07	20.37	1.30
13	19.40	20.50	19.30	20.40	19.50	20.40	19.40	20.43	1.03
14	19.30	20.40	19.40	20.50	19.40	20.50	19.37	20.47	1.10
15	19.20	20.40	19.40	20.40	19.40	20.50	19.33	20.43	1.10

TITRATION RESULT 2									
BOTTLE	RESULT 1A(ml)	RESULT 2A(ml)	RESULT 1B(ml)	RESULT 2B(ml)	RESULT 1C(ml)	RESULT 2C(ml)	AV.1(ml)	AV.2(ml)	AV.2 - AV.1(ml)
1	29.70	30.50	29.70	30.50	29.90	30.50	29.77	30.50	0.73
2	29.80	30.60	29.70	30.50	29.80	30.60	29.77	30.57	0.80
3	30.10	30.50	30.00	30.50	29.70	30.30	29.93	30.43	0.50
4	27.20	30.40	27.20	30.40	27.20	30.40	27.20	30.40	3.20
5	27.10	30.50	26.90	30.40	26.90	30.40	26.97	30.43	3.47
6	23.20	30.10	22.00	30.20	22.00	30.20	22.40	30.17	7.77
7	27.90	30.40	28.00	30.50	27.80	30.40	27.90	30.43	2.53
8	26.20	30.50	26.30	30.50	26.20	30.50	26.23	30.50	4.27
9	25.90	29.60	26.00	29.60	25.80	29.70	25.90	29.63	3.73
10	29.70	30.60	29.70	30.60	29.70	30.50	29.70	30.57	0.87
11	29.70	30.50	29.70	30.60	29.70	30.60	29.70	30.57	0.87
12	29.80	30.50	29.70	30.50	29.70	30.50	29.73	30.50	0.77
13	29.90	30.60	30.00	30.70	29.90	30.50	29.93	30.60	0.67
14	29.90	30.60	29.80	30.50	29.90	30.60	29.87	30.57	0.70
15	29.80	30.60	29.90	30.70	29.80	30.60	29.83	30.63	0.80

TITRATION RESULT 3									
BOTTLE	RESULT 1A(ml)	RESULT 2A(ml)	RESULT 1B(ml)	RESULT 2B(ml)	RESULT 1C(ml)	RESULT 2C(ml)	AV.1(ml)	AV.2(ml)	AV.2 - AV.1(ml)
1	29.30	30.60	29.50	30.60	29.20	30.50	29.33	30.57	1.23
2	29.00	30.60	29.10	30.60	29.00	30.60	29.03	30.60	1.57
3	28.50	29.70	28.50	29.60	28.40	29.60	28.47	29.63	1.17
4	25.30	30.30	25.40	30.40	25.50	30.40	25.40	30.37	4.97
5	25.50	30.10	25.70	30.10	25.60	30.00	25.60	30.07	4.47
6	25.00	29.00	24.90	29.00	24.60	28.90	24.83	28.97	4.13
7	27.00	30.10	26.90	30.10	27.00	30.00	26.97	30.07	3.10
8	28.10	30.60	28.00	30.60	27.90	30.50	28.00	30.57	2.57
9	27.90	30.60	27.90	30.50	27.90	30.60	27.90	30.57	2.67
10	28.40	30.70	28.30	30.60	28.30	30.60	28.33	30.63	2.30
11	28.40	30.50	28.40	30.60	28.20	30.60	28.33	30.57	2.23
12	28.50	30.60	28.50	30.50	28.30	30.40	28.43	30.50	2.07
13	28.20	30.50	28.10	30.50	28.10	30.50	28.13	30.50	2.37
14	27.80	30.40	27.80	30.50	27.60	30.40	27.73	30.43	2.70
15	27.90	30.60	27.50	30.60	0.00	0.00	27.70	30.60	2.90

TITRATION RESULT 4									
BOTTLE	RESULT 1A(ml)	RESULT 2A(ml)	RESULT 1B(ml)	RESULT 2B(ml)	RESULT 1C(ml)	RESULT 2C(ml)	AV.1(ml)	AV.2(ml)	AV.2 - AV.1(ml)
1	24.10	28.20	24.20	28.30	24.20	28.20	24.17	28.23	4.07
2	28.50	31.30	28.00	30.80	28.00	30.90	28.17	31.00	2.83
3	27.30	30.00	27.30	30.00	27.40	30.00	27.33	30.00	2.67
4	27.50	31.00	27.50	31.00	27.60	31.00	27.53	31.00	3.47
5	27.60	30.50	27.60	30.60	27.60	30.50	27.60	30.53	2.93
6	27.60	30.00	27.50	29.90	27.60	30.00	27.57	29.97	2.40
7	27.70	30.10	27.70	30.20	27.70	30.20	27.70	30.17	2.47
8	28.50	31.00	28.30	31.00	28.20	29.90	28.33	30.63	2.30
9	27.10	31.00	27.00	31.00	26.80	30.90	26.97	30.97	4.00
10	28.40	31.10	28.40	31.10	28.30	31.10	28.37	31.10	2.73
11	29.00	31.10	28.70	30.90	28.60	30.80	28.77	30.93	2.17
12	28.20	30.70	28.00	30.60	27.90	30.60	28.03	30.63	2.60
13	28.10	30.60	28.00	30.60	28.00	30.60	28.03	30.60	2.57
14	27.60	30.60	27.70	30.70	27.60	30.60	27.63	30.63	3.00
15	28.70	30.70	28.70	30.70	28.60	30.60	28.67	30.67	2.00

TITRATION RESULT 5							
BOTTLE	**RESULT 1A(ml)**	**RESULT 2A(ml)**	**RESULT 1B(ml)**	**RESULT 2B(ml)**	**AV.1(ml)**	**AV.2(ml)**	**AV.2 - AV.1(ml)**
1	28.50	30.30	28.60	30.40	28.55	30.35	1.80
2	28.90	30.40	29.00	30.40	28.95	30.40	1.45
3	29.10	30.40	29.00	30.40	29.05	30.40	1.35
4	26.10	29.90	26.00	29.90	26.05	29.90	3.85
5	26.70	29.70	26.80	29.80	26.75	29.75	3.00
6	28.40	29.40	28.40	29.40	28.40	29.40	1.00
7	28.80	29.90	28.70	29.80	28.75	29.85	1.10
8	26.90	30.40	26.80	30.40	26.85	30.40	3.55
9	26.50	30.30	26.40	30.20	26.45	30.25	3.80
10	27.80	30.50	27.70	30.50	27.75	30.50	2.75
11	27.30	30.30	27.30	30.30	27.30	30.30	3.00
12	26.80	30.30	26.80	30.30	26.80	30.30	3.50
13	27.30	30.30	27.20	30.20	27.25	30.25	3.00
14	27.50	30.50	27.50	30.50	27.50	30.50	3.00
15	27.60	30.10	27.60	30.00	27.60	30.05	2.45

TITRATION RESULT 6							
BOTTLE	**RESULT 1A(ml)**	**RESULT 2A(ml)**	**RESULT 1B(ml)**	**RESULT 2B(ml)**	**AV.1(ml)**	**AV.2(ml)**	**AV.2 - AV.1(ml)**
1	29.70	30.30	29.50	30.10	29.60	30.20	0.60
2	29.60	30.20	29.60	30.20	29.60	30.20	0.60
3	29.60	30.20	29.60	30.10	29.60	30.15	0.55
4	25.10	29.60	25.30	29.50	25.20	29.55	4.35
5	26.60	29.80	26.50	29.80	26.55	29.80	3.25
6	27.70	30.00	27.80	30.10	27.75	30.05	2.30
7	28.10	29.90	28.10	30.00	28.10	29.95	1.85
8	27.20	29.60	27.10	29.60	27.15	29.60	2.45
9	26.30	29.90	26.10	29.90	26.20	29.90	3.70
10	26.30	30.00	26.00	29.90	26.15	29.95	3.80
11	26.80	30.10	26.70	30.10	26.75	30.10	3.35
12	28.30	29.80	28.20	29.80	28.25	29.80	1.55
13	25.30	30.10	25.40	30.20	25.35	30.15	4.80
14	28.50	30.30	28.60	30.30	28.55	30.30	1.75
15	28.40	30.30	28.30	30.20	28.35	30.25	1.90

TITRATION RESULT 7							
BOTTLE	**RESULT 1A(ml)**	**RESULT 2A(ml)**	**RESULT 1B(ml)**	**RESULT 2B(ml)**	**AV.1(ml)**	**AV.2(ml)**	**AV.2 - AV.1(ml)**
1	29.50	30.10	29.30	30.10	29.40	30.10	0.70
2	30.00	30.30	30.00	30.20	30.00	30.25	0.25
3	29.70	29.90	29.60	29.90	29.65	29.90	0.25
4	27.70	29.40	27.70	29.40	27.70	29.40	1.70
5	29.60	30.20	29.60	30.20	29.60	30.20	0.60
6	29.40	30.10	29.20	30.10	29.30	30.10	0.80
7	29.00	30.10	29.00	30.20	29.00	30.15	1.15
8	27.10	30.10	26.90	29.90	27.00	30.00	3.00
9	27.30	30.00	27.30	30.00	27.30	30.00	2.70
10	26.80	30.10	26.80	30.20	26.80	30.15	3.35
11	27.70	30.10	27.70	30.20	27.70	30.15	2.45
12	29.20	30.00	29.40	30.20	29.30	30.10	0.80
13	27.20	30.00	27.20	30.00	27.20	30.00	2.80
14	29.40	30.10	29.30	30.10	29.35	30.10	0.75
15	28.90	30.60	29.00	30.60	28.95	30.60	1.65

TITRATION RESULT 8							
BOTTLE	**RESULT 1A(ml)**	**RESULT 2A(ml)**	**RESULT 1B(ml)**	**RESULT 2B(ml)**	**AV.1(ml)**	**AV.2(ml)**	**AV.2 - AV.1(ml)**
1	29.80	30.20	29.70	30.10	29.75	30.15	0.40
2	30.00	30.30	29.90	30.20	29.95	30.25	0.30
3	30.00	30.30	29.80	30.20	29.90	30.25	0.35
4	28.60	30.10	28.60	30.10	28.60	30.10	1.50
5	28.90	30.40	28.90	30.30	28.90	30.35	1.45
6	29.00	29.80	29.00	29.70	29.00	29.75	0.75
7	27.40	30.20	27.40	30.30	27.40	30.25	2.85
8	27.80	30.40	27.50	30.20	27.65	30.30	2.65
9	28.50	30.40	28.30	30.20	28.40	30.30	1.90
10	28.00	30.40	28.00	30.30	28.00	30.35	2.35
11	28.40	29.60	28.40	29.60	28.40	29.60	1.20
12	28.90	30.10	29.00	30.20	28.95	30.15	1.20
13	29.10	30.30	29.10	30.30	29.10	30.30	1.20
14	29.30	30.30	29.40	30.40	29.35	30.35	1.00
15	29.00	30.30	29.00	30.30	29.00	30.30	1.30

TITRATION RESULT 9							
BOTTLE	**RESULT 1A(ml)**	**RESULT 2A(ml)**	**RESULT 1B(ml)**	**RESULT 2B(ml)**	**AV.1(ml)**	**AV.2(ml)**	**AV.2 - AV.1(ml)**
1	19.80	20.10	19.40	20.30	19.60	20.20	0.60
2	19.80	20.20	19.90	20.20	19.85	20.20	0.35
3	18.80	20.00	18.90	20.20	18.85	20.10	1.25
4	18.20	20.10	18.30	20.20	18.25	20.15	1.90
5	18.50	20.20	18.50	20.20	18.50	20.20	1.70
6	19.20	20.20	19.20	20.20	19.20	20.20	1.00
7	17.20	19.20	17.10	19.30	17.15	19.25	2.10
8	18.10	20.10	18.10	20.20	18.10	20.15	2.05
9	18.50	20.20	18.50	20.20	18.50	20.20	1.70
10	18.00	20.20	16.30	20.30	17.15	20.25	3.10
11	18.50	20.20	18.70	20.40	18.60	20.30	1.70
12	18.10	20.20	18.00	20.20	18.05	20.20	2.15
13	16.30	18.80	16.40	18.90	16.35	18.85	2.50
14	20.50	22.60	20.50	22.70	20.50	22.65	2.15
15	18.70	20.30	18.70	20.30	18.70	20.30	1.60

TITRATION RESULT 10							
BOTTLE	**RESULT 1A(ml)**	**RESULT 2A(ml)**	**RESULT 1B(ml)**	**RESULT 2B(ml)**	**AV.1(ml)**	**AV.2(ml)**	**AV.2 - AV.1(ml)**
1	19.90	20.10	19.90	20.10	19.90	20.10	0.20
2	19.80	20.10	19.80	20.20	19.80	20.15	0.35
3	19.80	20.20	19.70	20.10	19.75	20.15	0.40
4	18.90	20.00	18.70	20.20	18.80	20.10	1.30
5	19.30	20.10	19.30	20.20	19.30	20.15	0.85
6	19.30	20.10	19.50	20.30	19.40	20.20	0.80
7	18.10	19.30	18.20	19.40	18.15	19.35	1.20
8	19.20	20.10	19.30	20.10	19.25	20.10	0.85
9	19.00	20.10	19.10	20.20	19.05	20.15	1.10
10	18.20	19.90	18.40	20.10	18.30	20.00	1.70
11	19.30	20.10	19.20	20.10	19.25	20.10	0.85
12	17.40	19.00	17.30	19.10	17.35	19.05	1.70
13	18.20	20.20	18.30	20.30	18.25	20.25	2.00
14	17.10	20.30	17.20	20.40	17.15	20.35	3.20
15	18.50	20.30	18.50	20.40	18.50	20.35	1.85

AMOUNT OF CARBON DIOXIDE(mg)										
BOTTLE	**DAY 7**	**DAY 18**	**DAY 30**	**DAY 41**	**DAY 51**	**DAY 62**	**DAY 73**	**DAY 83**	**DAY 93**	**DAY 104**
1	1136.93	806.85	1356.98	4474.35	1980.45	658.04	767.71	437.11	655.66	218.55
2	476.78	880.20	1723.73	3117.38	1595.36	658.04	274.18	327.83	382.47	382.47
3	880.20	550.13	1283.63	2934.00	1485.34	603.20	274.18	382.47	1365.96	437.11
4	1870.43	3520.80	5464.57	3814.20	4235.96	4770.77	1864.44	1639.15	2076.26	1420.60
5	2310.53	3814.20	4914.45	3227.40	3300.75	3564.37	658.04	1584.51	1857.71	928.85
6	2420.55	8545.28	4547.70	2640.60	1100.25	2522.48	877.38	819.58	1092.77	874.21
7	2860.65	2787.30	3410.78	2713.95	1210.28	2028.95	1261.24	3114.39	2294.81	1311.32
8	4621.05	4694.40	2823.98	2530.58	3905.89	2686.99	3290.19	2895.84	2240.18	928.85
9	4070.93	4107.60	2934.00	4401.00	4180.95	4057.90	2961.17	2076.26	1857.71	1202.05
10	1503.68	953.55	2530.58	3007.35	3025.69	4167.57	3674.04	2568.01	3387.58	1857.71
11	1467.00	953.55	2457.23	2383.88	3300.75	3674.04	2686.99	1311.32	1857.71	928.85
12	1430.33	843.52	2273.85	2860.65	3850.88	1699.93	877.38	1311.32	2349.45	1857.71
13	1136.93	733.50	2603.93	2823.98	3300.75	5264.30	3070.84	1311.32	2731.92	2185.54
14	1210.28	770.18	2970.68	3300.75	3300.75	1919.28	822.55	1092.77	2349.45	3496.86
15	1210.28	880.20	3190.73	2200.50	2695.61	2083.79	1815.41	1420.60	1748.43	2021.62

AMOUNT OF CARBON DIOXIDE(g)										
BOTTLE	**DAY 7**	**DAY 18**	**DAY 30**	**DAY 41**	**DAY 51**	**DAY 62**	**DAY 73**	**DAY 83**	**DAY 93**	**DAY 104**
1	1.1369	0.8069	1.3570	4.4744	1.9805	0.6580	0.7677	0.4371	0.6557	0.2186
2	0.4768	0.8802	1.7237	3.1174	1.5954	0.6580	0.2742	0.3278	0.3825	0.3825
3	0.8802	0.5501	1.2836	2.9340	1.4853	0.6032	0.2742	0.3825	1.3660	0.4371
4	1.8704	3.5208	5.4646	3.8142	4.2360	4.7708	1.8644	1.6392	2.0763	1.4206
5	2.3105	3.8142	4.9144	3.2274	3.3008	3.5644	0.6580	1.5845	1.8577	0.9289
6	2.4206	8.5453	4.5477	2.6406	1.1003	2.5225	0.8774	0.8196	1.0928	0.8742
7	2.8607	2.7873	3.4108	2.7140	1.2103	2.0289	1.2612	3.1144	2.2948	1.3113
8	4.6211	4.6944	2.8240	2.5306	3.9059	2.6870	3.2902	2.8958	2.2402	0.9289
9	4.0709	4.1076	2.9340	4.4010	4.1810	4.0579	2.9612	2.0763	1.8577	1.2020
10	1.5037	0.9536	2.5306	3.0074	3.0257	4.1676	3.6740	2.5680	3.3876	1.8577
11	1.4670	0.9536	2.4572	2.3839	3.3008	3.6740	2.6870	1.3113	1.8577	0.9289
12	1.4303	0.8435	2.2739	2.8607	3.8509	1.6999	0.8774	1.3113	2.3495	1.8577
13	1.1369	0.7335	2.6039	2.8240	3.3008	5.2643	3.0708	1.3113	2.7319	2.1855
14	1.2103	0.7702	2.9707	3.3008	3.3008	1.9193	0.8225	1.0928	2.3495	3.4969
15	1.2103	0.8802	3.1907	2.2005	2.6956	2.0838	1.8154	1.4206	1.7484	2.0216