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

ANAEROBIC DIGESTION OF MODIFIED BIOPLASTICS

MODIFITSEERITUD BIOPLASTIDE ANAEROOBNE KOMPOSTIMINE

MASTER THESIS

Student:	Oluwaseun Omowumi Olawoyin
Student code:	195453EABM
Supervisor:	Dr. Argo Kuusik (Researcher)
Co-Supervisor:	Pavlo Lyshtva (PhD student)

Tallinn 2021

AUTHOR’S DECLARATION

Hereby I declare, that I have written this thesis independently.

No academic degree has been applied for based on this material. All works, major viewpoints and data of the other authors used in this thesis have been referenced.

“.......” .... 20…..

Author: ....

/signature /

Thesis is in accordance with terms and requirements

“.......” .... 20….

Supervisor: ….........................

/signature/

Accepted for defence

“.......”....................20… .

Chairman of theses defence commission: ....

/name and signature/

Non-exclusive Licence for Publication and Reproduction of GraduationTthesis[1]

I, Oluwaseun Omowumi Olawoyin (date of birth: 01/10/1984) hereby

1. grant Tallinn University of Technology (TalTech) a non-exclusive license for my thesis Anaerobic digestion of modified bioplastics supervised by Dr. Argo Kuusik

1.1 reproduced for the purposes of preservation and electronic publication, incl. to be entered in the digital collection of TalTech library until expiry of the term of copyright;

1.2 published via the web of TalTech, incl. to be entered in the digital collection of TalTech library until expiry of the term of copyright.

1.3 I am aware that the author also retains the rights specified in clause 1 of this license.

2. I confirm that granting the non-exclusive license does not infringe third persons' intellectual property rights, the rights arising from the Personal Data Protection Act or rights arising from other legislation.

---

[1] *Non-exclusive Licence for Publication and Reproduction of Graduation Thesis is not valid during the validity period of restriction on access, except the university`s right to reproduce the thesis only for preservation purposes.*

______________ (*signature*)

______________ (*date*)

Department of Civil Engineering and Architecture

THESIS TASK

Student: Oluwaseun Omowumi Olawoyin 195453EABM

Study programme: EABM03/18- Environmental Engineering and Management

Supervisor: Researcher, Taltech. Dr. Argo Kuusik, +37253909716, argo.kuusik@ttu.ee

Co-supervisor: Ph.D. student, TalTech. Pavlo Lyshtva, +37258055196 pavlo.lyshtva@taltech.ee

Thesis topic:

(in English) Anaerobic digestion of modified bioplastics

(in Estonian) Modifitseeritud bioplastide anaeroobne kompostimine

Thesis main objectives:

1. Literature review
2. Setup and development of anaerobic digestion system according to standard
3. Analysis of biodegradability of materials

Thesis tasks and time schedule:

No	Task description	Deadline
1.	Introduction, aim of thesis and literature review	31/03/2021
2.	Data collation (measurements) and analysis	18/06/2021
3.	Results discussion, methodology, and review	02/07/2021

Language: ………… **Deadline for submission of thesis:** "……."………2021

Student: Oluwaseun Omowumi Olawoyin "……."……………………………2021

/signature/

Supervisor: Dr Argo Kuusik"......."...2021

/signature/

Co- Supervisor: Pavlo Lyshtva .... "......."....................2021

/signature/

Head of study programme: Prof Karin Pachel................... "...."...............2021

/signature/

Terms of thesis closed defence and/or restricted access conditions to be formulated on the reverse side

Table of Contents

PREFACE 8

LIST OF ABBREVIATIONS AND SYMBOLS 9

1 INTRODUCTION 11

2 LITERATURE REVIEW 12

- 2.1 Overview of traditional plastics 12
  - 2.1.1 Reasons for growth and benefits ---------- 13
  - 2.1.2 Application of plastics ---------- 14
  - 2.1.3 Production amounts ---------- 14
  - 2.1.4 Disadvantages of the use of plastics ---------- 16
  - 2.1.5 Legislations ---------- 16
- 2.2 Review of bioplastics 17
  - 2.2.1 Description, classification, and production ---------- 17
  - 2.2.2 Polylactic Acid (PLA) ---------- 20
  - 2.2.3 Polycaprolactone (PCL) ---------- 22
  - 2.2.4 Poly(butylene) Succinate (PBS) ---------- 23
- 2.3 Biodegradation of plastic materials 24
  - 2.3.1 Aerobic biodegradation ---------- 25
  - 2.3.2 Anaerobic digestion (AD) ---------- 27

3 MATERIALS AND METHODS 32

- 3.1 Biodegradation Measurement based on ISO 15985:2017 32
  - 3.1.1 Description of the testing process ---------- 32
  - 3.1.2 Materials preparation ---------- 34
- 3.2 Analytical methods 35
  - 3.2.1 AMPTS II ---------- 36
  - 3.2.2 Titration ---------- 37
- 3.3 Checks and measurements 37
- 3.4 Equations for calculations 37

4 RESULTS ANALYSIS 41

- 4.1 Titration results 41
- 4.2 Biogas results 44

4.3 Biodegradability calculation and results 49
5 SUMMARY 51
6 LIST OF REFERENCES 53

PREFACE

The proliferation of plastics has significantly eroded environmental quality. The improvement and preservation of this quality have become a significant research area and study in recent times. Plastics which is one of the significant contributors to environmental pollution have become a part of mans' everyday life with exposure ranging from birth to death hence the need for a replacement that is not just non-toxic and environmentally friendly from the cradle to the grave, but has similar characteristics and can perform similar functions.

To provide a feasible solution to the plastics menace, BIO-PLASTICS EUROPE, an organization at the front line of developing sustainable bio-based and biodegradable plastics that can replace conventional plastics, has produced modified bioplastics sent for examination. Testing the properties, especially the behavior at the end of life of these modified plastics at a laboratory scale, is the aim of this thesis.

This thesis topic was initiated as an additional testing method for the ongoing project testing on aerobic testing of biodegradable polymers by Dr. Victoria Voronova, the lecturer in charge of the BIO-PLASTICS EUROPE project TALTECH. This testing (anaerobic digestion) idea came up because these modified plastics may end up in systems with no oxygen, and there may be a need for recycling or energy recovery. The testing process and setup were perfected by Pavlo Lyshtva while assistance on the data collection, analysis, and interpretation of results was done by my supervisor Dr. Argo Kuusik.

The laboratory-scale experiment simulating the anaerobic environment performed on these modified bioplastics is necessary to determine their biodegradability before they are used for commercial and other purposes. This thesis is based on the experiment performed by anaerobic digestion of these modified bioplastics under controlled conditions, and it also includes the description of processes and the methods of calculating the biodegradability of these materials. The information obtained from the testing processes will help determine if the presented materials should be further modified and the type of recycling or end-of-life method appropriate for such product.

PLASTICS, BIOPLASTICS, ANAEROBIC TESTING, BIODEGRADABILITY, MASTER THESIS.

LIST OF ABBREVIATIONS AND SYMBOLS

AD	anaerobic digestion
AMPTS II	automatic methane potential testing system II
BDO	1-4 butanediol
BOD	biological oxygen demand
C	carbon
$C_6H_{12}O_6$	glucose
$CH_3CH_2CH_2COOH$	butyric acid
CH_4	methane
CHP	combined heat and power
CO_2	carbon dioxide
COD	chemical oxygen demand
EP	epoxides
EPS	expanded polystryene
EU	European union
H_2	hydrogen
H_2CO_3	carbonic acid
H_2O	water
H_2S	hydrogen sulphide
HCl	hydrochloric acid
K	potassium
K_2CO_3	potassim carbonate
KCl	potassium chloride
$KHCO_3$	potassium bicarbonate
KOH	potassium hydroxide
M_W	molecular weight
MSW	municipal solid waste
N and N_2	nitrogen
NaOH	sodium hydroxide
NH_3	ammonia
NO_2	nitrogen dioxide
O_2	oxygen
OFMSW	organic fraction of municipal solid waste
P	phosphorus
PA	polyamide

PBAT	polybutylene adipate terephtalate
PBT	polybutylene terephtalate
PBS	polybutylene succinate
PC	polycarbonate
PCL	polycaprolactone
PE	polyethylene
PEEK	polyether ether ketone
PET	polyethylene terephtalate
PHA	polyhydroxyalkanoates
PLA	polylactic acid
PP	polypropylene
PS	polystyrene
PTT	polytrimethylene terephthalate
PUR	polyurethane
PVC	polyvinyl chloride
S	sulphur
SA	succinic acid
SO_2	sulphur dioxide
ThOD	theoretical oxygen demand
TDS	total dry solids
TFS	total fixed solids
TS	total solids
VS	volatile solids

1 INTRODUCTION

Mass production of plastics began in the 1950s, and since then, the world without this polymer and its products seem unthinkable. The various properties of the plastic and its ability to produce different polymers with specified properties when enhanced by mixing with other monomers give it an edge of desirability. Plastics and its' enhanced or mixed polymers have found varying applications in diverse production sectors: from the production of packaging materials to automobile parts, medical applications, and fashion materials. The vast application has made the products pervasive such that man's exposure is from birth to death.

The market with the largest share of demand and usage of plastics is the single-use(packaging) market, i.e., the products used once and disposed of or discarded. This disposal has resulted in a large number of plastic materials finding their way into the environment. Hence, debris of plastics from wastes can be found on land, fresh and seawater, and air. This prevalence has led to a reduction in environmental quality, accumulation of solid waste, leaching of toxic chemicals present in plastics into the environment, and eventual effect on human and animal health.

Demand for a replacement for this polymer with excellent characteristics but has become a menace resulted in the production of bioplastics. Bioplastics can be defined by either the material from which they are formed(bio-based) or whether they decompose biologically(biodegradable). Nevertheless, the best type of bioplastics is the one that is both bio-based and biodegradable because, at production and end of life, it does not put much pressure on environmental quality and human health.

Bioplastics have begun to take some percentage of the plastics market. Therefore, the need to analyze their end-of-life properties, especially biodegradability.

This study examines the biodegradability of presented bioplastics in anaerobic conditions compared with reference material tested over time.

2 LITERATURE REVIEW

2.1 Overview of traditional plastics

Plastics are materials manufactured from the synthesis of different naturally occurring or man-made monomers. These monomers are particularly from non-renewable resources, especially hydrocarbons, coal, and natural gas. Plastics have become an essential part of today's society because of their range of applications, which is evident in every phase and aspect of human life due to their desirable physical and mechanical properties ranging from production cost and processing, versatility, and durability, weight, and, lifespan [1]. They can be configured into virtually any preferred shape through various methods such as rotation, injection, extrusion, compression, blowing, or thermo-forming. Their material properties are adjusted and enhanced by adding antioxidants and stabilizers during or after synthesis to achieve the desired color, opacity, strength, permeability, and porosity [2].

The global production rate of plastics has increased over the years from 2 million tonnes produced in 1950 to about 368 million tonnes in 2019 [3,4]. This growing plastics industry is one of the largest areas of crude oil usage [5], with the essential monomers produced from cracked or distilled crude oil. It has outpaced other manufactured materials industries. Most of the plastics produced from fossil hydrocarbon are not biodegradable and tend to stay in their disposed environment or are carried by elements into other environments where they stay for a long time, sometimes for fifty years or even a century, depending on which type of plastic [6–8].

As the plastics production and usage industry grows, the challenge of proper disposal of waste plastics also increases. Improper disposal has led to the leaking of this recalcitrant waste into water bodies, where they are eroded and broken down into smaller pieces and microplastics. These microplastics may be ingested by marine invertebrates and higher animals which causes bioaccumulation when such animals are consumed, resulting in sicknesses and diseases. Also, these microplastics are difficult to trace and clean up from the water bodies [9,10].

The impact of plastics from production to end of life includes

- green-house gas emissions during production and incineration (at disposal),
- residence time in the environment where it contributes to solid parts of MSW and land resource depletion,

- non-renewable resource depletion during extraction and manufacturing are significant concerns and reasons for seeking alternatives to conventional plastics [11].

The plastic family is a large family grouped into two extensive families (types) and other family types. The two major types are:

- Thermoplastics: These are called reversible plastics because they can be heated, melted, and re-molded as many times as possible. This unique characteristic is the reason for the name. They include Polyethylene Terephthalate (PET), Polyvinyl Chloride (PVC), Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polyether ether ketone (PEEK), Expanded Polystyrene (EPS) and, a host of others [12].
- Thermosets: These are the plastics family formed from cross-linking chemical reactions that result in parts that cannot be melted. These reactions cause the parts to have a permanent shape when heated to high temperatures. Thus, they decompose instead of melting [13]. They include Polyurethane (PUR), Epoxides (EP), Silicone, Vinyl Esters, Phenols (formaldehyde resins), Unsaturated polyester resins (UP), and others.

The other family types are bio-based plastics, biodegradable plastics, engineering plastics, epoxy resins, fluoropolymers, polyolefins [12].

2.1.1 Reasons for growth and benefits

The Plastics industry has experienced growth in leaps and bounds over the decades because of the following reasons:

- The dependence on Petroleum(feedstock), which is readily available.
- Broad and pervasive research.
- Various peculiar characteristics of plastic materials have made them famous, easy to work with, and desirable. These peculiarities include: cheap cost of production, lightweight, low density hence less energy needed for transportation, ability to cast and mold into different shapes and frames, production into different colors, some are recyclable (thermoplasts), excellent thermal and electrical insulation properties, varying degrees of resistance to chemicals and solvents [14].

2.1.2 Application of plastics

Plastics have a wide range of applications. Usage of which determines if it should be modified with additives. The uses are but are not limited to

- Electrical application, i.e., electrical and electronics.
- Building and construction, including housing and wiring installations.
- Packaging purposes
- Automotive: furniture fittings for domestic, commercial, automobile furniture purposes
- Medical applications: syringes, catheters, blood, and fluid bags.
- Agriculture: fishing nets, containers for artificial ponds, handles of farming tools.
- Recreational: sports industry (sporting gear and equipment).
- Fashion industry; shoe soles, boots, upper parts of shoes, bags, rain wears, visible clothing production.
- Plastics are also used to produce children's toys and accessories, children playing surfaces [3,14–16].

2.1.3 Production amounts

The amount of plastic and by-products produced since 1950 has grown since it was discovered [17]. This growth is shown in figure 2.1 below.

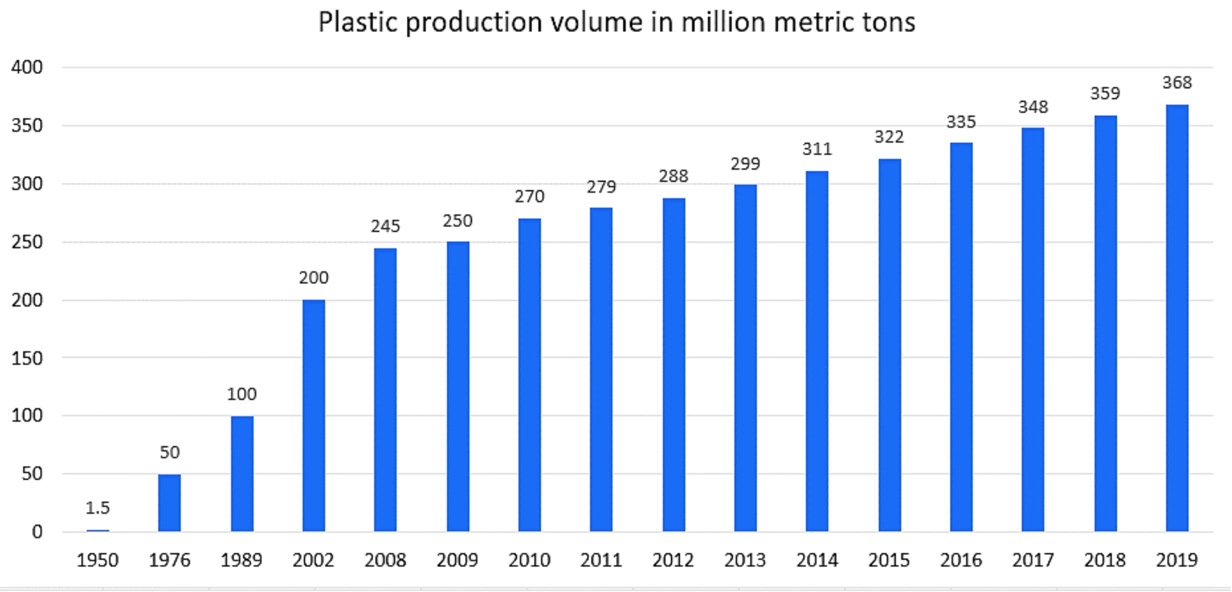


Figure 2.1 Growth in the plastics industry from 1950-2019

Different countries and regions of the world contribute to the production quota of the plastic market, as seen in figure 2.2 below. The Asia region has 51%, from which China is the major contributor here at 31%, while Japan and other Asian countries contributed 3% and 17% respectively [3].

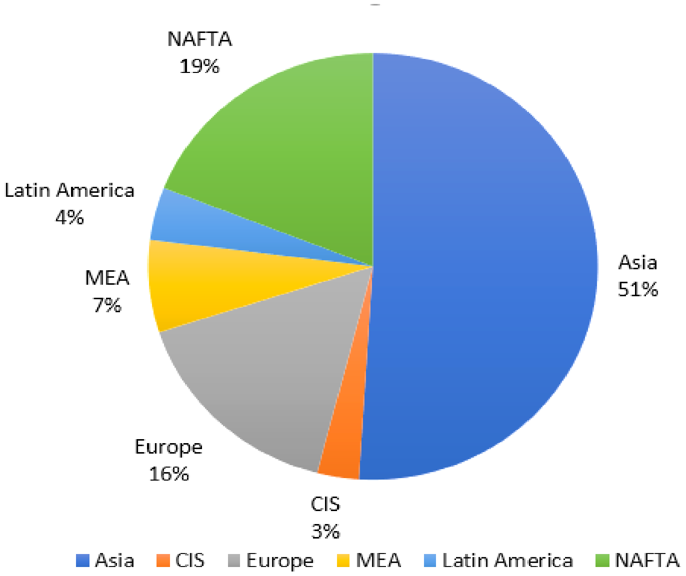


Figure 2.2. The different regions of the world and their plastic production figures [3]

In Europe, the high demand for plastics in the packaging industry can be seen in figure 2.3, with about 40% usage in this sector.

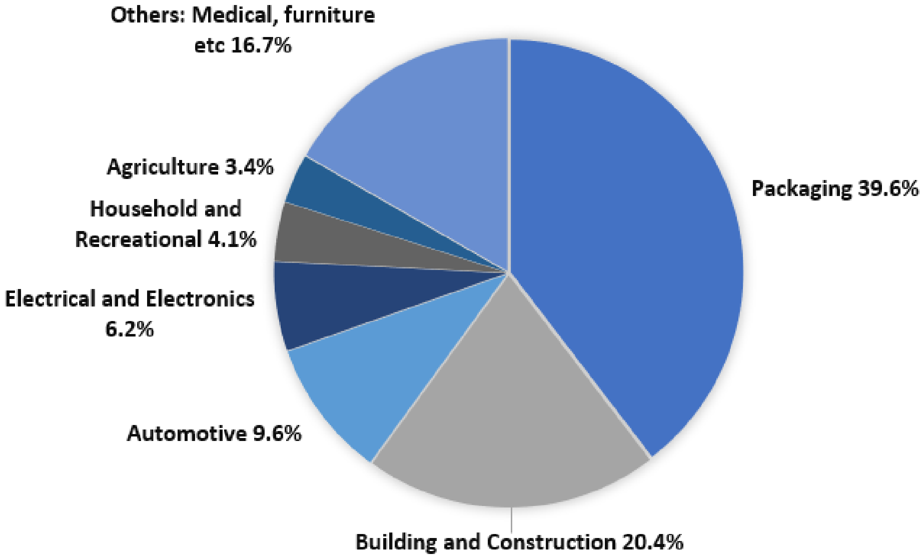


Figure 2.3. Demand for plastics produced in Europe [3]

This usage, as shown above, is reflected in the number of wastes generated from this area which is about 61% of total wastes generated, while just about 42% of this packaging waste was recycled in 2019, leaving the remaining percentage disposed of through other means. Plastic wastes generated are subjected to three main management methods during their end-of-life stage. These methods are recycling (mechanical recycling, dissolution, and chemical recycling), energy recovery (Alternate fuels, incineration with energy recovery), and landfill [7].

2.1.4 Disadvantages of the use of plastics

Despite the numerous unique qualities and advantages that plastics have, not forgetting the range of applications of plastic products, the consequences of rising demand and use of these polymers on the environment have become evident over the years. These effects include:

- Limited non-renewable resources.
- Persistence in the environment after disposal.
- Harmful effects on human health caused by gradual deposition of poisonous ingredients in the human body due to exposures to plastics from time of birth to death. This has led to health issues like aggressive behavior, early maturation, cancers.
- Undue use of land which should be used for agriculture in case of landfilling at disposal.
- Plastic wastes break down into different chemicals when they are disposed of at the landfill. Leachates from the dump may escape into groundwater and cause further pollution.
- Extra costs incurred from sorting during recycling.
- Leakage into water bodies resulting in clogging of waterways. The breakdown of plastics into smaller sizes that are ingested by marine animals is risky for the animals and leads to the bioaccumulation of toxic plastic materials in the food chain [18-20].

2.1.5 Legislations

Due to the proliferation and recalcitrance of these polymers, The European Union (EU) has set laws in place to deal with and manage the demand, usage, and disposal of plastics. The laws also highlight areas to focus on to make the environment sustainable. The EU legislation includes directives such as EU’s directive on single-use plastics, EU packaging and packaging waste directives, EU's strategy for plastics in a circular economy, and the

Directive of the EU 2019/904 on reducing the impact of certain plastics on the environment[21-22].
These directives highlight and focuses on:

- Design and production of plastics and their products with reusable, repairable, and recyclable materials and keeping the entire lifecycle of the products in mind at the time of design. The need for the development and promotion of sustainable materials.
- Discouragement of single-use plastics by promoting non-toxic, reusable products and re-use systems.
- Reduction of marine litter and prevention of dumping of wastes and other materials on the lands and oceans by promoting efficient and environmentally sound waste management services.
- Prohibition of placement on the market of single plastics that have suitable and sustainable substitutes.
- Consumer information and education on markings present on products containing plastics to avoid indiscriminate and improper disposal of waste.
- Improving the responsibility of the producers to cover costs of recycling and disposal [21-22].

2.2 Review of bioplastics

2.2.1 Description, classification, and production

The numerous challenges associated with conventional polymers significantly impacts the environment during its life cycle, finite availability and decline of fossil resources, damage to human health due to the varying degrees of exposure, and other damages done to water bodies and the animals in them have necessitated the research for suitable, environmentally friendly, and sustainable alternative [16]. This alternative is bioplastics. They are gotten from renewable resources and become the organic fraction of municipal solid waste when they degrade without leaving toxic residues [23].

Bioplastics are plastic materials that are either (partly or wholly) bio-based or biodegradable or have both properties [24]. They have a shorter carbon cycle in contrast to the carbon cycle present in the fossil-based polymers [5].

Bio-based means that the material is partly or wholly made from renewable organic materials of biological origin such as corn, sugarcane, cellulose, vegetable fats and oil, wood chips, marine animals and microorganisms, animals, algae, sawdust, and organic waste [24,25].

Biodegradable means that the plastic material can be converted to natural and simpler substances like carbon dioxide, water, and compost by actions of naturally occurring biological agents(microorganisms) in bioactive environments like landfills, composting sites, and even anaerobic digestion systems used for managing wastes [19,21,25].

The process of biodegradation needs suitable conditions like moisture, the right temperature, oxygen content, pH, which will determine the extent of decomposition [19,24,26], and also the chemical structure of the bio-polymer (polymer chain, crystallinity, and the complexity of polymer formula) [27].

However, a bio-based material may not be biodegradable and vice-versa because the biodegradation property does not depend on what the material is made from but its chemical structure. In other words, a bio-based plastic may be non-biodegradable, for example, polyvinyl chloride (PVC), while a fossil-based plastic can be biodegradable, for example, Polybutylene adipate terephthalate (PBAT) [19,28]. Nevertheless, studies are ongoing to improve the biodegradability of bioplastics in several environments [27]. The various divisions of the plastic family are shown in figure 2.4 below, where colored circles are the bioplastics.

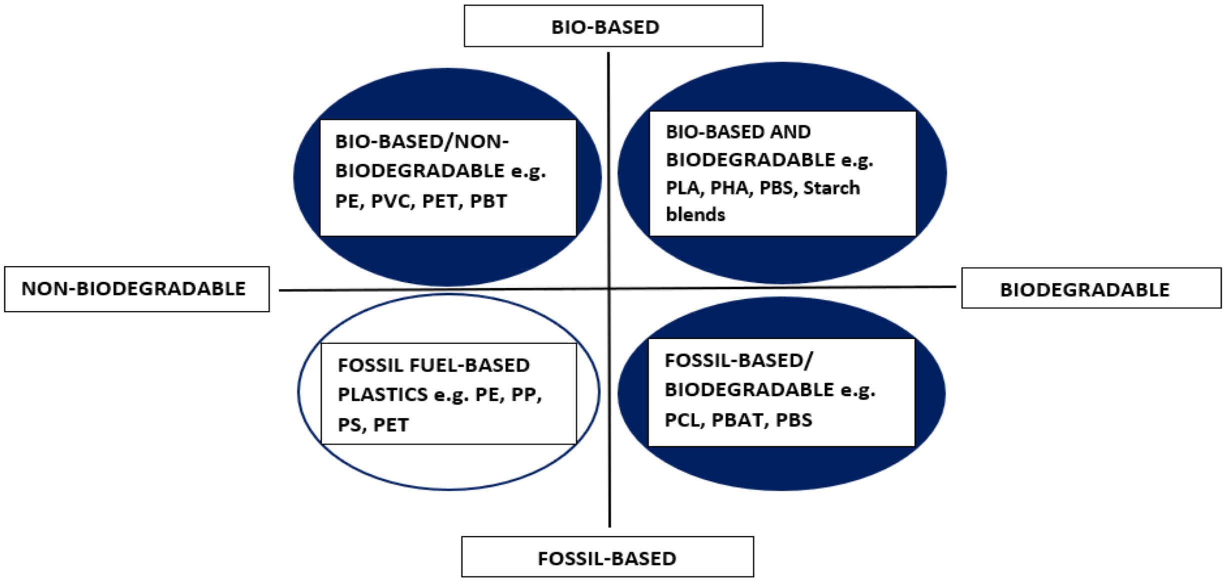


Figure 2.4. The division of the plastics family [5]

Nowadays, there are bioplastic substitutes with the same properties for every conventional plastic material in use, and they have also been put to use in various markets. These bioplastic alternatives have extra benefits that give them an edge over hydrocarbon-based plastics. Such benefits include better waste management options such as composting and anaerobic digestion, energy recovery options with biogas yield, reduced carbon footprints, and less dependence on non-renewable resources(fossil fuels) [19,28].

Currently, bioplastics produced is about 2.11million tons, and it constitutes about 1% of the total plastics family produced in 2020 and has been predicted to increase by 36% by 2025. Consequently, Europe is the largest market in the bioplastics industry and the second world producer at 26% [28,29]. The forecast of bioplastic production is illustrated in figure 2.5 below.

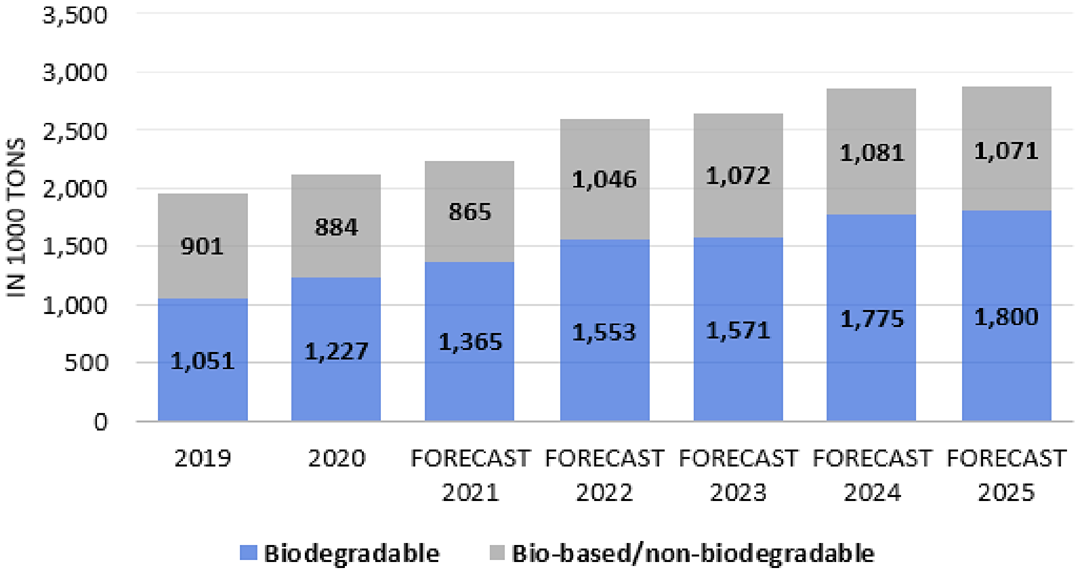


Figure 2.5 The number of bioplastics produced so far and the forecast [29]

In 2020, Biodegradable biopolymers made up 58.1% of bioplastics produced while non-biodegradable were 49.1%. The popular bioplastic materials are PLA, PBAT, PE, Starch-blends, and others [28]. Figure 2.6 below shows the different biopolymers and their percentage production.

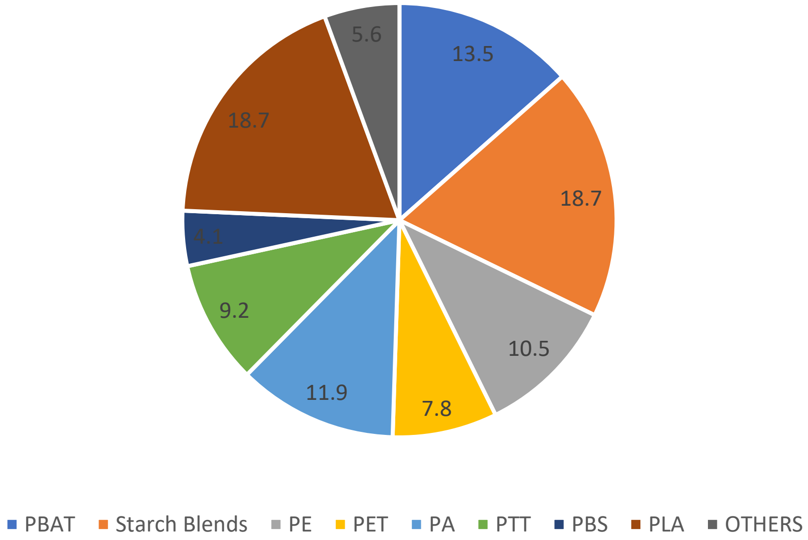


Figure 2.6. Different biopolymers and production capacities in 2020 [29]

2.2.2 Polylactic Acid (PLA)

Polylactic acid or Polylactide (PLA), depending on the formation, is a thermoplastic bio-polyester that is biodegradable and produced from renewable non-fossil feedstock such as starch, corn, potato, sugarcane, beet. It can be obtained from the action of bacteria on the carbohydrate found in these renewable sources by the fermentation process of dextrose(sugar) present in them to form the monomer (lactic acid). The lactic acid monomers produced are subjected to polycondensation to form Polylactic acid. Lactic acid, which occurs as a stereoisomer (L and D isomers), is an organic acid also known as 2-hydroxy propionic acid, which can either be produced by fermentation of sugars as mentioned above or chemical reactions [30-33]. If it is produced from fermenting sugars, it has 95% L-isomer and 0.5% D-isomers, while if it is from chemical reactions, it contains 50% each of L-isomer and D-isomer [34].

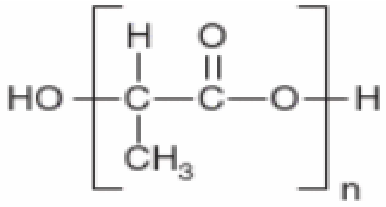
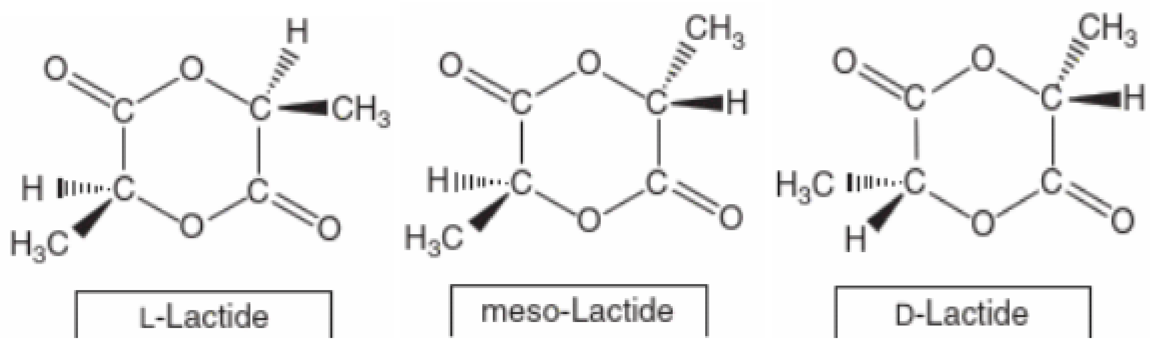


Figure 2.7. The structure of PLA [33]

L-Lactid Acid D-Lactid Acid

Figure 2.8. The stereoisomer of Lactic Acid [33]

PLA can also be formed chemically from ring-opening polymerization (ROP) of the ring form dimer of lactic acid known as lactide. Lactide abounds in three forms; L,L-Lactide, D,D-Lactide, and, D,L-Lactide(Meso- Lactide) [30,31,35].



L-Lactide meso-Lactide D-Lactide

Figure 2.9. The three forms of lactide [33]

PLA is a semi-crystalline polymer with chemical formula $(C_3H_4O_2)_n$ whose properties depend on the molecular constituents. It has a melting temperature between 130°C-180°C and a glass transition temperature of 50-80°C, good tensile, young modulus, and flexural strength. It is soluble in chloroform and other organic compounds, but the latter depends on the proportion of the different copolymers. Its barrier property is average which can be enhanced by mixing with other monomers. It decomposes thermally between temperatures 230°C and 260°C. The properties of PLA are determined by the molecular weight (M_w), degree of crystallinity, crystalline thickness, morphology, and the L/D(isomer) ratio [33,36].

PLA has a broad range of applications. Its production in large quantities from agricultural feedstock and blending or mixing with other bio-monomers help to yield varying products with different properties and features. It can be made into sheets, fibers, and films through film blowing, injection molding, and extrusion. It has a wide-ranging market prospect and has been commonly recognized as the capable material to replace plastics made from non-renewable resources such as PC, PET, PE, PP, and PS used to produce biomedical and packaging materials in this new age [32,37]. The areas of use are:

- Biomedical applications as it is compatible with living tissues.
- General products like bottles for holding liquids, casing for cosmetics, stationery casings, fiber for clothing materials.
- Toys
- Food-ware: cutlery, trays, plates.
- Electric and electronic uses: Phone and laptop casings, electric insulations [33].

PLA degrades by hydrolysis reaction [38]; the degradation (breaking down) occurs when it is exposed to moisture. This reaction occurs at the thermophilic (~58°C) temperature range and is dependent on pH, temperature, and moisture level. Then microorganism activities further degrade it into carbon dioxide, methane, and water [33,35].

2.2.3 Polycaprolactone (PCL)

PCL is a semi-crystalline with crystallinity at around 45% [39], a biodegradable aliphatic polymer obtained from non-renewable resources (fossil fuel). It is synthesized by polycondensation of 6-hydroxyhexanoic acid, which is a hydroxycarboxylic acid or the ROP of caprolactone in the presence of catalysts at high temperatures. PCL has different states depending on its molecular weight. For example, at M_w below several thousand, it is a waxy solid while it is a solid polymer at M_w above 20,000. It has a melting point temperature between 58-60°C [33,40] and a glass transition temperature of about -60°C [39,41,42].

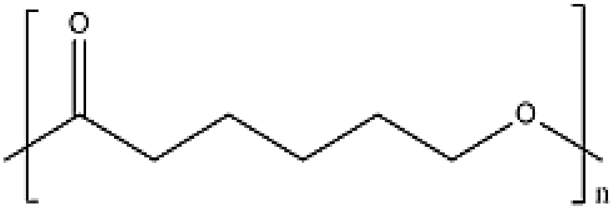


Figure 2.10 Molecular structure of polycaprolactone [43]

The thermal, mechanical, and physical properties of PCL are determined by the degree of crystallinity and its molecular weight, as shown in table 2.1. The molecular weight is vital and monitored during the synthesis if specific properties are desired. PCL can be quickly processed into fibers at temperatures below 200°C without thermal degradation and blended with other monomers and is readily miscible and bio-compatible with different monomers, and highly soluble in different organic compounds [33,40].

Table 2.1 Varying properties of PCL depending on the molecular weight [33]

Properties	PCL 1	PCL 2	PCL 3
Molecular weight Mw	37,000	50,000	80,000
Melting point (°C)	58-60	58-60	60-62
Tensile stress (kg/cm^2)	140	360	580
Elongation at break (%)	660	800	900

PCL has been found as a helpful biomaterial in the manufacture of medical materials and making medical devices, use in the delivery of vaccines and low molecular weight drugs, tissue engineering, bone repair, and fracture fixing pins. It is also helpful in the making of biodegradable packaging materials, adhesives, and microelectronics [33,36,40,41,44]. It is a significant and widely used macro-glycol in the synthesis of polyurethane [45]. PCL degrades in any environment, which could be sewage sludge, water, soil by hydrolysis and metabolic action of enzymes [33,40].

2.2.4 Poly(butylene) Succinate (PBS)

PBS is a long-chain synthetic, biodegradable polyester produced by condensation of succinic acid (SA) or dimethyl succinate and 1,4 butanediol (BDO). SA and BDO can be sourced from renewable and non-renewable sources. SA is a biobased chemical produced from sugars when produced from renewable resources, while BDO can be produced from reduction reactions on SA. Thus, a full biobased PBS can be synthesized from both biobased monomers [46,47].

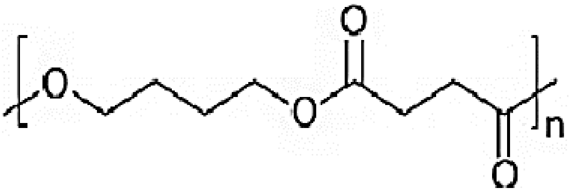


Figure 2.11 Molecular structure of PBS [47]

The synthesis of PBS can be done through different means, which include:

- Direct esterification of the monomers and polycondensation at high temperatures to get PBS with high molecular weight [33]. The M_w of produced PBS is vital because it determines some of its mechanical properties and its applications [48].
- ROP of cyclic monomers such as succinic anhydride and enzymatic esterification except that these two methods produce PBS with low M_w [47].

PBS is a crystalline polymer with a melting point temperature of 114°C, a glass transition temperature of -31°C, and good tensile strength at 34MPa. It shows moderate hardness and is quick to respond to changes in physical forces that can decrease M_w and mechanical damage; an example of such change is temperature change resulting in heating and cooling. It has similar properties and has been considered an alternative to polyolefins [33,46]. It is biodegradable in different environments in soil, water, activated sludge, and compost [38,47,49,50].

PBS has been copolymerized with other materials to enhance its abilities and properties, make up for its thermal weakness and increase its area of use. It and its copolymers are useful in agriculture for making fishing lines, ropes, and mulch films, also in medicine for drug delivery and tissue engineering, as well as in packaging materials like films, bottles, and bags. PBS is also useful in the automotive, electrical, and aerospace industries [47].

2.3 Biodegradation of plastic materials

Biodegradation is a natural process where the total mineralization of materials present in compounds occurs by biological actions of microorganisms such as fungi, algae, and bacteria to CO_2, H_2O, and biomass [51]. The process of biodegradation happens in three stages, namely:

- Bio-deterioration: This is the biological activity of microorganisms on the surface of polymer materials, causing a modification in their chemical, physical, and mechanical properties. The change here is majorly on their mechanical properties, and it induces porosity [27,51].
- Bio-fragmentation: Action of microorganisms on polymers which causes chains to be broken such that polymers are converted to oligomers and monomers by processes of hydrolysis, oxidation, and depolymerization [27,51].
- Assimilation: At this stage, microorganisms use products from bio-fragmentation, and they are converted to biodegradation by-products such as CO_2, H_2O and, biomass [27,51].

The biodegradability of a material can be evaluated by mass loss, rate of evolution and measurement of evolved CO_2 or CH_4, BOD(biological oxygen demand), or the ratio of biological oxygen demand to theoretical oxygen demand measurements (BOD/ThOD), surface morphology/erosion(visual analysis), spectroscopy, and molecular weight test of the material [23]. The total biodegradation of polymer has occurred when the polymer is completely converted to gaseous products and salts [51-52].

Various factors influence the ability of a polymer to degrade by a biological process, and they are the crystallinity of the polymer, chain configuration(linear or branch chained) and length of the chain of the polymer, chemical structure of the polymer, environmental conditions like moisture, pH, oxygen content/supply, temperature, and abundance of microorganisms [26,53].

Biodegradation occurs under aerobic and anaerobic conditions. During aerobic biodegradation, there is oxygen in excess, while anaerobic digestion occurs in the absence of oxygen.

2.3.1 Aerobic biodegradation

This is the degradation of materials by various microorganisms in an environment in the presence of excess oxygen to form carbon dioxide, water, minerals, and biomass. The aerobic environment could be soil, water(marine/fresh), compost. During aerobic biodegradation, the microorganisms use the polymer as a source of carbon for growth, and their metabolic processes yield CO_2. The amount of CO_2 produced during metabolic reactions and the fraction of carbon that is fused into biomass is dependent on the polymer type and concentration, physical characteristics of the environment, and the peculiarities of the species present [54]. The reaction below shows the biodegradation of polymer with carbon content shown.

$$C_{polymer}+O_2 \longrightarrow CO_2+H_2O+C_{residue}+C_{biomass}+\text{Salts [52]} \qquad (2.1)$$

- $C_{polymer}$ is the carbon content of polymer.
- $C_{residue}$ is the remaining polymer after biodegradation.
- $C_{biomass}$ is new material formed.

When the decomposition of organic materials by activities of microorganisms takes place under controlled conditions in an aerated environment, it is called composting. Composting can be done by individuals or on a large scale by industries. Composting in large quantities by industries occurs at a much faster process because it is better managed, and conditions required for composting are thoroughly monitored [55].

During the composting process, the microbes make use of oxygen and consume the organic matter present to generate heat in considerable quantities, CO_2 in large quantities as well as releasing water vapor. The composting process starts with the raw material been thoroughly mixed for homogeneity, and the microorganisms kick start the process by quickly consuming the degradable components of the materials and oxygen. This rapid consumption process/ metabolic activities result in the heat given off during the process. At the start, the temperature is normal, and then it rises rapidly through mesophilic to thermophilic temperature ranges. This rise/change in temperature is due to the metabolic processes of the microorganisms. As these activities slow down, the temperature also declines until it reaches ambient air temperature again. After the active composting period, the curing process follows. At this stage, the material continues to decompose slowly at mesophilic temperatures while the remaining microorganism consumes them. Then, the product/compost becomes stable and easy to handle [56]. The equation below shows the reaction that takes place during aerobic degradation.

$$\text{Organic matter} + S + O_2 \longrightarrow CO_2 + H_2O + NO_2 + SO_2 + \text{Heat} + \text{Compost [57]} \qquad (2.2)$$

To check if the composting process is taking place the way it should, pH, moisture, the concentration of CO_2 and O_2, and temperature are monitored. The pH value increases from 5 to 8 as the temperature increases from the mesophilic to the thermophilic level, then reduces to 6 as the composting process goes back to ambient temperatures. The temperature level also increases from 20^oC to 60^oC and then goes back to 20^oC. As this temperature and pH increase and decrease occur, moisture content decreases [52].

Municipal solid waste (MSW) is the largest source of organic waste, and it contributes the highest percent percentage of feedstock for composting. The organic fraction of MSW called the OFMSW, and other wastes such as wood, leaves, food waste are of particular interest in composting as it produces high-quality compost suitable for use as fertilizer, soil conditioner, used for conservation of soil moisture, and also help improve infiltration and reduce soil erosion. In addition, compost is used for land reclamation, organic farming, and horticulture [58].

The factors affecting composting are as follows:

- Oxygen content and aeration: Composting requires a large amount of oxygen, especially during the early stages. Proper aeration helps to evaporate excess moisture, help control temperature, and oxidize organic material. If the oxygen content is low, the process turns anaerobic [52,56].

- C to N ratio: The essential nutrients necessary for composting are carbon(C), nitrogen(N), potassium(K), and phosphorus(P), but the ratio of carbon to nitrogen present is of importance as carbon is needed for growth and energy and nitrogen is used for reproduction and production of proteins. The appropriate C:N ratio ensures that other nutrients are present in the required and adequate amounts. Ratios between 20:1 and 40:1 are acceptable for active composting [56].
- Moisture Content: For metabolic processes to take place actively, microbes need moisture. This should be kept within the 40-65% moisture content. If the moisture content is less than 40%, the composting process becomes inhibited, while if the moisture content is above 65%, water displaces the air present in the pore spaces needed for composting [56].
- Particle size: The rate of aerobic degradation increases with a smaller size of particles; hence polymers are suggested to be about 5cm in diameter [56].
- Temperature: Composting takes place over two major temperature ranges; mesophilic and thermophilic. The former temperature range is between $10^{o}C$ and $40^{o}C$, while the latter temperature starts from $40^{o}C$. Thermophilic temperature is desirable for composting because it destroys pathogens, fly larvae, and unwanted weed seeds in the composting material [56].
- Time: The factors listed above determine the length of time needed for the entire composting to take place. Under favorable conditions, composting is takes place quickly, and compost is left to mature before usage [56].

2.3.2 Anaerobic digestion (AD)

This is a biochemical, biodegradation process that involves the metabolic activities (oxidation) of microbes on organic materials and polymers in the absence of oxygen to yield water, ammonia, H_2S, biogas, and digestate [51]. The digestion process takes place at either mesophilic $37^{o}C$ or thermophilic $55^{o}C$ temperature ranges [58]. The AD of a polymer can be shown in the equation below

$$C_{polymer} \longrightarrow CO_2+H_2O+CH_4+C_{residue}+C_{biomass}+\text{Salts [52]} \qquad (2.3)$$

$C_{polymer}$ is the carbon content of the plastic polymer,

$C_{residue}$ is the carbon contained in the remaining polymer,

$C_{biomass}$ is the carbon content of biomass.

In general, AD can be shown as

$$OM+H_2O+Nutrients \longrightarrow DR + CO_2 + CH_4 + NH_3 + H_2S + Less\ heat\ [57] \qquad (2.4)$$

Where OM is Organic matter, and DR is Digestate residue.

The anaerobic digestion process is used extensively for organic waste treatments. These organic wastes include farm wastes, leftover food, sewage from homes, waste paper, wastewater, MSW, industrial organic waste, manure, and agricultural waste such as grasses, crop residue, and crops except woody wastes because of their high lignin content.[59]

AD occurs in four phases, and they are hydrolysis, acidogenesis, acetogenesis, and methanogenesis in that order. Each phase uses a different set of microorganisms that utilizes intracellular or extracellular enzymes to break down compounds present in the organic matter. These compounds are called substrates, and they are metabolized for cell growth and energy supply by microbes. The products of each phase are used as substrates in the next phase [60].

- Hydrolysis: This is the first stage of the AD process where composite matter is disintegrated and then dissolved by a set of microorganisms called hydrolytic bacteria. First, particulate protein, carbohydrate, and lipids are disintegrated from the organic material. Then, hydrolytic enzymes secreted by the hydrolytic bacteria convert these into amino acids, monosaccharides and, long-chain fatty acids, and glycerol in that order [60,61].
- Acidogenesis: Products from the hydrolysis phase are broken down by microorganisms to produce hydrogen, CO_2, alcohols, and volatile fatty acids. This step is also known as fermentation [62]. The equation below shows the breakdown of a monosaccharide, e.g., glucose into butyric acid by the acidogenic reaction [60,61].

$$C_6H_{12}O_6 \longleftrightarrow 2CH_3CH_2CH_2COOH+2CO_2+2H_2\ [60] \qquad (2.5)$$

- Acetogenesis: This third phase is achieved by acetogenic bacteria. It is a significant phase between acidogenesis and methanogenesis because volatile or long-chain fatty acids such as butyric acid formed by fermentation from acidogenesis phase are oxidized into acetic acid, CO_2, hydrogen and, water. This third step is important as higher volatile fatty acids cannot be used directly in the last phase(methanogenesis) [60].
- Methanogenesis: Here, products from the acetogenesis stage (acetic acid, hydrogen, and carbon dioxide are converted to CH_4, CO_2, and H_2O by methanogenic microorganisms. The organisms present at this stage are divided into two classes;

acetoclastic methanogens that convert acetate to methane and water and hydrogenotrophic methanogens that convert hydrogen and carbon dioxide to methane and water [60].

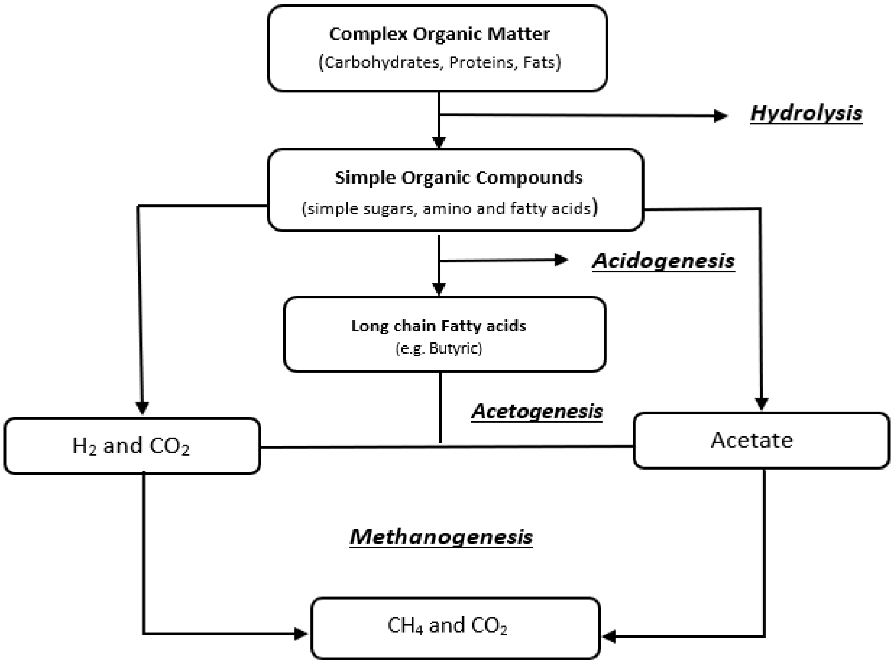


Figure 2.12 The stages of anaerobic digestion [63]

Sources of organic material useful in AD include OFMSW, residue from agricultural practices, food wastes, and sewage sludge [60].

The anaerobic digestion process takes place in a biogas digester which is a container or vessel constructed to facilitate the decomposition of feedstock (animal or other forms of wastes) into energy in the absence of oxygen. They serve as an excellent disposal system for wastes (e.g., human waste), thereby preventing contamination of the environment and the spread of diseases caused by pathogens, for example, diarrhea and cholera. They are efficient in rural areas (in developing countries), especially for cooking and heating purposes replacing firewood and reducing deforestation [64].

AD is influenced by factors such as pH, temperature, nutrients and trace elements, alkalinity, total and volatile solids, organic loading rate, presence of inhibitory or toxic substances, and retention time. These factors must be appropriately controlled and monitored so that the digestion environment is conducive for microorganisms while the production and quality of biogas and digestate are optimized [60]. Some of these conditions which determine the activities of the microorganisms that play a major role in the AD process are discussed briefly below.

- Temperature: Anaerobic digestion is mainly dependent on the temperature of the process. Even though the actions of microorganisms generate heat, it is not enough to keep the reactions going and favorable. So, the digesters or reactors are operated at specific temperatures favorable for microbial activities and organic material digestion. The temperature at which the digesters are operated is kept within three (3) ranges, and they are psychrophilic 5^oC-25^oC, mesophilic 30^oC-45^oC, thermophilic 50^oC-65^oC. Different bacteria dominate at different temperatures, and temperature fluctuation can cause a shift in balance and affect the production of methane by the methane-forming bacteria [63,65–67].
- Alkalinity and pH: This is a measure of the alkalinity or acidity at which the activities in the digester occur. Making sure the pH is acceptable is of utmost importance to the optimal performance of the system. At a pH of 5.0, acid-forming bacteria predominate while methane forming bacteria find pH above 6.2 convenient. The AD process is acidic at the beginning when organic material is loaded into the digester because of the formation of volatile acids. Then the pH increases as the methane-forming bacteria consume the acid. If loading of feedstock into the digester is done haphazardly, it may cause fluctuations in the pH at which reactions take place, thereby inhibiting methane production. To prevent this, organic material is loaded into the digester at designated times and in batches so that the pH is maintained. The stability of the digester is enhanced at alkalinity concentration, and methanogenesis is favored at pH 6.8-7.2 [63,65,66].
- Loading rate: This is the amount of organic materials/feedstock that is loaded into the anaerobic digester/reactor per unit volume. The ability of a digester to process feedstock and produce methane is dependent on its loading rate [63,66].
- Inhibitory or toxic substances: These are substances that disturb microbial activities in the digester and disturb methane production. They may be trace elements like zinc, copper, and nickel or a high concentration of alkali metals like potassium and sodium [63].

Anaerobic Digestion helps to conserve land space when compared to landfilling solid waste. AD also provides clean fuel from renewable feedstock hence an attractive strategy for waste management [68]. It helps reduce the organic load of wastes that can cause water and land pollution if released into the environment. Also, it produces a gas rich in methane that can be captured and used to produce energy [62].

The products of AD include:

- Digestate: This consists of both the liquid and solid parts. The solid digestate, also called biosolids, is a nutrient-rich, fibrous, and stable fraction whose end use is dependent on the nutrients and trace minerals present, which is also dependent on the feedstock used. While the liquid digestate, also known as liquor, is the liquid fraction of the digestate, it can also be obtained from dewatering the solid digestate. This liquid part is nutrient-rich as well and can be processed into a liquid biofertilizer [60,62].
- Biogas: This is the ultimate product of the AD process. It is entirely gaseous. It consists of Methane CH_4, which is the combustible part between 50-60% and 60-65%, Carbon dioxide CO_2 with amounts between 30-35% and 40-50%, and small amounts of Hydrogen H_2, Nitrogen N_2, Hydrogen sulphide H_2S, and water vapor H_2O with amounts between 5-10% [59,60]. The composition of substrate is an essential factor in determining the rate of methane yielded during the AD process. The more biodegradable the substrate is, the higher the biogas yield [69]. Also, the composition and characteristics of biomass, especially solid feedstock like wood, can be pretreated before the digestion process to enhance its biogas yield. These pretreatment processes aim at improving the biodegradability of the biomass. They include:
- Thermal treatments which can be beneficial in breaking down the chemical bond in cell walls, mechanical treatments done to reduce particle size and increase the surface area of feedstock for better exposure of cellular materials,
- Chemical pretreatments that require the addition of chemicals to cause a change in the chemical structure of organic molecules, and
- Biological pretreatments which is the addition of microorganisms to speed up reactions especially agricultural residues [60]. Biogas can be utilized in CHP (combined heat and power plants) by burning it to generate heat and power. Moreover, it can be upgraded to biomethane through the removal of trace gases and carbon dioxide. It can also be used as transport fuel [59,60].

3 MATERIALS AND METHODS

The interest in evaluating biodegradation of bioplastics with a focus on behavior in the environment at the end of life under conditions used in treating OFMSW is motivated by the rate of increase in production of bioplastics, especially those intended for single-use purposes(packaging) and agricultural films. Ensuring that these bioplastics are fit for biological recycling and can biodegrade under various conditions is a necessary factor to consider if they are to help reduce or replace conventional plastics.

The biodegradability tests can be conducted according to various standards under various exposure/environmental conditions. The standards include ASTM (American Society for Testing and Materials), ISO (International Organization for Standardization), and EN (European standards). These standards contain a description of terminologies and definitions, guidelines for testing, explanation of procedures, and suggestions on timing, sizes(sample), scale, and environmental conditions of tests and reagents to use. The contents also include equations for different calculations, validity, and interpretation of results [23,64,70].

There are various standards published by international standards to provide indications for biodegradability testing under aerobic and anaerobic conditions. However, for the purpose of this thesis which is AD testing, EVS-EN ISO 15985:2017 "Plastics-Determination of the ultimate anaerobic biodegradation under high-solids anaerobic-digestion conditions-Method by analysis of released biogas" [71] was chosen because of the ease of assemblage of materials needed, high chance of testing, and measurement of biogas evolved during the testing process.

3.1 Biodegradation Measurement based on ISO 15985:2017

3.1.1 Description of the testing process

Before the test started, fifteen digestion vessels made of glass with 500ml volume were thoroughly cleaned and weighed on a scale, and the weights were recorded. They are labeled appropriately. Then, the total dry solids (TDS) values of the compost, cellulose (reference material), and test materials were calculated by drying the materials at 105^{o}C for 8 hours in the furnace. Also, the volatile solids (VS) value was calculated by incinerating the residue after the TS to a temperature of 550^{o}C.

The compost was removed from the containing vessel and mixed thoroughly by hand to get a homogenous mixture. Then, the compost was weighed into different labeled digestion vessels, and test or reference materials were added and mixed carefully. The vessels were closed tightly to avoid gas loss. The mixtures were compacted evenly to a uniform density and then placed in the sample incubation unit of the AMPTS II (Automatic Methane Potential Testing System II), which is a hot water bath set at a consistent temperature of 52+/-2°C, the pH of the water in this unit is less than 2, for at least 15-40days till the evolution of gases reaches its plateau and tapers or stops. The time frame between mixing the compost and reference or test material to the time of connection to the gas measurement system was checked and monitored as it should not be more than 2 hours.

The fifteen (15) digestion vessels were labeled as:

- Three vessels for blank material (labeled A 1, 2, and 3).
- Three vessels for compost and reference material (labeled B 4, 5, and 6).
- Three vessels for test material 1 (labeled C 7, 8, and 9).
- Three vessels containing test material 2 (labeled D 10, 11, and 12)
- Three vessels containing test material 3 (labeled E 13, 14, and 15)

The vessels labeled A contained compost only, also known as the blank.

The vessels labeled B contained a mixture of compost and reference material. The reference material is cellulose. This was known as the reference; it was used to compare the vessels containing test materials. The CO_2 evolved in these vessels was compared with the vessels labeled C, D and, E.

The vessels labeled C, D, and E were made up of compost mixtures and the different test materials, namely PLA-MI, PBE 003, and NPSF-141, respectively. These vessels were clearly marked with the names of the test materials and numbers.

Visual appearances and shapes of the test materials were noted and recorded. Each of the test, reference, and blank vessels were in triplicates to aid proper monitoring and increase the results' trustworthiness because the mean value of figures recorded was used for calculations.

After the vessels containing the mixture of compost and materials were placed in the incubation unit of the AMPTS II, the tubes on the caps were connected to the bottles for draining water from the samples; these bottles contained acid for capturing other gases that are involved but not necessary in the experiment. Then, these tubes led to the bottles containing alkaline solution used for trapping CO_2, after which the evolved gas from these bottles went to the gas flow and volume measuring unit of the AMPTS II. At the gas flow and volume measuring unit, data was generated. The produced data was presented as tables and graphs, which were downloaded and interpreted accordingly to determine the cumulative gas produced. The properties and function description of the AMPTS II device are explained in the latter part of this chapter.

Table 3.1: Weights of Bottles and different materials

Bottle Number	Type	Empty Bottle(g)	Compost(g)	Material (g)	Full Bottle (g)
A1	Compost	339.60	250.20		589.80
A2	Compost	338.20	249.90		588.10
A3	Compost	339.70	250.10		589.80
B4	Cellulose	339.20	251.20	5.50	595.90
B5	Cellulose	338.80	250.10	5.50	594.40
B6	Cellulose	340.20	251.00	5.50	596.70
C7	PLA-MI	339.60	250.00	7.00	596.60
C8	PLA-MI	338.40	250.00	7.00	595.40
C9	PLA-MI	339.40	250.00	7.00	596.40
D10	PBE003	340.40	250.06	5.54	596.00
D11	PBE003	339.30	250.46	5.54	595.30
D12	PBE003	339.60	250.16	5.54	595.30
E13	NPSF141	338.90	250.20	5.10	594.20
E14	NPSF141	338.40	250.20	5.10	593.70
E15	NPSF141	338.80	250.00	5.10	593.90

3.1.2 Materials preparation

Test material preparation

The test materials were presented in granules. The sizes of the granules were less than 2cm by 2cm in surface area, so they were not resized. They were measured according to the standard after the TDS, and VS of the materials have been calculated.

Reference material preparation

The indicated particle size stated in the standard is a thin-layer, chromatography-grade cellulose of not less than 20 µm, which was also used for the process. TDS and VS of cellulose were calculated, and it was weighted according to ISO standards for mixture with compost.

Inoculum preparation (compost)

The compost was sourced from the Keila wastewater treatment plant and was kept for seven days at about 36°C to allow for further consumption of organic matter. It was later sorted by hand to remove inert materials like stones, metals, glass, hair, and sticks that can inhibit the process (This sorting was done under hygienic conditions as recommended in the standard), not forgetting that compost contains pathogenic materials. After sorting, sieving was done with two sieves of mesh sizes 0.5 and 1.0cm. The biochemical characteristics measured before use were pH, the volatile fatty acids, and ionized-ammonia nitrogen (NH_4^+-N) was also calculated according to standard.

3.2 Analytical methods

The pH of the compost and water present in the gas volume measuring unit of the AMPTS II was measured and analyzed using the pH meter. According to ISO 14855, one part of compost was mixed with five parts of distilled water and thoroughly shaken to measure the pH of compost. Then, the pH was measured immediately by dipping the electrode in the solution, and it was 5.61. While the TS, VS, volatile fatty acids, chemical oxygen demand (COD), total Kjeldahl nitrogen, TOC(total organic carbon), ammonium nitrogen were determined according to standard methods.

Figure 3.1 pH measuring instrument with electrode

Biogas produced was measured fully and automatically by the AMPTS II, which works by the principle of liquid dislodgment and buoyancy and may monitor extremely low gas flows; a digital pulse is generated when a defined volume of gas flows through the device. An integrated embedded data collection system is used to record, display, and analyze the results [72].

3.2.1 AMPTS II

AMPTS II was developed for online measurements of extremely low biogas and bio-methane flows produced from the anaerobic digestion of any biodegradable substrate at a laboratory [72]. The device is divided into three units: Unit A is a thermostatic water bath which is also the incubation unit where the digestion vessels are placed during the biodegradation process. Unit B is the CO_2 absorbing unit that holds trays 15 small glass bottles. The bottles contain an alkaline solution that is used to absorb acidic gases like CO_2 and H_2S. For example, a pH indicator, phenolphthalein, is added to the alkaline solution to indicate and help monitor the acid-binding capacity of the basic solution. Unit C is the gas volume measuring device that makes a ticking sound, and it is displaced upwards when the outlined volume of gas flows through. The data is captured automatically and reflects on the system where the software of the AMPTS II has been previously downloaded. Collected data is downloaded and shown as tables and graphs. For this experiment, AMPTS II was set up with an additional unit containing bottles 100ml each for collecting moisture from the digestion vessels.

Figure 3.2 AMPTS II showing the incubation unit and the bottles containing alkaline solution.

3.2.2 Titration

The gas released from the bottles passed through the alkaline solution (Potassium hydroxide (KOH)), which captured the CO_2 present. The KOH solution was titrated in two stages with hydrochloric acid (HCl), and the phenolphthalein indicator showed the mid-point by changing from pink to colorless, while methyl-orange indicator was added, changing from orange(yellow) to pink to indicate the endpoint.

3.3 Checks and measurements

Various periodic checks are performed, and they include:

- The water level in the thermostatic bath and filling up when necessary.
- The water level in the water bath for gas flow and volume measuring device and filling up as appropriate.
- Connecting tubes are checked to avoid gas leakage.
- The pH of the water bath for gas flow and volume measuring device is checked to be sure that alkaline solution is capturing enough CO_2 and is not lost to dissolution.

While for measurements, titration was done to check the CO_2 binding capacity of the KOH solution, and it was replaced as soon as it was low.

3.4 Equations for calculations

Determination of TS and VS

The samples of all materials to be tested (compost, cellulose, and test materials) are put in different crucibles and measured (m_{Wet}). The crucibles were put in the furnace and heated up for 8 hours at 105°C to remove all water content (m_{Dried}). At that point, it was allowed to cool down and measured. After this, the same samples were heated up to 550°C for 2hours so that organic matter present can be burnt off (m_{Burned}). When the samples cooled down, the weights were measured and recorded. The weight differences in the samples after heating to 105°C and 550°C showed the VS of the materials. The equations for calculations are shown below [73].

$$TS(\%) = \frac{mDried}{mWet} * 100 \tag{1}$$

$$VS(\%) = \frac{mDried - mBurned}{mDried} * 100 \tag{2}$$

Alternatively, the TS and VS can be calculated with the following steps: first, find the total dry solids (TDS) that is solids remaining after all moisture have been removed by weighing an empty crucible is measured with mass (E), material (compost or cellulose or test material) with mass(M), and mass of crucible and material after the temperature of 105°C (D). All the masses are in grams. The formula for calculating TDS is

$$TDS = \frac{D - E}{M} \tag{3}$$

An empty crucible with mass(E), material (compost or cellulose or test material) with mass(M), and the mass of crucible and material after heating at a temperature of 550°C. Then, the total fixed solids (TFS) are solids left after organic contents have been burnt. All masses are in grams.

$$TFS = \frac{G - E}{M} \tag{4}$$

And VS is calculated by

$$VS = TDS - TFS \tag{5}$$

The summary of TS and TVS percentages is shown in table 3.2 below.

Table 3.2 Percentage of TS and TVS

Material	**TS%**	**TVS%**
Compost	28.87	46.00
Cellulose	90.78	90.52
PLA MI	99.81	71.13
PBE 003	99.76	89.97
NPSF 141	99.74	97.83

Determination of the gaseous carbon

The amount of gaseous carbon C_g that evolved from each digestion vessel is calculated at first. Then the volumes of CH4 and CO2 evolved are converted to volumes at standard temperatures and pressure 273K and 1,013.25hPa, respectively, using the ideal gas equation:

$$constant = \frac{PV}{T} \tag{6}$$

P is the pressure in hPa

V is the volume in liters

T is the temperature in K.

The volume of biogas evolved is converted to the corresponding amount of gaseous carbon after the correction of volumes for water vapor pressure and atmospheric pressure during the test has been done. The equation for conversion is:

$$22.4ml\ of\ biogas\ at\ STP = 12mg\ of\ Cg \tag{7}$$

Determination of percentage biodegradation

First, the average of the amount in grams of the gaseous carbon evolved by the compost/control (from the duplicate bottles) is calculated, and this amount is subtracted from the average of the gaseous carbon evolved during biodegradation of the test material.

The percentage biodegradation is calculated by

$$\%biodegradation = \left(\frac{mC,g(test) - mC,g(blank)}{mCi}\right) * 100 \tag{8}$$

mC,g is the amount of gaseous carbon evolved in grams

mCi is the carbon initially in the test material, in grams

Determination of amount of CO_2 evolved.

The CO_2 evolved from the reactions which took place in the vessels was evaluated by the titration method. The equations of reaction and final calculations are according to ISO 19679-2020 and [74]. First, the CO_2 adsorbed in the KOH reacts with it according to this reaction:

$$2KOH+CO_2 \longrightarrow K_2CO_3+H_2O \tag{3.1}$$

The KOH solution used as adsorbing agents still has some unreacted KOH present with the K_2CO_3. The potassium carbonate (K_2CO_3) and KOH react with the HCl to give the first endpoint changing the color of the phenolphthalein indicator from pink to colorless.

$$KOH+HCl \longrightarrow KCl+H_2O \tag{3.2}$$

$$K_2CO_3+HCl \longrightarrow KHCO_3+KCl \tag{3.3}$$

Then, further addition of HCl causes potassium bicarbonate ($KHCO_3$) to react with HCl to give the endpoint where the methyl orange indicator changes from yellow to pink.

$$KHCO_3 + HCl \longrightarrow H_2CO_3 + KCl \tag{3.4}$$

At this stage, the volume of acid needed to calculate the amount of carbon dioxide produced is the difference in the volume of acid used at the midpoint and the volume at the endpoint [74]. This is shown by the formula below.

$$Volume\ of\ titrant(ml) * Molarity\ of\ acid * Molecular\ weight\ of\ CO2 = Mass\ of\ CO2\ (mg) \tag{9}$$

4 RESULTS ANALYSIS

The experiment lasted for 73 days, with titration done once in 3 days for the first few weeks. As the procedure progressed, titration was done once a week till the end. While biogas produced was measured automatically and the data downloaded was interpreted.

4.1 Titration results

The CO2 evolved from each bottle was measured during each titration, and the cumulative gas measured is shown in table 4.1 and figure 4.1 below.

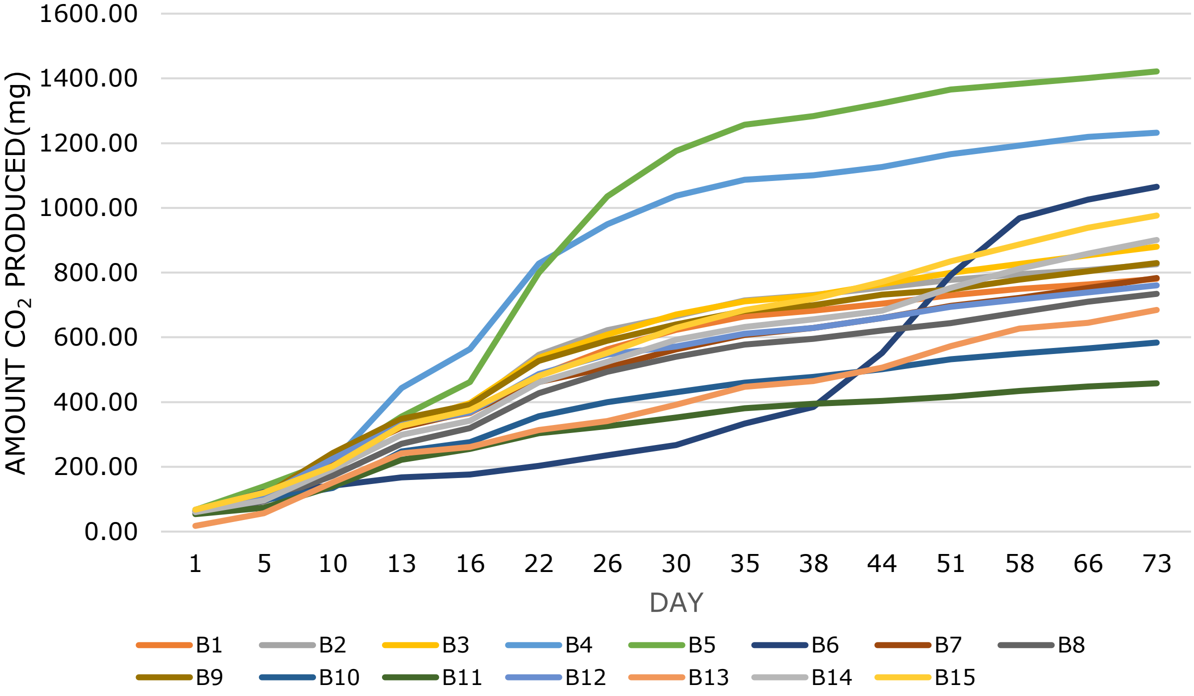


Figure 4.1: graph illustrating the cumulative CO2 produced in 73 days per bottle

At the start of the experiment, each bottle produced roughly the same amount of CO_2. Then, by the 16th day, bottle 4 containing cellulose+ compost at 563.92mg produced more CO_2 than the other bottles and continued till the 22nd day. On the 26th day, bottle 5 had produced 1035.26mg of CO_2; hence, it overtook bottle 4 and produced more CO_2 than the other bottles. By the 73rd day, Bottle 5 produced the highest amount of CO2 at 1421.67mg. These figures are shown in table 4.1.

Table 4.1: Cumulative CO2 production for 73 days per bottle

DAY	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15
1	57.21	62.79	63.08	67.48	66.90	63.37	61.61	56.04	62.79	57.21	53.99	64.25	17.60	60.15	67.78
5	101.22	110.76	121.61	123.81	139.51	122.35	107.82	93.45	110.76	96.82	73.79	110.47	57.21	96.46	119.71
10	210.81	185.58	207.43	213.60	220.20	142.01	222.25	172.67	242.79	135.33	139.81	224.89	151.69	193.28	201.86
13	329.63	331.47	333.52	442.89	355.53	167.09	321.27	271.69	348.41	247.56	221.66	329.19	241.80	298.90	326.41
16	365.70	388.59	396.46	563.91	462.03	176.77	369.68	320.10	391.98	276.60	255.55	367.92	261.16	342.15	374.82
22	482.33	545.71	537.07	827.97	798.71	203.18	462.11	427.92	527.31	355.82	303.96	486.75	313.97	460.98	480.44
26	563.01	622.73	608.58	949.00	1035.26	236.19	506.12	493.94	589.66	399.83	325.97	547.26	341.48	524.24	552.51
30	623.16	665.27	670.20	1037.02	1176.09	266.99	563.33	540.15	640.27	430.64	352.37	571.47	392.09	592.46	629.53
35	665.12	713.68	711.35	1087.05	1256.78	333.16	607.34	577.56	681.35	459.98	380.98	611.08	447.10	632.06	684.54
38	682.72	731.28	728.95	1100.25	1283.77	384.50	629.34	595.16	698.95	477.58	395.06	628.68	464.71	655.83	718.87
44	704.72	753.29	764.16	1126.66	1323.38	551.74	660.15	621.57	731.96	501.79	403.87	659.49	506.52	682.24	771.68
51	729.66	777.50	799.37	1166.27	1365.92	792.33	697.56	643.57	747.36	532.59	417.07	694.70	572.53	752.65	834.76
58	750.20	795.10	827.24	1192.67	1383.53	968.37	721.76	677.31	778.17	550.20	434.67	716.70	627.55	812.07	887.57
66	763.40	808.30	853.65	1219.08	1401.13	1025.58	752.57	710.32	804.58	566.34	447.88	738.71	645.15	858.28	938.92
73	781.01	825.91	880.05	1232.28	1421.67	1065.19	783.38	734.53	829.52	583.94	458.14	760.71	684.76	900.82	976.33

To further understand the CO_2 evolution results, the amount produced per bioplastic product/ day was also analyzed. The data is shown in table 4.2 and figure 4.2 below.

Table 4.2 Cumulative CO_2 production per bioplastic material(mg)

Day	Compost	Cellulose	PLA MI	PBE 003	NPSF 141
1	61.03	65.92	60.15	58.48	48.51
5	111.20	128.56	104.01	93.69	91.13
10	201.27	191.93	212.57	166.68	182.27
13	331.54	321.83	313.79	266.14	289.04
16	383.58	400.90	360.59	300.03	326.04
22	521.70	609.95	472.45	382.18	418.46
26	598.11	740.15	529.90	424.35	472.74
30	652.88	826.70	581.25	451.49	538.02
35	695.91	892.32	622.08	484.01	587.90
38	713.51	922.84	641.15	500.44	613.14
44	739.92	1000.59	671.23	521.71	653.48
51	768.03	1108.17	696.16	548.12	719.98
58	790.04	1181.52	725.75	567.19	775.73
66	807.64	1215.26	755.82	584.31	814.11
73	828.18	1239.71	782.47	600.93	853.97

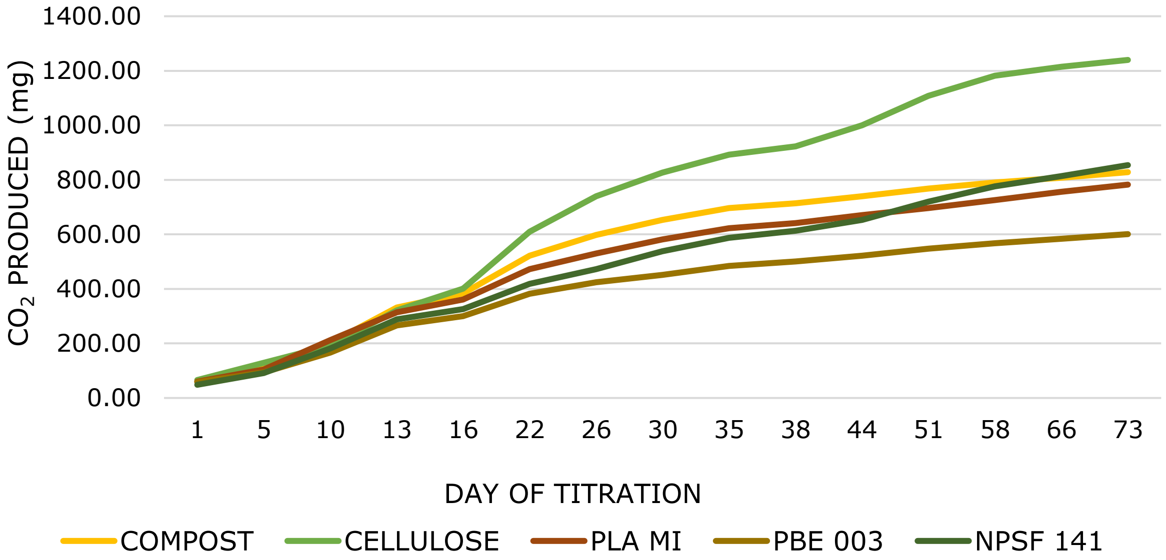


Figure 4.2 Cumulative CO_2 produced per day of titration

The graph and figure above show that the bottle labeled cellulose (compost+cellulose) produced a higher amount of CO_2 during the titration process, followed by NPSF 141, Compost, PLA MI, and PBE 003, respectively.

4.2 Biogas results

Biogas results were downloaded on the 72nd day; the average volume was calculated for each material bearing in mind that the results gotten are in triplicates. Table 4.3 and the graphical illustration shown in figure 4.3 below show the average cumulative biogas produced in ml.

Table 4.3 Cumulative gas volumes produced per day(ml)

Day	Compost	Compost+cellulose	Compost+PLA MI	Compost +PBE 003	Compost+NPSF 141
0	0.0	0.0	0.0	0.0	0.0
1	250.0	0.6	124.3	268.5	0.6
2	277.8	1.2	140.4	291.0	1.2
3	286.3	1.7	143.9	300.8	1.8
4	288.2	2.3	145.0	301.5	2.5
5	290.4	2.9	146.1	302.2	3.1
6	291.7	3.5	147.2	303.0	3.7
7	292.8	4.0	148.3	303.7	4.3
8	293.1	4.6	149.4	304.5	4.9
9	294.2	5.2	156.0	305.2	9.9
10	294.4	24.4	177.2	305.9	41.6
11	294.6	66.8	207.0	306.7	86.0
12	295.1	143.3	232.8	307.4	141.6
13	295.4	253.7	233.6	308.1	199.0
14	295.8	380.8	234.8	311.2	264.2
15	296.2	516.5	237.0	327.3	328.9
16	296.8	646.4	245.8	343.2	367.1
17	297.1	773.5	264.3	375.3	412.4
18	297.3	874.5	283.4	417.5	453.8
19	297.7	925.0	303.2	462.5	462.8
20	298.1	1002.5	328.1	510.9	504.3
21	298.4	1082.8	353.3	552.7	548.4
22	298.8	1171.9	382.1	592.8	602.0
23	299.2	1307.0	367.9	654.7	675.9
24	299.5	1438.7	423.3	708.5	743.8

25	299.9	1512.7	462.4	758.2	795.5
26	300.3	1587.9	502.8	804.1	844.7
27	300.6	1663.4	540.3	848.0	897.5
28	301.0	1728.7	573.7	886.1	943.6
29	301.3	1798.0	609.7	928.0	975.5
30	302.0	1908.6	672.2	930.8	1076.8
31	302.2	2018.5	727.8	933.5	1164.7
32	302.5	2084.3	761.8	936.3	1212.1
33	302.6	2127.3	764.6	972.8	1229.7
34	302.8	2156.3	770.0	1003.0	1239.8
35	302.9	2175.7	779.1	1025.3	1258.3
36		2225.2	625.1	1070.4	1305.5
37		2249.6	470.3	1093.6	1330.0
38		2266.3	470.7	1096.7	1331.0
39		2283.0	471.2	1099.6	1332.0
40		2295.0	471.6	1103.9	1333.0
41		2309.5	472.0	1108.1	1333.9
42		2324.9	472.4	1112.9	1334.9
43		2346.4	472.8	1131.6	1335.9
44		2383.8	473.3	1153.9	1336.8
45		2417.2	473.7	1176.4	1337.8
46		2445.3	474.2	1212.8	1339.1
47		2447.7	474.6	1224.3	1387.3
48		2450.1	475.0	1241.3	1440.1
49		2452.5	475.5	1260.0	1488.4
50		2458.2	475.9	1277.8	1533.4
51		2468.0	476.4	1292.3	1575.1
52		2480.1	476.8	1306.3	1616.4
53		2498.2	477.2	1328.0	1661.4
54		2499.6	477.7	1333.6	
55		2501.0	478.1	1347.6	
56		2502.4	478.6	1365.2	
57		2503.8	479.0	1374.6	
58		2505.2	479.4	1386.3	
59		2506.5	479.9	1401.8	
60		2519.6	480.3	1426.6	
61		2536.5	480.7	1429.6	
62		2555.0	481.2	1432.6	
63		2572.9	489.4	1438.4	
64		2585.7	505.5	1447.5	
65		2601.1	523.7	1456.3	

66		2616.5	542.8	1464.1	
67		2629.5	560.0	1471.6	
68		2639.4		1488.6	
69				1494.6	
70				1506.0	
71				1525.1	
72				1533.2	

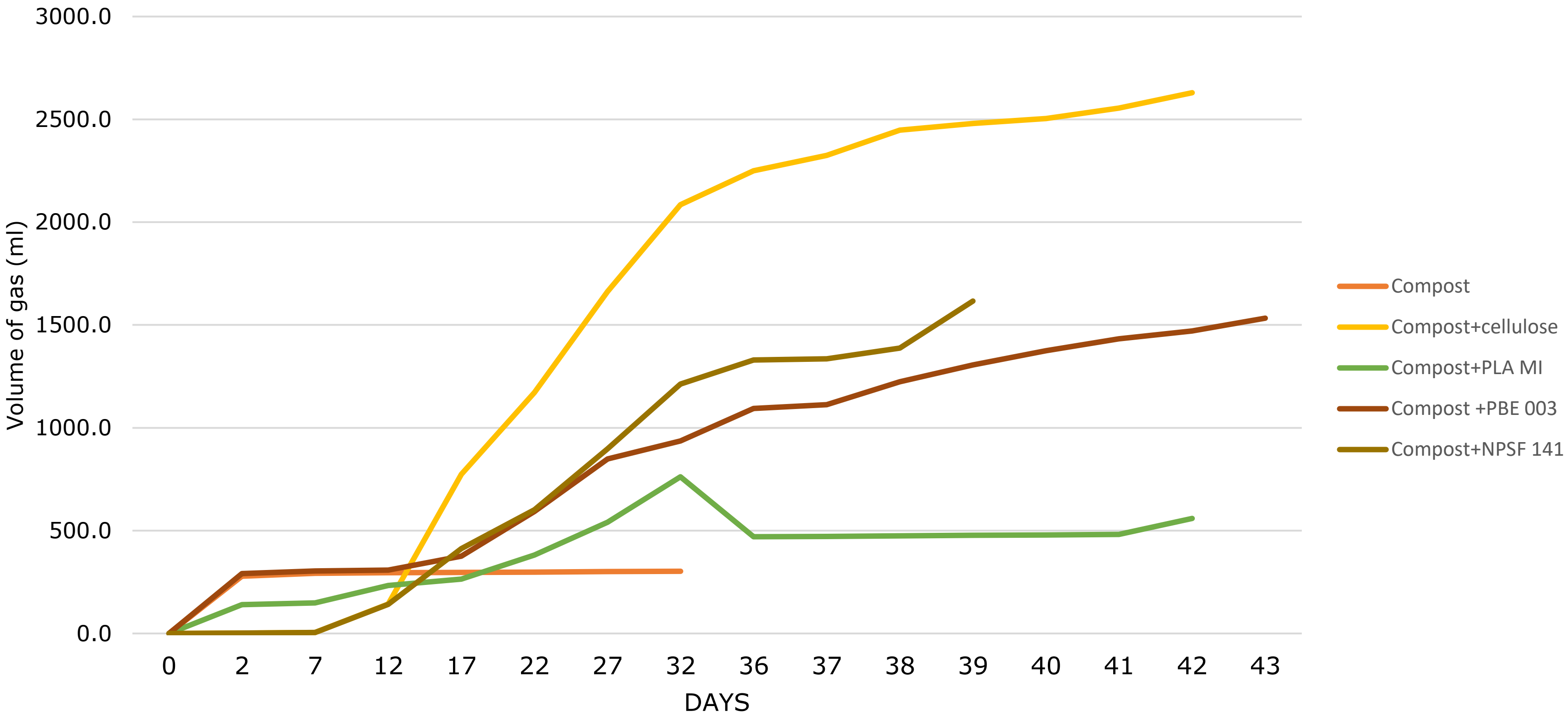


Figure 4.3 Cumulative volume of gas produced

From figure and graph 4.3 above, it can be deduced that the blank vessels did not evolve any gases from the 32nd day, and vessels containing compost+PLA MI dropped in the volume of gas produced from the 36th day. Compost+cellulose produced the highest volume of biogas, 2639.4ml of gas. This is followed by PBE 003, NPSF 141, and PLA MI with 1533.15ml, 1430.70ml, and 670ml, respectively, throughout the period.

Also, the cumulative amount of gases produced per material was both measured/calculated manually, and the data downloaded was summed up and presented in the table and figure 4.4 shown below

Table 4.4: Cumulative Biogas produced at 73days.

Material	**Cumulative amount of biogas (ml)**
COMPOST	723.62
CELLULOSE	3270.38
PLA MI	1068.36
PBE 003	1838.97
NPSF 141	1865.35

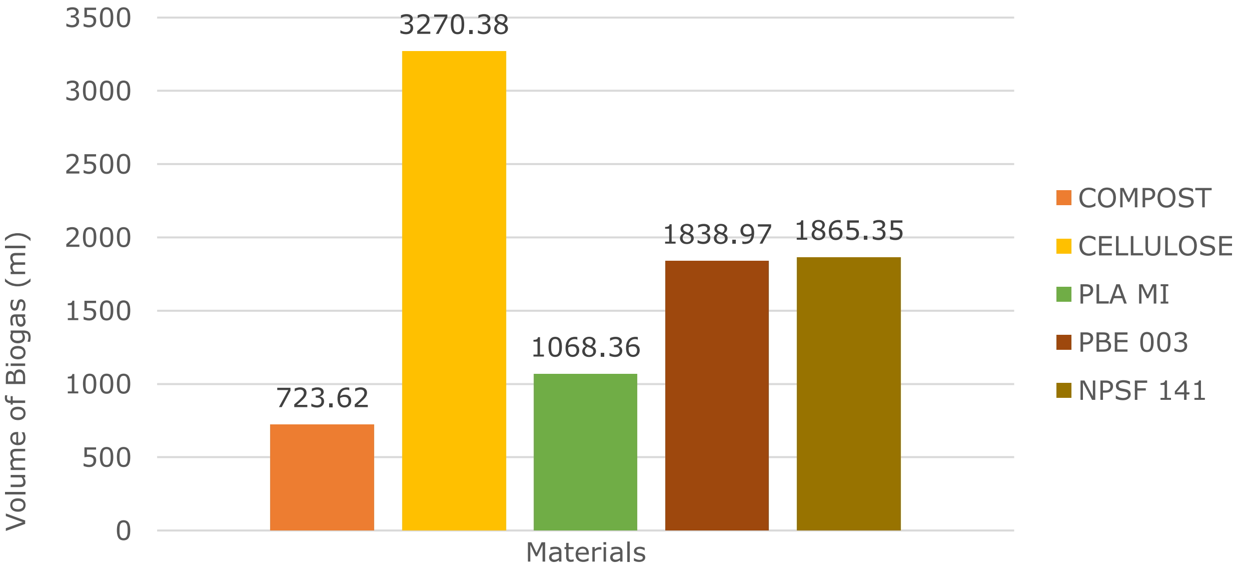


Figure 4.4 Cumulative volume of biogas produced.

From the table and figure 4.4 above, it is evident that Compost+cellulose produced the highest volume of biogas at 3270.38ml during the experiment period, followed by NPSF 141 at 1865.35ml and PBE 003 at 1838.97ml, respectively.

4.3 Biodegradability calculation and results

From table 4.4, the cumulative biogas sum produced in ml is seen. At this stage, the volume figures are converted from ml (gas) into mg(carbon) by merging equations 6 and 7 to realize equation 10, and the percentage biodegradation is calculated using equation 8 above. The calculation for each material is shown below and summarized in table 4.5.

$$Biogas\ mass = Biogas\ ml * \frac{273K}{(273+T)K} * (\frac{12mg}{22.4ml}) \tag{10}$$

Using Equation 10, the biogas mass for each material is:

Compost
$$Biogas\ mass = 723.62ml * \frac{273K}{(273+52)K} * \frac{12mg}{22.4ml} = 325.63mg$$

Cellulose
$$Biogas\ mass = 3270ml * \frac{273K}{(273+52)K} * \frac{12mg}{22.4ml} = 1471.67mg = 1.47g$$

PLA MI
$$Biogas\ mass = 1068.36 * \frac{273K}{(273+52)K} * \frac{12mg}{22.4ml} = 480.76mg$$

PBE 003
$$Biogas\ mass = 1838.97ml * \frac{273K}{(273+52)K} * \frac{12mg}{22.4ml} = 827.54mg$$

NPSF 141
$$Biogas\ mass = 1865.35ml * \frac{273K}{(273+52)K} * \frac{12mg}{22.4ml} = 839.41mg$$

According to equation 8, mci needs to be calculated. So,

$$mci = TOC * weight\ of\ sample\ material \tag{11}$$

Thus,

$$Cellulose\ mci = \ 0.44 * 5.5 = 2.42g$$

$$PLA\ MI\ mci = \ 0.39 * 7 = 2.73g$$

$$PBE\ 003\ mci = \ 0.53 * 5.54 = 2.94g$$

$$NPSF\ 141mci = 0.56 * 5.10 = 2.86g$$

Hence, from equation 8

Cellulose= $\%biodegradation = \left(\frac{1471.67mg-325.63mg}{2420mg}\right) * 100 = 47\%$

PLA MI=$\%biodegradation = \left(\frac{480.76mg-325.63mg}{2730mg}\right) * 100 = 5.7\%$

PBE 003= $\%biodegradation = \left(\frac{827.54mg-325.63mg}{2940mg}\right) * 100 = 17.1\%$

NPSF 141=$\%biodegradation = \left(\frac{839.41mg-325.63mg}{2860mg}\right) * 100 = 18\%$

Table 4.5: Summary of calculations

Material	**Biogas mass(mg)**	**Mci(g)**	**%biodegradation**
Compost	325.63		
Cellulose	1471.67	2.42	47
PLA MI	480.76	2.73	5.7
PBE 003	827.54	2.94	17.1
NPSF 141	839.41	2.86	18

According to ISO 15985:2017 the percentage biodegradation of reference material should be more than 70% after 15days to ensure the validity of test results.

At 15 days, the volume of biogas produced from the blank is 296.2ml, while compost+cellulose produced 516.5ml. The rate of biodegradation is calculated to ensure the validity of the test result.

$$\text{Compost: } Biogas\ mass = 296.2ml * \frac{273K}{(273+52)K} * \frac{12mg}{22.4ml} = 133.29mg$$

$$\text{Cellulose: } Biogas\ mass = 516.5ml * \frac{273K}{(273+52)K} * \frac{12mg}{22.4ml} = 232.43mg$$

Therefore,

$$\text{\%biodegradation @ 15 days=}\%biodegradation = \left(\frac{232.43mg - 133.29mg}{2420mg}\right) * 100 = 4.1\%$$

The rate of biodegradation of reference material at 15 days is 4.1% from the calculations above, and from this, it can be inferred that the experiment needs to be conducted again, preferably with compost that is not as acidic as the one used in this process.

5 SUMMARY

Following the proliferation of plastics since their mass production began in the mid-twentieth century and the consequential effects on human health and environmental quality, there has been a shift towards exploiting a more sustainable and environmentally friendly replacement in bioplastics. The experiment performed in this study was to evaluate the biodegradability of presented modified bioplastics under anaerobic conditions. This analysis was necessary to find out the behavior of these materials if used to make products that end up in an anaerobic environment.

Three modified bioplastics, namely PLA MI, PBE 003, and NPSF 141, were presented and analyzed using ISO 15985:2017. The reference material used for comparison is cellulose, while ordinary compost was the blank sample used for calculation purposes. The experiment lasted for 73 days, with both manual measurements of CO2 evolved done by titration and automatic measurements done by the AMPTS II device. The data obtained from all materials are presented in this thesis, and the various calculations are shown.

On the 73rd day, the volume of cumulative gas produced by blank, reference, and test materials (PLA MI, PBE 003, NPSF 141) was 723.63ml, 3270ml, 1068.36ml, 1838.97ml, and 1865.35ml, corresponding to 325.63mg,1471.67mg, 480.76mg, 827.54mg, and 839.41mg respectively. Also, the rate of biodegradation of the reference and test materials (PLA MI, PBE 003, NPSF 141) over the time of the experiment was calculated, and they are 47%, 5.7%,17.1%, and 18%.

For test validity, according to ISO 15985:2017, the test is considered valid if the percentage biodegradation of the reference material is more than 70% after 15days. On the 15th day, the rate of biodegradation of reference material was 4.1% and considered low. By the 73rd day, the percentage of biodegradation of reference material was 47%. This rate of biodegradation is low and could be caused by:

- The compost used for this experiment is acidic at a pH of 5.61; the recommended pH for anaerobic biodegradation is between 7.8-8.5 according to standard.
- Human error during titrations and measurement leading to mistake in calculations and subsequent results.
- Gas leakage from connecting tubes during titration procedures, also during replacement of basic solution.

- Experiment setup. Gas leakages might have occurred here as well because the AMPTS II setup was modified to accommodate vessels for titration.

As at the time of writing this thesis, this experiment was the second set done, and if further testing and experiment set occur, the standard should be strictly followed to get the desired rate of biodegradation.

6 LIST OF REFERENCES

[1] E. F. Gómez and F. C. Michel, "Biodegradability of conventional and bio-based plastics and natural fiber composites during composting, anaerobic digestion and long-term soil incubation," *Polym. Degrad. Stab.*, vol. 98, no. 12, pp. 2583–2591, Dec. 2013, doi: 10.1016/j.polymdegradstab.2013.09.018.

[2] A. Chamas *et al.*, "Degradation Rates of Plastics in the Environment," *ACS Sustain. Chem. Eng.*, vol. 8, no. 9, pp. 3494–3511, Mar. 2020, doi: 10.1021/acssuschemeng.9b06635.

[3] PlasticsEurope, "ISSUU - Plastics - the facts 2020 by PlasticsEurope," 2020. https://issuu.com/plasticseuropeebook/docs/plastics_the_facts-web-dec2020 (accessed Feb. 24, 2021).

[4] https://ourworldindata.org/plastic-pollution, "Plastic Pollution - Our World in Data." https://ourworldindata.org/plastic-pollution (accessed Mar. 01, 2021).

[5] G. Ross, S. Ross, and B. J. Tighe, "Bioplastics: New Routes, New Products," in *Brydson's Plastics Materials: Eighth Edition*, Elsevier Inc., 2017, pp. 631–652.

[6] R. Geyer, J. R. Jambeck, and K. L. Law, "Production, use, and fate of all plastics ever made," *Sci. Adv.*, vol. 3, no. 7, p. e1700782, Jul. 2017, doi: 10.1126/sciadv.1700782.

[7] https://issuu.com/plasticseuropeebook/docs/plastic_europe_study_final_spread, "The Circular Economy for Plastics – A European Overview by PlasticsEurope - issuu." https://issuu.com/plasticseuropeebook/docs/plastic_europe_study_final_spread (accessed Mar. 04, 2021).

[8] Chum H, "Polymers from Biobased Materials," 1991. https://app.knovel.com/web/toc.v/cid:kpPBM00001/viewerType:toc//root_slug:viewerType%3Atoc/url_slug:root_slug%3Apolymers-from-biobased?kpromoter=federation (accessed Feb. 23, 2021).

[9] M. C. Goldstein and D. S. Goodwin, "Gooseneck barnacles (Lepas spp.) ingest

microplastic debris in the north Pacific subtropical gyre," *PeerJ*, vol. 2013, no. 1, p. e184, Oct. 2013, doi: 10.7717/peerj.184.

[10] J. Hammer, M. H. S. Kraak, and J. R. Parsons, "Plastics in the marine environment: The dark side of a modern gift," *Reviews of Environmental Contamination and Toxicology*, vol. 220. pp. 1–44, 2012, doi: 10.1007/978-1-4614-3414-6_1.

[11] J. Zheng and S. Suh, "Strategies to reduce the global carbon footprint of plastics," *Nature Climate Change*, vol. 9, no. 5. Nature Publishing Group, pp. 374–378, May 01, 2019, doi: 10.1038/s41558-019-0459-z.

[12] https://www.plasticseurope.org/en/about-plastics/what-are-plastics/large-family, "A large family :: PlasticsEurope." https://www.plasticseurope.org/en/about-plastics/what-are-plastics/large-family (accessed Mar. 01, 2021).

[13] G. S. Bhat, "Plastics: Materials and Processing by A. Brent Strong," *Mater. Manuf. Process.*, vol. 12, no. 3, pp. 560–562, May 1997, doi: 10.1080/10426919708935166.

[14] M. Gilbert, "Plastics Materials: Introduction and Historical Development," in *Brydson's Plastics Materials: Eighth Edition*, 2016, pp. 2–18.

[15] https://www.chemicalsafetyfacts.org/plastics/, "Plastics | Uses, Benefits, and Chemical Safety Facts." https://www.chemicalsafetyfacts.org/plastics/ (accessed Feb. 26, 2021).

[16] H. Storz and K.-D. Vorlop, "Bio-based plastics: status, challenges and trends," *Landbauforsch · Appl Agric For. Res ·*, vol. 4, no. 63, pp. 321–332, 2013, doi: 10.3220/LBF_2013_321-332.

[17] https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/, "• Global plastic production 1950-2019 | Statista." https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/ (accessed Mar. 04, 2021).

[18] E. J. North and R. U. Halden, "Plastics and environmental health: The road ahead," *Reviews on Environmental Health*, vol. 28, no. 1. NIH Public Access, pp. 1–8, Apr.

2013, doi: 10.1515/reveh-2012-0030.

[19] R. Clift, H. Baumann, R. Murphy, and Et Al, "LEAD-journal.org - Managing Plastics: Uses, Losses and Disposal," 2019.

[20] J. R. Jambeck *et al.*, "Plastic waste inputs from land into the ocean," *Science (80-.).*, vol. 347, no. 6223, pp. 768–771, Feb. 2015, doi: 10.1126/science.1260352.

[21] https://ec.europa.eu/environment/topics/plastics/single-use-plastics_en, "Single-use plastics." https://ec.europa.eu/environment/topics/plastics/single-use-plastics_en (accessed Mar. 07, 2021).

[22] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32019L0904, "DIRECTIVE (EU) 2019/904 OF THE EUROPEAN PARLIAMENT." https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32019L0904 (accessed Mar. 07, 2021).

[23] F. Ruggero, R. Gori, and C. Lubello, "Methodologies to assess biodegradation of bioplastics during aerobic composting and anaerobic digestion: A review," *Waste Management and Research*, vol. 37, no. 10. SAGE Publications Ltd, pp. 959–975, Oct. 01, 2019, doi: 10.1177/0734242X19854127.

[24] https://www.european-bioplastics.org/bioplastics/, "Bioplastics – European Bioplastics e.V." https://www.european-bioplastics.org/bioplastics/ (accessed Mar. 08, 2021).

[25] https://bioplasticseurope.eu/about, "BIO-PLASTICS EUROPE | 'Bioplastics.'" https://bioplasticseurope.eu/about (accessed Mar. 10, 2021).

[26] S. Thakur, J. Chaudhary, B. Sharma, A. Verma, S. Tamulevicius, and V. K. Thakur, "Sustainability of bioplastics: Opportunities and challenges," *Current Opinion in Green and Sustainable Chemistry*, vol. 13. Elsevier B.V., pp. 68–75, Oct. 01, 2018, doi: 10.1016/j.cogsc.2018.04.013.

[27] S. M. Emadian, T. T. Onay, and B. Demirel, "Biodegradation of bioplastics in natural environments," *Waste Management*, vol. 59. Elsevier Ltd, pp. 526–536, Jan. 01, 2017, doi: 10.1016/j.wasman.2016.10.006.

[28] https://www.european-bioplastics.org/bioplastics/materials/, "Materials – European Bioplastics e.V." https://www.european-bioplastics.org/bioplastics/materials/ (accessed Mar. 12, 2021).

[29] https://www.european-bioplastics.org/market/, "Market – European Bioplastics e.V." https://www.european-bioplastics.org/market/ (accessed Mar. 12, 2021).

[30] S. Inkinen, M. Hakkarainen, A. C. Albertsson, and A. Södergård, "From lactic acid to poly(lactic acid) (PLA): Characterization and analysis of PLA and Its precursors," *Biomacromolecules*, vol. 12, no. 3. American Chemical Society, pp. 523–532, Mar. 14, 2011, doi: 10.1021/bm101302t.

[31] M. Murariu and P. Dubois, "PLA composites: From production to properties," *Advanced Drug Delivery Reviews*, vol. 107. Elsevier B.V., pp. 17–46, Dec. 15, 2016, doi: 10.1016/j.addr.2016.04.003.

[32] Q. Zhang, L. Shi, J. Nie, H. Wang, and D. Yang, "Study on poly(lactic acid)/natural fibers composites," *J. Appl. Polym. Sci.*, vol. 125, no. S2, pp. E526–E533, Sep. 2012, doi: 10.1002/app.36852.

[33] S. Ebnesajjad, *Handbook of Biopolymers and Biodegradable Plastics: Properties, Processing and Applications*. Elsevier Inc., 2012.

[34] E. T. H. Vink, K. R. Rábago, D. A. Glassner, and P. R. Gruber, "Applications of life cycle assessment to NatureWorks^{TM} polylactide (PLA) production," *Polym. Degrad. Stab.*, vol. 80, no. 3, pp. 403–419, Jan. 2003, doi: 10.1016/S0141-3910(02)00372-5.

[35] R. Auras, L. T. Lim, S. E. M. Selke, and H. Tsuji, *Poly(Lactic Acid): Synthesis, Structures, Properties, Processing, and Applications*. John Wiley and Sons, 2010.

[36] K. Hamad, M. Kaseem, H. Yang, … F. D.-E. P., and undefined 2015, "Properties and medical applications of polylactic acid: A review.," *academia.edu*, Accessed: Mar. 15, 2021. [Online]. Available: https://www.academia.edu/download/54965996/Properties_and_medical_applications_of_polylactic_acid.pdf.

[37] L. K. Ncube, A. U. Ude, E. N. Ogunmuyiwa, R. Zulkifli, and I. N. Beas, “Environmental impact of food packaging materials: A review of contemporary development from conventional plastics to polylactic acid based materials,” *Materials*, vol. 13, no. 21. MDPI AG, pp. 1–24, Nov. 01, 2020, doi: 10.3390/ma13214994.

[38] Y. Tokiwa and B. P. Calabia, “Biodegradability and biodegradation of polyesters,” *J. Polym. Environ.*, vol. 15, no. 4, pp. 259–267, Oct. 2007, doi: 10.1007/s10924-007-0066-3.

[39] S. Labidi, N. Azema, D. Perrin, and J. M. Lopez-Cuesta, “Organo-modified montmorillonite/poly(ε-caprolactone) nanocomposites prepared by melt intercalation in a twin-screw extruder,” *Polym. Degrad. Stab.*, vol. 95, no. 3, pp. 382–388, Mar. 2010, doi: 10.1016/j.polymdegradstab.2009.11.013.

[40] M. Labet and W. Thielemans, “Synthesis of polycaprolactone: A review,” *Chem. Soc. Rev.*, vol. 38, no. 12, pp. 3484–3504, Nov. 2009, doi: 10.1039/b820162p.

[41] A. G. A. Coombes, S. C. Rizzi, M. Williamson, J. E. Barralet, S. Downes, and W. A. Wallace, “Precipitation casting of polycaprolactone for applications in tissue engineering and drug delivery,” *Biomaterials*, vol. 25, no. 2, pp. 315–325, Jan. 2004, doi: 10.1016/S0142-9612(03)00535-0.

[42] S. Cesur, C. Köroğlu, and H. T. Yalçın, “Antimicrobial and biodegradable food packaging applications of polycaprolactone/organo nanoclay/chitosan polymeric composite films,” *J. Vinyl Addit. Technol.*, vol. 24, no. 4, pp. 376–387, Nov. 2018, doi: 10.1002/vnl.21607.

[43] M. Niaounakis, *Biopolymers: reuse, recycling, and disposal*. 2013.

[44] D. Homminga, B. Goderis, I. Dolbnya, and G. Groeninckx, “Crystallization behavior of polymer/montmorillonite nanocomposites. Part II. Intercalated poly(ε-caprolactone)/montmorillonite nanocomposites,” *Polymer (Guildf).*, vol. 47, no. 5, pp. 1620–1629, Feb. 2006, doi: 10.1016/j.polymer.2005.12.080.

[45] Karak N, *Biodegradable Polymers. In Vegetable Oil-Based Polymers - Properties, Processing and Applications*. 2012.

[46] N. Jacquel *et al.*, "Synthesis and properties of poly(butylene succinate): Efficiency of different transesterification catalysts," *J. Polym. Sci. Part A Polym. Chem.*, vol. 49, no. 24, pp. 5301–5312, Dec. 2011, doi: 10.1002/pola.25009.

[47] Kalia S and Avérous L, *Biodegradable and Biobased Polymers for Environmental and Biomedical Applications*. John Wiley & Sons, Inc., 2016.

[48] T. X. Jin, M. Zhou, S. D. Hu, F. Chen, Q. Fu, and Y. Fu, "Effect of molecular weight on the properties of poly(butylene succinate)," *Chinese J. Polym. Sci. (English Ed.*, vol. 32, no. 7, pp. 953–960, Jul. 2014, doi: 10.1007/s10118-014-1463-4.

[49] J. H. Zhao, X. Q. Wang, J. Zeng, G. Yang, F. H. Shi, and Q. Yan, "Biodegradation of poly(butylene succinate) in compost," *J. Appl. Polym. Sci.*, vol. 97, no. 6, pp. 2273–2278, Sep. 2005, doi: 10.1002/app.22009.

[50] M. Puchalski, G. Szparaga, T. Biela, A. Gutowska, S. Sztajnowski, and I. Krucińska, "Molecular and supramolecular changes in polybutylene succinate (PBS) and polybutylene succinate adipate (PBSA) copolymer during degradation in various environmental conditions," *Polymers (Basel).*, vol. 10, no. 3, p. 251, Mar. 2018, doi: 10.3390/polym10030251.

[51] A. Folino, A. Karageorgiou, P. S. Calabrò, and D. Komilis, "Biodegradation of wasted bioplastics in natural and industrial environments: A review," *Sustainability (Switzerland)*, vol. 12, no. 15. MDPI AG, p. 6030, Aug. 01, 2020, doi: 10.3390/su12156030.

[52] S. Grima, V. Bellon-Maurel, P. Feuilloley, and F. Silvestre, "Aerobic biodegradation of polymers in solid-state conditions: A review of environmental and physicochemical parameter settings in laboratory simulations," *J. Polym. Environ.*, vol. 8, no. 4, pp. 183–195, 2000, doi: 10.1023/A:1015297727244.

[53] Y. Tokiwa, B. Calabia, C. Ugwu, and S. Aiba, "Biodegradability of Plastics," *Int. J. Mol. Sci.*, vol. 10, no. 9, pp. 3722–3742, Aug. 2009, doi: 10.3390/ijms10093722.

[54] E. Castro-Aguirre, R. Auras, S. Selke, M. Rubino, and T. Marsh, "Insights on the aerobic biodegradation of polymers by analysis of evolved carbon dioxide in simulated composting conditions," *Polym. Degrad. Stab.*, vol. 137, pp. 251–271,

Mar. 2017, doi: 10.1016/j.polymdegradstab.2017.01.017.

[55] G. Kale, T. Kijchavengkul, R. Auras, M. Rubino, S. E. Selke, and S. P. Singh, "Compostability of Bioplastic Packaging Materials: An Overview," *Macromol. Biosci.*, vol. 7, no. 3, pp. 255–277, Mar. 2007, doi: 10.1002/mabi.200600168.

[56] Pace G. Michael, Miller E. Bruce, and Farrell-Poe L. Kathryn, "The Composting Process," Oct. 1995. https://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=1047&context=extension_histall (accessed Mar. 24, 2021).

[57] V. Bátori, D. Åkesson, A. Zamani, M. J. Taherzadeh, and I. Sárvári Horváth, "Anaerobic degradation of bioplastics: A review," *Waste Manag.*, vol. 80, pp. 406–413, Oct. 2018, doi: 10.1016/j.wasman.2018.09.040.

[58] E. Epstein, *Industrial Composting*. Environmental Engineering and Facilities Management, Taylor & Francis Group, 2011.

[59] A.-S. Nizami, *Anaerobic Digestion: Processes, Products and Applications*. 2011.

[60] L. Melville, A. Weger, S. Wiesgickl, and M. Franke, "Anaerobic Digestion," in *Transformation of Biomass: Theory to Practice*, vol. 9781119973, Wiley Blackwell, 2014, pp. 31–59.

[61] V. A. Vavilin, B. Fernandez, J. Palatsi, and X. Flotats, "Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview," *Waste Manag.*, vol. 28, no. 6, pp. 939–951, Jan. 2008, doi: 10.1016/j.wasman.2007.03.028.

[62] K. Stamatelatou, G. Antonopoulou, and G. Lyberatos, "Production of biogas via anaerobic digestion," in *Handbook of Biofuels Production: Processes and Technologies: Second Edition*, 1st ed., no. 15, 2011, pp. 266–304.

[63] J. A. Ogejo, Z. Wen, J. Ignosh, E. Bendfeldt, and E. R. Collins, "Biomethane Technology," *Virginia Coop. Ext.*, no. Publication 442-881, pp. 1–11, 2009, Accessed: Apr. 02, 2021. [Online]. Available: www.ext.vt.edu.

[64] G. Kale, T. Kijchavengkul, R. Auras, M. Rubino, S. E. Selke, and S. P. Singh, "Compostability of bioplastic packaging materials: An overview," *Macromolecular Bioscience*, vol. 7, no. 3. John Wiley & Sons, Ltd, pp. 255–277, Mar. 08, 2007, doi: 10.1002/mabi.200600168.

[65] K. Hagos, J. Zong, D. Li, C. Liu, and X. Lu, "Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives," *Renewable and Sustainable Energy Reviews*, vol. 76. Elsevier Ltd, pp. 1485–1496, Sep. 01, 2017, doi: 10.1016/j.rser.2016.11.184.

[66] J. Meegoda, B. Li, K. Patel, and L. Wang, "A Review of the Processes, Parameters, and Optimization of Anaerobic Digestion," *Int. J. Environ. Res. Public Health*, vol. 15, no. 10, p. 2224, Oct. 2018, doi: 10.3390/ijerph15102224.

[67] E. Kwietniewska and J. Tys, "Process characteristics, inhibition factors and methane yields of anaerobic digestion process, with particular focus on microalgal biomass fermentation," *Renewable and Sustainable Energy Reviews*, vol. 34. Elsevier Ltd, pp. 491–500, Jun. 01, 2014, doi: 10.1016/j.rser.2014.03.041.

[68] T. Forster-Carneiro, R. Isaac, M. Pérez, and C. Schvartz, "Anaerobic Digestion: Pretreatments of Substrates," in *Biogas Production: Pretreatment Methods in Anaerobic Digestion*, John Wiley and Sons, 2012, pp. 1–25.

[69] A. Mudhoo, *Biogas Production: Pretreatment Methods in Anaerobic Digestion*. John Wiley and Sons, 2012.

[70] S. Lambert and M. Wagner, "Environmental performance of bio-based and biodegradable plastics: The road ahead," *Chemical Society Reviews*, vol. 46, no. 22. Royal Society of Chemistry, pp. 6855–6871, Nov. 21, 2017, doi: 10.1039/c7cs00149e.

[71] "EVS-EN ISO 15985:2017." https://www.evs.ee/Download/ViewBrowsingServiceSubscription?productId=88087&language=EnglishLanguage (accessed Apr. 04, 2021).

[72] BioprocessControl, "AMPTS II and AMPTS II Light: Automatic Methane Potential Test System.," *Operation and Maintenance Manual*. 2016.

[73] Kuusik Argo, “Determining Biogas Yield from Industrial Biodegradable Waste - TalTech raamatukogu digikogu,” 2017. https://digikogu.taltech.ee/en/Item/060fd454-ad1e-40d5-892b-4583de20f85f (accessed Jun. 13, 2021).

[74] Crossno and Kalbus, “Determinations of Carbon Dioxide by Titration: New Experiments for General, Physical, and Quantitative Analysis Courses,” 1996. Accessed: Jul. 01, 2021. [Online]. Available: https://pubs.acs.org/sharingguidelines.