**TAL
TECH**

ANALYSIS OF CARBON DIOXIDE PRODUCTION AND BIODEGRADATION DEGREES OF (PHBV)-BASED PLASTICS AT A LABORATORY SCALE

(PHBV)-PÕHITSE PLASTIDE SÜSINIKDIOKSIIDI ERALDUMISE JA BIOLAGUNEMISE ASTMETE VÕRDLUS LABORATOORSETEL KATSETEL

MASTER THESIS

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Tallinn 2023

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THESIS TASK

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Thesis main objectives:
1. Literature review

2. Setup and development of aerobic biodegradability of plastic materials

3. Analysis of the amount of evolved carbon dioxide

4. Analysis of the level of biodegradability

5. Potential implications of materials usage compared to other blends

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PREFACE

This master thesis was motivated by the project "Bio-plastics Europe" with the objective of determining the ultimate aerobic biodegradability of plastic materials under controlled composting conditions. The test took place at Tallinn University of Technology (TalTech).

To make them more suitable for industrial applications, biodegradable polymers are often subject to modification. There are several viable waste treatment options for biodegradable polymer goods, but one of the most promising is anaerobic degradation. The biodegradability yields may be improved by studying the degradation of novel modified polymers.

This work has been carried out under the supervision of PhD student Pavlo Lyshtva.

Keywords: POLYHYDROXYBUTYRATE, CARBON DIOXIDE GENERATED, BIODEGRADABILITY, PLASTIC, COMPOSTING, AEROBIC, CONDITIONS, MASTER THESIS

LIST OF ABBREVIATIONS AND SYMBOLS

PHB   Phenolphthalein

PHBV   Poly(hydroxybutyrate-co-ydroxyvalerate)

PP   phenolphthaline

CO_2 Carbon-dioxide

NaOH   Sodium hydroxide

KOH   Potassium hydroxide

PET   Polyethylene terephthalate

PE   Polyethylene

PHA   Polyhydroxy acids

PLA   Polylactic acid

1 INTRODUCTION

Plastic is one of the most adaptable man-made substances. Due to its flexibility and durability, it is used in building materials, toys, appliances, and packaging. Plastics are now in high demand across all industries. About 36% of all plastics used today are used in the packaging business, with the building industry coming in a distant second with only 22%. The remaining 42% are employed in essential sectors such as electronics, furniture, transportation, agriculture, medicine, and toys. Plastics manufacturing is expected to increase by 100 percent by 2030 (Shaili Vadera, 2021).

Plastic has numerous applications; therefore, we have gotten hooked to single-use plastic goods that harm health, the environment, economy, and society (UN environmental programme, 2022). Plastic manufacturing has grown faster than other materials since the 1970s. If this historical pattern continues, worldwide primary plastic output will reach 1.100 million tons by 2050 (UN environmental programme, 2022). Less than 10% of plastic waste is recycled (15% recycled, 40% thrown away). Another 19% is burnt, 50% is thrown in landfills, and 22% ends up in uncontrolled dumpsites, open pits, or terrestrial or aquatic habitats, often in underdeveloped nations (OECD report, 2022). Plastic garbage at landfills may release harmful compounds into the soil, preventing water and air from reaching plant roots, causing plant growth and death, soil structure disruption, microbial community change, and climate change.

In recent years, biodegradable plastic has emerged as the most optimistic strategy for lowering overall waste volumes and improving waste administration. Due to their inability to decompose, petrochemical-derived plastics pose a serious threat to the environment. Because of this, governments, industry researchers and corporations have been inspired to scrutinize the potential of creating polymers from biodegradable resources and to develop new alternatives to the products that are already available on the market. PHB (Phenolphthalein) is seen as a viable potential replacement for petroleum-based plastic like polyethylene (PE) and polypropylene (PP) and polyvinyl chloride (PVC); because it biodegrades, decreasing pollution and environmental impact (McAdam et al., 2020). Moreso, it possesses thermoplastic properties regular with that of synthetic plastic. In 2021, the worldwide polyhydroxy butyrate market was predicted to be worth $102.4 million, and by 2028, that figure was projected to rise to $283.53 million (Credence Research, 2022). Small soil bacteria store energy in the form of carbon, which they utilize to ferment cellulose sources and generate PHB. And its usage is split into end-use industries: food & beverage, automotive, pharmaceutical, agricultural among others.

In recent years, biodegradation of PHB has gained popularity due to its widespread applicability and the fact that it may break down in both aerobic and anaerobic environments, both of which contribute greatly to sustainability. In an aerobic setting, it releases carbon dioxide and water; in anaerobic settings, it releases methane (CH_4), which may be burned to provide clean energy (Raja et al., 2017). Numerous scientists have examined various analytical methods by using the breakdown of the eco-friendly polymer PHB under aerobic settings. More PHB crystallinity was observed during enzymatic degradation of PHB films in aqueous solutions at 37 degrees Celsius and pH 7.4 (Kumagai et al., 2017). However, the enzymatic degradation rate showed a decreasing trend when compared to the degradation behavior of a blend of PHB with poly (ethylene oxide) under the same conditions. Fungal organisms may degrade PHB in a variety of environments, including ponds, soils, compost, sea water, hay, horse dung, and other organic matter (Lee et al., 2017). Analysis of PHB polymers over the last several years, however, has demonstrated that developments in these technologies will lead the road to standout polymers soon in several sectors.

The aim of this master's Thesis is to:
- Study the findings reported in the literature regarding the aerobic digestion of PHBV-based plastic under controlled conditions.
- Test the aerobic degradability of PHBV-based plastic under controlled composting conditions by analysis of evolved carbon dioxide.
- Compare the obtained results to those found in the literature.
- Conclusively determine if the PHBV-based plastics are suitable for aerobic degradation under controlled conditions when compared to other previously studied polymers.

2 LITERATURE REVIEW

2.1 Plastic pollution

Increased plastic pollution has an adverse effect on ecosystems, animals, and human health, among other aspects of the environment. Plastic pollution can cause harm to wildlife, damage to ecosystems, soil pollution, air pollution, microplastics, and enter the food chain, posing a risk to human health. Growing plastic pollution has serious consequences for the environment, and solving this problem is essential for the future of our planet and its inhabitants. Moreso, if current trends continue, the estimated emission rates for 2016 will almost double by the year 2025 (Macleod et al., 2021). While some nations have taken steps to lessen their plastic use and enhance their waste-management infrastructure, this is by no means the case everywhere. The implementation of the Basel convention to prevent the transport of plastic waste to countries with poor management systems or the European Union target to recycle more plastic as part of the transition to a circular economy are both examples of scenarios that involve concerted, joint global action, but they still predict annual increases in plastic emissions (Macleod et al., 2021).

As society, scientists and the economy gain more momentum, there is a considerable drive to develop strategies that are sustainable, among which has caused researchers to increase focus on waste and pollution prevention through the use of sustainable materials. Bioplastic is in recent years considered the futuristic approach to solving these problems. The subject of bioplastics is a combination of bio-based (green sources) and biodegradable plastics (clean removal) (Lambert et al., 2017).

Bio-based plastic contains organic carbon which comes from atmospheric species including plants, animals, agriculture, the marine environment, bacteria, fungus, and forestry materials. The biodegradability of these polymers is not guaranteed e.g., bio-based polyethylene (PE), bio-based polyethylene terephthalate (PET) (Lambert et al., 2017). Nevertheless, microbes break down biodegradable polymers like polybutylene adipate-co-terephthalate (PBAT) and polycaprolactone (PCL) by reducing the molar mass of the macromolecules that make them up (Lambert et al., 2017). Nevertheless, depending on the circumstances, bioplastics such as polyhydroxy butyrate (PHB), polylactic acid (PLA), cellulose (C), starch (Starch Blends), biodegradable polyethylene (PEBD), and polycaprolactone (PCL) are the most environmentally friendly.

Bioplastics can replace many polymers. Biobased, biodegradable, or both (Figure 2.1), they have the same features as ordinary plastics but often have extra benefits. Bioplastics lowers its carbon footprint by 42% and provides waste management choices (European Bioplastics, 2023) (Plastics Europe, 2022). Its use by Ford, Coca-Cola, Nike, and others in agriculture, catering, and packaging proves its worldwide commercial potential.
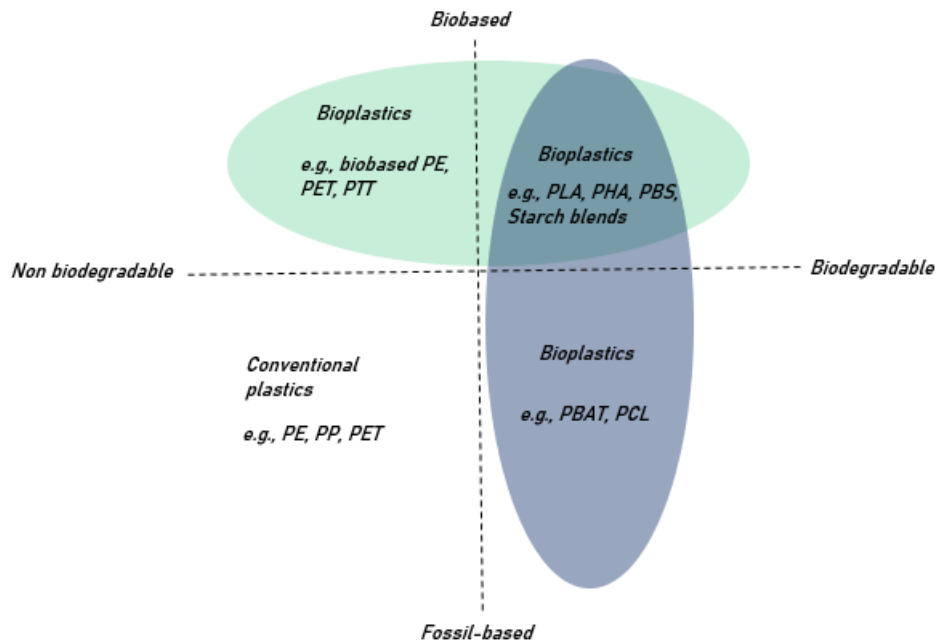
Figure 2.1 Family of bioplastic materials (European Bioplastic, 2022)

European Bioplastics and the nova-institute (2021) predict a global bioplastics production capacity increase from 2.23 million tonnes in 2022 to over 6.3 million tonnes in 2027. Bioplastics are anticipated to be worth USD 11.2 billion in 2021 and USD 46.1 billion by 2030. (European Bioplastic, 2023). PLA, PHA, starch blends, and others make up 51% (1.1 million tonnes) of bioplastics. PLAs and PHAs will produce about 3.5 million biodegradable plastics in 2027. (polyhydroxyalkanoates). 48% (1.1 million tonnes) of bioplastics are non-biodegradable. Drop-in biobased PE, PET, and PA (polyamides). Bio-based PET production stagnates as PP, PA, and PE expand (European Bioplastic, 2022).
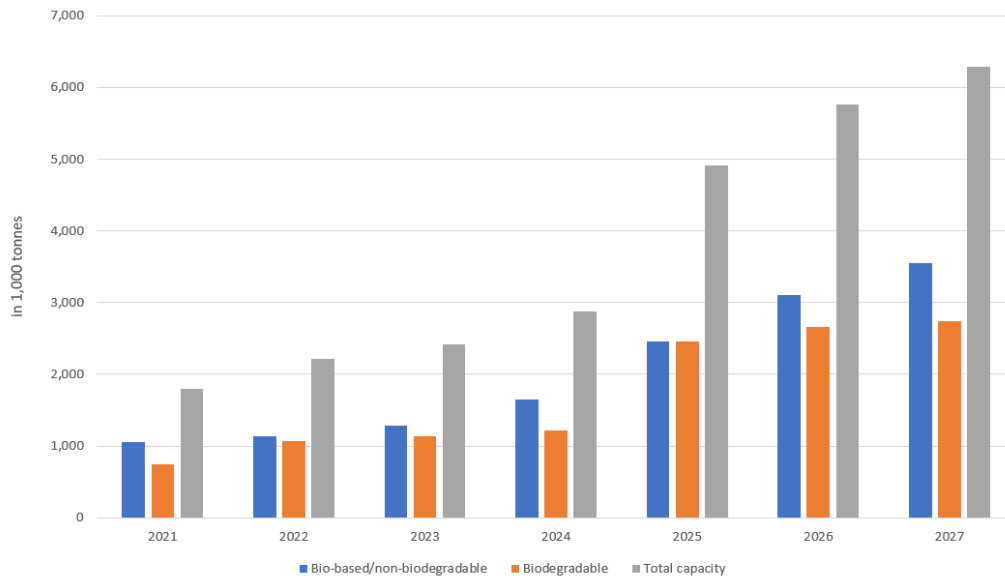
Figure 2.2 Bioplastic manufacturing worldwide (European Bioplastic, Nova-institute, 2022)

Figure 2.2 depicted above illustrates the projected growth in the manufacturing of biodegradable plastics in various sectors from 2021 to 2027, as estimated by a collaboration between European Bioplastics and nova-institute.

Bio-based/non-biodegradable 48.5% (PE, PET, PIA, PP, PEF, PTT)

Biodegradable 51.5% (PBAT, PBS, PLA, PHA, Starch blends, Cellulose)
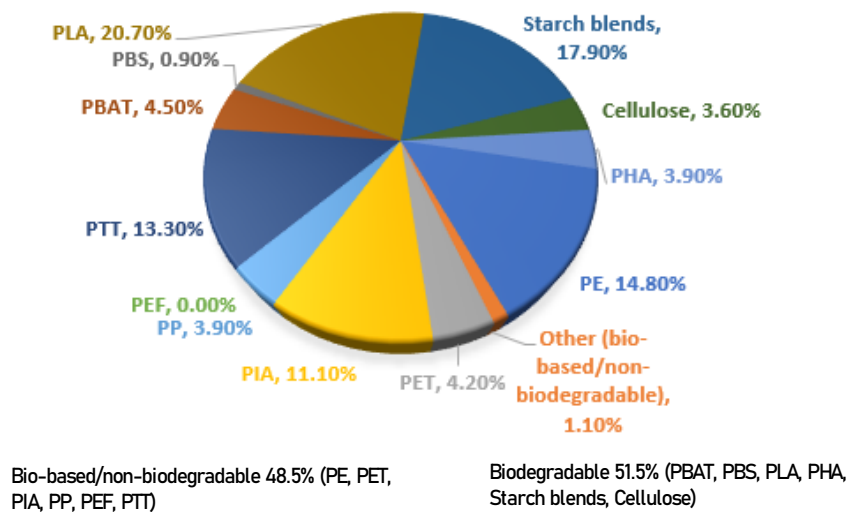
Figure 2.3 (a) Global production capacities of plastic (European bioplastics, nova-institute, 2022)

Furthermore, the above-mentioned figure 2.3(a) exhibits the distribution of various types of plastics, including bio-based/non-biodegradable and biodegradable ones, expressed in percentages, totaling an estimated 2.22 million tonnes as of 2022 in addition to the anticipated increase in the production of biodegradable plastics.

PLA, 37.90%

PBS, 0.30%
PBAT, 1.60%

PTT, 4.70%
PEF, 0.10%
PP, 6.00%

PIA, 18.70%

Starch blends, 6.30%

Cellulose, 1.50%

PE, 11.80%

PET, 1.80%

Other (bio-based/non-biodegradable), 0.40%

Bio-based/non-biodegradable 43.5%
(PE, PET, PIA, PP, PEF, PTT, Other)

Biodegradable 56.5% (PBAT, PBS, PLA, PHA, Starch blends, Cellulose)
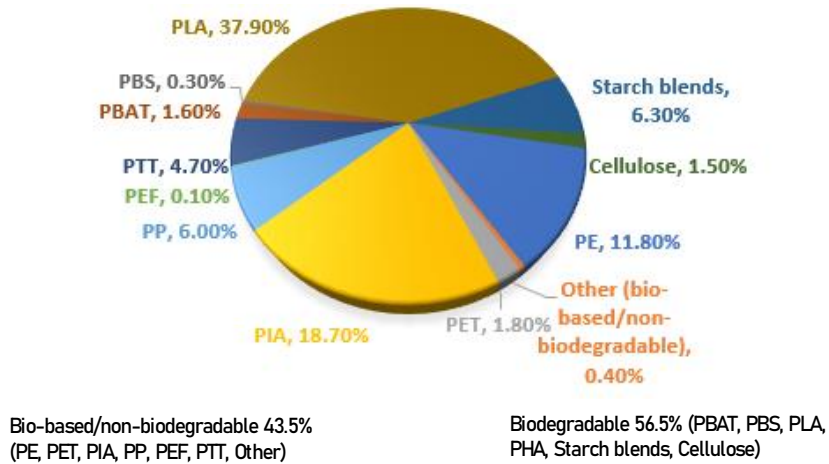
Figure 2.3(b) Global production capacities of plastic (European bioplastics, nova-institute, 2022)

Figure 2.3(b) provides an estimate for the global production of plastics in 2027, which is anticipated to be around 6.30 million tonnes, in continuation of the information presented in Figure 2.3(a).

Bioplastics is being adopted by various industries such as automobiles, consumer electronics, toys, textile, farming, and gardening. Bioplastics is expected to become the largest market in the packaging industry by 2022. Additionally, its potential usage is increasing, and it is being considered for use in other industries like construction (European Bioplastic, 2022).
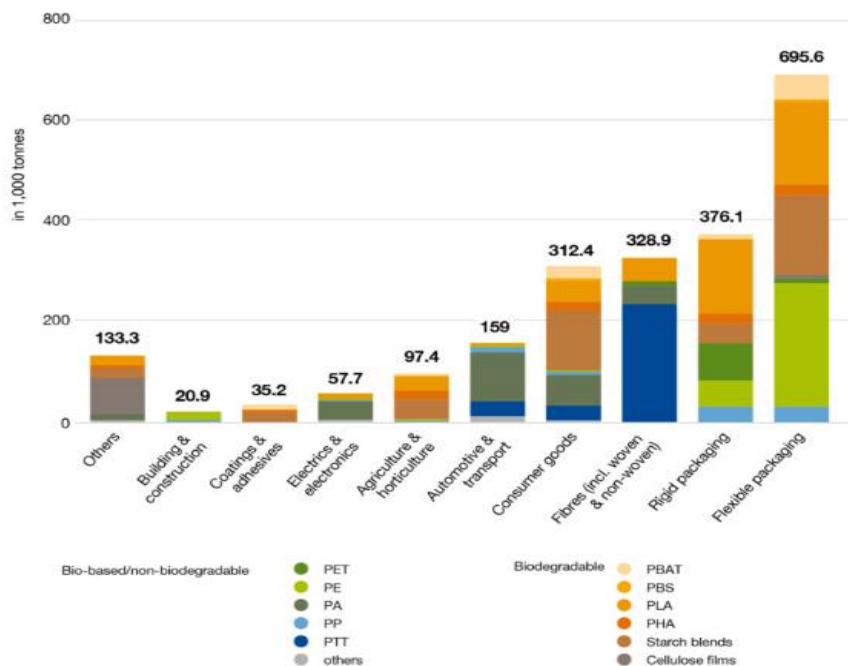

Figure 2.4 Global production capacities of bioplastic by market slice (European Bioplastics, nova-institute, 2022)

According to European Bioplastics and nova-institute (2022), Figure 2.4 depicts the global production of various types of bioplastics needed by the industries mentioned earlier, as per their market share. This highlights the bio-based/non-biodegradable and biodegradable plastics.

2.2 Waste management

2.2.1 Organic waste management

Composting seems to be one of the most promising approaches available for dealing with the issue of bioplastic waste, since it is one of the solid waste management strategies for biodegradable trash. Both biodegradation and composting involve microorganisms breaking down polymers, but the conditions under which the breakdown happens and the end products are different (Claudio et al., 2021). Composting creates soil fertilizer while biodegradation turns organic material into CO_2, new microbial biomass, and mineral salts under aerobic circumstances. Items made of bioplastic can be treated in composting plants along with organic waste (Dr. Gioia et al., 2021). Organic matter cannot be separated from plastics in certain applications, such as food waste collection bags. The fact that the item may be composted is a huge bonus in these circumstances, and the bioplastic has a good end of life in a composting facility.

Aerobic composting is a technique that utilizes air-dependent bacteria to decompose organic materials into compost. In this process, the microorganisms that need air include aerobic bacteria, fungi, and physical decomposers. Composting aerobically generates heat, water, and carbon dioxide (CO_2). CO_2 is categorized as a greenhouse gas, although it is far less dangerous than the methane produced by anaerobic composting (Compost Magazine, 2023).

Aerobic composting is an excellent method for reducing waste and producing a garden and farm soil supplement. It creates compost that is likely richer in nutrients than anaerobic composting. From an environmental standpoint, Methane has a 20-fold greater environmental impact than carbon dioxide (the main by-product of anaerobic degradation). The heat created during aerobic composting kills some hazardous bacteria and pathogens while encouraging the growth of beneficial microorganisms. Pathogens are eliminated when the compost cools, matures, and stabilizes. Unlike synthetic

fertilizers, the bulk of nutrients in compost are insoluble. This reduces soil nutrient loss, which may be damaging to the ecology (Compost Magazine, 2023).

2.2.2 Evolution of industrial composting in Europe

The Packaging and Packaging Waste Directive (PPWD) was first put out in 1994. Its main goal was to increase the amount of packaging waste that was reused and reduce the amount that was thrown away (landfilling) (European Bioplastic et al., 2015). In addition to mechanical recycling, biological (organic) recycling was also defined, and CEN was asked to create a standard for ''packaging recoverable through biodegradation and composting".
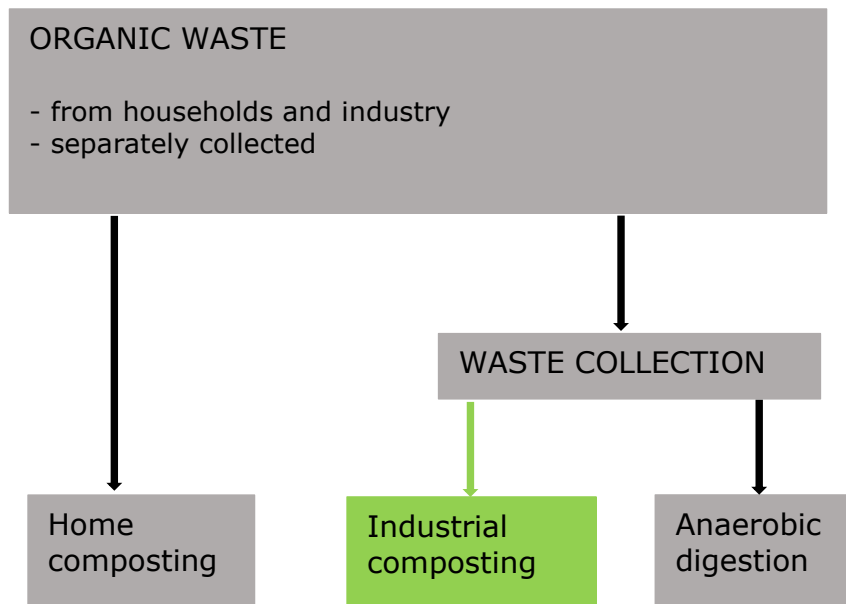
Figure 2.5 Options for organic waste management (European bioplastic et al., 2015)

The figure 2.5 above shows how organic waste has evolved over the years, describing in detail from which sources they were genrated and how they were sorted and collected for different purposes.

Industrial composting is a well-established and generally recognized aspect of the waste recovery infrastructure in a number of European nations, including Austria, Belgium, Germany, Italy, and the Netherlands. For other EU countries to get to the same level of industrial composting, they still need to catch up. But a good system for collecting trash separately is a must for organic recycling and industrial composting to work (European bioplastic et al., 2015).

2.2.3 Industrial composting mechanism

The process of industrial composting comprises two distinct stages: active composting or rotting, and curing (post-rotting). The duration of the active composting stage is determined by the type of composting being carried out (European bioplastic et al., 2015). In this stage, microorganisms break down the organic waste into carbon dioxide and water, while also utilizing it as a source of food and energy. This process generates heat, and as the temperature inside the compost pile rises, the microbes in the pile change from mesophiles to thermophiles.

The temperature in industrial composting heaps typically ranges from 50°C to 60°C. In the curing phase, the rate of decomposition slows down to a steady pace, and the compost continues to develop at temperatures in the lower mesophilic range of around 40°C, during which humic substances are formed (European bioplastic et al., 2015).

2.2.4 Industrial compostable of biodegradable plastic

The European Landfill Directive 1999/31/EC requires member states to implement alternative treatment of organic waste in order to reach established objectives for the reduction of landfill disposal. The most common method of organic waste recycling nowadays is industrial-scale composting (European bioplastic, 2009).

The amount of organic matter that is collected and treated varies a lot between EU countries. About 35%, or 17 million tpa, of the 49 million tpa of bio- and green-waste that could be recovered is currently collected separately in the EU15. This means that about 9 million tpa of compost are made each year (European bioplastic, 2009).

In the EU27 as a whole, 110 million tons of organic waste are made each year. The amount of waste that can be treated is 12 million tons for biowaste and 8 million tons for green waste. Every year, 10 million tons of compost are made (after J. Barth 2007).

Table 2:1 Amount of Bio-waste and Green waste in EU15 that is collected separately and turned into compost (European Bioplastic, 2015)

Country	Seperately collected + treated organic waste (Million tpa)				Recovery potential of organic waste (Million tpa)				Theoretical potential
	Biowaste	Greenwaste			Biowaste	Greenwaste			Total (M.tpa)
A(2000)	0,45	0,20			1,22	0,97			2,19
B Flanders (2000)	0,34	0,39							1,3
B Wallonia (1994)	0,12								0,16 in 2002
D (1999)	7,0								9,0
DK (1999)	0,037	0,65			0,1	0,66			0,76 in 2004
F (2000)	0,05	1,5			5,25	3,5			8,75
Fi (1998)	0,1								0,6
GR (1995)	-	-							1,8
I (1999)	1,5								9
IRE (1998)									0,44
Lux (1998)	0,03								0,06
NL (2001)	1,6	1,5			1,7	1,5			3,2
P (1995)		0,01							1,3
ES (2000)	0,03 (Catal.)	0,02 (Catal.)							6,6
SW (1999)	0,14	0,15			1,0	0,65			1,65
UK (2000)	0,039	1,0							3,2 in 2006
Sum	11,4	5,42							48,7
Treated Bio. + Greenwaste 16,9 M.tpa					Theoretical recovery potential 49 M.tpa				

The Table 2.1 shows the amount of biowaste and green waste that was collected separately and turned into compost in the European union. In the table, the amount of biowste and greenwaste collected seperately and treated across different years and the recovery potential of these (biowaste and green waste) organic waste.

When put into an industrial composting plant with other organic waste, biodegradable packaging and plastics biodegrade and don't cause any problems for the process, the product, or the environment. As of right now, both European and global standards for compostability of packaging and plastics are quite strictly controlled.

2.3 PHB as a biopolymer

PHB is a biopolymer. PHB's 3HB-to-4HB ratio depends on the bacteria and circumstances utilized to manufacture it. PHB's physical and chemical characteristics vary with monomer concentration (Rajan et al., 2017). PHB is becoming increasingly popular since it is a renewable and biodegradable alternative to oil-based polymers (Hankermeyer et al., 1999). If bacteria, for example, can break down a polymer into its constituent monomers, the substance may be biodegradable since it returns to its original condition. Since it can be generated from plant sugars and agricultural waste, PHB is a renewable resource. PHB is also found in Bacillus megaterium, Methyl bacterium rhodesianum,

Alcaligenes eutrophus, Methyl bacterium extorquens, Pasteurella putida, Sphaerotilus natans, Escherichia coli, and other bacteria (Hankermeyer et al., 2017).
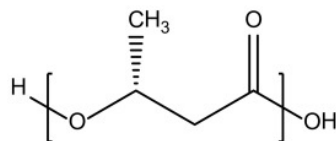

Figure 2.6 Structure of polyhydroxybutyrate (Rajan et al., 2017)

Figure 2.6 demonstrates the chemical structure of PHBV with the variations in monomers physical and chemical characteristics.

PHB might be used in lieu of synthetic polymers since it functions similarly to polypropylene. PHB is appealing to businesses since it is thermoplastic and biodegrades in compost. As PHB is superior to polyethene terephthalate and polypropylene in preventing oxygen vapor transmission, it has great promise as a food packaging material. PHB is comparable to polypropylene in terms of its properties (Hankermeyer et al., 2017).

Table 2.2 Properties of PHB and PP (Rajan et al., 2017)

Parameters	PHB	PP
Percentage of crystallinity (%)	60	50-70
Melting temperature (celcius)	177	176
Glass transition temperature (celcius)	2	-10
Tensile strength (MPa)	40	38
Elongation at break (%)	6	400
Density (g/cm3)	1.25	0.905
Tensile modulus MPa	3500	1900
UV resistance	Good	Poor

Table 2.2 above comparea different properties of PHBV polymers and polypropylene polymers and the significance of their different properties in terms of better environmental mangement decision making.

2.3.1 Phases of PHB

PHB granules are encased in a thick polymeric shell known as phasing, which defines the granule's size and protects it in life. After fermentation is complete, the culture medium is centrifuged to separate the bacteria from the polymer granules (Cai S. et al., 2012). With a solvent, the polymers may be readily removed from the cells (Cai S. et al., 2012).

Microorganisms store and consume carbon from substrate breakdown. The cell's cytoplasm accumulates 0.002–0.005 m granules (Santos J.A. et al., 2017). Granules comprise 70%–90% of a cell's dry bulk (Braunegg G. et al., 2012). These granules have a hydrophobic PHB core wrapped around a phospholipid coating. This activates PHA synthase and PHA depolymerase, which create and break down PHB, as indicated below.
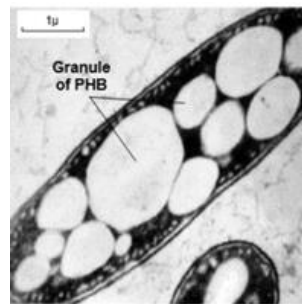

Figure 2.7 (a)In the cells of the bacterium Azobac- ter chroococcum, granules of PHB build up (Santos J.A. et al., 2017)
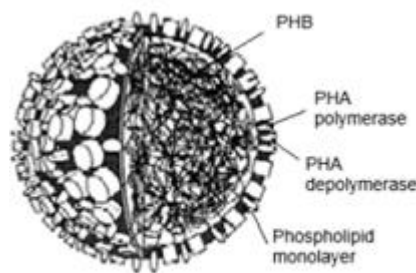
Figure 2.7 (b)Granules of PHB structure (Braunegg G. et al., 2012)

Once PHB builds up in the bacterial cell (Fig. 2.7: a), the polymer is removed and cleaned until it is dry and firm. It can be processed now. Polymerizing PHB under moderate processing conditions doesn't harm the environment, hence it's popular worldwide. The polymer becomes brittle with time, limiting its usage. Blending. To prevent PHB breakdown at 170°–180° C, we utilize lubricants such glycerol, acetyl citrate, or tributyrin (Rajan et al., 2018).

2.3.2 Production of PHB

The use of hybrid photosynthesis for photo augmented PHB production and the selection of appropriate feedstocks (Sirohi et al., 2020) have led to increases in PHB output (Xu et al., 2021; Kamravamanesh et al., 2017). Polymers with desirable properties may be used in a variety of applications, and genetic modification of the producer strain is an ongoing experiment in this direction (Zheng et al., 2020; Sohn et al., 2020). PHB may also be made by ring-opening polymerization (ROP) of -butyrolactone or via copolymerization of carbon monoxide and 1,2-propylene oxide (Reichardt and Rieger, 2012). In 2020, Martinez-Herrera et al. used ultrasonic to extract PHB with a low melting point and less than 50% crystallinity (Briassoulis et al., 2021).

2.3.3 Modifications of PHB blends

In 2021, the world made more than 400 million metric tons (Mt) of plastic made from petroleum, and the buildup of these non-biodegradable plastics in the environment is a worldwide concern. Polyhydroxybutyrate (PHB) has many advantages over traditional plastics made from petroleum because it is made from plants, biodegrades completely, and is not toxic. But it is still hard to make and use because it can't be bent very much and has a small processing window. It's also very sensitive to thermal processing conditions and has a small window of processing time when it's molten (Junior et al., 2022). A high processing temperature (close to or above Tm) is needed, which can lead to thermal degradation, which causes the molecular weight to go down and the melt strength of the crystalline domains to go down even more (Mekonnen et al., 2013, Weinmann et al, 2019).

Adding low-molecular-weight chemicals to the PHB that function as external eco-friendly plasticizers may fix this issue, at least in principle. The glass transition temperature (Tg) and the average melting temperature (Mt) might be lowered in the past by including plasticizers (Tm). Because of this, the material could be processed more easily using thermal means and was less prone to degrade when heated. The polymer structure is made more malleable and the inter-structure distance increases as a result of the addition of these substances. As a result, the material becomes more durable and adaptable (Mekonnen et al., 2013, Râpă et al., 2015, Seoane et al., 2018, Lee et al., 2021).

In the past few years, these biopolymers have been mixed with different plasticizers. These can be put into two groups: those with a low molecular weight, like citrates, glycerol, plant oils, and esters, and those with a high molecular weight, like polyethylene

glycol, propylene glycol, polyisobutylene, and aliphatic polyesters (de Sousa et al., 2022). Most plasticizers work best when they have a low molecular weight and groups that can interact with the polymer matrix (Lin K-W et al., 2016). They mix well with the PHB matrix, and their Tg goes down, their impact strength goes up, and their elongation at break is the greatest (Nosal et al., 2021). They mix well with the PHB matrix, and their Tg(glass temperature) goes down, their impact strength goes up, and their elongation at break is the greatest (Nosal et al., 2021). But there is also mass loss at processing temperatures (Seoane et al., 2018, Panaitescu et al., 2017) and the movement of these additives to the surface of the PHB, which causes the physical properties of the material to get worse. Oligomeric plasticizers have been used as an alternative. As PHB plasticizers, aliphatic polyester oligomers have a lot of promise (Nosal et at., 2021). They are biodegradable materials made from the polycondensation reaction of dicarboxylic acids and diols (Mochizuki et al., 1997). They have been used in the packaging, agriculture, and bio-medicine industries (Tsai et al., 2008).

2.3.4 Applications of PHB-based blends

Gram-negative bacteria like Pseudomonas spp. make PHB, while gram-positive bacteria like Bacillus spp., B. subtilis, B. thuringiensis, and B. cereus make it. The gram-negative bacteria A. eutropha can make PHB out of glucose, palm oil, molasses, and cereal grains. PHB is kept in the cytoplasmic fluid of the organism that makes it in granules with a diameter of 0.5 m that are called carbonosomes (Poltronieri et al., 2017; 11).

According to Juliana et al.,(2022) PHB is a polymer that is compatible with the body, and thus it does not get rejected. Moreover, the byproducts of its digestion are harmless. This polymer is capable of breaking down naturally, and it was produced using both chemical and biological techniques. However, it is not very durable due to its brittleness, which limits its practical applications. Juliana et al.,(2022) suggest that combining natural materials, synthetic biodegradable polymers, and/or non-biodegradable polymers can enhance the physical, mechanical, and thermal properties of the material.

Table 2.3 Application of PHB-based blends (Sreedevi et al., 2014)

PHB-based blends	Areas of Application
PHB/starch	Textile sector
PHB/chitosan	Drug delivery system
PHB/polyethylene glycol	Tissue engineering
PHB/polycaprolactone	Absorption properties
PHBV/starch	Production of disposable goods like food trays, cups, spoons, etc.
PHBV/polylactic acid	Biomedical devices and packaging goods.

Table 2.3 above shows possible blends of PHBV type plastic and the likely industries and sectors they are most applicable. As aforementioned these blends severly improves the durability and texture of this type of plastics.

Also, the current developments in the different blends of PHBV type plastic is shown in Table 2.4 with significant results whilst highlighting the specific properties investigated for the different blends.

Table 2.4 Current developments in PHB blends (Rajan et al., 2017)

No.	Blends	Preparation method	Properties investigated	Major results
1	PHB, low-density polyethylene (LDPE), and polyamide (PA)	Casting with chloroform as a solvent- ethanol solvent combination and melt/solid extrusion with a single-screw extractor.	Water movement, shape, and FTIR	Based on the results, a simple way to make blends that biodegrade quickly was suggested.
2	A natural terpene called D-limonene was added to Poly (lactic acid) (PLA) and PHB to make them more flexible (LIM)	After mixing in a HAAKE PolyLab mixer at 180°C, the material is shaped in a hot press at 180°C.	Calculations for DSC (Differential Scanning Calorimetry), TSC (Thermogravimetric Scanning Calorimetry), Measurements via FTIR (Fourier Transform Infrared Spectroscopy) and SEM (scanning electron microscopy)	Better water resistance and barrier properties, biodegradable in compost, and a blend that doesn't stick to each other.

			biodegradation, wettability, statistical analysis	
3	Maize starch, PHB	Mixing a solution in chloroform	Color, transparency, FTIR, SEM, and DSC.	Granules of starch are observed to be distributed heterogeneously. The possibility of using them as packaging
4	Nanofibers made from mixtures of PHB and PVA	Blend solution electrospinning	TEM, FTIR, DSC, SEM,	Blends were miscible, and PLA addition reduced the hydrolytic breakdown of PHB. Researchers recommended using the materials as scaffolds in cell culture studies.
5	PLA and PHB mix two different types of plasticizers: poly (ethylene glycol) (PEG) and acetyl- tri-n-butyl citrate (ATBC)	Melt-mixing and pressure molding	Degradation in conditions of composting, TGA, and SEM	As a nucleating agent, PHB slowed down the breakup, while plasticizers made it happen faster.
6	Poly(3-hydroxyoctanoate), or PHB (PHO)	Chloroform is used in solution casting to produce synthetic granules.	Analysis of density gradients, NMR, and DSC	It was shown that polymers might exist in two distinct phases while yet retaining their amorphous, elastomeric structure.
7	nanocellulose, polylactic acid (PLA), and phospholipid amide (PHB)	Microextrusion using a pair of screw nozzles	Conditions of composting, viscosity, mechanical properties, and statistical analysis of disintegration	As nanocellulose made the mixes less likely to disintegrate, it was recommended for use in food packaging.
8	Using poly (PHB/PLLA urethane) as a compatibilizer, we have poly[(R)3-hydroxybutyrate] (PHB) and poly (L-lactic acid) (PLLA).	Chloroform casting followed by vacuum dehydration	Anatomy, Mechanics, SAXS, DSC, WAXS, GPC, WAXS, DSC, WAX	Compatibilizer made the mixtures easier to mix and gave them better properties so they could be used as medical devices.
9	PHB and acrylonitrile-g- (ethylene-co-propylene-co- diene)-g-styrene (AES)	Extrusion with two screws and batch mixing	DSC, DMA, resistance to being hit, and SEM	Increase by ten times resistance to impact or 30 percent of AES
10	PHB and starch	Molding at 180 degrees Celsius in a hot press after mixing in a 175 degree C HAAKE PolyLab mixer	Optical microscopy, rheology, tensile testing, DSC and FTIR spectroscopy, DMA, and DSC thermal analysis	Enhancements in thermal stability, melt viscosity, and degradation resistance were attributed to the formation of hydrogen bonds between PHB and starch.

2.4 End of life options for PHB

Improving the environmental performance of bio-plastics relative to ordinary plastics has centered mostly on the end-of-life phase of industrially generated PHB (Polyhydroxybutyrate) plastic materials (K.P Rajan et al., 2017). Major issues at PHB plastics' end-of-life includes plastic biodegradation, composting and recycling options as explained below.

2.4.1 Plastic biodegradation

Biodegradation in an aerobic environment takes place between 6-12 weeks throughout the composting process. Industrial composting often takes place in industrial composting operations with carefully managed environmental conditions (including temperature, humidity, and air circulation). The chain structure of biodegradable polymers may be "digested" by microbes like bacteria or fungus and their enzymes. Three things are produced as a byproduct: water, carbon dioxide (CO_2), and a little amount of biomass (European Bioplastic, 2022).
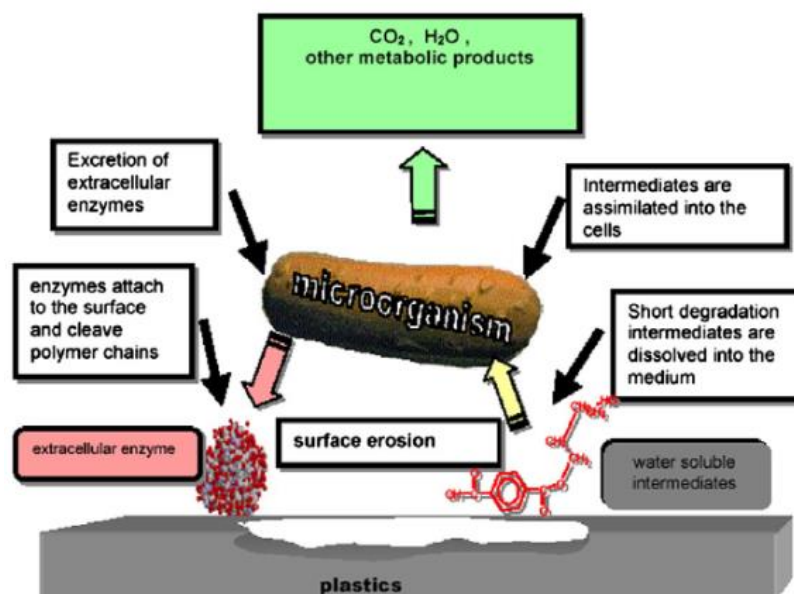

Figure 2.8 Mechanism for aerobic plastic biodegradation (Mueller, 2003)

PHB plastics are made to degrade, but the pace at which they do so and the degree to which they do so are both subject to the circumstances under which they are disposed of. If PHB plastics are disposed of in landfills or other places lacking oxygen, there is a possibility that they may not biodegrade as rapidly or fully as was planned. This is one

of the concerns. As a result of this, the breakdown of the plastics may result in the emission of methane, which is a powerful greenhouse gas (Shah et al., 2008).

2.4.2 Plastic composting

When plastic items are mixed with biowaste, it is clear that they can be composted. Under these conditions, it is not possible to recycle plastics or biowaste by hand. Using plastics that can be composted makes mixed trash suitable for organic recycling (composting), which makes it possible to switch from recovery to recycling (a treatment option which ranks higher on the European waste hierarchy). So, biowaste is taken out of other recycling streams or kept out of landfills. Separate collection is also made easier, which makes it easier to make more valuable compost (Plastics Europe, 2022).

While composting may be a useful method for getting rid of PHB plastics, it is crucial to do it properly. In the event that PHB plastics are not composted in the correct manner, they have the potential to give off methane in addition to other greenhouse gases and leach contaminants into the surrounding environment.

2.4.3 Recycling of plastics

PHB plastics might, in principle, be recycled; however, there are now only a limited number of tried-and-true recycling methods and facilities available. As a result of this, it is possible that many PHB plastics will continue to be disposed of in unclean ways, such as in landfills or incinerators, where they will likely contribute to pollution(Plastics Europe, 2022).

In order to enhance the environmental performance of PHB plastics in comparison to that of typical plastics, efforts have been concentrated on the development of improved end-of-life solutions, such as; Scientists now looking at ways to speed up the biodegradation of PHB polymers, particularly in anaerobic environments like landfills. Better composting techniques for PHB polymers are currently being developed. Research on PHB plastics' recycling infrastructure is underway to see how it may be improved and how far its reach can be extended. Although these initiatives are significant, lowering overall use of plastics, whether they are conventional or bio-based, is also a key approach for improving their environmental performance (Plastics Europe, 2022).

2.5 Biodegradation step and mechanism

Biodegradation has two different stages: depolymerization and mineralization. Chain cleavage is the first step in the process of breakup. At this stage, the polymer and microbes are in close contact with each other, which causes the macromolecules to break down into smaller chains (Grima et al., 2002).

The second stage is called mineralization. Once the oligomeric pieces are small enough, the cells take them in, where microorganisms bioassimilate them and turn them into minerals. This process of mineralization makes gases (CO_2, CH_4, N_2, and H_2), water, salts, minerals, and new biomass as byproducts (Grima et al., 2002).

2.5.1 Aerobic biodegradability of PHB

Bioplastic waste, made completely of natural materials, should be disposed of using an aerobic-anaerobic biodegradation method. Most businesses desire aerobic biodegradability (Situ Biosciences, 2022). Cost-effective. PHB degrades in soil, compost, and marine sediment. 0.5%–9.6% of colonies included PHB polymer-degrading bacteria. Most PHB-degrading bacteria are found at room temperature. PHB decomposes without oxygen and without hazardous consequences (Bugnicourt et al., 2014, Ong et al., 2017). Aerobic biodegradability means that microorganisms can break a substance apart in the presence of oxygen. Biodegradation, which is when bacteria break down matter, makes carbon dioxide, water, and new biomass. The chemical process is shown in the Formula (2.1) (Grima et al., 2002).

Many studies show that PHB breaks down in aerobic composting (Stevens, 2007). During industrial aerobic composting, PHAs with the following chemical structures were broken down: Poly(hydroxybutyrate-co-hydroxyvalerate) PHBV(40% mol HV) > PHBV(20% mol HV) > PHBV(3% mol HV) > PHB. 4HB and HV content are linked to biodegradation. After composting for 110 days, PHBV-40, PHBV-20, PHBV-3, P(3HB,4HB), and PHB biodegraded 90.5%, 89.3%, 80.2%, 90.3%, and 79.7%, respectively. Cellulose biodegraded 83.1%. (Weng et al., 2011).

2.5.2 PHB mixtures and combinations

PHB can be mixed with other polymers to make it better or cheaper, or to improve its qualities. Here are a few ways PHB can be mixed, mixed, or combined:

PHB may be combined with another biodegradable polyester, PCL (Polycaprolactone), to produce a material with superior mechanical characteristics and processability. PHB/PCL mixes are employed in a variety of applications, including packaging, biomedical devices, and tissue engineering. Also, it (PHB) be combined with starch to produce a biodegradable material that is less expensive and simpler to manufacture than pure PHB. Starch is a renewable and plentiful resource derived from plants such as maize, potatoes, and rice. Packaging, agricultural films, and other applications have made use of PHB/starch mixtures. PLA (Polylactic acid) is a biodegradable polyester made from renewable resources such as cornstarch, sugarcane, and cassava. As compared to pure PHB or PLA, PHB/PLA mixes may offer better mechanical characteristics and biodegradability. These mixtures have found uses in packaging, automotive, and biological fields. PHB may be mixed with nanofillers like clay, carbon nanotubes, or graphene to form PHB nanocomposites with enhanced mechanical and barrier characteristics. Packaging, biomedical, and environmental applications have all made use of PHB nanocomposites. These are just a handful of PHB mixes, mixtures, and combinations. Several additional materials may be mixed or combined with PHB to produce products with unique qualities and uses.

2.5.3 Mesophilic conditions

According to Cazaudehore et al. (2023), only PHB and TPS (thermoplastic starch) biodegraded quickly and significantly in mesophilic conditions at 38°C, while PCL and PLA biodegraded very slowly, and PBAT and PBS biodegraded very little or not at all at this temperature.

Experiments with anaerobic digestion at mesophilic temperatures revealed unsatisfactory kinetics for the majority of polymers. An experiment conducted by Naracic et al.(2018) reported the duration entended 500 days test showing biodegradation of TPS and PHB yielding exceptions of degradation as they were rapidly converted into methane. With TPS (Thermoplastic starch) undergoing 82.6% ± 7.8% degradation in 25-30 days.

Furthermore, during this experiment, two biodegradable polymers manufactured of PHB were broken down. Even though each of these plastics were manufactured of the same polymer, they broke down in quite distinct ways and in very different amounts of time. In around 50 days, 64.3% ± 0.6% of the PHB from Biomer broke down, whereas 80.1% ± 1.8% of the PHB from K.D. Feddersen broke down in just 25 days. These findings show that biodegradation may be affected by plastic qualities including crystallinity, molecular weight, accessible surface area, and others (Ahmed et al., 2018, Artham and Doble, 2008, Tokiwa et al., 2009).

Benn and Zitomer, (2018) found that four PHBs from different manufacturers biodegraded 67 % ± 19 % in mesophilic AD circumstances. Under mesophilic conditions, other biodegradable polymers had limited or no biodegradation. Even after a lengthy digesting time, PBS did not produce methane.

2.5.4 Thermophilic conditions

Studies of PHB degradation under thermophilic conditions have undergone a limited amount of research, however the rarest studies by researchers have shown good results of PHB degradation. Compared to the mesophilic test, the thermophilic test took less time since the samples of PLA, PHB, TPS, and cellulose attained their maximum methane outputs in 100 days. Under thermophilic circumstances (58°C), TPS, PHB, and PLA biodegraded quickly (100 days) and to high levels. At 58°C, it was not possible to convert PBS, PBAT, or PCL into methane. Bacteria that break down PHB (*Enterobacter and Cupriavidus*) and bacteria that use lactate (*Moorella and Tepidimicrobium*) seemed to play a big part in breaking down PHB and PLA, respectively (Cazaudehore et al., 2023). This is however the case for our PHB plastic tested in the laboratory which took 6 months.

Also, biodegradation of PBAT and PBS was minimal or nonexistent, with values of 1.7% ± 1.8% and 0.0% ± 1.8%, respectively, after 100 days. There have been reports of PBAT's poor biodegradability in other contexts (Svoboda et al., 2018) and PBS (Naranci et al., 2018, Yagi et al., 2013, Dvorackova et al., 2015).

3 MATERIALS AND METHODS

3.1 Materials

This research experiment focuses on carbon dioxide emission and the degree of biodegradation of plastics. As such, the sample plastic materials that will be used for testing are made of similar PHBV materials renamed as PHBV50-01 and PHBV50-02. These names are only sample names for the purpose of the experiment.

Both the PHBV50-01 and PHBV50-02 sample plastics, sold under the brand names PHi001 and PHi002, respectively, contain 50% natural rubber. The PHBV50-01 and PHBV50-02 sample plastics are insoluble in water and are generally resistant to hydrolytic breakdown, much like PHA (Polyhydroxyalkanoates) rubber. This distinguishes them from other biodegradable polymers, most of which are water-soluble. It is non-toxic, has high oxygen permeability, and turns to glass at 175 degrees Celsius, making it ideal for usage in the medical field. Its tensile strength is like that of polyethylene, measuring 40 MPa. We also used a thin layer chromatograph (TLC) grade microcrystalline cellulose with a particle size <20μm as the reference material for positive control. The plastic which was in pellet form were cut to smaller sizes of 2cm x 2cm which was the required size for easy fit into the test vessels.

To determine the biodegradability of the plastic, the total amount of carbon dioxide produced and the degree to which the test material was broken down was assessed at the end of the experiment under controlled aerobic conditions. The degree of breakdown is for daily degradation levels (see chapter 4). Furthermore, activated vermiculite (the contents of the bioreactors or vessels 0.5L were mixed with a spatula to allow air flow) was used as a substitute for natural compost, as it creates comparable conditions to composting the organic part of mixed municipal solid waste due to with the fact that suitable compost is difficult to find in Estonia. This vermiculite used as inoculum to simulate a typical composting environment, and careful regulation of temperature, airflow, and moisture content is necessary for optimal results.

The test outcome measures both biodegradability of plastics and the amount of evolved CO_2 through a two-step titration according to EVS-EN ISO 19679:2020 standard (see equation 3.4) of the PHBV50-01 and PHBV50-02 plastics (samples) used in the test.

3.2 Principle of the test system

The biodegradation of the polymers (PHBV50-01 and PHBV50-02) according to the ISO standard 14855-1 focuses on the determination of the ultimate aerobic biodegradability of the plastic materials under controlled composting conditions by method of analysis of evolved carbon using compost as the inoculum, but in the case of this experiment; the inoculum used was vermiculate (as aforementioned). However, the condition of temperature, humidity, aeration was all closely monitored to ensure consistency with the allowable ISO standards for pass fail degradation conditions.

In brief, the biodegradability test was carried out in a 0.5L gas-tight vessel where it is intensively composted under optimum oxygen, temperature and moisture conditions for a test period not exceeding 6 months.

During the aerobic biodegradation of the PHBV50-01 and PHBV50-02 sample, carbon dioxide, water, mineral salts, and new biomass are the ultimate by-products but, in this case, we only focused on the by-products of carbon dioxide, biodegradation of the PHBV50-01 and PHBV50-02 sample plastics. The evolved carbon was closely monitored daily at regular intervals within the 0.5L test vessels and blank vessels containing vermiculite to determine the cumulative carbon dioxide evolved. Also, we use the cumulative carbon dioxide evolved to enable us to calculate the percentage of biodegradability of the test samples (PHBV50-01 and PHBV50-02 plastics) given by ratio of the carbon dioxide produced by the test samples to the maximum theoretical amount of carbon dioxide that was produced by the test samples. Also, this maximum theoretical amount of CO_2 evolved is further calculated by the measured TOC (total organic carbon), total dry solids, material weight and carbon dioxde molecular mass (see equation 3.4).

A good reason for using vermiculite in this experiment is that the very small amount of CO_2 evolved from the blank vessels (almost 0) is captured. And these very small values may be due to inactivity by microbial organisms. Ultimately, this capture of tiny amount of CO_2 enables us precisely to evaluate low levels of degradation activity.

3.2.1 Test environment

Creating ideal incubation conditions was crucial to the experiment, which involved maintaining a temperature of 58℃ (± 2℃) and ensuring there were no vapors present that could hinder the growth of microorganisms. This was achieved by either keeping

the incubator in a dark environment or using diffused lighting. In this experiment, the incubator/bioreactor was kept in a dark environment.

3.2.2 Working principle of the test system.

As shown in Figure 3.1(a, b, and c), the adsorption system is supplied with synthetic air that is free of CO_2 or compressed air at a constant low pressure using an air supply device. We chose to use synthetic air because it allows us to remove CO_2 by passing it through a suitable CO_2 adsorption system. Additionally, we used a solution of NaOH in water as the adsorption system because it helps to maintain humidity levels.

The measurement of the gas flow according to the exact meter is very necessary to give us actual degrading measurement part-time. Here we used 0.5M HCl as the instrument for removing water.

According to the system, the exhaust air from each composting vessel is removed or absorbed in a carbon dioxide trap containing NaOH solution (this can be 0.2M, 0.25M) and the CO_2 is measured as dissolved inorganic carbon (DIC).
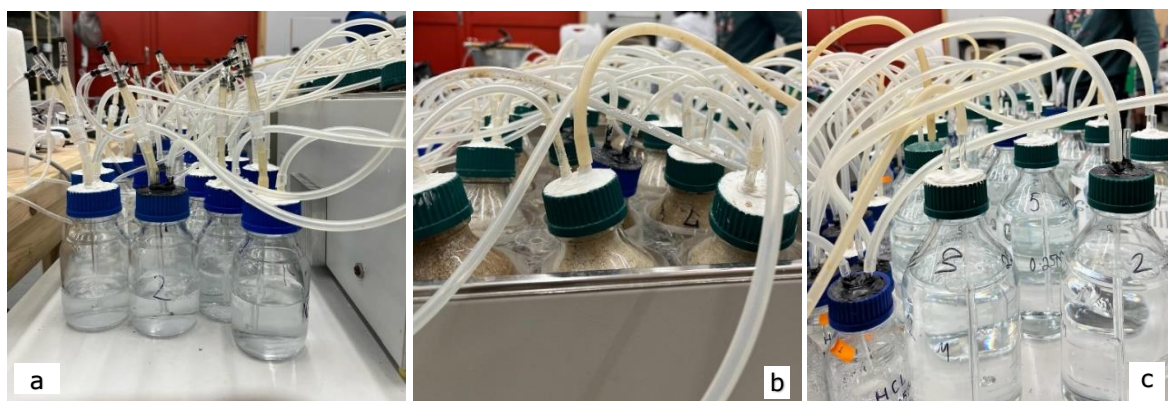

Figure 3.1 The seperate units of the system; (a): Air supply system supplying air into vessel containing NaOH solution which then extracts air through gas tubes to test mixtures, (b): Bioreactors for comprising test mixtures (activated vermiculite) with minimal air flow through the exhaust air tubes lid to prevent drying of the content, (c): CO_2 adsorption unit (Estonian Center for Standardisation, 2012)

3.2.3 Final set-up of the system

Once all of the bottles containing NaOH solution, bioreactors, HCl solution, and CO_2 absorption vessels are filled with products (sodium hydroxide and water, test mixture, hydrogen chloride, and potassium/sodium hydroxide solution, respectively), the incubation unit is assembled. The NaOH bottles are capped with two tiny apertures through which air is supplied and CO_2-free air is exhaled into the vessel of the bioreactor containing the sample mixture. In addition, the exhaust air reacts with the sample mixture to degrade the PHB polymers before the exhaution air is transported through pipelines into the CO_2 absorption receptacles, where final measurements are taken via titration.

The CO_2 free air supplied into the NaOH solution is connected to a power source. The thermostatic water bath of the incubation unit is filled with distilled water upto the required level. The final installation can be seen in the figure 3.2 below:

Figure 3.2 Final set up of the system

3.2.4 Incubation period

After starting up the test, the measurement of the amount of evolved carbon dioxide from the exhausting air from each of the composting vessel at different time intervals (usually 10 days in this experiment) using the two-step titration was done. The

frequency of the 10 days interval for measurement of evolved CO_2 is because we used the direct measurement. More often than the usual 10 days interval, we manually shake the composting vessels for the following reasons:

- to prevent extensive channeling
- to ensure uniform attack by microorganisms of the sample plastics, reference material and vermiculite.
- proper aeration of the vessels.
- more evenly distribution of moisture.

Also, there was visual inspection of the composting vessel to ensure the test mixture is well aerated and humidity is neither too high nor too low. Hence, there must not be any puddles or piles of debris that are not submerged. Water was also added to the composting vessel to maintain humidity (50% is the ideal humidity value) especially for extremely dry conditions when inspected. Furthermore, shaking the compost containers every week was done to cause the moisture to be distributed more evenly. And once any changes have been made, the carbon dioxide level was often monitored. Finally, tap water was added to the bioreactors when reduced below the required level. The essence is to maintain humidity. And this entire routine continues for the 6month period of the experiment.

3.3 Instruments used in the test

In this section, highlights of the aforementioned materials is stated along more detailed information of what they were used for and at what stage of the experimentation they were used.

3.3.1 The use of vermiculite

The type of vermiculite used was concrete type with initial density of 80kg/m^3 ± 16 kg/m^3 density with around 2% passed through a 0.5mm sieve at a size of 12mm and 14mm leaving 80%. In using vermiculite instead of compost as stated earlier, the vermiculite is firstly activated by inoculating it with a solution containing both organic and inorganic nutrients and/or mature compost which is then stirred thoroughly with the inoculate until homogeneity is achieved, and then put into the bioreactors. Each

bioreactor is then weighed whilst containing the mixed content and incubated in the sample incubation unit at 50 ℃ ± 2℃ for 3-4days as shown in Figure 3.2. The bioreactor is reweighed daily and tap water was added to it to maintain humidity. Also, the content of the bioreactor is mixed frequently (daily basis) with the use of a spatula.

Figure 3.1 A picture containing vermiculite undergoing activation in the incubation unit

After activating the vermiculite, the activated vermiculite, and the test materials (PHBV50-01 and PHBV50-02 sample plastics) is then put in the 0.5l composting vessels up to about ½ of the vessel, leaving enough room for manual shaking during the experiment to aid aeration. It is important to note that the amount of activated vermiculite and the PHBV50-01 and PHBV50-02 sample plastic put in the vessel is influenced by the size of the vessel. In this test the ratio of the dry mass of the activated vermiculite and the dry mass of the test material was 4:1.

3.3.2 Indicators preparation

The indicators are prepared for use in the two-step titration to determine biodegradability and amount of evolved CO_2 on every sample bottle.

- Methyl orange: To make a 1% weight by volume solution of methyl orange indicator, a petri dish was placed on a weighing balance and reset to zero. Then, the methyl orange chemical was transferred from its stock bottle to the dish using a spatula whilst carefully weighing out exactly 1 gram of the chemical and placing it into a beaker containing 20ml of methanol. The solution was mixed with a spatula until it was thoroughly combined. Next, a funnel was placed on top of a 100ml measuring flask and the solution was poured into the flask. Distilled water was added to the

flask until it reached the 100ml mark. The resulting solution was then a 1% weight by volume solution of methyl orange, which could be stored in any container and used as needed (Pharmaguddu, 2023).

- Phenolphthalein (P-P): The P-P indicator of 0.5% was prepared according to the following procedure; weighing 0.5g of P-P powder was done, the powder was then added to 50ml 100% (or 95% ethanol) and stirred well, allowing the proper mix of the powder with ethanol. The purpose of adding the P-P powder to ethanol is because it has very low solubility in water/acqueous solutions but dissolves well in ethanol. A dropper was then used to dispense it in the dispenser to ensure easy dropwise addition of the indicator.

3.3.3 Preparation of the test material and reference material

The total organic carbon (TOC) (described below) of both the reference material and sample plastic for testing was determined (in grams) and documented.

The PHBV50-01 and PHBV50-02 test materiasl were used in the form of simple shapes with dimensions of 2cm by 2cm as mentioned in the earlier literature (see Figure 3.2 below).
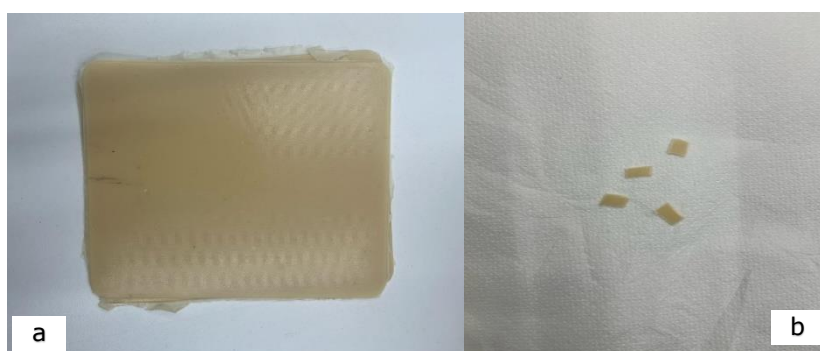

Figure 3.2 PHB plastic test material: (a) before shredding to little pieces (b)shredded pieces put into test bottle

Larger sizes of test material above the required dimension are further cut to a reduced size that meets the specification.

3.3.4 Total dry solids, total volatile solids and total organic carbon

Before the start of the experimentation, certain volume of the inoculate was assessed for total dry solids, calculation of the volatile solids after incineration at 550 ℃, and total organic carbon using the ISO 14855-1:2012 standard.

The total dry solids is the amount of water in the substance. So, we heat the substance at 105℃ for 9 hours (this is depended on the different methodology for carrying out the experiment, which can be done for several degrees in several hours e.g., 103 ℃ for 12 hours). At this temperature, the water evaporates and at the same time the material does not burn. The result is dry solids. For the material used in this experiment there is some percentage of total dry solids. In this experiment, PHBV50-01 sample plastic gives 99.7% of dry sample material left, PHBV50-02 sample plastic gives 99.72% of dry sample material left, and 95.6% of reference cellulose. The test material vermiculite was 13.87g for each vessel and 14.47g of cellulose per vessel when weighed.

When we then subtract the left-over of the vermiculite (which has undergone incineration at 550℃) from the TDS of that same sample, the result is the volatile solid. Doing this for all the vessels gives the TVS. This information also gives us an idea of the amount of organic matter present in the vessel (per vessel).

Furthermore, for every plastic type there is a specified or estimated percentage of organic compound present. For the test material of PHBV50-01 sample plastics, PHBV50-02 sample plastic and cellulose used; the percentage of TOC is 76%, 76%, and 44% respectively.

Mathematically, the formula for calculating TDS, TVS and TOC is shown below.

TS (%) = dry mass of solid ÷ wet mass of solid   (3.1)

VS (%) = (dry mass of solid − dry mass of oven deposits) ÷ wet mass of   (3.2)
solid

TOC (%) = 100 × [(dry mass of oven deposits − mass of burnt deposits) ÷   (3.3)
dry mass of oven deposits]

3.3.5 Start-up of the test

The number of composting vessels used in the experimentation is set up as follows;

a. Three vessels for the reference material
b. Three vessels for the blank/vermiculite
c. Three vessels for PHBV50-01 sample and
d. Three vessels for PHBV50-02 sample

After setting up these bottles, the bioreactor system and the air supply system (which is connected to a power source). The composting vessels were then placed into the testing environment (bioreactor) at a maintained temperature of 58°C with an accuracy of 2°C. The aeration process of the composting vessels was started using air pumps connected to power to pump CO_2 free air and saturated with water. This was achieved by passing air through smaller bottles containing NaOH solution.

A sufficiently high flow rate was maintained to support aerobic conditions in all composting vessels throughout the entire testing period. Regular inspections of the air flow were conducted at each outlet to confirm that there were no leaks in the system. The reference material was treated with the same procedures as the test material. In the blank vessels, the inoculum/vermiculite was the only content, with the same total dry solids content as the containers containing the test PHBV50-01 sample plastic and PHBV50-02 sample plastic.

3.4 Titration process and carbon dioxide determination

This study employed a two-step titration method to determine the CO_2 release in PHBV50-01 and PHBV50-02 sample. The initial step of the titration involved using the phenolphthalein indicator to observe the transformation of the CO_2-salt mixture, along with NaOH or KOH, from a colored state to a colorless state, indicating the completion of the acid-base reaction and the presence of salt. The data from this first stage was recorded in our database. In the subsequent stage, after the completion of the acid-base reaction, approximately 2-3 drops of methyl orange were added to titrate the salt. The interaction between salt and acid led to the decomposition of CO_2, resulting in a red coloration of the mixture, which was then documented as the final outcome. This procedure is repeated for 2-3 samples of the same bottle solution.

In the determining the carbon dioxide the values from the first step is deducted from the values of the second step for all bottles. The eventual carbon dioxide determination is calculated using the equation 3.4 below and the graph in figure 4.1 displays the resultant effect.

3.5 Calculation of the theoretical amount of carbon dioxide

Recall that in the earlier literature, it was stated that the calculation of the theoretical amount of evolved carbon dioxide is important because it enables us to calculate the degree of biodegradability.

Mathematically, the theoretical amount of evolved CO2 (ThCO$_2$) in grams per vessel of each test material is shown in the equation 3.4 below.

$$ThCO_2 = M_{TOT} * C_{TOT} * \frac{44}{12} \qquad\qquad (3.4)$$

M_{TOT} – the initial amount of dry solids, measured in grams, present in the composting vessels at the beginning of the test.

C_{TOT} – the ratio of total organic carbon content to the total dry solids in the test material, measured in grams.

44 and 12 – the molecular mass of carbon dioxide and the atomic mass of carbon, correspondingly.

3.6 Calculation of the percentage biodegradation

Haven calculated the cumulative amount of evolved carbon dioxide, we the use the equation 3.6 below to calculate the percentage biodegradability of the test material for each vessel.

Mathematically, it is expressed as:

$$D_t = \frac{(CO2)T - (CO2)B}{ThCO2} * 100 \qquad (3.5)$$

Where;

D_t - percentage degree of biodegradability.

$(CO_2)_T$ – the total quantity of carbon dioxide produced in each composting vessel containing the reference material, measured in grams per vessel.

$(CO_2)_B$ – the average total quantity of carbon dioxide emitted in the blank vessels, measured in grams.

$ThCO_2$ – the calculated quantity of carbon dioxide that can potentially be generated by the test material, measured in grams per vessel.

After calculating the theoretical amount of carbon dioxide, the resultant value enables us to calculate the degree of biodegradability.

If the disparities between individual findings are less than 20%, we determine the average biodegradation percentage. If this is not the case, utilizing the settings for each composting vessel individually is done.

3.7 Activities during and after the test

Under conditions that mimic a long aerobic composting process, the test method measures how biodegradable and broken up the test material is in the long run. If it's possible, the inoculum was steady, mature compost made by composting the organic part of solid city waste. The test material was mixed with the inoculum and put into a static composting tank (bottles in this case), where it digested quickly for up to six months under the best conditions for air, temperature, and wetness.

Also, the water bath/boiler temperature should always be at 58℃±2 to ensure efficiency in reactors the digestion. Often times when the water level in the boiler/bioreactors is decreased below the required level, tap water was added to it to maintain the teperature and humidity. Furthermore, the test mixtures vessels should be well mixed between 2-3 days regularly to enhance the flow of gases upstream and the efficient degradation of the test material. This can be done using electric mixers but, in our case, we did this manually by shaking the test mixture vessels, and also by adding water using a sringe.

After every interval of 10 days, the absorption vessels are changed with new absorption vessels solution along with bottles containing 0.5 HCl. With the samples of (1L bottles containing solution) using a 20 ± 0.03ml pipette transferred into a conical flask. This was done for 2-3 samples of the same solution. And then carry out the two step titration. The results are taken to identify the amount of biodegradability of the plastic material and the amount of evolved carbon (which is shown in the results Figure 4.3). And the test was carried out for six months.

Finally, once the test was over after the 6-month duration the entire setup was disconnected in no particular order. The CO_2 absorption vessels were disconnected and washed. The pH in the boiler/bioreactors was not measured in this situation. Furthermore, it is feasible to measure the total solids and volatile solids in each test mixture to ascertain the difference in volatile solids. We also measure the amount of degradation to see the difference in physical remains of the test materials. The rest of the test materials and bottles were disconnected, emptied, and washed/cleaned.

4 RESULT ANALYSIS

In this experimentation, we have four sets of vessels set up in the following order; sodium hydroxide solution which receives the compressed air used in the experiment (NaOH and water), bioreactor containing vessels of test mixture (PHBV plastics and vermiculite mix), hydrochloric acid solution (for water removal) and carbon dioxide absorption vessels for each bottle (containing either KOH or NaOH solution). The incubation unit is then set up.

The experimental procedure follows the guidelines outlined in section 3.7. The NaOH vessels are covered with a lid featuring two small openings, each equipped with a pipelike straw. One opening allows the supply of air, while the other permits the release of air into the vessels containing test mixtures placed in the bioreactor. The expelled air reacts with the test mixtures, leading to the degradation of PHBV polymers. Subsequently, the air, now containing water, is transported into the HCl vessels through a pipeline. The HCl solution removes the water, leaving only air, which accumulates in the CO_2 absorption vessels over a 10-day period. To measure the evolved CO_2, two to three samples are extracted from each CO_2 absorption vessel and subjected to a two-step titration process, as explained in section 3.4. The samples are then replaced after each 10-day interval.

For the determination of the total dry solids of our polymers, the PHBV plastics were heated at 105℃ for a period of 9 hours. This temperature allows the removal of water from the material without burning. The resulting material is then measured to give us the dry solid. However, the percentage of this materials can vary depending on what we are testing. In this experiment, PHBV50-01 sample plastic yielded 99.7% of dry sample material, PHBV50-02 sample plastic yielded 99.72%, and reference cellulose yielded 95.6%.

To identify the presence of organic matter in the samples, the total volatile solid is calculated for each vessel of test material by way of incinerating the leftover vermiculite at 550 and substracting the value from the total dry solids of the corresponding vessel. In our experiment, we investigated the PHBV50-01 sample plastic, PHBV50-02 sample plastic, and cellulose materials. The analysis revealed that these materials contain different percentages of total organic carbon (TOC). The PHBV50-01 sample plastic and PHBV50-02 sample plastic exhibited a TOC percentage of 76% each, indicating a high proportion of organic carbon in these materials. On the other hand, cellulose, which served as a reference, had a TOC percentage of 44%, indicating a relatively lower organic carbon content compared to the plastics.

4.1 Theoretical amount of carbon dioxide

The following parameters are need for the theoretical amount of CO_2 calculation as: the atomic mass of carbon, molecular weight of CO_2, total dry solids of test materials and cellulose, total organic carbon and material weight/vessel.

Table 4.1 Parameters require for theoretical amount of carbon dioxide calculation

Test material	Total dry solids (M_{TOT}) (%)	Total organic carbon (C_{TOT}) (%)	Material weight/vessel (g)
PHBV50-01	99.7	76	13.87
PHBV50-02	99.72	76	13.87
Reference cellulose	95.6	44	14.47

Using equation 3.4, the mathematical calculation for each test materials gives:

- For PHBV50-01:

$$ThCO_2 = ((99.7 \div 100) * 13.87) * (76 \div 100) * \frac{44}{12} = 38.535g$$

- For PHBV50-02:

$$ThCO_2 = ((99.72 \div 100) * 13.87) * (76 \div 100) * \frac{44}{12} = 38.543g$$

- For cellulose (reference):

$$ThCO_2 = ((95.6 \div 100) * 14.47) * (44 \div 100) * \frac{44}{12} = 22.317g$$

4.2 Carbon dioxide production

The experimental duration extended for a period of 180 days, during which distinct quantities of CO_2 were generated by various PHBV materials, as illustrated in the figures below. The CO_2 emissions were assessed using a CO_2 adsorption unit and employing the two-step titration method according to EVS-EN ISO 19679:2020 and subsequently documented in the database.

The following diagram depicts the portrayal of diverse materials employed in the experimental study. Specifically, the reference cellulose signifies conventional plastics derived from petroleum and petrochemical constituents, which manifest elevated levels of carbon dioxide on an average daily basis. Notably, the most pronounced average carbon dioxide concentration by this reference cellulose is observed on the 20th day of experimentation. Additionally, the control medium (vermiculite) emulates customary composting conditions, although vermiculite was selected due to its ready availability in the geographical vicinity. And it shows the natural composting conditions characterized by minimal carbon dioxide release, nearly approaching zero levels on the 90th day and zero on the 110th day.
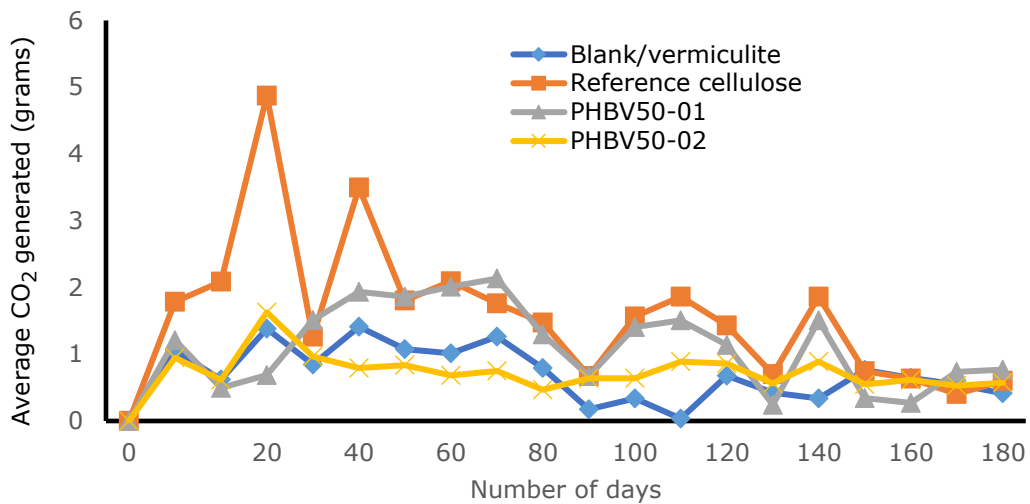
Figure 4.1 Average daily CO_2 generated (grams/vessel) by blank, reference cellulose, PHBV50-01 sample plastic and PHBV50-02 sample plastic materials

Figure 4.1 presents the average daily carbon dioxide production, revealing that the reference material exhibited the highest CO_2 production on day 20, while PHBV50-01 sample and PHBV50-02 sample shows emission levels closer to natural composting conditions depicted by blank (vermiculite).

Figure 4.2 depicted below illustrates the results, wherein PHBV50-01 sample plastic exhibit significantly reduced levels of CO_2 release on the 130th and 160th day, with PHBV50-02 sample plastic demonstrating a lower trend on a constant trajectory especially between the 30th to 80th day. The essence of comparing these samples (PHBV50-01 and PHBV50-02 samples) to the blank (vermiculite) is to demonstrate the behavioral expectations from these plastics.

Furthermore, it is important to look at the cumulative amount of CO_2 produced because this gives a broader idea of what we already explained in the previous figures. Hence, the cumulative carbon dioxide produced by materials shown in Figure 4.3 is significant evidence of the accumulation of their environmental impact over long period of time. The reference material (cellulose) shows greater environmental impact owing to the increased production of carbon dioxide, at a constant rate almost immediately. However, PHBV50-01 sample plastic shows lower emissions of carbon dioxide below the reference material and closer to that of the blank (vermiculite), evidently exhibiting tendencies of lower emissions over lengthy period compared to reference cellulose. Also, PHBV50-02 sample plastic maintains a constantly lower emission potential over long periods which is even lesser than blank vermiculite in 35th to 110th day.
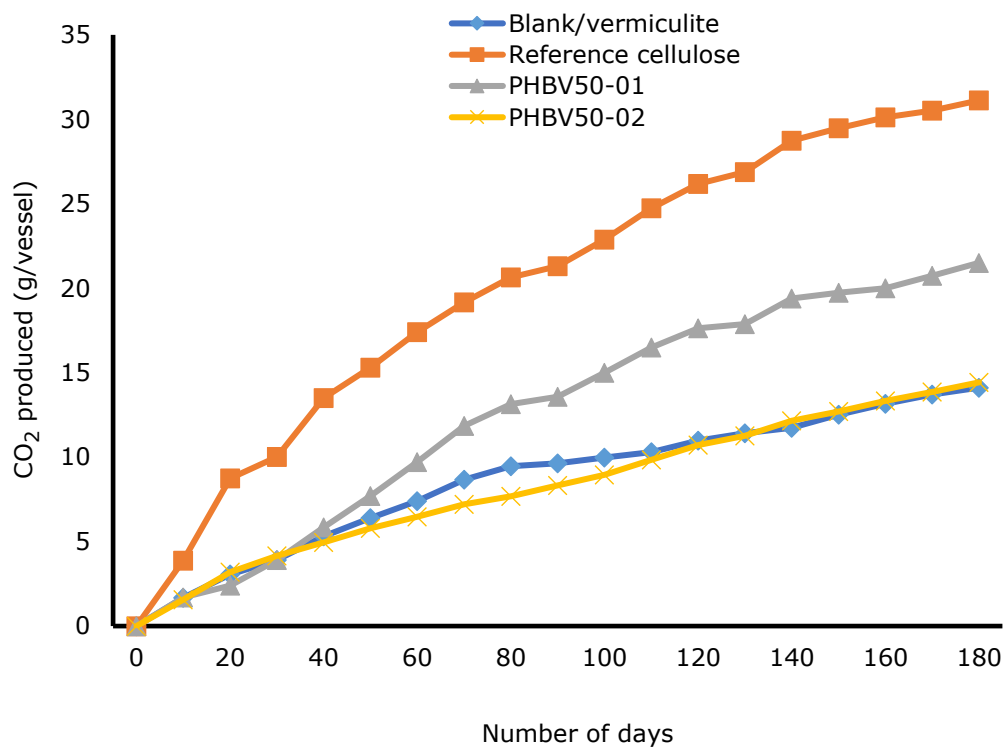
Figure 4.3 Cumulative CO_2 production/evolved in grams

Under ideal conditions (conditions of natural composting), the figure 4.3 shows that although there is an increase in carbon dioxide generated in the earlier stages of the experimentation by one of the samples, there is a constant trajectory of lower cumulative CO_2 generated by one of the samples (PHBV50-02 sample plastic). And it is important to note that the purpose of using two samples of the same properties is to give more accuracy to the findings to yield desired results especially in cases where there is little or no breakdown and/or CO_2 generation by one of the samples.

4.3 Degree of biodegradability

In recent years, a sub-focus of waste management has been initiated both to reduce the effects of waste materials on the natural environment by conserving raw materials used and to recover resources from wastes (Sharma, P. D. (2009)). Also, considering the abundance of plastics in the environment and their specificity in attacking plastics, biodegradation of plastics by microorganisms and enzymes seems to be the most effective process (Tokiwa Y. et al., 2009).

Here, the biodegradation rate of PHBV50-01 and PHBV50-02 samples were measured by the release of carbon dioxide. To ensure a fair comparison between the two measurements, several measurements tests was carried out simultaneously. After 180 days of incubation, it was impossible to continue measuring the specimens. The degradation had progressed too far, causing the films to break into smaller degrdable pieces that could be seen in figure 4.5 below. However, the method of measurement allowed the monitoring of biodegradation until the materials was completely transformed to ash/dust.

In this experiment, the reference material was used as the yardstick for measuring the supposed products our samples are competing with or improving. The graph (figure 4.4) is based on the highest reference value of degradation for the reference material (assumed to be 100%).

Indications from PHBV50-01 sample plastic show the continuous improved degradation of our PHBV-based plastic over time, implying improvement in the degree of biodegradation relative to reference material. Notice that the lag phase was in the first 20 days of the experimentation, which implies that there was over 10% degradation by the material. And subsequent display from the graph indicates that increased degradation of the PHBV50-02 sample plastic material over time. Also, the graphical display of figure 4.4 shows delayed and/or fluctuating lag phase, which may have been a result of delayed of reduced microbial population growth and/or adaptation of microbes to the new environment. But, in the progression of the test, the biodegradation increased.
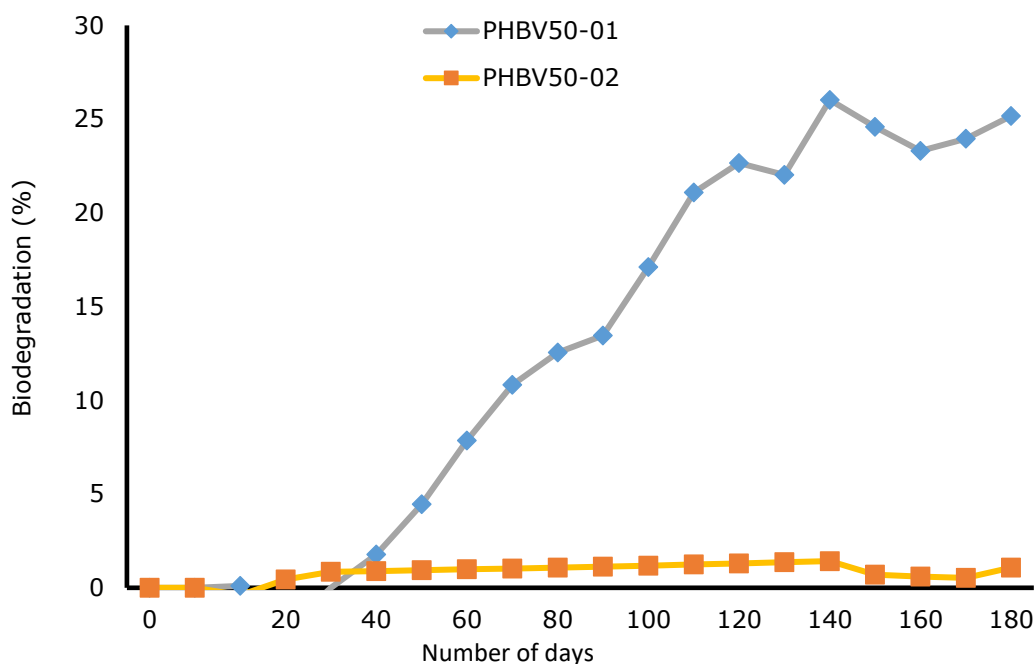
Figure 4.4 Degree of biodegradation of PHBV50-01 sample plastic and PHBV50-02 sample plastic based on maximum reference material

Figure 4.4 shows the percentage degree of biodegradation of the PHBV50-01 and PHBV50-02 samples. Here, the level of biodegradation for PHBV50-01 sample is much higher compared to the PHBV50-02 sample through the entire 180 days of the test. The degredation of PHBV50-02 is minimal however it can still be seen. It is important to also note that, if the sample materials (PHBV50-01 and PHBV50-02 samples) do not degrade more than the blank/vermiculite inoculum will have higher degree of degradation and produce more carbon dioxide than the test materials. Hence there can be negative 20% when our material is zero. Table 4.1 below shows the degree of biodegrading across different days.

4.4 Comparison of results

At the point the final values of CO_2 evolved from the sample materials was calculated, it becomes possible to draw comparisons between them and the values obtained in the literature for other polymer-based plastics under similar conditions. The values of TS,

VS, and TOC measured at the end of the experiment (day 180) were also used to compare the results at the end of the test.

Also, the degree of biodegradability of the polymers at the end of the test can also be compared to the results of those obtained in the literature using the Formular (3.4) with the results shown in Table 4.1. Because the chemical composition of the polymers used in the test is known as mentioned in section 3.1, cellulose was used as the reference material. Although, there are other reference materials but the reason for using cellulose is because it is common knowledge of how it degrades, and it meets the ISO certified standard for degradation (Mélanie et al., 2019).

Additionally, it was observed that PHB-based plastic underwent rapid biodegradation under mesophilic conditions at a temperature of 58℃ ± 2℃ within the initial 40 days of exposure (PHBV50-01 sample plastic). The biodegradation curve during this 0–40-day period can be attributed to a potential lack of adaptation of the microorganisms present in the activated vermiculite as aforementioned. Consequently, these microorganisms likely required additional time to synthesize the enzymes necessary for the degradation of PHBV bioplastics. After this initial lag phase, a consistent and progressive generation of CO_2 was observed throughout the later stages of the experiment.

4.4.1 Comparison of the materials by visual observation

Below is a graphical representation of the PHBV50-01 and PHBV50-02 samples material that was tested, showing the conditions of the material before the experimentation and afterwards. For clarity, in this figure, the both (PHBV50-01 and PHBV50-02 samples) polymer is made of identical features and components.
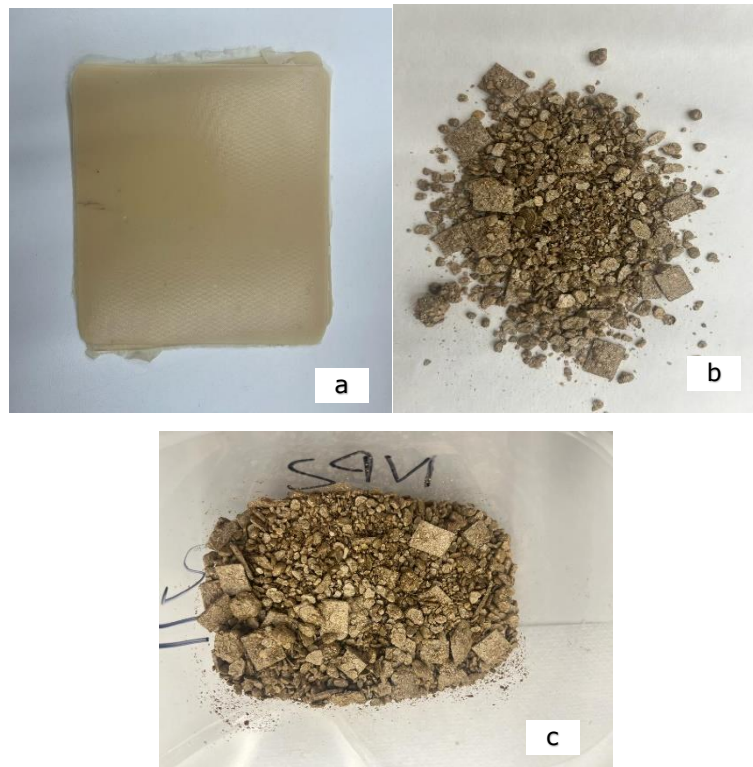
Figure 4.5 Graphics of sample materials. (a)before the experimentation (b) after the experimentation (PHBV50-01 sample) (c) after the experimentation (PHBV50-02 samples)

Figure 4.5 displayed above represents the deteriorated state of sample (PHBV50-01 and PHBV50-02 samples) PHBV plastic. It illustrates the comparative visual representation of biodegradability levels, with the "after" image exhibiting reduced density. Furthermore, when subjected to friction between two fingers, the "after" sample effortlessly disintegrates into a powdery form when held in the palm.
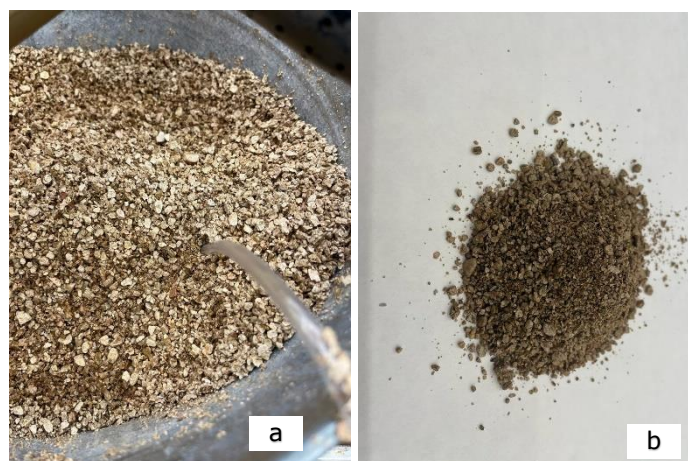
Figure 4.6 A pile of vermiculite (a)before experimentation (b) after experimentation

Figure 4.6 shows the look of activated vermiculite used in the test and the result after the test. From the picture (b) it shows a pile of vermiculite with reduced density haven

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undergone the experimentation process. Similarly, when subjected to friction between two fingers, the "after" sample effortlessly disintegrates into a powdery form when held in the palm.

4.4.2 Comparison of the materials by carbon dioxide evolution

Based on the analysis of evolved CO_2 and degree of degradation. A comparison between PHBV50-01 and PHBV50-02 samples reveals interesting insights.

In terms of evolved CO_2, PHBV50-01 sample plastic showed a higher amount of CO_2 release compared to PHBV50-02 sample plastic. This suggests that PHBV50-01 sample undergoes a more significant degradation process, resulting in a higher production of CO_2. on the other hand, PHBV50-02 sample plastic exhibited a relatively lower CO_2 release, indiating a comparatively slower degradation rate.

Furthermore, when considering the degree of degradation, PHBV50-01 sample displayed a more pronounced level of degradation compared to PHBV50-02 sample plastic. This cn be inferred from the higher amount of CO_2 released, indicating a more extensive breakdown of the plastic structure.

These findings highlight that PHBV50-01 sample is more susceptible to degradation compared to PHBV50-02 sample plastic. The reasons behind this discrepancy could be attributed to differences in the composition, additives, or environmental exposure of the two types of plastics. Understanding the variations in the degradation behavior of different plastics is crucial for assessing their environmental impact and informing decisions related to material selection and waste management strategies.

Further research and analysis can delve into the specific factors contributing to the divergent degradation patterns observed between PHBV50-01 sample and PHBV50-02 sample, providing valuable insights for the development of more sustainable plastic materials and waste.

4.4.3 Comparison with other studies

Garcia-Depraect et al. (2022) reported that at a temperature of 25 ± 1℃ the biodegradability of PHB and PHBV under aerobic conditions were 83 ± 1.6% and 87.4 ± 7.5% respectively, after 117 days. The biodegradation value for PHBV50-01 sample after 180 days was 20% and 1% for PHBV50-02 sample plastic.

Under thermophilic conditions at a temperature of 58℃ PHBV degraded quickly within 100 days to a very high level according to Cazaudehore et al. (2023). Conversely, the result of our experimentation shows a completely different outcome. It is important to note that the temperature of the heating environment hugely influences the degradability of the material undergoing testing.

Mélanie et al (2019), suggests that PHBV films biodegraded quickly within 60 to 80 days in a lab-scale composting conditions compared to PBSA despite having a higher molecular weight and degree of crystallinity. When compared to our PHBV50-01 sample polymer, which biodegraded within 20 to 40 days reaching its plateau phase in the 140th day. This suggests that different biodegradation mechanisms are involved in each polymer. This could also be influenced by the hydrolytic chain scission mechanism due to differences in water permeability, water diffusion, and molecular weight of the test materials.

5 CONCLUSION

In conclusion, this master's thesis aimed to investigate the aerobic digestion of the PHBV-based plastic under controlled conditions and compare the findings to the existing literature. The study involved testing the aerobic degradability of PHBV-based plastic the analysis of evolved carbon dioxide in controlled composting conditions.

By conductiong a thorough review of the literature, the study provided a comprehensive understanding of the current knowledge on the subject. The experimental results showed the level of degradation for one of the sample PHBV50-01 reaching 25.17% to increase as the test progesses, however the PHBV50-02 only degraded about 1%. When these results were then compared to the existing findings, it allows for a comprehensive evaluation of the aerobic degradation of PHBV plastics.

These findings further contribute to the body of knowledge surrounding PHBV plastics and their potential as suitable materials. The results also highlights the importance of controlled composting conditions in assessing the aerobic degradation of plastics. Overall, this research provides valuable insight into the aerobic digestion of PHBV-based plastic and serves as a foundation for the future studies in the field of biodegradable materials.

SUMMARY

The purpose of the masters thesis is to study and report the finding of aerobic degradability of PHBV-based plastics under controlled condition by analysis of evolved carbon dioxide, document the results and ascertain if they are suitable for aerobic degradation when compared to other polymers.

The research experiment focuses on the biodegradation of plastics and their carbon dioxide emissions. Two types of plastics, referred to as PHBV50-01 and PHBV50-02 sample, were used as samples for testing. These plastics are made of a material called PHBV, which is insoluble in water and resistant to hydrolytic degradation. They are also safe for use in the healthcare industry and have properties such as good oxygen permeability and high melting point.

To assess the biodegradability of the plastics, the experiment measured the amount of carbon dioxide produced and the degree of breakdown of the test materials under controlled aerobic conditions. Vermiculite was used as a substitute for natural compost to create conditions similar to composting mixed municipal solid waste. The experiment closely monitored the carbon dioxide evolution daily to calculate the percentage of biodegradability.

The test system followed the ISO standard 14855-1 for determining ultimate aerobic biodegradability. It employed a 0.5L gas-tight vessel where the plastics were composted under specific conditions for a maximum period of 6 months. Carbon dioxide, water, mineral salts, and new biomass were the by-products of biodegradation, but only carbon dioxide was monitored in this experiment.

The gas flow in the system was measured using synthetic air and an adsorption system with NaOH solution to remove carbon dioxide. Instruments such as HCl and indicators like methyl orange and phenolphthalein were used to measure and analyze the gases produced.

Vermiculite was activated by inoculating it with an inoculum solution containing organic and inorganic nutrients. The mixture was added to the bioreactors, and the content was incubated at $50°C$ for 3-4 days. The activated vermiculite and test materials were then placed in the composting vessels, with a ratio of 4:1 between vermiculite and test material.

Indicators like methyl orange and phenolphthalein were prepared for the two-step titration to determine biodegradability and evolved CO_2. The experiment also involved determining total organic carbon, total dry solids, and total volatile solids of the materials used.

The experiment maintained optimal incubation conditions with a temperature of 58℃ and no hindering vapors. Aeration was provided through air pumps connected to the composting vessels, and regular inspections were conducted to ensure proper airflow. The test and reference materials were treated similarly, while the blank vessels contained only vermiculite.

The incubation period involved measuring the evolved carbon dioxide from each composting vessel at regular intervals using two-step titration. The composting vessels were shaken periodically to prevent channeling, ensure uniform attack by microorganisms, provide proper aeration, and distribute moisture evenly. Visual inspections and adjustments were made to maintain appropriate humidity levels.

Overall, the experiment aim of analysing the PHBV-based plastics represented as PHBV50-01 and PHBV50-02 was acheived as it was determined that they are biodegradable under controlled aerobic condition.

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