

# EVALUATION OF BIODEGRADABLE POLYMERIC MATERIALS

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Master's thesis  
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2. J. Šerá, L. Serbruyns, B. De Wilde, M. Koutný. Accelerated biodegradation testing of slowly degradable polyesters in soil. Polym. Degrad. Stab., 171 (2020), Article 109031.
3. S.M. Emadian, T.T. Onay, B. Demirel. Biodegradation of bioplastics in natural environments. Waste Manag., 59 (2017), pp. 526-536, 10.1016/j.wasman.2016.10.006.
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## **ABSTRAKT**

S přibývajícímí zprávami o šíření petrochemických plastů v životním prostředí a o jejich ekologických rizicích jsou biologicky rozložitelné plasty považovány za optimální alternativu k tradičním plastům. Biologicky rozložitelné plasty jsou produktem z přírodních polymerů, jako jsou PHB a PLA. PHB a PLA v poslední době vzbudily zájem díky svým vlastnostem a schopnosti rozkládat se na přírodní prvky za méně než měsíce. Navzdory svým pozoruhodným vlastnostem mají tyto přírodní polymery některá omezení, jako je tepelná stabilita, antioxidační aktivita, biologická rozložitelnost a vysoká cena, kterou lze zvýšit jejich smícháním s jinými polymery.

Cílem této práce je posoudit vliv dalších polymerů na biodegradabilitu PHB a PLA pomocí mineralizační i mikroskopické metody.

Výsledky tohoto experimentu ukázaly, že organosolv a neutralizovaný alkalický lignin zlepšují vlastnosti PHB a zpomalují jeho degradaci. Míra mineralizace referenčního materiálu PHB dosáhla 106,66 %, zatímco míra mineralizace směsných polymerů byla 37,18 % pro PHB s 12 % organosolvového ligninu, resp. 41,95 % pro PHB s 12 % neutralizovaného alkalického ligninu.

Hodnocení vlivu ozařování na biodegradaci PLA ukázalo, že ozařování zvyšuje rozklad PLA, a tím i míru mineralizace. Míra mineralizace vzorků s delší dobou ozařování byla nejvyšší (118,56 %), následovaly vzorky s dobou ozařování 100 hodin (118,56 %) a neozařený vzorek, který měl hodnotu 77,57 %.

CO<sub>2</sub> vzorků smíchaných s esenciálními oleji bylo rovněž sledováno 50 dní a jasně se prokázala schopnost EO zpomalit růst mikroorganismů. Žádný ze směsných vzorků nedosáhl po 50 dnech inkubace v prostředí kompostu 60 % míry mineralizace.

**Klíčová slova:** biodegradace, kompost, PHB, PLA, hmotnostní analyzátor.

## **ABSTRACT**

With the increasing reports on the environmental distribution and ecological risks of petrochemical plastics, bio-degradable plastics are considered as the optimal alternative to the traditional plastics. Biodegradable plastics are product from natural polymers such as PHB and PLA. PHB and PLA have recently raised interest because of their properties and their ability to break down into natural elements in less than months. Despite their remarkable properties, these natural polymers have some limitations like the thermal stability, antioxidant activity, biodegradability, and high cost, which can be enhance by blending them with other polymers.

The goal of this thesis is to assess the effects of additional polymers on PHB and PLA biodegradability by using both mineralization and microscopic method.

Results of this experiment showed that organosolv and neutralised alkali lignin enhance PHB properties and slow down the deterioration. The mineralization rate of the reference PHB material reached 106.66% when the mineralization rate of blended polymers was respectively 37.18% for PHB with 12% of organosolv lignin and 41.95% for PHB with 12% of neutralized alkali lignin.

The assessment of irradiation on PLA biodegradation showed that irradiation enhanced PLA breakdown and therefore increase the mineralization rate. The mineralization rate of Samples with longer irradiation time was the highest (118.56%), follows by the samples with 100 hours irradiation time (118.56%) and non-irradiated sample which was 77.57%. CO<sub>2</sub> of samples blended with essentials oils were also monitored 50 days and ability of EOs on slowing down microorganisms' growth was clearly shown. None of the blended samples reached 60 % of mineralization rate after 50 days of incubation in compost environment.

Keywords: biodegradation, compost, PHB, PLA, mass analyzer.

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## INTRODUCTION

Using of Plastics has been from years now an indispensable part of our everyday life. As a ubiquitous material, they are found in nearly every item, electronic devices, furniture, clothing, vehicles and even in agriculture. According to data collated by the agency's experts, the current global consumption of plastics is more than 200 million tons, with an annual grow of approximately 5%, which represents one of the largest fields of application for crude oil (Siracusa et al., 2008). The crop production and livestock sectors were found to be one of largest users, accounting for 3.4 % (8-10 million tons) at a cost of around 6million dollars annually (Scarascia-Mugnozza et al., 2011).

The pressing issue is that they can take up to 450 to even 1000 years to degrade - making them non-renewable sources. Crude oil products are usually decomposed by combustion, which increases air pollution and causes greenhouse effects. It is therefore important to find a solution to this plastic infestation.

Many studies have been done and are underway to overcome this huge problem among which the use of biodegradable polymers such as PHB and PLA to produce bio-based plastics.

However, PHB and PLA have remarkably similar properties to those of non-biodegradable polymers but are expensive (relative to PP or PE). They are stiff, brittle in nature, and thermally unstable during processing. As a consequence of these properties, polymers blending has been used to improve PHB and PLA properties, and to low down the production costs (Mousavioun et al., 2013).

The strategy in this project is divided into three main aims:

First, the presentation of PHB and lignin properties, and then the evaluation of the degradation of the blends under soil environments. The goal is to obtain a biodegradable polymer that has satisfactory properties, and which could replace the petroleum-based mulch films.

The second aim is to evaluate effects of irradiation on PLA biodegradation. To reach that goal, the CO<sub>2</sub> production of PLA175 polymer and irradiated PLA 175 in industrial compost will be monitored and measured.

The third objective will be to assess the effect of essential oils (Eucalyptol, Limonene, and special additives) on both PHB and PLA.

The overall benefit of our research is to obtain eco-friendly materials which are biodegradable, have satisfactory properties and which could replace the fossil-base plastics or non-degradable products.

## **I. THEORETICAL PART**

## **1 ISSUE OF PLASTIC WASTE**

According to United Nations Environment Programme, approximately 7 billion of the 9.2 billion tonnes of plastic produced from 1950-2017 became plastic waste, ending up in landfills or dumped. Plastic is extremely useful in modern life, yet its increasing use may jeopardize human sustainability. Plastic production and disposal produce greenhouse gases and toxic waste. Toxins releases are piling up on land, oceans, lakes, rivers, and the consequent harm to human and ecological health. Most plastics are not recyclable, and the vast majority does not biodegrade. Furthermore, plastic products frequently degrade into extremely small fragments known as micro-plastics, which can pollute ecosystems and harm organisms (Evode et al., 2021).

### **1.1 Waste management system**

Researchers estimate that almost 7,000 million tons of virgin plastic were manufactured globally in 2015. 9 percent of that may have been recycled, 12 percent burnt, and the remainder remains in landfills, still in use, or in our environment. Approximately one-fourth of all plastic waste is never collected globally. Waste plastic is occasionally burned in the open in less developed countries, releasing harmful compounds into the air.

### **1.2 Plastics products**

Plastic primary constituents are derived from the processing of oil and natural gas such as PET, PP, PE. Different molecules are utilized to create several varieties of plastic, each with its own set of features and chemical structures. Manufacturers also incorporate additives to give specific items the desired characteristics. Colorants, plasticizers, flame retardants, stabilizers, fillers, reinforcing fibres, and biocides are examples of chemicals that may contain harmful substances such as lead, arsenic, and cadmium compounds, as well as BPA.

### **1.3 Petroleum derived plastics (non-biodegradable plastics)**

Strength, flexibility, light weigh, and low cost of traditional oil-derived plastics make them ideal materials for many applications, including packaging, medical devices, building, transportation, etc. The plastic industry generates approximately 300 million tons of plastics annually, which are used once and discarded after use. Discarded plastic waste,

owing to the durability and low degradability of these polymers, may take hundreds to thousands of years to decompose (Atiweh et al., 2021).

Thermoplastics polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP) and polystyrene (PS) are the most frequently used plastics in packaging and represented over 60 % of the total plastics demand in Europe in 2016. However, the bulk of plastics, coupled with a throw-away culture, causes pollution, accumulation of plastic trash, and the depletion of important resources. In the EU, 70 % of the collected plastic waste ends in landfills or is incinerated. The potential for recycling plastic waste remains largely unexploited, with very low global rates of recycling and plastic recycle representing only 6 % of total demand for plastics (Narancic & O'Connor, 2019). The high price and low quality of the recycled plastics relative to virgin plastics limits market applications. Polyethylene (PE) is one of the most used polymer in plastic production due to its availability, cost, and properties. PE is obtained by polymerization of ethylene monomers.

#### 1.4 Properties of polyethylene

PE is one of the most popular thermoplastic materials and belongs to polyolefin family. It is available in different crystalline structures, referred to as HDPE, LPE, UHMWPE, and LLDPE. PE properties can be divided into mechanical, chemical, electrical, optical, and thermal.

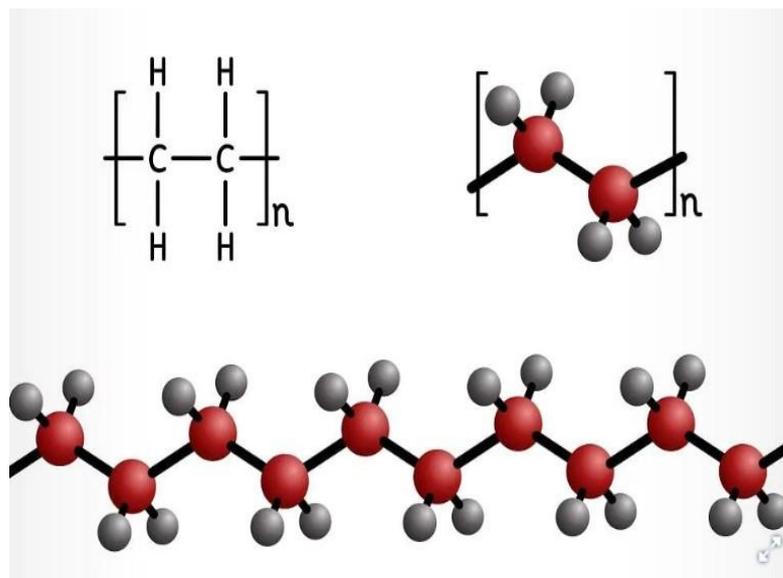


Figure 1 Chemical formula for polyethylene polymer

**a) Mechanical**

Low strength, hardness, friction, and stiffness are the defining characteristics of polyethylene's mechanical qualities. This polymer, however, has a high degree of ductility and impact resistance. Under constant pressure, it exhibits significant creep, which can be lessened by the inclusion of short fibbers. It feels waxy to the touch.

**b) Thermal**

PE is a thermoplastic comprised of tiny monomeric units. Material is limited by its low melting point compared to other thermoplastics. Low density polymers often have a melting point between 105 and 115 °C, while medium and high-density polyethylene typically have a melting point between 120 and 130 °C. The upper limit of melting of polyethylene is reported to be 144 to 146 °C. Usually, combustion occurs above 349 °C .

**c) Chemical**

Polyethylene (C<sub>2</sub>H<sub>4</sub>)<sub>n</sub> is made up of nonpolar, saturated, high-molecular-weight hydrocarbons and is created by polymerizing ethylene. PE has a remarkable level of chemical resistance, a high degree of crystallinity, and a symmetrical molecular structure. This merely implies that they are resistant to weak oxidizing and reducing agents and are not affected by powerful acids or bases. At normal temperature, crystalline samples do not dissolve. At high temperatures, polyethylene (apart from cross-linked polyethylene) may typically be dissolved in aromatic hydrocarbons like toluene or xylene or in chlorinated solvents like trichloroethane or trichlorobenzene.

Polyethylene absorbs almost no water and can become brittle when exposed to sunlight, carbon black is usually used as a UV stabilizer.

PE burns slowly with a blue flame having a yellow tip and gives off an odour of paraffin.

**1.5 Environmental impact of Plastics**

Most plastics in use today are made of polyethylene or PE. Plastics made from PE polymers hardly decompose because most bacteria cannot break them down.

UV light from the sun can break down PE plastics, but it takes more time and has long-lasting effects on both people and the environment. The use of heat or fire is another method of decomposition that is theoretically conceivable but results in large amounts of waste and toxic gas and takes a very long time to deteriorate. The third way is referred as disposable plastic, item, require hundreds to thousand years bio- degrade. In addition, after some time these plastics oxidize and give out toxic copper salt that pollute the land and

enter the food chain. These toxins are cancer-causing that can cause serious damage to the metabolism of living organisms after they are absorbed.

Steady exposure of plastics to heat melts those resulting in the emission of gases into the environment in a process known as out gassing. Incineration technique makes toxic exhaust be released into the atmosphere. When Inhale by humans, polluted air causes various sorts of illnesses like tuberculosis and different types of respiratory tract infections. To get rid of non-biodegradable products prompt on our planet, we need to track down a smart solution to address these issues. Nonetheless, substitute techniques can be embraced to replace conventional plastics with eco- friendly products.

## 2 BIODEGRADABLE POLYMERS

Biodegradable plastic is defined as moldable plastic material made up of chemical compounds that are derived from or synthesized by microbes such as bacteria or by genetically modified plants materials. According to ISO, these are polymers in which fragments of low molecular weight degrade. These fragments are formed by the action of naturally existing microorganisms (bacteria, fungi, algae). To be categorized as biologically degradable, materials must meet requirements defined by ASTM and ISO standards. Biodegradable polymers are commonly produced with renewable raw materials, micro-organisms, petrochemicals, or combinations of all three. Biopolymers have been recently raising interest and their applications are wide: medicine, agriculture, and packaging.

The oil-based plastics are very resistant to biodegradation, they will unavoidably accumulate in the environment once they are there, causing harmful environmental effects like the release of dangerous compounds and the polluting of ground waterways. The growing environmental awareness has led to the using of eco-friendly materials and the further study of biodegradable polymer materials.

### 2.1 Types and properties of biodegradable plastics

The development of bioplastics (plastics that are biodegradable and made of natural materials) provides businesses with eco-friendly alternatives for packaging and products, especially single-use items that generate a lot of waste. Although still in its early stages, the bioplastic sector is constantly expanding and will soon start to take the place of numerous disposable items made of petroleum-based plastic, including cups, cutlery, packaging, containers, straws, Mulch films. A material is considered “biodegradable” if it degrades within a relatively short period of time (less than a year). Bioplastics cover a wide range of bio-based polymers that have a variety of unique attributes and applications. Based on their origin and method of production, Bioplastics can be classified into four categories: directly extracted from biomass, synthesised from bio-derived monomers, biodegradable polymer synthesised from petrochemicals and produced directly by natural or genetically modified organisms.

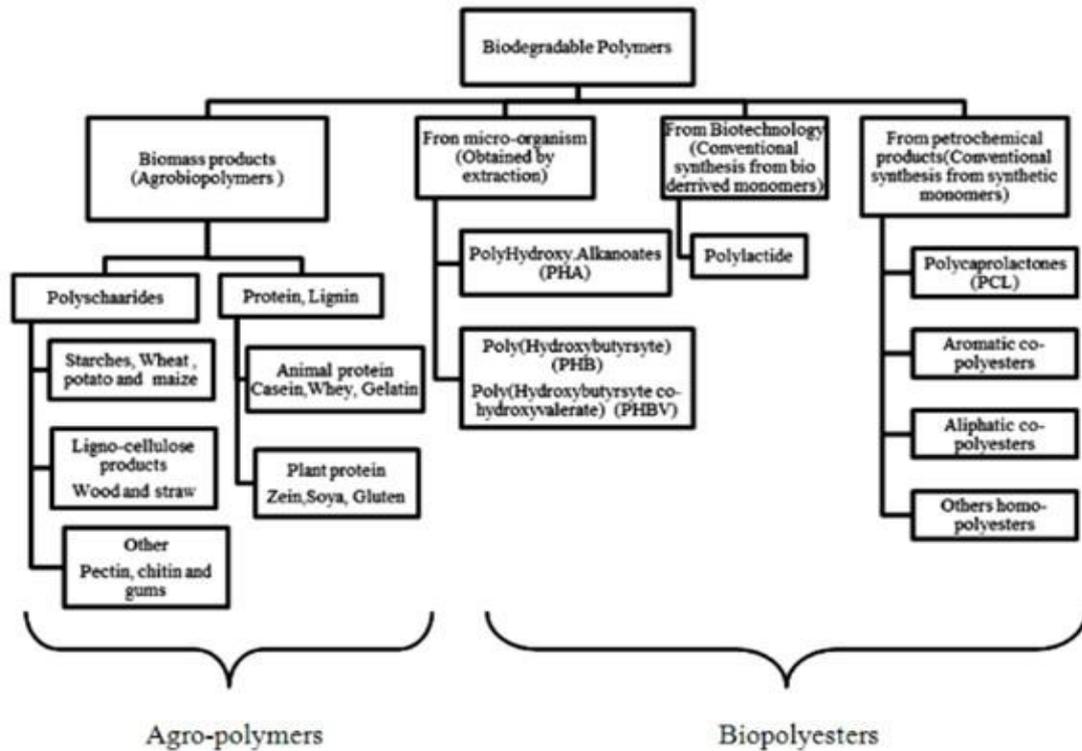


Figure 2 Classification of bioplastics-based origin and method of production

### 2.1.1 Directly extracted from biomass

Bio-based plastics can be either polysaccharides products or proteins.

The polysaccharides or polyoses are a group of carbohydrates polymerised in different forms from monosaccharides (simple sugars classified according to the number of carbon atoms). They exist naturally as starch and cellulose in plants and can be extracted in forms of starch granules or cellulosic fibres. Starch is one of the most abundantly available biopolymers derived from variety of natural resources, such as corn, wheat, potatoes, cassava, rice, etc. Starch is often mixed with biodegradable polyesters. Properties of starch such as granule shape, particle size, crystallinity, and amylase content, are dependent on their botanical origins.

Proteins are organic chains of amino acids that can be broken down by enzymes like proteases Protein-based polymers have been utilized as encapsulates, coatings, adhesives, and surfactants since the 1930s. Animal and plant proteins can be distinguished based on where they come from. Proteins' nutritious content makes them especially desirable for use in packaging edible meals. The protein origin are animal base proteins and plant-based proteins(Song et al., 2011).

### **2.1.2 Bioplastics synthesised from bio-derived monomers.**

Polylactic acid (PLA) is one of the most widely used and commercially accessible bioplastics in this group. Polylactic Acid or polylactide – is an aliphatic polyester made mostly from starch/sugar rich crops, but it can also be derived from petrochemicals. The difference in terminology simply indicates the synthesis method chosen to produce PLA from lactic acid fermented from starch or sugars (Song et al., 2011).

More recently, polyethylene, a non-biodegradable polymer, has also been produced using bioethanol produced from the fermentation of sugar. Ethanol converts to ethylene after a dehydration procedure. Ethanol can be obtained from sugar beet, corn, sugar cane and wheat grain. This plastic is not the best biodegradable and has the same physical and chemical characteristics as synthetic polythene.

The bio-derived monomers bioplastics are mainly Polylactic acid (PLA), Polyglycolic acid (PGA), and Polyethylene from bioethanol.

### **2.1.3 Biodegradable polymers from petrochemicals**

This group of materials consisting of polyvinyl alcohols (PVOH), aromatic co-polyesters, and aliphatic polyesters, are made from petrochemical monomers and have weak links that make them susceptible to enzymatic assault and biodegradation. They are frequently mixed with starch since most of them are significantly more expensive than the ordinary commodity polymers, which are rarely utilized alone for packaging purposes. Types of petrochemicals polymers are aliphatic polyesters and derivatives (Polycaprolactone, Polybutylene succinate and Polybutylene succinate adipate, Polyester amide), Aromatic polyesters and polyvinyl alcohol and polyethylene vinyl alcohol.

### **2.1.4 Polyesters directly produced from micro-organisms.**

Some aliphatic polyesters can be synthesised by certain microbes (where they act as energy storage material in cells) and are produced commercially using bioengineering methods to accumulate and extract the polymers. They are collectively termed polyhydroxy alkanates, including polyhydroxy butyrate, polyhydroxy valerate, polyhydroxy hexanoate and their copolymers. The properties of PHB are likely similar to those of polystyrene but more rigid and brittle than polypropylene (Song et al., 2011).

## 2.2 Biodegradation of bioplastics

The biodegradation process of biomaterial begins when the biodegradable polymer comes into touch with a microorganism-rich environment (soil, compost). Enzymes found in microorganisms speed up chemical decomposition reactions and are responsible for most natural degradation processes. A complete biodegradability is defined as the degradation of a specific chemical compound by the action of microorganisms in the presence of oxygen to CO<sub>2</sub>, water, mineral salts of all other elements present (mineralization), and new biomass, or in the absence of oxygen to methane (CH<sub>4</sub>), mineral salts, and new biomass (Aleksandra et al., 2020).

### 2.2.1 Biodegradation process

The biodegradation process is divided into four stages:

- **Biodeterioration** - the resistance and durability of materials deteriorate during this phase. This can happen as a result of bacteria and/or abiotic causes. As a result, the specific surface area increases and access to the polymer chains for depolymerization improves.
- **Depolymerization** - the process by which polymer chains are broken down into low-molecular compounds (oligomers, dimers, and monomers). Extracellular enzymes of bacteria and abiotic stimuli (heat and light) initiate this process.
- **Assimilation** - Water-soluble depolymerization products pass past the plasma membrane into the cytoplasm of bacteria. They are employed to generate energy, biomass, and primary and secondary metabolites in this environment.
- **Mineralization** - the release of end products (CO<sub>2</sub> / CH<sub>4</sub>, H<sub>2</sub>O) as a result of mineralization.

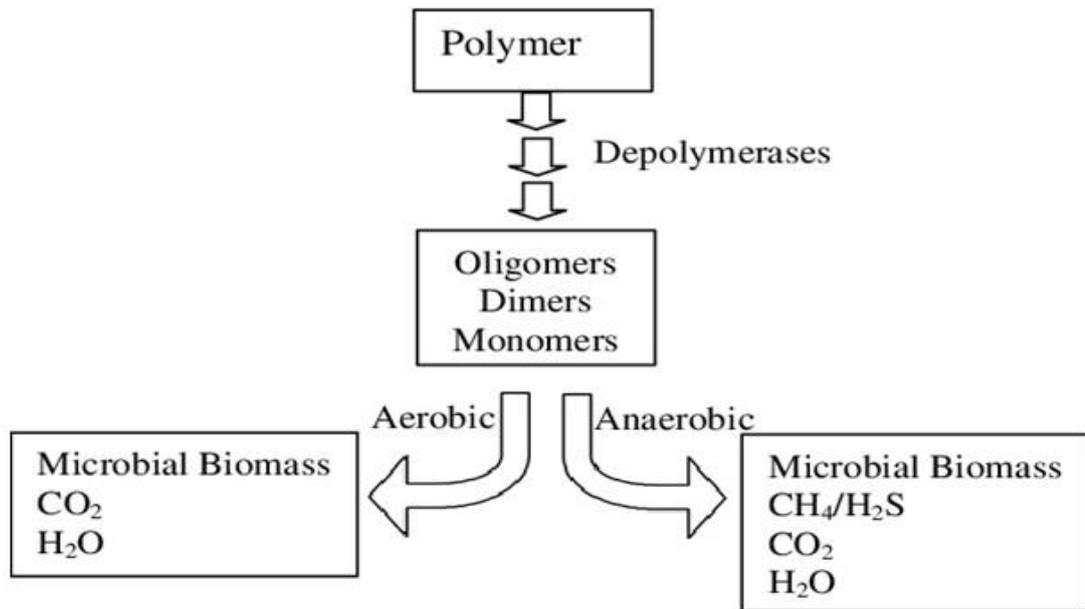


Figure 3 Schematic-diagram-of-polymer-degradation-under-aerobic-and-anaerobic-conditions

### 2.2.2 Factors influencing biodegradation.

The features of the environment in which biodegradation occurs, the quantity of chemicals entering the bio-degradation process, and the number of microorganisms present all have an impact on biodegradation. Each habitat has a separate optimal degradation mechanism. It is also critical to consider whether the given environment contains nutrients or inhibitors. Factors influencing biological degradation rate (Mebunii., 2022).

- **Biotic factors: microbe species, microbe population, and microbial enzymes.**

**The microbe species:** present in any environment, microbe species influences the biodegradation rate because different species of microbes have different metabolic capacities. Furthermore, the metabolic capability of any species of microbe will differ depending on the type of biodegradable materials to be destroyed. This means that one bacterium may be more effective at degrading one sort of organic waste while being useless at degrading another.

**The microbe's population:** this is an important factor because Greater numbers of microbes lead to greater rates of biodegradation, and so forth.

**Enzymes:** they are a powerful and essential biochemical tool used in biodegradation. Enzymes are supplied from the cells of microbes, which secrete these chemical substances.

The effectiveness of any microbial enzyme mainly depends on the nature of the biodegradable matter (substrate) in question.

- **Abiotic factors:** temperature, pH, aeration, salinity, humidity, and nutrients. These are the factors that do not directly depend on biological circumstances, organisms, or processes.

**Temperature:** Biodegradation is proportional to temperature in general. This indicates that when the environmental temperature is low, the rate of biodegradation is generally low, and when the temperature is moderate/relatively high, the rate of biodegradation is high. Microbial enzymes (as well as microbial populations) may be damaged at extremely high temperatures (over 50°C) or extremely low temperatures. At such high temperatures, biomass may degrade thermally.

**pH:** Because the survival, growth, multiplication, and efficacy of microorganisms vary with pH, pH influences biodegradation. In general, intermediate pH values are best for biodegradation. A pH range of 6.5 to 7.5 is ideal for bacteria development.

**Aeration:** this one of the most important components because microbial activities depend on oxygen. According to some studies 10-40% of oxygen is needed for effective aerobic degradation. Aeration is used in a variety of technological approaches to increase the rate and effectiveness of biodegradation to eliminate environmental toxins. These methods include bioventing, bio-sparging and trickling filtration. Aeration has been found to speed-up the biodegradation of materials like hydrocarbon and sewage.

**Salinity:** studies have shown that salinity is inversely proportional to biodegradation. This means that high salinity results in low rates of biodegradation and vice-versa.

**Humidity and moisture:** When other environmental/abiotic parameters such as temperature are low, moisture might accelerate biodegradation. Humidity and moisture have also been proven to accelerate the degradation of biodegradable polymers.

**Nutrients:** Nutrients are required to sustain the growth of microorganisms, which are then required to breakdown materials during the biodegradation process. Nutrients include nitrogen, potassium, carbon, and phosphorus. When the concentration of nutrients is increased, the number of microbes, as well as their metabolic effectiveness, will increase.

**Substrate characteristics:** biodegradation rates are affected by the characteristics of the substrate. These include chemical composition/complexity, and physical attributes like absorption, and hydro-affinity. Biodegradation is generally slower for substrates with high chemical complexity, poor absorption capacity, and hydrophobic properties.

Biodegradation, on the other hand, is faster for less complicated, hydrophilic, and absorptive substrate.

### **2.3 Application areas of biodegradable plastics**

Biodegradable plastics are commonly used for disposable items, such as packaging, crockery, cutlery, and foodservice containers. In principle they could replace many uses currently served by conventional plastics. However, cost and performance remain problematic. Their usage is financially favourable only if supported by specific regulations limiting the usage of conventional plastics.

### **2.4 Advantages of biodegradable plastics**

Biodegradable plastics are a highly desirable material due to its capacity to preserve fossil raw ingredients, total biological degradability, and composability. The usage of these plastics can also help to reduce the build-up of polymeric materials in the environment, lowering waste management costs. The process results in the production of H<sub>2</sub>O and CO<sub>2</sub>. Biodegradable plastics are being investigated as potential replacements for conventional plastics because, despite the emission of CO<sub>2</sub> into the atmosphere, they have a smaller environmental impact than petroleum-based plastics that build up in landfills and produce significant pollution Effects of non-biodegradable plastics on environment (*International scholars journals, Simon.2021*).

### **2.5 Disadvantages of biodegradable plastics**

Biodegradable materials, in general, have fewer advantages than synthetic polymers. Biodegradable plastics have both technological and economic problems. Low crystallization temperature, which results in a slower crystallization rate, relative thermal degradation under processing circumstances, and poor barrier characteristics are all technological aspects. Polysaccharide-based films, for example, have low barrier ability to water vapor but high barrier ability to oxygen and CO<sub>2</sub>. PLA has four times the water vapor transfer rate of ordinary polymer films. Creating a viable biodegradable material with ideal qualities is frequently a challenge. The worry of upsetting existing recycling projects is also an impediment to the introduction of these polymers.

Biodegradable polymers have been employed in a variety of applications over the last 10 years, but their expensive cost inhibits future development. The cost of making bioplastics ranges between 1.81 and 3.35 Euros per kilogram(Price et al., 2022).

Prices for biodegradable polymers can only be decreased by mass production, while demand in Europe is expected to be strong due to European rules encouraging the use of biodegradable polymers for packaging. Mixtures of these elements with a less expensive raw material like starch and lignin could provide a solution.

### 3 POLY-HYDROXYBUTYRATE (PHB)

Poly-hydroxybutyrate (PHB) is a poly-hydroxyalkanoate (PHA), a polymer belonging to the polyesters class that are of interest as bio-derived and biodegradable plastics. PHB is probably the most common promising, biocompatible material and polyhydroxyalkanoate that can substitute of fossil-based plastics.

#### 3.1 Production of PHB

PHB is produced in the cells of microorganisms, as product of microbial secondary metabolism, usually in conditions when the cells are subjected to nutrient stress or in an unfavourable environment such as carbon-excessive with limited nutrients, which is possible in both gram-positive and gram-negative bacteria.

There are several different approaches for the extraction and recovery of PHB materials and three main routes: the first method involves the ring opening polymerization of  $\beta$ -butyrolactone. The natural/transgenic plants are the second way. The biosynthesis of PHAs in transgenic plant cells is possible because of the general availability of acetyl-CoA, the primary substrate in PHA biosynthesis. The fermentation of bacteria is the third method of obtaining PHB compounds. More than 90% of the dry weight of the cells may contain PHA components when fermentation conditions are ideal (McAdam et al., 2020). This third approach is the most commonly method for producing PHB (Figure 4).

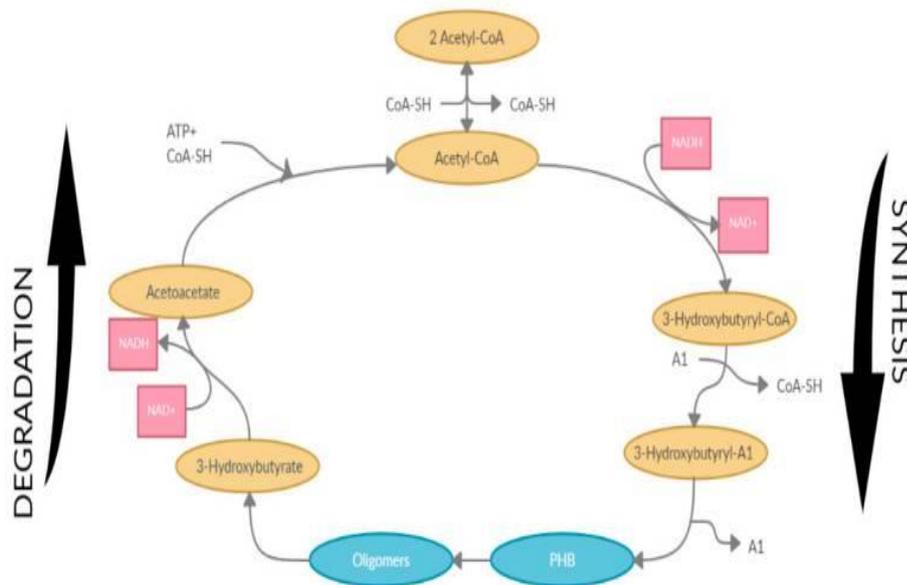


Figure 4 Polyhydroxy butyrate (PHB) synthesis and degradation process

### 3.2 Thermo-physical and chemical properties of PHB

PHB was the first PHA to be isolated and described. Due to its linear chain structure and presence of both amorphous and crystalline phases, PHB is highly crystalline. It can be found either as a virgin polymer or as part of copolymers and blends. It is manufactured industrially through bacterial fermentation and is produced as a carbon reserve in a wide range of generating bacterial strains. PHB is characterized by having a methyl functional group (CH<sub>3</sub>) and an ester linkage group (–COOR), it is these functional groups that are responsible for the material's thermoplastic, hydrophobic, high crystallinity, and brittle characteristics. In general, the higher the degree of crystallization results in a stiffer and stronger, yet more brittle material.

The polymer's tacticity, hardness, modulus, density, transparency, and the characteristics of cold drawing or ductile flows can all be affected by the degree of crystallinity. The tensile modulus of PHB is estimated to 3-3.5 GPa, Tensile Strength in the range of 20-40 MPa, Elongation at break 5-10%, Degree of Crystallinity from 50–60 %, Melting Temperature from 165–175°C and the glass transition temperature in the range of 5-9°C (McAdam et al., 2020).

### 3.3 PHB benefits

Polyhydroxy butyrate (PHB) offers many advantages over traditional petrochemically derived plastics. In addition to its complete biodegradability, PHB is formed from renewable resources. PHB completely degrades into CO<sub>2</sub>, H<sub>2</sub>O under aerobic condition and CH<sub>4</sub>/H<sub>2</sub>S, CO<sub>2</sub>, and H<sub>2</sub>O in absence of oxygen. Microbial synthesis of PHB is the best method for industrial production because it ensures the proper stereochemistry for biodegradation. PHBs are chosen as alternatives to produce biodegradable polymers due to their quick degradability in natural environmental circumstances. The average PHB degradation time is 20 days (McAdam et al., 2020).

### 3.4 PHB limitations

Several limitations currently exist when PHB is produced among which the high costs of production, low yield, susceptibility to degradation, technology complexities including extraction. To enhance these limitations, PHB blend with other polymers like starch, cellulose and lignin could provide a solution.

## 4 LIGNIN

The cell walls of wood, which is heterogeneous, hygroscopic, cellular, and anisotropic, are primarily made of three organic substances: cellulose (40–60wt%), hemicelluloses (25–35wt%), and lignin (15–30 wt%) (Xu & Ferdosian, n.d.).

Lignin is a class of complex organic polymers that have a crucial structural role in the tissues that support the most of plants. Lignin is particularly important in the formation of cell walls, especially in wood and bark, because they lend rigidity and do not rot easily. Lignin has different Physical, chemical, and thermal properties.

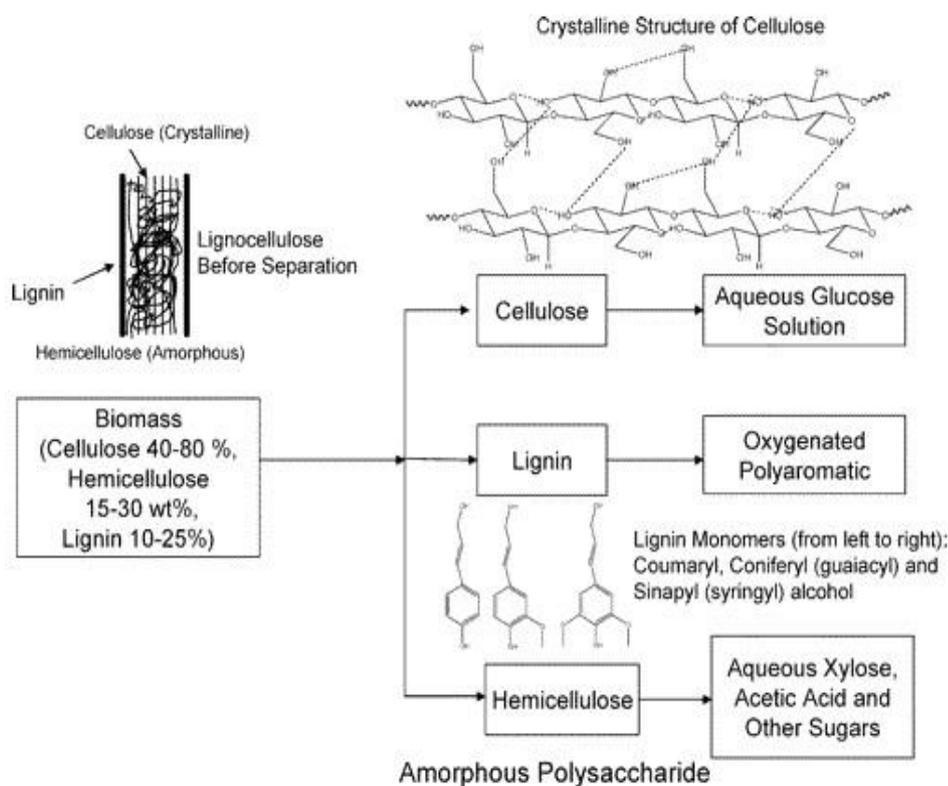


Figure 5 Schematic structure of wood cells (lignocellulose, cellulose, lignin, and hemicelluloses)

### 4.1 Lignin extraction methods

Lignin needs to be taken out of the plant before it can be used to create new products. In addition to the diversity of the repeat units and bonding patterns which characterise natural lignin, is the chemical alteration introduced by each method of removing lignin from the

plant. The techniques for lignin recovery from woody plants (Figure 5) alter the chemical and functional group composition of the lignin and greatly increase its heterogeneity.

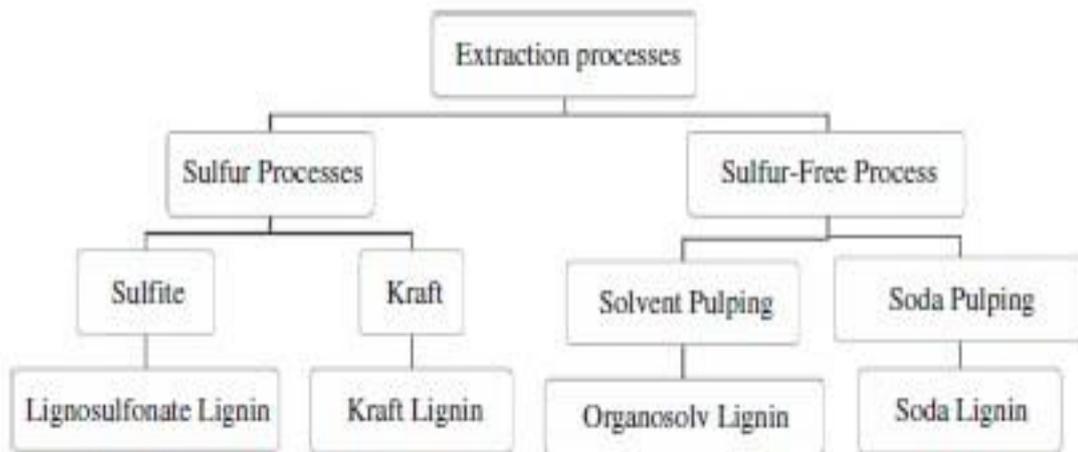


Figure 6 Various lignin extraction processes and their dominant products

The industrial processes for lignin extraction can be classified into two different categories: sulfur processes and sulfur-free processes. Figure 6 depicts several extraction techniques and the resulting compounds. The second-most abundant naturally occurring renewable polymer after cellulose is lignin, but unlike cellulose, which has a unique, well-defined structure, lignin has a three-dimensional network structure, and the structure is dependent on the type of plant, the environment, the time of year, etc. Lignin, a phenolic polymer created by radical coupling polymerization of three monolignols, has an amorphous macromolecular structure made up of three different phenyl-propanol's, including p-hydroxyl-phenyl propanol, guaiacyl-propanol, and syringyl-propanol (A. Nasrullah et al., 2017).

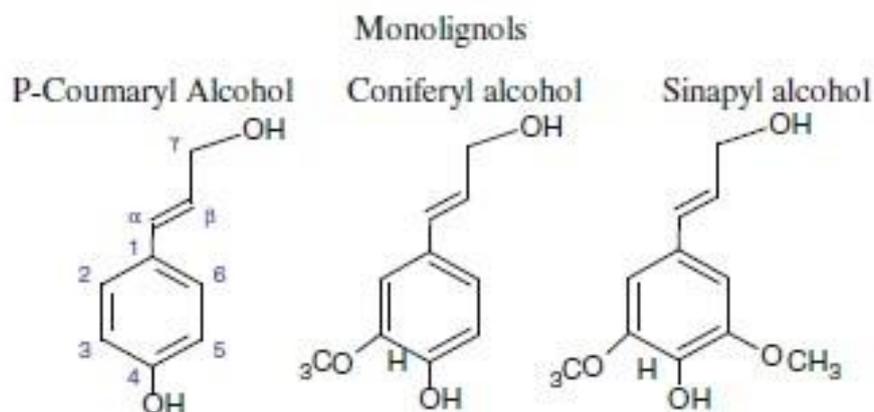


Figure 2 Structure of three lignin monomers

## 4.2 Types of lignin

Technical lignin is conventionally named after the separation (extraction) method, e.g., lignosulfonate lignin, kraft lignin, organosolv lignin, and soda lignin. Due to the partial degradation of lignin during the extraction process, the separation methods also have an impact on the molecular weight and polydispersity of lignin. In addition, the separation conditions which can include mechanical action, enzymes, or chemical reagents can influence the crosslinked structure of lignin and formed lignin fragments with different molecular weight distributions (*A. Nasrullah, ... H. Ajab, 2017*). Some important types of technical lignin are:

- Alkali lignin/kraft lignin
- Lingo sulfonate
- Organosolv lignin
- Milled wood lignin
- Klason lignin
- Hydrolytic lignin

## 4.3 Lignin properties

Different pulping processes and lignin extraction method to obtain the main physical and chemical properties include the following:

### 4.3.1 Lignin colour

The original form of lignin is a white or virtually colourless substance. We can observe lignin's colour alone, which is a result of processing, shows various hues with the division of the various preparation techniques.

### 4.3.2 Molecular weight distribution of lignin

Although lignin polymers are present, the relative molecular mass of tangled wood is much lower. Generally, only a few dry to tens of thousands, with only the original lignin reaching the hundreds of thousands. This contrasts with most polymers, which typically have relative molecular masses in the hundreds of thousands, millions, or even tens of millions. Strategies for separation and the degree of relative molecular mass.

An important feature of the polymer molecules having a polydispersity that is the molecular weight range of a certain size. The knotted nature of wood as a polymer also

revealed its molecular weight polydispersity. The typical molecular weight of ground wood lignin in softwood is 2000, while that in hardwood ground wood is lower, with sulfuric acid precipitated from black liquor grade wood trees between 330 and 63000, and 65% to 80% of lignin between 500 and 50000. The polydispersity of Stra14144w lignin molecular weight indicates that the dispersion coefficient is often more than 2.

#### 4.3.3 Lignin solubility

Lignin is an aggregate with many polar groups present in its structure, especially hydroxyl groups. Because of this, lignin has significant intramolecular and intermolecular hydrogen bond energies, making the original lignin insoluble in any solvent. Reduced production and degradation lead to lignin separation, which changes a variety of physical characteristics, including solubility resistance (Xu & Ferdosian, n.d.).

Alkali lignin is insoluble in water and insoluble in acidic and neutral media. However, it is soluble in a variety of lignin sulfonate aqueous solution PH values and solvents with strong hydrogen bonding, such as dioxane, acetone, methyl cellosolve, and others.

#### 4.3.4 Glass transition of lignin

In addition to lignin and copper amine acid lignin, the original separation of lignin and lignin most polymer material is a thermoplastic with a glass transition temperature and no specified melting point. The glass transition temperature, separation technique, relative molecular mass member, as well as the differences between the plant's wet and dry states, are all highly distinct (Xu & Ferdosian, n.d.).

#### 4.3.5 Chemical reactivity of lignin

A lignin molecule may include the following compounds: o-anisidine (a OCH<sub>3</sub>), ether bond (-O-), amino (a COOH), carbon-carbon double bonds, benzyl alcohol, hydroxy (PH-CH<sub>2</sub>OH), a phenolic hydroxyl group (PH one OH), carbonyl (C = O), and benzene, among others. The functional groups phenol polyethylene group, benzyl alcohol hydroxyl group, amino group, and carbon play a significant role in the reactivity of lignin. The structure can also be further alkylated, hydroxymethylated, esterified, or undergo an acylation reaction. Examples include the activation of a phenolic hydroxyl group, which can activate ortho- and para-electrophilic reactions, the methylation initial nucleophilic reaction, which results in ether chain cleavage, the magnetic reaction, which results in demethylation effect but

can be converted into water-soluble products, the UV absorption properties via the carbonyl group photochemical discoloration, and the magnetic reaction, which results in demethylation effect (Xu & Ferdosian, n.d.).

#### **4.3.6 Colloidal lignin chemistry**

PH value of at least 10, significant hydrophilicity, and alkaline pulping of black liquid lignin. In an alkaline solution, amorphous lignin molecules unite to form aggregates, which have interior hydrophobic groups and an external hydrophilic group that creates the aggregates' outer surface. The intermolecular condensation of lignin coalesces to produce precipitation as the pH is slightly lowered due to the acid addition, H<sup>+</sup>, and negatively charged groups on the electrophilic process (Xu & Ferdosian, n.d.).

#### **4.3.7 Lignin biodegradation**

Lignin is an aromatic polymer that makes up to 30% of the tissues in woody plants and gives them stiffness and resistance to biological attack. Lignin is a challenging substrate for enzymatic depolymerization due to its insoluble nature, complexity on a chemical level, and lack of hydrolysable connections. White-rot fungi can totally mineralize lignin, but brown-rot fungi just alter it while eliminating the carbohydrates in wood. Some fungi, usually basidiomycetes, are the only creatures capable of extensively biodegrading it. The function of several extracellular oxidative and reductive enzymes, including lignin peroxidase, manganese peroxidase, laccase, and cellobiose: quinone oxidoreductase, which have been isolated from ligninolytic fungi, is currently the subject of extensive research (Xu & Ferdosian, n.d.).

## 5 PHB AND LIGNIN BLENDS

To replace petroleum-based polymers, there are concerted efforts to manufacture biodegradable polymers. PHB and other comparable bacterial polyesters have drawn interest from all over the world due to its biodegradability, sustainability, durability, and flexibility. PHB is surprisingly comparable to isotactic polypropylene (PP) in terms of its characteristics, however it is more expensive (compared to PP), stiff and brittle, and thermally unstable when processed. These thermomechanical qualities are a result of the crystal structure and crystallization behaviour. As a result of these characteristics, polymer blending has been employed to change PHB, enhance its characteristics, and reduce production costs.

Research on thermal characteristics and miscibility of PHB and lignin blends revealed that PHB/lignin blends are thermally more stable than PHB over a wider temperature range. Knowing the mechanical and rheological characteristics of polymer blends is essential to determining modifications in the viscoelastic response and process ability conditions.

### 5.1 Blend preparation

Prior to use, lignin and PHB were dried for 12 hours at 100°C and 40°C, respectively, and stored in desiccators under vacuum. PHB/lignin blends with lignin contents ranging from 10% to 90% were mixed in a Thermo scientific micro compounder with a rotating twin screw mini extruder. To reduce PHB degradation, the extruder temperature was kept at 175°C for 2 minutes. Extruded strands of polymer blends were then cooled and pelletized. To avoid moisture absorption, the pellets were kept in desiccators. The previous studies on PHB/lignin blends showed that lignin up to 30 wt% content, is miscible in the PHB matrix (Mousavioun et al., 2013b).

### 5.2 Polymer film fabrication

Polymer films were created using a hot press instrument at 175°C and 7.5 bar pressure. A rectangular mould with a thickness of 100 micrometre was used to produce film samples of the same thickness. Compression moulding between two Teflon sheets was used to create film samples. The film was then removed and placed under vacuum (48 hours, 25°C) before standing for another 24 hours (25°C, 30% relative humidity) until it was atmospherically equilibrated (0.18-0.52g). The film was then aged for three weeks to allow

its crystallinity to reach an equilibrium value before being carefully inserted into slide frames.

### 5.3 Thermal properties and miscibility of PHB/Lignin

Research conducted by many scientists showed to increase the overall thermal stability of PHB/lignin blend. Although it reduces the initial onset temperature of PHB degradation. Thermal analyses(Mousavioun et al., 2013b).The glass transition temperature (T<sub>g</sub>) of a polymer blend indicates the material's miscibility. If blending produces a material with a lower T<sub>g</sub> than the brittle or stiff component, it means the blend has more flexible chains, lowering stiffness and improving processability. If, on the other hand, the blend has a higher T<sub>g</sub> and a higher melt viscosity, the processability would improve while the chain stiffness would remain unchanged. Immiscibility is indicated by two or more T<sub>g</sub>s. This is not to say that there are no interactions between them. The T<sub>g</sub> of PHB increases with increase in lignin content. However, its melting temperature (T<sub>m</sub>), ΔH<sub>m</sub>, X<sub>c</sub> and T<sub>mc</sub>, decreases with increase in lignin content, irrespective of the lignin loading. In general, when the lignin content ≤40 wt%, it raises the T<sub>mc</sub>(i.e., melt cold crystallization temperature) of PHB. However, when the lignin content is ≥50%, it reduces the T<sub>mc</sub> of PHB. (Weihua et al.,2004).

Thermal properties of lignin/PHB blends using the starting lignin material.

| Sample         | T <sub>g</sub> (°C) <sup>†</sup> | T <sub>m</sub> (°C) | ΔH <sub>m</sub> (J g <sup>-1</sup> ) | X <sub>c</sub> (%) | T <sub>mc</sub> (°C) |
|----------------|----------------------------------|---------------------|--------------------------------------|--------------------|----------------------|
| PHB            | 3                                | 172                 | 92                                   | 63                 | 89                   |
| Lignin content |                                  |                     |                                      |                    |                      |
| 10%            | 7                                | 174                 | 90                                   | 61                 | 132                  |
| 20%            | 9                                | 173                 | 85                                   | 58                 | 121                  |
| 40%            | 15                               | 170                 | 78                                   | 53                 | 98                   |
| 50%            | 18 (130)                         | 167                 | 34                                   | 23                 | 82                   |
| 60%            | 17 (148)                         | 165                 | 31                                   | 21                 | 86                   |
| 70%            | 21 (134)                         | 157                 | 9                                    |                    |                      |
| 80%            | 26 (125)                         | 152                 | 5                                    |                    |                      |
| 90%            | 43 (131)                         | 158                 | 3                                    |                    |                      |
| Lignin         | (130)                            |                     |                                      |                    |                      |

† T<sub>g</sub> with parentheses is lignin, and the one without is PHB.

Figure 8 Thermal properties of blends PHB/lignin

#### **5.4 Rheological properties of PHB/lignin blends**

Melt rheology of composite blends is critical for understanding the molten structural property relationship and whether they can be processed easily. The outcome of studies shows that the blend is stable and shows a linear viscoelastic response at strains of 10%. This relatively low value may be due to the system's particulate nature.

## 6 POLYLACTIC ACID (PLA)

Just like other biodegradable polymers, PLA has recently attracted much attention from academia and industries due to environmental concerns and non-composability issues that exist in petroleum-based polymers. Polylactic acid (PLA) is one of the well-developed commercial bio-based/bio-degradable polymers which is produced from the ring-opening polymerization using renewable resources such as corn-starch or sugarcane. PLA may be a promising substitute for petroleum-based polymers in a variety of commodity and engineering applications due to its comparable cost and properties to those of petroleum-based polymers. PLA is also an appropriate candidate for food packaging and other consumer products due to its non-toxic properties.

Despite the noted advantages of PLA, there are several limitations such as brittleness, low toughness, and low service temperature. To overcome some of these limitations, specifically the enhancement of ductility and toughness properties, PLA could be blended with other biodegradable polymers.

### 6.1 Production of PLA

Polylactic acid is primarily produced through two processes: condensation and polymerization. Ring-opening polymerization is the most common polymerization technique. Metal catalysts are combined with lactide in this process to produce larger PLA molecules. The condensation process is similar, with the main differences being the temperature during the procedure and the by-products (condensates) produced as a result of the reaction.

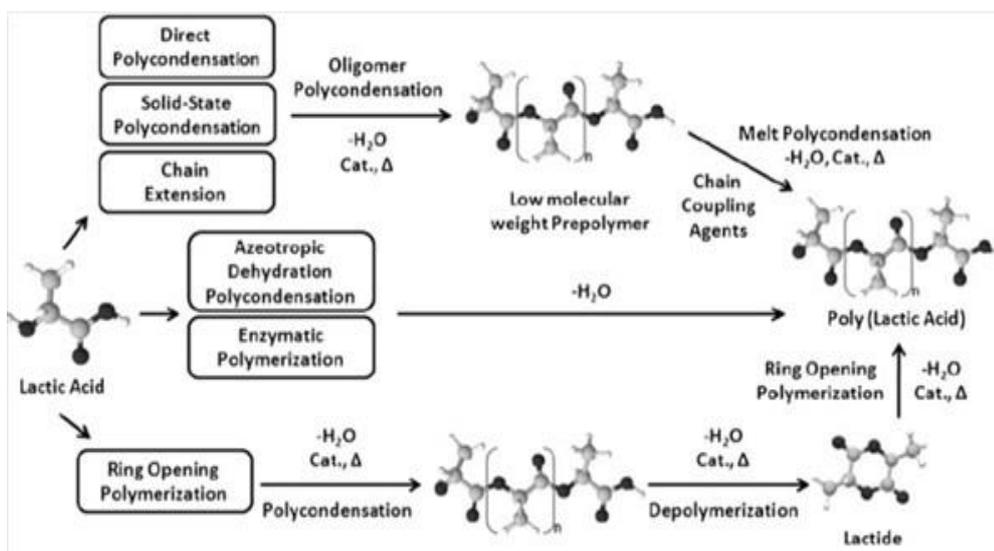


Figure 9 Main methods for PLA synthesis

## 6.2 Properties of PLA

PLA is a thermoplastic polyester (as opposed to "thermoset"), and the name refers to how the plastic reacts to heat. At their melting point, thermoplastic materials become liquid (150-160 degrees Celsius in the case of PLA). The ability of thermoplastics to be heated to their melting point, cooled, and reheated without significant degradation is a major advantage. Instead of burning, thermoplastics such as Polylactic Acid liquefy, allowing them to be easily injection moulded and then recycled. Thermoset plastics, on the other hand, can only be heated once (typically during the injection moulding process). The first heating causes thermoset materials to set (similar to a 2-part epoxy), resulting in an irreversible chemical change. If you tried to heat a thermoset plastic to a high temperature a second time it would simply burn. This characteristic makes thermoset materials poor candidates for recycling. The tensile strength of PLA is approximately 61-66 MPa, shrink rate 0.37-0.41% and the flexural strength 48-110 MPa (Oguz et al., 2019).

| Properties                             | PLA Polymer |
|--|-------------|
| Molecular Weight, g/mol                | 204453      |
| Melting Point, °C                      | 150-160     |
| Glass Transition Temperature, °C       | 58          |
| Specific Gravity                       | 1.24        |
| Melt Index, g/10 min (260 °C /2.16 kg) | 5.0 - 7.0   |
| Tensile Yield Strength, MPa            | 60          |
| Tensile Modulus, GPa                   | 3.5         |
| Tensile Elongation, %                  | 6.0         |
| Notched Izod Impact, J/m               | 12.81       |

Figure 10 Properties of PLA polymer

Some of his properties like brittle nature and poor heat resistance are constitute limits to that material. To enhance those properties, Irradiation by gamma rays, X-rays, ion beams, and electron beam is often employed.

### 6.3 Effect of UV irradiation on PLA

Samples were irradiated using SEPAP12/24. Its rotating carousel ensures uniformity of temperature and irradiance at exposed specimen surface. The SEPAP is equipped with four medium pressure mercury arc lamps in borosilicate envelopes that emit discrete radiation at 290,313,365,405,436,547 and 579nm.

Irradiation has effect on the mechanical, thermal, and morphological properties of PLA. Its mechanical properties are greatly dependent on the irradiation dose. One of the drawbacks for PLA that limits its industrial applications is the lower HDT. Therefore, improving the HDT of PLA via irradiation contribute the possibility of usage in automotive applications.

Percentage of crystallinity of PLA decreased upon heating. Irradiation can also turn PLA to a much brittle material, which may be a disadvantage for the applications where toughness is important, such as packaging. Irradiation also decrease the glass transition and the melting point of the PLA with increase of the absorbed dose caused by the lowering of the molar mass (Kodal et al., 2018).

## 7 ESSENTIAL OILS

Essential oils are a complex blend of plant volatiles. Terpenoids and phenolic chemicals dominate these compounds. These flavourful volatile chemicals are biosynthesised in specific cell types found in practically all sections of the plant, from the leaves or flower to the roots, depending on the genus.

EOs have many properties and can be used in aromatherapy, a kind of complementary medicine that uses smell to improve your health or applied topically to the skin. Boost mood. Improve job performance through reduced stress and increased attentiveness. Improve sleep. Kill bacteria, funguses, and viruses. Reduce anxiety and pain. Reduce inflammation. Reduce nausea. EOs have raised interest this recent decade as ingredient for biodegradable food packaging due to their natural origin and their functional properties, allowing for obtaining active materials aiming to extend shelf-life and add value to the product.

### 7.1 Types of essential oils

Essential oils are made up of a wide range of compositions that can be identified by their fragrance components. Different types of essential oils include *Azadirachta indica* (neem), *Lavandula angustifolia* (lavender), *Thymus vulgaris* (thyme), *Eucalyptus globulus* (eucalyptus), *Cinnamomum zeylanicum* (cinnamon), *Syzygium aromaticum* (clove), *Citrus limonum* (lemon), *Melaleuca alternifolia* (tea tree), *Brassica nigra* (mustard), and others (Bhavaniramya et al., 2019). Some of the most EOs used for food packaging are Eucalytol and limonene.

### 7.2 Methods for extraction of bio reactive of EOs

Bioactive compound can be extracted from leaves, fruits, or bark by solvent extraction, hydro-distillation, or supercritical fluid extraction. The Soxhlet extraction procedure, which uses multiple solvent combinations depending on the plant portion being extracted, can be used to extract solvents. A mixture of methanol and water in a ratio of 90:10 v/v and ethanol and water in a ratio of 70:30 is recommended for solvent extraction of phytochemicals from fresh leaves and fruits. This approach's yield is projected to be higher than that of any other method used. The rotatory evaporator is used to purify the oil extracts obtained by this process. In the Soxhlet extraction, organic solvents can also be

substituted for water. This is referred to as hydro-distillation. from plant materials (Surbhi et al.,2021).

### **7.3 Effects of essential oils (EOs) on biodegradable polymers**

Plant essential oils (EOs) are biologically active compounds, which are volatile in nature, safe, environmental-friendly, and have good consumer acceptance. Essential oils represent an interesting ingredient for biodegradable food packaging, mainly due to their natural origin and their functional (antioxidant/antimicrobial) properties. Idea of combining PHB and PLA, which are most widely biodegradable polymers use for food packaging with Eos has raise interest of scientists and many studies has been conducted with the aim of developing an active packaging (*Jiang, J., Gong, L., Dong, Q., Kang, Y., Osako, K., & Li, L. 2020*). The purpose is to obtain active materials aiming to extend shelf-life and add value to the product.

The incorporation of these essential oils considerably affected the crystalline structure of the polymer, increasing the degree of crystallinity of the films. EOs act as a plasticizer, have effect on mechanical and thermal properties of PLA and PHB as well as on their degradation(Rech et al., 2021).

## **II. EXPERIMENTAL PART**

## 8 AIM OF THIS THESIS

The aim of the practical part of this diploma thesis was to evaluate the biodegradability of some polymers in soil and industrial compost environment. The tested samples are divided in to four groups:

- Blended PHB/ Organosolv Lignin: it consists of a mixture of PHB and Organosolv lignin in different proportions (1%; 3%; 6% and 12%).
- Blended PHB/Neutralized Alkali Lignin: it consists of a mixture of PHB and neutralized alkali lignin in different proportions (1%; 3%; 6% and 12%).
- PLA with different UV irradiation times: samples are PLA 175, PLA 175 samples with different irradiation time (100 hours and 400 hours) and cellulose as a reference.
- Blended Polymers with some essential oils: EOs blended with PHB are Eucalytol, Limonene and PLA special additives.

The biodegradation rate is determined from the values of carbon mineralization in terms of CO<sub>2</sub> production, the amount of which was measured using a mass gas analyzer.

All results were evaluated together. The aim was to compare mineralization curves of individual samples and assess what effect some compounds have on biodegradation of PHB and PLA.

The second Assessment method was done by observing the surface of polymers through fluorescence microscopy.

## 9 CHEMICALS, INSTRUMENTS, SOLUTIONS AND MATERIALS

### 9.1 Chemicals

|   |   |
|---|---|
| $\text{KHCO}_3$   | Potassium bicarbonate                     |
| $\text{HCl}$  | Hydrogen chloride                         |
| $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$                           | Calcium chloride dihydrate                |
| $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ | Ammonium iron(II) sulfate                 |
| $\text{KH}_2\text{PO}_4$  | Potassium dihydrogen phosphate            |
| $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$                           | Magnesium sulfate heptahydrate            |
| $\text{NaCl}$   | Sodium chloride                           |
| $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$                | Disodium hydrogen phosphate dodecahydrate |
| $\text{NH}_4\text{Cl}$  | Ammonium chloride                         |

### 9.2 Instrumentation

- Analytical balances kern 770, Germany
- KERN 440-47 laboratory balance
- Automatic pipettes (Eppendorf AG)
- Desiccator
- Incubator
- Mass gas analyser UGA 300
- Fluorescence microscope BX53M
- Common laboratory glassware and equipment
- Moisture analyser (OHAUS)

## 9.3 Solutions

### 9.3.1 Preparation of mineral medium

Mineral medium serves as a source of elements important for the micro-organisms, but not as source of energy.

The mineral medium was prepared from the following solutions:

- $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  Calcium chloride dihydrate with a concentration of 1 g/l
- $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  Ammonium iron(II) sulphate with a concentration of 3 g/l
- $\text{A-KH}_2\text{PO}_4$  Potassium dihydrogen-phosphate with a concentration of 9.07 g/l
- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  Magnesium sulfate heptahydrated with a concentration of 10 g/l
- $\text{NaCl}$  Sodium chloride with the concentration of 50 g/l
- $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  Disodium hydrogen phosphate dodecahydrate with the concentration of 23.90 g/l
- $\text{NH}_4\text{Cl}$  Ammonium chloride with the concentration of 30 g/l
- 2.4ml Solution of trace elements

### 9.3.2 Test polymeric materials

The elemental analysis of each polymeric material was conducted in a specific laboratory at **Faculty of Technology at Tomas Bata University**. The composition of biodegradable polymers is shown in Tables below.

Table 1 Identification and composition of 1st series of samples (PHB/Organosolv lignin)

| samples            | PHB [%] | Organosolv Lignin [%] | Total carbone [%] |
|--------------------|---------|-----------------------|-------------------|
| PHB                | 100     | -                     | 41.5              |
| PHB/Org LIGNIN 1   | 99      | 1                     | 55.8              |
| PHB/ Org LIGNIN 3  | 97      | 3                     | 56.3              |
| PHB/ Org LIGNIN 6  | 94      | 6                     | 52                |
| PHB/ Org LIGNIN 12 | 88      | 12                    | 57.6              |

Tested samples are blended polymers with organosolv lignin at different concentration.

Table 2 Identification and composition of 2<sup>nd</sup> series of samples (PHB/Neutralized Alkali Lignin)

| <b>samples</b>     | <b>PHB [%]</b> | <b>Neutralized Alkali Lignin [%]</b> | <b>Total carbone [%]</b> |
|--------------------|----------------|--------------------------------------|--------------------------|
| PHB                | 100            | -                                    | 41.5                     |
| PHB/Akl LIGNIN 1   | 99             | 1                                    | 55.8                     |
| PHB/ Akl LIGNIN 3  | 97             | 3                                    | 56.3                     |
| PHB/ Akl LIGNIN 4  | 94             | 6                                    | 52                       |
| PHB/ Akl LIGNIN 12 | 88             | 12                                   | 57.6                     |

Tested samples are blended polymers with Neutralized Alkali Lignin at different concentration.

Table 3 Identification and composition of 3<sup>rd</sup> series of samples (Irradiated PLA)

| <b>samples</b> | <b>Cellulose [%]</b> | <b>PLA [%]</b> | <b>Irradiation time [Hour]</b> | <b>Total carbone [%]</b> |
|----------------|----------------------|----------------|--------------------------------|--------------------------|
| Cellulose      | 100                  | -              | -                              | 41.5                     |
| PLA175         | -                    | 100            | -                              | 55.8                     |
| PLA175-100hr   | -                    | 100            | 100                            | 56.3                     |
| PLA175-400hr   | -                    | 100            | 400                            | 52                       |

Tested samples were irradiated with UV light. cellulose was taken as a reference material.

Table 4. Identification and composition of 4<sup>th</sup> series of samples (PHB, PLA/ Essential Oils)

| <b>samples</b>     | <b>CELL [%]</b> | <b>PHB [%]</b> | <b>PLA [%]</b> | <b>Eucalyptol [%]</b> | <b>Limonene [%]</b> | <b>Additives [%]</b> | <b>Total carbone [%]</b> |
|--------------------|-----------------|----------------|----------------|-----------------------|---------------------|----------------------|--------------------------|
| Cellulose          | 100             | -              | -              | -                     | -                   | -                    | 41.5                     |
| PHB/20% Eukalyptol | -               | 80             | -              | 20                    | -                   | -                    | 55.435                   |
| PHB/20% Limonene   | -               | -              | 80             | -                     | 20                  | -                    | 56.065                   |
| PLA/ADD            | -               | -              | 80             | -                     | -                   | 20                   | 51.025                   |

Tested samples were blended different EOs. cellulose was taken as a reference material.

## 10 AEROBIC BIODEGRADATION OF SAMPLES

Aerobic biodegradation was determined at the laboratory of the Faculty of Technology of TBU in Zln. The percentage of carbon mineralization in terms of CO<sub>2</sub> production was used to assess the biodegradation rate of the samples.

### 10.1 Soil preparation

The soil freshly samples were collected from a zlin garden, sieved with a sieve with meshes less than 2mm to get rid of impurities. Soil samples were then used within a few days for biodegradation tests.

### 10.2 Compost preparation

Two compost samples were collected from Zlin - Compost Plant and used in the biodegradation. The compost was matured in an oven at 58°C for one week with regular soaking and aeration, which was accomplished with a pump that drove air into the compost container via a wash bottle filled with distilled water. This provided aerobic conditions as well as appropriate humidity. The compost was filtered through a sieve after maturation to eliminate big contaminants and to homogenize it.

### 10.3 Perlite preparation

Perlite with a fraction size of 2-4 mm was first washed with water to remove debris and extra dust. Following that, the washed perlite was dried in an oven at 100 °C.

### 10.4 Mineral medium preparation

#### 10.4.1 Composition of the mineral medium

For a litre of the mineral medium the composition was as follows:

- CaCl<sub>2</sub>·2H<sub>2</sub>O 10ml
- Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O 10ml
- A-KH<sub>2</sub>PO<sub>4</sub> 20ml
- MgSO<sub>4</sub>·7H<sub>2</sub>O 10ml
- NaCl 10ml
- B-Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O 80ml

- $\text{NH}_4\text{Cl}$  10ml
- solution of trace elements 2.4ml

All the above solutions were mixed in the volumetric flask with distilled water. The resulting mineral medium was then sterilized in an autoclave at 125°C for 30 minutes. It was stored in a refrigerator at 4 °C.

#### 10.4.2 Determination of the amount of mineral medium

To determine the amount of mineral medium that we need to add inside each bottle, it is necessary to measure the moisture percentage in soil and compost. To measure the moisture content, the sample start weight is recorded. Afterwards, a halogen lamp or other infrared radiator heats and dries the sample while the integrated balance continually records the sample weight. When the sample no longer loses weight, the instrument shuts off and the moisture content is calculated.



Figure 11 Moisture analyzer

##### a) Amount of mineral medium for Soil

The amount of mineral medium was determined according to the percentage of soil moisture.

Soil humidity: 33.34%

Soil dryness: 66.66%

100%.....15ml

66.66%.....x

$$x = \frac{66.66 * 15}{100}$$

$$x = \mathbf{10ml}$$

Inside each bottle the estimated amount of mineral medium introduced was 10 ml

**b) Amount of mineral medium for industrial compost used for mineralization of irradiated PLA**

The amount of mineral medium was determined according to the percentage of compost moisture.

Compost humidity: 60%

Compost dryness: 40%

100%.....2.5ml

40%.....x

$$x = \frac{40 * 2.5}{100}$$

$$x = \mathbf{1ml}$$

Inside each bottle the estimated amount of mineral medium introduced was 1ml

**c) Amount of the mineral medium for industrial compost used for mineralization of polymers with essentials oils**

The amount of mineral medium was determined according to the percentage of compost moisture.

Compost humidity: 45.32%

Compost dryness: 54.68%

100%.....3.3ml

54.68%.....x

$$x = \frac{54.68 * 3.3}{100}$$

$$x = \mathbf{1.8ml}$$

Inside each bottle the estimated amount of mineral medium introduced was 1.8 ml.

## 10.5 Incubation preparation

Experiments with biodegradation were conducted in soil for PHB and in compost for PLA. Before testing, samples were cut into approximately 5 x 5 mm pieces. The samples were tested in three series over the course 333 days. As bioreactors, glass bottles with a gas volume of 580 ml were used, into which individual components were gradually dosed accordingly:

### a. PHB/Lignin in soil

- 15 g of soil
- 5 g of perlite
- 10 mL of mineral medium
- Approximately 50 mg of polymer sample

### b. Irradiated PLA in compost

- 2.5 g of compost
- 1.5 g of perlite
- 1 mL of mineral medium
- Approximately 50 mg of polymer sample

### c. Polymers with essential oils in compost

- 3.3 g of compost
- 1.5 g of perlite
- 1.8 mL of mineral medium
- Approximately 100 mg of polymer sample

Inside each bottle appropriate amount of soil or compost were weights and added. The second step consisted of adding around 1.5g of perlite and calculate amount of mineral medium inside bottles. After adding the calculated amount of mineral medium, the three components were well mixed, and the bioreactors prepared were stored inside the incubator at 58 °C for compost and room temperature for soil. The bottles were closed with tops with septa made of silicone rubber (figure12). After monitoring activities of micro-

organisms for two weeks, the amount of sample was added inside each bottle accordingly. The exact weights of the samples were recorded for each bottle.



Figure 12 Prepared test bottle

## 10.6 Evaluation of biodegradation

The evaluation of the decomposition of the samples was performed using a UGA 300 mass gas analyzer. The production of CO<sub>2</sub>, a by-product of aerobic biodegradation, was evaluated using argon as a standard.

Parameters of the UGA 300 mass gas analyzer:

- Inlet: capillary (stainless steel, PEEK, glass lined with plastic), flow rate 1 – 10 ml per minute at atmospheric pressure
- Type of ionization: electron
- Analyzer type: quadrupole
- Detectors: Faraday cage/electron multiplier TBU in Zlín, Faculty of Technology 56
- Mass range: 1 – 300 atomic mass units (amu)
- Detection limits: < 10 ppm for Faraday cage, < 1 ppm for electron multiplier
- Operating pressure: 10<sup>-4</sup> mbar for Faraday cage, 10<sup>-6</sup> mbar for electron multiplier

### 10.6.1 Measurement of CO<sub>2</sub> produced in bioreactors

The device has been configured to measure the signal of only the selected gases, allowing you to know the device's current response to a specific gas. During the measurement, a graph of the device's (pressure) reaction over time is displayed. This mode allows you to watch how the signal stabilizes, making it easier to choose the best time to read the response. It is required to set the gases to be monitored before to each measurement. At the same time, a detector that will be employed by the device can be chosen; for our purposes, an electron multiplier was chosen as the detector.

To stabilize the signal, the gas mass analyzer must be turned on and started at least one hour before the test. The amount of CO<sub>2</sub> produced in the bioreactors could only be measured after they had cooled to room temperature. If the bottles were monitored at a higher temperature, the signal would change due to rapid cooling. Before the start of the measurement, the micro filter with a porosity of 0.45µm was replaced.

Following analysis, the bioreactors were always aerated with air driven via a pump-paddle and passed through a washing bottle with distilled water. This ensured an adequate supply of oxygen to re-establish the aerobic atmosphere while also removing the CO<sub>2</sub> created by microbes in the bioreactors.

Aeration of the samples was carried out for 3 to 5 minutes. The blown air was humidified, which partly ensured the humidification of the environment inside the bioreactors. After measurement, the septa were put back on the bioreactors and were returned to the thermostat where incubation continued.

### 10.6.2 Calibration

To monitor CO<sub>2</sub> during the experiment, a calibration curve has been created. The following solutions had to be prepared for calibration:

- Preparation of 1 M HCl solution

A solution of 1 M hydrochloric acid was prepared in a 1 litre volumetric flask. Approximately 400 ml of distilled water and 88.7 ml of 35% HCl were gradually dosed into a volumetric flask.

- Preparation of 1.1 g/l KHCO<sub>3</sub> solution

To prepare 100 ml of solution, approximately 1.1 g of KHCO<sub>3</sub> was weighed. The weigh-in was then quantitatively transferred to a volumetric flask and made up to the mark with distilled water.

Table 5 Volumes of KHCO<sub>3</sub> solution required for the preparation of calibration standards for a given CO<sub>2</sub> concentration.

| CO <sub>2</sub> [%] | V <sub>KHCO<sub>3</sub></sub> [ml] |
|---------------------|------------------------------------|
| 0                   | 0                                  |
| 0.2                 | 0.44                               |
| 0.45                | 0.98                               |
| 1                   | 2.19                               |
| 1.5                 | 3.28                               |
| 2                   | 4.37                               |
| 3                   | 6.56                               |
| 8                   | 17.50                              |
| 15                  | 39.50                              |

At each measurement, these volumes were recalculated based on the current pressure, temperature, and KHCO<sub>3</sub> weight, and then dosed into 580 ml gaseous bottles. The following equation was used in the calculations:

$$V_{KHCO_3} = \frac{\left(\frac{\varphi_{CO_2}}{100}\right) * V_{btl} * P_{atm}}{R * T * C_{KHCO_3}}$$

Where:

V<sub>KHCO<sub>3</sub></sub>-Volume of stock solution of KHCO<sub>3</sub> [ml]

φ<sub>CO<sub>2</sub></sub>-Volumetric concentration of CO<sub>2</sub> in calibration gas [%]

V<sub>btl</sub>- Gas volume of the biometric bottle [l]

P<sub>atm</sub>-Atmospheric pressure [Pa]

R-Molar gas constant [8,314 J.mol<sup>-1</sup>.K<sup>-1</sup>]

T-Temperature [°C]

C<sub>KHCO<sub>3</sub></sub>-Concentration of stock solution KHCO<sub>3</sub> [mol.l<sup>-1</sup>]

The tubes were filled with 10 ml of 1 M HCl and Exact amount of KHCO<sub>3</sub> (according to the concentration) solution was poured into bottles. Tubes of HCl were gently inserted inside each bottle and were then sealed with septa-fitted tops. The bottles' contents were carefully mixed. The CO<sub>2</sub> content was initially stabilized with such prepared standards before being measured around an hour later.

The calibration curve was created as a result of the CO<sub>2</sub> detector response being dependent on the CO<sub>2</sub> level of the calibration bottles.

### 10.6.3 Evaluation of the measured data

#### a) Daily carbon production in form of CO<sub>2</sub>

$$m_c = \frac{M_C * V_{btlts} * Atm}{R * T * C_{KCO_3}} * W_{(CO_2)} * \frac{V_{st}}{S_{st}} * \frac{S_s}{V_s}$$

Where:

$m_c$ -Amount of carbon produced in the form of CO<sub>2</sub> [mg]

$M_C$ -Molar mass of carbon [g/mol]

$V_{btlts}$ -Gas volume of the biometric bottle [ml]

$Atm$ -Atmospheric pressure [KPa]

$R$ -Molar gas constant [8,314 J.mol<sup>-1</sup>.K<sup>-1</sup>]

$T$ -Temperature [°C]

$W_{(CO_2)}$ - Amount of carbon dioxide in the calibration gas[%]

$V_{st}$ -Dosed volume of the gas phase standard [μl]

$S_{st}$ -Detector Signal for standard [μV.s]

$V_s$ -Dosed volume of the gas phase of the sample [μl]

$S_s$ -Detector signal for sample [μV.s]

#### b) Cumulative carbon production as CO<sub>2</sub>

$$m(c)c^{(2)} = m(c)c^{(1)} + mc^{(2)}$$

Where:

$m(c)c^{(2)}$  -Cumulative carbon production from the current measurement [mg]

$m(c)c^{(1)}$  -Cumulative carbon production from the previous measurement [mg]

$mc^{(2)}$  -Daily carbon production from the current measurement [mg]

**c) Formula for calculating substrate carbon production in the form of CO<sub>2</sub>**

$$m(s) = mc + \emptyset mc$$

Where:

m(s)-substrate carbon production expressed as CO<sub>2</sub> [mg]

mc-Daily carbon production as CO<sub>2</sub> [mg]

$\emptyset mc$ -Average daily carbon production expressed as CO<sub>2</sub> [mg]

**d) Percentage of carbon mineralisation in terms of CO<sub>2</sub> production**

$$Dc = \frac{m(s)c}{m_m * TC}$$

Where:

Dc-percentage carbon mineralisation in terms of CO<sub>2</sub> production [%]

m(s)c-Substrate carbon production in the form of CO<sub>2</sub> [mg]

m<sub>m</sub>-Weight of the tested material [mg]

TC-Total carbon content of the test material [%]

Three resulting values of Dc were determined for one sample.

**e) Arithmetic mean**

Three resulting values of Dc were determined for one sample and the arithmetic mean, and the sample standard deviation were calculated using the following formulae:

$$\bar{x} = \frac{\sum_{i=1}^n x_i}{n}$$

Where:

$\bar{x}$ -Arithmetic mean

x<sub>i</sub>- values of Dc or m(k)c, from individual parallel determinations

n-Number of measurements

**f) Sample standard deviation**

$$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}}$$

Where:

s-Sample standard deviation

The derived arithmetic mean values  $D_c$  was then used to create graphs of the time dependency of carbon mineralisation in terms of  $\text{CO}_2$  production for individual samples. The graphs also included error bars whose sizes corresponded to the sample standard deviations.

## 11 MICROSCOPIC TESTING METHOD

More testing was done to assess the biodegradation process. Prior to this testing, a burial experiment was performed to assess changes in the material during biological decomposition. Fluorescence microscopy was used to observe the surface of polymers.

Microscopy was done on samples taken from the bioreactors after the biodegradation studies were completed.

For the assessment of biodegradation, BX53M fluorescence microscopy was used for the capture of images of the surface of polymers. The samples were taken at the magnifications of 20x.

Before observing the surfaces through microscope, samples have been soaked in propidium iodide dye solution. The dye solution was a mixture of 1ml of distilled water, 1 $\mu$ l of solution A and 1 $\mu$ l of solution B. Each sample was first imbibed with drops of dye for 10seconds, washed with drop of distilled water, and covered with a cover glass. Each sample was then placed under the microscope and observed through live and dead backlight. Live light (green) was used to assess the presence of living microorganisms and dead light (red) for assessment of dead microorganisms. Both images were then combined.

## 12 RESULTS AND DISCUSSION

The goal of this diploma thesis was to conduct biodegradation tests on blended polymers in soil and compost environment. For increased clarity and ease of implementation of biodegradation tests, each sample was reproduced in three specimens. The overall of our experiment consisted of four series:

The first series consisted of PHB and blended PHB with different amount of neutralized Alkali Lignin.

The second series was PHB and blended PHB with different amount of Organosolv Lignin.

The third series consisted of measuring PLA and PLA at different irradiation with cellulose as a biodegradation.

The last series were polymers with three essential oils: Eucalyptol, Limonene, and a special additive.

The curves of biodegradation of individual samples as well as the resulting values of the percentage of carbon mineralization in terms of CO<sub>2</sub> production were evaluated.

### 12.1 Mineralization results

The CO<sub>2</sub> evolution measurement monitored the mineralization of the materials. PHB blended with different lignin soil, PLA irradiated in compost and PHB or PLA with essential oils on industrial compost. The mineralisation samples were studied for approximately 298-333 days at 25°C in soil and between 50- 188days58°C in compost. Standard biodegradation tests measure the quantity of CO<sub>2</sub> generated from finished polymeric products based on these facts. Direct evidence of the degree of biodegradation is provided by measuring released CO<sub>2</sub> and comparing it to the blank amount based on the carbon content. The validity of test methods like composting and soil burial biodegradation procedures can be confirmed by testing cellulose (positive reference) or testing PHB which are known to be rapidly biodegradable.

#### 12.1.1 Results and evaluation of 1<sup>st</sup> series of samples

PHB is eco-friendly and very biodegradable material which can degrade faster compared to other materials. The aim of our experiment was to assess the influence of organosolv lignin on bio-degradation rate of PHB.

The 1<sup>st</sup> series were selected following samples:

- PHB
- PHB/ Org Lignin 1
- PHB/ Org Lignin 3
- PHB/ Org Lignin6
- PHB/ Org Lignin 12

The experiment was conducted for 298 days in the laboratory under room temperature (25°C) with Mr. Ing. Ahmad Fayyaz Bakhsh collaboration. The testing PHB has been used as a reference to assess to mineralization rate of other polymers as shown in the figure 13.

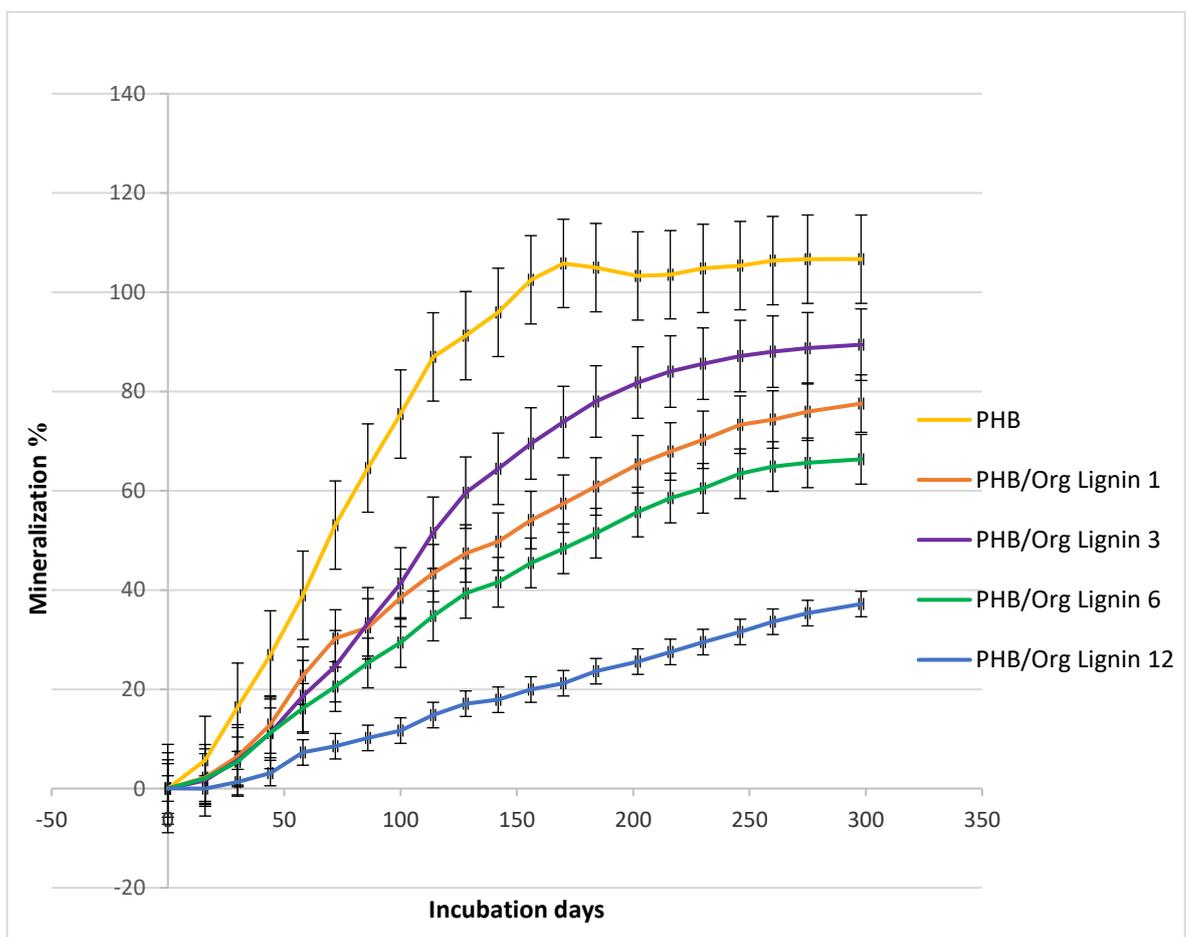


Figure 13 Mineralization of blended PHB with organosolv lignin at room temperature

From the chart, it shows that micro-organisms adapted very quickly, within 2 weeks and started the consumption of materials. From all samples, only PHB under reached the 106.66% of mineralization after 298 days, this could be explained by the increase in number of micro-organisms (they have more nutrients from samples) in comparison to blank.

Blended PHB samples with 1% organosolv Lignin (PHB/Org lignin1), 3% organosolv Lignin (PHB/Org lignin3) and 6% organosolv Lignin (PHB/Org lignin6) showed moderate retardation of biodegradation. The mineralization rate obtained after the incubation period was respectively 77.56% for PHB/OrgLignin 1, 89.43% for PHB/Org Lignin 3 and 66.34% PHB/Org Lignin 6 after incubation time. In comparison with other blended polymers, PHB blended with 12% organosolv Lignin (PHB/Org lignin12), has the highest influence and the retardation effect on biodegradation was more substantial (was less than 40 %).

This study suggests that organosolv Lignin can slow down the degradation of PHB. The more lignin was added the slower the blended polymer biodegraded.

### 12.1.2 Results and evaluation of 2<sup>nd</sup> series of samples

The aim of our experiment was to find out the influence of neutralized Alkali Lignin on bio-degradation rate of PHB.

The 2<sup>nd</sup> series were selected following samples:

- PHB
- PHB/Akl Lignin 1
- PHB/Akl Lignin 3
- PHB/Akl Lignin6
- PHB/Akl Lignin 12

The experiment was conducted for 333 days in the laboratory under room temperature (25°C) with Mr. Ing.Ahmad Fayyaz Bakhsh. The testing PHB has been used as a reference to assess to mineralization rate of other polymers as shown in the figure 14.

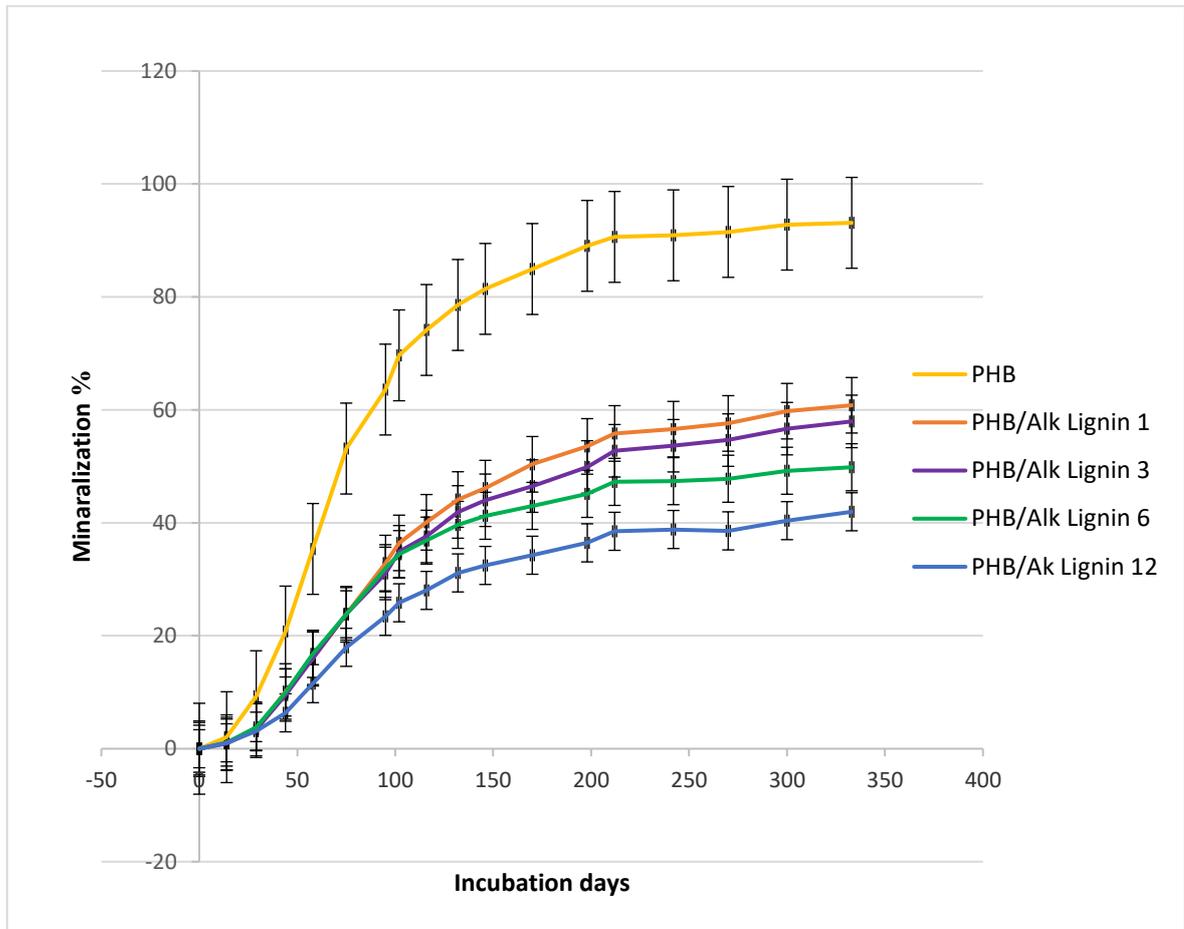


Figure 14 Mineralization of blended PHB with Neutralized Alkali at room temperature

The mineralization rate as shown on figure 14 of reference PHB reached 90.91% which were much different from other polymers. Mineralization rate of PHB blended with 1% and 3% of lignin reached respectively 60.81% and 57.95%. Mineralization rate of blended PHB with 6% neutralized Alkali Lignin (lignin6) and PHB with 12% neutralized Alkali Lignin (lignin12) was more substantial with respectively 49.84% for lignin 6 and 41.95% for lignin 12.

neutralized Alkali Lignin highly affects degradation of PHB, the more lignin was added the slower the blended polymer biodegraded.

### 12.1.3 Results and evaluation of 3<sup>rd</sup> series of samples

The aim of this experiment was to find out the influence of irradiation on bio-degradation rate of PLA.

The 3<sup>rd</sup> series were selected following samples:

- Cellulose
- PLA 175

- PLA 175-100 hr
- PLA 175-400 hr

Samples were exposed to UV irradiation at different irradiation times and incubated in the laboratory inside test bottles under industrial composting conditions (58 °C) for 174 days. Irradiations enhance PLA breakdown and therefore affects the mineralization rate as shown on figure 15.

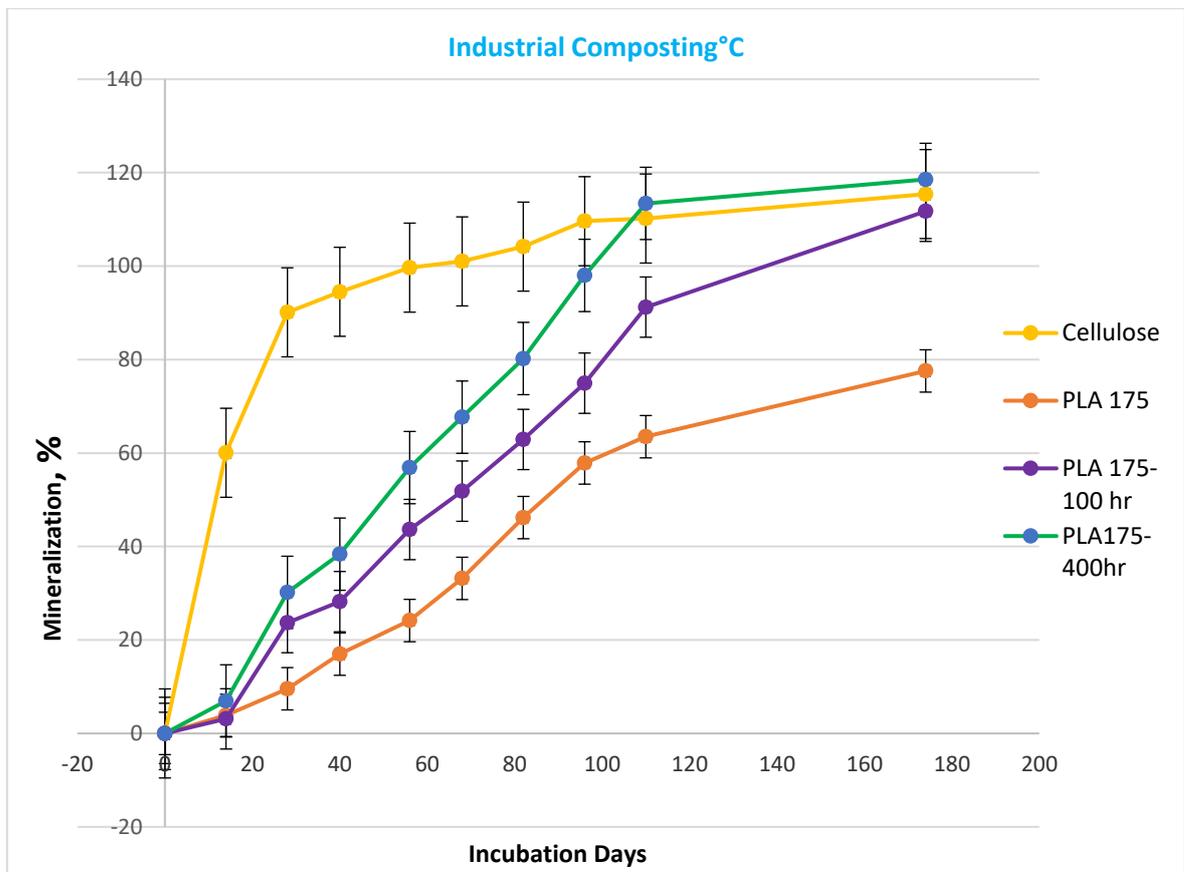


Figure 15 Irradiated PLA evolution during incubation in industrial compost at 58°C

From the chart, it shows that the mineralization rate of irradiated samples has been improved in comparison to the PLA 175. All samples except PLA 175 reached more than the 100% mineralization and this could be caused by the accumulation of CO<sub>2</sub> since samples were not aerated for a long period of time.

Reference sample (cellulose) has reached 115.41%. The mineralization of irradiated samples, PLA 175 with 100 hours irradiation and 400 hours irradiation are higher than standard PLA 175. PLA 175 with 100 hours irradiation reached 111.74% of

mineralization, while the mineralization of PLA 175 400 hours reached 118.56% after 110 days. Mineralization of standard PLA was slower, less than 80%.

Irradiation dose really has high effect in PLA mineralization and the longer the sample is irradiated the faster it biodegraded.

#### **12.1.4 Results and evaluation of 4<sup>th</sup> series of samples**

The aim of our experiment was to find out the influence of essential oils at high percentage on biodegradation rate of PHB and PLA.

The 4<sup>th</sup> series were selected following samples:

- Cellulose
- PHB/20% Eucalyptol
- PHB/20% limonene
- PHB/Additives

The experiment was conducted for 50 days in the laboratory under inside test bottles under industrial composting conditions (58 °C). The testing PHB has been used as a reference to assess to mineralization rate of other polymers as shown in the figure 17. Inhibitory effects of selected plant essential on the growth of microorganisms are clearly shown and the ability of inhibiting or slowing the growth of bacteria, yeasts, and moulds.

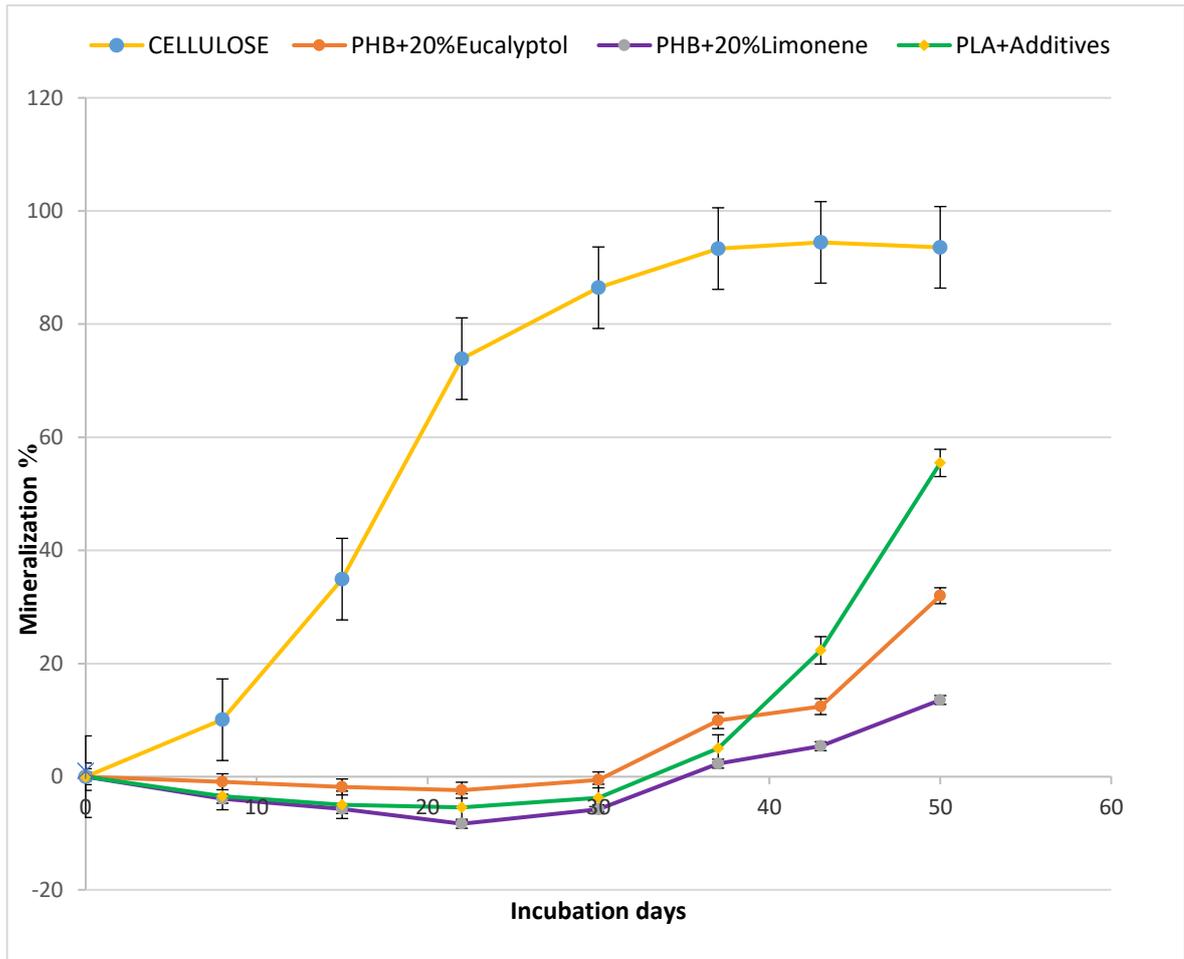


Figure 16 Blended polymers with essential oils evolution during incubation in industrial compost at 58°C

As shown on the chart, cellulose in comparison to blended polymers biodegraded faster and reached 93.58% after 50 days. Mineralization rate of blended polymers with EOs was slower than cellulose. Microorganisms took long time to adapt (around 30 days) and even some of them might have died within the process. After the incubation period (convex curves explanation). PHB blended with 20% Eucalyptol reached 31.98%, PHB with 20% limonene 13.56% and PLA with a special additive only 55.46%.

## 12.2 Fluorescence microscopy results

A fluorescence microscope type BX53M from Olumpus was used for the analysis of polymers surface. Samples surfaces were exposed to both green light and red light to assess the biodegradation and the presence of microorganisms.

### 12.2.1 Results of 1<sup>st</sup> series of samples

Surfaces of few samples of PHB blend with organosolv lignin were observed through fluorescence microscope.

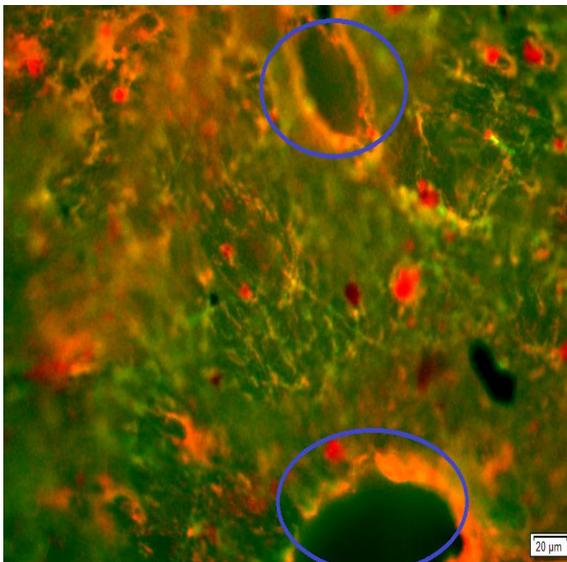


Figure 17 Microscope image of blended PHB/1% organosolv lignin

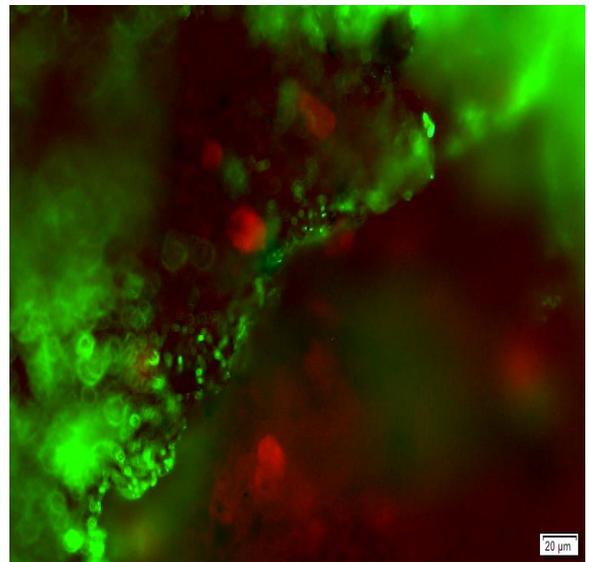


Figure 18 Microscope image of blended PHB/6% organosolv lignin

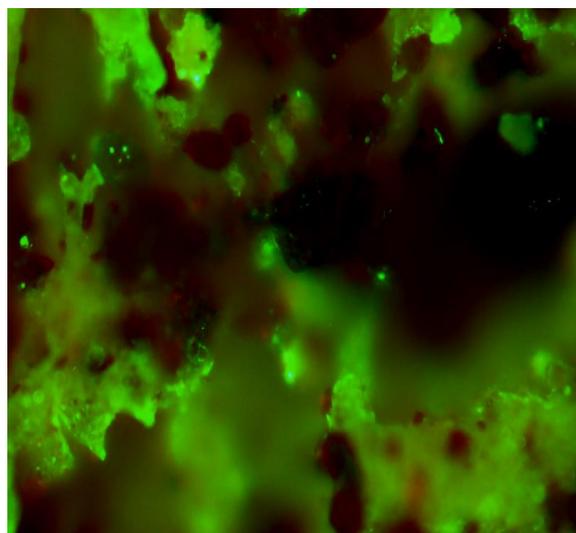


Figure 19 Microscope image of blended PHB/12% organosolv lignin

From the microscopic images, presence of bacterial colonies on incubated samples was observed. Living microorganisms are highly present (green spots) compared to dead microorganisms (red spots) in all samples. Some holes were observed on surface of blended PHB with 1% lignin which is a proof of the biodeterioration. Blended PHB with higher amount of lignin contained more living microorganisms than the one with lower amount of lignin which in contrary has considerable number of dead microorganisms.

### 12.2.2 Results of 2nd series of samples

Surfaces of samples of PHB blend with neutralized Alkali lignin were observed through fluorescence microscope.

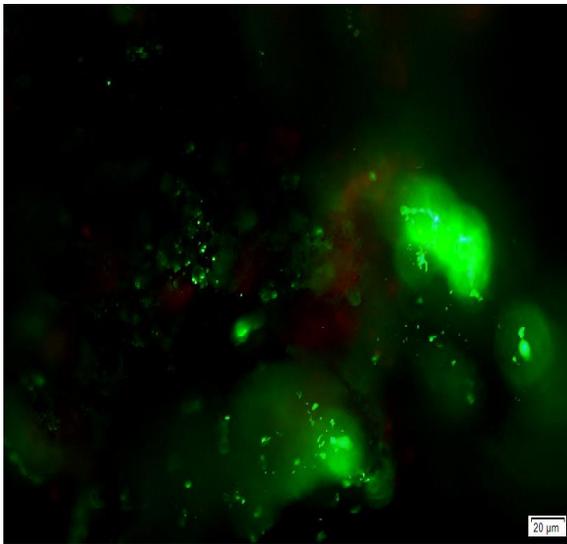


Figure 20 Microscope image of blended PHB/1% Neutralized Alkali lignin

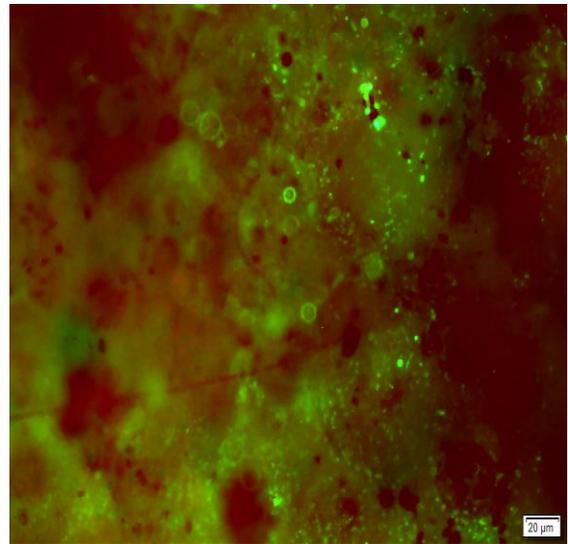


Figure 21 Microscope image of blended PHB/6% Neutralized Alkali lignin

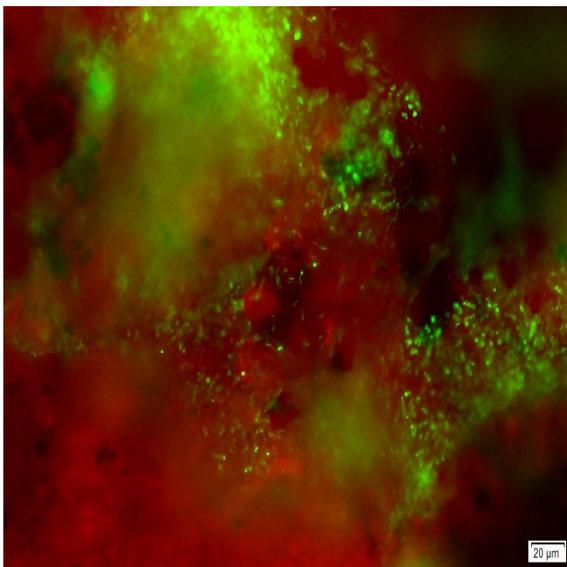


Figure 22 Microscope image of blended PHB/12% Neutralized Alkali lignin

Image of biofilms surface revealed presence of bacterial colonies on samples incubated. Surface containing 12% of neutralized alkali lignin showed higher amount of living microorganisms on surface (green spots) than polymer with 6% of neutralized alkali lignin.

### 12.2.3 Results of 3<sup>rd</sup> series of samples

Surfaces of samples of polymers blended with essential oils were observed through fluorescence microscope.

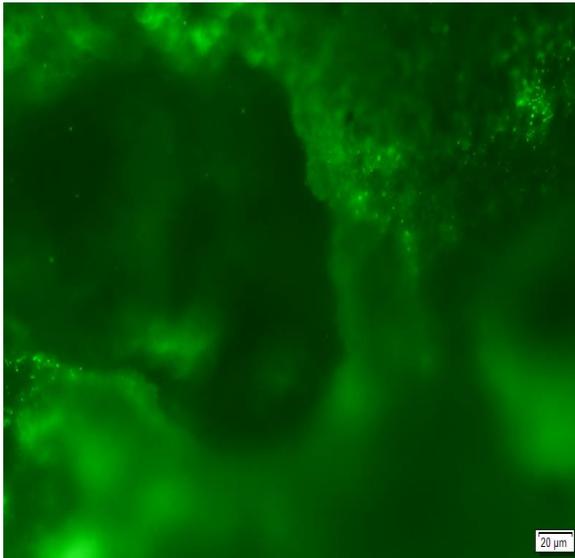


Figure 23 Fluorescence microscope image of blended PHB/20% Eucalyptol

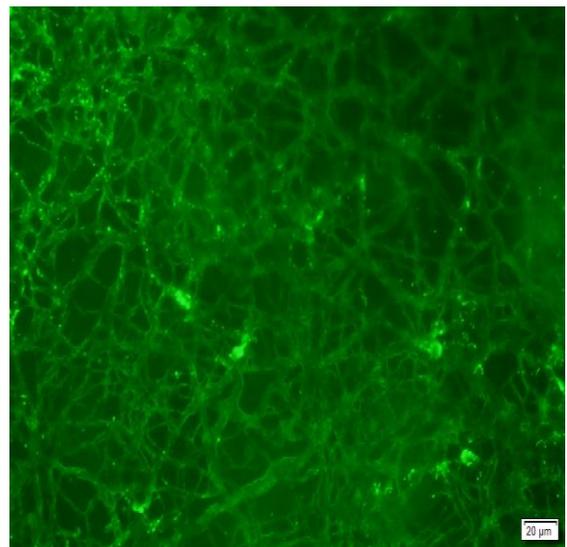


Figure 24 Fluorescence microscope image of blended PHB/20% Limonene

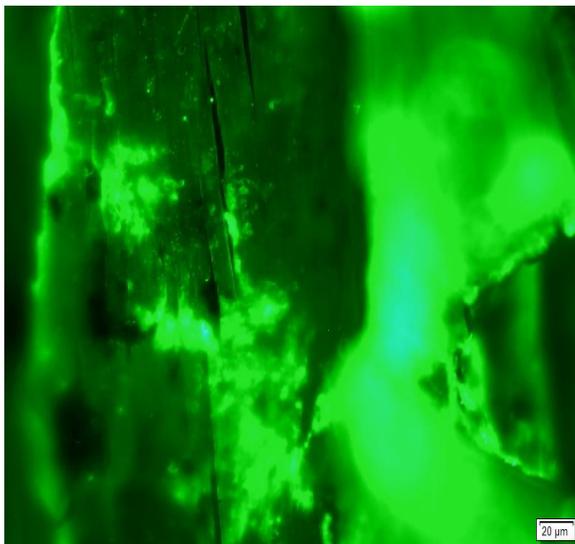


Figure 25 Microscope image of blended PLA/additives

Image of bio-films surface after biodegradation revealed presence of bacterial colonies on incubated samples but not on the entire surface because microorganisms are still colonising the surface of the material. This mainly confirmed the effectiveness EOs to slow down the growth of micro-organisms.

## CONCLUSION

The aim of this Thesis was to assess the curves and extent of biodegradation of blended polymers. The samples were subjected to a biodegradation experiment, the progress of which was also monitored using microscopic methods.

CO<sub>2</sub> of Four series of samples were monitored and measured to assess the biodegradability: The first series were blended PHB with organosolv lignin at different proportions, second were PHB with neutralized alkali lignin at different proportions, third irradiated PLA and fourth PLA, PHB with essential oils.

All samples were sent to another laboratory, TBU in Zlín, Faculty of technology, U15 for elemental analysis purpose. Only results of the elemental analysis of first and fourth series was provided, consequently total carbon of the first series for second and third (as shown on table 1, 2, 3 and 4).

The first series of samples was incubated inside 500 ml bottle at room temperature and the CO<sub>2</sub> production was monitored for 298 days. Chart Mineralization rate of samples shows that lignin affects PHB biodegradation. We observed progressive mineralization reduction between PHB (106.66%) and blended polymers (respectively 77.56%, 89.4312%, 77.56%, 66.34%, 37.18% for blended PHB with 1, 3, 6 and 12% of organosolv lignin).

The second series of samples were incubated for longer time, 333 days at the room temperature. Big difference on mineralization rate between PHB and blended polymer samples was noticed. The mineralization rate of PHB reached 93.12% when others were within 60.81 for PHB blended with 1% neutralized alkali lignin and 41.95% for blended PHB with 12% neutralized alkali lignin.

In comparison with PHB, PLA takes longer time to biodegrade, within 180 days under composting (Kalita et al., 2021). The third series were incubated for 174 days on industrial compost environment. The mineralization chart shows samples with highest irradiation time biodegrade faster than others. Samples were not aerated for over a month and some discrepancies on curves which might be explained because accumulation of CO<sub>2</sub>. Despite the discrepancies, the outcome of our experiment is that irradiation enhances PLA breakdown and increases the biodegradation rate.

The fourth series was also monitored under composting environment for 50 days. We noticed convex curves at the beginning of the experiment which means the biodegradation had not yet taken place and this might be because of the amount of Eos, 20% which could have been toxic to microorganisms or the nature of species of the inoculum. After a while

(30 days), the biodegradation started and the blended PLA with additives biodegraded faster than others. We could not monitor the samples till the end because of the short time left. Based on these results we might summarize by saying that at certain level, EOs might be toxic to some species but this must be verified. The advice is to reconduct another experience but because of short term we could not started.

Images of the samples stained with died solution and exposed to fluorescence lights show the presence of colonies of micro-organisms on surfaces. The presences of holes are also seen but not as expected.

The study of biodegradable polymers is critical in terms of its potential practical uses. The results found in the practical section of the thesis can be useful in the production and subsequent use of biodegradable mulch films and food packaging.

Minor errors on measurement process with the gas mass analyzer were addressed during the laboratory work.

**BIBLIOGRAPHY**

Siracusa, V., Rocculi, P., Romani, S., & Rosa, M. D. (2008). Biodegradable polymers for food packaging: a review. In *Trends in Food Science and Technology* (Vol. 19, Issue 12, pp. 634–643). <https://doi.org/10.1016/j.tifs.2008.07.003>

Narancic, T., & O'Connor, K. E. (2019). Plastic waste as a global challenge: Are biodegradable plastics the answer to the plastic waste problem? In *Microbiology (United Kingdom)* (Vol. 165, Issue 2, pp. 129–137). Microbiology Society. <https://doi.org/10.1099/mic.0.000749>

Doppalapudi, S., Jain, A., Khan, W., & Domb, A. J. (2014). Biodegradable polymers-an overview. In *Polymers for Advanced Technologies* (Vol. 25, Issue 5, pp. 427–435). John Wiley and Sons Ltd. <https://doi.org/10.1002/pat.3305>

Atiwesh, G., Mikhael, A., Parrish, C. C., Banoub, J., & Le, T. A. T. (2021). Environmental impact of bioplastic use: A review. *Heliyon*, 7(9). <https://doi.org/10.1016/j.heliyon.2021.e07918>

Song, J., Kay, M., & Coles, R. (2011). Bioplastics. In *Food and Beverage Packaging Technology: Second Edition* (pp. 295–319). Wiley-Blackwell. <https://doi.org/10.1002/9781444392180.ch11>

Simon Sampson. (2021). Effets of non-biodegradable plastics on environment. *International Scholars Journals*.

McAdam, B., Fournet, M. B., McDonald, P., & Mojicevic, M. (2020). Production of polyhydroxybutyrate (PHB) and factors impacting its chemical and mechanical characteristics. In *Polymers* (Vol. 12, Issue 12, pp. 1–20). MDPI AG. <https://doi.org/10.3390/polym12122908>

Xu, C., & Ferdosian, F. (n.d.). *Green Chemistry and Sustainable Technology Conversion of Lignin into Bio-Based Chemicals and Materials*. <http://www.springer.com/series/11661>

Payam Mousavioum. (2011). *Properties of Lignin and Poly(hydroxybutyrate) Blends*.

Mousavioum, P., Halley, P. J., & Doherty, W. O. S. (2013). Thermophysical properties and rheology of PHB/lignin blends. *Industrial Crops and Products*, 50, 270–275. <https://doi.org/10.1016/j.indcrop.2013.07.026>

Oguz, H., Dogan, C., Kara, D., Ozen, Z. T., Ovali, D., & Nofar, M. (2019). Development of PLA-PBAT and PLA-PBSA bio-blends: Effects of processing type and PLA crystallinity on morphology and mechanical properties. *AIP Conference Proceedings*, 2055. <https://doi.org/10.1063/1.5084813>

Fogašová, M., Figalla, S., Danišová, L., Medlenová, E., Hlaváčiková, S., Vanovčanová, Z., Omaníková, L., Baco, A., Horváth, V., Mikolajová, M., Feranc, J., Bočkaj, J., Plavec, R., Alexy, P., Repiská, M., Příkryl, R., Kontárová, S., Báreková, A., Sláviková, M., ... Kadlečková, M. (2022). PLA/PHB-Based Materials Fully Biodegradable under Both Industrial and Home-Composting Conditions. *Polymers*, 14(19). <https://doi.org/10.3390/polym14194113>

Vidotto, M., Mihaljević, B., Žauhar, G., Vidović, E., Maltar-Strmečki, N., Klepac, D., & Valić, S. (2021). Effects of  $\gamma$ -radiation on structure and properties of poly(lactic acid) filaments. *Radiation Physics and Chemistry*, 184. <https://doi.org/10.1016/j.radphyschem.2021.109456>

Kodal, M., Wis, A. A., & Ozkoc, G. (2018). The mechanical, thermal and morphological properties of  $\gamma$ -irradiated PLA/TAIC and PLA/OvPOSS. *Radiation Physics and Chemistry*, 153, 214–225. <https://doi.org/10.1016/j.radphyschem.2018.10.018>

Benyathiar, P., Selke, S., & Auras, R. (2016). The Effect of Gamma and Electron Beam Irradiation on the Biodegradability of PLA Films. *Journal of Polymers and the Environment*, 24(3), 230–240. <https://doi.org/10.1007/s10924-016-0766-7>

Kalita, N. K., Damare, N. A., Hazarika, D., Bhagabati, P., Kalamdhad, A., & Katiyar, V. (2021). Biodegradation and characterization study of compostable PLA bioplastic containing algae biomass as potential degradation accelerator. *Environmental Challenges*, <https://doi.org/10.1016/j.envc.2021.100067>

Jiang, J., Gong, L., Dong, Q., Kang, Y., Osako, K., & Li, L. (2020). Characterization of PLA-P3,4HB active film incorporated with essential oil: Application in peach preservation. *Food Chemistry*, 313. <https://doi.org/10.1016/j.foodchem.2019.126134>

Huang, P. Y., Zhang, Y., Ouyang, H., Yang, F., & Lee, S. (2022). Photo-response of polylactide-poly(methyl methacrylate) blends: Effect of ultraviolet irradiation. *Materials Chemistry and Physics*, 277. <https://doi.org/10.1016/j.matchemphys.2021.125528>

Atarés, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. In *Trends in Food Science and Technology* (Vol. 48, pp. 51–62). Elsevier Ltd. <https://doi.org/10.1016/j.tifs.2015.12.001>

Muniyasamy, S., Ofosu, O., John, M. J., & Anandjiwala, R. D. (2016). Mineralization of poly(lactic acid) (PLA), Poly(3-hydroxybutyrate-co-valerate) (PHBV) and PLA/PHBV blend in compost and soil environments. *Journal of Renewable Materials*, 4(2), 133–145. <https://doi.org/10.7569/JRM.2016.634104>

Oussalah, M., Caillet, S., Saucier, L., & Lacroix, M. (2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella* Typhimurium, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Control*, 18(5), 414–420. <https://doi.org/10.1016/j.foodcont.2005.11.009>

Sharma, S., Barkauskaite, S., Jaiswal, A. K., & Jaiswal, S. (2021). Essential oils as additives in active food packaging. In *Food Chemistry* (Vol. 343). Elsevier Ltd. <https://doi.org/10.1016/j.foodchem.2020.128403>

Mousavioun, P., George, G. A., & Doherty, W. O. S. (2012). Environmental degradation of lignin/poly(hydroxybutyrate) blends. *Polymer Degradation and Stability*, 97(7), 1114–1122. <https://doi.org/10.1016/j.polymdegradstab.2012.04.004>

Saleh, H. M. (n.d.). *Biodegradation: Process Mechanism*. <https://www.researchgate.net/publication/312491332>

Nanda, S., Patra, B. R., Patel, R., Bakos, J., & Dalai, A. K. (2022). Innovations in applications and prospects of bioplastics and biopolymers: a review. In *Environmental Chemistry Letters* (Vol. 20, Issue 1, pp. 379–395). Springer Science and Business Media Deutschland GmbH. <https://doi.org/10.1007/s10311-021-01334-4>

Plastics-the Facts 2022 OCTOBER 2022. (n.d.).

Surbhi, Kumar, A., Singh, S., Kumari, P., & Rasane, P. (2021). Eucalyptus: phytochemical composition, extraction methods and food and medicinal applications. In *Advances in Traditional Medicine*. Springer. <https://doi.org/10.1007/s13596-021-00582-7>

Rech, C. R., Brabes, K. C. S., Silva, B. E. B., Martines, M. A. U., Silveira, T. F. S., Alberton, J., Amadeu, C. A. A., Caon, T., Arruda, E. J., & Martelli, S. M. (2021). Antimicrobial and Physical–Mechanical Properties of Polyhydroxybutyrate Edible Films Containing Essential Oil Mixtures. *Journal of Polymers and the Environment*, 29(4), 1202–1211. <https://doi.org/10.1007/s10924-020-01943-0>

Pischedda, A., Tosin, M., & Degli-Innocenti, F. (2019). Biodegradation of plastics in soil: The effect of temperature. *Polymer Degradation and Stability*, 170. <https://doi.org/10.1016/j.polymdegradstab.2019.109017>

Liu, L., Xu, M., Ye, Y., & Zhang, B. (2022). On the degradation of (micro)plastics: Degradation methods, influencing factors, environmental impacts. In *Science of the Total Environment* (Vol. 806). Elsevier B.V. <https://doi.org/10.1016/j.scitotenv.2021.151312>

Price, S., Kuzhiumparambil, U., Pernice, M., & Ralph, P. (2022). Techno-economic analysis of cyanobacterial PHB bioplastic production. *Journal of Environmental Chemical Engineering*, 10(3). <https://doi.org/10.1016/j.jece.2022.107502>

Siotto, M., Tosin, M., DegliInnocenti, F. *et al.* Mineralization of Monomeric Components of Biodegradable Plastics in Preconditioned and Enriched Sandy Loam Soil Under Laboratory Conditions. *Water Air Soil Pollut* 221, 245–254 (2011). <https://doi.org/10.1007/s11270-011-0787-8>

Ho, K.-L. G., & PomettoIii, A. L. (1999). Temperature Effects on Soil Mineralization of Polylactic Acid Plastic in Laboratory Respirometers 1. In *Journal of Environmental Polymer Degradation* (Vol. 7, Issue 2).

Evode, N., Qamar, S. A., Bilal, M., Barceló, D., & Iqbal, H. M. N. (2021). Plastic waste and its management strategies for environmental sustainability. *Case Studies in Chemical and Environmental Engineering*, 4. <https://doi.org/10.1016/j.cscee.2021.100142>

**LIST OF ABBREVIATIONS**

|                   |  |
|-------------------|--|
| PHB               | Polyhydroxy butyrate                       |
| PLA               | Polylactic acid                            |
| PP                | Polypropylene                              |
| PE                | Polyethylene                               |
| PHA               | Polyhydroxy alkanoates                     |
| Eos               | Essentials oils                            |
| PS                | Polystyrene                                |
| PET               | Polyethylene terephthalate                 |
| HDPE              | High-density polyethylene                  |
| LDPE              | Low-density polyethylene                   |
| UHMWPE            | Ultra-high-molecular-weight polyethylene   |
| LLDPE             | Linear low-density polyethylene            |
| ASTM              | American Society for Testing and Materials |
| PGA               | Polyglycolic acid                          |
| T <sub>g</sub>    | Glass transition temperature               |
| T <sub>m</sub>    | Melting temperature                        |
| X <sub>c</sub>    | Degree of crystallinity                    |
| UV                | Ultraviolet                                |
| HCl               | Hydrogen chloride                          |
| KHCO <sub>3</sub> | Potassium bicarbonate                      |

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