TAL TECH

BIOMETHANE PRODUCTION FROM THE ANAEROBIC DIGESTION OF POLYLACTIC ACID (PLA)-BASED POLYMERS UNDER MESOPHILIC CONDITIONS

BIOMETAANI TOOTMINE POLLÜPIIMHAPE POLÜMEERIDEST ANEAEROOBSEL KÄÄRITAMISEL MESOFIILSETES TINGIMUSTESS

MASTER THESIS

Student:	Iñigo Lasheras Minchinela
Student code:	201685EABM
Supervisor:	Pavlo Lyshtva (PhD student)
Co-supervisor:	Dr. Viktoria Voronova (Senior lecturer)

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Author: Iñigo Lasheras Minchinela
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Water and Environmental Engineering Research Group

THESIS TASK

</div>

Student: Iñigo Lasheras Minchinela, 201685EABM

Study programme, EABM03/18 - Environmental Engineering and Management

main speciality:

Supervisor(s): PhD student, TalTech. Pavlo Lyshtva, +37258055196,

pavlo.lyshtva@taltech.ee

Co-supervisor: Senior Lecturer, TalTech. Dr. Viktoria Voronova,

viktoria.voronova@taltech.ee

Thesis topic:

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1. Literature review
2. Setup and development of anaerobic digestion system of degradable polymers
3. Analysis of the methane produced by the polymer and their biodegradability

Thesis tasks and time schedule:

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1.	Introduction, aim of thesis and literature review	14/03/2022
2.	Preparation of the test	18/03/2022
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Student: Iñigo Lasheras Minchinela   18 May 2022
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Supervisor: …………………   …………………….   "......."…………………2022
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PREFACE

This master thesis was motivated by the project "Bio-plastics Europe" with the objective of testing the methane production and biodegradability of modified PLA-based polymers by anaerobic digestion. The test took place at Tallinn University of Technology (TalTech).

Biodegradable polymers are normally modified in order to improve their characteristics and be used for industrial purposes. Anaerobic degradation is one of the most promising waste treatment methods for products made from biodegradable polymers. Therefore, it is essential to study the degradability of new modified polymers to check the improvement in biodegradability yields.

This work has been carried out under the supervision of PhD student Pavlo Lyshtva and the co-supervision of Dr. Viktoria Voronova.

Keywords: BIOPLASTIC, POLYLACTIC ACID, ANAEROBIC DIGESTION, BIOMETHANE, MASTER THESIS

LIST OF ABBREVIATIONS AND SYMBOLS

AD	anaerobic digestion
AMPTS II	Automatic Methane Potential Test System II
BMP	biochemical methane potential
C/N	carbon to nitrogen ratio
CH_4	methane
CO_2	carbon dioxide
DP	direct polycondensation
H_2S	hydrogen sulfide
LA	lactic acid
MS	modified starch
N_2	molecular nitrogen
NaOH	sodium hydroxide
NH_3	ammonia
PA	polyamide
PBAT	polybutylene adipate terephthalate
PBS	polybutylene succinate
PCL	polycaprolactone
PE	polyethylene
PET	polyethylene terephthalate
PHA	polyhydroxyalkanoate
PHB	poly 3-hydroxybutyrate
PHBV	3-hydroxybutyrate-cohydroxyvalerate
PLA	poly(lactic acid) or polylactic acid
PP	polypropylene
PS	polystyrene
PTT	polytrimethylene terephthalate
ROP	ring-opening polymerization
TS	total solids
VS	volatile solids

1   INTRODUCTION

Plastics have become essential in the industry as well as in the current society. This can be reflected in an increase in the use of plastics from only 1,5 million metric tons in 1950 to 367 million metric tons in 2020 (PlasticsEurope, 2021). The increasing demand for plastics can be explained due to being low-priced, lasting, lightweight, chemical resistant, and having high properties as thermal and electrical insulator. Additionally, the wide diversity of polymers available on the market makes them present in every industry (Andrady & Neal, 2009). It has been estimated that around 42% of the plastics manufactured every year (or primary plastics) are used in packaging, followed by the construction industry with 19% (Geyer et al., 2017).

Plastic waste has become a serious environmental problem owing to the massive production of plastics and their corrosion-resistant properties. From 1950 to 2015, Geyer et al. (2017) estimated that approximately 60% of the total primary plastics globally produced during those years have been discarded in landfills, dumpsters, or directly into the environment. In addition, 10% have been incinerated and the rest have been recycled or are still in use. Poor management of plastic waste causes plastics to accumulate in most ecosystems globally (Phelan et al., 2022). Water bodies have been seriously affected by plastic pollution, especially oceans where large plastic items and microplastics have been accumulated (Li et al., 2016). Plastic waste can also seriously harm soils since plastic waste can be accumulated in terrestrial systems. Agricultural soils can be especially affected due to the presence of plastic particles in the sewage sludge used as fertilizer or because of the contamination of freshwater used as irrigation (Hurley et al., 2020).

Bioplastics are considered one of the most promising solutions to improve plastic waste management and reduce waste accumulation. The possibilities to produce polymers from renewable resources and biodegrade the residues have made many researchers and companies interested in investigating and developing new possibilities to be used in the current market. Poly(lactic acid) or PLA is one of the most popular bioplastics available in the market since it is biobased, can be biodegraded, and it possesses similar characteristics to conventional plastics like polystyrene (PS) and polyethylene terephthalate (PET). PLA global production has rapidly increased recently and it is expected to continue to increase in the coming years (European Bioplastics, 2021). PLA is produced from starchy and cellulosic feedstocks, and it is widely used in packaging use, as well as in textile, agricultural, and medical industries.

The biodegradation of PLA has acquired interest during the last years, especially by anaerobic digestion due to obtaining high yields of methane that can be used as a source of clean energy. The anaerobic digestion of different PLA-based polymers has been studied by many authors during the last decades, evaluating different conditions and times. However, the results obtained are not entirely satisfactory since in many cases the production of biogas at mesophilic temperatures (35-38 ºC) was low or non-existent, while at thermophilic temperatures (55-58 ºC) the biomethane obtained is generally higher (Cazaudehore et al., 2022). Nonetheless, digestion times in experiments normally exceed the time used in anaerobic digesters in waste treatment plants, indicating that it is currently not feasible to totally degrade PLA together with organic waste (Narancic et al., 2018).

The aim of this Master Thesis is to:
- Analyse the results obtained in the literature about the anaerobic digestion of PLA-based polymers.
- Test the anaerobic digestion of three different PLA-based polymers under mesophilic conditions for 49 days, measuring the methane production of the polymers and their biodegradability.
- Compare the results obtained in the test to those found in the literature.
- Reach conclusions if the studied polymers are suitable for anaerobic degradation under mesophilic conditions and if they have better results than pure PLA and other polymers previously studied.

2 LITERATURE REVIEW

2.1 Bioplastics

In recent years, bioplastics have been proposed as a possible solution to the environmental problem derived from traditional plastic waste. Within the term bioplastic, two branches can be distinguished, bio-based plastics and biodegradable plastics (Figure 2.1). To be considered a bioplastic, the polymer must meet one or both characteristics. Bio-based means that the polymer is totally or partially made from renewable sources (e.g., polysaccharides, lipids, proteins) or synthesized from renewable resources as in the case of polyesters produced from derived monomers (Flieger et al., 2003; Madhavan Nampoothiri et al., 2010). Some examples are the bio-based polymers analogous to petroleum-derived such as bio-polyethylene (Bio-PE), bio-polypropylene (Bio-PP), bio-polyamide (Bio-PA), and bio-poly(ethylene terephthalate) (Bio-PET) (Siracusa & Blanco, 2020).

On the other hand, biodegradable plastics are those polymers that have the ability to break down into simpler substances by the action of bacteria. Those simpler substances (e.g., water, carbon dioxide, minerals) are environmentally accepted, avoiding the production of any type of waste (Ashter, 2016). Polymers such as polybutylene adipate terephthalate (PBAT) and polycaprolactone (PCL) are produced from fossil materials but can be degraded. However, from an environmental point of view, the most interesting bioplastics are those that meet both characteristics, including poly(lactic acid) (PLA), polyhydroxyalkanoate (PHA), polybutylene succinate (PBS), and starch blends.

The renewable origin of their feedstock and the possibility of being degraded have made bioplastics gain popularity around the world. According to European Bioplastics (2017), the global bioplastic production in 2017 was 2,054 million tons. The most recent data shows that global production has risen to 2,417 million tons in 2021 and it is expected to increase to an estimated value of 7,593 million tons in 2026 (European Bioplastics, 2021). The main market segments of bioplastics are packaging (flexible and rigid), consumer goods, fibers, and agriculture. In economic terms, the global bioplastics market size is projected to grow from USD 10,7 billion in 2021 to USD 29,7 billion by 2026. Asia is the main current bioplastic market followed by Europe and North America. This market growth is expected owing to the growing demand from the packaging industry worldwide alongside the restrictions and regulations in the use of traditional plastics, especially single-use plastics (MarketsandMarkets, 2021).
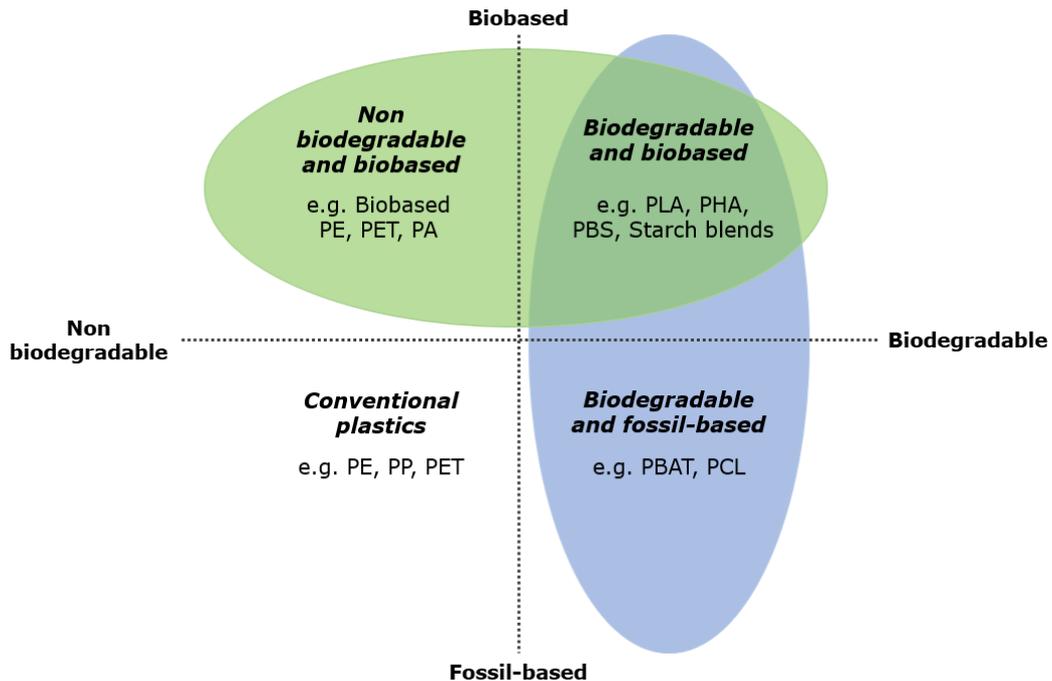
Figure 2.1 Classification of bioplastics (European Bioplastics, 2021)

Biodegradable plastics, including PLA, PHA, and starch blends, dominate the market with 64% of the total bioplastics production in 2020 (Figure 2.2). The share of biodegradable plastics has increased during the last years owing to their advantage in waste treatment. Additionally, their production is expected to increase to almost 70% of the total production in 2026, while the production of bio-based, non-biodegradable plastics is predicted to further decrease to slightly over 30%, where biobased PE, PET, PA, and polytrimethylene terephthalate (PTT) will dominate (European Bioplastics, 2021).
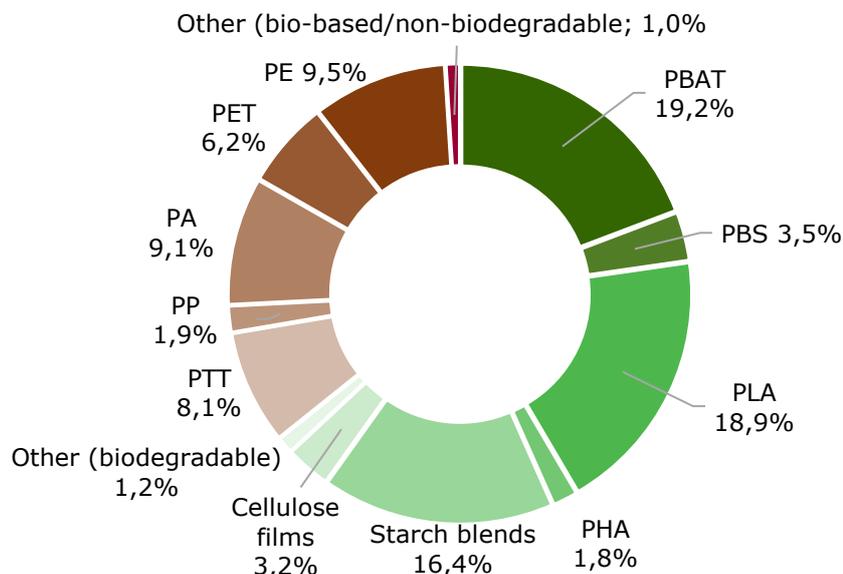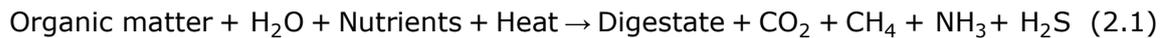

Figure 2.2 Global production capacities of bioplastics in 2021 (European Bioplastics, 2021)

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2.2 Biogas production

Solid waste management methods for biodegradable waste such as composting and anaerobic digestion (AD) are the most interesting options to deal with the bioplastic waste. Industrially, from an environmental and energy point of view, anaerobic digestion seems to be the most promising due to the production of biogas. Biogas has a high content of methane (biomethane) that can be used to produce energy. Furthermore, it is expected an important development of biogas plants in the future (Bátori et al., 2018).

AD is a biochemical endothermic process in which, in an oxygen-free environment, biomass is digested by particular kinds of bacteria. This process is widely used in industry for the treatment of agricultural wastes, food wastes, and sewage sludges (Cazaudehore et al., 2022). The general reaction that occurs in the anaerobic digesters has been represented in Formula (2.1) (Xu et al., 2019).

$$\text{Organic matter} + H_2O + \text{Nutrients} + \text{Heat} \rightarrow \text{Digestate} + CO_2 + CH_4 + NH_3 + H_2S \quad (2.1)$$

From the AD process, solid and gaseous products are obtained. A solid and rich in nutrients digested is obtained from the non-degraded solid matter. In some cases, this digestate may be immediately used in agriculture but generally it is further treated by aerobic composting and stabilization to obtain complete maturity of the end product (European Bioplastics, 2015). From the gaseous products, biogas is obtained. Biogas is a mixture of 50-70% methane (CH_4), 50-30% carbon dioxide (CO_2), and some traces of molecular nitrogen (N_2), ammonia (NH_3), and hydrogen sulfide (H_2S) (Rufai, 2019). The biogas obtained can be directly converted into heat and electricity in a cogeneration heat and power unit (Walla & Schneeberger, 2008), or it can be upgraded to biomethane by increasing the amount of methane in the gas. Biomethane can be introduced into the national gas grid or used as transportation fuel, being a substitute for fossil fuels (gasoline, diesel) with much lower greenhouse gas emissions (Sahota et al., 2018).

Anaerobic digestion technologies are normally classified into wet and dry systems, depending on the concentration of total solids (TS) used in the digestion process (Tchobanoglous et al., 1993). In the wet AD processes, water is added to the feedstock so that the solid matter concentration is typically TS <15%. Completely mixed tank digesters are normally used for these processes (Angelonidi & Smith, 2015). In the case of dry AD processes, the solid matter concentration is higher (TS 20-40%). Dry AD processes can be differentiated between continuous and batch processes, depending on their operation

system (André et al., 2018). In Europe, for the treatment of organic wastes, dry technologies are mainly used in the AD plants (Mattheeuws, 2015). Depending on the temperature of operation anaerobic digesters can be divided into mesophilic (35-38 °C) or thermophilic (55-58 °C) (Cazaudehore et al., 2022).

2.2.1 Evolution of anaerobic digestion plants in Europe

In Europe, the number of operating biogas plants has increased during the last decades, as well as the amount of energy produced from the biogas obtained (Figure 2.3). Germany is the main leader in biogas production with a much larger capacity than the following countries like Italy, France, or the United Kingdom. The dominant feedstock type for biogas production varies depending on the country. However, agricultural feedstocks are the main driving force of the European biogas market, including energy crops, farm residues, plant residues, and livestock manure (European Biogas Association, 2020).
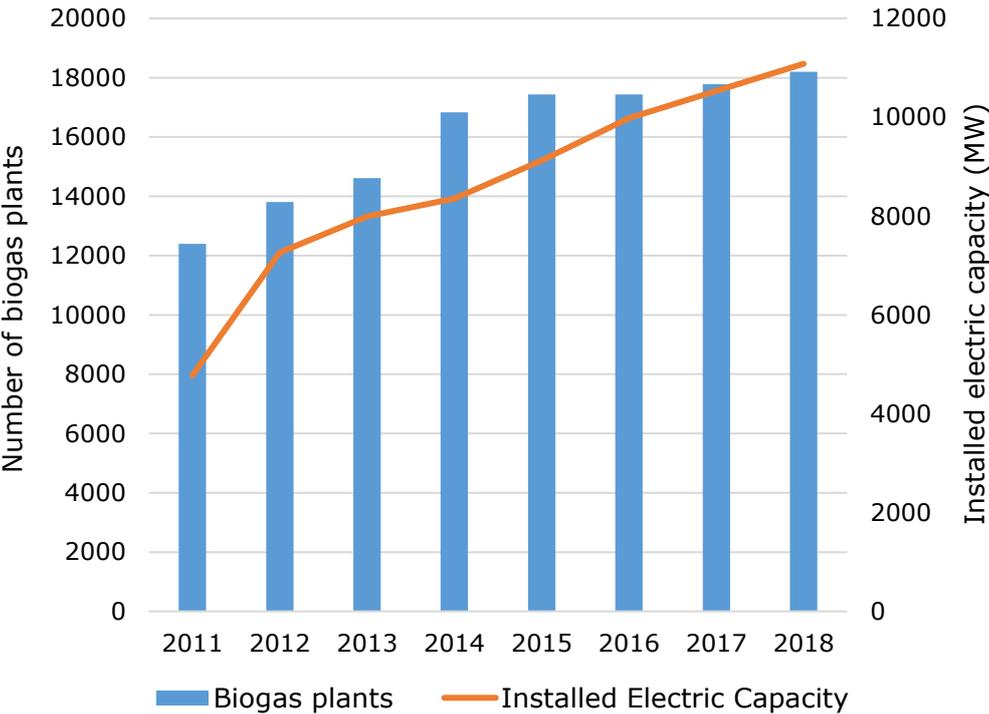
Figure 2.3 Evolution of the number of biogas plants and the installed electric capacity from biogas in Europe (European Biogas Association, 2020)

2.3 Bioplastics in anaerobic digesters

The inclusion of biodegradable plastics in the anaerobic industrial waste treatment presents different benefits (European Bioplastics, 2015). Firstly, biodegradable plastics increase the amount of waste in the digester, increasing the final biogas production. Additionally, bioplastics used in food waste-related uses such as waste bags or cutlery may be directly collected with other organic waste, considering that anaerobic digestion is widely used in the treatment of kitchen and catering waste due to the moisture and the reek. Also, if biodegradable plastics are used in laminated paper and cardboard items, they can be directly recovered in anaerobic digestion systems without the need for prior treatment. Finally, in order to prevent ammonia production and nutrient limitation, the carbon to nitrogen ratio (C/N) for AD is recommended to be between 20:1 and 30:1 (Hawkes, 1980). In general, biowaste C/N is low (6:1 to 16:1) so the addition of bioplastics like PLA with a high C/N ratio may be a solution to improve the digestion process (European Bioplastics, 2015).

However, the AD of biodegradable plastics on an industrial scale still has some challenges that must be solved in order to be applied in reality. Compostable bioplastics have different degradation times, and they will not degrade to the same extent, being necessary for different technologies. Additionally, although PLA can be anaerobically biodegraded and produce high yields of biogas, its digestion time is much longer than the operation time of anaerobic digestion facilities (21 days) (Bernat et al., 2021). PLA and other synthetic polyesters are far less reactive in anaerobic degradation compared to bacterial polyesters such as poly 3-hydroxybutyrate (PHB) or starch blends, which complicates the possibility of a general process (El-mashad et al., 2012; Shrestha et al., 2020). One of the main challenges for the future is the collection of waste bioplastics since new technologies should be developed to efficiently sort the biodegradable plastics from non-biodegradables (Abraham et al., 2021).

Some developments are still required to introduce biodegradable plastic wastes in commercial anaerobic digesters. Pre-treatment technologies should be developed and improved to accelerate the degradation process and obtain higher biogas yields. Additionally, it must be considered the possible impact of the bioplastics on the quality and safety of the digestate produced. Different valorisation routes have been studied to give a further use to the digestate from AD. However, the most common uses of digestate are related to agronomy (Nkoa, 2014). Nkoa (2014) studied the benefits and environmental risks of using anaerobic digestates for soil fertilization, concluding that only digestates that

are totally compatible can be applied to crop fields. The impact of bioplastics in the digestate from anaerobic digesters has not been properly studied yet.

Physical contaminants such as microplastics as well as toxic chemicals can remain in the digestate, affecting the AD performance and impacting the properties of the digestate (Cazaudehore et al., 2022). Considering that biodegradable plastics may become a significant fraction of biogas plant feedstock (Zhang et al., 2018), a deep study should be undergone to determine the possible risks and effects of bioplastics in the valorised digestate.

2.3.1 Experimental anaerobic digestion of biopolymers

AD has been recently studied as a possible end-of-life method for biodegradable plastic waste. The plastics biodegradability under mesophilic and thermophilic conditions, in batch or in continuous systems, and in different media with different parameters have been studied for the main bioplastic available in the market (Cazaudehore et al., 2022). Authors like Abraham et al. (2021) and Cazaudehore et al. (2022) observed that biodegradability and biomethane production widely vary among polymers. Biopolymers from the family of PHAs such as PHB and 3-hydroxybutyrate-cohydroxyvalerate (PHBV) present high biodegradation values under mesophilic conditions, close to a complete degradation in some cases. Starch blends also present good biodegradability under mesophilic conditions. However, extensively used bioplastics such as PLA, PCL, PBS, and PBAT generally present low degradation under mesophilic conditions, being non-existent in many cases. Under thermophilic conditions, the biodegradability and biomethane production of PLA and PCL greatly increase, while PBS and PBAT only slightly increase.

Lucas et al. (2008) described three different stages in the anaerobic degradation process in most biodegradable polymers: biodeterioration, biofragmentation, and assimilation. The biodeterioration process is due to the activity of microorganisms that can grow on the surface or/and inside the polymer, modifying the mechanical, physical, and chemical properties of the material. Microorganisms involved in this stage are truly diverse and mainly belong to bacteria, protozoa, algae, and fungi. Their development strongly depends on environmental conditions. Then, during the fragmentation stage, the polymer bonds are cleaved to obtain monomers and/or oligomers that can be further absorbed by molecules (Laycock et al., 2017).

Polymers are broken down into small fragments by the action of microbial enzymes that convert polymers into metabolized monomers. Different microorganisms can secrete enzymes on the surface of the polymer causing the breakdown of the polymer chain by different methods such as hydrolysis or oxidation (Laycock et al., 2017). Finally, those lower-molecular-weight monomers can easily cross the microbial membrane for the assimilation stage. In this final stage, atoms from the fragments of polymeric materials are integrated inside microbial cells so that microorganisms can grow and reproduce while consuming nutrient substrate. As a result of this stage, gases are emitted, including methane and CO_2 (Lucas et al., 2008). In the measurement of biomethane from biodegradable plastics AD, these phases are reflected in the called lag phase. The lag phase was defined as the period between the initiation of the biochemical methane potential assay and the time when biomethane was produced from the polymer in reality (Benn & Zitomer, 2018). The lag phase strongly varies depending on the digested polymer and the conditions and reflects the duration of the first two stages until the beginning of the methane production.

The anaerobic degradability of most biodegradable polymers is significantly dependent on several factors. Abiotic (i.e., pH, moisture, and temperature) and biotic (i.e., the presence of microorganisms or enzymes) factors have a major influence on the final degradation of the polymer. Many authors have focused their research on comparing the biogas yield in the AD of the same bioplastic products but in mesophilic and thermophilic conditions (Bernat et al., 2021; Vargas et al., 2009; Vasmara & Marchetti, 2016). The interaction of microorganisms and the polymer is also of significant importance for the final performance. Different microorganisms were found under mesophilic and thermophilic conditions for the different biodegradable plastics, including different types of bacteria, protozoa, algae, and fungi (Shah et al., 2008).

The surface conditions (surface area, hydrophilic or hydrophobic conditions), the first-order structures (molecular weight, chemical structure), and the high order structures (crystallinity, melting temperature, glass transition temperature) of the polymer also have a great influence on the AD process (Tokiwa et al., 2009). Crystallinity highly affects anaerobic digestion since amorphous parts become readily accessible to microorganisms, being easier and faster degraded than the crystalline ones. The molecular weight of the polymer is negatively correlated with the biodegradation rate. Šmejkalová et al. (2016) observed how under similar conditions, a reduction in molecular weight of the polymer was associated with an enhancement in the AD rate. Surface area was also found to have a relation to the biodegradation rate so that the more surface the polymer pieces have, the

faster the degradation is, since the microorganisms have more interaction area. For that reason, size reduction in most cases accelerated methane production, although the final biomethane production potential does not vary (Yagi et al., 2009).

Nowadays, most of the biodegradable plastics used in the market exhibit a low degree of biodegradability and low methane production, especially under mesophilic conditions. Additionally, the kinetics of the biodegradation process is slow compared to the conventional substrates used in industrial anaerobic digesters (e.g., manures, biowastes, sludges). This can be reflected in some experiments where a high biogas output was obtained, but the degradation times were much longer than the retention times used in commercial plants (Narancic et al., 2018).

2.3.2 Strategies to increase methane production from bioplastics

In order to increase the biomethane production yield as well as the biodegradability kinetics different strategies can be implemented: pretreatment technologies, acclimation of inoculum, and the incorporation of additives into the polymers.

Pretreatment technologies have been widely investigated to increase the biodegradability of different substrates used in anaerobic digestion such as sludges, manures, or municipal solid wastes (Brémond et al., 2018). Depending on their nature, pretreatment techniques can be classified into four main categories: mechanical, chemical, thermal, and biological (Carlsson et al., 2012). Mechanical pretreatments are widely applied in commercial plants, using methods such as grinding, shredding, or extrusion to obtain a more appropriate operation particle size. In the case of digestion of bioplastics, Yagi et al. (2009) found that a reduction in the particle size of the bioplastic was related to faster degradation kinetics, although the final value of biomethane obtained was the same. However, the same author found that slower biomethane production rates were obtained from small pieces of PLA film than from large pieces (Yagi et al., 2012). This result could be explained since the small pieces floated on the sludge, reducing the surface area in contact with the microorganisms.

Chemical pretreatments like alkaline treatments are not generally used on an industrial scale due to high costs and possible damage to the environment. Thermal pretreatment is more common and in the case of biodegradable plastics like PLA, it has been studied the effect of thermal and thermochemical pretreatments, obtaining favorable results. Vargas et al. (2009) found that a steam pretreatment (3 h, 120 ºC) could enhance the biomethane production both in mesophilic and thermophilic conditions, being more prominent the

increase in the mesophilic case since originally there was hardly any methane production. In the case of chemical pretreatments in biodegradable plastics, Benn & Zitomer (2018) studied the impact of alkaline pretreatment (addition of sodium hydroxide until pH = 10-12) in the methane potential of different bioplastics under mesophilic conditions. It was found that alkaline pretreatment is especially beneficial for PLA since methane potential increased from 1,33 L $CH_4 \cdot kg^{-1}$ to 114 L $CH_4 \cdot kg^{-1}$. It was also found that the lag time was reduced compared to the untreated PLA.

The addition of additives into the plastic composition is a quite promising method to enhance the biodegradability kinetics of biopolymers. The addition of fibers, $CaCO_3$, lactic acid monomer, or enzymes has shown a possibility not only to reduce the production cost but to enhance some properties such as thermal stability and biodegradability. The inoculum acclimation has not been specifically studied in the case of biodegradable plastics but it has been found that the inoculum origin and its acclimation have a great influence on the anaerobic biodegradability of organic wastes (Cazaudehore et al., 2022).

2.4 Polylactic acid

Poly(lactic acid) or polylactide (PLA) is a synthetic bio-based polymer derived from starchy and cellulosic feedstocks, considered biodegradable and compostable (Garlotta, 2001). PLA is produced from the monomer of lactic acid (LA) (Figure 2.4). It is a thermoplastic with mechanical properties similar to widely used petrochemical-based plastics such as polystyrene (PS) and polyethylene terephthalate (PET) (Auras et al., 2003).
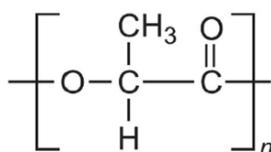

Figure 2.4 Monomeric subunit of PLA (Karamanlioglu et al., 2017)

Lactic acid is a naturally occurring organic acid that can be produced by chemical synthesis or fermentation. It was originally industrially produced by chemical synthesis from petrochemicals. However, the interest in the fermentation method has increased during the last decades due to the use of renewable resources, being a more environmentally friendly process (Madhavan Nampoothiri et al., 2010). The feedstock used as a carbon source for microbial production of lactic acid can be either sugar in pure form (e.g., glucose, sucrose, lactose) or sugar-containing materials (e.g., molasses, whey, sugar cane

bagasse). Different food products or residues have been proposed as an alternative to refined sugars in order to reduce lactic acid production costs (John et al., 2006). In Table 2.1 some of the possible feedstocks are presented beside their lactic acid yield.

PLA is nowadays one of the main bioplastics produced globally and it represents 18,9% of the total bioplastics produced annually (European Bioplastics, 2021). The main global producer of PLA is the American company NatureWorks™ which uses plants like corn, cassava, sugar cane, or beets to produce the called Ingeo™ polylactide. In Europe, Futerro S.A. (Belgium) and Corbion N.V. (Netherlands) are the main leaders in the PLA market (Polymer Database, 2022).

Table 2.1 Feedstock used for the production of lactic acid (John et al., 2008; Madhavan Nampoothiri et al., 2010)

Substrate	Lactic acid yield
Wheat and rice bran	126 g/l
Corn cob	90 g/l
Pre-treated wood	48-62 g/l
Cellulose	0,89 g/g
Barley	0,87-0,98 g/g
Cassava bagasse	0,9-0,90 g/g
Wheat starch	0,77-1 g/g
Whole wheat	0,93-0,95 g/g
Potato starch	0,87-0,97 g/g
Corn, rice, wheat starches	<0,70 g/g
Corn starch	0,935 g/g

PLA is derived from renewable resources, and it is biodegradable and compostable, which make PLA an attractive biopolymer. Its biodegradability provides several end-of-life options, including industrial composting, anaerobic digestion, and chemical recycling, apart from traditional plastic end-of-life methods such as mechanical recycling or incineration (Momeni et al., 2021). Additionally, it is biocompatible, so it has important biomedical uses. PLA can be easily processed by standard methods such as injection molding, sheet extrusion, film extrusion, or blow molding (Auras et al., 2003). Its good mechanical properties and the different processing possibilities allow PLA to be used for different applications in packaging, as well as other applications such as electronics, synthetic fibers, tapes, wrapping films, and 3-D printers (Singhvi et al., 2019).

Despite that, PLA has several drawbacks which limit its use, and which are the reason it cannot be a direct substitute for synthetic polymers nowadays. Although some mechanical properties such as tensile strength and elastic modulus are comparable to other polymers,

PLA is a very brittle material so it cannot be used in applications that need deformation at higher stress levels (Hiljanen-Vainio et al., 1996). In addition, PLA does not solve the problem of plastic waste contamination. It was found that the degradability of PLA in soil showed small or no sign of degradation in the polymer after times even longer than two years (Calmon et al., 1999; Kamiya et al., 2007; Shogren et al., 2003). Similar results were obtained when PLA was degraded in seawater and freshwater (Bagheri et al., 2017).

During the last decades, it has been compared the environmental performance of PLA-based products compared with petroleum-based plastics such as PP and PET (Madival et al., 2009; Rezvani Ghomi et al., 2021). The life cycle assessment of similar products made with the different polymers shows that PLA significantly reduces fossil resource depletion (Moretti et al., 2021). However, PLA has higher impacts compared to the other polymers in terms of photochemical ozone formation, acidification, water depletion, and terrestrial eutrophication because of the use of plantations to obtain the raw materials (Moretti et al., 2021). Nowadays, the contribution of PLA to climate change is comparable to the other plastics so the global environmental performance does not really improve that of other plastics (Rezvani Ghomi et al., 2021).

However, there is a significant potential to considerably reduce the environmental impacts of PLA production. In the future, it may be possible to increase the feedstock production yield by improving agricultural practices, as well as to reduce emissions by optimizing the application of fertilizers (Morão & de Bie, 2019). The use of agro-industrial residues would reduce the impact as well as the production costs (de Albuquerque et al., 2021). Authors such as Moretti et al. (2021) and Shen (2011) have defended the fact of the need to improve the waste management of PLA, following a circular product life and avoiding being landfilled. This circular product life lies in the empowerment of processes such as recycling, composting, and anaerobic digestion. An improvement in the treatment of PLA waste could considerably reduce its environmental impact

2.4.1 Synthesis and production of PLA

Lactic acid (chemically known as 2-hydroxy-propionic acid) is the basic building block for the production of PLA. Before its use in the manufacture of PLA, LA was industrially used as a solvent for metal cleaning, as a humectant, a detergent, and for tanning leather. Additionally, LA is present in many products daily consumed such as yogurt and cheese as well as it is naturally present in the body of mammals (Sin & Tueen, 2019b). The molecular structure of pure LA has two optically active isomeric forms (or stereoisomers) called L(+)-

form and D(-)-form, represented at the top of Figure 2.5. Different lactate dehydrogenase enzymes present in living organisms synthesize these two isomeric forms.

Industrially, there are two main synthesis routes to produce PLA from LA, the direct polycondensation (DP) route and the ring-opening polymerization (ROP) route (Figure 2.6). DP is a simpler method in which PLA is produced directly from LA without intermediary products. However, the PLA obtained has a low molecular weight, being unusable for industrial purposes because of its fragile and glassy properties. For that reason, coupling agents are needed to increase the molecular weight of the polymer (Sin & Tueen, 2019b). Some authors (Cadar et al., 2011; Kim & Woo, 2002) studied the possible effects of using catalysts to increase the molecular weight of the PLA obtained.
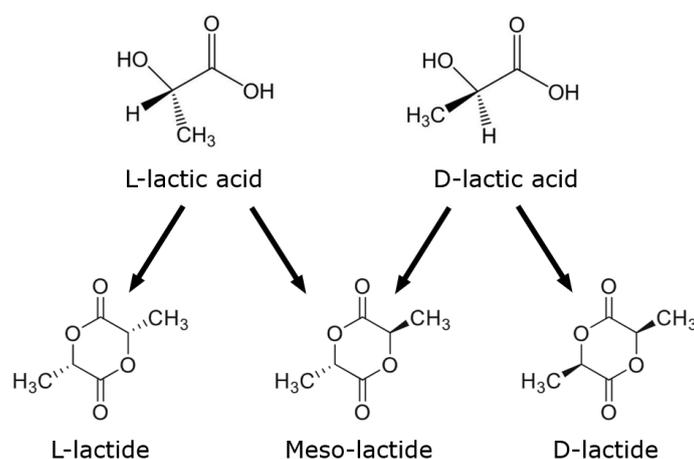

Figure 2.5 Steoreoforms of lactides (Madhavan Nampoothiri et al., 2010; Sharmin & Rudd, 2017)
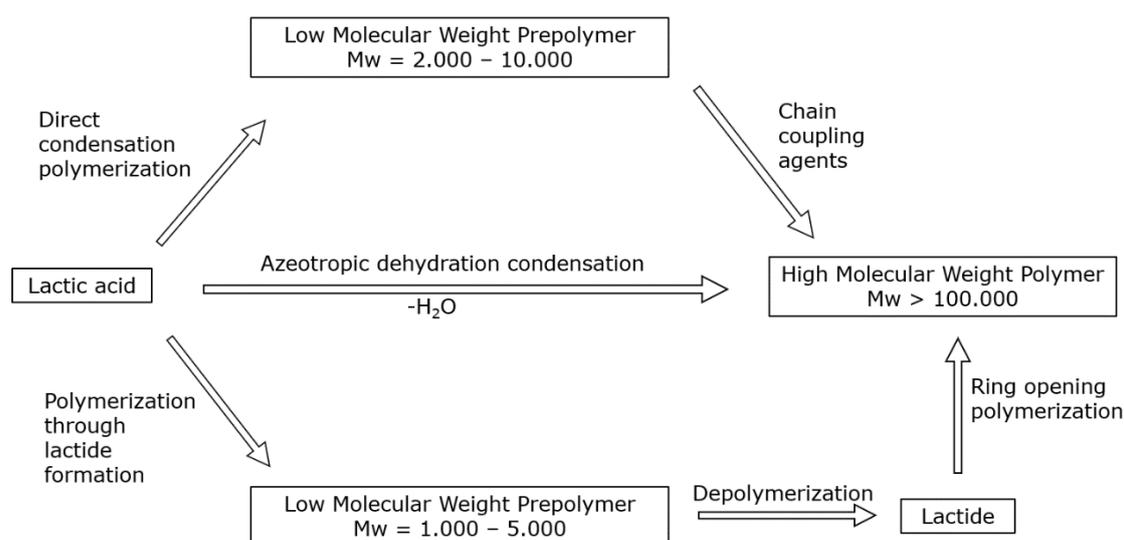

Figure 2.6 Main reaction pathways for producing poly(lactic acid) from lactic acid (Hartmann, 1998; Sin & Tueen, 2019b)

Despite requiring extra steps compared to the DP, the ROP route has been generally applied in the industrial production of PLA. ROP route has gained attention from industries because of its better reaction control and the possibility of obtaining high-molecular-weight PLA that can be industrially used. In this method, a low-molecular-weight prepolymer is also produced to later obtain lactide as an intermediate substance. Three different stereoisomers of lactide are produced from lactic acid: L-lactide, D-lactide, and meso-lactide (dimerized from D- and L-lactic acids) which are shown at the bottom of Figure 2.5 (Sharmin & Rudd, 2017). Finally, a high-molecular-weight PLA is obtained from the polymerization of lactide without the need for coupling agents.

The final properties of PLA depend on its molecular weight and processing temperature but mainly on the content of both LA stereoisomers within PLA chains (Raquez et al., 2013). At atmospheric temperature, the high-molecular-weight PLA may be amorphous or semicrystalline. PLA crystallinity depends on the concentration of L-, D-, and meso-lactide in the structure. The crystallinity of PLA is defined by the concentration of L-lactide so that the structure can be amorphous (50%-93%) or semicrystalline (>93%) (Nesic et al., 2020). Semicrystalline form possesses attractive mechanical properties similar to PP or PET polymers.

Different processing methods can be used to produce final products based on PLA. Extrusion, injection molding, blow molding, thermoforming, and melt spinning are among the most common, using technologies similar to traditional synthetic plastics. Apart from the conventional polymer processing methods, PLA has promising properties to be used in other methods such as 3D printing or electron beam irradiation (Sin & Tueen, 2019c; Sp & Rudolph, 2017).

2.4.2 Modifications

PLA possesses some characteristics that limit its use in several market applications. To overcome those conditions, researchers have studied different methods to modify the PLA and improve its performance according to the necessity (Figure 2.7). However, in many cases, the modifications of PLA to improve one or several characteristics of the polymer also worsen its behaviour in other aspects, for example decreasing the biodegradability. Critical factors that influence the biodegradability of polymers include chemical components, compositions, and morphological structure (Wang et al., 2005).

Two main types of modifications are generally employed for PLA, bulk and surface modifications (Puthumana et al., 2020). Bulk modifications are carried out to improve the toughness, the degradation behavior, the crystallinity, and the processability of the polymer (Rasal et al., 2010). Surface modifications are often needed to control the roughness, hydrophilicity, and topography of the PLA. Surface modification methods can be divided into permanent (covalent attachment, e.g., photografting, chemical conjugation using wet chemistry) and non-permanent (non-covalent attachment, e.g., coating, entrapment) (Rasal et al., 2010).
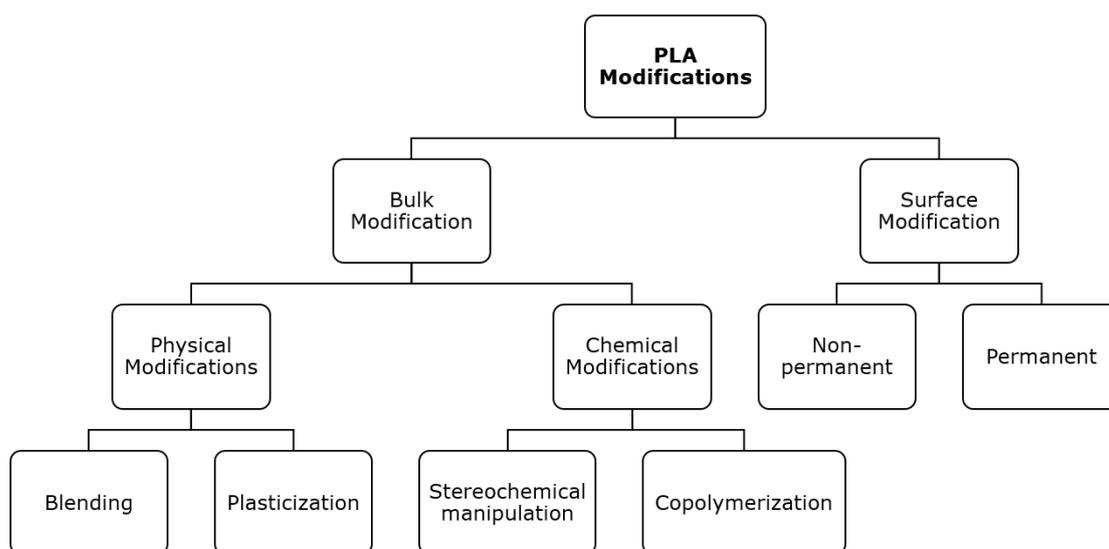

Figure 2.7 Modification methods for PLA (Puthumana et al., 2020)

Bulk modification can be further classified into physical and chemical modifications. Among the physical modifications, blending is widely used to improve the mechanical properties of the polymer. Different biodegradable and non-biodegradable plasticizers and polymers have been used to increase ductility, improve the processability, and lower the glass transition temperature of PLA (Mascia, 1992). Biodegradable blends such as amorphous polyhydroxyalkanoates (PHA) or modified starch (MS) have been extensively studied since they can improve mechanical properties without compromising the biodegradability of the final product, or in some cases improving the biodegradability rate (Burzic et al., 2019; Gao et al., 2011).

Chemical modifications are also typically used in PLA production. The stereochemical composition depends on the ratio of L-lactide, D-lactide, and meso-lactide, and has great effects on the melting point, crystallization rate, mechanical properties, and biodegradability. A 1:1 mixture of pure poly(L-lactide) and poly(D-lactide) exhibited a higher melting temperature and better mechanical properties than either pure polymer

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(Garlotta, 2001). Amorphous PLA was found to have a higher degradation grade than crystalline PLA (Kolstad et al., 2012). Copolymerization is also a well-studied chemical modification in which copolymers can be formed by the combination of PLA and other polymers to obtain superior properties. PLA can be copolymerized with a wide range of monomers or polyesters by different methods. Through polycondensation of lactic acid with other monomers, low molecular weight copolymers can be produced, and through ring-opening copolymerization of lactide with cyclic or linear monomers to obtain high molecular weight copolymers (Rasal et al., 2010).

2.4.3 PLA market and applications

The production of an aliphatic polyester from lactic acid was first raised in 1932 by William Carothers. The objective was to produce a biodegradable implantable polymer that could be used as scaffolds in tissue engineering, medical devices, and drug carriers (Bu et al., 2019). Nonetheless, the product obtained from lactic acid had low molecular weight and inadequate mechanical properties. The company DuPont managed to patent a higher-molecular-weight product in 1954. In 1972 it was the first time high-strength, biocompatible fibers based on lactic acid were used for medical resorbable sutures. During those years, PLA use was restricted to medical applications (Williams, 1982). Additionally, the high production cost of the petrochemical-derived precursor of lactic acid also restricted its development and use in other fields. Nevertheless, the production of lactic acid by bacterial fermentation of starch considerably reduced PLA production costs, enabling commercial-scale production for non-medical applications such as packaging (Lunt, 1998).

During the last decade, PLA production has increased worldwide (Figure 2.8) with a market estimation of 456.813 tons in 2026. This value is expected to increase to 789.672 tons in 2026, according to European Bioplastics (2021). This growth can be mainly explained by the favorable government policies regarding the use of bioplastics as well as the increase in the use of bioplastics in the packaging industry. The demand for PLA in food packaging has greatly increased in recent years, especially in North America and Europe (Mordor Intelligence, 2021). However, the accelerated production that is predicted for the next few years is mainly due to the increasing demand for packaged foods in the Asian Pacific market, especially in China and India (Grand View Research, 2021).
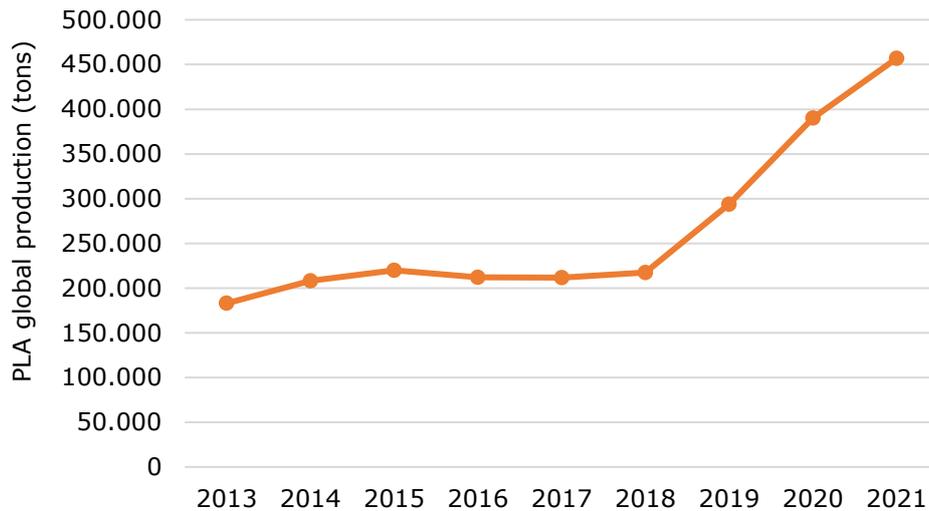
Figure 2.8 Evolution of the PLA world production capacity (tons) (European Bioplastics, 2021)

Nowadays packaging is the main application of PLA with a wide difference (Figure 2.9). Some of the most common packaging products are water or juice bottles, shampoo bottles, packing bags, clear film overwrap for trays, trays for meat and fish, or shopping bags. Additionally, PLA is widely used in the textile industry as fiber in diapers, cloths, comforters, or pillows. PLA is also used in disposable cutlery, casing for cosmetics, toys, and electronics covers (Sin & Tueen, 2019a). Despite the fact PLA production in the medical industry is not as representative as in other industries, PLA has multiple applications as a biomaterial such as tissue engineering, cardiovascular implants, medicine carrier, and dental niches (DeStefano et al., 2020). PLA is also used in agriculture as mulch film to modify the temperature of the soil, limit the growth of the plants, and prevent the loss of moisture (França et al., 2019).
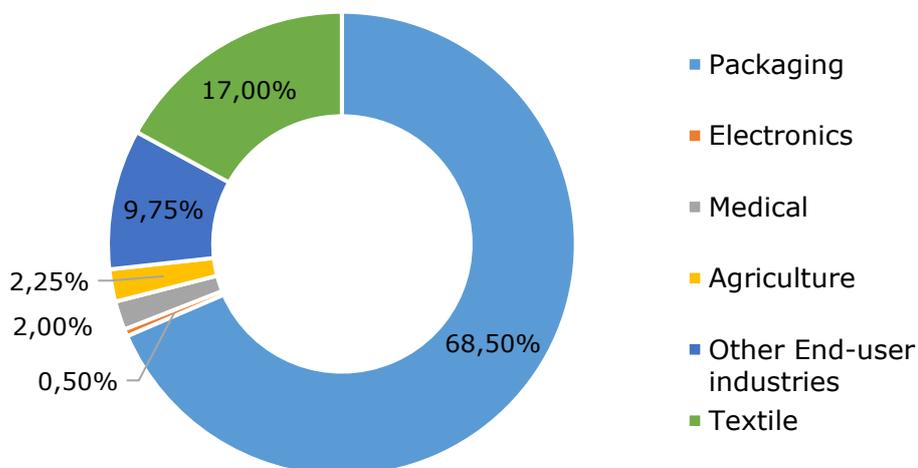

Figure 2.9 Global 2020 PLA market, revenue (%), by end-user industry (Mordor Intelligence, 2021)

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2.4.4 End-of-life of PLA

The end-of-life of the industrially produced PLA has been one of the main concerns to really improve the environmental performance of bioplastic compared to traditional plastics. According to the European Commission (2008) in the European Waste Directive 2008/98/EC, there are five levels of waste hierarchy: 1) prevention, 2) reuse, 3) recycling, 4) other recovery, and 5) disposal. Lightweighting of PLA has been made in order to reduce waste as well as to decrease production costs of PLA. Nonetheless, this also reduces the mechanical functionality of the products, so extensive research has been carried out to produce lightweight PLA materials with good mechanical properties (Castro-Aguirre et al., 2016). In the case of packaging PLA, reuse on a small scale can be done since PLA products and packaging generally maintain their functionality and safety.

PLA, especially when used in packaging, can be recycled since it presents low contamination. Mechanical recycling would be the cheapest way to recycle PLA waste. Nevertheless, nowadays economic costs involved in recycling plastics other than HDPE and PET can be not favorable, especially because of the lack of infrastructure in the collection and sorting of PLA. Chemical recycling is feasible since PLA is hydrolyzed to LA at elevated temperature to further be polymerized to high-molecular-weight PLA. Unfortunately, the costs of this process may be much higher than the mechanical recycling (Chariyachotilert et al., 2012).

Biodegradation is probably the most attractive waste treatment method owing to the biodegradability of the polymer. Composting may be a solution to degrade the polymer into CO_2, water, and biomass that can be used as compost in the further growth of new agricultural crops. At present, there are only a few composting facilities that deal with biodegradable plastics. The main concern is the difficulty of distinguishing conventional plastics and bioplastics with the current quality control. As in the case of recycling, collecting and sorting PLA waste from other MSW is the biggest challenge in avoiding PLA to finish landfilled like other plastic materials (Rossi et al., 2015).

Despite presenting similar problems when collecting and separating the PLA, anaerobic digestion seems to be a more promising option than composting due to the possibility of obtaining energy in the form of biogas (Bátori et al., 2018). The anaerobic digestion of PLA has not been studied as thoroughly as aerobic degradation (composting). However, the results obtained seem promising, obtaining high biogas yields, especially when working at elevated temperatures.

Even if it is lower in the waste treatment hierarchy, incineration can be attractive since it reduces the volume of waste at the same time some energy is recovered from waste to produce heat and/or electricity, reducing the dependency on using fossil fuels. PLA and other polymers are suitable for thermal energy recovery since their calorific value is comparable to other waste such as wood or paper (Figure 2.10) (Laußmann et al., 2010). One of the main problems of PLA waste incineration, as in the incineration of general waste, is the air polluting emissions. PLA oxidation products are mainly CO_2 and PHAs to a lesser extent. In comparison with other plastics, the amounts of PHAs emitted from PLA combustion are considerably lower than in the combustion of other plastics. Additionally, no residue remains after combustion, suggesting that PLA is suitable for general waste incineration (Chien et al., 2010).

Landfilling is the last preference according to the hierarchy of waste treatment. However, it is still extensively used in such a way that thousands of million tons of waste are sent to landfills every year. PLA biodegradation closely depends on the temperature and moisture. For that reason, very little or no biomethane is produced in landfill conditions, being very stable for a long time (Quecholac-Piña et al., 2020). A better degradation may be obtained if chemical hydrolysis is carried out prior to degradation (Kolstad et al., 2012).
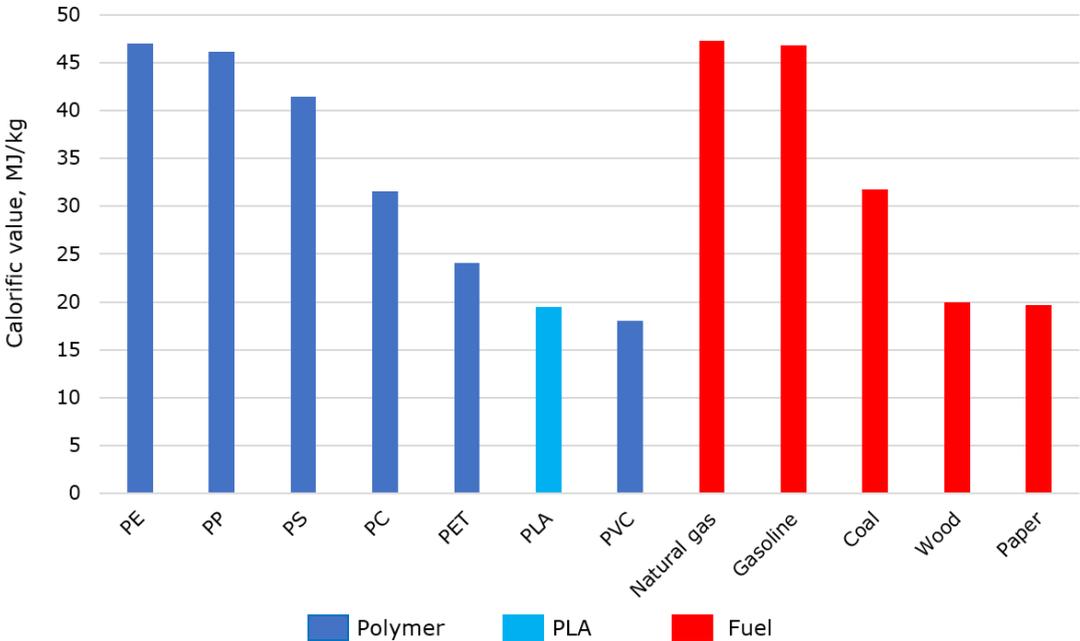

Figure 2.10 Calorific values of different polymers and fuels (Costiuc et al., 2011)

2.5 Anaerobic digestion of PLA

As it was explained in Section 2.3, anaerobic digestion is a promising method to treat biodegradable plastic waste. In the case of PLA, AD has great interest since energy can be produced from biomethane obtained as well as compost can be used in agriculture to grow more agricultural products. These agricultural products can be further used to produce the polymer itself, following the circular economy concept (Figure 2.11).

During the anaerobic digestion, the interaction of microorganisms and the polymer has significant importance for the final performance. The temperature of the process has a great influence on the degradation of the polymer. For that reason, different results are obtained under mesophilic and thermophilic conditions for the same polymers (Bernat et al., 2021). Different microorganisms were found in PLA anaerobic degradation under mesophilic and thermophilic conditions. In addition, other properties such as crystallinity highly affect the degradation of PLA-based polymers since, in PLA crystalline parts with a high molecular weight, it is not possible to find complete degradation due to its resistance to bacterial growth (Hobbs et al., 2019).

Figure 2.11 Lifecycle of a PLA-based product with anaerobic digestion as an end-of-life method (Xiao et al., 2012)

2.5.1 Mesophilic conditions

The degradation and methane production from PLA under mesophilic conditions has been widely studied, much more than in the case of other degradable polymers (Cazaudehore et al., 2022). A summary of the results found in the literature on PLA degradation in mesophilic anaerobic digestion can be found in Table 4.2. Itävaara et al. (2002) studied the biodegradability of pure PLA under mesophilic conditions, obtaining a biodegradability lower than 5% after 40 days of the experiment. Other authors have studied the anaerobic degradation of PLA-based polymers, founding low degradation in test times of 20 to 40 days, obtaining biodegradation levels below 20% (Greene, 2018; Vargas et al., 2009).

At longer degradation times, Yagi et al. (2014) found degradation of 29-49% after 277 days and Bernat et al. (2021) 66% after 280 days. Additionally, Bernat et al. (2021) found that the lag phase before the polymer started to produce methane was up to 40 days. After that, the methane production rate stayed constant during the first 40-60 days of methane production and later it increased the rate until the end of the experiment. After the 280 days of the test, the methane production did not plateau, showing that the maximum volume of methane production was not reached.

Massardier-Nageotte et al. (2006) and Benn & Zitomer (2018) did not find degradation of the polymer for 28 and 40 days respectively, showing that probably the lag phase for longer than the experiment time. Other authors such as Vasmara & Marchetti (2016) and Narancic et al. (2018) showed that rigid pieces of PLA-derived polymers did not produce methane in 90 and 56 days respectively.

Microorganisms such as *Xanthomonadaceae bacterium* and *Mesorhizobium* sp*.* were found in the digestion of PLA in mesophilic conditions (Yagi et al., 2013, 2014). The slow biodegradation rate under mesophilic conditions may be explained by the lack of participation of the microorganism in the AD of PLA since they do not have the ability to biodegrade high molecular weight polymers (Yagi et al., 2014).

2.5.2 Thermophilic conditions

The degradation of PLA under thermophilic conditions has not been as much studied as in the mesophilic case. Nevertheless, different authors have investigated this, obtaining more favourable results than under mesophilic conditions (see Table 4.3). Under thermophilic conditions, microorganisms such as *Bacillus Infernus, Propionibacterium*, and *Ureibaccillus* were identified, being much more active than in the mesophilic case (Yagi et al., 2014).

Additionally, higher temperatures increase the mobility of the polymeric chains so that the accessibility to biological and chemical degradation is facilitated for microorganisms (Lucas et al., 2008).

Higher levels of biodegradation were found under thermophilic conditions compared to mesophilic conditions. Itävaara et al. (2002) found degradation of pure PLA of 60% after 40 days, much higher than the less than 5% obtained under mesophilic conditions in the same period of time. High biodegradability levels between 82% and 90% were reported by Hegde et al. (2018), Yagi et al. (2013), and Narancic et al. (2018). However, other authors showed maximum degradation of PLA of 40-56% in similar experiment times (Vargas et al., 2009, Vasmara & Marchetti, 2016).

Bernat et al. (2021) found that the degradation lag phase under thermophilic conditions was much shorter than under mesophilic conditions (8-10 days against 40 days). Additionally, the production rate under thermophilic conditions was higher and, in this case, the methane production reached a plateau after 40 days. Unlike under mesophilic conditions, in this case a maximum value of methane production was reached, being close to the maximum theoretical value.

3   MATERIALS AND METHODS

3.1 Materials

3.1.1 Biodegradable polymers

Three different biopolymers have been tested in this study: PLA-Fil, PLA-Cut, and PLA-Pa. Some of the main characteristics of the polymers are summarized in Table 3.1. PLA-Fil is a thermoplastic resin based on PLA used as mulch film in agriculture. It is produced from renewable vegetal resources and shows improved softness and elongation at break compared to standard PLA. This polymer is transparent and can be processed on conventional extrusion equipment to produce the films. PLA-Cut is denser, and it has considerably higher flexural and tensile modulus than the previous case. These characteristics make it interesting to be processed by injection molding and to produce cutlery and other similar products. PLA-Pa has similar mechanical characteristics to PLA-Fil, but this polymer is used for rigid packaging.

Table 3.1 General characteristics of the bioplastics used in the AD process

	PLA-Fil	**PLA-Cut**	**PLA-Pa**
Size and shape	Pellet 3x2	Pellet 3x3	Pellet 2,5x2,5
Processing method	Film extrusion	Injection molding	Injection molding
Density	$1,21$ g·cm^{-3}	$1,40$ g·cm^{-3}	$1,24$ g·cm^{-3}
Flexural Modulus	1837 MPa	5100 MPa	1800 MPa
Tensile Modulus	1850 MPa	5700 MPa	1850 MPa
Melt temperature	150-160 ºC	180-200 ºC	180-200 ºC
Use	Mulch film	Cutlery	Rigid packaging

3.1.2 Positive control sample

In most of the biochemical methane potential (BMP) tests it is included a positive (or reference) control with a known BMP. It allows to control the correct functioning of the experimental setup, execution, and the performance of the inoculum (Koch et al., 2020). Positive control substances must satisfy a number of characteristics such as affordability, easy availability, consistent composition, reasonably fast degradation, and high reproducibility (Koch et al., 2020).

Compounds such as starch or cellulose are widely used as positive control substances. In this BMP test powder cellulose has been used as a positive control substance. The methane production of cellulose is 350 ± 29 mL CH$_4$·g^{-1} VS (Raposo et al., 2011).

3.2 Instruments

The equipment used in this test has the objective of measuring the biochemical methane potential of the different substrates. The Automatic Methane Potential Test System (AMPTS$^{®}$) II from Bioprocess Control Instruments AB was used (Bioprocess Control, 2016). The instrument setup can be divided into three units (Figure 3.1), the Sample Incubation Unit (A), the CO_2-absorbing Unit (B), and the Gas Volume Measuring Device (C). The Sample Incubation Unit has 15 vials containing small amounts of biodegradable plastics samples (substrate) with anaerobic inoculum (sludge), incubated at the desired temperature controlled by a thermostatic water bath.

The biogas produced in each incubation vial passes through an individual vial containing an alkaline solution in the CO_2-absorbing Unit. The alkaline solution is a 3 M sodium hydroxide (NaOH) solution so that several acid gas fractions such as CO_2 and H_2S are retained by chemical interaction with NaOH, and only CH_4 is allowed to pass. It is needed to add in the solution a pH indicator into each vial for controlling the acid-binding capacity of the solution. In this case, phenolphthalein has been used as pH indicator. The pH indicator will turn from fuchsia to colorless when the CO_2 binding capacity of the NaOH solution decreases below optimal.

In the Gas Volume Measuring Device, the methane released from the CO_2-absorbing Unit is measured using a wet gas flow measuring device. This device works based on the principle of liquid displacement and buoyancy so that it can measure ultra-low gas flows with a multi-flow cell arrangement composed of 15 cells, one per incubation sample. Every time a volume of gas flows through the device, a digital pulse is generated so that results can be recorded, displayed, and analyzed by an integrated data acquisition system.

The test was carried out with tests in triplicates for statistical significance: three reactors were used as blanks (containing only inoculum), three reactors were used as reference control reactors (filled with inoculum and cellulose as substrate), and for each polymer three reactors were filled with inoculum and the polymer as substrate.
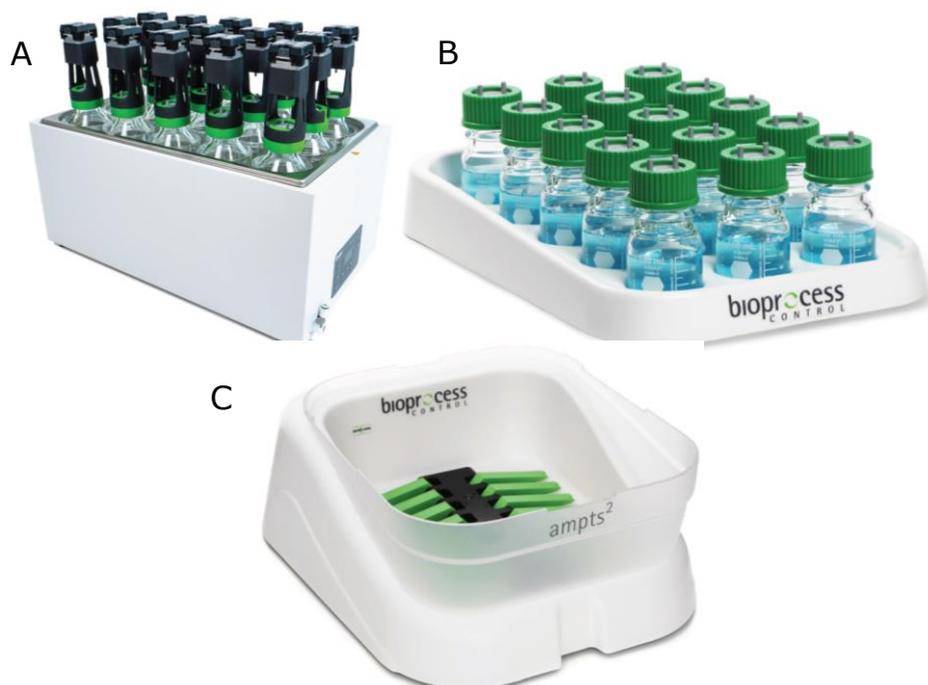
Figure 3.1 Main units of the equipment; A: Sample Incubation Unit, B: CO2-absorbing Unit (in the picture thymolphthalein was used as pH indicator while in the real unit the color of the indicator is fuchsia due to the phenolphthalein), C: Gas Volume Measuring Device (Bioprocess Control, 2016)

3.3 Preparation of the inoculum

Sludge obtained from Keila wastewater treatment plant was used as inoculum in the test. In order to be used in anaerobic digestion, the sludge was previously conditioned according to the indications from the AMPTS II manual (Bioprocess Control, 2016). The sludge needed to be dried for 2-3 days to remove the excess water content and all the visible wood pieces were removed manually. Before the incubation, the pH had to be checked, and the total solids and volatile solids were measured to ensure that it was appropriate for the process.

3.3.1 Total solids and volatile solids calculation

Before starting the BMP test, the sludge was characterized with regard to total solids (TS) and volatile solids (VS) according to the AMPTS II manual (Bioprocess Control, 2016). Same measurements and processes were done in the case of the substrates (cellulose and polymers). To calculate those values, it was necessary first to weigh a sample. After this, the sample was heated up in an oven to 105 ºC for 8 hours in order to remove all water content and obtain a totally dry sample. After being weighed, the dried sample was further heated up in the oven to 550 ºC for 1 hour 45 minutes in order to burn the organic matter.

Finally, the remains of the sample were weighed to know the non-volatile part of the biomass.

TS can be calculated as the ratio between the amount of dried sample (m_{Dried}) and the initial amount of wet sample (m_{Wet}), as indicated in Formula (3.1). VS is calculated as the ratio between the difference in the amount of sample after drying (m_{Dried}) and after burning (m_{Burned}) and the initial amount of the sample (m_{Wet}), as indicated in Formula (3.2). The same measurements and calculations must be done for the substrates, in this case, the three polymers studied, and the cellulose used as reference control. The results of TS and VS obtained from the sludge samples, as well as from the polymers and the cellulose used are represented in Table 3.2.

$$TS\ (\%) = \frac{m_{Dried}}{m_{Wet}} \qquad (3.1)$$

$$VS\ (\%) = \frac{m_{Dried} - m_{Burned}}{m_{Wet}} \qquad (3.2)$$

where m_{Dried} – is the mass of the sample after 105 ºC for 8 hours, g,

m_{Wet} – is the mass of the initial wet sample, g,

m_{Burned} – is the mass of the sample after 550 ºC for 1 hour 45 minutes, g.

Table 3.2. Characteristics of the sludge and the biopolymers

	TS (%)	VS (%)
Sludge	33,86	28,93
Cellulose	90,78	90,52
PLA-Fil	99,74	97,83
PLA-Cut	99,77	80,20
PLA-Pa	99,81	71,13

3.3.2 pH measurement

The pH of the inoculum must be checked to ensure that it is not acid since it may inhibit the biodegradation process. The optimum pH range in an anaerobic digester is 6,8 to 7,2 although it can tolerate a wider range, from 6,0 to 8,0 (Mao et al., 2017).

The measurement has been done according to the standard ISO 14855-1 (International Organization for Standardization [ISO], 2012). A pH meter was used to measure the sludge used as inoculum in the reactors. One part of the inoculum was mixed with 5 parts of distilled water and fully mixed. Then, the electrode of the meter was submerged in the

solution to immediately measure the acidity. The pH result was 6,08, which is inside the range in which anaerobic digestion occurs.

3.4 Preparation of the system

Knowing the TS and VS values of the inoculum and substrates the reactors were prepared following the AMPTS II manual recommendations (Bioprocess Control, 2016). An inoculum to substrate ratio of 2:1 (based on VS) was chosen for all the substrates. 500 mL bottles were used as reactors filled with 300 g of inoculum and substrate mix. In each case, the mass of inoculum (m_{IS}) and substrate (m_{sS}) are calculated according to Formulas (3.3) and (3.4), knowing the respective VS values (VS_I and VS_s). The results are represented in Table 3.3.

$$\frac{m_{IS} \times VS_I}{m_{sS} \times VS_s} = 2 \tag{3.3}$$

$$m_{sS} + m_{IS} = 300 \tag{3.4}$$

where m_{IS} – is the mass of the inoculum, g,

m_{sS} – is the mass of the substrate, g,

VS_I – volatile solids of the inoculum, %,

VS_s – volatile solids of the substrate, %.

Table 3.3 Mass and VS of inoculum and substrate in the different reactors

	m_{IS} (g)	m_{sS} (g)	VS_I (g)	VS_s (g)
Blank	300	0	86,80	0
Cellulose	258,66	41,34	74,84	37,42
PLA-Fil	261,35	38,65	75,62	37,81
PLA-Cut	254,16	45,84	73,54	36,77
PLA-Pa	249,30	50,70	72,13	36,07

3.4.1 Indicator preparation

Phenolphthalein has been used in this case as pH indicator. The color of the Phenolphthalein solution is fuchsia when pH is between 8,3 and 10,5, while out of those values is colorless (Lee et al., 2012). Phenolphthalein is an organic compound so that it has very low solubility in water. However, it dissolves well in Ethanol (National Center for Biotechnology Information, 2022).

In this case, a 0,5% Phenolphthalein indicator solution has been prepared. Firstly, 0,5 g of Phenolphthalein powder is weighed and added to 50 mL 100-95% Ethanol. The solution must be stirred well until the powder is properly dissolved in Ethanol. 100 mL of distilled water is finally added to the solution (Zumdahl & DeCoste, 2012).

3.4.2 NaOH solution preparation

For the CO_2-absorption unit a 3 M NaOH solution is prepared by mixing NaOH with distilled water according (Bioprocess Control, 2016). For its preparation, 240 g of pure NaOH is mixed with distilled water up to 2 L. After weighting the necessary amount of NaOH, it is mixed with approximately three-quarters of the required total volume of distilled water. The dissolution of NaOH highly generates heat so it is recommended to add a small amount of supplementary water followed by mixing. Finally, when NaOH is completely dissolved, the remaining water is added and mixed well. To handle NaOH it is necessary to work in an environment with good ventilation due to the hazardous gases it releases in the dissolution.

5 mL of previously prepared pH-indicator solution is added per liter of 3 M NaOH solution. 100 mL bottles are filled with about 80 mL NaOH solution. As these bottles should be substituted once the CO_2 binding capacity of the NaOH solution decreases below optimal, extra NaOH solution was made. Phenolphthalein turns colorless when the pH of the solution goes below 8,3 and the bottle must be replaced (Bioprocess Control, 2016).

3.4.3 Final installation

Once all the reactors and CO_2-absorption bottles are filled they can be installed in the AMPTS II units (Bioprocess Control, 2016). Rector bottles should be placed in their respective places in the Sample Incubation Unit. Bottles filled with NaOH solution containing the pH indicator are also placed in the CO2-absorption unit. Each reactor bottle cap has an opening that is directly connected to its respective NaOH solution bottle using flexible plastic tubes. NaOH solution bottles have two openings, one connected to the reactors and the other connected to the respective entrance of the Gas Volume Measuring Device using also flexible plastic tubes.

The thermostatic water bath of the Sample Incubation Unit is filled with distilled water as well as the water bath of the Gas Volume Measuring Device until the indicated levels. Both the Sample Incubation Unit and the Gas Volume Measuring Device are connected to a

power source. The Gas Volume Measuring Device must also be connected to internet. The thermostatic water bath temperature is programmed to remain constant at 38 ºC during the whole test (Bioprocess Control, 2016). The final installation can be seen in Figure 3.2.
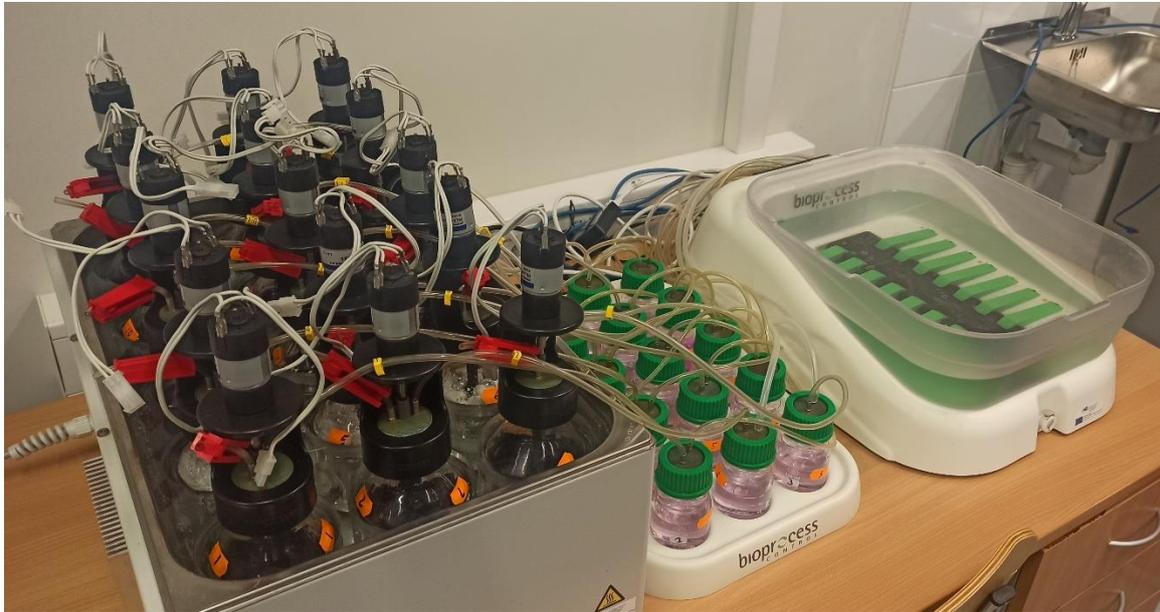

Figure 3.2 Final appearance of the system

3.4.4 Software and data processing

The AMPTS II software it makes possible to collect methane production data throughout the test duration (Bioprocess Control, 2016). The cells in the Gas Volume Measuring Device detect the volume of methane produced in the reactors and it is stored so that it is possible to represent the evolution of the methane production. The software can calculate the normalized accumulated gas volume (NmL) and the normalized flow rate (NmL/day) in each reactor. However, it is not possible to directly measure BMP from the substrate so that must be further calculated.

The BMP can be defined as the volume of methane produced per amount of organic (or volatile) substrate material added to the reactor (Filer et al., 2019). Owing to the fact that in the experimental sample bottles there are also organic residues present in the inoculum, the biogas produced from those residues has to be subtracted from the total accumulated methane volume of the reactor with a substrate. For that reason, the methane production in the blank reactors (only containing inoculum) has to be measured. In order to compare the results of the different substrates, it is necessary to obtain the results per amount of organic substrate material. For that reason, the volume of methane produced only from the substrate should be divided by the amount of organic (or volatile in this case) substrate

material added to the reactor. The volume of methane (normalized) from the substrates is calculated according to Formula (3.5).

$$BMP = \frac{V_S - V_B \frac{m_{IS}}{m_{IB}}}{m_{VS,sS}}$$ (3.5)

where BMP - is the normalised volume of methane produced per gram VS of substrate added, NL·g^{-1} VS,

V_S – is the accumulated volume of methane produced from the reactor with a sample, L,

V_B – is the mean value of the accumulated volume of methane produced by the three blanks (inoculum), L,

m_{IS} – is the total amount of inoculum in the sample, g,

m_{IB} – is the total amount of inoculum in the blank, g,

$m_{VS,sS}$ – is the amount of organic material (i.e., volatile solids) of substrate contained in the sample bottle, g.

The final biodegradation of the biopolymer can be calculated as the percentage of methane conversion, as represented in Formula (3.6). Previously, the theoretical methane production under the standard conditions (at 0 ºC and 1 atm) must be calculated using the Buswell Formula (3.7), and the BMP calculated from the experimental measures (Buswell & Mueller, 1952).

$$Biodegradation\ (\%) = \frac{\text{Experimental methane production (BMP)}}{\text{Theoretical methane production}}$$ (3.6)

$$\text{Theoretical methane production} \left(L\ CH_4 \cdot g^{-1}\ C_x H_y O_z N_n S_s\right)^* = \frac{22.4 \cdot \left(\frac{x}{2} + \frac{y}{8} - \frac{z}{4} - \frac{3n}{8} - \frac{s}{4}\right)}{12x + y + 16z + 14n + 32s}$$ (3.7)

3.4.5 Activities during and after the test

During the test, several activities must be done to ensure the correct functioning of the system according to the AMPTS II manual (Bioprocess Control, 2016). The temperature in the water bath should stay at 38 ± 2 ºC throughout the test to ensure appropriate digestion in the reactors. Distilled water must be added when the water level decreases below the recommended level in order to ensure the even distribution of the heat in all the reactors.

Additionally, the inside of the reactor bottles should be mixed every 2-3 days to improve the degradation process and allow the formed gas to flow up. An electric mixer can be used for the mixing process. However, in this case, the mixing procedure has been done manually by shaking the bottles. The final duration of the test is 49 days although the data has been extracted several times in order to check the correct functioning of the system.

Once the test period is over, the thermostatic water bath is disconnected. The reactors are also disconnected from the CO_2-absorption bottles and the pH in every reactor is measured as explained in 3.3.2 pH measurement. It is also possible to measure TS and VS in each reactor in order to check the reduction of VS. The rest of the parts of the equipment should be disconnected and the water bath and the Gas Volume Measuring Device emptied and cleaned.

4   RESULTS ANALYSIS

4.1 Accumulated volume of methane production

During the 49 days of the test, the methane produced in the different reactors has been measured throughout the days and stored in the database. The data collected from the triplicate reactors has been summarized in one only through the arithmetic mean. In Figure 4.1 it can be seen a representation of the accumulated data directly collected in the Gas Volume Measuring Device.

The amount of methane produced in the cellulose reactors was considerably larger than the others due to its already known high biodegradability under anaerobic conditions (Koch et al., 2020). For a better representation of the accumulated volume of methane in the biopolymers and blank reactors, they have been closely represented in Figure 4.2. It could be observed how during the first days of degradation the amount of biomethane produced in the blank reactors was higher than in the case of the polymers. Only after days 8 and 10 the values in PLA-Cut and PLA-Pa respectively surpassed the blank ones. In the case of PLA-Fil, those values stayed below the blank ones until day 38.
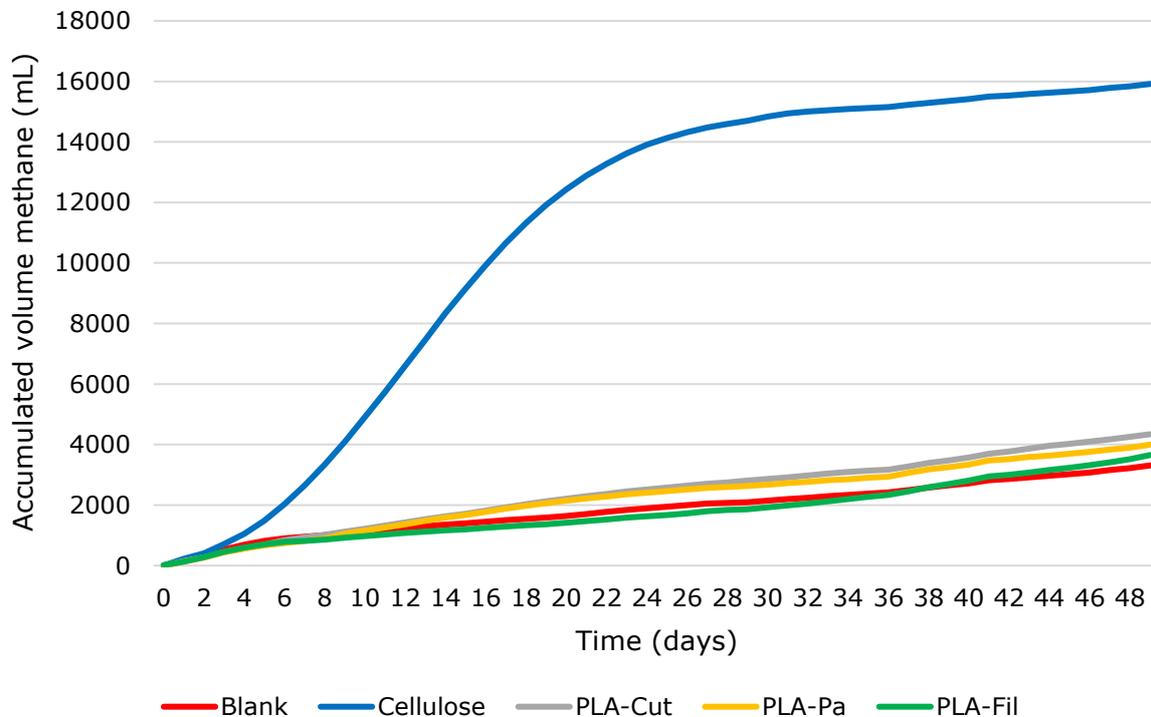

Figure 4.1 Average of the methane production for the different reactors (including samples of substrate, control samples, and blank reactors)
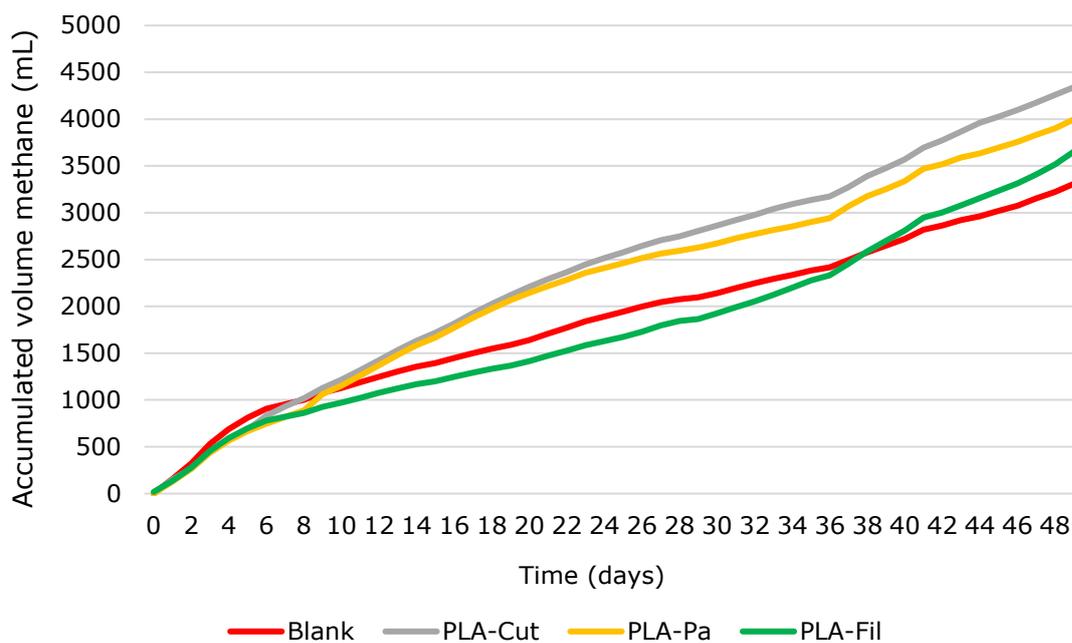
Figure 4.2 Average of methane of the polymer reactors compared to the inoculum-only reactors

4.2 Specific methane production from the substrates

Although the accumulated volume of methane produced in the reactors can be directly measured, it did not provide clear information about the methane production from the substrates. Additionally, it was not possible to compare the volumes obtained among the substrates and with other similar tests. It was possible to calculate the specific methane production of the substrates per gram of volatile solids using Formula (3.5) for the accumulated volume of methane each day.

The calculation of the BMP allowed the comparison of methane production among different substrates since it is calculated based on the volatile solids of the substrate. The representation of the specific methane production for the samples and control reactors can be found in Figure 4.3. It can be seen how the methane production from the cellulose has followed the expected evolution according to previous studies (Raposo et al., 2011). Methane was produced from cellulose from the first day of the experiment, rapidly increasing the production ratio during the first week. During the following 10 days, the production ratio stayed stable and, after day 18, it started decreasing until the volume of methane stayed at a constant value. However, the final methane production was under the theoretical values established by Raposo et al. (2011) (350 ± 29 mL $CH_4 \cdot g^{-1}$ VS). The obtention of values lower than expected was due to problems in the measurement of

methane by the instrument. For that reason, 350 mL $CH_4 \cdot g^{-1}$ VS has been used as a reference and all measured values have been adapted to the reference value.

The methane production from the polymers was much lower than in the cellulose case. For that reason, a better representation of the specific methane production from the polymers can be found in Figure 4.4. During the first days of the test, no methane was produced in any of the three cases. The lag phase was the shortest in the case of PLA-Cut in which methane was not measured until the fifth day of the test. PLA-Pa lag phase lasted until the seventh day while in the case of PLA-Fil methane was not produced until the 26th day.

PLA-Cut methane production was pretty uniform during the first days producing from 1,87 to 1,20 $NmL \cdot g^{-1}$ $VS \cdot day^{-1}$. After day 20 the methane production from the polymer decreased to values below 1,00 $NmL \cdot g^{-1}$ $VS \cdot day^{-1}$ for 15 days and increased the production again from day 36 until the end of the test. A similar evolution was found in the PLA-Pa case. Despite starting to produce methane two days later, the methane production ratio from PLA-Pa was higher than in the PLA-Cut case during the first days, reaching similar specific methane production between days 15 and 20. As in the previous case, there was an important decrease in the methane production after day 20 but, in this case, the production values of PLA-Pa were even lower than PLA-Cut. Although an increasing production could also be found on day 36, the production ratio was lower than from PLA-Cut, obtaining a lower final BMP value. In both cases, there was a peak in the methane production on day 36. This was due to an adjustment in the water content of the water bath, which affected the amount of methane produced that day but not the final volume of methane.

PLA-Fil presented much lower methane production than the other two polymers. After the lag phase, the methane production rate did not reach 1,00 $NmL \cdot g^{-1}$ $VS \cdot day^{-1}$ until day 37. In the following days, the methane production continued to grow regularly, with no indication that the ratio could decrease and remain stabilized at a value.

It could be seen that the methane production of none of the polymers followed a distribution similar to that of cellulose. Although the polymers did not show a stable methane production as in the cellulose case, it could be seen how the methane volume rapidly increased during the first production days. This methane production ratio did not seem to decrease to stabilize at a value as the methane produced from cellulose, especially in the case of PLA-Fil which had the highest production rate on the last day of testing. This is due to the fact that the biodegradability of the polymers had not ended within the period

of the test, and they could continue producing methane for more days. Additionally, the fact that the methane production ratio did not decrease during the last days of the test (as in the case of cellulose), indicates that the biodegradation would continue for more weeks.
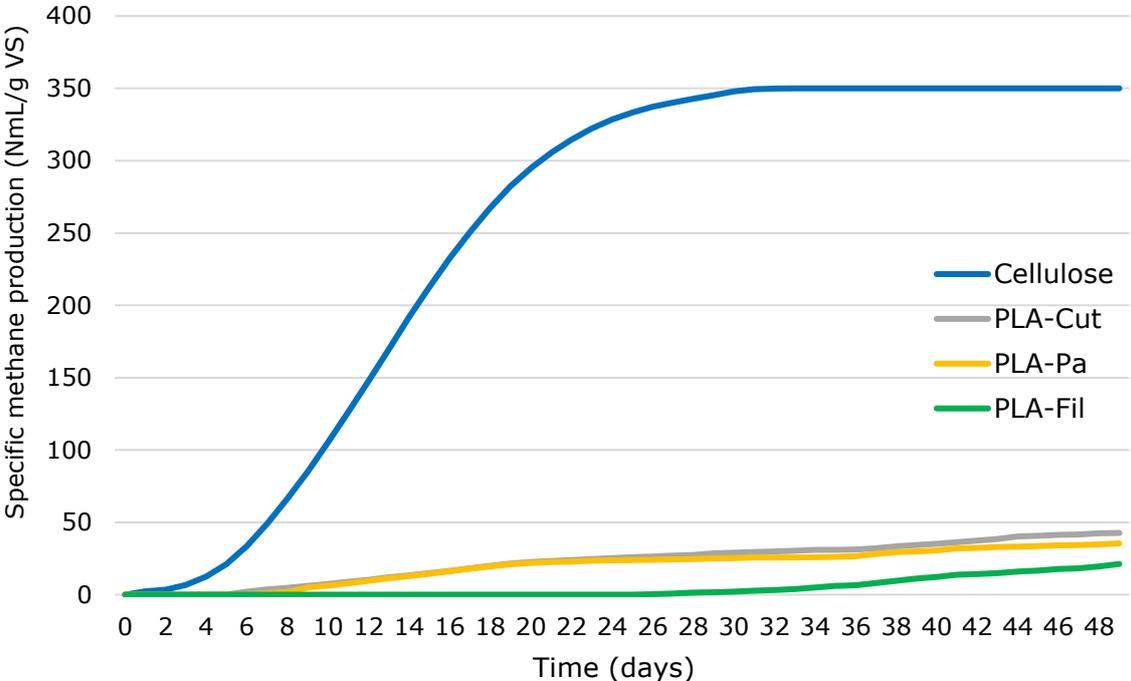

Figure 4.3 Specific methane production for the samples and control reactors (the gas from the inoculums has been subtracted and the data has been expressed as volume of CH_4 per gram VS)
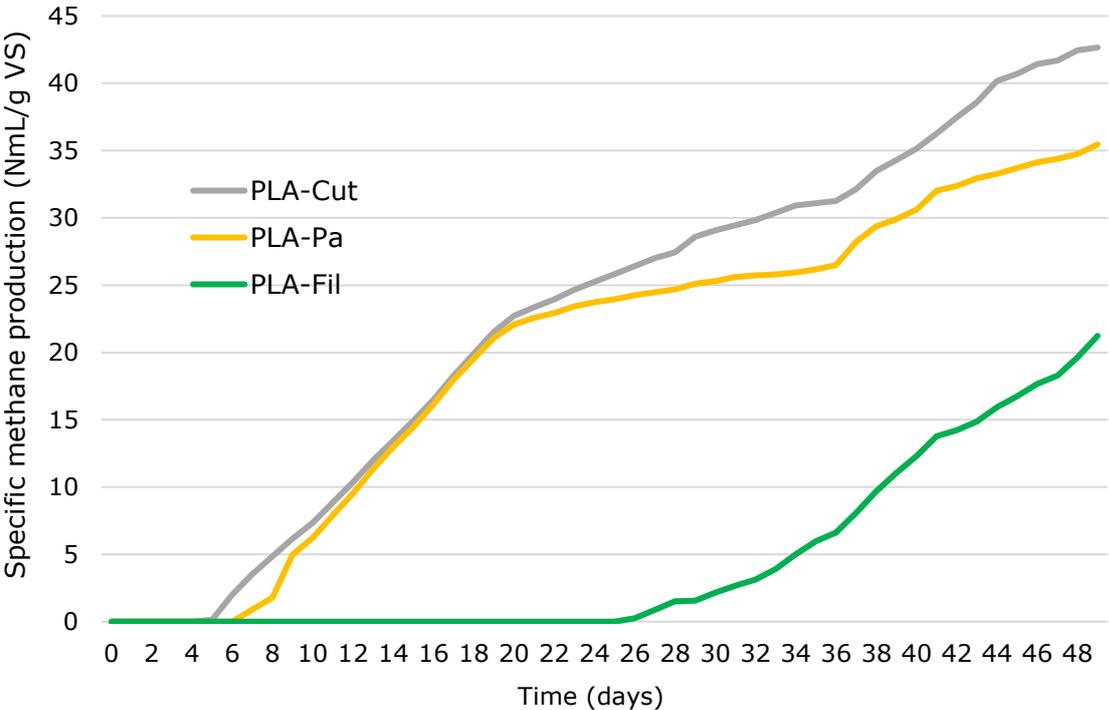

Figure 4.4 Specific methane production for only the biopolymer samples

4.3 Accumulated volume per day

To better see when the methane was produced throughout the days, in Figure 4.5 it was represented the accumulated volume of methane each day as a percentage of the total methane produced. This daily percentage can be calculated according to Formula (4.1). In the cellulose case, it could be seen how the rate of methane produced each day increased for 14 days, and then, after reaching a maximum, the rate values decreased during the following days until reaching 0% methane production after day 33. This distribution is similar to that obtained in previous studies (Raposo et al., 2011), concluding that the conditions were appropriate for anaerobic digestion although the values obtained were lower than expected.

$$\text{Accumulated volume per day (\%)} = \frac{\text{Daily accumulated methane volume}}{\text{Total accumulated methane volume}} \qquad (4.1)$$

However, the production of methane per day in the case of polymers was quite different and much more irregular than in the case of cellulose. PLA-Cut methane production rapidly increased during the first production days. After that, it could be seen a dome as in the case of cellulose but without a recognizable peak, maintaining similar production rates from day 6 to 20. After day 20 the production volume decreased and stayed more or less stable at low values until day 36 when the value increased again. This sudden increasing production rate coincides with the peak that can be seen in Figure 4.4 on day 36. After day 36, the values continued slightly increasing until day 44 when they started to decrease again.

PLA-Pa methane production rate followed a similar evolution to PLA-CUT. In the beginning, the methane production rapidly reached 9% of the total production on day 9. However, the production rate rapidly decreased and stayed at values around 5%, forming a similar dome as in the PLA-Cut. Day 20 was also the beginning of the decrease in the production rate, staying stable at low values until day 36. In this case, the second peak was larger, increasing the production rate from less than 1% to almost 5% in one day. After that, the production rate continuously decreased until the end of the test.

Finally, PLA-Fil methane production fast increased from the beginning of the degradation on day 26 in a quite irregular way. After 12 days of increasing, the production ratio reached the maximum (7,6%) although it decreased again during the following days. However,

after day 42, the production rate increased again reaching the last day of the test at 7,6% again.
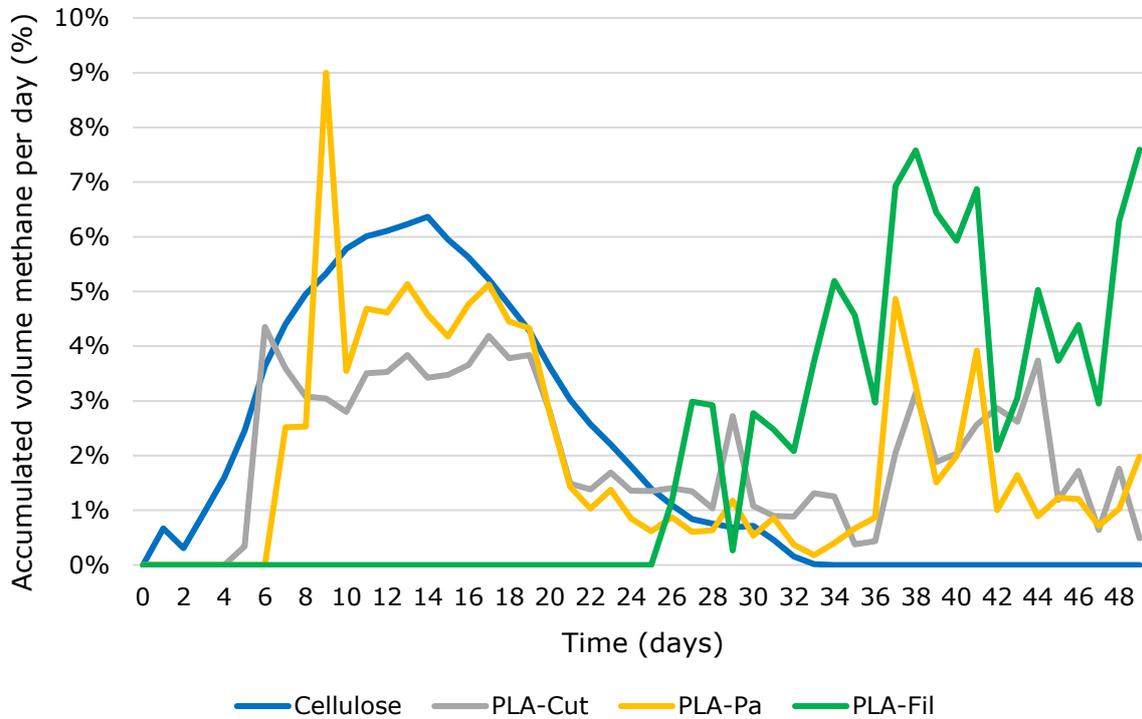

Figure 4.5 Daily methane production as % of the total accumulated volume (the gas from the inoculum has been subtracted from the data)

Although PLA-Cut and PLA-Pa showed a behaviour that can be compared with that of the cellulose during the first production days, the behaviour the rest of the test was totally different. None of the polymers increased the production ratio in a regular way but with ups and downs. Additionally, once the peak of production was reached, the values remained similar for at least 10 days until the production rate decreased more rapidly than in the case of cellulose. Besides, although the production from the polymers decreased, it never reached 0 and after some days of low production, the rate increased again. This shows that neither PLA-Cut nor PLA-Pa was close to the maximum production and that, even though it was irregular, methane would be produced for more days, even weeks. PLA-Fil showed a different distribution since methane production was very irregular during the whole test, showing constant ups and downs. Nevertheless, it could be seen how at no time the methane production ratio showed a decreasing trend and at the end of the test it was still in full growth.

4.4 Comparison of results

Once the final values of methane production from the samples have been obtained, it is possible to compare them with the values obtained in the literature for pure PLA and other PLA-based polymers under similar conditions. The values of BMP measured on the last day of the test (day 49) were used to compare the final results. In Table 4.1 it can be found the BMP values obtained for each substrate at the end of the test.

Additionally, the biodegradability of the polymers at the end of the test can also be used to compare the results with those obtained in the literature. Biodegradability is calculated following Formula (3.6) and the results can be seen in Table 4.1. Due to the lack of knowledge of the chemical composition of polymers used in the test, pure PLA ($C_3H_4O_2$) was used as a reference. It is possible to obtain the theoretical methane production of PLA using Formula (3.7), as can be seen in Formula (4.2).

$$\text{Theoretical methane production (L CH}_4\text{·g}^{-1}\text{ VS PLA)} = \frac{22.4 \cdot (\frac{3}{2}+\frac{4}{8}-\frac{2}{4})}{12\cdot3+4+16\cdot2} \qquad (4.2)$$
$$= 0{,}467 \text{ L CH}_4\text{·g}^{-1}\text{ VS PLA}$$

Among the studied degradable polymers, PLA-Cut has shown the best results, followed by PLA-Pa and finally PLA-Fil. PLA-Cut had the shortest lag phase and the highest production rate throughout the test, obtaining a higher final BMP value. Although PLA-Pa started with a higher methane production rate, the BMP value was not higher than in the case of PLA-Cut on any day. In addition, from day 20, the production rate was much lower than the PLA-Cut rate, obtaining a considerably lower final value. PLA-Fil lag phase was 25 days, being considerably longer than in the other two cases. Additionally, its production rate was not high enough to reach the production of the other polymers. Since the methane production did not reach a plateau in any case, the maximum BMP of the polymers is unknown, and a longer experiment should be carried out to determine it.

At 37 ºC, Itävaara et al. (2002) reported biodegradation of pure powder PLA lower than 5% after 40 days of the test under mesophilic conditions. The biodegradation values after 40 days of PLA-Cut and PLA-Pa were higher than pure PLA, which indicates improvement in the biodegradability of the modified polymers. However, methane production of PLA-Fil was very low compared to the other polymers, indicating that this polymer is not suitable for mesophilic anaerobic digestion.

The obtained results have been compared to those obtained in the literature under similar conditions (Table 4.2). Unlike the results obtained by Massardier-Nageotte et al. (2006) and Benn & Zitomer (2018), methane production was found in this test in similar experiment times. Additionally, the lag phase in the three studied cases was shorter than the 40 days found by Bernat et al. (2021).

Compared to other studies with higher methane production, the final results obtained were lower than those obtained from Vargas et al. (2009) and Yagi et al. (2009). However, it should be noted that Vargas et al. (2009) obtained 19,3% biodegradability after 56 days of test and the polymer was pre-treated by steam exposition to obtain better results. In the case of Yagi et al. (2009), the test lasted longer (77 days) and the daily methane production rate was higher than in any polymer study in this case.

As in the cases in the literature, none of the polymers studied reached the maximum methane production since the methane measurement continued on the last day of the test. None of the polymers studied is suitable for anaerobic digestion under mesophilic conditions since the degradation rates were not high enough for the polymers to completely degrade within the times used in anaerobic digestion on an industrial scale. Additionally, the polymer residue could negatively affect the compost obtained since it could not be used in many applications.

Table 4.1 Biochemical Methane Potential and biodegradation of the studied substrates and of pure PLA (according to the bibliography in the pure PLA case)

	Cellulose	PLA-Cut	PLA-Pa	PLA-Fil	Pure PLA
BMP (L $CH_4 \cdot kg^{-1}$ VS)	350,0	42,7	35,5	21,2	-
Biodegradation after 49 days	-	9,1%	7,6%	4,6%	-
Biodegradation after 40 days	-	7,5%	6,6%	2,6%	<5%

The results can also be compared to experiments carried out under thermophilic conditions (Table 4.3). It can be seen how the results obtained in any test under thermophilic conditions are much higher than those obtained in this study. It is demonstrated that under thermophilic conditions the degradation of PLA and derived polymers is much greater than under mesophilic conditions. In addition, the values obtained under thermophilic conditions are the maximum experimental values of methane production and the methane production times are much shorter. A new study of the three polymers should be carried out under thermophilic conditions in order to be directly compared with the rest of the results.

Table 4.2 Results from anaerobic digestion under mesophilic conditions of PLA-based polymers

Plastic	T (ºC)	Time (days)	BMP (L CH$_4$· kg^{-1} VS)	Biodegradation (%)	Reference
Pure PLA	37	40	-	< 5%	Itävaara et al. (2002)
PLA (Fabri-Kal) * + steam exposition	37	56	2 / 90	0,4% / 19,3%	Vargas et al. (2009)
PLA (Unitika)	37	90	-	7%	Yagi et al. (2014)
PLA (Nature Works)	37 ± 2	20	-	< 5%	Greene (2018)
LA (H-400)	37	77	-	14%	Yagi et al. (2009)
PLA (plastic cup)	37± 0,5	280	282	66%	Bernat et al. (2021)
PLA (comercial ítems)	37± 1	250	130	-	Battista et al. (2021)
PLA-Pa / PLA-Cut / PLA-Fil	38	49	42,7 / 35,5 / 21,2	9,1% / 7,6% / 4,6%	This study

Table 4.3 Results from anaerobic digestion under thermophilic conditions of PLA-based polymers

Plastic	T (ºC)	Time (days)	BMP (L CH4· kg-1 VS)	Biodegradation (%)	Reference
Pure PLA	52	40	-	60%	Itävaara et al. (2002)
PLA (Ingeo)	55	90	285	56%	Vasmara & Marchetti (2016)
PLA (Fabri-Kal)	58	56	187	40%	Vargas et al. (2009)
PLA (4043D)	55 ± 2	80	-	88%	Narancic et al. (2018)
PLA (plastic cup)	58 ± 0,5	50	417	90%	Bernat et al. (2021)
PLA (H-400)	55	30	-	60%	Yagi et al. (2009)
PLA (Unitika)	55	80	408	82%	Yagi et al. (2013)
PLA (4043D)	52 ± 2	36	409	90%	Hegde et al. (2018)

5   CONCLUSIONS

The anaerobic degradation under mesophilic conditions of PLA-Cut, PLA-Pa, and PLA-Fil has shown promising results, obtaining after 49 days of test BMP values of 42,7, 35,2 and 21,2 L $CH_4 \cdot kg^{-1}$ VS. The methane obtained and the final biodegradability are quite above most results previously obtained in the literature, although they are below others. Additionally, the biodegradability values of PLA-Cut and PLA-Pa are higher than the degradability of pure PLA, showing an improvement over the original material.

However, the time of the test has been longer than the digestion times in industrial anaerobic degradation facilities. The degradation of the studied polymers before 30 days was not enough to satisfy the demands of industrial anaerobic digestion under mesophilic conditions. For that reason, the studied polymers could not be introduced in real digesters along with other biodegradable waste such as food or agricultural waste.

The results of the test may be improved by using a higher pH in the inoculum being more appropriate for anaerobic digestion. Additionally, more modern equipment would allow obtaining more precise data and closer to reality. The usage of enzymes in the degradation and the pre-treatment of the polymers would be interesting to further study since higher methane production may be obtained. A further study of the methane production of the degradable polymers under thermophilic conditions is of great interest since it has been proved that PLA is better degraded under higher temperatures. Additionally, degradation times would be shorter so that it might be possible to degrade the polymers in industrial facilities.

SUMMARY

Conventional plastics have several problems that concern the environment and that must be solved in the future. The accumulation of plastic waste in landfills and the environment, including plastics and microplastics in the oceans, poses a great danger for many species as well as for human health. Additionally, plastic production is predicted to increase, so more plastic waste is expected to have to be treated in the coming decades.

Bioplastics have been proposed as one of the most promising solutions. Bioplastics that are bio-based and biodegradable such as PLA are expected to decrease the plastic waste at the same time they are produced from renewable sources. Those polymers can be degraded in the environment so that they are converted into non-toxic substances.

However, nowadays bioplastics have a number of disadvantages that prevent them from being direct substitutes for conventional plastics. Many times, the physical properties of bioplastics do not satisfy the necessities, so they need to be modified. The modification can negatively affect the biodegradability of the polymers, not being adequate for waste treatment processes. Anaerobic degradation is probably the most interesting waste treatment method since methane can be produced, being used to produce clean energy.

The methane production of three PLA-based by anaerobic degradation has been studied to be compared with other similar tests. The polymers have been introduced into reactors along with sludge under mesophilic anaerobic conditions (38 ºC) for 49 days.

According to the results obtained, the modified PLA-based polymers showed better or similar biodegradation than pure PLA under similar conditions. Additionally, the results of methane production were higher than in other studied cases. However, degradation times were longer than in industrial processes. Biodegradability in short times was low and not feasible for industrial anaerobic degradation under mesophilic conditions.

LIST OF REFERENCES

Abraham, A., Park, H., Choi, O., & Sang, B. I. (2021). Anaerobic co-digestion of bioplastics as a sustainable mode of waste management with improved energy production – A review. *Bioresource Technology*, *322*(October 2020), 124537. https://doi.org/10.1016/j.biortech.2020.124537

Andrady, A. L., & Neal, M. A. (2009). Applications and societal benefits of plastics. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1526), 1977–1984. https://doi.org/10.1098/rstb.2008.0304

André, L., Pauss, A., & Ribeiro, T. (2018). Solid anaerobic digestion: State-of-art, scientific and technological hurdles. *Bioresource Technology*, *247*(September 2017), 1027–1037. https://doi.org/10.1016/j.biortech.2017.09.003

Angelonidi, E., & Smith, S. R. (2015). A comparison of wet and dry anaerobic digestion processes for the treatment of municipal solid waste and food waste. *Water and Environment Journal*, *29*(4), 549–557. https://doi.org/10.1111/wej.12130

Ashter, S. A. (2016). Fundamentals on Biodegradability. *Introduction to Bioplastics Engineering*, 61–80. https://doi.org/10.1016/b978-0-323-39396-6.00004-x

Auras, R. A., Harte, B., Selke, S., & Hernandez, R. (2003). Mechanical, physical, and barrier properties of poly(lactide) films. *Journal of Plastic Film and Sheeting*, *19*(2), 123–135. https://doi.org/10.1177/8756087903039702

Bagheri, A. R., Laforsch, C., Greiner, A., & Agarwal, S. (2017). Fate of So-Called Biodegradable Polymers in Seawater and Freshwater. *Global Challenges*, *1*(4), 1700048. https://doi.org/10.1002/gch2.201700048

Bátori, V., Åkesson, D., Zamani, A., Taherzadeh, M. J., & Sárvári Horváth, I. (2018). Anaerobic degradation of bioplastics: A review. *Waste Management*, *80*, 406–413. https://doi.org/10.1016/j.wasman.2018.09.040

Battista, F., Frison, N., & Bolzonella, D. (2021). Can bioplastics be treated in conventional anaerobic digesters for food waste treatment? *Environmental Technology & Innovation*, *22*, 101393. https://doi.org/https://doi.org/10.1016/j.eti.2021.101393

Benn, N., & Zitomer, D. (2018). *Pretreatment and Anaerobic Co-digestion of Selected PHB and PLA Bioplastics*. *5*(January), 1–9. https://doi.org/10.3389/fenvs.2017.00093

Bernat, K., Kulikowska, D., Wojnowska-baryła, I., & Zaborowska, M. (2021). Thermophilic and mesophilic biogas production from PLA-based materials : Possibilities and limitations. *Waste Management*, *119*, 295–305. https://doi.org/10.1016/j.wasman.2020.10.006

Bioprocess Control. (2016). *AMPTS II and AMPTS II Light: Automatic Methane Potential Test System*.

Brémond, U., de Buyer, R., Steyer, J.-P., Bernet, N., & Carrere, H. (2018). Biological pretreatments of biomass for improving biogas production: an overview from lab scale to full-scale. *Renewable and Sustainable Energy Reviews*, *90*, 583–604. https://doi.org/https://doi.org/10.1016/j.rser.2018.03.103

Bu, Y., Ma, J., Bei, J., & Wang, S. (2019). Surface Modification of Aliphatic Polyester to Enhance Biocompatibility. *Frontiers in Bioengineering and Biotechnology*, *7*(May), 1–10. https://doi.org/10.3389/fbioe.2019.00098

Burzic, I., Pretschuh, C., Kaineder, D., Eder, G., & Smilek, J. (2019). Impact modification of PLA using biobased biodegradable PHA biopolymers. *European Polymer Journal*, *114*(January), 32–38. https://doi.org/10.1016/j.eurpolymj.2019.01.060

Buswell, A. M., & Mueller, H. F. (1952). Mechanism of methane fermentation. *Industrial & Engineering Chemistry*, *44*(3), 550–552.

Cadar, O., Cadar, S., Şenilă, M., Majdik, C., & Roman, C. (2011). Synthesis of poly(l-lactic acid) by direct polycondensation. *Studia Universitatis Babes-Bolyai Chemia*, *2*, 57–64.

Calmon, A., Guillaume, S., Bellon-Maurel, V., Feuilloley, P., & Silvestre, F. (1999). Evaluation of Material Biodegradability in Real Conditions–Development of a Burial Test and an Analysis Methodology Based on Numerical Vision. *Journal of Environmental Polymer Degradation*, *7*(3), 157–166. https://doi.org/10.1023/A:1022849706383

Carlsson, M., Lagerkvist, A., & Morgan-Sagastume, F. (2012). The effects of substrate pre-treatment on anaerobic digestion systems: A review. *Waste Management*, *32*(9), 1634–1650. https://doi.org/https://doi.org/10.1016/j.wasman.2012.04.016

Castro-Aguirre, E., Iñiguez-Franco, F., Samsudin, H., Fang, X., & Auras, R. (2016). Poly(lactic acid)—Mass production, processing, industrial applications, and end of life. *Advanced Drug Delivery Reviews*, *107*, 333–366. https://doi.org/10.1016/j.addr.2016.03.010

Cazaudehore, G., Guyoneaud, R., Evon, P., Martin-Closas, L., Pelacho, A. M., Raynaud, C., & Monlau, F. (2022). Can anaerobic digestion be a suitable end-of-life scenario for biodegradable plastics? A critical review of the current situation, hurdles, and challenges. *Biotechnology Advances*, *56*(February), 107916. https://doi.org/10.1016/j.biotechadv.2022.107916

Chariyachotilert, C., Joshi, S., Selke, S. E., & Auras, R. (2012). Assessment of the properties of poly(L-lactic acid) sheets produced with differing amounts of postconsumer recycled poly(L-lactic acid). *Journal of Plastic Film and Sheeting*, *28*(4), 314–335. https://doi.org/10.1177/8756087911434337

Chien, Y.-C., Liang, C., Liu, S.-H., & Yang, S.-H. (2010). Combustion kinetics and emission characteristics of polycyclic aromatic hydrocarbons from polylactic acid combustion. *Journal of the Air & Waste Management Association*, *60*(7), 849–855. https://doi.org/10.3155/1047-3289.60.7.849

Costiuc, L., Patachia, S., Baltes, L., & Tierean, M. (2011). Investigation on energy density of plastic waste materials. *Journal of Solid Waste Technology and Management*, 930–939.

de Albuquerque, T. L., Marques Júnior, J. E., de Queiroz, L. P., Ricardo, A. D. S., & Rocha, M. V. P. (2021). Polylactic acid production from biotechnological routes: A review. *International Journal of Biological Macromolecules*, *186*(May), 933–951. https://doi.org/10.1016/j.ijbiomac.2021.07.074

DeStefano, V., Khan, S., & Tabada, A. (2020). Applications of PLA in modern medicine. *Engineered Regeneration*, *1*(September), 76–87. https://doi.org/10.1016/j.engreg.2020.08.002

El-mashad, H. M., Zhang, R., & Greene, J. P. (2012). Anaerobic Biodegradability of Selected Biodegradable Plastics and Biobased Products. *Journal of Environmental Science & Engineering A*, *1*(December), 108–114.

European Biogas Association. (2020). *European Biogas Association Statistical Report: 2019 European Overview*. https://www.europeanbiogas.eu/eba-statistical-report-2019-european-overview/

European Bioplastics. (2015). *Anaerobic digestion*.

European Bioplastics. (2017). *Bioplastics market data 2017*. https://www.european-bioplastics.org/global-market-for-bioplastics-to-grow-by-20-percent/

European Bioplastics. (2021). *Bioplastics market data*. https://www.european-bioplastics.org/market/

European Commission. (2008). *DIRECTIVE 2008/98/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 19 November 2008 on waste and repealing certain Directives*.

Filer, J., Ding, H. H., & Chang, S. (2019). Biochemical Methane Potential (BMP) Assay Method for Anaerobic Digestion Research. *Water*, *11*(5). https://doi.org/10.3390/w11050921

Flieger, M., Kantorová, M., Prell, A., Řezanka, T., & Votruba, J. (2003). Biodegradable plastics from renewable sources. *Folia Microbiologica*, *48*(1), 27–44. https://doi.org/10.1007/BF02931273

França, D. C., Almeida, T. G., Abels, G., Canedo, E. L., Carvalho, L. H., Wellen, R. M. R., Haag, K., & Koschek, K. (2019). Tailoring PBAT/PLA/Babassu films for suitability of agriculture mulch application. *Journal of Natural Fibers*, *16*(7), 933–943. https://doi.org/10.1080/15440478.2018.1441092

Gao, H., Hu, S., Su, F., Zhang, J., & Tang, G. (2011). *Mechanical , Thermal , and Biodegradability Properties of PLA / Modified Starch Blends*. https://doi.org/10.1002/pc

Garlotta, D. (2001). A Literature Review of Poly (Lactic Acid) A Literature Review of Poly (Lactic Acid). *Journal of Polymers and the Environment*, *9*(2), 63–84.

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances*, *3*(7), 3–8. https://doi.org/10.1126/sciadv.1700782

Grand View Research. (2021). *GVR Report cover Polylactic Acid Market Size, Share & Trends Analysis Report By End-use (Packaging, Textile, Agriculture, Automotive & Transport, Electronics), By Region (North America, APAC, Europe), And Segment Forecasts, 2021 - 2028*. https://www.grandviewresearch.com/industry-analysis/polylactic-acid-pla-market

Greene, J. (2018). Biodegradation of biodegradable and compostable plastics under industrial compost, marine and anaerobic digestion. *Ecol Pollut Environ Sci*, *1*, 13–18.

Hartmann, M. H. (1998). High Molecular Weight Polylactic Acid Polymers. *Biopolymers from Renewable Resources*, 367–411. https://doi.org/10.1007/978-3-662-03680-8_15

Hawkes, D. L. (1980). *Factors affecting net energy production from mesophilic anaerobic digestion*.

Hegde, S., Dell, E., Lewis, C., Trabold, T. A., & Diaz, C. A. (2018). Anaerobic biodegradation of bioplastic packaging materials. *The 21st IAPRI World Conference on Packaging*.

Hiljanen-Vainio, M., Varpomaa, P., Seppälä, J., & Törmälä, P. (1996). Modification of poly(L-lactides) by blending: Mechanical and hydrolytic behavior. *Macromolecular Chemistry and Physics*, *197*(4), 1503–1523. https://doi.org/10.1002/macp.1996.021970427

Hobbs, S. R., Parameswaran, P., Astmann, B., Devkota, J. P., & Landis, A. E. (2019). Anaerobic Codigestion of Food Waste and Polylactic Acid : Effect of Pretreatment on Methane Yield and Solid Reduction. *Advances in Materials Science and Engineering*.

Hurley, R., Horton, A., Lusher, A., & Nizzetto, L. (2020). Plastic waste in the terrestrial environment. In *Plastic Waste and Recycling*. Elsevier Inc. https://doi.org/10.1016/b978-0-12-817880-5.00007-4

International Organization for Standardization [ISO]. (2012). *Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions — Method by analysis of evolved carbon dioxide — Part 1: General method (ISO Standard No. 14855)*.

Itävaara, M., Karjomaa, S., & Selin, J.-F. (2002). Biodegradation of polylactide in aerobic and anaerobic thermophilic conditions. *Chemosphere*, *46*(6), 879–885. https://doi.org/https://doi.org/10.1016/S0045-6535(01)00163-1

John, R. P., Gangadharan, D., & Madhavan Nampoothiri, K. (2008). Genome shuffling of Lactobacillus delbrueckii mutant and Bacillus amyloliquefaciens through protoplasmic fusion for L-lactic acid production from starchy wastes. *Bioresource Technology*, *99*(17), 8008–8015. https://doi.org/10.1016/j.biortech.2008.03.058

John, R. P., Madhavan Nampoothiri, K., & Pandey, A. (2006). *and Fermentation of Cassava Bagasse for L - (+) -Lactic Acid Production*. *134*, 263–272.

Kamiya, M., Asakawa, S., & Kimura, M. (2007). Molecular analysis of fungal communities of biodegradable plastics in two Japanese soils. *Soil Science and Plant Nutrition*, *53*(5), 568–574. https://doi.org/10.1111/j.1747-0765.2007.00169.x

Karamanlioglu, M., Preziosi, R., & Robson, G. D. (2017). Abiotic and biotic environmental degradation of the bioplastic polymer poly(lactic acid): A review. *Polymer Degradation and Stability*, *137*, 122–130. https://doi.org/10.1016/j.polymdegradstab.2017.01.009

Kim, K. W., & Woo, S. I. (2002). *Synthesis of High-Molecular-Weight Poly (L -lactic acid) by Direct Polycondensation*. 2245–2250.

Koch, K., Hafner, S. D., Astals, S., & Weinrich, S. (2020). Evaluation of Common Supermarket Products as Positive Controls in Biochemical Methane Potential (BMP) Tests. *Water*, *12*(5). https://doi.org/10.3390/w12051223

Kolstad, J. J., Vink, E. T. H., De Wilde, B., & Debeer, L. (2012). Assessment of anaerobic degradation of Ingeo™ polylactides under accelerated landfill conditions. *Polymer Degradation and Stability*, *97*(7), 1131–1141. https://doi.org/10.1016/j.polymdegradstab.2012.04.003

Laußmann, C., Land, U., Münster, B., Endres, G. H.-J., F. Hannover, G. U., Giese, A.-S., & Kitzler, A. (2010). Disposal of Bio-Polymers via Energy Recovery. *Papierveredelung*, 42–43.

Laycock, B., Nikolić, M., Colwell, J. M., Gauthier, E., Halley, P., Bottle, S., & George, G. (2017). Lifetime prediction of biodegradable polymers. *Progress in Polymer Science*, *71*, 144–189. https://doi.org/https://doi.org/10.1016/j.progpolymsci.2017.02.004

Lee, H. J., Kim, D. G., Lee, J. H., & Cho, M. S. (2012). *A Study for Carbonation Degree on Concrete using a Phenolphthalein Indicator and Fourier-Transform Infrared Spectroscopy*. 152–158.

Li, W. C., Tse, H. F., & Fok, L. (2016). Plastic waste in the marine environment: A review of sources, occurrence and effects. *Science of the Total Environment*, *566–567*, 333–349. https://doi.org/10.1016/j.scitotenv.2016.05.084

Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., & Nava-saucedo, J. (2008). Chemosphere Polymer biodegradation : Mechanisms and estimation techniques. *Chemosphere*, *73*, 429–442. https://doi.org/10.1016/j.chemosphere.2008.06.064

Lunt, J. (1998). Large-scale production, properties and commercial applications of poly lactic acid polymers. *Polymer Degradation and Stability*, *59*(1–3), 145–152. https://doi.org/10.1016/s0141-3910(97)00148-1

Madhavan Nampoothiri, K., Nair, N. R., & John, R. P. (2010). An overview of the recent developments in polylactide (PLA) research. *Bioresource Technology*, *101*(22), 8493–8501. https://doi.org/10.1016/j.biortech.2010.05.092

Madival, S., Auras, R., Singh, S. P., & Narayan, R. (2009). Assessment of the environmental profile of PLA, PET and PS clamshell containers using LCA methodology. *Journal of Cleaner Production*, *17*(13), 1183–1194. https://doi.org/https://doi.org/10.1016/j.jclepro.2009.03.015

Mao, C., Zhang, T., Wang, X., Feng, Y., Ren, G., & Yang, G. (2017). Process performance and methane production optimizing of anaerobic co-digestion of swine manure and corn straw. *Scientific Reports*, *7*(1), 9379. https://doi.org/10.1038/s41598-017-09977-6

MarketsandMarkets. (2021). *Bioplastics & Biopolymers Market by Type (Non-Biodegradable/Bio-Based, Biodegradable), End-Use Industry (Packaging, Consumer Goods, Automotive & Transportation, Textiles, Agriculture & Horticulture), Region - Global Forecast to 2026*. https://www.marketsandmarkets.com/Market-Reports/biopolymers-

bioplastics-market-
88795240.html?gclid=Cj0KCQiA6NOPBhCPARIsAHAy2zAHXNYS9IKo13dE8tDzFIG0uTCejb
rxu7NmVOZDX92JKnmHnP1J3tMaAjW2EALw_wcB

Mascia, L. (1992). An Overview of Additives and Modifiers for Polymer Blends: Facts , Deductions , and Uncertainties. *Advances in Polymer Technology*, *11*(4), 237–248.

Massardier-Nageotte, V., Pestre, C., Cruard-Pradet, T., & Bayard, R. (2006). Aerobic and anaerobic biodegradability of polymer films and physico-chemical characterization. *Polymer Degradation and Stability*, *91*(3), 620–627. https://doi.org/10.1016/j.polymdegradstab.2005.02.029

Mattheeuws, B. (2015). *State of the Art of Anaerobic Digestion of Municipal Waste in Europe in 2015*.

Momeni, S., Ghomi, E. R., Shakiba, M., Shafiei-Navid, S., Abdouss, M., Bigham, A., Khosravi, F., Ahmadi, Z., Faraji, M., Abdouss, H., & Ramakrishna, S. (2021). The effect of poly (Ethylene glycol) emulation on the degradation of pla/starch composites. *Polymers*, *13*(7), 1–19. https://doi.org/10.3390/polym13071019

Morão, A., & de Bie, F. (2019). Life Cycle Impact Assessment of Polylactic Acid (PLA) Produced from Sugarcane in Thailand. *Journal of Polymers and the Environment*, *27*(11), 2523–2539. https://doi.org/10.1007/s10924-019-01525-9

Mordor Intelligence. (2021). *Bio-polylactic acid (PLA) market - Growth, trends, Covid-19 impact and forecast (2022-2027)*. https://mordorintelligence.com/industry-reports/bio-polylactic-acid-pla-market

Moretti, C., Hamelin, L., Jakobsen, L. G., Junginger, M. H., Steingrimsdottir, M. M., Høibye, L., & Shen, L. (2021). Cradle-to-grave life cycle assessment of single-use cups made from PLA, PP and PET. *Resources, Conservation and Recycling*, *169*(December 2020). https://doi.org/10.1016/j.resconrec.2021.105508

Narancic, T., Verstichel, S., Reddy Chaganti, S., Morales-Gamez, L., Kenny, S. T., De Wilde, B., Babu Padamati, R., & O'Connor, K. E. (2018). Biodegradable Plastic Blends Create New Possibilities for End-of-Life Management of Plastics but They Are Not a Panacea for Plastic Pollution. *Environmental Science and Technology*, *52*(18), 10441–10452.

https://doi.org/10.1021/acs.est.8b02963

National Center for Biotechnology Information. (2022). *PubChem Compound Summary for CID 4764, Phenophthalein*. https://pubchem.ncbi.nlm.nih.gov/compound/Phenolphthalein

Nesic, A., Castillo, C., & Castan, P. (2020). *Bio-based packaging materials*. https://doi.org/10.1016/B978-0-12-818493-6.00008-7

Nkoa, R. (2014). Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. *Agronomy for Sustainable Development*, *34*(2), 473–492. https://doi.org/10.1007/s13593-013-0196-z

Phelan, A. (Anya), Meissner, K., Humphrey, J., & Ross, H. (2022). Plastic pollution and packaging: Corporate commitments and actions from the food and beverage sector. *Journal of Cleaner Production*, *331*(October 2021), 129827. https://doi.org/10.1016/j.jclepro.2021.129827

PlasticsEurope. (2021). *Plastics-the Facts 2021*. https://plasticseurope.org/knowledge-hub/plastics-the-facts-2021/

Polymer Database. (2022). *POLYLACTIC ACID OR POLYLACTIDE (PLA)*. https://polymerdatabase.com/Polymer Brands/PLA.html

Puthumana, M., Santhana Gopala Krishnan, P., & Nayak, S. K. (2020). Chemical modifications of PLA through copolymerization. *International Journal of Polymer Analysis and Characterization*, *25*(8), 634–648. https://doi.org/10.1080/1023666X.2020.1830650

Quecholac-Piña, X., Hernández-Berriel, M. del C., Mañón-Salas, M. del C., Espinosa-Valdemar, R. M., & Vázquez-Morillas, A. (2020). Degradation of plastics under anaerobic conditions: A short review. *Polymers*, *12*(1), 109.

Raposo, F., Fernández-Cegrí, V., la Rubia, M. A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J. C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M., Uellendahl, H., … de Wilde, V. (2011). Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *Journal of Chemical Technology \& Biotechnology*, *86*(8), 1088–1098.

https://doi.org/https://doi.org/10.1002/jctb.2622

Raquez, J., Habibi, Y., Murariu, M., & Dubois, P. (2013). Polylactide (PLA)-based nanocomposites. *Progress in Polymer Science*, *38*(10–11), 1504–1542. https://doi.org/10.1016/j.progpolymsci.2013.05.014

Rasal, R. M., Janorkar, A. V, & Hirt, D. E. (2010). Poly(lactic acid) modifications. *Progress in Polymer Science Poly (Lactic Acid) Modifications*, *35*, 338–356. https://doi.org/10.1016/j.progpolymsci.2009.12.003

Rezvani Ghomi, E., Khosravi, F., Saedi Ardahaei, A., Dai, Y., Neisiany, R. E., Foroughi, F., Wu, M., Das, O., & Ramakrishna, S. (2021). The life cycle assessment for polylactic acid (PLA) to make it a low-carbon material. *Polymers*, *13*(11), 1–16. https://doi.org/10.3390/polym13111854

Rossi, V., Cleeve-Edwards, N., Lundquist, L., Schenker, U., Dubois, C., Humbert, S., & Jolliet, O. (2015). Life cycle assessment of end-of-life options for two biodegradable packaging materials: sound application of the European waste hierarchy. *Journal of Cleaner Production*, *86*, 132–145. https://doi.org/https://doi.org/10.1016/j.jclepro.2014.08.049

Rufai, I. A. (2019). *A Review of the Evolution and Development of Anaerobic*. *September*.

Sahota, S., Shah, G., Ghosh, P., Kapoor, R., Sengupta, S., Singh, P., Vijay, V., Sahay, A., Vijay, V. K., & Thakur, I. S. (2018). Review of trends in biogas upgradation technologies and future perspectives. *Bioresource Technology Reports*, *1*, 79–88. https://doi.org/10.1016/j.biteb.2018.01.002

Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2008). Biological degradation of plastics : A comprehensive review. *Biotechnology Advances 26*, *26*, 246–265. https://doi.org/10.1016/j.biotechadv.2007.12.005

Sharmin, N., & Rudd, C. D. (2017). Structure, thermal properties, dissolution behaviour and biomedical applications of phosphate glasses and fibres: a review. *Journal of Materials Science*, *52*(15), 8733–8760. https://doi.org/10.1007/s10853-017-0784-4

Shen, J. J. (2011). *Comparative Life cycle Assessment of Polylactic acid (PLA) and Polyethylene terephthalate (PET) Jennifer J. Shen*. 1–16.

Shogren, R. L., Doane, W. M., Garlotta, D., Lawton, J. W., & Willett, J. L. (2003). Biodegradation of starch/polylactic acid/poly(hydroxyester-ether) composite bars in soil. *Polymer Degradation and Stability*, *79*(3), 405–411. https://doi.org/https://doi.org/10.1016/S0141-3910(02)00356-7

Shrestha, A., van-Eerten Jansen, M. C. A. A., & Acharya, B. (2020). Biodegradation of bioplastic using anaerobic digestion at retention time as per industrial biogas plant and international norms. *Sustainability (Switzerland)*, *12*(10). https://doi.org/10.3390/su12104231

Sin, L. T., & Tueen, B. S. (2019a). 1 - Overview of Biodegradable Polymers and Poly(Lactic Acid). In *Polylactic Acid*. https://doi.org/10.1016/b978-0-12-814472-5.00001-7

Sin, L. T., & Tueen, B. S. (2019b). 2 - Synthesis and Production of Poly(Lactic Acid). In *Polylactic Acid*. https://doi.org/10.1016/b978-0-12-814472-5.00002-9

Sin, L. T., & Tueen, B. S. (2019c). 9 - Processing of Poly(Lactic Acid). In L. T. Sin & B. S. Tueen (Eds.), *Polylactic Acid (Second Edition)* (Second Edi, pp. 307–324). William Andrew Publishing. https://doi.org/https://doi.org/10.1016/B978-0-12-814472-5.00009-1

Singhvi, M. S., Zinjarde, S. S., & Gokhale, D. V. (2019). Polylactic acid: synthesis and biomedical applications. *Journal of Applied Microbiology*, *127*(6), 1612–1626. https://doi.org/10.1111/jam.14290

Siracusa, V., & Blanco, I. (2020). Bio-polyethylene (Bio-PE), Bio-polypropylene (Bio-PP) and Bio-poly(ethylene terephthalate) (Bio-PET): Recent developments in bio-based polymers analogous to petroleum-derived ones for packaging and engineering applications. *Polymers*, *12*(8). https://doi.org/10.3390/APP10155029

Šmejkalová, P., Kužníková, V., Merna, J., & Hermanová, S. (2016). Anaerobic digestion of aliphatic polyesters. *Water Science & Technology*, 2386–2393. https://doi.org/10.2166/wst.2016.088

Sp, Y., & Rudolph, N. (2017). *Processing of Poly (lactic Acid)*. https://doi.org/10.1007/12

Tchobanoglous, G., Theisen, H., & Vigil, S. (1993). *Integrated Solid Waste Management*. McGraw-Hill, Inc.

Tokiwa, Y., Calabia, B. P., Ugwu, C. U., & Aiba, S. (2009). Biodegradability of Plastics Bioplastics. *International Journal of Molecular Sciences*, *10*, 3722–3742. https://doi.org/10.3390/ijms10093722

Vargas, L. F., Welt, B. A., Teixeira, A., Pullammanappallil, P., Balaban, M., & Beatty, C. (2009). *BIODEGRADATION OF TREATED POLYLACTIC ACID (PLA) UNDER ANAEROBIC CONDITIONS*. *52*(3), 1025–1030.

Vasmara, C., & Marchetti, R. (2016). Biogas production from biodegradable bioplastics. *Environmental Engineering & Management Journal (EEMJ)*, *15*(9).

Walla, C., & Schneeberger, W. (2008). The optimal size for biogas plants. *Biomass and Bioenergy*, *32*(6), 551–557. https://doi.org/https://doi.org/10.1016/j.biombioe.2007.11.009

Wang, S., Cui, W., & Bei, J. (2005). Bulk and surface modifications of polylactide. *Analytical and Bioanalytical Chemistry*, *381*(3), 547–556. https://doi.org/10.1007/s00216-004-2771-2

Williams, D. F. (1982). Biodegradation of surgical polymers. *Journal of Materials Science*, *17*(5), 1233–1246. https://doi.org/10.1007/BF00752233

Xiao, L., Wang, B., Yang, G., & Gauthier, M. (2012). Poly(Lactic Acid)-Based Biomaterials: Synthesis, Modification and Applications. *Biomedical Science, Engineering and Technology*, *January*. https://doi.org/10.5772/23927

Xu, F., Li, Y., Wicks, M., Li, Y., & Keener, H. (2019). Anaerobic Digestion of Food Waste for Bioenergy Production. In P. Ferranti, E. M. Berry, & J. R. Anderson (Eds.), *Encyclopedia of Food Security and Sustainability* (pp. 530–537). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-08-100596-5.22433-5

Yagi, H., Ninomiya, F., Funabashi, M., & Kunioka, M. (2009). Anaerobic Biodegradation Tests of Poly (lactic acid) under Mesophilic and Thermophilic Conditions Using a New Evaluation System for Methane Fermentation in Anaerobic Sludge. *International Journal of*

Molecular Sciences, *10*, 3824–3835. https://doi.org/10.3390/ijms10093824

Yagi, H., Ninomiya, F., Funabashi, M., & Kunioka, M. (2012). Anaerobic Biodegradation of Poly (Lactic Acid) Film in Anaerobic Sludge. *Journal of Polymers and the Environment*, *20*(3), 673–680. https://doi.org/10.1007/s10924-012-0472-z

Yagi, H., Ninomiya, F., Funabashi, M., & Kunioka, M. (2013). Thermophilic anaerobic biodegradation test and analysis of eubacteria involved in anaerobic biodegradation of four speci fi ed biodegradable polyesters. *Polymer Degradation and Stability*, *98*, 1182–1187. https://doi.org/10.1016/j.polymdegradstab.2013.03.010

Yagi, H., Ninomiya, F., Funabashi, M., & Kunioka, M. (2014). Mesophilic anaerobic biodegradation test and analysis of eubacteria and archaea involved in anaerobic biodegradation of four speci fi ed biodegradable polyesters. *Polymer Degradation and Stability*, *110*, 278–283. https://doi.org/10.1016/j.polymdegradstab.2014.08.031

Zhang, W., Heaven, S., & Banks, C. J. (2018). Degradation of some EN13432 compliant plastics in simulated mesophilic anaerobic digestion of food waste. *Polymer Degradation and Stability*, *147*, 76–88. https://doi.org/https://doi.org/10.1016/j.polymdegradstab.2017.11.005

Zumdahl, S. S., & DeCoste, D. J. (2012). *Chemical principles*. Cengage Learning.