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Improving the circularity of biodegradable bioplastics by producing biogas: a full-scale assessment

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Abstract

Polylactic acid (PLA) is one of the commonly used bioplastics, promoted as a sustainable alternative to conventional plastics. Their anaerobic co-digestion with the organic fractions of municipal solid waste (OFMSW) is a promising end-of-life scenario, as it reduces pre-treatment and increases process efficiency and biogas production.

However, PLA shows poor biodegradability under anaerobic digestion (AD) conditions, requiring long retention times (HRTs) compared to industrial OFMSW plants' HRTs. This limits their integration into biogas plants and their potential for integration in the circular bioeconomy. To overcome HRT's limitations of PLA, Microbial acclimation has emerged as an eco-friendly strategy to enhance bioplastic degradation; however, its potential to degrade PLA in co-digestion with OFMSW remains unexplored.

Moreover, PLA commercial products are present in the waste stream with varying thicknesses and shapes due to their diverse applications; however, the impact of product thickness on the anaerobic degradation performance of these products, when the same total surface area is maintained, is poorly understood.

In this regard, this thesis addresses these gaps by investigating, in the first study, microbial acclimation as a strategy to enhance PLA biodegradability and biogas production under mono- and co-digestion, as well as the microbial communities enriched during PLA acclimation in different settings.

Moreover, in the second study, the role of product thickness on their anaerobic degradation performance was studied. The first study results demonstrate that microbial acclimation significantly improves PLA biogas yield in mono-digestion by up to 152% (831 ± 11 NL $kgVS^{-1}$) and biogas production rate from 27 to 47 NL $kgVS^{-1}$ d^{-1}, with a reduction in the lag phase by 5

days. This enhancement was linked to the enrichment of the PLA-degrading bacteria *Tepidanaerobacter*. While in PLA + OFMSW co-digestion, biogas production increased by 69% (827 ± 69 NL $kgVS^{-1}$), the biogas production rate increased to 58 NL $kgVS^{-1}$ d^{-1}, accompanied by a 7-day reduction in the lag phase.

In the second study, the effect of PLA product thickness on their anaerobic biodegradation performance was investigated using four commercial products (Cup50, Cup100, Spoon450, Fork750) while maintaining the same total surface area. The findings showed that, in run1, cumulative biogas production differed significantly ($p \leq 0.05$) between all PLA products of varying thickness. The thinnest product, Cup50, produced the highest biogas yield (836 ± 81 NL $kgVS^{-1}$), which decreased with PLA thickness: Cup100 (731 ± 25 NL $kgVS^{-1}$ >Spoon450 (633 ± 23 NL $kgVS^{-1}$) > Fork750 (470 ± 15 NL $kgVS^{-1}$). after acclimation (run2), cumulative biogas production was similar among the products ($p > 0.05$). Nevertheless, the biogas production rate increased from run1 to run2. Kinetic analysis (modified Gompertz) showed that microbial acclimatation increased the degradation rate, resulting in a reduction in the time needed to produce 90% of the total producible biogas (t_{90}) from 108 to 42 days for Cup50 and from 98 to 45 days for Cup100, with these retention times being comparable to those that operate at full-scale plant. However, thicker products showed a different kinetic behavior (zero-order) after acclimation, with t_{90} reduced from 112 to 92 days in Spoon450 and Fork750 from 146 to 86 days, which still exceeding the full-scale plant HRT. However, microbial acclimation recovered 45% of their biogas potential within 45 days. Additionally, incomplete PLA decomposition suggests that AD and composting can provide free bioplastic digestate.

Moreover, combining microbial acclimation strategies with insights into product thickness paves the way for more efficient and sustainable PLA management and renewable energy recovery in the circular bioeconomy.

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Thesis Structure

The thesis was organized as follows: **Chapter 1** provided background, a literature review, research gaps, thesis aims, and structure. Where **Chapter 2:** First research paper (published), Investigating microbial acclimation as a strategy to improve PLA biodegradability and biogas yield in mono and co-digestion with OFMSW under thermophilic anaerobic digestion conditions. **Chapter 3:** Second research paper (to be submitted), evaluate the influence of product thickness on PLA degradation under unacclimated and acclimated conditions. **Chapter 4:** Conclusions, summarizing the primary outcomes and the future work.

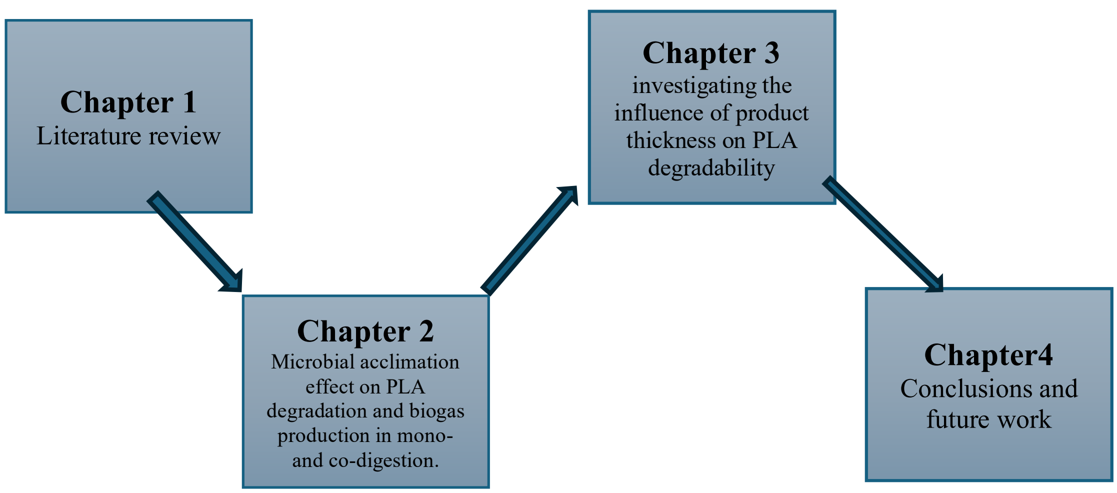

Figure 1- Thesis structure

Chapter 1

1. Introduction

1.1. Plastic problem

Plastic is a fundamental material in modern life, as it is widely used in many industries from adhesives, coatings, foams, pipes, tanks, packaging materials to textile and industrial fibers, agricultural films, automobile parts and construction due to its lightweight, versatility, durability and low production (Idumah & Nwuzor, 2019; Leal Filho et al., 2019; Van Eygen et al., 2017). However, its unsustainable production, limited degradability, and poor waste management are major problems (Walker & Fequet, 2023). This linear "produce, use, and dispose" economy has resulted in a massive accumulation of plastic waste (Johansen et al., 2022), with only 9% being recycled ,12% incinerated and 79% ending up in landfills or leaking into natural ecosystems (Geyer et al., 2017). Furthermore, the global plastic waste production is expected to be triple (OECD, 2022), which can cause serious environmental problems according.

Plastics can fragment slowly into micro- and nano-plastics (Amparán et al., 2025), and these microplastics have been detected in various ecosystems, including seas, freshwater systems, sediments, soils, and the atmosphere (Sunny et al., 2025) , with numerous negative implications for human health (Lee et al., 2023) and aquatic ecosystems (Thacharodi et al., 2024). Conventional plastics largely follow a linear economy model of “produce, use, and dispose,” which has led to their massive accumulation in the environment (Johansen et al., 2022).

In response to this challenge, a transition to a circular economy (CE) has gained traction. It should be applied across the entire plastics value chain to ensure circular design, production, use and waste management (Johansen et al., 2022). Bioplastics have the potential to address these concerns within this framework (Atiwesh et al., 2021). Compared to conventional plastics, bioplastics have several advantages, including reduced carbon footprints, improved material properties, and support for a circular economy through the use of renewable resources, as well as providing an alternative end-of-life option due to their biodegradability (Rosenboom et al., 2022)

1.2. Circular Economy and Bioplastics

The "take, make, and dispose" model of the modern linear plastic economy is a primary driver of global plastic pollution (Johansen et al., 2022). In contrast, a Circular Economy (CE) aims to eliminate waste and encourages practices such as recycling, reuse, composting, and anaerobic digestion (Kirchherr et al., 2023). Within this framework, bioplastics have been identified as a crucial element for achieving circularity (Ritzen et al., 2023). Their integration is based on three key aspects : (i) their production from renewable feedstocks (Zhao et al., 2023); (ii) their production is often associated with lower greenhouse gas emissions compared to conventional plastics, thereby supporting climate mitigation strategies (Islam et al., 2024); and (iii) bioplastics are developed with multiple end-of-life options, such as industrial composting or anaerobic digestion, which broaden the range of recovery pathways available and make them more compatible with circular systems (Rosenboom et al., 2022). However, the integration of bioplastics into the circular economy requires more than just renewable origins and a lower carbon footprint (Ritzen et al., 2023). True circularity depends on effective management of materials across their entire lifecycle, from design and production to use and recovery (Ritzen et al., 2023; Rosenboom et al., 2022). A critical factor is that the sustainability and circularity of bioplastics depend on their recovery at end-of-life (Ritzen et al., 2023). Without efficient management systems, Bioplastics may still contribute to plastic pollution (Ritzen et al., 2023). Consequently, they can only play a sustainable role in the circular economy if supported by robust recovery strategies and infrastructure. This highlights the critical importance of assessing end-of-life options in evaluating the potential of bioplastics within a circular economy framework.

1.3. Bioplastics: Definitions and Market

The term "bioplastics," as defined by European Bioplastics, , is a bioplastic if it is either biobased, biodegradable, or composed of both these properties (Leal Filho et al., 2019). Biobased refers to materials that are derived entirely or partially from renewable biomass sources. At the same time, "biodegradability" involves the microbial conversion of these materials into water, carbon dioxide, methane, and biomass (Abang et al., 2023).

Bioplastics can be classified into three categories (Nizamuddin et al., 2024). The first category comprises those bio-based and not biodegradable, such as bio-polyethene bio-PE) and bio-polyethene terephthalate (bio-PET). The second category encompasses biobased and biodegradable materials, including polylactic acid (PLA) and polyhydroxyalkanoates (PHA). The third category comprises bioplastics derived from petrochemical feedstocks but still biodegradable, such as polybutylene adipate terephthalate (PBAT) and polycaprolactone (PCL) (Faizan Muneer et al., 2021).

Global bioplastic production is expected to reach 5.726 million tonnes by 2029, a sharp increase from the estimated 2.469 million tonnes in 2024 (European Bioplastics, 2024). The production of bio-based, biodegradable plastics is expected to rise from 1.390 million tonnes in 2024 to 3.778 million tonnes by 2029 (European Bioplastics 2024). These bioplastics are used for food packaging, medicinal implants, and building construction (European Bioplastics, 2024).

1.4. Polylactic Acid (PLA)

PLA is considered a promising bio-based and biodegradable bioplastic that contributes to a sustainable future(Abdelshafy et al., 2023). In 2024, PLA accounted for 37% of biobased biodegradable plastic production, with this share projected to rise to 42% by 2029 (European Bioplastics, 2024). It is used in many industries, such as including packaging (e.g., films, containers, cutlery), medical applications (e.g., sutures, implants, drug delivery systems), agriculture (e.g., mulch films, pots), textiles, and 3D printing (European Bioplastics, 2024). Notably, the high PLA market share primarily contributes for polymerization (European Bioplastics, 2024). PLA is synthesized from lactic acid (LA) monomers, which act as the main building blocks in the polymerizations process (Södergård et al., 2022). Lactic acid is typically produced by fermentation from renewable first-generation feedstocks such as corn, wheat and potatoes (Liang et al., 2015). However, recent studies have extensively explored the production of lactic acid from various wastes as second-generation feedstocks. These include lignocellulosic waste (Díaz-Orozco et al., 2025) and food waste (Song et al., 2024). This shift can reduce production costs and promote a circular economy by valorizing waste streams(Islam et al., 2024; Kruczek et al., 2025). After lactic acid production and purification, into PLA primarily through ring-opening polymerization, the most widely used approach (Mou et al., 2025). The resulting polymer can then be processed through various methods, such as injection moulding, sheet and film extrusion, blow moulding, foaming, fibre spinning, and thermoforming (Castro-Aguirre et al., 2016).

From a sustainability perspective, PLA is particularly valuable because it is both biobased and biodegradable. It is important to note, however, that without adequate waste management, PLA can still accumulate in the environment and contribute to plastic pollution, undermining its ecological benefits (Cucina et al., 2021).

1.5. End-of-Life Management of PLA

Effective end-of-life (EoL) management is just as crucial as increasing PLA production to improve the overall sustainability of bioplastics throughout their life cycle(Islam et al., 2025). The PLA life cycle includes four main stages: (1) collection and conversion of feedstocks, (2) processing, (3) usage, and (4) end-of-life (EoL) management (Rezvani Ghomi et al., 2021). PLA waste may undergo several end-of-life (EoL) pathways, including mechanical recycling, chemical recycling, landfilling, incineration, composting, and anaerobic digestion (AD) (Islam et al., 2025). Each scenario has its own advantages and drawbacks, often shaped by technical, environmental, and economic factors.

Mechanical recycling allows the recovery of secondary raw materials for the production of new objects with similar properties (Babaremu et al., 2022). However, the presence of contaminants such as conventional plastics significantly reduces the quality of recycled bioplastics (Al-Salem et al., 2009). Moreover, a separate recycling stream requires a critical mass of biopolymers, estimated at ~200 million kg per year, to be economically viable (Cornell, 2007). This pathway also involves potential costs and logistical challenges connected with recovery and separation (Zaborowska & Bernat, 2023).

Chemical recycling, in contrast, breaks polymers down into smaller molecules, such as monomers and oligomers, through various chemical processes (Harasymchuk et al., 2024). However, it remains unattractive due to its high energy demands and a lack of a complex pre-sorting stage (Schade et al., 2024).

Landfilling is a widespread disposal method due to its simplicity and low cost, as it does not require pretreatment or sorting (Folino et al., 2020). Nevertheless, it is considered the least desirable option (Folino et al., 2020). A large share of bioplastics still ends up in landfills due to limited sorting and composting infrastructure (Ali et al., 2023). The anaerobic process in landfills produces fugitive methane (CH_4), a greenhouse gas with a warming potential 21 times greater than that of CO_2 (Gómez & Michel, 2013). It can also release microplastics into the environment(Wojnowska-Baryła et al., 2022). Incineration, another traditional option, reduces waste volume but produces greenhouse gases, particulates, ash, and toxic emissions, thereby contributing to air pollution and posing human health risks (Ali et al., 2023).

Biodegradation-based strategies, such as composting and anaerobic digestion, are increasingly considered as more sustainable alternatives.

Composting is the controlled aerobic conversion of organic waste into CO_2, H_2O, heat, minerals, and humus for soil application. Composting is the controlled aerobic conversion of organic waste into CO_2, H_2O, heat, minerals, and humus for soil application (Fredi & Dorigato, 2021). However, it requires ample land space, and is linked to greenhouse gas emissions, leachate formation, odour pollution, and net energy use (Lin et al., 2018). Anaerobic digestion (AD) , however, has emerged as one of the most promising routes for the end-of-life management of bioplastics such as PLA

(Abraham et al., 2021). AD facilitates the effective processing of bioplastic waste due to its minimal space requirements, reduced environmental pollution, and renewable energy production (Abraham et al., 2021).

1.6. Anaerobic Digestion (AD): Process and Potential

The AD process begins with hydrolysis, which is considered the rate-limiting step of AD, where the degradation of complex organic material, such as carbohydrates, proteins, and fats, happens to form soluble monomers like sugars, amino acids, and long-chain fatty acids (Franca & Bassin, 2020; Surendra et al., 2014). The subsequent transformation of monomers into short-chain fatty acids and alcohols is referred to as acidogenesis. These products will be transformed further to acetic acid, hydrogen, and carbon dioxide in a process known as acetogenesis. Finally, biogas will be generated through the conversion of hydrogen, carbon dioxide, and acetic acid into methane and carbon dioxide, at the last stage of the process called methanogenesis.

Biogas typically consists of 60–70% methane and 30–40% carbon dioxide (Franca & Bassin, 2020; Sikora et al., 2017), and it can be purified to biomethane, achieving a purity of 95–99% making it suitable for use as vehicle fuel or integration into natural gas grids by advanced upgrading methods (Alengebawy et al., 2024). Digestate is another product of AD, a nutrient-rich by-product containing stabilized organic matter and essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K). which can be used as organic fertilizer, promoting sustainable agricultural practices (Alengebawy et al., 2024; Zielińska & Bułkowska, 2024).

AD can operate under two temperatures mesophilic and thermophilic, which have different effects on PLA degradation (Fernández-Rodríguez et al., 2013), and under different hydraulic retention times (HRTs), depending on the type of feedstock being treated. Process HRTs are typically around 30-50 days for biowastes and 50-120 days for agricultural wastes (Cazaudehore et al., 2022). This is a critical operational parameter. for ensuring efficient degradation process.

Bioplastic waste is usually collected with the OFMSW, and as a result, the content of bioplastics in the waste can potentially reach the concentrations of 8–10 % of OFMSW by weight, posing the problems of bioplastic waste management, degradation, and leakage in the environment (Cucina et al., 2021). In standard AD practices, operators often depackaged food waste from bags, to prevent its negative impacts on AD performance such as clogging the pipes and incomplete degradation (Dolci et al., 2021; Taneepanichskul et al., 2022). The co-digestion of bioplastics with food waste can offer multiple advantages, including reducing the loss of food waste and enhancing the profitability of the biogas plant (Dolci et al., 2021). Moreover, it can also help by balancing the ratio of nitrogen and carbon, which enhances process performance. Bioplastics are rich in carbon and can increase the C: N ratio during anaerobic digestion with substrates that have a lower C: N ratio, thereby resulting in increased biomethane production (Stroot et al., 2001).

Additionally, it can also avoid the inhibition caused by the accumulation of volatile fatty acids (VFAs), which often occur due to the rapid hydrolysis of highly degradable food waste fractions —a common issue in food waste feedstocks, as demonstrated in the study(Cazaudehore et al., 2023). In terms of energy recovery, co-digestion of PLA with food waste can enhance methane and biogas production, thereby offering a promising approach to improving the circulation of PLA waste (Yu et al., 2023). In terms of co-digestion, (Lu et al. (2022) investigated the degradation of PLA with kitchen waste (KW) at 55 °C. They reported a production of 693 ± 10 L CH4 $kgVS^{-1}$ under thermophilic conditions after 30 days, while the theoretical CH4 potential of PLA (100 % PLA degradation) (i.e., 1092 ± 8 L CH4 $kgVS^{-1}$) was reached after 120 days. While (El-Mashad, 2012) observed no biogas production over 43 days from PLA-based cups alone in a co-digestion with food waste at 50 °C. However, when the substrate was changed to PLA-based straws, a biogas production of 0.024 L gVS^{-1} was achieved. Both studies suggest that PLA did not fully degrade until the complete degradation of food waste had occurred. Therefore, there is a need for a strategy to shorten PLA's HRTs and effectively facilitate the co-digestion of PLA and OFMSW and improve AD's workflow, process, microbiology, and end-products quality.

1.7. Factors Affecting PLA Biodegradation in AD

The anaerobic degradation of PLA is influenced by both the digestion conditions and the properties of the biopolymer (Falzarano et al., 2023). Operational parameters, such as temperature, have an effect on PLA, as thermophilic conditions can enhance PLA degradation and consequently biogas and methane production compared to mesophilic conditions (Battista et al., 2021; Bernat et al., 2021; Cazaudehore et al., 2021; Cazaudehore et al., 2023) (Table 1). This effect occurs when the temperature reaches close to or at the glass transition temperature (Tg) of PLA, the polymer chains will be freely mobilized. These facilities penetrate microbes and enzymes (Cazaudehore et al., 2021; Cucina et al., 2021).

Table 1. Summary of PLA anaerobic digestion studies under mesophilic and thermophilic conditions.

Sample	Temp (°C)	Inoculum source	Time (days)	Methane yield	Degradation (%)	Reference
PLA cup (< 1 mm)	35	Pig slurry mixed with synthetic medium	90	–	0%	(Vasmara & Marchetti, 2016)
PLA film with 150 µm thick cut it in 20 × 20 mm squares	37	Wastewater treatment plant	32	59.38 N ml CH_4 g VS^{-1}	11%	(Gadaleta et al., 2024)

PLA (Unitika) 125-250 µm	37	Digester treating cow manure & green waste	277	-	29-49%	(Yagi et al., 2014)
PLA (Ingeo 2003D, NatureWorks) 0,15 mm	35	Lab inoculum with nutritive media	40	1 L CH_4 kg^{-1} ThOD	0%	(Benn & Zitomer, 2018)
PLA (Nature Plast), ground in liquid N_2 using centrifugal mill	38	Agricultural byproduct digestate	500	–	80.3 %	(Cazaudehore et al., 2023)
PLA (Total Corbion), ground in liquid N_2 using centrifugal mill				–	74.7	
PLA-based cups	55	Municipal wastewater digester	100	453 L CH_4 kg VS^{-1}	100	(Zaborowska et al., 2023)
PLA cutlery (2.5 × 2.5 cm)	55	Sewage sludge	30	154 ± 31 ml CH_4 g VS^{-1}	21.5	(Cucina et al., 2022)
PLA dish (2.5 × 2.5 cm)				108 ± 27 ml CH_4 g VS^{-1}	19.1	
PLA (Nature Plast), ground in liquid N_2 using centrifugal mill	55	Agricultural byproduct digestate	100	–	74.6	(Cazaudehore et al., 2023)
PLA (Total Corbion), ground in liquid N_2 using centrifugal mill				–	74.6	
PLA commercial products (≈1 × 1 cm squares)	55	Trickling filters & activated sludge	385	507 ± 24 L CH_4 kg VS^{-1}	100	(Olaya-Rincon et al., 2025)

For instance,(Cazaudehore et al., 2023) evaluate the degradability of PLA-based material in AD at 38 °C and 58 °C achieving a degradation of 75 % in 500 and 100 days, respectively. At the same time, Other studies reported no degradation of PLA after 40 days at 35 °C (Benn & Zitomer, 2018). Additionally, Vasmara & Marchetti, 2016) evaluate the degradability of PLA plastic cups (< 1 mm) at 35°C, noting that no methane production occurred after 90 days. These studies indicate the lower degradability of PLA under both mesophilic and thermophilic conditions, as well as the incompatibility of their degradation time with the industrial full-scale plant HRTS. On the other hand, the chemical and physical properties of bioplastic materials are key factors affecting their degradation behaviour (Ali et al., 2023). One of the important properties is its Molecular weight; the higher the molecular weight, the slower the degradation rate (Ranakoti et al., 2022). Another important property is crystallinity. PLA can be crystalline (ordered) or amorphous (disordered), or semi-crystalline (a combination of both). However, most PLA applications use the semicrystalline form due to its strength and durability(Senila et al., 2025). Moreover, the crystalline form is more resistant to degradation by bacteria, while the amorphous form is easily degraded. In the case of semicrystalline, degradation begins with the amorphous portion, until the embedded crystalline region is exposed to its enzyme (Carboué et al., 2022). The thickness and surface area also strongly influence degradation. Where thicker materials are, the more it takes to degrade (Rujnić-Sokele & Pilipović, 2017). Surface area, as degradation occurs on the surface of the polymer (Dotson et al., 2024). Therefore, the larger the surface area, the higher the degradation rate, as a greater surface area is exposed to microbial activity and enzymes (Ciuffi et al., 2024). Moreover, (Zhao et al., 2024) examined the effect of PBAT/PLA blend bags of two thicknesses (30 µm and 40 µm) and dimensions (20 × 20 mm, 2 × 2 mm, and 1 × 1 mm) during AD co-digestion with food waste (FW) under mesophilic conditions, demonstrating that 30 µm bag produced more methane (i.e. 260 ± 2 mL g VS^{-1}) compared to 40 µm bag(i.e. 197 ± 4 mL g VS^{-1}) after 38 days. Furthermore, Bracciale et al. (2023) investigated the degradation under thermophilic AD condition of four commercial PLA-based items (cups and plates) with thicknesses of 133 μm and 205 μm for the cups (PLA4, PLA1, respectively) and 257 μm and 257 μm for plates (PLA2, PLA3), all having the same size (1.5 × 1.5 cm). They highlighted that thinner products degraded faster in both early and late process stages, but that all items ultimately achieved similar biodegradation degrees (86–100 %) and biogas yields (1620–1830 mL/g TOC) at the end of the test, which ranged from 38–45 days except one case, lasting until 52 days. These results underscore that PLA can be completely degraded, but the kinetic rate can vary depending on the item's thickness. Although this study provides valuable insight into the role of thickness on biodegradation of PLA-based products, it examined a limited range of thicknesses (133–257 μm) and product types (cups and plates), and it did not consider the role of higher thicknesses in driving PLA degradation.

1.8. Microbial Communities in PLA Degradation

The microorganisms associated with AD have been intensively investigated in recent years. However, little is known about the specific microbial consortia involved in the anaerobic digestion of bioplastics, particularly PLA. Understanding the composition of these microbial communities is crucial to enhance the biodegradability of bioplastics in AD and achieve a more efficient co-digestion with food waste (Peng et al., 2022) In the studies by (Yagi et al., 2013, 2014) anaerobic digestion of different bioplastics (PLA, PCL, and PHB) was carried out under mesophilic and thermophilic conditions. Several microorganisms associated with the anaerobic digestion process of these polymers were successfully identified; however, their specific roles in the process remain unknown.

Under mesophilic conditions, *Xanthomonadaceae bacteria and Mesorhizobium* sp. participated in the digestion of PLA, while under thermophilic conditions, *Ureibacillus* was specific to PLA digestion. Additionally, Bacillus *infernus* and *Propionibacterium* were successfully identified as microorganisms associated with anaerobic digestion of all the tested polymers. Further research has identified other key genera. (Tseng et al., 2019) highlighted the importance of the genus *Tepidimicrobium* as a key genus in AD of PLA, after examining the microbial communities involved in AD PLA under thermophilic conditions using PCR-DGGE. Later, Subsequently,(Tseng et al., 2020) were able to isolate and characterise a strain of *T. xylanilyticum* from the same digester, observing that it suppressed the physicochemical depolymerisation of PLA due to lactate accumulation, thereby directly providing methanogens with CO_2, H_2, and acetate.

Other studies have pointed to different key players. (Cazaudehore, Guyoneaud, Lallement, et al., 2022) reported a correlation between the genus *Tepidanaerobacter* and the enhanced methane production from PLA in the thermophilic reactors. Supporting this, (Clagnan et al., 2023) successfully enriched the genus *Tepidanaerobacter* through microbial acclimation over three subsequent runs of exposure to PLA, a strategy that was correlated with improved biogas and methane production.

1.9. Strategies to Enhance PLA Degradation in AD

PLA has low biodegradability under both mesophilic and thermophilic conditions, often requiring 100–500 days for complete digestion (Table 1). This slow rate poses a significant challenge for designing efficient organic waste management systems, creating an urgent need for strategies to enhance its degradation.

1.9.1. Pretreatment technologies

Pretreatment is one of the potential strategies to enhance bioplastics degradation in AD. Three main categories of pretreatment can be used: mechanical, thermal, chemical or a combination of different strategies (Yasin et al., 2022). Recently, many researchers have investigated the use of pretreatments to enhance the biodegradability of PLA and its blends in AD (Ashraf Joolaei et al., 2024; Cazaudehore et al., 2022; Ferrentino et al., 2025).

For instance, (Nie et al., 2024) assess the impact of thermo-alkaline pretreatment (48 h, 70 °C, 1% w/v NaOH) on the anaerobic digestion of PLA pellets. The methane yield achieved was 334 ± 22 NmL/g VS, which was similar to untreated PLA, indicating that thermo-alkaline pretreatment under these conditions did not significantly enhance the biodegradability of PLA.

In contrast,, (Wang et al., 2011) applied a novel two-step strategy that coupled hyper-thermophilic treatment with ammonia addition, followed by thermophilic anaerobic digestion. This method achieved high methane conversion ratios of 82% and 77% for two types of PLA within 22 days. Moreover, the impact of alkaline pretreatment (21 °C, pH > 11, 15 days) on enhancing the methane potential of crystalline and amorphous PLA in co-digestion with food waste was investigated by (Hobbs et al., 2019). Alkaline pretreatment resulted in reached near complete for both amorphous and crystalline PLA at 97% and 99%, respectively

Despite the improvement of such strategies, they are often associated with high costs and high energy consumption (Yasin et al., 2022).

1.9.2. Microbial Acclimation

In AD systems, microbial acclimation is a sustainable strategy for enhancing the degradation of various bioplastics, including PLA and starch-based bioplastics (SBs) (Clagnan et al., 2023).

For instance, (Clagnan et al., 2023) demonstrated that acclimating microbial communities to PLA and SBS substrates in thermophilic AD improved biogas yield by 97% and 52 % respectively, after three runs of acclimation, highlighting the potential of microbial acclimation to improve both bioplastic degradation and biogas production.

1.10. Gaps Analysis

Co-digestion of PLA with OFMSW is an ideal end-of-life scenario for their disposal; however, it is limited by the slow degradation of PLA and longer HRTs compared to those of OFMSW, thereby hindering their integration into a full-scale AD plant. To enhance PLA degradability; many strategies has been proposed such as pretreatments. However, they are often associated with high costs and energy consumption. as promising eco-friendly strategy, Microbial acclimation has been considered for enhancing bioplastic degradation. However, their potential has not been explored to enhance the co-digestion of PLA with OFMSW, and the microbial community involved in this process remains poorly understood.

Moreover, the waste stream has contained a wide range of PLA commercial products, such as cups, forks, spoons, and other items, which vary in designs as well as shapes, thicknesses, and surfaces for specific applications, which can influence their biodegradation during the AD process. However, most studies have investigated a limited range of product types and thicknesses, without controlling the total surface area. The role of product thickness in PLA degradability remains unclear, as well as the effect of microbial acclimation on the biodegradability of PLA of varying thickness.

Addressing these gaps is crucial to enhancing PLA degradation performance and enabling the effective their integration into the AD system, contributing to the circular bioeconomy framework.

2.Thesis Aim and Objectives

The aims of the PhD thesis are, firstly, to enhance the anaerobic degradation of PLA in mono- and co-digestion using microbial acclimation under thermophilic conditions. Secondly, it investigates the effect of product thickness on PLA anaerobic degradation performance under acclimated and unacclimated conditions, thereby enhancing their integration into the AD system and promoting their circular management.

Thesis Objectives:

- **Study 1 (Chapter 2):** Investigate microbial acclimation as a strategy to improve PLA biodegradability and biogas yield in mono and co-digestion with OFMSW under thermophilic anaerobic digestion conditions.
- **Study 2 (Chapter 3):** Assess the influence of product thickness on PLA degradation under unacclimated and acclimated conditions
- **Conclusion and Future Work (Chapter 4)**: Summarizing the thesis key outcomes and future perspectives.

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Chapter 2

The following chapter is based on a published paper that addresses part of the objectives introduced in Chapter 1.

Microbial acclimation of thermophilic anaerobic digestate enhances biogas production and biodegradation of polylactic acid in combination with the organic fraction of municipal solid waste (OFMSW)

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Abstract

Bioplastics are a promising alternative to conventional plastics. Their anaerobic co-digestion with the organic fractions of municipal solid waste (OFMSW) is an ideal end-of-life scenario reducing pre-treatment and increasing efficiency and biogas production. Bioplastic degradation is limited under anaerobic digestion (AD) as it requires longer hydraulic retention time (HRT) compared to industrial OFMSW plants' HRTs. Here, three AD runs were conducted sequentially under thermophilic conditions to investigate the effects of inoculum acclimation on enhancing the degradation of polylactic acid (PLA) and OFMSW in mono and co-digestion (PLA+OFMSW). In PLA mono-digestion, microbial acclimation increased biogas production up to +152% (831 ± 11 NL $kgVS^{-1}$) and biogas production rate from 27 to 47 NL $kgVS^{-1}$ d^{-1} with a 5-day reduction of the lag phase. This improvement was associated with the enrichment of the PLA-degrading bacteria *Tepidanaerobacter*. In PLA+OFMSW co-digestion, biogas production increased of +69% (827 ± 69 NL $kgVS^{-1}$), the biogas production rate increased to 58 NL $kgVS^{-1}d^{-1}$ with a lag phase reduction of 7 days. An increase of both protein degraders (*Halocella* and *Acetomicrobium*) and *Tepidanaerobacter* was achieved. In OFMSW mono-digestion, acclimation increased cumulative biogas production to +22% (719 ± 25 NL $kgVS^{-1}$) with no biogas production rate and lag phase modifications, indicating an already adapted community. A variance in *Methanothermobacter* and *Metanoculleus* abundances across treatments was linked to different biomethane productions. Microbial acclimation is a valid and economical approach to enhance biogas production and PLA degradability, alone or with OFMSW, further reducing HRTs enabling sustainable bioplastic and OFMSW waste management.

Keywords

Anaerobic digestion; Biogas; Bioplastic; Microbial acclimation; Organic fraction municipal solid waste (OFMSW); Polylactic acid (PLA).

1. Introduction

Bioplastics are emerging as a viable alternative to conventional petroleum-based plastics and currently, they account for ~0.5% of plastics produced annually. The annual production of bioplastics is expected to increase by 66% by 2029 (European Bioplastics, 2024). PLA is the most commercially available bioplastic and accounts for 37.1% of global bioplastics production in 2024 and expected to increase to 42.3% by 2029 (European Bioplastics, 2024). PLA is used in the manufacturing of consumer goods and disposable products such as cutlery, glasses, dishes, and packaging, but also in construction, agriculture, medical applications, and fibre production (Cucina et al., 2021a). Currently, bioplastic waste is usually collected with the OFMSW and as a result, the content of bioplastics in the waste can potentially reach the concentrations of 8–10% of OFMSW by weight, posing the problems of bioplastic waste management, degradation, and leakage in the environment (Cucina et al., 2021b). In the context of circular economy, the increasing production of PLA needs therefore to be combined with a suitable waste management system to improve bioplastic sustainability.

Among the possible end-of-life scenarios, AD is a promising technology for bioplastic biodegradation (Abraham et al., 2021, Cucina et al., 2021a). Anaerobic digestion is typically used to process various types of waste such as OFMSW, at both mesophilic (35–37°C) or thermophilic (50–60°C) temperatures (Samoraj et al., 2022), to produce biomethane and digestate (or compost) that can be further used as an energy source and bio-based fertilizer, respectively (Kumar et al., 2020). Currently, in AD plants, all plastics (and as a consequence bioplastics) are de-packaged to avoid clogging of the system and the production of a digestate containing microplastics (Cazaudehore et al., 2023a; Taneepanichskul et al., 2022). This de-packaging process results in a significant loss of OFMSW, which reduces the profitability of AD (Cazaudehore et al., 2023a). Furthermore, hypothesising an absence of plastics within the OFMSW waste, an optimised co-digestion of bioplastics and OFMSW will eliminate the need for sorting and separating, thereby reducing the overall costs associated with the AD process. Industrial AD plants treat OFMSW normally at short hydraulic retention times (HRTs), i.e. between 15 and 30 days (Cazaudehore et al., 2022c). On the other hand, PLA needs longer HRTs to be completely degraded under mesophilic and thermophilic conditions, which are not compatible with the HRTs used for OFMSW. Cazaudehore et al. (2023b) assessed the degradability of PLA-based material in AD at 38°C and 58°C achieving a degradation of 75% in 500 and 100 days, respectively. Other studies reported no degradation of PLA after 60 days at 38°C, while 93% degradation was achieved at 58°C in 120 days (Jin et al., 2022). These data indicate that both thermophilic conditions and long HRTs promote PLA degradation. Thermophilic conditions are more performant since PLA reaches its glass transition (i.e., polymeric structure changes from crystalline to amorphous) at high temperatures, becoming more accessible to microbial activity and leading to a higher polymer biodegradability (Cazaudehore et al., 2021; Cucina et al., 2021b). However, even with the transition to an amorphous structure, PLA biodegradation can hardly meet industrial OFMSW HRTs.

In terms of co-digestion, a recent study investigating the degradation of PLA with kitchen waste (KW) at 55°C reported a production of 693 ± 10 L CH_4 $kgVS^{-1}$ under thermophilic conditions after 30 days, while the theoretical CH_4 potential of PLA (100% PLA degradation) (i.e., 1092 ± 8 L CH_4 $kgVS^{-1}$) was reached after 120 days (Lu et al., 2022). El-Mashad et al. (2012) observed no biogas production over 43 days from PLA-based cups alone in a co-digestion with food waste at 50°C, when changing the substrate form cups to PLA-based straws a 0.024 L gVS^{-1} production was achieved. Both studies seem to indicate that PLA did not degrade entirely until food waste completely degraded. It is therefore necessary to further research methodologies to shorten PLA's HRTs and to enable PLA and OFMSW co-digestion and improve AD's workflow, process, microbiology, and end-products quality. To overcome HRT limitations of PLA in both mono and co-digestion, various strategies can be used, such as PLA pre-treatments, the addition of additives to PLA, inoculum acclimatization or bioaugmentation (Cazaudehore et al., 2022a). Acclimatization has been successfully applied to AD of various organic wastes (e.g. olive mill effluent and extruded food waste) to improve degradability and CH_4 production (Gonçalves et al., 2011). Recently, inoculum acclimatization was successfully applied to PLA mono-digestion under thermophilic conditions, leading to an increase of PLA-degradation by 97% compared to a non-acclimated inoculum (from 395 to 779 NL $kgVS^{-1}$ of biogas) (Clagnan et al., 2023). Nevertheless, little is known about the bacterial and archaeal communities that lead to bioplastic degradation in AD, especially during acclimatization. Understanding the composition of these microbial consortia is crucial to improve biodegradability of bioplastics in AD and achieve a more efficient co-digestion with OFMSW (Peng et al., 2022).

In this context, this study aims at investigating the effects of microbial communities' acclimatization on PLA mono- and co-digestion with OFMSW, therefore using acclimatization as a low-cost approach to improve PLA degradation. Main objectives were to: *i.* acclimatize digestate microbial communities to PLA and OFMSW under thermophilic anaerobic mono- and co-digestion through a series of batch runs; *ii.* investigate the effect of acclimatization on biodegradation rate and biogas production, and *iii.* characterize the bacterial and archaeal community structure to identify the microbial drivers of acclimatization.

2. Materials and methods

2.1. Substrates: inoculum, OFMSW and PLA

The digestates used as inocula were collected from a full-scale AD plant that treats OFMSW operating under thermophilic conditions (55±2°C) (Lombardy Region, Italy). Within this plant, bioplastic is mechanically separated from the OFMSW on arrival. OFMSW is then processed by a bio-pulper (mechanical pulping) to reach a slurry-like final product that is then anaerobically digested. Digestate was collected across Spring 2023 before each AD run and immediately sieved with a 2 mm mesh to retain possible undigested materials. The digestate was then pre-incubated at 55 ± 2°C until no biogas production was detected.

Pulped OFMSW was sampled from the same plant from which digestate was sampled once at the beginning of the experimental period. After sieving with a 2 mm mesh the OFMSW was stored at -80°C. Frozen OFMSW was then defrosted when needed and used across the whole experimental period as OFMSW substrate. Digestates and OFMSW were characterized for total solids (TS), volatile solids (VS), pH, total organic carbon (TOC), total ammonium nitrogen (NH_4^+-N), volatile fatty acids (VFA), total alkalinity (TA), and the ratio of VFA to TA here referred as FOS/TAC (see section 2.3).

PLA-based cups, labelled as compostable by TÜV Austria (Austria), were purchased from an Italian supermarket (Milan, Lombardy Region, Italy). The PLA cups were then cut into 2.5×2.5 cm squares as recommended by standard tests for the assessment of anaerobic degradability of bioplastics (ISO 14855-2:2018) and used as PLA substrate.

2.2. Batch test experimental design

Three AD runs were conducted sequentially to achieve inoculum acclimation and assess its efficiency of PLA degradation with and without OFMSW. All AD runs were operated under thermophilic conditions (55 ± 2°C) using 500 mL glass bottles filled with digestate (300 g) and substate. Bottles were manually shacked daily to ensure optimal mixing. Five treatments were set up: 1) PLA mono-digestion (PLA): digestate plus PLA (3 g fresh weight (FW)); 2) PLA and OFMSW co-digestion (PLA+OFMSW): digestate plus PLA (1.5 g FW) and OFMSW (6.3 g FW) with PLA representing 50% on a carbon basis of the total substrate; 3) OFMSW mono-digestion (OFMSW): digestate plus OFMSW (12.6 g FW); 4) digestate as negative control; and 5) digestate plus microcrystalline cellulose (3g FW) as positive control. Substrate quantities were selected to ensure a carbon ratio between the substrate and digestate of 0.33 according to Cucina et al. (2021b; 2022). Although the inoculum-to-substrate ratio (ISR) was initially calculated based on fresh weight following the protocol previously established in (Clagnan et al., 2023), ISR values were also recalculated using VS to align with common reporting standards in anaerobic digestion research (**Table S1)**.

Bottles were purged with pure N_2 for 1 minute to ensure an anaerobic environment and tightly capped before the beginning of each run.

For the first AD run, all the bottles were placed at 55 ± 2°C in a static incubator. Three replicates were used for each treatment while five for PLA and PLA+OFMSW; these two additional bottles were used as controls to track residual biogas production across the three runs. After the first run, all bottles were opened and the digestate from PLA and PLA+OFMSW treatments were sieved to 2 mm to retain eventual plastic residues (none retrieved). Replicates of each treatment were then mixed and used both as inoculum for the second run and characterization analyses.

For the second AD run, four bottles were filled with 150 mL of digestate coming from the first AD run, 150 mL of fresh digestate and the same substrate weights from the previous run were adopted. A fifth bottle was kept as a control without any substrate addition to check the background methane production of potential PLA residues in the digestate after run 1. This control will be eliminated at the end of run 2. For run 3, only three replicates were used, following the same protocol proposed for the second run with a fourth bottle maintained as a control.

Although batch runs have been performed keeping constant all variables, fresh inoculum taken directly from full scale plant before each run could be different affecting AD processes (**Table 1**). To account for this variability and ensure data comparability across different runs, all runs have been carried on until maximum cellulose biogas production was achieved, i.e. 600 NL $kgVS^{-1}$ (Clagnan et al., 2023).

Bottles were regularly analysed to determine biogas production both quantitatively and qualitatively. The volume of biogas produced was measured by withdrawing gas with a 100-mL syringe (Chickering et al., 2018). Biogas production of blank and control bottles was subtracted from the biogas production of every sample. Biogas composition was analysed through gas chromatography (Agilent Technologies 3000A 2-channel Micro GC (G2801A, USA)) twice a week.

Digestate samples collected at the end of each run were then used for chemical (TS, VS, VFA, TA, TOC, and NH_4^+-N) characterization (see section 2.3).

Table 1. Chemical characterization of inoculum and substrates.

Parameter	Digestate			OFMSW	PLA
	Run 1	Run 2	Run 3		
Total solids (%)	5[a] ± 0b[b]	5 ± 0.1b	4.1 ± 0.1a	17.2 ± 0.3	99.4 ± 0.0
Volatile solids (% dry matter)	56.9 ± 0.1a	49.5 ± 2.3a	55.6 ±1.6a	86.3 ± 0.1	100 ± 0.0
Total organic C (% dry matter)	20.6 ± 2.7a	25.2 ± 0.5ab	28.1 ± 0.1b	58.1 ± 4.6	
pH	8.6 ± 0.0a	9.3 ± 0.0b	8.5 ± 0.0a	6.1 ± 0.0	
Titrable acidity (gCH_3COOH L^{-1})	1.8 ± 0.9a	4.2 ± 0.4b	1.8 ± 0.4a	3.2 ± 0.5	
Total alkalinity (g$CaCO_3$ L^{-1})	27.8 ± 0.3a	30.6 ± 0.8a	35.9 ± 0.8b	9.9 ± 0.0	
FOS/TAC	0.31 ± 0.2a	0.48 ± 0.1a	0.24 ± 0.1a	1.6 ± 0.3	
Ammonium-N (gN-NH_3 L^{-1})	3 ± 0.1a	3 ± 0.1a	2.9 ± 0.1a	0.4 ± 0.1	

[a]Av. ± St. Dev. (n=3)

[b]Letters indicate statistical differences ($p \leq 0.05$) across the three runs digestates for each parameter according to Tukey test.

2.3. Chemical characterisation

Digestates were characterized before and after each AD run (**Table 1 and 2)**. Chemical analyses on digestates and substrates were carried out following standard procedures; TS and VS were determined according to standard procedures of the American Public Health Association (Rice et al., 2017). TOC was analysed using COD 15,000 test kits (range of 1.0–15.0 g LO_2^{-1}) (Nanocolor, Macherey Nagel, Germany) and then converted to C by stoichiometric calculations. pH was determined using a pH-meter (Eutech ™ pH 700, Thermo Fisher Scientific, Waltham, MA, USA). Ammonium-N was measured with the Nanocolor Ammonium100 (4-80 mg L^{-1} NH_4^+-N) kit (Nanocolor, Macherey Nagel, Germany), and absorbance was measured with a PF-12 Plus photometer (Macherey Nagel, Germany) using the supernatant layer of each sample after centrifugation at 6,000 rpm for 15 min (Chiappero et al., 2021). VFA, TA and FOS/TAC were determined by titration as described by Di Maria et al. (2014).

All chemical analyses were conducted in triplicate. Averages and standard deviations were calculated through Microsoft Excel 2021 and its analysis toolbox. Significant differences for normal parameters were determined using analysis of variance (ANOVA) followed by the Tukey test using SPSS version 29.1 software.

Table 2. Digestate characterization at the end of each run.

Parameter	Run 1				Run 2				Run 3			
	Cellulose	OFMSW	PLA	PLA+OFMSW	Cellulose	OFMSW	PLA	PLA+OFMSW	Cellulose	OFMSW	PLA	PLA+OFMSW
Total solids (%)	3.6[a] ± 0a[b]	3.9 ± 0.5a	3.7 ± 0a	3.8 ± 0.2a	3.6 ± 0a	3.2 ± 0.4a	3.5±0.1a	3.5 ± 0a	3.7 ± 0.1a	3.7 ± 0.4a	3 ± 0.1a	3.2 ± 0a
Volatile solids (% dry matter)	53.1 ± 0.3a	61.4 ±13.6a	51.1 ± 0.4a	54.1 ± 2.4a	50.1 ± 0.6a	50.2 ± 0.1a	51 ± 0a	47.3 ± 2.5a	54.9 ± 0.2a	49.9 ± 1.8a	49.7 ± 0.4a	44.8 ± 7a
Total organic C (% dry matter)	41.9 ± 0.4a	39.6 ± 0a	37 ± 4.3a	46.4 ± 2.5a	35.9 ± 1.5a	36 ± 0.4a	34 ± 2a	35.4 ± 0.8a	54.9 ± 0.2a	49.9 ± 1.8a	49.7 ± 0.4a	44.8 ± 7a
pH (pH unit)	8.6 ± 0a	8.6 ± 0a	8.5 ± 0a	8.41 ± 0a	8.6 ± 0a	8.5 ± 0a	8.5 ± 0a	8.4 ± 0a	8.5 ± 0a	8.5 ± 0a	8.3 ± 0a	8.4 ± 0a
Titratable acidity ($gCH_3COOH\ L^1$)	2.2 ± 0.3ba	2.8 ± 0b	1.8 ± 0a	2 ± 0.1a	1.8 ± 0.1c	1.4 ± 0b	1.1 ±0a	1.9 ± 0.1c	1.5 ± 0.1a	1.48 ±0.2a	1.1 ± 0a	1.6 ± 0.1a
Total alkalinity ($gCaCO_3\ L^{-1}$)	28.7 ± 0.1a	28.7 ± 0.1a	30.6 ± 0.5a	36.1 ± 2b	28.4 ± 0.2a	28 ± 0a	28.5 ± 0.9a	29.4 ± 0.2a	30.7 ± 0.8a	30.7 ± 1.6a	30.5 ± 1.8a	30.7 ± 0.4a
FOS/TAC	0.4 ± 0ba	0.5 ± 0b	0.3 ± 0a	0.3 ± 0a	0.3 ± 0b	0.2 ± 0ab	0.2 ± 0a	0.3 ± 0b	0.2 ± 0a	0.2 ± 0.1a	0.2 ± 0a	0.2 ± 0a
Ammonium-N ($gN\text{-}NH_4^+\ L^{-1}$)	3.2 ± 0a	3.2 ± 0.2a	3.2 ± 0.1a	3.2 ± 0a	2.6 ± 0a	2.9 ± 0.1a	2.7 ± 0a	2.4 ± 0.5a	3.2 ± 0a	3.4 ± 0.2a	3.5 ± 0.2a	3.6 ± 0.4a

[a](Av. ± St. Dev., n=3).

[b]Letters indicate statistical differences ($p \leq 0.05$) across the three runs for each parameter according to Tukey test.

2.4. Kinetic analysis and calculations

A modified Gompertz model was applied to determine the kinetic parameters of biogas production potential of the substrates for each run (Mu et al., 2021). Maximum biogas production rates (R_{max}) and lag phases (λ) were calculated from experimental data and subsequently applied in the following equation to evaluate the model's suitability:

$$G(t) = \mathrm{B0} \times exp\left(-exp\left(\frac{R_{max} \times e}{\mathrm{B0}} \times (\lambda - t) + 1\right)\right)$$

where *G(t)* is the cumulative biogas production (NL $kgVS^{-1}$), B0 is the biogas production potential (NL $kgVS^{-1}$), R_{max} is the maximum biogas production rate (NL $kgVS^{-1}$ d^{-1}), λ is the lag phase (days), *t* is the digestion time (days), and *e* = 2.7183.

B0 parameters were taken from literature assuming that batch experiments were carried out under optimal conditions. As reported in **Section 2.2.**, to confirm optimal environmental conditions, cellulose was used as positive control. Under optimal conditions, cellulose total production is 600 NL $kgVS^{-1}$. **Figure S1** shows that cellulose degradation reached plateau, and that experimental data had a good fitting to model curves (i.e., R^2=0.974); it can be therefore assumed that all runs were performed under optimal environmental conditions. The selected model and B0 were thus used if model fitting parameters where higher than literature (R^2=0.967) if inhibition occurred (R<0.967) data were excluded for kinetics application, as reported in the section 3.1.).

To build the model, B0 (the biogas production potential) for PLA in mono-digestion was estimated as the average (i.e. av. 840 L $kgVS^{-1}$) of the biogas productions reported by Bernat et al. (2021), where PLA-based cups were anaerobically digested at 58°C under different organic loading rates. These data were chosen over the commonly used theoretical biogas values calculated by using the Buswell equation for pure PLA (934 L $kgPLA^{-1}$) at 35°C and

1 atm (Buswell et al., 1952) as the Buswell equation does not account for the use of substrate, and/or other pathways of conversion of organic material, for bacterial biomass production (de Lemos Chernicharo, 2007). It is assumed that 10% of the total carbon contained in the polymer is utilised for bacteria biomass production (García-Depraect et al., 2023) and therefore that this 10% does not contribute to the theoretical biogas yield value as calculated by Buswell equation. The use of the Buswell equation was therefore discarded as it would have led to an overestimation of the theoretical biogas yield.

For the calculation of the B0 of OFMSW, the maximum biogas production was estimated as the average (i.e. av. 755 ± 68 (n=6)) of the anaerobic bio-gasification potential (ABP) data obtained from different OFMSW samples collected from various plants across the Lombardy region in Italy (Schievano et al., 2009; Tambone et al., 2010).

The B0 for the co-digestion treatment was calculated by multiplying the cumulative biogas production of the mono-digestion treatments previously described by the corresponding VS percentage of each substrate added in the co-digestion treatment.

To investigate the effects of co-digestion on the degradability of PLA and biogas production, theoretical cumulative biogas productions were calculated for each run of co-digestion by using the cumulative biogas production obtained from PLA and OFMSW mono-digestions within the following equation:

$$B_{co} = (B_{PLA} \times V_{PLA}) + (B_{OFMSW} \times V_{OFMSW})$$

where B_{co} is the theoretical biogas production of co-digestion (NL kgVS^{-1}), B_{PLA} is the biogas production of PLA mono-digestion (NL kgVS^{-1}), V_{PLA} is the amount of VS of PLA added within a bottle (g VS), Bo_{FMSW} is the biogas production of OFMSW mono-digestion (NL kgVS^{-1}), and V_{OFMSW} is the amount of VS of OFMSW added within a bottle (g VS).

2.5. 16S rRNA next-generation sequencing

DNA extraction was performed on all inocula and each digestate after each run. Samples (~40 ml) were pelleted at 13000 rpm for 20 min. From each pellet, DNA was extracted using the DNeasy® PowerSoil® Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA yield and purity were quantified on a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific) while quality was determined through gel electrophoresis 1% (w/v) 1×TAE agarose gels. DNA was stored at -80°C until analysis.

The NGS was performed at Novogene Co. Ltd (Cambridge, UK). Sequencing targeted the V3 and V4 regions of the bacterial 16S rRNA gene using primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) (Yu et al., 2005) and V4 region of the Archaeal 16S rRNA gene using primers U519F (CAGYMGCCRCGGKAAHACC) and 806R GGACTACNSGGGTMTCTAAT) (Porat et al., 2010). The generated DNA libraries were sequenced with an Illumina NovaSeq PE250, and the generated nucleotide sequences are available at the NCBI SRA repository (BioProject accession number: PRJNA1088852). The sequences resulting from the NGS were quality-checked through the FastQC software and analysed using DADA2 for R as per https://benjjneb.github.io/dada2/tutorial.html (Callahan et al., 2016). For the taxonomic assignment, the SILVA database v. 138.1 was used as a reference(McLaren et al., 2021).

All microbial statistical analyses were performed on R studio (version 4.3.1) as by (Clagnan et al., 2023) while MaAslin2 analyses were carried out as by https://huttenhower.sph.harvard.edu/maaslin/.

3. Result and discussion

3.1 Impact of acclimation on biogas production under mono- and co-digestion conditions.

3.1.1 Cellulose

Cellulose was degraded similarly across the three runs and final biogas productions were close to theoretical production and previous research (Clagnan et al., 2023) indicating a functioning and reproducible system (**Table 3**).

3.1.2 OFMSW mono-digestion

Such as reported in Section 2.2., fresh inoculum taken directly from full scale plant before each run could be different therefore affecting AD processes (**Table 1**). To ensure data comparability across different runs, AD runs were carried on until the maximum cellulose (control) biogas production was achieved, i.e. 600 NL $kgVS^{-1}$ (Clagnan et al., 2023). Doing so AD length differed across runs (**Table 3**) and it was generally lower for run 1 due to non-acclimation (**Figure 1**).

The first run of OFMSW mono-digestion resulted in a production of 597 ± 71 NL $kgVS^{-1}$(341 ± 42 $NLCH_4$ $kgVS^{-1}$). At the end of run 2 and 3 (i.e. after acclimatation), a significantly higher production of cumulative biogas was achieved (719 ± 25 NL $kgVS^{-1}$ and 517 ± 8 $NLCH_4$ $kgVS^{-1}$ for run 2; 730 ± 15 NL $kgVS^{-1}$ and 535 ± 10 $NLCH_4$ $kgVS^{-1}$ for run 3; i.e. +20% and +22% when compared to run 1; $p < 0.05$) (**Table 3**).

Differently from run 2 and 3, run 1 showed a curve of biogas production typical of inhibition and partial biogas production due to the toxic effects of VFAs and/or ammonium-N accumulation (Liu et al., 2016) (**Figure 1**). In this study ammonium toxicity was excluded as its content was similar in runs where this pattern was not seen. On the other hand, the content of titratable acidity was higher ($p < 0.05$) in run 1 than the other runs, i.e 2.8 ± 0.0 g

CH_3COOH L^{-1} for run 1 vs. 1.4 ± 0.0 and 1.48 ± 0.22 g CH_3COOH L^{-1}, for run 2 and 3 respectively (**Table 2**). Anaerobic digesters generally operate stably at VFA concentrations below 1–2 g L^{-1} (Angelidaki et al., 2005). In our study, run 1 showed a titratable acidity up to 2.8 ± 0.0 g CH_3COOH L^{-1}, which exceeded the common range indicated, leading to the partial inhibition of the methanation bacteria activity such as indicated by the reduced biomethane percentage, i.e. 57.1 ± 2 % of run 1 with respect runs 2 and 3 (i.e., 71.9 ± 3 and 73.3 ± 2 % respectively) **(Table 3)**. Due to this partial inhibition occurred of run 1, the kinetic parameters for this run were not considered in the subsequent discussion ($R^2 < 0.967$).

The modified Gompertz model fit biogas production (R^2 = 0.994 and 0.982) (**Table 4**). Biogas production rate (R_{max}) and the lag phase remained constant between runs 2 and 3 (i.e. R_{max} of 39 and 38 NL $kgVS^{-1}d^{-1}$ and lag phases of 7 and 8 d) indicating that the microbial community was already optimized and adapted to efficiently degrade this substrate (**Table 4**).

Table 3. Biogas production and composition during the three anaerobic digestion runs

	Parameter	**Run 1** (29d) [a]	**Run 2** (42d)	**Run 3** (37d)
Cellulose	**Biogas** ($NLkgVS^{-1}$)	651[b] ± 34a[c]	593 ± 39a	576 ± 27a
	Biomethane ($NLkgVS^{-1}$)	413 ± 32a	397 ± 19a	391 ± 16a
	Biomethane (% v/v)	63.4 ± 1	66.9 ± 2	67.9 ± 5
OFMSW	**Biogas** ($NLkgVS^{-1}$)	597 ± 71a	719 ± 25b	730 ± 15b
	Biomethane ($NLkgVS^{-1}$)	341 ± 42a	517 ± 8b	535 ± 10b
	Biomethane (% v/v)	57.1 ± 2	71.9 ± 3	73.3 ± 2
PLA	**Biogas** ($NLkgVS^{-1}$)	330 ± 23a	831 ± 11c	710 ± 11b
	Biomethane ($NLkgVS^{-1}$)	227 ±14a	466 ± 22c	362 ± 5b
	Biomethane (% v/v)	68.8 ± 2	56.1 ± 3	51.0 ± 1
PLA+OFMSW (Experimental)	**Biogas** ($NLkgVS^{-1}$)	488 ± 51a	827 ± 69b	749 ± 9b
	Biomethane ($NLkgVS^{-1}$)	326 ± 31a	485 ± 42b	430 ± 12b
	Biomethane (% v/v)	66.8 ± 2	58.7 ± 2	57.4 ± 2
PLA+OFMSW (Theoretical)	**Biogas** ($NLkgVS^{-1}$)	432 ± 31a	788 ± 16b	721 ± 11c
	Biomethane ($NLkgVS^{-1}$)	293 ± 18a	486 ± 13b	428 ± 5b

[a]The duration of the run (d) depended on the achievement of max cellulose biogas production, i.e. about 600 NL kg VS^{-1}.
[b](Av. ± St. Dev.; n=3).
[c]Letters indicate statistical differences ($p \leq 0.05$) across the three runs for each parameter according to Tukey test.

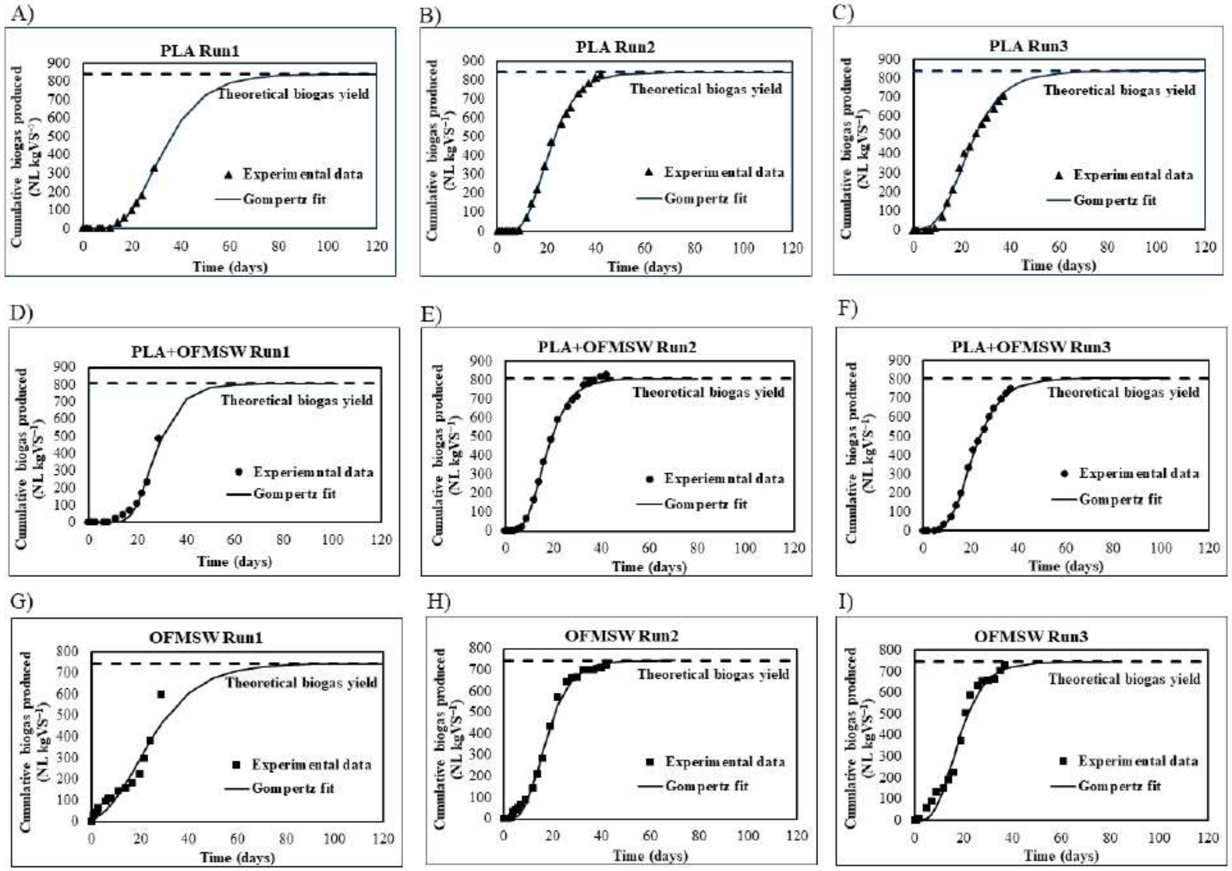
Table 4. Kinetic analysis of biogas production during the three anaerobic digestions runs.

	Parameter	Run 1	Run 2	Run 3
OFMSW	$\mathbf{B_0}$ (NL kgVS^{-1})	755	755	755
	$\mathbf{R_{max}}$ (NL kgVS^{-1}d^{-1})	20	39	38
	$\boldsymbol{\lambda}$ (d)	5	7	8
	$\mathbf{R^2}$	0.891	0.994	0.982
	$\mathbf{t_{90}}^{a}$ (d)	n.d.	31	32
PLA	$\mathbf{B_0}$ (NL kgVS^{-1})	840	840	840
	$\mathbf{R_{max}}$ (NL kgVS^{-1}d^{-1})	27	47	39
	$\boldsymbol{\lambda}$ (d)	17	12	12
	$\mathbf{R^2}$	0.997	0.996	0.985
	$\mathbf{t_{90}}$ (d)	54	33	38
PLA+OFMSW	$\mathbf{B_0}$ (NL kgVS^{-1})	806	806	806
	$\mathbf{R_{max}}$ (NL kgVS^{-1} d^{-1})	42	58	51
	$\boldsymbol{\lambda}$ (d)	17	10	13
	$\mathbf{R^2}$	0.967	0.994	0.985
	$\mathbf{t_{90}}$ (d)	40	27	32

[a]t_{90}: time required to get 90 % of the total producible biogas.

Figure 1. Experimental and predicted biogas yields of each substrate based on Gompertz model across the three runs and treatments:

PLA mono-digestion (A, B, C), co-digestion (PLA+ OFMSW) (D, E, F), and OFMSW mono-digestion (G, H, I).



A)
PLA Run1
B)
PLA Run2
C)
PLA Run3
D)
PLA+OFMSW Run1
E)
PLA+OFMSW Run2
F)
PLA+OFMSW Run3
G)
OFMSW Run1
H)
OFMSW Run2
I)
OFMSW Run3
Cumulative biogas produced (NL kgVS⁻¹)
Time (days)
Theoretical biogas yield
Experimental data
Experiemntal data
Gompertz fit

3.1.3 PLA mono-digestion

In run 1, the cumulative biogas produced from PLA in mono-digestion was of 330 ± 23 NL $kgVS^{-1}$227 ± 14 $NLCH_4$ $kgVS^{-1}$) (**Table 3**). This production is comparable to data previously reported by Clagnan et al. (2023) where PLA-based glasses mono-digested under similar AD conditions produced 395 ± 3 NL $kgVS^{-1}$ (233 $NLCH_4$ $kgVS^{-1}$). Moreover, Cucina et al. (2022) reported a slightly lower CH_4 production achieving 215 ± 11 and 279 ± 20 NL $kgVS^{-1}$ after 60 days under similar conditions for PLA-based dishes and cutlery, respectively. Similarly, Vasmara and Marchetti (2016) obtained a slightly lower CH_4 yield of 282 NL $kgVS^{-1}$for PLA-based cups after 90 days under thermophilic condition. The differences in the degradation time observed between the studies could be explained by variations in the types or sources of the inoculum, as well as differences in the composition and physical properties of the PLA materials.

In run 2, biogas production increased to 831 ± 11 NL $kgVS^{-1}$(466 ± 22 $NLCH_4$ $kgVS^{-1}$), i.e. +152% compared to run 1 ($p < 0.05$). At the end of run 3, there was a decrease in biogas production to 710 ± 11 NL $kgVS^{-1}$ (362 ± 5 $NLCH_4$ $kgVS^{-1}$; i.e., -15% compared to run 2; $p < 0.05$) although it was still higher than in run 1 (+115%) (**Table 3**). The lower productivity of run 3 was associated to the shorter degradation time (37 days for run 3 instead of the 42 days of run 2). The ratio of biomethane to biogas, expressed as methane concentration (% v/v), was between 68.8 ± 2 to 51.0 ± 1% (**Table 3**); while some variations were observed, the values remained within the range commonly reported for AD processes involving mixed substrates (50-70%), indicating that methane production was consistently maintained throughout the experiment (Taramasso et al., 2024).

Again, the modified Gompertz model fitted biogas production (R^2 = 0.996 and 0.985 for run 2 and run 3, respectively) (**Table 4**). The PLA biogas production rate (R_{max}) showed an increase from 27 NL $kgVS^{-1}d^{-1}$ (lag phase of 17 d; $R^2 = 0.997$) in run 1 to 47 and 39 NL $kgVS^{-1}d^{-1}$ to run 2 and 3, respectively (lag phases of 12 d for both runs) (**Table 4**) (**Figure 1**). The increase in the biogas production rate and the decrease in the lag phase indicated that microbial adaptation occurred within this treatment (**Table 4**).

For PLA mono-digestion, acclimation seems to be an efficient approach to improve the biodegradability of PLA. The degradation here obtained was similar to the degradability results obtained by PLA pre-treatment in literature. For example, Zaborowska et al. (2023) reported that under thermophilic AD conditions, PLA-based cups pre-treated by hydrothermal and alkaline treatments lead to a CH_4 production of 448 $NLCH_4$ $kgVS^{-1}$. Similarly, Jin et al. (2023) achieved a comparable CH_4 production (462.6 $NLCH_4$ $kgVS^{-1}$) through alkali pre-treatment and thermophilic AD of ground PLA pellets. Alkaline and thermal pre-treatments are however associated with significant costs, energy consumption and environmental issues, affecting the overall sustainability of the process. On the other hand, microbial acclimatization is cost-effective and environmentally friendly and has the potential to be a strategy to promote the circular economy of bioplastics (Clagnan et al., 2023; Cucina et al., 2023).

3.1.4 PLA and OFMSW co-digestion

Co-digestion of PLA and OFMSW produced 488 ± 51 NL $kgVS^{-1}$(326 ± 31 $NLCH_4$ $kgVS^{-1}$) in run 1. At the end of run 2, a significant increase in biogas production was achieved (827 ± 69 NL $kgVS^{-1}$), i.e. +69% if compared to run 1 ($p < 0.05$). No significant difference was seen between runs 2 and 3 (**Table 3**). Biomethane concentration for PLA+FW co-digestion was consistently lower than that observed for OFMSW alone, indicating that CH_4 content depended by the substrate added, i.e. OFMSW containing fat produced biogas with more

CH_4 (REF) than PLA, so that when PLA was added to OFMSW the CH_4 content decreased (see Table 3 run 2 and 3, run 1 was not considered to inhibition occurred).

Theoretical production, calculated starting form PLA and OFMSW mono-digestion, gave similar results to the experimental results indicating reproducibility and validating both the experimental work and data acquired. These results were a further indication that OFMSW and PLA degrade similarly in both mono- or co-digestion (**Table 3**).

The modified Gompertz kinetics showed an increase of the biogas production rate from 42 NL $kgVS^{-1}d^{-1}$ in run 1 (R^2 of 0.967) to a rate of 58 NL $kgVS^{-1}d^{-1}$ in run 2 (R^2 of 0.994). A reduction of the lag phase was also shown from 17 days to 10 d for run 1 and 2, respectively **(Table 4**) (**Figure 1**). Run 3 (R^2 of 0.985) showed a slightly reduction of the R_{max} when compared to run 2 (51 NL $kgVS^{-1}d^{-1}$) and a lag phase of 13 days **(Table 4**). These data indicated that the biogas production rate increased with acclimatation. It can be assumed that for run 1 the contribution to biogas production came above all from OFMSW degradation as microbial adaptation was not required for an efficient degradation (see section 3.1.2) and less from PLA degradation. In run 2 and run 3, the adaptation of the microbial population to PLA, started to contribute to biogas production reducing lag phase. Interesting the lag phase trend observed for PLA+OFMSW was the same of PLA mono-digestion. These results seem to confirm that microbial adaptation is needed to maximize PLA degradation.

3.2. Prokaryotic communities' characterization

The composition and dynamics of the bacterial communities of all treatments (i.e., inoculum, negative control, cellulose, OFMSW, PLA, and PLA+OFMSW) were investigated for the three batch runs performed (i.e., run 1, run 2, and run 3) through a 16S rRNA sequencing analysis with a double couple of primers one dedicated to Bacteria and one to Archaea.

3.2.1 Bacteria

Bacterial NGS analysis produced between 151,934 and 219,794 input reads while between 118,915 and 179,089 reads after DADA2 assignment (**Figure S2**).

When looking at the phyla composition, all samples showed a similar composition. Similarly to Clagnan et al. (2023), main phyla (above 2%) across most samples were Firmicutes, Cloacimonadota, Bacteroidota and Synergistota with Proteobacteria characterizing the run 3 of all treatments (**Figure S3**). Proteobacteria, Bacteroidota, Firmicutes, and Synergistota have been shown to enhance substrate (often lignocellulosic) degradation (i.e. hydrolysis, acidogenesis and acetogenesis) (Li et al., 2024). Firmicutes and Bacteroidetes have been linked to process performance and its parameters such as organic loading rate, volatile fatty acids concentration, and methane production (Chen et al., 2016), while Cloacimonadota to mutual metabolic interactions with methanogens (Feng et al., 2023).

When looking at the structure at genus level (abundance >5%), the inoculum was characterized by *Fastidiosipila* at both run 1 (14%) and run 2 (12%), a proteolytic and VFAs-producing bacteria (Wang et al., 2023), with the addition of the thermotolerant organic matter degrader *Sinibacillus* (10%) at run 2 (Zhen et al., 2021), while run 3 showed the presence of the denitrifier *Pusillimonas* (10%) (Fang et al., 2023), *Sinibacillus* (7%), and the carbohydrates fermenter *Lentimicrobium* (5%) (Li et al., 2022) (**Figure 2**).

The negative controls were characterized by a similar genera (>5%) composition of proteolytic bacteria, denitrifiers and starch, casein, and tributyrin hydrolysers with high NH_3 tolerance (Perman et al., 2022) (**Figure 2**).

The positive control (cellulose) showed again a similar composition with the addition of an enrichment in *Halocella* increasing from run 2 (14%) to run 3 (27%) (**Figure 2**). *Halocella* are hemicellulose and starch degraders whose growth seems to be impaired by the presence of PLA (Zheng et al., 2023; Wahid et al., 2021) and it was retrieved for the same treatment also in Clagnan et al. (2023).

The OFMSW treatment was characterized by *Fastidiosipila* (12%) and *Halocella* (6%) at run 1. At run 2 *Fastidiosipila* (8%) was followed by *Caldicoprobacter* (7%), another glucose-based acidogen (Xiao et al., 2024), with a combination of *Lentimicrobium* (7%), *Halocella* (5%) and *Caldicoprobacter* (5%), at run 3 (**Figure 2**). The PLA treatment was characterized again by *Fastidiosipila* (12%) with the addition of *Tepidanaerobacter* (9%) at run1. Run 2 showed again the presence of *Fastidiosipila* (7%) and *Acetomicrobium* (6%) with an increase *Tepidanaerobacter* (14%). *Tepidanaerobacter* (23%) showed a further increase in run 3 accompanied by *Lentimicrobium* (8%) (**Figure 2**). Similarly to *Lentimicrobium*, *Tepidanaerobacter, a* lactate-degrading bacterium, is positively linked to propionic and butyric acid accumulation (Xiao et al., 2024) and has been shown to positively correlate also with the increasing methane production from PLA in the thermophilic reactors (Cazaudehore et al., 2022b; Clagnan et al., 2023). Its increase in abundance over time can be seen as an indication of acclimation of the community.

Generally, co-digestion of PLA and OFMSW showed a mixed composition of genera (>5%) between PLA and OFMSW mono-digestion. Run 1 showed the presence of *Fastidiosipila* (15%), while at run 2 *Fastidiosipila* (8%) was followed by *Acetomicrobium* (6%) and *Lentimicrobium* (5%). At run 3, *Acetomicrobium* (6%) and *Lentimicrobium* (7%) were followed by *Tepidanaerobacter* (8%) and the H_2-producing bacteria *Clostridium* sensu stricto 1 (7%) (**Figure 2**).

Across most samples, genera belonging to the family of W27 and the order of MBA03 were further encountered. They are frequently observed in cellulose-based biogas reactors, and

MBA03 has been found to be a potential syntrophic acetate-oxidizer which follows similar trends to hydrogenotrophic methanogens (Perman et al., 2022).

When looking at the most enriched genera across all treatments, a significant enrichment in *Tepidanaerobacter* can be further confirmed for PLA and PLA+OFMSW. Conversely, OFMSW and PLA+OFMSW showed a further enrichment in the proteolytic bacterium *Keratinibaculum* (**Figure 3)**. The increase in protein and starch degraders is most likely driven by a dual cause, the substrate high in starch and protein (due also to the increasing biomass from digestate recycling) and secondly as protein degraders are directly contributing to PLA degradation as multiple bacterial proteases have been proven to be enzymatically active for PLA hydrolysis (e.g. the hydrolytic activity of Bacteroidales protease) (Zhu et al., 2023).

Figure 2. Bacterial (a) and archaeal (b) communities' composition at genus level with a > 5% cut-off (Av., n=2).

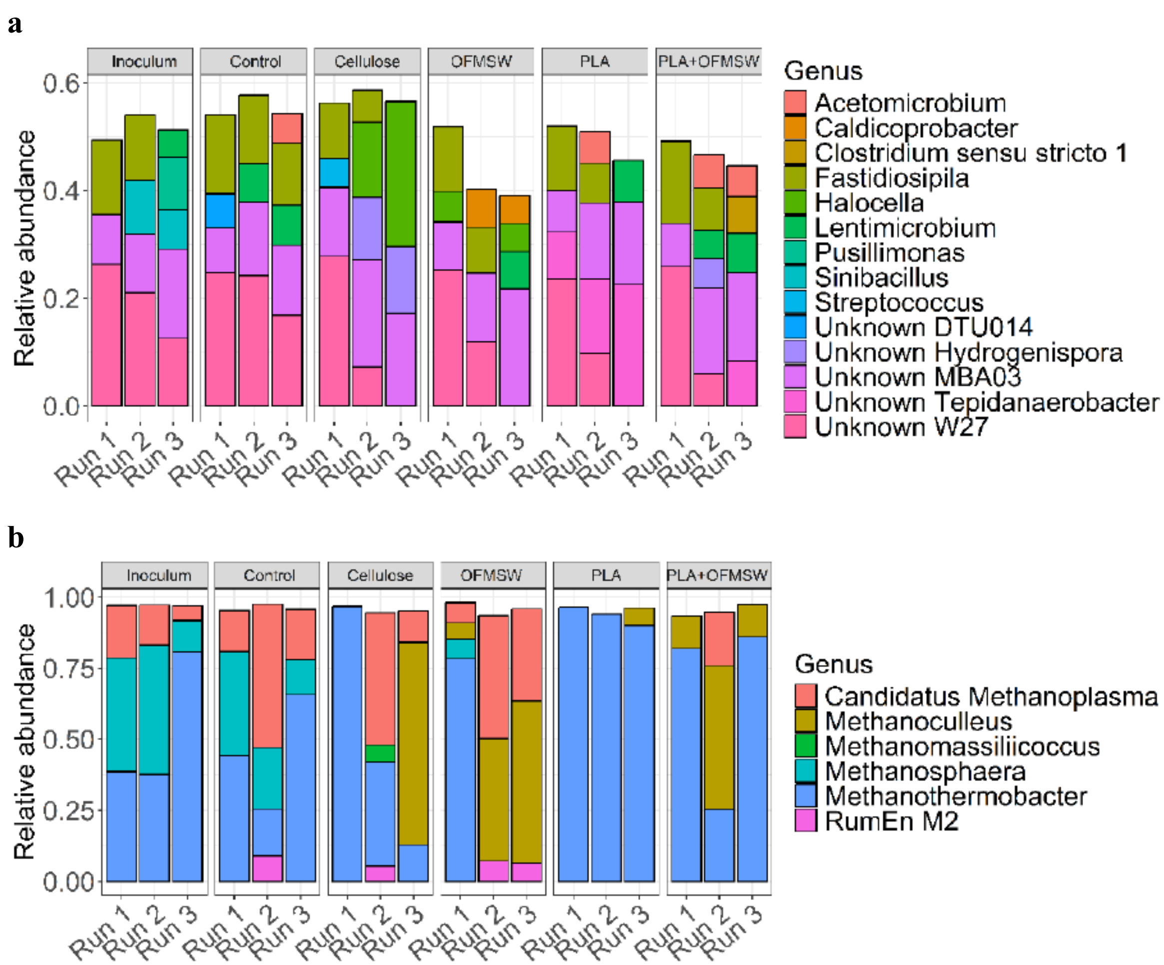
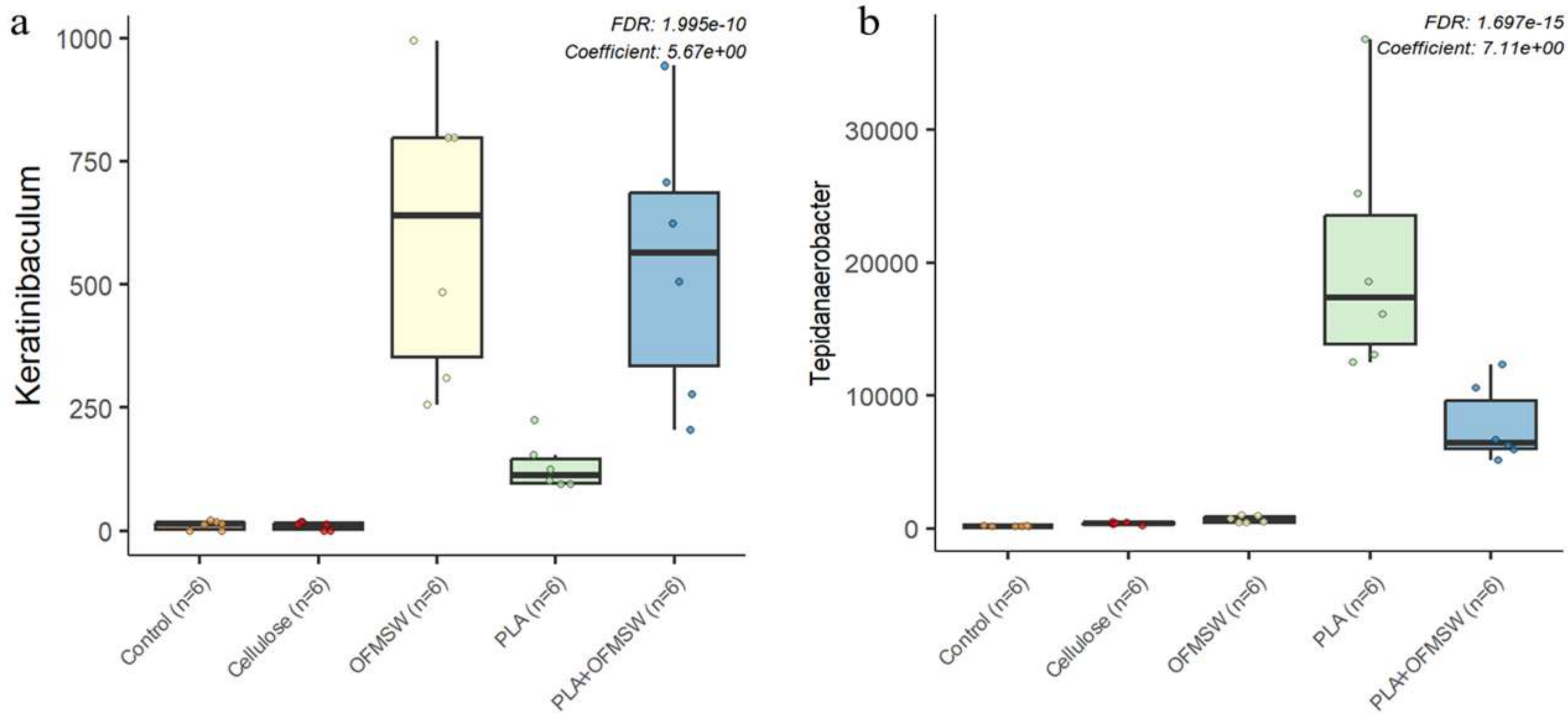


Figure 3. Selection of the most enriched genera across treatments from MaAsLin2 analysis.



a
FDR: 1.995e-10
Coefficient: 5.67e+00
Keratinibaculum
1000
750
500
250
0
Control (n=6)
Cellulose (n=6)
OFMSW (n=6)
PLA (n=6)
PLA+OFMSW (n=6)
b
FDR: 1.697e-15
Coefficient: 7.11e+00
Tepidanaerobacter
30000
20000
10000
0
Control (n=6)
Cellulose (n=6)
OFMSW (n=6)
PLA (n=6)
PLA+OFMSW (n=6)

3.2.2 Archaea

Archaeal NGS produced between 184,132 and 219,745 input reads while between 108,131 and 187,393 reads after DADA2 assignment (**Figure S2**).

Archaeal phyla composition (above 2%), included three main phyla, all containing methanogens: (i) Euryarchaeota, generally hydrogenotrophic, acetoclastic, methylotrophic, or H_2-dependent methylotrophic (Kumar et al., 2021), (ii) Halobacterota, mainly aerobic halophiles that possibly evolved from anaerobic methanogens (Martijn et al., 2020), and (iii) Thermoplasmatota that thrives under energy limitation thanks to a metabolic potentials for the degradation of aromatic and halogenated organic compound and alkane utilization (Zheng et al., 2022) (**Figure S3**).

Considering the most abundant archaeal genera (above 5%), the inocula and the negative controls showed a similar composition with the presence of mainly three genera: a candidatus *Methanoplasma*, and two hydrogenotrophic methanogens *Methanosphaera* (Ros et al., 2017) and *Methanothermobacter* (Tian et al., 2015) (**Figure 2**). Candidatus *Methanoplasma* is an understudied methanogen which has been found in the AD processes that is however correlated to low methane production (Vendruscolo et al., 2020). On the other hand, *Methanothermobacter* has been found to outcompete other methanogens thanks to its syntrophic relationship with fatty acid-oxidizers (Yan et al., 2020).

The positive control (cellulose) showed *Methanothermobacter* as main genera at run 1 (97%), which was retrieved also at run 2 (37%) together with candidatus *Methanoplasma* (46%). In run 3, the community switched to *Methanoculleus* (71%), a genus that has been found in literature to positively correlate with high ammonia levels and high methane production (Ziganshin et al., 2016) (**Figure 2**).

Similarly to cellulose, OFMSW showed *Methanothermobacter* (78%) at run 1 with a small presence of candidatus *Methanoplasma* (7%), *Methanoculleus* (6%) and *Methanosphaera* (7%) while both run 2 and 3 were characterized by a higher abundance of candidatus *Methanoplasma* (43% and 32%, respectively) and *Methanoculleus* (43% and 57%, respectively), with traces of *RumEn M2* (7% and 6%, respectively) (**Figure 2**).

PLA was characterized across all runs by *Methanothermobacter* (90-97%) with only a small abundance of *Methanoculleus* at run 3 (6%) (**Figure 2**).

PLA+OFMSW showed, both at run 1 and 3 a high abundance of *Methanothermobacter* (82% and 86%, respectively) together with *Methanoculleus* (both 11%). Run 2 showed a higher abundance of *Methanoculleus* (51%) than *Methanothermobacter* (25%) with also the presence of candidatus *Methanoplasma* (19%) (**Figure 2**).

Ammonia released from AD usually leads to suboptimal conditions with methanogens being the most vulnerable to high NH_4^+-N concentrations (Yan et al., 2020). High NH_4^+-N suppressed acetoclastic methanogenesis to the advantage of hydrogenotrophic methanogens, as seen in this study. A genomic analysis of hydrogenotrophic methanogens has found that Methanomassiliicoccales and *Methanothermobacter* carry out methanogenesis from methanol and formate (respectively) and H_2/CO_2 which is highly exergonic and might lead to a higher ammonia tolerance than *Methanoculleus* from acetate and H_2/CO_2 (Yan et al., 2020). However, a more recent study has shown how *Methanoculleus* is consistently enriched under inhibitory levels of NH_4^+-N pushing CH_4 productions over other genera such as *Methanothermobacter* (Finn et al., 2023). The same study highlighted how the adaptation of a community leads to a higher methanogenesis under suboptimal NH_4^+-N concentrations and how a successful and robust system is the results not of specific taxa but of the constant interaction and redundances between tolerant methanogens and other tolerant taxa (i.e. broadscale tolerance) (Finn et al., 2023). In this context, when looking at CH_4 production, PLA showed a trend of lower diversity among the three treatments (OFMSW, PLA+OFMSW and PLA) (**Figure S4**) and a lower abundance of *Methanoculleus,* which might have led to the trend of lower production especially in run 3.

3.3. PLA degradation vs. microbial acclimatation

These results appear of great interest considering the upscale potential to a full-scale plant treating OFMSW wastes containing bioplastics. In particular, full scale anaerobic digestion must be able to (i) completely degrade PLA therefore avoiding or limiting the content of PLA in the final digestate and (ii) maximize biogas production to which PLA is contributing (Abraham et al., 2021; Cazaudehore et al., 2022a).

Literature reported that acceptable AD performance in continuous stirred-tank reactors (CSTR) allows the achievement 90% of producible biogas (t_{90}) from OFMSW at a HRT of 30 days(Schievano et al., 2011). In this work, kinetics analyses performed using experimental data indicated that microbial adaptation allowed the reduction of the t_{90} from 54 and 40 days to 33-38 and 27-32 days, for PLA mono- and co-digestion respectively (**Table 4**). These enhanced (t_{90}) values obtained from PLA mono- and co-digestion are in line with the HRT typically applied within the real-scale OFMSW treating plant cited above.

These results showed that microbial acclimation is an efficient strategy to achieve a faster PLA biodegradation characterised by a shorter lag phase and an increase of the biogas production rate, indicating the potential to apply this concept to CSTRs at full-scale leading to a more sustainable bioplastic waste management in the context of circular economy. This study is of interest when considering future upscaling to full-scale. Microbial acclimation can be applied through two main approaches: pre-acclimation of the inoculum to the target substrate prior to reactor start-up and gradual in situ acclimation during continuous digestion by gradually increasing the organic loading rate. While no full-scale CSTR studies have yet implemented these strategies for bioplastics, their effectiveness has been demonstrated for other complex substrates. For instance, Wojcieszak et al. (2017) showed that both pre-acclimation and in situ acclimation enhanced biogas production and process stability during maize silage digestion, while Gonçalves et al. (2012) demonstrated similar improvements using pre-acclimated inoculum with olive mill wastewater. These findings strongly support the potential applicability of microbial acclimation strategies to the industrial-scale AD of bioplastics. Overall, microbial acclimation is promising and effective strategy for enhancing biogas production from PLA, both in mono-digestion and co-digestion systems. Furthermore, its application in the co-digestion of bioplastics with OFMSW will further avoid the need for de-packaging, and relative loss of OFMSW, thus increasing process profitability.

Conclusions

Microbial acclimation was a strong enhancer of PLA biodegradation and biogas production through thermophilic AD both alone and in co-digestion with OFMSW. After microbial acclimation, biogas productions and the kinetic rate of both PLA mono-digestion and co-digestion were enhanced and linked to an enrichment in PLA degrading bacteria such as *Tepidanaerobacter*; these were also characterized by a high abundance of hydrogenotrophic Archaea member such as *Methanothermobacter* and *Metanoculleus*. The time required to degrade at least 90 % of PLA was reduced after microbial acclimatation leading to a degradation time that is comparable with HRTs commonly used in CSTRs of full-scale AD plants. These results suggest that microbial acclimation is a useful strategy to improve PLA degradation efficiency especially in co-digestion with OFMSW therefore the use of this technique might lead, after further targeted research, to its use in AD full-scale plant improving PLA waste management.

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Supplementary tables

Table S1. ISR values for different substrates in the three AD runs

Sample	Parameter	Run 1	Run 2	Run 3
Cellulose	ISR*	2.90	2.78	2.02
OFMSW	ISR	4.64	4.44	3.23
PLA	ISR	2.89	2.80	2.03
PLA+OFMSW	ISR	3.58	3.43	2.50

*ISR = Inoculum-to-Substrate Ratio, calculated on a volatile solids (VS) basis

Supplementary figures

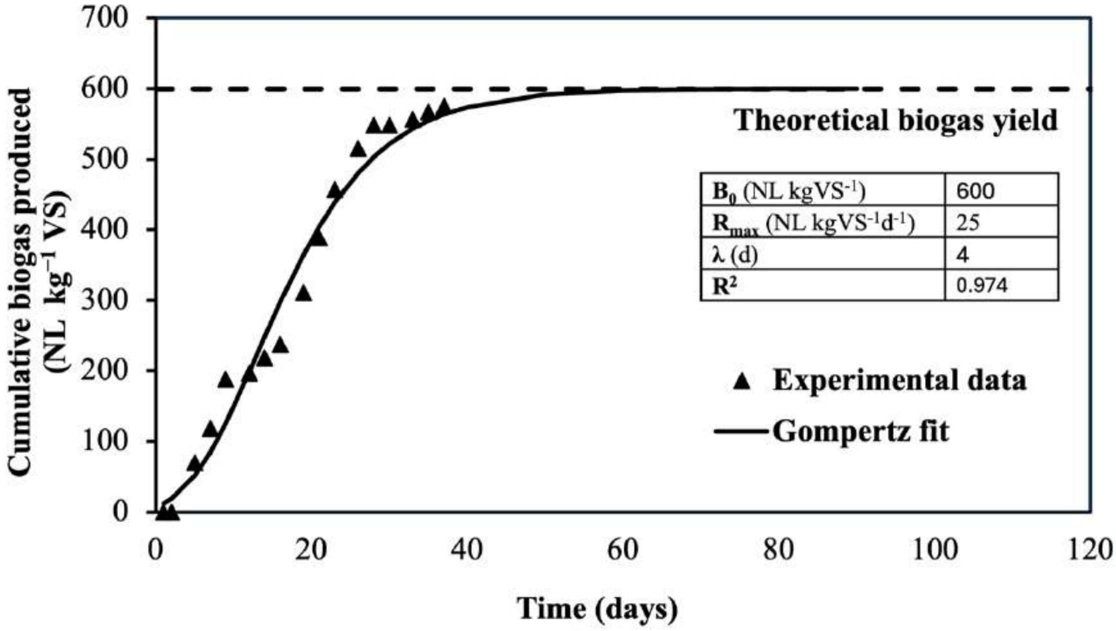


Figure S1. Experimental and predicted biogas yields of cellulose based on Gompertz model.

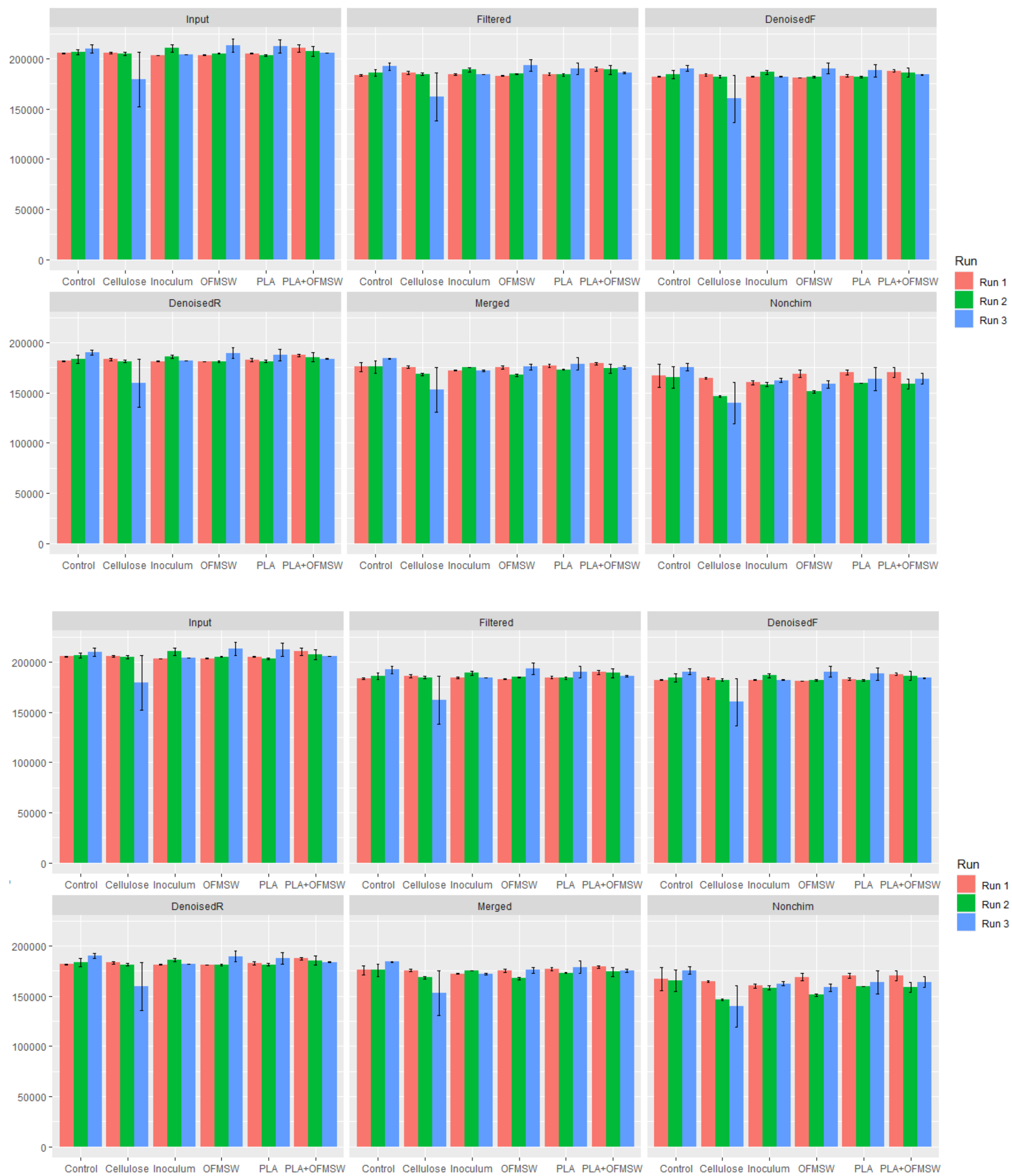
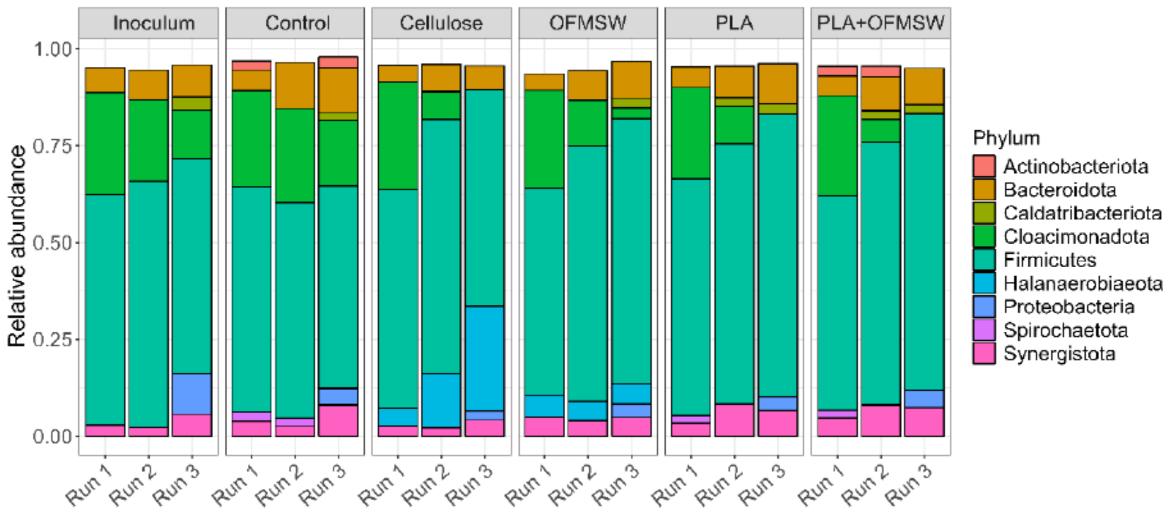


Figure S2. Number of reads retained at each DADA2 step for archaea (top) and bacteria (bottom) (Av., n=2).

a



b

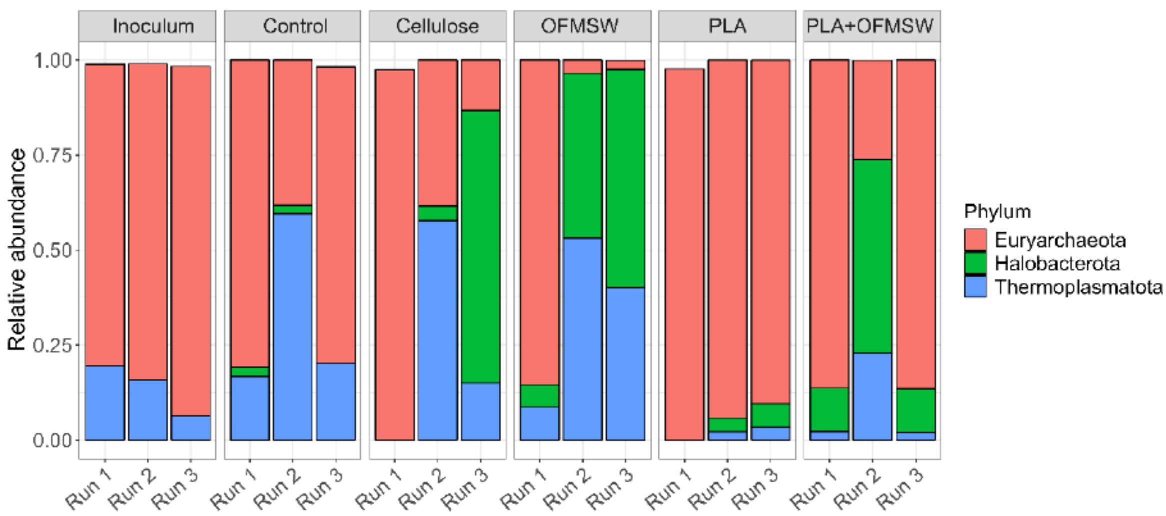


Figure S3. Bacterial (a) and archaeal (b) communities' composition at phylum level with a cut-off > 2% (Av., n=2).

Figure S4. Prokaryotic observed richness, alpha diversity community indexes and evenness for archaea (top) and bacteria (bottom) (Av., n=2).

Chapter 3

The following chapter is based on a manuscript currently ready to be submitted that addresses second part of the objectives introduced in Chapter 1.

Enhancing Polylactic Acid (PLA) degradation under anaerobic condition by microbial acclimatation: the effect of thickness.

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Abstract

In the present study, the anaerobic degradation of four PLA-based products (Cup50, Cup100, Spoon450, Fork750) with varying thicknesses was evaluated through two sequential thermophilic anerobic digestion (AD) runs for 105 days, while maintaining a constant total surface area across all sample products. In run1, cumulative biogas production differed significantly ($p \leq 0.05$) between all PLA products of varying thickness. The thinnest product, Cup50, produced the highest biogas yield (836 ± 81 NL $kgVS^{-1}$), which decreased with PLA thickness: Cup100 (731 ± 25 NL $kgVS^{-1}$ >Spoon450 (633 ± 23 NL $kgVS^{-1}$) > Fork750 (470 ± 15 NL $kgVS^{-1}$). After acclimation (run2), cumulative biogas production was similar among the products ($p > 0.05$); however, comparing the two runs, the biogas production rate increased from run1 to run2. Kinetic studies (modified Gompertz) indicated that the degradation rate increased because of microbial acclimatation, so that the time required to produce 90 % of the total producible biogas (t_{90}) reduced from 108 to 42 days for Cup50 and from 98 to 45 days for Cup100, with this retention time comparable to those operating at full-scale. In contrast, thicker products showed a different kinetic (zero-order) after acclimation, with shortened t_{90} in Spoon450, from 112 to 92 days and for Fork750, from 146 to 86 days, which were still exceeding the full-scale plant hydraulic retention time (HRT). However, 45% of their biogas potential was recovered within 45 days by microbial acclimation, which added to biogas produced by thinner PLA products, making the application of AD to PLA management interesting. Furthermore, the incomplete degradation of PLA suggests a coupling of AD with composting, allowing for the production of free bioplastic digestate.

Keywords: Anaerobic digestion (AD); Kinetic models; Thickness; Polylactic acid (PLA).

1. Introduction

In response to environmental problems associated with traditional plastics, bioplastics have emerged as a sustainable solution (Atiwesh et al., 2021). Among these promising bioplastics, PLA is considered the most extensively produced biopolymer, currently representing 37.1% of the global bioplastics market and is expected to increase to 42.3% by 2029 (Ishimwe, 2024). PLA has contributed to many sectors, including disposable cutlery, food packaging, textiles, and 3D printing (Cucina et al., 2021a; Ishimwe, 2024). Establishing suitable waste management systems for its disposal has become a critical issue with its increasing production. PLA waste can be processed through multiple end-of-life options, such as mechanical recycling, chemical recycling, incineration, composting, and AD (Folino et al., 2020). However, mechanical and chemical recycling face several challenges, including the need for effective separation of bioplastics from conventional plastics, high energy consumption, and costs (Folino et al., 2020). In contrast, AD is considered the most environmentally favorable valorization option for biodegradable waste, such as PLA, since it has a lower footprint than both composting (both at home and industrially) and incineration (Hermann et al., 2011), and it aligns with circular economy principles by converting PLA waste into methane (Abraham et al., 2021; Song et al., 2022; Swetha et al., 2023; Vardar et al., 2022).

AD is a well-established technology for treating food waste from source-separated collection, allowing for the production of energy/biofuels, and biofertilizers (Morales-Polo et al., 2018; Pilarska et al., 2023). The advent of bioplastic as a substitute for single-use plastic and food packaging and bags determined the presence in the source separated food waste-stream of bioplastic materials that started to be very consistent in terms of quantity, i.e. 3.7 % (Marchelli & Fiori, 2025), and is expected to increase to 8-10% fraction in the near future (Cucina et al., 2021a).

The increasing presence of bioplastics in organic waste streams raises questions about their fate during the biological treatment of organic waste. While composting standards (e.g., EN 13432) require bioplastics to be degraded at least 90% by weight within six months and disintegrate into smaller fragments less than 2 mm within three months upon contact with organic materials and must be less than 10 % of the initial mass to be certified as compostable (Campanale et al., 2024), AD needs to be studied for its ability to completely degrade PLA while contributing to biogas production and producing PLA-free biofertilizer (Battista et al., 2021; Folino et al., 2020). PLA biodegradation during AD has been recently studied at both lab and full-scale processes (Cucina et al., 2022a), and results indicated that the AD process, as usually performed at full scale, is not able to degrade PLA completely (Cucina et al., 2022a).

As a consequence, new strategies such as bioplastic pretreatments (i.e., mechanical, chemical, and physical treatments) need to be developed to make bioplastics more degradable under anaerobic conditions, thereby shortening the time required for their complete degradation. These pretreatments, unfortunately, are costly and energy-demanding, making them economically unsustainable (Cazaudehore et al., 2022; Mat Yasin et al., 2022). Another strategy enhancing bioplastic degradability is represented by microbial acclimation, which involves selecting a microbial population suitable for degrading bioplastics, thereby improving biogas production in a short time, i.e., HRT compatible with full-scale processes (Elboghdady et al., 2025). where improved PLA biogas production under thermophilic conditions was observed after two sequential AD runs performed to select a suitable microbial population, i.e. PLA degraded by +152% and biogas yields rose from 330 to 831 NL $kgVS^{-1}$, with t_{90} of 33 days.

Nevertheless, PLA products, such as cups, forks, spoons, and other items, present different designs for specific applications, as well as various shapes, thicknesses, and surfaces, which can influence their biodegradation during the AD process (Cazaudehore et al., 2022). In particular, the thickness of these products is a key factor that affects the biodegradation kinetics rate in AD, and so the time required for the complete PLA degradation (Bracciale et al., 2023; Cazaudehore et al., 2022). (Zhao et al. 2024) evaluated the effect of PBAT/PLA blend bags of two thicknesses (30 μm and 40 μm) and dimensions (20 × 20 mm, 2 × 2 mm, and 1 × 1 mm) during AD co-digestion with food waste (FW) under mesophilic conditions, showing that 30 μm materials produced more methane (i.e. 260 ± 2 mL g^{-1} VS) than 40 μm materials (i.e. 197 ± 4 mL g^{-1} VS) after 38 days. In another study, (Bracciale et al., 2023) investigated the degradation under thermophilic AD conditions of four commercial PLA-based items (cups and plates) with thicknesses of 133 μm and 205 μm for the cups (PLA4, PLA1, respectively) and 257 μm and 257 μm for plates (PLA2, PLA3), all having the same size (1.5 × 1.5 cm). They concluded that thinner products degraded faster in both early and late process stages, but that all items ultimately achieved similar biodegradation degrees (86–100 %) and biogas yields (1620–1830 mL/g TOC) at the end of the test, which ranged from 38–45 days except one case, lasting until 52 days. These results demonstrated that PLA can be completely degraded, but the kinetic rate can vary depending on the item thickness. Although this study provides valuable insight into the role of thickness on biodegradation of PLA-based products, it considered only a limited range of thicknesses (133–257 μm) and product types (cups and plates), and it did not consider the role of higher thickness in driving PLA degradation.

Since polymer biodegradation starts at the surface, where microbes form biofilms containing extracellular enzymes (Chinaglia et al., 2018), the biodegradation rate is often considered proportional to the available surface area (Chamas et al., 2020) and a "take-away" mechanism consisting in the polymer hydrolysis mediated by extracellular enzymes producing lactic acid monomers was suggested (Cucina et al., 2021b). Therefore, (Cucina et al., 2021b) highlighted the importance of considering surface area rather than total mass when estimating the biodegradation rate of 3D plastic materials, such as PLA.

This present study aims to investigate the anerobic biodegradability of PLA products (cups, spoons, and forks) characterized by different thickness ranges (50, 100, 450, and 750 μm) under unacclimated and acclimated microbial conditions. To ensure that thickness was the only factor influencing biodegradation, all products tested were prepared with the same total surface area.

2. Materials and methods

2.1 Digestate and commercial PLA products

Anaerobic digestate (inoculum) was collected before each AD run from an AD full-scale plant (Lombardy Region, Italy) operating under thermophilic conditions (55 ± 2 °C) and processing the organic fraction of municipal solid waste (OFMSW). Digestate was incubated at 55 ± 2 °C until no significant biogas production was observed and then chemically characterized before each AD run (Table S1). Four commercial PLA-based bioplastics products were selected based on their thicknesses: forks of 750 μm (Fork750), spoons of 450 μm (Spoon450), and two varieties of cups, i.e. one of 100 μm and one of 50 μm (Cup100 and Cup50, respectively). These PLA-based products, certified as compostable by TÜV Austria, were collected from Italian supermarkets. These products were analyzed by Fourier Transform Infrared Spectroscopy (FT-IR) (Section 2.3) Characteristic peaks at 3,000, 1,750, 1,400, and 1,100 cm^{-1} identified the material as PLA (Clagnan et al., 2023; Figure S1; Table S2). The thicknesses of these products were measured using a caliper. The surface area of the cup was measured directly, and that of the spoon and fork (head part) was calculated using the ImageJ program (Version 1.54, Madison, USA). To standardize surface area exposure, the two cups (Cup50 and Cup100) were then cut into 2.4 × 2.4 cm pieces, with corresponding weights recorded for each piece.

2.2 Experimental design

Two AD runs (batches) were conducted sequentially to investigate the effect of varying thicknesses of commercial PLA-based products on AD performance, in terms of biogas and methane production, while maintaining a consistent surface area across all sample products.

All runs were carried out in 500 mL batch glass bottles following (Elboghdady et al., 2025). A total surface area of 39 cm^2 was selected for each PLA-based product to ensure consistent exposure to biodegradation (e.g. microbial activity). To achieve this constant surface (39 cm^2), the amount of bioplastic added to each bottle was adjusted starting from 1% (w/w) of the total digestate for Fork750 (i.e. 3.41 g) (Clagnan et al., 2023) and decreasing for the other samples in the following order: Spoon450 (2.06 g); Cup100 (0.80 g); and Cup50 (0.47 g). This variation in weight (due to the varying thicknesses of products) was necessary to maintain a fixed surface area (i.e., 39 cm^2) across treatments, enabling the comparison of degradation performance by separating the effect of material thickness from surface availability.

In run1, all batch bottles were filled with 300 mL of digestate, as indicated in Elboghdady et al. (2025), and the above-mentioned corresponding weight of each PLA product Additionally, a positive control was prepared by adding 1.8 g of cellulose powder (Sigma, USA) to the digestate, thereby achieving an inoculum-to-substrate ratio (ISR) of 2 (Rodriguez-Chiang and Dahl, 2014). Blank bottles containing only 300 mL of digestate were also included to subtract the biogas production of inoculum from all samples (Elboghdady et al., 2025). For all samples, six replicates were used. After 55 days, three bottles were opened for all six experimental treatments. The bottles for the PLA treatments were sieved at 2 mm to account for PLA residues. All digestates were collected for chemical and microbial characterization and to set up the subsequent run2. The other replicates were kept running until complete substrate degradation (reaching the plateau) to assess long-term patterns.

Bioplastics residues were removed, rinsed with water, and dried at 40 °C until a constant weight was achieved. For run2, bottles (n=3) were filled with 150 mL of fresh digestate, 150 mL of digestate from run1of each product bottle and the same corresponding weight used in run1for each treatment. Extra bottles (n = 1) were kept as blanks (without the addition of substrates for each treatment) to track possible biogas production from the residues of run1.

After 105 days, run2 was ended, and the same procedure described for run1 was followed to collect samples of digestate and bioplastics.

2.3 Chemical and spectroscopic analyses

Digestate inocula and samples collected at the end of each run were chemically analysed according to standard procedures. Total solids (TS) and volatile solids (VS) were measured according to Rice et al. (2012) guidelines. Total organic carbon (TOC) was assessed using COD 15,000 test kits (range: 1.0–15.0 g LO_2^{-1}) (Nanocolor, Macherey Nagel, Germany) and then converted to C by stoichiometric calculations. pH was measured using a pH-meter (Eutech ™ pH 700, Thermo Fisher Scientific, Waltham, MA, USA). Ammonium-N was determined with the Nanocolor Ammonium100 (4-80 mg L^{-1} NH_4^+-N) kit (Nanocolor, Macherey Nagel, Germany), and absorbance was measured with a PF-12 Plus photometer (Macherey Nagel, Germany) using the supernatant layer of each sample after centrifugation at 6,000 rpm for 15 min (Chiappero et al., 2021). Volatile fatty acids (VFAs) and total alkalinity (TA) were quantified by titration following the method described by Di Maria et al. (2014). All chemical analyses were performed in triplicate. Averages and standard deviations were calculated using Microsoft Excel 2021 and its analysis toolbox. Significant differences in normal parameters were determined using analysis of variance

(ANOVA) followed by the Tukey test and an independent sample t-test, as implemented in SPSS version 29.1 software.

Spectroscopic characterization of the bioplastics' residues retrieved after each run was again performed by FT-IR spectroscopy using a Shimadzu IRAffinity-1S and Miracle Pike ATR unit (Shimadzu Italia srl, Milano, Italy). Spectral data were collected across 4000–500 cm^{-1} with 2 cm^{-1} resolution, and peak areas calculated using LabSolutions IR software. Before analysis, bioplastics' residues were dried and then their surface was cleaned by gentle brushing with a toothbrush to ensure the removal of all surface deposits.

2.4 Kinetics

A modified Gompertz model was used to estimate the kinetic parameters of biogas production potential of each product in each run (Mu et al., 2021). Maximum biogas production rates (*Rmax*) and lag phases (λ) were calculated from experimental data and subsequently applied in the following equation to evaluate the model's suitability with $B_0$was 840 NL $kgVS^{-1}$ as was demonstrated in (Elboghdady et al., 2025)

$$G(t)= B_0\times exp\ (-exp\ (R_{max}\times e\ /\ B_0\times(\lambda-t)\ +1))$$

Where G(t) is the cumulative biogas production (NL $kgVS^{-1}$), B_0 is the biogas production potential (NL $kgVS^{-1}$), R_{max} is the maximum biogas production rate (NL $kgVS^{-1}$ d^{-1}), λ is the lag phase (days), *t* is the digestion time (days), and e =2.7183.

3. Result and discussion

3.1 Effect of PLA thickness on biogas production

Thickness is one of the key parameters that affect both the biodegradation rate and the time required to complete bioplastic degradation (Folino et al., 2020). Since thicker bioplastic materials have a lower surface area * g^{-1} bioplastic available for microbial colonization and enzyme action, consequently extending the duration required for complete degradation (Folino et al., 2020; Waisarikit et al., 2025). Cumulative biogas production of PLA products was monitored for 105 days in run1, until all treatments reached a plateau **(Figure 1).** Cumulative biogas production differed significantly ($p \leq 0.05$) between all PLA products of varying thickness **(Table 1).** The thinnest product, Cup50, produced the highest biogas yield (836 ± 81 NL $kgVS^{-1}$, 67.08% ± 0.12 CH_4), which decreased with PLA thickness: Cup100 (731 ± 25 NL $kgVS^{-1}$, 66.2% ± 0.83CH_4) >Spoon450 (633 ± 23 NL $kgVS^{-1}$, 57.5% ± 0.24CH_4) > Fork750 (470 ± 15 NL $kgVS^{-1}$, 55.8% ± 0.04CH_4). These results indicated that the thickness was the main driver affecting bioplastic degradation as well as biogas yield, since the same total surface area was maintained among all the samples **(Table 1).** This result was confirmed by the negative correlation found between PLA thickness and biogas production ($r = 0.97$; $p< 0.05$; $n = 4$).

In run2, the effect of thickness was less evident on cumulative biogas production after 105 d, as all PLA stuff produced similar cumulative biogas amount, with no significant difference ($p > 0.05$) (**Table 1).** These results can be explained by considering that microbial acclimation enhances the degradation of PLA. Previously, our work indicated that the improvement in PLA degradation was associated with the enrichment of the media with the genus *Tepidanaerobacter* (Clagnan et al., 2023; Elboghdady et al., 2025), which was identified as a lactate-utilizing bacterium in thermophilic anaerobic digestion systems (Sekiguchi et al., 2006). *Tepidanaerobacter* was reported to be a key genus during the thermophilic anaerobic degradation of PLA-based materials (Cazaudehore et al., 2021; Tseng et al., 2019), playing a crucial role by consuming lactate and preventing its accumulation in the medium, thereby preventing an inhibitory effect due to PLA depolymerisation (Tseng et al., 2020).

The kinetics in run1 for all PLA products were controlled by microbial growth and adaptation as described by the modified Gompertz model (sigmoid curve). The sigmoidal curve pattern was characterised by a lag phase (microbial colonisation), an exponential phase (maximum degradation rate), and finally a plateau, when the material was depleted completely (Mohammadianroshanfekr et al., 2024). During the lag phase, microbial communities begin to adapt, colonise the polymer surface, and start excreting extracellular enzymes (Larrañaga & Lizundia, 2019), These enzymes cannot penetrate the polymer matrix due to their small size and instead act on the surface, initiating a surface erosion process (removal processes) (Cucina et al., 2021b). Then, short chains of oligomers, dimers, and monomers are produced due to the continuous erosion of material surface by enzymatic cleavage. These products become soluble as their molecular weight is lowered, and then they diffuse from the polymer to be assimilated into various metabolic pathways (Larrañaga & Lizundia, 2019).

In the run2, the kinetic analysis revealed that the thinner products (Cup50 and Cup100), as for run1, showed a sigmoidal pattern; however, R_{max} increased a lot, becoming similar to that measured after biomass acclimatation in previous work (Elboghdady et al., 2025). This pattern, indicated that the acclimated microbial community rapidly colonized, fragmented and metabolized polymer mass, leading to a rapid increase in the degradation rate (R_{max} increased) (Elboghdady et al., 2025). On the contrary, the kinetics observed for Spoon450 and Fork750 were different from run1, as they followed a linear regression, indicating that the reaction rates obey pseudo-zero-order kinetics, i.e. the degradation rate remained constant and independent from the total bioplastics amount indicating that degradation did not depend on substrate availability (Cucina et al., 2021b) (**Table 2).** This kinetic allowed these products to achieve similar biogas production in run2 to that of the thinner product after 105 days **(Figure 1).** This different trend can be explained by taking into consideration that PLA-polymer biodegradation depends on the total polymer surface availability, instead of total mass (Chamas et al., 2020; Chinaglia et al., 2018), and that microbial acclimatation enhances PLA surface erosion and fragmentation (Larrañaga & Lizundia, 2019) , making available continuously polymer surface to be then degraded (**Table 2) (Figure 3).**

Similarly, Bracciale et al. (2023) demonstrated that the thickness of different PLA products (with a thickness range of 133 µm to 257 µm) affected the degradation kinetics; however, contrary to this work, there was no effect on total biogas yield. This discrepancy was likely due to the greater range of thickness used in this study (ranging from 50 µm to 750 µm), as well as variations in material form and composition.

3.2 Effect of microbial acclimation on biogas production

Cumulative biogas production of thinner product (Cup50) under acclimated conditions (run2) after 105 days was similar to that of run1, while for higher thickness PLA products (i.e., Cup100), biogas yield was higher in run2 than run1 ($p \leq 0.05$) **(Table 2) (Figure 1).** However, the acclimation significantly enhanced the degradation kinetics for both PLA samples. The maximum production rate R_{max} increased sharply, from 11 to 38 NL $kgVS^{-1}$ d^{-1} for Cup50 and from 13 to 34 NL $kgVS^{-1}$ d^{-1} for Cup100. Consequently, t_{90} was shortened from 108 to 42 days for Cup50 and from 98 to 45 days for Cup100. These figures were similar to those previously reported for PLA cup degradation in batch after microbial acclimatation (Elboghdady et al., 2025), indicating, again, that microbial adaptation reduced the degradation time.

A significant enhancement in cumulative biogas production was observed, particularly for the thicker products under acclimated conditions (run2). The biogas yield for Spoon450 increased by 30%, from 633 ± 23 in run1 to 827 ± 41 NL $kgVS^{-1}$ in run2, while Fork750 showed an 80% increase, from 470 ± 15 in run1 to 846 ± 7 NL $kgVS^{-1}$ in run2. Due to their shift in kinetic model (from sigmoidal to linear), a direct comparison of degradation rate was not possible for these products. Therefore, 90% degradation, i.e. t_{90}, was used to compare the improvement fairly. The t_{90} decreased from 112 to 92 days for Spoon450, while for Fork750 it lowered from 146 to 86 days, demonstrating the effectiveness of microbial acclimation in enhancing the overall degradation process period. However, despite this improvement, the degradation period for these thicker products was still longer than that of typical industrial AD plants treating food waste, which operate with HRTs of 25-40 days (Papa et al., 2023).

Figure 1. Cumulative Biogas Production from different PLA-based products during AD run1and run2

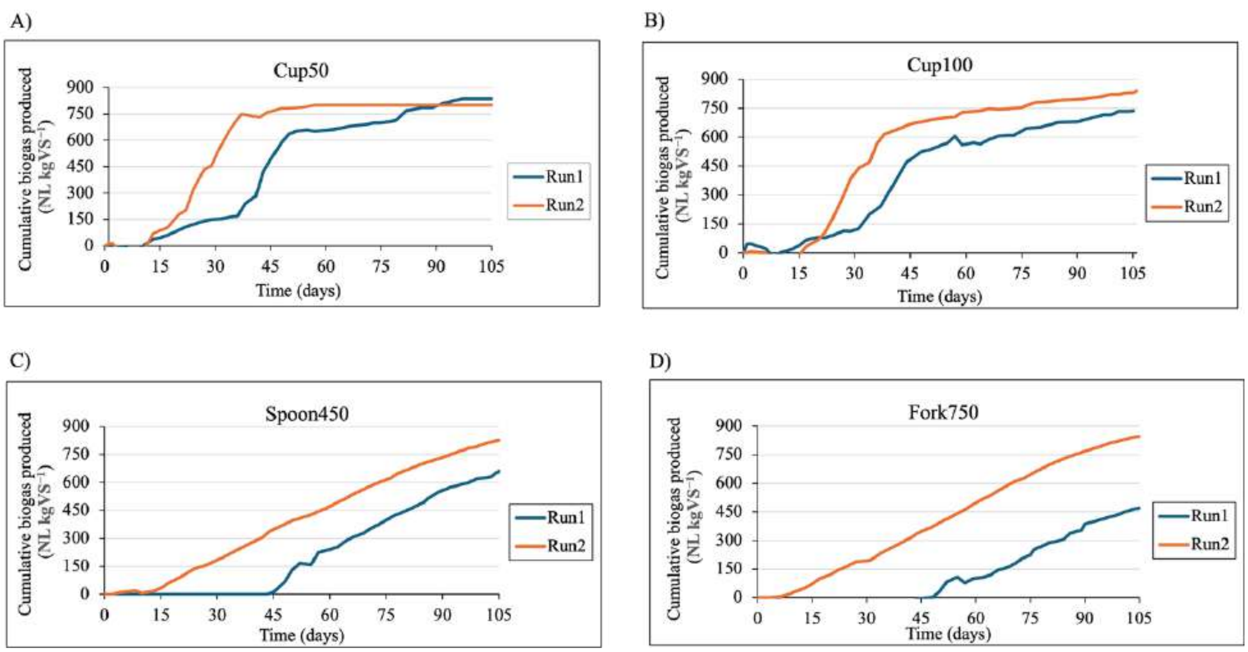


Table1. Biogas production and composition during the two anaerobic digestion runs

	Parameter	Run1	Run2
Cup50	**Biogas** (NL $kgVS^{-1}$)	836[a] ± 81a[b]D[c]	800 ± 26aA
	Biomethane (NL $kgVS^{-1}$)	560 ± 55a	521 ± 17a
	Biomethane (% v/v)	67.08 ± 0.12	65.05 ± 1.24
Cup100	**Biogas** (NL $kgVS^{-1}$)	731 ± 42aC	840 ± 48bA
	Biomethane (NL $kgVS^{-1}$)	484 ± 28a	531± 31a
	Biomethane (% v/v)	66.20 ± 0.83	63.24 ± 0.21
Spoon450	**Biogas** (NL $kgVS^{-1}$)	633±23aB	827 ± 41bA
	Biomethane (NL $kgVS^{-1}$)	363 ± 13a	491± 25b
	Biomethane (% v/v)	57.50 ± 0.24	59.32 ± .049
Fork750	**Biogas** (NL $kgVS^{-1}$)	470 ± 15aA	846 ± 7bA
	Biomethane (NL $kgVS^{-1}$)	262 ± 8.5a	468 ± 4b
	Biomethane (% v/v)	55.80 ± 0.04	55.37 ± 0.20

a) (Av. ± St. Dev.; n=3).
b) small letters indicate statistical differences ($P \leq 0.05$) between the two runs biogas production for the same item according to t test.
c) Capital letters indicate statistical differences ($P \leq 0.05$) between biogas production from all PLA products of run1 according to Anova test.

Table2.kinetic analysis of biogas of PLA products for run1 and run2

	Parameter	Run 1	Run 2
Cup50	B_0 (NL $kgVS^{-1}$)	840	840
	R_{max} (NL $kgVS^{-1}d^{-1}$)	11	38
	λ (d)	17	16
	R^2	0.969	0.988
	t_{90} [b] (d)	108	42
Cup100	B_0 (NL $kgVS^{-1}$)	840	840
	R_{max} (NL $kgVS^{-1}$ d^{-1})	13	34
	λ (d)	20	17
	R^2	0.961	0.994
	t_{90} (d)	98	45
Spoon450	B_0 (NL $kgVS^{-1}$)	840	n.a[c]
	R_{max} (NL $kgVS^{-1}$ d^{-1})	14	n.a
	k[a] (d^{-1})	n.a	8.66
	λ (d)	40	n.a
	R^2	0.969	0.993
	t_{90} (d)	112	92
Fork750	B_0 (NL $kgVS^{-1}$)	840	n.a
	R_{max} (NL $kgVS^{-1}$ d^{-1})	10	n.a
	k[a] (d^{-1})	n.a	8.75
	λ (d)	49	n.a.
	R^2	0.965	0.992
	t_{90} (d)	146	86

[a)] Kinetic constant calculated for the zero-order kinetic (Cucina et al., 2021)
[b)] t_{90}: time required to get 90 % of the total producible biogas.
[c)] Not applicable.

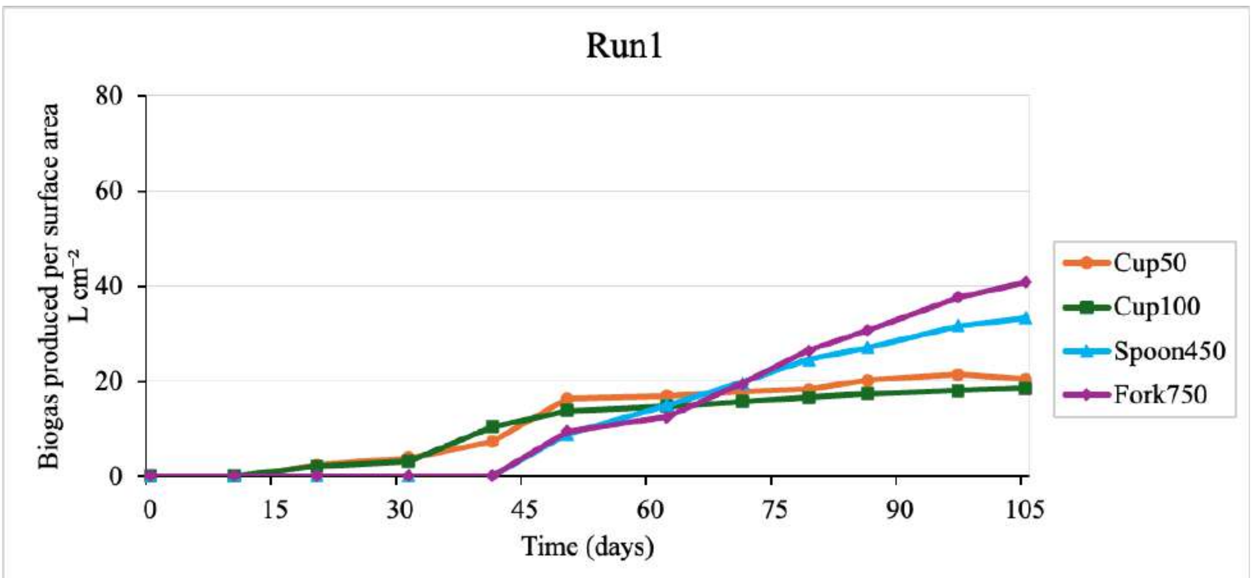
3.3 Effect of PLA surface area on biogas production

Surface area is a key factor in the anaerobic biodegradation of PLA because it influences the extent of microbial attachment and colonisation, as well as facilitates enzymatic hydrolysis. A larger surface area typically results in a faster degradation rate (Chinaglia et al., 2018). In this study, PLA products with different thicknesses and identical total surface areas were compared under pre-acclimated (run1) and further acclimated (run2) conditions. Interestingly, when biogas production was normalised to the initial surface area at the end of the trials (105 d), thicker products produced the highest biogas production per surface area unit, and the thinnest produced the lowest **(Figure 2)**. These results were unexpected, as similar production was predictable, considering the "take away" mechanism (Cucina et al., 2021b). Anyway, from **Figure 2,** it can be seen that at the early stage of the AD process, the degradation kinetic was similar for all PLA items; then degradation rate increased a lot for the highest thickness PLA products. One explanation of these behaviours is that the fragmentation of the bioplastic increased its surface area for microorganisms (Bher et al., 2022; Lu et al., 2022)

By day 55, visual inspection of Fork750 and Spoon450 revealed apparent physical fragmentation, indicating that the initial surface area had been altered during anaerobic digestion **(Figure 3).** Fragmentation increased the effective surface area exposed to hydrolytic enzymes and microbial colonization which enhanced and sustained PLA degradation and biogas production (Lu et al., 2022). So, when biogas production was referred to the starting surface, it was overestimated. Since Fork750 has a higher volume, it leads to the production of more fragments, which is accompanied by a significant increase in the available surface area, differing from the initial surface provided

(Figure 3). For the thinner PLA products, which have a lower surface area-to-volume ratio, fragmentation affected total surface area to a lesser extent, i.e., biogas production referred to the surface area increased much more than that observed for PLA products with a large thickness. Moreover, the complete degradation of the thin fragment contrasted with the total surface increase, so that biogas production per surface unit reached a plateau after 50 days **(Figure 2).**

Figure 2. Cumulative biogas production normalized by the surface area for run1and 2.



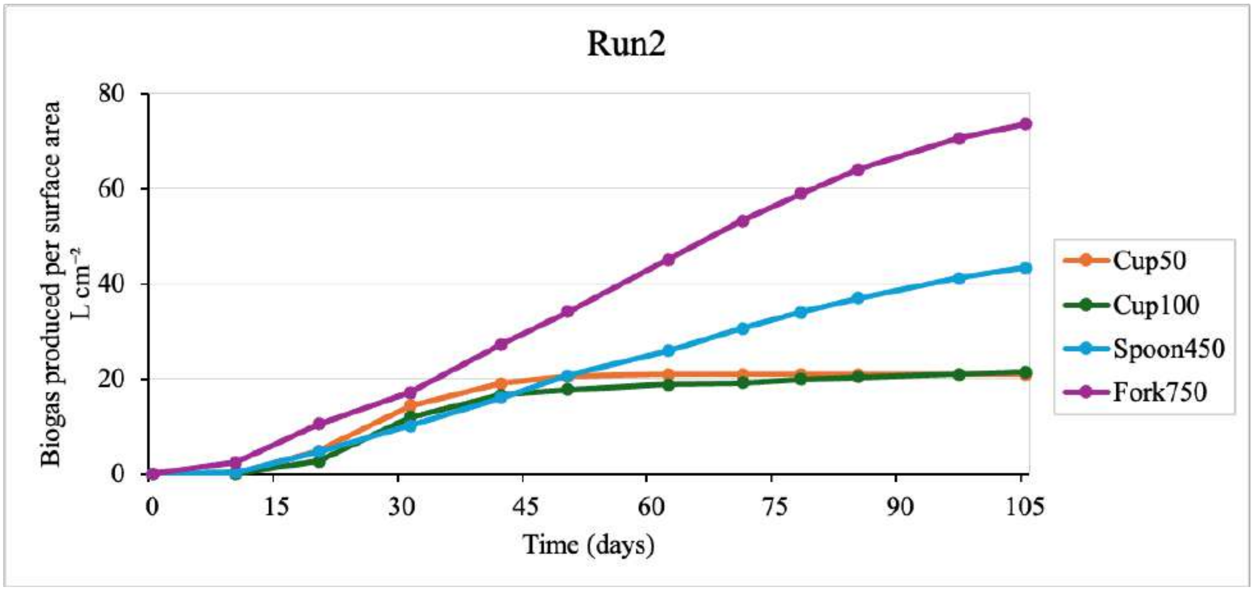

Run2
Biogas produced per surface area L cm⁻²
Time (days)
0
20
40
60
80
0
15
30
45
60
75
90
105
Cup50
Cup100
Spoon450
Fork750

Figure 3. Photographs of Spoon450 and Fork750 before and after 55 days of anaerobic digestion. (1) Spoon450 before digestion; (2) Spoon450 after 55 days of digestion; (3) Fork750 before digestion; (4) Fork750 after 55 days of digestion.

4. PLA degradation under anaerobic digestion: a full-scale proposal based on lab scale

The complete integration of bioplastics (e.g., PLA) into the circular economy requires a new approach that enables them to produce new products or generate energy (Parveen et al., 2024). End-of-life management of bioplastics involves composting processes that result in the complete degradation of bioplastics and closure of the material's lifecycle (Huang et al., 2025). Nevertheless, bioplastic degradation under aerobic conditions led to CO2, water and microbial biomass production (Nizamuddin et al., 2024), which cannot be fully considered "as products". The implementation of waste management, introducing an anaerobic digestion step before composting, proves to be useful because, under anaerobic conditions, bioplastics (PLA) can be transformed into biomethane, i.e., a useful molecule for producing energy or biofuel. Nevertheless, the limited degradation of bioplastics under anaerobic conditions (Cucina, 2022b) limits the application of AD to bioplastics in real-world, i.e., full-scale plants treating waste. Enhancing bioplastic (PLA) degradation during AD can be achieved by mechanical and chemical pretreatment (Mat Yasin et al., 2022) or by adapting microbial population, as previously indicated (Clagnan et al., 2023).

In this work, microbial acclimatisation was used to study its effect in degrading different PLA products (i.e. different shapes and thicknesses) in order to obtain the first data to understand the feasibility of PLA product degradation in a full-scale AD plant. To assess the process feasibility, the time required to achieve 90% of the total biogas yield (t_{90}) (Elboghdady et al., 2025) was considered, because for the successful integration of AD in the bioplastic management the t_{90} must be compatible with the HRT of full-scale AD plants (Cucina et al., 2022a; 2022b).

In the present study, microbial acclimation shortens the t_{90} to 42-45 days (Table 2) for the thin products (Cup50 and Cup100), respectively, achieving full compatibility with typical full-scale HRTs. By this alignment, these products can be effectively processed within conventional waste management systems. In contrast, for thicker products like forks and spoons, microbial acclimation recovered around 45% of the total biogas potential within 45 days, compared to negligible production in unacclimated conditions. Despite incomplete degradation of thicker PLA products, the biogas produced was not negligible and, when added to that produced by thinner PLA products, represented an improvement in the total biogas/biomethane obtainable from PLA degradation under anaerobic conditions. Nevertheless, the partial degradation of these products, led to the generation of PLA residues in the digestate, which poses challenges for their valorisation and safe agricultural application. To effectively close the loop, a post-treatment step, such as composting, is recommended to ensure the complete degradation of residual PLA (Papa et al., 2023). Combining these two approaches (AD and composting) may facilitate bioenergy recovery, minimise the leakage of PLA fragments into the environment, and lead to the creation of an effective end-of-life system that supports PLA integration into the circular economy framework.

While this present work demonstrated the effectiveness of microbial acclimation in batch systems and provided valuable insights into the degradation efficiency and kinetics of PLA of varying thicknesses, such tests remain a preliminary step in the process. Transferring these findings into continuous or pilot-scale conditions is essential, as these systems provide a realistic insight into the process (Cazaudehore et al., 2022). Enabling continuous or semi-continuous feeding and monitoring of multiple parameters, including biological, operational, and performance, which provides valuable insights into process performance and stability over extended periods [(Cazaudehore et al., 2022)]. To date, no studies have investigated the degradation of PLA in thermophilic continuous stirred tank reactor (CSTRs) under acclimated conditions.

However, the successful implementation of microbial acclimation in continuous systems has been demonstrated for other recalcitrant substrates, such as Maize Silage (Wojcieszak et al., 2017).

For the future work, assessing the applicability of microbial acclimation in CSTRs should be evaluated to promote better integration and the circularity of PLA waste.

5. Conclusion

The integration of bioplastics into the circular economy requires the possibility of obtaining products from their management and reuse. Anaerobic digestion can represent an opportunity because it allows for the production of biomethane from PLA degradation. This work showed that microbial acclimatisation allowed enhancing PLA degradation and biogas production compatible with full-scale AD processes. Although thicker PLA products were not completely degraded, the biogas produced was interesting (i.e. 45 % of the total producible biogas), suggesting the integration of AD with subsequent composting to manage organic waste with PLA products. Further study is needed to upgrade the lab-scale study performed under batch conditions to a completely stirred tank reactor (CSTR) approach at both lab and full scale.

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Supplementary tables

Table S1. Chemical characterization of inoculum and substrates.

Parameter	Digestate		PLA based products			
	Run 1	Run 2	Cup50	Cup100	Spoon450	Fork750
Total solids (%)	1.9[a] ± 0.1a[b]	1.6 ± 0.2a	99.4 ± 0.0	99.7 ± 0.4	99.9 ± 0.0	99.9 ± 0.0
Volatile solids (% dry matter)	62.6 ± 0.5a	62.5 ± 2.3a	100 ± 0.0	99.7 ± 0.3	99.6 ± 0.2	99.8 ± 0.2
Total organic C (% dry matter)	49 ± 2.8a	43 ± 3.2a				
pH	8.6 ± 0.0	8.8 ± 0.0				
Titrable acidity ($gCH_3COOH\ L^{-1}$)	1.42 ± 0.0b	1.0 ± 0.12a				
Total alkalinity ($gCaCO_3\ L^{-1}$)	13.3 ± 0.4b	10.5 ± 0.4a				
FOS/TAC	0.11 ± 0.0a	0.10 ± 0.01a				
Ammonium-N ($gN\text{-}NH_3\ L^{-1}$)	2.3 ± 0.1a	2.7 ± 0.0b				

[a]Av. ± St. Dev. (n=3)
[b]Letters indicate statistical differences ($P \leq 0.05$) across the two runs digestates for each parameter according to t test.

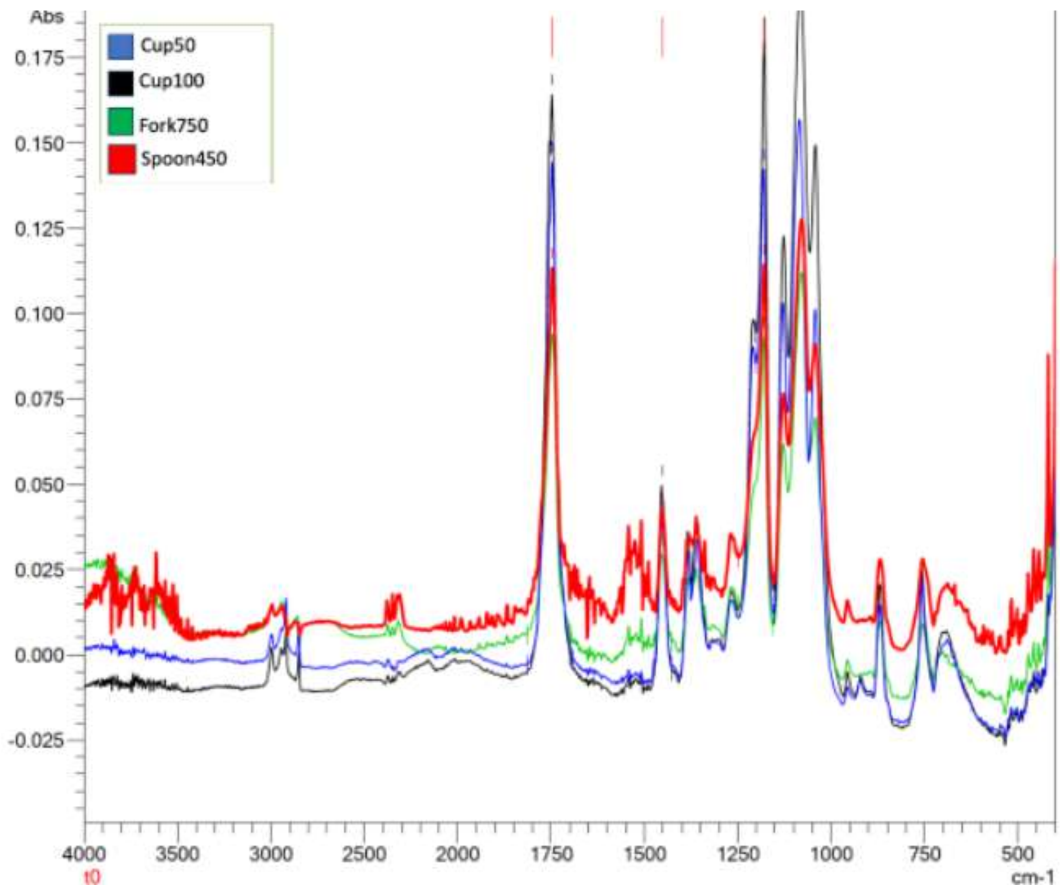
Table S2. Digestate characterization at the end of each run.

	Run 1					Run 2				
Parameter	**Cellulose**	**Cup 50**	**Cup100**	**Spoon 450**	**Fork750**	**Cellulose**	**Cup 50**	**Cup100**	**Spoon 450**	**Fork750**
Total solids (%)	3.2 [a] ± 0.1a[b]	2.1 ± 0.3a	2.2 ± 0.2a	2.2 ± 0.4a	2.5 ± 0.8a	1.8 ± 0.7a	1.7 ± 0.2a	1.6 ± 0.7a	1.7 ± 0.9a	1.7 ± 0.5a
Volatile solids (% dry matter)	62.2 ± 0.0a	58.8 ± 0.7a	58.3 ± 1.2a	58.8 ± 1.3a	58.3 ± 0.5a	56.5 ± 0.7a	54.4 ± 0.1a	49.6 ± 4.3a	55 ± 0.8a	55.3 ± 0.6a
Total organic C (% dry matter)	31 ± 4.14a	43 ± 2.53b	26 ± 0.76a	45.4 ± 1.2b	41 ± 1b	18 ± 2b	13 ± 0.4ab	17 ±1.8b	15 ± 1.5ab	11 ± 0.9a
pH (pH unit)	8.6 ± 0.0	8.6 ± 0.0	8.5 ± 0.0	8.41 ± 0.0	8.41 ± 0.0	8.2 ± 0.0	8.3 ± 0.0	8.2 ± 0.0	8 ± 0.0	8 ± 0.0
Titrable acidity (gCH_3COOH L^1)	0.92 ± 0.0a	1.25 ± 0.2a	1.83 ± 0.4a	1.42 ± 0.5a	1.09 ± 0.0a	0.76 ± 0.0a	1.17 ± 0.1a	3.91± 0.9b	0.59 ± 0.2a	0.72 ± 0.1a
Total alkalinity ($gCaCO_3$ L^{-1})	11.3 ± 0.3a	11.3 ± 1.4a	11.4 ± 0.1a	10.6 ± 0.5a	10.2 ± 0.0a	12.6 ± 0.1a	10.5 ± 0.1a	11.8 ± 1a	10.6 ± 0.1a	10.7 ± 1.1a
FOS/TAC	0.08 ± 0.0a	0.11 ± 0.01a	0.16± 0.03a	0.13 ± 0.04a	0.11 ± 0.0a	0.06 ± 0.0a	0.11 ± 0.01a	0.34 ± 0.11b	0.06 ± 0.02a	0.07 ± 0.0a
Ammonium-N (gN-NH_4^+ L^{-1})	2.4 ± 0.0a	2.5 ± 0.0a	2.4 ± 0.1a	2.4 ± 0.0a	2.4 ± 0.0a	2 ± 0.3a	2.3 ± 0.2a	2.1 ± 0.1a	1.8 ± 0.1a	2.4 ± 0.0a

[a](Av. ± St. Dev., n=3).
[b]Letters indicate statistical differences (P ≤ 0.05) across the three runs for each parameter according to Tukey test.

Supplementary figures

Figure S1. FT-IR spectra of Four PLA based products



Chapter 4

4.1. Conclusions and Future recommendations

This thesis aims to investigate, in the first study, the effects of microbial acclimatisation on PLA mono- and co-digestion with OFMSW. The second study evaluated the effect of thickness on PLA anaerobic degradation, while maintaining a constant total surface area under both acclimated and unacclimated conditions.

The key finding of the first study demonstrates that microbial acclimation enhances biogas production and the kinetic rate of PLA under both mono-digestion and co-digestion and is associated with an increase in PLA-degrading bacteria, including *Tepidanaerobacter.* This was also linked with a significant presence of hydrogenotrophic Archaea, such as *Methanothermobacter* and *Metanoculleus*, and it led to a reduction of the degradation time required to produce at least 90% of biogas of PLA under both mono-digestion and co-digestion, leading to a degradation time comparable to HRTs commonly used in full-scale AD plants.

Microbial acclimation has been demonstrated as an effective strategy for enhancing PLA degradation efficiency, particularly in co-digestion with OFMSW. It can be applied in full-scale AD plants to enhance PLA waste management after further target research and promote their integration into the circular economy framework.

In the second study, the results highlight that Product thickness is a crucial factor in PLA anaerobic degradation. As in run 1 (unacclimated), the thinner products produce the highest cumulative biogas production compared to the thicker products. However, after acclimation (run2), the cumulative biogas production becomes similar among all products. However, the thinner products, after acclimation (following the modified Gompertz model), achieve a higher biogas production rate and shorten t_{90}, which becomes comparable to full-scale HRTs. The thicker products shifted from modified Gompertz to zero-order kinetics, with a reduced t_{90} but still exceeding the industrial HRT. Moreover, thicker PLA products were achieved (i.e. 45 % of the total producible biogas) after acclimation, compared to unacclimated within 45 days, suggesting the integration of AD with subsequent composting to manage organic waste with thick PLA products.

While this thesis focused on the application of microbial acclimation for enhancing the degradation of PLA at a lab scale, Future research efforts can focus on transitioning to continuous systems (CSTRs) and moving beyond the lab batch system. These CSTR systems will mimic industrial conditions by providing realistic insights into process performance and stability over extended periods.

In conclusion, applying microbial acclimation along with careful consideration of product thickness is required for the efficient anaerobic digestion of PLA and to reach degradation rates comparable to those of the industrial full-scale AD plants. Moreover, paves the way for enabling the practical integration of PLA waste into AD within a circular bioeconomy.