EVALUATING BIOGAS POTENTIALS OF BIOPLASTIC DEGRADATION THROUGH ANAEROBIC CO-DIGESTION

by

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ABSTRACT

EVALUATING BIOGAS POTENTIALS OF BIOPLASTIC DEGRADATION THROUGH ANAEROBIC CO-DIGESTION

Biodegradable plastics offer a promising approach to mitigating the adverse impacts of plastic pollution, as they are capable of breaking down either naturally or under controlled conditions. This study investigates the degradation of two widely used bioplastics—polylactic acid (PLA) and polybutylene adipate terephthalate (PBAT)—to assess their influence on biogas production and during anaerobic co-digestion (AD) with food waste under mesophilic conditions. Although the setups containing PLA did not yield clear, comparative results regarding its contribution to biogas production, the experiments involving PBAT produced meaningful outcomes for both inoculum to substrate ratio (ISR) that are ISR of 2:1 and 1:1 conditions. Specifically, PBAT contributed an 8.92% increase in total biogas production in the ISR of 2:1 setup and a 5.00% increase in the ISR of 1:1 setup when compared to the respective control groups. Moreover, gas composition analysis performed using gas chromatography (GC) showed that cumulative biomethane production from PBAT-containing setups reached 438.6 mL for ISR of 2:1 and 15,110.2 mL for ISR of 1:1 by the end of the experiments. Accordingly, the biodegradation rates of PBAT bioplastic in these setups were calculated as 0.78% and 10.05%, respectively.

ÖZET

ANAEROBİK KOŞULLARDA BİYOPLASTİK BOZUNMASININ BİYOGAZ POTANSİYELİ TAKİBİ İLE DEĞERLENDİRİLMESİ

Biyoplastikler, doğal yollarla ya da kontrollü koşullar altında parçalanabilme özellikleri sayesinde plastik kirliliğinin olumsuz etkilerini azaltmak için umut verici bir yaklaşım sunmaktadırlar. Bu çalışmada, en fazla üretimi yapılan iki biyoplastik türü olan polilaktik asit (PLA) ve polibütilen adipat tereftalat (PBAT)'ın, mezofilik koşullar altında gıda atığı ile birlikte yürütülen anaerobik çürütme sürecindeki biyogaz üretimine etkisini değerlendirmek amacıyla gerçekleştirilmiştir. PLA içeren setler, biyogaz üretimine katkı sağlayan net karşılaştırmalı sonuçlar sunmasa da PBAT ile gerçekleştirilen deneylerde anlamlı sonuçlar elde edilmiştir. Özellikle, PBAT içeren sistemlerde; inokülüm/substrat=2 ile kurulan sette, kontrol grubuna göre %8,92 oranında, inokülüm/substrat=1 ile kurulan sette ise %5,00 oranında toplam biyogaz artışına katkı sağlanmıştır. Ayrıca, GC ile yapılan gaz kompozisyonu analizleri sonucunda, deneylerin sonunda PBAT içeren setlerde kümülatif biyometan üretiminin inokülüm/substrat=2 için 438,6 mL ve inokülüm/substrat=1 için 15.110,2 mL olduğu gözlemlenmiştir. Buna bağlı olarak, bu sistemlerde PBAT biyoplastiğinin biyobozunma oranları sırasıyla %0,78 ve %10,05 olarak hesaplanmıştır.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS/ABBREVIATIONS

Abreviation	Explanation
AA	Adipic acid
AD	Anaerobic digestion
Ag_2SO_4	Silver sulfate
APHA	American Public Health Association
Avg.	Average
Bio-PE	Bio-polyetheylene
Bio-PET	Bio-polyethylene terephtalate
Bio-PP	Bio-polypropylene
BDO	Butanediol
BMP	Biochemical methane potential
BPC	Bioprocess Control
BRS	BioReactor Simulator
C	Carbon
$C_6H_{12}O_6.H_2O$	Glucose monohydrate
cm^3	Cubic centimeter
C:N	Carbon to nitrogen ratio
CH_4	Methane
CO_2	Carbon dioxide
COD	Chemical oxygen demand
$CuSO_4$	Copper (II) Sulfate
D-	Dextrorotatory
EtOH	Ethanol (Ethyl alcohol)
EU	European Union
Eq.	Equation
FW	Food waste
g	Gram
GC	Gas chromatography
GHG	Greenhouse gas
gVS	Grams of volatile solids
H	Hydrogen
He	Helium

H_2O	Water
H_2SO_4	Sulfuric acid
H_3BO_3	Boric acid
H_3PO_4	Phosphoric acid
$HgSO_4$	Mercury (II) sulfate
Inoc.	Inoculum
ISO	International Organization for Standardization
ISR	Inoculum to substrate ratio
$K_2Cr_2O_7$	Potassium dichromate
K_2SO_4	Potassium sulfate
KHP	Potassium hydrogen phthalate
L	Liter
L-	Levorotatory
mg	Miligram
mm	Milimeter
mol	Mol
mL	Mililiter
MSc	Master of science
MW	Molecular weight
N	Nitrogen
NaOH	Sodium hydroxide
NH_3	Ammonia
NH_3-N	Ammoniacal nitrogen
NH_4^+	Ammonium
NP	Natural Polymers
OFMSW	Organic fraction of municipal solid waste
PBAT	Polybutylene adipate terephthalate
PCL	Polycaprolactone
PHA	Polyhydroxyalkanoate
PLA	Polylactic acid
sCOD	Soluble chemical oxygen demand
SM	Standard Method
TA	Terephthalic acid
TKN	Total Kjeldahl nitrogen
TPA	Terephthalic acid

TS	Turkish Standards Institution
TS	Total solids
VFAs	Volatile fatty acids
VS	Volatile solids
WBA	World Biogas Association
Ygas	Gas yield
μm	Micrometer

1. INTRODUCTION

In today's world, plastic as a raw material or main component is preferred and used for many applications in different sectors such as food-beverage, textile, electronics, construction etc. Since their initial development in the 1950s, plastics have played a vital role in daily life and have served as a key player across all industrial sectors where they have been utilized (Folino, Pangallo, & Calabro, 2023). Thus, they are considered as remarkable materials and are extensively utilized in everyday life. Their demand continues to rise due to their affordability, light weight, durability, energy efficiency, and the ability to be customized with various functional and performance characteristics according to specific end-use requirements. They have become the preferred material across a wide range of sectors, serving diverse applications such as packaging, agriculture, electronics, and more.

Accordingly, excessive usage of the plastic in those sectors has resulted in a worldwide pollution in the soil, water/marine life and air at a varying rate (Bellasi et al., 2020). The lifespan of conventional plastics is estimated to range from several hundred to thousands of years. Discarded plastics infiltrate the food chain, turning sections of marine environments into layers of plastic debris. Globally, plastic waste obstructs drainage systems, leads to animal fatalities due to ingestion, and ultimately makes its way into the food chain (Goel et al., 2021). Widespread occurrence of plastic wastes seems to be one of the greatest environmental problems in the world. Therefore, improperly disposed waste plastics pose significant environmental challenges, including land, marine, and water pollution. To compete with these problems, researchers and developers put in some innovative, sustainable and realistic solutions (Emadian et al., 2017). One of those alternatives is using biodegradable plastic that can be degraded via natural processes or in closed and controlled systems.

Plastics contain multiple polymers under the category of biodegradable plastics. Bioplastics can be classified into three types according to European Bioplastics which are biobased and biodegradable plastics, biobased and non-biodegradable plastics, fossil based biodegradable plastics (European Bioplastics, n.d.). Bioplastics are defined as polymers that are either derived from renewable resources, such as plants, or possess the capability to biodegrade or undergo composting processes (Cucina et al., 2021; Rahman and Bhoi, 2021; Ülger-Vatansever, Onay & Demirel, 2024). Unlike conventional plastics and their associated environmental impacts, bioplastics have emerged as a potential alternative. Using biodegradable plastics alternatively in the sectors which have high demand of conventional plastics is evaluated as a promising and environment-friendly choice. By this

way, biodegradable plastics may supply energy or heat for production sites and households, at the end of their lives, which could be processed via several technologies. For instance, anaerobic digestion (AD) is one of these sustainable technologies which aim to recover energy through biogas generation (Abraham et al., 2021).

Due to the lower stability of biodegradable plastics compared to conventional polymers, anaerobic digestion (AD) is regarded as a promising method for converting the former type of plastics into value-added products, utilizing organic matter, microorganisms, and abiotic factors such as pH, temperature, and moisture. Integrating the co digestion of food wastes and some bioplastics provides an effective strategy to reduce the volume of waste entering municipal waste systems, to diminish landfill-related greenhouse gas emissions, and to facilitate the generation of renewable energy (Hobbs et al., 2019). Therefore, the inoculum to substrate ratio (ISR), defined as the ratio of volatile solids in the inoculum to those in the substrate, is another critical parameter in anaerobic digestion; accurate assessment of a substrate's biochemical methane potential (BMP) strongly depends on ISR (Raposo et al., 2011).

Under varying environmental or controlled conditions, the degradation of bioplastics mixed with food waste in anaerobic environments, absence of oxygen, leads to the production of CO_2, CH_4, water, biomass, and residual material. Microorganisms are unable to directly transport the larger polymeric materials into the cells at the beginning. To start the degradation process, microorganisms must first release extracellular enzymes, which break the polymers externally and plastic biodegradation primarily occurs through surface erosion (Goel et al., 2021).

In this study, considering the likelihood that bioplastics used in packaging may enter the same waste stream with food waste, the biodegradation rates of two commonly used bioplastics—polylactic acid (PLA) and polybutylene adipate terephthalate (PBAT)—were examined under mesophilic conditions through an anaerobic co-digestion process with food waste. The study also evaluated how different inoculum to substrate ratios (ISR) in anaerobic digesters contribute bio-methane production, resulting in a sustainable value-added product.

2. LITERATURE REVIEW

2.1. Plastic Pollution

Plastic pollution is considered one of the most pressing environmental issues in today's world, due to the vast production and use of plastics and their improper post-use management. A significant number of plastics do not follow appropriate disposal practices, resulting in pollution in marine and terrestrial environments (Alvarez-Mendez et al., 2023).

Studies have shown that 70% of plastic waste in the European Union (EU) is utilized for landfill and incineration (Battista et al., 2021; Cheng Yu et al., 2023). Inadequate management of plastic wastes have made their usefulness a critical problem, due to their persistence in ecosystems and the possible emission of toxic compounds during the degradation process (Folino et al., 2023).

With improper plastic waste management, other problems are introduced into our lives. A range of human health complications, including cancer, respiratory issues, and reproductive disturbances, can be attributed to the ingestion of plastics via contaminated food or the inhalation of plastics through dust or air (Allouzi et al., 2021).

The decomposition of plastic wastes can extend to a hundred or thousand years when it is disposed of in natural environments. As a result of this alarming challenge, there has been a recent push to replace synthetic plastics with biodegradable or bioplastics, which can undergo biodegradation (Song et al., 2009).

2.2. Solution for Plastic Pollution: Bioplastics in Anaerobic Digestion Process

2.2.1. Bioplastics and Biodegradation: Biodegradable and Biobased Plastics

Research has indicated that bioplastics would be a more sustainable and environmentally beneficial alternative for conventional petroleum-based ones (Muthusamy & Pramasivam, 2019; Naser et al., 2021).

Bioplastics are the polymers which can be defined as biodegradable and/or biobased. Therefore, bioplastics are categorized in three main sections which are biobased and biodegradable plastics,

biobased/partially biobased and nonbiodegradable plastics, and fossil resource-based and biodegradable plastics (Vardar et al., 2022). Therefore, bioplastics generally are categorized in two main groups which are biobased plastics and biodegradable plastics by European Bioplastics. Biobased plastics express the materials that are manufactured from biomass, which can be sugarcane, corn, and cellulose such as bio-polypropylene (bio-PP) and bio-polyethylene terephthalate (bio-PET). Biodegradable plastics that can be break-up into smaller organics and gases, such as polycaprolactone (PCL), and polybutylene adipate terephthalate (PBAT). Both categories share plastics that are bio-based and biodegradable, such as starch blends, polylactic acid (PLA), and polyhydroxyalkanoate (PHA) (Batori et al., 2018).

The term “biodegradable” refers to a component or material that is susceptible to degradation under specific conditions by microorganisms. Those microorganisms convert biodegradable plastics, a complex polymer, into methane, water, carbon-dioxide and some solid molecules in anaerobic conditions (Dilkes-Hoffmann et al., 2019). There are many biodegradable plastics which are natural polymers with high quantities in renewables and they can also be synthesized from petroleum (Vroman & Tighzert, 2009).

Bioplastics are considered as "biobased" when they are derived from natural resources, and they are termed "biodegradable" when microorganisms can break down their polymeric structure into natural compounds such as water and carbon dioxide (Gadaleta et al., 2024). In relation to this matter, it should be emphasized that: not all bioplastics possess biodegradability; and certain petrochemical-derived plastics can also be categorized as bioplastics because of their biodegradable nature.

Biodegradability is determined by the chemical structure of the material, rather than its origin, whether it is derived from natural or synthetic sources. The bioplastic network of European Bioplastics that is given in Figure 1 below.

The biodegradation of bioplastics depends on biotic and abiotic conditions (temperature, moisture, etc.) and chemical and physical properties of the polymer. The biodegradation is measured by monitoring two factors in general: the change in the weight of the bioplastics and the volume of carbon dioxide generated during the degradation processes (Emadian et al., 2017). Furthermore, visual analysis can be utilized to assess the biodegradation of bioplastics (Bracciale et al., 2023).

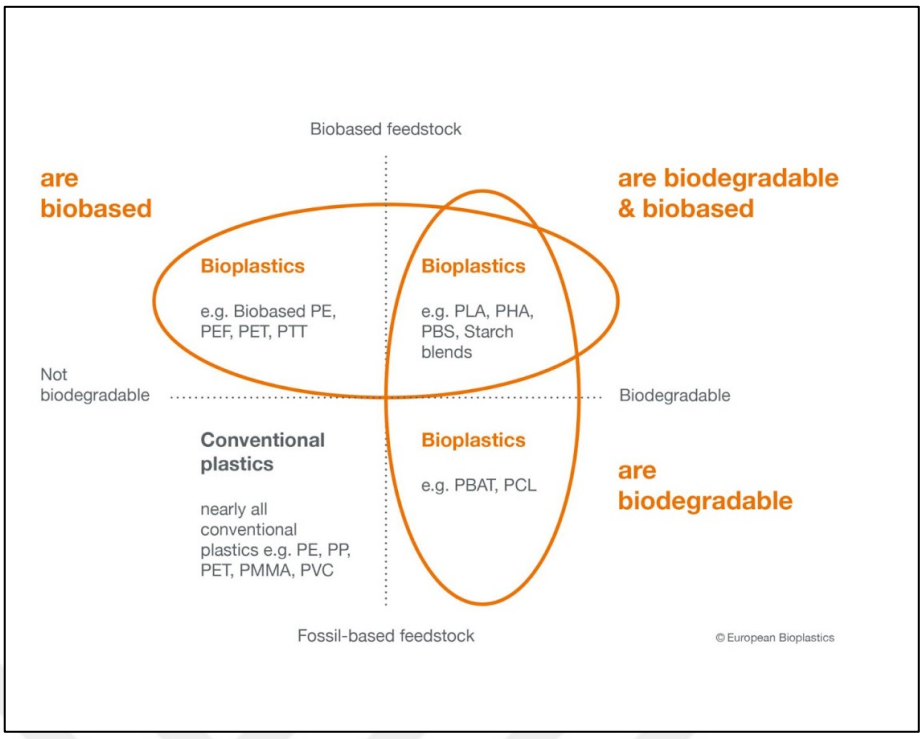


Figure 2.1. Network of bioplastics (European Bioplastics, n.d).

In response to the issues related to the production and disposal of plastic products, bioplastics have been developed in recent decades as a practicable replacement for conventional plastics. Shifting to use of bioplastics instead of conventional ones across the globe is a significant contributor to reducing the global carbon footprint, alleviating climate change, and addressing plastic-related pollution (Meereboer, Misra, & Mohanty, 2020). The overall advantages of biodegradable bioplastics embrace lower carbon footprints, less dependence on oil and its fragments, and better managing their end-of-life through appropriate waste management practices (Cucina et al., 2022).

In this regard, managing waste of bioplastics is vital, especially with respect to the circular economy steps, especially for determining strategies of their end-of-life, such as recycling, incineration, landfilling, and biodegradation (Folino et al., 2023).

Because of the diverse nature of bioplastics, sorting, processing, and recycling may not be the most robust recovery method that is why recycled bioplastics tend to experience a reduction in quality (Rosenboom, Langer, & Traverso, 2022). Through biodegradation, biocompounds are expected to be converted into harmless materials such as monomers, CO_2, and H_2O. In addition, biological treatment processes like anaerobic digestion systems can yield value-added products, such as biomethane, which is more environmentally friendly than petroleum-based plastics as shown in Figure 2. Additionally, compared to other biodegradation methods, anaerobic digestion process where biomethane can be produced for energy utilization as an end-of-life treatment for bioplastics is

correlated with a lower global warming potential (Zhao, Cornish, and Vodovotz, 2020). PLA and PBAT are the biobased and biodegradable bioplastics respectively that have been analyzed in this research via observing contributions on biomethane productions in the AD process.

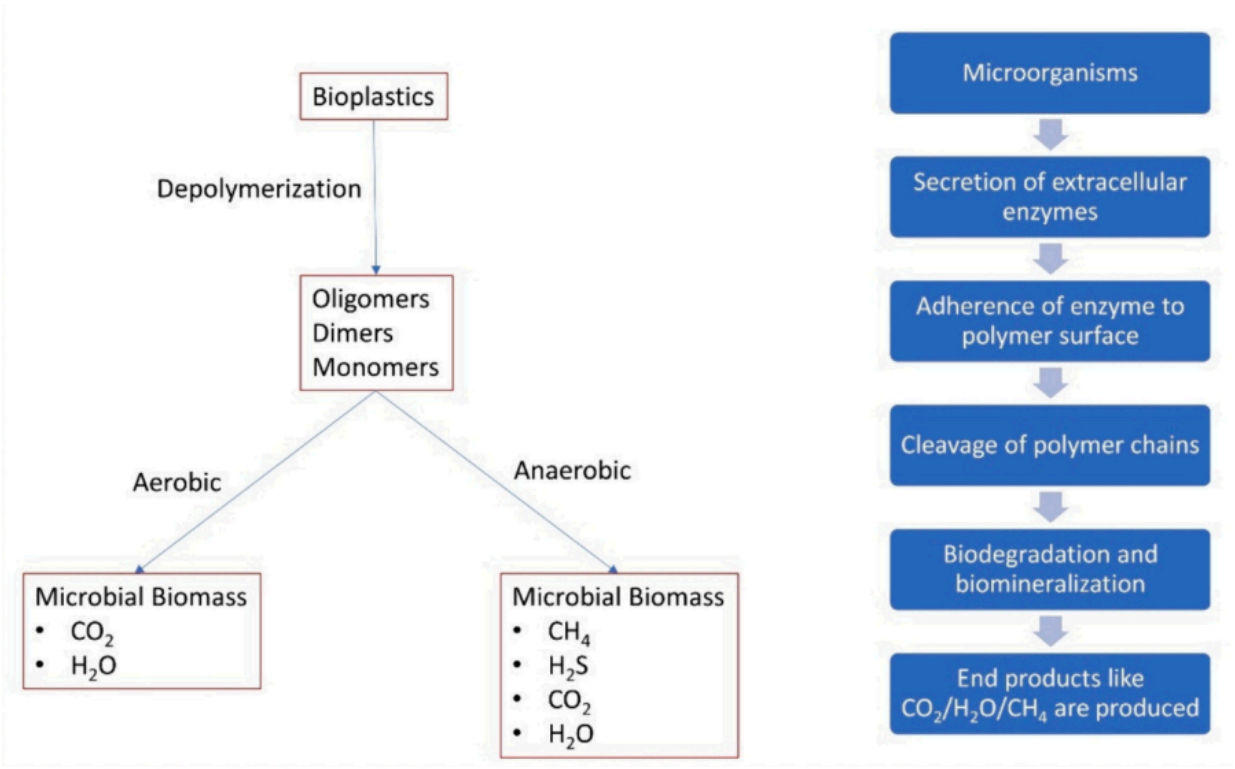


Figure 2.2. Biodegradation pathway of bioplastics (Rasheed et al., 2024).

2.2.2. Anaerobic Digestion Process

Anaerobic digestion (AD) is a biological process occurring under absence of oxygen in which various microorganisms use organic materials as a feedstock to produce mainly biogas and digestates. There are four fundamental steps in the AD process which are hydrolysis, acidogenesis, acetogenesis and methanogenesis with the presence of several microorganisms enabling complicated chemical reactions to happen in an oxygen-free medium (Abraham et al., 2021). At each step, certain bacteria groups play a role in these complex interactions (Uddin & Wright, 2023).

AD starts with conversion of large compounds into smaller ones which is called hydrolysis. Accordingly, water and some enzymes break down the bigger polymeric molecules into monomer or oligomer scales and then, they are converted into short-chain acids. Macro organic matter or materials that are also complex polymers are consumed by the microorganisms in the process after they are converted into smaller molecules like fatty acids via hydrolysis and acetogenesis (Vardar et al., 2022). Then, acetic acid is formed as an intermediate product to produce the desired products which are

methane and carbon dioxide (Nsair et al., 2020). At the last stage, which is called methanogenesis in AD, short carbon chains are transformed to carbon dioxide and methane by the interactivity of methanogenic microorganisms. The flow of the anaerobic process is given in Figure 2.3 below.

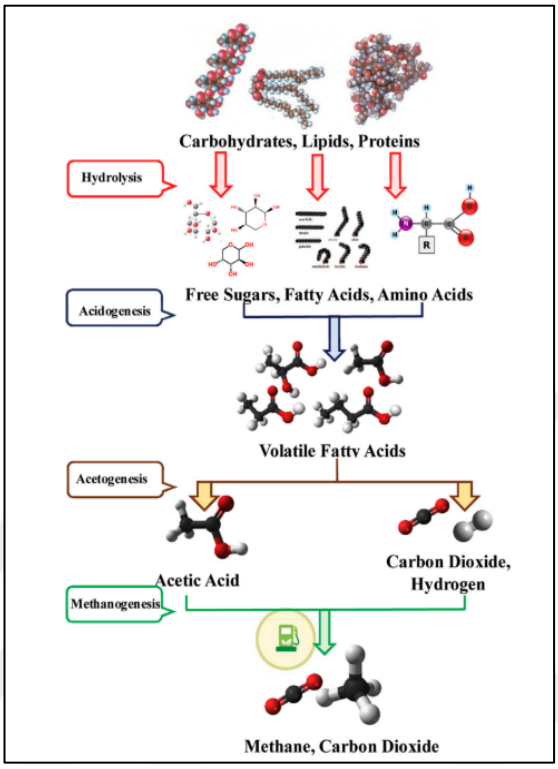


Figure 2.3. Flow scheme in an anaerobic digestion process (Momayez et al., 2019).

Complex microbial populations are fundamental to the operation of anaerobic digestion (De Vrieze et al., 2018). The decomposition of the bioplastic occurred through anaerobic biodegradation, resulting in the mineralization of the used material into CO_2, H_2O, NH_4^+, and biomass due to biological activity (Urbanek et al., 2017).

Selection of feedstock is an important decision to operate an anaerobic digestion process. Moreover, during this process there are some vital parameters that have to be observed such as C:N ratio, volatile solids (VS), total solids (TS), ammonia content, pH and other elemental values (Quecholac-Pina et al., 2020). Bioplastics are not good raw materials in AD with mono-digestion method due to their high carbon content. Thus, digestion of bioplastics is not well-enough for an AD process that needs to use a feedstock with high C:N ratio (Abraham et al., 2021).

Accordingly, co-digestion of bioplastics with other feedstocks like food waste in the AD process is a more suitable method for the higher bioplastic degradation (Mohee et al., 2008). In parallel with more bioplastic degradation in the mixture of food waste and bioplastics, this study

focuses on evaluating a method to quantify biomethane production of PLA and PBAT from co digestion of both biopolymers with food waste.

Anaerobic digestion holds a key position in circular waste management, particularly for the treatment of organic wastes (Gadaleta et al., 2024). The implementation of biodegradable bioplastics for food packaging applications and food waste collection to be used in AD processes may present a viable solution to mitigate the problem of plastic contamination. Therefore, anaerobic digestion represents a promising method for bioplastics recovery, enabling the production of biomethane, minimizing carbon emissions, and ensuring the circular use of these products (Cucina et al., 2022).

Taking its final products and energy of AD systems into consideration; anaerobic digestion is assessed as an advantageous method when compared to its alternatives. Furthermore, anaerobic digestion (AD) may serve as a more effective and beneficial end-of-life solution, promoting energy recovery as part of the food–water–energy nexus (Kakadellis et al., 2022). Accordingly, major pros and cons of the anaerobic digestion processes are summarized in the Table 2.1 below.

Table 2.1. Characteristics of Anaerobic Digestion (Kakadellis et al., 2022).

Technology	**Process Description**	**Advantages**	**Disadvantages**
Anaerobic digestion (AD)	Degradation of organic waste by microorganisms in the absence of oxygen in a closed chamber	+ Allows for energy production alongside nutrient and organic matter recovery + Products are substitutes for fossil-based natural gas and synthetic fertilizers	- Capital and operational costs can be prohibitive - Highly sensitive process - CH_4 content of biogas can be low for some substrates - Digestate storage (e.g., lagoons) can be costly - Possible restrictions on digestate application timings

2.2.2.1. Hydrolysis stage. Hydrolysis, the initial step of the anaerobic digestion process, involves the degradation of insoluble and complex organic substances—such as lipids, proteins, polysaccharides, and nucleic acids—into simpler, soluble compounds like monosaccharides and amino acids, which are then either metabolized for energy or utilized as precursors for the biosynthesis of other vital compounds.

As noted by Uddin and Wright (2023), hydrolytic enzymes such as amylase, cellulase, lipase, protease, and pectinase play a critical role in the degradation process, as large organic molecules are not directly available for microbial utilization. Hydrolytic bacteria, characterized by their rapid growth rates, secrete extracellular enzymes that decompose these complex macromolecules into smaller, absorbable units, thereby supplying essential nutrients and energy.

2.2.2.2. Acidogenesis Stage. In the anaerobic digestion process, acidogenesis constitutes the second step, where fermentative microbes facilitate the breakdown of amino acids, sugars, and some fatty acids derived from the hydrolytic stage.

Simple carbohydrates, fatty acids, and amino acids are generally metabolized into organic acids and alcohols during this stage (Gerardi, 2003). Hydrolytic products are chiefly converted into short-chain volatile fatty acids (e.g., acetic, propionic, formic, and lactic acids), various alcohols (such as ethanol and methanol), and ketones (notably glycerol and acetone). Moreover, carbon dioxide, hydrogen, ammonia, and minor amounts of other compounds emerge as byproducts (Uddin & Wright, 2023).

2.2.2.3. Acetogenesis Stage. The acids and alcohols formed in the acidogenic phase are subsequently utilized by other microorganisms during the third step of anaerobic digestion. Volatile fatty acids (VFAs) and alcohols undergo oxidation to produce substrates essential for methanogenesis, including acetate, hydrogen, and carbon dioxide. Longer-chain VFAs are oxidized into acetate and hydrogen (Adekunle & Okolie, 2015).

2.2.2.4. Methanogenesis Stage. In the methanogenesis phase, methanogenic microorganisms transform intermediate products into methane and carbon dioxide under strictly anaerobic conditions. As the slowest biochemical reaction within anaerobic digestion, methanogenesis is pivotal to the overall process. Successful methanogenic activity depends on environmental factors such as a relatively higher pH and a low redox potential, the latter complicating laboratory cultivation efforts (Meegoda et al., 2018).

2.2.3. Conditions for Anaerobic Digestion

The anaerobic degradability of bioplastics is largely influenced by their composition and the conditions of the AD process, mainly operating temperature (Abraham et al., 2021). Moreover,

factors such as moisture, oxygen concentration, and pH play crucial roles in determining the biodegradation behavior of bioplastics (Ülger-Vatansever et al., 2024).

Due to the effects of microorganisms on the system biotic factors are also considered in this issue. Accordingly, the degradation rate of bioplastics is not only dependent on their material characteristics but also on specific environmental factors, including the presence of microorganisms such as bacteria and fungi (Folino et al., 2023). Additionally, it was also indicated that observing biodegradation of a biodegradable plastic is strictly dependent on the reaction environment (Narancic et al., 2018).

The effect of temperature has also been discussed in the literature in many perspectives. Two operation temperature scales are used in AD systems in general which are mesophilic (around 35-38°C) and thermophilic (around 55-60°C). Studies conducted recently have confirmed the feasibility of degradation of biopolymers alongside municipal sludge in mesophilic conditions, producing CH_4 that can be utilized in systems combined with heat and power (Guo et al., 2011; Hobbs et al., 2019). Thus, anaerobic systems feeding with food wastes results in higher operating costs at thermophilic ranges due to the high-water nature of food wastes (Angelonidi & Smith, 2015). On the other hand, mesophilic anaerobic digestion is regarded as the most practical and financially advantageous system for the co-digestion of biodegradable bioplastics and food waste (Kakadellis et al., 2022).

Another parameter, inoculum to substrate ratio, is ISR corresponds to the ratio of the volatile solids (VS) from the inoculum (residual organic material and actively degrading microorganisms) to the volatile solids from the substrate. According to the research, accurate evaluation of a substrate's biochemical methane potential (BMP) in an AD largely depends on key factors such as the inoculum to substrate ratio (ISR), the diversity of the inoculum, storage practices, and the production of endogenous methane (Raposo et al., 2011).

2.3. Food Wastes in Anaerobic Digestion

The organic fraction of municipal solid waste (OFMSW)—comprising food waste from households and similar sources like institutional canteens and food markets—is increasingly viewed as a valuable resource, offering the potential to support circular and energy-positive waste management strategies as shown in Figure 2.4 below.

At present, biodegradable and compostable certified bioplastics are managed within the biowaste stream, undergoing treatment through organic waste management methods like anaerobic digestion, composting, or integrated anaerobic digestion and composting processes (Cucina et al., 2022). Anaerobic co-digestion of food waste with sludge inocula such as municipal wastewater, brewery effluent, grass silage, and farm residues has been effectively performed, demonstrating the potential advantages of utilizing municipal anaerobically digested inocula (Hobbs et al., 2019).

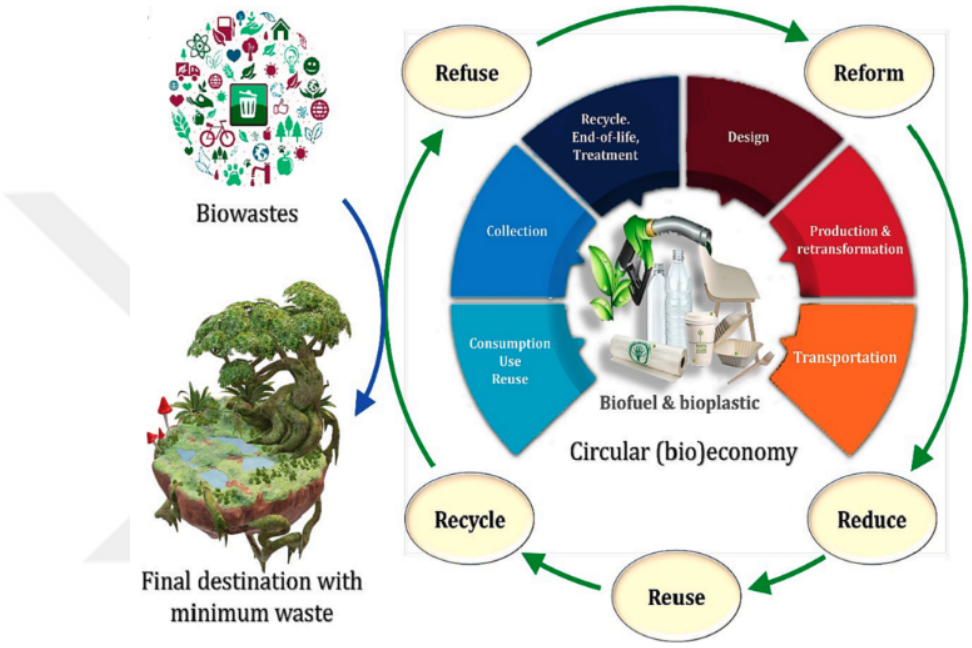


Figure 2.4. Circular economy concept (S.Ali et al., 2022).

Throughout the biowastes, food wastes are abundantly preferred substrates in anaerobic digestion systems. Despite growing interest, the potential for co-digesting biodegradable bioplastics with food waste in anaerobic digestion processes remains largely under-researched, with limited studies available on the topic (Batori et al., 2018). The advantages of a combined collection and processing system for food waste and biodegradable bioplastics are of interest, primarily due to the current lack of detailed knowledge about the biodegradation of bioplastics during anaerobic digestion, particularly alongside food waste. Such insights are critical for validating the anaerobic co-digestion of biodegradable bioplastics as a sustainable waste management strategy (Hedge et al., 2021; Kakadellis et al., 2022).

According to the research, due to its high energy content and a decay rate of approximately 0.14 per year, food waste is considered an excellent candidate for energy recovery technologies such as anaerobic digestion (AD) (De la Cruz & Barlaz, 2010).

Anaerobic digestion (AD) is increasingly regarded as the most suitable and sustainable technology to address the large volumes of food waste produced annually, as highlighted by the World Biogas Association, owing to its multiple benefits (WBA Global Bioenergy Statistics, 2018). Although prioritizing food waste reduction is essential for achieving maximum greenhouse gas (GHG) emissions savings throughout the food production lifecycle, the primary advantage of AD lies in its capacity to recover the chemical energy embedded in food wastes (Dilkes-Hoffman et al., 2018). The process generates biogas—a renewable energy source—comprising approximately 50–70% methane, 30–50% carbon dioxide, and minor amounts of other gases.

2.4. Candidate Bioplastics: PLA and PBAT

The definition of bioplastics usually includes both bio-based and biodegradable materials; however, not all biodegradable plastics are derived from biological sources. For instance, poly (butylene adipate-co-terephthalate) (PBAT) is a biodegradable polymer produced from petrochemicals. In contrast, bio-based plastics, such as polylactic acid (PLA), are synthesized from renewable biological resources and are generally biodegradable (Alvarez-Mendez et al., 2023). Chemical structures, subunits and some other information about both polymers are given in the Figure 2.5 below.

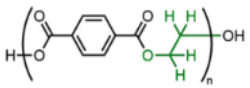
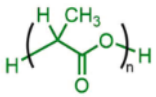
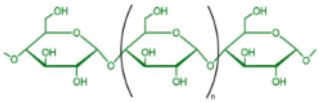
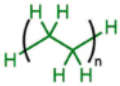
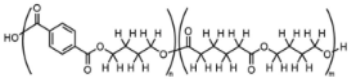
Polymer	Monomer/Subunit	Common Feedstocks	Biodegradable	Chemical Structure
bio-PET	Ethanol (EtOH) and terephthalic acid (TPA)	Corn, sugar beet, sugarcane, wheat (EtOH) and fossil-based (TPA)	N	
PLA	Lactic acid	Corn, sugarcane	Y †	
Starch-based polymer	Starch (α-linked D-glucose)	Corn, potato, wheat, cassava, sugarcane	Y	
bio-PE	EtOH	Corn, sugar beet, sugar cane, wheat	N	
PBAT	Adipic acid, 1,4-butanediol (BD) and dimethyl-terephthalate	Fossil based ‡	Y	

Figure 2.5. Details of some common biopolymers (Kakadellis et al., 2022).

PLA is a linear aliphatic polyester that is transformed from renewable sources. Thus, it can be synthetized via lactic acid condensation reaction or lactide ring-opening polymerization (Eichorn &

Gandini, 2010). As a biodegradable, bio-based aliphatic polyester, polylactic acid (PLA) can be obtained through sugar fermentation. The biodegradation of PLA within anaerobic digestion (AD) systems is characterized by a complex interaction between the polymer's chemical properties, microbial activity, and the operational parameters of the system.

The dominant raw material in the production of bio-based, biodegradable plastics is PLA, which is synthesized through the polycondensation of lactic acid and/or the ring-opening polymerization of lactide in which PLA finds applications in numerous sectors, such as plasticulture, textiles, the medical field, and packaging industries (Goel et al., 2021).

Polybutylene adipate terephthalate (PBAT) is a biodegradable random copolyester composed of adipic acid, 1,4-butanediol, and terephthalic acid. It is widely utilized in applications such as coatings for paper and cardboard, packaging in the form of plastic films and bottles, and foam products (Goel et al., 2021)

The most produced and utilized biodegradable bioplastics (with a projection study) are starch-based blends, with PBAT and PLA-based plastics following closely behind in terms of production as shown in the Table 2.2.

Table 2.2. Global bioplastic production and foresight (S.Ali et al., 2022).

Bioplastic type	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**	**2023**	**2024**
				Bio-based/biodegradable (%)				
Starch-blend	18.8	18.2	21.3	18.7	16.4	NA	15.8	18.5
PLA	10.3	10.3	13.9	18.7	18.9	NA	16.2	13.1
PHA	2.4	1.4	1.2	1.7	1.8	NA	4.5	6.6
PBS	4.9	4.6	4.3	4.1	3.5	NA	3.8	3.8
PBAT	5.0	7.2	13.4	13.5	19.2	NA	7.7	11.6
Other	1.5	1.5	1.4	1.4	4.4	NA	1.2	1.3
Total (%)	42.9	43.2	55.5	58.1	64.2	NA	49.2	54.9
Total production (MT)	0.88	0.91	1.05	1.24	1.55	3.7	4.1	4.4

Although PLA has been shown to be anaerobically digestible, the complete breakdown of PLA-based products like cups and thin films remains uncertain. Furthermore, limited research exists on whether these materials can degrade at similar rates to food waste under mesophilic conditions (Hobbs et al., 2019). On the other hand, PLA (poly lactic acid) stands out as one of the most thoroughly investigated polymers for substituting petroleum-based plastics (Boey et al., 2021).

The co-digestion of food waste with PLA can help divert municipal waste, lower landfill-related GHG emissions, and support renewable energy production. However, under mesophilic anaerobic digestion (35–40 °C), PLA degrades extremely slowly, and various publications have documented an absence of biodegradation even after 40 to 100 days of treatment (Benn and Zitomer, 2018; Cazaudehore et al., 2022a; Vargas et al., 2009; Vasmara and Marchetti, 2016).

The role of microbial communities is fundamental in the anaerobic degradation of PLA. Incorporating PLA into anaerobic digesters modifies the microbial consortia, which directly impacts biogas generation efficiency (Zhang et al., 2018). While specific microbes capable of degrading PLA have been identified, their activity is sensitive to digester conditions like temperature and hydraulic retention time (Wang et al., 2012). Moreover, co-digestion with food waste has demonstrated increased methane yields, indicating synergistic substrate effects (Vasmara & Marchetti, 2016).

PBAT, a co-polyester derived from butanediol (BDO), adipic acid (AA), and terephthalic acid (TA), has been specifically developed to promote hydrolytic susceptibility and biological degradability by introducing aliphatic moieties into aromatic polyester chains (Jian et al., 2020).

The biodegradation rate of PLA is strongly influenced by its chemical structure. PLA exists in three enantiomeric forms: levorotatory (L-), dextrorotatory (D-), and meso (a combination of L- and D- forms). A higher proportion of D-isomers reduces the crystallization rate, which has been identified as a key factor for enhancing PLA biodegradation (Ranakoti et al., 2022). On the other hand, the biodegradability of PBAT is primarily dependent on the proportion of TA in the polymer; higher TA content results in slower degradation (Jian et al., 2020). Additionally, the oligomers and monomers produced during the biodegradation process are fully metabolized without leaving residual products (Alvarez-Mendez et al., 2023).

3. AIM OF THE STUDY

The main objective of this study is to investigate the biomethane contribution of bioplastics—specifically PLA and PBAT—through anaerobic co-digestion with food waste under mesophilic conditions. To achieve this, biogas production from each bioplastic was evaluated by varying the inoculum to substrate ratio (ISR) based on grams of volatile solids (gVS). In addition, the impact ISR on the performance of anaerobic co-digestion reactors was examined in detail via changing the ISR from 2 to 1. An additional analytical step was conducted to assess biogas composition, providing insight into how ISR influences biomethane generation.

The specific objectives of the study are as follows:

- To evaluate the effect of PLA and PBAT bioplastics on biogas contribution during anaerobic digestion
- To enhance understanding of how different ISRs influence biogas contribution to the control setups during anaerobic co-digestion of food waste under mesophilic condition
- To assess the biochemical methane potential (BMP) and determine the biodegradability of PLA and PBAT bioplastics in an anaerobic environment

4. MATERIALS AND METHODS

4.1. Substrates and Inoculum Selection

The key aspects of bioplastic biodegradation are the reaction rates and the extent of degradation, which are largely influenced by the operational setup, inoculum and substrate types. In this study, a controlled, oxygen-free environment was constructed where 3 types of organic substrates and inoculum (food waste, bioplastics, namely PLA and PBAT, and inoculum) are broken down into biogas.

Food waste was collected from Boğaziçi University's dining hall as a waste. The food waste was a mix of the following foods: olive oil, sunflower oil, rice, bulgur pilaf, and chickpeas. To form a homogeneous mixture for this study, the food waste was prepared by mixing the whole food waste, followed by chopping and grinding by a kitchen mixer. After achieving the desired consistency of samples, samples were stored at a temperature-controlled room at 4^{o}C to be used in the analyses and reactor setups. They were brought to room temperature prior to use. After sampling of the food waste samples, TS and VS analyses were immediately carried out.

The activated inoculum to be used in the anaerobic process for BMP tests was collected from mesophilic digesters operated by the Istanbul Metropolitan Municipality in Kemerburgaz Biomethanization Plant, İstanbul, Türkiye, which are shown in Figure 4.1. In this facility, fruit/vegetable leftovers, cooked food leftovers, bakery products, milk and dairy products, pre-packaged foods, kitchen and canteen wastes and other 20 02 01 coded 'biodegradable wastes' (see in Figure 4.2.) are sterilized and processed. 20 liters of inoculum were collected from Istanbul Metropolitan Municipality Kemerburgaz Biomethanization Plant for each setup. Therefore, inoculum samples from the reactor of the plant were kept under 4^{o}C for further analyses.

Figure 4.1. Digesters, Istanbul Metropolitan Municipality Kemerburgaz Biomethanization Plant.

Figure 4.2. Waste collection site, Istanbul Metropolitan Municipality Kemerburgaz Biomethanization Plant.

PLA and PBAT were selected for the degradation experiments in this study due to their widespread production and use in the bioplastics market, the limited existing knowledge regarding PBAT, and the lack of sufficient information on their pellet forms in the literature. The plastic samples of PLA and PBAT in the form of pellets as shown in Figure 4.3 have been added to the mixture consisting of inoculum and substrate. The bioplastics were provided by two manufacturers, Karex

Polymers and Sunar NP have provided the PLA and PBAT samples, respectively, with the purpose of academic studies. The characteristics of the bioplastics are presented in Table 4.1 below.

Figure 4.3. PLA pellets (on the left) and PBAT pellets (on the right).

Table 4.1. Characteristics of bioplastics.

Parameter	PLA	PBAT
TS (%)	99.66	99.79
VS (%)	99.64	99.76
Ash (%)	0.014	0.024
C %	58.78	72.12
H %	8.58	11.09
N %	0.00	0.00
O %	32.63	16.77

4.2. Characterization Analyses for Substrates and the Inoculum

4.2.1. Chemicals and Reagents

4.2.1.1. Reagents of pH. The pH meter was calibrated using buffer solutions with pH values of 4.01, 7.00, and 10.01.

4.2.1.2. Reagents of chemical oxygen demand (COD) and soluble chemical oxygen demand (sCOD). COD and sCOD analyses were performed using potassium dichromate ($K_2Cr_2O_7$), sulfuric acid (H_2SO_4), mercury (II) sulfate ($HgSO_4$), and silver sulfate (Ag_2SO_4). For the calibration curve, potassium hydrogen phthalate (KHP) was used at concentrations of 0, 100, 200, 400, 600, and 900 mg/L.

4.2.1.3. Reagents of total Kjeldahl nitrogen (TKN). TKN analysis was conducted using potassium sulfate (K_2SO_4), copper (II) sulfate ($CuSO_4$), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), boric acid (H_3BO_3), methyl red indicator, 95% ethyl alcohol, and methylene blue.

4.2.2. Analytical Methods

pH, total solid (TS), volatile solid (VS), chemical oxygen demand (COD), soluble chemical oxygen demand (sCOD), elemental analysis (C-H-N) and TKN were analyzed before running the experiment for physicochemical characterization of the substrates. Moreover, all parameters were analyzed once more after the experiment was completed to observe the changes during the AD process. All parameters, and the methods were used are given in Table 4.2.

Table 4.2. Parameters to be analyzed.

Parameters	Method/Standard/Reference
pH	TS EN ISO 10390
TKN	Gerhardt TKN Analysis & SM 4500-NH3
COD	SM 5220 D
sCOD	SM 5220 D
TS	SM 2540 B
VS	SM 2540 E
Carbon	Elemental Analysis/Laboratory Procedure
Hydrogen	Elemental Analysis/Laboratory Procedure
Nitrogen	Elemental Analysis/Laboratory Procedure

4.2.2.1. pH. The pH of the samples was determined following the TS ISO 10390 standard (Turkish Standards Institution, 2021). Inolab pH 7110 pH meter (see in Figure 4.4), previously calibrated with buffer solutions of pH 4.01, 7.00, and 10.01, was used for the measurements. During the pH measurement, the samples were stirred using a magnetic stirrer.

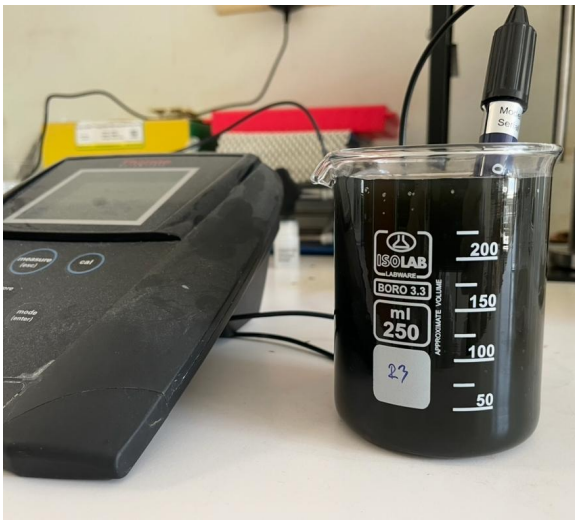


Figure 4.4. pH measurements in the laboratory.

4.2.2.2. Total solids (TS) and volatile solids (VS). Total solids and volatile solids of the samples were measured based on the Standard Methods for the Examination of Water and Wastewater, sections 2540 B and 2540 E, respectively (APHA, 2012). A 50 mL sample was placed into a clean crucible, then dried in an oven at 105 °C for 24 hours. After cooling in a desiccator, the crucible was weighed to determine the TS. For VS analysis, the same crucible containing the dried residue was ignited in a furnace at 550 °C for 15 minutes as shown in Figure 4.5 below, then cooled in a desiccator and weighed again.

Figure 4.5. Crucibles used in TS and VS analyses.

4.2.2.3. Chemical oxygen demand (COD) and soluble chemical oxygen demand (sCOD). The determination of chemical oxygen demand (COD) and soluble COD (sCOD) in samples were performed according to Standard Method 5220 D (APHA, 2012). For COD analysis, firstly the sample was diluted. Subsequently, a high-range digestion reagent and sulfuric acid were added as

shown in Figure 4.6. The sealed vials were gently mixed and digested at 150 ± 2 °C for 2 hours in a preheated block digester (VELP Eco 25). After digestion, the vials were cooled in the dark, and the absorbance was measured at 600 nm using a Hach DR/2010 spectrophotometer. For sCOD, samples were filtered using a 0.45 µm pore size filters. The filtrate was diluted if necessary and analyzed with the same protocol.

Figure 4.6. Equipments for COD and sCOD analyses.

4.2.2.4. Total Kjeldahl nitrogen (TKN). The measurements of Total Kjeldahl Nitrogen (TKN) in the samples were carried out following the guidelines of Standard Method 4500-NH_3 (APHA, 2012) and the Gerhardt Analytical Systems' Manual for Kjeldahl Nitrogen Determination. For the digestion process, 50 mL of the sludge sample was placed in a Kjeldahl quartz tube, to which sulfuric acid, potassium sulfate, and copper sulfate were added. The mixture was digested at 400 °C for min. 1.5 hours using a Gerhardt Kjeldatherm digestion unit. After cooling, deionized water was added before moving on to the distillation step. In this step, 50 mL of boric acid was added to an Erlenmeyer flask and placed on one side of the Vapodest 200 distillation system, while the digested sample was set on the other side (see in Figure 4.7). Distillation was initiated by adding 32% sodium hydroxide. The distillate was titrated with a standardized sulfuric acid solution until a color change from green to purple occurred. The amount of TKN was determined from the titrant volume.

Figure 4.7. Gerhardt device for Kjeldahl nitrogen determination and titration unit.

4.2.2.5. Elemental analysis (C, H, N). Carbon (C), hydrogen (H), and nitrogen (N) were the elements analyzed in the bioplastic samples. The dried material was then scraped using a metal spoon and finely ground using a porcelain mortar and pestle. The elemental composition of the ground sludge was subsequently analyzed using a Costech ECS 4010 elemental analyzer.

4.3. Experimental Design, Reactor Setups and Data Collection

4.3.1. The Reactor System

The AD process was performed in batch mode through the reactor system, involving a total of nine glass reactors (the experiment reactors and water bath are shown in Figure 4.8). The equipment (namely BRS III) used was supplied by BPC Instruments AB (Sweden-based company). Each reactor (R1-R9) has a working maximum volume of 2 L, it is equipped with an internal stirrer which ensures a complete mixing, and it can be controlled by using software on PC in the system.

During operation of the reactors stirring was provided by built-in stirrers of the reactor system. Biogas flow of the reactors was recorded by built-in sensors in the system via the Flow Cell Unit of the equipment with the help of BioReactor Simulator-3 and Aurora Software. Units of the research reactor system (stirrer, motor, glass reactor, etc.) are given in Figure 4.9. below.

Figure 4.8. Water bath integrated experiment reactors.

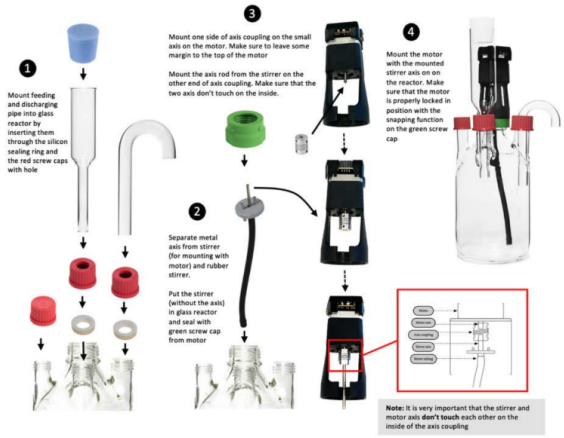


Figure 4.9. Units of a reactor in this research (BPC Instruments, n.d).

A schematic illustration of the reactor and the Flow Cell Unit is shown in Figure 4.10 below. Additionally, the Figure 4.10 presents the complete experimental system, including the water bath, reactors, Flow Cell Units, and the computer used for monitoring.

Biochemical Methane Production (BMP) tests were conducted to measure the possible amount of biogas that can be produced out of each sample by anaerobic digestion. (BMP) tests were performed by using a volumetric gas production method via BRS III Complete System.

The BRS III Complete System is a laboratory-scale instrument developed for evaluating biogas production from various substrates using anaerobic digestion. As depicted in Figure 4.11 below, the system consists of nine individual, gas-tight glass digesters, each with a working volume capacity of up to 2 liters. These digesters are submerged in a water bath to ensure operation under mesophilic

conditions. Each digester is equipped with a rotary stirrer to enable continuous mixing of the substrate at 37 °C.

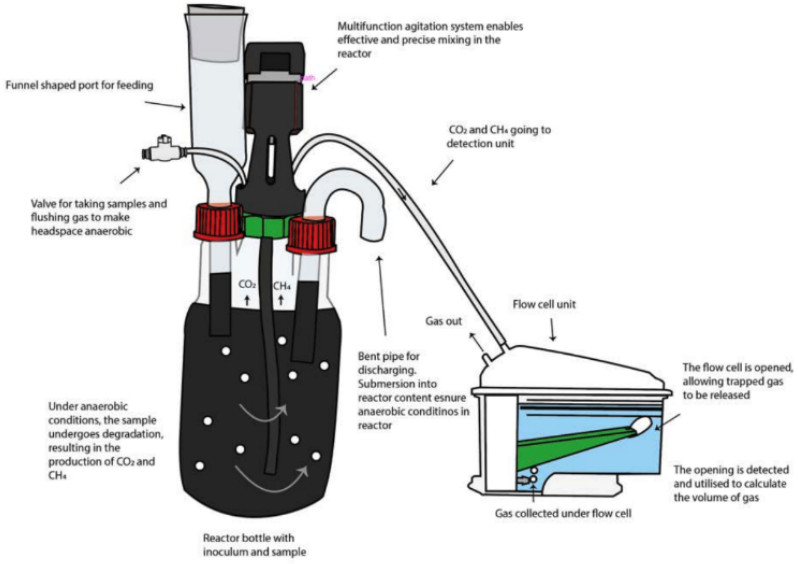


Figure 4.10. Schematic diagram of a reactor connected to Flow Cell Unit.

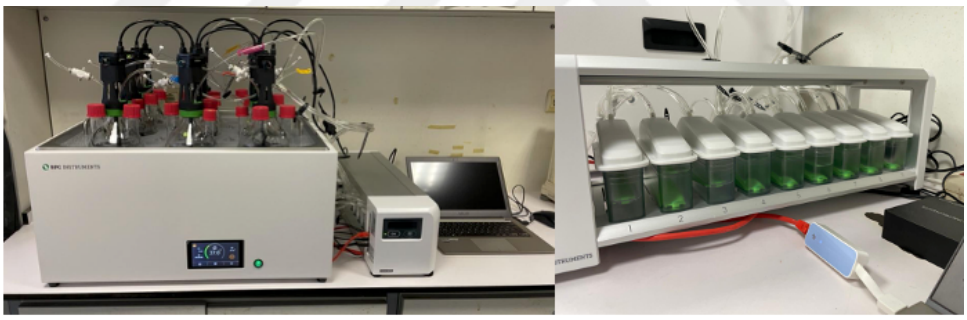


Figure 4.11. Water bath, reactors with BMP equipment in the laboratory.

Upon system activation, biogas—mainly composed of methane and carbon dioxide—is generated. The integrated data acquisition system (see an example screenshot from the computer software in Figure 4.12 below) continuously monitors and records the gas flow rate for each reactor. Accordingly, the automated setup allows biogas production data to be continuously recorded and retrieved via the software for detailed analysis.

Therefore, the BRS III system required regular monitoring to ensure proper functionality and to identify any potential operational issues. Additionally, the water level in the thermostatic water bath had to be checked frequently and replenished as necessary.

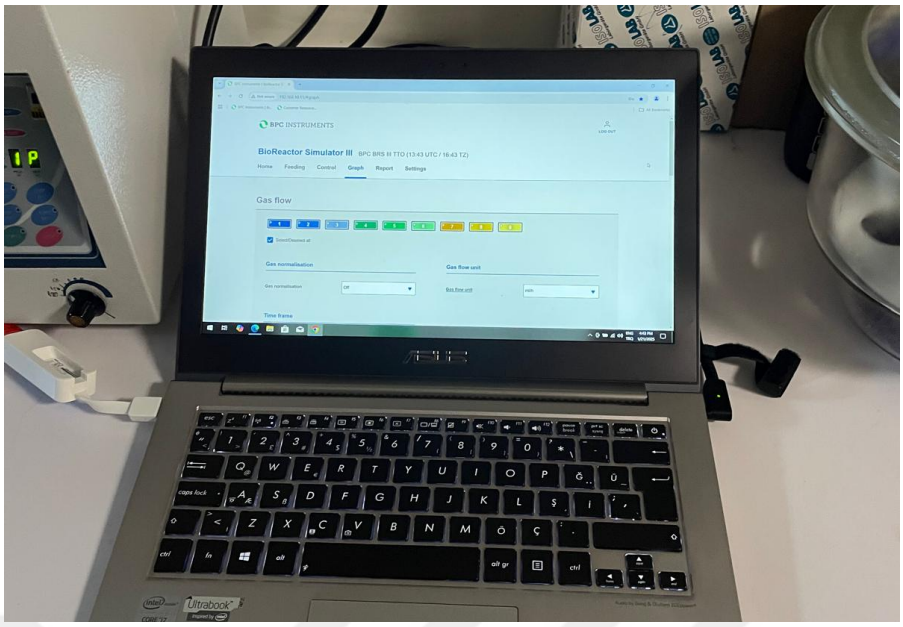


Figure 4.12. BRS III control panel on the software.

4.3.2. Experiment Setups

After taking inoculum samples from the plant, a trial setup (as shown in Figure 4.13 below) was constructed in the laboratory with known glucose, $C_6H_{12}O_6.H_2O$, (D (+)-Glucose Monohydrate, manufactured by ISOLAB) that has MW: 198.17 g/mol, density: 0.62 g/cm^3. The trial setup has been operated to observe the activity of the inoculum for further experiment setups.

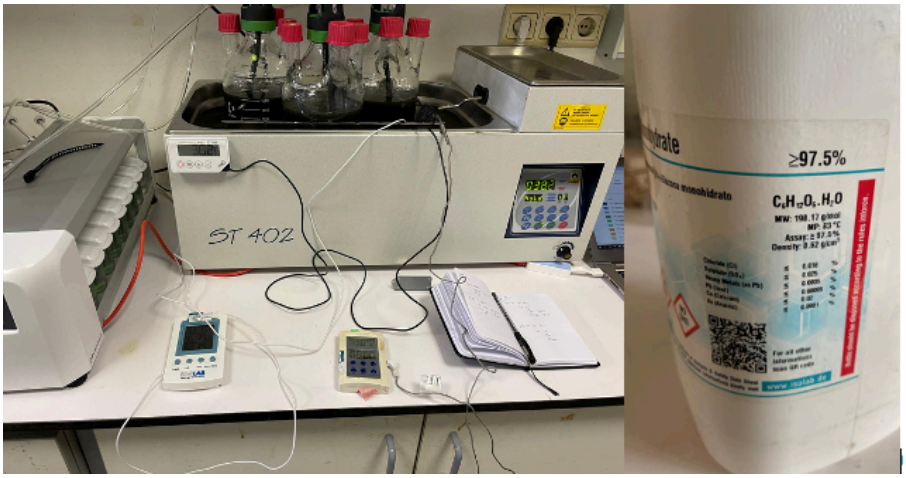


Figure 4.13. Trial setup configuration, Institute of Environmental Sciences.

Once the trial glucose setup was completed, the analyses proceeded with the reactors consisting of inoculum, food waste and polymers. Determination of volatile solids (VS) for both the inoculum and food waste in these sets, were carried out to determine substrate amount in grams of VS. All

experiments were conducted under mesophilic conditions (around 37°C). For each run, including Trial Setup, Setup-1, Setup-2, and Setup-3, 1.6 liters of inoculum were used in each reactor, considering the headspace in the reactor bottles. Around 3g plastic samples (for both PLA and PBAT) were placed in the reactor as pellet forms in each setup.

For Trial Setup and Setup-3, an inoculum to substrate ratio (ISR) of 1, and an ISR of 2 for the other setups were selected. Required amounts of food waste were calculated for each reactor. After adding and thoroughly mixing the inoculum, food waste, and plastics, all reactors were sealed to maintain anaerobic conditions. Nitrogen gas was injected for 150 seconds into the sampling unit channel of the reactors before they were placed in hot water baths.

In this study, Setup-1 reactors (except for reactors containing PBAT polymer that were conducted as triplicate) were carried out as duplicates. Setup-2 and Setup-3 reactors were carried out with 3 replicates. Tables 4.3, 4.4 and 4.5 demonstrate the contents of the reactors for each setup.

In Setup-1, the substrate (food waste) was mixed with the inoculum with an inoculum to substrate ratio (ISR) of 2:1. At the beginning of the experiment, pH of all digesters was aimed to be in the range of 6.8-7.5.

Table 4.3. Reactors design for Setup-1.

Reactor No	Content	Replicate Name
R1	Only Inoculum	Control-1
R2	Only Inoculum	Control-2
R3	Inoculum + Food Waste	FW-1
R4	Inoculum + Food Waste	FW-2
R5	Inoculum + Food Waste + PLA	PLA-1
R6	Inoculum + Food Waste + PLA	PLA-2
R7	Inoculum + Food Waste + PBAT	PBAT-1
R8	Inoculum + Food Waste + PBAT	PBAT-2
R9	Inoculum + Food Waste + PBAT	PBAT-3

In Setup-2, the substrate (food waste) was mixed with the inoculum (anaerobic seed sludge), and ISR was 2:1. At the beginning of the experiment the pH of all digesters was aimed to be in the range of 6.8-7.5.

Table 4.4. Reactors design for Setup-2.

Reactor No	Content	Replicate Name
R1	Only Inoculum	Control-1
R2	Only Inoculum	Control-2
R3	Only Inoculum	Control-3
R4	Inoculum + Food Waste	FW-1
R5	Inoculum + Food Waste	FW-2
R6	Inoculum + Food Waste	FW-3
R7	Inoculum + Food Waste + PLA	PLA-1
R8	Inoculum + Food Waste + PLA	PLA-2
R9	Inoculum + Food Waste + PLA	PLA-3

In Setup-3, the substrate (food waste) was mixed with the inoculum (anaerobic seed sludge), and inoculum to substrate ratio was 1:1. At the beginning of the experiment the pH of all digesters was aimed to be in the range of 6.8-7.5.

Table 4.5. Reactors design for Setup-3.

Reactor No	Content	Replicate Name
R1	Only Inoculum	Control-1
R2	Only Inoculum	Control-2
R3	Only Inoculum	Control-3
R4	Inoculum + Food Waste	FW-1
R5	Inoculum + Food Waste	FW-2
R6	Inoculum + Food Waste	FW-3
R7	Inoculum + Food Waste + PBAT	PBAT-1
R8	Inoculum + Food Waste + PBAT	PBAT-2
R9	Inoculum + Food Waste + PBAT	PBAT-3

4.3.3. Total Biogas Production and BMP Analyses

4.3.3.1. Software for biogas data collection and gas chromatography analysis. In this study, biogas production from waste materials co-digested at mesophilic temperatures were determined via biomethane potential (BMP) assessments. Experiments were performed using reactors connected to a Flow Cell Unit and software that enabled the tracking of gas flow rates. The experimental results obtained using BioReactor Simulator-3 and Aurora Software are presented in the Excel document in Table 4.6 below.

Table 4.6. Example output of the BMP instrument as an excel document.

LINE	1	2	3
NAME	Inoculum 1	Inoculum 2	Inoculum 3
START OF EXPERIMENT (UTC)	2025-03-21 12:25:25	2025-03-21 12:25:25	2025-03-21 12:25:25
END OF EXPERIMENT (UTC)	Running	Running	Running
TIME (UTC) INTERVAL: DAY	(ml/day)	(ml/day)	(ml/day)
2025-03-21 00:00:00	0.0	0.0	0.0
2025-03-22 00:00:00	1369.5	1293.5	1354.4
2025-03-23 00:00:00	1641.2	1549.9	1605.3
2025-03-24 00:00:00	978.5	899.8	963.7
2025-03-25 00:00:00	1175.9	1088.6	1147.0
2025-03-26 00:00:00	1349.4	1251.2	1315.3
2025-03-27 00:00:00	1415.4	1332.9	1393.6
2025-03-28 00:00:00	1366.5	1277.2	1340.8
2025-03-29 00:00:00	1101.9	1057.5	1077.0

Average cumulative biogas differences between bioplastic-contained reactors and control reactors were analyzed to observe the contribution of selected bioplastics into biogas production. However, to observe the direct effect of bioplastics on the biomethane production biogas composition has been determined by Gas Chromatograph (GC) in this study.

The composition of biogas, specifically the concentrations of CO_2, N_2, and CH_4, was determined using a gas chromatography system (HP Agilent 6850) equipped with a 30 m × 0.53 mm column and a thermal conductivity detector, employing helium (He) as the carrier gas as shown in Figure 4.14. Gas samples were collected from the headspace of the reactors using a 0.25 mL Hamilton airtight syringe. The analysis of biogas composition was performed following the procedure outlined by Sohoo et al. (2021).

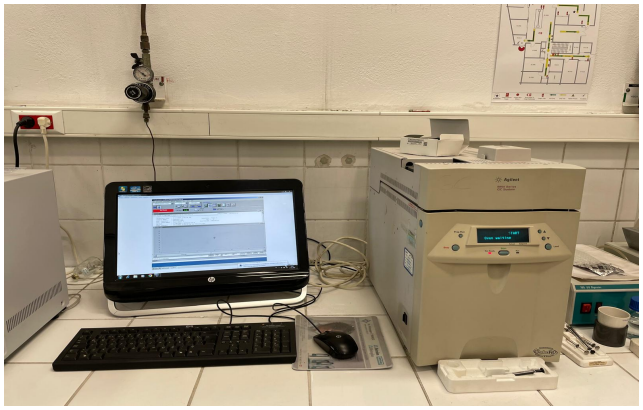


Figure 4.14. Gas chromatography, HP Agilent 685.

4.3.3.2. Calculation of biomethane contribution of bioplastics and biodegradation rates. The method relies on comparing the gas production of reactors containing bioplastics (both PLA and PBAT) with those containing inoculum and food waste. By analyzing the differences in cumulative biogas generation, the extent to which bioplastics contribute to gas production through degradation can be assessed.

The reactors containing inoculum and food waste serve as control units to evaluate the influence of bioplastics on biogas production. Thus, reactor performance was validated by calculating the gas yield, *Ygas*, using the Eq. 4.1:

$$\mathrm{Ygas} = \frac{(\mathrm{Pgas_{substrate}} - \mathrm{Pgas_{inoculum}})}{(\mathrm{OM_{substrate}})} \quad (4.1)$$

where Ygas (mL gVS^{-1}) represents the gas yield; OM_substrate (gVS) is the amount of volatile solids in the substrate added to the reactor; Pgas_substrate (mL) is the total gas volume generated by the reactor; and Pgas_inoculum (mL) refers to the gas volume attributed to the inoculum, calculated as the product of the inoculum's volatile solids (gVS) in each test reactor and the average gas yield of the inoculum (mL gVS^{-1}), as determined from the blank reactors.

To evaluate the biomethane contribution of bioplastics, biogas composition was analyzed using gas chromatography. Assessing the methane content within the total biogas mixture allows for the investigation of bioplastic biodegradation rates during anaerobic digestion. This is achieved through the application of Eq. 4.2 below for calculating the theoretical biomethane potential, and Eq. 4.3 below for estimating the degree of biodegradation with using outputs of elemental analysis for PLA and PBAT (Buswell and Mueller, 1952; Cazaudehore et al., 2022b).

$$\mathrm{BMPth\ (\ NL(CH_4).kg^{-1}(CxHyOzNnSs))} = \frac{22.4 \times (\frac{x}{2} + \frac{y}{8} - \frac{z}{4} - \frac{3n}{8} - \frac{s}{4})}{12x + y + 16z + 14n + 32s} \quad (4.2)$$

$$\mathrm{Biodegradation\ (\%)} = \frac{BMP_{\mathrm{exp}}}{BMP_{\mathrm{th}}} \times 100 \quad (4.3)$$

In this methodology, the direct contribution of bioplastics to methane production is determined by subtracting the methane yield of the food waste–inoculum set (as a control) from the methane yield of the bioplastic-containing reactors (Ruggero et al., 2019; Yagi et al., 2014; Zhang et al., 2018). The

resulting methane volume is then divided by the gVS amount of bioplastic used in the experiments to calculate the Experimental BMP (BMPexp) value. This value is subsequently compared to the Theoretical BMP (BMPth), which is calculated using Equation 1 based on C-H-N elemental analysis. The ratio of the Experimental BMP to the Theoretical BMP provides the final biodegradation percentage of used bioplastic in the process.

5. RESULTS AND DISCUSSION

This study aims to explore the impact of anaerobic digestion on the degradation of bioplastics—namely PLA and PBAT—when co-digested with food waste. Although better performance was observed under thermophilic conditions, most real plants operate at mesophilic temperatures (Folino et al., 2023). Hence, working under mesophilic conditions was prioritized for this study. The research focuses on evaluating biogas generation from different setups composed of inoculum, food waste, and bioplastics, with particular attention to the differences in gas volumes between bioplastic-containing reactors and control reactors. Gas composition analyses were conducted using gas chromatography (GC) to determine the specific contribution of biomethane from bioplastic degradation.

Alongside gas measurements, several physicochemical parameters—including COD, sCOD, TS, VS, and pH—were analyzed at the beginning and end of each digestion cycle. These data were essential for interpreting the system behavior, assessing the stability of the digestion process, and identifying any experimental deviations or inconsistencies.

5.1. Characterization of the Inoculum, Feed and Reactor Ingredients

The results of the characterization experiments conducted for the inoculum and food waste used in the experimental setups are presented in Table 5.1 below. Additionally, the amounts of inoculum, and food waste to be added to the reactors are also presented in the same table.

Table 5.1. Inoculum and food waste characteristics and reactor setups.

Experiment	Parameter	Substrates	
		Inoculum	**Food Waste**
Setup-1 Batch Experiment (ISR = 2)	pH	8.05	4.8
	TS (%)	1.03	28.55
	VS (%)	0.28	24.65
	Volume (mL) added	1,600	–
	Weight (g) added	–	9.08
	gVS	4.48	2.24
Setup-2 Batch Experiment (ISR = 2)	pH	7.85	4.8
	TS (%)	1.04	28.55
	VS (%)	0.28	24.65
	Volume (mL) added	1,600	–
	Weight (g) added	–	9.09
	gVS	4.48	2.24
Setup-3 Batch Experiment (ISR = 1)	pH	6.98	4.8
	TS (%)	1.96	28.55
	VS (%)	0.98	24.65
	Volume (mL) added	1,600	–
	Weight (g) added	–	63.61
	gVS	15.68	15.68

The experiments were designed based on stabilization stages reported in the literature for anaerobic digestion systems, considering two different inoculum-to-substrate ratios (ISR=1 and ISR=2) as key setup parameters. During the setup of the experiments, commonly studied ISR values in the literature were reviewed as shown in Table 5.2 below.

Table 5.2. Inoculum to substrate ratios studied in the literature.

ISR	**Substrate**	**Condition**	**Reference**
ISR=1	Food Waste	Mesophilic	Gandhi et al., 2022
ISR=0.25	Organic Substrates	Mesophilic	Ahmadi-Pirlou et al., 2019
ISR=2.85	Organic Substrates	Mesophilic	Cazaudehore et al., 2023
ISR=2	Organic Substrates	Mesophilic	Gadaleta et al., 2024
ISR=1 and ISR=2	Food Waste	Mesophilic	Khadka et al., 2022
ISR=2	Food Waste	Mesophilic	Kosheleva et al., 2023
ISR=1	Food Waste	Mesophilic	Cucina et al., 2021
ISR=2	Food Waste	Mesophilic	Nachod et al., 2021

While ISR of 1 is generally accepted as the standard for achieving maximum or optimal methane production, some studies also recommend using ISR of 2. Based on these insights, reactors in this study were initially set up with ISR of 2, and the final setup was carried out using ISR of 1.

Along with the determination of ISR values for the setups, the reactor contents were prepared based on their gVS values as demonstrated in the following studies: Owamah et al. (2021), Hamzah et al. (2023), Kosheleva et al. (2023), and Khadka et al. (2022). In all setups, the inoculum volume was kept constant at 1600 mL, and the corresponding amounts of food waste were calculated according to the selected ISR ratio. Specifically, the inoculum had a VS content of 0.28%, corresponding to 4.48 gVS in Setup-1 and Setup-2, while this value was calculated as 15.68 gVS for Setup-3. Additionally, the amount of food waste to be added was determined using the measured VS content of 24.65% for food waste. Consequently, 2.24 gVS of food waste was added to Setup-1 and Setup-2 based on an ISR of 1. Considering the unstable gas production values observed in the first two setups, an alternative inoculum was selected for subsequent experiments and 15.68 gVS of food waste was added to Setup-3 due to the higher VS content of the inoculum used (while the TS% value of inoculum was 1.03-1.04 in Setup-1 and Setup-2, the value was 1.96% for alternative inoculum in Setup-3).

5.1.1. Reactor Design for Setup-1

Following the verification of microbial activity in the selected inoculum using a glucose-fed trial experiment, biogas production was confirmed through monitoring on the BPC bioreactor system. Subsequently, Setup-1 was initiated, comprising duplicate reactors containing only inoculum (R1 & R2), duplicate reactors with inoculum and food waste (R3 & R4), duplicate reactors with inoculum, food waste, and PLA bioplastic (R5 & R6), and triplicate reactors with inoculum, food waste, and PBAT bioplastic (R7, R8 & R9) in which ISR of 2.

Initially, each reactor was loaded with 1600 mL of inoculum. The quantity of food waste added to the reactors (excluding R1 and R2) was calculated based on the volatile solids (VS) content, which are 0.28% for inoculum and 24.65% for food waste. Accordingly, 9.08 g (2.24 gVS) of food waste was added to the relevant reactors. Additionally, 3 g of pellet PLA was introduced into reactors R5 and R6, while 3 g of pellet PBAT was added to reactors R7, R8, and R9. The detailed configuration of the reactors in Setup-1 is presented in Table 5.3 below.

Table 5.3. Reactor details of Setup-1.

Reactor No	Name	ISR	Content
R1	Inoculum 1	2	1,600 mL inoculum
R2	Inoculum 2	2	1,600 mL inoculum
R3	Inoculum + FW 1	2	(1,600 mL inoculum) + 9.08 g FW
R4	Inoculum + FW 2	2	(1,600 mL inoculum) + 9.08 g FW
R5	Inoculum + FW + PLA 1	2	(1,600 mL inoculum) + 9.08 g FW + 3 g PLA
R6	Inoculum + FW + PLA 2	2	(1,600 mL inoculum) + 9.08 g FW + 3 g PLA
R7	Inoculum + FW + PBAT 1	2	(1,600 mL inoculum) + 9.08 g FW + 3 g PBAT
R8	Inoculum + FW + PBAT 2	2	(1,600 mL inoculum) + 9.08 g FW + 3 g PBAT
R9	Inoculum + FW + PBAT 3	2	(1,600 mL inoculum) + 9.08 g FW + 3 g PBAT

5.1.2. Reactor Design for Setup-2

The same procedure used in Setup-1 was followed for determining the reactor contents in Setup-2. The same food waste and inoculum were used to ensure consistency between the two setups. Based on volatile solids (VS), the inoculum-to-substrate ratio (ISR) was again set to 2. Detailed information about the reactors in this setup is presented in Table 5.4 below.

Table 5.4. Reactor details of Setup-2.

Reactor No	Name	ISR	Content
R1	Inoculum 1	2	1,600 mL inoculum
R2	Inoculum 2	2	1,600 mL inoculum
R3	Inoculum 3	2	1,600 mL inoculum
R4	Inoculum + FW 1	2	(1,600 mL inoculum) + 9.09 g FW
R5	Inoculum + FW 2	2	(1,600 mL inoculum) + 9.09 g FW
R6	Inoculum + FW 3	2	(1,600 mL inoculum) + 9.09 g FW
R7	Inoculum + FW + PLA 1	2	(1,600 mL inoculum) + 9.09 g FW + 3 g PLA
R8	Inoculum + FW + PLA 2	2	(1,600 mL inoculum) + 9.09 g FW + 3 g PLA
R9	Inoculum + FW + PLA 3	2	(1,600 mL inoculum) + 9.09 g FW + 3 g PLA

5.1.3. Reactor Design for Setup-3

Studies have shown that co-digestion of food waste with bioplastics results in lower biogas production compared to digestion of food waste alone (El-Mashad et al. 2012; Nachod et al. 2021). Similarly, considering the irregular gas production patterns and the lack of consistent data observed in Setup-1 and Setup-2, a third experimental setup (Setup-3) was developed using an alternative

inoculum collected from a different location within the same facility. In the alternative inoculum collected from the facility, the volatile solids (VS) content was calculated to be 0.98%. Unlike the previous setups, 63.61g of food waste (15.68 gVS) was used in Setup-3.

Since biogas generation from PLA was nearly absent in Setup-2, only the PBAT bioplastic group was included in Setup-3, in addition to the control groups. Moreover, to contribute meaningfully to the literature and allow for comparison with Setup-1, the inoculum-to-substrate ratio (ISR) in Setup-3 was adjusted from 2 to 1. Following the same preparation steps as in the previous setups, reactors were assembled and operated under this new configuration. Detailed information about the reactors in this setup is presented in Table 5.5 below.

Table 5.5. Reactor details of Setup-3.

Reactor No	Name	ISR	Content
R1	Inoculum 1	2	1,600 mL inoculum
R2	Inoculum 2	2	1,600 mL inoculum
R3	Inoculum 3	2	1,600 mL inoculum
R4	Inoculum + FW 1	2	(1,600 mL inoculum) + 63.61 g FW
R5	Inoculum + FW 2	2	(1,600 mL inoculum) + 63.61 g FW
R6	Inoculum + FW 3	2	(1,600 mL inoculum) + 63.61 g FW
R7	Inoculum + FW + PBAT 1	2	(1,600 mL inoculum) + 63.61 g FW + 3 g PBAT
R8	Inoculum + FW + PBAT 2	2	(1,600 mL inoculum) + 63.61 g FW + 3 g PBAT
R9	Inoculum + FW + PBAT 3	2	(1,600 mL inoculum) + 63.61 g FW + 3 g PBAT

5.2. Changes in Physicochemical Parameters in the Reactors

Across all setups, both TS and VS percentages showed a decrease following the anaerobic digestion process. Details of TS and VS for each run are provided in Table 5.6 below.

The reductions in VS are indicative of active microbial processes and biogas generation. In Setup-1 and Setup-2, which were configured with ISR of 2, the initial VS content of 0.28% decreased to a range between 0.14% and 0.23%, corresponding to a reduction of 21–50%. Contrary to previous findings by Bong et al. (2017), which associated ISR of 2 with more stable methane production, GC analyses in this study revealed inconsistent methane trends in these setups. In Setup-3, operated with ISR of 1, reactors that started with a VS content of 0.98% also exhibited a consistent decreasing trend, with final VS values ranging from 0.20% to 0.34%. The average VS reduction in this setup was calculated to be between 60% and 80%. Unlike the findings of Bouallagui et al. (2005), who

suggested that methanogens could be inhibited by high food waste concentrations, an increase in methane levels was observed in this setup according to GC results. Furthermore, the hypothesis proposed by Chen et al. (2008), which suggests increased methanogen inhibition at lower ISR values, could not be clearly confirmed in this study.

Table 5.6. TS & VS analyses.

Setup Number	Reactor Number	Before AD		After AD	
		TS (%)	VS (%)	TS (%)	VS (%)
Setup-1 Batch Experiment (ISR = 2)	R1	1.03	0.28	0.91	0.21
	R2			0.90	0.20
	R3			0.89	0.19
	R4			0.87	0.16
	R5			0.96	0.23
	R6			0.96	0.23
	R7			0.89	0.20
	R8			0.92	0.21
	R9			0.93	0.20
Setup-2 Batch Experiment (ISR = 2)	R1	1.04	0.28	0.91	0.20
	R2			0.82	0.14
	R3			0.89	0.19
	R4			0.89	0.18
	R5			0.94	0.22
	R6			0.91	0.19
	R7			0.91	0.21
	R8			0.92	0.21
	R9			0.91	0.20
Setup-3 Batch Experiment (ISR = 1)	R1	1.96	0.98	0.97	0.20
	R2			1.08	0.25
	R3			0.97	0.21
	R4			1.16	0.28
	R5			1.13	0.30
	R6			1.15	0.31
	R7			1.16	0.29
	R8			1.13	0.28
	R9			1.21	0.34

Despite the observed reductions in VS, the irregularities in gas production may be attributed to pH-related factors. As noted by Rajagopal et al. (2013), methane production may be negatively affected when pH levels are higher than 8. Across all setups, the final pH values (see Table 5.7 below)

were recorded within the range of 7.7 to 8.4. Although Setup-3 showed lower initial pH levels compared to the other two setups, no indication of pH inhibition was observed in its gas production performance. Additionally, the increase in pH following the anaerobic digestion process—due to the degradation of protein content in the food waste—aligns with the outcomes observed in this study.

Table 5.7. pH analyses.

Setup Number	Reactor Number	Content	pH	
			Before AD	After AD
Setup-1 Batch Experiment (ISR = 2)	R1	Inoculum only	8.06	8.11
	R2	Inoculum only	8.04	8.10
	R3	Inoculum + FW	7.98	7.96
	R4	Inoculum + FW	7.96	8.13
	R5	Inoculum + FW + PLA	7.95	7.84
	R6	Inoculum + FW + PLA	7.98	8.40
	R7	Inoculum + FW + PBAT	7.97	7.94
	R8	Inoculum + FW + PBAT	7.97	7.84
	R9	Inoculum + FW + PBAT	7.99	7.85
Setup-2 Batch Experiment (ISR = 2)	R1	Inoculum only	7.87	8.31
	R2	Inoculum only	7.84	8.20
	R3	Inoculum only	7.83	8.08
	R4	Inoculum + FW	7.85	7.82
	R5	Inoculum + FW	7.88	7.79
	R6	Inoculum + FW	7.88	7.81
	R7	Inoculum + FW + PLA	7.81	7.98
	R8	Inoculum + FW + PLA	7.82	7.87
	R9	Inoculum + FW + PLA	7.83	8.01
Setup-3 Batch Experiment (ISR = 1)	R1	Inoculum only	6.98	8.00
	R2	Inoculum only	6.98	8.06
	R3	Inoculum only	6.99	8.04
	R4	Inoculum + FW	7.03	8.06
	R5	Inoculum + FW	6.98	8.03
	R6	Inoculum + FW	7.01	8.03
	R7	Inoculum + FW + PBAT	6.94	8.10
	R8	Inoculum + FW + PBAT	6.94	8.26
	R9	Inoculum + FW + PBAT	6.98	8.08

Another important parameter, TKN (Total Kjeldahl Nitrogen) (see in Table 5.8 below), represents the total organic nitrogen and ammonium nitrogen present in the reactors. Increases in TKN values were observed in R6 of Setup-1 and in R7, R8, and R9 of Setup-3. Previous studies, such

as those by Kang et al. (2022), reported that nitrogen conversion processes progress slowly during the anaerobic digestion of PLA, leading to ammonia accumulation. Additionally, it has been reported in the review study by Yenigün and Demirel (2013) that the breakdown of proteins in organic waste may lead to an increase ammonia content, which could exert an inhibitory effect on the bacterial community in producing biogas step. These observations align with the findings in the PLA reactor of Setup-1.

Table 5.8. TKN analyses.

Setup Number	Reactor Number	Content	(mg/L NH_3-N)	
			Before AD	After AD
Setup-1 Batch Experiment (ISR = 2)	R1	Inoculum only	2710.4	1724.8
	R2	Inoculum only	2688.0	1657.6
	R3	Inoculum + FW	2665.6	1848.0
	R4	Inoculum + FW	2632.0	1803.2
	R5	Inoculum + FW + PLA	2329.6	1836.8
	R6	Inoculum + FW + PLA	2296.0	3539.2
	R7	Inoculum + FW + PBAT	2273.6	1713.6
	R8	Inoculum + FW + PBAT	2396.8	1568.0
	R9	Inoculum + FW + PBAT	2363.2	1556.8
Setup-2 Batch Experiment (ISR = 2)	R1	Inoculum only	1702.4	1590.4
	R2	Inoculum only	1691.2	1624.0
	R3	Inoculum only	1646.4	1568.0
	R4	Inoculum + FW	2430.4	1579.2
	R5	Inoculum + FW	2307.2	1624.0
	R6	Inoculum + FW	2396.8	1657.6
	R7	Inoculum + FW + PLA	2419.2	1691.2
	R8	Inoculum + FW + PLA	2363.2	1680.0
	R9	Inoculum + FW + PLA	2430.4	1534.4
Setup-3 Batch Experiment (ISR = 1)	R1	Inoculum only	1758.4	1534.4
	R2	Inoculum only	1724.8	1579.2
	R3	Inoculum only	1724.8	1523.2
	R4	Inoculum + FW	1926.4	1848.0
	R5	Inoculum + FW	1948.8	1881.6
	R6	Inoculum + FW	1892.8	1870.4
	R7	Inoculum + FW + PBAT	1814.4	1993.6
	R8	Inoculum + FW + PBAT	1848.0	2016.0
	R9	Inoculum + FW + PBAT	1904.0	2004.8

In the AD process, a decrease in COD (Chemical Oxygen Demand) indicates the biological degradation of organic compounds and the occurrence of biogas production. Among the reactors containing bioplastic, only R6 in Setup-1 showed an increase in COD values as shown in Table 5.9 below. All other bioplastic-containing reactors exhibited a decrease in COD after the anaerobic digestion process. Regarding the anomaly observed in Setup-1, Kang et al. (2022) and Álvarez-Méndez et al. (2023) reported that PLA degrades only to a limited extent under mesophilic conditions, potentially leading to COD accumulation. This observation may serve as an important indicator of the fluctuations in gas production observed in the R6 reactor of Setup-1.

Table 5.9. COD analyses.

Setup Number	Reactor Number	Content	COD (g/L)	
			Before AD	After AD
Setup-1 Batch Experiment (ISR = 2)	R1	Inoculum only	40.26	5.06
	R2	Inoculum only	41.26	6.57
	R3	Inoculum + FW	43.27	5.94
	R4	Inoculum + FW	19.66	4.81
	R5	Inoculum + FW + PLA	26.95	5.06
	R6	Inoculum + FW + PLA	35.49	45.50
	R7	Inoculum + FW + PBAT	30.21	7.95
	R8	Inoculum + FW + PBAT	30.46	6.44
	R9	Inoculum + FW + PBAT	30.46	8.58
Setup-2 Batch Experiment (ISR = 2)	R1	Inoculum only	4.09	5.19
	R2	Inoculum only	3.84	5.69
	R3	Inoculum only	3.09	6.69
	R4	Inoculum + FW	12.38	7.57
	R5	Inoculum + FW	22.43	4.56
	R6	Inoculum + FW	16.90	5.06
	R7	Inoculum + FW + PLA	15.65	7.07
	R8	Inoculum + FW + PLA	17.15	6.57
	R9	Inoculum + FW + PLA	15.40	3.43
Setup-3 Batch Experiment (ISR = 1)	R1	Inoculum only	20.63	3.80
	R2	Inoculum only	21.01	4.06
	R3	Inoculum only	20.13	3.68
	R4	Inoculum + FW	21.76	5.19
	R5	Inoculum + FW	23.14	6.57
	R6	Inoculum + FW	21.13	5.69
	R7	Inoculum + FW + PBAT	19.75	6.82
	R8	Inoculum + FW + PBAT	17.24	7.82
	R9	Inoculum + FW + PBAT	18.75	6.19

Regarding the sCOD (soluble Chemical Oxygen Demand) parameter (the results are given in Table 5.10 below), all reactors in Setup-1 and Setup-3 showed a decrease compared to initial values, whereas the opposite trend was observed in R1, R2, and R4 of Setup-2. A decrease in sCOD during

anaerobic digestion typically indicates microbial activity and the conversion of intermediate products (such as VFAs) into biogas. The gas production behavior observed in Setup-2 contradicts the findings reported by Bouallagui et al. (2005), suggesting that the gas production issues in this setup may be interpreted through the sCOD parameter.

Table 5.10. sCOD analyses.

Setup Number	Reactor Number	Content	sCOD (g/L)	
			Before AD	After AD
Setup-1 Batch Experiment (ISR = 2)	R1	Inoculum only	9.37	1.07
	R2	Inoculum only	10.62	1.12
	R3	Inoculum + FW	15.14	1.32
	R4	Inoculum + FW	15.90	1.42
	R5	Inoculum + FW + PLA	16.15	1.37
	R6	Inoculum + FW + PLA	14.89	2.93
	R7	Inoculum + FW + PBAT	12.13	1.27
	R8	Inoculum + FW + PBAT	13.39	1.72
	R9	Inoculum + FW + PBAT	13.13	1.72
Setup-2 Batch Experiment (ISR = 2)	R1	Inoculum only	2.55	3.78
	R2	Inoculum only	1.17	3.58
	R3	Inoculum only	3.43	3.08
	R4	Inoculum + FW	1.80	2.33
	R5	Inoculum + FW	4.81	2.23
	R6	Inoculum + FW	8.95	2.53
	R7	Inoculum + FW + PLA	5.06	2.83
	R8	Inoculum + FW + PLA	4.31	2.73
	R9	Inoculum + FW + PLA	5.81	1.62
Setup-3 Batch Experiment (ISR = 1)	R1	Inoculum only	10.66	3.83
	R2	Inoculum only	10.31	2.93
	R3	Inoculum only	9.86	3.08
	R4	Inoculum + FW	11.87	1.87
	R5	Inoculum + FW	12.27	2.33
	R6	Inoculum + FW	12.47	2.53
	R7	Inoculum + FW + PBAT	12.12	2.23
	R8	Inoculum + FW + PBAT	12.42	1.52
	R9	Inoculum + FW + PBAT	12.02	1.87

5.3. BMP Assessment of Reactors

5.3.1. Outputs from Setup-1

Following the construction of the reactors, the experiment was initiated under anaerobic conditions. Upon commencement, biogas production was simultaneously monitored and recorded using the BPC system and accompanying software. To assess the composition of the produced gas, gas chromatography (GC) analyses were also initiated on Day 1.

The experiment for Setup-1 continued for 17 days, during which a plateau in cumulative biogas production was observed across all reactors. Biogas outputs were meticulously tracked via the BPC software. Cumulative gas production of the reactors is given in Table 5.11 below. According to the data, the inoculum-only reactors (R1 and R2) showed increasing biogas production until Day 5, after which the gas generation stabilized. As expected, the lowest gas production was recorded in these two reactors, which served as control units in line with the approach adopted in the following studies (Alvarez-Mendez et al., 2023; Kang et al., 2022) in alignment with the main methodology of the study.

Table 5.11. Cumulative gas production in Setup-1.

	Cumulative Gas Production (mL)								
Day	**Reactor-1**	**Reactor-2**	**Reactor-3**	**Reactor-4**	**Reactor-5**	**Reactor-6**	**Reactor-7**	**Reactor-8**	**Reactor-9**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	96.78	97.46	161.18	162.00	222.41	280.76	166.97	170.39	217.88
2	111.73	112.42	535.46	319.48	711.44	568.02	338.01	622.93	708.35
3	114.15	116.78	1,093.42	482.48	1,340.70	653.23	429.34	1,174.37	1,187.58
4	116.57	121.89	1,422.78	539.68	1,563.81	683.01	524.61	1,450.57	1,413.50
5	118.99	127.97	1,536.31	556.75	1,679.01	793.32	524.61	1,535.72	1,550.73
6	129.57	142.73	1,574.33	607.38	1,754.47	821.83	524.61	1,588.57	1,609.00
7	130.50	146.78	1,577.90	607.38	1,804.68	821.83	524.61	1,625.61	1,639.41
8	131.42	146.78	1,577.90	607.38	1,824.10	821.83	524.61	1,649.13	1,672.95
9	132.34	146.78	1,577.90	607.38	1,829.01	821.83	524.61	1,667.56	1,683.78
10	133.26	146.78	1,577.90	607.38	1,831.35	821.83	524.61	1,677.11	1,699.35
11	134.18	146.78	1,577.90	607.38	1,833.69	821.83	524.61	1,686.31	1,705.17
12	135.10	146.78	1,577.90	607.38	1,836.03	821.83	524.61	1,695.66	1,708.94
13	136.03	146.78	1,577.90	607.38	1,838.82	821.83	524.61	1,699.05	1,712.71
14	136.95	146.78	1,577.90	607.38	1,841.87	821.83	524.61	1,699.05	1,723.93
15	137.87	146.78	1,577.90	607.38	1,844.93	821.83	524.61	1,699.05	1,730.67
16	138.21	146.78	1,577.90	607.38	1,851.82	821.83	524.61	1,699.05	1,734.82
17	138.21	146.78	1,577.90	607.38	1,855.22	821.83	524.61	1,699.05	1,738.20

In contrast, reactors containing food waste exhibited varying gas production trends. When cumulative biogas volumes were compared, the order from the lowest to the highest production was as follows: R5, R9, R8, R3, R6, R4, R7, R2, and R1. Although a sequential trend was expected between R3 (1,577.90 mL) and R4 following the control reactors, lower-than-anticipated biogas production was observed in R4 (607.38 mL), R6 (821.83 mL), and R7 (524.61 mL) as shown in Figure 5.1 below.

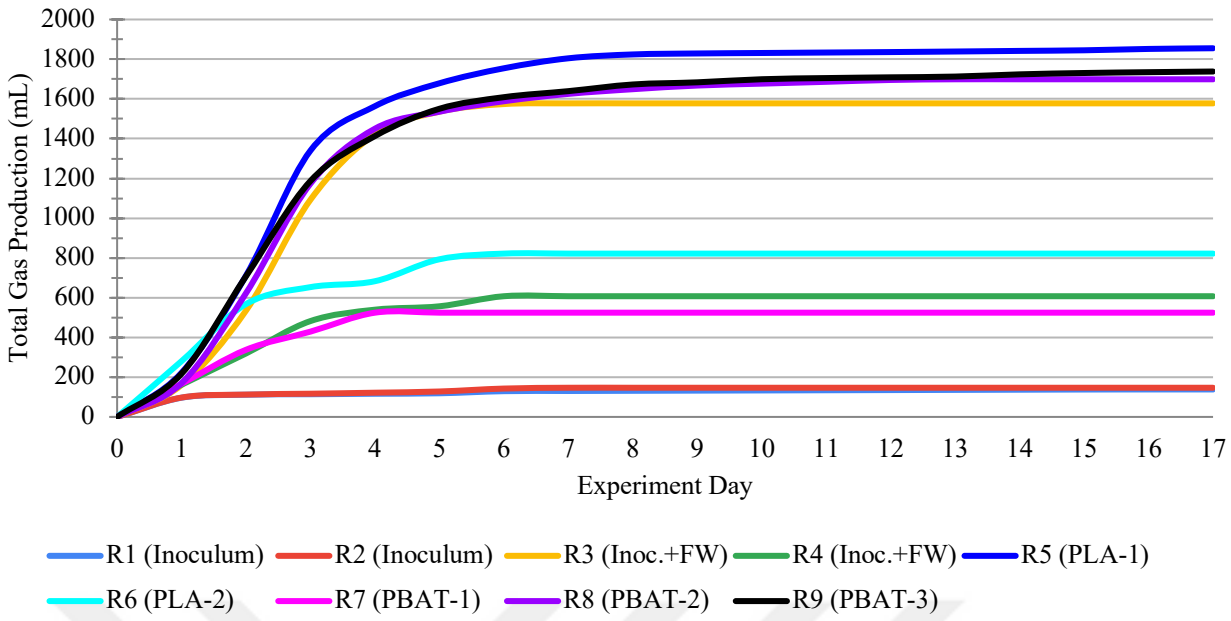


Figure 5.1. Cumulative gas production trends for Setup-1.

PLA film and pellet samples showed significantly different biogas yields under mesophilic conditions reported by Zhang et al. (2018). In line with literature findings that classify PLA as having higher biodegradability in anaerobic digestion systems, the highest biogas yield among bioplastic-containing reactors was observed in R5 (1,855.22 mL), which included PLA.

No noticeable effect of PBAT on biogas production was observed after 28 days of anaerobic digestion under mesophilic conditions (Massardier-Nageotte et al. 2006). On the other hand, PBAT-containing reactors in this setup demonstrated the next highest gas productions in R9 (1,738.20 mL) and R8 (1,699.05 mL) in the setup, respectively.

Although smaller-sized materials and films often performed better than larger samples, several studies reported inconsistent behavior in their degradation patterns correlated with biogas productions (Vardar et al., 2022). Accordingly, irregularities and inconsistencies observed in some duplicate and triplicate reactor sets—likely caused by selection of material type rather than experimental conditions—certain reactors were excluded from further calculations in this study.

In order to ensure reliable and interpretable results, specific reactors were selected for data evaluation in Setup-1. To evaluate the effects of PLA and PBAT in the bioplastic-containing reactor group on the food waste-inoculum set, the biogas difference per unit VS was used in the selection of the data sets. The following method was used for this calculation: the amount of gas produced in the

bioplastic-added groups was subtracted by the amount of gas produced in the inoculum group, and this difference was divided by the VS value of the food waste substrate initially used. Reactors that could be positioned within the value range observed in the literature were included in the calculations. Additionally, for the food waste reactor group, the amount of gas produced per unit gVS was also examined based on literature consistency in which 551.4 mL CH_4/gVS, 535 mL CH_4/gVS, 450 mL CH_4/gVS, 435 mL CH_4/gVS, 199 mL CH_4/gVS (Tanimu et al., 2014; Uddin & Wright, 2023; Zhang et al., 2019). Thus, based on the method, the specific biogas yields for the reactors in Setup-1 were calculated as follows: 640.80 mL/gVS for R3, 764.00 mL/gVS for R5, 694.89 mL/gVS for R8, and 712.37 mL/gVS for R9. When methane composition data from GC analysis are considered, the corresponding methane yields were calculated as 251.45 mL CH_4/gVS for R3 (39.24% CH_4), 368.55 mL CH_4/gVS for R5 (48.24% CH_4), 333.06 mL CH_4/gVS for R8 (47.93% CH_4), and 341.44 mL CH_4/gVS for R9 (47.93% CH_4). All results remained within the expected ranges reported in the literature. This approach was applied to ensure that only valid data that surpassed the lower limit values reported in the literature. Reactors that fell outside this range or produced negative values were excluded from the calculations, and the results were presented accordingly.

Included reactor groups are R1 and R2 for the inoculum-only group, R3 for the inoculum and food waste group, R5 for the PLA group, and R8 and R9 for the PBAT group for further discussion. The selection of these reactors was informed by consistency in biogas production data. Moreover, the interpretation and discussion of results focused on the methane yield parameter (mL CH_4/gVS), which will be explained in greater detail in Section 5.4.

The research showed that a commercial bag made of PLA and PBAT had low biodegradation under anaerobic conditions, at mesophilic (35 °C) temperatures, with no biogas contribution (Peng et al., 2022). However, the cumulative gas production values obtained from the selected reactors presented in Table 5.12 below in which 142.50 mL for inoculum group; 1,577.90 mL for inoculum-food waste group; 1,718.62 mL for PBAT group, and 1,855.22 mL for PLA group.

Table 5.12. Average biogas production (mL), Setup-1.

Day	R1 & R2 (Inoc.)	R3 (Inoc.+FW)	R5 (PLA)	R8 & R9 (PBAT)
0	0.00	0.00	0.00	0.00
1	97.12	161.18	222.41	194.14
2	112.07	535.46	711.44	665.64
3	115.47	1,093.42	1,340.70	1,180.97
4	119.23	1,422.78	1,563.81	1,432.04
5	123.48	1,536.31	1,679.01	1,543.22
6	136.15	1,574.33	1,754.47	1,598.79
7	138.64	1,577.90	1,804.68	1,632.51
8	139.10	1,577.90	1,824.10	1,661.04
9	139.56	1,577.90	1,829.01	1,675.67
10	140.02	1,577.90	1,831.35	1,688.23
11	140.48	1,577.90	1,833.69	1,695.74
12	140.94	1,577.90	1,836.03	1,702.30
13	141.40	1,577.90	1,838.82	1,705.88
14	141.87	1,577.90	1,841.87	1,711.49
15	142.33	1,577.90	1,844.93	1,714.86
16	142.50	1,577.90	1,851.82	1,716.93
17	142.50	1,577.90	1,855.22	1,718.62

PLA did not significantly contribute to biogas production, except for the PLA film samples in Zhang et al. (2018). However, the reactor containing PLA pellets in Setup-1 exhibited the highest biogas production within the system, which produced a comparatively higher amount of biogas. Although the PBAT set produced relatively less biogas compared to the PLA set overall, the second highest biogas yield was still observed in the PBAT reactor. The trend obtained from the experimental data is visualized below in Figure 5.2.

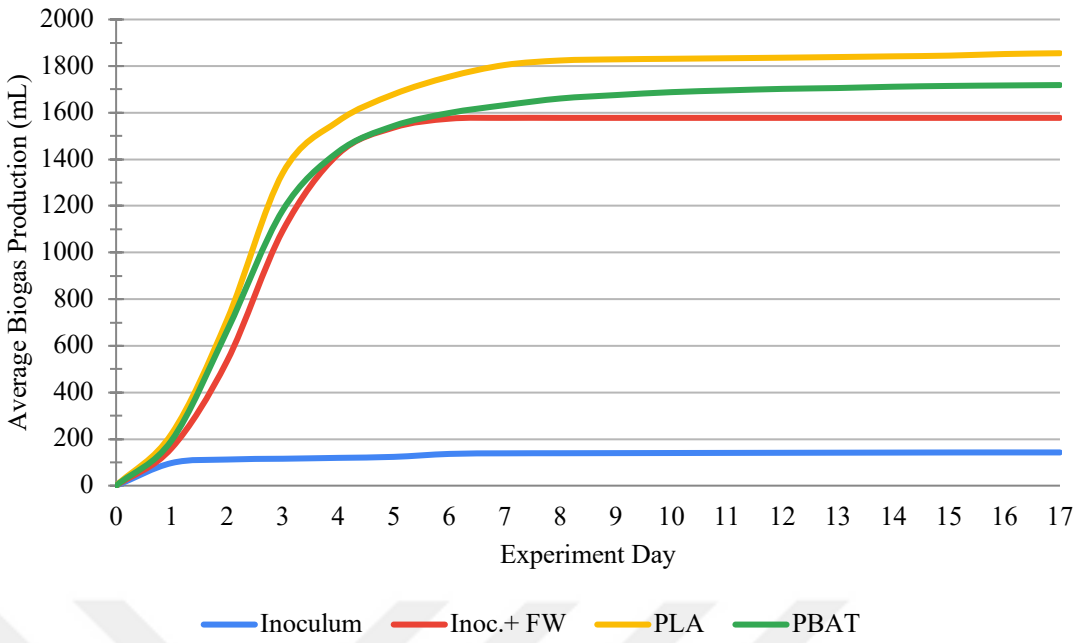


Figure 5.2. Average biogas production, Setup-1.

5.3.2. Outputs from Setup-2

In Setup-1, following the observation of a plateau in cumulative biogas production, a second experimental setup (Setup-2) was designed to clarify the uncertainties encountered. Similarly, Wang et al. (2012) also reported that 8 days of anaerobic digestion had no observable effect on PLA films.

A potential reason for this result is the sensitivity of methanogenic bacteria to high pH levels, which may have negatively impacted their activity towards the end of the process. Moreover, while previous studies (Fotidis et al., 2013; Patel et al., 2017) suggest that a stable anaerobic digestion process typically occurs within a pH range of 6.5–7.2, the final pH values recorded in this setup ranged from 7.79 to 8.31 for reactors R1 through R9, specifically: 8.31, 8.20, 8.08, 7.82, 7.79, 7.81, 7.98, 7.87, and 8.01.

In the previous setup, microbial community activity was verified using control groups composed of inoculum-only and inoculum-food waste mixtures, this time established in triplicate to enhance reliability. In Setup-2, the experimental design focused solely on monitoring the biogas production from PLA bioplastic; therefore, the PLA group was also established in triplicate, and subsequent analyses were conducted from this perspective.

Many PLA-based materials showed poor performance under both mesophilic and thermophilic anaerobic digestion conditions (Bandini et al., 2020; Shrestha et al., 2020). A similar situation was observed in this setup.

In the control group reactors (R1 and R2), which were prepared to monitor microbial community activity, no biogas production was detected. Additionally, reactor R3 showed an almost negligible value, with a total biogas production of only 27.52 mL. The total biogas production values for Setup-2 are presented in Table 5.13 below.

Table 5.13. Cumulative gas production in Setup-2.

	Cumulative Gas Production (mL)								
Day	**Reactor-1**	**Reactor-2**	**Reactor-3**	**Reactor-4**	**Reactor-5**	**Reactor-6**	**Reactor-7**	**Reactor-8**	**Reactor-9**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.00	0.00	27.52	0.00	183.50	166.36	0.00	0.00	0.00
2	0.00	0.00	27.52	0.00	609.88	552.58	15.26	0.00	0.00
3	0.00	0.00	27.52	63.79	1,485.97	1,059.85	145.68	20.06	0.00
4	0.00	0.00	27.52	119.61	1,779.18	1,472.65	259.79	35.47	0.00
5	0.00	0.00	27.52	119.61	1,909.85	1,772.42	355.11	92.30	0.00
6	0.00	0.00	27.52	119.61	1,958.68	1,844.10	358.84	92.30	0.00
7	0.00	0.00	27.52	119.61	1,962.17	1,870.74	358.84	92.30	0.00
8	0.00	0.00	27.52	119.61	1,965.66	1,883.76	358.84	92.30	0.00
9	0.00	0.00	27.52	119.61	1,965.77	1,883.76	358.84	92.30	0.00

As presented in Table 5.13 above, the issue observed in the inoculum control group regarding gas production did not improve throughout the monitoring period; therefore, the experiment for Setup-2 was terminated after 9 days.

In parallel with findings of several studies (El-Mashad et al., 2012; Yagi et al., 2014; Benn and Zitomer, 2018; Zhang et al., 2018), it was found that PLA showed almost no degradation when subjected to mesophilic conditions (35–37°C) over anaerobic digestion periods. In the PLA reactor group (R7, R8, and R9), however, a pattern similar to the inoculum control group was noted—very limited biogas production was observed in R7 (358.84 mL) and R8 (92.30 mL), and no gas production was recorded in R9 as shown in Figure 5.3 below.

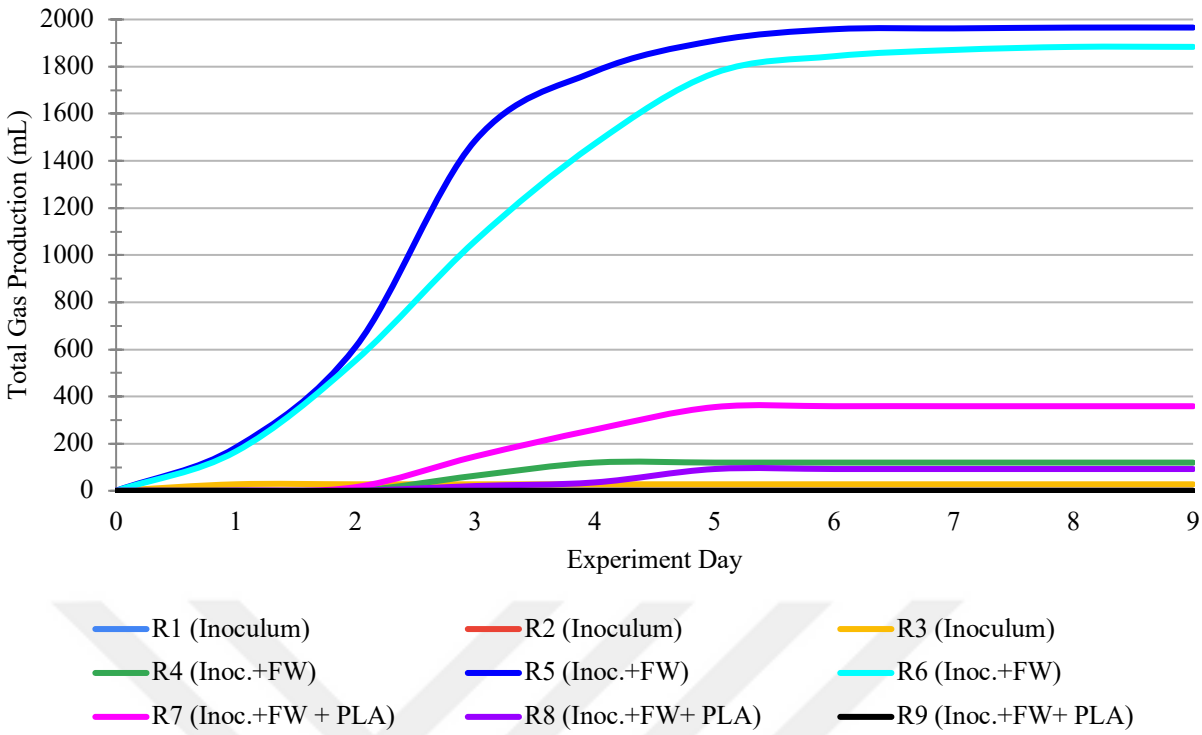


Figure 5.3. Cumulative gas production trends for Setup-2.

In contrast to the problem seen in the inoculum control group, significant gas production was observed in the food waste-inoculum group. By the end of Day 9, reactor R5 had produced 1,965.77 mL of biogas, while reactor R6 reached 1,883.76 mL. Furthermore, the cumulative biogas production output for this part of the setup—calculated as the average of the relevant reactors (1,924.76 mL biogas generation at the end)—has been presented below in Figure 5.4.

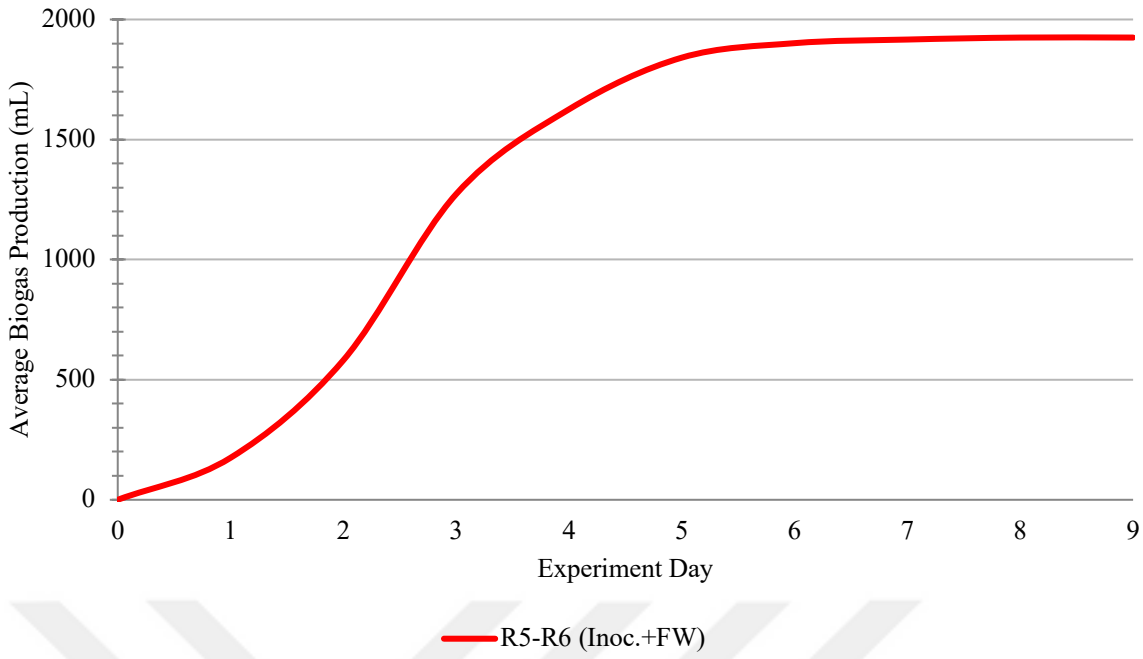


Figure 5.4. Average biogas production of R5 & R6, Setup-2.

Due to these findings, the Gas Chromatography (GC) analyses—which were a core part of Setup-1 and central to this study—were not applied to Setup-2.

5.3.3. Outputs from Setup-3

Upon the initiation of processing, meaningful results were obtained in the biogas production values observed. In Setup-3, the reactors R1, R2, and R3, established for microbial activity monitoring, exhibited very similar biogas production values: 11,675.11 mL; 10,587.53; 11,455.06 mL respectively. Similarly, in the food waste-inoculum group, reactors R5 (25,514.83 mL) and R6 (26,025.78 mL) also showed comparable biogas production results. Additionally, within the PBAT group, reactors R7 and R9 produced similar gas production values (27,586.76 mL and 26,531.24 mL respectively), and in the triplicate setup, significant results were obtained from at least two reactors in each group. The total biogas production values for Setup-3 are presented in Table 5.14 below.

Table 5.14. Cumulative gas production in Setup-3.

Cumulative Gas Production (mL)									
Day	**Reactor-1**	**Reactor-2**	**Reactor-3**	**Reactor-4**	**Reactor-5**	**Reactor-6**	**Reactor-7**	**Reactor-8**	**Reactor-9**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1,369.48	1,293.53	1,354.41	1,549.41	2,668.53	2,860.36	2,007.33	256.89	2,572.97
2	3,010.66	2,843.46	2,959.74	4,654.78	7,892.57	8,784.31	6,547.96	2,212.39	8,063.15
3	3,989.19	3,743.22	3,923.42	5,038.42	9,776.05	11,748.93	9,529.12	2,213.79	10,392.91
4	5,165.05	4,831.78	5,070.46	5,147.83	11,647.32	14,674.21	11,999.49	2,215.18	12,721.12
5	6,514.47	6,083.02	6,385.75	5,208.50	13,751.27	16,921.36	14,226.92	2,216.58	15,205.75
6	7,929.84	7,415.89	7,779.33	5,481.63	16,066.14	19,544.75	16,797.00	2,217.97	17,732.05
7	9,296.38	8,693.10	9,120.12	6,410.25	18,450.45	22,704.52	19,413.90	2,219.37	20,315.52
8	10,398.26	9,750.61	10,197.14	6,708.52	20,680.34	23,628.96	19,965.53	2,220.77	22,864.85
9	10,698.76	10,044.16	10,503.29	8,332.55	23,123.33	24,207.05	21,057.26	2,282.71	24,570.39
10	10,966.85	10,260.13	10,780.33	9,365.60	23,890.22	24,569.90	22,118.30	2,543.35	25,045.34
11	11,114.96	10,357.16	10,919.93	9,451.08	24,272.08	24,852.10	23,693.03	3,047.69	25,375.84
12	11,239.05	10,423.77	11,040.09	9,583.22	24,573.75	25,092.67	25,260.58	3,527.69	25,647.64
13	11,341.24	10,467.39	11,135.88	9,729.78	24,816.93	25,294.39	26,629.68	3,615.31	25,853.67
14	11,433.77	10,499.26	11,219.02	9,845.51	25,014.62	25,479.82	26,973.87	3,615.31	26,033.23
15	11,526.23	10,533.40	11,303.98	9,882.48	25,198.07	25,666.28	27,177.81	3,615.31	26,213.20
16	11,611.06	10,568.37	11,390.74	9,882.48	25,366.17	25,845.79	27,390.86	3,615.31	26,379.24
17	11,675.11	10,587.53	11,455.06	9,882.48	25,514.83	26,025.78	27,586.76	3,615.31	26,531.24

The trends of biogas production for each reactor during the 17-day period are presented in Figure 5.5 below. Reactor 4, within the food waste-inoculum group, exhibited the lowest cumulative biogas production, with a total of 9,882.48 mL in the group. Due to its lower gas production compared to the average gas amount from the inoculum control group (11,239.23 mL), it was determined that reliable data could not be obtained from this reactor. Likewise, even if the same environmental conditions are maintained, the type of inoculum cannot be standardized because it varies greatly depending on its origin (Folino et al., 2023). Similarly, an issue was also observed in Reactor 8, which reflected the lowest biogas production in the PBAT set (3,615.31 mL). Thus, reactor 8 showed very low biogas production than inoculum set and inoculum-food waste reactors, leading to its exclusion as a reliable data source. Based on the final characterization analyses, the pH values for R4 and R8 were recorded as 8.06 and 8.26, respectively. Similar to the previous setup, it is possible that the methanogenic bacteria were also impaired in these reactors, which showed poor gas production in this setup.

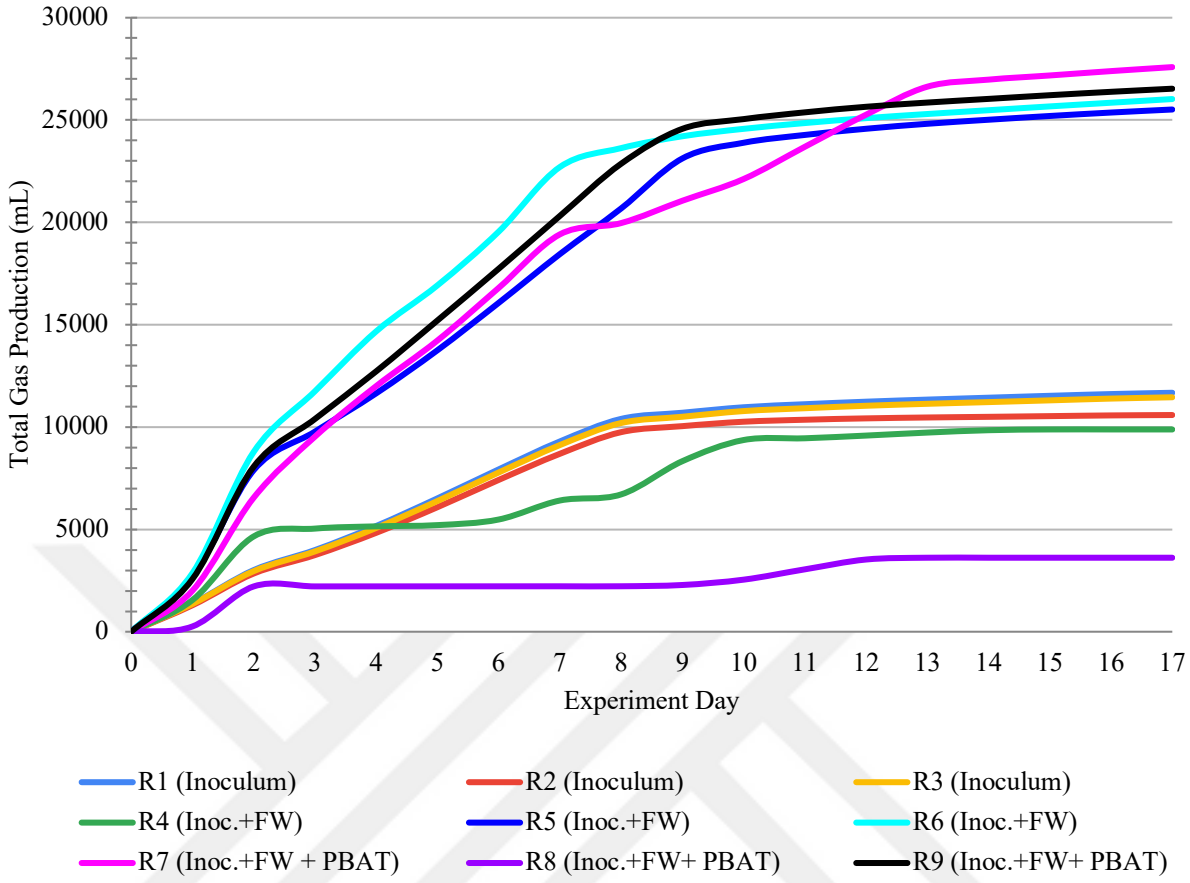


Figure 5.5. Cumulative gas production trends for Setup-3.

To evaluate the effect of PBAT in the bioplastic-containing reactor group (R7, R8 & R9) on the food waste-inoculum set, the biogas difference per unit VS, which was previously applied in Setup-1 and Setup-2, was used in the selection of the data sets. The values for R5 and R6 are 910.43 mL biogas/gVS and 943.02 mL biogas/gVS. Moreover, R7 (1042.57 mL biogas/gVS) and R9 (975.26 mL biogas/gVS) have been also selected for further analyses. Comparison of averages of the reactors is presented in Table 5.15 below.

Trends of this comparison can be followed in Figure 5.6 below. In the PBAT set, biogas production followed a trend very similar to that of the inoculum-food waste group until around Day 11. After that point, a slight increase in gas production was observed in the PBAT group. While the inoculum control group began to plateau after Day 10, both the PBAT and the inoculum-food waste groups reached the plateau phase after Day 12.

Table 5.15. Average biogas production (mL), Setup-3.

Day	R1–R2–R3 (Inoc.)	R5–R6 (Inoc.+FW)	R7–R9 (PBAT)
0	0.00	0.00	0.00
1	1,339.14	2,764.44	2,290.15
2	2,937.95	8,338.44	7,305.55
3	3,885.28	10,762.49	9,961.02
4	5,022.43	13,160.77	12,360.31
5	6,327.75	15,336.31	14,716.34
6	7,708.35	17,805.45	17,264.53
7	9,036.53	20,577.49	19,864.71
8	10,115.34	22,154.65	21,415.19
9	10,415.40	23,665.19	22,813.83
10	10,669.10	24,230.06	23,581.82
11	10,797.35	24,562.09	24,534.43
12	10,900.97	24,833.21	25,454.11
13	10,981.50	25,055.66	26,241.67
14	11,050.68	25,247.22	26,503.55
15	11,121.20	25,432.17	26,695.51
16	11,190.06	25,605.98	26,885.05
17	11,239.23	25,770.31	27,059.00

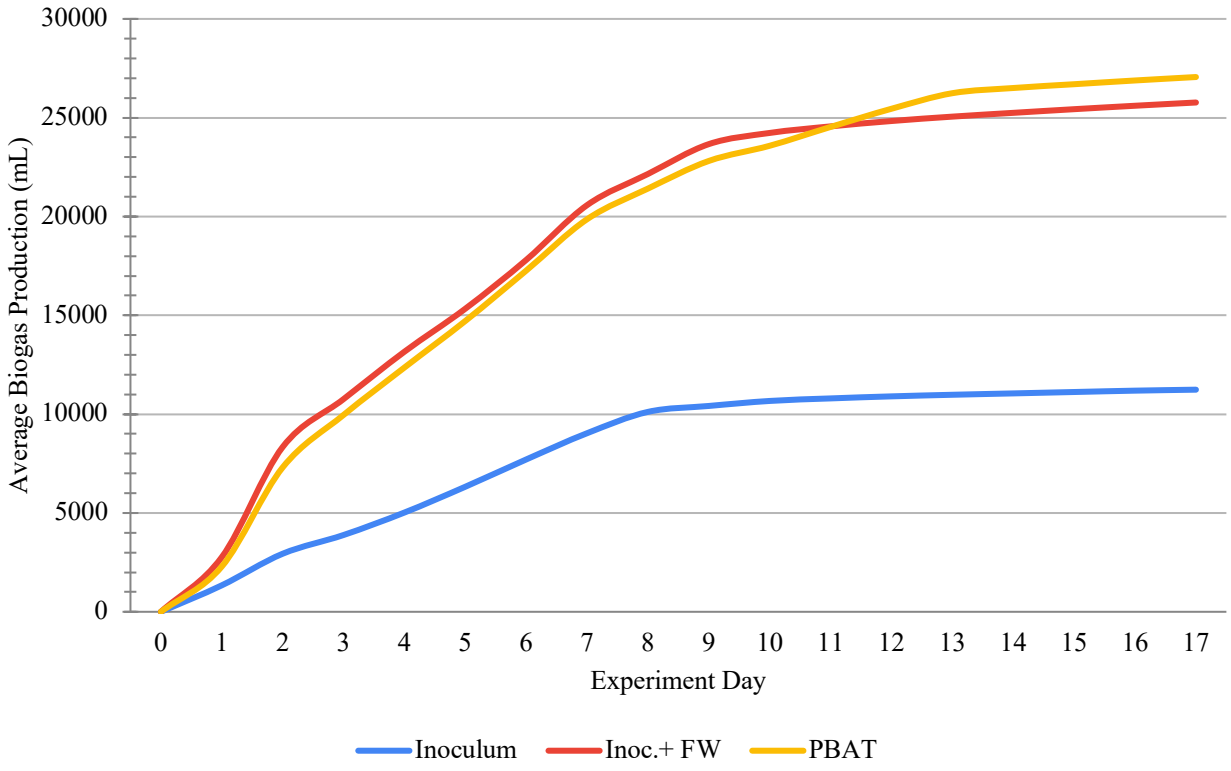


Figure 5.6. Average biogas production, Setup-3.

5.4. Comparison of PBAT Sets and GC Results

This section focuses on discussing the biogas contribution data obtained from the PBAT set in Setup-1 and Setup-3, specifically in relation to the ISR parameters (ISR=2 and ISR=1). In line with the literature, differences in degradation observed with various inoculum-to-substrate (ISR) ratios may provide insights into the ideal feedstock materials and mixtures for both degradation and biogas production (Dilawar & Eskicioglu, 2022; Hegde et al. 2021). In Setup-1 (ISR=2), the PBAT set was monitored through reactors R8 and R9, while in Setup-3 (ISR=1), reactors R7 and R9 were considered for analysis.

As previously described, the specific biogas yield (mL biogas/gVS) was calculated by subtracting the biogas produced in the inoculum-only reactors from the total biogas in the bioplastic-added reactors and then dividing this value by the VS content of the food waste. The comparative results of these calculations are presented below in Table 5.16.

Table 5.16. Specific biogas yield (mL biogas/gVS).

	Setup-1 (ISR = 2)		**Setup-3 (ISR = 1)**	
Day	**R8 (PBAT)**	**R9 (PBAT)**	**R7 (PBAT)**	**R9 (PBAT)**
0	0.00	0.00	0.00	0.00
1	32.71	53.91	42.61	78.69
2	228.06	266.19	230.23	326.86
3	472.73	478.62	359.94	415.03
4	594.35	577.80	444.97	490.99
5	630.47	637.16	503.77	566.20
6	648.40	657.52	579.63	639.27
7	663.83	669.99	661.82	719.32
8	674.12	684.75	628.20	813.11
9	682.14	689.39	678.69	902.74
10	686.20	696.13	730.18	916.85
11	690.10	698.52	822.43	929.75
12	694.07	700.00	915.79	940.48
13	695.38	701.47	997.97	948.48
14	695.17	706.28	1 015.51	955.52
15	694.97	709.08	1 024.02	962.50
16	694.89	710.86	1 033.21	968.70
17	694.89	712.37	1 042.57	975.26

As shown in Figure 5.7 below, a noticeable increase in specific biogas yield was observed when the ISR ratio was reduced from 2 to 1. In Setup-1 (ISR of 2), the specific biogas yields were calculated as 694.89 mL biogas/gVS for R8 and 712.37 mL biogas/gVS for R9. On the other hand, in Setup-3 (ISR=1), these values increased to 1,042.57 mL biogas/gVS for R7 and 975.26 mL biogas/gVS for R9. When the average values are compared, the shift from ISR=2 to ISR of 1 resulted in a 43.38% increase in specific biogas production — from 703.64 mL/gVS to 1,008.91 mL/gVS.

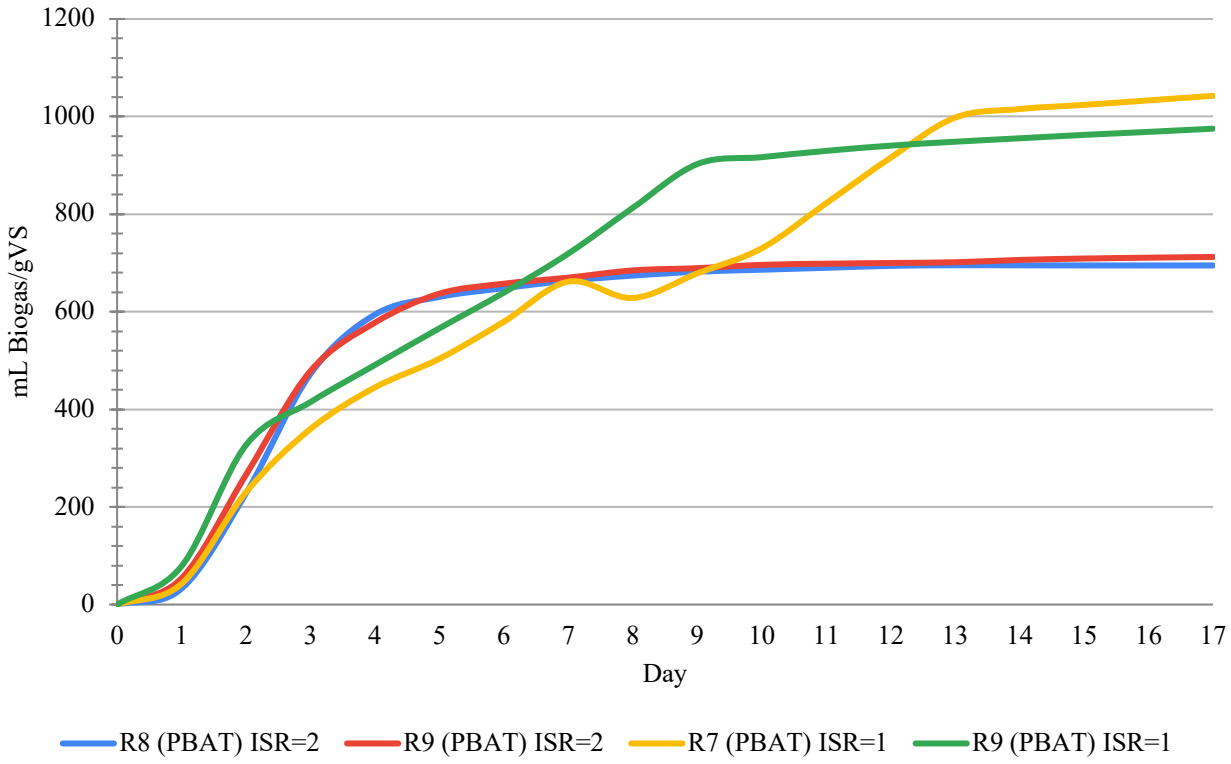


Figure 5.7. Trends for PBAT sets: ISR=1 versus ISR=2.

To evaluate the direct contribution impact of PBAT bioplastic on cumulative biogas production, the average gas yields from the PBAT group reactors at ISR of 2 and ISR of 1 were compared with those from reactors containing only inoculum and food waste. After 17 days of anaerobic digestion, the PBAT group at ISR of 2 produced 1,718.62 mL of biogas. Assuming that 1,577.90 mL originated from the inoculum–food waste mixture, the remaining 140.73 mL was attributed to the PBAT component. This corresponds to a proportional increase of 8.92%. On the other hand, at ISR of 1, an increase in the VS content of the inoculum and the amount of added food waste resulted in a biogas production of 27,059.00 mL in the PBAT group. In the same setup, the inoculum–food waste group produced 25,770.31 mL of biogas. The difference between the two groups was calculated as 1,288.69 mL, corresponding to a proportional increase of 5.00%. Details are given in Table 5.17 below.

Table 5.17. Contribution effect of PBAT on cumulative biogas production.

	Setup-1			**Setup-3**		
	ISR = 2			ISR = 1		
Day	PBAT Avg. (mL)	Inoc.+FW Avg. (mL)	Differences (mL)	PBAT Avg. (mL)	Inoc.+FW Avg. (mL)	Differences (mL)
0	0.00	0.00	0.00	0.00	0.00	0.00
1	194.14	161.18	32.96	2290.15	2764.44	-474.30
2	665.64	535.46	130.17	7305.55	8338.44	-1032.88
3	1180.97	1093.42	87.55	9961.02	10762.49	-801.47
4	1432.04	1422.78	9.26	12360.31	13160.77	-800.46
5	1543.22	1536.31	6.92	14716.34	15336.31	-619.98
6	1598.79	1574.33	24.45	17264.53	17805.45	-540.92
7	1632.51	1577.90	54.62	19864.71	20577.49	-712.78
8	1661.04	1577.90	83.14	21415.19	22154.65	-739.45
9	1675.67	1577.90	97.77	22813.83	23665.19	-851.36
10	1688.23	1577.90	110.33	23581.82	24230.06	-648.23
11	1695.74	1577.90	117.85	24534.43	24562.09	-27.65
12	1702.30	1577.90	124.40	25454.11	24833.21	620.90
13	1705.88	1577.90	127.98	26241.67	25055.66	1186.02
14	1711.49	1577.90	133.59	26503.55	25247.22	1256.33
15	1714.86	1577.90	136.96	26695.51	25432.17	1263.33
16	1716.93	1577.90	139.04	26885.05	25605.98	1279.07
17	1718.62	1577.90	140.73	27059.00	25770.31	1288.69
Change (%)		**8.92**			**5.00**	

To further examine the biodegradation of bioplastics, GC analyses were conducted (seen in Table 5.18 and Table 5.19 below) and incorporated into the calculations. This allowed for the evaluation of biogas yields in terms of methane content.

Table 5.18. GC analysis for Setup-1 (ISR=2).

GC Analysis (ISR=2)		
Day	PBAT Avg.	Inoculum + FW Avg.
	CH_4 (%)	CH_4 (%)
Day 1	6.45	11.56
Day 2-5	26.78	28.47
Day 6	25.57	16.25
Day 7	34.86	22.59
Day 8	47.93	11.90
Day 9	39.17	37.41
Day 10-12	47.37	39.24
Day 13	47.74	31.68
Day 14	47.62	25.31
Day 15	46.61	16.93
Day 16	45.70	13.74

Table 5.19. GC analysis for Setup-3 (ISR=1).

GC Analysis (ISR=1)		
Day	PBAT Avg.	Inoculum + FW Avg.
	CH_4 (%)	CH_4 (%)
Day 1-3	47.05	48.72
Day 4	56.09	61.43
Day 5-6	56.52	68.48
Day 7	66.49	68.09
Day 8-11	65.09	60.39
Day 12	65.73	62.34
Day 13-14	65.62	62.69
Day 15-16	63.95	59.11

Cumulative methane generations from inoculum, food waste-inoculum and PBAT groups in Setup-1 (ISR=2) can be observed in Table 5.20 below.

Table 5.20. Cumulative methane production, ISR=2.

Day	Inoculum (mL CH_4)	Inoculum + FW (mL CH_4)	PBAT (mL CH_4)
0	0.0	0.0	0.0
1	0.7	18.6	12.5
2	1.2	125.2	138.8
3	1.4	284.0	276.7
4	1.5	377.8	344.0
5	1.7	410.1	373.7
6	2.3	416.3	387.9
7	2.4	417.1	399.7
8	2.5	417.1	413.4
9	2.5	417.1	419.1
10	2.5	417.1	425.1
11	2.5	417.1	428.6
12	2.6	417.1	431.7
13	2.6	417.1	433.4
14	2.6	417.1	436.1
15	2.6	417.1	437.1
16	2.6	417.1	438.6

At the end of the process, ISR of 2—where 2.24 gVS of food waste was added—resulted in the production of 438.6 mL CH_4 in PBAT group, 417.1 mL CH_4 in inoculum-food waste group, and 2.6 mL CH_4 in inoculum group.

The contribution volume of methane from PBAT bioplastics used for biodegradation calculations was calculated as follows: by subtracting the methane produced in the control group (food waste–inoculum) from that in the PBAT group, a net methane contribution of 21.5 mL was

obtained in ISR of 2 setup. This corresponds to a 5.15% increase in methane attributable to the presence of bioplastic.

On the other hand, under ISR of 1, with 15.68 gVS of food waste, cumulative methane productions resulted in the production of 15,110.2 mL CH_4 in PBAT group, 14,832.0 mL CH_4 in inoculum-food waste group, and 6,787.5 mL CH_4 in inoculum group are shown in Table 5.21 below.

Table 5.21. Cumulative methane production, ISR=1.

Day	Inoculum (mL CH_4)	Inoculum + FW (mL CH_4)	PBAT (mL CH_4)
0	0.0	0.0	0.0
1	663.0	1,346.9	1,077.6
2	1,454.6	4,062.7	3,437.6
3	1,923.7	5,243.8	4,687.1
4	2,638.2	6,717.0	6,033.0
5	3,514.8	8,206.8	7,364.5
6	4,441.9	9,897.6	8,804.6
7	5,356.3	11,785.1	10,533.5
8	6,075.9	12,737.6	11,542.7
9	6,276.0	13,649.8	12,453.1
10	6,445.2	13,990.9	12,953.1
11	6,530.8	14,191.4	13,573.1
12	6,599.6	14,360.4	14,177.6
13	6,652.3	14,499.9	14,694.4
14	6,697.6	14,620.0	14,866.2
15	6,743.1	14,729.3	14,989.0
16	6,787.5	14,832.0	15,110.2

The methane contribution volume was observed as follows by using the same approach as in the earlier setup: a net methane contribution of 278.2 mL was obtained. This corresponds to a 1,87% increase in methane attributable to the presence of bioplastic.

The trends in cumulative methane production for both ISR setups are presented in Figure 5.8 and Figure 5.9 below.

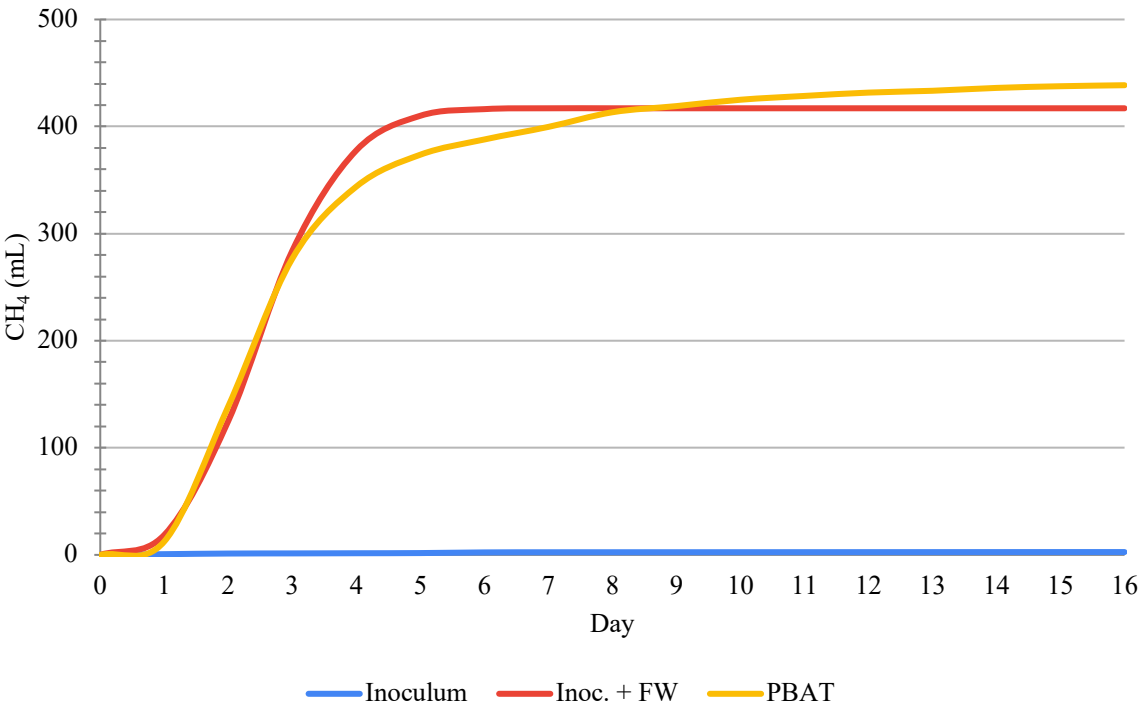


Figure 5.8. Cumulative methane production from Setup-1.

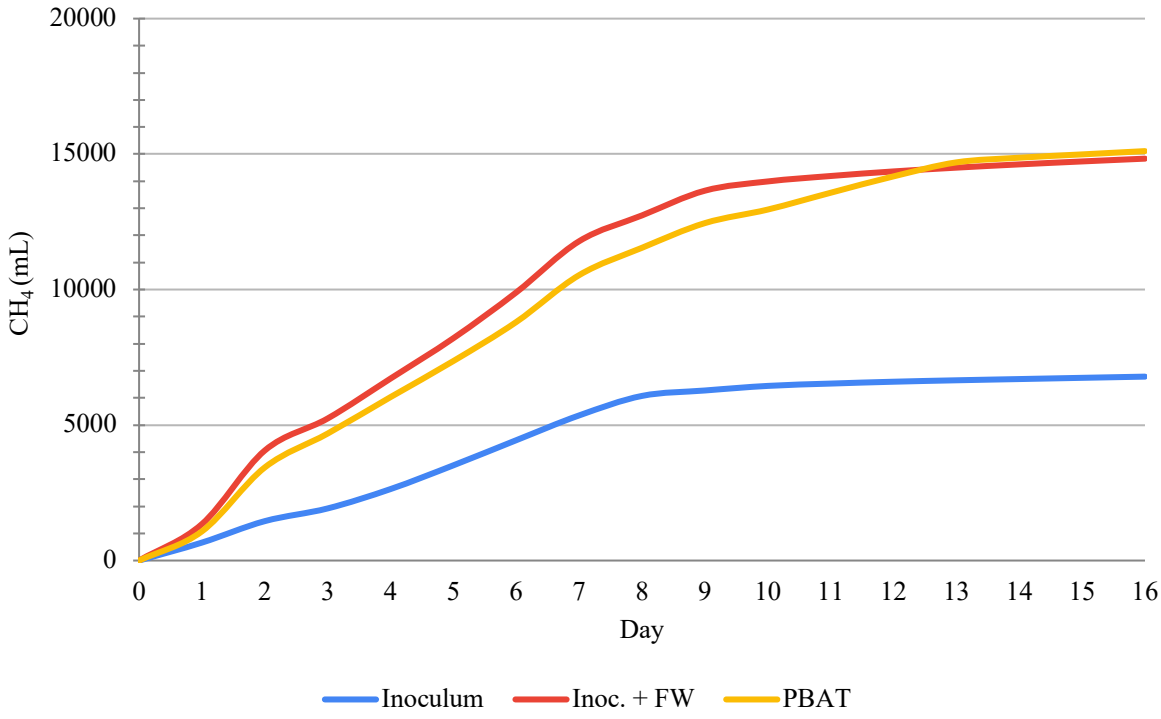


Figure 5.9. Cumulative methane production from Setup-3.

Regardless of grain size and plastic type, mixing bioplastics with food wastes is expected to enhance biomethane production and improve stability in the anaerobic digestion (AD) process (Ye et al., 2013). Therefore, results from this study indicated that there was not a significant enhancement

in methane production when the bioplastics were mixed with food waste in both ISR values for 2 and 1.

5.5. Biodegradability of PBAT

As Vardar et al. (2022) explained, the respirometry method is commonly used to measure the amount of biogas produced in a sealed reactor containing the test polymer and to calculate its proportion relative to the theoretical biogas yield. Hence, this method provides a biodegradability ratio by comparison with a control reactor and is considered the most widely adopted approach in the field. The elemental analysis results required for the application of the method are presented below in Table 5.22 for the PBAT bioplastic.

Table 5.22. Characteristics of PBAT.

Parameter	Value (%)
TS	99.79
VS	99.76
Ash	0.024
C	72.12
H	11.09
N	0.00
O	16.77

Using the data obtained from elemental analysis, the theoretical BMP potential of PBAT bioplastic was calculated as 925 mL CH_4/gVS, based on Eq. 4.2 previously presented in Section 4.3.3.2. Biodegradation calculations were performed using Eq. 4.3, also provided in the same section. Thus, based on VS analysis, PBAT was found to contain 99.76% volatile solids, corresponding to 2.99 gVS. In Setup-1, the direct contribution of PBAT to methane production was recorded as 21.5 mL CH_4, calculated from the difference between 438.6 mL (PBAT containing group) and 417.1 mL (inoculum + food waste group). Similarly, for Setup-3, the methane contribution of PBAT was calculated as 278.2 mL CH_4 using the same approach.

Furthermore, the biodegradation percentages were calculated as the ratio of experimental yield to theoretical yield. The following approach was used to calculate the biodegradation percentage: the amount of methane produced in the PBAT groups was subtracted from the methane produced in the inoculum + food waste control groups, and the resulting values were then divided by the gVS amount

of the PBAT bioplastic. Using this method, the experimental BMP for PBAT was calculated as 7.20 mL CH_4/gVS in Setup-1 (ISR of 2) and 92.94 mL CH_4/gVS in Setup-3 (ISR of 1). Subsequently, the ratio of experimental to theoretical BMP values yielded biodegradation percentages of 0.78% for Setup-1 and 10.05% for Setup-3.

Among these results, the 10.05% biodegradation rate obtained under ISR of 1, supported by the contribution of food waste co-digestion, exhibited a higher degradation trend to the 5.9% biodegradation rate reported under mesophilic conditions in the study conducted by Ren et al. (2019).

Most existing studies on the degradation of biodegradable plastics under mesophilic conditions have concentrated on polymers such as PLA, PHA, cellulose, or starch. However, Svoboda et al. (2018) reported only 2.2% biodegradation for PBAT films under mesophilic conditions. In a similar constructed study, Peng et al. (2022) investigated microbial populations during the digestion and co-digestion of a PLA/PBAT blend with food waste under mesophilic conditions in which their findings showed no significant differences in performance between reactors containing bioplastics and the control reactors. Similarly, in another study involving a PBAT-based blend, Dolci et al. (2021) reported BMP values ranging from 56.5 to 101.2 mL/gVS. Considering the contributions observed in the present experiment, it can be concluded that the ISR of 1 condition had a comparable effect under the conditions described in that study.

In another study (Cazaudehore et al., 2023), the BMP contribution of PBAT polymer under mesophilic mono digestion conditions was reported as 159.7 mL CH_4/gVS, which is higher than the methane contributions observed in the co-digestion systems of the present study, 7.20 mL CH_4/gVS for ISR of 2 and 92.94-mL CH_4/gVS for ISR of 1.

On the other hand, consistent with the results and observations presented in this research, the accuracy of biodegradation measurements using this method may be limited, as part of the substrate's carbon is not converted into biogas but is instead incorporated into microbial biomass during the anaerobic digestion process (Buswell and Mueller, 1952; Cazaudehore et al., 2022a; Shah et al., 2008).

6. CONCLUSION AND RECOMMENDATION

In this study, the biodegradation of bioplastics—specifically polylactic acid (PLA) and polybutylene adipate terephthalate (PBAT), which were widely used in packaging applications based on global production volumes was investigated through co-digestion with food waste within an anaerobic digestion (AD) system.

BMP analyses demonstrated that bioplastics have contributed to the biogas production. For PBAT, biogas production in Setup-1 has increased from 640.80 mL/gVS to 703.63 mL/gVS by 9.8% contribution with the 0.28% VS of inoculum and 2.24gVS food waste (ISR of 2:1). Thus, by using an alternative inoculum (0.98% VS), biogas contribution of PBAT in Setup-3 (ISR of 1:1) was observed as an increase by 8.9% from 926.73 mL/gVS to 1,008.91 mL/gVS with 15.68gVS food waste. For PLA, a contribution on biogas yield was also observed in Setup-1, with an increase of 19.3%, (from 640.80 mL/gVS to 764.61 mL/gVS). However, the contribution effect of PLA bioplastic on biogas production during anaerobic co-digestion process could not be consistently demonstrated within this study because the triplicate reactors in second setup—using the same inoculum and food waste composition—failed to produce measurable gas.

Individual contribution of PBAT bioplastic to the total biogas volumes were evaluated by comparing biogas volumes of control reactors (inoculum + food waste) with reactors containing PBAT. This analysis revealed an increase in total biogas from 1,577.90 mL to 1,718.62 mL for the Setup-1 and a 5% increase from 25,770.31mL to 27,059.00 mL in the Setup-3 with contribution of PBAT bioplastic. Gas composition analysis using GC indicated the followings:

- Methane production in the inoculum + food waste reactors (control) was measured as 417.0 mL CH_4 in the Setup-1, while reactors containing PBAT bioplastic within the same setup produced 438.6 mL CH_4 (an increase of 5.15%). On the other hand, methane production reached 14,832.0 mL CH_4 in the inoculum + food waste reactors in the Setup-3, whereas the PBAT-containing reactors produced 15,110.2 mL CH_4 (an increase of 1.88%).

- Direct contribution of PBAT on methane production was 7.20 mL CH_4/gVS in the Setup-1 operated with an inoculum that has TS (1.03%), while it reached 92.94 mL CH_4/gVS in Setup-3 operated with an alternative inoculum that has higher TS (1.96%) value.

The biodegradation percentages observed under different conditions during the anaerobic digestion process at mesophilic conditions are as follows:

- Biodegradation rate of 0.78% was observed for PBAT bioplastic at Setup-1, whereas a higher rate of 10.05% was recorded at Setup-3.

- Increasing the food waste input from 2.24 gVS to 15.68 gVS, along with raising the VS content of the inoculum from 0.28% to 0.98%, resulted in an approximately 12.9-fold increase in the biodegradation percentage on PBAT.

Future recommendations derived from the findings of this study are summarized below:

- Monitoring of reactor performance showed that PLA may positively influence biogas production in mesophilic anaerobic co-digestion systems. Therefore, repeating these tests with more replicates under same conditions of this work in future studies is recommended.

- Future studies on PBAT bioplastic could be conducted under thermophilic conditions, which have yielded better results for other plastic types in the literature.

- Instead of batch reactors, continuous systems could be employed to observe long-term changes in plastic biodegradation and better evaluate the contribution effects of PBAT on biogas production during food waste co-digestion.

- Furthermore, in addition to food waste, alternative biomass sources from different organic waste streams could be explored to investigate the interactions between PBAT and various substrates.

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