INVESTIGATION OF BIODEGRADABILITY OF POLYETHYLENE UNDER ANAEROBIC CONDITIONS AND EVALUATION OF ITS POTENTIAL FOR BIOGAS PRODUCTION.

POLİETİLENİN OKSİJENSİZ ŞARTLARDA BİYOBOZUNURLUĞUNUN İNCELENMESİ VE BİYOGAZ ÜRETİMİ POTANSİYELİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

INVESTIGATION OF BIODEGRADABILITY OF POLYETHYLENE UNDER ANAEROBIC CONDITIONS AND, EVALUATION OF ITS POTENTIAL FOR BIOGAS PRODUCTION.

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In recent decades, the increase in plastic pollution has been a growing environmental problem. One of the most resistant contaminants nowadays is polyethylene (PE); due to its high resistance to degradation is easily accumulated in nature. However, several techniques can be used to break it down and, there are recent studies on this issue.

In the present study, the main objective was to explore the possibility of biodegrading PE under anaerobic conditions after being treated by three different techniques and evaluate the potential of the material for biogas production. The techniques (pre-treatments) used on PE were photo-oxidation with UV radiation exposure (POxUV), microwave-assisted oxidation with $KMnO_4$ (MAOx) and thermo-oxidative degradation with $K_2S_2O_8$ (TOD). The material of study was PE in two of its more common forms, low-density and, high-density polyethylene (LDPE and, HDPE, respectively) in the form of commercial plastic bags (films).

After applying the pre-treatments on the samples of PE it was found that only MAOx had oxidized LDPE samples, reducing the hydrophobicity of the material and, thus, only these were fit for biodegradation.

The anaerobic degradation was done for a total of 125 days at thermophilic conditions (55°C). Two out of eight samples were subjected to co-digestion using glucose and, acetic acid as co-substrates. After the experimental time was finished, the samples were retrieved and analyzed. It was found that pre-treated PE can indeed be biodegraded to some extent. The FTIR showed a general decrease in the transmittance values as well as the appearance or intensification of a signal at 1377 cm^{-1} that indicates CH_3 formation and, consequently, some degree of chain scission. Regarding the biogas production, among the samples, there was a cumulative biogas production overall minimum of 536.8 mL and, an overall maximum of 1474.5 mL. At the end of the experimental time, the PE samples' cumulative biogas production was still increasing, which means that there was a potential for further degradation although at a very slow rate.

Keywords: polyethylene, pre-treatment, oxidation, anaerobic, biodegradation, biogas.

ÖZET

POLİETİLENİN OKSİJENSİZ ŞARTLARDA BİYOBOZUNURLUĞUNUN İNCELENMESİ VE BİYOGAZ ÜRETİMİ POTANSİYELİNİN DEĞERLENDİRİLMESİ

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Son yıllarda plastik kirliliğindeki artış, büyüyen bir çevre sorunu haline gelmiştir. Günümüzde en kalıcı kirleticilerden biri polietilen (PE) olup bozulmaya karşı yüksek direnci nedeniyle doğada kolayca birikir. Ancak son zamanlarda polietilen atıkları parçalayabilen teknikler üzerinde çalışmalar yapılmaya başlanmıştır.

Bu çalışmada temel amaç, üç farklı teknikle muamele edildikten sonra anaerobik koşullarda PE'nin biyolojik olarak parçalanma olasılığını araştırmak ve malzemenin biyogaz üretimi için potansiyelini değerlendirmektir. Bu amaçla kullanılan teknikler (ön işlemler), UV radyasyonuna maruz kalma ile foto-oksidasyon (POxUV), $KMnO_4$ ile mikrodalga destekli oksidasyon (MAOx) ve $K_2S_2O_8$ ile termal oksidatif bozunmadır (TOD). Çalışmada polietilenin en yaygın iki formu olan, ticari plastik torbalar (filmler) biçiminde, düşük yoğunluklu ve yüksek yoğunluklu polietilen (sırasıyla LDPE ve HDPE) kullanılmıştır.

Ön muamelelerin PE numunelere uygulanmasından sonra, sadece MAOx yöntemi ile LDPE numunelerin oksitlenmiş olduğu, malzemenin hidrofobikliğini azaldığı ve dolayısıyla sadece bunların biyolojik bozunma için uygun olduğu görülmüştür.

Anaerobik bozunma, termofilik koşullarda (55°C) toplam 125 gün süreyle gerçekleştirilmiştir. Sekiz numuneden ikisi, ko-substratlar olarak glikoz ve asetik asit kullanılarak birlikte bozunmaya tabi tutulmuştur. Deney süresi bittikten sonra numuneler alınmış ve analiz edilmiştir. Ön işleme tabi tutulmuş PE'nin gerçekten de bir dereceye kadar biyolojik olarak parçalanabileceği görülmüştür. FTIR, transmitans değerlerinde genel bir düşüşün yanı sıra CH_3 oluşumunu ve sonuç olarak polimerik zincir kesilmesini gösteren 1377 cm-1'de bir sinyalin ortaya çıktığı veya yoğunlaştığı gözlenmiştir. Biyogaz üretimi ile ilgili olarak, numuneler arasında toplamda minimum 536,8 mL ve toplamda maksimum 1474,5 mL kümülatif biyogaz üretimi bulunmuştur. Deney süresinin sonunda, PE numunelerinin kümülatif biyogaz üretimi artmaya devam etmekte olup, bu durum çok yavaş bir hızda olmasına rağmen daha fazla bozulma potansiyeli olduğu anlamına gelmektedir.

Anahtar kelimeler: polietilen, ön muamele, oksidasyon, anaerobik, biyobozunurluk, biyogaz.

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SYMBOLS AND ABBREVIATIONS

Symbols

CO_2	Carbon dioxide
CH_4	Methane
CH_2	Methylene
CH_3	Methyl group
$KMnO_4$	Potassium permanganate
$K_2S_2O_8$	Potassium persulfate
$CaCO_3$	Calcium carbonate
mL	Milliliter
g, mg	Gram, milligram
nm	Nanometer

Abbreviations

PE	Polyethylene
HDPE	High-density polyethylene
LDPE	Low-density polyethylene
LLDPE	Linear low-density polyethylene
VFA	Volatile fatty acids
AD	Anaerobic digestion
ACoD	Anaerobic Co-digestion
VS	Volatile solids
UV	Ultraviolet
MAOx	Microwave-assisted oxidation with $KMnO_4$
TOD	Thermo-oxidative degradation with $K_2S_2O_8$
POxUV	Photo-Oxidation with UV radiation

1. INTRODUCTION

One of the main environmental problems in the last decades is related to plastic pollution. This is a situation created by the over-production of plastic products for a wide variety of applications. Plastics do not have the property of biodegradability, meaning that they do not degrade into nature in a relatively short time without harming the environment. The reason behind the lack of this property is the molecular structure of these products and it has been a challenge for the scientific community to modify the characteristics of plastics through techniques that can alter them at a molecular level.

In the context of environmental hazard, the first general objective of this study is to investigate the possibility of microbial degradation of PE, which is known as non-biodegradable polymer, under anaerobic digestion (AD) conditions, after the application of several primary degrading techniques (from now on referred to as pre-treatments). The second general objective consists of exploring the potential of this biodegradation process for biogas production. The specific objective of the study is to compare the effects of the selected pre-treatments on PE and evaluate their influence on its biodegradation.

1.1. Polymers

A polymer can be defined as "a type of molecule, a very, very large molecule" [1]. This type of molecule is built by units (or *building blocks*) called monomers which repeat themselves in a chain. Polymers can be of natural, synthetic, or semi-synthetic origin. Some examples of natural polymers include starch, chitin, nylon, cellulose, DNA and RNA. Synthetic polymers can be derived from petrochemical activities or produced in the laboratory through chemical processes. Among them, polystyrene, polypropylene and polyester are found.

Plastics are certain types of polymers that are characterized by their wide range of mechanical behaviors. They can be categorized as flexible and rigid plastics according to properties such as tensile strength, crystallinity, modulus, and resistance to deformation. One of the most evident differences among them is the elongation; flexible plastics can undergo high

elongations (20-800%) having low resistance to deformation, while rigid plastics cannot overcome 3% of elongation without rupturing [2].

1.2. Worldwide Waste of Plastics

According to Geyer [3], pollution caused by certain types of plastics has increased in the past 60 years as is shown in Figure 1.1. Plastic production is one of the biggest industries worldwide, because of its high functionality in terms of chemical, mechanical, and thermal resistance. Simultaneously, this is the very same reason why the accumulation of plastic waste is a huge environmental problem. Some methods such as incineration and pyrolysis are widely used to degrade them but at risk of generating greenhouse or hazardous gases as products.

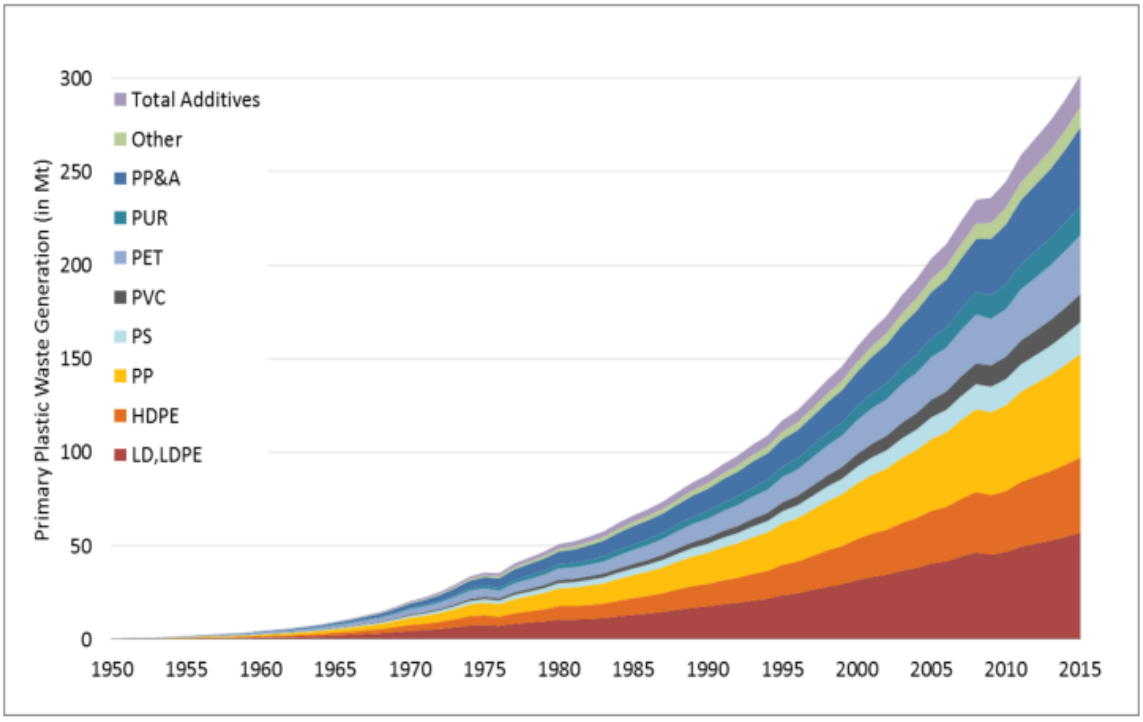

Figure 1.1. Global primary waste generation in million metric tons according to polymer type from 1950 to 2015. [3]

As shown in Figure 1.1, PE is one of the most important wastes (LDPE, and HDPE), with a generation of almost 100 million metric tons between 1950 and 2015. This polymer can be

found in numerous plastic applications, being one of the most important and hazardous for the environment in the form of films, and particularly, plastic bags.

In Turkey, the use of plastic (PE) bags reach 250 billion tons per month. Because of the risk that they represent for nature, Turkey, as well as some European countries have assigned prices to plastic bags in markets and supermarkets. According to the Ministry of Environment and Urbanism, plastic bags are being sold for 25 kuruş, around USD 0.044, since 2019 [4]. In Colombia, plastics represent 13% of the daily waste and around 2'714.000 units of that waste are plastic bags. To reduce the use of these items, the Government imposed a tax of COP$20 (approximately USD 0.005) on the plastic bags, which has been applied from the 1st of July 2017 [5]. The measure decreased their usage by 30% by August 2018 [6]. In both countries the waste of plastic bags is an environmental issue and despite the government efforts to reduce their impact, still, great efforts are needed to minimize it. Is in this context that this project has been developed and for its purposes, PE bags have been used as the object of study.

1.3. Polyethylene

PE is the polymer resulting from the polymerization of the ethylene monomer, also called vinyl. When radical chain polymerization is carried out at conditions of high pressures between 120 and 300 MPa and temperatures above the crystalline melting temperature (Tm) of PE, LDPE is obtained. This type of PE is characterized by the presence of both long and short branches, high flexibility, and low crystallinity. The mechanism of radical chain polymerization is shown in Figure 1.2, where R* represents a reactive species, such as a free radical, an anion, or a cation [2].

On the other hand, when the reaction occurs under low pressures (up to 8MPa) and catalyst presence, coordinate polymerization occurs, producing HDPE also known as linear PE. Pressure and temperature conditions vary depending on the phase of the process. HDPE has a very low degree of branching, which means high crystallinity and low flexibility [2].

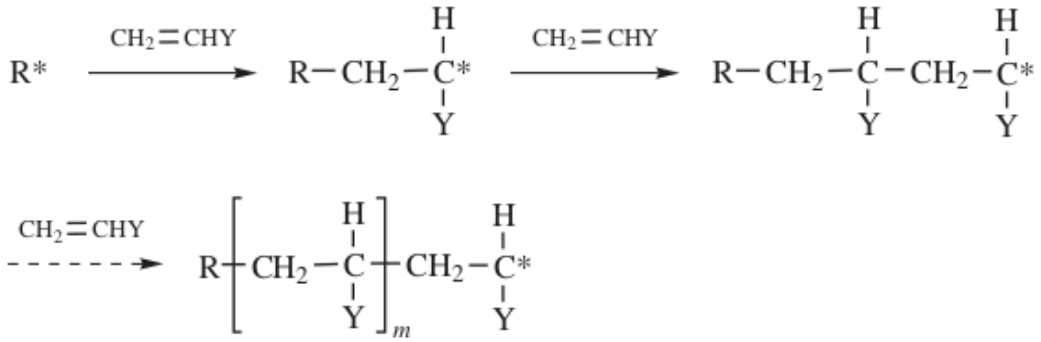

Figure 1.2. Mechanism of radical chain polymerization for the ethylene monomer [2]

Due to its molecular structure, both HDPE and LDPE are highly resistant to degradation. In the case of biodegradation, as will be explained next, the main obstacle is the presence of CH_2, which makes the PE surface hydrophobic and because of this, the attachment of microorganisms to it for biodegradation purposes is not possible.

1.4. Biodegradation

The biodegradation process can be understood as the chemical degradation of a material caused by the activity of microorganisms such as fungi, bacteria, and algae that result in products that can be easily assimilated by nature, such as CO_2, oxygen, or biomass [7]. Biodegradation can be performed under different conditions and environments that determine the products to be obtained. In general, biodegradation can be of two types: aerobic, or with the presence of oxygen; or anaerobic, with oxygen absence. From aerobic biodegradation CO_2, water and biomass are obtained. Meanwhile, anaerobic degradation can produce additional CH_4 to the aerobic products if conditions are methanogenic, or hydrogen sulfide (H_2S) if conditions are sulfidogenic [8].

In general terms, biodegradation consists of 4 steps: bio-deterioration, the alteration of physical and chemical properties of the material caused by the attachment of microorganisms to its surface; bio-fragmentation, the breakdown of the main chains of the material due to enzymatic activity that leads to the formation of lower molecular weight compounds; assimilation, the utilization of the smaller compounds by the microorganisms as a carbon and

energy source; and mineralization, the formation of oxidized metabolites (CO_2, water, CH_4) [8, 9].

1.4.1. Anaerobic Degradation

AD is a process in which microorganisms take energy and grow through the metabolization of organic material in an environment without oxygen. The process consists of four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis, to be explained later. Each one of these steps requires a specific group of microorganisms and the outcome of this process is the production of biogas, a mixture of mainly CH_4 (55-75% vol) and CO_2 (25-45% vol) [10, 11].

AD can be carried out in a wide range of temperatures, at restricted pH values and avoiding toxic agents that affect the process of methanogenesis (such as -VFAs-, heavy metals, ammonia, cations, among others). Depending on the temperature range, AD can be psychrophilic (10-20°C), under which long retention times are required; mesophilic (20-40°C), where a relative maximum CH_4 production is reached at 35-37°C; or thermophilic (50-60°C), where maximum methanogenic activity occurs at 55°C or higher. It is important to note that higher temperatures advantage the CH_4 production, especially when reluctant materials are involved in the process. On the other hand the pH values affect directly the methanogenesis process, which can occur only at neutral pH (values between 6.5 and 7.5) [11].

As mentioned before, the process consists of four phases. The first one, hydrolysis, is the conversion of non-soluble polymers into smaller soluble compounds due to the action of exoenzymes excreted by hydrolytic fermentative bacteria. The produced compounds are usually alcohols, VFAs, lactic acid, CO_2, ammonia, among others. The second phase, acidogenesis, consists of the transformation of the soluble compounds to VFAs and CO_2. The third phase, acetogenesis, is the production of acetic acid, H_2 and CO_2 from the previous products, for methanogenic bacteria to transform into CH_4. In this phase, also propionic and butyric acid are produced, but the action of the acetogenic bacteria decomposes them into acetic acid as well. Finally, methanogenesis is the conversion of the acetogenesis products into CH_4. This can occur through two processes: conversion to acetic acid or methanol from

acetate-using microorganisms or conversion of H_2 and CO_2 from hydrogen-using microorganisms. The first group of methanogens (acetoclastic) are a few species, but they produce about 60-70% of the CH_4. The second group, called hydrogenotrophic, represent a vast majority of the methanogenic species. They are responsible for the hydrogen consumption and transformation, throughout the digestion, and especially in the final stage. The four phases of AD are coupled: the microorganisms participating are constantly guaranteeing that VFA and hydrogen are completely converted, avoiding inconvenient changes of pH and consequently, favoring the methanogenesis [10].

To guarantee a successful AD, a series of factors need to be taken into consideration. This process depends on both environmental parameters, such as temperature, pH and amount of VS, total solids, organic loading rate, C/N (carbon to nitrogen) ratio and particle size of the waste; and operational factors, like solid retention time, the number of stages of the process, digester design and mixing. Among the environmental factors, often the most difficult ones to overcome, the C/N ratio is fundamental. Too high or low values of C/N indicate consumption of nitrogen by the methanogens or excess of ammonia in the system, neither of which is desirable. To maintain stability in the system, the values of C/N should be in a range of 20-30. Additionally, numerous compounds can cause inhibition of the process (volatile and long-chain fatty acids, potassium, sulfide, heavy metals, hydrogen in excess) and other that are nutrients, for the most needed for the methanogenesis to occur properly; iron, nickel, and cobalt are essential, while manganese, molybdenum, aluminum, selenium and boron in traces enrich the medium [12]. Ideally, nutrients need to be balanced for the methanogens to use, inhibitors should not be present, the substrate's physical and chemical characteristics should ease the process; however, this is not always the case.

1.4.2. Anaerobic Co-digestion

The simultaneous treatment of different feedstocks (substrates) in the same process of AD is known as Anaerobic Co-digestion (ACoD). ACoD has proven to be an effective alternative to overcome the difficulties arisen from the anaerobic mono-digestion. As seen before, AD on its own needs a careful balance of many factors to be successful, and thanks to the use of several substrates, many of these factors can be controlled easier. Some of the benefits of using ACoD instead of mono-digestion include the dilution of toxic compounds, the

improvement of the buffer capacity and nutrient's balance, the increase of organic loading rate due to the biodegradable material content, and consequently, the increase of the CH_4 yield of the process, due to the synergistic effects created by the interaction of the co-substrates and the inoculum [12, 13].

Plenty of studies have shown the efficacy of ACoD. The mixing of organic feedstocks has shown important improvements in CH_4 yields and system stability. In every case, the synergy between the co-substrates improves the overall performance of the process. However, the optimal operational conditions will depend exclusively on the co-substrates and the feedstocks to be used must be investigated for each specific case. All types of organic substrates containing lipids, carbohydrates, proteins, cellulose and hemicellulose can be used for ACoD. The digestion of lipids results in the highest biogas yield but the slowest biodegradability; carbohydrates and proteins biodegrade faster but result in lower biogas yields [13].

Many studies prove the effectiveness of ACoD in many aspects. One of the most widely studied aspects of ACoD is the C/N ratio; one of the factors that affect directly the CH_4 yields. Li [14] studied the C/N ratio in the co-digestion of pre-treated corn stalks and cattle manure, finding that the optimal value for biogas production was 1/3 (manure VS/corn stalks VS) and obtaining 4.9-7.4% more biogas production. Cabbai [15] studied the co-digestion of sewage sludge with source selected organic fraction of municipal solid waste (SS-OFMSW) and found an increase of 47% of CH_4 production for sewage sludge mono-digestion when using C/N ratio within the range of 6-15.4. Cavinato [16] obtained a gas production rate improvement of 47% in the waste activated sludge and biowaste co-digestion at 55°C when compared to mesophilic, supporting previously reported superior performances of processes under thermophilic conditions. A study carried out by Silvestre [17] showed that co-digestion of sewage sludge and crude glycerin under mesophilic conditions result in an increase in CH_4 production of 148% due to the steady performance of the process, related to the characteristics of the glycerin used as co-substrate.

These are very few of the investigations on ACoD and there are still many more to be done due to the many challenges created by it. The important number of factors involved, both in the development of the digestion and in the technical aspects implies that there are still many

challenges to overcome. From the selection of the appropriate co-substrates, their availability, biodegradability, and characterization; to performance factors such as the maintenance of the system's stability through nutrient balance, buffering capacity and microbiological stability and the possibility of controlling and monitoring any inhibitory effects represent important challenges. Additionally, to take the ACoD to an industrial scale, the need for accurate mathematical models is fundamental. There are several models to predict ACoD, however, none is universal, mainly due to the difficulties derived from feedstocks characterization and carbon metabolization mechanisms [18].

1.5. Biodegradation of PE

As mentioned before, PE is highly resistant to biodegradation. This is a consequence of two factors, mainly: its hydrophobicity and its high molecular weight. For PE to biodegrade, both need to be overcome. Microorganisms with cellular hydrophobicity (hydrophobic amino acids and mycolic acid in the fimbrial structure) or surfactant action result useful in this task, and consequently, surface attachment is possible. On the other hand, the high molecular weight needs to be reduced for the microorganisms to use the polymer as an energy source; they can attack structures of up to 50 carbons. Once size reduction occurs, the resulting oligomers, dimers and monomers can be reduced to carboxylic acid for the microorganisms to metabolize them. These two processes, molecular weight, and hydrophobicity reduction are often outcomes of simultaneous and complementary effects of both natural and artificial factors, such as sunlight (UV irradiation), humidity, temperature, and enzymatic action. Once they have been overcome, biofilm growth starts; a matrix of microorganisms (from the same or different species) through which nutrients, gases, enzymes, water and other resources are transported all over the surface of the polymer [9, 19].

PE biodegradation has been reported under a variety of conditions. Villa-Carvajal [20] biodegraded PE under aerobic conditions after several pre-treatments with UV radiation and thermal exposition. The biodegradation was enhanced by the microorganisms *Brevibacillus borstelensis* (ATCC 51668) which takes PE as a carbon source. These microorganisms are strictly aerobic gram-positive motile bacteria that can grow in a wide range of temperature (they are thermophilic) and degrade LDPE at 37°C [21].

According to Swift [22], polymer-degrading microbial species can be bacteria (*Pseudomonas, Streptococcus, Staphylococcus, Micrococcus, Moraxella*) and fungi (*Aspergillus niger, Aspergillus glaucus*), *Actinomycetes sp*. and *Saccharomonospora* genus). Most of them are strictly aerobic microorganisms, being exceptions *Staphylococcus aureus* (ATCC 25923) and *Actinomycetes sp* (ATCC 15214).

Due to the reluctant nature of PE, any previous physical or chemical treatments improve the effectiveness of the process, by making it faster than under natural conditions. Processes such as photo-oxidation, hydrolysis and physical disintegration and techniques like exposure to high temperatures or chemical oxidation and the use of surfactants favor the increase of the surface availability for microbial growth in PE. Its degradation can be followed through the measurement of different property changes such as glass transition temperature reduction, crystallinity reduction, molecular weight reduction and weight loss, CO_2 evolution and biofilm formation [9].

Some attempts to biodegrade PE under anaerobic conditions for different purposes have been done. Alassali [23] assessed the biodegradation of PE after AD and composting. They aimed to evaluate the level of quality decrease of PE after being subjected to these treatments to check its suitability for mechanical recycling. To do so, some mechanical, physical, and chemical properties of PE were analyzed after its exposure. They found that the deterioration of the initial properties was lower than 50%, below the suggested quality standards, meaning that PE was suitable for mechanical recycling. On the other hand, Selke [24] investigated the biodegradation of PE blended with biodegradation-promoting additives in both aerobic and anaerobic conditions. They subjected the samples to UV degradation before the biodegradations and followed the biogas production for anaerobic conditions and the CO_2 evolution in aerobic conditions. They found no evidence to support that the additives used to promote and/or enhance the biological degradation of PE are effective for that purpose. To the best of our knowledge, no studies are exploring the AD of PE for biogas production.

Among all, to fulfill the objectives of the present study, three techniques were selected for the primary degradation of PE: one of physical nature (POxUV), the second one of chemical nature (MAOx) and the third one of physicochemical nature (TOD). These techniques have been proven successful in the degradation of PE at different effectiveness rates. It is aimed

to compare their effects on the PE for posterior biodegradation. At the time of the pre-treatment's application, there were no investigations that have applied pre-treatments to enhance or improve the biogas production in the AD of PE.

1.5.1. Photo-oxidation

Photo-oxidation is a technique that uses a type of light to induce an oxidation reaction. Light can be of the visible spectrum, UV radiation (A or B), or infrared. In the case of UV, when applied to PE, the result is the creation of free radicals that absorb oxygen and form carbonyl groups. If additional exposure is present, it will lead to Norrish type I and/or II degradation that ultimately reduces the molecular weight of the polymer by forming oligomers [8]. Figure 1.3 summarizes the photo-degradation mechanism. In general terms, it consists of an initiation caused by the action of energy (UV radiation, in the case of interest) that creates free radicals; followed by the propagation, which can be of chain transfer or chain branching and a termination reaction that stabilizes the polymers due to the decrease of the overall number of radicals. The final stage is chain scission, which ultimately reduces the molecular weight of the polymer [25].

Degradation of PE under UV radiation has been extensively studied by the usage of different conditions to facilitate posterior biodegradation. As mentioned earlier, Villa-Carvajal [20] exposed LDPE samples to 68 h of UV radiation at 254 nm of wavelength as the sole and combined (with thermal exposure) pre-treatment to determine its biodegradation by the microorganism *Brevibacillus borstelensis*. They found that the samples pre-treated with photo-oxidation showed higher biodegradation rates reflected in % of weight loss when compared to the samples with only thermal exposure pre-treatment. Martínez-Romo [26] studied the effects of UV-B radiation in HDPE and biodegradable PE by exposing them for periods up to 60 days. It was found that the formation of oxidation products occurs mainly in HDPE instead of biodegradable PE; photo-oxidation is slower in the second one due to the presence of carbonyl groups that come from manufacturing. Suresh [27] studied the changes in mechanical and surface properties of LDPE blended with an oxo-biodegradable polymer additive when exposed to photo-oxidation. The samples were irradiated with UV-B lamps at 30°C at different time intervals for periods up to 49 days and it was found that the degree of reduction in mechanical strength and surface property changes was significant; there was an

increase in wettability related to the formation of carbonyl groups by photo-oxidation. Finally, Jeon [28] studied the biodegradation of photo-oxidized LLDPE as a function of UV intensity, irradiation time and temperature, finding that PE incorporated with Fe-stearate as a photo-degradation catalyst showed significant decay in its tensile properties and molecular weight when increasing the UV irradiation temperature and time.

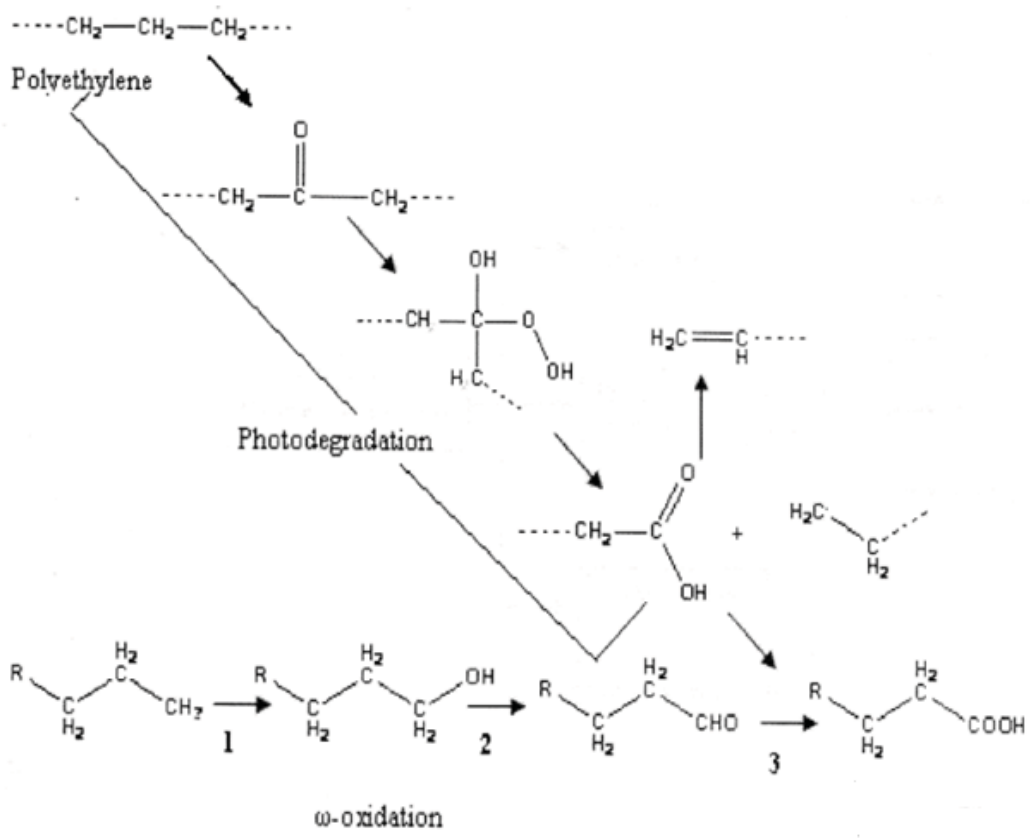

Figure 1.3. Photo-degradation mechanism. Taken from [8].

1.5.2. Microwave-Assisted Chemical Oxidation

Chemical oxidation uses oxidizing agents to attack the surface of PE and enhance its degradability, among them nitric acid (HNO_3), $KMnO_4$, $K_2S_2O_8$, and benzoyl peroxide. Meanwhile, the use of microwave energy represents an attractive alternative for degradation due to the volumetric heating that results in lower reaction times.

Microwave-assisted chemical oxidation has been used to treat PE in different scenarios. One of them is presented by Backstrom [29] in which PE is converted to functional chemicals through this process using nitric acid as the oxidizing agent. The concentration of the oxidant and the microwave irradiation time were the main factors affecting the degradation rate of PE, being able to completely transform it into acids such as succinic, malonic, glutaric, propionic, acetic, adipic and pimelic. Roy [30] reported the microwave-assisted oxidation of PE with $KMnO_4$ and $K_2S_2O_8$ "to increase the susceptibility of the polymer to photodegradation and thermal degradation". In the first case, the microwave irradiation decomposes the $KMnO_4$ and generates the oxygen that reacts with PE to form free radicals and ultimately decomposes to hydroxide or carbonyl groups in the polymeric chain (Figure 1.4).

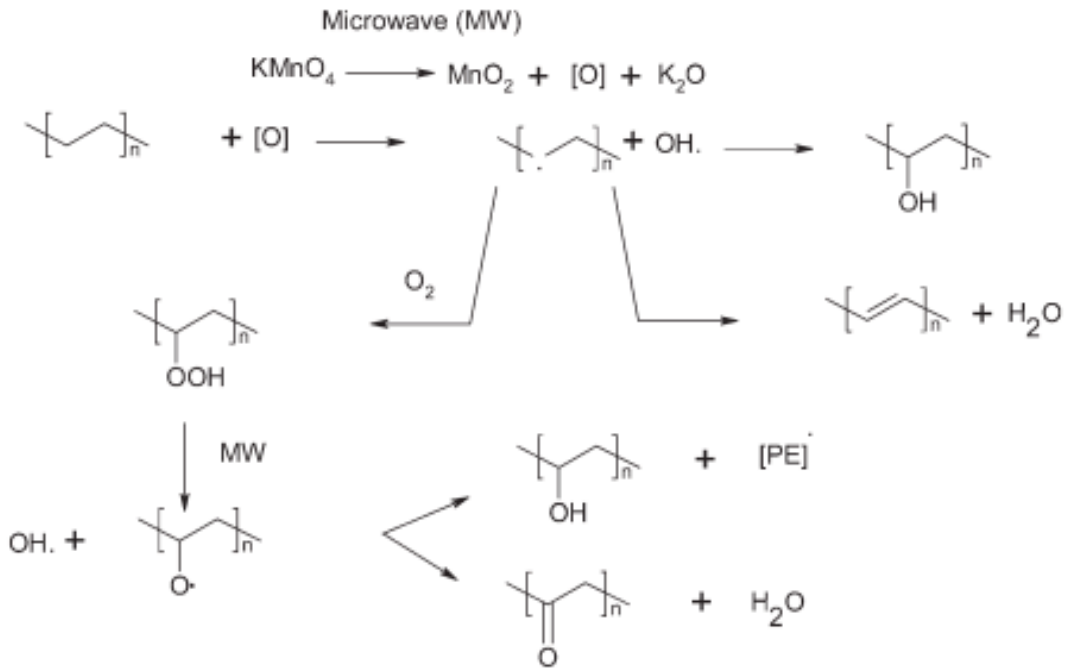

Figure 1.4. Scheme for the oxidation of PE with KMnO4. Taken from [30]

On another hand the $K_2S_2O_8$ dissociates into sulfate radical anions that lead to the formation of hydroxide radicals in aqueous media. The OH^- plays the role of electron acceptor from the PE chain, creating free radicals (Figure 1.5). They found that the oxidizing agents did not generate remarkable changes in the mechanical properties but did increase the oxygen

content in the polymer chain, seen in the presence of carboxylic acids, aldehydes, and/or esters.

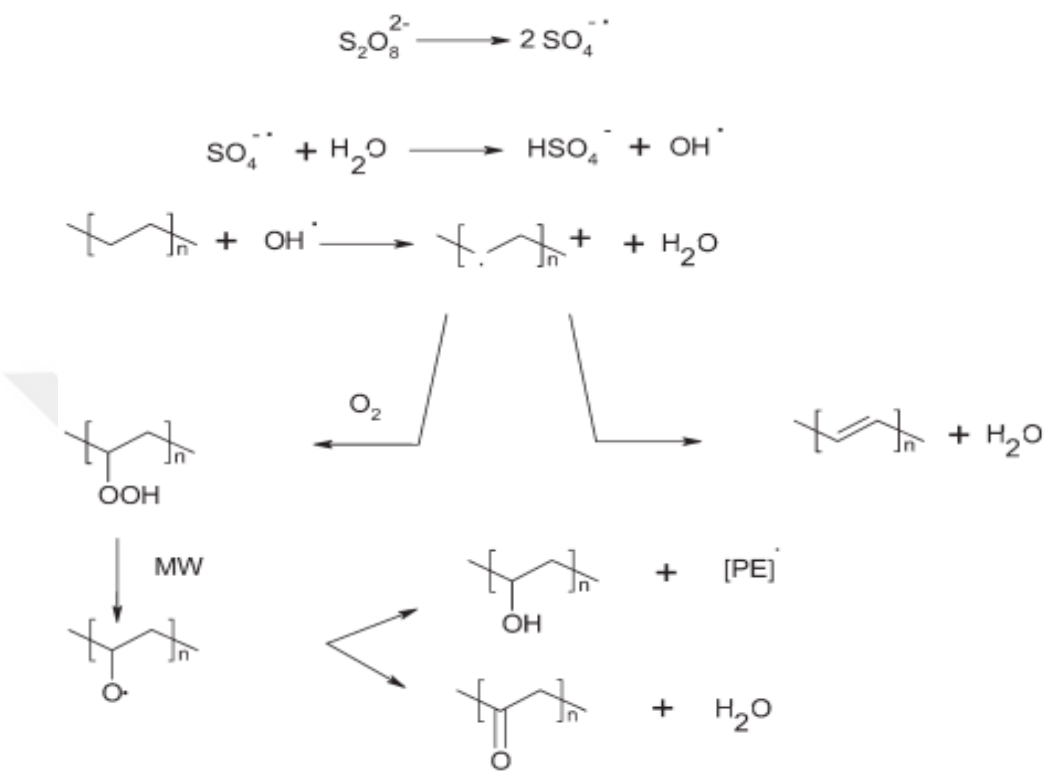

Figure 1.5. Scheme for the oxidation of PE with K2S2O8. Taken from [30]

1.5.3. Thermo-Oxidative Degradation

This technique consists of the combination of an oxidizing agent and thermal exposure. Many researchers have used blends of PE instead of an oxidant and the thermal exposure occurs inside an air oven at relatively high temperatures. Such is the case of Roy [31], in which cobalt carboxylates were blended into LDPE films to evaluate its effect on the thermal degradation of the material, carried out in an air oven at 70°C for 600h. Maryudi [32] studied the thermal exposure of HDPE molded with manganese laureate, also in an air oven at 70°C for up to 1000h. Antunes [33] exposed blends of HDPE, manganese stearate and two antioxidants to 3 different temperatures (60, 70 and 80°C) in air circulation ovens. In every case, degradation was evidenced in the change of mechanical properties.

There is another case of TOD that is worth mentioning, however, the material used was not PE but poly(ethylene oxide), a water-soluble polymer. In this study, Vijayalakshmi [34] aimed to study the kinetics of the oxidation process in presence of $K_2S_2O_8$ in two scenarios: thermal exposure and microwave-assisted degradation. They found that in the case of thermal exposure, where temperatures from 40 to 70°C were used, the oxidant drastically reduced the stability of the polymer and degradation rates increased with the temperature. This investigation was taken as a guide for the application of thermo-oxidative degradation pre-treatment since it was decided that it is valuable to assess such conditions in a non-soluble material as it is PE. The required modifications to the methods shall be explained in the following chapter.

2. MATERIALS AND METHODS

The experimental work of this study is divided into two general parts and the material of study is PE in two of its variations: HDPE and LDPE. The first part consists of the pre-treatment of HDPE and LDPE films, for primary degradation. Such methods are photo-oxidation with UV radiation (POxUV), microwave-assisted oxidation with $KMnO_4$ (MAOx) and thermo-oxidative degradation with $K_2S_2O_8$ (TOD). The second part is the biodegradation process under anaerobic conditions in which the determination of volatile solids (VS) is fundamental for the definition of the parameters for the process.

2.1. Characteristics of PE.

The HDPE and LDPE films were taken from commercial plastic bags since the aim of this project is to evaluate the degradation potential of this material to mitigate its impact on the environment. The films were cut depending on the pre-treatment technique to be used, as will be specified in each case. The films were characterized with FTIR spectroscopy before any pre-treatment, to identify their initial molecular content. Both HDPE and LDPE samples show the following peaks: at around 2915cm^{-1} and 2847cm^{-1} that correspond to CH_2 stretching C-H bonds; 1472cm^{-1} and 1462cm^{-1} that correspond to CH_2 bending C-H bonds; at around 730cm-1 and 718cm-1 that correspond to CH_2 rocking vibration. HDPE samples additionally showed a peak at 874-871cm^{-1} corresponding to $CaCO_3$. The presence of this compound is not unusual in PE matrixes. PE might include different types of inorganic nano-fillers, such as $CaCO_3$ nanoparticles, to improve mechanical and physical properties [35]. Additionally, the presence of these compounds in the polymer matrix has proven improvement in the photodegradation of the material [36].

2.2. Pre-Treatments

2.2.1. Photo-Oxidation with UV Radiation (POxUV)

HDPE and LDPE from plastic bags were cut in approximately 15 cm x 7.5 cm films, having weights between 0.13 and 0.20 g and were exposed to 60, 70, 80 and 90 h of continuous UV radiation at ~2.5 cm of distance from the radiation source (UV-A Phillips TL 60W/10R lamps with a 350-400 nm range emission) in an Accelerated Weathering Test Chamber with a Temi880 programmable controller (Figure 2.1). There was no humidity involved in the experiments due to the intention to evaluate the sole effect of the UV radiation. The radiation intensity was 2 W/m^2 and the temperature was set at 20°C, both at the beginning of every experiment. The temperature was left to increase according to the radiation exposure time. After the exposure time, the samples were retrieved and weighed.

Figure 2.1. Accelerated Weathering Tester and programmable controller.

2.2.2. Microwave-Assisted Oxidation with $KMnO_4$ (MAOx)

This pre-treatment was done according to the methodology proposed by Roy [30]. HDPE and LDPE were cut into 0.5 g films and were dipped in 12 mL of a 0.5M aqueous $KMnO_4$ solution, according to a molar ratio of 3:1 (repeating units of PE to KMnO4), that has been proposed by the authors as the most effective one for oxidation purposes. The samples were placed in wide-mouthed bottles, closed with their respective caps, and exposed to microwave

heating for 1, 1.5, 2, and 2.5 minutes in a microwave oven at 180 W of power. After exposure, the samples were retrieved from the bottles, washed with distilled water, and dried with paper towels (Figure 2.2). Finally, every sample was weighed. The calculations needed for the development of this pre-treatment can be found in Appendix 6.2.

Additionally, a statistical relevance evaluation was done, in which the weight change was taken as the response and the factor was the exposure time. The experimental design and the results obtained from the statistical analysis done in Minitab Statistical Software 18 can be found in Appendix 6.1.

Figure 2.2. PE samples with KMnO4 inside the bottle before (left) and after (right) microwave exposure.

2.2.3. Thermo-Oxidative Degradation with $K_2S_2O_8$ (TOD)

For this pre-treatment, the study made by Vijayalakshmi [34] was taken as a guide. The methodology used was modified since the material under study in this case is not water-soluble. First, two solutions of 0.5 and 1 g/L of $K_2S_2O_8$ were prepared. HDPE and LDPE films were cut, having weights between 0.7 and 1.3 g. The solutions were placed in a water bath with temperature control (see Figure 2.3), at 60, 70, 80 and 90°C with a temperature variation of ±1°C in wide-mouthed glass bottles with caps. Once the solutions reached the

temperature, the films were introduced in the bottles, capped, and left to react. After one hour of reaction, the samples were retrieved from the bottles, washed with distilled water, dried in paper towels, and weighed.

For this pre-treatment, a statistical relevance evaluation was done as well, in which the weight change was taken as the response and the factors were the temperature and the concentration of the oxidant. The experimental design and the results obtained from the statistical analysis can be found in Appendix 6.1.

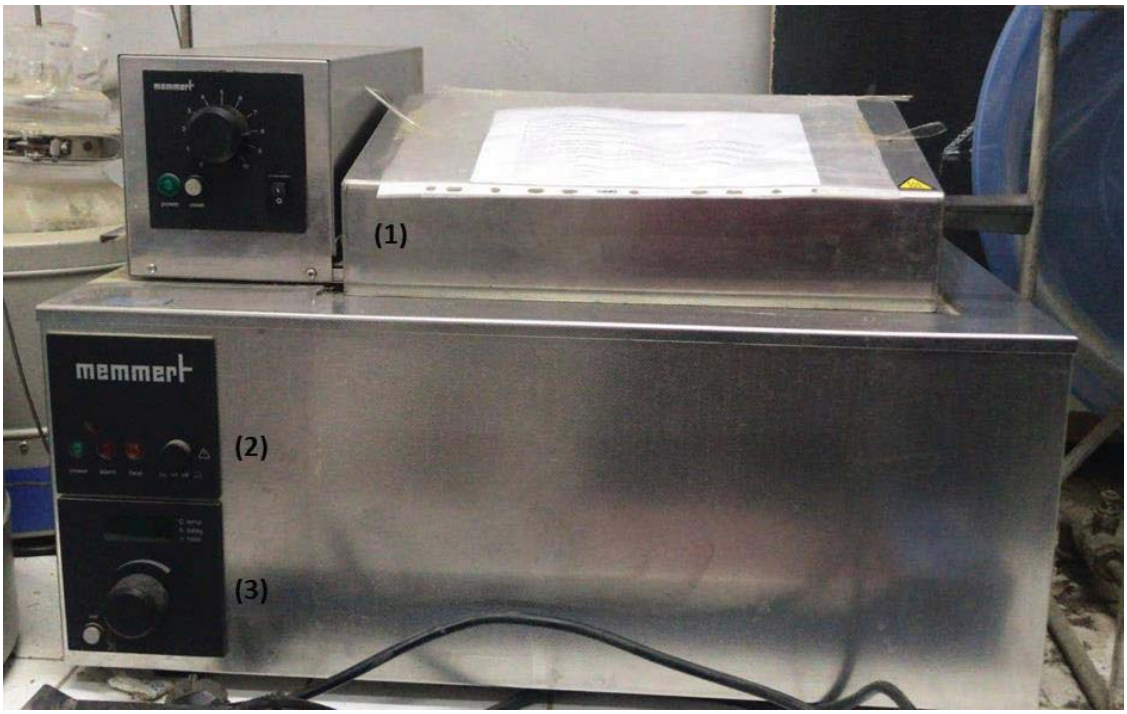

Figure 2.3. Device used for TOD. (1) water bath, (2) on/off button, (3) temperature control panel.

2.3. Biodegradation Experiments

The AD experiments were performed for a total of 125 days, at thermophilic conditions (55°C of temperature) and constant agitation (50 rpm). The PE samples were used in an amount of 0.145 g and three runs were made for each, resulting in a total of 24 samples for AD. For the

biodegradation experiments, it was decided to perform two co-digestion, using glucose and acetic acid as co-substrates for the PE, to evaluate whether there was or not any positive effect on the digestion of PE. Before the digestions, the determination of VS was done to calculate the amount of sample and inoculum needed for the experiments.

2.3.1. Microbial Inoculum

The sludge seed used in the experiments as inoculum was obtained from a local biogas plant of Ankara. The sludge was heated at a rate of 1-1.5°C per day until it reached the desired temperature (55°C). Also, it was fed occasionally, with glucose to keep the microorganisms alive. For AD to occur properly, the pH of the environment needs to be close to neutral. For this reason, it was necessary to adjust the pH of the inoculum. To do so, a 1 M H_3PO_4 solution was prepared and added to the inoculum slowly until pH was between 7 and 7.5.

2.3.2. Anaerobic Digestion

To perform the AD, the ASTM D5511-02 standard was used as a guide. According to the VS content, the amounts of sample and inoculum, and consequently the S:I ratio were defined. The parameters defined for the experimental set up are found in Table 2.1.

Table 2.1. Parameters used for the AD experiments.

Parameter	Value
Temperature	55°C / 328 K
Time	125 days
PE samples	8
Amount of PE sample	0.140 g VS (145 mg in total)
Amount of co-substrate	~ 0.150 g
Amount of inoculum	60 mL
Space of bottles	40 mL
S:I ratio	0.073
Agitation	50 rpm

The PE samples were weighed and cut into small pieces to maximize the contact surface. Then, they were put into 100 mL bottles with the corresponding amount of inoculum and approximately 100 mg of $NaHCO_3$ was added to each bottle to maintain the pH throughout the experiments. The bottles were closed with a rubber stopper and a holed cap, as seen in Figure 2.4, and the air was extracted with a syringe until a negative pressure was measured.

After assuring the absence of oxygen, the bottles were put into the incubator at thermophilic conditions, over a moving platform that provided agitation to the system (Figure 2.4).

At the end of the experimental time, the bottles were taken outside the incubator and left to reach room temperature. The pressure and the pH were measured one last time. Finally, the samples were retrieved, washed, dried, and weighed. A couple of pieces of each sample were prepared and sent for FTIR analyses.

Figure 2.4. Bottles used for anaerobic degradation.

Figure 2.5. Bottles placed in the incubator (left). Incubator's controllers and display of temperature (1), time (2) and agitation (3) (right).

2.4. Analytical methods

2.4.1. Fourier-Transformed Infrared Spectroscopy (FTIR)

The equipment used was a Thermo Scientific NICOLET 6700 FTIR spectrometer, operated by a specialist. The PE films were subjected to this analysis before any pre-treatment as well as after pre-treatments and after the anaerobic degradation to evaluate the molecular changes derived from the procedures. Several guides were used to read the obtained spectra.

2.4.2. Determination of Volatile Solids

This procedure was done based on the EPA Method 1648: Total, Fixed, and Volatile Solids in Water, Solids and Biosolids. Briefly, the method consists of first, weight porcelain dishes inside of which later, the samples are cut and weighed. Then, the samples are taken to an oven at 105°C for 24 h. After this time, the samples are left in a desiccator until they reach room temperature. Once this happens, the samples are weighed again and taken to a muffle

furnace at 550°C for one hour. When this time is finished, they are taken out and left to cool down in a desiccator again. Finally, they are weighed for the last time. This procedure was done for the PE samples, the inoculum, and the co-substrates.

The data and calculation regarding this step can be found in Appendix 6.3.

2.4.3. Biogas production measurement

Biogas production was measured indirectly with the pressure of the bottles. The pressure was measured manually with a manometer every day for the first two weeks and twice per week after. The percentage of CO_2 was not measured due to the lack of an appropriate measuring device. The ideal gas law (Equation 1) was used to calculate the amount of biogas produced by each sample.

$$V_{STP} = V_{gas} * \left(\frac{P_{gas}}{P_{STP}}\right) * \left(\frac{T_{STP}}{T_{gas}}\right) \qquad Equation\ 1$$

Where STP indicates standard conditions (1.03 bar and 273 K), P_{gas} is the measured pressure and T_{gas} is the temperature of the experiments (55°C) and V_{gas} is the volume of the headspace of the bottles (40 mL). The production of biogas was expressed as mL of biogas.

3. RESULTS AND DISCUSSION

3.1. Characterization of HPDE and LDPE samples.

To see any structural changes after the application of pre-treatments and the AD, the initial samples were characterized through FTIR. The spectra are shown in Figures 3.1 and 3.2.

As mentioned earlier, the main peaks for the samples are, from left to right: at around 2915cm^{-1} and 2847cm^{-1} that correspond to CH_2 stretching C-H bonds; 1472cm^{-1} and 1462cm^{-1} that correspond to CH_2 bending C-H bonds; at around 730cm-1 and 718cm-1 that correspond to CH_2 rocking vibration. HDPE samples additionally showed a peak at 874-871cm^{-1} corresponding to $CaCO_3$.

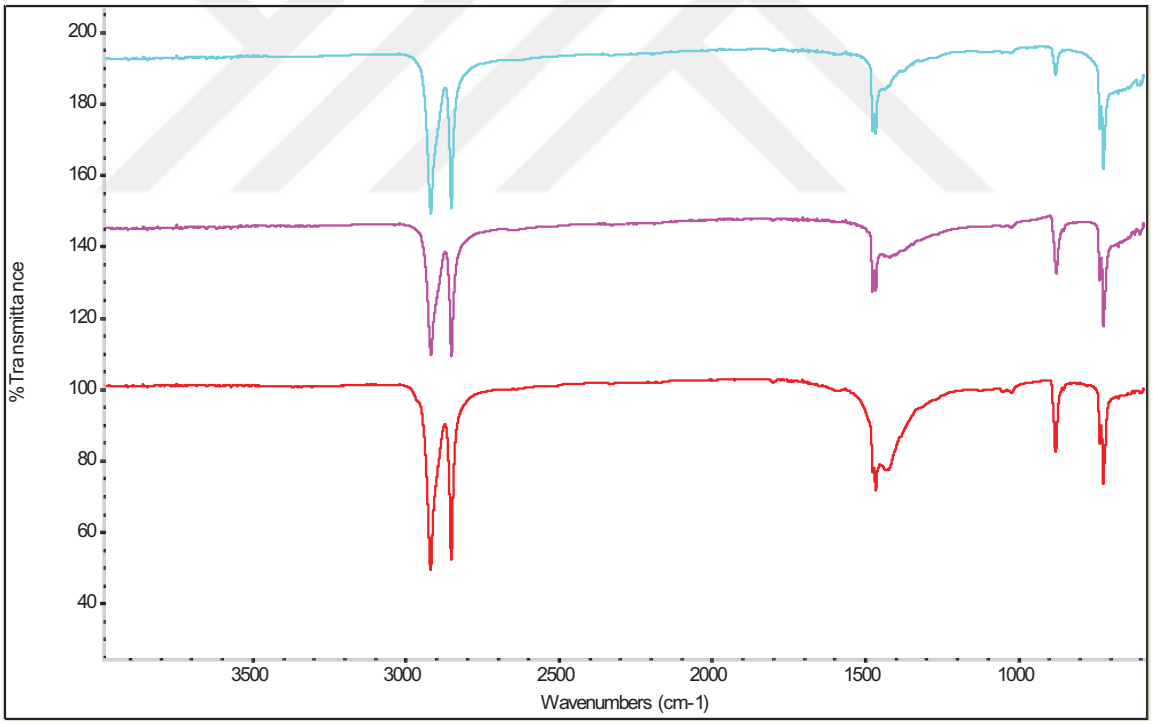

Figure 3.1. FTIR spectrum of HDPE samples

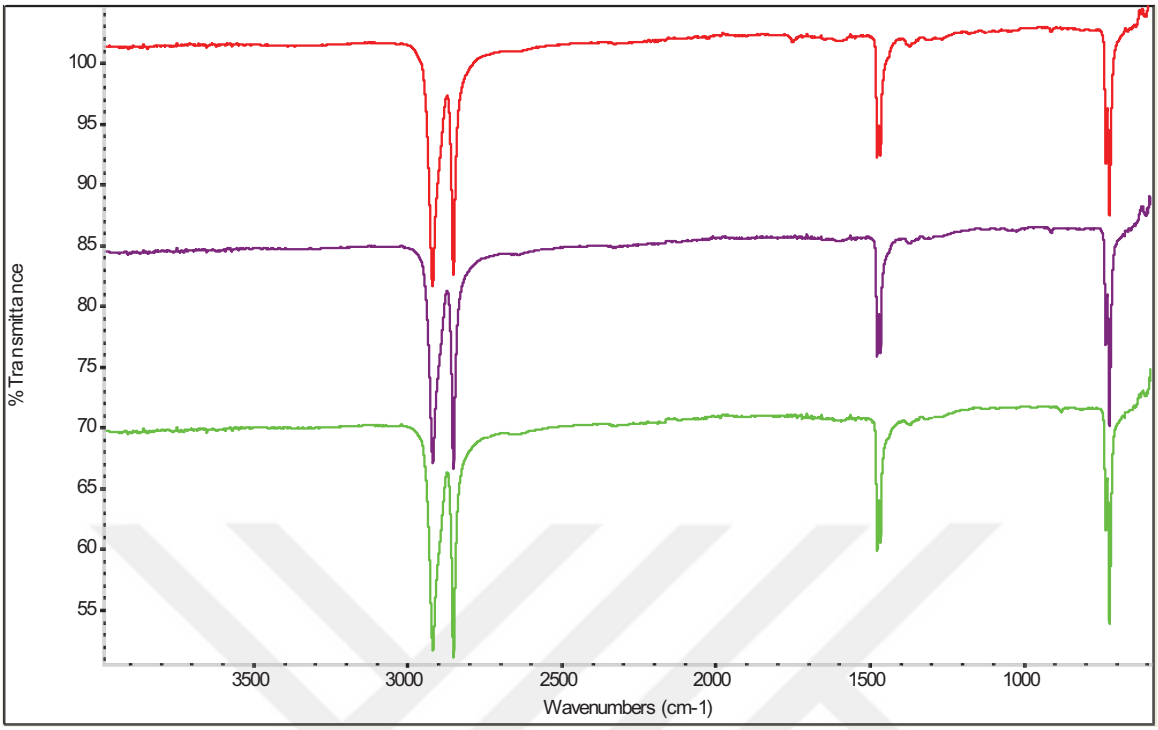

Figure 3.2. FTIR spectrum of LDPE samples.

3.2. Pre-treatments

3.2.1. Photo-Oxidation of PE Films with UV Radiation (POxUV).

The application of UV radiation to the samples showed no significant changes in the FTIR spectra nor the weight of the samples. As can be seen in Table 3.3, the percentages of weight change are smaller than 1% which shows that they were not affected by the UV radiation. From the results obtained it can be said that even though the radiation intensity used was the highest adjustable in the Accelerated Weathering Tester, the time of exposure was not long enough to induce a satisfactory grade of photo-oxidation. When compared to some literature references, the exposure times in different studies vary from 35 hours up to 90 days [37, 38]. It was expected to find any carbonyl group formation by the end of the pre-treatment; however, this was not the case: the spectra of LDPE samples showed no changes whatsoever.

For HDPE samples, there were some changes related to the width of the peak at ~1460cm^{-1}; after exposure, it was broadened, and a new peak appeared at ~1420cm^{-1} (Figure 3.5).

Changes in the shape and the intensity of the peaks can be caused by the increase of the groups present at a specific wavelength and the occurrence of interactions such as H bonding.

Table 3.1. Weight data of POxUV samples

Sample	Initial Weight (mg)	Final Weight (mg)	Weight Change %
LD60	159	159.5	0.31 %
HD60	151	151	0.00 %
LD60rep	193.5	193.8	0.16 %
HD60rep	171.3	171.2	-0.06 %
LD70	160	160	0.00 %
HD70	185	186	0.54 %
LD70rep	164	163.8	-0.12 %
HD70rep	220.5	220.5	0.00 %
LD80	134	133.7	-0.22 %
HD80	176	176.5	0.28 %
LD80rep	151.6	151.3	-0.20 %
HD80rep	211.9	212	0.05 %
LD90	161	161.6	0.37 %
HD90	197	196.8	-0.10 %
LD90rep	161.9	161.6	-0.19 %
HD90rep	206.8	207.2	0.19 %

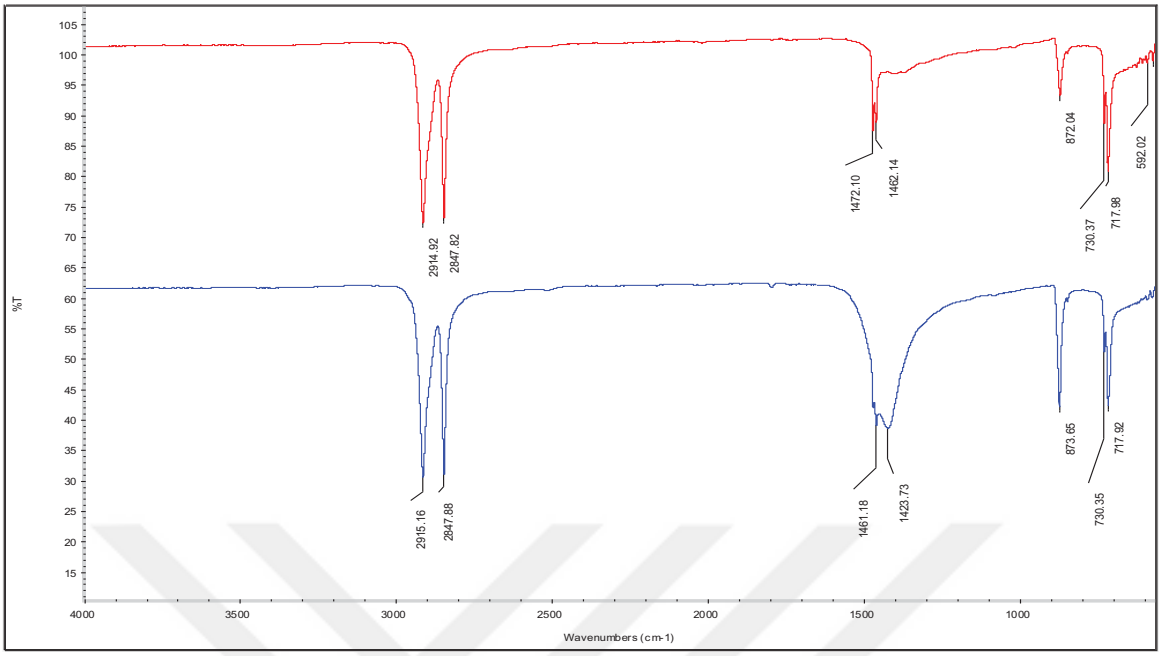

Figure 3.3. FTIR spectrum of HD60 (above) and HD60rep (below) samples after POxUV.

It was expected to observe a significant change in FTIR spectra of HDPE after UV radiation due to the presence of CaCO3 in the polymeric matrix, as explained before. Unfortunately, no significant changes in FTIR spectra hence no carbonyl group formation was observed in HDPE films, like LDPE films. The complete set of FTIR spectra can be found in Appendix 6.6.

3.2.2. Microwave-Assisted Oxidation of PE Films with $KMnO_4$ (MAOx).

The MAOx pre-treatment, opposite to TOD, showed interesting results in terms of structural changes. It is important to mention that some tests were replicated more than twice to discard errors and corroborate the results. Samples LD1, LD1 run3 (confirmation run), LD1 run5 (confirmation run), LD1 run6 (confirmation run), LD1.5, LD1.5 rep2 (confirmation run), LD2, LD2 rep3, and LD2 run3 (confirmation run) showed an interesting change of shape in the area between 3650 and 3200cm^{-1}: a curve, shaped like a wide tongue, which corresponds to the presence of O-H bond as it can be seen in Figure 3.3. The complete spectra obtained after MAOx can be found in Appendix 6.5.

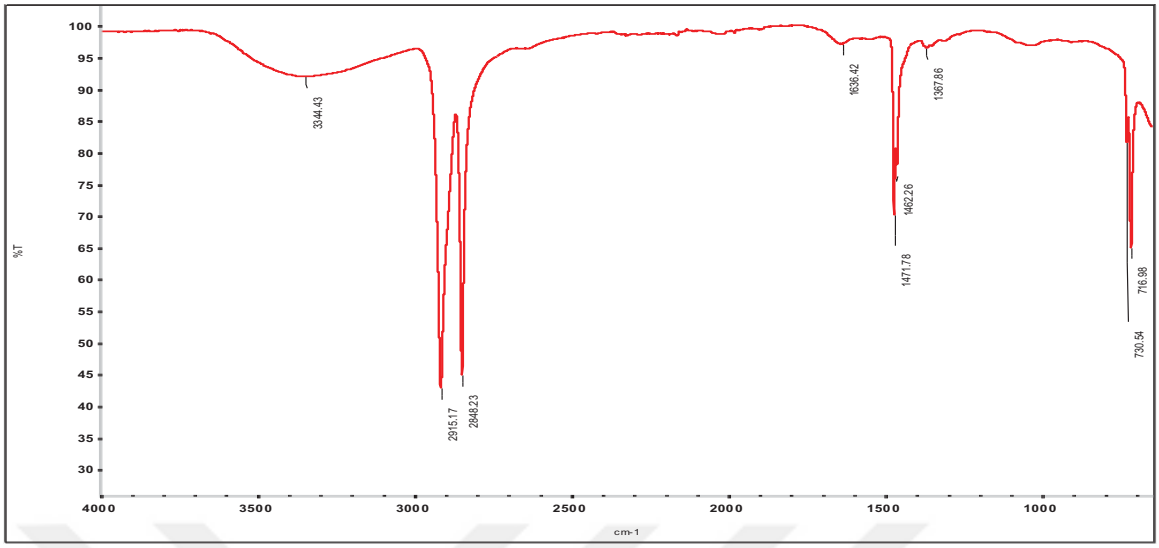

Figure 3.4. FTIR spectrum of sample LD1.5 rep2

The presence of the O-H bond in PE represents the formation of water or polymeric alcohol. In either case, it is a promising result because it means that the natural hydrophobicity of PE was decreased, showing the effectivity of the oxidation at some molecular extent. All the above-mentioned samples were considered for the AD stage.

Table 3.2 shows the weight changes of the samples. Some runs needed to be repeated to corroborate FTIR results. The weight changes were in general not significant. Some samples showed a weight increase superior to 50%. This was not necessarily coherent with the changes in the FTIR spectra; after the pre-treatment, some samples got wrinkled by the effects of the heat, the oxidant, and the size of the samples itself, making it possible that the solution got trapped and dried between these wrinkles, making impossible its removal with washing and increasing the overall weight of the sample.

Same as in the TOD pre-treatment, in MAOx the samples were subjected to oxidation. In this case, following the methodology of Roy [30], the oxidant agent was KMnO4 and the samples were dipped in a solution 0.5M following a molar ratio of 3:1 (repeating units of PE to KMnO4). It was seen that even though the physical change was immediate and noticeable (Figure 3.4), opposite to LDPE samples, HDPE samples showed no significant structural

alterations that could be seen in the FTIR spectra. This could be a result of the exposure time (from 1 to 2.5 minutes at 180W), which compared with the literature (10 minutes) is quite low. However, some trial samples were subjected to higher exposure times (5-7 minutes) in the KMnO4 solution and could not be retrieved: they were completely burned and unfit for washing and weight measuring.

Table 3.2. Weight data of MAOx samples

Sample	Initial Weight (mg)	Final Weight (mg)	Weight Change %
LD1	501.8	539.3	7 %
LD1 rep	506.9	545	8 %
LD1 run3	509	510	0 %
LD1 run4	507	547	8 %
LD1 run5	509	513	1 %
LD1 run6	506	514	2 %
LD1.5	507.7	546.2	8 %
LD1.5 rep	504.5	531	5 %
LD1.5 rep2	506.2	907.8	79 %
LD2	501.1	1022	104 %
LD2 rep	500.4	612.3	22 %
LD2 rep2	508.4	818	61 %
LD2 rep3	501.6	564.8	13 %
LD2 run3	507	551	9 %
LD2.5	507.2	510.6	1 %
LD2.5 rep	502	788	57 %
LD2.5 rep2	503.1	668.7	33 %

Figure 3.5 Sample HD2.5 rep before and after MAOx.

Additionally, and again like in TOD, the system was heterogeneous because of the film form of the samples and the aqueous form of the oxidant. This represents a limitation for the surface area available for reacting; the size of the films was slightly large (~5cm) to ease the retrieval after oxidation. For future experimentation, it is recommended to reduce the size of the samples to maximize the surface area and the reaction rate. Finally, it would be worthy to study the effect of different molar ratios in the HDPE oxidation with KMnO4, or of other oxidants: the conditions used in the literature were applied to LDPE films, however, HDPE has higher crystallinity and requires more energy to weaken and break the bonds in the polymeric chain.

Additionally, a statistical analysis (ANOVA one-way) was run for these samples, using the exposure time as the factor and the weight change as the response variable. It was found that the treatment applied to the samples caused no (statistically) significant changes in the response variable. (Results in Appendix 6.1.)

3.2.3. Thermo-Oxidative Degradation of PE Films with $K_2S_2O_8$ (TOD).

After applying TOD, it was found that none of the samples showed any sign of carbonyl formation. FTIR spectra showed some new peaks in samples LD11rep (1742-1704cm^{-1}

indicating the presence of esters and/or lactones and carboxylic acid, respectively), HD21rep and HD41rep (1421cm^{-1} presence of carboxylic acid), HD32rep and HD41 (1425cm^{-1} and 1427cm^{-1} indicators of carboxylic acid presence). However, due to the inconsistency of these results between two runs of the same sample under the same conditions, the experiments were replicated once more. To conclude the presence of carboxylic acid, the spectra should show peaks at 3000-2200cm^{-1} (OH stretching), 1725-1700cm^{-1} (carbonyl stretching) and around 1420-1200cm^{-1}(carboxyl torsion/stretching).

The new spectra showed no significant changes when compared to the initial samples nor any of the previously mentioned peaks. For this reason, the tested samples were discarded and accepted as mistaken. Although there were some shape changes in the FTIR spectra of the HDPE samples, none of them relevant to the objectives of this investigation. For these reasons, none of the samples of this pre-treatment were considered for the AD stage. FTIR spectra corresponding to the complete set of experiments can be found in Appendix 6.4. Additionally, as shown in Table 3.1, although there was a little weight loss, especially for LDPE at low temperatures, the change in the weight of the samples was not enough to consider the samples for further degradation under anaerobic conditions (in general, less than 20%). Also, the samples showed no visible physical changes.

The application of this pre-treatment was not successful in showing degradation signs. It is relevant to consider that the conditions taken from the literature were not appropriate for the material under study. For future studies, it would be worth it to use higher concentrations of the oxidant and maximize the surface area of PE. Additionally, a statistical analysis (ANOVA) was run for these samples: the system was defined as a factorial design, using the temperature and the concentration of the oxidant as the factor and the weight change as the response variable. It was found that only the temperature influenced the weight change of the samples. The concentration of the oxidant and the interaction between the two factors are statistically insignificant to the response variable. (Results in Appendix 6.1).

Table 3.3. Weight data of TOD samples.

T (°C)	Sample	Initial Weight (g)	Final Weight (g)	Weight Change %
50	LD11	1.098	1.294	18 %
	LD12	0.965	1.153	19 %
	HD11	1.268	1.260	-1 %
	HD12	1.277	1.267	-1 %
	LD11 rep	0.789	0.962	22 %
	LD12 rep	0.992	1.136	15 %
	HD11 rep	1.320	1.313	-1 %
	HD12 rep	0.990	1.136	15 %
60	LD21	0.725	0.814	12 %
	LD22	0.824	0.912	11 %
	HD21	2.007	1.941	-3 %
	HD22	1.580	1.570	-1 %
	LD21 rep	0.719	0.737	3 %
	LD22 rep	1.277	1.330	4 %
	HD21 rep	1.120	1.134	1 %
	HD22 rep	1.112	1.119	1 %
70	LD31	0.990	1.009	2 %
	LD32	1.446	1.518	5 %
	HD31	1.469	1.472	0 %
	HD32	1.926	1.939	1 %
	LD31 rep	1.219	1.221	0 %
	LD32 rep	0.932	0.948	2 %
	HD31 rep	1.012	1.004	-1 %
	HD32 rep	1.096	1.084	-1 %
80	LD41	0.778	0.567	-27 %
	LD42	0.778	0.774	-1 %
	HD41	1.099	1.1428	4 %
	HD42	1.168	1.1451	-2 %
	LD41 rep	0.716	0.7213	1 %
	LD42 rep	0.728	0.7182	-1 %
	HD41 rep	1.308	1.3158	1 %
	HD42 rep	1.097	1.077	-2 %

In general terms, and regarding the three pre-treatments, it is recommended to consider measuring the changes of molecular weight after the application of the primary degrading techniques, as well as after the AD. It was not possible to perform this property's measurement in the present study.

3.3. Anaerobic Degradation

After acclimatizing the inoculum and adjusting its pH, the samples were placed inside the bottles, the inoculum was added, and the AD was performed for 125 days at 55°C and 50 rpm of agitation. From the results obtained in the pre-treatments and the additional runs made, it was defined that 8 samples would be subjected to AD: LD1, LD1 run3, LD1 run5, LD1 run6, LD1.5, LD1.5 run2, LD2 run2, and LD2 run3. All the samples were exposed to the MAOx pre-treatment. At the end of the name, they were identified as runs; r1, r2 and r3. The samples LD1 and LD1 run3 were subjected to ACoD; for runs 1, glucose was used as co-substrate; for runs 2, acetic acid was used; and runs 3 were left only with PE for comparative purposes. There were in total 24 pre-treated samples, a positive control (cellulose), a negative control (untreated LD), positive controls for the co-substrates and a blank.

ACoD was included in the investigation to evaluate whether having organic co-substrates could improve the overall AD of PE, as it happens with other organic substrates. It was expected to find higher biogas production volume in a shorter time with co-digested samples than with those without any co-substrate. Glucose and acetic acid were selected as co-substrates due to their availability and readiness for biodegradation; both are easily biodegradable materials that could provide the microorganisms with the necessary nutrients for their growth.

As mentioned before, the total experimental time was of 125 days. The degradation of organic constituents takes from 15 to 30 days to complete, and in the beginning, the experiments were planned to continue for 60 days to give time for PE degradation. However, given the persistent nature of PE (high molecular weight and general hydrophobicity), even after being oxidized to some extent, it is still a material of slow degradation, and 60 days were not enough. After 107 days of experimentation, it was decided to retrieve one run of each sample

(except for those with ACoD) and send them to FTIR analysis to evaluate what was the state of the samples from a molecular perspective.

3.5.1. FTIR spectra after 107 days of AD

From the samples sent to analysis after 107 days, it was observed that the signals of degradation they had before AD disappeared. Most of the samples had a signal of OH group in the zone from 3600 to 3000cm^{-1}, indicating a decrease of hydrophobicity of the PE. The disappearance or reduction of this peak indicates that the functional group was decomposed from the PE chain, indicating that there was a microbial attachment to the PE surface and degradation to some extent.

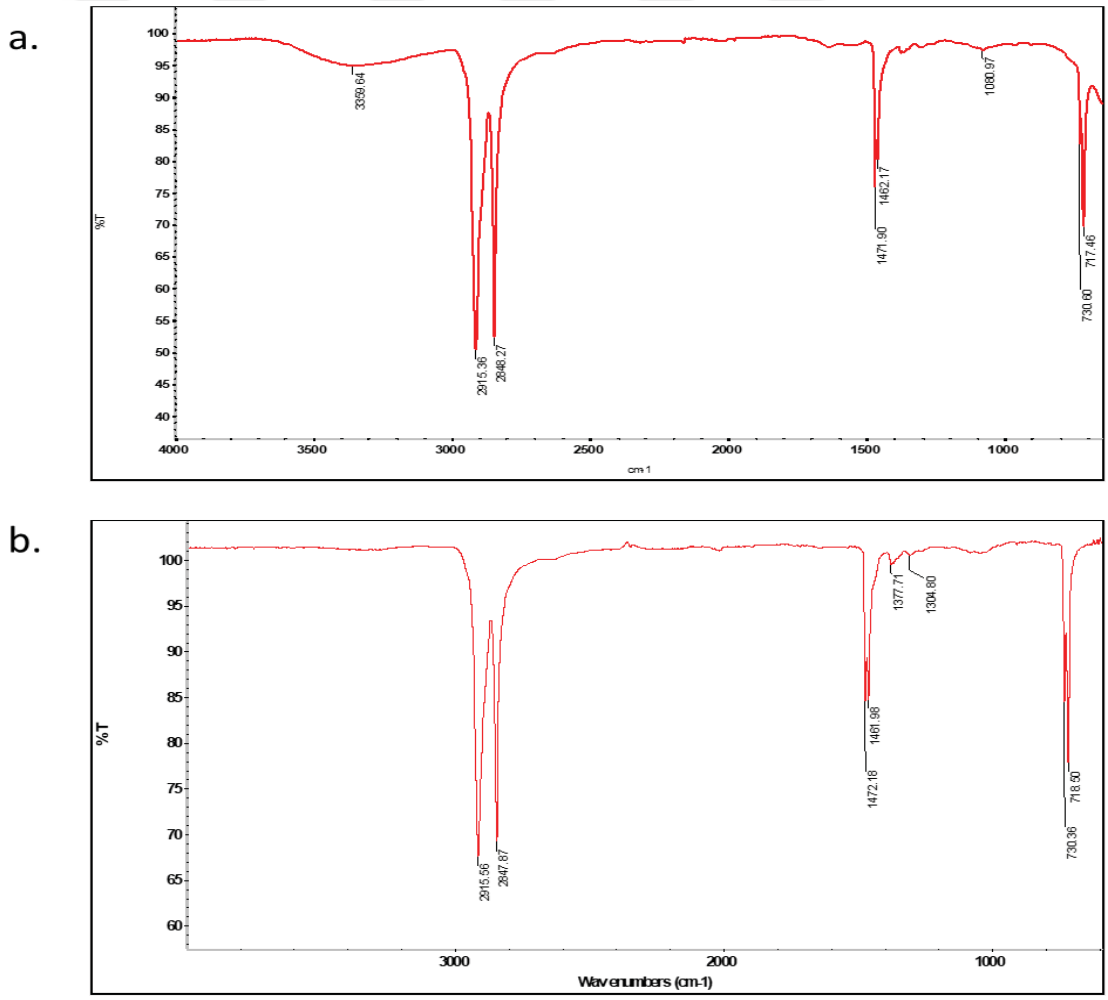

Figure 3.6. FTIR spectra of sample LD1.5 before (a) and after (b) AD

Additional to the disappearance of the OH signals, it was common among most of the samples a small new signal at around 1377cm^{-1}, which corresponds to the CH_3 group. The appearance of this signal or the increase in the transmittance percentage values are indicators of chain scission and the formation of oligomers. The changes in sample LD1.5 r1 can be seen in Figure 3.6.

3.5.2. FTIR spectra after 125 days of AD

Once it was determined that biodegradation was occurring to some extent, it was decided to finish the AD of the rest of the samples, even though the biogas production did not reach a stationary stage, as it will be shown later. The samples were taken out of the incubator and retrieved from the bottles, after measuring the final pH of each one. After washing and weighing, some pieces were sent for FTIR analysis.

The spectra showed very similar behavior to the samples taken out at 107 days. In general terms, and as it can be seen in Figure 3.7 for sample LD2 run2, for the samples of PE, any signal of OH disappeared or was significantly reduced, the signal of CH_3 formation was present or the zone around the signal was broadened, and values of transmittance increased in an average of 12%, indicating an increase in chain scission and reduction of molecular weight through the formation of smaller compounds. It is also important to note that, even though the samples showed some interesting changes after the application of the pre-treatment, such as OH group formation and broadening of peaks after each treatment, when compared to the initial FTIR spectra of LDPE, the main peaks seen in the characterization of the samples remain the same, meaning that there was not a significant overall alteration of the molecular structure of the PE.

Regarding the samples subjected to ACoD, the only visible change was the consumption of the OH group, as can be seen in Figure 3.8. There were no visible changes in the peak of CH_3 formation, although there was a slight increase in the transmittance values. According to these results, adding a co-substrate does not improve the overall performance of the biodegradation to a significant extent, as it has been proved to happen with many other organic compounds. The complete set of spectra corresponding to the samples after AD can be found in Appendix 6.7.

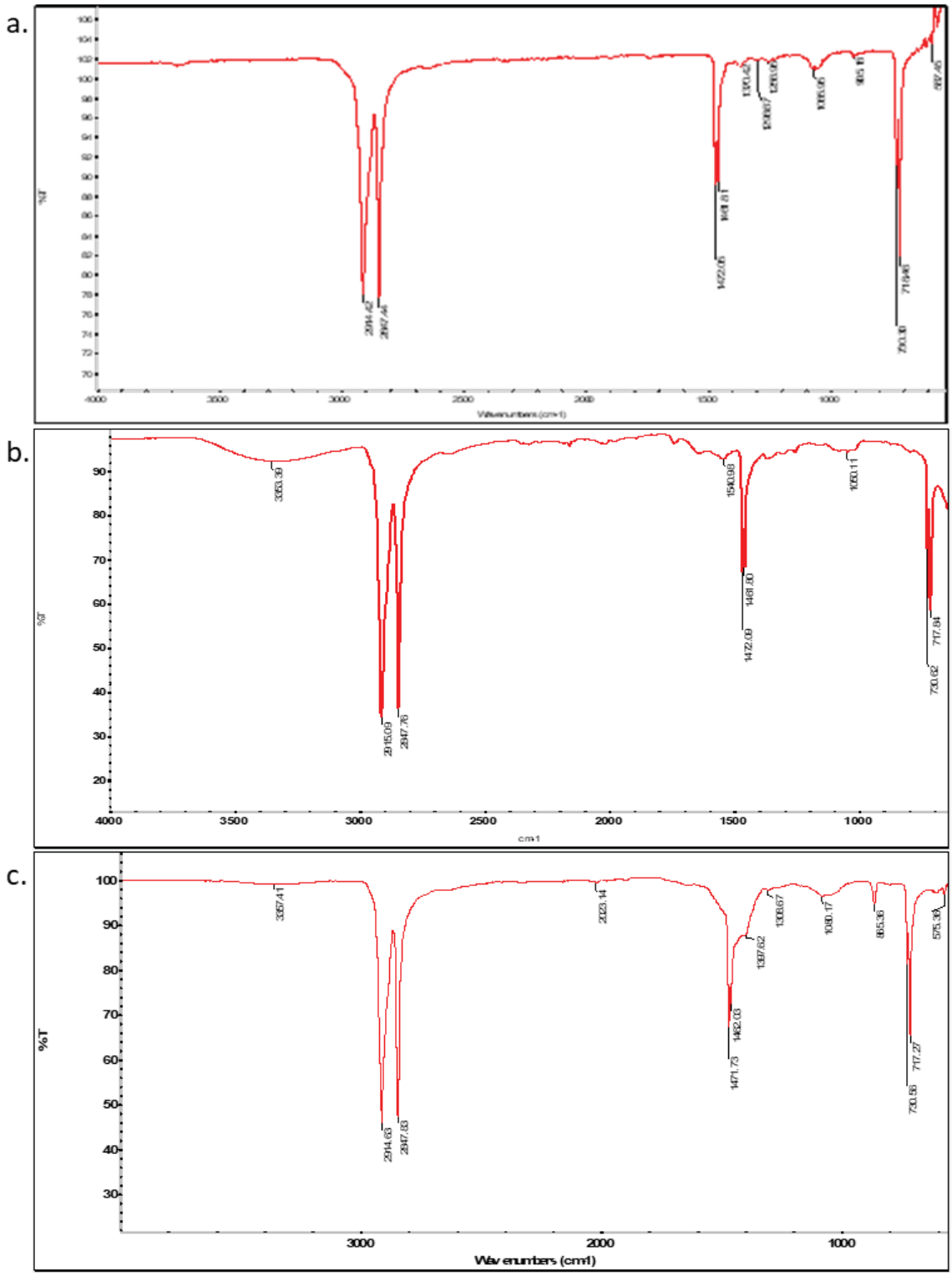

Figure 3.7. FTIR spectra of sample LD2 run2 r1 before MAOx (a), after MAOx (b) and after AD (c).

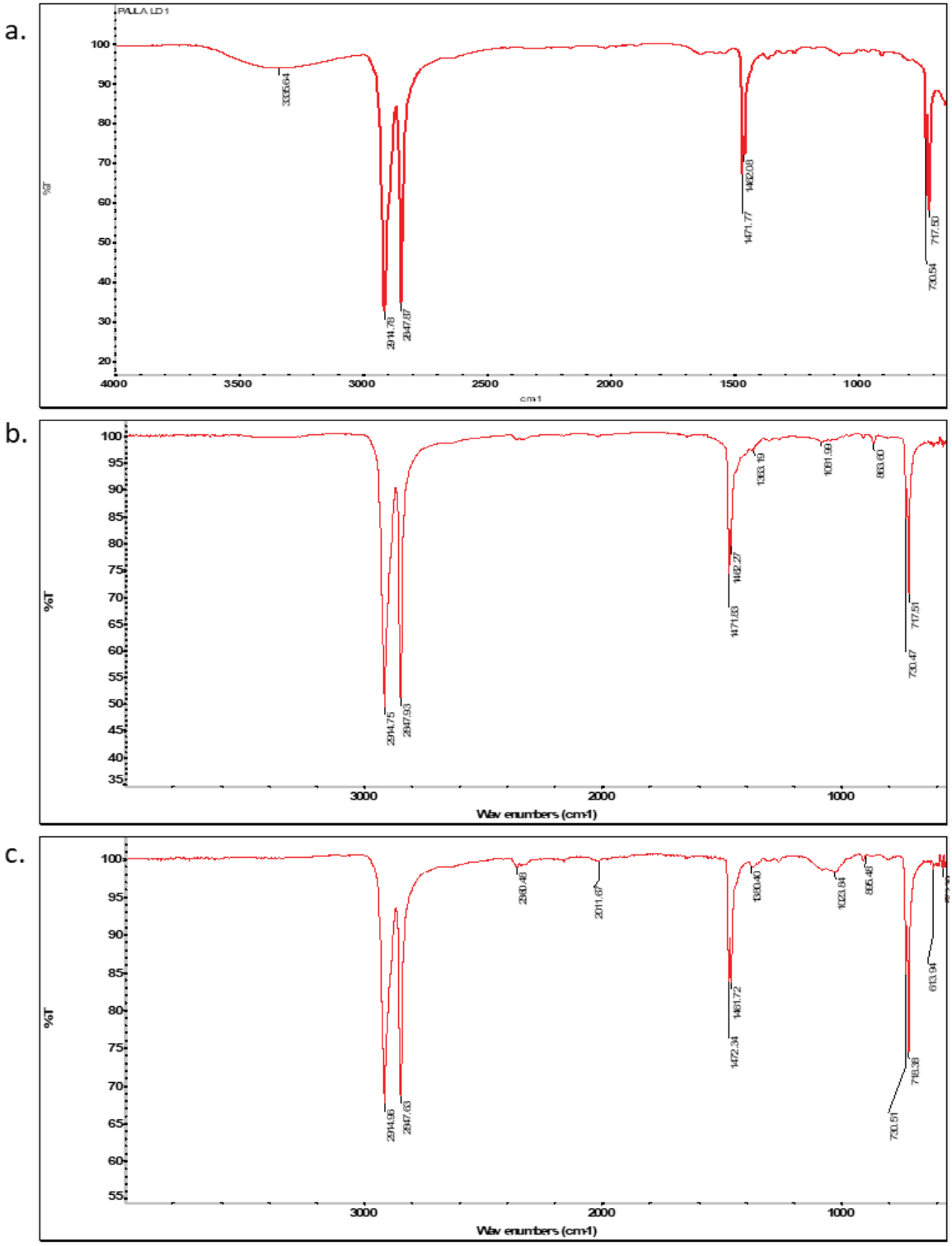

Figure 3.8. FTIR of sample LD1 before AD (a), and after AD with co-substrates glucose (b) and acetic acid (c).

3.5.3. Anaerobic digestion of PE

One of the objectives of this investigation was to explore the potential of PE for biogas production. This idea emerged from the concern of the serious environmental issues caused by PE accumulation in nature. Being able to biodegrade PE and recover energy from this process through AD would be an ideal option to redirect the impact of this polymer towards something positive. Up to date and to the best of our knowledge, there are no attempts of biodegrading PE to produce energy.

To define the temperature conditions to be used in the process, it was considered the nature of PE. Due to its high resistance to degradation, it was decided to use thermophilic instead of mesophilic conditions; a higher temperature could improve the overall process. The inoculum used was an anaerobic sludge seed with 0.033 VS (g/g) and the PE had 0.963 VS (g/g). Given the low density of PE and the amount of sample available for use, the S:I was found after defining the experimental conditions, with a value of 0.073.

To follow the biogas production of the samples, the pressure of the bottles was measured once a day for the first two weeks and twice per week after. The pressure was used to calculate the volume of biogas at standard conditions and then added up, to find the total volume of biogas produced throughout the experimental time. Finally, this data was expressed as mL of biogas. Figure 3.9 and Figure 3.10 show the daily and cumulative biogas production of the treated PE samples, respectively. The daily and cumulative biogas production can be seen in more detail in Appendix 6.8.

The daily production of biogas in PE samples shows that for samples with higher exposure times (LD1.5 and LD2), the biogas production increases later in time, at around 70 days, while the samples with the lower exposure time (LD1), show a peak of production in an earlier stage, at around 40 days of digestion (except for the sample LD1). Most of the samples show two production peaks. This could be a sign of the first stage of production due to consumption of the organic matter in the inoculum, which is readily biodegradable, and a later production due to PE decomposition. The maximum biogas production recorded from PE samples corresponds to sample LD1 r3 and is ~15 mL at 41 days. Regarding the samples LD1.5 and LD2, most of them did not show a decrease in the production as the experimental

time finished, contrary to most of the LD1 samples; their biogas productions showed a decreasing tendency.

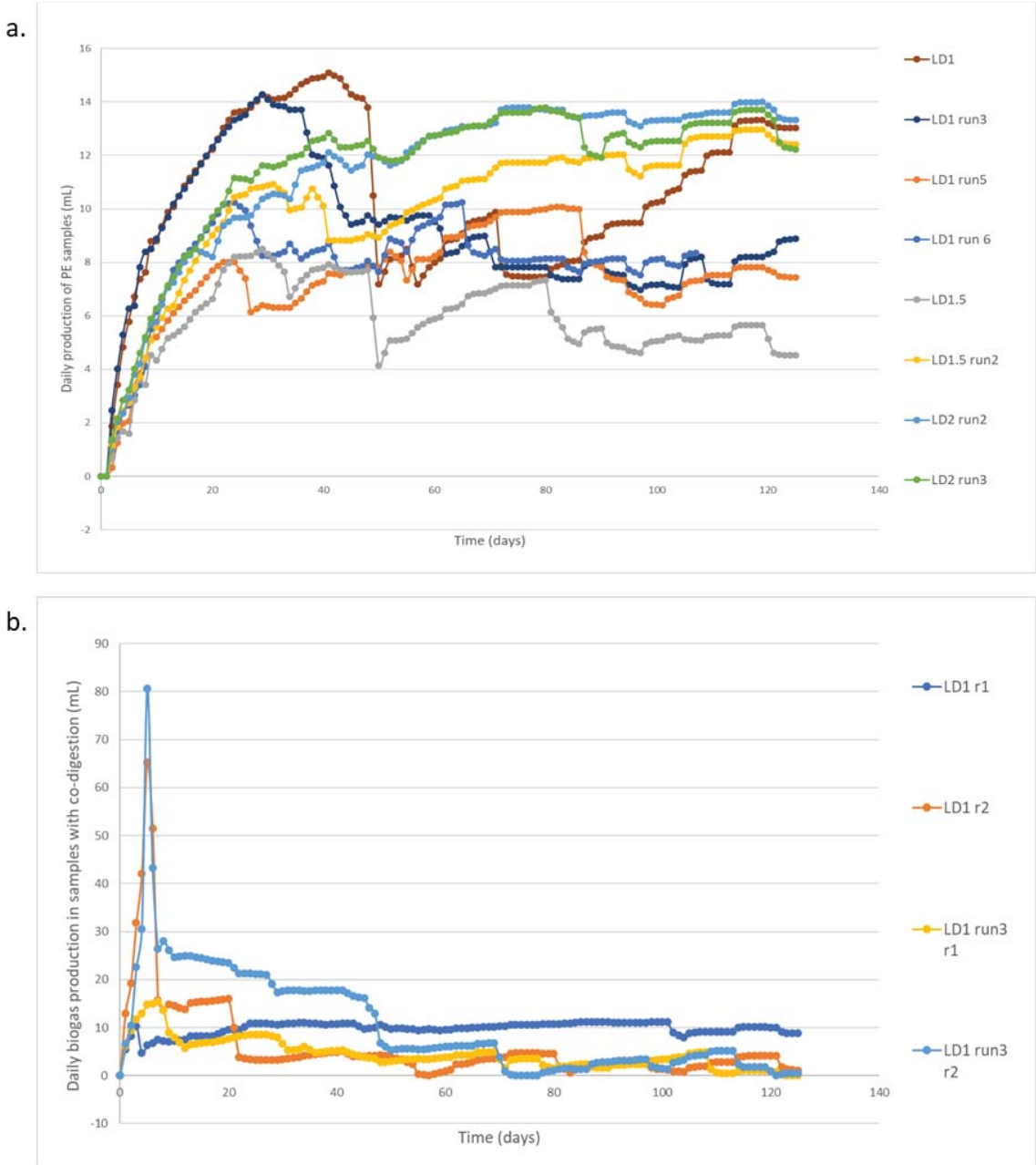

Figure 3.9. Daily Biogas production of PE samples without (a) and with co-substrates (b).

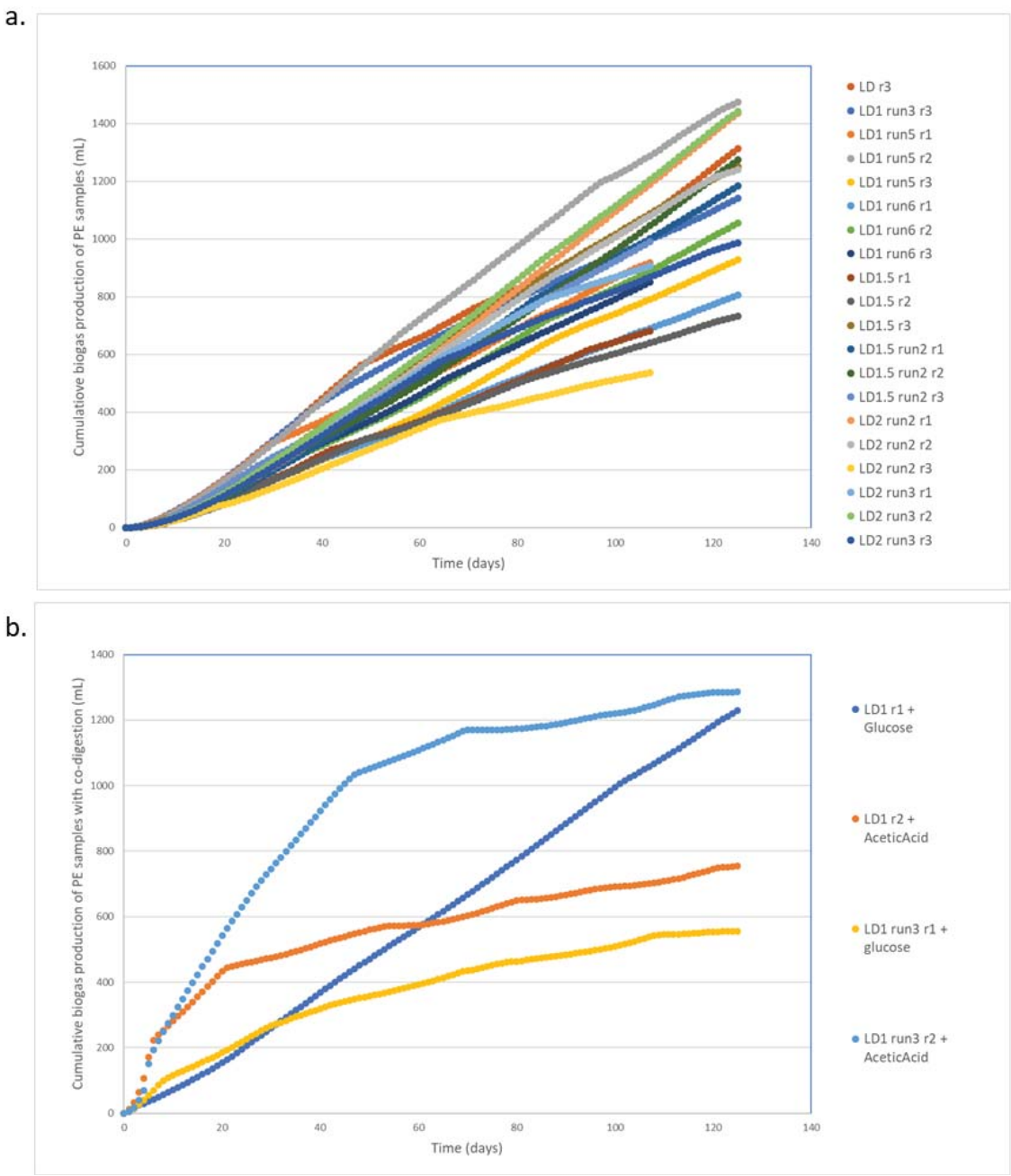

Figure 3.10. Cumulative Biogas production of PE samples without (a) and with co-substrates (b).

In the case of samples with ACoD, there are two clear peaks of production, corresponding to the samples with acetic acid, very early in the experimental time: ~65 mL and ~80 mL after 5 days. In these cases, the production diminished continuously after the peaks. For glucose,

the biogas production reached a maximum in the beginning: ~10 mL and ~15 mL in the first week. Then, the production was continuous around the same range of values, and in the final days of experimentation, the biogas production started to drop. One of the co-digested samples end with no production by the final day, while the other was still producing biogas; this would mean that for the former case, the samples' oxidized surface was consumed faster. Finally, the samples without co-substrate (r3 in both cases) showed an initial peak (15mL, day 41 and ~14 mL, day 29), and then the production continued around the same values (~10mL daily), which was higher than most of the co-digested samples. By the final days of the experiments, these samples did not show signs of a production decrease.

Regarding the cumulative production graph, none of the PE samples reached a stationary stage: the production was continuous and even on the final day, there was no sign of reaching it for most of the samples. The behavior of the samples was consistent. Some of the samples with ACoD were closer to reach the stationary stage; those with acetic acid had a more consistent behavior than those with glucose. From these results, it can be said that PE samples needed a longer time to reach a greater degradation. As seen in the FTIR results, there was some degree of degradation, given the increase of CH_3 group presence. It is not possible to assess the maximum biogas production of the PE samples without allowing the experiments to run for a longer time. It is suggested to let the AD continue for at least 6 months.

From the ACoD samples, it was found that acetic acid showed better performance as a co-substrate than glucose, generating a higher biogas production, on average. However, when compared to the other yields, it does not represent a significant increase; there are yields in the same range and even higher from samples without ACoD. This is consistent with the FTIR results: the spectra showed no significant changes in the molecular structure of PE after being co-digested with glucose or acetic acid. No formation of CH_3 was seen for either of these samples, contrary to what happened to most samples without co-digestion. The interactions between PE, the co-substrates and the inoculum might have caused some inhibitory effects in the long term of the AD. PE has an inert nature and the few parts of it that were oxidized could have withdrawn its degradation when interacting with another substrate. The co-digestion of biodegradable substrates results in the overall improvement of the process due to factors such as the balance of nutrients and the dissolution of toxic

compounds. This might not be the case when the co-substrates are different. Regarding acetic acid, it was comparatively better than glucose, but still not remarkable; this might have been a consequence of the concentration of acetate that was available for biogas production. It could be of interest to explore further effects of this co-substrate in the PE ACoD in future studies by increasing the number of samples.

In Table 3.5 there is a summary of the important data regarding the daily and cumulative production of biogas from the tested samples. The values of the cumulative production of the samples at the end of the experimental time can be seen in Figure 3.11.

Table 3.4. Summary of the biogas productions obtained in the batch AD studies.

Sample	**Max mL in a day**	**Average cumulative biogas production in mL**
ACoD glucose	15.5	757.1
ACoD acetic acid	80.6	885.2
LD1	15.3	1060.9
LD1.5	13.4	1019.0
LD2	14.0	1091.6

There are no remarkable changes between values of biogas production of the samples were subjected to different exposure times in the MAOx pre-treatment. According to this, treating the PE samples with different oxidation times does not cause a significant difference in the volume of biogas produced after AD. It is recommended for future studies to increase the number of samples pre-treated and apply an experimental design that allows running statistical tests over the results, to evaluate the actual statistical significance of using different levels of exposure time in the pre-treatment in the biogas production. Additionally, the production of biogas in co-digested samples was lower, on average, than those without co-substrates. When individually seen, the biogas productions vary greatly between the samples with the same co-substrate; for this reason, the data is not conclusive. It is important to emphasize that only two samples of treated PE were exposed to ACoD and the discussion of their results cannot be generalized until more complete experimentation is done. It is not possible to state a general conclusion from this behavior given the few numbers of samples

subjected to these conditions. The results obtained show that co-digestion of PE with acetic acid or glucose is not effective neither for enhancing the average biogas production nor for reducing the time of the process.

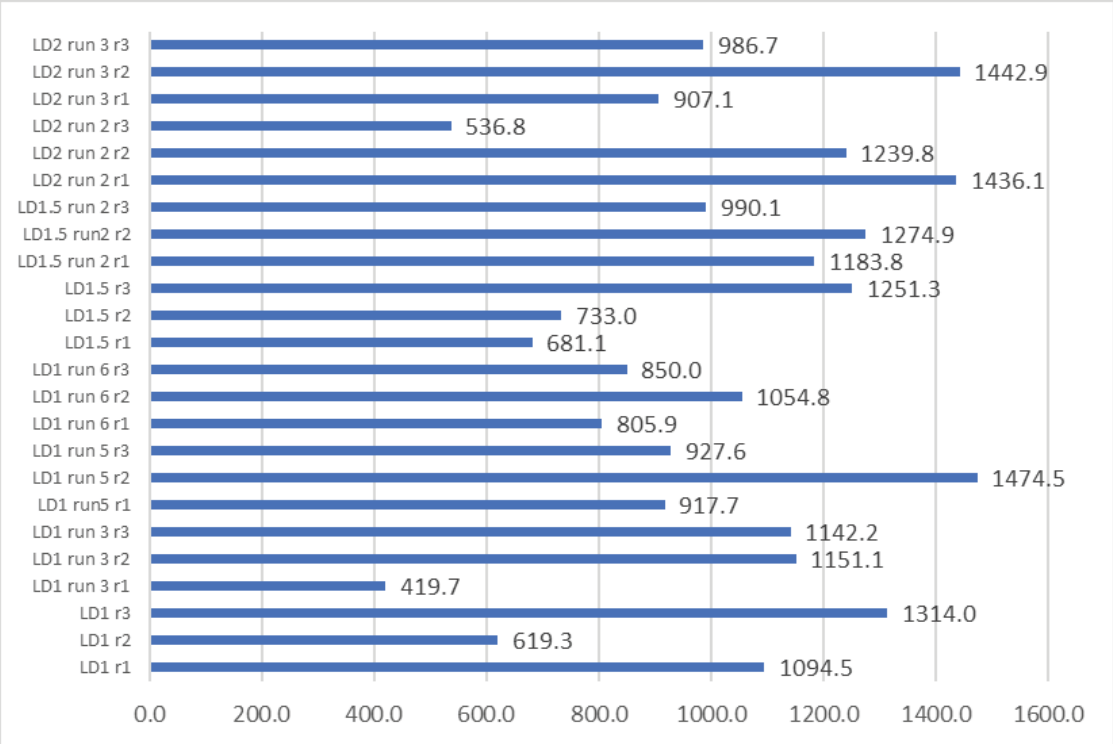

Figure 3.11. Cumulative biogas production in mL

Finally, as seen in the graph above, the biogas production values showed an overall minimum of 536.8 mL, an overall maximum of 1474.5 mL, and an average production of ~1060 mL. In the study made by Selke [24] and mentioned earlier, samples of PE blended with biodegradation-promoting additives were exposed to AD environments in both mesophilic and thermophilic conditions for 464 days. For the thermophilic conditions, the set temperature was 50°C and they found an average accumulated production of biogas of 900 mL after this time. It is important to note that the conditions used in this study were not the same as the ones used in our project, and that the results presented above correspond to the total instead of the net biogas production.

Regarding some macromolecular properties, even though the experimental time was longer when compared to organic materials, the physical changes in most of the samples were unnoticeable and the weights of the samples showed an average of 1% change; none of the samples showed changes higher than 10%. The samples' weight was not a relevant variable to measure, since it was not capable of reflecting the extent of the material's degradation which is small. It was not possible to assess to what extent the molecular weight was reduced. It is highly recommended to measure the molecular weight of the samples at the beginning and after each treatment to assess if they were successful in terms of chain scission even in any small amount. The data of weight changes can be seen in Appendix 6.9.

4. CONCLUSIONS

In the present study, the main objectives were to investigate the possibility of biodegradation of PE under anaerobic conditions after a pre-treatment with different techniques and to explore the potential of this material for biogas production.

PE in the form of LDPE and HDPE commercial bags, was exposed to three different oxidizing techniques. The pre-treatment applied were of different natures: physical oxidation (POxUV), chemical oxidation (MAOx), and physicochemical oxidation (TOD). After applying the pre-treatments, it was seen that only MAOx was successful in causing some degradation. From this study, it can be concluded that:

1. TOD pre-treatment did not generate any considerable oxidation in the samples. Neither LDPE nor HDPE was affected by this technique, as can be seen from the FTIR spectra and the weight changes. The reason why no effect was observed can be attributed to the concentration of oxidant used (0.5 and 1 g/L) and the size of the samples (films). It is recommended to increase the concentration of the oxidant and the surface area of the samples to improve the contact between PE and the oxidant.
2. POxUV pre-treatment did not show any sign of carbonyl formation. The sole exposure to UV radiation is not effective in generating photo-oxidation in a time up to 90h. For future studies, it is recommended to increase the time of exposure and to use UV-B lamps, to increase the radiation as well. Additionally, it could be of interest to study the combined effect of UV radiation and humidity.
3. MAOx pre-treated samples showed clear signs of degradation, both at macroscopic and molecular levels. In general, $KMnO_4$ was an effective oxidant for this procedure and most of the samples showed hydrophobicity decrease in the form of OH bond formation, as seen in the FTIR spectra of most of the LDPE samples. HDPE samples showed no significant molecular modifications, although there were evident physical changes. For these samples, it would be recommended to study with higher concentrations of oxidant to generate changes in the molecular structure and to

increase the formation of carbonyl groups. It is also important to note that decreasing the size of the samples might improve the degree of oxidation due to surface area enlargement.

From the pre-treatments, and due to the results obtained, only samples of LDPE from the MAOx technique were considered for the AD stage. The AD was performed on 8 samples for a total of 125 days at thermophilic conditions (55°C). Two of the samples were subjected to co-digestion with glucose and acetic acid as co-substrates. The slowness of the process was expected due to the nature of the material under study and after 107 days, one run of each sample was retrieved and analyzed under FTIR. The spectra obtained showed a small degree of degradation and the experiments were stopped after 125 days. After obtaining and analyzing the FTIR spectra of all the PE samples it can be concluded that:

1- The PE samples were degraded to some extent: the peaks of OH bond found after the MAOx pre-treatment disappeared after the AD, denoting that it was used by the microorganisms for attachment to the PE surface.
2- There was chain scission in the PE molecular structure: this was seen in the appearance of a small peak in 1377 cm^{-1} in most of the samples, which is a sign of CH_3 formation. Additionally, there was a general increase in the transmittance percentage values of the peaks, also an indicator of chain scission.

Finally, regarding the AD of PE:

PE is a highly persistent material, and it requires longer than 125 days to be degraded under anaerobic conditions, even after being oxidized previously. Despite this, it is possible to generate biogas out of oxidized PE. From the PE samples subjected to AD, it was found a minimum and maximum cumulative biogas production of 536.8 mL and 1474.5 mL, respectively, at the end of the experimental time. It can also be said that:

1. ACoD of PE with acetic acid showed better performance in terms of biogas production than with glucose. However, when compared to samples without ACoD, the cumulative biogas production was not the best. This was consistent with the FTIR spectra that showed no significant changes in the polymeric chain, including the lack

of CH_3 formation. This could be studied to a further extent. The few samples available for analysis in the present study are not enough to give general conclusions regarding this aspect.

2. According to the data obtained, there was no visible difference in the biogas production of samples pre-treated with different exposure times. The application of a pre-treatment was fundamental to make microbial attachment possible, but with the data obtained, it is not possible to say that the different levels of the applied technique had a significant effect on the biogas production. For this reason, it is highly recommended to make an experimental design with the appropriate number of samples, that allows evaluating whether using different levels of exposure time in the pre-treatment cause any effect in the biogas production.
3. The weight changes of the samples did not show important alterations after the applied treatments. Weight was not a relevant variable to measure in this study, given that the molecular changes caused by the treatments, although important for the scope of this investigation, could not be reflected in it.

Some final recommendations for future studies, additional to the ones previously given, include the measurement of the molecular weight of the samples after each stage of treatment; this way it is possible to assess whether the primary degrading techniques have the expected effect or not. As mentioned earlier, it was not possible to measure the percentage of CH_4 in the biogas due to the lack of an appropriate device, but the CH_4 must be measured directly or indirectly.

Despite the limitations of the present study, it is still possible to highlight that, given that PE is a non-biodegradable material, after a proper degrading technique, is capable to be degraded to some extent and produce some biogas. Taking this into account, the outcomes of this investigation could serve as a starting point of future studies on the conversion of one of the most polluting materials into a form of energy.

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6. APPENDIXES

6.1. Experimental Designs and Statistical Analysis

To test the effects of three different techniques on the primary degradation of PE for posterior anaerobic digestion, the following experimental designs were proposed. Each one of them is presented in a table with the names assigned to the samples, the statistical model used (if applicable) and the results obtained from the analysis.

Pre-treatment 1: Photo-Oxidation with UV radiation

As proposed by several authors, the exposure of PE films to UV radiation for prolonged times can photo-degrade the material. For such purpose, LDPE and HDPE were exposed to UV radiation starting from 60 hours up to 90 hours, according to the following design:

Table 6.1. Experimental design for POxUV

Sample	60 h	70 h	80 h	90 h
LDPE	LD60	LD70	LD80	LD90
HDPE	HD60	HD70	HD80	HD90

The samples corresponding to the second run were named equally plus “rep”. there were 16 experiments in total. It was not possible to perform a statistical analysis because there were only two runs for each sample and a total of 16 experiments wouldn’t fulfill the requirements of the normal distribution of the population, which is mandatory for statistical relevance. Instead, the results were analyzed individually.

Pre-treatment 2: Microwave-Assisted Oxidation with $KMnO_4$

As mentioned in chapter 2, this pre-treatment was performed according to the method suggested by Roy et al (2010). The corresponding experimental design is as shown in Table 2. Initially, there were 16 experiments, however, it was necessary to repeat some of the tests. For every run, the samples were named equally plus “rep” and “repX” where X is the number of the run (2, 3…).

Table 6.2. Experimental design for MAOx

Sample	1 min	1.5 min	2 min	2.5 min
LDPE	LD1	LD1.5	LD2	LD2.5
HDPE	HD1	HD1.5	HD2	HD2.5

For these experiments, the ANOVA one-way was performed. This test is robust for the normality assumption, meaning that it can tolerate quite well violations to it. It is important to state that this test evaluates the significance of a null hypothesis according to the p-value (0.05). For this test, the null hypothesis H_0 is that the means of two or more groups are equal, whilst the alternative hypothesis H_1 is that they are different. In this case, the groups are the weights of the samples according to the oxidation time, thus there are four groups, as seen in Table 3. The results of the analysis are presented below.

Table 6.3. Data used to feed the ANOVA test

Group 1		Group 2		Group 3		Group 4	
Time min	**ΔW**	**Time min**	**ΔW**	**Time min**	**ΔW**	**Time min**	**ΔW**
1	37,5	**1,5**	38,5	**2**	520,9	**2,5**	3,4
1	38,1	**1,5**	26,5	**2**	63,2	**2,5**	286,0
1	1,0	**1,5**	401,6	**2**	44,0	**2,5**	165,6
1	40	**1,5**	0	**2**	1		
1	4	**1,5**	-2	**2**	1		
1	8	**1,5**	18	**2**	2		

One-way ANOVA: Weight change versus Time min

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal

Significance level α = 0,05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Time min	3	1,0. 1,5. 2,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time min	2	22288	11144	0,50	**0,619**
Error	15	337442	22496		
Total	17	359729			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
149,987	6,20%	0,00%	0,00%

Means

Time min	N	Mean	StDev	95% CI
1,0	6	21,43	18,88	(-109,08. 151,95)
1,5	6	80,4	158,1	(-50,1. 210,9)
2,0	6	105,4	205,3	(-25,2. 235,9)

Pooled StDev = 149,987

According to these results, the p-value of the ANOVA is 0.619 and H_0 is accepted. The meaning of this is that the means of the groups are equal, and consequently, the treatment applied to the samples causes no (statistically) significant changes in the response variable.

Pre-treatment 3: Thermo-Oxidative Degradation with $K_2S_2O_8$

For this pre-treatment it was necessary to combine two factors: the oxidant, $K_2S_2O_8$ in two levels (concentration) and the temperature in four levels (from 50 to 80°C). Hence, the experimental designs resulted as shown in Table 4. Because of the presence of two factors, each sample was named after the type of PE (LDPE or HDPE), then a number from 1 to 4 indicating the temperature, and finally a second number, 1 or 2, indicating the concentration of the oxidant.

Table 6.4. Experimental design for TOD

Concentration	**50°C**	**60°C**	**70°C**	**80°C**
0.5 g/L	LD11	LD21	LD31	LD41
	HD11	HD21	HD31	HD41
1 g/L	LD12	LD22	LD32	LD42
	HD12	HD22	HD32	HD42

For these tests, a factorial design was established, and the ANOVA was performed, to evaluate the statistical significance and the effect of the factors individually and combined on the weight change of the samples. In this case, H_0 evaluates three possibilities: that the means of the first factor are equal; that the means of the second factors are equal; and that there are no interactions between factor 1 and factor 2. Consequently, H1 has three possibilities as well: that at least one of the means of factor 1 is different; that at least one of the means of factor 2 is different; and that there is an interaction between factor 1 and factor 2. The level of confidence is 95% and consequently, the p-value is 0.05.

In Table 5 there is the data analyzed and after it, the ANOVA results.

Table 6.5. Data set of the factorial design.

Group 1			Group 2			Group 3			Group 4		
T (°C)	**C (g/L)**	**ΔW**	**T (°C)**	**C (g/L)**	**ΔW**	**T (°C)**	**C (g/L)**	**ΔW**	**T (°C)**	**C (g/L)**	**ΔW**
50	1	196,0	60	1	89,0	70	1	18,7	80	1	-211,0
50	1	-8,5	60	1	-65,8	70	1	2,8	80	1	43,8
50	1	173,0	60	1	18,1	70	1	1,8	80	1	5,3
50	1	-7,1	60	1	14,2	70	1	-8,3	80	1	7,8
50	2	188,0	60	2	88,0	70	2	71,9	80	2	-4,2
50	2	-9,8	60	2	-10,0	70	2	12,5	80	2	-22,9
50	2	144,0	60	2	53,0	70	2	16,3	80	2	-9,8
50	2	146,0	60	2	7,4	70	2	-12,3	80	2	-19,6

Multilevel Factorial Design

Design Summary

Factors:	2	Replicates:	1
Base runs:	8	Total runs:	8
Base blocks:	1	Total blocks:	1

Number of levels: 4. 2

Design Table (randomized)

Run	Blk	A	B
1	1	1	2
2	1	2	2
3	1	3	1
4	1	1	1
5	1	3	2
6	1	4	1
7	1	2	1
8	1	4	2

General Factorial Regression: Weight change versus ... t concentration

Factor Information

Factor	Levels	Values
Temperature	4	50. 60. 70. 80
Oxidant concentration	2	1. 2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	74548	10649,6	2,08	0,086
Linear	4	74425	18606,3	3,64	0,019
Temperature	3	70177	23392,3	4,57	**0,011**
Oxidant concentration	1	4248	4248,1	0,83	**0,371**
2-Way Interactions	3	122	40,8	0,01	0,999
Temperature*Oxidant concentration	3	122	40,8	0,01	**0,999**
Error	24	122841	5118,4		
Total	31	197389			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
71,5429	37,77%	19,62%	0,00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	28,4	12,6	2,24	0,034	
Temperature					
50	74,3	21,9	3,39	0,002	1,50
60	-4,1	21,9	-0,19	0,851	1,50
70	-15,5	21,9	-0,71	0,487	1,50
Oxidant concentration					
1	-11,5	12,6	-0,91	0,371	1,00

Temperature*Oxidant concentration					
50 1	-2,8	21,9	-0,13	0,898	1,50
60 1	1,2	21,9	0,05	0,958	1,50
70 1	2,3	21,9	0,11	0,916	1,50

Regression Equation

Weight change = 28,4 + 74,3 Temperature_50 - 4,1 Temperature_60 - 15,5 Temperature_70
- 54,7 Temperature_80 - 11,5 Oxidant concentration_1
+ 11,5 Oxidant concentration_2 - 2,8 Temperature*Oxidant concentration_50 1
+ 2,8 Temperature*Oxidant concentration_50 2
+ 1,2 Temperature*Oxidant concentration_60 1
- 1,2 Temperature*Oxidant concentration_60 2
+ 2,3 Temperature*Oxidant concentration_70 1
- 2,3 Temperature*Oxidant concentration_70 2
- 0,7 Temperature*Oxidant concentration_80 1
+ 0,7 Temperature*Oxidant concentration_80 2

According to the p-values of the individual factors and the combined effect, only the temperature (0.011) rejects H_0, meaning that it is the only factor that shows an effect on the weight change of the samples. The concentration of the oxidant and the interaction between the two factors are insignificant to the response variable. Regarding the regression model proposed, it could predict the response. However, it is preferred to refine the model and discard the insignificant elements.

6.2. Calculations for MAOx Pre-Treatment

This specific pre-treatment has been taken from the methodology developed by Roy et al (2010), as described in the article "*Surface Oxidation of Low-Density Polyethylene Films to Improve Their Susceptibility Toward Environmental Degradation*". The authors recommend a molar ratio of 3:1 (repeating units of PE to KMnO4), stating that it has been reported as the most effective composition for the oxidation purpose (Roy et al, 2010). The amount of sample to be used per experiment is 0.5 g of LDPE and HDPE. Now, what is the molecular weight corresponding to one repeating unit, meaning a monomer of PE? Since the monomer is constituted by two atoms of carbon and four atoms of hydrogen, its molecular weight (MW) is

$$PE\ Monomer\ MW = 2 * \left(12\left(\frac{g}{mol}\right)\right)_{C\ MW} + 4 * \left(1\left(\frac{g}{mol}\right)\right)_{H\ MW} = 28\frac{g}{mol}$$

In 0.5 g of PE, there are 0.018 moles. To maintain the molar ratio of 3:1, the corresponding moles of $KMnO_4$ are 0.0059 ~ 0.006 moles. Now, according to this, how many grams are there in 0.006 mol of $KMnO_4$?

$$[g\ of\ KMnO_4] = 0.006\ mol\ \times 158.034\frac{g}{mol} = 0.95\ g$$

With these grams, a 0.5 M KMnO4 solution needs to be prepared. The volume of solution needed per sample will be

$$Concentration\left[\frac{mol}{L}\right] \times Volumen[L] \times Molecular\ Weight\left[\frac{g}{mol}\right] = Amount\ [g]$$

$$V = \frac{0.95\ g}{0.5\frac{mol}{L}\ \times 158.034\frac{g}{mol}} = 0.012\ L \sim 12\ mL$$

Then, 12 mL of the solution is needed per experiment. According to the experimental design specified in the previous appendix, 16 experiments were be done for this pre-treatment, meaning that the amount of solution needed is 192 ~ 200 mL. To prepare the solution, the amount of KMnO4 powder needed is

$$0.5\frac{mol}{L} \times 0.2\ L\ \times 158.034\frac{g}{mol} = 15.8\ g\ of\ KMnO_4$$

6.3. Data and Results of Determination of Volatile Solids Experiments

The total solid (TS) and volatile solid (VS) content is calculated by using the following equations:

$$TS\left(\frac{g}{g}\right) = \frac{W_i - W_{ts}}{W_s} \qquad VS\left(\frac{g}{g}\right) = \frac{W_{ts} - W_{vs}}{W_s}$$

Where Wi is the initial weight, that contains the weights of both the dish and the sample, Ws is the weight of the sample, Wts is the weight after the first heating (total fixed solids) and Wvs is the weight after the second heating. In the following table, there is the data collected in the experiments performed for DSV. The weights are in grams.

Table 6.6. Data obtained from the determination of volatile solids

Sample	Wdish	Ws	Wts	TS (g/g)	Wvs	VS (g/g)
LD2.5	86.593	87.078	87.067	0.023	86.600	0.963
Inoculum	95.599	138.576	98.314	0.937	96.876	0.033
Acetic acid	84.691	114.442	84.694	1.000	84.674	0.001
Glucose	86.613	89.627	89.346	0.093	86.614	0.906

The inoculum VS ratio was 0.032 g VS/mL. The amounts of substrate and inoculum are determined by the S:I ratio and the calculations are as follows:

$$Substrate\ (g\ VS) = Substrate\ (g) * VS\ \left(\frac{g}{g}\right)$$

$$Inoculum\ (g\ VS) = \frac{Substrate\ (g\ VS)}{S{:}I}$$

$$mL\ of\ Inoculum = \frac{Inoculum\ (g\ VS)}{Inoculum\ ratio\ \frac{g\ VS}{mL}}$$

The S:I ratio, selected according to the amount of sample available for use, was 0.073. The amount of substrate was set as 0.140 g VS (0.145 g total) and the volume of inoculum was ~60 mL.

6.4. FTIR Spectra of samples after TOD.

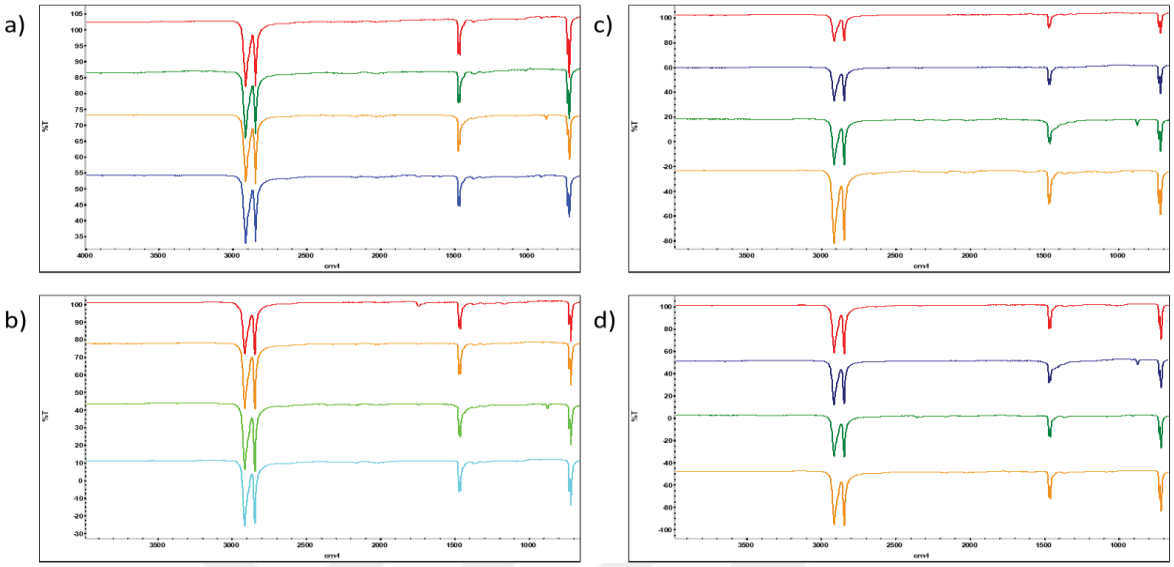

Figure 6.1. Spectra of samples LDPE at K2S2O8 concentration of 0.5 (a, b) and 1 g/L (c, d). a) from above to below: LD11, LD21, LD31, LD41. b) from above to below: LD11rep, LD21rep , LD31rep, LD41rep. c) from above to below: LD12, LD22, LD32, LD42. d) from above to below: LD12rep, LD22rep, LD32rep, LD42rep.

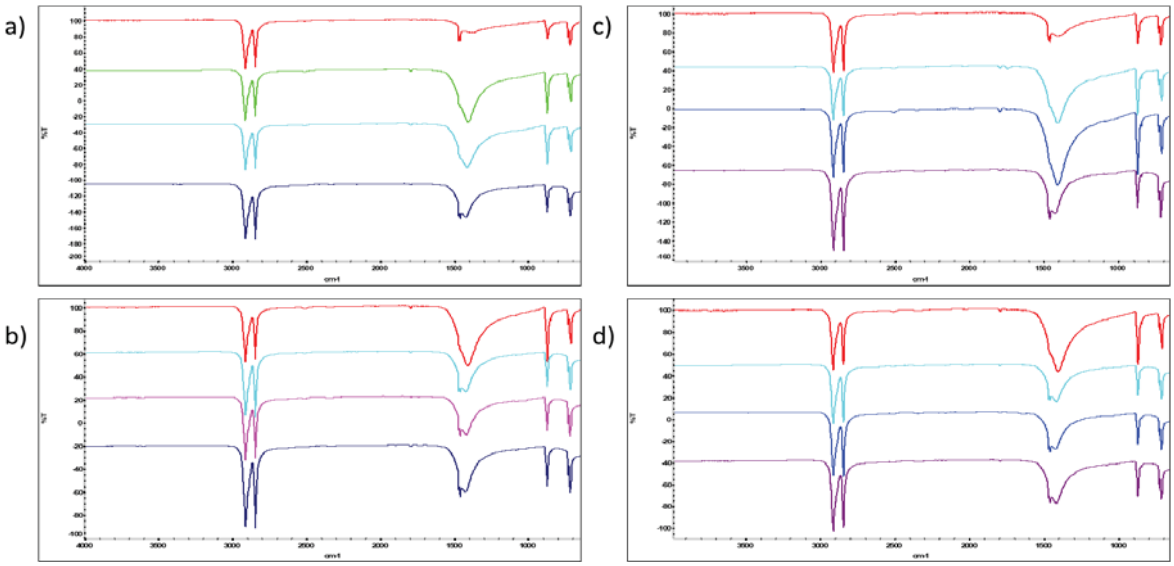

Figure 6.2. Spectra of samples HDPE at K2S2O8 concentration of 0.5 (a, b) and 1 g/L (c, d). a) from above to below: HD11, HD21, HD31, HD41. b) from above to below: HD11rep, HD21rep, HD31rep, HD41rep. c) from above to below: HD12, HD22, HD32, HD42. d) from above to below: HD12rep, HD22rep, HD32rep, HD42rep .

To read the values of the peaks, the spectra of samples LD12/LD12rep and HD12/HD12rep are given as follows:

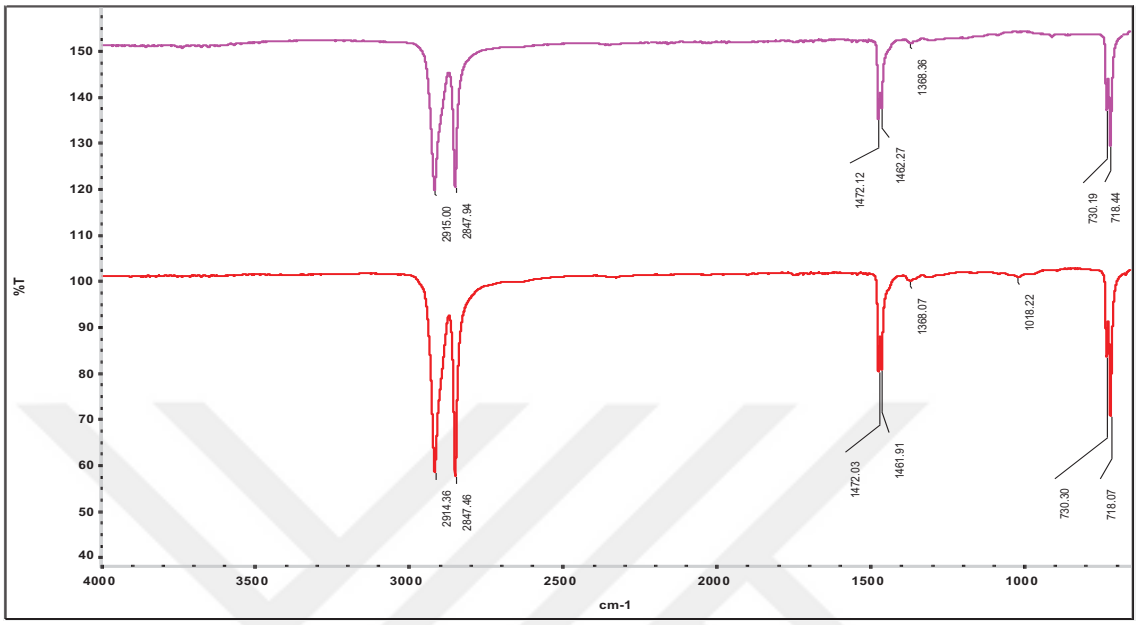

Figure 6.3. Spectra of samples LD12 (above) and LD12rep (below) with peak values.

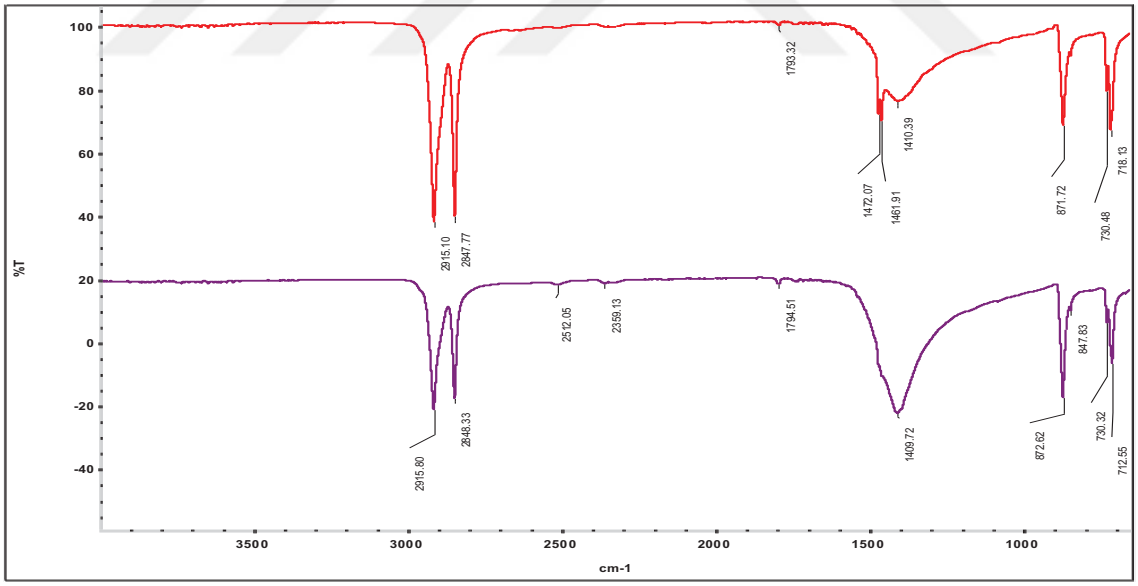

Figure 6.4. Spectra of samples HD12 (above) and HD12rep (below) with peak values.

6.5. FTIR spectra of samples after MAOx.

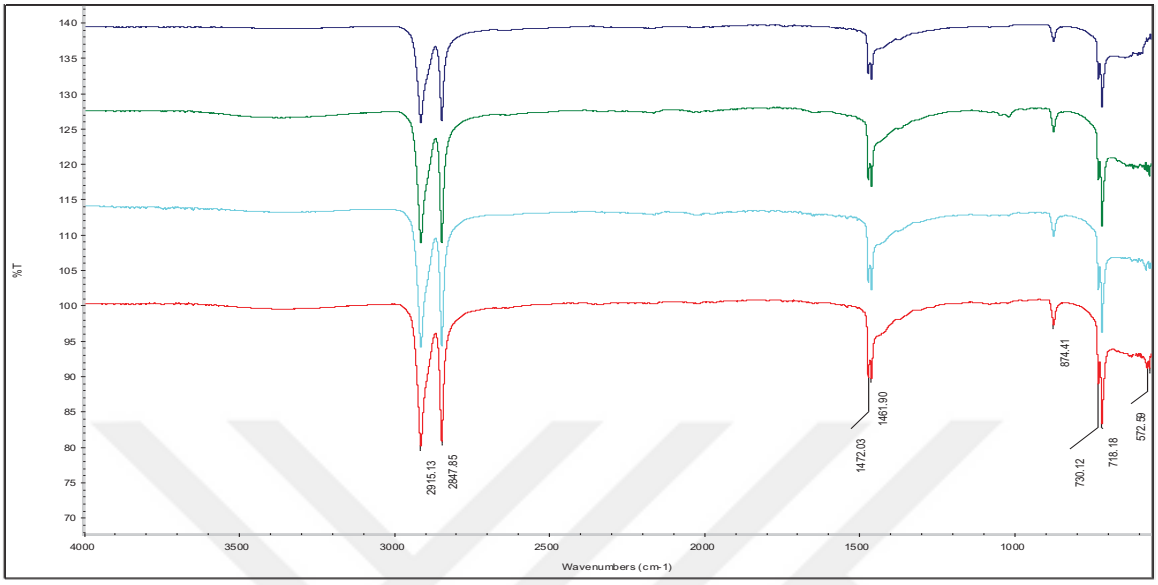

Figure 6.5. Spectra of HDPE samples with peak values. From above to below: HD1, HD1.5, HD2, HD2.5.

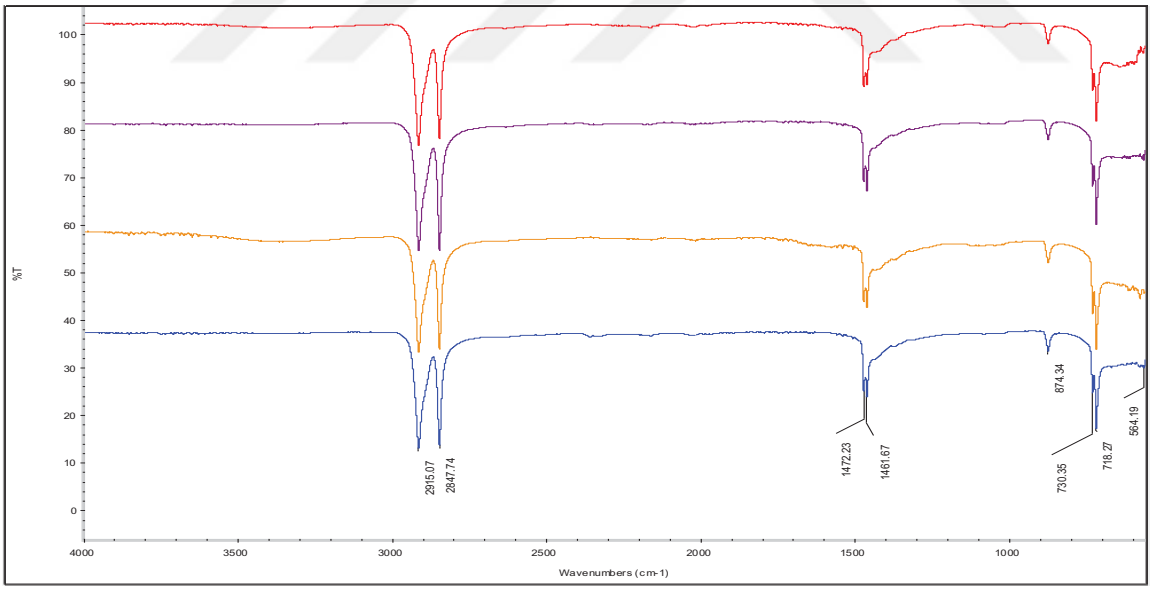

Figure 6.6. Spectra of HDPE replicate samples with peak values. From above to below: HD1rep, HD1.5rep, HD2rep, HD2.5rep.

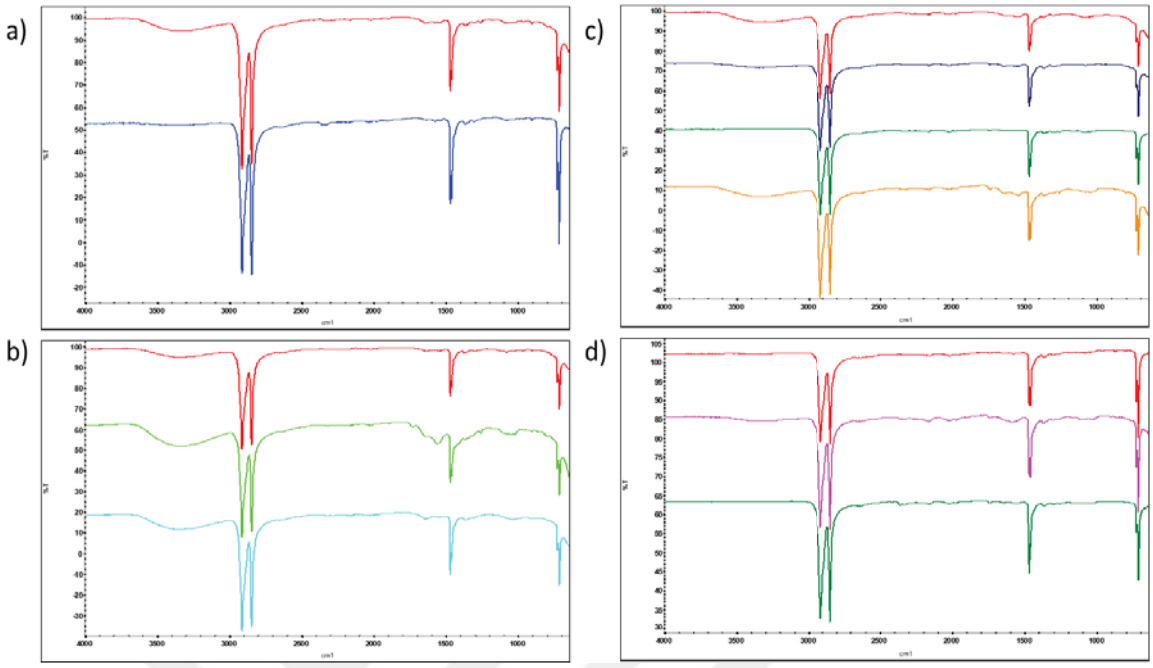

Figure 6.7. Spectra of LDPE samples. From above to below: a) LD1, LD1rep; b) LD1.5, LD1.5rep1, LD1.5rep2; c) LD2, LD2rep1, LD2rep2, D2rep3; d) LD2.5, LD2.5rep1, LD2.5rep2.

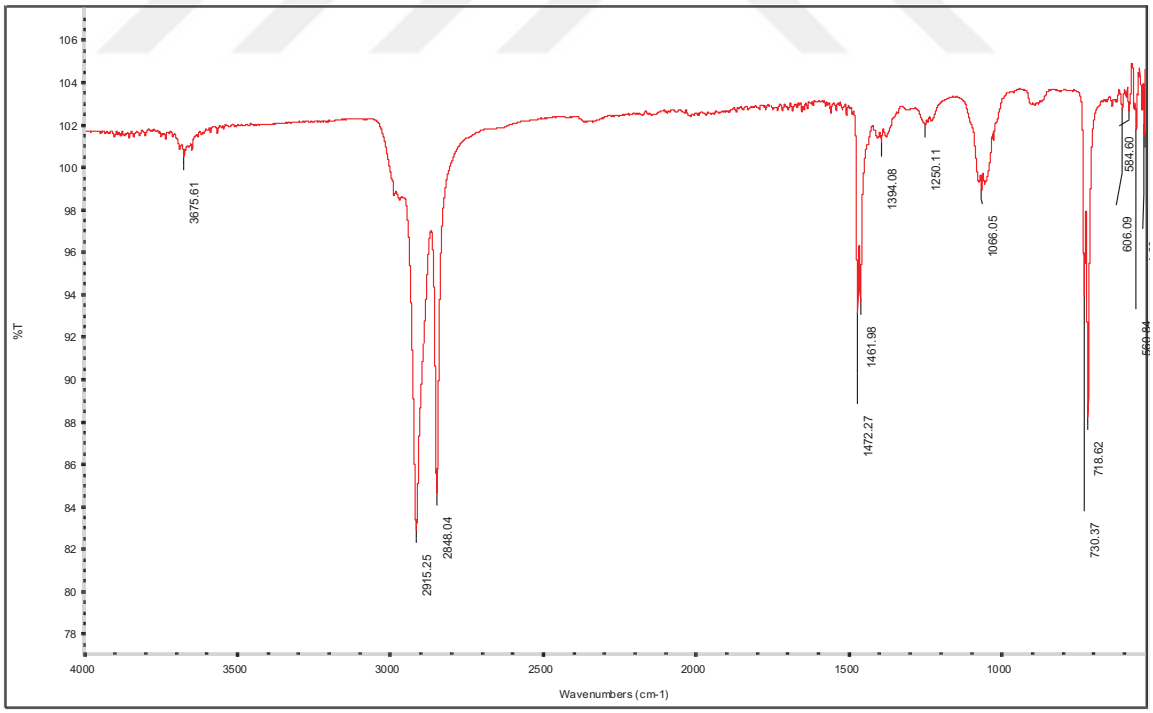

Figure 6.8. Spectrum of sample LD1 run3 with peak values.

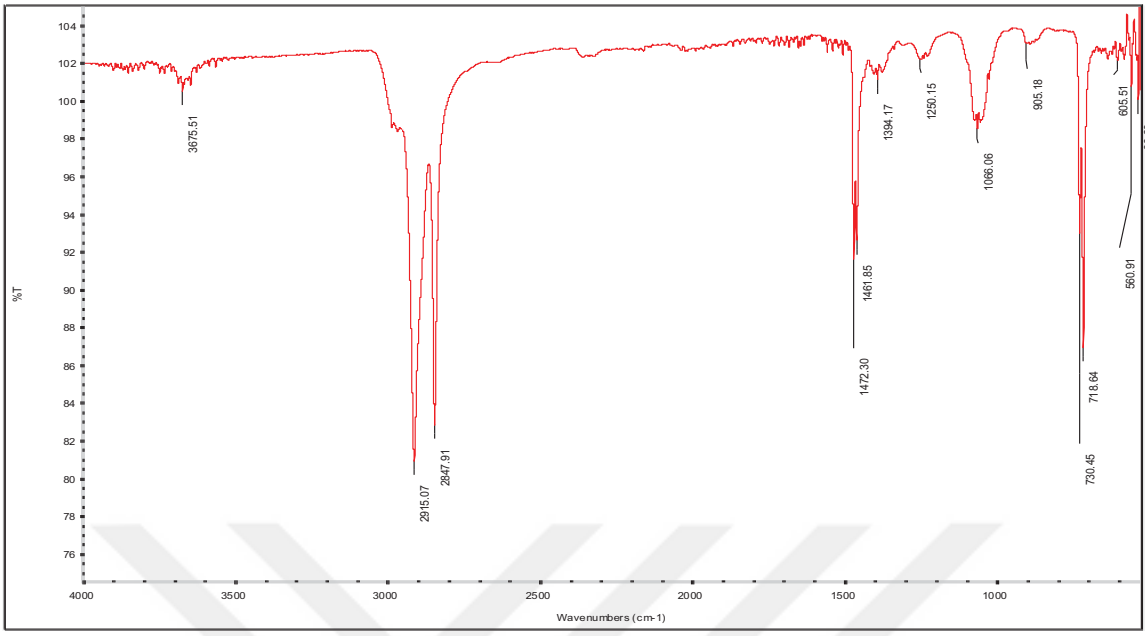

Figure 6.9. Spectrum of sample LD2 run3 with peak values.

Samples LD1 run5 and run6 showed a very small sign around 2360 cm^{-1} corresponding to CO_2; although very slight, it is a sign of a degree of oxidation and were considered for AD.

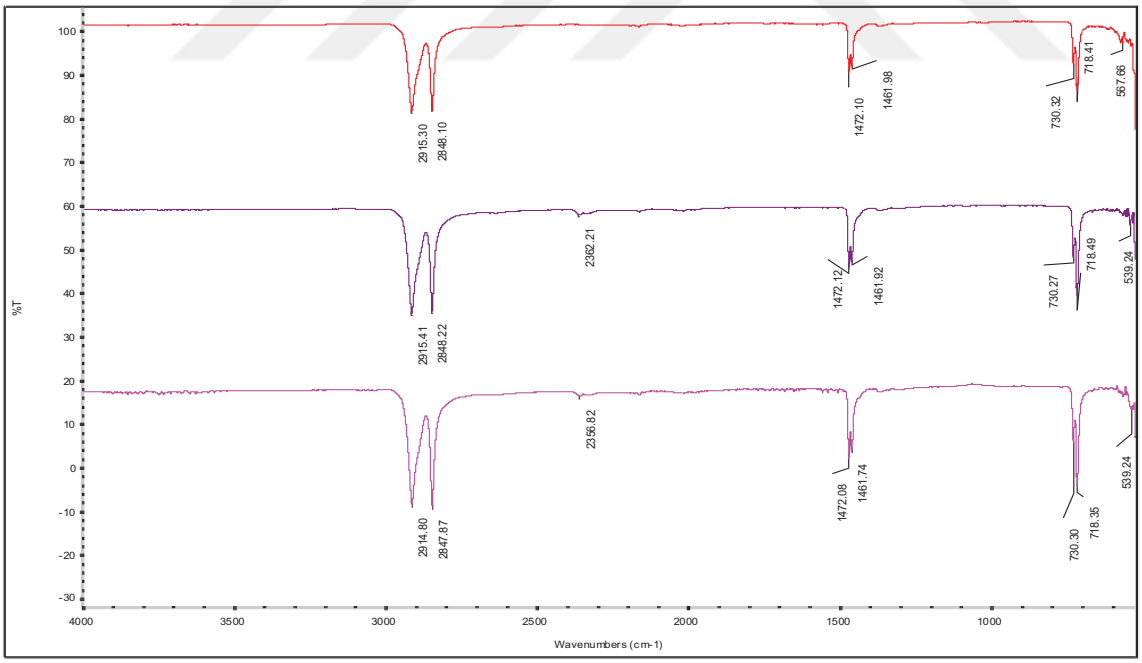

Figure 6.10. Spectra of extra runs of LD1. From above to below: LD1 run4, LD1 run5, LD1 run6.

6.6. FTIR spectra of samples after POxUV.

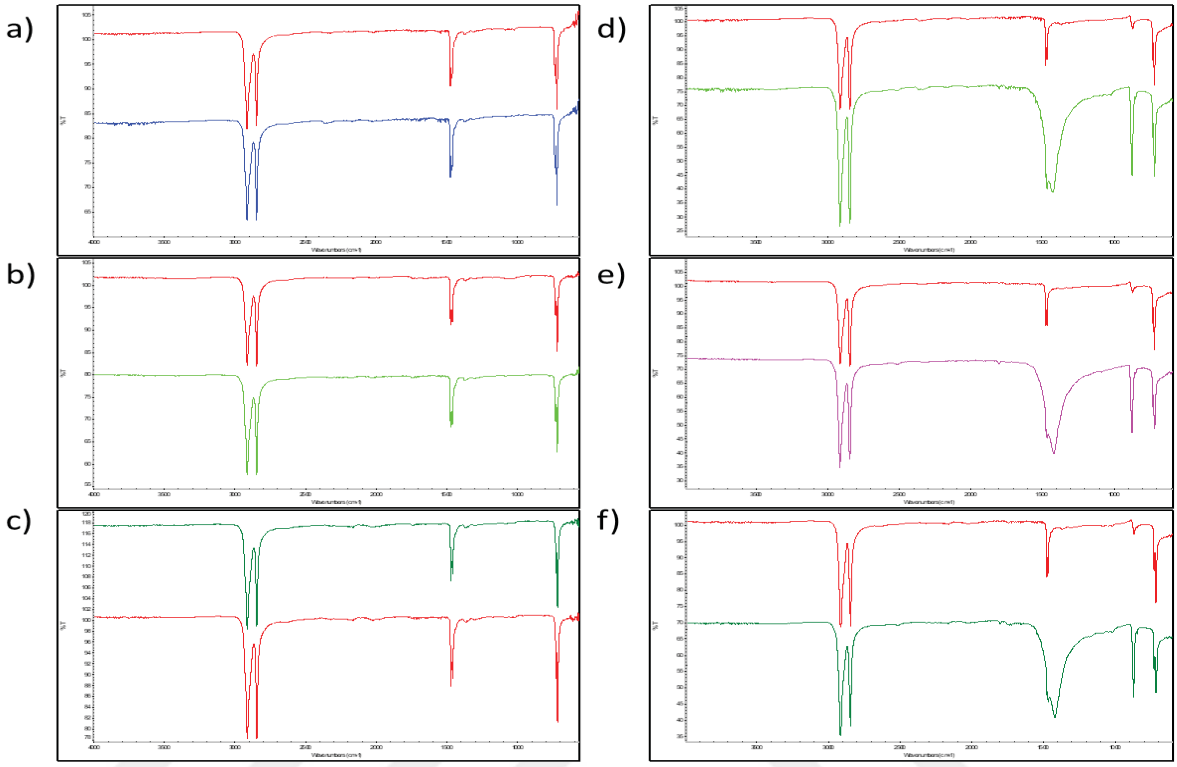

Figure 6.11. Spectra of LDPE and HDPE samples and replicates. From above to below: a) LD70 and LD70rep; b) LD80 and LD80rep; c) LD90 and LD90rep; d) HD70 and HD70rep; e) HD80 and HD80rep; f) HD90 and HD90rep.

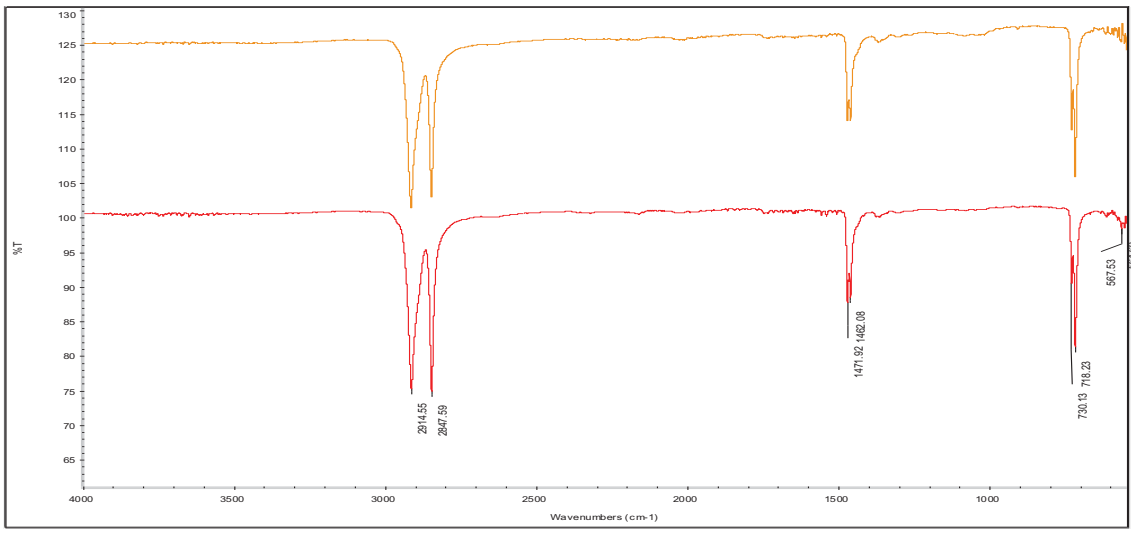

Figure 6.12. Spectra of LD60 (above) and LD60rep (below) with peak values.

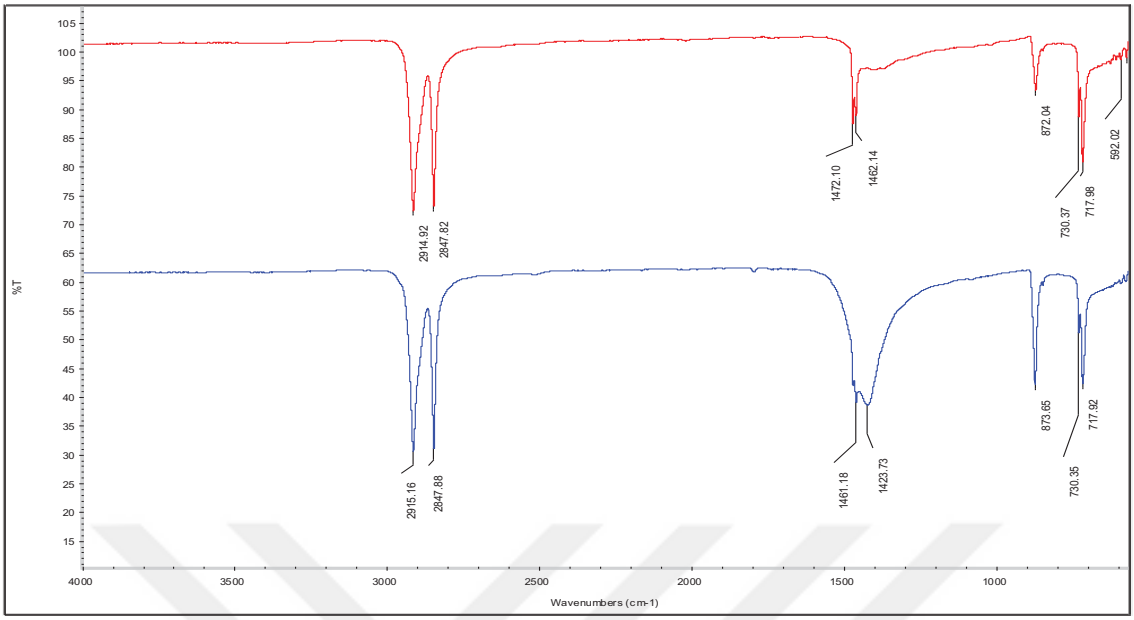

Figure 6.13. Spectra of HD60 (above) and HD60rep (below) with peak values.

6.7. FTIR spectra of samples after AD.

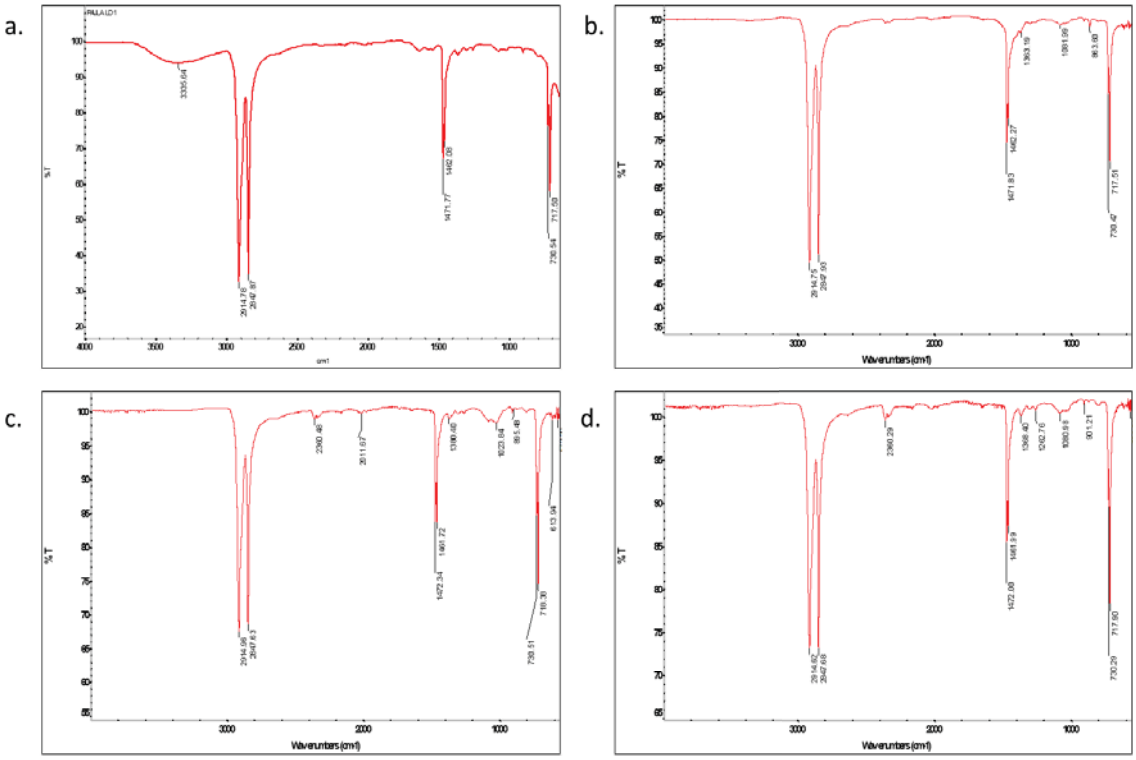

Figure 6.14. FTIR spectra of sample LD1 before AD (a), and after AD of r1(b), r2(c), r3(d)

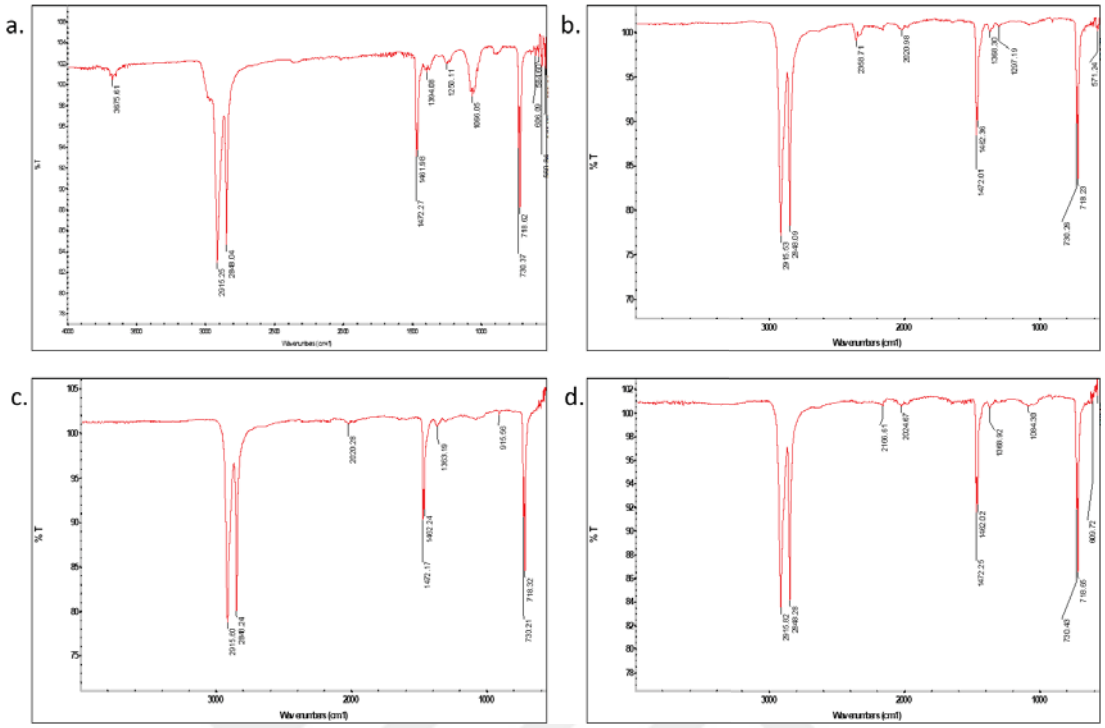

Figure 6.15. FTIR spectra of sample LD1 run3 before AD (a), and after AD of r1(b), r2(c), r3(d)

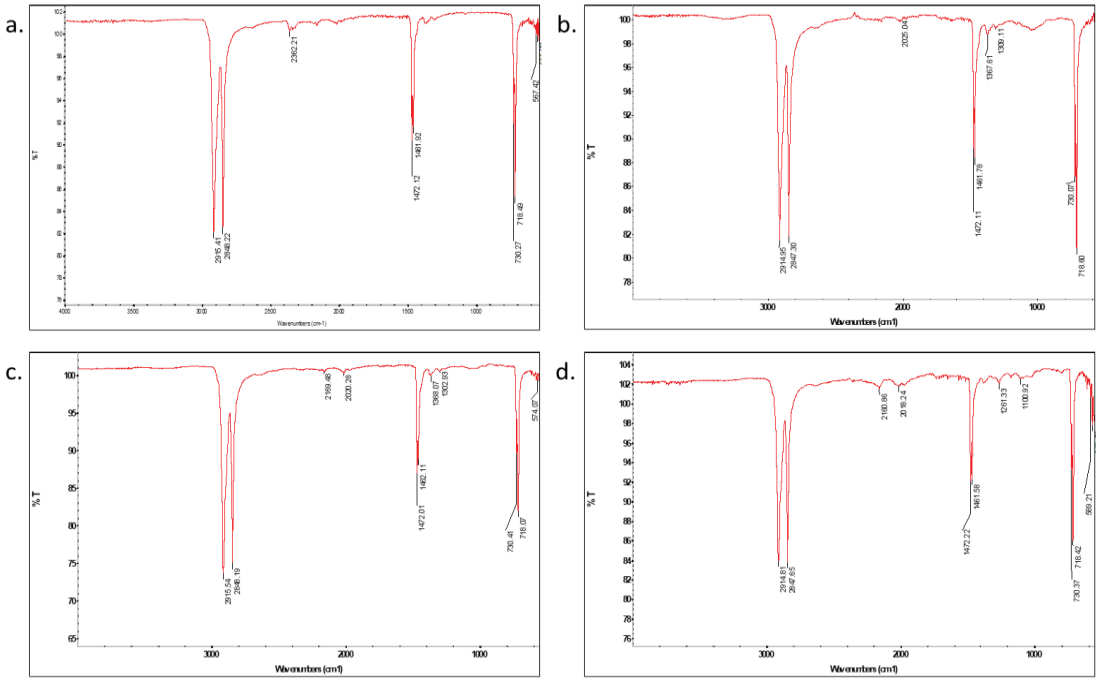

Figure 6.16. FTIR spectra of sample LD1 run5 before AD (a), and after AD of r1(b), r2(c), r3(d)

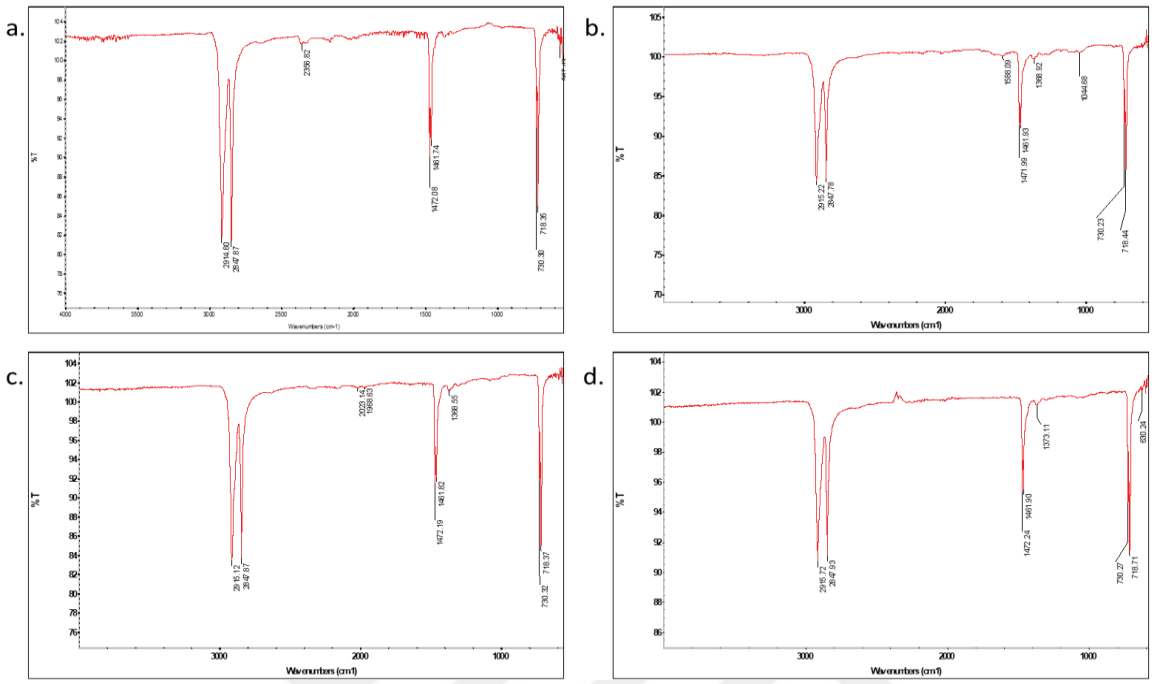

Figure 6.17. FTIR spectra of sample LD1 run6 before AD (a), and after AD of r1(b), r2(c), r3(d)

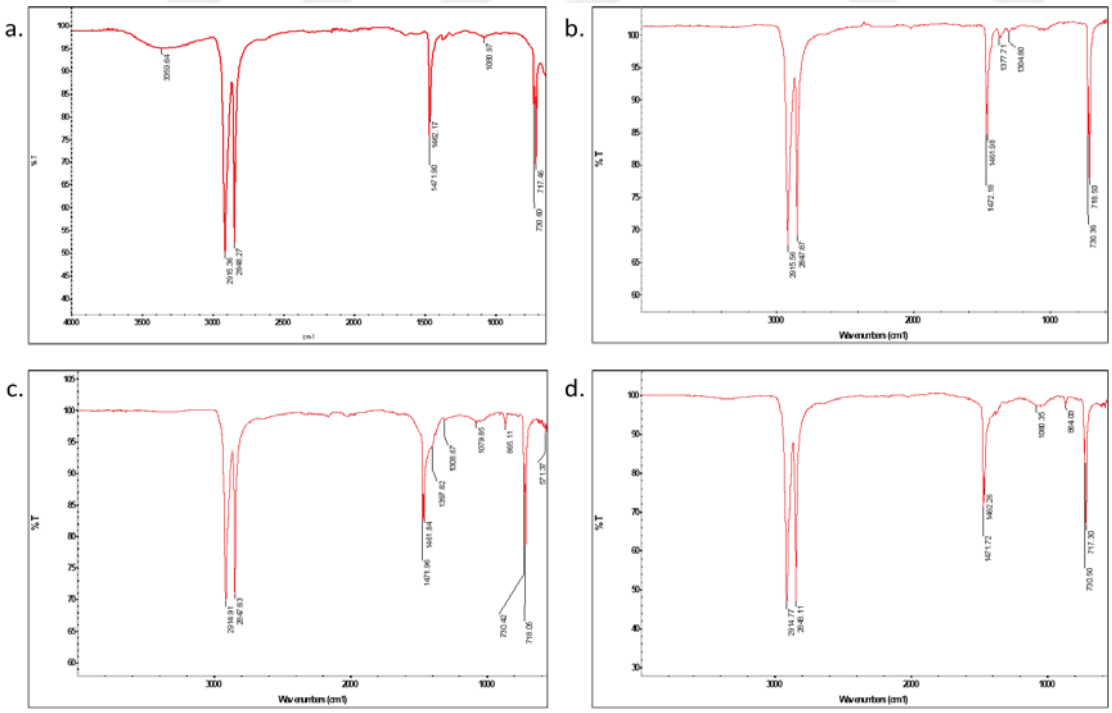

Figure 6.18. FTIR spectra of sample LD1.5 before AD (a), and after AD of r1(b), r2(c), r3(d)

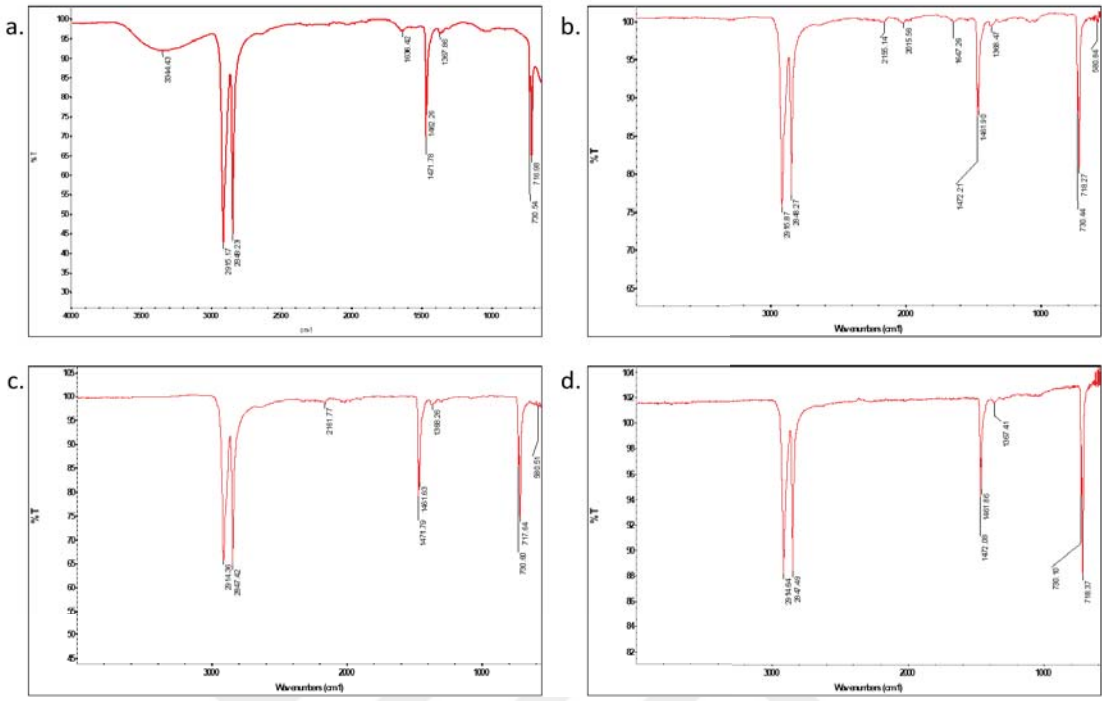

Figure 6.19. FTIR spectra of sample LD1.5 run2 before AD (a), and after AD of r1(b), r2(c), r3(d)

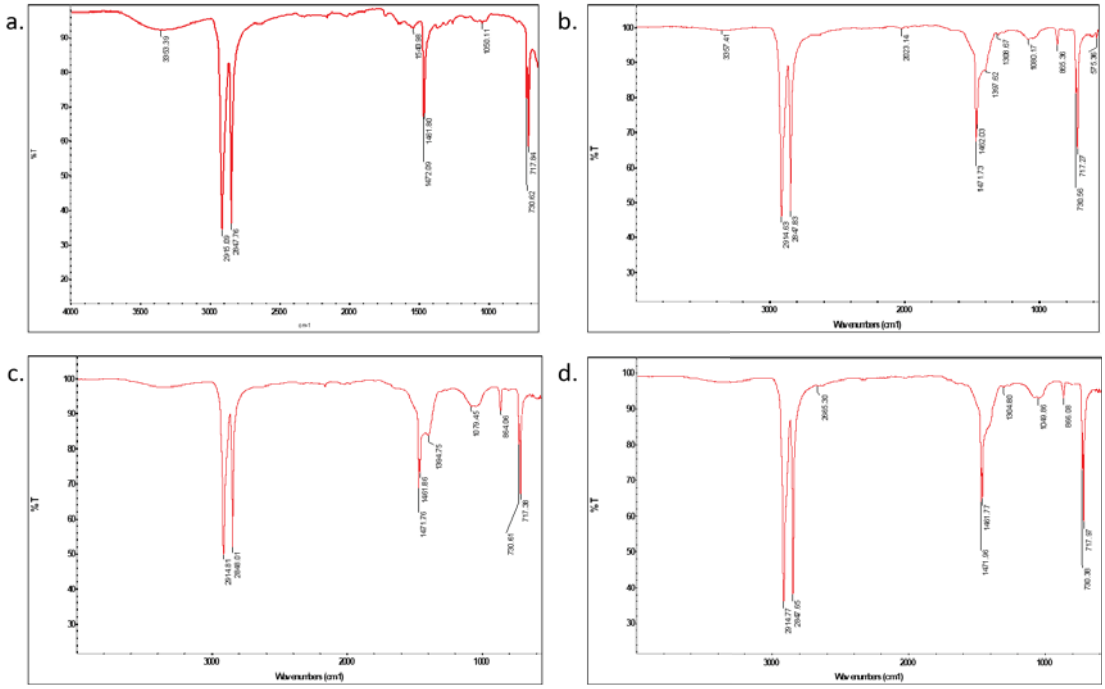

Figure 6.20. FTIR spectra of sample LD2 run2 before AD (a), and after AD of r1(b), r2(c), r3(d)

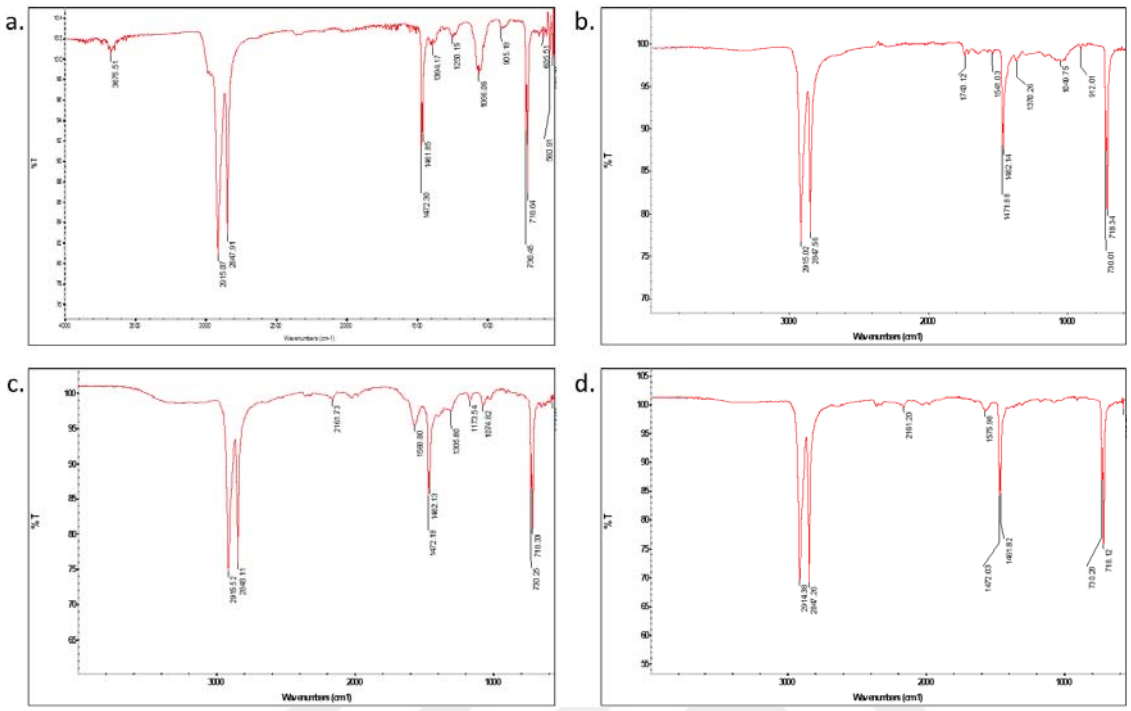

Figure 6.21. FTIR spectra of sample LD2 run3 before AD (a), and after AD of r1(b), r2(c), r3(d)

6.8. Daily and cumulative biogas production of PE samples

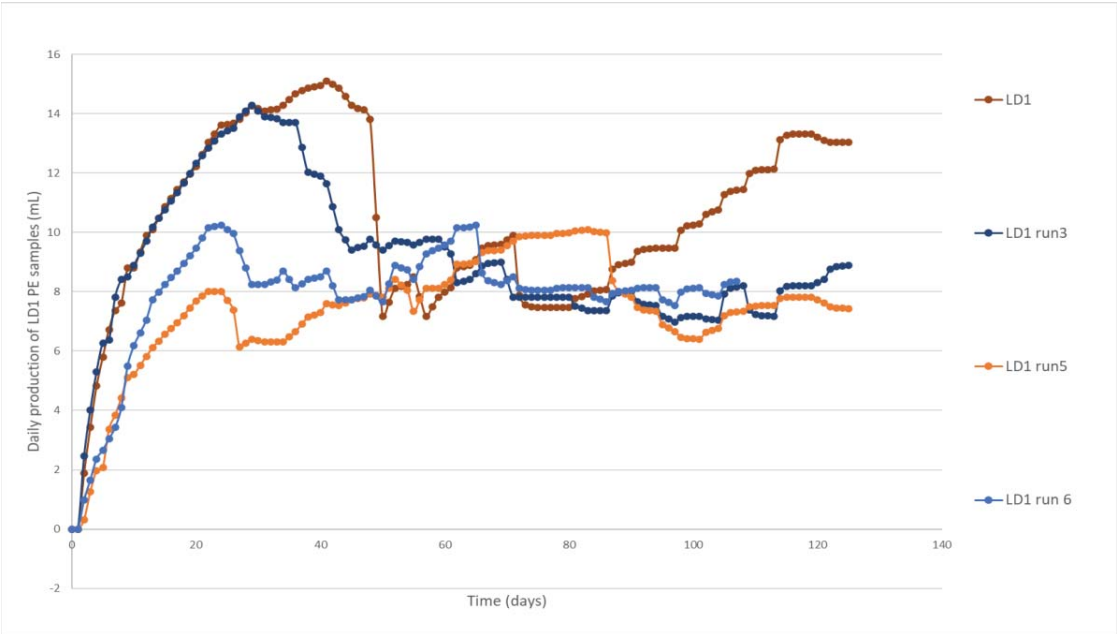

Figure 6.22. Daily biogas production of LD1 samples.

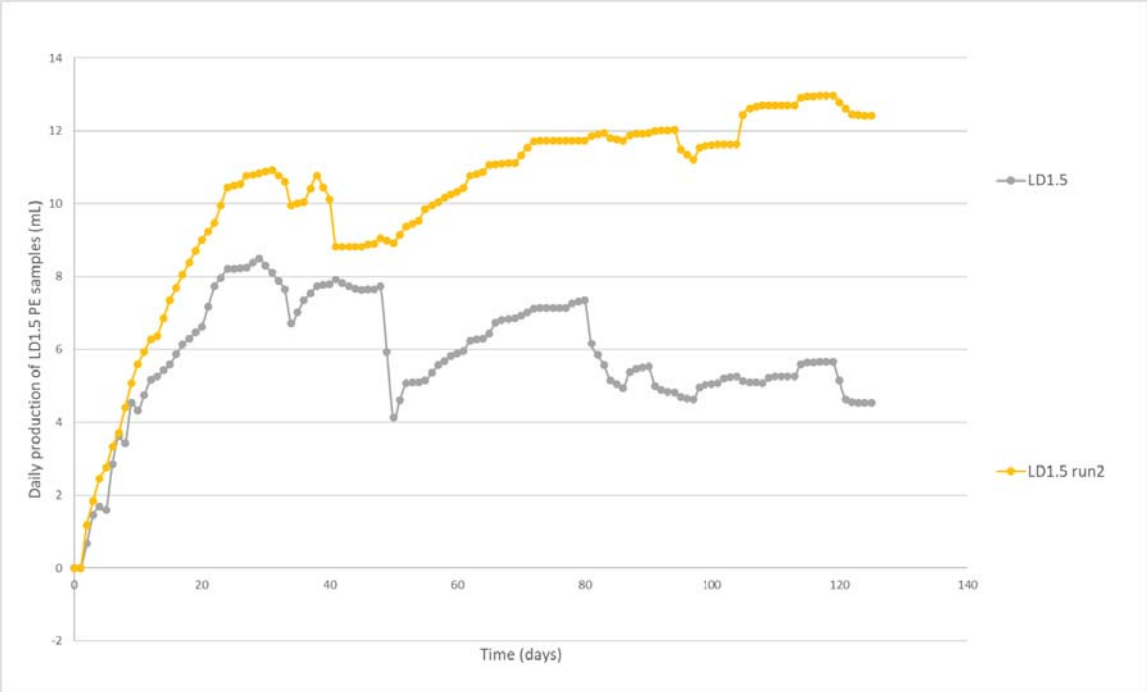

Figure 6.23. Daily biogas production of LD1.5 samples.

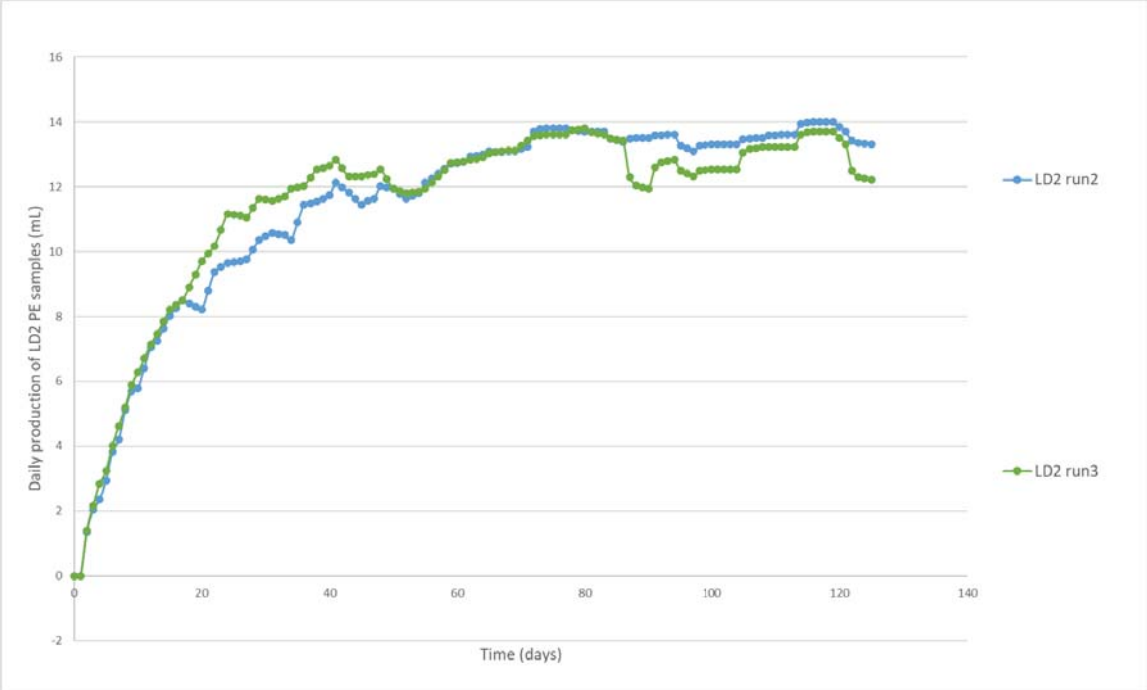

Figure 6.24. Daily biogas production of LD2 samples.

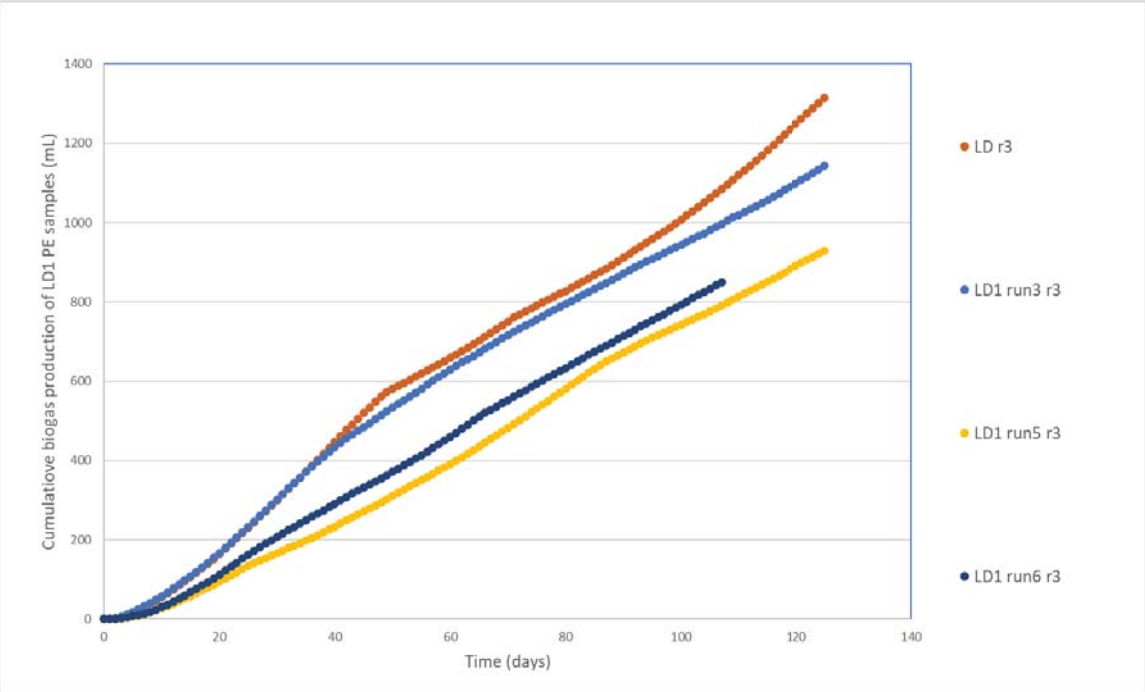

Figure 6.25. Cumulative biogas production of LD1 samples.

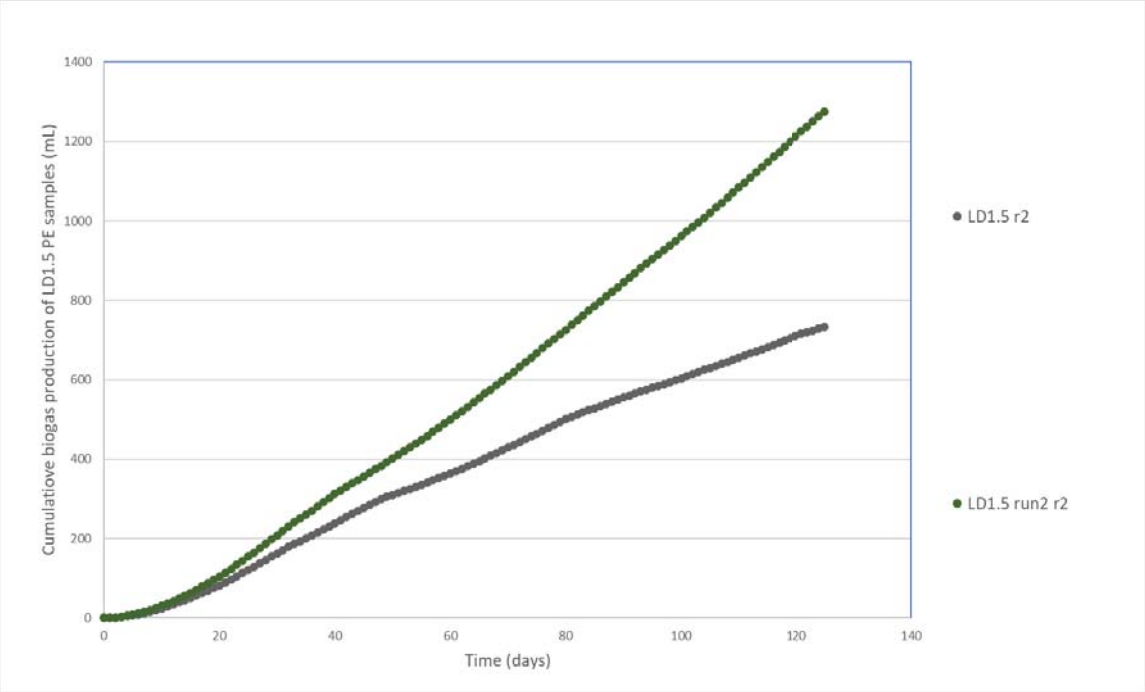

Figure 6.26. Cumulative biogas production of LD1.5 samples.

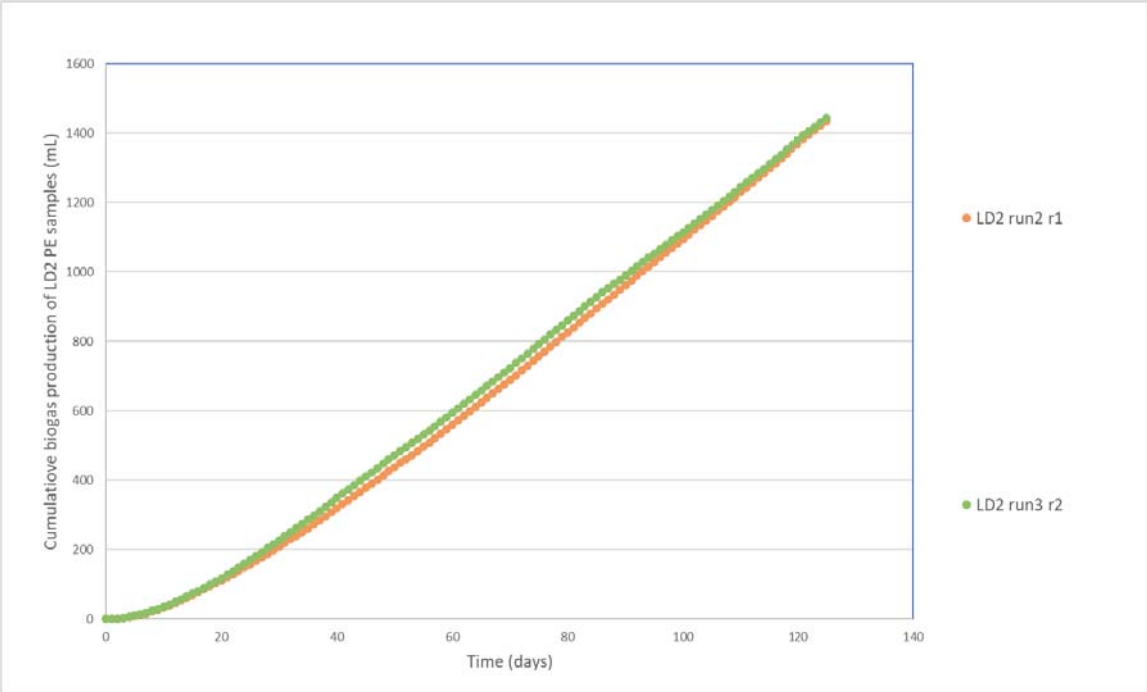

Figure 6.27. Cumulative biogas production of LD2 samples.

6.9. Weight change of samples after AD

It is important to recall that the initial weight of PE of samples was set as 0.145 g.

Table 6.7. Weight change of samples after AD.

Samples	W final PE (g)	W change
LD1 r1	0.144	-1%
LD1 r2	0.144	-1%
LD1 r3	0.145	0%
LD1 run 3 r1	0.147	1%
LD1 run 3 r2	0.147	1%
LD1 run 3 r3	0.146	1%
LD1 run5 r1	0.135	-7%
LD1 run 5 r2	0.149	3%
LD1 run 5 r3	0.154	6%
LD1 run 6 r1	0.155	7%
LD1 run 6 r2	0.151	4%
LD1 run 6 r3	0.147	1%
LD1.5 r1	0.145	0%
LD1.5 r2	0.15	3%
LD1.5 r3	0.148	2%

LD1.5 run 2 r1	0.147	1%
LD1.5 run 2 r3	0.142	-2%
LD2 run 2 r1	0.145	0%
LD2 run 2 r2	0.148	2%
LD2 run 2 r3	0.139	-4%
LD2 run 3 r1	0.145	0%
LD2 run 3 r2	0.149	3%
LD2 run 3 r3	0.152	5%