ANAEROBIC DIGESTION OF LDPE/LLDPE BLEND FILM AND PET SHEET WITH PRO-DEGRADING ADDITIVES AT 35 AND 50°C

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ABSTRACT

ANAEROBIC DIGESTION OF LDPE/LLDPE BLEND FILM AND PET SHEET WITH PRO-DEGRADING ADDITIVES AT 35 AND 50°C

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Low density polyethylene/linear low density polyethylene blend film and polyethylene terephthalate sheet incorporated with pro-degrading additives from Symphony Environmental Ltd., Wells Plastics Ltd., and EcoLogic LLC were evaluated in an anaerobic digestion environment for 16 months together with negative (blank) and positive controls (cellulose) in general accordance with ASTM D5526-12. Total biogas production of cellulose was significantly higher than that of the remaining samples. Total biogas production of samples containing plastics and the negative control were not significantly different from each other. Pro-degrading additives tested in the study did not increase the biodegradation of these plastic materials.

To my family

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

C	=	carbon
C/N	=	carbon-nitrogen ratio
CH_4	=	methane
CO_2	=	carbon dioxide
d	=	days
E	=	Ecologic
g	=	gram
H_2O	=	water
LDPE	=	low density polyethylene
LLDPE	=	linear low density polyethylene
mL	=	milliliters
mm	=	millimeters
NaOH	=	sodium hydroxide
O_2	=	oxygen
PET	=	polyethylene terephthalate
S	=	Symphony
UV	=	ultraviolet light/radiation
W	=	Wells
wt%	=	Weight percent

CHAPTER 1: INTRODUCTION

1.1 Background

1.1.1 Functions of packaging

The three functions of packaging are protection, utility, and communication [1]. For food and beverage packaging, protection is probably the most important because of its direct effects on products' shelf-lives. Packaging helps prevent spoilage due to environmental, chemical, and physical hazards associated with the production, transportation, and distribution of food and beverages. In 2010, food and beverage packaging accounted for 69% of the global market for consumer packaging. Categorizing by materials, plastic topped all other packaging materials with 37% of the global consumer packaging market by value [2]. Because of its protective function, plastic packaging is generally inert to biological and chemical changes, and continues to exist in the environment hundreds to thousands of years past its useful life [3]. This creates severe problems for waste management around the world.

1.1.2 Low density polyethylene/linear low density polyethylene and polyethylene terephthalate

Two common packaging plastics for food and beverages are low density polyethylene/linear low density polyethylene (LDPE/LLDPE) and polyethylene terephthalate (PET). LDPE/LLDPE is widely used in plastics bags, film for bakery goods, shrink films, overwrap, pallet stretch wrap, and milk/juice cartons [4]. PET food applications include containers for carbonated beverages, water, and juice [5]. Since their introduction in the twentieth century, packaging plastics' production, consumption, and waste generation has

increased significantly [6]. Since 1980, the amount of PET from bottles and jars generated in the U.S. municipal solid waste (MSW) has increased tenfold from 260 to 2,670 thousand tons. In 2010, LDPE/LLDPE from containers and packaging generated in the U.S. MSW accounted to 3,480 thousand tons, with 12.1% recovery. In the same year, PET from containers and packaging generated in MSW totaled 3,380 thousand tons, only 23.1% of which was recovered [7].

1.1.3 Legislation and public opinions in the United States and around the world on plastic waste

In 2012, Barnosky et al. reported in *Nature* that the Earth's ecological system is "approaching a planetary-scale critical transition as a result of human influence". Similar to localized ecosystem shifts that are suddenly and irreversible, the Earth's state shift will have detrimental effects on our lives [8]. These concerns about the impacts of people on the Earth have focused more attention on the issue of disposal of plastics. Legislation has been introduced around the world to deal with the plastic waste problem, although the approach has not been systematic. The best known legislation is the ban of the plastic bag, the most ubiquitous of all packaging. In Bangladesh, the plastic bag ban started around the capital city of Dhaka in 2002 and quickly spread nationwide. Shoppers were encouraged to use alternatives such as jute, paper, and reusable cloth bags [9]. In 2002, Ireland began to tax plastic shopping bags at a rate of €0.15 initially and increased to €0.22 per bag. Bag use was down 90% shortly after the ban, with strong support from the public and the retail industry [10]. In 2007, San Francisco became the first city in the US to ban plastic checkout bags in large supermarkets and retail pharmacies. On September 2012, the ordinance was upheld by the San Francisco Superior Court banning "non-compostable plastic checkout bags [in] all retail stores and food establishments, and imposing a

10-cent charge on other bags provided to consumers" [11]. Since 2010, shoppers in Washington, D.C. buying food or alcohol must pay a $0.05 bag fee for each plastic bag used [12]. In Australia, a bag ban took effect in the state of South Australia in May 2009, the Northern Territory in September 2011, and the Australian Capital Territory in November 2011. Since the ban in each state or territory, retailers are only allowed to provide compostable or biodegradable bags that meet Australia's standard to customers [13–15]. In 2012, The United Arab Emirates (UAE) banned all disposable plastic bags with the exception of those made from oxo-biodegradable plastic, in compliance with UAE Standard 5009:2009 [16].

1.1.4 Common biodegradable plastics and pro-degrading additives in the market

To cope with changes in legislation and consumer perception, two prominent trends that have emerged in plastics manufacturing are producing biodegradable plastics from biomass sources (biodegradable bioplastics) and adding degradation-promoting additives to petroleum-based plastics. With the advance of technology, bioplastics' properties and processability are improving but still somewhat inferior to those of traditional petroleum-based plastics. Some examples of commercial biodegradable packaging materials based on raw materials from crops are Mater-Bi, NatureWorks Polylactide, Bioska, Bioplast, Solanyl, Potatopac, Greenfil and Eco-Foam [17]. Because of the drawbacks in processability of biodegradable bioplastics, degradation-promoting additives are being marketed as the better option [18]. Many degradation-promoting additives are oxo-biodegradable additives, most often stearates incorporated with transition metal ions such as $Fe^{3+,}$ Mn^{2+}, or Co^{2+} [19]. Some examples of degradation-promoting additives on the market include Totally Degradable Plastic Additives [20], VIBATAN 04089 [21], d2w [22], Eco-One [23], Reverte [24], and EcoPure [25].

1.1.5 Skepticism of biodegradable technology

In the UAE, Wells Plastics Reverte and Symphony's d2w were certified to be in compliance with UAE Standard 5009:2009 [26,27]. With the ban of plastic bags in the UAE in 2012, only bags made from plastics incorporated with Wells Plastics' and Symphony's or other approved suppliers' additives are allowed to circulate in the country. Around the world, retailers such as U.S.'s Yoke's, United Kingdom's Co-operative Food, and Vietnam's Saigon Co-op supermarket chains also picked up oxo-biodegradable plastic bags [28–30].

However, there have been a variety of criticisms about the oxo-biodegradable technology. In a report to the United Kingdom's Department for Environment, Food and Rural Affairs, researchers from Loughborough University (Leicestershire, UK) highlighted consumers' confusion about oxo-biodegradable claims, the inability of oxo-biodegradable plastics to be composted, and their effects on recycling and composting facilities [31]. As the result of this finding, the UK's Co-operative Food supermarket chain decided to stop using oxo-biodegradable plastic bags [32]. Some plastic trade associations also expressed concerns and doubts about the oxo-biodegradable technology. The Flexible Packaging Association and the Society of the Plastics Industry Bioplastics Council published positions on degradation-promoting additives that asked manufacturers to include scientific data from recognized third parties to corroborate claims such as "biodegrades in landfills" or "oxo-biodegradable". Claims must be tested according to accepted industry standards such as ASTM D6400, ASTM D6868, ASTM D7081 or EN 13432 [33,34]. In addition, degradation-promoting additives do not have the support of recyclers because of the belief that common plastics incorporated with biodegradable additives can contaminate recyclers' processing operations. In its 2010 strategy paper, the European Plastics Recyclers Association calls bioplastics and oxo-biodegradables unsustainable. They also

asked for collection of these materials to be in a separate stream because of the fear that bioplastics and oxo-biodegradable plastics will have damaging effects on mechanical recycling [35]. In addition, the Association of Postconsumer Plastic Recyclers shared the same concerns about the largely unknown effects of oxo-biodegradable plastics on recycled materials [36].

In October 2012, the Federal Trade Commission (FTC) amended its Guides for the Use of Environmental Marketing Claims. The guide was originally published in 1992, amended in 1996 and 1998 subsequently. The guide advises manufacturers and marketers to possess data to qualify their environmental marketing claims. Without these data, manufacturers can be found by the FTC to deceive consumers which can result in orders prohibiting their deceptive marketing as well as fines [37,38]. FTC has been regularly taking actions against companies for deceptive environmental claims. Examples of companies that have received fines from the FTC include Amazon.com Inc., Leon Max Inc., Sears, Roebuck and Co., Kmart Corporation, Tender, and Dyna-E [39,40].

1.2 Motivation

There is great interest among environmentally responsible companies in using degradation-promoting additives for plastic packaging. LDPE/LLDPE film was chosen because Bimbo Bakeries USA, one of the project's sponsors, was interested in the additives' application in bread bags. Bimbo Bakeries is the largest bakery company in the US whose brands include Arnold, Bimbo, Boboli, Sara Lee, Thomas', Oroweat, and many others [41]. In addition to LDPE/LLDPE film, PET sheet was chosen as PET is widely used for carbonated soft drinks, water, ketchup, and many other beverages and food. This is of particular interest of member companies of the Center for Packaging Innovation and Sustainability (CPIS), Michigan State

University (East Lansing, MI USA). CPIS, the main sponsor of this project, “is a global leader in research and outreach related to packaging innovation and sustainable systems, resulting in positive environmental effects on the global footprint of packaging and related systems across the supply chain” [42]. CPIS’s members are The Coca-Cola Company, ConAgra Foods, The Dow Chemical Company, Abbott Laboratories, World Wildlife Fund, H. J. Heinz Company, and AkzoNobel [43].

In 2010, with 12.1% and 23.1% recovery for LDPE/LLDPE and PET from containers and packaging in U.S. MSW, a significant amount was discarded. Most of these plastics were discarded to landfills or combusted [7]. Therefore, it is the project’s interest to study the biodegradation of LDPE/LLDPE and PET with pro-degrading additives in landfill conditions.

1.3 Goal and objectives

The goal of this study was to investigate the performance of degradation-promoting additive systems from Symphony Environmental Ltd., Wells Plastics Ltd., and EcoLogic LLC in an anaerobic digestion environment for 16 months. The objective was to study the biodegradation of LDPE/LLDPE and PET in an anaerobic digestion environment conditions by measuring total biogas production.

CHAPTER 2: LITERATURE REVIEW

2.1 LDPE, LLDPE, their production and general properties

LDPE was discovered by the Imperial Chemical Industries in 1933. It is produced by the free-radical-initiated polymerization process from ethylene monomers. Ethylene monomers are mainly manufactured by manufactured from natural gas or high temperature cracking of crude oil. As a result of the free-radical polymerization process, LDPE has a large amount of long-chain branching. The molecular weight, molecular weight distribution, frequency of short-chain branches, and frequency and length of the long-chain branches of LDPE affect its physical and extrusion properties [44].

LLDPE was introduced for commercial use in the late 1970s by Union Carbide and Dow Chemical. LLDPE is produced by the copolymerization of ethylene and α-olefins. As a result, LLDPE has a narrower molecular weight distribution than LDPE and does not contain long-chain branching. Because of its nature as a copolymer, LLDPE's properties are strongly dependent on comonomer content. The four most common comonomers are 1-hexene (40%), 1-butene (35%), 1-octene (25%), and 4-methyl-1-pentene (only a small fraction) [45]. The difference in structure of LDPE, LLDPE, and single-site-catalyzed LLDPE is illustrated in figure 2-1. A comparison of blown film properties between LDPE and LLDPE is shown in table 2-1.

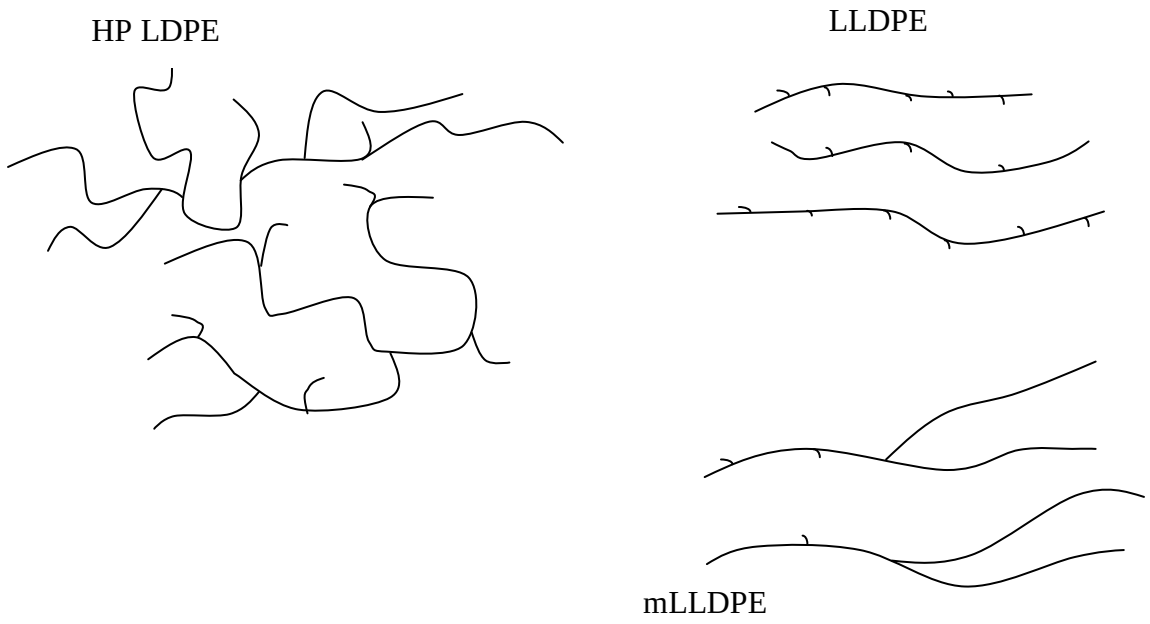


Figure 2-1. Differences in structures of LDPE, LLDPE, and single-site-catalyzed LLDPE, adapted from [45].

Table 2-1. A comparison of blown film properties between LDPE and LLDPE, adapted from [44].

Property	ASTM test method	HP-LDPE	HP-LDPE	LLDPE	LLDPE	LLDPE
Melt index, g/10 min	D1238	2.5	0.2	1.0	1.0	1.0
Density, g/cm^3	D1505	0.921	0.923	0.918	0.918	0.918
Comonomer		None	None	Butene	Hexene	Octene
Dart drop, N/mm	D1709	29	71	39	77	97
Puncture energy, kJ/m		27	22	71	76	-
Elmendorf tear, N/mm (=dyn/cm)	D1922					
MD		62	35	54	131	143
XD		43	39	131	226	309
Tensile strength, MPa	D882					
MD		20	19	35	36	45
XD		19	21	26	32	35
Haze, %	D1003	6	25	17	20	12
Gloss, 45°	D2457	70	30	53	50	60

2.2 PET, its production and general properties

High molecular weight PET was first successfully synthesized in England in 1942 by J. Rex Whinfield and W. Dickson. However, commercialization of PET did not commence until after World War II ended. At first, PET was manufactured to be used exclusively as synthetic fibers. PET was not widely used as a molding resin in cold molds, with temperatures less than 130 °C, due to its low crystallization rate. However, during the late 1960s, specific nucleating agents, whose development was spearheaded by Akzo and DuPont, removed this technical disadvantage. PET is made by the reaction of ethylene glycol and terephthalic acid or dimethyl terephthalate. Terephthalic acid is produced by air-oxidizing p-xylene in acetic acid under moderate pressure with the help of catalysts. Since the 1967-1972 period, direct esterification using pure terephthalic acid has been favored over the dimethyl terephthalate method due to the improved polymerization and purification processes of terephthalic acid. However, in recent years, due to an increase in recycling of PET, the dimethyl terephthalate method has had a resurgence. Dimethyl terephthalate can be made from the methanolysis and glycolysis of waste PET [46].

The degree of crystallinity of PET dictates its thermochemical properties. The usual melting temperature is from 260-265 °C but can reach 280 °C for highly annealed samples. PET is semipermeable to oxygen and carbon dioxide. The stretch blow molding process used to create PET bottles involves radial and axial drawing, which causes strain-induced crystallization. This crystallization improves the mechanical strength and reduces the permeability of the bottles [46].

2.3 The concept of polymer biodegradation

According to ASTM, a "biodegradable plastic is a degradable plastic in which the degradation results from the action of naturally-occurring micro-organisms such as bacteria, fungi, and algae" [47]. Biodegradation of polymers is typically a surface erosion process. The long chains and water-insolubility of polymers make them unsuitable for being transported directly into the microorganisms to be digested. The process starts with the secretion of extracellular enzymes by the microorganisms. The products of this stage are then transported into the microorganisms to be digested. End products include water, carbon dioxide, methane, and new biomass [48]. The materials must not have negative impacts on either the disposal processes or the environment [49].

2.4 Factors affecting biodegradation of polymers

Biodegradation is affected by the exposure conditions and the characteristics of the polymer. Figure 2-2 illustrates the relationship. Exposure conditions can be classified as abiotic and biotic. Abiotic factors include but are not limited to temperature, pH, moisture, and UV exposure. Microbial activity tends to increase at higher temperature and moisture content. However, extremely high temperature can slow down and stop the microbial activity. pH level can affect hydrolysis of polymers. In addition, pH values below or above the range that microorganisms can tolerate can slow down or stop the microbial activity. Another factor, UV exposure, affects biodegradation by causing main chain scissions (hastens up biodegradation) and introducing crosslinking (slows down biodegradation) [50]. Biotic factors include but are not limited to extracellular enzymes, hydrophobicity, and biosurfactants. Extracellular enzymes are used by the microorganisms to depolymerize the polymer outside the cell wall [48,50].

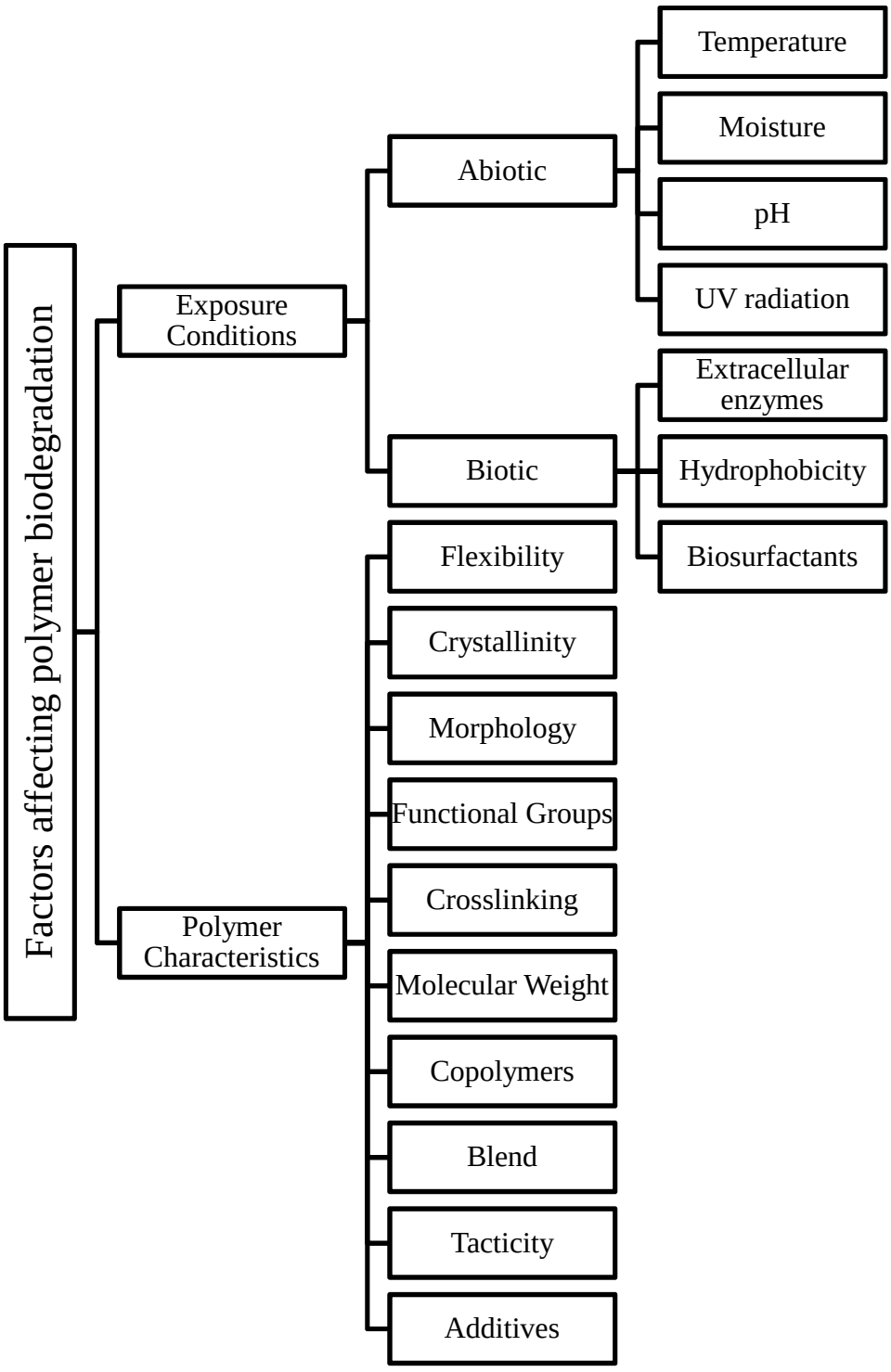


Figure 2-2. Factors affecting polymer biodegradation, adapted from [50].

Polymer biodegradation is also dictated by the characteristics of the polymer itself. These characteristics include but are not limited to flexibility, crystallinity, morphology, functional groups, crosslinking, molecular weight, copolymers, blends, tacticity, and additives. A rise in conformational flexibility of a polymer increases the accessibility of microorganisms and water to the polymer. On the other hand, crystallinity can affect the biodegradation greatly by affecting the accessibility of water. The more crystalline the polymer is, the more difficult it is for water to diffuse through the polymer. For this same reason, adding a copolymer causes molecular irregularity, which in turn decreases the crystallinity and increases biodegradation. However, it should be noted that addition of copolymer can increase the rigidity of the polymer and reduce its biodegradability. Hydrolysable functional groups act as sites for hydrolysis for many polymers. Crosslinking reduces the accessibility of microorganisms and water to the polymer chains. Since only low molecular weight polymer molecules can be transported into the cell wall for digestion, high molecular weight polymers take longer to biodegrade [50].

2.5 History of biodegradable polymers and common approaches in making polymers biodegradable

Because polyethylene is hydrophobic, usually incorporated with antioxidants and stabilizers during processing, and has high molecular weight but no functional groups, it is not considered a biodegradable polymer [51]. Many attempts have been made since the 1970s to achieve biodegradable polyethylene. One of the approaches is to use polyethylene-starch blends. Starch, is a relatively cheap commodity which primarily comes from cereal crops. However, even though starch comes from renewable sources, there are concerns about the sustainability of using starch for plastics manufacturing and the conflict with food production. Starch-based

blends are more expensive and there is currently not adequate infrastructure for recycling and composting polyethylene-starch blends [52]. A different approach is to incorporate oxo-biodegradable additives into polyethylene. In addition to initiating the free-radical oxidation of polyethylene, these additives can modify the surface of polymer to be hydrophilic [53]. Common oxo-biodegradable additives contain transition metal stearates. Transition metals commonly used are manganese (Mn^{2+}/Mn^{3+}), iron (Fe^{2+}/Fe^{3+}), and cobalt (Co^{2+}/Co^{3}) [54].

2.6 Oxo-biodegradation mechanism

Figure 2-3 illustrate the free-radical oxidation process of polyethylene. The cycle starts from the top of the diagram with the creation of free radical $P^{\cdot}$ from PH due to shear stress or catalyst residues. The free radical $P^{\cdot}$ reacts with oxygen to form $POO^{\cdot}$. $POO^{\cdot}$ then reacts with a polymer molecule to form a new radical $P^{\cdot}$ and POOH. The pro-degrading additive catalyzes the conversion of POOH to $PO^{\cdot}$ and $^{\cdot}OH$. $PO^{\cdot}$ will be converted to biodegradable functional fragments Fs(O)x. $^{\cdot}OH$ will react with a new polymer molecule PH to form POH that will eventually be converted by further oxidation and fragmentation. In summary, in one cycle, the radical will react with two polymer molecules PHs to form new radicals. These new radicals will travel in the same process all over again.

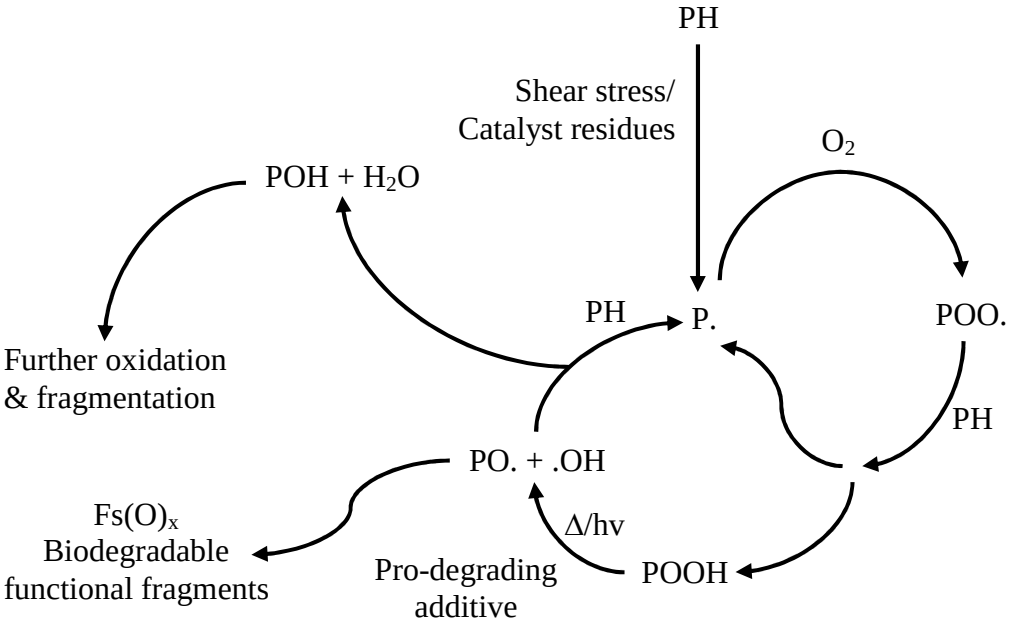


Figure 2-3. Mechanism of free-radical oxidation of polyethylene, adapted from [55].

2.7 Pro-degrading additives used in the project and their mechanisms

d2w additive from Symphony is claimed to work according to an oxo-biodegradation mechanism [22]. On the other hand, Reverte from Wells plastics uses a hybrid mechanism. In the first stage, the company claims that the additive catalyzes the oxo-biodegradation of the polymer. In the second stage, the microbial growth is said to be promoted by the additive [24]. The mechanism for Eco-One additives from Ecologic is not explained in detail. The company asserts that the additives promote the formation of biofilm on the surface of the plastics, and expand the molecular structure of the plastic so that microorganisms can penetrate and digest the plastics [23].

2.8 Publicly known/evidence about biodegradation with these or other additive systems

Corti et al. reported that combining abiotic treatment such as prolonged thermal and sunlight exposure, and oxo-biodegradable additive promotes the biodegradation of LLDPE films containing oxo-biodegrdable additives inoculated with fungal strains known for their ability to use oxidized LDPE as the only carbon source. Films with oxo-biodegradable additives also have higher carbonyl indices and produce more CO_2 in fungal biodegradation tests [51]. Billingham et al. reported that LDPE incorporated with EPI's TDPA oxo-biodegradable additive degraded rapidly in thermal aging. In experiments monitored by FTIR spectroscopy, tensile testing, and size exclusion chromatography, films with oxo-biodegradable additives lose strength and polymer chain length quickly, and produce oxidation products [56]. Chiellini et al. studied the effects of temperature and relative humidity on oxidation and cleavage of the macromolecules by measuring weight variation (using an analytical balance), film wettability (by measuring contact angle on glass slides), carbonyl index (using FTIR), molecular weight (using size exclusion chromatography), and extractability with polar solvents of oxidized thermal-aged samples using EPI's TDPA additive. They concluded that the TDPA oxo-biodegradable additive was effective in initiating the oxidative degradation of the polymer [57]. Vogt and Kleppe showed that after exposure to light, polyethylene and polypropylene with 2% Renatura pro-oxidant additive continued to degrade under dark thermal conditions. The thermal oxidative degradation increases with the increase in light exposure [58].

2.9 Anaerobic digestion: mechanism, inhibitors, and other influencing factors

Anaerobic digestion is a complicated biological process. As illustrated in figure 2-4, there are multiple reactions running in series and parallel to each other. There are four main stages.

The first stage is the hydrolysis of complex organic materials such as proteins, carbohydrates, and lipids into amino acids, carbohydrates, fatty acids, and alcohols. The second stage is the fermentation of amino acids and carbohydrates. The fermentation of amino acids produces short-chain fatty acids, succinate, aminovalerate, and H_2. The fermentation of soluble carbohydrates results in ethanol, acetate, H_2, and CO_2.The third stage is the anaerobic oxidation of long-chain fatty acids and alcohols. The end products are acetate and propionate. The fourth stage is the anaerobic oxidation of short-chain fatty acids such as propionate and butyrate to acetate and H_2.The last stage is methanogenesis. The end products are CH_4 and CO_2 [59].

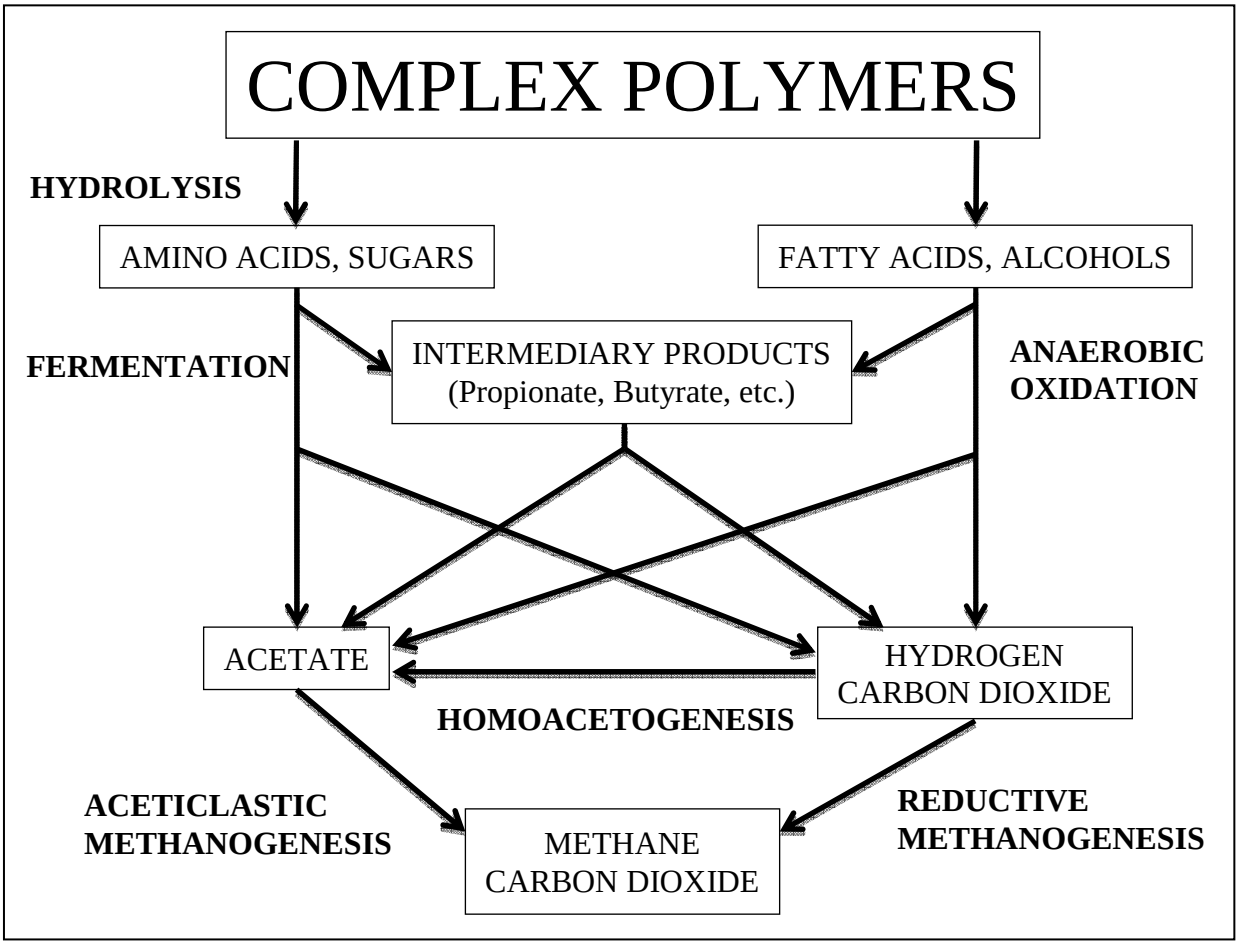


Figure 2-4. Anaerobic digestion process, adapted from [59].

There are many factors that can inhibit an anaerobic digestion process. Ammonia exists in large quantity in animal waste due to decomposition of organic nitrogen. The mechanisms for ammonia inhibition include intracellular pH changes, increase in maintenance energy requirements, and specific enzyme reaction inhibition. Ammonia inhibition affects methanogens the most. Light metal ions such as Na, K, Mg, Ca, and Al also inhibit the anaerobic digestion process. A high concentration of salt causes the cells of the bacteria to lose water due to osmotic pressure. Light metal ions come from the breakdown of biomass or are added to adjust pH. At low or moderate levels, these micronutrients can speed up bacterial growth. However, at high levels, light metal ions can inhibit or even halt the anaerobic digestion process. Organic chemicals can also inhibit an anaerobic digestion process. Agricultural waste contains high amounts of lignocellulosic content in stalks, straws, and bark. Methanogens are highly vulnerable to lignin and lignin derivatives [60]. Temperature can also affect the kinetics of the anaerobic digestion process. Higher temperature (below or equal to the optimum temperature) leads to higher microbial activity. However, temperature beyond the optimum temperature decreases microbial activity [59].

2.10 Testing standards

Testing standards for biodegradation of plastic materials under anaerobic conditions include:

- ASTM D5526-12: Standard Test Method to Determine Anaerobic Biodegradation of Plastic Materials under Accelerated Landfill Conditions.
- ASTM D5511-12: Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials under High-Solids Anaerobic-Digestion Conditions.

- ASTM D5210-92(2007): Standard Test Method for Determining the Anaerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge.
- ASTM D7475-11: Standard Test Method for Determining the Aerobic Degradation and Anaerobic Biodegradation of Plastic Materials under Accelerated Bioreactor Landfill Conditions.

Table 2-2. Overview of testing standards for biodegradation of plastic materials under anaerobic conditions.

Test standards	Purpose	Data obtained
ASTM D5526-12	Anaerobic Biodegradation of Plastic Materials Under Accelerated Landfill Conditions	Test duration, % biodegradation as a function of time, % CH_4 and % CO_2 in evolved gas [61].
ASTM D5511-12	Anaerobic Biodegradation of Plastic Materials Under High-Solids Anaerobic-Digestion Conditions	Test duration, % biodegradation as a function of time, % CH_4 and % CO_2 in evolved gas [62].
ASTM D5210-92(2007)	Anaerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge	Test duration, % of gas evolution as a function of time, molecular weight of plastic before and after the exposure, weight loss of the specimen, inoculum's soluble solid organic carbon content [63].

Table 2-2. (cont'd)

ASTM D7475-11	Aerobic Degradation and Anaerobic Biodegradation of Plastic Materials under Accelerated Bioreactor Landfill Conditions	Temperature range of the test as a function of time, test duration, % biodegradation as a function of time, % CH_4 and % CO_2 in headspace, changes in molecular weight, weight, tensile, and other properties of the samples [64].

2.11 ASTM D5526-12

The ASTM D5526-12 test method simulates biologically active landfills where moisture and temperature are controlled, and gas recovery is promoted. There are seven steps in the method. The first step is to choose and evaluate the test material. The second step is to obtain a pretreated municipal-solid-waste medium and an anaerobic inoculum. The third step is to place the material in an anaerobic static batch fermentation. It is noted that the medium should contain more than 30% solids. The fourth step is to quantify the total carbon (in CO_2 and CH_4 evolved) as a function of time. The fifth step is to clean and test the exposed material. The sixth step is to calculate the degree of biodegradability. The last step is to assess the degree of biodegradability when the conditions are less than optimum [61].

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

This chapter describes the production of materials, and methodology employed to study the biodegradation of LDPE/LLDPE film and PET sheet in an anaerobic digestion environment. The major challenge of this research was to provide enough nutrients for the anaerobic microorganisms to thrive while keeping the closed system's pH and environmental parameters at optimal growth conditions.

3.2 Plastics manufacturing and properties

The polymeric film and sheet used in this study were extruded at the School of Packaging, Michigan State University (East Lansing, MI). The resins for the LDPE/LLDPE film, DOWLEX 2045G (LLDPE) and DOW 501I (LDPE), were donated by the Dow Chemical Company (Midland, MI). LDPE and LLDPE resins were blended in a 70/30 ratio by weight. LDPE/LLDPE blend was then mixed with 1 and 5 wt% of degradation promoting masterbatch additives d2w (Symphony Environmental Ltd., Borehamwood, Hertfordshire, UK), Reverte (Wells Plastics Ltd., Stone, Staffordshire, UK), and Eco-one EL10 (EcoLogic LLC, Oakbrook Terrace, IL). The film was extruded on a Killion KLB 100 blown film extruder (Davis-Standard LLC, Pawcatuck, CT) with a screw diameter of 25.4 mm (2 inch), screw length/ diameter ratio of 24:1, and a 2 in diameter circular die. The temperature profile of the extruder was 215-215-212-212-210-204 ºC (420-420-415-415-411-410-400 ºF) for barrel zones 1, 2, 3, clamp ring, adapter, die 1, and die 2, respectively. A screw speed of 14 rpm and take up speed of 10 feet per minute were used. The diameter of the film was controlled at 10 cm at a blow up ratio of 2. The overall

thickness of the LDPE/LLDPE control film was 0.9 ± 0.2 mil. Table 3-1 shows the overall thickness of the produced film.

PET resin, provided by EcoLogic LLC (Oakbrook Terrace, IL), was mixed with 1 and 5 wt% of degradation promoting masterbatch additives Reverte (Wells Plastics Ltd., Stone, Staffordshire, UK) and Eco-one EC 80 (EcoLogic LLC, Oakbrook Terrace, IL). The resin was placed in a vacuum oven at 110 °C for 24 hours for drying. After drying, the resin was stored under vacuum and was cooled down to room temperature. Resin was removed from storage just before extrusion to prevent any regain of moisture. PET sheet was manufactured by cast film extrusion using a Microextruder model RCP-0625 (Randcastle Extrusion Systems, Inc., Cedar Grove, NJ). The microextruder has a 1.5875 cm (0.625 inch) diameter 24/1 L/D ratio extruder with 34 cc volume. The extrusion system was equipped with a 20 cm (8 in) wide coat hanger die, Eurotherm temperature control system for the extruder (Eurotherm, Ashburn, Virginia), a chill roll with Sterling M50-3-2-2 cooling system (Sterling, New Berlin, WI) and a Bronco II take up roll from Seco AC/DC drives (Warner Electric, Braintree, MA). The temperature profile of the extruder was 218-226-257-254-254 ºC (425-500-495-490-490 ºF) for feed zone, barrel zones 2, 3, transfer tube, and die, respectively. A screw and take up speed of 60 rpm were used. The chill roll temperature was controlled at 71 ºC (160 ºF) and was set at a speed of 15 rpm. The chill roll was placed very close to the die exit so that the film was quenched rapidly in order to prevent crystallization of the film, resulting in a highly amorphous film. Table 3-1 shows the overall thickness of the produced sheet.

Table 3-1. Average thickness of the LDPE/LLDPE film and PET sheet produced.

	Percent loading of additive, %	Ecologic, mil	Wells Plastics, mil	Symphony, mil
LDPE/LLDPE	1	1.0 ± 0.3	1.0 ± 0.2	1.3 ± 0.3
	5	1.1 ± 0.3	1.1 ± 0.1	1.0 ± 0.2
PET	1	11.2 ± 1.1	9.4 ± 0.5	N/A
	5	12.4 ± 0.8	9.0 ± 0.3	N/A

Note: LDPE/LLDPE 0 wt% thickness was 0.9 ± 0.2 mil, and PET 0 wt% thickness was 9.2 ± 0.6 mil.

The total amount of carbon, nitrogen and hydrogen content for each sample was determined by a CHN analyzer from Perkin Elmer (Waltham, Massachusetts). Values are provided in Table 3-2.

Table 3-2. Carbon, nitrogen, and hydrogen content for samples.

Sample Name	C, wt%	H, wt%	N, wt%
LDPE Control	84.8 ± 1.4	14.5 ± 0.5	0.1 ± 0.1
LDPE Ecologic 1 wt%	85.2 ± 0.5	14.4 ± 1.0	0.1 ± 0.0
LDPE Ecologic 5 wt%	84.6 ± 0.4	14.7 ± 0.2	0.1 ± 0.0
LDPE Wells 1 wt%	85.6 ± 0.5	14.7 ± 0.6	0.1 ± 0.0
LDPE Wells 5 wt%	85.5 ± 0.4	15.1 ± 0.2	0.1 ± 0.1
LDPE Symphony 1 wt%	85.5 ± 0.4	15.1 ± 0.2	0.1 ± 0.0
LDPE Symphony 5 wt%	85.2 ± 0.6	14.9 ± 0.3	0.1 ± 0.1
PET Control	62.1 ± 0.6	4.2 ± 0.1	0.1 ± 0.0
PET Ecologic 1 wt%	61.9 ± 0.8	4.2 ± 0.0	0.1 ± 0.1
PET Ecologic 5 wt%	61.8 ± 0.9	4.2 ± 0.1	0.1 ± 0.1
PET Wells 1 wt%	61.7 ± 0.9	4.1 ± 0.2	0.1 ± 0.1
PET Wells 5 wt%	61.5 ± 1.2	4.0 ± 0.2	0.1 ± 0.1

3.3 Anaerobic digestion inoculum and dairy manure

This experiment was done in general accordance with ASTM D5526-12. This test method simulates biologically active landfills where moisture and temperature are controlled, and gas recovery is promoted. The standard was used as a starting point. There were deviations that will be discussed here. Even though the standard only covers tests at 35 ± 2°C, the tests were run at 35 and 50 °C. In addition, pretreated-household waste was replaced by dairy manure. Manure was used because of its high biological activity. The standard requires that the total solid content be more than 30%. However, a total solid content of 5% was used because lower solid content could lead to higher yield [65]. The standard suggested cellulose (analytical grade for thin-layer chromatography) as a positive control. In the test, we used a powder form of cellulose as well as corn starch.

LDPE/LLDPE film and PET sheet produced were exposed to anaerobic digestion environments at 35 and 50 °C. The anaerobic inoculum was obtained directly from an operational in-house anaerobic digester at Michigan State University. In this digester, pretreated household waste was replaced by fresh dairy manure as permitted by ASTM D5526-12. The manure was obtained from the Michigan State University dairy farm and added with water to create a 5% (w/v) total solids mixture. The weights of the manure used for the mixtures are included in Appendix J. Manure was used as the only nitrogen source. Carbon sources were manure and plastics. Manure also acts as a buffer to maintain the pH within the optimal range (6.8 – 7.2) so that microorganisms can grow and thrive. The treatments and controls were mounted on orbital shakers model Innova 2050 (New Brunswick Scientific, Edison, New Jersey) at 95 ± 5 RPM, and placed in incubators model 11-690 (Fisher Scientific, Hampton, New Hampshire) at 35 and 50°C.

3.4 Sample preparation

LDPE/LLDPE film and PET sheet with or without additives were cut into 0.635 cm x 0.635 cm (0.25 in x 0.25 in) pieces using a sample cutter and scissors. They were then weighed before being inserted into 125 mL serum bioreactors. The weight of each sample is listed in Appendix A. These serum bioreactors were airtight and fitted with septa for measuring gas production. The weight of each component of the mixture was calculated to yield a C/N ratio within the optimum 20 – 30 range. Table 3-3 shows the composition of each treatment and control.

Table 3-3. Composition for treatments and controls.

	Inoculum, mL	Manure, mL	Cellulose or starch, g	Plastic sample, g
Negative control (blank)	7.5	75		
Positive control 1 (cellulose)	7.5	75	0.550	
Positive control 2 (cellulose)	7.5	75	1.100	
Treatment (LDPE/LLDPE)	7.5	75		2.250
Treatment (PET)	7.5	75		3.085

Initially, positive controls containing 4.337 g of starch or cellulose were digested rapidly resulting in uncontrollable drop in pH below 5 (Appendix L contains gas evolution data for these bioreactors). This created an unfavorable living environment for microorganisms inside the bioreactors. Therefore, a new experiment with just the negative control (blank) and two positive controls was conducted adding 0.55 and 1.10 g of cellulose for the positive controls using the same manure and inoculum. The theoretical total gas evolution of a bioreactor containing manure is calculated to be 1.21 L and 1.27 L at 35°C and 50°C, respectively. The theoretical total gas evolution of a bioreactor containing 0.55g cellulose is calculated to be 1.57 L and 1.65 L at 35°C

and 50°C, respectively. The theoretical total gas evolution of a bioreactor containing 1.10g cellulose is calculated to be 1.93 L and 2.03 L at 35°C and 50°C, respectively. The formula used for the theoretical values is included in Appendix I.

3.5 Biogas measurement

The generation of total gas in mL (i.e., methane, carbon dioxide and other minor gases) from the LDPE/LLDPE and PET samples without and with additives was quantified, and compared to both positive and negative controls. The gas production was measured using the water displacement method (as depicted in Figure 3-1) initially every 3 days (for the first 100 days) and then after every 7 days. A glass water reservoir with a capacity of 1000 mL was filled with 800 mL of water. Two metal tubes were inserted through a rubber stopper attached to the opening of the water reservoir. One tube was fitted securely inside Tygon tubing, also connected to a needle on the other end. To measure the gas, the needle was inserted into the septum on top of the bioreactors, and the tube connected to the Tygon was placed inside a graduated cylinder to collect the displaced amount of water. The system was entirely airtight except the openings where biogas entered and water siphoned out. The excess pressure inside the bioreactor pushed the water level inside the water reservoir down and siphoned water out of the tubing. The measurement was terminated when the whole system returned to atmospheric pressure. The needle was then removed from the septum of the bioreactor. The AutoCAD drawings of the bioreactor and the gas measuring apparatus are included in Appendix K.

Bioreactors were taken out of the chamber in batches of three in order to keep the inner temperature of the bioreactor close to the original temperature as much as possible. Each

measurement took 20 seconds to finish. The water was refilled when the water level reached the 40% mark. Gas measurements are in Appendix B and C.

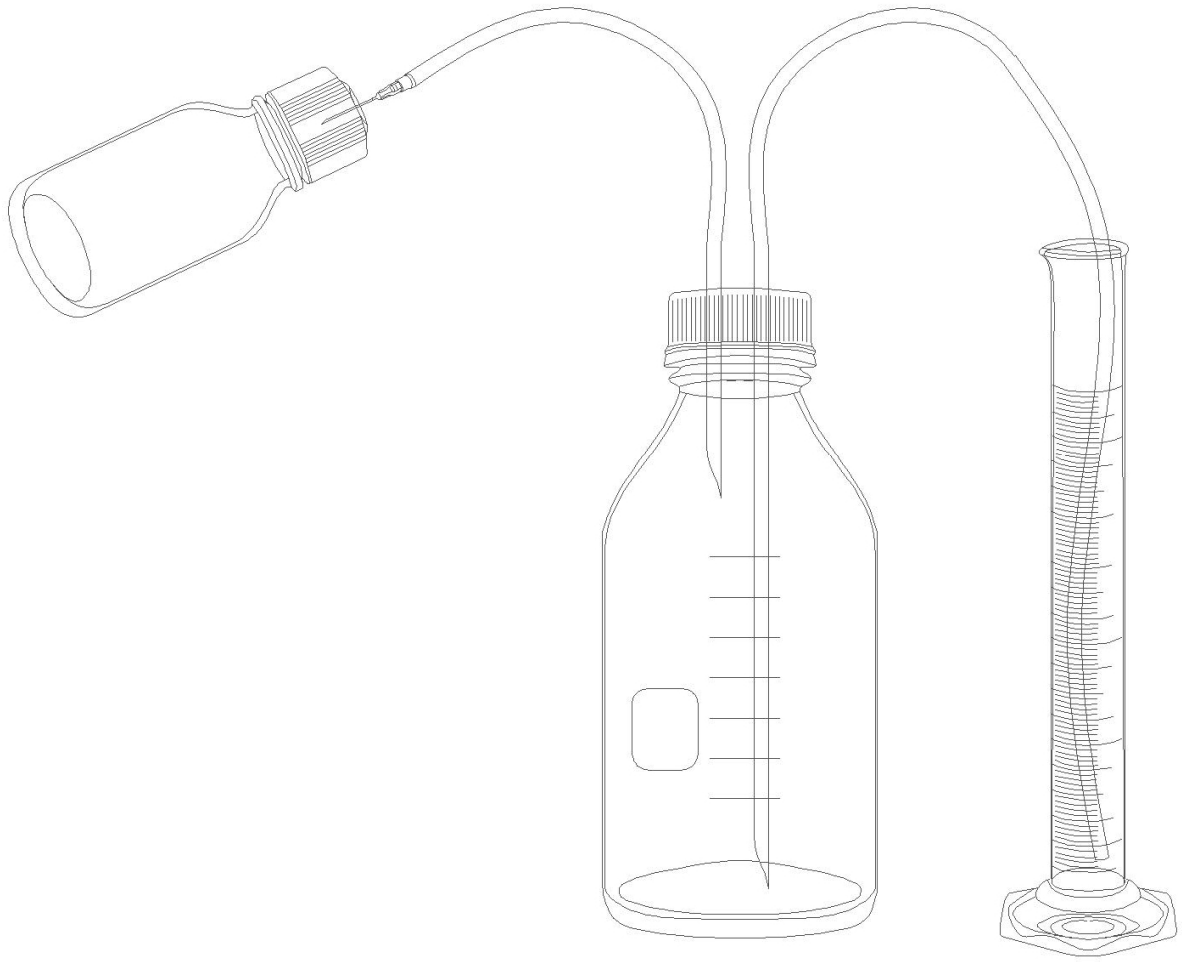


Figure 3-1. Gas production measuring apparatus.

3.6 Optical microscopy

After the main experiment is finished, plastic samples from bioreactors 2, 13, 23, 34, 52, 59, 70, and 75 were retrieved. Biofilms on the surface of the plastic samples were examined under a compound microscope model Eclipse 50i (Nikon Instruments Inc., Melville, NY) with

10 x and 100 x objective for 100 x and 100 x total system magnification. Images were captured with Nikon's NIS-Elements D 3.00.

3.7 Spiking of the bioreactors

After 464 days of running the initial experiment, 0.55 g of corn starch was added to one replicate of LDPE/LLDPE 0 wt%, LDPE/LLDPE Ecologic 5 wt%, LDPE/LLDPE Symphony 5 wt%, LDPE/LLDPE Wells 5 wt%, PET Ecologic 5 wt%, PET Wells 5 wt%, and blank at each incubation temperature. The biogas production as well as the pH level was monitored for the following 50 days.

3.8 pH determination

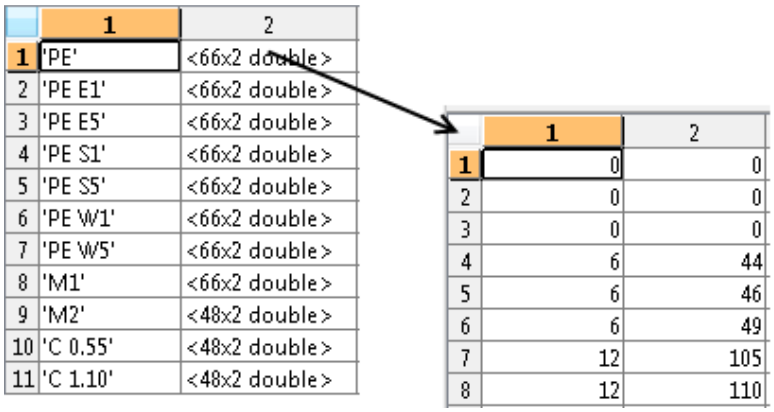
The pH of each bioreactor was checked several times during the study to ensure that it was close to 6.9. A controlled environment anaerobic chamber model 855 from Plas Labs, Inc. (Lansing, MI) was used to conduct this determination. The headspace gas (85% nitrogen, 10% hydrogen, and 5% carbon dioxide) was supplied by Airgas Inc. (Radnor Township, Pennsylvania). The bioreactors were shaken before being opened. A pH meter model Accumet AB15 (Fisher Scientific) with an Ag/AgCl electrode was inserted into the opening. If the pH was lower than 6.7, NaOH 10% solution was added to bring it close to a pH of 6.9.

At low concentration, Na^+ can stimulate anaerobic bacteria growth. However, at higher concentrations, Na^+ slows down and even inhibits bacteria growth by disrupting their metabolisms [60]. The half maximal inhibitory concentration of Na^+, the amount of Na^+ needed

to inhibit the growth of anaerobic bacteria by half, is 5.6 to 53 g/L [65]. In the experiment, the total amount of NaOH added to each bioreactor was less than 4 g/L.

3.9 Statistical analysis and data management

Statistical analyses were performed using MATLAB (The MathWorks Inc., Natick, MA). One-way analyses of variance (ANOVA) were performed, and Tukey's honestly significant difference (HSD) test was used to determine differences ($p \leq 0.05$) among treatments and controls at day 252 (for manure 2nd run and cellulose samples) and 464 (for manure 1st run and the rest). The MATLAB code for ANOVA analysis is included in Appendix H. Gas measurement data were recorded in Excel files, transferred into MATLAB, and saved as MAT-files ("LDPE 35C.mat", "LDPE 50C.mat", "PET 35C.mat", "PET 50C.mat", "LDPE 35C spike.mat", "LDPE 50C spike.mat", "PET 35C spike.mat", and "PET 50C spike.mat"). Each MAT-file contains a cell array named "data" or "spikedata". In each cell array, there are two columns. The first cell array's column contains the name of the samples. The corresponding rows on the second column contain matrices. Each matrix contains two columns (day and corresponding total accumulative gas).



	1	2
1	'PE'	<66x2 double>
2	'PE E1'	<66x2 double>
3	'PE E5'	<66x2 double>
4	'PE S1'	<66x2 double>
5	'PE S5'	<66x2 double>
6	'PE W1'	<66x2 double>
7	'PE W5'	<66x2 double>
8	'M1'	<66x2 double>
9	'M2'	<48x2 double>
10	'C 0.55'	<48x2 double>
11	'C 1.10'	<48x2 double>

	1	2
1	0	0
2	0	0
3	0	0
4	6	44
5	6	46
6	6	49
7	12	105
8	12	110

Figure 3-2. Data structure diagram of a cell array.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Total gas evolution

One-way analyses of variance (ANOVA) were performed, and Tukey's HSD test was used to determine differences ($p \leq 0.05$) among treatments and controls at day 252 (for manure 2nd run and cellulose samples) and 464 (for manure 1st run and the rest). Appendix E contains boxplots accompanying the ANOVA operations. Appendix D contains ANOVA tables listing sum of squares, mean squares, degree of freedom for treatment and errors, f-ratios, and p-values. Because all p-values (LDPE/LLDPE 35°C vs. controls, LDPE/LLDPE 50°C vs. controls, PET 35°C vs. controls, and PET 50°C vs. controls) are smaller than $\alpha = 0.05$, there were significant differences in total gas evolution among treatments and controls at each temperature. Tukey's HSD test was then used for pair-wise comparisons. As shown in Tables 4-1, 4-2, 4-3, 4-4, the total accumulated gas of cellulose 1.10g and 0.55g were significantly higher compared to blanks and plastic samples at each temperature. It must be noted that cellulose samples evolved significantly more biogas in a shorter period of time even though the amounts of carbon in the cellulose samples were less than a quarter of those in the plastic samples. On the other hand, there was no significant difference in gas production between the blanks and the plastic samples. In addition, at 35 °C, there was no significant difference in gas production between cellulose 1.10g and cellulose 0.55g samples. However, at 50 °C, the gas production of cellulose 1.10g samples was statistically significantly higher than that of the cellulose 0.55g samples.

Table 4-1. Average accumulated gas volume at day 252 (for manure 2nd run and cellulose samples) and 464 (for manure 1st run and LDPE/LLDPE samples) at 35°C.

Samples	Mean
Cellulose 1.10g	1945 a
Cellulose 0.55g	1818 a
LDPE/LLDPE Symphony 1 wt%	1443 b
LDPE/LLDPE Wells 1 wt%	1375 b
LDPE/LLDPE Ecologic 1 wt%	1373 b
LDPE/LLDPE Symphony 5 wt%	1359 b
LDPE/LLDPE Ecologic 5 wt%	1349 b
LDPE/LLDPE Wells 5 wt%	1319 b
Manure (1st run)	1293 b
Manure (2nd run)	1279 b
LDPE/LLDPE 0 wt%	1266 b

Note: Samples not connected by the same letter are significantly different.

Table 4-2. Average accumulated gas volume at day 252 (for manure 2nd run and cellulose samples) and 464 (for manure 1st run and LDPE/LLDPE samples) at 50°C.

Samples	Mean
Cellulose 1.10g	1973 a
Cellulose 0.55g	1618 b
LDPE/LLDPE Wells 1 wt%	1150 c
Manure (2nd run)	1088 c
LDPE/LLDPE Wells 5 wt%	1062 c
LDPE/LLDPE Symphony 5 wt%	1057 c
LDPE/LLDPE Ecologic 5 wt%	1024 c
LDPE/LLDPE Symphony 1 wt%	995 c
Manure (1st run)	955 c
LDPE/LLDPE Ecologic 1 wt%	952 c
LDPE/LLDPE 0 wt%	941 c

Note: Samples not connected by the same letter are significantly different.

Table 4-3. Average accumulated gas volume at day 252 (for manure 2nd run and cellulose samples) and 464 (for manure 1st run and PET samples) at 35°C.

Samples	Mean
Cellulose 1.10g	1945 a
Cellulose 0.55g	1818 a
PET Wells 1 wt%	1359 b
PET Ecologic 5 wt%	1348 b
PET Wells 5 wt%	1329 b
PET 0 wt%	1318 b
PET Ecologic 1 wt%	1296 b
Manure (1st run)	1293 b
Manure (2nd run)	1279 b

Note: Samples not connected by the same letter are significantly different.

Table 4-4. Average accumulated gas volume at day 252 (for manure 2nd run and cellulose samples) and 464 (for manure 1st run and PET samples) at 50°C.

Samples	Mean
Cellulose 1.10g	1973 a
Cellulose 0.55g	1618 b
PET Wells 1 wt%	1184 c
Manure (2nd run)	1088 c
PET Wells 5 wt%	1048 c
PET 0 wt%	1003 c
PET Ecologic 5 wt%	997 c
PET Ecologic 1 wt%	995 c
Manure (1st run)	955 c

Note: Samples not connected by the same letter are significantly different.

Figures 4-1 and 4-2 show the total gas evolution in mL of LDPE/LLDPE samples and controls at 35 and 50 °C. Figures 4-3 and 4-4 show the total gas evolution in mL of PET samples

and controls at 35 and 50 °C. These figures were generated using MATLAB (code in Appendix F).

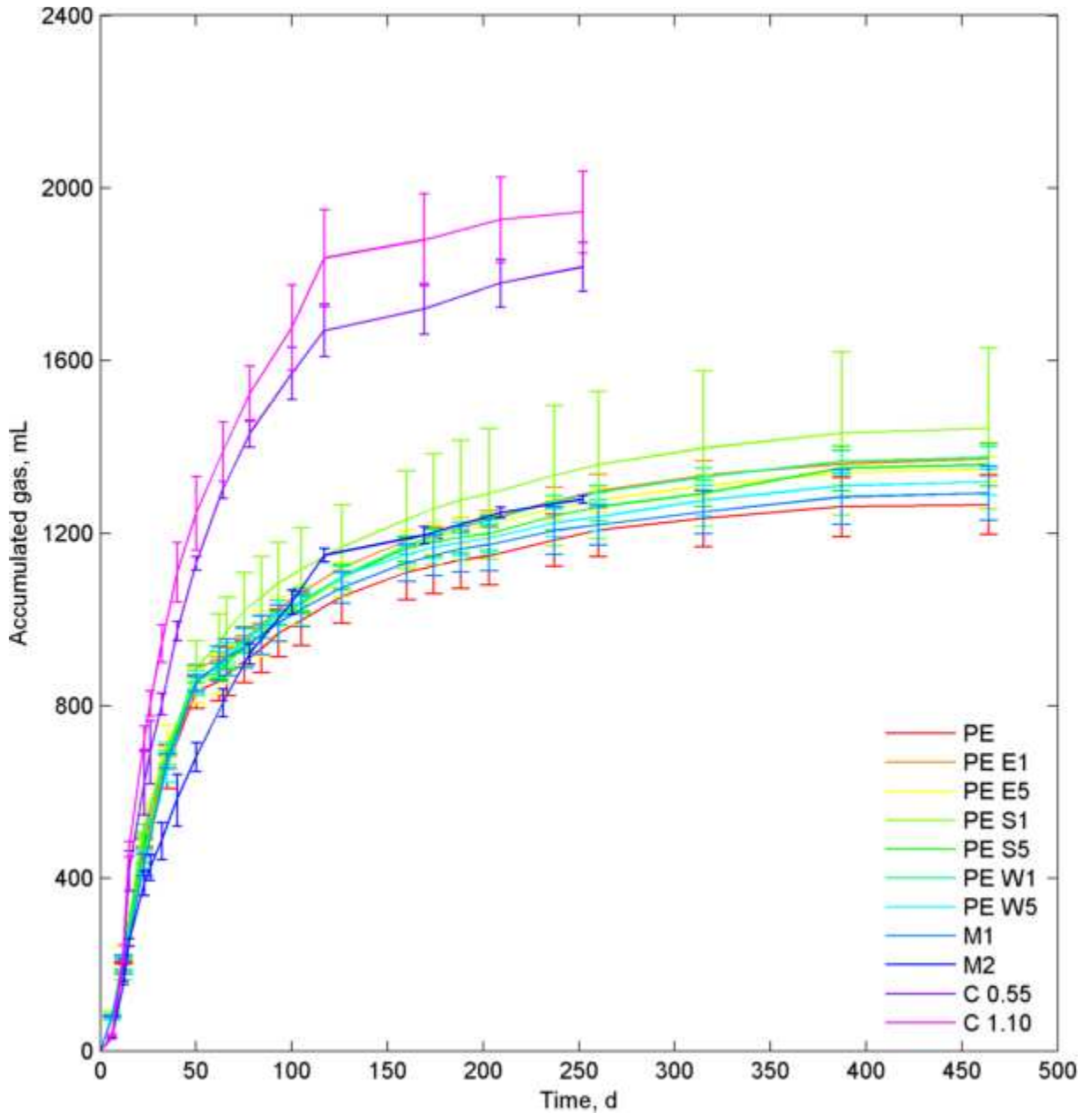


Figure 4-1. Accumulated gas in mL at 35°C for LDPE/LLDPE Ecologic 1 & 5 wt%, LDPE/LLDPE Symphony 1 & 5 wt%, LDPE/LLDPE Wells 1 and 5 wt%, cellulose 0.55g and 1.10 g (positive controls), and blanks (manure 1st and 2nd run).

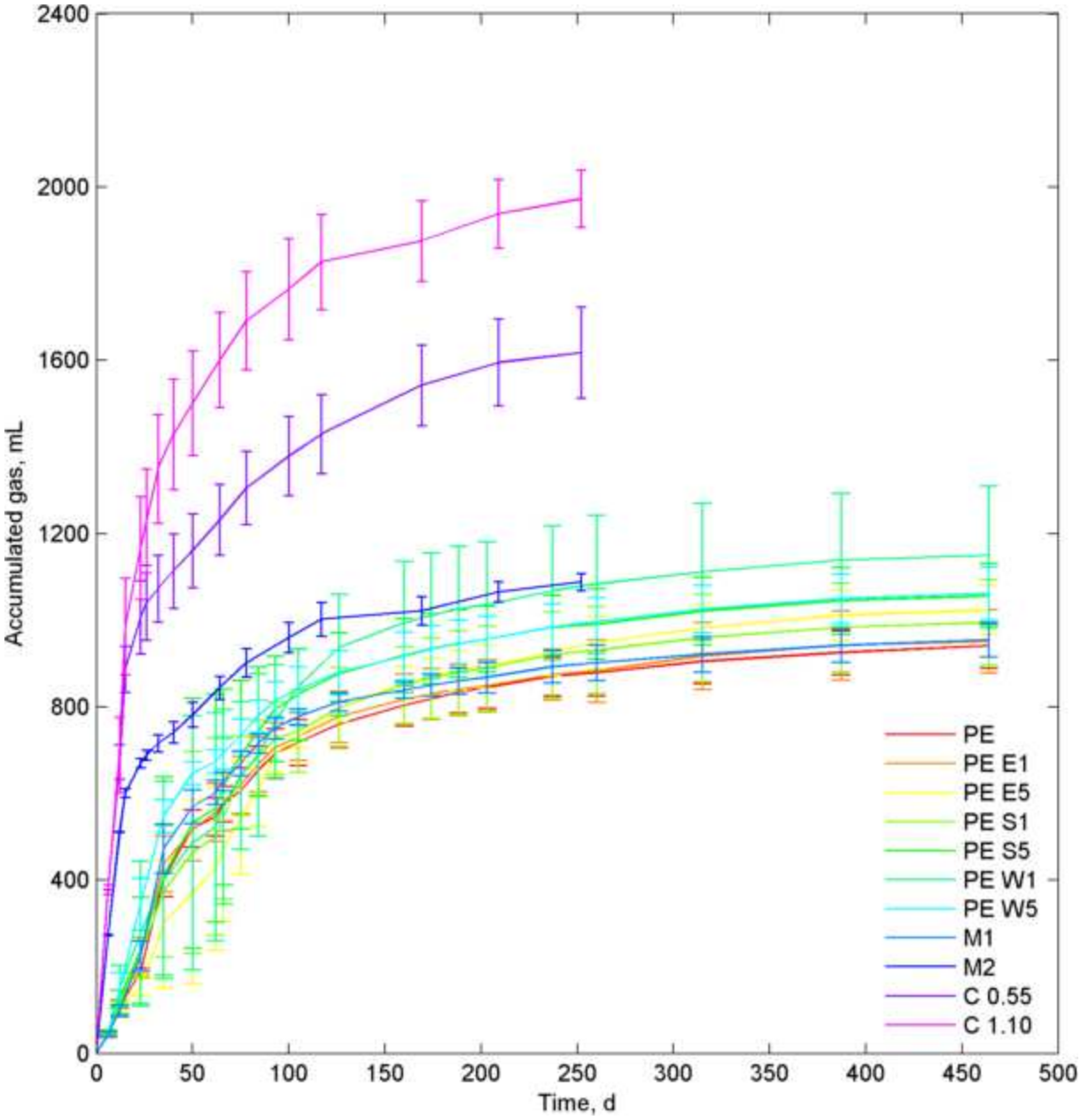


Figure 4-2. Accumulated gas in mL at 50°C for LDPE/LLDPE Ecologic 1 & 5 wt%, LDPE/LLDPE Symphony 1 & 5 wt%, LDPE/LLDPE Wells 1 and 5 wt%, cellulose 0.55g and 1.10 g (positive controls), and blanks (manure 1st and 2nd run).

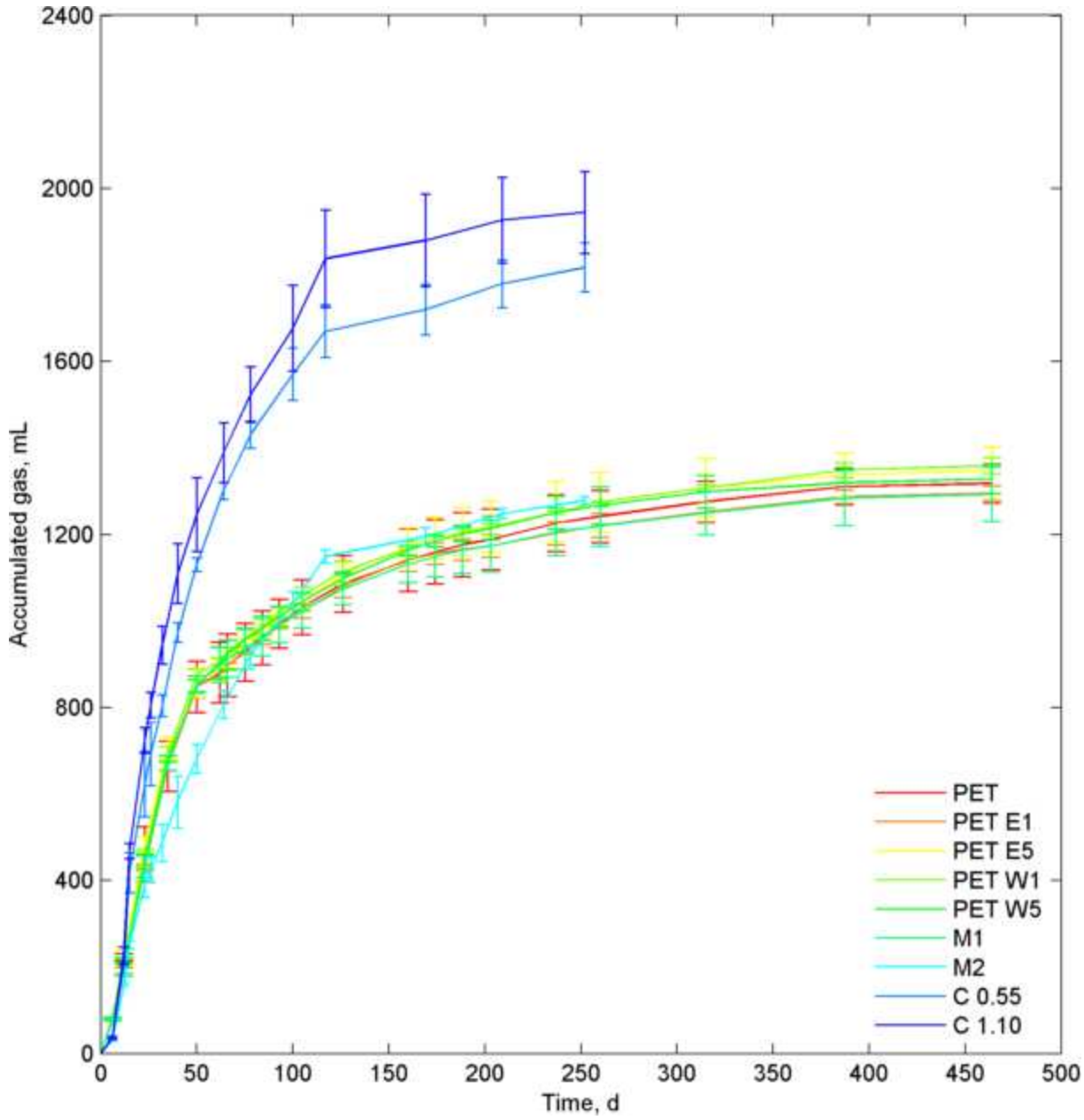


Figure 4-3. Accumulated gas in mL at 35°C for PET Ecologic 1 & 5 wt%, PET Wells 1 & 5 wt%, cellulose 0.55g and 1.10 g (positive control), and blanks (manure 1st and 2nd run).

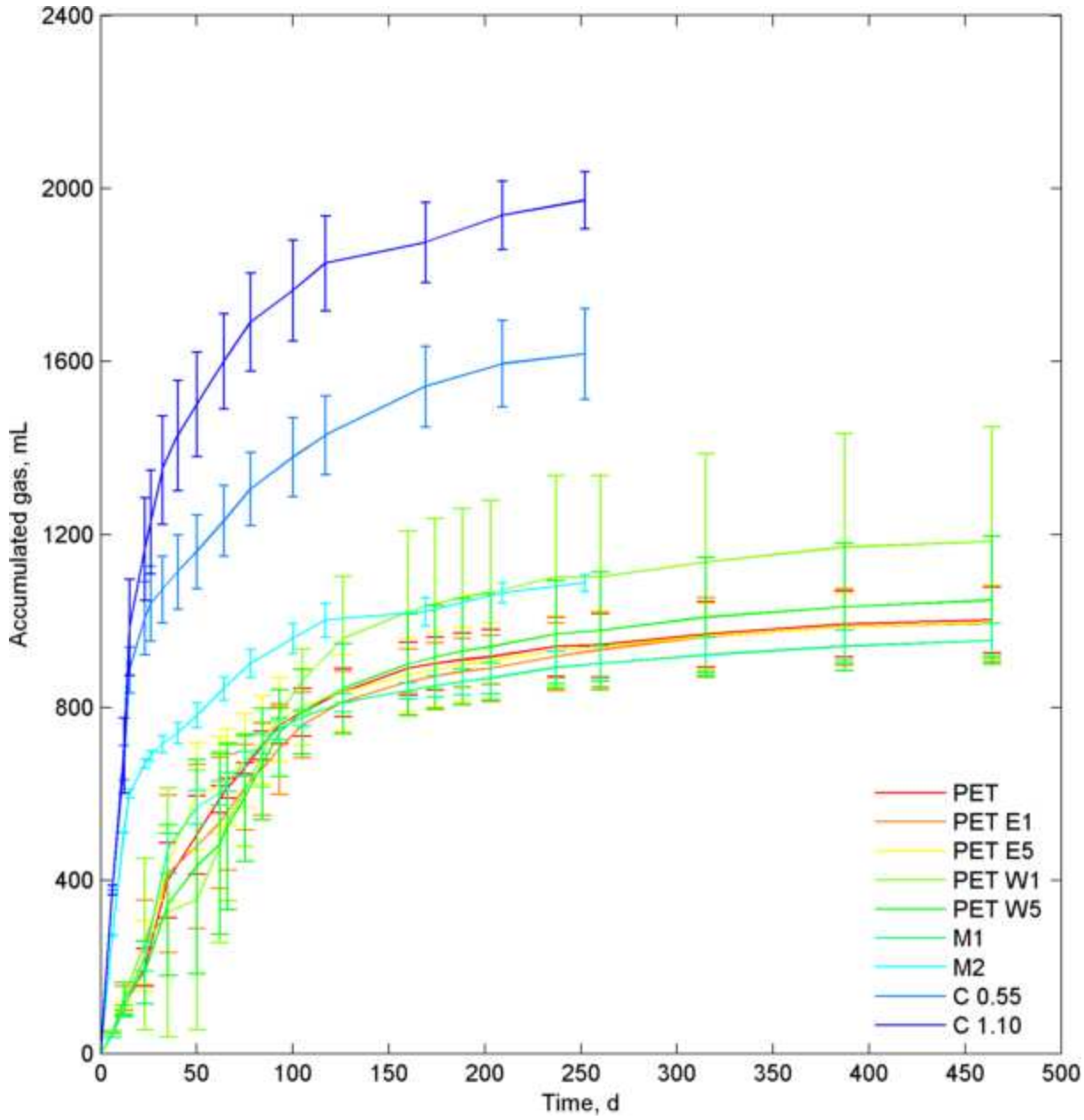


Figure 4-4 Accumulated gas in mL at 50°C for PET Ecologic 1 & 5 wt%, PET Wells 1 & 5 wt%, cellulose 0.55g and 1.10 g (positive control), and blanks (manure 1st and 2nd run).

4.2 Spiking

Figures 4-5 and 4-6 showed the spikes in gas production after corn starch was introduced into bioreactors containing LDPE/LLDPE 0 wt%, LDPE/LLDPE Ecologic 5 wt%,

LDPE/LLDPE Symphony 5 wt%, LDPE/LLDPE Wells 5 wt%, PET Ecologic 5 wt%, PET Wells 5 wt%, and blank. The increase in gas production proved that the microorganisms inside the bioreactor could still grow if enough digestible nutrients were present.

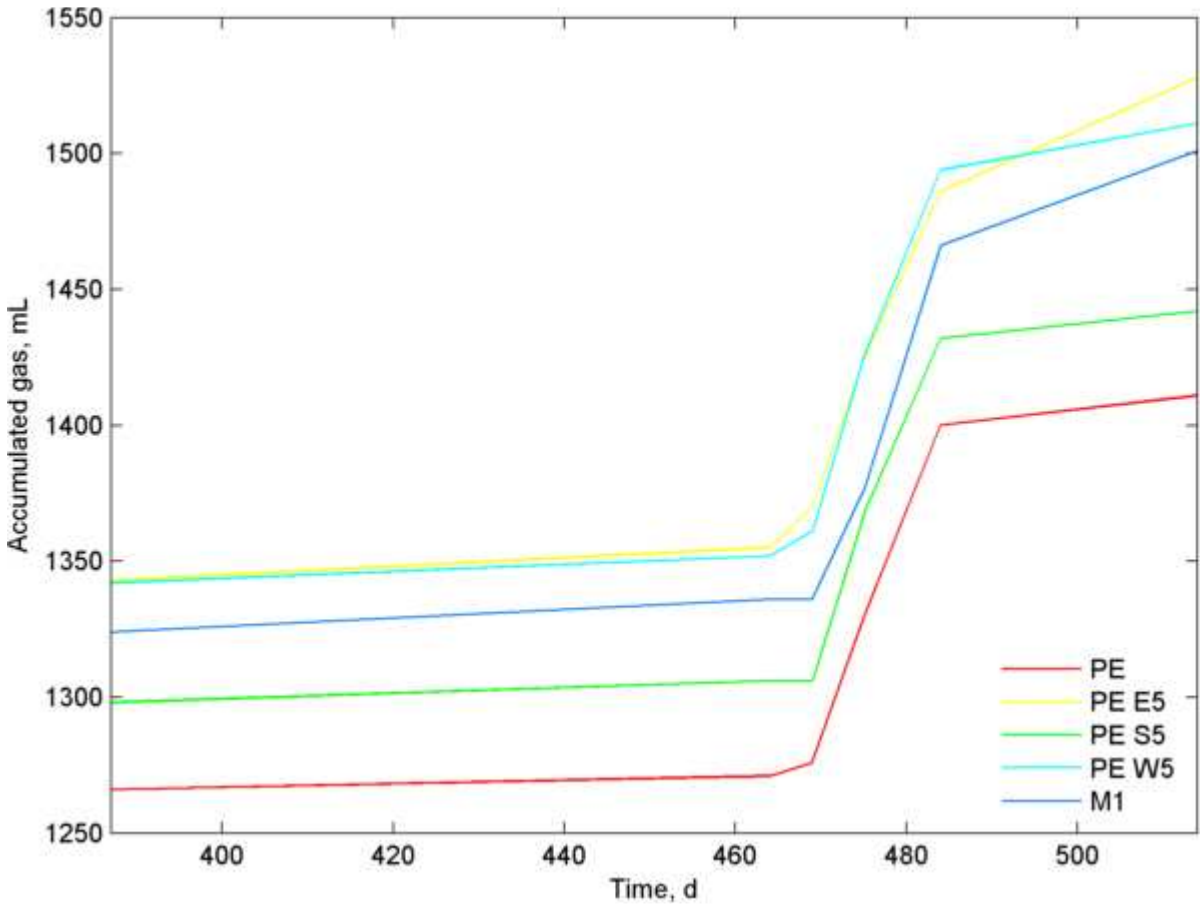


Figure 4-5. Spikes in accumulated gas evolution at 35°C for LDPE/LLDPE 0 wt%, LDPE/LLDPE Ecologic 5 wt%, LDPE/LLDPE Symphony 5 wt%, LDPE/LLDPE Wells 5 wt%, and blank (bioreactor 1, 9, 14, 21, and 44).

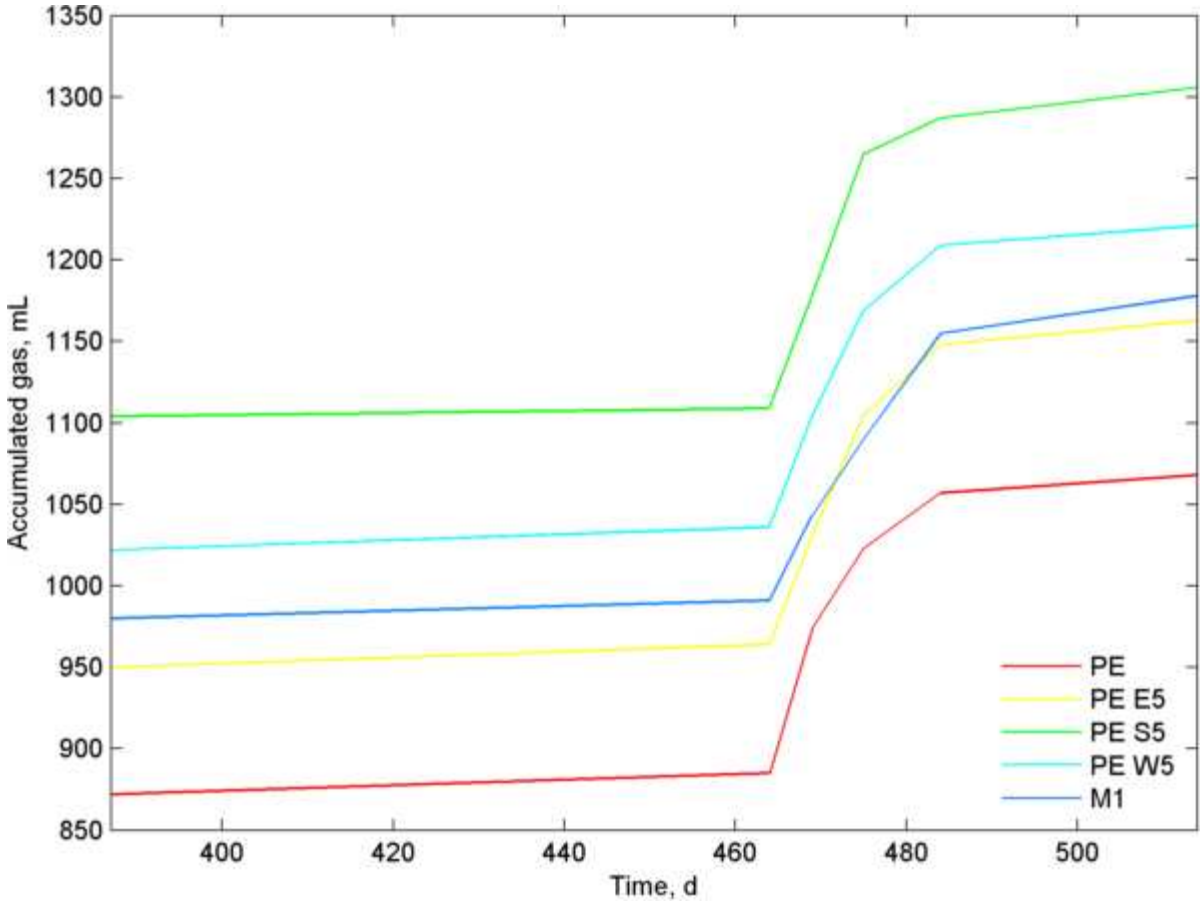


Figure 4-6. Spikes in accumulated gas evolution at 50°C for LDPE/LLDPE 0 wt%, LDPE/LLDPE Ecologic 5 wt%, LDPE/LLDPE Symphony 5 wt%, LDPE/LLDPE Wells 5 wt%, and blank (bioreactor 53, 60, 64, 71, and 94).

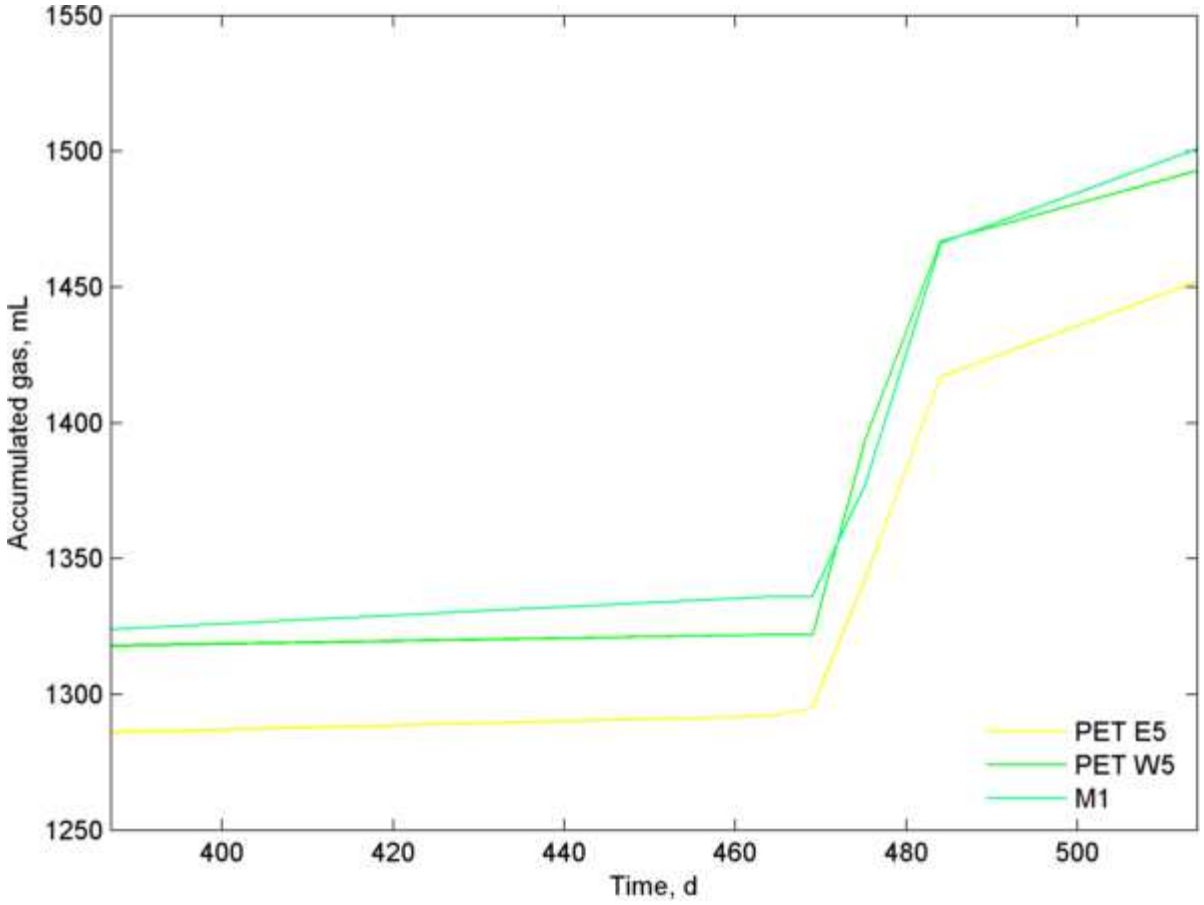


Figure 4-7. Spikes in accumulated gas evolution at 35°C for PET Ecologic 5 wt%, PET Wells 5 wt%, and blank (bioreactor 28, 36, and 44).

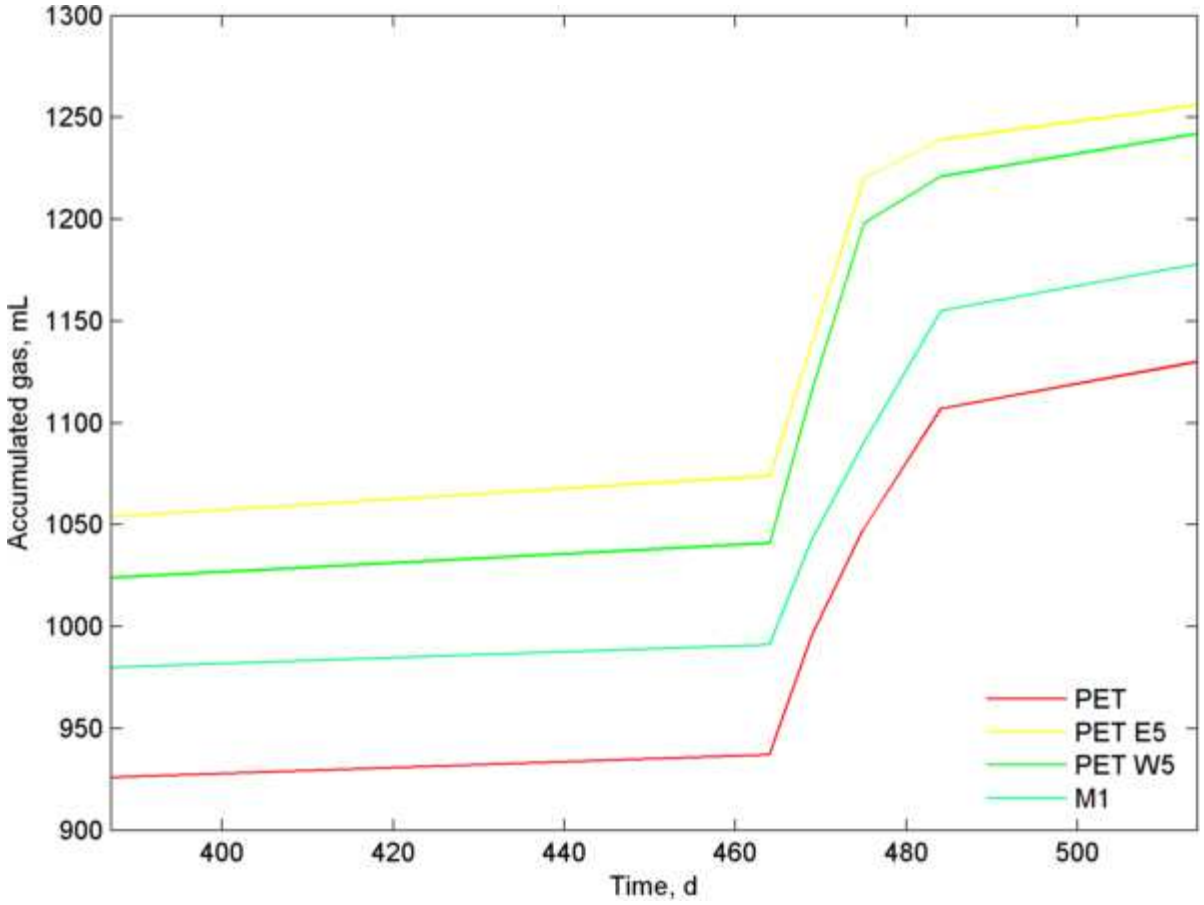


Figure 4-8. Spikes in accumulated gas evolution at 35°C for PET Ecologic 5 wt%, PET Wells 5 wt%, and blank (bioreactor 73, 81, 85, and 94).

4.3 Optical microscopy

Optical microscopy confirmed with visual inspection that even though biofilms formed on the surfaces of the plastic samples retrieved, the films were very thin and negligible.

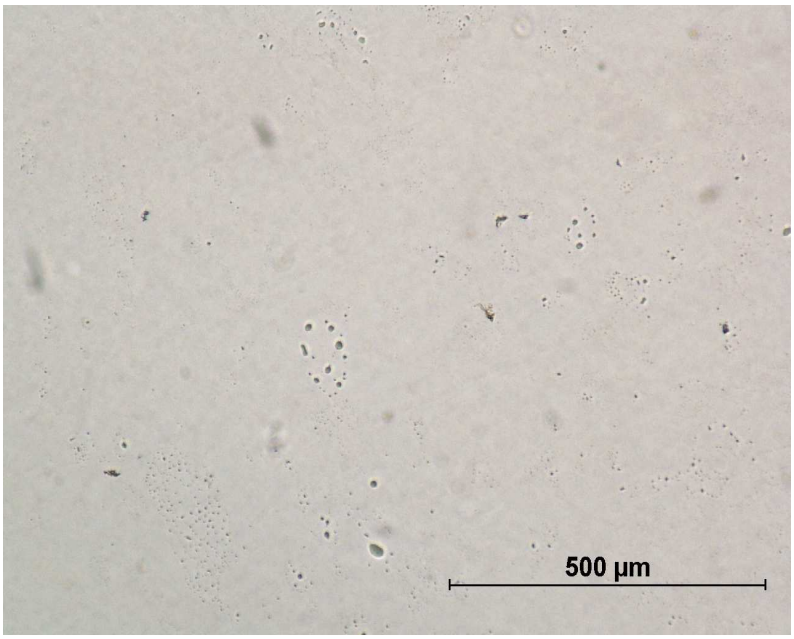


Figure 4-9. Optical microscopy of the surface of a LDPE/LLDPE Symphony 5 wt% sample from bioreactor #13 (10x objective, 100x total).

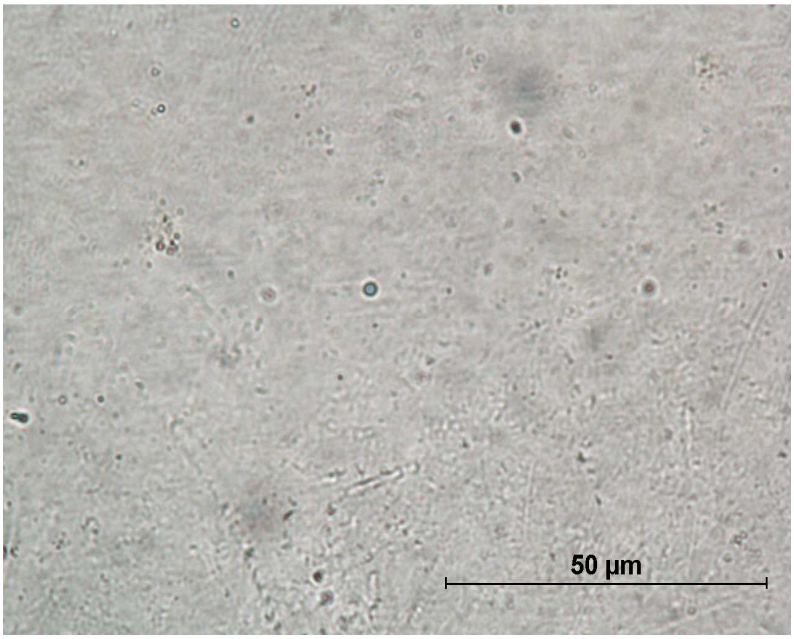


Figure 4-10. Optical microscopy of the surface of a LDPE/LLDPE Symphony 5 wt% sample from bioreactor #13 (100x objective, 1000x total).

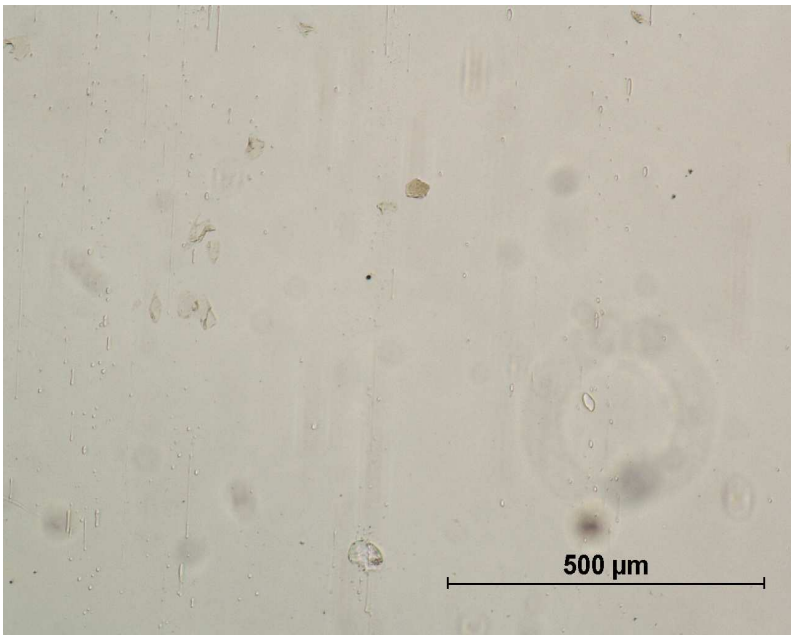


Figure 4-11. Optical microscopy of the surface of a PET Wells 5 wt% sample from bioreactor #34 (10x objective, 100x total).

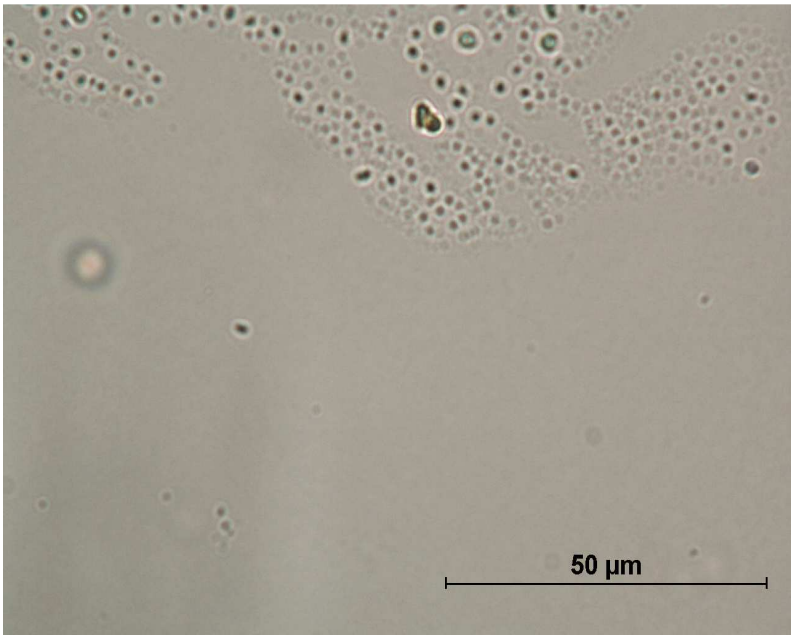


Figure 4-12. Optical microscopy of the surface of a PET Wells 5 wt% sample from bioreactor #34 (100x objective, 1000x total).

CHAPTER 5: CONCLUSIONS

5.1 Overall conclusions

The working hypothesis was that the additive systems significantly promote biodegradation of the polymers into which they are incorporated. This study was run for an extended amount of time. However, we did not find any evidence of significant degradation of plastics incorporated with pro-degrading additives. We have concluded that the additive systems from Symphony Environmental Ltd., Wells Plastics Ltd., and EcoLogic LLC do not promote significant biodegradation for either LDPE/LLDPE or PET under the anaerobic digestion test conditions as determined by total gas evolution. This particular study does not prove or disprove the effects of these additive systems under other test conditions.

5.2 Recommendations for future work

In the future, more research should be done using other additive systems under anaerobic digestion or aerobic composting conditions. Mechanisms of the biodegradation process can be studied by constructing mathematical models of the gas production, and comparing their parameters and corresponding confidence intervals to each other. To construct models that accurately emulate the response of the system, the concentration of the growth limiting substrate as well as other critical response variables should be measured. If there is evidence that the additives promote biodegradation of the plastics, the properties of the degraded samples should be compared against the properties of samples from the same batch that have not gone through the degradation process. For example, properties such as intrinsic viscosity, glass transition temperature, and melting temperature (using differential scanning calorimetry) can be measured.

In addition, Fourier transform infrared spectroscopy, UV/Vis spectroscopy, and scanning electron microscopy can be used to study the chemical structure changes and surface erosion of the plastics.

APPENDICES

APPENDIX A: WEIGHT OF SAMPLES

Table A-1. Weight for LDPE/LLDPE samples (35 °C).

Bioreactor	Sample	Weight of plastic sample (g)
1	LDPE/LLDPE 0 wt%	2.2474
2	LDPE/LLDPE 0 wt%	2.2501
3	LDPE/LLDPE 0 wt%	2.2413
4	LDPE/LLDPE Ecologic 1 wt%	2.2481
5	LDPE/LLDPE Ecologic 1 wt%	2.2469
6	LDPE/LLDPE Ecologic 1 wt%	2.2434
7	LDPE/LLDPE Ecologic 5 wt%	2.2453
8	LDPE/LLDPE Ecologic 5 wt%	2.2469
9	LDPE/LLDPE Ecologic 5 wt%	2.2539
10	LDPE/LLDPE Symphony 1 wt%	2.2522
11	LDPE/LLDPE Symphony 1 wt%	2.2433
12	LDPE/LLDPE Symphony 1 wt%	2.2527
13	LDPE/LLDPE Symphony 5 wt%	2.2478
14	LDPE/LLDPE Symphony 5 wt%	2.2461
15	LDPE/LLDPE Symphony 5 wt%	2.2477
16	LDPE/LLDPE Wells 1 wt%	2.2458
17	LDPE/LLDPE Wells 1 wt%	2.2459
18	LDPE/LLDPE Wells 1 wt%	2.2494
19	LDPE/LLDPE Wells 5 wt%	2.2492
20	LDPE/LLDPE Wells 5 wt%	2.2492
21	LDPE/LLDPE Wells 5 wt%	2.2476

Table A-2. Weight for PET samples (35 °C).

Bioreactor	Sample	Weight of plastic sample (g)
22	PET 0 wt%	3.0817
23	PET 0 wt%	3.0840
24	PET 0 wt%	3.0809
25	PET Ecologic 1 wt%	3.0811
26	PET Ecologic 1 wt%	3.0838
27	PET Ecologic 1 wt%	3.0875
28	PET Ecologic 5 wt%	3.0820
29	PET Ecologic 5 wt%	3.0847
30	PET Ecologic 5 wt%	3.0833
31	PET Wells 1 wt%	3.0865
32	PET Wells 1 wt%	3.0812
33	PET Wells 1 wt%	3.0839
34	PET Wells 5 wt%	3.0851
35	PET Wells 5 wt%	3.0864
36	PET Wells 5 wt%	3.0875

Table A-3. Weight for cellulose samples (35 °C).

Bioreactor	Sample	Weight of sample (g)
O1	Cellulose 0.55g	0.5540
O2	Cellulose 0.55g	0.5510
O3	Cellulose 0.55g	0.5536
P1	Cellulose 1.10g	1.1029
P2	Cellulose 1.10g	1.1052
P3	Cellulose 1.10g	1.1028

Table A-4. Weight for LDPE/LLDPE samples (50 °C).

Bioreactor	Sample	Weight of plastic sample (g)
52	LDPE/LLDPE 0 wt%	2.2470
53	LDPE/LLDPE 0 wt%	2.2491
54	LDPE/LLDPE 0 wt%	2.2438
55	LDPE/LLDPE Ecologic 1 wt%	2.2487
56	LDPE/LLDPE Ecologic 1 wt%	2.2479
57	LDPE/LLDPE Ecologic 1 wt%	2.2464
58	LDPE/LLDPE Ecologic 5 wt%	2.2420
59	LDPE/LLDPE Ecologic 5 wt%	2.2463
60	LDPE/LLDPE Ecologic 5 wt%	2.2449
61	LDPE/LLDPE Symphony 1 wt%	2.2442
62	LDPE/LLDPE Symphony 1 wt%	2.2432
63	LDPE/LLDPE Symphony 1 wt%	2.2486
64	LDPE/LLDPE Symphony 5 wt%	2.2464
65	LDPE/LLDPE Symphony 5 wt%	2.2460
66	LDPE/LLDPE Symphony 5 wt%	2.2447
67	LDPE/LLDPE Wells 1 wt%	2.2473
68	LDPE/LLDPE Wells 1 wt%	2.2497
69	LDPE/LLDPE Wells 1 wt%	2.2434
70	LDPE/LLDPE Wells 5 wt%	2.2478
71	LDPE/LLDPE Wells 5 wt%	2.2465
72	LDPE/LLDPE Wells 5 wt%	2.2483

Table A-5. Weight for PET samples (50 °C).

73	PET 0 wt%	3.0883
74	PET 0 wt%	3.0828
75	PET 0 wt%	3.0852
76	PET Ecologic 1 wt%	3.0848
77	PET Ecologic 1 wt%	3.0825
78	PET Ecologic 1 wt%	3.0854
79	PET Ecologic 5 wt%	3.0807
80	PET Ecologic 5 wt%	3.0832
81	PET Ecologic 5 wt%	3.0873
82	PET Wells 1 wt%	3.0809
83	PET Wells 1 wt%	3.0850
84	PET Wells 1 wt%	3.0845
85	PET Wells 5 wt%	3.0814
86	PET Wells 5 wt%	3.0803
87	PET Wells 5 wt%	3.0849

Table A-6. Weight for cellulose samples (50 °C).

R2	Cellulose 0.55g	0.5567
R2	Cellulose 0.55g	0.5528
R3	Cellulose 0.55g	0.5543
S1	Cellulose 1.10g	1.1008
S2	Cellulose 1.10g	1.1054
S3	Cellulose 1.10g	1.1076

APPENDIX B: ACCUMULATED GAS MEASUREMENT

Table B-1. Accumulated gas measurement for LDPE/LLDPE 0 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #1	Bioreactor #2	Bioreactor #3		
0	0	0	0	0	0.0
6	75	77	76	76	1.0
12	205	205	206	205	0.6
23	470	488	486	481	9.9
35	682	693	601	659	50.2
50	831	872	796	833	38.0
62	873	892	806	857	45.2
66	891	907	818	872	47.4
75	923	939	847	903	49.2
84	952	974	871	932	54.2
93	980	1017	909	969	54.9
105	1012	1049	935	999	58.2
126	1067	1106	987	1053	60.7
160	1122	1165	1041	1109	63.0
174	1137	1182	1055	1125	64.4
188	1149	1197	1067	1138	65.7
203	1160	1208	1076	1148	66.8
237	1188	1243	1123	1185	60.1
260	1210	1267	1145	1207	61.0
315	1241	1296	1167	1235	64.7
387	1266	1330	1190	1262	70.1
464	1271	1331	1195	1266	68.2

Table B-2. Accumulated gas measurement for LDPE/LLDPE Ecologic 1 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #4	Bioreactor #5	Bioreactor #6		
0	0	0	0	0	0.0
6	81	81	72	78	5.2
12	214	205	203	207	5.9
23	504	502	489	498	8.1
35	684	687	689	687	2.5
50	882	893	886	887	5.6
62	904	910	912	909	4.2
66	921	929	934	928	6.6
75	950	954	964	956	7.2
84	979	990	989	986	6.1
93	1012	1029	1030	1024	10.1
105	1056	1059	1072	1062	8.5
126	1120	1113	1127	1120	7.0
160	1189	1171	1189	1183	10.4
174	1208	1189	1213	1203	12.7
188	1225	1204	1233	1221	15.0
203	1239	1213	1250	1234	19.0
237	1268	1250	1310	1276	30.8
260	1277	1274	1343	1298	39.0
315	1307	1323	1373	1334	34.4
387	1339	1346	1397	1361	31.7
464	1343	1362	1413	1373	36.2

Table B-3. Accumulated gas measurement for LDPE/LLDPE Ecologic 5 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #7	Bioreactor #8	Bioreactor #9		
0	0	0	0	0	0.0
6	82	86	75	81	5.6
12	227	241	217	228	12.1
23	570	544	507	540	31.7
35	754	679	722	718	37.6
50	870	801	868	846	39.3
62	911	821	887	873	46.6
66	932	848	909	896	43.4
75	979	888	949	939	46.4
84	1007	908	987	967	52.3
93	1037	943	1026	1002	51.4
105	1064	981	1058	1034	46.3
126	1122	1048	1110	1093	39.7
160	1205	1123	1170	1166	41.1
174	1225	1153	1189	1189	36.0
188	1238	1176	1206	1207	31.0
203	1247	1196	1217	1220	25.6
237	1280	1233	1260	1258	23.6
260	1302	1245	1284	1277	29.1
315	1336	1282	1313	1310	27.1
387	1363	1312	1343	1339	25.7
464	1374	1318	1355	1349	28.5

Table B-4. Accumulated gas measurement for LDPE/LLDPE Symphony 1 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #10	Bioreactor #11	Bioreactor #12		
0	0	0	0	0	0.0
6	89	71	87	82	9.9
12	193	163	207	188	22.5
23	471	403	452	442	35.1
35	711	651	692	685	30.7
50	961	856	847	888	63.4
62	1025	899	907	944	70.6
66	1064	929	926	973	78.8
75	1122	978	960	1020	88.8
84	1160	1004	992	1052	93.7
93	1193	1038	1022	1084	94.4
105	1227	1065	1060	1117	95.0
126	1280	1125	1096	1167	98.9
160	1363	1172	1160	1232	113.9
174	1405	1187	1178	1257	128.5
188	1437	1199	1194	1277	138.9
203	1467	1203	1207	1292	151.3
237	1522	1235	1244	1334	163.2
260	1556	1250	1269	1358	171.4
315	1605	1286	1301	1397	180.0
387	1650	1312	1334	1432	189.1
464	1658	1324	1348	1443	186.3

Table B-5. Accumulated gas measurement for LDPE/LLDPE Symphony 5 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #13	Bioreactor #14	Bioreactor #15		
0	0	0	0	0	0.0
6	83	76	81	80	3.6
12	223	191	203	206	16.2
23	503	451	446	467	31.6
35	698	676	666	680	16.4
50	868	858	856	861	6.4
62	895	882	861	879	17.2
66	909	911	885	902	14.5
75	943	940	921	935	11.9
84	969	966	956	964	6.8
93	1022	992	996	1003	16.3
105	1057	1017	1041	1038	20.1
126	1118	1069	1108	1098	25.9
160	1190	1133	1170	1164	28.9
174	1208	1145	1187	1180	32.1
188	1221	1151	1201	1191	36.1
203	1231	1156	1212	1200	39.0
237	1273	1207	1241	1240	33.0
260	1296	1230	1257	1261	33.2
315	1322	1260	1296	1293	31.1
387	1354	1298	1401	1351	51.6
464	1370	1306	1402	1359	48.9

Table B-6. Accumulated gas measurement for LDPE/LLDPE Wells 1 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #16	Bioreactor #17	Bioreactor #18		
0	0	0	0	0	0.0
6	81	82	82	82	0.6
12	219	215	218	217	2.1
23	526	517	507	517	9.5
35	687	712	707	702	13.2
50	831	900	855	862	35.0
62	868	928	891	896	30.3
66	884	947	911	914	31.6
75	927	982	949	953	27.7
84	956	1006	988	983	25.3
93	987	1042	1022	1017	27.8
105	1014	1074	1044	1044	30.0
126	1072	1127	1102	1100	27.5
160	1153	1190	1170	1171	18.5
174	1183	1211	1191	1195	14.4
188	1205	1228	1208	1214	12.5
203	1225	1244	1220	1230	12.7
237	1272	1289	1258	1273	15.5
260	1293	1312	1278	1294	17.0
315	1326	1352	1314	1331	19.4
387	1362	1394	1344	1367	25.3
464	1375	1401	1350	1375	25.5

Table B-7. Accumulated gas measurement for LDPE/LLDPE Wells 5 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #19	Bioreactor #20	Bioreactor #21		
0	0	0	0	0	0.0
6	78	79	74	77	2.6
12	190	220	194	201	16.3
23	420	463	449	444	21.9
35	633	693	639	655	33.0
50	844	885	851	860	21.9
62	881	900	892	891	9.5
66	899	924	918	914	13.1
75	935	951	955	947	10.6
84	969	980	984	978	7.8
93	1008	1013	1012	1011	2.6
105	1034	1046	1052	1044	9.2
126	1085	1106	1107	1099	12.4
160	1131	1150	1173	1151	21.0
174	1140	1171	1190	1167	25.2
188	1146	1186	1204	1179	29.7
203	1151	1196	1217	1188	33.7
237	1188	1235	1250	1224	32.3
260	1208	1235	1270	1238	31.1
315	1244	1269	1315	1276	36.0
387	1287	1302	1342	1310	28.4
464	1299	1305	1352	1319	29.0

Table B-8. Accumulated gas measurement for PET 0 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #22	Bioreactor #23	Bioreactor #24		
0	0	0	0	0	0.0
6	80	84	83	82	2.1
12	227	228	213	223	8.4
23	497	498	388	461	63.2
35	707	688	598	664	58.2
50	886	879	780	848	59.3
62	912	930	800	881	70.4
66	928	950	815	898	72.4
75	960	973	851	928	67.0
84	989	1005	890	961	62.3
93	1015	1038	930	994	56.9
105	1053	1083	961	1032	63.6
126	1108	1138	1012	1086	65.8
160	1162	1200	1060	1141	72.4
174	1177	1225	1079	1160	74.4
188	1190	1243	1097	1177	73.9
203	1200	1253	1114	1189	70.1
237	1238	1285	1156	1226	65.3
260	1252	1296	1177	1242	60.2
315	1282	1321	1226	1276	47.8
387	1310	1353	1270	1311	41.5
464	1317	1363	1274	1318	44.5

Table B-9. Accumulated gas measurement for PET Ecologic 1 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #25	Bioreactor #26	Bioreactor #27		
0	0	0	0	0	0.0
6	82	76	84	81	4.2
12	208	198	211	206	6.8
23	430	433	422	428	5.7
35	670	673	652	665	11.4
50	871	852	822	848	24.7
62	884	872	872	876	6.9
66	904	890	893	896	7.4
75	925	929	940	931	7.8
84	944	961	965	957	11.2
93	982	993	998	991	8.2
105	1009	1020	1039	1023	15.2
126	1056	1078	1107	1080	25.6
160	1117	1139	1171	1142	27.2
174	1136	1148	1184	1156	25.0
188	1150	1153	1194	1166	24.6
203	1161	1156	1203	1173	25.8
237	1189	1186	1234	1203	26.9
260	1213	1198	1251	1221	27.3
315	1245	1234	1278	1252	22.9
387	1272	1288	1303	1288	15.5
464	1277	1304	1306	1296	16.2

Table B-10. Accumulated gas measurement for PET Ecologic 5 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #28	Bioreactor #29	Bioreactor #30		
0	0	0	0	0	0.0
6	79	83	78	80	2.6
12	204	241	223	223	18.5
23	469	505	478	484	18.7
35	691	735	690	705	25.7
50	848	895	831	858	33.2
62	873	915	875	888	23.7
66	894	932	909	912	19.1
75	928	961	949	946	16.7
84	957	987	982	975	16.1
93	989	1015	1016	1007	15.3
105	1026	1054	1051	1044	15.4
126	1080	1112	1133	1108	26.7
160	1133	1168	1218	1173	42.7
174	1145	1188	1243	1192	49.1
188	1153	1204	1263	1207	55.0
203	1158	1217	1277	1217	59.5
237	1185	1248	1325	1253	70.1
260	1205	1276	1343	1275	69.0
315	1237	1315	1372	1308	67.8
387	1286	1340	1388	1338	51.0
464	1292	1348	1403	1348	55.5

Table B-11. Accumulated gas measurement for PET Wells 1 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #31	Bioreactor #32	Bioreactor #33		
0	0	0	0	0	0.0
6	82	80	78	80	2.0
12	217	216	206	213	6.1
23	469	461	436	455	17.2
35	711	691	676	693	17.6
50	890	872	867	876	12.1
62	916	907	900	908	8.0
66	942	925	920	929	11.5
75	980	959	943	961	18.6
84	1004	989	971	988	16.5
93	1032	1016	1010	1019	11.4
105	1082	1055	1044	1060	19.6
126	1141	1112	1091	1115	25.1
160	1183	1170	1149	1167	17.2
174	1200	1188	1170	1186	15.1
188	1215	1200	1188	1201	13.5
203	1226	1210	1202	1213	12.2
237	1256	1251	1248	1252	4.0
260	1274	1278	1274	1275	2.3
315	1300	1311	1313	1308	7.0
387	1332	1367	1347	1349	17.6
464	1341	1377	1360	1359	18.0

Table B-12. Accumulated gas measurement for PET Wells 5 wt% (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #34	Bioreactor #35	Bioreactor #36		
0	0	0	0	0	0.0
6	77	75	78	77	1.5
12	198	210	184	197	13.0
23	458	425	450	444	17.2
35	688	675	680	681	6.6
50	840	844	866	850	14.0
62	872	893	944	903	37.0
66	895	910	961	922	34.6
75	945	937	988	957	27.4
84	971	963	1015	983	28.0
93	1003	993	1038	1011	23.6
105	1039	1027	1068	1045	21.1
126	1099	1080	1113	1097	16.6
160	1155	1160	1176	1164	11.0
174	1170	1190	1195	1185	13.2
188	1181	1215	1212	1203	18.8
203	1185	1236	1227	1216	27.2
237	1211	1288	1254	1251	38.6
260	1232	1315	1254	1267	43.0
315	1266	1340	1290	1299	37.8
387	1284	1356	1318	1319	36.0
464	1299	1365	1322	1329	33.5

Table B-13. Accumulated gas measurement for manure only (1st run) (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #43	Bioreactor #44	Bioreactor #45		
0	0	0	0	0	0.0
6	81	84	80	82	2.1
12	194	225	211	210	15.5
23	403	444	444	430	23.7
35	652	683	680	672	17.1
50	831	862	858	850	16.9
62	867	884	894	882	13.7
66	883	894	913	897	15.2
75	898	920	941	920	21.5
84	928	951	965	948	18.7
93	955	984	1003	981	24.2
105	991	1013	1037	1014	23.0
126	1045	1063	1072	1060	13.7
160	1091	1128	1132	1117	22.6
174	1101	1152	1155	1136	30.3
188	1107	1172	1175	1151	38.4
203	1109	1185	1187	1160	44.5
237	1146	1220	1223	1196	43.6
260	1167	1237	1241	1215	41.6
315	1193	1266	1289	1249	50.1
387	1211	1324	1356	1297	76.2
464	1222	1336	1369	1309	77.1

Table B-14. Accumulated gas measurement for manure only (2nd run) (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor Q1	Bioreactor Q2	Bioreactor Q3		
0	0	0	0	0	0.0
6	32	34	29	32	2.5
12	160	163	154	159	4.6
15	262	248	247	252	8.4
23	410	401	356	389	28.9
26	443	441	392	425	28.9
32	464	536	461	487	42.5
40	524	644	578	582	60.1
50	679	717	650	682	33.6
64	800	843	780	808	32.2
78	912	948	902	921	24.2
100	1044	1066	1011	1040	27.7
117	1164	1151	1133	1149	15.6
169	1217	1191	1178	1195	19.9
209	1262	1244	1238	1248	12.5
252	1289	1274	1273	1279	9.0

Table B-15. Accumulated gas measurement for cellulose 0.55g (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor R1	Bioreactor R2	Bioreactor R3		
0	0	0	0	0	0.0
6	35	33	32	33	1.5
12	205	204	215	208	6.1
15	375	412	467	418	46.3
23	546	626	697	623	75.5
26	616	701	761	693	72.9
32	805	780	830	805	25.0
40	971	952	997	973	22.6
50	1142	1112	1136	1130	15.9
64	1322	1284	1297	1301	19.3
78	1467	1419	1407	1431	31.7
100	1637	1557	1519	1571	60.2
117	1735	1662	1614	1670	60.9
169	1782	1715	1665	1721	58.7
209	1837	1775	1727	1780	55.1
252	1874	1817	1762	1818	56.0

Table B-16. Accumulated gas measurement for cellulose 1.10g (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor S1	Bioreactor S2	Bioreactor S3		
0	0	0	0	0	0.0
6	35	40	38	38	2.5
12	245	208	228	227	18.5
15	483	449	473	468	17.5
23	756	699	718	724	29.0
26	836	777	804	806	29.5
32	961	895	978	945	43.8
40	1052	1091	1187	1110	69.5
50	1153	1266	1320	1246	85.2
64	1315	1402	1452	1390	69.3
78	1465	1513	1592	1523	64.1
100	1565	1753	1712	1677	98.9
117	1716	1861	1937	1838	112.3
169	1759	1922	1960	1880	106.8
209	1814	1972	1996	1927	98.9
252	1837	1985	2013	1945	94.6

Table B-17. Accumulated gas measurement for LDPE/LLDPE 0 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #52	Bioreactor #53	Bioreactor #54		
0	0	0	0	0	0.0
6	46	44	49	46	2.5
12	110	105	113	109	4.0
23	192	177	194	188	9.3
35	452	387	374	404	41.8
50	567	482	510	520	43.3
62	595	511	532	546	43.7
66	617	535	577	576	41.0
75	666	565	589	607	52.8
84	716	605	639	653	56.9
93	754	641	682	692	57.2
105	774	669	711	718	52.8
126	812	707	761	760	52.5
160	852	758	800	803	47.1
174	868	773	817	819	47.5
188	881	787	832	833	47.0
203	893	798	843	845	47.5
237	917	827	867	870	45.1
260	932	827	877	879	52.5
315	955	855	905	905	50.0
387	973	872	931	925	50.7
464	988	885	951	941	52.2

Table B-18. Accumulated gas measurement for LDPE/LLDPE Ecologic 1 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #55	Bioreactor #56	Bioreactor #57		
0	0	0	0	0	0.0
6	51	41	48	47	5.1
12	100	95	128	108	17.8
23	210	195	273	226	41.4
35	490	365	457	437	64.8
50	565	435	547	516	70.4
62	591	480	600	557	66.8
66	609	506	624	580	64.2
75	660	544	658	621	66.4
84	701	595	707	668	63.0
93	727	642	752	707	57.7
105	739	674	782	732	54.4
126	776	719	838	778	59.5
160	815	760	877	817	58.5
174	826	774	891	830	58.6
188	835	784	902	840	59.2
203	841	793	912	849	59.9
237	871	818	935	875	58.6
260	871	818	960	883	71.8
315	910	845	998	918	76.8
387	931	869	1027	942	79.6
464	937	887	1031	952	73.1

Table B-19. Accumulated gas measurement for LDPE/LLDPE Ecologic 5 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #58	Bioreactor #59	Bioreactor #60		
0	0	0	0	0	0.0
6	49	52	53	51	2.1
12	103	102	115	107	7.2
23	132	163	171	155	20.6
35	157	452	289	299	147.8
50	157	582	379	373	212.6
62	230	606	443	426	188.6
66	290	624	505	473	169.3
75	400	653	577	543	129.8
84	515	686	637	613	88.1
93	652	735	688	692	41.6
105	716	765	719	733	27.5
126	804	823	762	796	31.2
160	886	868	814	856	37.5
174	909	878	826	871	41.9
188	928	886	836	883	46.1
203	943	893	844	893	49.5
237	984	920	876	927	54.3
260	1010	944	890	948	60.1
315	1036	988	923	982	56.7
387	1066	1018	950	1011	58.3
464	1077	1030	964	1024	56.8

Table B-20. Accumulated gas measurement for LDPE/LLDPE Symphony 1 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #61	Bioreactor #62	Bioreactor #63		
0	0	0	0	0	0.0
6	56	47	49	51	4.7
12	120	107	115	114	6.6
23	165	267	255	229	55.7
35	200	477	445	374	151.5
50	200	550	642	464	233.2
62	247	568	687	501	227.6
66	339	583	704	542	185.9
75	562	621	741	641	91.2
84	612	657	780	683	87.0
93	671	686	825	727	84.9
105	671	711	851	744	94.5
126	740	748	905	798	93.0
160	813	787	952	851	88.7
174	826	801	972	866	92.4
188	837	812	989	879	95.8
203	845	822	1001	889	97.4
237	878	851	1034	921	98.8
260	887	861	1046	931	100.2
315	916	888	1074	959	100.3
387	940	910	1100	983	102.1
464	948	929	1109	995	98.9

Table B-21. Accumulated gas measurement for LDPE/LLDPE Symphony 5 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #64	Bioreactor #65	Bioreactor #66		
0	0	0	0	0	0.0
6	48	49	54	50	3.2
12	150	114	118	127	19.7
23	380	174	161	238	122.9
35	640	409	181	410	229.5
50	753	638	206	532	288.4
62	767	664	269	567	262.9
66	785	704	358	616	226.8
75	822	752	496	690	171.6
84	869	783	578	743	149.5
93	897	828	662	796	120.8
105	914	860	712	829	104.6
126	942	919	767	876	95.1
160	983	953	822	919	85.6
174	996	965	842	934	81.5
188	1007	974	858	946	78.3
203	1016	982	870	956	76.4
237	1044	1005	901	983	73.9
260	1056	1017	901	991	80.6
315	1086	1044	934	1021	78.5
387	1104	1073	957	1045	77.5
464	1109	1089	972	1057	74.0

Table B-22. Accumulated gas measurement for LDPE/LLDPE Wells 1 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #67	Bioreactor #68	Bioreactor #69		
0	0	0	0	0	0.0
6	45	47	47	46	1.2
12	117	212	115	148	55.4
23	229	462	139	277	166.7
35	433	612	159	401	228.2
50	572	728	159	486	294.0
62	592	745	234	524	262.3
66	617	766	324	569	224.9
75	658	803	464	642	170.1
84	709	865	493	689	186.8
93	738	910	653	767	130.9
105	761	935	846	847	87.0
126	800	969	1041	937	123.7
160	844	1010	1128	994	142.7
174	858	1031	1145	1011	144.5
188	871	1046	1160	1026	145.6
203	881	1057	1170	1036	145.6
237	909	1092	1205	1069	149.4
260	909	1119	1223	1084	160.0
315	937	1153	1245	1112	158.1
387	969	1177	1270	1139	154.1
464	971	1197	1281	1150	160.3

Table B-23. Accumulated gas measurement for LDPE/LLDPE Wells 5 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #70	Bioreactor #71	Bioreactor #72		
0	0	0	0	0	0.0
6	49	49	50	49	0.6
12	111	148	187	149	38.0
23	281	358	397	345	59.0
35	526	528	592	549	37.5
50	676	626	639	647	25.9
62	705	654	664	674	27.0
66	737	669	676	694	37.4
75	777	704	722	734	38.0
84	821	750	766	779	37.2
93	864	780	801	815	43.7
105	900	806	816	841	51.6
126	939	849	851	880	51.4
160	981	887	885	918	54.9
174	997	901	901	933	55.4
188	1008	913	918	946	53.5
203	1018	922	928	956	53.8
237	1047	949	956	984	54.7
260	1060	963	968	997	54.6
315	1089	992	997	1026	54.6
387	1115	1022	1012	1050	56.8
464	1132	1036	1019	1062	60.9

Table B-24. Accumulated gas measurement for PET 0 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #73	Bioreactor #74	Bioreactor #75		
0	0	0	0	0	0.0
6	47	49	50	49	1.5
12	113	105	105	108	4.6
23	195	159	245	200	43.2
35	365	338	499	401	86.2
50	522	408	588	506	91.1
62	572	569	625	589	31.5
66	597	605	640	614	22.9
75	648	674	659	660	13.1
84	687	749	701	712	32.5
93	731	807	739	759	41.8
105	746	851	771	789	54.8
126	799	900	808	836	55.9
160	842	959	872	891	60.8
174	850	970	886	902	61.6
188	856	979	897	911	62.6
203	861	986	906	918	63.3
237	879	1015	930	941	68.7
260	879	1024	930	944	73.6
315	905	1053	951	970	75.7
387	926	1076	978	993	76.2
464	937	1087	984	1003	76.7

Table B-25. Accumulated gas measurement for PET Ecologic 1 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #76	Bioreactor #77	Bioreactor #78		
0	0	0	0	0	0.0
6	54	48	47	50	3.8
12	160	118	107	128	28.0
23	355	258	158	257	98.5
35	545	494	208	416	181.6
50	595	583	261	480	189.5
62	620	622	359	534	151.3
66	630	642	404	559	134.1
75	648	695	506	616	98.4
84	687	749	540	659	107.3
93	732	793	589	705	104.7
105	770	830	679	760	76.0
126	813	885	741	813	72.0
160	867	932	779	859	76.8
174	882	944	791	872	77.0
188	894	954	801	883	77.1
203	903	961	809	891	76.7
237	930	990	834	918	78.7
260	940	1018	844	934	87.2
315	977	1047	868	964	90.2
387	999	1069	893	987	88.6
464	1013	1073	899	995	88.4

Table B-26. Accumulated gas measurement for PET Ecologic 5 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #79	Bioreactor #80	Bioreactor #81		
0	0	0	0	0	0.0
6	50	51	52	51	1.0
12	122	105	112	113	8.5
23	217	150	312	226	81.4
35	489	340	487	439	85.5
50	599	470	717	595	123.5
62	619	509	734	621	112.5
66	638	528	753	640	112.5
75	673	572	789	678	108.6
84	708	634	834	725	101.1
93	774	675	869	773	97.0
105	804	712	890	802	89.0
126	835	747	923	835	88.0
160	882	788	957	876	84.7
174	897	799	969	888	85.3
188	910	809	980	900	86.0
203	917	815	991	908	88.4
237	944	845	1006	932	81.2
260	954	854	1016	941	81.7
315	977	883	1035	965	76.7
387	1001	903	1054	986	76.6
464	1013	905	1074	997	85.6

Table B-27. Accumulated gas measurement for PET Wells 1 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #82	Bioreactor #83	Bioreactor #84		
0	0	0	0	0	0.0
6	46	47	46	46	0.6
12	107	107	170	128	36.4
23	143	134	482	253	198.4
35	149	173	658	327	287.2
50	149	218	700	356	300.2
62	291	421	721	478	220.5
66	380	492	738	537	183.1
75	483	600	738	607	127.6
84	613	698	758	690	72.9
93	790	767	794	784	14.6
105	947	814	828	863	73.1
126	1127	874	874	958	146.1
160	1238	917	911	1022	187.1
174	1268	933	920	1040	197.3
188	1293	946	926	1055	206.4
203	1312	956	931	1066	213.1
237	1373	983	951	1102	234.9
260	1373	983	951	1102	234.9
315	1426	1013	970	1136	251.8
387	1472	1053	989	1171	262.3
464	1487	1066	999	1184	264.5

Table B-28. Accumulated gas measurement for PET Wells 5 wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #85	Bioreactor #86	Bioreactor #87		
0	0	0	0	0	0.0
6	38	44	50	44	6.0
12	98	169	113	127	37.4
23	170	269	126	188	73.2
35	425	454	156	345	164.3
50	634	508	156	433	247.7
62	670	526	259	485	208.5
66	707	541	326	525	191.0
75	747	571	456	591	146.6
84	818	610	581	670	129.3
93	847	648	730	742	100.0
105	871	682	819	791	97.6
126	905	723	903	844	104.5
160	941	768	991	900	117.0
174	955	784	1014	918	119.5
188	964	795	1032	930	122.0
203	971	805	1045	940	122.9
237	991	836	1083	970	124.8
260	991	836	1107	978	136.0
315	1013	869	1145	1009	138.0
387	1024	890	1184	1033	147.2
464	1041	904	1200	1048	148.1

Table B-29. Accumulated gas measurement for manure only (1st run) wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #94	Bioreactor #95	Bioreactor #96		
0	0	0	0	0	0.0
6	50	39	42	44	5.7
12	110	83	104	99	14.2
23	246	187	244	226	33.5
35	412	482	524	473	56.6
50	553	542	615	570	39.4
62	583	590	635	603	28.2
66	606	629	650	628	22.0
75	643	668	700	670	28.6
84	698	709	740	716	21.8
93	740	735	781	752	25.2
105	772	761	798	777	19.0
126	809	792	834	812	21.1
160	844	818	856	839	19.4
174	863	822	865	850	24.3
188	880	825	873	859	29.9
203	895	828	881	868	35.3
237	923	851	905	893	37.5
260	941	859	905	902	41.1
315	963	881	920	921	41.0
387	980	903	942	942	38.5
464	991	912	962	955	40.0

Table B-30. Accumulated gas measurement for manure only (2nd run) wt% (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor N1	Bioreactor N2	Bioreactor N3		
0	0	0	0	0	0.0
6	272	275	273	273	1.5
12	511	512	511	511	0.6
15	611	590	603	601	10.6
23	679	658	673	670	10.8
26	699	676	691	689	11.7
32	736	696	715	716	20.0
40	765	716	742	741	24.5
50	807	750	789	782	29.1
64	868	815	850	844	27.0
78	916	865	925	902	32.4
100	976	920	984	960	34.9
117	1027	957	1023	1002	39.3
169	1038	984	1044	1022	33.0
209	1089	1044	1064	1066	22.5
252	1111	1075	1079	1088	19.7

Table B-31. Accumulated gas measurement for cellulose 0.55g (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor O1	Bioreactor O2	Bioreactor O3		
0	0	0	0	0	0.0
6	382	390	380	384	5.3
12	692	628	700	673	39.5
15	903	828	931	887	53.3
23	1051	909	1061	1007	85.0
26	1086	941	1094	1040	86.1
32	1112	984	1122	1073	77.0
40	1162	1014	1165	1114	86.3
50	1210	1062	1210	1161	85.4
64	1279	1137	1280	1232	82.3
78	1359	1208	1349	1305	84.4
100	1443	1274	1421	1379	91.9
117	1495	1325	1470	1430	91.8
169	1592	1435	1600	1542	93.0
209	1646	1480	1660	1595	100.1
252	1669	1497	1687	1618	104.9

Table B-32. Accumulated gas measurement for cellulose 1.10g (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor P1	Bioreactor P2	Bioreactor P3		
0	0	0	0	0	0.0
6	374	370	391	378	11.2
12	594	765	708	689	87.1
15	872	1094	991	986	111.1
23	1043	1279	1178	1167	118.4
26	1103	1341	1245	1230	119.7
32	1220	1471	1358	1350	125.7
40	1289	1539	1460	1429	127.8
50	1370	1608	1526	1501	120.9
64	1480	1695	1626	1600	109.8
78	1570	1794	1710	1691	113.2
100	1641	1872	1780	1764	116.3
117	1710	1928	1844	1827	110.0
169	1783	1968	1877	1876	92.5
209	1863	2020	1932	1938	78.7
252	1915	2045	1959	1973	66.1

APPENDIX C: SPIKING GAS MEASUREMENT DATA

Table C-1. Spiking gas measurement for bioreactors at 35 °C.

Day	Bioreactors						
	#1	#9	#14	#21	r# 28	#36	#44
0	0	0	0	0	0	0	0
5	5	15	0	9	3	0	0
11	58	69	61	73	49	70	40
20	129	131	126	142	125	145	130
50	140	173	136	159	160	171	165

Table C-2. Spiking gas measurement for bioreactors at 50 °C.

Day	Bioreactors							
	#53	#60	#64	#71	#73	#81	#85	#94
0	0	0	0	0	0	0	0	0
5	89	66	69	68	59	66	76	52
11	138	141	156	133	111	146	157	99
20	172	184	178	173	170	165	180	164
50	183	199	197	185	193	182	201	187

APPENDIX D: ANOVA TABLES

Table D-1. ANOVA table for LDPE/LLDPE samples and controls at 35 °C.

Source	SS	df	MS	F	Prob>F
Columns	1541352	10	154135.2	27.62279	3.63E-10
Error	122760	22	5580		
Total	1664112	32			

Table D-2. ANOVA table for PET samples and controls at 35 °C.

Source	SS	df	MS	F	Prob>F
Columns	1524829	8	190603.7	75.25039	2.98E-12
Error	45592.67	18	2532.926		
Total	1570422	26			

Table D-3. ANOVA table for LDPE/LLDPE samples and controls at 50 °C.

Source	SS	df	MS	F	Prob>F
Columns	3223681	10	322368.1	48.36137	1.23E-12
Error	146648	22	6665.818		
Total	3370329	32			

Table D-4. ANOVA table for PET samples and controls at 50°C.

Source	SS	df	MS	F	Prob>F
Columns	2968047	8	371005.8	25.62477	2.55E-08
Error	260611.3	18	14478.41		
Total	3228658	26			

APPENDIX E: BOXPLOTS

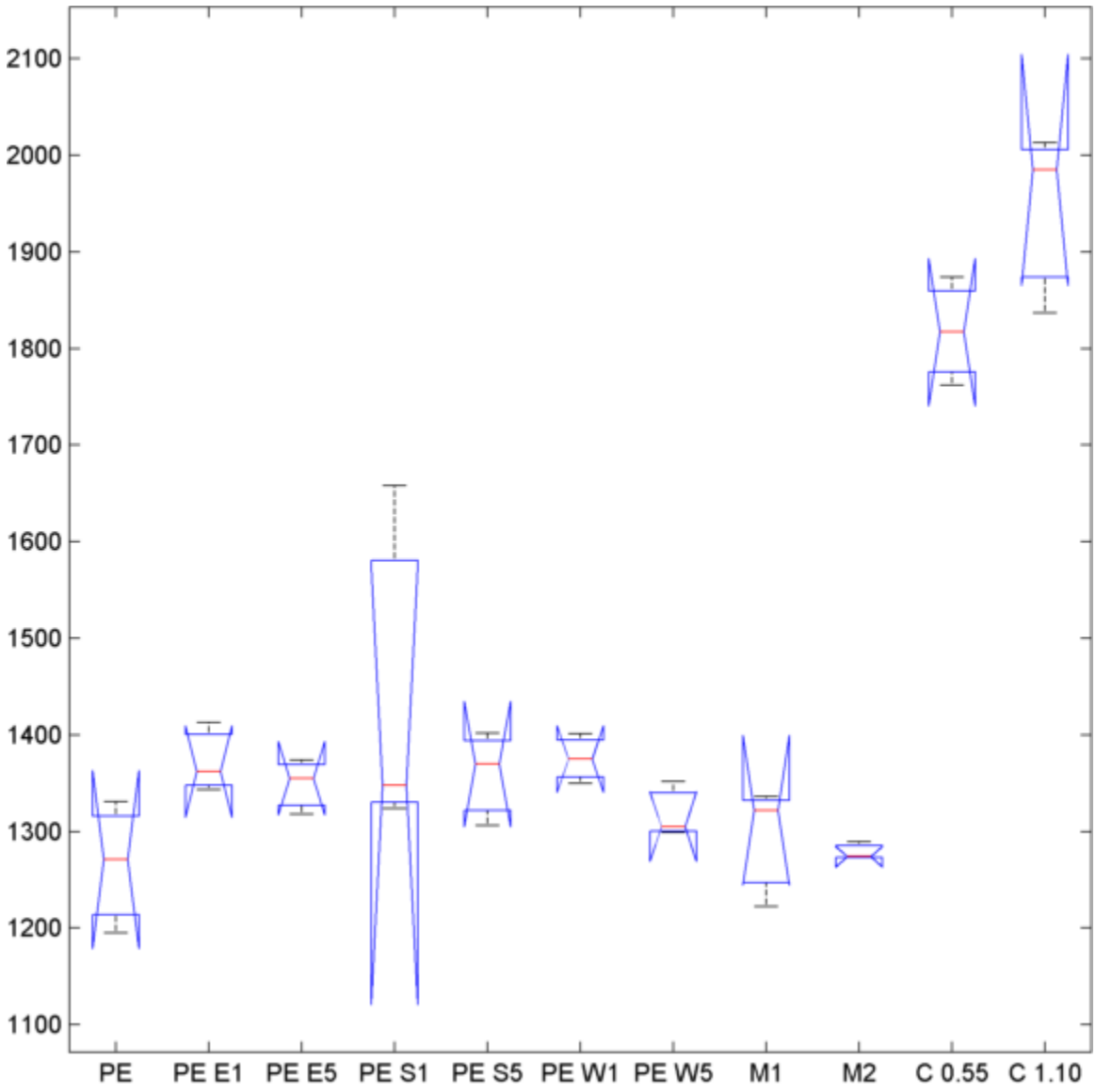


Figure E-1. Boxplots for LDPE/LLDPE samples and controls at 35 °C.

In the box plot, the central line is the median. The edges of the box are the 25th and 75th percentiles. The whiskers are extended to include the most extreme data points. Two medians are

significantly different ($\alpha = 0.05$) if their intervals (from the lower the upper extremes of the notches) do not overlap.

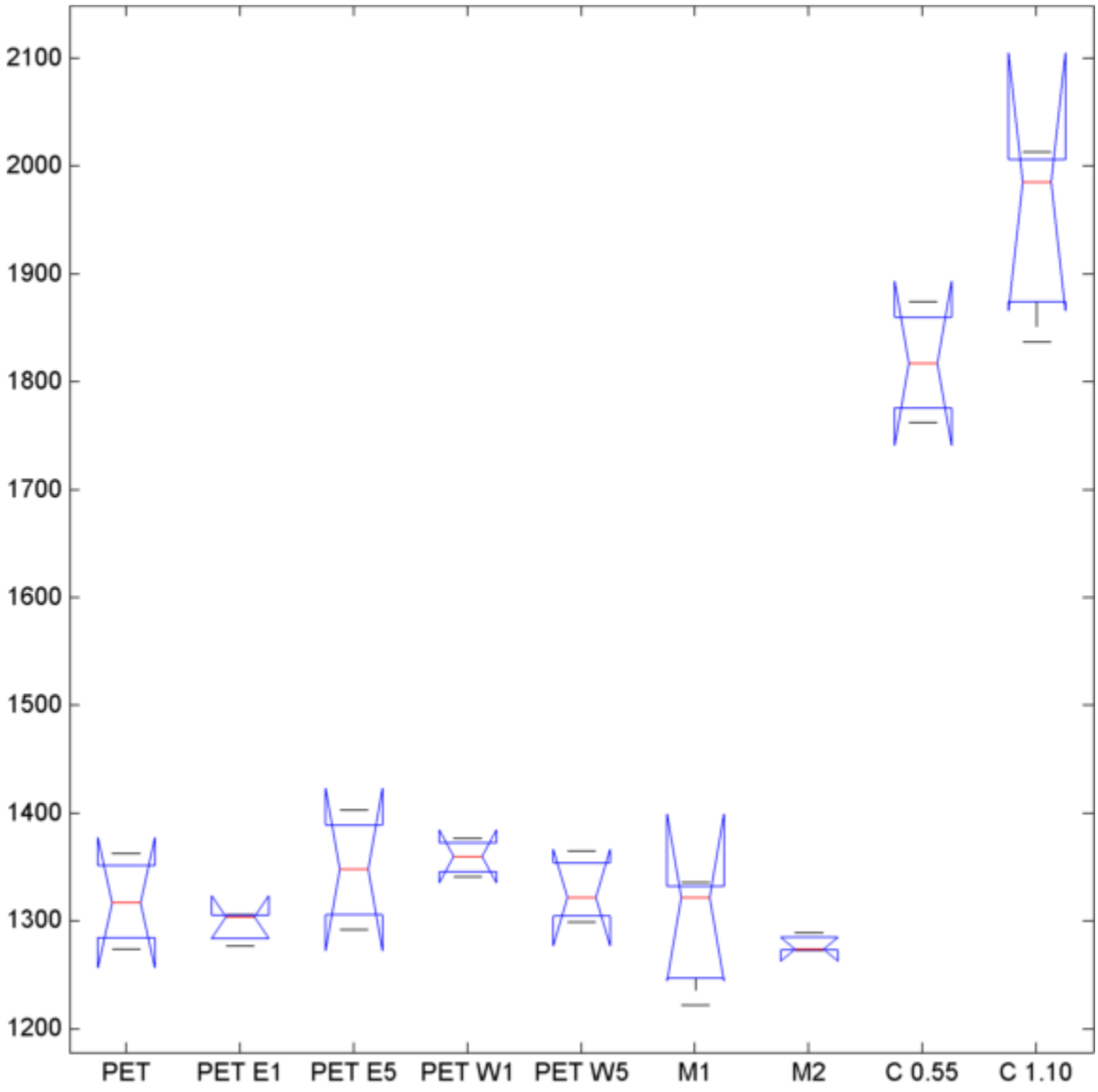


Figure E-2. Boxplots for PET samples and controls at 35 °C.

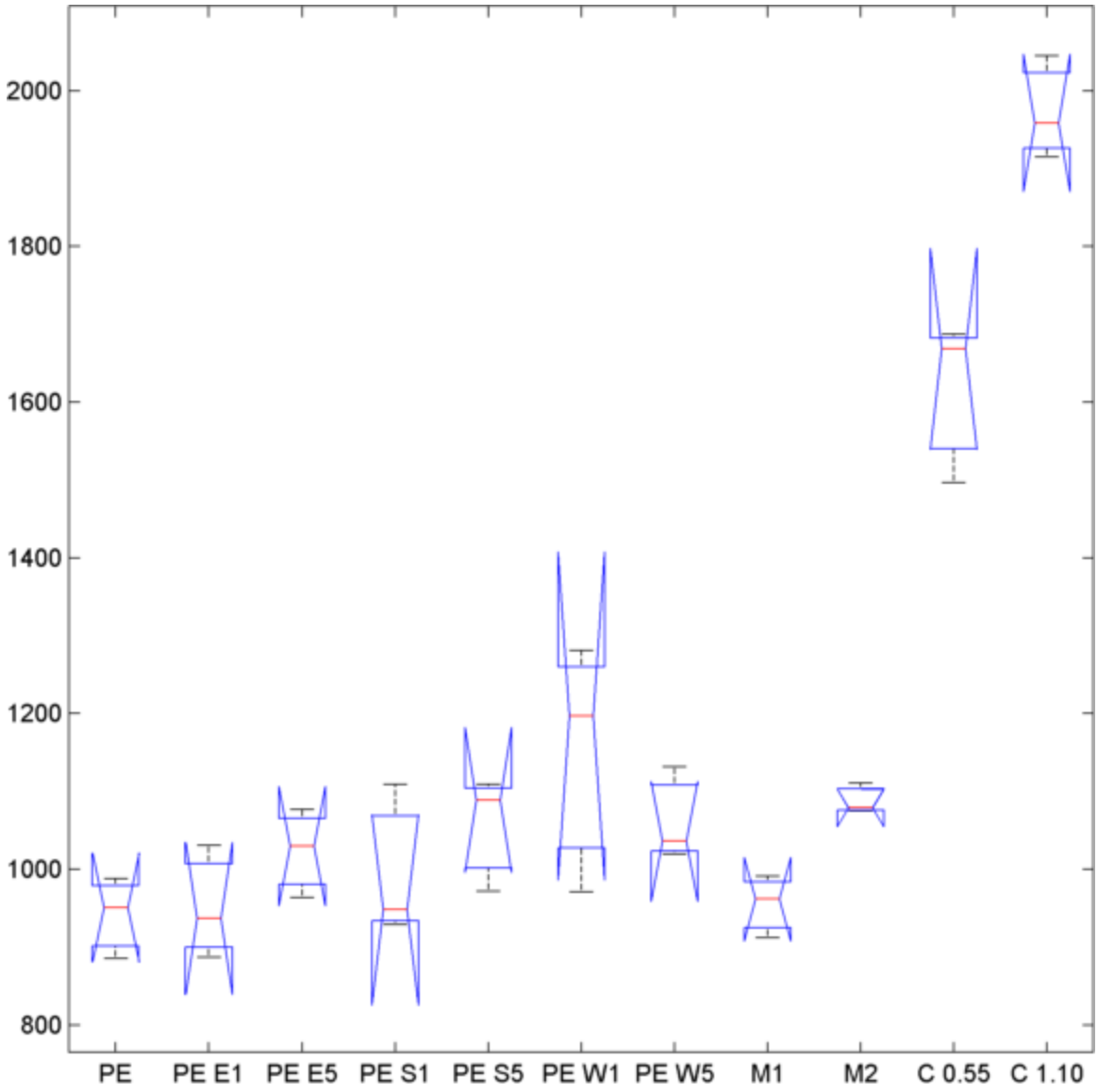


Figure E-3. Boxplots for LDPE/LLDPE samples and controls at 50 °C.

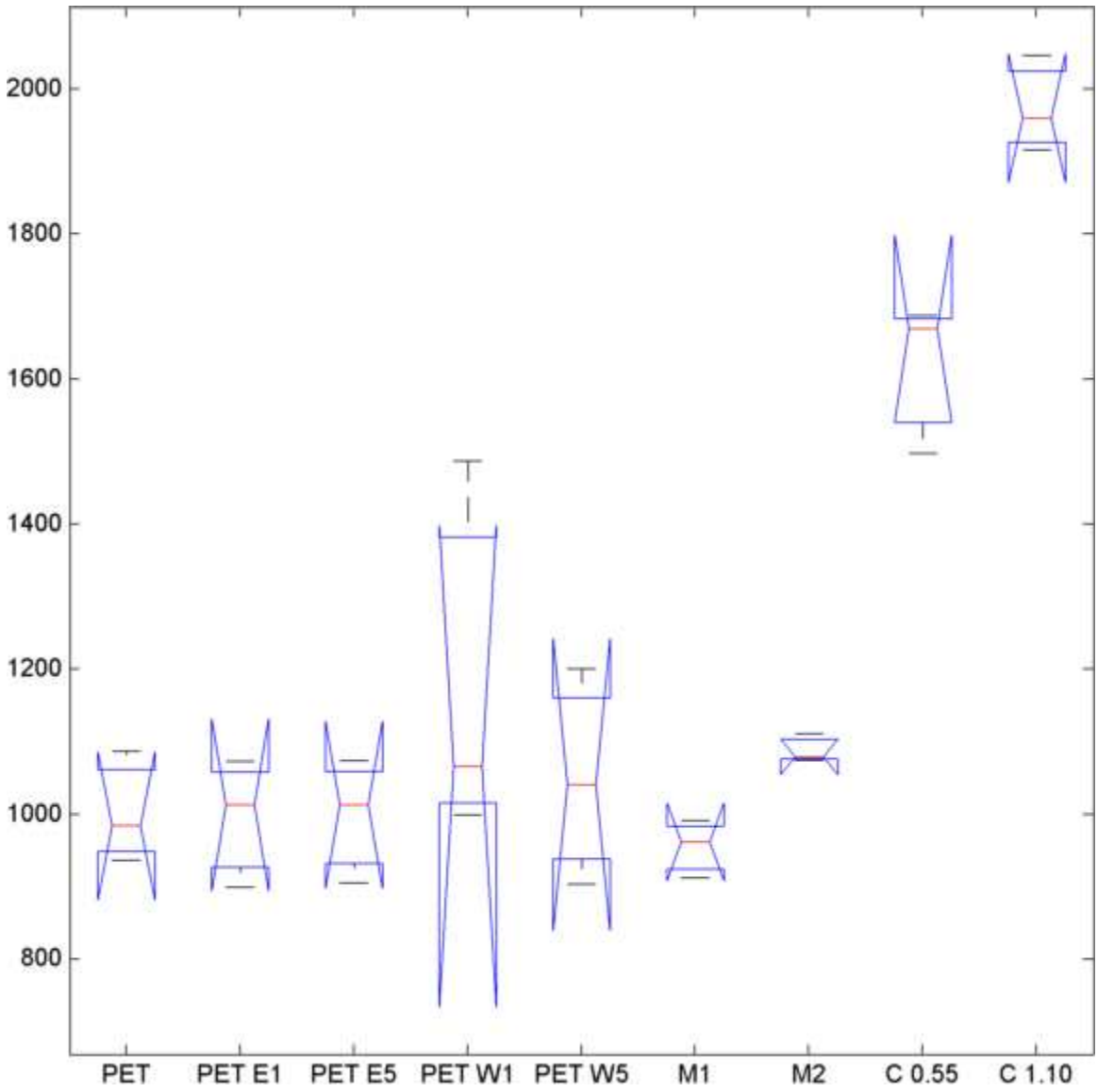


Figure E-4. Boxplots for PET samples and controls at 50 °C.

APPENDIX F: MATLAB CODE FOR PLOTTING MAIN EXPERIMENT DATA

```
clear
close all
clc
addpath(fullfile(pwd,'export_fig'))
format compact

cmap = hsv(12);

%% Interface for choosing dataset:
fprintf('Choose a dataset:\n')
fprintf('  1. LDPE 35C\n')
fprintf('  2. LDPE 50C\n')
fprintf('  3. PET 35C\n')
fprintf('  4. PET 50C\n')
choice = input('Enter a number: ');

switch choice
    case 1
        fileName = 'LDPE 35C';
    case 2
        fileName = 'LDPE 50C';
    case 3
        fileName = 'PET 35C';
    case 4
        fileName = 'PET 50C';
    otherwise
        halt
end

%% Plotting main graph:
load(fullfile(pwd,'Data',[fileName '.mat']))
nOfSamples = size(data,1);
figure('name',fileName);
hold on
box on

for sampleNumber = 1:nOfSamples
    data{sampleNumber,3} = zeros(0,2);

    for n = 1:(numel(data{sampleNumber,2}(:,1))/3)  % Iterate through each three replicates
        a = 3*(n-1) + 1;    % Position of the first replicate
        b = 3*(n-1) + 3;    % Position of the last replicate
        data{sampleNumber,3}(end+1,1) = data{sampleNumber,2}(a,1);  % Copy the dates
```

```
        data{sampleNumber,3}(end,2) = mean(data{sampleNumber,2}(a:b,2));
        data{sampleNumber,3}(end,3) = std(data{sampleNumber,2}(a:b,2));
    end

    H1(sampleNumber) = errorbar(data{sampleNumber,3}(:,1),data{sampleNumber,3}(:,2),...
        data{sampleNumber,3}(:,3),'LineStyle','-','Color',...
        cmap(sampleNumber,:),'LineWidth',0.9);
end

hold off
set(gcf, 'Position', [100 10 700 850])
xlim([0,500])
ylim([0,2400])
set(gca,'YTick',0:400:2400)
xlabel('Time, d')
ylabel('Accumulated gas, mL')
H1_legend = legend(H1,data(:,1),'location','SouthEast');
set(H1_legend, 'Box', 'off')
set(H1_legend,'FontSize',10);
set(gcf, 'Color', 'w');

%% Save figure as a high quality png:
export_fig(fullfile(pwd,'Images',sprintf('%s.png',fileName)),'-png');
```

APPENDIX G: MATLAB CODE FOR PLOTTING SPIKING EXPERIMENT DATA

```
clear
close all
clc
addpath(fullfile(pwd,'export_fig'))
format compact

cmap = hsv(12);

%% Interface for choosing dataset:
fprintf('Choose a dataset:\n')
fprintf('  1. LDPE 35C\n')
fprintf('  2. LDPE 50C\n')
fprintf('  3. PET 35C\n')
fprintf('  4. PET 50C\n')
choice = input('Enter a number: ');

switch choice
   case 1
      fileName = 'LDPE 35C';
   case 2
      fileName = 'LDPE 50C';
   case 3
      fileName = 'PET 35C';
   case 4
      fileName = 'PET 50C';
   otherwise
      halt
end

%% Plotting spiking data:
load(fullfile(pwd,'Data',[fileName ' spike.mat']))
nOfSamples = size(spikedata,1);
legend_string = cell(0,1);
hold on
box on

for sampleNumber = 1:nOfSamples
   if ~isempty(spikedata{sampleNumber,1})
      plot(spikedata{sampleNumber,2}(21:end,1),...
         spikedata{sampleNumber,2}(21:end,2),'-',...
         'Color',cmap(sampleNumber,:),'LineWidth',0.9);
      legend_string{end+1,1} = spikedata{sampleNumber,1};
   end
```

```
end

hold off
set(gcf, 'Position', [100 10 700 500])
xlim([387,514])
xlabel('Time, d')
ylabel('Accumulated gas, mL')
H1_legend = legend(legend_string,'location','SouthEast');
set(H1_legend, 'Box', 'off')
set(H1_legend,'FontSize',10);
set(gcf, 'Color', 'w');

%% Save figure as a high quality png:
export_fig(fullfile(pwd,'Images',sprintf('%s spike.png',fileName)),'-png');
```

APPENDIX H: MATLAB CODE FOR STATISTICAL ANALYSIS

```
clear
close all
clc
addpath(fullfile(pwd,'export_fig'))
format longG
format compact

%% Interface for choosing dataset:
fprintf('Choose a dataset:\n')
fprintf('  1. LDPE 35C\n')
fprintf('  2. LDPE 50C\n')
fprintf('  3. PET 35C\n')
fprintf('  4. PET 50C\n')
choice = input('Enter a number: ');

switch choice
    case 1
        fileName = 'LDPE 35C';
    case 2
        fileName = 'LDPE 50C';
    case 3
        fileName = 'PET 35C';
    case 4
        fileName = 'PET 50C';
    otherwise
        halt
end

%% Loading data:
load(fullfile(pwd,'Data',[fileName '.mat']))
nOfSamples = size(data,1);
group = cell(0,1);

% Import data from day 464 from main experiment:
X = zeros(0,nOfSamples);

for sampleNumber = 1:nOfSamples-3
    X(1,sampleNumber) = data{sampleNumber,2}(64,2);
    X(2,sampleNumber) = data{sampleNumber,2}(65,2);
    X(3,sampleNumber) = data{sampleNumber,2}(66,2);
    group{sampleNumber,1} = data{sampleNumber,1};
end
```

```
% Import data from day 252 from positive control experiment:
for sampleNumber = nOfSamples-2:nOfSamples
   X(1,sampleNumber) = data{sampleNumber,2}(46,2);
   X(2,sampleNumber) = data{sampleNumber,2}(47,2);
   X(3,sampleNumber) = data{sampleNumber,2}(48,2);
   group{sampleNumber,1} = data{sampleNumber,1};
end

%% ANOVA:
% p: p-value.
% table: ANOVA table.
% stats: structure stats used to perform a follow-up multiple comparison test.
% Note: Notches in the boxplot provide a test of group medians.
[p,table,stats] = anova1(X,group);
fprintf('\nANOVA table:\n')
disp(table)
set(gcf, 'Color', 'w');
set(gcf, 'Position', [50 10 700 700])
export_fig(fullfile(pwd,'Images',sprintf('boxplot %s.png',fileName)),'-png');

%% Multiple comparison test using Tukey's HSD:
% 'alpha' = 0.05: Set alpha value to be 0.05.
% 'ctype' = 'hsd': Use Tukey's honestly significant difference criterion.
% 'estimate' = 'anova2': Either 'column' (the default) or 'row' to compare
% column or row means.
figure
[c,m,h,group] = multcompare(stats,'alpha',0.05,'ctype','hsd','estimate','anova2');
set(gcf, 'Color', 'w');
set(gcf, 'Position', [780 10 700 700])
export_fig(fullfile(pwd,'Images',sprintf('HSD %s.png',fileName)),'-png');

% Mean estimates and the standard errors:
fprintf('\nMean estimates and the standard errors:\n')
fprintf('  [Sample]        [Estimates]              [SE]\n');
disp([group num2cell(m)])

% Display the comparison results:
for row = 1:size(c,1);
   if 0>c(row,3) & 0<c(row,5)
      c(row,6) = 0;
   else
      c(row,6) = 1;
   end
end

fprintf('\nDisplay the comparison results:\n')
```

```
fprintf(' [Sample 1]    [Sample 2]  [lower limit of CI]    [est. dif. in means]    [upper limit of CI]    [Sig. dif.?]\n');
disp([group(c(:,1)),group(c(:,2)),num2cell(c(:,3:6))])
```

APPENDIX I: MAXIMUM THEORETICAL GAS EVOLUTION FORMULA

Manure was mixed with water to achieve a total solid content of 50 g/L. Chemical Oxygen Demand (COD) of manure was measured to be 74.1 g/L. Since the same manure was used to feed the bioreactor where the inoculum came from, it was assumed that the COD of the inoculum was approximately 74.1 g/L. The COD reduction rate of manure/inoculum was assumed to be 30%. The COD reduction rate of cellulose was assumed to be 100%. Stoichiometrically, 1 g of COD reduction is converted into 0.395 L methane at 35°C, 1 atm [66]. Biogas produced was assumed to be 60% CH_4 and 40% CO_2.

$$V_{gas} = \frac{k \times COD \times \%COD\_reduction \times \frac{0.395\ L\ CH_4}{1g\ COD} \times (V_{manure} + V_{inoculum})}{\%V_{methane}/V_{total}}$$

V_{gas}: total gas produced.

k: conversion factor based on ideal gas law (k = 1 if at 35 °C; k = 1.04868 if at 50 °C).

COD: COD of manure and inoculum (74.1 g/L).

%COD_reduction: percentage of COD reduction (30%).

V_{manure}: Volume of manure (0.075 L).

$V_{inoculum}$: Volume of inoculum (0.0075 L).

$\%V_{methane}/V_{total}$: percentage of methane in total gas volume (60%).

APPENDIX J: WEIGHT OF MANURE

Table J-1. Manure mixture for main experiment.

Replicates	Weight of aluminum pan, g	Weight of wet manure and pan, g	Weight of dry manure and pan, g	Weight of wet manure needed, g
1	1.2752	14.6424	3.4459	3079.0
2	1.279	20.4102	4.3647	3100.0
3	1.2966	20.3788	4.2399	3241.6
Average				3140.2

Note: Ten liters of 5% (w/v) manure was prepared by mixing 3140.2 g wet manure and adding DI water to the required volume.

Table J-2. Manure mixture for positive control experiment.

Replicates	Weight of aluminum pan, g	Weight of wet manure and pan, g	Weight of dry manure and pan, g	Weight of wet manure needed, g
1	1.3272	12.9255	3.0934	328.34
2	1.3317	12.1992	2.9245	341.14
3	1.3421	13.8742	3.2469	328.96
Average				332.82

Note: A liter of 5% (w/v) manure was prepared by mixing 332.82g wet manure and adding DI water to the required volume.

APPENDIX K: AUTOCAD DRAWINGS OF GAS MEASURING APPARATUS

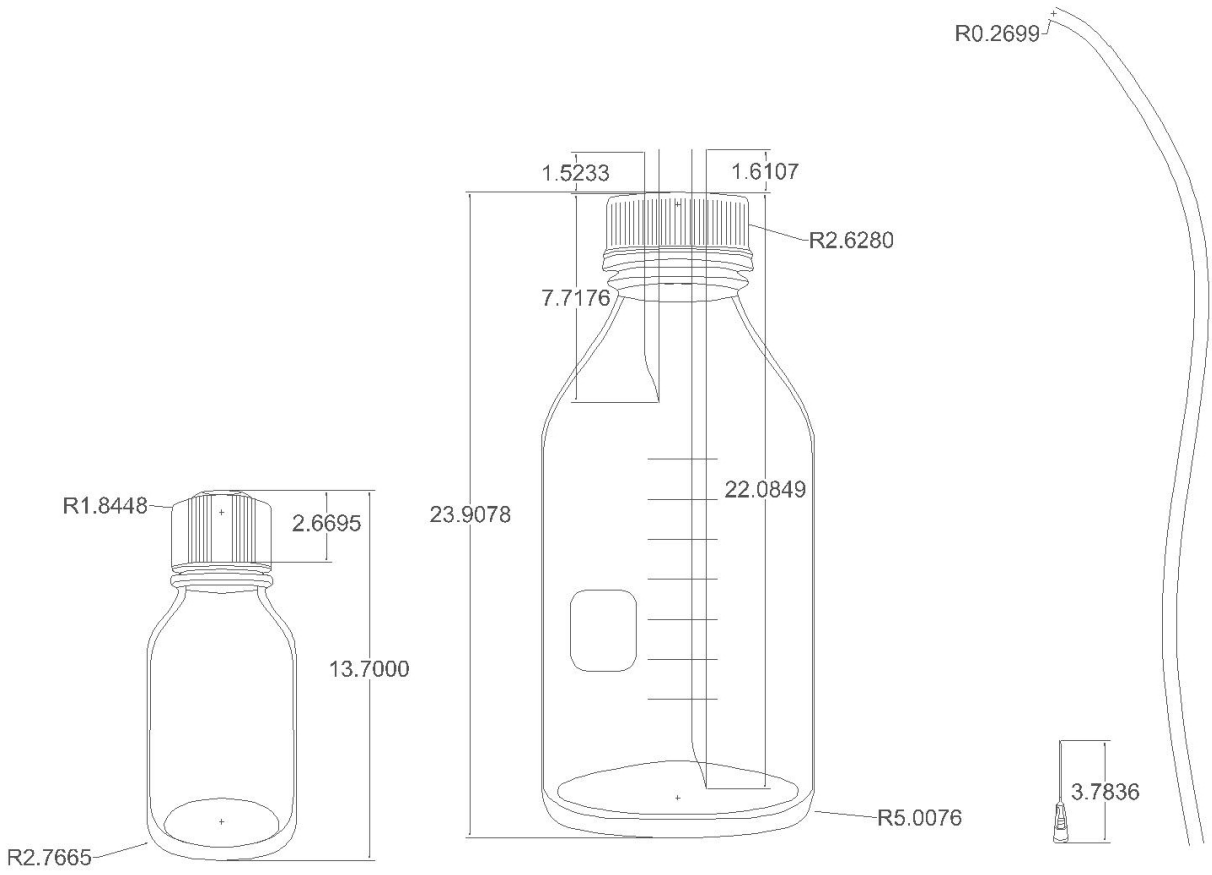


Figure K-1. Dimensions of the components of the gas measuring apparatus.

APPENDIX L: GAS MEASUREMENT FOR ORIGINAL POSITIVE CONTROLS

Table L-1. Accumulated gas measurement for starch (original positive control) (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #37	Bioreactor #38	Bioreactor #39		
6	101	103	106	103	2.5
12	149	158	155	154	4.6
23	229	227	235	230	4.2
35	229	252	260	247	16.1
50	234	277	260	257	21.7

Table L-2. Accumulated gas measurement for cellulose (original positive control) (35 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #40	Bioreactor #41	Bioreactor #42		
6	85	82	87	85	2.5
12	132	167	164	154	19.4
23	187	228	235	217	25.9
35	217	228	265	237	25.1
50	252	253	295	267	24.5

Table L-3. Accumulated gas measurement for starch (original positive control) (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #88	Bioreactor #89	Bioreactor #90		
6	90	76	95	87	9.8
12	140	154	183	159	21.9
23	140	165	218	174	39.8
35	180	195	238	204	30.1
50	220	195	238	218	21.6

Table L-4. Accumulated gas measurement for cellulose (original positive control) (50 °C).

	Accumulative gas (mL)			Average accumulative gas (mL)	Standard deviation
Day	Bioreactor #91	Bioreactor #92	Bioreactor #93		
6	65	63	69	66	3.1
12	122	157	102	127	27.8
23	200	245	124	190	61.2
35	280	310	173	254	72.0
50	308	340	208	285	68.9

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