Laboratory and field scale biodegradability assessment of biocomposite cellphone cases for end of life management

by

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The following individuals certify that they have read, and recommend to the College of Graduate Studies for acceptance, a thesis/dissertation entitled:

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the degree of Master of Applied Science.

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Abstract

The increase in production of biobased plastics as a replacement for fossil fuel-based plastics has created the need for studies to assess their degradation under various conditions. In our case, the biodegradability of cellphone cases was determined under laboratory scale anaerobic and composting assays (58^{o}C) as well as under field scale (60-67^{o}C) composting conditions. The anaerobic assays were conducted under mesophilic (38^{o}C) and thermophilic (55^{o}C) conditions.

In the laboratory scale composting assays, two trials were conducted. The first trial was conducted for 46 days using cellphone cases with dimensions of 7 × 3.5 × 0.2 and 4.6 × 3.5 × 0.2 cm. The second trial was conducted for 34 days and the cellphone cases were 2 × 2 × 0.2 and 4 × 4 × 0.2 cm. The highest biodegradation (21%) was achieved in trial 1 by the 4.6 × 3.5 × 0.2 cm phone cases. The field scale composting conditions achieved 55% weight loss of cellphone cases in 80 days.

During initial anaerobic assay optimization, microcrystalline cellulose was used as a positive control and three different anaerobic seed (inocula) originated from full-scale anaerobic sludge digesters (mesophilic 1, mesophilic 2, and thermophilic) were assessed. A range of food to microorganism ratios (0.5-5 g chemical oxygen demand (COD)/ g volatile solids (VS)) for the microcrystalline cellulose was tested. It was determined that 0.5 g COD/ g $VS_{Inoculum}$ was the optimal food to microorganism ratio to yield the highest methane production.

The subsequent anaerobic biodegradation assays contained three different sized cellphone cases conducted under mesophilic conditions (grinded, 2 × 2 × 0.2 and 4 × 4 × 0.2 cm pieces) for 169 days. The size of cellphone cases did not cause a significant difference in biodegradation under anaerobic conditions. The biodegradation of grinded cellphone cases was also tested under thermophilic conditions for up to 105 days. Mesophilic and thermophilic anaerobic digestion

conditions had similar levels of cellphone case biodegradation (6-8%), which was significantly lower than that of composting. The results agree with literature stating that aerobic processes are more effective to break down complex substrates than their anaerobic counterparts.

Lay Summary

Nowadays, fossil-based plastics are commonly used due to their beneficial features, such as high durability and flexibility as well as being inexpensive. Unfortunately, the plastics also have a low biodegradability and tend to accumulate in landfills, terrestrial, and aquatic environments. Thus, biobased plastics have been created as an alternative to the fossil-based plastics. These bioplastics can degrade in a variety of environments. This study focused on the biodegradation of biocomposite cellphone cases with flax under laboratory and field scale composting conditions. The biodegradation was also assessed under anaerobic conditions to determine if there was an increase in methane production.

Preface

The research presented in this thesis is the original work performed by the author. This thesis was supervised by Dr. Cigdem Eskicioglu at the Bioreactor Technology Group in the School of Engineering, University of British Columbia. Preliminary results from this thesis were presented at the Inaugural Eminence Cluster Trainee Poster Session (February 25, 2020) and the Biocomposite Research Network advisory meeting and research day (May 11, 2020).

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List of Abbreviations

AD	Anaerobic digestion
ANOVA	Analysis of variance
ASTM	American society for testing and materials
ATBC	Acetyl-tri-n-butyl citrate
BMP	Biochemical methane potential
BTG	Bioreactor Technology Laboratory
CBHB	Cellulose-based high barrier
CBHS	Cellulose-based heat sealable
CBnHS	Cellulose-based non heat sealable
CBM	Cellulose-based metallised
CDF	Cellulose diacetate film
COD	Chemical oxygen demand
COVID	Coronavirus
CSTR	Continuous stirred tank reactor
DAS	Dialdehyde starch
DIN	Deutsches Institut für Normung e.V
EG	Ethylene glycol
EPA	Environmental protection agency
EVA	Ethylene-vinyl acetate
GC	Gas chromatograph
HDPE	High-density polyethylene
HRT	Hydraulic retention time

ISO	International standards organization
LA	Lactic acid
LCFA	Long chain fatty acids
LDPE	Low-density polyethylene
MA	Malic acid
MCC	Microcrystalline Cellulose
MPN	Most probable number
OLR	Organic loading rate
OMRR	Organic matter recycling regulation
PBS	Polybutylene succinate
PCL	Polycaprolactone
PEG	Polyethylene glycol
PFF	Poultry feather fiber
PGA	Polyglycolic acid
PHA	Polyhydroxyalkonoate
PHB	Polyhydroxybutyrate
PHH	Poly-3-hydroxyhexanoate
PHO	Poly-3-hydroxyoctanoate
PHV	Poly-3-hydroxyvalerate
PLA	Polylactic acid
PLL	Poly-L-lactic acid
PP	Polypropylene
SA	Succinic acid

SEM	Scanning electron microscope
SRT	Sludge retention time
STP	Standard temperature and pressure
TPDAS	Thermoplastic dialdehyde starch
TPS	Thermoplastic starch
TPU	Thermopolyurethane
TS	Total solids
UV	Ultraviolet
UNI	Ente Nationale Italiano di Unificazione
VFA	Volatile fatty acids
VS	Volatile Solids
WWTP	Wastewater Treatment Plant

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Chapter 1: Introduction

Nowadays plastics made from crude oil are commonly used as they are inexpensive, flexible, and sturdy; however, the plastics have a high durability that prevents them from being biodegraded (Sivan, 2011; Karamanlioglu and Robson, 2013). This arises from their hydrophobicity and high stability. Currently plastics are being produced at an alarmingly high rate of 180 tons per year, with polyethylene accounting for 140 tons per year (Adamcová et al., 2013). Since the 1950s, over 7800 million metric tons of plastic have been produced, with over half of that amount being produced from 2004 to 2017 (Geyer et al., 2017). Annual plastic production was reported to be approximately the entire weight of the human population in 2015 (Worm et al., 2017). Moreover, plastics can take up 20-30% of the volume at landfills. Theoretically, the waste in landfills should be able to decompose; however, the poor degradability of plastics prevents this from occurring and has resulted in a reduction of the life of landfills. (Ishigaki et al., 2004).

Plastic waste has ended up accumulating in terrestrial and aquatic environments, leading to costly clean-ups (Barnes et al., 2009). Furthermore, when exposed to ultraviolet (UV) radiation, these plastics become fragments called microplastics (Sivan, 2011). The small size of the microplastics makes them more difficult to remove from environments. Both macro- and microplastics can be ingested by animals; however, microplastics have a higher chance of being ingested, due to their size. Once ingested, these plastics can block the digestive tracts of animals. The animals can also be harmed by the chemical additives found in many plastics (Barnes et al., 2009).

The deleterious impact of plastics on the environment has led to the development of biodegradable plastics (Karamanlioglu and Robson, 2013). These bioplastics have the possibility to degrade under a variety of conditions including aerobic (composting), anaerobic, and aquatic

conditions. While there are numerous bioplastics available, some such as polylactic acid are more popular and have higher biodegradation performance under composting conditions. This has been determined from numerous composting studies assessing the biodegradation of a variety of bioplastics.

1.1 Motivation of Research

The production of various bioplastics has created a need to determine their biodegradation and end of life fate under various environments including aerobic and anaerobic conditions. As these bioplastics are being created in place of traditional fossil based plastics, it is important to determine their biodegradation behavior and how large the improvements are in comparison to conventional plastics. In our case, we wish to determine the biodegradation of compostable cellphone cases made with agricultural material, such as flax.

1.2 Objectives

The proposed research was intended to determine the end of life fate of the phone cases under uncontrolled and controlled conditions. The specific objectives of this study are as follows:

- Determine the biodegradability (via carbon dioxide production) and weight loss of the cellphone cases under laboratory scale aerobic bioreactor (composting) conditions.
- Determine the weight loss of the cellphone cases under field scale aerobic bioreactor (composting) conditions.
- Determine the biodegradability of the cellphone cases under laboratory scale anaerobic bioreactor conditions via biogas (methane) production

1.3 Thesis Organization

The first chapter, Chapter 1: Introduction, describes the main topic of the research as well as the motivation and the objectives. Chapter 2: Literature review provides a general knowledge of

various bioplastics such as polylactic acid, thermoplastic starch, polycaprolactone, and polyhydroxyalkonoate. The composting process and the standards used for laboratory scale composting experiments were also discussed. Moreover, a general overview of the results from laboratory scale composting and field scale experiments was provided. Following this, anaerobic digestion was described as well as the parameters that effected the efficiency of anaerobic digestion. These parameters include mixing, microbiology, temperature, sludge retention time, feeding regime, and the organic loading rate. Lastly an overview of the biodegradation of various bioplastics under laboratory scale anaerobic conditions was provided. Chapter 3: Materials and methods describes the experiments performed, the materials and equipment used in the assays, and the testing procedures for sample characterization. These experiments included laboratory scale anaerobic and aerobic assays as well as field scale aerobic assays. Chapter 4: Results and discussion provided the results of the experiments and discussed the results. Chapter 5: Conclusion, Limitations, and Future Work, summarizes the results and discusses the possible limitations and possibilities for further research. A flow chart of the thesis organization is provided in Figure 1.1.

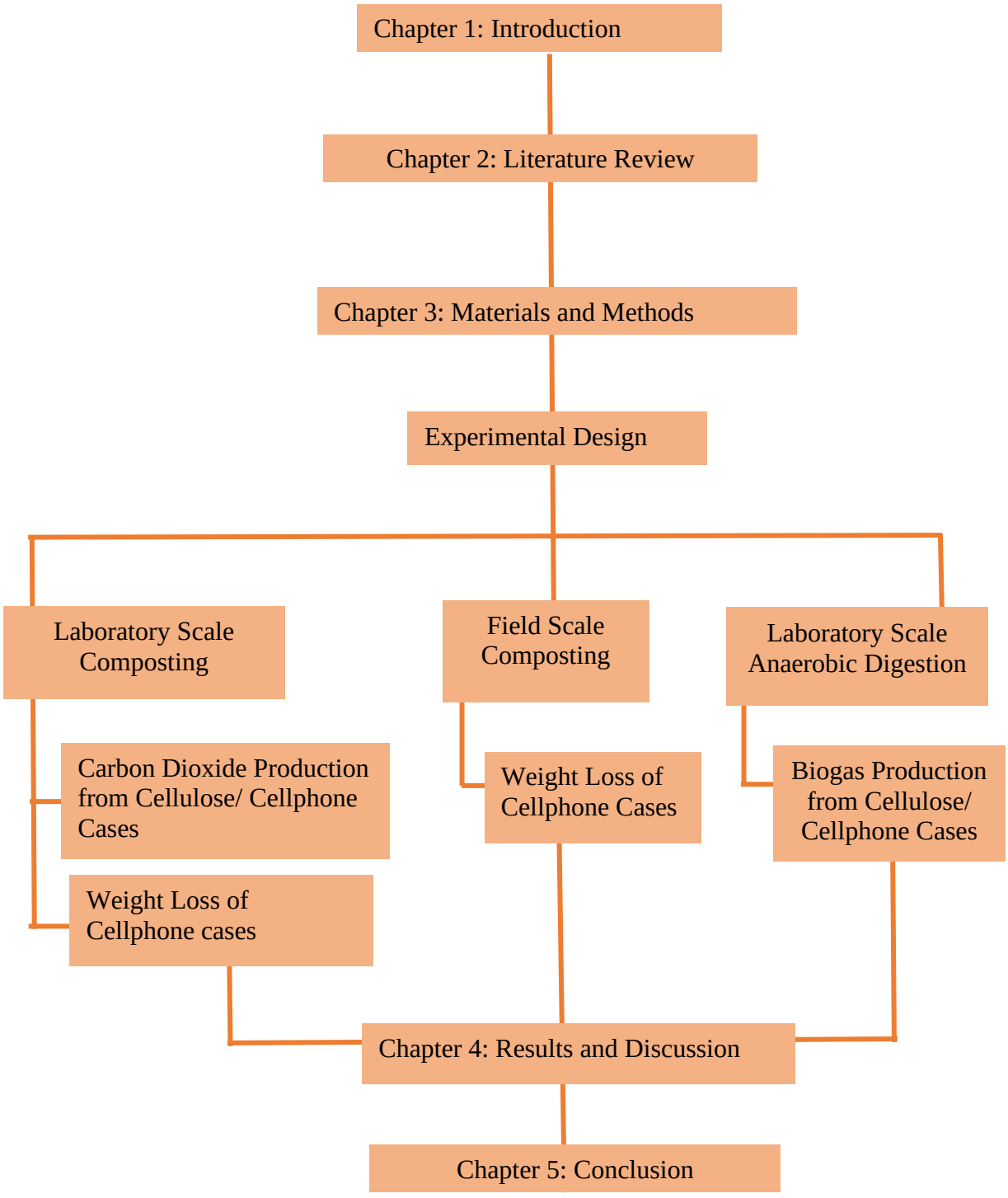

Figure 1-1. Thesis organization flow chart

Chapter 2: Literature Review

2.1 Bioplastics

Biodegradable plastics are an alternative to conventional plastics, that have fewer negative effects on the environment. It is important to note that a biodegradable plastic is not necessarily a compostable plastic (Briassoulis et al., 2010). According to the American Society for Testing and Materials (ASTM) standard D6400-04, a compostable plastic is "a plastic that undergoes degradation by biological processes during composting to yield carbon dioxide, water, inorganic compounds, and biomass at a rate consistent with other known compostable materials and leaves no visually distinguishable or toxic residues". On the other hand, a biodegradable plastic is "a plastic that degrades because of the action of naturally occurring microorganisms such as bacteria, fungi, and algae" (Vaverková et al., 2012). Currently, there are two main types of biodegradable polymers: aliphatic polymers and aliphatic aromatic co-polymers. Aliphatic polymers degrade better than aromatic polymers. Unfortunately, they can be fragile and have a low durability (Adamcová et al., 2017). On the other hand, aromatic polyesters have a high durability and are available at a low cost; however, microorganisms cannot degrade them well (Eubeler et al., 2010). Thus, aliphatic aromatic co-polymers have been created, that have combined the durability of aliphatic polymers and the biodegradability of aromatic polymers (Adamcová et al., 2017). Another method to improve the mechanical properties of aliphatic polymers is to combine different aliphatic polymers. Details about common bioplastics are discussed in the following sections.

2.1.1 Polyglycolic Acid

Polyglycolic acid (PGA) comes in a semi-crystalline form that has good mechanical properties and is commonly used in medical applications due to its biocompatibility (Figure 2.1). It has a hydrophilicity which allows it to degrade quickly. These properties are taken advantage of, when

utilizing PGA in sutures, devices used to fix bones, (ie. rods and plates), carriers of drugs, and cell culture scaffolds (Vieira et al., 2010). PGA has been reported to have a glass transition temperature (T_g), melting temperature (T_m), crystallinity (X_{cr}), and elongation of 35-40^{o}C, 220-225^{o}C, 45-55%, and 15-20% respectively (Middleton and Tipton, 2000).

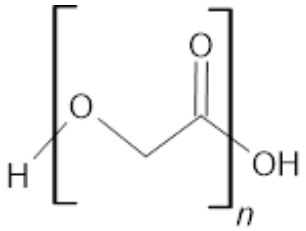

Figure 2-1. Chemical structure of PGA

2.1.2 Polyhydroxyalkonoate

Polyhydroxyalkonoates (PHA) (Figure 2.2) such as polyhydroxybutyrate (PHB) poly-4-hydroxybutyrate (P4HB), poly-3-hydroxyvalerate (PHV), poly-3-hydroxyhexanoate (PHH), poly-3-hydroxyoctanoate (PHO) are aliphatic polymers (Bátori et al., 2018). The average glass transition temperature, melting temperature, crystallinity degree, tensile strength, and elongation at break of PHAs have been reported to be 2^{o}C, 160-174^{o}C, 40-60%, 15-40 MPa, and 1-15% respectively (Bugnicourt et al., 2014). The most commonly used and studied PHA is PHB. PHAs can be used in packaging materials, diapers, and paints (Ahmed et al., 2018). They can be produced by bacteria via the fermentation of sugars and fats. PHB is produced by microorganisms such as, *Alcaligenes latus*, *Cupriavidus necator*, and *Pseudomonas putida*, under stressed conditions including feast/famine or nutrient limited conditions. The PHB can then be extracted from granules present in the microorganisms (Venkiteshwaran et al., 2019). Usually, an extracellular enzyme from a microorganism is required to break down PHBs along their surface. For instance, enzymes used to break down PHB come from microorganisms such as *Alcaligenes faecalis, Rhodospirillum rubrum, Bacillus megaterium, Acinetobacter beijerinckii,* and *Pseudomonas lemoignei* (Bátori et

al., 2018). Another bacterium associated with the breakdown of PHAs is *Pseudomonas stutzeri*, which uses a serine hydrolase. Other microbial genera involved with their biodegradation include *Bacillus, Burkholderia, Nocardiopsis, Cupriavidus, Mycobacterium,* and *Micromycetes* (Ahmed et al., 2018).

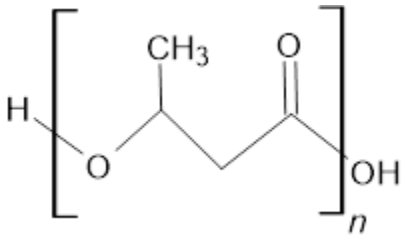

Figure 2-2. Chemical structure of PHA

2.1.3 Polycaprolactone

Polycaprolactone (PCL) (Figure 2.3) is a synthetic aliphatic polyester that can be found in catheters, blood bags, and some forms of packaging (Eubeler et al., 2010). PCL can also be used as a carrier in drug release systems because of its biocompatibility and permeability to drugs (Ponjavic et al., 2019). PCL has been reported to have a glass transition temperature, melting temperature, and elongation of -60^{o}C, 60-65^{o}C, and 15-20% respectively (Middleton and Tipton, 2000). PCL can be degraded well by microorganisms that use lipases and esterases; however, it has a low melting temperature and high cost that has made manufacturers resistant to utilizing it by itself in commercial applications (Bátori et al., 2018; Eubeler et al., 2010). Fortunately, PCL has demonstrated high compatibility with other biopolymers (Narancic et al., 2018). Some microorganisms associated with its biodegradation include, *Clostridium botulinum, Clostridium acetobutylicum,* and *Fusarium solani*. *Aspergilluss* p.ST-01 can also degrade PCL into products such as butyric, succinic, and valeric acids (Ahmed et al., 2018).

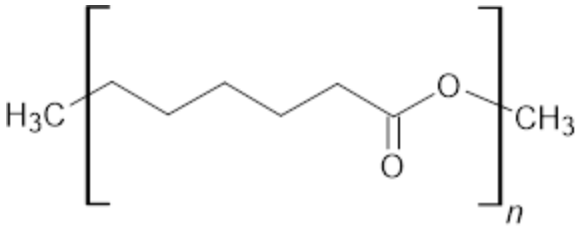

Figure 2-3. Chemical structure of PCL

2.1.4 Polybutylene Succinate

Polybutylene succinate (PBS) (Figure 2.4) is a synthetically produced aliphatic polyester that is considered to be biodegradable (Bátori et al., 2018). It is used in compostable bags, mulching films, nonwoven sheets, textiles catering products, and foams. It claims to be biodegradable in both aerobic and anaerobic conditions. It also has a melting temperature that is similar to polyethylene (Di Lorenzo et al., 2017). PBS has been reported to have a melting temperature of 111-115ºC and a glass transition temperature of -32 to -39ºC (Bautista et al., 2015; Gao et al., 2017; Rudnik, 2013).

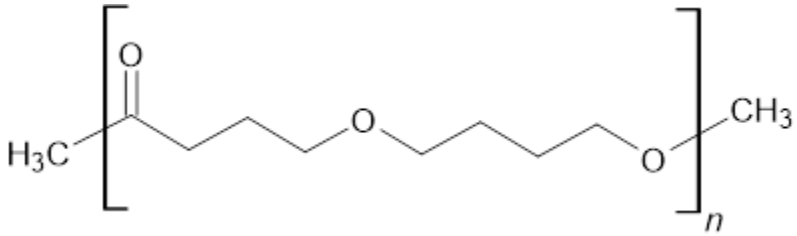

Figure 2-4. Chemical structure of PBS

2.1.5 Thermoplastic Starch

Thermoplastic starch (TPS) is completely biobased and biodegradable; however, it can also be quite brittle. TPS results from the thermal and chemical processing of starch granules. If this process occurs in the presence of water, then the resulting TPS will be very brittle; however, if a plasticizer (ie. glycerol, propylene glycol, glucose, or sorbitol) is present, then its flexibility will increase (Janssen and Moscick, 2009). The biodegradation of TPS results from the microbial biodegradation of glycosidic links between the sugar units (Sessini et al., 2019). Microorganisms

associated with TPS biodegradation include *Micromonospora*, *Nocardia,* and *Streptomycete,* belonging to the phylum *Actinomyctes* (Du et al., 2008).

2.1.6 Polylactic Acid

Polylactic acid (PLA) (Figure 2.5) is an aliphatic polyester that is easy to produce, non-toxic, and compostable (Karamanlioglu and Robson, 2013). PLA is an extremely popular bioplastic that is most commonly used in packaging. It can come in two enantiomeric forms: L-lactide and D-lactide. The L-lactide is slower to degrade and is brittle; thus, it is often combined with PCL to improve its degradability and make the overall bioplastic less brittle (Vieira et al., 2010). Pure PLA has been reported to have a melting temperature of 150-180^{o}C, a glass transition temperature of 52-65^{o}C, and elongation of 3-10% (Middleton and Tipton, 2000; Karamanlioglu and Robson, 2013; Södergård and Stolt, 2002; Carmona et al., 2015).

Normally the biodegradation of PLA involves two steps. First the rate limiting step occurs in which PLA is broken down into smaller fragments in the presence of water. This occurs through the chemical hydrolysis of its ester bonds (Stloukal et al., 2015; Karamanlioglu and Robson, 2013). The fragments are then taken up by microorganisms and eventually converted into water, carbon dioxide, and biomass (Stloukal et al., 2015). Microorganisms that can degrade PLA include *Amycolatopsis*, *Bacillus licheniformis* (from soil), and *Cryptococcus* species (Bátori et al., 2018). Other microorganisms involved in its biodegradation include *Bacillus brevis*, *Amycolatopsis* species, *Penicillium Roquefor*, and *Geobacillus thermoleovoran* (Ahmed et al., 2018; Castro-Aguirre et al., 2018).

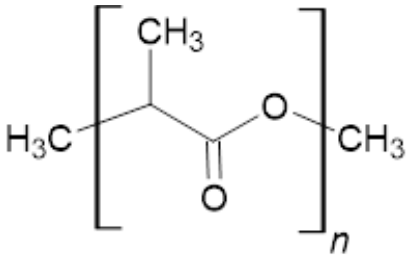

Figure 2-5. Chemical structure of PLA

The importance of temperature and microorganisms for PLA biodegradation were documented when researchers compared the tensile strength of PLA after biodegradation in different conditions. These conditions included microbe rich soil and compost as well as sterile water, soil, and compost at different temperatures (23, 37, 45, 50, and 55°C). It was found that the loss of tensile strength was faster in microbe rich compost at high temperatures than in sterile conditions at high temperatures (45 and 50°C). There was also little to no tensile strength loss at every condition when the temperature was low (23 and 37°C) (Karamanlioglu and Robson, 2013).

2.2 Bioplastic Degradation

The bioplastics mentioned above have the potential to degrade in several managed (controlled) and unmanaged (uncontrolled) environments, such as aquatic environments, industrial composting conditions, and anaerobic digestion. Most of research so far has been conducted under composting conditions, as bioplastics usually exhibit excellent biodegradation under those conditions. Of the three conditions, aquatic environments are the least promising potential environments for end of life bioplastic biodegradation. Research has looked at various environments because bioplastics that can degrade across a wide range of environments offer more economical and environmental advantages (Narancic et al., 2018).

2.2.1 Aerobic Degradation

Composting, being the most commonly used aerobic biodegradation process, involves the biodegradation of organic waste using bacteria and fungi. This process can be conducted in

enclosed reactors, windrows, or compost piles (Metcalf and Eddy, 2014). The thermophilic conditions (55-60^{o}C) of the compost allow for destruction of pathogens that may be present in the waste (Onwosi et al., 2017; Pandey et al., 2016). The final compost can be put into two categories: Class A or Class B. If the Class A compost does not come from biosolids (treated solids residue from wastewater treatment plants), then the compost can be distributed on land as soil amendment with no volume restriction; however, if the Class A compost comes from biosolids then more stringent rules apply. The compost must have a reduction in fecal coliforms (< 1000 most probable numbers or MPN per gram of total solids) and the pile temperature must be held at 45°C for at least 14 days. Moreover, there are limits on trace metals: arsenic, cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, selenium, and zinc. On the other hand, Class B compost allows for the presence of a larger number of fecal coliforms (< 2 000 000 MPN per gram of total solids); however, there are more restriction regarding the land application of Class B compost (OMRR, 2018). A detailed discussion on the composting process is provided in section 2.4.

2.2.2 Anaerobic Digestion

Anaerobic digestion involves the biodegradation of organic matter to methane rich biogas in the absence of oxygen. The methane produced from this process can be used to provide electricity and heat to the wastewater treatment plant or nearby residences depending on the scale of the plant. Digesters can be operated at either mesophilic (35-42^{o}C) or thermophilic (45-60^{o}C) temperatures. This process also produces biosolids which can be applied to land as biofertilizer (Metcalf and Eddy, 2014). A detailed discussion on AD process is provided in section 2.9.

2.2.3 Aquatic Degradation

Human behavior has led to the release of plastics into aquatic environments. These plastics then deleteriously impact aquatic life (Accinelli et al., 2017). Thus, research has been conducted on the potential biodegradability of bioplastics in aquatic environments. For instance, Narancic et al., (2018) looked at the degradability of a wide range of bioplastics in fresh water and marine environments. They concluded that only TPS and PHB are biodegradable in aquatic environments. This means that replacing petroleum-based plastics with any bioplastics, will not mitigate their impact on marine life (Accinelli et al., 2017).

2.3 Composting Process

Composting is an aerobic process that uses microorganisms for the decomposition and humification of organic matter. The process consists of three stages with microbial succession occurring as the stages progress. The first stage is the moderate temperature stage (20-40^{o}C) in which mesophilic microorganisms predominate. The mesophilic organisms quickly degrade the compounds that are soluble and easily degraded. As the mesophiles degrade compounds, they generate heat which causes an increase in temperature. Once the temperature increases past 40^{o}C, thermophilic organisms start to take over. This leads to the next stage, the high temperature stage (>40^{o}C). Due to the high temperature, weed seeds and pathogens are eliminated. During this stage, the thermophiles break down carbohydrates, proteins, and fats using extracellular hydrolytic enzymes. Once these compounds start to decrease in quantity, the thermophiles have less substrate and the temperature starts to decrease. This allows mesophiles to predominate once again in the final stage: the curing stage (10-40^{o}C). The organic matter in the compost also stabilizes (matures), so that it can be used by plants as soil amendment (Mehta et al., 2014; Onwosi et al., 2017).

Composting is considered to be a good waste management option that helps to reduce the amount of materials sent to landfills and subsequently the amount of greenhouse gas emissions from landfills (Cerda et al., 2018). Moreover, compost can be added to soil to increase its fertility and immobilize metals present in soil (Onwosi et al., 2017; Cerda et al., 2018).

There are also potential issues that can arise from composting. This includes the absence of a general consensus on the point at which compost matures. This means that compost that is not mature could be added to soil and end up negatively affecting the plants. Another problem is the emission of gases, such as hydrogen sulfide, that may have a deleterious impact on the environment or have a foul odor. Furthermore, composting can lead to the generation of leachates that have a high organic load and may contain problematic compounds such as heavy metals and chlorinated compounds (Onwosi et al., 2017). There is no opportunity to recover bioenergy from composting and finally, aeration and moisture addition requirements make composting an energy intensive waste management process.

2.3.1 Windrow Composting

Windrow composting is a large-scale composting method that involves placing organic waste into long thin piles that are mixed at regular intervals. The compost is placed on composting pads typically composed of materials such as gravel and rocks. Oxygen enters the system when the piles are mixed using windrow turners. A benefit to turning the compost is the possible reduction of greenhouse gas emissions through the prevention of methane production via anaerobic decomposition. The temperature of the piles is important to monitor to guarantee that during the thermophilic phase, the temperature reaches 55^{o}C, to ensure the destruction of pathogens and weeds (Haug, 1993).

2.3.2 Aerated Static Pile Composting

A variety of materials can be degraded using the aerated static pile method, including plant materials, kitchen waste, and manure; however, the material needs to be mixed with a bulking agent (i.e. sawdust, rice straw, wheat straw, palm tree waste, grass clippings, etc.) first to increase the porosity of the compost and aid in maintaining the structure when the material is formed into a pile (Abdoli et al., 2019; Haug, 1993; Onwosi et al., 2017). This method requires no mixing of the compost, but oxygenated air is required, which is provided using induced draft or forced aeration (Haug, 1993). The temperature can be controlled through the airflow, with a higher airflow causing a decrease in temperature and vice versa (Abdoli et al., 2019).

2.4 Composting Microbiology

Studies have illustrated that numerous types of microorganisms are present during the composting process. Bacteria are the major players in the composting process, as their activity results in the majority of the biodegradation and increase in temperature. The types of bacteria present during the moderate temperature phase (10-40^{o}C) include hydrogen oxidizing, sulfur oxidizing, and nitrogen fixing bacteria. Some genera of these bacteria include *Escherichia, Aeromanoas, Enterococcus, Bacillus,* and *Klebsiella* (Mehta et al., 2014. Other studies have also found the presence of *Lactobacillus* and *Acetobacter* species (Partanen et al., 2010). The high temperature phase (>40^{o}C) consists mostly of *Bacillus* species, such as *B. subtilis, B. polymyxa,* and *B. licheniformis*; although *Actinobacter* species have also been found (Mehta et al., 2014; Partanen et al., 2010). Mesophilic bacteria in their vegetative state have also been found in the high temperature stage (Mehta et al., 2014).

The most abundant bacteria phyla that have been commonly found during the composting process are *Proteobacteria, Firmicutes, Bacteroidetes,* and *Actinobacteria* (Antunes et al., 2016).

This finding is in agreement with the research mentioned above, as *Escherichia*, *Aeromanoas*, and *Klebsiella* belong to the *Proteobacteria* phylum, while *Enterococcus* and *Bacillus* belong to the *Firmicutes* phylum. A study by Antunes et al. (2016) used shotgun DNA, 16S rRNA gene amplicon, and metatranscriptome high-throughput sequencing on compost consisting of solid waste and plant materials. They claimed that the most abundant bacteria were *Rhodothermus marinus, Thermobispora bispora, Symbiobacterium thermophilum, Sphaerobacter thermophilus*, and *Thermobifida fusca*. As a side note, they also found a previously undiscovered bacterial genus, belonging to the order of *Bacillales*. They also determined that the microbes present in the compost, depend on the starting material and the composting procedure, as well as the techniques the researchers used to identify the microbes.

The composition of the compost plays a large part in the composition of the microbial community. A study contrasted the microbial communities in compost composed of manure, hay plus manure, or hardwood plus manure, using Illumina sequencing. They found that the presence of archaea (most commonly *Crenarchaeota* and *Euryarchaeota*) was rare and that the most plentiful bacteria phyla were *Proteobacteria* and *Bacteroidetes* with *γ-proteobacteria* being the most common taxon for the former. A wide range of phyla including *Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Gemmatimonadetes, Planctomycetes, α-Proteobacteria, β-Proteobacteria, δ-Proteobacteria, γ-Proteobacteria*, and *Verrucomicrobi* were found with varying abundance across the different compost compositions. There were four main fungal phyla found: *Ascomycota*, *Basidiomycota, Chytridiomycota*, and *Zygomycota*, with *Ascomycota* being the most abundant. The research indicated that the microbial composition of compost varies greatly, with certain phyla continuously found to be abundant.

The type of composting process also plays an important role in the microbial community composition. A study assessed windrow, aerated static piles, and vermicompost (employed earthworms at mesophilic temperatures), with the former two having more similar compositions. The main phyla that were discovered were *Bacteroidetes, Chlorobi, Chloroflexi, γ-Proteobacteria*, and *Verrucomicrobia*. All three composting methods found *Bacteroidetes* as the most abundant bacteria phyla. Windrow composting had the phyla *Chloroflexi* also in high abundance. The fungal communities found included the phyla: *Ascomycota, Basidiomycota, Zygomycota*, with the phyla *Ascomycota* having the highest abundance (Neher et al., 2013).

As previously documented, the microbial community changes with time, but here it was found that the phyla present across time, especially after the thermophilic phase, were heavily influenced by the type of composting occurring during the curing phase. For instance, *Bacteroidetes* were found in high abundance throughout windrow composting with a slight decrease followed by a slight increase; on the other hand, *Firmicutes* were abundant during the thermophilic stage in aerated static pile composting and vermicomposting but were replaced by *Bacteroidetes* as time went by. Furthermore, *Actinobacteria* seemed to decrease across time for all of the processes (Neher et al., 2013).

2.5 Composting Standards

Currently, there are several standards at both the international and national levels that define norms for testing the compostability of a plastic. The majority of the standards define substrate (organic material treated), inoculum (microbial culture used for biodegradation), and temperature conditions of industrial composting or anaerobic digestion facilities operated under controlled conditions, while a few standards are available for home composting applications and none for field scale composting.

The issue of the first version of ASTM D6400: Standard Specification for Compostable Plastics established the criteria for plastics and products made from plastics to be labelled as compostable in municipal and industrial composting facilities in 1999. The standard identifies complete biodegradation, disintegration, and safety as three assessment criteria and references other ASTM standard documents for testing (i.e., ASTM D5338, 5152, 5951) (Briassoulis et al., 2010). For instance, it references ASTM D5338 which establishes the degree and rate of biodegradation of plastics under controlled composting conditions, in a laboratory. This ASTM requires the monitoring of aeration, temperature, and humidity. The plastic must completely mineralize into carbon dioxide, water, and biomass, in order for it to be considered compostable. The amount of biodegradation is determined by calculating how much of the original carbon content was converted into carbon dioxide (ASTM Standard D5338 – 15, 2015). ASTM D6400 is also considered to be very similar to standards set out in Europe, Japan, Korea, China, and Taiwan (Briassoulis et al., 2010).

Even though there are many ASTM standards, there are a couple of key points that they have set out for plastics to be considered ‘‘compostable in municipal and industrial composting facilities’’. The key points are as follows: (i) 60% of materials made up of single polymers must mineralize within 6 months, (ii) polymers made up of more than one polymer must show 90% mineralization within 6 months, (iii) the plastic must degrade into fragments, with fewer than 10% of those fragments being caught on 2 mm sieves, and (iv) following land application, the materials cannot be toxic or prevent plant growth and heavy metal content of the polymers should be less than 50% of the threshold prescribed by the Environmental Protection Agency (EPA) (Briassoulis et al., 2010).

There are also international standards for the biodegradability of plastics, set out by the International Standards Organization (ISO). These standards are similar to that of the ASTM, CEN (European) and the Deutsches Institut für Normung e.V (DIN) (German) standards. One of the main ISO standards is ISO 17088:2008 which identifies procedures and requirements for plastics that can be treated via composting. This standard is based on two ISO standards: ISO 14855-1:2005 and ISO 14855-2:2007. Both standards evaluate the biodegradability of plastics using the amount of carbon dioxide evolved under controlled composting conditions. ISO 14855-1:2005 uses a solid phase respirometric test system to measure the amount of evolved carbon dioxide; whereas, ISO 14855-2:2007 uses a gravimetric method. The two standards also differ in terms of the amount of compost and test item used. Furthermore, ISO 14855-1 and ASTM D5338 have similar guidelines but with some technical differences (Briassoulis et al., 2010). For instance, the ISO standard does not require a negative control (a control group that is expected to produce a null result), while the ASTM standard does (Briassoulis et al., 2010; Lipsitch et al., 2010).

There are only a few standards involving home composting. For instance, in Belgium the tests involve following standards such as ISO 14851, 14852, 14855, etc., but with a lower temperature of 20-30°C, and 90% biodegradation requirement within 12 months. There is also the Ente Nationale Italiano di Unificazione (UNI) 11183, which is a national norm that establishes tests for plastics to be considered compostable under room temperature. Plastics that are compostable under this norm are home compostable (Briassoulis et al., 2010).

2.6 Laboratory Scale Biodegradation Tests under Composting Conditions

Numerous studies have conducted various laboratory scale biodegradation tests in aerobic conditions (usually in compost), using various bioplastics, most commonly PLA (Table 2.1). Most of the studies looked at the degradation of the bioplastics through the production of carbon dioxide.

However, few studies also looked at the microbial content of the compost. Although it is not requited by the standards, it would be beneficial to examine the microbial content of the compost as certain microorganisms degrade certain bioplastics better than others; thus, different composts with different microbial contents could have more ideal microorganisms present.

Table 2.1. Overview of laboratory scale composting biodegradation studies

Source	Material (% w/w)	Time (days)	Biodegradation (%)	Form	Setup and Method	Compost	Temperature/TS[a]%/VS[b]%	Standard
Narancic et al. (2018)[c]	PLA	75	100	20 × 20 × 0.2 cm rectangle	80 g of test material and 1200 g inoculum was used. Gas from reactors was continuously analyzed using gas chromatograph. CO_2 was absorbed using potassium hydroxide (KOH) and titrated.	Municipal solid waste	58 ± 2°C/50-55/>30 of dry solids	ISO 14855
	PHB	45	110					
	Polyhydroxyoctanoate (PHO)	124	105					
	PBS	207	92					
	PCL	45	125					
	PLA/PCL(80/20)	75	110					
	PLA/PBS(80/20)	75	100					
	PLA/PHB(80/20)	75	98					
	PLA/PHO(85/15)	145	95					
	PHB/PCL(60/40)	46	120					
	PHB/PBS(50/50)	88	99					
	PHB/PHO(85/15)	75	118					
	PCL/TPS(70/30)	46	105					
	PBS/TPS(60/40)	85	90					
	PCL/PHO (85/15)	75	110					
Kalita et al. (2020)	(1) PLA	140	85		500 g of compost and 50 g of test material were placed in 3 L glass composting vessels. The vessels were connected to a gas chromatograph system to measure CO_2	Compost consisting of dry leaves, cattle manure, sawdust, and vegetable waste	58°C ± 5°C	ASTM D5338-15
	(2) PCL	110	90					
	(3) PLA/PCL(80/20)	140	75					
	(4) PLA/PCL(90/10)	140	70					
	(5) PLA/PCL/MCC(80/20/1)	140	90					
	(6) PLA/PCL/MCC(80/20/3)	140	90					
	(7) PLA/PCL/MCC(90/10/1)	140	90					
	(8) PLA/PCL/MCC(90/10/3)	140	75 (biodegradation order: (6), (1), (4), (2), (5), (3), (7), (8)					

Source	Material (% w/w)	Time (days)	Biodegradation (%)	Form	Setup and Method	Compost	Temperature/TS[a]%/VS[b]%	Standard
Ahn et al. (2011)	PLA Bioplastic B (Poultry feather fiber/ (PFF)/PLA/starch (5/80/15)) Bioplastic C (PFF/urea/glycerol (50/25/25)	60 60 60	13 ± 3 53 ± 2 39 ± 3	0.5 cm square pieces	CO_2 was absorbed by sodium hydroxide (NaOH) pellets in the bottles.	Dairy manure compost	58°C/	
Balaguer et al. (2016)	PLA (4.5% D-lactic acid isomer, 0.24% residual monomer) PLA + layered silicate modified nano clay (Clay 1) PLA + Calcium carbonate nanoparticles (nano-$CaCO_3$) PLA + Nano silicon dioxide (nano-SiO_2) Microcrystalline Cellulose Microcrystalline Cellulose (MCC)	130 85 60 130 45	72 90 90 80 >70	25 mm × 25 mm pieces and 500 µm grounded pieces (10/90) Powder (20 µm diameter)	CO_2 from biodegradation tests analyzed using Pac Check Model 650 EC	Mature compost from municipal urban waste treatment plant and vermiculite (1 g)	58 ± 2°C	EN 13432: 2000 ISO 14855: 2012 (compost and biodegradation)
Ranjan et al. (2010)	MCC PLA soy straw wheat straw PLA/wheat/straw (70/30) PLA/soy straw (70/30)	45 100 45 58 70 70	>70 85 90 98 90 90	Powder Ground up	700 g of compost and 50 g of test material. The CO_2 was collected using 0.5 N NaOH, which was then titrated intermittently.	Mature compost (master Gardener-Canada)	58 ± 5°C	ASTM D5338

Source	Material (% w/w)	Time (days)	Biodegradation (%)	Form	Setup and Method	Compost	Temperature/TS[a]%/VS[b]%	Standard
Sessini et al. (2019)[d]	TPS ethylene-vinyl acetate (EVA) B40TPS (40%wt TPS) B50TPS (50%wt TPS) B40TPS/ 1% Cloisite-Na^+ ($CLNa^+$) B50TPS + 1% $CLNa^+$	60 150 150 150 150 150	100 0 22 28 8 20	15 mm × 15 mm squares	The materials were placed in a textile mesh and buried 4-6 cm in perforated plastic boxes containing synthetic waste. The weight loss was determined at regular intervals.	Solid synthetic waste composed of: 10% of compost (Compo, Spain), 30% rabbit food, 10% starch, 5% sugar, 4% corn oil, 1% urea, and 40% sawdust	50-60 °C/50	ISO 20200: 2015
Zhao et al. (2005)	PBS	90	(1) 14.1 (2) 71.9 (3) 60.7	(1) Powder (42 μm) (2) Granule (3 mm) (3) Film (1 cm by 1 cm, thickness : 40 um)	Compost and test material were mixed at a ratio of 1:6 (dry weight). The CO_2 was trapped using $Ba(OH)_2$ and then titrated.	Aerated municipal solid waste compost from Nangong Compost Factory (Beijing, China)	58 ± 2°C	ISO 14855
Iovino et al. (2008)	PLA TPS PLA/TPS(75/25) PLA/TPS/coir (natural fiber)(52/17/30) PLA/TPS/ maleic anhydride(MA)(75/25/1) PLA/TPS/coir/MA(52/17/30/1)	90 90 90 90 90 90	55 87 61 59 57 54	1 cm^2 squares, thickness : ~ 1 mm	The compost and the test material were mixed in a 6:1 (w/w) in 2 L glass flasks. The CO_2 was trapped in 50 mL of 1 M KOH and titrated with 1 M HCl	Mature compost (2 months old) from vegetable refuse.	58 ± 2°C/48/45	ISO 14855

Source	Material (% w/w)	Time (days)	Biodegradation (%)	Form	Setup and Method	Compost	Temperature/TS[a]%/VS[b]%	Standard
Jašo et al. (2015)[d]	PLA Thermopolyurethane (TPU) PLA/TPU (80/20) PLA/TPU (60/20) PLA/TPU (50/50) PLA/TPU (40/60) PLA/TPU (20/80)	70	95 27 30 55 55 30 15	Finely milled (ca. 100 mm)	Test material (0.2 g) was added to the compost in 500 mL chambers connected to an automated and fully computerized closedcircuit Micro-Oxymax Respirometer System, to monitor CO_2 production.	Locally purchased compost adjusted to 60% (w/w).	58 ±2 °C	ASTM D5338
Arrieta et al. (2014)[d]	PLA PLA-/polyethylene glycol (PEG) PLA/acetyl-tri-n-butyl citrate (ATBC) PLA/PHB/PEG PLA/PHB/ATBC	28	All material had weight loss higher than 90%. PLA-PEG had the fastest rate and PLA-PHB-PEG had the slowest rate.	30 × 30 × 0.2 mm films	The materials were placed in an iron mesh and buried 6 cm deep in plastic reactors containing the solid synthetic wet waste.	Solid synthetic waste consisting of 10% of compost at pH 6.5, 30% rabbit food, 10% starch, 5% sugar, 1% urea, 4% corn oil and 40% sawdust.	58 °C/55	ISO-20200
Du et al. (2008)[e]	TPS thermoplastic dialdehyde starch (TPDAS) 6 TPDAS30 TPDAS50 TPDAS70 TPDAS95 MCC	56 56 56 56 56 56 45	73 66 56 45 26 6 74	Fine powder	A pressurized air control system, with CO_2 removed, was used to provide air into the composting system. The CO_2 was collected using a 0.2 mol NaOH trap system.	Mature compost from organic fraction of municipal waste	58 ±2 °C	ISO 14855

Source	Material (% w/w)	Time (days)	Biodegradation (%)	Form	Setup and Method	Compost	Temperature/TS[a]%/VS[b]%	Standard
Cadar et al. (2012)	MCC PLA 1 (commercial) PLA 2 (synthesized in lab) Copolymer 3: lactic acid (LA) (1.0 mol)/ethylene glycol (EG) (0.5 mol)/malonic acid (MA) (0.5 mol) Copolymer 4: LA (0.1 mol)/EG (2.0 mol)/MA (2.0 mol) Copolymer 5: LA (1.0 mol)/EG (0.5 mol)/succinic acid (SA) (0.5 mol) Copolymer 6: LA (0.1 mol)/EG (2.0 mol)/SA (2.0 mol)	110 110 110 110 110 110 110	(A)76 (B)83 (A)72 (B)86 (A)69 (B)83 (A)69 (B)76 (A)37 (B)42 (A)69 (B)75 (A)33 (B)38	Powder	Compost stored for 7 days at 5°C 2 L glass flasks with 6:1 compost to test material (dry mass), using 50 mL/min flow rate (A) Titration method: CO_2 absorbed using 0.125 mol/L $Ba(OH)_2$, which was then titrated with 0.1 mol/L HCL to determine CO_2 produced. (B) Automatic method: CO_2 absorbed using 0.05 mol/l NaOH CO_2 trap and total organic carbon content was determined	3-month-old mature compost from organic domestic waste (33% vegetable waste, 30% fruits peels, 15% wood chips, and 12% compost)	58 °C/51/29	ISO 14855-1: 2005
Suthapakti et al. (2018)	MCC PLL PLA/TPU (PCL based TPU) (90/10) PLA/TPU/PLA+PCL(PLLCL) (90/10/10)	90	85 78 65 85	Powder (<500 µm)	The materials were placed with compost in 2 L glass vessels. The CO_2 was measured as dissolved inorganic carbon after absorption in NaOH.		58 ± 2 °C/50-55%	ISO 14855-1:2005

Source	Material (% w/w)	Time (days)	Biodegradation (%)	Form	Setup and Method	Compost	Temperature/TS[a]%/VS[b]%	Standard
Arrieta et al. (2018)[d]	Polyurethane (PU) (made from synthesized PCL and hexamethylene diisocyanate	50	87	15 mm × 15 mm squares	The materials were buried at 4–6 cm depth in perforated plastic boxes containing a solid synthetic wet waste. The weight loss was determined at regular intervals.	Solid synthetic wet waste: 10% of compost (Mantillo, Spain), 30% rabbit food, 10% starch, 5% sugar, 1% urea, 4% corn oil and 40% sawdust	58°C/50	ISO 20200
	PU + L-lysine	50	89					

[a]Total solids
[b]Volatile solids
[c]Biodegradation is in relative comparison to biodegradation of cellulose
[d]Disintegration
[e]Numbers indicate dialdehyde starch (DAS) carbonyl content

In most of these studies, cellulose is used as a positive control (a control group that is expected to produce a certain extent of biodegradation result within a defined time period, such as 70% in 45 days). Cellulose is a good indicator of the validity of the composting assays due to the ubiquitous nature of microorganisms that can degrade it. Typically, cellulose is converted into glucose via the following equation.

$$(C_6H_{10}O_5)_n + n\,H_2O \rightarrow n\,(C_6H_{12}O_6) \quad (1.1)$$

Following this reaction, the glucose is converted into pyruvate via glycolysis. The pyruvate is then oxidized to acetyl-CoA, which then enters the citric acid cycle and leads to the production of carbon dioxide. The overall reaction is seen in the following equation.

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow CO_2 + 6\,H_2O \quad (1.2)$$

Even though cellulose has been used as a positive control in numerous studies, that does not mean that the same biodegradation results will be achieved across studies or even by the same compost source. For instance, a study conducted by Castro-Aguirre et al. (2017) had seven biodegradation tests with the same compost source sampled over the course of a year. The biodegradation of cellulose varied between 50-85%. Of the seven tests, five reached 70% or greater biodegradation of cellulose (one of those reached approximately 67%). Thus, even if the same compost is used, various factors such as oxygen availability, total solids (TS), volatile solids (VS), pH, temperature, and carbon content can influence the biodegradation results. For instance, it is important to have enough oxygen as composting is an aerobic process; however, too much air flow can cause drying of compost and reduce water availability. Water is important as it is a distribution medium for the microorganisms and the nutrients and affects microbial activity. Normally 50-60% moisture is preferred for biodegradation; however, too much water can cause anaerobiosis by reducing the available airspace. This is combatted by adding inorganic material (i.e. vermiculite)

to increase porosity. The VS values of compost are recommended to be less than 30% to prevent the microorganisms from preferring the carbon present in the compost to that present in the test material. Even so, in the study mentioned above with seven biodegradation tests, there was not a clear relationship between these factors and the biodegradation of cellulose (TS: 41.5-60.9%, VS: 23.1-44.6%). For instance, the authors achieved approximately 60% biodegradation of cellulose by day 45 when the TS and VS were 53.3% and 26.4% respectively. On the other hand, another trial achieved 80% biodegradation of cellulose when the TS and VS were 41.5% and 43.2%. The carbon to nitrogen ratio is recommended to be between 10-40. If it is too high, then it can lower biodegradability as nitrogen is a limiting factor, but if it is too low then the nitrogen can turn into ammonia and volatize leading to odor problems. A wide range in pH (7-9) is allowed as compost tends to have a natural buffering capacity; however, a neutral pH is preferred as an acidic pH may cause inhibition, while an alkaline pH can cause the loss of nitrogen in the form of ammonia (Castro-Aguirre et al., 2017). Castro-Aguirre et al. (2017) have thus stated that biodegradation tests involving compost can have low reproducibility and that it is difficult to compare biodegradation results from different experiments. It is also beneficial to implement a second positive control if the test substance is suspected to not to be easily biodegradable or act similar to cellulose. If researchers want to know more than if a material is compostable but also the mechanisms of biodegradation and effects on the environment, then tests other than those that analyze the carbon dioxide production, such ecotoxicity tests, are recommended.

2.7 Field Scale Composting Tests

In addition to laboratory scale composting studies, there have also been studies that have investigated the biodegradation of various products under industrial composting conditions. As mentioned above, currently there is no standard for placing test material directly into industrial

composting conditions; however, it is beneficial to conduct these tests to obtain a general idea on how quickly the materials would degrade in real life conditions. Moreover, many composting facilities are hesitant to accept biopolymers because the conditions of the laboratory based tests present an ideal environment for the biopolymers to degrade in, while the actual field scale conditions are not necessarily ideal conditions as they are difficult to control/adjust and more variable (Zhang et al., 2017). In some cases, biopolymers that degraded well in laboratory scale tests, degraded poorly in field scale tests at composting facilities (Briassoulis et al., 2010). Thus, it is necessary for field scale tests to be conducted for the biopolymer to be accepted into the composting facility. It is also beneficial to conduct these tests using various concentrations of the biopolymer at various composting facilities (Zhang et al., 2017).

It can be difficult to find a suitable composting facility that will allow experimental tests to be conducted in their facility. The field scale tests are usually conducted in green waste composting facilities. Many of these experiments are also conducted in outdoor conditions which makes it difficult to control conditions such as moisture content. Moreover, outdoor composting processes can be affected by weather conditions such as temperature and precipitation, i.e. low precipitation can lead to lower biodegradation. Instead of placing the items in a composting facility, the items can be placed in large composting bins; however, the results may not be the same as that of a composting facility, as the conditions in the bins are more consistent (Musiol et al., 2016). In some cases, the consistent conditions may lead to higher degradability of the products while in other cases, low biodegradability has been observed (Musiol et al., 2011; Musiol et al., 2016). In comparison to the amount of laboratory based composting studies, there are only a few studies that have incorporated both field scale and laboratory scale studies on the same product, using the same compost. The results of field scale composting studies can be seen in Table 2.2.

Table 2.2. Overview of field scale composting studies.

Source	Material	Form	Method	Time	Weight Loss	Compost
Adamcová et al. (2017)	(1) Sponge (Renewable resources and organic cotton mesh) (2) Sponge (cellulose, cotton, mesh, water, salts, and pigments) (3) Sponge (70% cellulose, 30% cotton) (4) Sponge (75% cellulose, 25% cotton) (5) Cellulose filter paper (the sponges were in duplicates)		Placed materials in wooden frames (width = 280 mm, length = 340 mm and height = 50 mm) with polyethylene mesh on the top and bottom. Placed wooden frames 1 meter deep. Visually inspected the samples every 2 weeks. Recorded air temperature and precipitation and compost temperature. Measured the weight loss	12 weeks	(1) 20% (2) 83% (3) 97% (4) 100% (5) 100%	Bio-waste in a static pile with aeration in Boskovice-Doubravy
Adamcová et al. (2013)	Plastic bags: (1) BIO-D Plast (2) HDPE + TDPA (100% degradable) (3)(100% degradable) (4) Starch (Compostable) (5) Starch and PCL (6) (Compostable) (7) Natural material (Compostable) (8) Cellulose filter paper (blank)		Samples were placed in wooden frames (width: 280 mm, length: 340 mm and height: 50 mm) with 1 by 1 mm polyethylene mesh on the top and bottom of the frames. Samples were 1m from the surface of the pile and 1.5 m from the ground. The samples were visually inspected.	15 weeks	(1), (2) and (3): no degradation (6) & (7): 90% (5) high degradation (4) lower degradation than (5)	Composting Plant
Sikorska et al. (2008)	(1) Biomixed E (BTA) (2) Biomixed ELB10 (BTA + 10% PLA) (3) Biomixed ELB30 (BTA + 30% PLA) (4) Biomixed ELB5A5 (BTA + 5% PLA + 5% a-PHB)	Filaments with diameter = 1 mm and length = 10cm	Placed materials 1 m below the surface of the compost pile. Monitored visual changes, weight loss, polydispersity changes, and composition of the products.	14 days	(1) 19% (2) 4% (3) 10 % (4) 6%	A composting pile (40% leaves, 30% branches, and 30% grass) with an average temperature of 57^{o}C and an average pH of 7.4.

Source	Material	Form	Method	Time	Weight Loss	Compost
Zhang et al. (2017)	(1) Kraft control (Fiber (tree-based)) (2) Spoon (PLA) (3) Fork (Crystallized PLA) (4) Knife (Crystallized PLA) (5) Bio foam Tray (Blown PLA) (6) Lid (Crystallized PLA) (7) Fabrika Cup (Amorphous PLA) (8) D&W box (Amorphous PLA) (9) D&W lid (Amorphous PLA) (10) Cellulose bag (Fiber (cellulose)) (11) BESICS Sleeve (Fiber (tree-based)) (12) BESICS Bowl (Fiber (tree-based)/single lined with PLA) (13) Eco-tainer Bowl (Fiber (tree-based)/double lined with PLA) (14) BESICS fiber ware (Fiber (bagasse based)) (15) BESICS Wrap (Fiber (tree-based)/single lined with PLA) (16) Clamshell (Fiber (bagasse based))		25 L double bagged polypropylene mesh bags were filled with the sample and representative compost. Low (10% by dry volume) and high (20% by dry volume) concentrations of the samples were used. The mesh bags were buried in the compost piles. The temperature and moisture of the compost piles were measured. The disintegration criteria was modified from the ASTM D6868-11, 2011 standard: less than 10% of the dry weight or surface area should remain and can be sieved through a 3.2 mm screen. (Also measured concentration of microorganisms)	(A) 4 months (after pathogen reduction phase) (B) and (C) over 2 months (D) 5 months (4 months was curing phase)	Disintegration varied by material type, not concentration (except for (C)). (A) (1), (10)-(17): 0 to 13% -(2)-(9): 74 to 100%. -Kraft paper (control): 4 to 6% -PLA products: 91 to 94% -Fiber (tree, bagasse and cellulosic materials) products: 1 to 4% -Fiber with PLA based products: 11 to 12% (B) - Low disintegration (mean: ~31%) -(8), (10), (12), (15): 66 to 100% - -Cellulose products: 86 to 93% (C) Higher concentrations of sample had higher (16%) disintegration -High concentrations: all (except (1):66% and (13):75%) :>90% (D) 100% disintegration for all	-Revolution Resource Recovery (Lytton, BC): (A)Turned windrow compost with yard waste (30%) and food waste (60%) and a temperature of 50-60^{o}C -Yes Harvest Power (Richmond, BC) (B) Anaerobic digestion (30% yard waste and 70% commercial food waste) then (C) static pile (90% yard waste and 10% residential food waste) -Whistler Composting Facility (Whistler/Squamish, BC): (D)In-vessel composting then roofed windrow (56% woody material/yard waste, 35% bio-solid and 9% food waste)

Source	Material	Form	Method	Time	Weight Loss	Compost
Sikorska et al. (2015)	(1) PLA (commercial polylactide grade 2002D (NatureWorks®, USA)) (2) PLA/a-PHB blend (15% mol a-PHB (poly[(R,S)-3-hydroxybutyrate)	4 cm x 3 cm rectangles	The samples (triplicates) were all placed together in a cage, 1m below the surface of the composting pile, during the months of July to September. The samples were inspected on day 7, 21, and 70.	70 days		A static composting pile (40% leaves, 30% branches, and 30% grass) at the Sorting and Composting Station in Zabrze (Upper Silesia, Poland). The pile was 30 m by 33 m by 4 m. Kitchen waste (exact quantity unknown) was added to the pile. The compost temperatures were 52, 54, and 59^{o}C for day 7, 21, and 70 respectively. The average outdoor temperature was 16.5^{o}C with rainfall of 0.1 mm
Greene (2007)	(1) Avicel cellulose control (2) Cup (PLA) (3) Knife (PLA) (4) Container (PLA) (5) Kraft paper control (6) Trash bag (corn starch) (7) Plate (sugarcane)		Samples were placed in perforated plastic agricultural bags and buried in the compost piles. The temperature and moisture of the compost in the bag were measured. The weather conditions were also recorded.	20 weeks (i) 2 weeks (ii) 7weeks (iii) 20 weeks: ASTM 6400 timeframe	(1)(i) 29% (ii) 100% (iii) 100% (2) (i) 28% (ii) 100%, (iii) 100% (3) (i) 48% (ii) 100% (iii) 100% (4) (i) 12% (ii) 100% (iii) 100% (5) (i) 28% (ii) 52% (iii) 88% (6) (i) 20% (ii) 31% (iii) 84% (7) (i) 15% (ii) 19% (iii) 78%	Conducted at Chico municipal compost facility that used aerobic windrow composting. The windrows are 8 ft tall and 13 ft wide. The composting piles consist of green yard-waste (lawn clippings, leaves, wood, sticks, weeds, and pruning).

Source	Material	Form	Method	Time	Weight Loss	Compost
Musiol et al. (2011)	(1) CONS-PET biodegradable bags (13% PLA, and BioPlaneta) polylactide (PLA), aliphatic aromatic co-polyester terephthalic acid/adipic acid/1,4-butanediol (BTA) and commercial additives) (2) BioPlaneta (20% PLA, aliphatic aromatic co-polyester terephthalic acid/adipic acid/BTA and commercial additives)		The samples were placed in metal cages with holes, that were placed 1 m below the surface in a (A) composting pile and a (B) container system. Visual changes and weight loss of the samples were determined.	3 weeks	[a](1)(A) 40% (B) 20% [a](2)(A) 31% (B)17% (1)(A)20% (B) 9% (2)(A) 6% (B)2%	The composting pile (40% leaves, 30% branches, and 30% grass) was in Zabraze and had an average pH of 6.9 and temperature of 64^{o}C The container system (18% leaves, 22% branches, 23% grass, and 37% domestic waste) had an average temperature of 60^{o}C
Kale et al. (2006)	(1) 500 mL spring water bottles (96% L-lactide and 4% D-lactide with bluetone additive) (2) Poly (lactide) trays (94% L-lactide and 6% D-lactide.) (diameter = 0.24 m, height = 0.046 m) (3) Deli containers (94% L-lactide and 6% D-lactide)	(1) height:0.2 m, base diameter: 0.065 m (2) diameter: 0.24 m, height: 0.046 m (3) 0.195 m by 0.17 m by 0.04 m	The samples (duplicates) were placed in wooden boxes (0.6 m by 0.3 m by 0.1 m) with a 0.011 mm mesh gauge. The boxes were 1.2 m above the ground and 1 m below the surface of the compost pile	45 days	(1) Degradation time of up to 35 days (2) and (3) degradation time of up to 45 days	A compost pile at the Michigan State University composting facility (East Lansing, MI). 11.6 m^3 of cow manure and 7.8 m^3 of wood shaving combined with waste feed in 2:1. in a rectangular bay of 3.6 m by 36.5 m by 1.8 m. Turned every 3 days for 3 weeks. Then moved to a pile of 6 m by 24 m by 3 m on an asphalt pad. The initial pile temperature, relative humidity, and pH: 65 ± 5C, 63 ± 5%, and 8.5 ± 0.5, respectively.

[a]Molecular weight loss

2.8 Anaerobic Digestion

Anaerobic digestion has become a preferred method for organic waste stabilization as it has a low energy requirement and produces methane, which is a valuable source of bioenergy. Anaerobic digestion is the degradation of organic matter in the absence of oxygen to produce biogas. This process involves four main steps: (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, (4) methanogenesis. The first step is the rate limiting step which involves the degradation of high molecular weight compounds into soluble, low molecular weight compounds such as amino acids, long chain fatty acids (LCFAs), and monosaccharides. Hydrolytic microorganisms such as *Butyrivibrio, Micrococci, Bacteroides, Clostridia*, *Selenomonas*, *Micrococci, Fusobacterium*, and *Streptococcus* are responsible for releasing enzymes to such as cellulase, amylase, and protease to break down the high molecular weight compounds. The low molecular weight compounds are broken down in the next stage by fermentative bacteria (i.e. *Lactobacillus, Streptococcus, Escherichia coli, Salmonella, Bacillus,* etc.) into volatile fatty acids (VFAs), ammonia, carbon dioxide, alcohols, aldehydes, and hydrogen sulfide (Haandel and Lubbe, 2007; Merlin et al., 2014). Acetogenesis involves the degradation of organic acids and alcohols by acetogenic bacteria into mainly acetic acid and H_2. In the last stage, acetoclastic and hydrogenotrophic methanogenic archaea produce methane. The acetoclastic methanogens split the acetate into methane and carbon dioxide. The hydrogenotrophic methanogens produce methane (and water) by oxidizing H_2 and using carbon dioxide as a carbon source. Methane can be produced from another (uncommon) route involving methylotrophic methanogens which covert methanol and hydrogen into methane and water (Metcalf and Eddy, 2014). The hydrogenotrophic methanogens usually belong to one of the following orders: *Methanococcales, Methanobacteriales*, or *Methanomicrobiales*. The acetoclastic methanogens usually belong to the order of *Methanosarcinales*, within the family of

Methanosarcinaceae or *Methanosaetaceae* (Grady, 2011). The formation of methane using hydrogen only accounts for 30% of methane production due to the limited amount of hydrogen in anaerobic digesters. Thus, the acetoclastic methanogen pathway is the most dominant pathway as it accounts for the remaining 70% of methane production (Gerardi, 2003).

A variety of biomass can be used as a substrate for anaerobic digestion to produce biogas; however, the main components of the substrate should have carbohydrates, proteins, fats, and cellulose. Thus, the methane yield and the biogas composition will depend on the type of feedstock used. Anaerobic digesters have been reported to digest various substances including sewage sludge and animal manure (Weiland, 2010). Moreover, co-substrates can be added to the digesters to increase cost-efficiency (one digester for several materials), biogas yield, and organic content, supply missing nutrients, and dilute inhibitory compounds such as ammonia and heavy metals (Luostarinen et al., 2009). Co-substrates can also be used to alter the moisture content of the feedstock (Mata-Alvarez et al., 2000). Some examples of co-substrates include food waste, municipal biowaste, energy crops, cattle slurry, and organic waste from industries related to agriculture. The biogas yield of the various co-substrates depends on their composition. For instance, lipids have a biogas yield (1200-1250 Nm^3/tonne of TS) but take longer to degrade because of their low bioavailability (Weiland, 2010). Lipid products such as LCFAs can also have inhibitory effects on methanogenesis (Luostarinen et al., 2009). The LCFA toxicity is due to the surfactant effect that damage the cell membrane of the microorganisms (Long et al., 2012). LCFAs can also adsorb to cell membranes and decrease the transfer of substrates (Nzila et al., 2019). On the other hand, carbohydrates and proteins degrade much faster; however, they do not yield as much biogas as lipids (790-800 and 700 Nm^3/tonne of TS, respectively) (Weiland, 2010). The following sections describe the parameters that affect the efficiency of anaerobic digestion.

2.8.1 Organic Loading Rate

The organic loading rate (OLR) is the amount of organic substrate added to an anaerobic digestion process per effective (liquid) volume of digester per day. It is an important design and operation parameter. The OLR cannot be too low or too high or else the microorganisms will starve, or the acids will accumulate and possibly halt fermentation, respectively. Generally, the advantage of high OLRs is a reduction in required volume and therefore capital cost of the digester (Jain et al., 2015). There is no universal OLR that can be used for all anaerobic digester conditions as the optimum OLR varies with temperature and substrates. For example, a study that looked the digestion of pig manure using OLRs ranging from 1.13 to 3.03 g VS/L/day at 35^{o}C found that the optimum OLR was 1.89 g VS/L/day, as it achieved the maximum methane yield (Duan et al., 2019). On the other hand, another study determined that the optimum OLR for raw food waste, food solid waste, and food liquid waste were 7, 9, and 4 g VS/L/d respectively in dual solid-liquid semi-continuous flow anaerobic digesters at 35^{o}C (Zhang et al., 2013). Another study looked at the methane production during the thermophilic (55^{o}C) and mesophilic (37^{o}C) digestion of chicken manure at OLRs of 1.6 and 2.5 kg VS/L/day. The study found a higher amount methane production at an OLR of 2.5 kg VS/L/day under mesophilic conditions (252 vs 245 mL CH_4/g TS). While the methane yield was higher at 1.6 kg VS/L/day under thermophilic conditions (200 vs 94 mL CH_4/g TS), but still less than the methane yield under mesophilic conditions. This may have been caused by the high free ammonia concentrations, especially at high OLRs, more toxic to thermophilic than mesophilic methanogens (Bi et al., 2019). Lastly, it has been reported that an OLR between 0.5-1.6 kg VS m^3/day is a safe range for the anaerobic digestion of municipal sludge at wastewater treatment plants (Jain et al., 2015); although, at lab scale some studies showed that anaerobic

digesters utilizing municipal sludge can be operated without process failure up to 5 kg VS m^3/day (Kor-Bicakci et al., 2019).

2.8.2 Hydraulic Loading Rate / Sludge Retention Time

As other important design and operation parameters, the hydraulic retention time (HRT) is the average time the substrate (mostly in liquid or slurry form) is kept in the digester, while the solid retention time (SRT) is the average time that microbial cultures, such as the acidogens and methanogens (considered in solid/floc form) are kept in the digester. If there is no recycling occurring within the digester, then the SRT and the HRT are fairly equal (Appels et al., 2008) and SRT term is mostly used for digesters. It is calculated by dividing the liquid (effective) volume of digester by the daily volumetric flowrate of the substrate. The SRT must be long enough to allow hydrolysis and fermentation to occur (Liu et al., 2020). For instance, if the SRT is too short then there may be an incomplete breakdown of some compounds (i.e. lipids) and methanogenic microorganisms may be washed out (Appels et al., 2008). Moreover, a short SRT can lead to an accumulation of VFAs, as the growth rate of methanogenic archaea is slower than that of fermentative bacteria. Furthermore, the SRT can be shorter at thermophilic than mesophilic temperatures as the growth rate of thermophilic methanogens is 2-3 times higher than their mesophilic homologues (Ferrer et al., 2010). Liu et al. (2020) have reported that an SRT of 20-30 days is acceptable for the digestion of municipal sludge. The SRT requirements for bioplastics in AD is expected to be much longer given the more complex molecular structure.

2.8.3 Mixing

Mixing is another important parameter of anaerobic digestion that influences several other parameters including a pH and temperature uniformity, availability of substrates and nutrients to microorganisms, and the distribution and proximity of the microorganisms with respect to

substrate (food) source within the bioreactor. Mixing is typically a continuous process achieved by mechanical pumping/stirring, gas recirculation, liquid/slurry recirculation, or a combination of the methods mentioned above. Mixing also provides several other advantages including a reduction of floating solids, sedimentation, clogging, crust and foam formation, facilitation of biogas release from substrates, and promotion of mass heat transfer (Kariyama et al., 2018). Mixing can use 29-54% of the energy input requirements of an anaerobic digester. Research has demonstrated that a low mixing speed can optimize the methanogen community while decreasing the energy demands of mixing (Ma et al., 2019). For instance, one study found higher biogas production at 50 revolutions per minute (rpm) compared to 150 rpm during the digestion of rice straw at 35^{o}C and an HRT (= SRT) of 30 days. They also found an increase in the efficiency of the hydrolysis of cellulose and hemicellulose at 50 rpm due to the formation of microbe-substrate aggregates (Kim et al., 2017). Another study found increased biogas production at 25 rpm compared to 150 rpm during the digestion of the organic fraction of municipal solid waste at 37^{o}C (Lindmark et al., 2014a). On the other hand, some studies have determined that increasing the mixing intensity can improve the hydrolysis and acidification processes during the digestion of sewage sludge and lignocellulose. For instance, one study found that mixing intensities of 90 and 120 rpm improved the hydrolysis and acidification efficiency (Ma et al., 2019). Furthermore, a mixing intensity that is too high can result in shear stress, a reduction in biogas production, and a reduction in the formation of flocks. The flocks allow the microorganisms to come in close proximity to one another (Lindmark et al., 2014a). Lastly, intermittent mixing (i.e. 15 min ON and 45 min OFF) has been shown to produce similar biogas yields as continuous mixing. A benefit of intermittent mixing is the lower energy requirements (Lindmark et al., 2014b).

2.8.4 Temperature

Digester operating temperature is another important parameter during anaerobic digestion as it influences the metabolism and growth of microorganisms as well as the physiochemical properties of the substrate components (Appels et al., 2008). The methane producing microorganisms are more active at either mesophilic (35-42^{o}C) or thermophilic (45-60^{o}C) temperatures (Gerardi, 2003; Weiland, 2010), although it is possible to generate methane in psychrophilic temperature range (<15-20^{o}C) at much slower rates. An increase in temperature is typically associated with an increase in the enzymatic reactions of microorganisms, solubility of organic compounds, and the destruction of pathogens (Appels et al., 2008). However, thermophilic temperatures are also associated with an increase in free ammonia (inhibits microorganisms) and a decrease in microbial diversity (Appels et al., 2008; Weiland, 2010). The thermophilic microorganisms are also more sensitive to fluctuations in temperature than mesophilic microorganisms; however, methanogens typically have a higher growth rate at thermophilic temperatures (Weiland, 2010). One study found the highest biogas production at 45^{o}C, when looking at temperatures of 10, 20, 37, 45 and 55^{o}C. The substrate used in this study was maize straw and cattle slurry (Lin et al., 2016). Another study found an increase in biogas production along the temperature increments of 25, 35, 35, and 50^{o}C, but a decrease in biogas production at 55^{o}C during the digestion of swine manure (HRT = SRT = 30 days).

2.8.5 Microorganisms

The microbial diversity varies across anaerobic reactors, as it is influenced by various parameters including temperature, substrate, and OLR. For instance, one study compared the methanogenic community across mesophilic (35^{o}C, OLR = 2.8 kg VS/m^{3}/d) and thermophilic (55^{o}C, OLR = 5.2 kg VS/m^{3}/d) reactors treating agricultural waste (i.e. cow manure, corn silage,

and vegetable waste). They found that the composition of the communities across the different temperatures was similar but there were differences in the structure (Franke-Whittle et al., 2014). Generally, archaeal communities in mesophilic reactors are more diverse and evenly distributed (Li et al., 2014). *Methanothermobacter* were the dominant methanogens in thermophilic reactors, while *Methanosarcina, Methanoculleus, Methanobacterium,* and *Methanosaeta* were the dominant methanogens in mesophilic reactors. *Methanosarcina* and *Methanosaeta* are acetoclastic methanogens (although *Methanosarcina* is also capable of hydrogenotrophic methanogenesis) while *Methanobacterium, Methanothermobacter*, and *Methanoculleus* are hydrogenotrophic methanogens (Franke-Whittle et al., 2014; Ghasimi et al., 2015). Thus, the hydrogenotrophic methanogens were more dominant than acetoclastic methanogens in the thermophilic reactors. This dominance indicates that acetate degradation, under thermophilic conditions, occurs via a syntrophic relationship between acetate oxidizers (convert acetate into carbon dioxide and hydrogen) and hydrogenotrophic methanogens (Franke-Whittle et al., 2014). Another anaerobic digester study utilizing municipal sludge as the substrate found similar results in which the main methanogens were *Methanothermobacter* (hydrogenotrophic methanogens) and *Methanosaeta* (acetoclastic methanogens) under thermophilic (55±1^{o}C, SRT = 6 days) and mesophilic conditions (35±1^{o}C, SRT = 6 days), respectively. The dominant bacterial cultures under mesophilic conditions were Candidate Division WWE1 (Wastewater of Evry 1), *Firmicutes, Proteobacteria*, and *Bacteroidetes* which had an abundance of 46.4%, 14.8%, 12.2%, and 10.0% respectively. On the other hand, *Firmicutes* and *Thermotogae*, with an abundance of 42.3% and 15.2% respectively, were the dominant bacterial phyla under thermophilic conditions (Kor-Bicakci et al., 2020).

Pap et al. (2015) looked at the change in microbial community when the temperature was changed from mesophilic to thermophilic (day 1, 20, 80 at temperature of 37, 55, 55^{o}C,

respectively) in an anaerobic digester that used maize silage as a substrate. *Bacteroidetes*, *Firmicutes*, *Synergistetes*, and *Proteobacteria* were the main bacterial phyla throughout the experiment; however, their abundance varied across the experiment. *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were the dominant phyla on day 1 and 20, while *Firmicutes* (66.5% total bacteria) and *Synergistetes* were the dominant phyla on day 80; thus, day 1 and 20 represented a mesophilic bacterial community, while day 80 represented a restructured thermophilic bacterial community with *Clostridium* being the most abundant genus of *Firmicutes*. The study indicated that syntrophic oxidizing bacteria and hydrolyzing bacteria (i.e. *Clostridium and Caldicellulosiruptor*) became dominant under thermophilic conditions. The dominant archaea on day 1 and 20 was *Methanosaeta* (69.3% of total archaea at day 1), while the major archaea on day 80 were *Methanosarcina*, *Methanothermobacter*, and *Methanoculleus* (28.3%, 19.3%, 20.1% of total archaea, respectively); thus, acetoclastic methanogens dominated the mesophilic digester and hydrogenotrophic methanogens dominated the thermophilic digester. As a side note, an increased amount of Fe-hydrogenase was observed at day 20 and 80. This indicated the importance of dissolved hydrogen used for hydrogenotrophic methanogenesis under thermophilic conditions.

Another study found *Bacteriodes* (46%-90% relative abundance) and *Anaerolinea* (5-24% relative abundance) as the dominant genera of bacteria in mesophilic (35^{o}C) reactors. The study varied the reactor OLR from 2.5 kg chemical oxygen demand (COD)/m^3/day to 5.5 kg COD/m^3/day and found that an increase in abundance of the *Bacteroides* (fermenting genera) was related to an increase in VFA formation which is linked to an increase in OLR. The dominant archaea in the mesophilic digester were *Methanosaeta*. The study used the fine sieved (6 mm mesh size) fraction from raw municipal wastewater as their substrate (Ghasimi et al., 2015). Merlino et al. (2013) found *Firmicutes, Bacteroidetes*, and *Synergiste*s as the dominant bacteria and

Methanosarcinales as the dominant archaea when swine manure and fruit and vegetable waste was used as a substrate under thermophilic conditions (55^{o}C, HRT = 25 days).

2.8.6 Feeding Regime

Continuously (or semi-continuously) fed and batch bioreactors can be used for anaerobic digestion. Continuously fed reactors are fed throughout the day while batch reactors are only fed once a day. Continuously fed reactors most closely replicate full-scale anaerobic digestion; however, in the case of an experiment with multiple scenarios, it can be quite costly and time consuming to test all desired scenarios. Thus, batch reactors can be used during preliminary studies to evaluate a wide range of scenarios and narrow down important variables to be assessed further. Unfortunately, they do not stimulate full-scale reactors as well as the continuously fed reactors (Owen et al., 1979).

Biochemical Methane Potential (BMP) assays are small scale (100 – 1000 ml) batch experiments that determine the biodegradability and methane potential of various substrates (Filer et al., 2019). Two of the most important parameters are the inoculum source and the food to microorganism ratio (F/M). The F/M can be stated as the VS of the substrate per the VS of the inoculum (Yoon et al., 2014). The F/M in BMPs replaces the OLR in continuously fed reactors (anaerobic digesters) and its value is often related to the properties of the substrate (Pellera and Gidarakos, 2016). Numerous studies have focused on the optimization of the F/M as it is influenced by the substrate characteristics of the organic material (Pellera and Gidarakos, 2016; Yoon et al., 2014).

The general process for BMP assays is as follows. In an assay, the substrate is mixed with the inoculum (initial microbial population) in a serum bottle with approximately 100 ml total liquid volume at the desired F/M. The inoculum normally comes from a full-scale bioreactor at steady-

state utilizing similar substrate; therefore, contains a mixed culture acclimatized to similar substrate at similar operating temperature range. Nutrients (mainly N, P) can also be provided in the case the substrate is a highly carbonaceous substance instead of municipal sludge containing all C, N, P elements. The bottles are then purged with nitrogen gas, to remove any oxygen, before they are sealed. They are then incubated at the same temperature as the full-scale bioreactor and constantly mixed. The biogas can be measured via a manometer or liquid displacement and its composition can be determined using a gas chromatograph (Angelidaki and Sanders, 2004).

2.9 Anaerobic Degradation of Bioplastics

The studies that have examined the biodegradation of bioplastics have focused more on aerobic composting conditions, as they usually degrade the fastest in composting conditions; however, these aerobic conditions require a constant input of energy due to the addition of moisture and compressed air (air may not need to be added if the compost pile is simply being turned). Furthermore, the compost piles need to constantly be turned to reduce the heat in the system, to ensure that the temperature remains at an optimal range for the microorganisms present in the compost. Anaerobic digestion (involving the digestion of ie. residual sludge from municipal wastewater treatment plants), on the other hand is not considered as much as composting in terms of bioplastic disposal; however, it has several advantages such as a shorter processing time and the production of methane that can be used as energy Thus, anaerobic digestion uses less energy than composting and can be a potential alternative to composting. Moreover, anaerobic conditions can be used to stimulate accelerated conditions in biologically active anaerobic landfills.

In most cases, the biodegradation of bioplastics in anaerobic conditions is slower than in composting conditions. For instance, PLA has been shown to degrade poorly under anaerobic conditions. This is due to fact that PLA needs to be hydrolyzed at temperatures around 50^{o}C, before

microorganisms can break it down; thus, PLA has shown little degradation in mesophilic anaerobic conditions (Bátori et al., 2018). For instance, one study found no significant methane production when they looked at the biodegradation of PLA pellets in inoculum (from a digestor treating maize sileage) under mesophilic (35^{o}C) batch conditions for 175 days. The biogas was measured using a manometer and the percent biodegradation was determined from the amount of carbon dioxide and methane produced (Kolstad et al., 2012).

Another study examined the biodegradation of PLA powder (125–250 mm) under batch conditions with mesophilic (37^{o}C) sludge (from cow manure and vegetable waste) that was adapted to 55^{o}C. The PLA biodegradability in diluted and undiluted sludge was 79% (100 days) and 91% (75 days), respectively. The biogas was collected in a gas sampling bag (2–5 L) and its volume was measured via a glass syringe. The percentage of carbon dioxide and methane in the biogas was determined using a gas chromatograph. The percent biodegradability was determined by adding the volume of carbon dioxide and methane produced and subtracting the volume of the water vapor (Yagi et al., 2009a).

The same authors conducted the same test under mesophilic (37^{o}C) and thermophilic conditions (55^{o}C) with undiluted sludge. They found that the PLA powder (125–250 mm) achieved 60%, 80%, and 90% biodegradability in 30, 40, and 60 days, respectively. On the other hand, the PLA only achieved 21% biodegradation under mesophilic conditions and biodegradation did not start until day 55 (Yagi et al., 2009b). The authors then conducted another study under the same thermophilic conditions to test PLA films of various sizes. The films with size of >500 mm, 1×1 cm, and 15×34 cm exhibited 75%, 80%, and 84% biodegradation respectively, in 40 days. The lower biodegradability of the smaller PLA films was due to the films sticking together (via static electricity) and reducing the surface area available for biodegradation (Yagi et al., 2012).

The biodegradation of PCL under anaerobic conditions is varied, with some studies stating that little to no biodegradation occurs at 35^{o}C, while others have obtained results contrary to this statement (Bátori et al., 2018). For instance, one study determined that there was 7.6% PCL (10×90 mm) biodegradation in mesophilic sludge (37^{o}C) (collected from an anaerobic laboratory scale reactor fed with wastewater from the sugar industry). The percentage biodegradation was determined as the net gas production per the theoretical value (calculated using carbon content of sample) (Abou-Zeid et al., 2001). Another study found that a mixture of 55% PCL, 30% starch, and 15% aliphatic polyesters achieved 83% biodegradation in 139 days at mesophilic (35^{o}C) temperatures. This study was conducted using BMP assays that involved serum bottles filled with 20 mL anaerobic sludge (as inoculum) and 80 mL nutrient medium. The authors determined the biogas volume using glass syringes and the biogas composition was determined via a gas chromatograph. The biodegradability percentage was determined as the amount of methane produced per the theoretical methane generation (Cho et al., 2011). Yagi et al. (2009a) (as previously mentioned) found that PCL showed 90% biodegradation in mesophilic sludge that was adapted to thermophilic conditions.

The study conducted by Cho et al. (2011) also found only 2% biodegradation of PBS in 100 days. Furthermore Yagi et al. (2013) conducted a study (under previously mentioned conditions involving adapted sludge) in which PBS did not degrade; however, PHB, PCL, and PLA had 90% (14 days), 80% (50 days), and 75% (75 days) biodegradation respectively. All the aforementioned bioplastics were in the forms of powders (125–250 mm).

A study by Narancic et al. (2018) used ISO 15985 standards to determine the end of life fate of various bioplastics and their blends (mentioned in Table 2.1) under static thermophilic (52±2^{o}C) conditions, that stimulated landfilling conditions. The 15 g of the bioplastics were added to 1000

g inoculum from a digester treating the organic fraction of household waste. The biogas was collected in an inverted glass cylinder in water and its composition was determined via a gas chromatograph. The biodegradation percentage was determined from the amount of methane and carbon dioxide produced. All the bioplastics, except PBS, PHO, and PBS/TPS (60/40 percent by mass) were able to degrade under the anaerobic conditions. More specifically, the results can be seen in Table 2.3. A review article by Bátori et al. (2018) concluded that the order of anaerobic degradability of bioplastics from worst to best is PLA, PCL, followed by PHB.

Table 2.3. Anaerobic relative biodegradation of bioplastics in study conducted by Narancic et al. (2018)

Bioplastic[a]	**Relative anaerobic biodegradation (%)[b]**	**Degradation time (days)**
PLA/PCL (80/20)[c]	90	121
PLA/PBS (80/20)	85	121
PLA/PHB (80/20)	105	80
PLA/PHO (85/15)	90	66
PHB/PHO (85/15)	90	66
PHB/PCL (60/40)	105	80
PHB/PBS (50/50)	75	121
PCL/PHO (85/15)	85	66
PCL/TPS (70/30)	70	80
PLA	90	80
PCL	95	127
PHB	93	127
TPS	80	127

[a]PLA: Polylactic acid, PCL: Polycaprolactone, PBS: Polybutylene succinate PHB: Polyhydroxybutyrate, PHO:Polyhydroxyoctanoate, PBS: Polybutylene succinate, TPS: Thermoplastic starch
[b]Relative biodegradability (per percent cellulose biodegradation)
[c]Percentage by weight

One study looked at the biodegradation of PHB in anaerobic co-digesters with primary municipal sludge at 35^{o}C. The PHB was either untreated or treated at pH 12 at 55^{o}C. They found

that the treatment of PHB reduced the lag phase of PHB biodegradation by 5 days; however, there was not a significant difference between the biodegradation of untreated and treated PHB (86% and 91% respectively in 20 days) to methane. Moreover, the authors observed a change in bacterial community abundance after the PHB was added to the digesters. More specifically, the abundance of *Cloacimonetes* and *Chloroflexi* increased and decreased, respectively. There was no significant change in archaeal community composition (Venkiteshwaran et al., 2019). The change in bacterial communities and lack of change in archaeal communities has also been observed in other studies when the substrate was changed (Yue et al., 2013). Interestingly, the bacteria that increased in abundance have not previously been associated with PHB biodegradation (Venkiteshwaran et al., 2019).

A study by Zhang et al. (2018) looked at the biodegradation of nine EN 13432 compliant bioplastics and two negative controls (uncoated polypropylene (PP) film and a plain low-density polyethylene (LDPE) film) under batch and semi-continuous flow (SRT = 50 days) mesophilic anaerobic digesters utilizing food waste as co-substrate. The nine bioplastics included a cellulose-based metallised film (CBM), a cellulose-based heat sealable film (CBHS), a cellulose-based high barrier heat-sealable film (CBHB), a cellulose-based non heat sealable film (CBnHS), a cellulose diacetate film (CDF), two different starch-based film blends, a PLA film, and PLA blend pellets. The results from the semi-continuous flow digesters indicated that cellulose based films and the PLA film had significant weight loss, while the starch blends and the PLA blend pellets showed little biodegradation. The four cellulose blends (CBM, CBHS, CBHB and CBnHS) showed significant calorific recovery of methane (74.3%, 86.6%, 84.0%, and 80.4% respectively); however, the PLA film and CDF had little calorific methane recovery (18.8% and 8.9% respectively). This suggested that while these two bioplastics may have had significant weight loss

in the semi-continuous flow digesters, there was probably little biodegradation occurring. The authors also suggested that some bioplastics may need a longer SRT in continuously fed anaerobic digesters, in order have enough time to degrade.

2.10 Summary of Literature Review

The negative effects of fossil-based plastics have led to the creation of bioplastics such as PHA, PGA, PCL, PLA, and TPS. The biodegradation of these bioplastics has been investigated under various environments including aerobic and anaerobic conditions. The aerobic conditions mostly involve laboratory scale composting assays, with very limited amount of studies conducted at field scale. While numerous studies have conducted composting assays, they so far had an issue of reproducibility due to difficulty to control variables (temperature, moisture, maturity of compost soil); however, some bioplastics that degrade well under composting conditions include PLA, PHB, and PCL. Moreover, factors such as composting source can affect the microbial composition of the compost which in turn can affect the biodegradation of the bioplastics as some specific microorganisms are better at degrading certain bioplastics. The weight loss of bioplastics has also been assessed under field scale conditions to better stimulate real life conditions, as the bioplastics may not have the same biodegradation levels in field scale and laboratory scale composting conditions.

In general, most bioplastics degrade better under aerobic (composting) conditions than anaerobic conditions; however, they can be potentially used to increase methane production during anaerobic digestion. There are numerous factors that are important to anaerobic digestion including mixing, sludge retention time, organic loading rate, and temperature which collectively influence microbial community.

This study intents to assess the biodegradation of cellphone cases with flax under the aforementioned conditions. The anaerobic assays will involve assessing the degradation of both microcrystalline cellulose (as positive control) and various sized cellphone cases under mesophilic (38^{o}C) and thermophilic (55^{o}C) conditions. Furthermore, composting assays under field scale (60-67^{o}C) and laboratory (58^{o}C) conditions will be used to determine the degradation of the cellphone cases under aerobic conditions.

Chapter 3: Materials and Methods

This study was performed to determine the end of life fate of phone cases with flax under uncontrolled and controlled conditions. This chapter provides the list of equipment used to conduct the research. The methods used in the laboratory scale composting and anaerobic assays as well as the field scale composting are discussed. Moreover, analytical test procedures are described in detail.

3.1 Equipment

The list of equipment used throughout this study is listed in Table 3.1.

Table 3.1. List of equipment

Equipment	Model number & manufacturer
Analytical balance	XS204DR, Mettler Toledo
Centrifuge	Sorvall Legend XT, Thermo Scientific
Dual channel pH/ion meter	Accumet excell XL25, Fisher Scientific
Gas Chromatograph –A	7890A, Agilent (equipped with a 25 m column and a flame ionization detector)
Gas chromatograph – B	7820A, Agilent (equipped with a 3 m packed column and a thermal conductivity detector)
Gas monometer	Custom built
Incubator/shaker	Innova 44R, New Brunswick Scientific
Microcentrifuge	Sorvall Legend Micro 21, Thermo Scientific
Muffle furnace	W-13, Paragon Industries
pH probe	13-636-XL25, Fisher Scientific
UV-Vis Spectrophotometer	Genesys 10, Thermo Electron Corporation
Thermotron temperature-controlled chamber	S-1.5-3200, Thermotron

3.2 Experimental Procedure

3.2.1 Cellphone Cases

The cellphone cases tested in the present study were made up of a material containing 35% of biobased components with an addition of about 5% flax. The exact composition of the cases is proprietary information and therefore it is not disclosed. The main body of the cellphone cases were approximately 14 cm × 7 cm × 0.2 cm and the portion (thickness) covering the sides of the phones was about 1 cm. The total weight of the cellphone case was approximately 32.5 g.

3.2.2 Laboratory Scale Composting Assays

The laboratory scale testing was conducted at Bioreactor Technology Laboratory (BTG) at UBC's Okanagan Campus. The composting conditions were maintained as per ASTM D5338-15. Two types of compost soil were used in this experiment: Glengrow compost (utilizing plant materials, i.e. lawn trimmings and pruning) and Ogogrow compost (utilizing hog fuel (by-product of lumber mills), wood ash, and municipal biosolids). Both compost soils were collected from the composting facilities operated by the City of Kelowna. During the preliminary testing at BTG, only the Ogogrow compost was able to achieve 70% biodegradation (recommended by ASTM D5338-15) of microcrystalline cellulose (positive control) by 45 days. Therefore, Ogogrow compost was used in the further experiments to determine the biodegradability of the cellphone cases. As a side note, the microorganisms in different composts may be acclimated to different substrates, for instance the microorganisms in the Glengrow compost, may be better acclimatized to plant-based materials, such as flax.

Before the experiments, the compost was sieved through a 10 mm sieve and TS, VS, and pH of the sieved compost were determined. The carbon and nitrogen content of the sieved compost were also determined. After the end of the experiment, the weight loss of the cellphone cases was

determined by drying in a temperature-controlled chamber (Thermotron) at 35^{o}C for 48 hours. Additionally, some of the degraded phone cases were analyzed using scanning electron microscopy (SEM).

The laboratory scale composting apparatus was designed by BTG and built by the School of Engineering machine shop (Figure 3.1). The full-scale composting process was simulated at the laboratory scale in 1.5 L jars with lids that had holes for an air inlet line, air outlet line, and a glass thermometer (to monitor temperature) (Figure 3.2).

Figure 3-1. Composting apparatus set up at BTG laboratory

Figure 3-2. Composting jar without (left) and with compost (right)

The custom-built composting system had a capacity of incubating a total 12 jars during each run. The composting assays had three main operating conditions in terms of type of material (substrate) assessed (i.e., blank, positive control, and test material) each studied in triplicates. The blanks consisted of jars filled only with compost. The positive control was microcrystalline cellulose with a known biodegradation extent as per ASTM D5338-15. The test material was the cellphone cases cut into $1/4^{th}$ (7 × 3.5 × 0.2 cm), $1/6^{th}$ (4.6 × 3.5 × 0.2 cm), 2 × 2 × 0.2 cm, and 4 × 4 × 0.2 cm pieces. Normally the ASTM D5338-15 requires the test material to be in the form of powder, granules, or film with maximum surface area of around 2 × 2 cm; however, in this case larger pieces were used to stimulate sizes that were more similar to those that would be composted in real life conditions, potentially by consumers themselves. Each composting condition involved equal amounts of compost with beneficial microbial cultures (around 500 grams) and amounts of microcrystalline cellulose, roughly the same weight as the cellphone cases. There was a flow meter (rotameter) on each line carrying pressurized air from a compressor into the composting vessels. The air was humidified prior to entry into the composting vessels, by passing it through an air

sparger placed in a tube of heated water. This helped prevent the compost soil from drying out. The vessels were held in a stainless-steel water bath at 58 ± 2°C, as per ASTM D5338-15, with a lid to prevent evaporation/heat loss. Each jar was shaken manually at least once a week. The exhaust air from each compost vessel was sampled via a glass syringe from a sampling port on the air line. The carbon dioxide in the sampled exhaust air along with other gases (oxygen and nitrogen) were measured via an Agilent 7820A gas chromatograph (GC), twice a day.

The cumulative CO_2 production from the cases in compost jars were determined from the measurements of exhaust air flowrate, GC gas composition results, and were converted to standard temperature and pressure conditions (STP: 0^oC, 1 atm, respectively). The amount of cumulative CO_2 production (g) measured for each bottle was divided by 44 g/mol (carbon dioxide molar mass) and multiplied by 12 g/mol (carbon molar mass) to convert it to total gaseous carbon (g). The percent biodegradation of the cases was then be determined by subtracting the average amount of carbon produced in the blank control ($C_{(g)}$ (control)) from the average amount of carbon produced by the jars with the cell phone cases ($C_{(g)}$ (test)). This value was then divided by the amount of carbon originally present in the cell phone case ($C_{initial}$) and then multiplied by 100 (Equation 3.1) (ASTM D5388-15). The relative biodegradation of the cellphone cases was also determined by dividing the biodegradation of the cellphone cases by the biodegradation of the positive control.

$$\%\ \text{biodegradation} = \frac{\text{mean } C_{(g)}(\text{test}) - \text{mean } C_{(g)}(\text{control})}{C_{\text{initial}}} \times 100 \qquad (3.1)$$

3.2.3 Field Scale Composting

The field scale composting was performed at the Glengrow composting facility (Figure 3.3). The facility is 10 hectares and annually receives approximately 50,000 tonnes of organic waste (based on the previous data from 2015-2019).

Figure 3-3. Glengrow composting facility

The compost in this facility is mainly composed of plant materials like lawn trimmings, brought in by Central Okanagan residents. Before the composting, any plastic or metal material from the incoming plant materials are removed by the facility staff. The plant materials are placed in piles called windrows for composting, which are turned about once a month to ensure that there is enough oxygen for the microorganisms that are degrading the organic material. The carbon to nitrogen ratio in the pile is usually kept 30 to 1 and the ideal moisture level for the piles are kept 65% (water is added to obtain that level). Ideally, the temperature of each pile is also measured every two weeks. When the composting process is complete, the piles are screened to remove any pieces of wood that are larger than half an inch (Glengrow, 2018). The field scale composting was performed by placing the cellphone cases in a large compost pile in outdoor conditions from October 2019 to January 2020. A bulldozer was used to dig up the compost at around 1 m below the top of the pile. Each cellphone case (pre-weighed) was placed in a small mesh bag with compost from the pile. Six of these small mesh bags were placed in larger mesh bags filled with compost, also from the pile. There were three replicates of the large mesh bags. Each large bag was tied up using a nylon rope. The nylon rope extended from the bags to the top of the compost

pile, where they were tied around poles with flags attached them (to indicate the general area of the bags) (Figure 3.4).

Figure 3-4. Field scale composting set up

The compost pile was covered up once all the bags have been placed in the compost and the temperature of the compost near each bag was determined using a temperature probe. The composting pile was revisited at day 46 and 80. At these points, the compost was dug up once again by a bulldozer to obtain a cellphone case from each large bag. The temperature of the compost was also determined. Compost samples in triplicate were taken from soil around each bag, to determine the TS and VS contents at BTG laboratory.

3.2.4 Laboratory Scale Anaerobic Digestion Assays

The anaerobic digestion of microcrystalline cellulose and cellphone cases was conducted using small scale batch-fed biochemical potential (BMPs) assays to determine the biodegradation (methane generation potential). There are no full-scale anaerobic digesters in Kelowna; therefore, testing was conducted at laboratory scale only.

The first phase of the BMP assays consisted of blanks (anaerobic inoculum only) and positive controls (microcrystalline cellulose, inoculum, and water) in 160 ml serum bottles (Figure 3.5). The assays were conducted first with two mesophilic and one thermophilic inocula to assess the biodegradation of microcrystalline cellulose at various F/M ratios (0.5, 1, 2, 5 g COD/g $VS_{Inoculum}$). The COD of the cellulose was determined based on the following reaction.

$$C_6H_{10}O_5 + 6\,O_2 \rightarrow 6\,CO_2 + 5\,H_2O \quad (3.2)$$

The total molecular weight of the oxygen was then divided by the total molecular weight of the cellulose (Equation 3.3)

$$COD = \frac{g\ O_2\ needed}{g\ substrate\ consumed} \quad (3.3)$$

These BMPs were used to determine which inoculum would be best suited for the following biodegradation studies with the cellphone cases. The pH and VFA of these BMPs were also assessed at various points to ensure that the pH remained neutral and the VFA did not accumulate to levels inhibitory to the microorganisms.

Figure 3-5. Biochemical methane potential (BMP) assays using serum bottles (160 mL)

The second phase was used to determine the biodegradation of the cellphone cases under mesophilic and thermophilic conditions. Each experiment consisted of bottles labelled as: blank (inoculum), control (inoculum and mixed sludge), and test material (cellphone case, mixed sludge,

inoculum). For the mesophilic conditions, cellphone cases of various sizes (grinded, cut into 2 × 2 × 0.2 cm, and 4 × 4 × 0.2 cm pieces) were placed in 500 mL Wheaton bottles (Figure 3.6). The F/M was 1 g VS $_{\text{Mixed Sludge}}$ /g VS $_{\text{Inoculum}}$ in each bottle and about 5 g cellphone case/g VS $_{\text{Mixed Sludge}}$ was added to each bottle. The substrate refers to the mixed sludge which was used as a co-substrate. In this case, the mixed sludge was added to the BMP bottles to stimulate a full-scale anaerobic digestor and to provide nutrients to the assays. For the thermophilic conditions, grinded cases were placed in 160 mL serum bottles. The thermophilic digester (at bench scale at BTG lab) produced far less inoculum volume than the mesophilic digester (at full-scale); thus, the smaller serum bottles were used for the thermophilic BMP assays. The F/M was 1, 2, and 3 g VS $_{\text{Mixed Sludge}}$ /g VS $_{\text{Inoculum}}$ and the dosage of the cases was about 5 g cellphone case/g VS $_{\text{Mixed Sludge}}$ and 10 g cellphone case/g VS $_{\text{Mixed Sludge}}$, 3 g cellphone case/g VS $_{\text{Mixed Sludge}}$ and 6 g cellphone case/g VS $_{\text{Mixed Sludge}}$, and finally 2 g cellphone case/g VS $_{\text{Mixed Sludge}}$ and 3 g cellphone case/g VS $_{\text{Mixed Sludge}}$, respectively. Lastly, some of the degraded cellphone cases were analyzed using scanning electron microscopy.

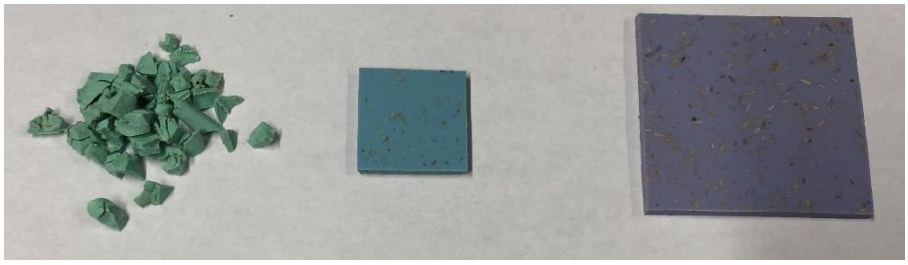

Figure 3-6. Various sizes of cell phone cases (from left to right: grinded, cut pieces with 2 × 2 × 0.2 cm, and 4 × 4 × 0.2 cm)

3.2.4.1 Biochemical Methane Potential Assays

The BMP assay procedure was adapted from previous work (Angelidaki et al., 2009; Holliger et al., 2016). The required amount of mesophilic and thermophilic inocula for the assays was collected from the digesters maintained at 38^{o}C and 55^{o}C, respectively, and incubated in the corresponding temperature shakers. The inoculum was starved for 7 days prior to each assay in order to allow it to degas (Angelidaki et al., 2009). A buffering solution consisting of sodium

carbonate and potassium bicarbonate was added to each bottle, to help maintain a neutral pH. Nutrient solution was also added to BMP assays with microcrystalline cellulose. The composition of the nutrient solution was based on experiments from Eskicioglu et al. (2017). After adding the appropriate substances, each bottle was purged with N_2 gas for 5 minutes. Then it was sealed with a butyl rubber and aluminum cap. The bottles were incubated in a shaker at 38°C or 55°C at 90 rpm. The bottles were placed horizontally in the shaker to ensure the solutions were mixed well and monitored until BMP assays stopped producing biogas (Figure 3.7).

Figure 3-7. Temperature controlled shaker

3.2.4.2 Anaerobic Inocula

Similar to composting assays, different sources of anaerobic inoculum were assessed in this study as the biodegradability of substrates can vary between various sources of inocula. This is due to the different microbial populations, the adaptation of the substrate, and the initial activity of the microorganisms (Filer et al., 2019). There were three types of inocula used in this study: two mesophilic and one thermophilic. Mesophilic inoculum 1 came from a bench scale anaerobic sludge digester operated at the BTG laboratory. The reactor had a 2 L volume and operated at an SRT of 15 days in a semi-continuous feeding mode (fed once a day, seven days a week). It was

kept at 38°C in an automated shaker (Innova 44R, New Brunswick Scientific) rotating at 90 rpm. This reactor had inoculum originating from a wastewater treatment plant (WWTP) in Penticton and was fed with sludge from a WWTP in Kelowna. Mesophilic inoculum 2 came from a full-scale anaerobic digester located at Lulu Island WWTP (Vancouver, British Columbia, Canada). The thermophilic inoculum came from a bench scale anaerobic sludge digester operated at the BTG laboratory. The reactor had a 2 L volume and operated at an SRT of 15 days in a semi-continuous feeding mode (fed once a day, seven days a week). It was kept at 55°C in an automated shaker rotating at 90 rpm. This reactor had inoculum originating from the Annacis Island WWTP (Vancouver, British Columbia, Canada) and was fed with sludge from a Lulu Island WWTP. Its biogas was collected using a 2 L Tedlar® bag and measured using a custom designed manometer. The properties of the mesophilic inoculum 1 and the thermophilic inoculum were analyzed weekly for TS, VS, COD, pH, VFAs, ammonia and gas composition (N_2, O_2, CH_4 and CO_2).

3.2.4.3 Co-substrate

The municipal mixed sludge, from Lulu Island WWTP, was used as a co-substrate for BMP assays. This sludge was used as it was the feed for both thermophilic sludge digester and the mesophilic sludge digester (for mesophilic inoculum 2) from which inocula were obtained for BMP assays. Benefits of co-substrates include an increase in biogas yield, and organic content, supply missing nutrients, and dilution of inhibitory compounds such as ammonia and heavy metals (Luostarinen et al., 2009). The mixed sludge was a mixture of 65 ± 5% primary and 35 ± 5% secondary clarifier sludge, based on volume. Moreover, it was shipped every 14 days from Lulu Island WWTP in Vancouver British Columbia, Canada. The properties of the mixed sludge were analyzed weekly for TS, VS, COD, pH, VFAs, and ammonia.

3.3 Scanning Electron Microscopy (SEM)

The non-degraded and degraded cellphone cases were prepared for SEM imaging. The bioplastics were cut into 1 cm × 1 cm × 0.2 cm pieces and coated with 10 nm platinum using a Cressington Sputter Coater (Cressington Scientific Instruments, UK). The platinum was added as a conductive material as the bioplastics were not conductive. The samples were then adhered to a stub using carbon tape (a conductive adhesive). The SEM images were performed on a TESCAN Mira3 XMU Scanning Electron Microscope (TESCAN, Czech Republic) equipped with an Oxford Instruments X-Max energy dispersive spectrometer detector (Oxford Instruments, UK) from the Fipke Laboratory for Trace Element Research at the UBC Okanagan Campus. The SEM was also equipped with an Energy Dispersive Spectrometer that was used to determine the elemental composition of the cellphone case.

3.4 Fourier Transform Infrared Spectrometry (FTIR)

The non-degraded cellphone cases were prepared for fourier transform infrared spectrometry attenuated - total reflectance (FTIR-ATR) analysis by freezing the samples using liquid nitrogen, then grinding the samples up using a coffee grinder. The spectra of the grounded cellphone cases were collected using a Nicolet iS20 FTIR spectrometer with a diamond ATR crystal. The spectra were recorded from 400 to 4500 cm^{-1}. There were 32 scans conducted per sample.

3.5 Analytical Methods

3.5.1 Total Solids and Volatile Solids

The TS and VS of the digestate, feed, and compost were determined based on APHA 2540 B and 2540 E procedures (APHA 2005). Initially, the crucibles were prepared by soaking them in diluted sulfuric acid (20%). The soaked crucibles were then heated at 550°C and stored in a desiccator. The samples were weighed in the crucibles and then placed in the oven to dry at 98°C

overnight. After most of the water was evaporated, the temperature was increased to 105°C for 2 hours. The crucibles with samples were then burned at 550°C for 2 hours and 30 minutes for the compost, digester effluent (digestate) and digester feed samples, respectively. The percentage weight (% wt.) of TS and VS were calculated according to equations 3.2 and 3.3.

$$\text{Total Solids } \left(\%, \frac{g}{g}\right) = \left(\frac{\text{Wet mass (g)} - \text{Dry mass (g)}}{\text{Wet mass (g)}}\right) \times 100 \quad (3.4)$$

$$\text{Volatile Solids } \left(\%, \frac{g}{g}\right) = \left(\frac{\text{Dry mass (g)} - \text{Burned mass (g)}}{\text{Wet mass (g)}}\right) \times 100 \quad (3.5)$$

3.5.2 Chemical Oxygen Demand

The Closed Reflux Colorimetric Method (Standard Methods 5220 D) was used to determine the total COD of the digestate and feed of the inoculum digestors (APHA 2005). The samples were initially diluted with deionized water to ensure they were within the range of the method (100-700 mg COD/L). A benchtop homogenizer (Kinematica™ Polytron™, PT 10-35 GT) was used to mix the diluted samples for 5 minutes at 7000 rpm. Afterwards, 2.5 mL of the mixed sample was added to 12 mL glass vials. Then 3 ml of digestion solution (mixture of mercuric sulfate, potassium dichromate, and concentrated H_2SO_4) and 1.5 mL of catalyst solution was added. The prepared mixtures were placed in a Thermotron oven at 150°C for 3 hours. The digested samples were then cooled in a dark location. The absorbance of the samples was measured using a spectrometer at 600 nm. The COD concentration of the samples was determined using a calibration curve that was prepared by measuring the absorption of known amounts of potassium hydrogen phthalate and an ultra-pure water blank. An example calibration curve is provided in Appendix A.

3.5.3 pH

The pH of the effluent of the inoculum digesters and the mixed sludge was determined by following the Standard Methods 4500-Hþ B (APHA 2005). The pH of the compost was also determined by using a 1:5 mixture of compost to deionized water. The pH was measured using an

Accumet™ Excel XL25 pH/mV/Temperature/ISE meter (Thermo Fisher Scientific, Inc., Ottawa). The pH probe was calibrated by measuring pH standards of 4, 7, and 10. Thus, samples with unknown values were measured against the values of the known accurate standards, which were stored into the memory of the pH meter.

3.5.4 Ammonia

The first step in determining the ammonia concentration of the anaerobic inoculum and sludge was to centrifuge the samples at 10000 rpm for 30 minutes. The supernatant was then used to measure the ammonia by colorimetry according to the Environmental Protection Agency (EPA) method 350.1. The supernatant was first diluted using ultra-purified water into a volumetric flask. Ultra-purified water is prepared by treating water with softening, reverse osmosis, and ultraviolent disinfection processes. Following these steps, 0.61 mL of the diluted mixture was pipetted into 10 mL glass culture tubes in triplicates. Along with the diluted samples, 0.61 mL of six calibration standards (0.1 – 7.5 mg/L) were also pipetted to separate tubes. The standards were prepared by diluting ammonium chloride to varying degrees (0.1, 0.25, 0.5, 1, 2.5, 5, and 7.5 mg/L) with ultra-purified water. To each tube, 2.0 mL of complexing solution (33 g/L potassium sodium tartrate and 24 g/L sodium citrate), 0.86 mL of alkaline phenol solution (34 g/L sodium hydroxide and 10.56 g/L phenol), 0.01 mL of a 12% sodium hypochlorite solution, and 0.39 mL of a 500 mg/L nitroferricyanide solution were added. The solutions were then stored in the dark for 1 hour. Lastly, the absorbance of each sample was measured using a spectrophotometer at a wavelength of 660 nm. The ammonia nitrogen concentration of the samples was determined using a calibration curve that was prepared by measuring the absorption of the standards. An example calibration curve is provided in Appendix A.

3.5.5 Volatile Fatty Acids

The VFAs (acetic, propionic, and butyric acid) of each sample were determined by first centrifuging the samples at 10000 rpm for 30 minutes and filtering the supernatant using a 0.2 μm nylon filter. If the samples were from the BMP assays, then they were filtered using Nanosep® centrifugal tubes (Pall Corporation, New York, USA). Following this step, 0.5 mL of filtrate was added to a 2 mL glass vial with 0.5 mL of internal standard solution (iso-butyric acid (2%), formic acid, sulfuric acid and sodium hydroxide). A main standard (2 g/L acetic, 2 g/L propionic, and 2 g/L butyric) was also prepared. The main standard (0.5 mL) was mixed in a glass vial with 0.5 mL of the internal standard (Ackman, 1972). The above mixture was used as a quality control to verify the GC performance. The VFA was analyzed by injecting the samples into an Agilent 7890A Gas Chromatograph (GC) with a capillary column (Agilent 19091F-112, HP-FFAP polyethylene glycol TPA column length x ID: 25 m × 320 mm), a flame ionization detector, and an autosampler. The inlet temperature was set to 220^{o}C with a helium flow rate of 25.2 mL/min. The oven temperature was initially 70^{o}C for 0.2 minutes, followed by an increase in temperature to 200^{o}C for 6.5 minutes. The column flow was set at 2.52 mL/min. The flame ionization detector had its fuel, oxidizer, and make-up gas flows at 40 mL/min of hydrogen, 400 mL/min of air, and 25 mL/min of nitrogen, respectively.

3.5.6 Total Organic Carbon and Nitrogen

The total organic carbon and nitrogen of the compost were determined by Element laboratories. Upon receival, the samples were first dried then sieved using a 2 mm sieve. Then 1 g of the compost sample was weighed and inserted into a furnace at 500°C for two hours, to burn off the organic carbon. Following this step, 0.1-0.2 g of the dry sample and the ashed (heated to 500°C) sample were analyzed separately in the LECO Truspec Analyzer. The analysis of the

dried sample determines the nitrogen content and the total carbon. The analysis of the ashed sample determines the inorganic carbon content. The organic carbon content is determined by subtracting the inorganic carbon content form the total carbon content.

3.5.7 Gas Volume and Composition

The biogas generated by the thermophilic anaerobic digester (from which one of the inocula was obtained for BMP assays) was collected using a Tedlar™ bag, that was emptied every day using a custom build manometer. The biogas pressure in the BMP bottles was measured using a handheld digital manometer (LEO 2, Keller, Winterthur, Switzerland). This manometer was calibrated at the factory and had an accuracy full-scale of < 0.1% at room temperature.

The gas composition (CH_4, H_2, N_2 and O_2) of the headspace in BMP bottles was measured using a method that was established by van Huyssteen (1967). Initially, 1 mL of gas was sampled from the BMPs using an Agilent gas tight syringe and purging it to 0.6 mL. The gas sample was then injected into a GC (Agilent 7820A GC) with a packed column (Agilent G3591- 8003/80002) and a thermal conductivity detector. The inlet, outlet, and oven temperatures were 100, 150, and 70^{o}C respectively. The carrier gas of the GC was helium (from Air Liquide (Kelowna)) with a flowrate of 25 mL/min. The gas composition of the sample from the lab scale composting apparatus was examined in a similar fashion. The same method was used for the headspace analysis in the compost jars, except the Agilent syringe was purged to 0.4 mL.

Chapter 4: Results and Discussion

The results of this study are divided into four sections. Section one assesses the biodegradability and weight loss of the cellphone cases under laboratory scale composting conditions. Section two assesses the weight loss of the cellphone cases under field scale composting conditions. Section three investigates the anaerobic biodegradability of microcrystalline cellulose and the cellphone cases under mesophilic and thermophilic conditions. Lastly, section four presents the SEM and FTIR images of the cellphone cases.

4.1 Laboratory Scale Composting Assays

The results of the analysis of the compost soil used to set up the composting assays are summarized in Table 4.1. The soils for trials 1 and 2 came from the Ogogrow composting facility.

Table 4.1 Weight loss of whole cellphone cases under field scale composting conditions

Parameters	Compost trial 1	Compost trial 2
Carbon to nitrogen ratio (-)[a]	15.6	
Nitrogen (% w/w)[a]	5	
Total organic carbon (% w/w)[a]	46.1	
Total solids (% w/w)[b]	50.8 (±1.8)	47.1 (±2.4)
Volatile solids (% w/w)[b]	40.0 (±1.8)	37.4 (±3.4)
pH[b]	8.06 (±0.12)	7.83 (±0.05)

[a] Average of duplicate measurements
[a] Average (±standard deviation) of triplicate measurements

The VS of the compost, in both trials, is considered somewhat high (> 30%); however, this is likely due to some of the wood chips that remained after the compost was sieved. In this case, the wood chips were used to help increase the porosity of the compost. The carbon and nitrogen content for the compost in trial 2 were not determined as these parameters were conducted by an off campus commercial laboratory (Element laboratories) and the accuracy of the results was

uncertain, due to the small (0.1-0.2 g) sample size. The TS% for the compost in trial 2 was a little low (optimum: 50-55%). Every other parameter was within the optimal range for the compost soil.

The carbon dioxide production of the cellphone cases in the first trial is shown in Figure 4.1. The first composting trial achieved 71 ± 11% biodegradation for microcrystalline cellulose used as positive control after 46 days. This trial would be considered valid by the ASTM D5338-15, which states that the biodegradation of microcrystalline cellulose should be at least 70% in 45 days with less than a 20% deviation in biodegradation. In comparison, the microcrystalline cellulose biodegradation results by various studies listed in Table 2.1, achieved 70% or greater biodegradation by day 45.

The biodegradation of the cellphone cases cut into 7 × 3.5 × 0.2 cm and 4.6 × 3.5 × 0.2 cm pieces was 20 ± 8% and 21 ± 13%, respectively after 46 days. The relative (to the positive control) biodegradation of the 7 × 3.5 × 0.2 cm and 4.6 × 3.5 × 0.2 cm pieces was 28% and 30%, respectively. There was not a significant difference in biodegradation between the different sized phone cases in this trial by day 46 ($p = 0.61 > 0.05$), nor at any other point in the trial (Appendix B). The weight loss of 7 × 3.5 × 0.2 cm and 4.6 × 3.5 × 0.2 cm pieces of cellphone cases was 34 ± 8% and 31 ± 2% after 46 days under lab scale composting conditions at 58 ± 2°C. After 46 days, % biodegradation and % weight loss results were not identical (around 20% versus 30%), which indicated that the majority of weight loss may be attributed to organics converted to carbon dioxide, which was expected but other factors may also have played a role.

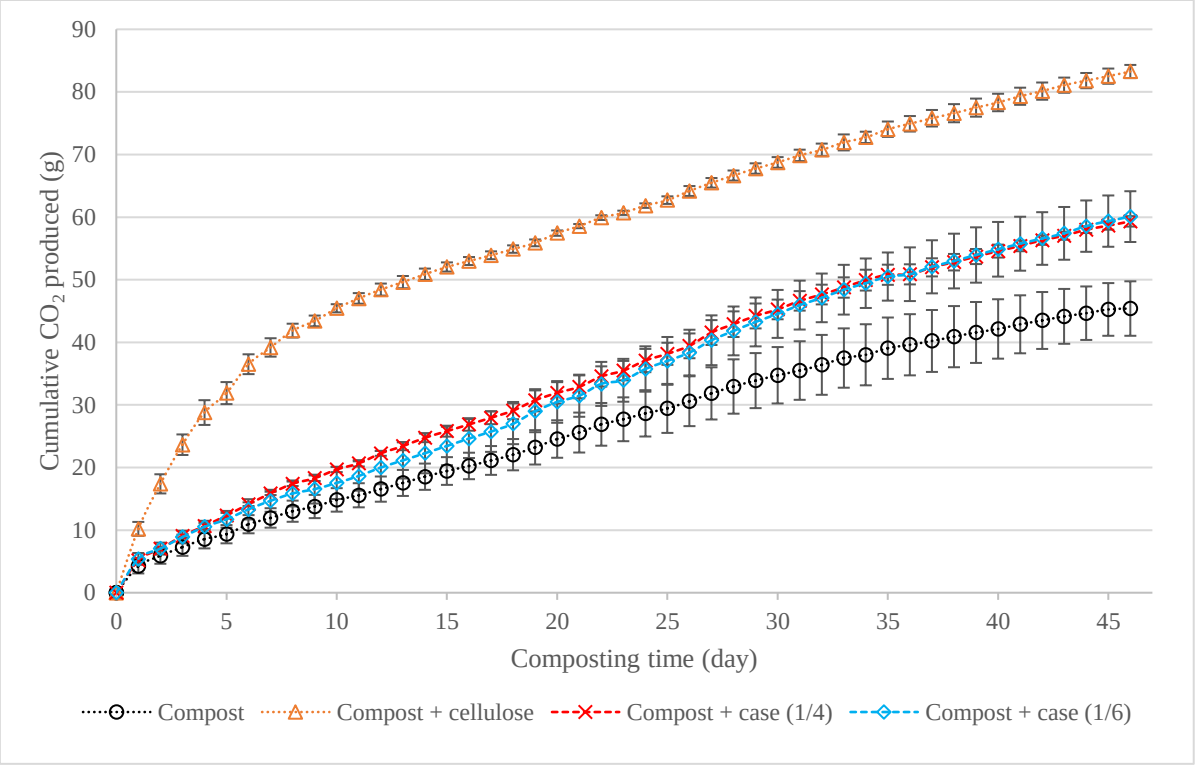

Figure 4-1. Cumulative carbon dioxide production at standard temperature (0°C) and pressure (1 atm) in trial 1 of laboratory scale composting assays at 58 ± 2°C with cellphone cases cut into 1/4th and 1/6th pieces. *Cases cut into 1/4th and 1/6th pieces correspond to 7 × 3.5 × 0.2 cm and 4.6 × 3.5 × 0.2 cm pieces, respectively. Cellulose is included as positive control. Points represent averages and error bars are standard deviation of triplicate assays.*

The second composting trial achieved 62 ± 14%, 10 ± 9%, and 12 ± 18% biodegradation of the microcrystalline cellulose, the 2 × 2 × 0.2 cm and the 4 × 4 × 0.2 cm phone cases, respectively after 34 days (Figure 4.2). As a side note, this trial was ended on day 34, as the laboratory was shutdown due to the COVID-19 pandemic. Since the trial had to be shutdown earlier than day 45, the validity of the positive control could not be conclusively determined; however, there is a good chance that it would have reached 70% biodegradation by day 45, as it had already reached 62% biodegradation. In contrast, trial 1 achieved 67 ± 11% biodegradation of microcrystalline cellulose by day 34. There was not a significant difference in the amount of carbon dioxide produced by the two different sized phone cases by day 34 in both trial 1 and trial 2 ($p=0.95>0.05$ and $p=0.84>0.05$, respectively). There was a significant difference on day 6 to day 14 in trial 2 (Appendix B), as the

2 × 2 × 0.2 cm phone cases produced more carbon dioxide than the 4 × 4 × 0.2 cm phone cases. This may be attributed to the smaller size of the former. It is also possible that the size difference here was only enough to impact the initial degradation; therefore, the degradation rate not the extent of the biodegradability was impacted. The weight loss of 2 × 2 × 0.2 cm and 4 × 4 × 0.2 cm pieces of cellphone cases was 21 ± 1% and 26 ± 0% after 34 days. Interestingly, in both trials the weight loss of the cases was about 10% more than the biodegradation. This points to the fact that weight loss does not mean biodegradation. It may also be attributed to the fact that the biodegradation results might be more accurate if the gas measurements were taken automatically rather than manually. As a side note, the increase in standard deviation for the 4 × 4 × 0.2 cm cases on day 27 and onward was due to one of the jars with this treatment breaking on day 27.

The relative biodegradation of the 2 × 2 × 0.2 cm and 4 × 4 × 0.2 cm phone cases in this trial (trial 2) was 16% and 20%, respectively after 34 days. In comparison, in trial 1, the relative biodegradation of the cellphone cases cut into 7 × 3.5 × 0.2 and 4.6 × 3.5 × 0.2 cm pieces, after 34 days, was 26% and 25%, respectively. The 4 × 4 × 0.2 cm (trial 2) and the 4.6 × 3.5 × 0.2 cm (trial 1) pieces were similar in size and did not have a statistically significant difference in biodegradation by day 27 ($p=0.52>0.05$).

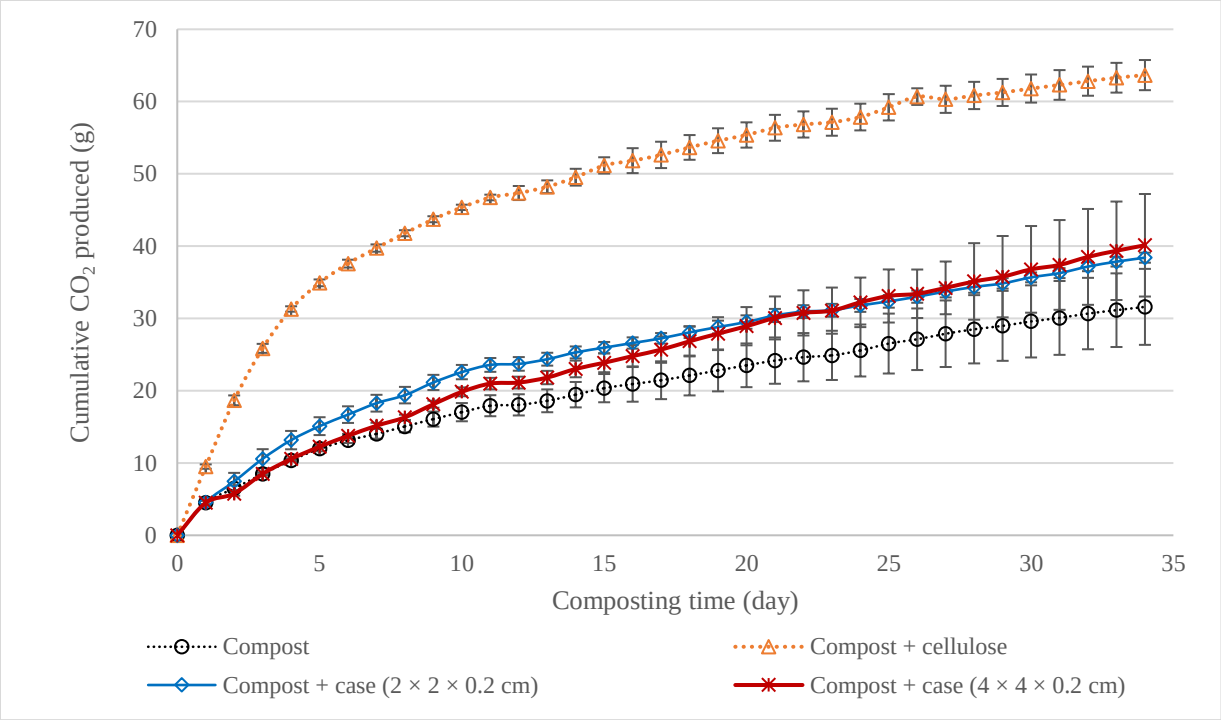

Figure 4-2, Cumulative carbon dioxide production at standard temperature (0°C) and pressure (1 atm) in trial 2 of laboratory scale composting assays 58 ± 2°C with cellphone cases. *Cellulose is included as positive control. Points represent averages and error bars are standard deviation of triplicate assays (except for the assays with 4 cm x 4 x 0.2 cm on day 27 onwards due to one of the triplicate jars breaking).*

There was not a statistically significant difference between the 7 × 3.5 × 0.2 cm (trial 1), 4.6 × 3.5 × 0.2 cm (trial 1), and 2 × 2 × 0.2 cm (trial 2) pieces using a single factor ANOVA test ($p=0.09>0.05$) by day 34. In this case, the ANOVA did not include the 4 × 4 × 0.2 cm pieces as one of the jars broke on day 27, resulting in a high standard deviation. There was a statistically significant difference between the biodegradation of the 7 × 3.5 × 0.2 cm (trial 1) pieces and the 2 × 2 × 0.2 cm pieces (trial 2) ($p=0.02<0.05$) by day 34, even though the phone case pieces were smaller in trial 2. This may be because the compost used in these trials was from different periods of time. As mentioned previously, the same source of compost can produce different results at different points in time. For instance, one study found that the biodegradation of microcrystalline cellulose ranged from 50-85% when the same compost source was used from different point in

time; thus, they stated that it is difficult to compare biodegradation results from different tests. Various factors including pH, TS, VS, and the carbon to nitrogen ratio C/N can affect the biodegradation results (Castro-Aguirre et al., 2017). In this case, the difference could be attributed to the higher moisture content present in the compost of the second trial, as it may have reduced the available airspace for the microorganisms.

Biodegradation often occurs via surface erosion; thus, some researchers have proposed that biodegradation is more rapid when the test material is in smaller pieces (Yang et al., 2005). In these trials, it appears that the size of the phone cases does not make a big difference in biodegradation. Moreover, in real life scenarios, the cellphone cases are more likely to be placed whole in composting piles; thus, the cellphone case sizes from trial 1 are closer to real life scenarios.

According to ASTM D5338-15 the incubation time of 45 days may be extended if significant biodegradation is being observed; thus, in this case trial 1 was ended on day as the CO_2 production was starting to decrease by day 46 and the biodegradation of the cellphone cases were low compared to other bioplastics (mentioned in Table 2.1). The lower biodegradation in comparison to other bioplastics is likely due to the low biobased content of the cases. In comparison, some studies have found the biodegradation of PBS (20 × 20 × 0.2 cm) to achieve approximately 20% relative biodegradation by day 45 (Narancic et al., 2018). Another study found that PBS powder (42 μm) achieved 10% biodegradation in 45 days (Zhao et al., 2005). It is also important to note that specific microorganisms (as previously mentioned in the literature review), have been found to degrade specific bioplastics. Since the compost microbiology is influenced by various factors such as compost source, some composts may have the microorganisms that are better at degrading various bioplastics while other do not. Furthermore, the low reproducibility of biodegradation, particularly composting, tests, in general, means that different studies will likely achieve different results.

While there is possibility for the cases to biodegrade more after 45 days, their low biobased content, likely means that they will not degrade at the same rate as pure bioplastics such as PLA. In comparison one study demonstrated that when thermopolyurethane (TPU) (fossil based plastic) was added to PLA, the general trend was a lower biodegradation percentage when TPU was increased (Jašo et al., 2015) (Table 2.1).

4.2 Field Scale Composting Tests

The weight loss of the cellphone cases was lower (10%) at field scale at day 46 (60-66^{o}C) than the biodegradation in the composting (58±2^{o}C) assays (up to 21%); however, this was likely due to the low moisture content and pH of the compost at the beginning of the trials (Tables 4.2; 4.3). The cellphone cases were removed from the composting pile on day 46, to provide a comparison to the laboratory scale composting assays. They were not sampled earlier to avoid disruption of microbial activity. The field scale tests were continued until day 80 to see if there was an increase in weight loss under a longer period of time, especially since the original conditions of the Glengrow compost pile were not optimal (low pH and moisture).

The weight loss reached 55% by day 80, but that may be because the cellphone cases (buried in compost piles) became too small to be able to recover all of the disintegrated pieces used to determine the weight loss. Thus, weight loss may not mean all biodegradation and it is impossible to validate it by comparing the results to carbon dioxide production as it is not possible to trace carbon dioxide production from cellphone cases at the field scale. This was also seen in the laboratory scale composting assays in which the weight loss was 10% more than the biodegradation. In another trial conducted the previous year, to test the methodology, a cellphone case achieved 34% weight loss when placed in composting piles. The phone cases were first placed in a compost pile with an average moisture content of 47% and an average temperature of 71^{o}C,

for 35 days. The case was then placed in another compost pile with an average moisture content of 62% and an average temperature of 55^{o}C, for 43 days. Two piles were used as the phone case needed to be moved as the initial pile needed to be turned over. These results demonstrate the variability in results that occur in field scale conditions. As seen in Table 4.2, the cellphone cases can absorb up to 27% water. This absorption in water in combination with the high temperature (60-67^{o}C) of the composting piles may have caused the high disintegration of the cases. In comparison, one study found the weight loss of PLA (2 × 2 × 0.2 cm) in compost, soil, and sterile water at 50^{o}C to be 68%, 64%, and 57%, respectively in 4 weeks. Moreover, the weight loss of PLA in compost extract and soil extract (no microorganisms) were 53% and 57%, respectively. In this case, chemical hydrolysis, in the presence of water, can cause PLA degradation to occur at high temperatures, with microorganisms being able to use the products of PLA degradation (Karamanlioglu and Robson, 2013); however, in our case, the cellphone cases do not have a high biobased content. Thus, their degradation without the microbial consumption of the products, may cause the formation of microplastics.

Table 4.2. Weight loss of whole cellphone cases under field scale composting conditions

Composting time (day)	Original weight of cases (g)	Wet weight after composting (g)	Weight after drying for 48 hours (g)	Water absorption of cases (%)	Weight loss of cases (%)
46	32.63 (±0.18)[a]	35.13 (±0.28)	29.52 (±0.50)	16 (±2)	10 (±2)
80	32.53 (±0.21)	20.35 (±4.73)	14.72 (±2.48)	27 (±6)	55 (±8)

[a] Data represent arithmetic mean of triplicate measurements (±standard deviation)

Table 4.3. Characteristics of compost around cellphone cases under field scale composting conditions

Composting time (day)	Total solids (% w/w)	Volatile solids (% w/w)	Moisture (%)	Temperature (ºC)	pH
0	70.8 (±3.1)[a]	34.8 (±2.4)	29.2 (±3.1)	60	5.41 (±0.21)
46	56..0 (±9.0)	27.2 (±4.1)	44.0 (±9.1)	66	7.63 (±0.70)
80	54.9 (±4.3)	26.0 (±4.1)	45.1 (±4.3)	67	7.98 (±0.92)

[a] Data represent arithmetic mean of 27 measurements (standard deviation)

4.3 Microbial Characteristics of Anaerobic Inoculum

In this study, the genomic analysis of the microorganisms present in the inocula was outside the scope and budget of this project; however, other reports from ongoing projects at the BTG laboratory with similar anaerobic digesters have conducted microbial analyses in partnership with researchers from Microbiology and Immunology at UBC's Vancouver Campus. The structure of the microbial communities was determined based on the 16S rRNA amplicon profiling on the Illumina miSeq platform. These reports include microbial analyses from mesophilic (35±1ºC, SRT = 6 days) and thermophilic (55±1ºC, SRT = 6 days) digesters. The digesters were fed with 67% thickened waste activated sludge and 33% fermented primary sludge from the West Kelowna WWTP. The main methanogens were *Methanothermobacter* (hydrogenotrophic methanogens) and *Methanosaeta* (acetoclastic methanogens) under thermophilic and mesophilic conditions, respectively. The dominant bacterial cultures under mesophilic conditions were Candidate Division WWE1 (Wastewater of Evry 1), *Firmicutes, Proteobacteria*, and *Bacteroidetes*. On the other hand, *Firmicutes* and *Thermotogae,* were the dominant bacterial phyla under thermophilic conditions (Kor-Bicakci et al., 2020). The variety of dominant bacteria phyla likely decreased in diversity under thermophilic because microbial diversity tends to decrease under thermophilic conditions. The microorganisms from mesophilic inoculum 1 may be similar to the mesophilic microorganisms in this study as the same mixed sludge was used to feed both digesters.

Another study analyzed the methanogenic population in mesophilic (38°C) BMP assays with F/M ratios od 2.2, 3.2, and 4.4 g VS $_{Substrate}$/g VS $_{Inoculum}$. In this case, the inoculum was from a second stage (methane phase) digester (SRT=28 days) and the substrate was the effluent from a first stage (acid phase, SRT=2 days) digester (which was fed with the same mixed sludge (65 ± 5% primary and 35 ± 5% secondary clarifier sludge) from Lulu Island WWTP, Vancouver, as mesophilic inoculum 2. The dominant methanogens were *Methanomicrobiaceae*, *Methanospirillaceae*, and *Methanosarcinacea* (acetoclastic methanogen) (BTG 2020, unpublished). *Methanomicrobiaceae* and *Methanospirillaceae* belong to the order *Methanomicrobiales* (hydrogenotrophic methanogens). The methanogens in this study may be similar to the to the methanogenic archaea present in the mesophilic inocula 2, as the first phase digestor used the same type of mixed sludge; however, the two inocula had different SRTs. Differences in SRT can cause changes in the microbial community structure.

Another research project looked at the microbial population in thermophilic (55°C) BMP assays under F/M ratios of 2.2 and 4.4 g VS $_{Substrate}$/g VS $_{Inoculum}$. This research used the same inoculum and mixed sludge as the thermophilic acclimatized reactor used in this study, the only difference was that the SRT was 20 days instead of 15 days. They researchers found that *Thermotogae*, *Protobacteria*, *Synergistes,* and *Coprothermobactereota* were the dominant bacterial phyla under thermophilic conditions. Moreover, the phyla *Firmicutes* became dominant on day 20 in F/M 2.2 g VS $_{Substrate}$/g VS $_{Inoculum}$. Similarly, to the study conducted by Kor-Bicakci et al. (2020), they also found *Methanothermobacter* to the most dominant methanogens under thermophilic conditions (BTG 2020, unpublished). The results regarding methanogenic archaea were in accordance with literature, since hydrogenotrophic methanogens are more dominant than acetoclastic methanogens under thermophilic conditions (Franke-Whittle et al., 2014).

The thermophilic microbial analyses of the bacterial population had similarities such as the abundance of *Thermotogae*, which are often dominant bacteria in thermophilic anaerobic cultures. They excrete hydrolytic enzymes to degrade polysaccharides into acetate, carbon dioxide, and hydrogen. The phyla *Protobacteria* and *Synergistes* were also present in the study by Kor-Bicakci et al. (2020), although to a lesser extent. These differences in microbial diversity was likely due to the differences in substrate and operating conditions (i.e. SRT).

4.4 Performance of Anaerobic Biodegradability Assays

The results of the weekly analyses of the anaerobic inocula and co-substrate used to set up BMP assays are summarized in Table 4.4. The parameters of the inoculum were within typical literature ranges; however, the alkalinity of the thermophilic inoculum was slightly high (Appels et al., 2008). The pH for the thermophilic inoculum was higher than the mesophilic inoculum. This was likely due to the lower solubility of CO_2 at higher temperatures (Olaya et al., 2020). The ammonia concentration for the thermophilic inoculum was higher than that of the mesophilic inoculum which was as expected.

Table 4.4. Properties of municipal mixed sludge (co-substrate) and anaerobic inocula

Parameters	Municipal mixed sludge	Thermophilic inoculum	Mesophilic inoculum 1[a]
pH	5.33 (±0.57)	7.88 (±0.12)	7.43 (±0.10)
Total solids (% w/w)[b]	4.2 (±0.4)	1.9 (±0.2)	2.3 (±0.0)
Volatile solids (% w/w)	3.6 (±0.4)	1.3 (±0.2)	1.7 (±0.0)
Ammonia (mg/L)	446 (±141)	1343 (±19)	913 (±49)
Alkalinity (mg as $CaCO_3$/L)	1369 (±254)	5535 (±418)	3800 (±380)
COD (g/L)	56.9 (±10.4)	22.9 (±3.1)	24.0 (±0.3)
Volatile fatty acids (mg/L)[c]	2418 (±517)	47 (±8)	30 (±10)

[a]Abbott and Eskicioglu (2020)
[b]Data represent arithmetic mean of triplicate measurements (±standard deviation)
[c]Summation of acetic, propionic and butyric acids

4.4.1 Performance of Anaerobic Assays with Cellulose (Positive Control)

Like the composting assays, the BMP assays also used microcrystalline cellulose as a positive control. In anaerobic degradation, cellulose is normally the positive control as it is relatively cheap and involves all the anaerobic degradation steps (Koch et al., 2017). Unfortunately, the results are rarely 100% accurate. This is due to approximately 10% of cellulose being used by the microorganisms to grow and generate heat; thus, the positive control is expected to achieve 80% biodegradation. As a side note, cellulose controls are uncommon in BMP papers even though they verify the BMP method accuracy (Filer et al., 2019).

During anaerobic digestion, cellulose is hydrolyzed according to the following reaction to produce the primary product of glucose. This process involves breaking the β-1, 4-glycosidic linkages in cellulose (Anukam et al., 2019).

$$(C_6H_{10}O_5)_n + n\,H_2O \rightarrow n\,(C_6H_{12}O_6) + n\,(H_2) \quad (4.1)$$

During acidogenesis, the glucose can be broken down into various products including ethanol (CH_3CH_2OH), propionic acid (CH_3CH_2COOH), and acetic acid (CH_3COOH). Although ethanol generally forms when the pH is less than 5. The products from this stage are used in the following reactions during acetogenesis and methanogenisis to ultimately produce carbon dioxide and methane (Anukam et al., 2019).

$$C_6H_{12}O_6 \rightarrow 2\,CH_3CH_2OH + 2\,CO_2 \quad (4.2)$$

$$C_6H_{12}O_6 + 2\,H_2O \rightarrow 2\,CH_3CH_2OOH + 2\,H_2O \quad (4.3)$$

$$C_6H_{12}O_6 \rightarrow 3\,CH_3COOH \quad (4.4)$$

4.4.1.1 Optimization of F/M Ratio in Mesophilic Anaerobic Digestion Utilizing Cellulose

Cellulose degrades relatively quickly; thus, too high of a concentration can cause an accumulation of VFAs in anaerobic digesters. Typically, the methanogenic archaea degrade the VFAs as substrate, but an accumulation can decrease the pH and inhibit the methanogens. On the other hand, too low of a concentration of microcrystalline cellulose can lead to starvation of both VFA producing fermentative bacteria and methane producing archaea. Thus, a large range of organic loading quantified by F/M ratios (0.5 to 5 g COD of cellulose/g $VS_{Inoculum}$ under mesophilic conditions**)** was tested in order to find the optimal ratio that achieved 80% biodegradation with the lowest VFA accumulation (highest VFA to methane conversion) for cellulose. The lower end of F/M ratio was chosen based on the optimal F/M ratio for glucose (0.5 g COD/g $VS_{Inoculum}$), which degrades quickly and requires a low F/M ratio. The highest F/M ratio was chosen as a value that would most likely cause VFA inhibition; thus initially, very low and high values were chosen in hopes of narrowing down the correct F/M ratio in the middle.

As seen in Figure 4.3, all of the F/M ratios, except for the highest organic loading ratio (F/M of 5 g COD/g $VS_{Inoculum}$), reached (or close to) 80% biodegradation of microcrystalline cellulose in mesophilic inoculum 1. The F/M of 1 g COD/g $VS_{Inoculum}$ achieved 75% biodegradation.

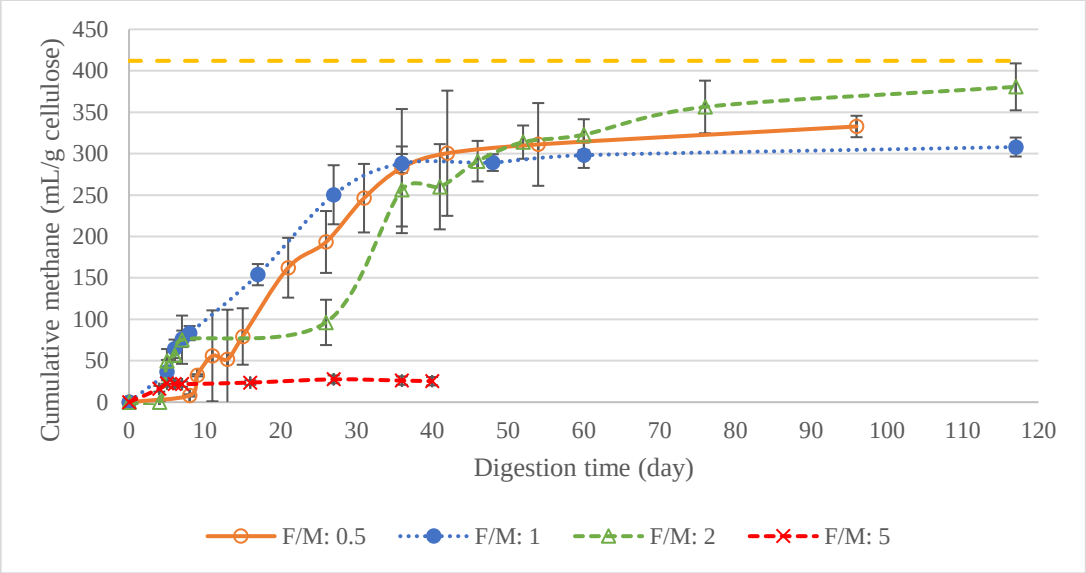

Figure 4-3. Cumulative methane production per grams of microcrystalline cellulose at standard temperature (0°C) and pressure (1 atm) at F/M ratios ranging from 0.5 to 5 g COD/ g VS in mesophilic (38°C) inoculum 1. *Straight dashed line at the top of the graph represents theoretical methane yield of cellulose based on chemical formula. Points represent averages and error bars are standard deviation of triplicate assays.*

In the BMP bottles, on day 6, the VFA concentration of assays set up with F/M of 1, 2, and 5 g COD/g VS $_{Inoculum}$ were 3282 ± 166, 4524 ± 500, and 6582 ± 66 mg/L, respectively, while the VFA concentration of bottles with F/M of 0.5 g COD/g VS $_{Inoculum}$ on day 8 was 1642 ± 4 mg/L (Figure 4.4). This corresponded to the following pH values in assays on day 6 for F/M of 1, 2, and 5 g COD/g VS $_{Inoculum}$: 6.77 ± 0.06, 5.56 ± 0.04, and 5.30 ± 0.01, respectively. The pH in BMP assays with F/M of 0.5 g COD/g VS $_{Inoculum}$ on day 8 was 7.09 ± 0.11 (Figure 4.5). The pH of anaerobic digestion processes can vary between 6-8.3; however, methanogens tend to perform the best at neutral pH values (6.5-7.5) (Angelidaki and Sanders, 2004). Thus, the pH values for F/M of 5 g COD/g VS $_{Inoculum}$ were far too low and inhibited the microorganisms, as can be seen by the low methane production (Figures 4.3; 4.4). On the other hand, the F/M of 2 g COD/g VS $_{Inoculum}$ had a low pH of 5.56, but it increased to 6.75 ± 0.12 by day 36; thus, some microbial inhibition occurred on day 6, but the VFAs were slowly consumed by the methanogens. This can be seen in

Figure 4.3, from day 6 to 36, when the assays experienced a dip in methane production. The pH of F/M of 0.5 and 1 g COD/g VS $_{Inoculum}$ were at acceptable levels; however, F/M of 0.5 g COD/g VS $_{Inoculum}$ maintained a more neutral pH and a lower VFA concentration. Thus, the BMP assays with F/M of 0.5 g COD/g VS $_{Inoculum}$ appeared to have a more optimal pH and a lower VFA concentration indicating the highest conversion of VFA to methane. As a side note, the F/M of 0.5 g COD/g VS $_{Inoculum}$ was ended sooner than the other assays, as it was started afterwards the other assays and had achieved 80% biodegradation by day 96 (Figure 4.3).

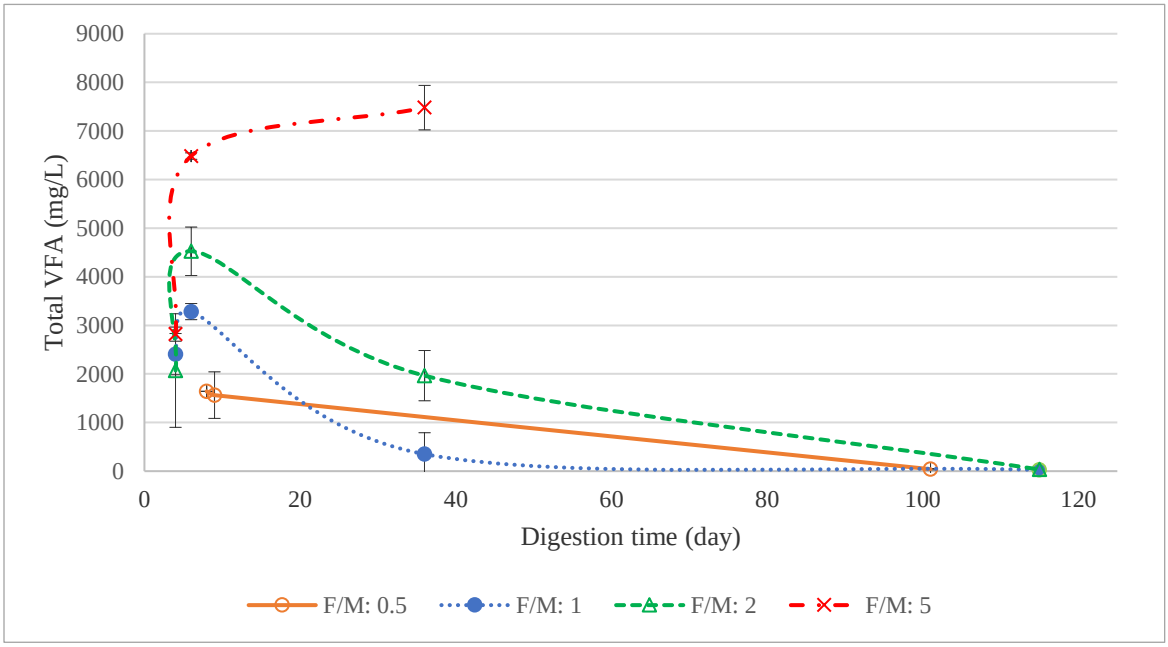

Figure 4-4. Total volatile fatty acids (VFA) concentration during the degradation of microcrystalline cellulose at standard temperature (0°C) and pressure (1 atm) at F/M ratios ranging from 0.5 to 5 g COD/ g VS in mesophilic (38°C) inoculum 1. *Points represent averages and error bars are standard deviation of triplicate assays.*

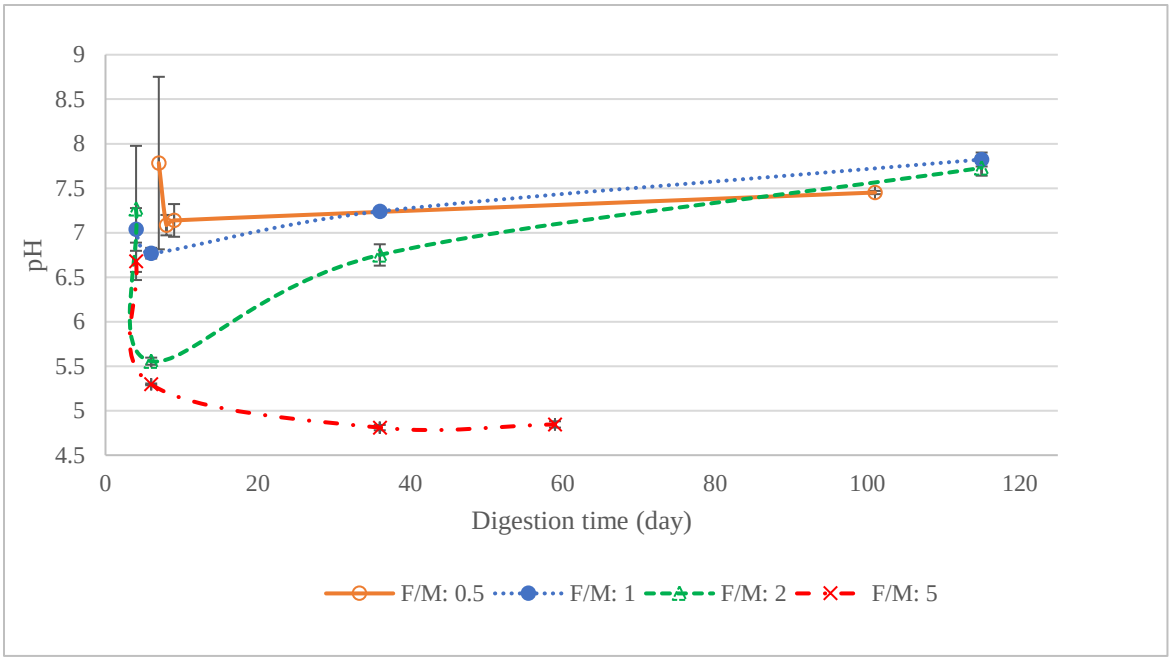

Figure 4-5. pH monitored during the degradation of microcrystalline cellulose at standard temperature (0°C) and pressure (1 atm) at F/M ratios ranging from 0.5 to 5 g COD/ g VS in mesophilic (38°C) inoculum 1. *Points represent averages and error bars are standard deviation of triplicate assays.*

4.4.1.2 Optimization of F/M Ratio in Thermophilic Anaerobic Digestion Utilizing Cellulose

The biodegradation of microcrystalline cellulose was also conducted under thermophilic (55ºC) conditions, as the rate of reactions typically occurs at a faster rate when the temperature is increased. In this case, the F/M of 0.5 g COD/g VS $_{Inoculum}$ achieved 80% degradation while F/M of 1 g COD/g VS $_{Inoculum}$ did not (Figure 4.6). While the pH in BMP assays at day 3 for F/M of 0.5 and 1 g COD/g VS $_{Inoculum}$ (7.48 ± 0.04 and 6.82 ± 0.04), were both within the acceptable range, the pH of assays with F/M 0.5 g COD/g VS $_{Inoculum}$ was more neutral. Moreover, the F/M of 0.5 maintained a lower VFA concentration than F/M of 1 g COD/g VS $_{Inoculum}$ on day 3 (2411 ± 29 and 4530 ± 709 mg/L, respectively). A neutral pH is more important under thermophilic than mesophilic conditions as thermophilic microorganisms are more sensitive to environmental disturbances. This is due to the reduction of microbial diversity at thermophilic temperatures and

subsequent reduction in resilience (Labatut et al., 2014). Thus, this may explain why the F/M of 1 g COD/g VS $_{Inoculum}$ did not achieve 80% biodegradation.

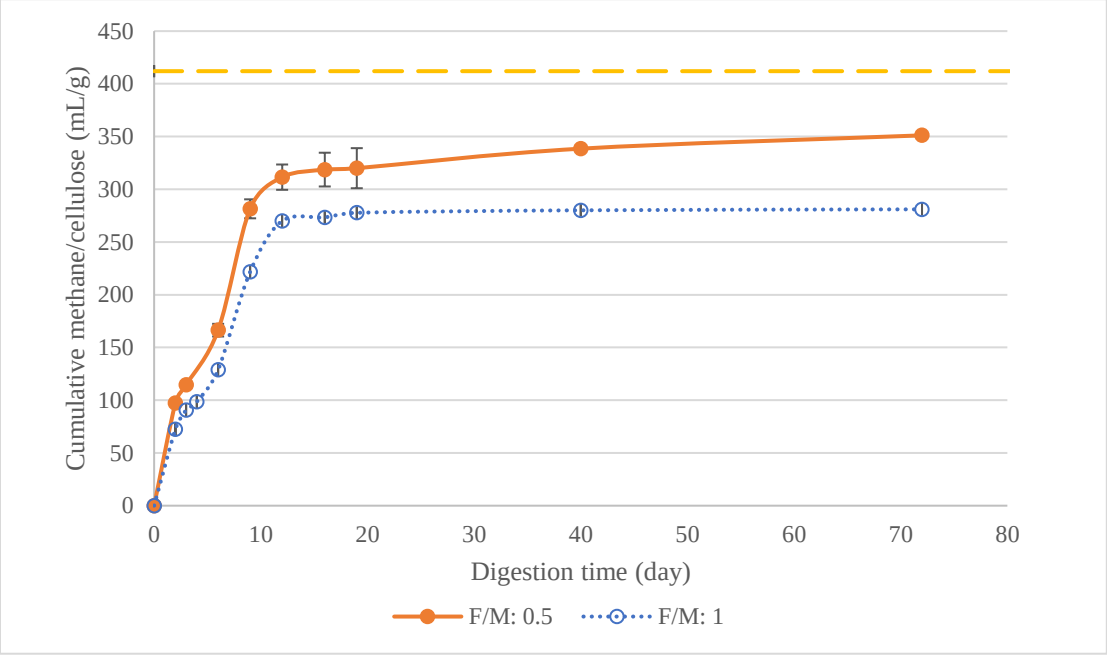

Figure 4-6. Cumulative methane production per grams of microcrystalline cellulose at standard temperature (0°C) and pressure (1 atm) at F/M ratios of 0.5 and 1 g COD/ g VS in thermophilic (55°C) inoculum. *Straight dashed line at the top of the graph represents theoretical methane yield of cellulose based on chemical formula. Points represent averages and error bars are standard deviation of triplicate assays.*

4.4.1.3 Comparison of Cellulose Degradation among Inocula

Different sources of inoculum can result in different substrate degradation values. This is due to different microbial populations, the adaptation of the substrate, and the initial activity of the microorganisms (Filer et al., 2019). For instance, one study achieved a similar methane yield among various inocula (from an agricultural biogas plant treating manure, from a WWTP digester, and from a biowaste treatment plant) at mesophilic temperatures (38^{o}C); however, the rate of microcrystalline cellulose biodegradation varied (Koch et al., 2017). Thus, the biodegradation of microcrystalline cellulose was tested in different inocula.

In this study, mesophilic inoculum 1, mesophilic inoculum 2, and thermophilic inoculum achieved 81%, 80%, and 77% biodegradation of cellulose by day 96, 22, and 19, respectively (Figure 4.7). As seen in Figure 4.7, mesophilic inoculum 2 and thermophilic inoculum had the highest rates of methane production under the organic loading of F/M: 0.5 g COD/g VS $_{Inoculum}$; thus, they were used in the following experiments involving the biodegradation of the cellphone cases under anaerobic conditions. Moreover, mesophilic inoculum 2 and thermophilic inoculum were treating the same sludge (from Lulu Island WWTP, Vancouver), which made them a better choice of inoculum in comparison to mesophilic inoculum 1 (which used sludge from a WWTP in West Kelowna).

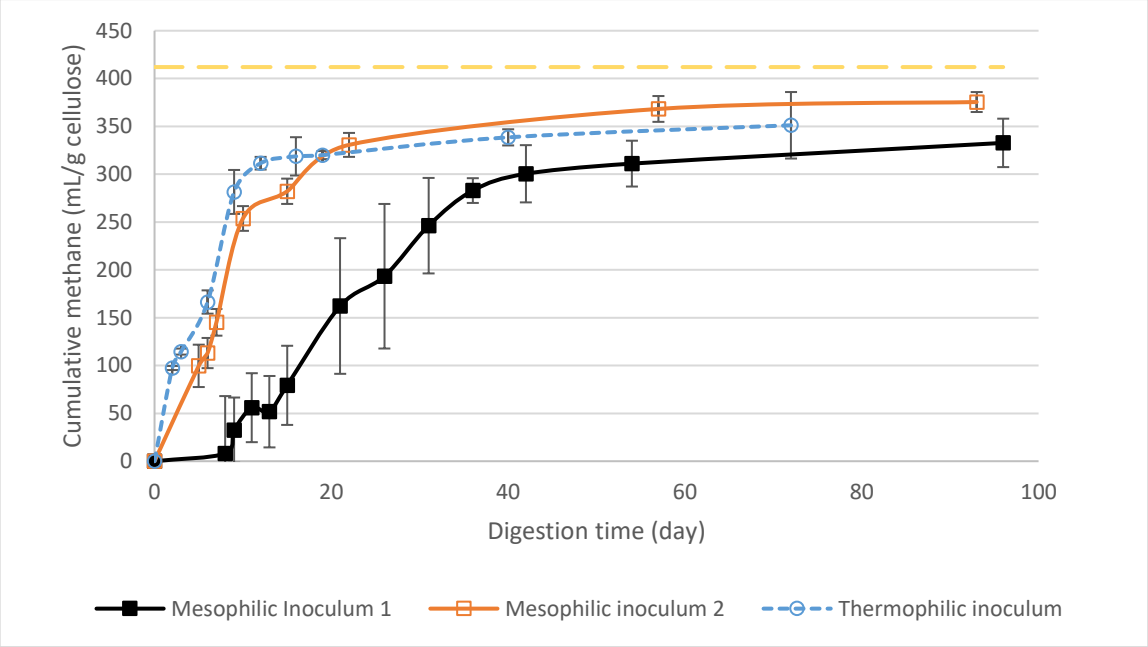

Figure 4-7. Cumulative methane production per grams of microcrystalline cellulose by mesophilic inoculum 1, mesophilic inoculum 2, and thermophilic inoculum at standard temperature (0°C) and pressure (1 atm) at F/M ratio of 0.5 g COD/ g VS. *Straight dashed line at the top of the graph represents theoretical methane yield of cellulose based on chemical formula. Points represent averages and error bars are standard deviation of triplicate assays.*

Cellulose degradation is normally characterized by a short lag phase followed by a rise in methane production (Koch et al., 2017). In this study, the initial lag period of cellulose degradation was shorter in the thermophilic inoculum than the mesophilic inoculum 2 (Figure 4.7). In

comparison, one study achieved 86% and 91% biodegradation of cellulose under mesophilic (35^{o}C) and thermophilic (55^{o}C) conditions, respectively, using sewage fine sieved fraction as inoculum in BMPs. This study found that thermophilic conditions had a higher rate (an apparent hydrolysis rate of 0.77 ± 0.02 and 1.54 ± 0.02 1/d for mesophilic and thermophilic conditions, respectively) and a smaller lag phase for cellulose biodegradation (Ghasimi et al., 2016). Another study achieved 84%, 90%, 93% and 80%, 87%, and 87% biodegradation of cellulose in mesophilic (38^{o}C) and thermophilic (55^{o}C) batch conditions that corresponded to OLRs of 5.5 ± 0.2 kg VS/m^3, 11.2 ± 0.3 kg VS/m^3 and 16.7 ± 0.4 kg VS/m^3, respectively. The thermophilic inoculum was from a continuously operated plug-flow fermenter, treating maize and grass silage at an OLR of 1.5 kg VS/m^3/d. The mesophilic inoculum came from a CSTR anaerobic digester in a biogas plant with an OLR of 2.5 kg VS/ m^3/d which was fed manure, maize silage, grass silage, and corn whole-crop-silage. In their case, they found that while thermophilic and mesophilic temperatures achieved similar methane yields, the biodegradation was faster under mesophilic conditions due to a larger concentration of active mesophilic bacteria. They also found that the there was a decrease in digestion rate when the OLRs increased. This was more obvious under thermophilic conditions (Golkowska and Greger, 2013).

4.4.3 Performance of Anaerobic Assays with Cellphone Cases

The following section provides the results for the biodegradation of the cellphone cases under anaerobic conditions. All the anerobic assays had to be ended approximately 10-30 days earlier than intended duration due to shut down of the laboratory as a result of the COVID-19 pandemic. In this case the F/M ratios were calculated as g VS $_{\text{Mixed Sludge}}$/ g VS $_{\text{Inoculum}}$, as this format has been used by several other studies.

4.4.3.1 Mesophilic Anaerobic Assays

The results of the methane production from various sized cellphone cases, under mesophilic (38ºC) conditions, at F/M ratio of 1 g VS $_{\text{Mixed Sludge}}$/ g VS $_{\text{Inoculum}}$, is shown in Figure 4.8. The biodegradation for the grinded, 2 × 2 × 0.2 cm, and 4 × 4 × 0.2 cm phone cases was 8 ± 0%, 8 ± 0%, and 8 ± 1%, respectively after 169 days. Moreover, there was significantly lower extent of biodegradation of the cellphone cases under anaerobic conditions than under composting conditions, as by day 42, the grinded, 2 × 2 × 0.2 cm, and 4 × 4 × 0.2 cm phone cases biodegraded by 3 ± 1%, 3 ± 0%, and 3 ± 0%, respectively. In comparison, during the first composting trial, up to 21% biodegradation occurred by day 46 when the cellphone case pieces were much larger. As mentioned, in the literature review, it is not uncommon for bioplastics to degrade less under anaerobic conditions than under composting conditions.

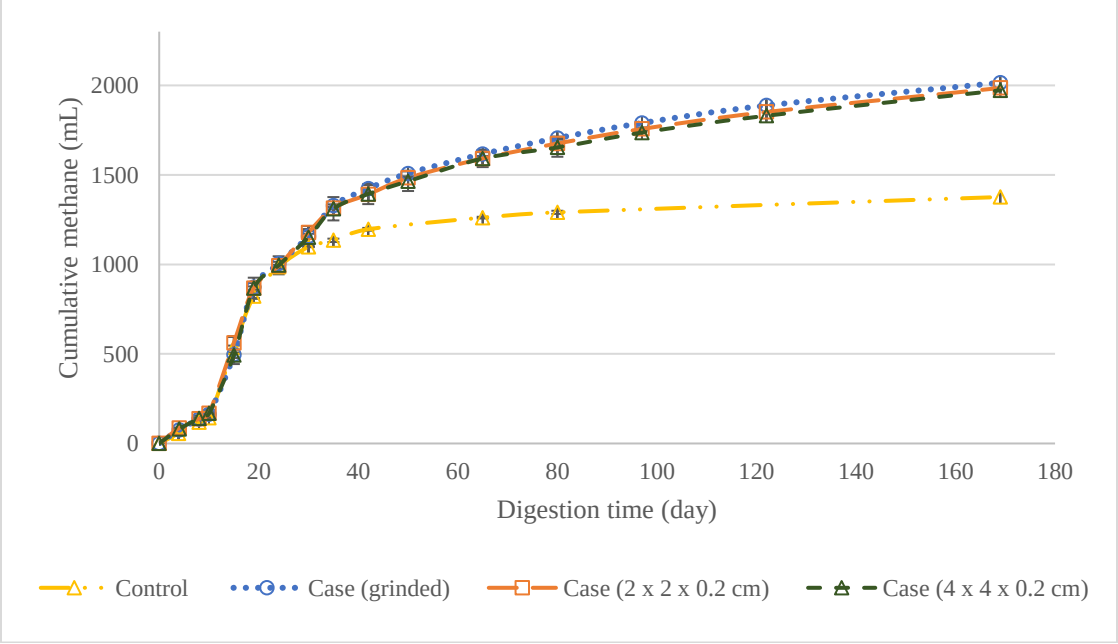

Figure 4-8. Cumulative methane production from cellphone cases under mesophilic (38°C) conditions at standard temperature (0°C) and pressure (1 atm) (F/M: 1 g VS/g VS). *Control assays contain mesophilic inoculum + mixed sludge and others contain inoculum + mixed sludge + cases. Points represent averages and error bars are standard deviation of triplicate assays.*

The results for the statistical analysis comparing the carbon dioxide and methane production (in grams carbon) between the mesophilic anaerobic assays with the cellphone cases and the controls (inoculum + mixed sludge), are reported in Appendix B. As indicated by the statistical analysis, all the assays did not continuously have a significant difference between the control until day 35. The biodegradation of the cellphone cases was seen more clearly in Figure 4.8 after day 35, likely due to a reduction in the co-substrate at that point, as the co-substrate was more readily degradable than the cellphone cases. Furthermore, a single factor ANOVA tested indicated that there was not a significant difference between the anaerobic mesophilic biodegradation of the different sized cellphone cases ($p = 0.47 > 0.05$) on day 169. Thus, the following thermophilic assays were conducted using the grinded cellphone cases, as size did not make a big difference as well as the fact that the serum bottles were smaller and could not contain cellphone that were larger in size. The lack of significant difference was likely due to the low biodegradation of the cellphone cases. Lastly, as seen in Figure 4.9, the pH of the assays with cases and controls were neutral from day 4 to 15, as they ranged from 7.15 to 7.56; thus, the cellphone cases did not degrade quickly like microcrystalline cellulose and cause a decrease in pH that negatively effected the microorganisms.

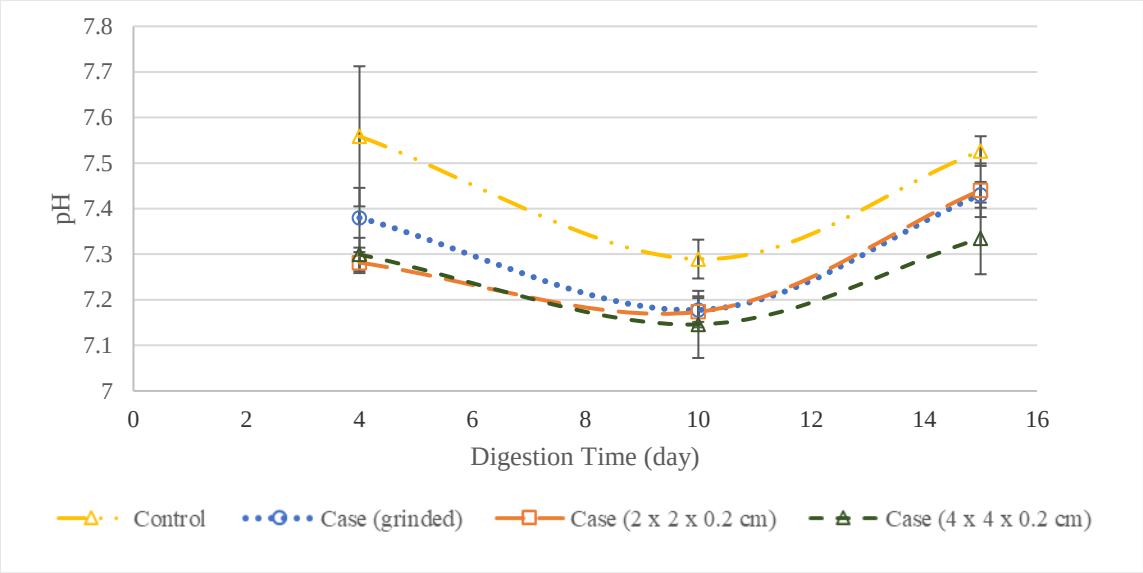

Figure 4-9. pH monitored during the degradation phone cases under mesophilic (38°C) conditions at standard temperature (0°C) and pressure (1 atm) (F/M: 1 g VS/g VS). *Control assays contain mesophilic inoculum + mixed sludge and others contain inoculum + mixed sludge + cases. Points represent averages and error bars are standard deviation of triplicate assays.*

4.4.3.2 Thermophilic Anaerobic Assays

The results of the methane production from the grinded cellphone cases, under thermophilic (55°C) conditions, is shown in Figure 4.10. The biodegradation for the BMP assays with cellphone cases added at 5 g of grinded case/g VS $_{\text{Mixed Sludge}}$ and 10 g of grinded case/g VS $_{\text{Mixed Sludge}}$, was 8 ± 1% and 6 ± 1%, respectively by day 105. On the other hand, by day 38, the biodegradation of the grinded cases added to bottles at 5 g of grinded case/g VS $_{\text{Mixed Sludge}}$ and 10 g of grinded case/g VS $_{\text{Mixed Sludge}}$ was 3 ± 2% and 2 ± 1%, respectively.

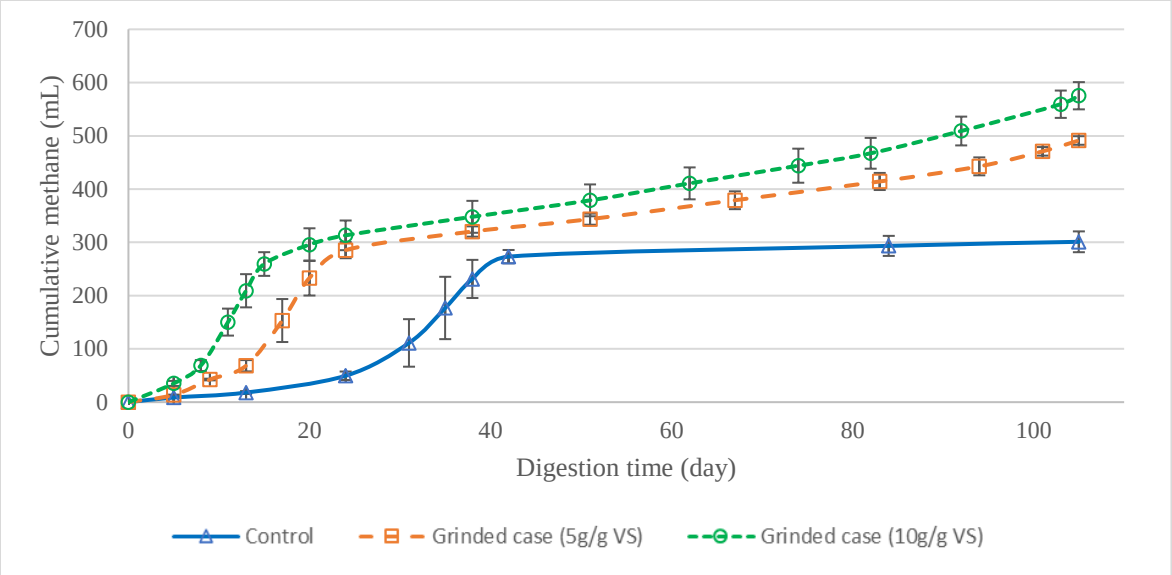

Figure 4-10. Cumulative methane production from grinded cellphone cases under thermophilic (55°C) conditions at standard temperature (0°C) and pressure (1 atm) (F/M: 1 g VS/g VS). *Control assay contain thermophilic inoculum + mixed sludge and others contain inoculum + mixed sludge + cases. Points represent averages and error bars are standard deviation of triplicate assays.*

The results for the statistical analysis comparing the carbon dioxide and methane production (in grams carbon) between the thermophilic assays with the cellphone cases are reported in Appendix B. As indicated by the statistical analysis, the assays were significantly different from each other (except from day 13 to 20). The lower biodegradation of in BMP bottles with 10 g of grinded case/g VS $_{\text{Mixed Sludge}}$ may be due to the larger mass of phone case pieces, that may have provided less surface area overall. It appears that there was not a vast difference between the biodegradation in the mesophilic and thermophilic trials. Therefore, unlike PLA, the cellphone cases do not need thermophilic conditions to degrade anaerobically.

As can be seen in Figure 4.11, the pH in the BMP assays ranged from 7.10 to 8.36. The pH of the assays with 10 g of cellphone cases/g VS $_{\text{Mixed Sludge}}$ was 7.20 ± 0.67 on day 5, while the assays with 5 g of cellphone cases/g VS $_{\text{Mixed Sludge}}$ and the controls had pH of 8.14 ± 0.13 and 8.37 ± 0.02, respectively. The pH of the assays and the controls declined in order from 10 g cellphone cases/g VS $_{\text{Mixed Sludge}}$, 5 g cellphone case/ g VS $_{\text{Mixed Sludge}}$, and finally the controls. It appears that the F/M

of 1 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$ was not at a level that was inhibitory to the microorganisms as the pH was initially higher than the assays with the cases; thus, it is unlikely that the slight lag in methane production in the controls (compared to the assays) was due to a high VFA concentration. This can be corroborated by the fact that the VFAs follow the same pattern as the pH (Figure 4.12). The VFA concentration was quite high for the control and BMP bottles with 5 g grinded cases/g VS $_{\text{Mixed Sludge}}$ on day 24 (3735 ± 422 mg/L) and day 13 (4023 ± 244 mg/L) respectively; however, these values did not appear to cause a decrease in methane production. It seems that the presence of the cellphone cases decreased the lag phase of the controls, with the higher concentration of phone cases causing a faster production of VFAs. As a side note, the biodegradation for the assays with 5 g/ VS $_{\text{Mixed Sludge}}$ and 10 g/ VS $_{\text{Mixed Sludge}}$ grinded cellphone case assays on day 5 was 0.2% and 0.8%, respectively.

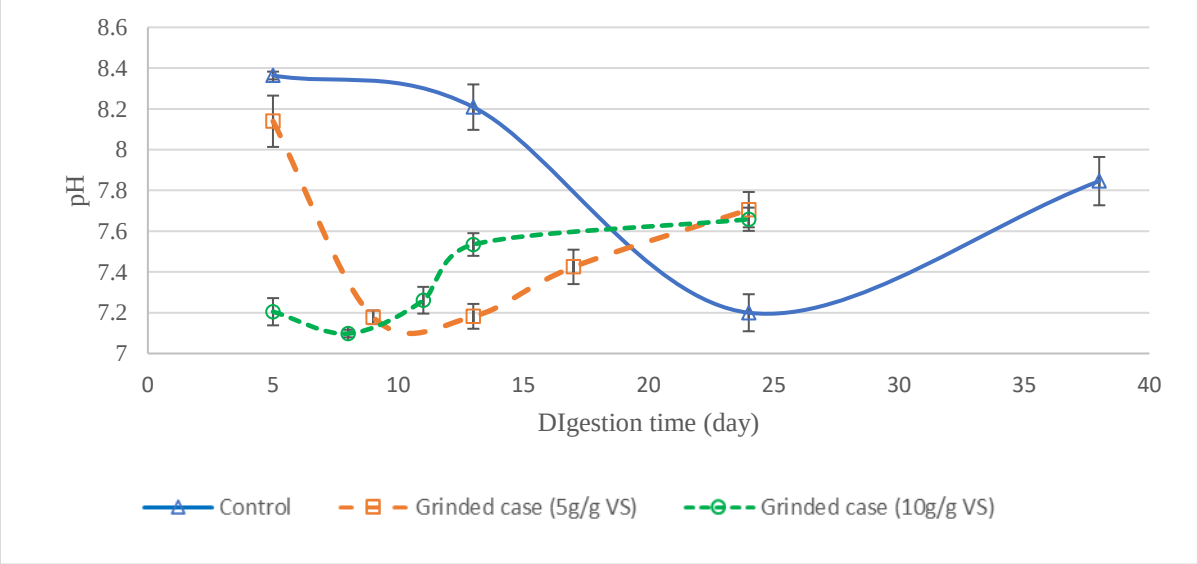

Figure 4-11. pH monitored during the degradation grinded cellphone cases under thermophilic (55°C) conditions at standard temperature (0°C) and pressure (1 atm) (F/M: 1 g VS/g VS). *Control assays contain thermophilic inoculum + mixed sludge and others contain inoculum + mixed sludge + cases. Points represent averages and error bars are standard deviation of triplicate assays.*

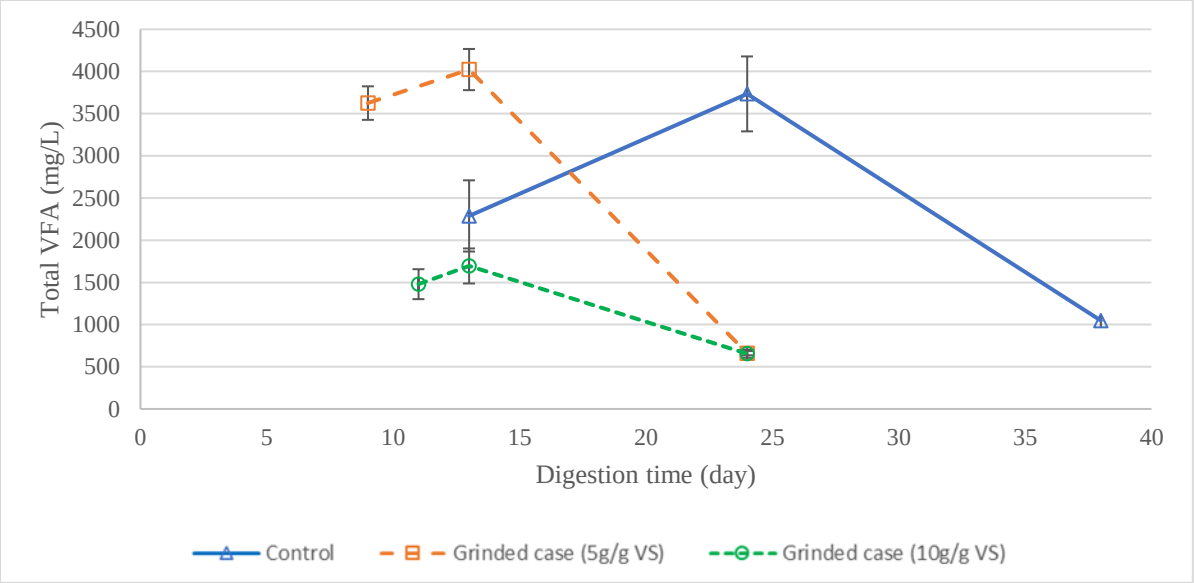

Figure 4-12. Total volatile fatty acids monitored during the degradation grinded cellphone cases under thermophilic conditions (55°C) at standard temperature (0°C) and pressure (1 atm) (F/M: 1 g VS/g VS). *Control assay contain thermophilic inoculum + mixed sludge and others contain inoculum + mixed sludge + cases. Points represent averages and error bars are standard deviation of triplicate assays.*

The lag phase in the controls may be due to the amount of time (about 3-4 weeks) it took to collect the inoculum for the assays and caused the inactivation of microorganisms. It could also be caused by the F/M of 1 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$ being too low; thus, to test this theory, the F/M of 2 and 3 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$ were also tested. As seen in Figure 4.13, the F/M of 2 and 3 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$ also had long lag phases, with the lag phase of F/M 2 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$ being shorter. Therefore, the F/M of 1 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$ was not too low. The presence of the cellphone cases appears to reduce the lag phase in the methane production while also enabling the microorganisms to tolerate high VFAs, but not reducing them.

The F/M of 2 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$ had a long lag phase, with methane production starting to increase on day 34, while the assays with cellphone cases had higher methane production and a shorter lag phase. On the other hand, the F/M of 3 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$ continuously had low methane production. The assays with 3 g cellphone cases/g VS $_{\text{Mixed Sludge}}$ also did not produce a lot of methane, while the assays with 2 g cellphone cases/g VS $_{\text{Mixed Sludge}}$

had a high production of methane (Figure 4.13). The concentration of the cellphone cases seemed to have a larger effect on the F/M of 3 than F/M of 2 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$, likely because of the higher loading rate associated with a higher VFA concentration (ie. F/M 3 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$ had a VFA concentration of 3256 ± 36 mg/L on day 5); however, as previously mentioned, the cellphone cases do not decrease the VFA. Furthermore, there was not a significant difference between the cellphone case assays at F/M 2 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$ (except for day 21 and 52), but there was a significant difference between the cellphone cases assays at F/M 3 g VS $_{\text{Mixed Sludge}}$/g VS $_{\text{Inoculum}}$ (Appendix B). Lastly, we were unable to determine the biodegradation of the cellphone cases under the aforementioned conditions due to the lack of biogas production in the controls.

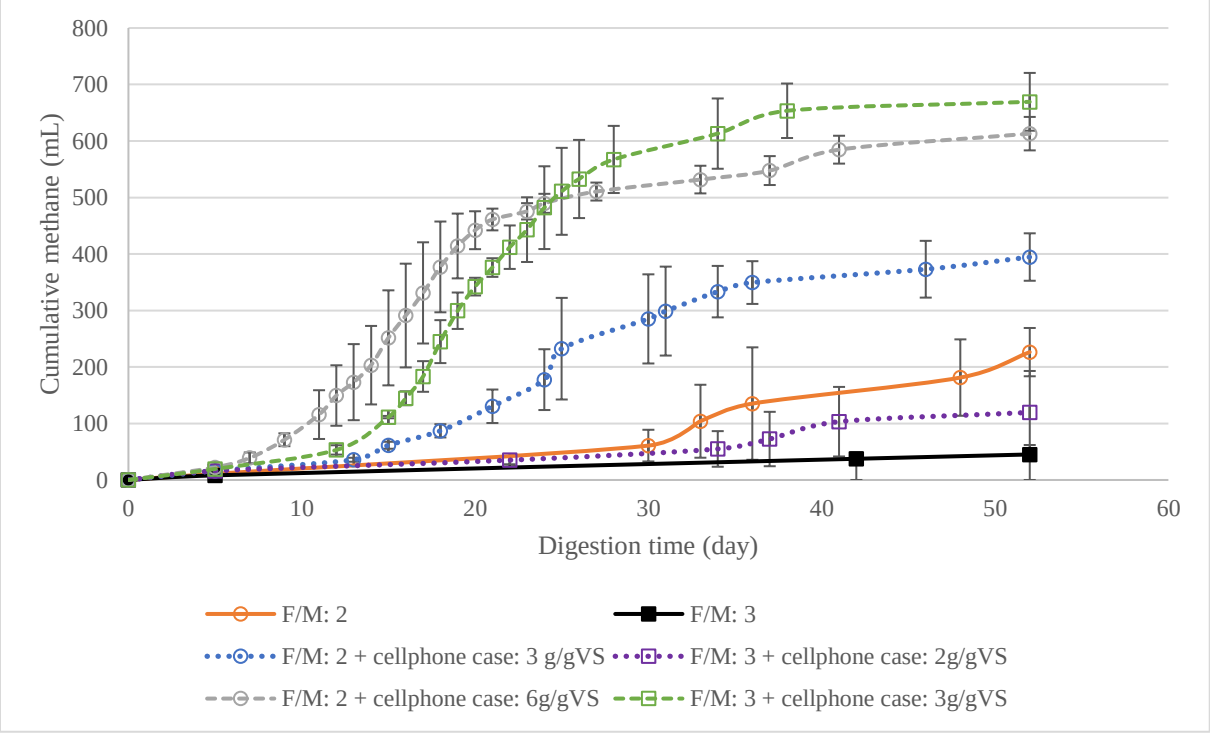

Figure 4-13. Cumulative methane production from grinded cellphone cases under thermophilic (55°C) conditions at standard temperature (0°C) and pressure (1 atm) (F/M ratios: 2 and 3 g VS/g VS). *Control assays contain thermophilic inoculum + mixed sludge and others contain inoculum + mixed sludge + cases. Points represent averages and error bars are standard deviation of triplicate assays.*

4.5 FTIR

The FTIR spectrum is an easy and convenient method to find out the surface functional groups of the biomaterials and bioplastics. The spectra of cellphone case showed five major bands in the wavelength range of 3300–3400 cm^{-1}, 2850-3000 cm^{-1}, 1700–1800 cm^{-1}, 1000–1350 cm^{-1} and 730 cm^{-1}. The smaller band at 3335 cm^{-1} was assigned to the O-H stretching of hydroxyl functional group due to the adsorption of water molecules. The peak in the region of 2957 cm^{-1} was associated with the asymmetric stretching of aliphatic functional group (C-H). The peaks in the region of 1413 cm^{-1} and 1534 cm^{-1} were due to the presence of sulphate (S=O) and nitro (N=O) groups present in the cellphone cases. The peaks at 1731 cm^{-1}, 1270 cm^{-1} and 1215 cm^{-1} were associated with the -C=O and C-O-C stretch of the cellulosic ethers. The peak at the 729 cm^{-1} was associated with the in-plane and out-of-plane aromatic ring deformation vibrations (Figure 4.14). These functional groups indicate that cellphone cases are made up of several components such as bioplastic elements and cellulose (flax shive).

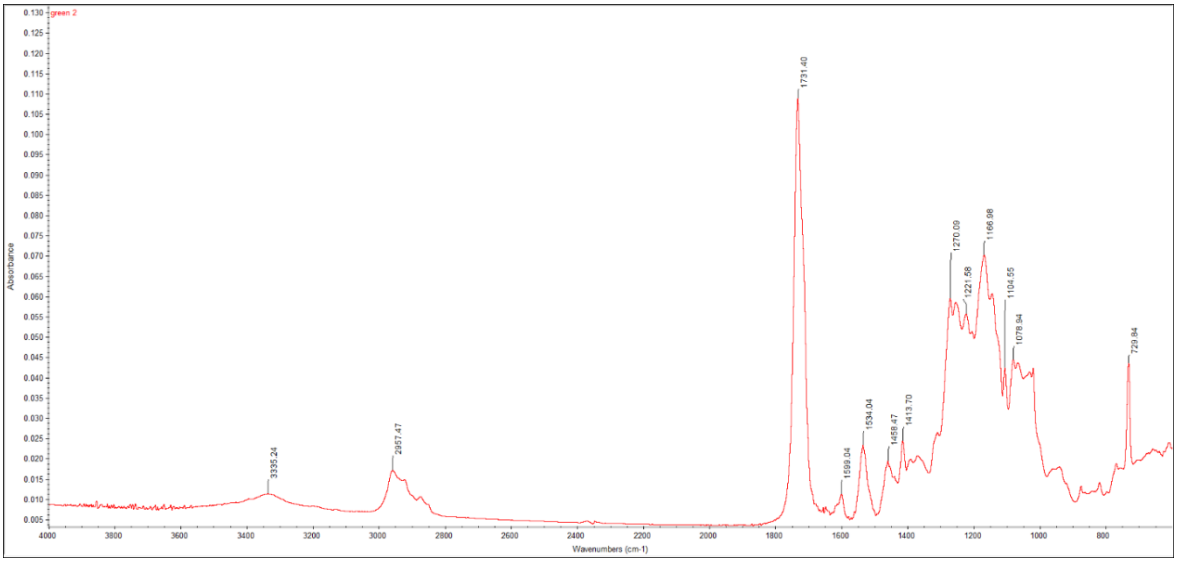

Figure 4-14. FTIR-ATR spectrum of grounded up cellphone cases

FTIR spectra of cellulosic materials were reported to have the characteristic peaks at 3323 cm^{-1} (O-H), 2890 cm^{-1}(C-H), and 1020-1160 cm^{-1} (Ma and Wang et al., 2015), where the intensity

of O-H and C-O peaks tend to be larger than other peaks. Our results were in agreement with literature; however, we got a smaller peak at 3335 cm^{-1}, likely because the cellphone cases contained only about 5% flax shive. The slight deviation in peak position and intensity indicates that cellulose-based materials may have undergone reactions during the bioplastic formation that might have caused shifts and reduction in peaks from the original positions. Some of the peaks in Figure 4.14 correspond to following characteristic peaks of thermoplastic polyurethane: 3349 cm^{-1} (N–H), 2926 cm^{-1} (C–H), 1725 cm^{-1} (C=O), and 1217 cm^{-1}(C-O) (Samimi et al., 2018). Thus, it is likely that the cellphone cases contained a significant amount of thermoplastic polyurethane. However, it is difficult to determine what type of bioplastic was added to the cellphone cases solely based on the FTIR results, as other bioplastics tend to have similar peaks. For instance, PLA has peaks between 2998-2847 cm^{-1} (C-H), ~1745 cm^{-1}, (C=O), 1187 cm^{-1} (ester C-O) and ~1072 cm^{-1} (C-O-C) (Pop et al., 2018). PCL has absorption bands at 2949 cm^{-1} and 2865 cm^{-1} (C-H), 1,727 cm^{-1} (C=O), 1293 cm^{-1} (C–O and C–C), and 1240 cm^{-1} (C-O-C) (Chong et al., 2015). Thus, further, research is required to determine the exact composition of cellphone cases.

4.6 SEM

The following SEM images were used to determine the change in surface morphology of cellphone cases after composting and anaerobic degradation. As seen in Figure 4.15, prior to degradation, the surface of the cellphone cases was quite smooth. The 2 × 2 × 0.2 cm phone cases after 34 days under laboratory scale composting conditions had several cracks along the surface (Figure 4.16). The 2 × 2 × 0.2 cm cellphone cases after 169 days of anaerobic degradation under mesophilic conditions did not show as may cracks as the cellphone cases after composting; however, the surface layers appear to have eroded as the flax can be seen beneath the surface. (Figure 4.17). This is in accordance with the biodegradation values from the composting and BMP

assays. Additional SEM images can be seen in Appendix C. As a side note, the arrows in the SEM images indicate the location of the flax. Lastly, the elemental analysis determined the presence of both carbon and oxygen in the cellphone cases, with carbon being present at 63%.

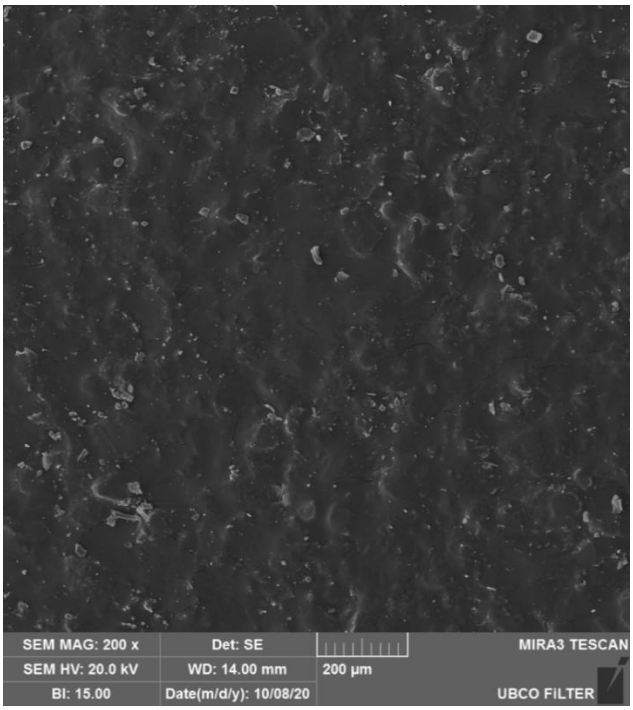

Figure 4-15. SEM image of cellphone case at magnification of 200x

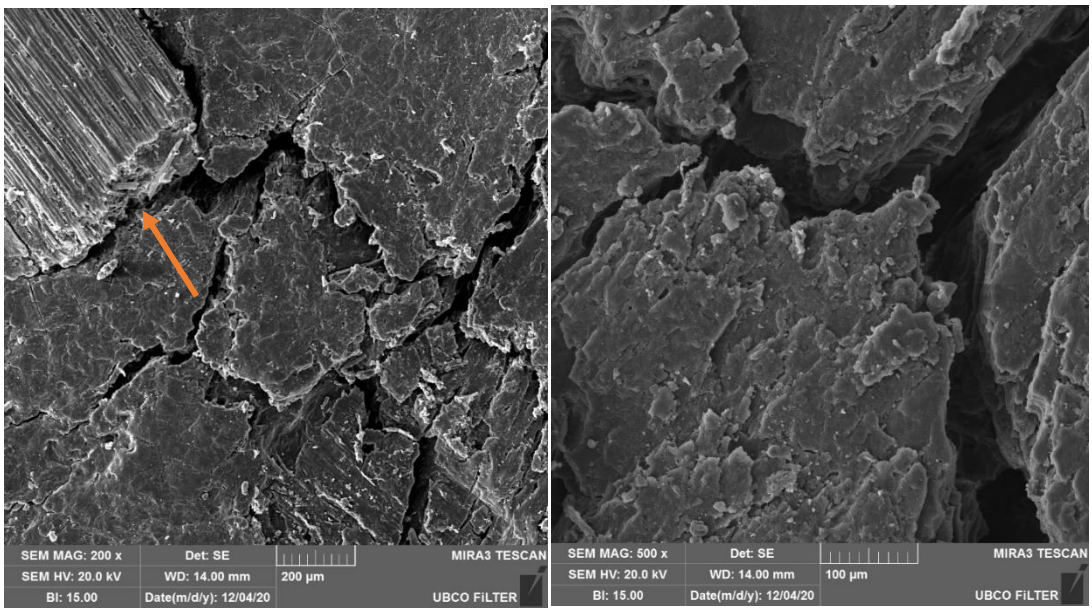

Figure 4-16. SEM image of 2 × 2 × 0.2 cm cellphone cases after composting assays for 34 days at magnification of 200x (left) and 500x (right). The arrow indicates flax.

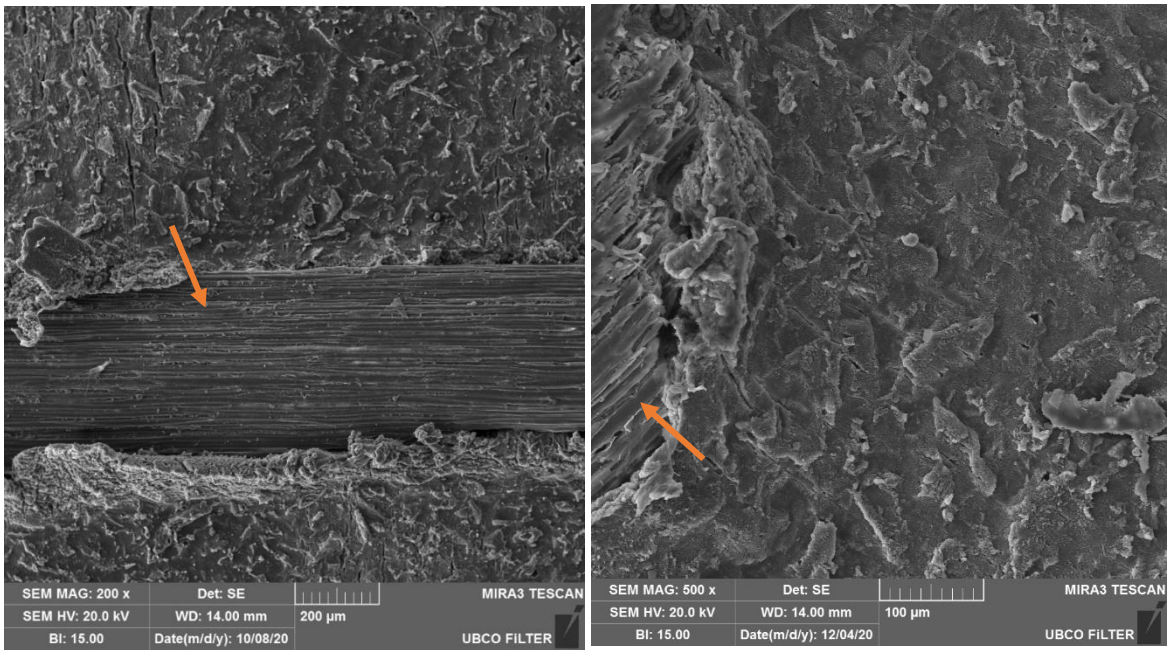

Figure 4-17. SEM image of 2 × 2 × 0.2 cm cellphone cases after mesophilic anaerobic degradation assays for 169 days at magnification of 200x (left) and 500x (right). The arrows indicate flax

Chapter 5: Conclusions, Limitations, and Future Work

In this research, the biodegradability of cellphone cases was determined under laboratory scale anaerobic and composting assays. The anaerobic assays were conducted under mesophilic (38^{o}C) and thermophilic (55^{o}C) conditions to determine if temperature had an effect on anaerobic biodegradation. Experiments were also conducted under field scale (60-67^{o}C) composting conditions to determine weight loss of cellphone cases under real life conditions. The following conclusions were determined from the collection and analysis of the data.

Like most bioplastics, the cellphone cases biodegraded better under aerobic (composting) conditions than anaerobic degradation conditions. However, the biodegradation (up to 21% after 46 days for cellphone cases cut into 4.6 × 3.5 × 0.2 cm) of the cellphone cases under laboratory scale (58^{o}C) composting conditions was less than pure bioplastics such as PLA. The field scale composting conditions achieved 55% weight loss of cellphone cases in 80 days; however, disintegration did not correspond to biodegradation levels observed from lab scale assays from carbon dioxide production. The mineralisation is an important part of the biodegradation process, but it does not mean microbial consumption of the products.

The comparison of microcrystalline cellulose degradation in three inocula, under anaerobic conditions, determined that mesophilic inoculum 2 (originated from Lulu Island WWTP in Vancouver) and thermophilic inoculum (originated from Annacis Island WWTP in Vancouver) had higher microcrystalline cellulose degradation of 80%, and 77% by day 22 and 19, respectively. Moreover, the optimal F/M for cellulose was 0.5 g COD/g VS $_{\text{Inoculum}}$, as the VFA concentrations were the lowest (i.e. 1642 mg/L on day 8). Mesophilic and thermophilic anaerobic digestion conditions had similar levels of cellphone case biodegradation (6-8%).

Moreover, size of cellphone cases (grinded, 2 × 2 × 0.2, and 4 × 4 × 0.2 cm) did not appear to effect biodegradation under anaerobic conditions.

Overall, the cellphone cases, assessed in a size range of range of 2 × 2 × 0.2 to 7 × 3.5 × 0.2 cm did not biodegrade a significant amount (up to 21% after 46 days) under laboratory scale composting conditions and may do better under the aforementioned conditions if its biobased content were to increase.

5.1 Limitations and Future Work

The study has indicated that the degradation of the cellphone cases under composting and anaerobic conditions is not as high as other bioplastics. One possibility would be to increase the biobased content of the cellphone cases to see if that will increase its biodegradation. Another option would be to recycle the cellphone cases instead of composting them.

It is recommended that further composting assays have continuous automatic gas measurements to provide more accurate results for the laboratory scale composting assays. The field scale assays were conducted at a facility that only treated green waste; however, a better comparison would have been to conduct the analysis at a composting facility treating municipal solids. Unfortunately, due to stricter regulations, that is not possible, in at least regions near the BTG laboratory. Moreover, it is recommended to conduct assays under aquatic conditions to determine the biodegradation percentage resulting from the microorganisms that are naturally present on the cellphone cases.

Lastly, the cellphone cases provided interesting results in which the assays with the cellphone cases, under thermophilic conditions, produced biogas faster than the controls. It would be interesting to further investigate the mechanisms that allow the microorganisms to produce more biogas under these conditions (assuming that the cellphone cases produce very little of that biogas).

Lastly, the COVID-19 pandemic resulted in the shutdown of several composting and anerobic assays without being able to analyze any of the inoculum or substrates.

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Appendices

Appendix A. Sample Calibration Curves

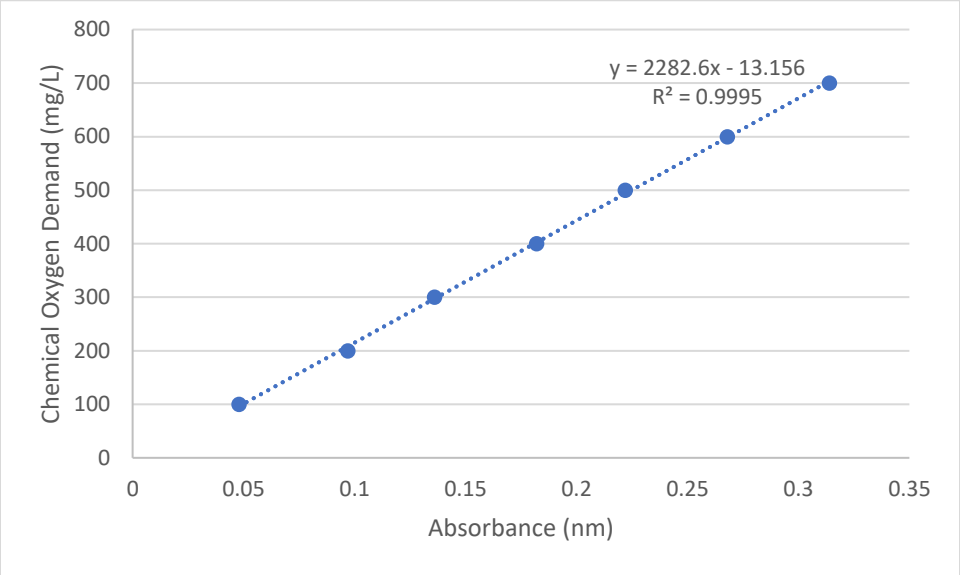


Figure A-1. Calibration curve of chemical oxygen demand

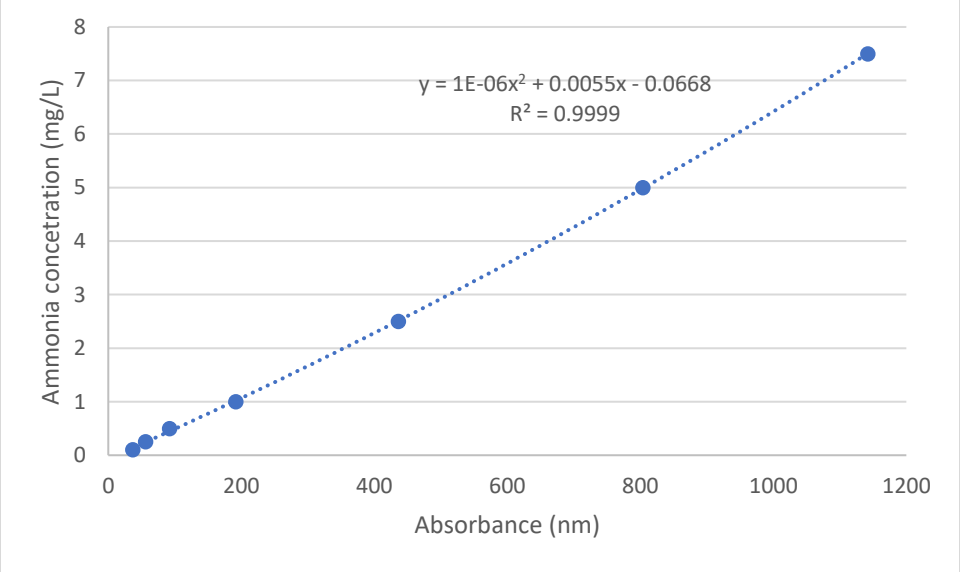


Figure A-2. Calibration curve of ammonia nitrogen

Appendix B. Statistical Analysis

Table B- 1. Statistical analysis of cumulative carbon dioxide production during composting assays comparing cellphone cases in each trial, using the two sample Student's t-test.

Cumulative time (days)	P value, 2 Tails[a]	
	Compost trial 1[b]	Compost trial 2[c]
1	0.645	0.612
2	0.881	0.116
3	0.926	0.101
4	0.926	0.065
5	0.982	0.051
6	0.604	0.038
7	0.551	0.029
8	0.425	0.030
9	0.374	0.021
10	0.365	0.019
11	0.319	0.018
12	0.313	0.017
13	0.319	0.017
14	0.347	0.034
15	0.355	0.058
16	0.363	0.109
17	0.391	0.192
18	0.419	0.308
19	0.451	0.429
20	0.560	0.597
21	0.645	0.713
22	0.664	0.781
23	0.740	0.832
24	0.634	0.994
25	0.713	0.891

Cumulative time (days)	P value, 2 Tails[a]	
	Compost trial 1[b]	Compost trial 2[c]
26	0.787	0.993
27	0.803	0.976
28	0.781	0.940
29	0.824	0.918
30	0.862	0.898
31	0.930	0.899
32	0.948	0.879
33	0.993	0.861
34	0.949	0.842
35	0.988	
36	0.923	
37	0.846	
38	0.822	
39	0.798	
40	0.781	
41	0.764	
42	0.739	
43	0.755	
44	0.724	
45	0.663	
46	0.612	

a: Null hypothesis H0: $\mu 1 = \mu 2$ and alternate hypothesis H1: $\mu 1 \neq \mu 2$
b: 1/4 cellphone cases ($\mu 2$) and 1/6 cellphone cases ($\mu 1$)
c: $2 \times 2 \times 0.2$ cm ($\mu 2$) and $4 \times 4 \times 0.2$ cm cellphone cases ($\mu 1$)

Table B- 2. Statistical analysis of cumulative carbon dioxide and methane production with grinded cellphone cases (μ2) controls (μ1), under mesophilic conditions using the two sample Student's t-test (F/M: 1 g VS/g VS).

Cumulative time (days)	P value, 2, tails[a]		
	Grinded cellphone cases	2 × 2 × 0.2 cm phone cases	4 × 4 × 0.2 cm phone cases
4	0.015	0.012	0.020
8	0.103	0.000	0.162
10	0.121	0.000	0.060
15	0.792	0.086	0.889
19	0.192	0.003	0.035
24	0.224	0.013	0.037
30	0.091	0.001	0.064
35	0.035	0.000	0.000
42	0.022	0.000	0.000
65	0.006	0.000	0.000
80	0.005	0.000	0.000
169	0.001	0.000	0.000

a: Null hypothesis H0: $\mu 1 = \mu 2$ and alternate hypothesis H1: $\mu 1 \neq \mu 2$

Table B- 3. Statistical analysis of cumulative carbon dioxide and methane production with 5 g cellphone case/ g VS $_{\text{Mixed Sludge}}$ (μ2) and 10 g cellphone case/ g VS $_{\text{Mixed Sludge}}$ (μ1), under thermophilic conditions, using the two sample Student's t-test (F/M: 1 g VS/ g VS).

Cumulative time (days)	P value, 2, tails[a]
5	0.022
9	0.043
13	0.618
20	0.117
24	0.024
38	0.001
51	0.000
83	0.000
105	0.000

a: Null hypothesis H0: $\mu 1 = \mu 2$ and alternate hypothesis H1: $\mu 1 \neq \mu 2$

Table B- 4. Statistical analysis of cumulative carbon dioxide and methane production with 3 g cellphone case/ g VS $_{\text{Mixed Sludge}}$ (μ2) and 6 g cellphone case/ g VS $_{\text{Mixed Sludge}}$ (μ1), under thermophilic conditions, using the two sample Student's t-test (F/M: 2 g VS/g VS).

Cumulative time (days)	P value, 2, tails[a]
5	0.012
13	0.108
15	0.162
18	0.063
21	0.015
24	0.191
34	0.084
52	0.030

a: Null hypothesis H0: $\mu 1 = \mu 2$ and alternate hypothesis H1: $\mu 1 \neq \mu 2$

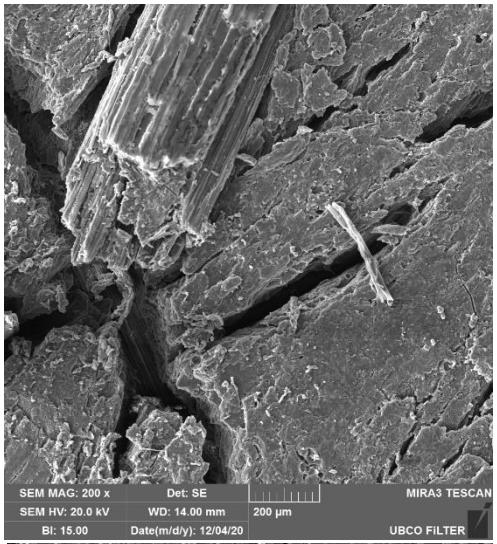
Table B- 5. Statistical analysis of cumulative carbon dioxide and methane production with 2 g cellphone case/ g VS $_{\text{Mixed Sludge}}$ (μ2) and 3 g cellphone case/ g VS $_{\text{Mixed Sludge}}$ (μ1), under thermophilic conditions, using the two sample Student's t-test (F/M: 3 g VS/g VS).

Cumulative time (days)	P value, 2, tails[a]
5	0.000
22	0.001
34	0.001
37	0.003
52	0.014

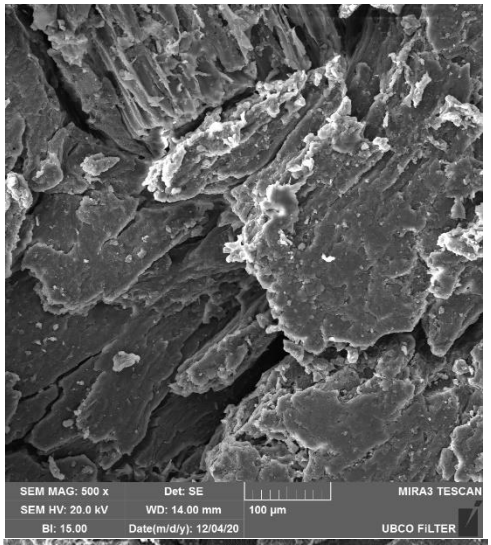
a: Null hypothesis H0: $\mu 1 = \mu 2$ and alternate hypothesis H1: $\mu 1 \neq \mu 2$

Appendix C. SEM Images

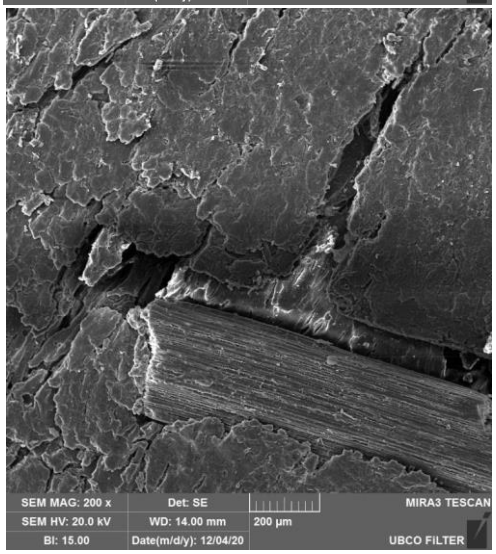
a)



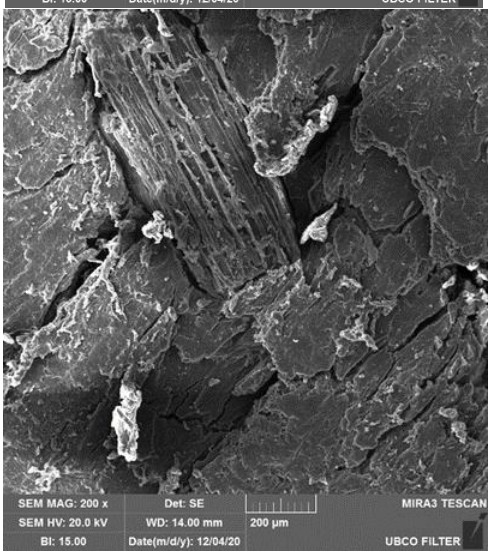
b)



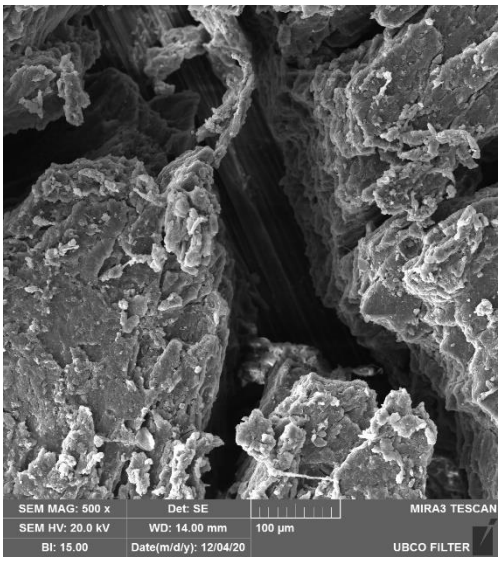
c)



d)



e)



f)

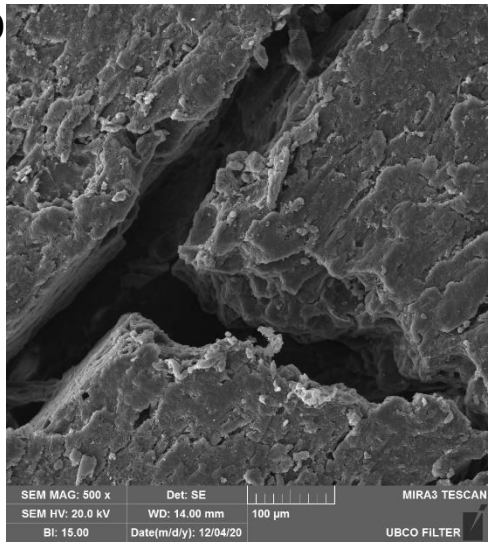
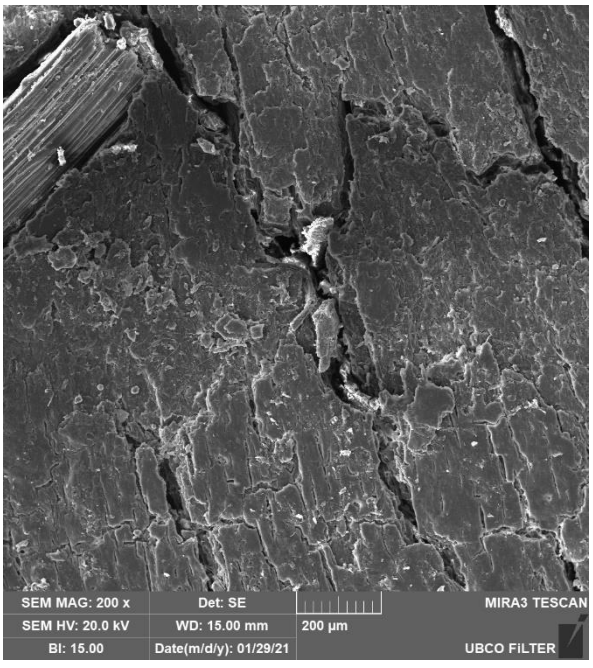


Figure C-1. SEM image of 2 × 2 × 0.2 cm cellphone cases after composting assays for 34 days at magnification of 200x (a, b, c) and 500x (d, e, f)



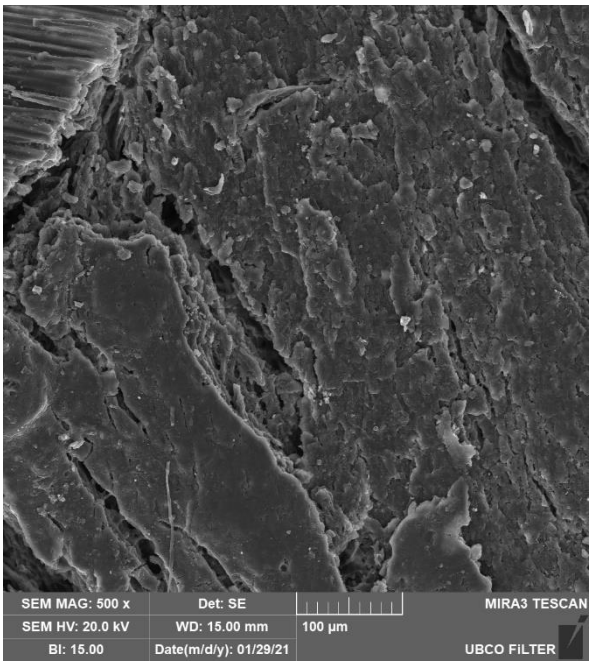


Figure C-2. SEM image of 4 × 4 × 0.2 cm cellphone cases after composting assays for 34 days at magnification of 200x

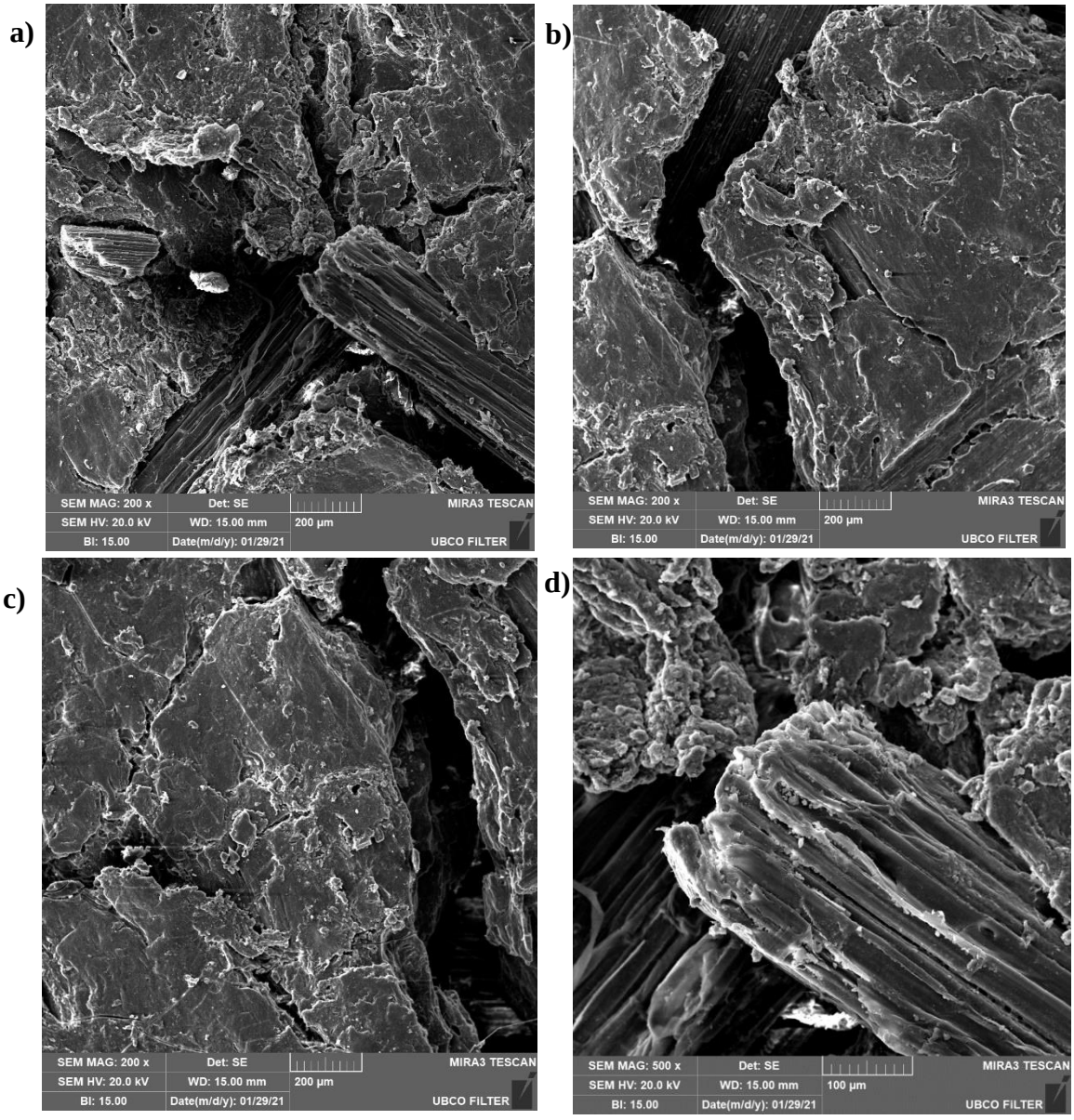
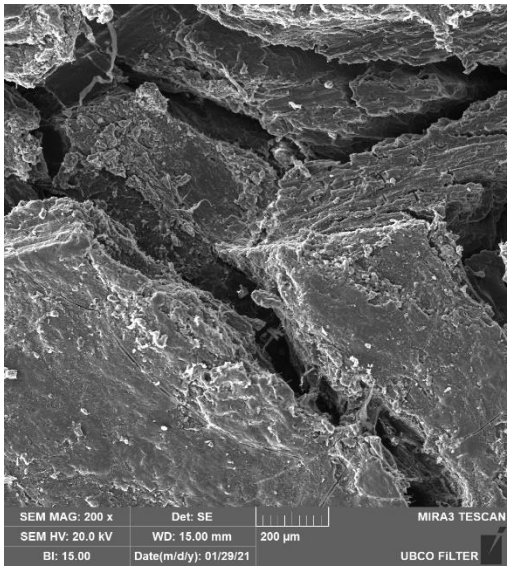
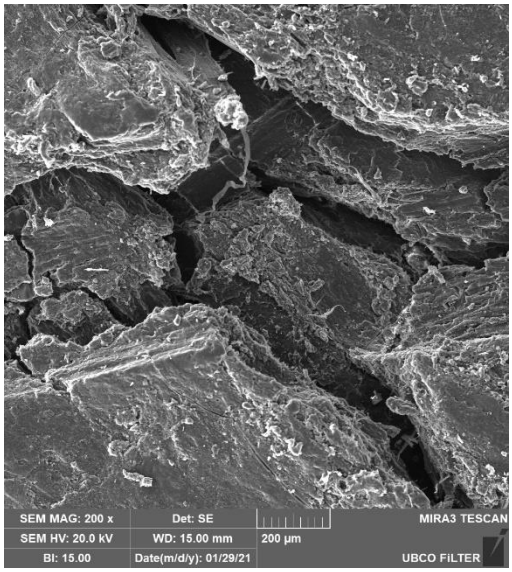


Figure C-3. SEM image of 7.5 × 3.5 × 0.2 cm cellphone cases after composting assays for 46 days at magnification of 200x (a, b, c) and 500x (d)

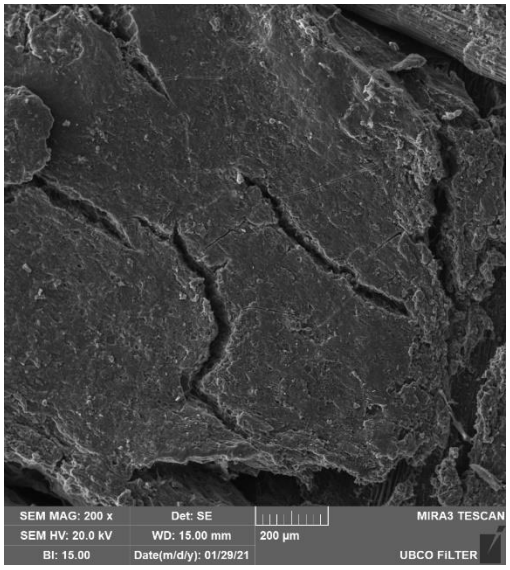
a)


SEM MAG: 200 x
Det: SE
MIRA3 TESCAN
SEM HV: 20.0 kV
WD: 15.00 mm
200 µm
BI: 15.00
Date(m/d/y): 01/29/21
UBCO FiLTER

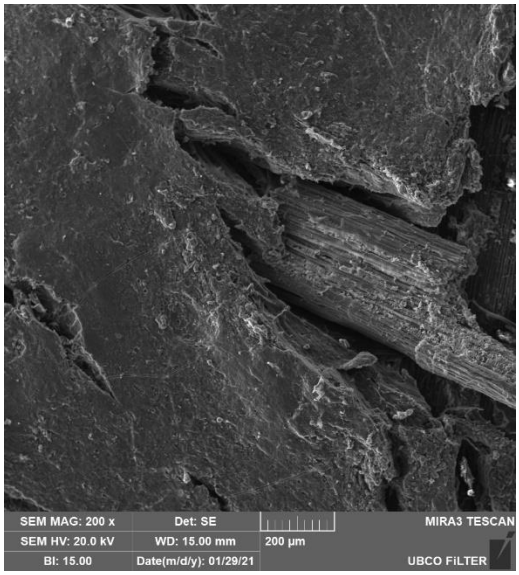
b)


SEM MAG: 200 x
Det: SE
MIRA3 TESCAN
SEM HV: 20.0 kV
WD: 15.00 mm
200 µm
BI: 15.00
Date(m/d/y): 01/29/21
UBCO FiLTER

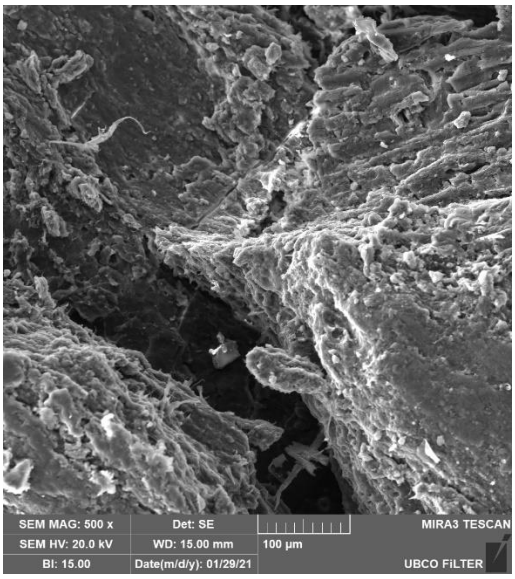
c)


SEM MAG: 200 x
Det: SE
MIRA3 TESCAN
SEM HV: 20.0 kV
WD: 15.00 mm
200 µm
BI: 15.00
Date(m/d/y): 01/29/21
UBCO FiLTER

d)


SEM MAG: 200 x
Det: SE
MIRA3 TESCAN
SEM HV: 20.0 kV
WD: 15.00 mm
200 µm
BI: 15.00
Date(m/d/y): 01/29/21
UBCO FiLTER

e)



f)

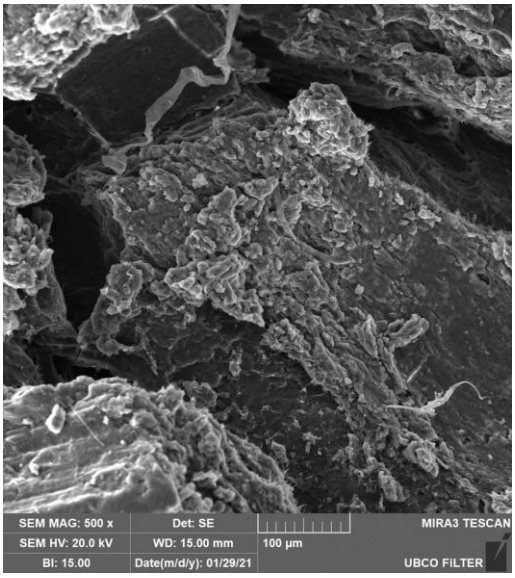


Figure C-4. SEM image of 4.6 × 3.5 × 0.2 cm cellphone cases after composting assays for 46 days at magnification of 200x (a, b, c, d) and 500x (e, f)

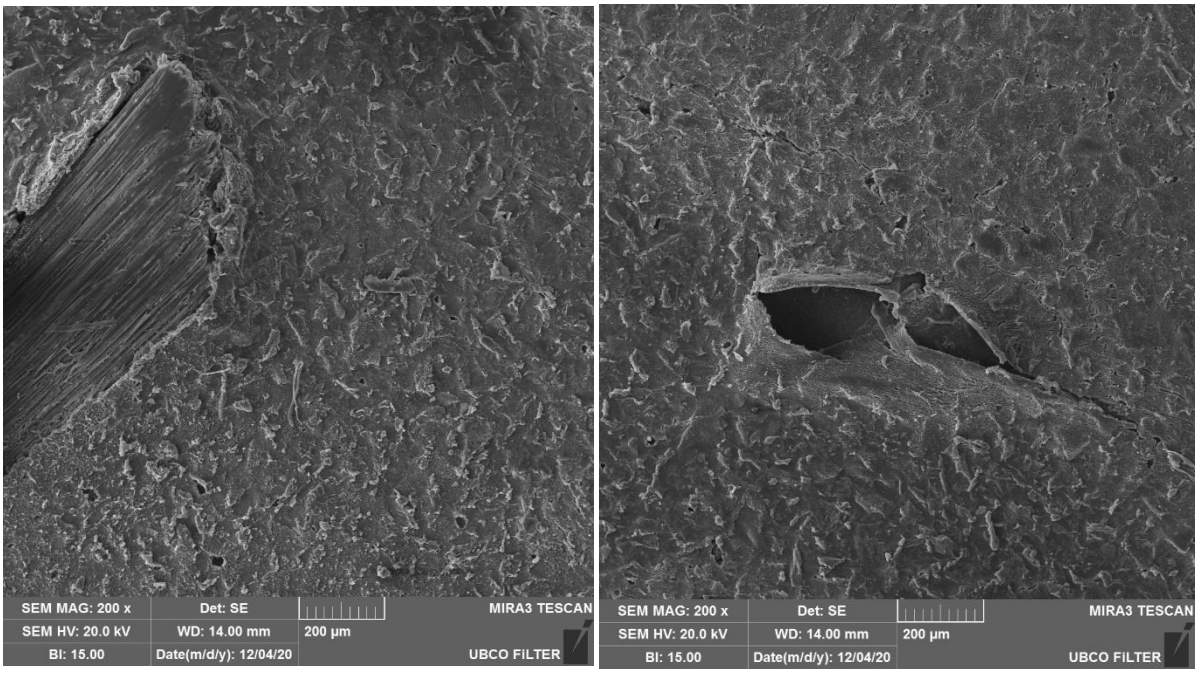


Figure C-5. SEM image of 2 × 2 × 0.2 cm cellphone cases after anaerobic assays for 169 days at magnification of 200x

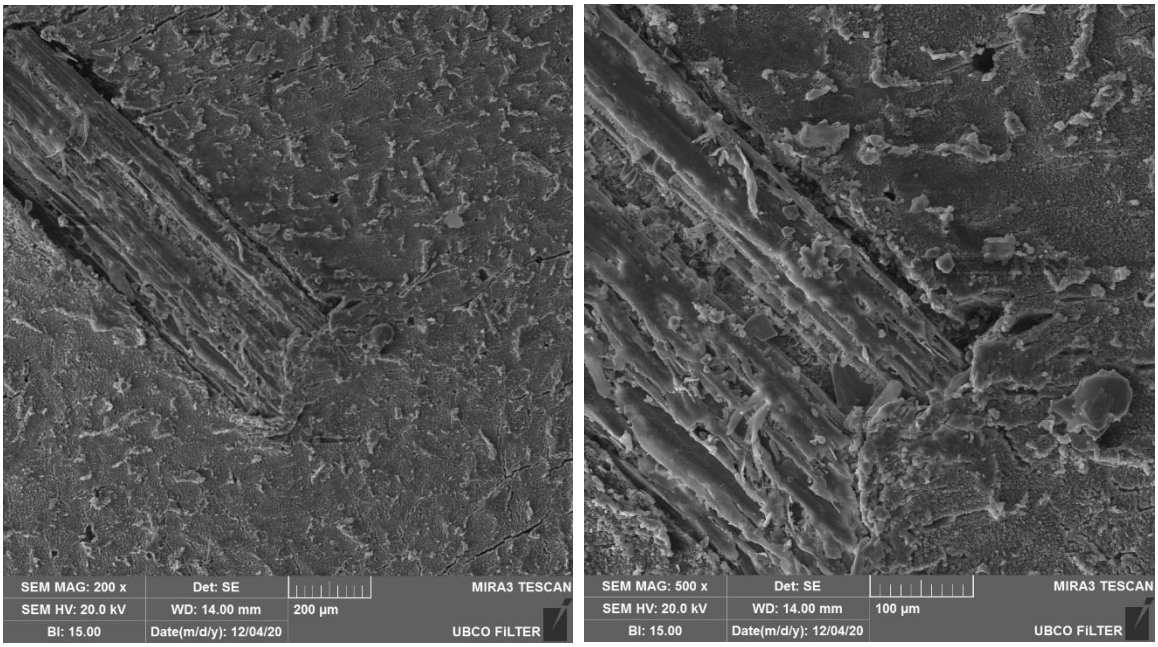


Figure C-6. SEM image of 4 × 4 × 0.2 cm cellphone cases after anaerobic assays for 169 days at magnification of 200x (left) and 500x (right)