Master's Thesis

Degradation of bioplastic in anaerobic conditions

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20.06.2019

JYVÄSKYLÄN YLIOPISTO, Matemaattis-luonnontieteellinen tiedekunta
Bio- ja ympäristötieteiden laitos
Ympäristötiede ja -teknologia

Hirvonen Mikael: Biomuovin anaerobinen hajoaminen
Pro gradu -tutkielma: 57 s., 2 liitettä (9 s.)
Työn ohjaajat: Prof. Tuula Tuhkanen ja FT Kai Sormunen
Tarkastajat: Prof. Tuula Tuhkanen ja dos. FT Sami J. Taipale
Kesäkuu 2019

Hakusanat: biohajoaminen, biokaasu, muovi, mädätys, thermoplastinen tärkkelys

Muovien hajoamattomuus on synnyttänyt ympäristöongelman, jota yritetään ratkaista biohajoavien muovien avulla. Samalla mädätys kerää suosiota orgaanisen jätteen käsittelymenetelmänä biokaasupotentiaalin ja maanparannusaineeksi soveltuvan mädätysjäännöksen ansiosta. Muovien on kuitenkin havaittu haittaavan mädätyslaitosten toimintaa Muovien biohajoamistutkimukset ovat myös painottuneet aerobisiin oloihin, kuten kompostointiin ja maaperäkokeisiin. Tämä tutkimus pyrkii tarkastelemaan muovin määrää biojätteessä ja termoplastisesta tärkkelyksestä valmistetun biomuovin hajoamista anaerobisissa oloissa. Tutkimus tehtiin mesofiilisena märkämädätyskokeena. Tässä tutkimuksessa tarkastelulla biomuovilla on EN13432-sertifikaatti, joka osoittaa tuotteen hajoavan teollisessa kompostoinnissa. Tutkimukseen kuului kaksi aikajännettä; 30 ja 90 vuorokautta. Tutkimusmateriaalit olivat termoplastinen tärkkelys, LLDPE ja paperi, joista LLDPE:tä käytettiin negatiivisena kontrollina, eli materiaalina, jonka ei odotettu hajoavan ja paperi taas oli positiivinen kontrolli. Materiaalien hajoamista tarkasteltiin visuaalisten havaintojen, massahäviön ja tuotetun biokaasun avulla. Termoplastinen tärkkelys menetti keskimäärin 14,9 ± 0.3 % massastaan 30 vuorokaudessa ja 21 ± 1 % 90 vuorokaudessa. Biokaasun tuottoon perustuvat mineralisaatioasteet ovat erittäin epävarmoja, koska materiaalien hiilipitoisuudesta ei ollut varmaa tietoa ja reaktorit kärsivät osin merkittävästä happi-inhibitiosta. Vähäinen biohajoaminen on mahdollista, sillä suurin saavutettu mineralisaatioaste termoplastiselle tärkkelykselle oli 14 % 30:ssä vuorokaudessa, mutta lukeman todettiin olevan virhemarginaalissa. Pidemmän aikajakson hyödyistä suurempana mineralisaatioasteena ei saatu todisteita, vaikka termoplastinen tärkkelys menettikin 6 prosenttiyksikköä enemmän massaa 90 vuorokaudessa 30 vuorokauden kokeeseen verrattuna, mikä oli tilastollisesti merkitsevä ero. Biokaasun tuotanto hidastui 30:n vuorokauden jälkeen, joten suuremman massahäviön 90 vuorokauden kokeessa ajateltiin johtuvan suurelta osin ympäristötekijöistä. Tässä työssä tarkastellun termoplastisen tärkkelyksen ei todettu hajoavan merkittävästi anaerobisisssa olosuhteissa EN13432 sertifikaatista huolimatta, mikä osoittaa etteivät aerobisissa olosuhteissa hajoavat muovit hajoa mädätyksessä.

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Department of Biological and Environmental Science
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Hirvonen Mikael: Degradation of bioplastic in anaerobic conditions
MSc thesis: 57 p., 2 appendices (9 p.)
Supervisors: Prof. Tuula Tuhkanen and Ph.D. Kai Sormunen
Inspectors: Prof. Tuula Tuhkanen and Adj. Prof. Ph.D. Sami J. Taipale

June 2019

Keywords: anaerobic digestion, biodegradation, biogas, plastic, thermoplastic starch

Plastics once applauded for their durability are now causing environmental harm for the same reason. To mitigate this issue, biodegradable plastics have been developed. Degradation studies however have mostly been conducted in aerobic conditions such as composting and soil. Anaerobic digestion has some advantages over aerobic digestion, for example anaerobic digestion produces methane rich biogas that can be used as a fuel. Plastics have been noted to be problematic for anaerobic digestion. This study aims to examine plastic contents in biowaste and provide insight on anaerobic degradability of thermoplastic starch, a kind of plastic made out of starch. The study was conducted as a wet digestion batch experiment in 37 °C with retention times of 30 and 90 days. Digestate from Mustankorkea anaerobic digestion plant was used as inoculum and leftover food from Ylistö restaurant was used to provide nutrients for the microbes. Sample materials were thermoplastic starch, LLDPE and paper. Degradability was assessed as relative mass loss and as amount of biogas produced out of theoretical maximum while also observing the materials visually. Theoretical maximum biogas yield was calculated assuming all carbon in a sample was converted into biogas. Thermoplastic starch lost on average 14.9 ± 0.3 % of mass in 30 days and 21 ± 1 % in 90 days. Meanwhile paper was completely disintegrated in 30 days and LLPDE gained mass possibly due to biofilm formation. Statistically signifigant increase in mass loss shows that degradation of thermoplastic starch continued throughout the last 60 days of test, albeit at reduced rate, despite minimal microbial activity. Plastics yielded less biogas than the mixture of inoculum and food waste resulting in the mineralization degrees being mostly negative. A low level of mineralisation may be achievable for the TPS studied here, as the highest mineralisation degree reached was 14 % in 30 days. However the margin of error for mineralisation degrees in this study is remarkable due to uncertainty regarding the carbon contents of the sample materials and notable oxygen inhibition, so 14 % is quite likely within the margin of error.

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DEFINITIONS

Inoculum: Substance used to provide necessary microbes for anaerobic digestion

Substrate: Feed material for the microbes.

ABBREVIATIONS

LLPDE: Linear low-density polyethylene

TPS: Thermoplastic starch

VS: Volatile solids

1 INTRODUCTION

Plastics have become an essential part of daily life due to their cheap production costs and beneficial physical and chemical qualities (Cho et al. 2011). Plastics are used in several applications such as packaging and building (Geyer et al. 2017). Unfortunately conventional plastics are also resistant to biodegradation, which causes the plastics to remain in the environment (Mohee and Unmar 2007). Instead of biodegradation, plastics may be degraded into microplastics that are smaller than 5 mm pieces of plastics and are a growing environmental concern (Collignon et al. 2014, Wright and Kelly 2017). To mitigate this, biodegradable options for conventional plastics have been developed (Mohee and Unmar 2007). Starch has been noted as a promising resource for bioplastics, because starch is affordable and commonly available (Torres et al. 2011). Infact starch based polymers are among the most manufactured bioplastics (European bioplastics 2018a).

So far bioplastic biodegradability studies have mainly focused on aerobic conditions such as compost and soil. Suitability of bioplastic bags for sorting biowaste depends on biowaste processing method. For example in Finland waste management company Kiertokapula does not recommend using biodegradable plastic bags in many of its client municipalities, because the collected biowaste is used to produce ethanol (Kiertokapula 2019). Additionally plastics have been reported to cause issues with anaerobic digestion plants (Yle 2019a). This also includes biodegradable plastic bags, which have been noted to hinder the biogas plants' operation by getting stuck on crushers and conveyors (Yle 2019b). Conventional plastics can even be preferred over biodegradable bags, since conventional plastics can be more easily removed (Yle 2019b).

Therefore this study aims to assess the amount of plastic in biowaste stream and shed some light on bioplastic biodegradability in anaerobic conditions by comparing degradation of a starch-based bioplastic to those of paper and fossil fuel based LLDPE. Furthermore the effect of retention time was examined by running parallel sets of reactors for 30 and 90 days. The research questions of this study were 'does a EN13432 certified starch based bioplastic degrade in anaerobic digestion, and if yes, to what extent does it degrade?' and 'does longer retention time increase degradation?'. The hypothesis was that the examined bioplastic is more degradable than LLDPE but less degradable than paper, which were used as control materials. Furthermore, it was hypothetised that 90 day retention time would allow more degradation to occur than 30 day retention time.

2 LITERATURE

2.1 Bioplastics

Conventional fossil fuel based plastics have become a major part of modern lifestyle (Cho et al. 2011). Plastics have many beneficial properties, such as cheap manufacture costs, durability and light weight, which have lead to plastic being used for many purposes (Cho et al. 2011). In biggest plastic using sectors are packaging, building and construction and textiles, which had shares of 35.9 %, 16.0 % and 14.5 % respectively of global primary plastic prodution in 2015 (Geyer et al. 2017). Plastics resistance to degradation has however lead to plastic accumulation in the environment, which has caused a need for biodegradable plastics (Mohee and Unmar 2007).

European bioplastics defines bioplastics as plastics that are either at least partly produced from biomass, are biodegradable or fall under both categories (European bioplastics 2018b). Therefore bioplastics cover a plethora of plastic

materials ranging from nonbiodegradable biobased equivalents of conventional plastics, such as polyethylene and polyamides, to biodegradable plastics derived from fossil fuels, for example polycaprolactone (European bioplastics 2018b) (Figure 1).

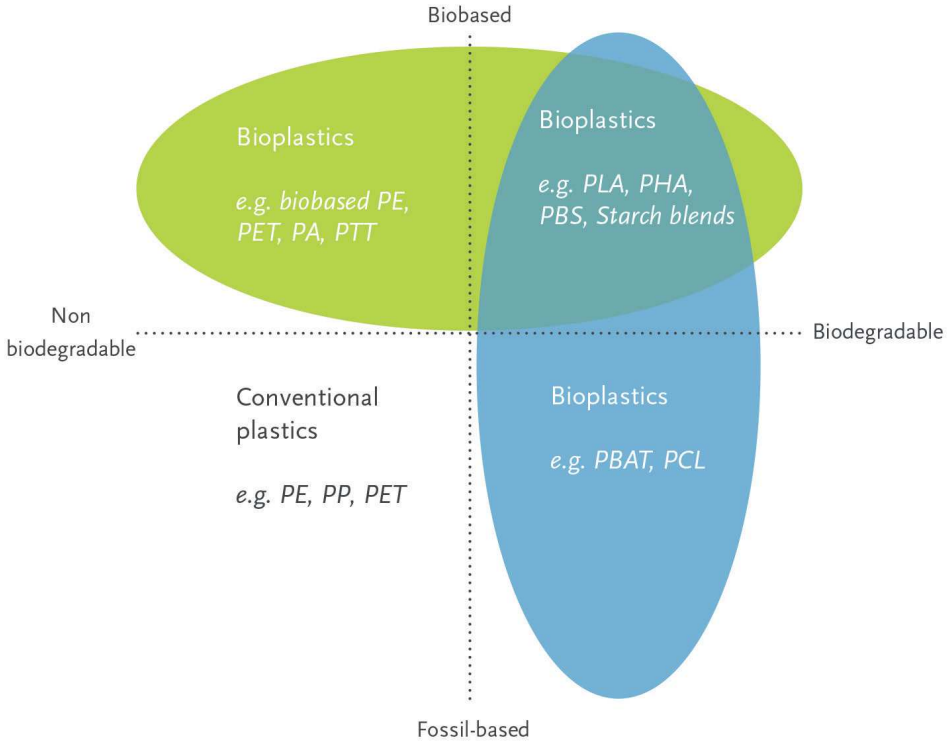

Figure 1. Bioplastic classification system according to European bioplastics (2018b). The abbreviations are PA: polyamide, PBAT: polybutylene adipate terephthalate, PBS: polybutylene succinate, PCL: polycaprolactone, PE: polyethylene, PET: polyethylene terephthalate, PHA: polyhydroxyalkanoate, PLA: polylactic acid, PP: polypropelene and PTT: polytrimethylene terephthalate.

With an annual production capacity of 2.1 million tons, bioplastics make out less than one percent of yearly total plastic production of approximately 335 million tons (European bioplastics 2018a). However bioplastic production is expected to increase reaching 2.6 million tons in 2023 (European bioplastics 2018a). Coupled with the rising popularity of anaerobic digestion as a means of biowaste treatment, an increase in bioplastics ending up in anaerobic digestion plants can also be expected (Zhang et al. 2018). Ideally anaerobically degradable bags could

be used as a feedstock in anaerobic digestion, which would remove the need to separate the bags from the biowaste (Zhang et al. 2018).

Fossil based plastics can also be manufactured using renewable resources (Prieto 2016). These bio-based versions of conventional plastics are called drop-in polymers and they include polyethylene, polypropylene and polyethylene terephthalate, which are among the most common kinds of plastic (Lackner 2015). Although drop-in polymers do not alleviate the littering problem, they can reduce mankind's dependancy on fossil fuels (Lackner 2015). Bioplastic production is leaning towards drop-in polymers (European bioplastics 2018a).

2.1.1 Sources of bioplastics

Bioplastics can be manufactured using a plethora of resources ranging from plants and microbes to animals (Belgacem and Gandini 2008a). Plant fibers and cellulose are an useful feedstock for bioplastic production (Lackner 2015). Fibers can be used in composites as they are or they can be utilised in cellulose extraction (Sun 2013). Cellulose on the other hand can be used to manufacture plastics such as cellophane (Lackner 2015). Cellulose can also be converted into cellulose esters, which are used in plastic production (Lackner 2015).

Bacteria have gained some attention as a source of polymers (Gandini and Belgacem 2008b). Commercial utilisation of these polymers is a quite new prospect despite the ability of bacteria to synthezise these polymers is well known. Polyhydroxyalkanoates and bacterial cellulose have gained the most attention out of polymers of bacterial origin (Gandini and Belgacem 2008b).

Polyhydroxyalkanoates (PHAs) are a group of polyesters that are biodegradable and possess great physical qualities (Gandini and Belgacem 2008b). Around 250 bacteria are known to be capable of synthesizing PHA. (Lackner 2015). The kind of PHA that is produced depends on the bacteria, the substrate the bacteria is fed

and growing conditions of the bacteria (Chodak 2008). PHA production is small scale and thus applications are also limited. Nevertheless there are potential uses for PHAs for example in medicine and packaging. (Chodak 2008).

Bacterial cellulose is chemically identical to its plant equivalent. In terms of morphology however, bacterial cellulose is different from plant cellulose. Bacterial cellulose has more crystalline structure and lacks compounds that accompany plant cellulose such as lignin and hemicelluloses. (Pecoraro et al. 2008). Bacterial cellulose can be used in multiple applications for example as artificial skin for burns, in oil and toxin absorbers and as an emulsion stabilizer in cosmetics. (Pecoraro et al. 2008).

Proteins are a group of compounds naturally present in animals, plants and bacteria (Zhang and Zeng 2008). Proteins are made of chains of amino acids, which form intricate three dimensional structures. Important protein sources include soy protein, zein, which is separated from corn, wheat gluten and casein, which is a major component in milk. Several kinds of proteins, for example soy protein, zein and collagen, have been investigated as a material for edible films. Edible films are useful as a protective layer on food products. (Zhang and Zeng 2008).

Polylactic acid is among the most common sources of bioplastic. As the name suggests, polylactic acid is a polymeric form of lactic acid. Lactic acid can be produced by bacterial fermentation or chemical synthesis. During carbohydrate fermentation bacteria generate lactic acid from sugars. (Avérous 2008, Lackner 2015.) Polylactic acid has seen potential applications in medical use, packaging, and everyday items such as cups and utensils (Avérous 2008). However, polylactic acid does have some issues that need to be solved for polylactic acid to achieve greater use, namely polylactic acid's poor mechanical and barrier qualities. (Avérous 2008, Lackner 2015).

Lignin too is a potential source of materials for bioplastics. Lignin is a complex polymer that is found in plants (Gellerstedt and Henriksson 2008). Research has been conducted regarding kraft lignin based polyurethane (Gandini and Belgacem 2008c, Agrawal et al. 2014). Additionally lignin can be combined with fibres, such as flax and cellulose, and additives to form a plasticlike material (Agrawal et al. 2014). Suberin, which is a polyester present in some tree species, has also been noted as a source for polyurethane (Silvestre et al. 2008).

Hemicelluloses are a group of polysaccharides that are abundant in plant cell walls. They comprise of several sugar units and the composition of hemicelluloses depends on the plant species (Spiridon and Popa 2008). Hemicelluloses have found use in food industry as emulsifiers. One of the hemicelluloses, gluconoroxylan, has been shown to exhibit low oxygen permeability. Thus such films could be used in food packaging. (Spiridon and Popa 2008.)

2.1.2 Thermoplastic starch

Starch is a complex carbohydrate that forms a energy reserve in vascular plants (Carvalho 2013). Starch is a promising resource for bioplastic production as starch is widely available, abundant and affordable (Torres et al. 2011). Starch consists of two polysaccharides: amylose and amylopectin (Shanks & Kong 2012). Both amylose and amylopectin are made of glucose units linked together. Amylose is a linear molecule consisting of approximately 100–10000 glucose units (Khan et al. 2017). The lenght of the glucose chain depends on the plant species (Khan et al. 2017). Amylopectin on the other hand is a branched molecule that contains short chains of glucose units (Khan et al. 2017). The ratio of amylose to amylopectin varies based on the plant species. Starch also contains slight amounts of lipids and proteins (Khan et al. 2017). However in its natural form starch is granular and heating dry starch up leads to the starch being thermally degraded before the melting point is reached (Shanks & Kong 2012, Mohammadi Nafchi et al. 2013).

Therefore native starch requires some refining to yield a starch based plastic (Mohammadi Nafchi et al. 2013).

In order to create a plasticky product, starch is mixed with a plasticizer and the mixture is heated and subjected to mechanical strain. During the process starch granules absorb the plasticizer via the amorphous sections of starch granules at first and eventually the plasticizer penetrates the crystalline sectors. Finally starch is dissolved into the plasticizer forming an amorphous gel. (Happonen and Törmälä 1995, Combrzyński et al. 2012.) The gel can then processed into plastic products using the same methods that used to produce conventional plastics, for example blow molding (Mohammadi Nafchi et al. 2013).

Plasticizer is a compound that is used to increase the flexibility and malleability of a material (Mohammadi Nafchi et al. 2013). Plasticizers have low molecular weight and volatility (Vieira et al. 2011). Choice of plasticizer can alter the thermoplastic starch's properties (Vu and Lumdubwong 2016). Water is commonly used as a plasticizer in thermoplastic starch manifacturing (Mohammadi Nafchi et al. 2013). However water is ill-suited to be the only plasticizer, because water results causes the thermoplastic starch to be fragile (Liu et al. 2009). Possible plasticizers include but are not limited to glycerol, sorbitol, urea and glucose (Vieira et al. 2011, Mohammadi Nafchi et al. 2013, Vu and Lumdubwong 2016).

Starch source can also affect the qualities of thermoplastic starch. Some of the variability can be explained by plants having different amylose to amylopectin ratios. Starch with high amylose content has been found to produce thermoplastic starch exhibiting higher strength and elongation (Zullo and Iannace 2009). Zullo and Iannace (2009) studied the effect of starch source and plasticizer on the properties of thermoplastic starch. Maize, potato and wheat starch were the examined starch sources. Glycerol and a mixture of urea and formamide were

used as plasticizer. Zullo and Iannace found that potato starch produced the stiffest thermoplastic starch. As for plasticizers, urea-formamide -mixture produced thermoplastic starch with higher elongation at break and lower stiffness than glycerol (Zullo and Iannace 2009.)

Packaging is the main application for thermoplastic starch (Shanks and Kong 2012). Interest in thermoplastic starch as a packaging material is mostly driven by starch's biodegradability (Shanks and Kong 2012). Unfortunately pure thermoplastic starch is hydrophilic and has poor mechanical qualities, which restricts its suitability for packaging and as a result TPS is more suitable for packaging dry products (Shanks and Kong 2012). Hence thermoplastic starch is commonly used as a part of a polymer blend or a composite instead (Tokiwa et al. 2009, Shanks and Kong 2012).

Polymer blending means mixing several polymers together in order to create a polymer blend with advantageous properties (Makhijani et al. 2015). The polymers that are blended need to be reasonably similar in chemical composition for the polymers to form a homogenous blend. Otherwise the polymers will remain in their respective phases (Mohammadi Nafchi et al. 2013.) Compatibilizers can be used to promote the blending. (Hahladakis et al. 2018.) Composites on the other hand make use of some filler material that is incorporated into the polymer matrix. Filler materials can be for example clay, glass particles or natural fibres. (Shanks and Kong 2012.)

Retrogradation is another issue with thermoplastic starch (Mohammadi Nafchi et al. 2013). Retrogradation is a process, in which starch regains crystallinity over time (Zullo and Iannace 2009). As the crystallinity increases, the thermoplastic starch becomes more brittle (Shanks and Kong 2012). Choice of plasicizer can affect retrogradation: polyols such as glycerol are more suspectible to retrogradation, as they consist of small molecules that are easily separated from

amylose and amylopectin chains (Zullo and Iannace 2009). According to Zullo and Iannace (2009) urea-formamide mixture on the other hand reduced retrogradation.

Starch is a competetive feedstock for anaerobic digestion in terms of methane yield, since its theoretical methane potential is about 410 l/kg VS (Hansen et al. 2004). Hansen et al. (2004) were able to reach 84 % of the theoretical potential in their study, which is approximately 340 l/kg VS and therefore is on a similar level to methane potentials of many crops. For example methane potentials for clover, potatoes and and grass have been reported as 300–350 l/kg VS, 276–400 l/kg VS and 298–467 l/kg VS respectively (Murphy and Thamsiriroj 2013).

Bioplastic methane potentials seem to be a fairly new field of research though, as only one article regarding bioplastic methane potentials was found in literature search. Among the pioneers of this field, Vasmara and Marchetti (2016), found starch based Mater-Bi® to yield mere 33 liters of methane per kilogram of volatile solids as the sole feedstock material in mesophilic conditions. Thus starch based bioplastics are not necessarily great sources for biogas, but it should be borne in mind that biogas plants being filled with just starch based bioplastics is a highly improbable occurence and so starch based bioplastics can still provide some extra biogas, especially as co-digestion of several feedstocks has been noted to potentially increase biogas yields (Yen and Brune 2007, Vasmara and Marchetti 2016).

2.1.3 Bioplastic disposal

Possibly the simplest way to dispose of bioplastics is landfilling (Niaounakis 2013). However, nowadays landfilling is generally frowned upon as a waste treatment method. In fact legislation has been set to reduce the amount of waste ending up on landfills, for example the EU landfill directive requires that the member states reduce the amount of organic waste on landfills (Directive 1999/31/EC 1999). Plants sequester carbon from the atmosphere, so technically

landfilling bioplastics made from said plant could act as a carbon sink and therefore help to reduce carbon emissions (Niaounakis 2013). However this is most likely foiled as anaerobic conditions form in a sealed landfill and methane starts to form. Many landfills lack an landfill gas capturing system, so methane escapes to the atmosphere. Methane is more powerful greenhouse gas than carbon dioxide, which cancels the benefits of a carbon sink. (Niaounakis 2013).

2.1.4 Recycling

Recycling means using waste to create reusable objects. Recycling can be divided into four levels: primary, secondary, tertiary and quartary recycling. Primary recycling means reusing an object as is or as a resource for a similar new item. Secondary recycling takes place when an object is modified to serve a new purpose, for example when a plastic canister is used to create a fence. Tertiary recycling is also known as chemical recycling and it occurs when a plastic is chemically broken down to smaller molecules, which can then be used to create new plastic. Quartary recycling means energy recovery by incineration. (Eskelinen et al. 2016.)

The heat and pressure that polymers are subjected to during the processing pose a threat to polymers' structure. Therefore reprocessing recycled material compromises the structure even further. Reprocessing can cause the polymer to exhibit undesired qualities such as changes in colour. Suitability of a polymer for recycling is analysed by putting materials through the manufacturing process again and examining any changes in structure and morphology. For example, many studies have been conducted regarding the recyclability of polylactic acid. (Niaounakis 2013).

Recycling of bioplastics is a fairly new field of research, which has seen an increase of interest in the 2000's (Soroudi and Jakubowicz 2013). Recycling of bioplastics has raised some arguments both in defence of and against recycling (Niaounakis

2013). The main pro-recycling arguments are that recycling reduces the amount of resources and energy needed to produce the plastics. Moreover, some bioplastics are not biodegradable and therefore could cause similar littering issues as conventional plastics are causing now. (Niaounakis 2013.) Recycling is paramount for bioplastics' sustainability, as Piemonte (2011) found mechanical recycling to be the best method of disposal for bioplastics in terms of saving energy and resources. Thus recycling appears to be preferable over incineration, composting and anaerobic digestion (Piemonte 2011).

The naysayers on the hand argue that bioplastics can cause issues with conventional plastic recycling streams. Bioplastics also constitute a rather small material stream, which is not enough to faciliate profitable recycling. (Niaounakis 2013). Even the recycling of conventional plastics has quite much room for improvement, despite recycling methods for conventional plastics being more established (Sivan 2011). For example the recycling rate of plastic packaging waste in Norway, Switzerland and the 18 EU member states in 2016 was 40.8 %, whereas with a share of 38.8 % almost as much of plastic packaging was incinerated (PlasticsEurope 2018). Steps are taken in the right direction though. Finland, among few other countries, for example have restricted landfilling plastics (Finland's state council's degree on landfills 2013, PlasticsEurope 2018).

Drop-in bioplastics are easier to recycle, as they are chemically identical to their conventional fossil fuel based counterparts and thus do not cause issues with existing plastic recycling schemes (European bioplastics 2015a). Other bioplastics however can contaminate conventional plastic recycling, hence for example Suomen Pakkauskierrätys Rinki Ltd, which manages recycling of packaging waste in Finland, recommends disposing of biodegradable plastics among biowaste (Arikan and Oszoy 2015, Rinki 2019). The variety in bioplastics poses a challenge as efficient ways of identifying and separating the bioplastics are required for bioplastic recycling to be feasible (Arikan and Ozsoy 2015).

Mechanical recycling the most used recycling method for plastics (Shen and Worrell 2014). Mechanical recycling involves several steps, namely sorting the waste, cutting it to smaller pieces, cleaning the pieces and finally the pieces can be refined into new products (Shen and Worrell 2014). The chemical structure of the polymers stays more or less intact during physical recycling (Niaounakis 2013).

Chemical recycling can be more well-suited for recycling polymer blends than mechanical recycling. Blends contain several polymers, which ideally should be separated from one another in order to avoid contamination. Research have been conducted on developing polymer-specific chemical recycling methods that could separate the polymers from a blend. (Soroudi and Jakubowicz 2013.) Downside of chemical recycling is that the required materials and investment can make the recycled matter more expensive than virgin material (Ragaert et al. 2017).

2.2 Biodegradation

Objects are subjected to various degradation processes during their lifetime and after disposal. These degradation pathways can be divided into biodegradation and abiotic degradation (Lucas et al. 2008). Biodegradation is a degradation process faciliated by microbes (Lucas et al. 2008). Degradation can occur due to abiotic reasons, such as heat, ultraviolet radiation and physical wear and tear. Abiotic degradation and biodegradation often accompany one another, as sterile conditions are an exception on Earth. Abiotic degradation mechanisms can be a prerequisite for biodegradation, as changes in polymer structure may render the polymer more easily accessible for microbes (Lucas et al. 2008). Biodegradability of a polymer depends on the polymer's chain lenght, complicatedness of polymer's structure and crystallinity of the polymer (Emadian et al. 2017). Less is more in this regard, as polymers that consist of short molecular chains, have simple structures and exhibit low crystallinity are more readily biodegraded.

Biodegradation is also highly dependant on environmental factors, such as oxygen content, pH, water content and temperature. (Emadian et al. 2017).

Biodegradability has caused some confusion with products being labeled degradable without specifying conditions (European bioplastics 2015b). Additionally oxo-degradable products have emerged in the plastic market. Oxo-degradable plastics make use of additives, namely metal salts, that under exposure to heat and/or sunlight promote fragmentation of the plastic with the hope that these small fragments could then be mineralized by microbes. However, as summarised by Deconinck and De Wilde (2013), there is no clear consensus as to whether or not oxo-degradable plastics are also biodegradable. Therefore instead of faciliating biodegradation, these additives may only promote fragmentation of the plastic, which results in the plastic getting shattered into little pieces and hence only adds to the growing environmental and health concern of microplastics (European bioplastics 2015b).

Biodegradation is undertaken by all kinds of microbes with over 90 types of microbes having been recognized (Emadian et al. 2017). Microbes suited for many environments are represented in biodegradation capable microbes, such as aerobic and anaerobic bacteria, photosynthetic bacteria, archeabacteria and lower eukaryotic. (Emadian et al. 2017) During biodegradation the matter is converted into methane, carbon dioxide and microbial biomass (Cho et al. 2011). The first step of biodegradation is called biodeteriation, which breaks the polymer's surface allowing microbes to have better access to the polymer. The process usually begins with microbes colonizing the surface (Tokiwa et al. 2009). Plastics consist of quite large molecules, which microbes cannot utilise as such (Lucas et al. 2008). Therefore the polymers are first degraded outside the cells (Lucas et al. 2008).

Microbes can also degrade polymers physically as they attach themselves to the polymer surface (Lucas et al. 2008). Some microbes can secrete a gluelike

substance, which allows them to adhere to a surface. This substance can penetrate pores on the surface changing the pores's size and distribution. Filamentous microbes's ability to generate mycelia can provide an additional source of biodeteriation, because as the mycelium grows it has to dig through the polymer (Lucas et al. 2008).

Microbes also have chemical means to degrade materials at their disposal (Lucas et al. 2008). The substances secreted by microbes can ease the interaction between hydrophobic and hydrophilic phases. The slime microbes secrete can gather air pollutants, which in turn can promote microbial growth and therefore also promote biodeteriation (Lucas et al. 2008).

Biofragmentation is the next step in biodegradation. As above fragmentation means breaking polymers down into a mixture of smaller monomers and oligomers, whereas the term "biofragmentation" is used to specify fragmentation caused by microbes. Biofragmentation is achieved through enzymes and free radicals. (Lucas et al. 2008.) Free radicals are atoms or molecules that have an unpaired valence electron. Enzymatic oxidative reactions can yield free radicals, which can then further oxidate the polymer and thus promote biofragmentation (Lucas et al. 2008).

Enzymes on the other hand are a group of proteins that promote certain chemical reactions by lowering the activation energy requirement (Lucas et al. 2008). Enzymes are separated into two groups based on their secretion: microbes produce constitutive enzymes throughout their lives, whereas production of inducive enzymes is triggered, when a cell recognizes presence of suitable substrate. Released enzymes can occur as soluble compounds in the medium or adsorbed onto a particle such as sand or soil organic matter. Enzymes that are adhered to a particle are called fixed enzymes. Fixed enzymes often exhibit increased catalytic activity. (Lucas et al. 2008). Enzymatic degradation is a major

degradation pathway for starch based plastics. (Azevedo et al. 2003). Azevedo et al. (2003) studied the degradation of starch-polyethylene-vinyl alcohol and starch-poly-ε-caprolactone blends by various enzymes and enzyme mixtures and found mass losses of 40–45 % for starch-polyethylene-vinyl alcohol blend and 15–20 % for starch-(poly-ε-caprolactone) blend.

The last step of biodegradation is called assimilation. Assimilation takes place when a microbe feeds on surrounding molecules. Once the polymer molecular size is reduced, molecules can pass through the cell walls (Lucas et al. 2008). Some molecules can enter the cell with ease through specific membrane carriers whilst other molecules may not be able to enter the cell at all. However, some of the molecules unable to penetrate cell can still be transformed into another molecule via biotransformation reactions, and thus permeate the cell wall. Inside the cell, the molecules are used in cellular metabolism providing energy and nutrients. A single strain of microbes may not be able to complete all the steps of biodegradation by itself. Therefore biodegradation is a joined effort of several types of microbes, where degradation products of one strain are used by another strain. (Lucas et al. 2008.)

2.2.1 Measuring methods for biodegradation

Biodegradation of plastics can be determined in many ways (Lucas et al. 2008). Visual observations of holes, cracks and colour changes with naked eye or some magnifying object such as microscope are probably the most obvious method. Although visual changes in a polymer are not definitive proof of biodegradation, because abiotic degradation pathways can also cause such changes, these observations can indicate microbial attack on the polymer surface (Lucas et al. 2008).

Degradation can alter materials's mechanical properties, which can be taken as a sign of degradation (Lucas et al. 2008). For example tensile strength in highly

dependant on polymer's molar mass, hence lowered tensile strength can indicate degradation. As is the case with visual observation, separating changes in mechanical properties caused by microbial metabolism from changes caused by abiotic reasons is impossible (Lucas et al. 2008).

Mass loss is commonly used as sign of degradation (Lucas et al. 2008). Again, definitive proof of biodegradation specifically is not gained. Used in cooperation with chemical analysis of intermediate products, mass loss can provide insights about degradation process (Lucas et al. 2008).

During metabolism carbon is converted into gaseous compounds, which are mostly carbon dioxide in aerobic conditions and methane and carbon dioxide in anaerobic conditions (Lucas et al. 2008). Therefore measuring the release of these compounds can be used to determine the degree of biodegradation. Because this method is based on the end products of metabolism, it is believed to yield accurate results of actual biodegradation taking place. In order to make the most of gas based methods, the carbon content of the polymer should be known and background noise caused by additional carbon sources should be accounted for if not eliminated (Lucas et al. 2008).

However additional carbon sources can be beneficial to the microbes, which is why for example food waste may be added into an anaerobic reactor to kickstart the microbial activity (El-Mashad et al. 2012). Radio labelling has been suggested as a method to separate carbon dioxide and methane that originate from polymer samples from carbon dioxide and methane originating from other sources (Lucas et al. 2008). Disadvantage of radiolabelling however is that radiolabelled materials are not always available and if they are available they are often costly (Lucas et al. 2008).

Clear zone formation tests are used to find strains of microbes that are able to degrade a specific polymer (Lucas et al. 2008). The polymer is added onto a agar

plate as small particles, which turns the agar opaque (Lucas et al. 2008). If a strain is able to depolymerize that polymer, clear zones should form around the colony (Lucas et al. 2008).

2.3 Anaerobic digestion

Anaerobic digestion is a process, in which microbes degrade organic matter in the absence of oxygen (Wilkie 2005). Therefore anaerobic digestion is a potential treatment method for easily degradable waste streams, such as food waste, animal slurry and sewage sludge (Deublein and Steinhauser 2011). Anaerobic degradation yields biogas, which mostly consists of methane (CH4) and carbon dioxide (CO2), and digestate (Rajagopal et al. 2013). Biogas can be used as a renewable fuel, while digestate makes for a fertiliser (da Costa Gomez 2013, Al Seadi et al. 2013).

Anaerobic digestion can be divided into four main steps: hydrolysis,acidogenesis, acetogenesis and methanogenesis in chronological order (Chen et al. 2014). During hydrolysis complex compounds, such as carbonhydrates, proteins and lipids are broken down to amino acids, sugars and fatty acids that have more simple structures thus increasing the substrate's solubility (Deublein and Steinhauser 2011). Hydrolysis is advanced by enzymes that hydrolysing microbes excrete (Deublein and Steinhauser 2011). Carbohydrates can be hydrolysed within hours whereas fats and proteins can take several days to hydrolyse (Weiland 2010).

During acidogenesis the end products of hydrolysis are degraded further into volatile fatty acids, hydrogen, carbondioxide and acetate. The microbes in charge of acidogenesis are known as acidogenic microbes. Acidogenic microbes are diverse to accommodate the variety of starting compounds. (Deublein and Steinhauser 2011.)

In acetogenesis acetogenic bacteria turn volatile fatty acids into acetate, carbon dioxide and hydrogen (Chen et al. 2014). During methanogenesis methanogenic microbes mainly use acetate, carbon dioxide and hydrogen to produce methane (Deublein and Steinhauser 2011).

The two major pathways for producing methane are aceticlastic and hydrogenotrophic methanogenesis (Yenigün and Demirel 2013). Aceticlastic methanogenesis takes place when methane is produced out of acetate, whereas in hydrogenotrophic methanogenesis carbon dioxide and hydrogen gas are used to produce methane (Yenigün and Demirel 2013). Aceticlastic methanogenesis is the major pathway as it accounts for roughly 70 % of produced methane in methanogenesis, whilst the remaining 30 % of methane is produced of hydrogen and carbon dioxide (Deublein and Steinhauser 2011). Therefore sufficient acetate production is paramount for methane production and acetogenesis and acidogenesis may be the rate limiting steps in anaerobic digestion of easily degradable matter (Pavlostathis and Giraldo-Gomez 1991). Hydrolysis is often the rate limiting step for feedstocks whose degradation is challenging, such as woody plants (Zheng et al. 2014).

2.3.1 Anaerobic digestion parameters

The success of anaerobic digestion depends on several parameters (Weiland 2010). Temperature is one of the main parameters. Mesophilic and thermophilic temperature ranges are commonly used: the former being generally reported to be around 35–42 °C whereas the latter is considered to be around 45–70 °C (Weiland 2010). Additionally anaerobic digestion can occur in below 20 °C, which is called psychrophilic anaerobic digestion (Meher 1994, Saady and Massé 2013). Methanogenic microbes, whose optimum temperature is above thermophilic range, have also been found (Banks and Heaven 2013). However these microbes have not been in much use in large scale anaerobic digestion plants (Banks and

Heaven 2013). Generally speaking, the higher the temperature is the more efficient the digestion process, because high temperatures tend to speed up chemical reactions (Weiland 2010). High temperature used in thermophilic digestion also can more effectively sanitise the feedstock, which is an advantage when dealing with pathogenic feedstocks such as manure and sewage sludge (Smith et al. 2005). Unfortunately unlike composting, anaerobic digestion does not generate much heat, which means that reaching and maintaining a high temperature requires external heating and insulation and thus increases costs (Bachmann 2013).

pH is also important for anaerobic digestion (Weiland 2010). pH should be around neutral, as the methane formation takes place in 6.5–8.5 range. Optimal conditions however are slighty alkaline, in the 7–8 range. Outside the 6.5–8.5 range inhibition starts to occur, which decreases the methane yields (Weiland 2010). The intermediate products that form during the process and release free hydrogen ions are the main contributor to pH change in anaerobic digestion (Deublein and Steinheuser 2011). The reactors can resist pH change to some extent because of buffering systems that are in place (Weiland 2010). Bicarbonate buffering system cancels acidification, while ammonia buffering system stops the pH from raising too much (Deublein and Steinheuser 2011). Naturally there is a limit to buffering systems's capabilities and as such they can eventually succumb to changes caused by for example acidic feedstocks, changes in temperature changes or overfeeding (Deublein and Steinhauser 2011).

Feedstock compostion plays a key role in anaerobic digestion (Weiland 2010). Importance of feedstock composition stems from the fact that microbes need a balanced source of macronutrients, that is carbon, nitrogen, phosphorus, hydrogen and sulfur, and some micronutrients in order to thrive (Drosg et al. 2013). Most organic materials are suitable for anaerobic digestion, including sugars, cellulose, fats, and hemicelluloses (Weiland 2010). Some organic materials, such as wood, however are degraded too slowly in anaerobic conditions for anaerobic digestion

to be practical (Weiland 2010). Carbon to nitrogen ratio is used as way to estimate the suitability of a feedstock for anaerobic digestion, as carbon and nitrogen are the most needed elements for microbe metabolism (Drosg et al. 2013). Plenty of research has gone into finding out the optimal C:N ratio, which has been reported to be around 15:1–30:1 (Weiland 2010). If carbon is too dominant in the feedstock's chemical composition, the microbes will suffer from lack of nitrogen. Too much nitrogen on the other hand will give rise to ammonia inhibition. (Deublein & Steinhauser 2011). Co-digestion of several feedstocks can effectively balance the nutrient ratio (Yen and Brune 2007). For example co-digestion of algal sludge and waste paper has been researched, the algal sludge having relatively high nitrogen content while paper is rich in carbon (Yen and Brune 2007).

The quantity of feedstock added to a reactor is expressed as organic loading rate, which shows the amount of total or volatile solids added per reactor volume per day. Chemical oxygen demand can be used as a surrogate quantity for volatile solids (Banks and Heaven 2013). From an economic viewpoint, the higher the organic loading rate the better, as high loading rates allow the whole potential of the reactor to be utilised. Overfeeding can however cause the reactor to fail, so reactors are often run at below optimal loading rates just to be safe (Ward et al. 2008).

2.3.2 Technical solutions

Several technical solutions have been invented to provide alternative methods for anaerobic digestion (Weiland 2010). One of them is altering the consistency of the feedstock, which depends on the feedstock's solids content. In dry digestion the total solids content of the feedstock is approximately 15–35 %, whereas for wet digestion the total solids content is kept below 10 % (Weiland 2010). Wet digestion's benefits include the possibility to use pumps for moving the feedstock (Angelonidi and Smith 2015). Additionally mixing and controlling the conditions

within the reactor are easier when the feedstocks have sludgelike consistency (Deublein and Steinhauser 2011).

Water consumption is a natural disadvantage for wet digestion (Angelonidi and Smith 2015). Water requirement leads to both wastewater being generated and digestate needing to be dried before further refining (Guendouz et al. 2010). Furthermore wet digestion requires more energy, which can be up to 45 % of the produced energy while dry digestion consumes less than 15 % (Guendouz et al. 2010). Wet digestion also requires larger reactors than dry digestion, as dry digestion allows for larger organic loading rate (Guendouz et al. 2010).

The feedstock can be added to the reactors in batches or as a continuous stream (Bachmann 2013). In batch feeding the reactor is filled with feedstock and allowed to degrade it before removing most of the digestate and adding the next batch (Weiland 2010). The reactor is not completely emptied because leaving some digestate is essential for maintaining the microbe cultures in the reactor. Continuosly fed reactor on the other hand require that some of the digested material is constantly removed in order to avoid reactor overflowing (Deublein and Steinheuser 2011). Therefore retention time, in other words the time the feedstock spends in the reactor, needs to be long enough to faciliate maximum digestion (Abbasi et al. 2012). However too long retention time does not generally increase methane yields, because the majority of digestate's methane potential is exhausted.

Anaerobic digestion reactors come in many shapes and sizes (Rajeshwari et al. 2000). Reactors are categorized based on the location of the microbial flora, which can float freely in the feedstock or be fixed on a growth medium (Rajeshwari et al. 2000). Fixed film reactors are easier to construct, allow for higher organic loading rates and are less sensitive to toxicant loads (Rajeshwari et al. 2000). Limitations for fixed film reactors include excess biofilm growth, which can lead to clogging

(Rajeshwari et al. 2000). Additionally the biofilm growth does not necessarily increase the microbes ability to digest matter, as only the microbes within about 1 mm of the surface of the biofilm can take part in the digestion process (Deublein and Steinhauser 2011).

Number of stages is another classification system (Comparetti et al. 2013). In a single stage reactor all phases of anaerobic digestion occur in the same reactor, whereas a multistage reactor separates the phases into several chambers. The benefit of a single stage system lies in its simplicity. Multistage systems on the other hand are more versatile allowing the conditions to be set for each stage separately. (Bachmann 2013). The best possible conditions for hydrolysis are not the same as the optimum conditions for methanogenesis (Weiland 2010). For example slightly acidic conditions are preferred during hydrolysis and acidogenesis while alkaline conditions are better for methanogenesis (Weiland 2010). Therefore multistaged systems can be more efficient (Bachmann 2013).

2.3.3 Inhibitory agents

Anaerobic digestion is suspectible to a variety of inhibition sources (Chen et al. 2008, Chen et al. 2014). Inhibition is a complicated subject, as some compounds are necessary in small amounts but inhibitory in large doses. Some inhibitory agents are formed during anaerobic digestion as intermediate products and thus cannot be avoided completely. Furthermore, microbes can grow accustomed to their surroundings and thus develop resistance against inhibitory compounds to some extent. Antagonistic effects between inhibition sources are also possible. For example, heavy metals and sulfides can both inhibit anaerobic digestion, but together they can form heavy metal precitipates hence limiting each other's availability. (Deublein and Steinhauser 2011.)

Oxygen is a widely acknowledged inhibitory agent for anaerobic digestion, because of strictly anaerobic microbes that are involved in acetogenesis and

methanogenesis (Botheju and Bakke 2011). Moreover, presence of oxygen can result in aerobic degradation processes taking place instead, which stops methane production. Low oxygen concentration can be enough to cease methane production according to Scott et al. (1983), who examined the suspectibility of two methanogenic microbe cultures to oxygen. The cultures originated from a rumen and an anaerobic digestor. They reported that methane production of both cultures stopped at 30 nM concentration. Deublein and Steinhauser (2011) report that 0.1 mg/l oxygen concentration is the inhibition threshold for methatogenic microbes.

Oxygen is not quite as bad as it is made out to be though (Botheju and Bakke 2011). Anaerobic digestion reactors can exhibit notable tolerance for oxygen. Some of the tolerance has been attributed to the diverse microbial flora in the reactor, which can contain facultative fermenting microbes. These microbes can utilise dissolved oxygen reducing the toxic load on strictly anaerobic microbes. Microbes can also form protective aggregates such as biofilms and flocs, which prevent oxygen from reaching anaerobic microbes. Facultative or aerobic microbes will thrive near the surface of the aggregate consuming the oxygen, while anaerobic conditions remain deeper in the aggregate (Botheju and Bakke 2011).

Ammonia is a well-known cause of inhibition for anaerobic digestion (Yenigün and Demirel 2013). Ammonia in anaerobic digestion originates from the degradation of nitrogen containing matter, namely proteins and urea (Chen et al. 2008). Suggested inhibition mechanisms for ammonia include ammonia altering the pH in cells, raising the maintenance energy requirement and inhibiting enzyme reactions (Chen et al. 2008). Ammonia is present in reactors as ammonium ions (NH_4^+) and free ammonia (NH_3). Free ammonia is considered the main culprit for ammonia inhibition due to its ability to permeate through cell walls (Chen et al. 2008).

Sulfate is also noted as an inhibitory agent (Chen et al. 2008). Sulfate is a commonly found in industrial waste waters. Two inhibition mechanisms are known. The main mechanism is that sulfate reducing bacteria compete against other microbes for feedstock. Sulfide, which is the reduced form of sulfate, is toxic to several groups of bacteria, which causes an another source of inhibition (Chen et al. 2008).

Fatty acids and amino acids form an unavoidable inhibition source. They occur naturally in many suitable feedstock materials. Additionally fatty acids and amino acids are formed as intermediate products when fats and proteins are hydrolysed. Depending on the pH in the reactor, fatty acids and amino acids can occur in dissociated and undissociated form. Undissociated acids are more effective inhibitors, due to their increased ability to enter cells. Inside the cells undissociated acids can denature proteins. Fortunately, being intermediate products, fatty acids and amino acids are degraded further over time thus gradually lessening their concentrations. (Deublein and Steinhauser 2011.)

Heavy metals are necessary trace minerals for bacteria, but large doses are toxic. Luckily inhibition thresholds are rarely reached, although heavy metals are accumulated in the microbial biomass over time. Sulfides can be used to precipitate heavy metals, which renders heavy metals unaccesible to microbes. Additionally some heavy metals such as copper, cadmium and lead can be bound into metal complexes by adding polyphosphates into the feedstock. (Deublein and Steinhauser 2011.)

3 METHODS AND MATERIALS

3.1 Inoculum and substrates

Digestate from Mustankorkea biogas plant was used as the inoculum. Mustankorkea Ltd is a waste management company that takes care of waste management for municipalities of Jyväskylä, Laukaa, Muurame and Toivakka in central Finland (Mustankorkea 2019a). Mustankorkea anaerobic dry digestion plant began working in summer 2017 (Mustankorkea 2019b). Leftover food from Ylistö restaurant at University of Jyväskylä's campus at Ylistönniemi was also added to each reactor to provide some nutrients and promote microbial activity.

The test samples were a bioplastic bag made of thermoplastic starch (TPS) (Pirkka® biowaste bag, Plastiroll Ltd), a linear low density polyethylene (LLDPE) bag (Rainbow® freezer bag, unknown manufacturer) and a paper bag (Rainbow® biowaste bag, Pyroll Group Ltd). Paper bag acted as a positive control, that is a material that should degrade quite easily, while the LLDPE bag was used as a negative control, in other words a material that was not expected to degrade much. The TPS bag is biodegradable in industrial composting as defined by the EN 13432 standard, which is to say that the minimum of 90 % of organic matter of the bag is converted to carbon dioxide in six months and 90 % of bag's mass is fragmented into smaller than 2 mm pieces in three months (European bioplastics 2019). The bags were cut into 10 cm wide pieces in order to find a size that would allow meeting the sample volatile solids requirement by adding several pieces of same size into a reactor without having to cut the samples into unrealistically small pieces. The dimensions of paper bags and LLDPE bags were similar and thus both paper and LLDPE bags were cut into approximately 24 cm by 10 cm pieces. The TPS bags on the other hand were larger and as such were cut into approximately 36 cm by 10 cm pieces (Figure 2).

Figure 2. Example of pieces of a bioplastic bag pieces added to a reactor

Two biowaste samples courtesy of Mustankorkea of approximately 15 kg were acquired and their composition was examined. The samples were received on 4th and 5th of June 2018. Digestate was also acquired on June 5th 2018. The samples and digestate were stored in a cold room in 4 °C until examinations. The examination was done by manually mixing the samples and taking subsamples of 1–2 kg, which were then sorted manually. Five subsamples were analyzed from both biowaste samples. Plastic content of the samples was calculated as the ratio of total plastic recovered from all subsamples of a given sample to the total mass of examined subsamples.

3.2 Analytics

The volatile solids of paper, inoculum and food waste were determined according to Finnish standard method (SFS 3008). The volatile solids of the plastics were

determined using the ASTM D5630-13 method. The volatile solids measurements were done in triplicates.

The composition of produced biogas was analysed using a GeoTech GA 2000 gas analyser fitted with infrared and electrochemical detector, which measured the methane, carbon dioxide and oxygen content of the biogas. The gas volume was measured using the water displacement method. The volume of gas used in the compositional analysis was estimated using the duration of the measurement and the typical flow rate of 0.3 l/min mentioned in the analyser manual (Geotech). The compositional analysis usually took 30 seconds per reactor. If the reactor had not produced enough biogas to allow for a 30 second measurement, the latest readings and the time passed were recorded when possible. The room temperature was also monitored and the air pressure data was collected from the nearest weather station at Tikkakoski airport roughly 18 km away from downtown Jyväskylä (Finnish Meteorological Institute 2018).

pH was measured at the end of the 30 and 90 day experiments to check for signs of inhibition. Measurements were taken using a WVR pH 100 pH-meter.

3.3 The reactor set up

The reactors were set up according to El-Mashad et al. (2012) with some modifications. The experiments began on July 31st 2018. The reactors consisted of a one litre glass bottles, which were sealed with rubber stoppers and connected via a tube to a gas bag (Figure 3). 230 g of inoculum was weighed for each reactor based on wet weight. The amounts of food waste and test samples were calculated based on the materials' volatile solids contents. Feedstock volatile solids to inoculum volatile solids ratio of 0.5 was used based on the recommendation of VDI 4630 method (Equation 1).

$$m_{feedstock} = \frac{0.5 \times m_{inoculum} \times VS_{inoculum}}{VS_{feedstock}} \quad , \tag{1}$$

where m stands for mass and VS denotes volatile solids contents of the material. The background control group contained food waste and inoculum, so feedstock consisted of food waste. The experiment reactors on the other hand contained either paper, TPS or LLDPE in addition to food waste, so the feedstock portion of volatile solids was halved between the test material and food waste causing the individual ratios of test materials and food waste VS to inoculum VS to be 0.25.

Examples:

$$\text{The mass of food waste in control reactors}: m_{food\ waste} = \frac{0.5 \times 230\,\text{g} \times 0.0766}{0.229} = 38.5\,\text{g}$$

$$\text{The mass of food waste in experimental reactors}: m_{food\ waste} = \frac{0.25 \times 230\,\text{g} \times 0.0766}{0.229} = 19.3\,\text{g}$$

$$\text{The mass of TPS in experimental reactors}: m_{TPS} = \frac{0.25 \times 230\,\text{g} \times 0.0766}{0.986} = 4.5\,\text{g}$$

Once the feed materials and the inoculum had been weighed and added into reactors, the reactors were filled with tap water to volume of 750 ml. Then the reactors were purged with nitrogen gas for two minutes in order to ensure anaerobic conditions and closed with stoppers. Finally the gas bags were attached and the reactors were moved to the incubation chamber.

The anaerobic experiment took place in 37 °C. The degradation of inoculum and mixture of food waste and inoculum was examined using six reactors. The degradation of test materials on the other hand was measured in triplicates for both 30 day and 90 day tests. Reactors were labelled 1.1–1.3 for each sample material in the 30 day experiment and 2.1–2.3 for the 90 day experiment. The reactors were mixed by manual shaking before and after the gas composition analysis.

Figure 3. An example of the reactor set-up.

3.4 Degradation measurements

Degradation degrees of the samples were determined as relative change in mass (Equation 2) and as amount of biogas generated compared to the theoretical maximum. Additionally changes in materials' appearance were examined.

$$D_m = \frac{m_b - m_a}{m_a} \times 100\% \quad , \tag{2}$$

where D_m stands for degradation degree based on mass loss, and m_a and m_b are the sample masses before and after the experiment respectively. Mass loss was calculated on a per reactor basis using the total mass of all strips added to a each reactor. After the experiment, the strips were recovered, rinsed with water and air-dried over night before weighing.

Biogas yield was used to calculate the mineralization degrees according to Yagi et al. (2009). As they point out, some of the carbon dioxide may be dissolved into the sludge, which means the degradation degrees are possibly under-estimations of

true values (Yagi et al. 2009). At the end of both 30 and 90 day experiments the cumulative biogas yields were calculated. The volume of gaseous carbon, that is mainly carbon dioxide and methane, in the biogas was calculated and transformed into NTP-volumes (Normal temperature and pressure: 293.15 K and 101325 Pa) according to the ideal gas law (Equation 3).

$$V_{\mathrm{NTP}} = V \times \frac{293{,}15\,\mathrm{K}}{T} \times \frac{p}{103125\,\mathrm{Pa}} \quad , \tag{3}$$

where V_{NTP} is the volume of gas in NTP conditions, V is the gas volume in prevailing conditions, T is the prevailing temperature in Kelvins and p is the prevailing air pressure in Pascals.

Biogas generated by inoculum and food waste was accounted for using a control group of six reactors. The average biogas yield per kg of volatile solids was calculated for the control group, which was then used to estimate how much of the biogas in the experiment reactors originated from the inoculum and food waste (Equation 4). The biogas yield from inoculum and food waste was substracted from the biogas yields of sample materials (Equation 5).

$$Y_{\mathrm{inoculum+food}} = \frac{Y_{control,av}}{VS} \times \left(m_{food\,waste} \times VS_{food\,waste} + m_{inoculum} \times VS_{inoculum} \right) \quad , \tag{4}$$

where $Y_{\mathrm{inoculum+food\ waste}}$ is the volume of biogas generated by inoculum and food waste in an experimental reactor, $Y_{\mathrm{control,av}}/\,VS$ is the background control reactors' average biogas yield per mass of volatile solids and $m_{\mathrm{food\ waste}}$ and m_{inoculum} are the masses of both materials added to a reactor and $VS_{\mathrm{food\ waste}}$ and $\mathrm{VS}_{\mathrm{inoculum}}$ are the volatile solids contents of both materials expressed as a real number between zero and one.

$$Y_{sample} = Y_{total} - Y_{inoculum+food\,waste} \quad , \tag{5}$$

where Y_{sample} is the volume of biogas estimated to have been originated from the sample material and Y is the total biogas yield of a reactor.

Theoretical maxima for each sample type were estimated by assuming all carbon in the sample is transformed into gas. The carbon content of each sample material was estimated to be 55 % of their respective volatile solids contents according to Adams et al. (1951) (Equation 6).

$$C = \frac{VS}{1.8} \quad , \tag{6}$$

where C is the carbon content of the material as a percentage and VS stands for volatile solids content of the material as a percentage. The theoretical maximum biogas yield was therefore calculated according to Equation 7.

$$Y_{\text{theoretical}} = \frac{m_{\text{sample}} \times C_{sample} \times M_{carbon} \times V_{NTP,mol}}{100\,\%} \quad , \tag{7}$$

where $Y_{\text{theoretical}}$ is the theoretical maximum biogas yield, M_{carbon} is carbon's molar mass (12.01 g/mol) and $V_{\text{NTP,mol}}$ is the molar volume of ideal gas in NTP conditions (24.054 l/mol). The mineralization degrees D_{b} were given by Equation 8.

$$D_{\text{b}} = \frac{Y_{\text{sample}}}{Y_{\text{theoretical}}} \times 100\,\% \tag{8}$$

3.5 Statistical analysis

Statistical analysis was performed in order to estimate if longer retention time resulted in significantly higher mass loss for TPS. In the end statistical analysis of mineralization degrees was deemed futile, as samples carbon content could not be accurately quantified and some reactors failed, which reduced the amount of representative reactors to below three, so statistical analysis was not feasible

anyway. Statistical analysis was performed on R (version 3.5.1). The normality and equality of variance were studied using Shapiro-Wilk's test of normality and Bartlett's test of homogeneity of variances. Statistical significance of the difference between retention times was examined using Student's t test. *P*-values of below 0.05 were considered to express statistical significance.

4 RESULTS

4.1 Biowaste composition

The biowaste samples provided a glimpse into the varying composition of biowaste. Prior to taking sample A, Mustankorkea had received a delivery of plants from a garden center, which resulted in the sample mostly consisting of soil. Sample B was taken the following day and included more foodstuffs such as potatoes and orange peels (Figure 4). The variability also caused a slight difference in labels for the sorted materials (Tables 1 and 2). Sample B was in the middle of decaying process, so half-decayed mush was a major component in sample B. The mush made recognizing components more difficult, so only plastic and paper could be effectively indentified and the rest was considered to be organic matter. Therefore some impurities may have also been concealed inside the mush.

Figure 4. Biowaste samples. Sample A is on the left and B on the right.

Table 1: Composition of subsamples from biowaste sample A.

Subsam-ple	Subsam-ple mass (kg)	Soil (g)	Organic material (g)	Plastic (g)	Rocks (g)	Paper and cardboard (g)	Metal (g)
1	2.4	1600	524.26	42.04	4.24	36.46	N.R*
2	2.1	1200	293.86	13.13	5.27	11.38	0.21
3	1.2	572.44	283.71	2.56	4.41	17.09	0.38
4	1.1	572.15	215.97	4.06	5.32	34.16	0.26
5	0.8	477.4	141.63	4.28	8.35	18.51	0.08

* N.R stands for not recovered

Table 2. Composition of subsamples from biowaste sample B

Sub-sample	Subsample mass (kg)	Organic material (g)	Plastic (g)	Paper and cardboard (g)
1	0.9	799.27	11.44	5.97
2	1.9	1609.8	19.01	88.12
3	1.7	1437.89	29.44	39.40
4	1.2	1157.59	10.25	22.48
5	2.2	1612.2	9.00	38.40

The organic material in both samples included various materials ranging from recognizable pieces of fruit and vegetables to leaves, plant roots, fish skeletons, egg shells and decaying mush. Paper and cardboard were also somewhat difficult to define, since some pieces of cardboard included plastic sheets.

Plastic content was around 1 % in both samples. Most of the plastic pieces found were plastic films and sheets such as fragments of plastic bags. A few pieces of dense plastics can however quite easily increase the mass of plastics in biowaste, such as pieces of plastic plant pots, which were found due to the delivery of plants Mustankorkea had received prior to sampling. Additionally a few pieces of aluminum foil were found, but the amounts were negligible.

4.2 Volatile solids

The volatile solid contents are presented in Table 3. Plastics had volatile solid contents of nearly 100 %, which highlights the discrepancy that might occur between biodegradability and high volatile solids content. That is, materials may contain high amounts of volatile solids and therefore organic material, but that does not mean the organic material is easily accessible to microbes.

Table 3. Average volatile solids contents and standard deviations of inoculum, food waste, paper and plastics.

Material	VS (%)
Inoculum	7.659 ± 0.046
Food waste	22.896 ± 0.569
Paper	93.249 ± 0.326
LLDPE	99.192 ± 0.003
TPS	98.576 ± 0.252

4.3 Degradation based on mass loss

After the experiment the reactors were opened and examined for sample pieces. The remaining samples were rinsed with water and air-dried over night before weighing (Table 4 and 5). The pieces were also examined for visual signs of degradation such as changes in colour and holes.

Table 4. Mass losses in 30 day experiment. m_a and m_b denote the total mass of the material at the beginning and at the end of the experiment respectively. Δm denotes change in mass as a percentage of original mass.

Sample	m_a (g)	m_b (g)	Δm (%)
TPS1.1	4.32	3.69	-14.58
TPS1.2	4.33	3.68	-15.01
TPS1.3	4.42	3.75	-15.16
Paper1.1	4.94	0	-100
Paper1.2	5.11	0	-100
Paper1.3	5.07	0	-100
LLDPE1.1	5.03	5.18	2.98
LLDPE1.2	4.81	4.99	3.74
LLDPE1.3	4.81	4.90	1.87

Table 5. Mass losses of each sample in 90 day experiment. m_a and m_b denote the total mass of the material at the beginning and at the end of the experiment respectively. Δm denotes change in mass as a percentage of original mass.

Sample	m_a (g)	m_b (g)	Δm (%)
TPS2.1	4.38	3.51	-19.86
TPS2.2	4.36	3.45	-20.87
TPS2.3	4.35	3.40	-21.84
Paper2.1	5.07	0	-100
Paper2.2	4.90	0	-100
Paper2.3	5.18	0	-100
LLDPE2.1	4.88	5.00	2.46
LLDPE2.2	4.98	5.03	1.00
LLDPE2.3	5.00	5.07	1.40

The most notable visual change was a change in colour. Some holes also appeared, particularly in the 90 day experiment, but the holes could be also partly attributed to the strips being torn while rinsing and detaching from the table after drying (Figure 5). TPS lost on average 14.9 ± 0.3 % of its' mass in 30 days and 21 ± 1 % in 90 days. TPS mass losses turned out to be normally distributed and homoscedastic (Tables 6 and 7), so parametric Student's t-test could be used to examine the statistical signifigance of the difference between time scales. Student's t-test produced a statistically signicant result (t = -9.9668, df = 4, p-value = 0.0006, where t is the test statistic and df stands for degrees of freedom). Therefore significant degradation took place during the extended experiment according to the mass losses.

Figure 5. On the left there is a picture of a TPS strip before the experiment. In the middle there is a picture of a TPS strip after 30 days of anaerobic digestion and on the right is a picture of a TPS strip after 90 days of anaerobic digestion.

Table 6. Results of Shapiro-Wilk normality test. W is the test statistic for Shapiro-Wilk.

Timescale (d)	W	p-value
30	0.92608	0.4741
90	0.99986	0.9771

Table 7. Results of Bartlett's test of homogeneity of variances. Bartlett's K^2 is the test statistic for Bartlett's test of homogeneity of variances.

Bartlett's K^2	*df*	*p*-value
1.8894	1	0.1693

Paper bags disintegrated in 30 days to such an extent that no pieces could be distinguished and recovered and thus 100 % mass loss was considered. LLDPE on the other hand gained mass during the experiment: on average 3 ± 1 % in 30 days and 1.6 ± 0.8 % in 90 days. The mass gain may be due to biofilm formation onto the surface. According to Sivan (2011) microbes need to be hydrophobic in order to attach to hydrophobic plastic surfaces. In addition, microbes have been found to become increasingly hydrophobic when there is a lack of a carbon source (Sivan 2011).

4.4 Mineralization degrees

Biogas volume and chemical composition were monitored throughout the experiment in order to estimate mineralization degrees. Only the carbonaceous fraction of biogas, that is methane and carbon dioxide combined, was taken into consideration for this purpose. Methane and carbon dioxide generated by food waste and inoculum was accounted for using a background control set. After substracting the background biogas production, the excess CO_2 and CH_4 volume was compared to the theoretical maximum CO_2 or CH_4 volume that a material could produce, which was calculated by estimating the carbon content of the sample materials.

A noticeable lag phase took place at the beginning of the experiment with reactors taking 7–10 days to reach biogas's typical methane concentrations of 50 % or more (da Costa Gomez 2013) (Appendix 1). The lag phase was most likely caused by unacclitimized inoculum that had been stored for around two months. The pH

values were in the 7.6–8.0 range at the end of the experiments (Appendix 2). Since the pH was only measured at the end of the experiments, the pH values shed little light on the whole degradation process. Nonetheless the pH values were within optimal range, so pH related inhibition taking place at the end of the experiment can be ruled out.

Eliminating oxygen contamination proved difficult, which resulted in reactor failures as represented by high variability in biogas yields (Tables 8 and 9, Appendix 1). The reactors were purged again and stoppers were changed if the oxygen concentration reached 10 %, as that was considered a clear sign that something was wrong. Reactors were also purged, if the gas bags fell from the tube or the stoppers were blown away by pressure build up. Threshold of 10 % is arguably quite high as it is approximately half of the oxygen content in ambient air. Judging by the data (Figure 6), 50 % methane concentrations were accompanied by oxygen concentrations of below 8 %. The ratio of methane to carbon dioxide in biogas depends on the materials chemical composition as demonstrated by Buswell and Mueller (1952) (Equation 9). Thus some substrates might not reach 50 % methane content even in optimal conditions, and maximum oxygen content that still reached 50 % methane content cannot be used as an universal inhibition threshold for all materials.

$$C_cH_hO_o+\left(c-\frac{h}{4}-\frac{o}{2}\right)H_2O\rightarrow\left(\frac{c}{2}-\frac{h}{8}+\frac{o}{4}\right)CO_2+\left(\frac{c}{2}+\frac{h}{8}-\frac{o}{4}\right)CH_4 \quad (9)$$

Due to the reactor failures (Tables 8 and 9), only the background control reactors that produced at least 50 % of the best performing reactor's biogas yield in the background control group were included in calculating the average biogas yield per volatile solids. Placing the threshold on 50 % is admittedly quite lenient, but since the best biogas yield from background was in a leaque of its own and experimental reactors also experienced inhibition, it was considered best to allow

some variability and not compare the experimental reactors to the optimal background biogas yield. Thus food waste and inoculum combined produced 210 ± 70 l/kg VS of CH_4 and CO_2 in 30 days and 220 ± 80 l/kg VS of CH_4 and CO_2 in 90 days. Slight increase in biogas production was therefore noted between 30 and 90 day retention times, but the increase is negligible compared to the standard deviations.

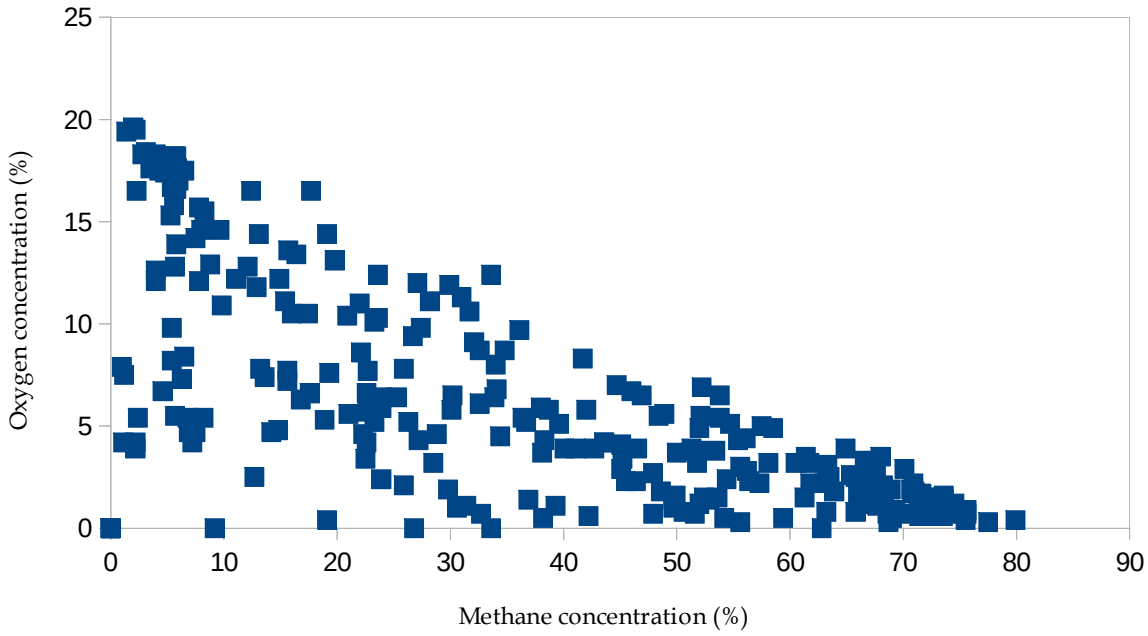

Figure 6. Methane and oxygen concentrations from all biogas compositional analyses.

Table 8. Cumulative volumes in NTP conditions of CH_4 and CO_2 generated by each reactor in 30 days. Background reactors were named 'control1.1'–'control2.3', reactors containing TPS were called 'TPS1.1'–'TPS2.3' etc. Thus each row contains the reactor of that specific number in each reactor group. All six background reactors were run for the whole 90 days. In the experimental reactor groups reactors named 1.1–1.3 represent the 30 day experiment reactors and reactors named 2.1 – 2.3 represent the 90 day experiment group of each sample type.

Reactor group	Background	TPS	LLPDE	Paper
Reactor number	V_{NTP} (l)	V_{NTP} (l)	V_{NTP} (l)	V_{NTP} (l)
1.1	2.61	5.37	4.67	6.20
1.2	4.43	2.39	4.41	5.93
1.3	3.67	5,16	2.34	8.49
2.1	8.19	1.36	3.96	8.07
2.2	4.66	4.14	2.90	4.81
2.3	4.97	3.03	5.04	6.63

Table 9. Cumulative volumes in NTP conditions of CH_4 and CO_2 generated by each reactor in 90 days.

Reactor group	Background	TPS	LLPDE	Paper
Reactor	V_{NTP} (l)	V_{NTP} (l)	V_{NTP} (l)	V_{NTP} (l)
1.1	2.69	-	-	-
1.2	4.81	-	-	-
1.3	4.06	-	-	-
2.1	8.97	1.40	4.35	8.87
2.2	4.75	4.43	3.09	4.89
2.3	5.07	3.15	5.54	6.99

Biogas production reached a plateau at around 30 days, so the changes in mineralization degrees between 30 and 90 day experiments were most likely caused by different levels of oxygen contamination between 30 day and 90 experiment groups. The change was most prominent for the TPS group whose 30

day experiment reactor group produced more biogas than 90 day experiment group did to such an extent that average biogas yield dropped notably after disassembeling the 30 day reactor group (Figure 7).

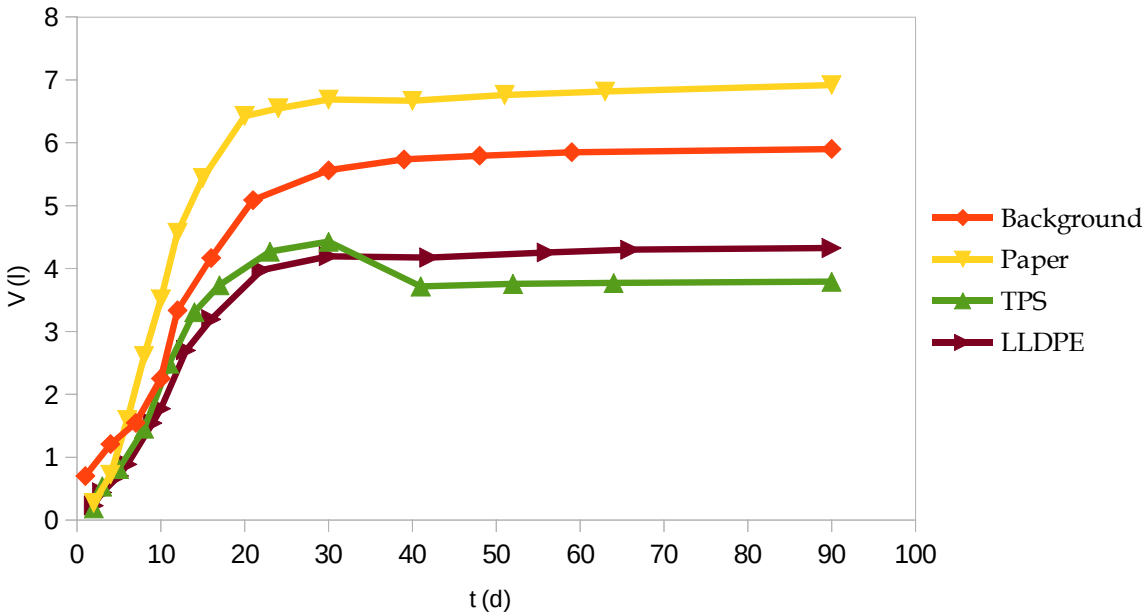

Figure 7. Average cumulative biogas yields for each material. The yields are in NTP volumes and only include CH_4 and CO_2. Reactors in each group that produced less than 50 % of that group's best biogas yield were not taken into account in calculating the averages.

The mineralization degrees were mostly negative for plastics signifying that food waste and inoculum produced more biogas on average than the samples did (Table 10). Some mineralization can be possible for the TPS examined here, as the best performing reactor in the TPS groups produced a mineralization degree of 14 % in 30 days. The TPS 90 day experiment failed to yield a single positive mineralization degree, so this study is inconclusive in regards to longer retention times allowing higher mineralization. Only two reactors from TPS reactor groups gave a positive result, which made calculation of averages quite useless.

Similar levels of mineralization were achieved for LLPDE: only one reactor showed a positive result with mineralization degrees of 7.6 and 11.5 % in 30 and 90 days respectively. Paper was unsurprisingly the most degradable material reaching maximum mineralization degrees of 71 and 76 % in 30 and 90 days respectively.

Table 10. Mineralization degrees for each material. Reactors containing TPS were called 'TPS1.1'–'TPS2.3', reactors with paper were named 'Paper1.1–Paper2.3' etc. Thus each row contains the mineralization degree of the sample with that specific number in each reactor group. Reactors named 1.1–1.3 for each material represent the reactors that were run for 30 days and the reactors named 2.1 – 2.3 were run for 90 days.

Experiment duration	30 days			90 days		
Reactor number	TPS (%)	Paper (%)	LLDPE (%)	TPS (%)	Paper (%)	LLDPE (%)
1.1	14.0	31.7	-0.9	-	-	-
1.2	-49.5	23.9	-4.8	-	-	-
1.3	7.6	71.1	-43.7	-	-	-
2.1	-66.8	66.3	-10.7	-71.7	76.2	-8.6
2.2	-11.0	-0.4	-30.6	-10.8	-4.5	-32.2
2.3	-35.2	36.7	7.5	-38.7	38.1	11.5

5 DISCUSSION

Although the composition of municipal solid waste has been studied in great detail across the globe (e.g. Burnley 2007, Ogwueleka 2009 and Zhang et al. 2010), the composition of biowaste has not received that much attention (Malamis et al. 2015). Compositional studies of biowaste are scarce and impurities such as metals,

plastics and glass are often grouped together (Malamis et al. 2015). Thus there is not much reference material in literature at the moment.

In one of the few studies available, Zhang et al. (2013) studied the composition of biowaste in Finland, United Kingdom, Italy and Portugal. For example, in Finland, plastics made up 0.2 % of the sample and biodegradable bags made up 1.6 %. The plastic shares of approximately 1 % found in this study therefore agree quite well with findings of Zhang et al. (2013), considering that biodegradable bags were not separated in the present study. In another example, in Italy plastic containers made up 0.3 % while plastic films had a share of 2.2 % and biodegradable bags made up 3.7 % (Zhang et al. 2013).

Starch exhibits hydrophilic behaviour and has quite limited mechanical properties, which is why starch is usually used in blends with other polymers (Carvalho 2013). Biodegradability of some of these blends have been studied. Cho et al. (2011) reported biodegradability degree of 83 % measured as methane yield for thermoplastic starch-polycaprolactone blend in anaerobic conditions. In composting conditions Du et al. (2008) found a biodegradation degree 73 % based on carbon dioxide production. As these reports are based on gas yields, it can be assumed that the carbon was actually used in microbial metabolism.

The TPS studied in this experiment did not degrade much in anaerobic conditions. It has however been proven to degrade in industrial composting according to the EN13432 standard, which goes to show that requirements of aerobic and anaerobic degradation differ, and aerobically degradable plastics might not be anaerobically degradable and vice versa (Zhang et al. 2018).

In this study TPS lost six percentage points more mass in 90 days than in 30 days. Considering the low biogas yield and, by extension, low microbial activity during the last 60 days of experiment, the data suggests that six percentage point increase might be mostly due to abiotic factors. The increase in mass loss was considered

statistically significant, so in terms of mass loss extending the retention time can improve degradation.

As for mineralization degree of TPS, maximum mineralization degree reached was 14 % in 30 days, which suggests that some level of mineralization could be possible. It should however be noted that 14 % is quite likely within the margin of error, as the materials' carbon content could not be accurately determined and the oxygen inhibition was highly variable both between reactor groups and between reactors of each reactor set. Only two TPS containing reactors produced more biogas than background, so in this study the biogas yields were lower than those reported by Vasmara and Marchetti (2016). As none of the three TPS containing reactors in the 90 day experiment produced enough biogas to reach a positive mineralisation degree, the results of this study are inconclusive in regards to longer retention times allowing for higher mineralization degrees.

LLDPE produced similar results: the only reactor that showed a positive mineralization degree belonged to the 90 day reactor group and thus yielded mineralization degrees of 7.5 % and 11.5 % in 30 and 90 days respectively. Possible reasons for low biogas yields from plastics could be plastic strips trapping some of the gas despite mixing and varying levels of oxygen contamination that the reactors faced. Moreover, food waste exhibited great biogas potential as the biogas yield of the best performing background control reactor was second to only the highest biogas yield of the paper containing reactors in the 30 day experiment and in the 90 day experiment background control reactor yielded the most biogas. Hence there is a possibility that biogas production of the plastics was masked by the food waste or even that plastics impeded biogas production. Nonetheless it is difficult to estimate how much each different factors, such as oxygen inhibition and leaks in the gas bags, contributed to the variability of biogas yields.

One issue with the reactor set-up was that plastics floated at the top of reactor due to their low density. That quite possibly rendered the afloat portion of plastic strips inaccessible to microbes. The floating strips of plastics may have also obstructed the nitrogen flow during purging and thus contributed to oxygen contamination.

Unsurprinsingly paper was the most degradable material. Thus in terms of biodegradability, paper is a preferable material for household scale biowaste bags. However it is worth noting that biodegradability is only one aspect of environmental friendlyness of a product. For example, according to the life cycle assessment by Mattila et al. (2009) paper and plastic shopping bags produce nearly identical greenhouse gas emissions having emissions of 14–51 grams of CO_2 equivalent per bag and 15–48 grams of CO_2 equivalent per bag respectively.

There is some uncertainty related to contributions of test materials to the methane concentrations in comparison to the food waste's and inoculum's contributions. All test materials had at least one reactor reaching 50–80 % methane concentrations (Appendix 1), which suggests that at least in terms of concentrations methane production was not hampered by plastics or paper in such small quantities. Reports of plastics hindering anaerobic degradation have made news in Finland, so large amounts can still pose a threat (Yle 2019a). Nevertheless, due to unknown chemical compositions of test materials, it is difficult to estimate, whether the high methane content was caused by degradation of the test materials or the food waste and inoculum.

6 CONCLUSIONS

Plastics end up in biowaste where they can cause problems for biogas plants. In Finland biowaste seems to be fairly clean of impurities, although biowaste

composition is a scarcely studied subject and therefore there is not much literature available for comparison. Furthermore, judging by the two samples studied in this thesis, biowaste composition can change quite notably even on a daily basis. Therefore biowaste composition studies have so far merely scratched the surface.

As hypothetised, thermoplastic starch was placed between paper and LLDPE in terms of biodegradability. However, the thermoplastic starch used in this study was found to be quite undegradable in anaerobic conditions. Thus there is a possibility that in large amounts the TPS could for example create blockages in anaerobic reactors, which might necessitate removal of the TPS from biowaste. Nonetheless compostability of the TPS studied here should ensure that if the digestate is appropriately composted, any fragments of the TPS are degraded.

The 90 day experiment produced statistically significantly higher mass loss for TPS than that of 30 day experiment, which suggests that considerable degradation took place during the last 60 days of experiment. As biogas production and, by extension, microbial activity were low after 30 days, the increased mass loss may have been mostly caused by abiotic factors. Nevertheless 21 % mass loss is still somewhat low degradation.

TPS exhibited low level of mineralization in properly anaerobic conditions, but considering that this is based on a single reactor, there is little evidence to support that claim. Ensuring anaerobic conditions proved difficult, which coupled with uncertainty about the materials' carbon content and lack of statistical analysis mean that the mineralization degrees are subject to substantial error and should be taken with a grain of salt, ergo the mineralization degrees for both plastics are most likely within the margin of error. Mass loss yielded more consistant figures, although some level of uncertainty also accompanies mass loss as a method of assessing degradability due to the impossibility of diffrentiating mass loss caused by biodegradation from that caused by abiotic reasons.

As could be expected, paper was the most degradable material. Thus in terms of biodegradability, paper is a preferable material for household scale biowaste bags. However it is worth noting that biodegradability is only one aspect of environmental friendlyness of a product.

Bioplastics are still in their infancy, with a marginal share of total plastic production. Anaerobically degradable bioplastics can offer several benefits over conventional plastics, such as reduced use of fossil fuels in manufacturing phase and potential feedstock for biogas production, which can provide incentive for bioplastic development. Nevertheless, given the variety of starch based bioplastics, let alone bioplastics in general, and the anaerobic digestion methods, further research is required to determine the best suited bioplastic for anaerobic digestion.

ACKNOWLEDGEMENTS

I thank my master's thesis supervisors Tuula Tuhkanen and Kai Sormunen for their assistance with my master's thesis and for giving me an opportunity to combine my enthusiasm for renewable energy with my master's thesis. I would like to thank laboratory technicians Mervi Koistinen, Emma Pajunen and Nina Honkanen for helping me with the laboratory equipment. I am thankful to Iida Pulkkinen for showing me the ropes of anaerobic digestion experiments. I am grateful to Maa- ja vesitekniikan tuki ry for the grant they gave me.

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APPENDIX 1. BIOGAS VOLUME AND COMPOSITION MEASUREMENTS

Reactor group: Background 1												
Reactor:	1.1				1.2				1.3			
Days passed	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)
1	1.0	42.4	7.9	0.84	1.1	50.1	4.2	1.63	1.2	38.7	7.5	0.20
4	5.7	28.0	12.8	0.93	14.2	63.3	4.7	0.73	13.2	49.8	7.8	0.18
7	8.0	15.8	14.6	0.49	24.0	46.8	6.4	0.44	22.7	32.9	5.7	0.10
10	20.9	12.2	10.4	0.44	43.6	35.3	4.2	0.88	23.6	12.9	12.4	0.05
12	34.8	11.3	8.7	1.46	65.4	22.8	2.6	1.56	51.8	14.2	3.2	1.66
16	31.7	9.3	10.6	1.24	70.1	16.9	2.9	2.18	40.1	13.4	3.9	0.99
21	19.1	7.3	14.4	1.14	57.5	18.5	5.0	1.38	61.4	19.3	3.5	1.19
30	12.4	5.3	16.5	1.05	41.7	15.2	8.3	0.80	56.1	18.7	4.4	1.10
38	5.9	3.6	17.6	0.60	17.4	9.8	10.5	0.50	22.6	13.3	6.6	0.60
48	3.8	3.0	18.0	0.15	15.4	9.1	11.1	0.20	19.3	12.5	7.6	0.15
59	3.1	2.7	18.4	0.15	11.1	7.7	12.2	0.28	15.6	11.7	7.7	0.28
90	1.4	2.0	19.4	0.15	7.5	6.4	14.2	0.10	14.9	6.8	12.2	0.21

Volumes are corrected for gas lost during composition measurement and converted into NTP-volumes.

Reactor group: Background 2												
Reactor	2.1				2.2				2.3			
Days passed	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)
1	2.2	53.0	4.2	1.18	2.4	50.6	5.4	1.18	2.2	53.5	3.9	1.23
4	19.1	77.9	0.4	0.78	14.8	61.4	4.8	0.83	18.9	58.6	5.3	0.68
7	38.2	61.4	0.5	0.59	25.3	44.2	6.4	0.69	36.7	40.1	5.2	0.30
10	59.4	37.9	0.5	1.17	45.1	31.8	4.1	1.08	53.4	27.7	3.8	1.03
12	75.5	22.9	0.4	1.32	63.3	20.8	3.1	1.22	66.6	18.4	3.3	1.07
15	79.9	19.6	0.4	1.38	46.9	14.9	6.5	0.94	64.9	17.3	3.9	1.04
21	77.5	23.0	0.3	1.48	32.6	14.8	8.7	0.99	52.2	15.6	6.9	1.14
30	75.6	22.9	0.9	0.90	8.8	7.9	12.9	0.13	29.9	9.9	11.8	0.40
38	71.4	26.8	0.6	0.40	5.3	6.6	15.3	0.18	16.4	7.0	13.4	0.25
48	68.6	26.6	0.7	0.17	3.5	4.2	17.6	0.10	9.6	4.5	14.6	0.15
59	66.2	25.9	1.2	0.10	9.8	9.0	10.9	0.20	5.6	3.4	15.8	0.17
90	62.8	23.5	0.0	0.15	5.8	6.2	13.9	0.15	4.0	2.6	18.3	0.13

Volumes are corrected for gas lost during composition measurement and converted into NTP-volumes.

Reactor group: thermoplastic starch 30 day experiment												
Reactor	1.1				1.2				1.3			
Days passed	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)
3	7.5	45.9	4.7	1.12	7.8	19.6	12.1	0.20	8.2	37.3	5.4	0.98
5	31.4	62.6	1.1	0.34	-	-	-	0.15	30.6	61.6	1.0	0.49
8	50.6	46.1	0.8	0.74	27.4	22.8	9.8	0.40	51.6	45.5	0.7	0.74
11	69.0	29.4	0.5	1.32	38.3	17.7	4.3	1.32	68.7	30.1	0.3	1.18
14	75.6	22.8	0.7	1.04	61.7	21.1	3.2	0.84	73.0	23.5	1.0	0.94
17	73.9	21.8	1.1	0.49	55.4	19.6	4.3	0.30	73.6	22.6	1.6	0.44
23	73.5	22.0	1.5	0.79	48.9	18.8	5.6	0.54	70.7	22.1	1.9	0.84
30	66.4	23.8	1.8	0.18	32.6	18.0	6.1	0.23	68.3	23.9	1.6	0.30

Volumes are corrected for gas lost during composition measurement and converted into NTP-volumes. - denotes failed measurement

Reactor group: thermoplastic starch 90 day experiment												
Reactor	2.1				2.2				2.3			
Days passed	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)
2	4.0	17.5	12.1	0.78	6.9	33.9	4.7	0.73	5.4	33.0	9.8	0.20
5	-	-	-	0.07	28.5	53.5	3.2	0.65	27.2	53.1	4.3	0.63
8	33.6	14.9	12.4	0.25	46.5	35.6	3.9	0.84	47.9	39.3	2.7	0.50
11	36.1	9.9	9.7	0.88	67.8	26.9	1.2	0.98	63.1	26.1	2.2	0.83
14	31.0	6.6	11.3	0.94	71.6	22.2	1.7	0.69	67.6	20.0	2.9	0.79
17	15.7	3.9	13.6	0.59	70.9	19.5	2.2	0.44	58.5	17.6	4.9	0.54
23	17.7	2.7	16.5	0.89	68.0	18.0	3.5	0.59	53.8	15.0	6.5	0.13
30	6.5	1.9	17.5	0.18	54.7	16.6	5.1	0.18	34.0	15.3	8.0	0.15
41	5.8	1.8	18.2	0.30	52.1	17.6	5.5	0.30	25.9	13.6	7.8	0.13
52	5.6	1.6	18.2	0.12	21.0	12.0	5.6	0.12	22.1	11.2	8.6	0.12
64	2.2	0.9	19.5	0.18	7.8	3.0	15.7	0.12	3.9	2.3	17.7	0.22
90	2.0	1.2	19.6	0.13	23.6	10.0	10.3	0.08	9.2	6.0	-	0.10

Volumes are corrected for gas lost during composition measurement and converted into NTP-volumes. - denotes failed measurement

Reactor group: paper 30 day experiment												
Reactor	1.1				1.2				1.3			
Days passed	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)
2	6.5	28.1	8.4	0.59	6.3	30.1	7.3	0.64	7.2	35.5	4.2	0.64
4	22.3	54.4	4.6	0.19	17.6	46.4	6.6	0.54	23.9	58.7	2.4	0.63
6	28.8	50.3	4.6	0.88	26.3	48.9	5.2	0.93	36.9	57.7	1.4	0.98
8	30.2	38.0	6.5	1.24	34.4	43.7	4.5	0.99	42.2	56.3	0.6	1.09
10	41.1	39.2	3.9	1.03	42.7	36.8	3.9	0.93	54.2	43.7	0.5	0.93
12	50.0	30.7	3.7	1.61	51.7	28.5	3.8	1.42	63.2	34.0	0.8	1.37
15	52.0	23.0	4.9	1.43	39.6	19.2	5.1	1.58	69.7	28.7	0.7	1.33
20	46.0	19.1	6.7	1.53	44.7	18.9	7.0	1.43	73.5	25.5	0.6	1.53
24	28.2	12.7	11.1	0.15	27.1	11.4	12.0	0.25	71.0	21.2	1.8	0.49
30	12.9	9.7	11.8	0.20	12.1	8.4	12.8	0.35	66.5	22.1	2.1	0.20

Volumes are corrected for gas lost during composition measurement and converted into NTP-volumes.

Reactor group: paper 90 day experiment												
Reactor	2.1				2.2				2.3			
Days passed	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)
2	12.7	43.4	2.5	0.69	13.6	33.2	7.4	1.18	-	-	-	-
4	32.7	62.1	0.7	0.63	22.7	33.4	7.7	0.88	23.3	27.1	5.2	1.17
6	39.3	57.5	1.1	0.93	34.1	32.4	6.8	1.27	45.5	43.4	2.3	1.27
8	47.9	49.8	0.7	1.09	36.4	36.4	5.4	1.43	49.9	43.2	1.6	1.43
10	55.6	43.3	0.3	1.13	38.0	31.5	5.9	1.08	52.9	39.8	1.4	1.13
12	65.8	32.0	0.8	1.42	32.1	22.4	9.1	0.78	54.4	33.6	2.4	0.88
15	72.0	27.2	0.6	1.09	23.3	14.0	10.1	0.84	55.6	28.8	3.0	0.59
20	74.5	24.2	0.7	1.33	19.8	8.2	13.1	0.89	60.5	22.9	3.2	1.13
24	-	-	-	-	8.3	5.6	15.5	0.25	26.7	12.4	9.4	0.25
30	66.0	24.8	1.7	0.25	13.1	7.9	14.4	0.50	38.7	22.4	5.8	0.35
40	63.9	27.4	1.8	0.35	5.8	5.2	16.6	0.25	33.9	22.2	6.4	0.25
51	61.3	28.1	1.5	0.20	5.4	4.5	16.7	0.20	16.8	16.6	6.3	0.25
63	56.4	26.0	2.3	0.10	4.3	3.8	17.5	0.25	15.6	14.5	7.2	0.22
90	48.4	13.1	5.5	0.36	2.8	3.4	18.3	0.26	16.0	10.9	10.5	0.26

Volumes are corrected for gas lost during composition measurementand converted into NTP-volumes. - denotes failed measurement

Reactor group: LLDPE 30 day experiment												
Reactor	1.1				1.2				1.3			
Days passed	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)
3	5.7	38.4	5.5	1.12	7.3	39.1	5.3	0.83	2.3	1.4	16.5	0.34
6	23.9	45.2	5.9	0.73	29.8	58.2	1.9	0.44	6.0	0.8	17.0	0.20
10	52.4	39.5	1.5	1.03	53.6	40.1	1.5	0.88	45.1	23.0	2.9	0.59
13	52.0	19.9	1.2	1.18	71.4	26.1	0.9	1.13	65.8	21.8	2.5	0.54
16	73.6	21.0	1.2	0.70	73.6	21.2	1.4	0.60	53.8	16.2	5.4	0.70
22	74.5	21.0	1.2	1.00	73.3	20.8	1.5	0.95	68.9	20.6	1.9	0.85
30	67.0	21.8	1.8	0.30	63.5	21.7	2.5	0.30	56.2	22.0	2.8	0.25

Volumes are corrected for gas lost during composition measurement and converted into NTP-volumes.

Reactor group: LLDPE 90 day experiment												
Reactor	2.1				2.2				2.3			
Days passed	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)	CH_4 (%)	CO_2 (%)	O_2 (%)	V (l)
2	5.4	28.9	8.2	0.54	6.8	37.3	5.4	0.49	4.6	35.5	6.7	0.73
5	25.9	57.2	2.1	0.53	22.6	51.5	4.2	0.63	22.5	56.2	3.4	0.63
9	49.7	42.0	1.4	0.79	48.6	39.8	1.8	0.69	49.7	43.8	1.0	1.28
13	67.7	28.4	1.1	1.28	46.4	19.3	2.3	1.18	69.3	27.8	0.9	1.42
16	70.8	21.8	1.7	0.45	68.2	21.4	2.1	0.35	72.6	22.1	1.4	0.55
22	71.2	21.2	1.7	0.80	67.0	20.9	2.1	0.50	72.8	22.1	1.3	0.95
30	58.1	21.3	3.2	0.30	45.2	21.3	3.4	0.13	61.8	22.8	2.2	0.30
42	51.3	23.0	3.9	0.35	38.1	22.7	3.7	0.12	57.3	25.5	2.2	0.35
56	43.6	22.5	4.2	0.15	30.1	21.9	5.8	0.10	47.9	25.1	2.7	0.13
65	4.8	2.4	17.4	0.13	22.0	15.3	11.0	0.13	42.0	20.0	5.8	0.13
90	26.8	-	-	0.08	4.0	9.0	12.6	0.13	33.6	-	-	0.13

Volumes are corrected for gas lost during composition measurementand converted into NTP-volumes. - denotes failed measurement.

APPENDIX 2. AVERAGE pH VALUES AND STANDARD DEVIATIONS OF THE DIGESTATE AT THE END OF THE EXPERIMENTS

Reactor group	Background control	Paper	TPS	LLDPE
Experiment duration (d)	pH	pH	pH	pH
30	7.65 ± 0.04	7.80 ± 0.03	7.74 ± 0.02	7.72 ± 0.03
90	8.01 ± 0.08	7.93 ± 0.15	8,02 ± 0.10	7.88 ± 0.07