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MASTER THESIS

Critical issues in biodegradability of bioplastics

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ABBREVIATIONS AND SYMBOLS

ABS	Absorbance and/or Acrylonitrile butadiene-stirene
AD	Anaerobic Digestion
AE	Aquatic Environment
AFM	Atomic Force Microscope
ASTM	American Society for Testing and Materials
ATR	Attenuated Total Reflection Mode
BIOB	Biobased
BIODEG	Biodegradable
BOD	Biochemical Oxygen Demand
BP/BPs	Bioplastic/Bioplastics
BS	British Standards
CA	Cellulose acetate
CAB	Cellulose acetate butyrate
CAP	Cellulose acetate propionate
CENELEC	European Committee for Electrotechnical Standardization
CFU	Colony Forming Unit
$\mathbf{CH_4}$	Methane
CN	Cellulose nitrate
$\mathbf{CO_2}$	Carbon-dioxide
COD	Chemical Oxygen Demand
DAFNAE	Department of Agronomy Food Natural Resources Animals and Environment
DIN	Deutsche Institut für Normung
DNA	Deoxyribonucleic acid
DOC	Dissolved Organic Carbon
DS	Dissolved Solids
DSC	Differential Scanning Calorimetry
EN/CEN	European Committee de Normalisation
EoL	End of Life
ETSI	European Telecommunication Standards Institute
FT-IR	Fourier-transform infrared spectroscopy
FW	Fresh water
GCVs	Gross Calorific Values
GHG	Greenhouse gasses
HDPE	High density poly-ethylene
$\mathbf{H_2O}$	Water
IC	Inorganic Carbon
IEC	International Electronic Commission
ISO	International Organisation for Standardization
JIS	Japanese Industrial Standards committee
JPBA	Japan BioPlastic Association

K	potassium
LDPE	Low density poly-ethylene
MCL-PHA	medium chain length Polyhydroxyalcanoathes
MITI	Ministry of International Trade and Industry
MLVSS	Mixed liquor suspended solids
MP	Microplastic
N, $\mathbf{N_2}$	Nitrogen
$\mathbf{NO_2^-}$	Nitrite
$\mathbf{NO_3^-}$	Nitrate
NPOC	Non Purgeable Organic Carbon
NVS	Non-Volatile Solids
OECD	Organization for Economic Cooperation and Development
OFMSW	Organic Fraction of Municipal Solid Wastes
OPPTS	Office of Prevention, Pesticides and Toxic Substances
P	Phosphorous
P(3HB-CO-3HV-CO-3HB)	Poly(3-hydroxybutyrate-co-3- hydroxyvalerate-co-3-hydroxybutyrate)
P(3HB-CO-4HB)	Poly(3-hydroxybutyrate-co-4- hydroxybutyrate)
PA	Polyamide
PA 11	Amino-undecanoic acid-derived polyamide
PA 12	Laurolactam-derived polyamide
PA 6	Polyamide 6
PA 66	Polyamide 66
PART. BIOB	Partially-Biobased
PBA	Poly(butylene adipate)
PBAF	Poly(butylene adipate-co- furandicarboxylate)
PBAT	Poly(butylene adipate-co-terephthalate)
PBDF	Poly(butylene diglycolate -co- furandicarboxylate)
PBF	Poly(butylene furandicarboxylate)
PBFGA	Poly(butylene furandicarboxylate-co-glycolate)
PBS	Poly(butylene succinate)
PBSA	Poly(butylene succinate-adipate)
PBSC	Poly(butylene succinate-co-ε-caprolactone)
PBSE	Poly(butylene sebacate)
PBSET	Poly(butylene sebacate-co-terephtalate)
PBSF	Poly(butylene succinate-co- furandicarboxylate)
PBSGA	Poly(butylene succinate-co- glycolate)
PBSL	Poly(butylene succinate-adipate)
PBST	Poly(butylene succinate-co-terephthalate)
PBT	Poly(butylene terephthalate)
PCA	Plate Count Agar
PCL	Poly(e-caprolactone)
PDLA	Poly (D-lactic acid)
PE	Polyethylene

PEO	Polyethilene oxide
PES	Poly(ethylene succinate)
PET	Poly(ethylene terephthalate)
PGA	Polyglycolide
PGM	Poly(glycerol maleate)
PHA	Polyhydroxyalcanoathes
PHB	Poly(hydroxy butyrate)
PHBHH	Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)
PHBV	Poly(3-hydroxybutyrate-co- 3-hydroxyvalerate)
PHV	Polyhydroxyvalerate
PLA	Polylactic acid
PLGA	Poly(lactic-co-glycolic acid)
PLLA	Poly (L-lactic acid)
PLS	Poly(lactic acid-slat-salicylic acid)
PMPA	Poly(2-methyl-1,3-propanediyl adipate)
PMPG	Poly(2-methyl-1,3-propanediyl glycolate)
PP	Polypropylene
PPC	Poly(propylene carbonate)
PS	Polystyrene
PTMAT	Poly(methylene adipate-co-terephthalate)
PTT	Poly(trimethylene terephthalate)
PU	Polyurethane
PUR	Polyurethanes
PVA, PVAI, PVOH	Poly(vinyl alcohol)
PVC	Poly(vinil chloride)
RI	Respirometric Index
SBR	Stirene-butadiene rubber
SC	Sub-committees
scl-PHA	Small chain length Polyhydroxyalcanoathes
SE	Surface erosion
SEC	Size-exclusion chromatography
SI	Supernatant Inoculum
SOC	Soluble Organic Carbon
SS	Suspended Solids
SUP	Single Use Plastic
SW	Salt water
TC	Technical committees and/or Total Carbon
Tc	Crystallisation temperature
TDC	Total direct count
Tg	Glass transition temperature
Th$\mathbf{CO_2}$	Theoretical Carbon Dioxide
ThOD	Theoretical Oxygen Demand
TM	Test Medium
Tm	Melting temperature

TOC	Total Organic Carbon
TPS	Thermoplastic starch
TSS	Total Suspended Solids
UI	Unaltered inoculum
UNI	Ente Nazionale Italiano di Unificazione
UV	Ultraviolette
VS	Volatile Solids
WG	Working groups
WL	Weight loss
WOS	Web Of Science

ABSTRACT

In recent decades, concerns about the sustainability of plastic have emerged considering both the intense consumption of non-renewable resources for its production and the pollution generated in the environment due to its low degradability.

In this context, bioplastics have been introduced as environmentally friendly materials with properties similar to those of conventional plastics. But the question is, are bioplastics valid substitutes for conventional plastics, from an environmental point of view? Does bioplastics really biodegrade if left in the environment?

Several issues need to be solved when discussing about “biodegradable bioplastics”. The main problem is that there is not an unique definition of “biodegradability”, and among the existing ones there is none that sets standards to be reached in order to label a substance as biodegradable.

A scientific review made with the aim of clarifying the state of the discussion at the research level showed that, since there is not a clear definition of “biodegradability”, the researchers used so various test methods with different test durations, temperatures, test medium composition, tested material forms and sizes, to understand the biodegradability of the bioplastics. Different methodologies for the calculation of the degree of biodegradation have been used too. This prevents from any possible comparison of the biodegradability level.

In this not well-defined framework, it has been decided to carry out a set of biodegradation tests, following some indications of the International Standard Organization, in order to evaluate the biodegradability of PLA in aqueous environment by performing a 28-day BOD test.

1. INTRODUCTION

Plastics have led to a true revolution in the everyday life of man and have provided significant benefits for many applications in different industrial sectors; however, plastic has also developed into a significant environmental problem, due to the incredibly high amount of waste produced, to its low biodegradability in the environment and to the intense consumption of non renewable resources for its production.

Approximately 5.800 million tons of plastic have been discarded since 1950, the 79% of which have been landfilled or dispersed into the environment (Ritchie and Roser, 2018), causing a high presence of plastic pollution in every environmental compartment. Plastic litter has been found even in the most remote locations of the planet Earth, like the Poles, the Mount Everest and the Marianas Trench (Gwinnet et al., 2021a).

Most of the plastic waste is abandoned in the environment by humans, it reaches first, the waterways, such as rivers and canals, within cities which acts as “plastic highways", transporting trash from cities to the sea. Approximately 80% of marine plastic pollution derives from terrestrial litter (Canal & River Trust and Coventry University, 2019; GESAMP, 2019; Munari et al., 2021). When dispersed in the aquatic environment the plastic, if floating, can accumulate generating plastic islands; can be mechanically broken down into micro or nano plastics by the action of UV radiation, wind and waves; can be ingested by living organisms, causing their death, or bioaccumulating in the food chain; moreover, can became a disease vector (Shruti et al., 2019).

Another issue related to plastic is that it comes from oil, which is a non-renewable resource. Mentioning (Rujnić-Sokele et al., 2017), by 2050, the plastics industry will consume 20% of all oil, leading to a net depletion of this resource, if the current significant growth in plastics usage continues as predicted.

Despite this discouraging framework, however, polymeric materials are essential for many applications and indispensable in many sectors. It is possible to try to limit the problem of plastics by redesigning the entire supply chain of plastics, starting from a vision of sustainable design (eco-design), or by replacing it with other bio-derived materials. It is also possible to apply extended producer responsibility policies and eliminate single-use products in order to reduce the problem of marine litter.

Over the past decades, various forms of bioplastics have been created with the intention of partially replacing plastic products. Bioplastics are not just one single substance, they comprise of a whole family of materials with differing properties and applications. According to European Bioplastics association, a plastic material is defined as a bioplastic if it is either bio-based, biodegradable, or features both properties (European Bioplastics, 2021). Given the production growth trend expected for these biopolymers it would be better to solve some misconceptions not only related to the term “bioplastic” but also to the terms “biodegradable” and “compostable” (Harrison et al., 2018). The prefix “bio” doesn’t indicate that the polymer is biodegradable, their biodegradability doesn’t imply their compostability, but their compostability imply their biodegradability. Another issue is related to the fact that no existing standard defines “biodegradability” in general terms. In fact, some plastics are considered as “biodegradable” on the market, not referring to a specific standard which define the biodegradability in general terms, but according to standards established for composting or for anaerobic digestion processes (like ISO 18606:2018 or EN 13432:2000). For the aquatic environment, many different internationally recognized standards have been set to assess the capability of a plastics to be biodegraded, but these methodologies do not provide clear targets to be satisfied in order to label a bioplastic as biodegradable. Moreover, the different methodologies give great leeway regarding the ranges to be chosen for the environmental parameters (such as temperature, timeframe, inoculum concentration) and on the bioplastic shape and size.

All these considerations highlight the fact that bioplastics are biodegradable only under specific environmental conditions, which are difficult to be reached in a natural environment (like the aquatic ones). So, regrettably, if biodegradable bioplastics are discharged in an environment with a lack in the ideal elements for the occurrence of the biological degradation, their biodegradation may not occur or may occur only partially (EEA, 2020; Napper, et al., 2019; Nazareth, et al., 2019). In this situation, biodegradable bioplastics may produce fragments known as micro(bio)plastics, which do not address the problem of pollution caused by conventional microplastics because their fate and effects on biota are currently unknown (Emadian et al., 2017; Shruti et al., 2019; Napper et al., 2019).

In this thesis the author tried to understand what are the biodegradation rates of different bioplastics in the aquatic environment (considering both salt and fresh water) through:

- the collection of literature data obtained through a scientific review, with the aim of collecting information on the types of biodegradability tests performed by different research laboratories and of extrapolating the range of parameters that can be considered useful for defining the biodegradability of the different groups of bioplastics;
- the implementation of three biodegradation tests in fresh water, following the test method presented in BS EN ISO 14851: 2019, for testing the biodegradability of polylactic acid granules (being one of the most produced bioplastics) under laboratory conditions.

This report will continue with an introductive chapter (Chapter 2) on bioplastics (origins, description of different families, numbers and market data, description of the biodegradation process) and on the existing bioplastics certifications (compostable products and biobased products). At the end of this introductive part, Chapter 3 will present a discussion related to the meaning of the terms "biodegradability", "compostability" and "bioplastics" and an analysis of the current bioplastic waste treatments and related issues. After that, the Materials and Methods section (Chapter 4) will show the procedure followed for the achievement of the abovementioned aims of this thesis: the procedures followed for the scientific review and for the setting of the laboratory experiments will be reported. Chapter 5 will show the results obtained through the literature review and through the lab experiments, and a discussion of these is also presented. The thesis will end with the presentation of the conclusions and the future perspectives (Chapter 6).

2. BIOPLASTICS

2.1 The origins

The first bioplastic was created in 1856 from cellulose nitrate and camphor or galalithe, a biodegradable polymer derived from a mixture of formalin and casein, the milk protein. Then, the work of pioneers in chemurgy (the chemical and industrial use of organic raw materials) enabled Henry Ford to make plastics car parts derived from soybeans. As petroleum became a major source of chemicals and fuel in the early 1900s, the history of plastics underwent a significant transition. The first synthetic polymer-based plastics simply replaced the early bioplastics, such as polylactic acid (PLA), which was discovered around 1890. The manufacture of plastics significantly increased after World War II and has since continued to rise. Cellophane, a sheet material made from cellulose, is one well-known bioplastic that has endured the expansion of the synthetic plastics industry. It is still used in candy, cigarette, and other product packaging even though its production reached its peak in the 1960s (Rujnić-Sokele et al., 2017). In the 1970s the environmental movement stimulated a greater development of bioplastics materials and in 1983 the first bioplastics company was started, Marlborough Biopolymers, which used a battery-based bioplastic called BIOPAL. In 1989, Dr. Patrick R. Gruber further develop PLA, discovering how to make it from corn. One year later, the leading company in the bioplastics sector called Novamont was created. It produced a starch based bioplastic called Mater-Bi. Towards the end of the 90's the development of thermoplastic starch and BIOPLAST from the research and production of the BIOTEC company resulted in the BIOFLEX film. This has led to the production, with bioplastic material, of films, garbage bags, mulch sheets, hygiene products, diaper films, air bubble films, protective clothing, gloves, double rib bags, labels, barrier tapes; trays, flowerpots, freezer products and packaging, glasses, pharmaceutical packaging; injection molding, disposable cutlery, cans, containers, finished pieces, CD holders, cemetery items, toys, writing materials. In 2007 the market tested the first 100% biodegradable plastic called Mirel by Metabolix inc, produced by the fermentation of corn sugar and by genetically modified bacteria (Wikipedia: Bioplastics, 2022).

Nowadays, new bioplastic materials are constantly invented with the consequence of the growth in their production rate.

2.2. The different families

Bioplastics are not just one single substance, they comprise of a whole family of materials with differing properties and applications. According to European Bioplastics, a plastic material is defined as a bioplastic if it is either bio-based, biodegradable, or features both properties (European Bioplastics, 2021). The term 'bio-based' means that the material or product is derived from biomass (plants). Biomass used for bioplastics stems from e.g. corn, sugarcane, or cellulose. Biodegradation is a chemical process during which microorganisms that are available in the environment convert materials into natural substances such as water, carbon dioxide, and methane. The ability of a material to biodegrade is tied to its chemical structure rather than its resource base. In other words, whereas 100% of plastics made from biomaterials may not degrade, 100% of polymers made from fossil fuels can (European Bioplastics, 2021).

The family of bioplastics is divided into three main groups based on the origin of the raw material and on the environmental fate (Figure 2.2.1):

- Biobased and biodegradable, such as PLA, PHA and CA;
- Biobased and not biodegradable, such as biobased PE, PET, PA and PTT;
- Partially biobased and biodegradable;
- Partially biobased and not biodegradable;
- Fossil based and biodegradable such as PBAT, PCL and PGA.

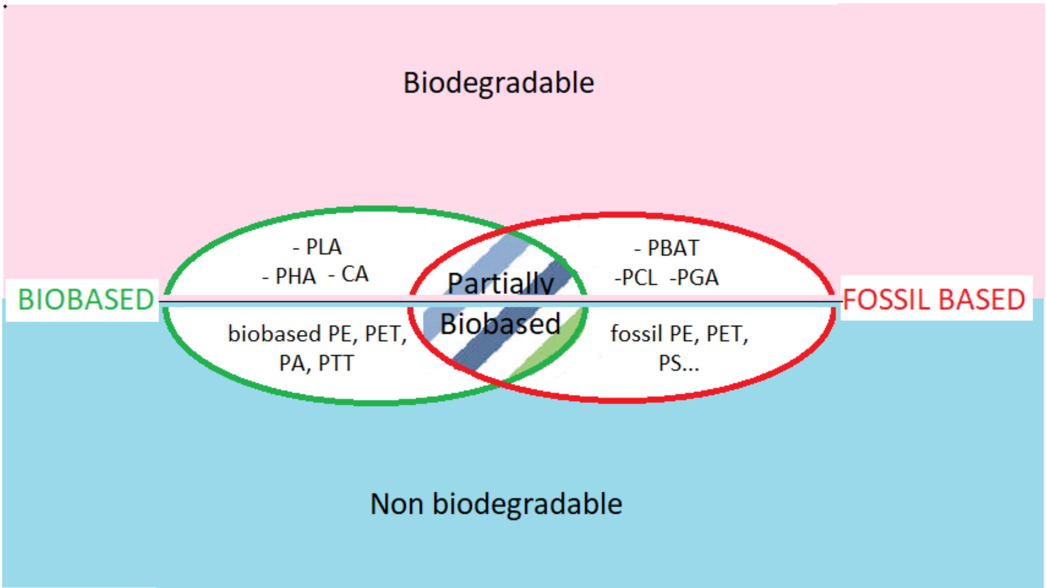


Figure 2.2.1: Graphic representation of the different groups of existing bioplastics (adapted from: European Bioplastics, 2021).

In Table 2.2.1 most of the existing bioplastics have been divided according to the category they belong to.

Table 2.2.1: Classification of biopolymers according on the origin of the raw material and environmental fate (Cescot Veneto and Università degli Studi di Padova, 2020).

	Biodegradable	**Non-Biodegradable**
Bio-based/Partially bio-based	*Bio-based*: CA, CAB, CAP, CN, PHB, PHBV, PLA, starch, chitosano. *Partially:* PBS, PBAT, PLA blends, starch blends.	*Bio-based*: PE (LDPE), PA 11, PA 12, PET, PTT. *Partially:* PBT, PET, PTT, PVC, SBR, ABS, PU, resine epossidiche.
Fossil based	PBS, PBSA, PBSL, PBST, PCL, PGA, PTMAT, PVOH	PE (LDPE, HDPE), PP, PS, PVC, ABS, PBT, PET, PA 6, PA 66, PU, resine epossidiche, gomma sintetica

2.2.1 Bio-Based and Non-biodegradable bioplastics

They are essentially a “bio-similar” copy of the conventional (fossil-based) plastics and are not biodegradable or compostable. They are most commonly produced from bioethanol. As the value-added chain only requires adaptation at the outset, while the properties of the products remain identical to their fossil versions, they are also referred to as ‘drop-in’ bioplastics or as ‘bio-blend’ and sometimes can contain starch or other biodegradable components in smaller amounts to only accelerate their fragmentation. However, once fragmented into microplastics, these bioplastics essentially have the same environmental impact as fossil-based plastics. Drop-in bioplastics include commodity plastics like biobased Polyethylene (PE), Propylene (PP), Polyvinyl chloride (PVC) and Polyethylene terephthalate (PET). Other non-biodegradable technical/performance polymers included in this category are bio-based polyamide (PA), polyesters like poly(trimethylene) terephthalate (PTT) and polybutylene terephthalate (PBT), polyurethanes (PUR) and polyepoxides typically used as textile fibres, whose operating life lasts several years but are clearly not biodegradable. Their use is most diverse. Some typical technical applications are textile fibres (seat covers, carpets), automotive applications like foams for seating, casings, cables, hoses, and covers. Drop-ins and conventional plastic differ in their price and environmental footprint. Drop-ins are more expensive due to lower processing capacity, lower investment in research and development and higher price of raw material compared to conventional plastics. The only conceivable advantage of drop-ins is their lower environmental footprint. Conventional plastic production introduces new carbon-dioxide (CO_2) but the CO_2 released during the manufacture of drop-ins could be captured by the plants providing the raw material, thus theoretically completing the cycle (Bhagwat et al., 2020).

2.2.2 Bio-Based and Biodegradable bioplastics

Bioplastics belonging to this category could be produced from plant biomass, microbial fermentation products and animal-derived polymers to emulate the life cycle of biomass producing CO_2 and water while conserving fossil resources. Plant-derived raw materials (which include vegetable oil, starch from wheat, rice, barley, oat and soy sources, fibres obtained from pineapple, jute, hemp, henequen leaves and banana stem, etc.) are used to extract thermoplastic starch, lignin, rubber, cellulose, etc. required for bioplastic production. Bioplastics derived from microbial sources are mainly polyesters [e.g. poly (3-hydroxybutyric acid)] which are storage polymers enzymatically produced by certain microbes to support their survival and growth when subjected to different nutrient and environmental stressors. Given the high production and recovery cost, the use of microbial fermentation for bioplastic production is still very limited. Animal-derived products such as chitin, silk, wool, casein, gelatine, gluten and fats are also be used in bioplastic production. Commercial bioplastics belonging to this category include Polylactic acid (PLA), Polyhydroxyalcanoathes (PHAs) and Starch-blends (Bhagwat et al., 2020).

2.2.2.1 Poly(lactide) (PLA)

Also known as polylactate, PLA (Figure 2.2.2) is a biodegradable thermoplastic polymer derived from natural lactic acid contained in corn, sugar cane or sugar beet. It is usually prepared by a polycondensation reaction of D- or L-lactic acid, also the ring opening polymerization reaction of lactide can be used to prepare PLA. It is a hydrophobic, semi-crystalline polymer with a glass transition temperature (Tg) of about 60,8°C. Above the Tg, the chains in the amorphous regions of the polymer become flexible, enhancing the degradation process. It resembles transparent polystyrene and it has a good aesthetic (characterized by gloss and transparency), brittleness and less thermal stability are the main drawbacks of PLA. It is plasticized to bring improvement in its chain mobility and enhance crystallization. Of the three possible isomeric forms, poly (L-lactic acid) and poly (D-lactic acid) are both semi-crystalline in nature, while poly (D, L-lactic acid) is amorphous (Waseem et al., 2021).

It's typical uses includes: plastic bags, plant pots, diapers, bottles, cold drink containers, plates, glasses, cutlery and film, packaging and paper coatings. With regard to PLA bottles are suitable only for still, non-sparkling water, because they do not guarantee the barrier effect and consequently are unable to retain CO_2. Sectors of application: electronics, medical products, building and construction, agricultural field, textiles and films (Cescot Veneto and Università degli Studi di Padova, 2020).

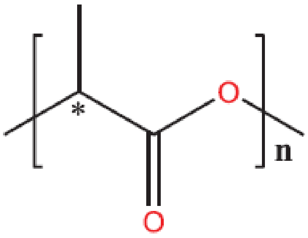


Figure 2.2.2: Chemical structure of PLA (Waseem et al., 2021).

2.2.2.2 Polyhydroxyalkanoates (PHAs)

PHAs are naturally occurring polymers. These substances are found as intracellular inclusions within the cytoplasm of many prokaryotic organisms. They are formed by the fermentation of sugars or lipids obtained from soybean, corn, or palm oil. There are about 250 strains of microbe that have the capacity to produce Polyhydroxyalkanoates. The most commonly used for the industrial production is *Cupriavidus necator* strain (Waseem et al., 2021). There have been claimed to be 150 different varieties of PHA polymers and their constituent hydroxyalkanoic acids. They are generally classified in two categories depending on the number of carbon atoms in their monomer units:

- small chain length (scl)-PHA when the monomer units contain from 3 to 5 carbon atoms;
- medium chain length (mcl)-PHA with monomer units possessing from 6 to14 carbon atoms (Kynadi and Suchithra, 2014).

The general structure of PHAs consists of 3-hydroxy fatty acids as shown in Figure 2.2.3.

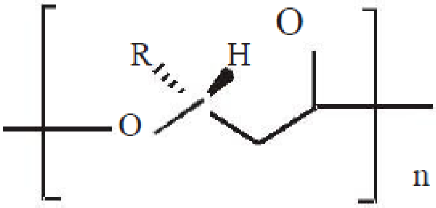


Figure 2.2.3: Chemical structure of Polyhydroxyalkanoates (Waseem et al., 2021).

The change in the pendant group R, which can range from methyl (C1) to tridecyl, causes the monomer to differ (C13). Table 2.2.2 displays the most popular polymers with their various R groups (Waseem et al., 2021).

Table 2.2.2: Different types of PHAs, according to their R group (Waseem et al., 2021).

R Group	***Full name***	***Short***
CH_3	Poly(3-hydroxybutyrate)	PHB
CH_2CH_3	Poly(3-hydroxyvalerate)	PHV
$CH_2CH_2CH_3$	Poly(3-hydroxyhexanoate)	PHHx

The most known PHA is poly(hydroxy butyrate) (PHB), shown in Figure 2.2.4. It has properties similar to those of PP, although it is more rigid and brittle (Waseem et al., 2021).

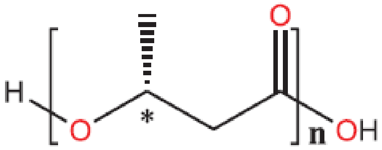


Figure 2.2.4: Chemical structure of poly(3-hydroxybutyrate), where n is the number of the 3-hydroxybutyrate units (Waseem et al., 2021).

Some of the mechanical properties (elasticity modulus, tensile strain, and tensile strength) of PHAs are quite comparable to that of bones. Thus, such types of PHAs are promising for use as implant materials. However, this intriguing biopolymer's main drawback is its high price, hence PLA or starch-based bioplastics are favoured (Kynadi and Suchithra, 2014).

The main uses of PHB are: products like bottles, bags, wrapping film and disposable nappies, as a material for tissue engineering scaffolds and for controlled drug release carriers. The main uses of PHBV are films and paper coatings; other possible markets include biomedical applications, therapeutic delivery of worm medicine for cattle, and sustained release systems for pharmaceutical drugs and insecticides (Kynadi and Suchithra, 2014; Cescot Veneto and Università degli Studi di Padova, 2020).

2.2.2.3 Starch-blends

Starch is an organic compound of the carbohydrate class, characterized by a large number of polymerized glucose units joined together by α-glycosidic bond and consisting of 4/5 of amylopectin (Figure 2.2.5a) and 1/5 of amylose (Figure 2.2.5b). Starch is the reserve carbohydrate of plants, stored as an energy source, synthesized enzymatically starting from glucose, in turn produced by chlorophyll photosynthesis. So, starch is fully biodegradable and abundant in nature. This raw material is cheap and can be worked using the conventional technologies used to working conventional plastics (Wikipedia: Starch, 2022).

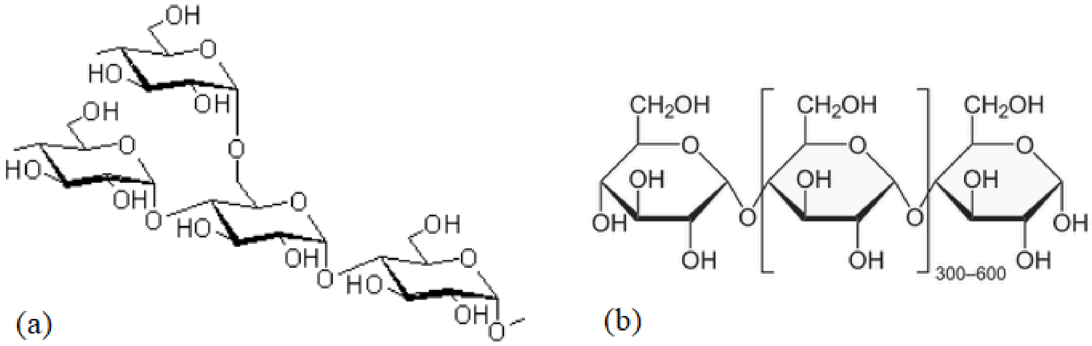


Figure 2.2.5: Chemical structure of amylopectin (a) and amylose (b) (Wikipedia: Starch, 2022).

Starch alone has low water resistance and is not very robust, so it is generally mixed with other biopolymers (that can be biobased, or fossil based) to improve its properties, so that, this family, is not composed by mixtures made up only of 100% biodegradable polymers (Waseem et al., 2021). The typical blends generally used and their principal uses are synthesized in the Table 2.2.3.

Table 2.2.3: Starch-blend types and relative applications (Cescot Veneto and Università degli Studi di Padova, 2020).

Starch-blend	**Applications**
Starch-PVA	Replacement for LDPE films in applications where barrier properties are not critical; water soluble laundry bags; biomedical and clinical fields; replacement for polystyrene foams as bulk filling material; and packaging material.
Starch-PLA	Food packaging; electronic devices; membranes (in the chemical and automotive industry); textile industry (fibers are used of PLA); medical applications; and packaging material.
Starch-PBS	Antimicrobial packaging material.
Starch-PHB	Biomaterial in medical applications.

2.2.3 Fossil-Based and Biodegradable bioplastics

Some fossil-based polymers used for bioplastic production inherently possess a certain amount of biodegradability. These polymers include aliphatic polyesters like polyglycolic acid (PGA), poly-caprolactone (PCL), polybutylene succinate (PBS), polyvinyl alcohol (PVOH) and aromatic polyesters like polybutylene terephthalate (PBAT). They are typically used in conjunction with starch or other bioplastics since the latter's performance for a given application is enhanced by the former's biodegradability and mechanical attributes. Currently, petrochemical manufacturing procedures are still used to create these biodegradable plastics. However, there are some variations of these materials that are partially bio-based (Bhagwat et al., 2020).

2.2.3.1 Poly(glycolide) (PGA)

PGA (Figure 2.2.6) is a simple linear aliphatic thermoplastic polyester. It is formed through the ring opening polymerization reaction of glycolide. This polymerization is catalyzed through different catalysts such as antimony trioxide, antimony trihalides, zinc lactate, and stannous octoate. However, the latter is one of the commonly used initiators because of its functioning as a food stabilizer too. These polymerizations are generally carried out in nitrogen atmosphere. PGA has a crystallinity of 45–55% and does not dissolve in water. PGA's high molecular mass forms are insoluble in solvents like acetone, dichloromethane and ethyl acetate. Its low molecular mass forms are more soluble. Its melting point is 220–225 °C, with 35–40 °C as its glass transition temperature. PGA displays outstanding mechanical properties, although, its low solubility and rapid degradation restricts its biomedical applications. Due to these limitations, glycolide copolymers containing caprolactone, trim ethylene carbonate, and lactide have been created for the manifacturing of medical devices. Its main uses are: specialized applications, controlled drug releases, implantable composites and bone fixation parts (Waseem et al., 2021).

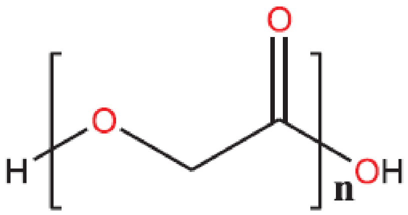


Figure 2.2.6: Chemical structure of PGA (Waseem et al., 2021).

2.2.3.2 Poly(ε-caprolactone) (PCL)

PCL (Figure 2.2.7) is prepared by ring opening polymerization reaction of ε-caprolactone in the presence of catalysts such as stannous octoate, titanium isopropoxide, dibutyltin dimethoxide, etc. It has a low melting point of around 60 °C. Its glass transition temperature is around −60 °C. Because of PCL's biocompatibility with different materials, it can be blended with starch for lowering cost and increasing biodegradability. In physiological conditions, PCL undergoes degradation by the hydrolysis of its ester bonds. It is an important polymer for its use as a long term implantable biomaterial. In fact, PCL was recognized as a biodegradable and non toxic material, and a promising candidate for controlled release applications, especially for long-term drug delivery. The main uses of PCL are: long-term items, mulch and other agricultural films, fibers containing herbicides to control aquatic weeds, seedling containers, slow release systems for drugs and plasticizer to poly(vinyl chloride) (PVC), (Waseem et al., 2021).

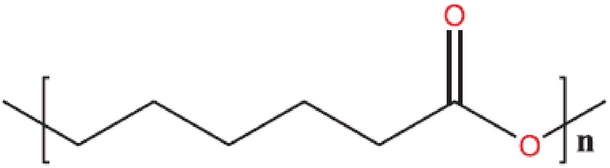


Figure 2.2.7: Chemical structure of PCL, where n is the number of units of ε-caprolactone (Waseem et al., 2021).

2.2.3.3 Poly(alkenedicarboxylate)s

Poly(alkenedicarboxylate)s are biodegradable aliphatic thermoplastic polymers. They are generally prepared by polycondensation reactions between glycols (ethylene glycol and 1,4-butanediol) and aliphatic dicarboxylic acids (succinic acid and adipic acid). The most known poly(alkenedicarboxylate)s are poly(butylene succinate) PBS and its compolymers, poly(ethylene succinate) (PES) and poly(butylene succinate-co-adipate) (PBSA), as shown in Figure 2.2.8.PBS belongs to the category of partially bio-based polymers, as it is composed of butylene glycol (which is bio-based) and succinic acid (which is fossil-derived). It is a thermoplastic material, with white crystalline appearance. Its melting point and glass transition temperature are ≈ 90 to 120 °C and −45 to −10 °C, respectively. The mechanical properties of PBS are equivalent to that of poly(ethylene) and poly(propylene). Its main properties are biodegradability, melt processability, thermal and chemical resistance. PBS is a promising polymer with a wide range of possible uses since it can be treated into melt blow, multifilament, monofilament, flat, and split yarn for textiles and injection moulded items. PBSA is a combination of 1,4-butane diol, succinic acid, and adipic acid. It is prepared by adding adipic acid to source materials during PBS synthesis. Although usually synthesized from fossil fuel, it can also be produced from bio-based feedstock. PBSA degrades faster than PBS. The latter has higher crystallinity and is better suited for molding, while the former has lower crystallinity and is better suited to film applications. PES has a melting point ranging from 103 to 106 °C (Waseem et al., 2021).

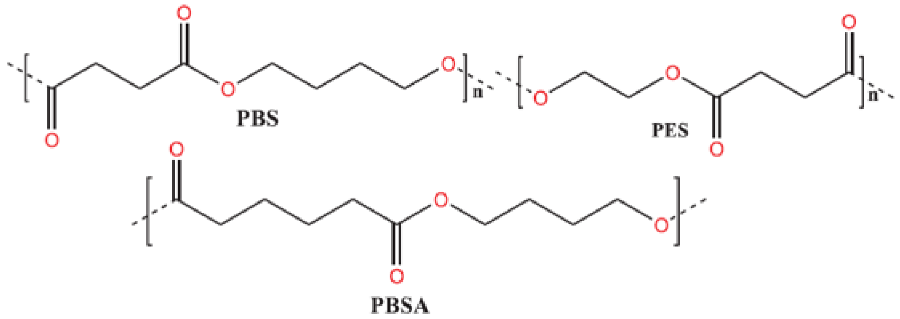


Figure 2.2.8: Chemical structure of PBS, PES and PBSA, where n is the number of units of butylene succinate, ethylene succinate and butylene succinate-co-adipate, respectively (Waseem et al., 2021).

2.2.3.4 Poly(butylene adipate-co-terephthalate) (PBAT)

PBAT, also known as polybutyrate (Figure 2.2.9), is a semi-aromatic thermoplastic copolyester that can be easily moulded and thermoformed. It is a biodegradable random copolyester of adipic acid, 1,4-butanediol and terephthalic acid. PBAT shows appreciable thermal and mechanical properties when terephtalic acid content is more than 35% mol in the copolymers. The biodegradation of PBAT decreases promptly when the concentration of terephtalic acid is higher than 55%. Due to its adaptability and toughness, it is utilized as a biodegradable substitute for low-density polyethylene (ethylene). It is considered as a good candidate for the toughening of Thermoplastic Starch (TPS). It finds uses in the production of biodegradable plastic bags, wraps and for agricultural films (Waseem et al., 2021).

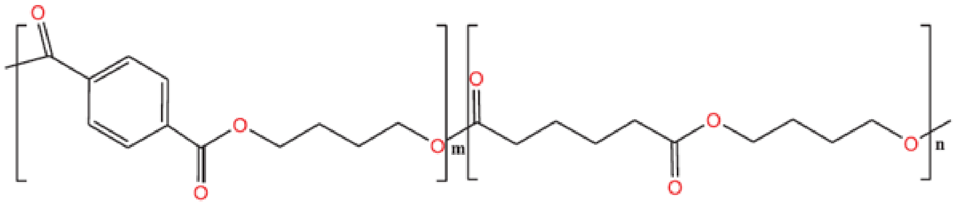


Figure 2.2.9: Chemical structure of PBAT, wherem and n are the number of polyester units of dimethyl terethalate and adipic acid, respectively (Waseem et al., 2021).

2.2.3.5 Poly(vinyl alcohol) (PVA, PVOH, or PVAI)

PVA (Figure 2.2.10) is a colourless, crystalline, odourless and water soluble synthetic polymer. It is synthesized by hydrolysis of poly(vinylacetate). The molecular weight and degree of hydrolysis, which determine the polymer's water solubility, are used to categorise it into different categories. It is non-toxic, resilient to oils, grease, and solvents. It is a ductile, strong and flexible polymer, and works as barrier to high oxygen levels and fragrance. PVA is a crucial component in many medical applications, including contact lenses, cartilage replacements, and eye drops due to its biocompatibility, low affinity for protein adhesion, and low toxicity. Its main uses are: packaging and bagging applications which dissolve in water to release products such as laundry detergent, pesticides, and hospital washables, in papermaking, textiles and several coatings (Waseem et al., 2021).

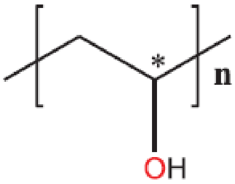


Figure 2.2.10: Chemical structure of PVA (Waseem et al., 2021).

2.3 Some numbers and market data

In 2020, the global bioplastics production capacity was equal to 2.08 million tonnes. It is estimated that the global bioplastics production capacity will increase up to approximately 7.60 million tonnes in 2026 (Figure 2.3.1). Hence, the share of bioplastics in global plastic production will bypass the threshold of two percent for the first time (European Bioplastics and nova-Institute, 2021).

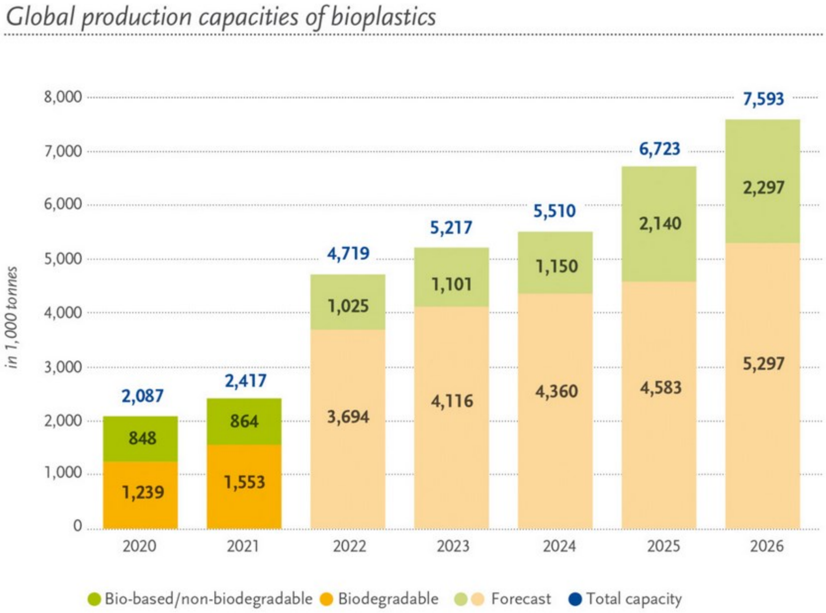


Figure 2.3.1: Global production capacity of bioplastics from 2020 to 2026 (European Bioplastics and nova-Institute, 2021).

PBAT, bio-based PBS, and bio-based PAs are the main drivers of this impressive growth. The production of polylactic acid will also continue to grow due to further investments in PLA production sites in Asia, in the US, and in Europe, but its market share will decrease. Instead, the market share of PHAs will increased in 2026. Production capacities of bio-based polyolefins, such as PP, will further grow as well, as can be seen from Figure 2.3.3, where a comparison of the global production capacity of bioplastics in 2021 and in 2026 is shown (European Bioplastics and nova-Institute, 2021).

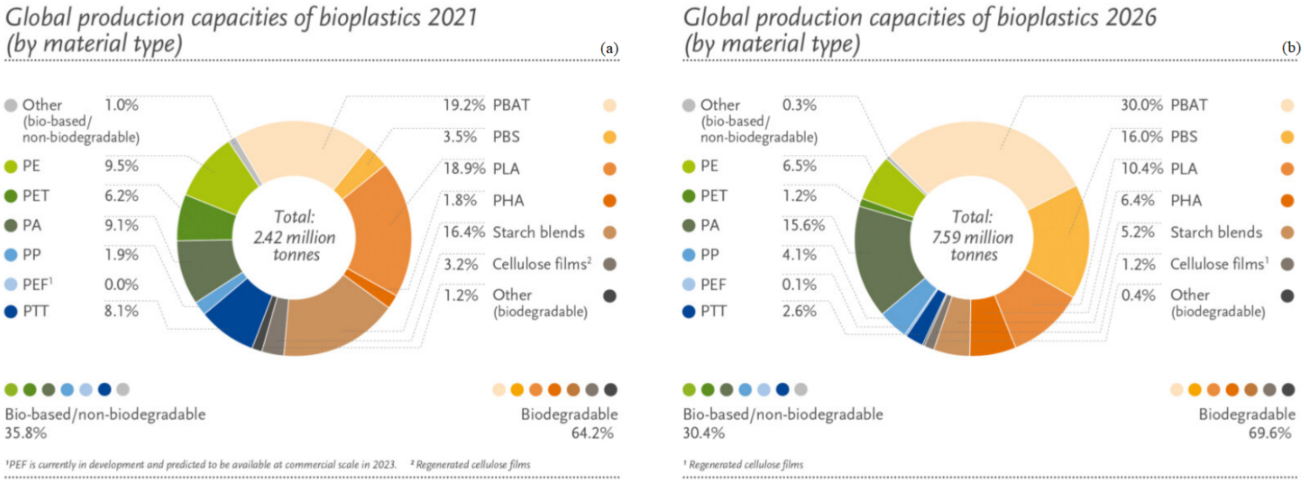


Figure 2.3.2: Global production capacities of bioplastics in 2021(a) and in 2026 (b) (European Bioplastics and nova-Institute, 2021).

The market sectors of application of bioplastics includes packaging, catering products, consumer electronics, automotive, agriculture/horticulture, toys, textiles in tissue engineering and medicine (Figure 2.3.3). Packaging remains the largest field of application for bioplastics, with almost 48 percent (1.15 million tonnes) of the total bioplastics market in 2021. However, the portfolio of application continues to diversify. Segments, such as automotive & transport or building & construction, significantly increased their share. Bio-based plastics do not only help to make cars lighter in order to save fuel, but they provide additional means to reduce carbon emissions and the impact on the environment (European Bioplastics and nova-Institute, 2021).

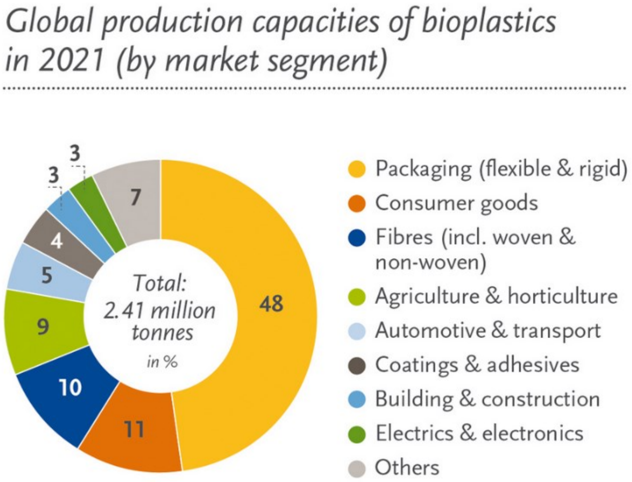


Figure 2.3.3: Global production capacities of bioplastics in 2021, by market segment (European Bioplastics, nova-Institute, 2021).

With a view to the regional capacity development, Asia further strengthened its position as major production hub with almost 50 percent of bioplastics currently being produced in that region. Presently, almost a fourth of the production capacity is still located in Europe. However, Europe's share and that of other world regions will significantly decrease within the next five years. In contrast, Asia is predicted to have passed the 70 percent by 2026 (Figure 2.3.4).

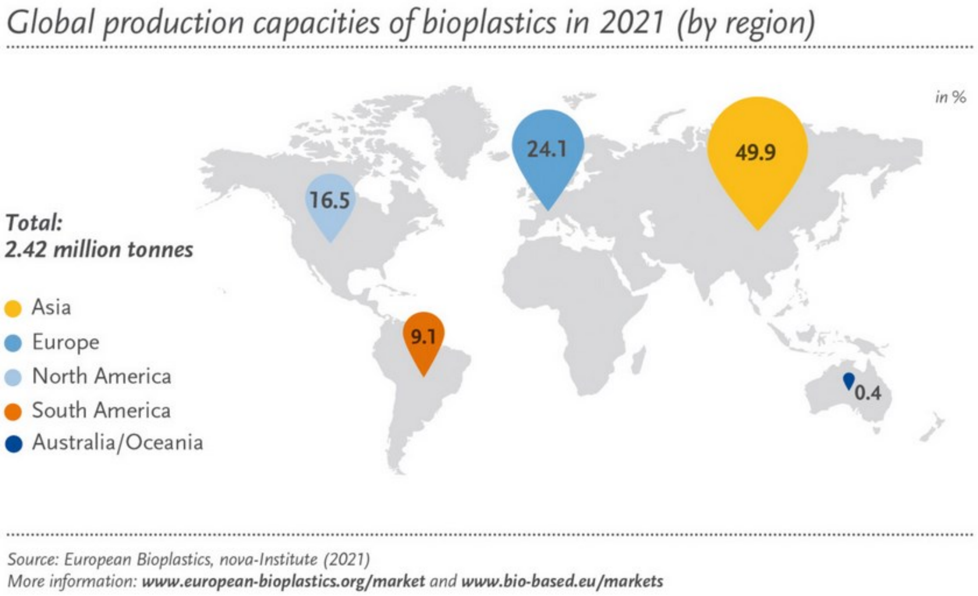


Figure 2.3.4: Global production capacities of bioplastic in 2021, by region (European Bioplastics and nova-Institute, 2021).

In 2021, the global production capacities of bioplastics required approximately 0.7 million hectares of land to grow the renewable feedstock, which account for just only over 0.01 per cent of the global agricultural area. Metaphorically speaking, this ratio correlates to the size of an average cherry tomato compared to the Eiffel Tower. Alongside the estimated significant growth of global bioplastics production in the next five years, the land use share for bioplastics will increase to below 0.06 percent (Figure 2.3.5). This clearly shows that there is no competition between the renewable feedstock for food, feed, and the production of bioplastics (European Bioplastics and nova-Institute, 2021).

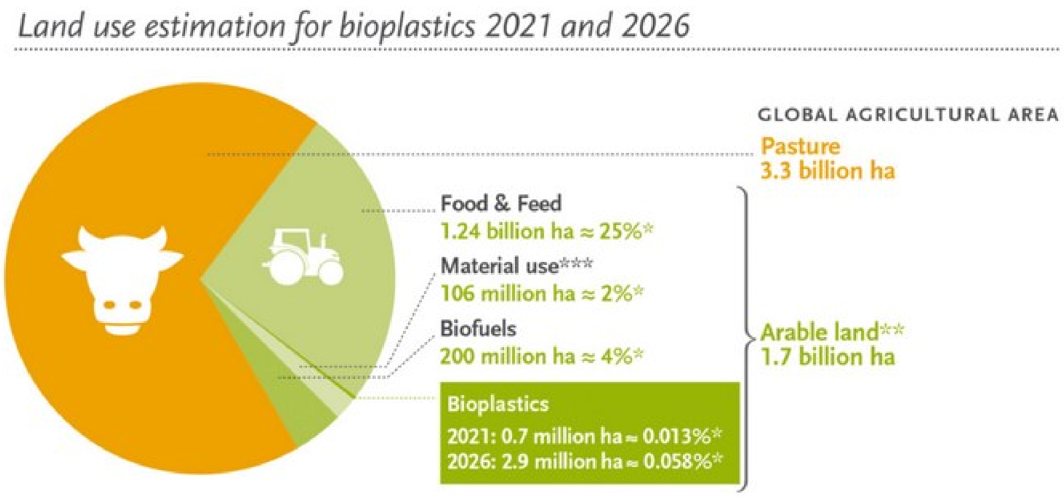


Figure 2.3.5: Land use estimation for bioplastics in 2021 and in 2026 (European Bioplastics and nova-Institute, 2021).

2.4 The biodegradation process

The polymer biodegradation process occurs in four sequential steps: 1) biodeterioration, 2) depolymerization, 3) bioassimilation, and 4) mineralization, as schematically represented in Figure 2.4.1. In the first step, the development of a microbial biofilm causes superficial degradation, which results in the disintegration of the polymeric material into smaller pieces. Extracellular enzymes, which are secreted by the biofilm's microorganisms, catalyse the polymer chain's depolymerization into oligomers, dimers, or monomers (step 2). Then, the small molecules produced are uptaked into the microbial cells and the primary and secondary metabolites are produced, through a process called bioassimilation (step 3). Finally, these metabolites are mineralized in the final stage, forming and releasing into the environment end products such CO_2, CH_4, H_2O, and N_2 (Haider et al., 2019).

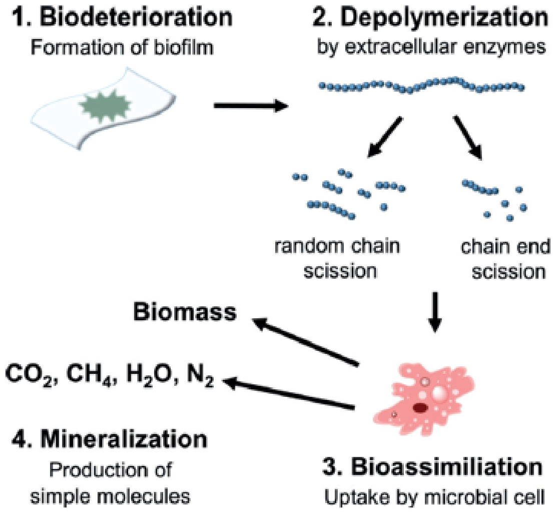


Figure 2.4.1: Schematic representation of the four steps involved in the biodegradation process (Haider et al., 2019).

The polymers biodegradability is dependent on both polymer properties, abiotic and biotic factors. The term “abiotic” describes parameters like mechanical stress, light or temperature, while the term “biotic” the involvement of naturally occurring microorganisms like bacteria, fungi, and algae (Haider et al., 2019).

The main polymer properties influencing the biodegradability are (Haider at al., 2019; Kale et al., 2015; Wierckx et al., 2018):

- Chemical structure and length of chain;
- Shape and size: larger surface area will promote degradation. For example, the rate of degradation of polymer foils increases with decreasing thickness of the foil.
- Crystallinity; enzymatic hydrolysis initially occurs in the amorphous regions, so the rate of enzymatic degradation decreases with increasing crystallinity.
- Surface chemistry; the surface chemistry and charge of the plastics would influence the microbial colonization and the biodegradation process.
- Complexity of the molecular formula; higher the complexity higher the difficulty with which microorganisms manage to break the polymer chain.
- Molecular weight; polymer with high molecular weight has a decreased degradability, due to a lower flexibility of the polymer and to greater glass transition temperature of the polymer.
- Glass transition temperature (Tg); for instance, for PLA, temperatures above its glass transition temperature (Tg=55–62 °C) are usually required for the onset of degradation, making the degradation process difficult at ambient temperatures.

Polymers with a shorter chain, a bigger amorphous part, and a less complex formula are more susceptible to biodegradation by microorganisms.

Speaking about the biotic and abiotic factors influencing the biodegradation, the temperature is a key environmental parameter, it influences biochemical reactions and taxonomic composition of microbial communities, controlling reproduction, growth and distribution of decomposing microorganisms. In compost and in soil, where higher temperatures than those find in water are available for the degradative reactions, the rate of biodegradation is higher. Additionally, the concentration and diversity of microbial communities is higher in soil and in compost rather than in water, with a faster rate of biodegradation in those media (Figure 2.4.2). For instance, $10^7 - 10^8$ colony-forming units per gram of material are reported for compost and only 10^6 in the soil. For seawater, an estimate based on direct bacteria counts numbers about 10^6 cells/mL. Microorganism diversity is crucial, but so is their capacity to break down various polymer substrates. Other factors impacting biodegradation of materials include salinity, humidity, oxygen presence, pH and UV radiation (Viera et al., 2021).

Undegraded plastics can linger in the environment for a very long period since, under natural circumstances, biodegradation is a very sluggish process that does not promote a quick entry into the degradation cycles of the living organisms. However, the probability for bioplastics of producing fragments of different sizes (meso, macro, micro, and nano) and forms in environments during deterioration is high, given that the fragmentation of bigger portions of bioplastics is unavoidable and a fundamental route for degradation (Haider et al., 2019; Kubowicz and Booth, 2017; Lambert and Wagner, 2016).

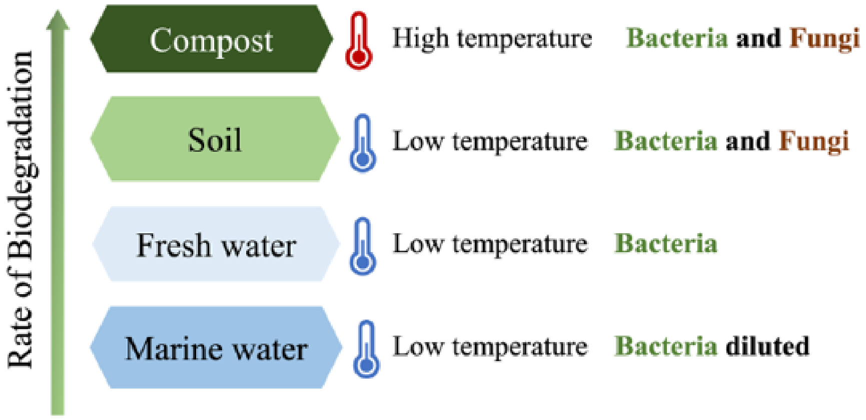


Figure 2.4.2: Rate of biodegradation in different environments (Viera et al., 2021).

The major pathway of degradation for polymers containing heteroatoms like esters, anhydrides, amides, or urethanes is hydrolysis Water and moisture are crucial for biodegradation since bacteria need them for development and reproduction. Moreover, polymer degradation in water environments starts with water uptake, followed by random cleavage of the ester bonds in the polymer chain. The hydrolysis of the material proceeds either via a bulk or surface erosion mechanism (Figure 2.4.3). Surface erosion refers to a reduction in surface thickness, whereas bulk erosion refers to degradation that happens uniformly over a polymeric object's thickness (Haider et al., 2019).

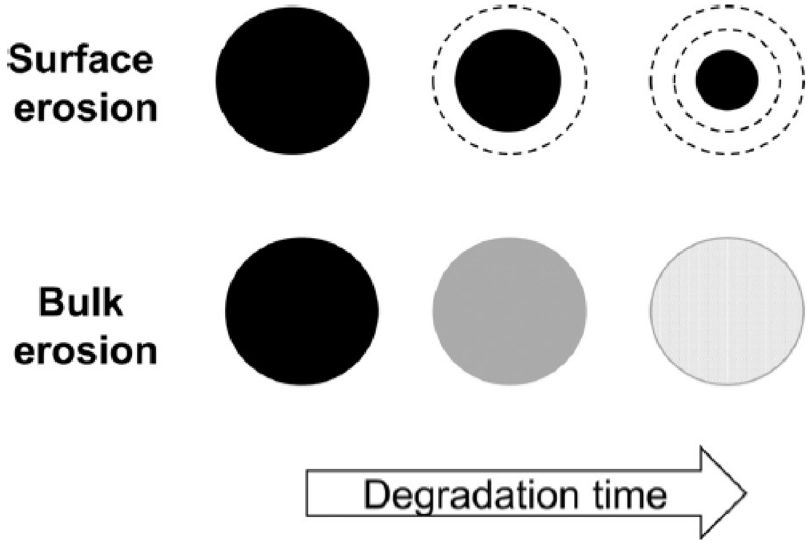


Figure 2.4.3: Graphic representation of surface and bulk erosion processes (Haider et al., 2019).

Surface erosion takes place when the rate of hydrolysis exceeds the rate of diffusion of water into the bulk, or when a catalyst (e.g. enzymes) cannot penetrate the bulk polymer. Surface erosion is the predominating mechanism for hydrophobic and semi-crystalline polymers. In contrast, bulk erosion occurs when the rate of diffusion of water exceeds the rate of the hydrolysis reaction. Temperature and pH levels are two external elements that have an impact on the hydrolysis rate. A rise in temperature accelerates the process. Enzymes are crucial in the catalysis of hydrolysis in natural environments. They mainly work at moderate temperatures and a neutral pH and they can increase reaction rates by 10^8 to 10^{20} times. Due to their relatively high molecular weights of several kDa, enzymes are unable to penetrate the polymer matrix and, as a result, enzymatic hydrolysis often happens via surface erosion (Haider et al., 2019).

The degradation process can be analytically observed by using different methods, which depict different stages in biodegradation: the polymer specimen's weight loss can be a sign of both full mineralization and the generation of water-soluble breakdown products; the CO_2 evolution or the oxygen consumption are a definite sign of the degree of mineralization that has occurred (Haider at al., 2019). By comparing the stable peaks of a Fourier-Transform Infrared Spectroscopy (FT-IR) spectrum it is possible to understand if the corresponding bonds have been broken or altered, observing a modification of these peaks; andthrough the Differential Scanning Calorimetry (DSC) analysis it is possible to observe whether the melting and glass transition temperatures of a polymer have changed, thus implying its degradation.

2.5 Certifications Bodies & Bioplastics certifications

Many certification bodies exist that are involved in creating and issuing standards that must be followed to certify a certain property of bioplastic. These standards are a set of rules that a product must comply with before it can obtain a certain label. A certificate is a proof that a product can biodegrade under the conditions outlined in the standard in the case of biodegradable polymer materials, while in the case of materials derived from renewable resources is a proof that the product contains a certain amount of renewable content (Horvat et al., 2012).

In this chapter, the main certification bodies are presented.

2.5.1 Certification Bodies

The most important Standardization Bodies in the World are presented in Table 2.5.1, with a specification of the geographical level at which they work, their acronym and their full name.

Table 2.5.1: Main global Standard Certification Bodies.

Level	Acronym of the Body	Description
International	ISO	International Organisation for Standardization
Intergovernmental international organization	OECD	Organization for Economic Cooperation and Development
European	CEN	European Committee de Normalisation
National (Italy)	UNI	Ente Nazionale Italiano di Unificazione
National (USA)	ASTM	American Society for Testing and Materials
National (Australia)	AS	Australian Standard
National (Germany)	DIN	Deutsche Institut für Normung (German Institute for Standardization)
National (French)	AFNOR	Association Française pour la Normalisation (French Standardization Association)
National (Japan)	JIS	Japanese Industrial Standards committee
National (England)	BSI	British Standards Institution

2.5.1.1 ISO

ISO is an independent, non-governmental international organization with a membership of 167 national standards bodies. It was born in London in 1947, now it is based in Geneva. It aims to promote standardization activities in the world to facilitate the exchange of goods and services.

Within it, the standardization work is carried out in technical committees (TC), which are divided into sub-committees (SC), which, in turn, are divided into working groups (WG) and study groups. The Plastic Committee TC/61 works in the standardization of aquatic tests and working group 22 of TC 61/SC 5 has the task of preparing the international standards for this environment (ISO website, 2022).

2.5.1.2 OECD

OECD is an intergovernmental international organization consisting of 38 member countries. It was born in 1948, it is based in Paris. It was formed to administer American and Canadian aid under the Marshall Plan for the reconstruction of Europe after World War II. The organization mainly plays the role of a consultative assembly which allows an opportunity to compare political experiences for the resolution of common problems, the identification of commercial practices and the coordination of local and international policies of the member countries. The OECD sets international standards and codes in collaboration with Member countries (OECD website, 2022).

2.5.1.3 CEN

CEN is an officially recognized standardization body within the European Union. It was born in 1961, it is based in Bruxelles. CEN's National Members are the National Standardization Bodies of the 27 European Union countries, United Kingdom, Republic of North Macedonia, Serbia and Turkey plus three countries of the European Free Trade Association (Iceland, Norway and Switzerland). It has the same internal working division of ISO. CEN provides a platform for the development of European Standards and other technical documents in relation to various kinds of products, materials, services and processes. CEN standards are compulsory for European Union countries and the standards are transferred to individual national standardization structures. This facilitates manufacturers’ entry to the European market once they comply with the standard requirements. CEN has an agreement for technical co-operation with the International Organization for Standardization, with the aim of preventing duplication of effort and of reducing time when preparing standards. As a result, new standards projects are jointly planned between CEN and ISO. Furthermore, European and American certification organizations both recognize each other’s standards in the field of polymers, plastics, and compostable products. The following technical committees work for the development of standards relating to plastics biodegradation: CEN/TC 249 WG 9 - Biobased and biodegradable plastics; CEN/TC 249 WG 24 - Environmental Aspects; CEN/TC 261 SC 4 WG 2 - Degradability and organic recovery of packaging and packaging materials (this group is in duty of EN13432); CEN/TC 411 Bio-based products WGs 1,3,4,5 (CEN website, 2022).

2.5.1.4 UNI

UNI is the national standard body of Italy. It was born in 1921, it has two offices, the principal in Milan and another one in Rome. UNI represents Italy in European and World standardization organizations (CEN and ISO, respectively) and organizes the participation of national delegations in supranational standardization work. When an ISO standard is issued, it is first implemented at an European level, becoming an EN/ISO standard and then, at a national level, becoming a UNI/EN/ISO standard. If a standard is developed directly at a national level (in Italy) it is marked as “UNI xxx”; if it is developed at an European level, the abbreviation is “UNI EN xxx”; if a standard is formulated internationally and then adopted in Italy, it is signed as “UNI ISO xxx” (UNI website, 2022).

2.5.1.5 ASTM

ASTM is the United Nations standardization body. It was born in 1898, it is based in Pennsylvania. ASTM International, is a globally recognized leader in the development and delivery of voluntary consensus standards. It has over 30,000 members, from over 140 countries. ASTM is one of the major technical contributors to ISO and it maintains a solid leadership in the definition of materials and test methods in nearly all industries, with a monopoly in the petroleum and petrochemical industry. The D20.96 Committee worked intensively on test methods for water-insoluble polymers and plastic materials (ASTM website, 2022).

2.5.1.6 AS

AS is the Australian national Standards bodies, it was born in 1922, it is based in Sydney. It has 73 members representing groups interested in the development and application of technical standards and related products and services. AS represent the Australia in the International Organization for Standardization. Standards Australia develops internationally aligned Australian standards and participates in standard-related activities (AS website, 2022).

2.5.1.7 DIN

DIN is recognized as the national standards body of Germany from 1975. It was born in 1917, it is based in Berlin. In 1951 it became a member of the International Organization for Standardization. More than 36,000 experts from industry, research, consumer protection and from the public sector bring their expertise to work on standardization projects managed by DIN. It works in the framework of the world organization of standards (ISO) and of the European one (CEN) and in the International Electronic Commission (IEC) and in the European Committee for Electrotechnical Standardization (CENELEC). It organizes the transposition of international standards for Germany. DIN Committees “Biodegradable Plastics” and “Degradable Packaging” reflect the activities at CEN and ISO level in the development of standards regarding the plastic degradability. The first standard in the field of composting and biodegradation of plastics was issued by DIN in 1997 (DIN V54900) and was later replaced by the European standard EN 13432 (DIN website, 2022).

2.5.1.8 AFNOR

AFNOR is the French national organization for standardization and it is an International Organization for Standardization member body. It was born in 1926, its head-office is La Plaine Saint-Denis. It counts 1480 members (AFNOR website, 2022).

2.5.1.9 JIS

The Japanese Industrial Standards Committee is the national standardization organization of Japan. It was born in 1949, its headquarters is in Tokyo. It is an ISO member. Since 1990, the government and the Ministry of International Trade and Industry (MITI) have tried to encourage the development of biodegradable plastics; the Japanese industries created a research group called "Biodegradable Plastics Society" to coordinate the work on the development of biodegradable polymers and the assessment of polymer biodegradability. The method based on the "Modified MITI Test" (OECD 302C-1981) is recognized as a test method for measuring the biodegradability of plastics. In Japan, GreenPla verifies biodegradable plastics using the ISO methods and its evaluation is based upon the pre-established criteria by Japan BioPlastic Association (JPBA), with a development of a “Green Pla” biodegradable plastics certification system (JIS website, 2022).

2.5.1.10 BSI

The British Standards Institution is a British standardization organization. It is a member of the International Organization for Standardization and of the European Telecommunication Standards Institute (ETSI). It was founded in 1901 in England as the first standardization body in the world. Its head office is in London. BSI is a global network, with 3 regional hubs with head offices in the UN, USA and Hong Kong and 76 offices around the world and has over 80,000 customers in 182 countries (BSI website, 2022).

2.5.2 Certification organizations for bioplastics

The two most significant certifying bodies for bioplastics in Europe are Vinçotte (Belgium) and DIN CERTCO (Germany). The first gives certificates for products created from biodegradable materials, based on four standards that are extremely similar one to another. In addition to this certification, Vinçotte also provides certifications for plastics that can be composted at home and for plastics that degrade in soil and water. Both organizations certify materials made of renewable resources based on the ASTM D6866 standard. Certificates for biodegradable products are also issued by the Biodegradable Products Institute (BPI) in the United States, the Japan BioPlastics Association in Japan as well as by other certification organizations (Horvat et al., 2012).

2.5.2.1 Certification of compostable products

Compostability is a characteristic of packaging or plastic products that enable them to decompose during the composting process. The standard EN 13432:2000 requires that a product or packaging can be considered compostable if it respects the following characteristics (Figure 2.5.1) (EN 13432:2000, 2000):

a. **Biodegradability**: within 6 months, the 90% of the material must be assimilated by microorganisms and then converted into CO_2; the actual metabolic conversion of the compostable material into carbon dioxide is measured through the standard test method EN ISO 14855:2012.

b. **Disintegrability**: within 3 months the mass of the material must consist of at least 90% of fragments smaller than 2 mm, i.e., less than 10% of the material must remain on a sieve with a 2 mm mesh (10 mesh). Disintegrability is measured with a pilot scale composting test (EN 14045:2003).

c. **Absence of negative effects on the composting process**: requirement verified with a composting test on a pilot scale.

d. **Low levels of heavy metals and absence of toxicity**: the compost obtained must be tested for its phyto-toxicity and its eco-toxicity.

The certificate, certification number and certification label can only be applied to a finished product.

Biodegradability. It is the ability of the compostable material to be converted into CO2, water and biomass under the action of microorganisms. A biodegradation level of at least 90% must be achieved in 6 months.

Disintegrability. That is the fragmentation and loss of visibility of the material in the final compost, so as to make it indistinguishable from the compost itself. Over 90% by weight of the tested material must be reduced to fragments of less than 2 mm within 3 months.

Absence of negative effects on the composting process.

Low levels of heavy metals and no negative effects on the final compost. The measurements are made both on the starting material (which must show levels of heavy metals lower than the prudential ones reported by the standard), and by means of germination and plant growth tests on the final compost, to highlight any ecotoxic effects.

Figure 2.5.1: Requirements of the EN 13432:2000 (Mater-bi, 2022).

The “Seedling” logo, developed by CEN CERTCO and owned by European Bioplastics, certifies that the material complies with the EN 13432 standard. Since April 2012, the Belgian certification organization, Vinçotte, has the right to assign the Seedling logo (Horvat et al., 2012).
In Table 2.5.2 and 2.5.3, the main certification organizations and their certificate labels for biodegradable plastics are presented.

Table 2.5.2 Labels attesting compostability (Horvat et al., 2012).

Country	**Organization**	**Standards**	**Certification label**
Germany	DIN CERTCO	EN 13432, ASTM D6400, ISO 17088, EN 14995	® compostable
Germany	DIN CERTCO	EN 13432, ASTM D6400, ISO 17088, EN 14995 and, if applicable, AS 4736	COMPOSTABLE EN 13432 DIN Geprüft
Germany	DIN CERTCO	AS 5810	HOME COMPOSTABLE AS 5810 DIN Geprüft

Belgium	Vinçotte	EN 13432, EN 14995	OK compost VINÇOTTE compostable
Belgium	Vinçotte	Special Vinçotte process based on EN 13432 at low temperatures	HOME OK compost VINÇOTTE
USA	BPI	ASTM D6400	COMPOSTABLE Biodegradable Products Institute | US COMPOSTING COUNCIL

Table 2.5.3: Labels attesting other biodegradability characteristics (Horvat et al., 2012).

Country	Organization	Standards	Certification label
Belgium	Vinçotte	Special Vinçotte process based on ISO 14851 or ISO 14852 (degradation in freshwater environment)	WATER OK bio-degradable VINÇOTTE
Belgium	Vinçotte	Special Vinçotte process based on ISO 17556 or ASTM D 5988 or ISO 11266 (degradation in soil)	SOIL OK bio-degradable VINÇOTTE
Belgium	Vinçotte	Special Vinçotte process based on ASTM D7081 (degradation in marine environment)	MARINE OK bio-degradable TÜV AUSTRIA MARINE

2.5.2.2 Certification of biobased products from renewable materials

A material can be classified as bio-based according to the carbon content (biobased carbon content) or to the biomass content (biobased mass content) of its products. The determination of the biobased carbon content is done through the measuring of the activity of the 14_C isotope. Materials made from fossil fuels and renewable resources are mostly made of carbon, which occurs in nature as the three isotopes: 12_C, 13_C, and 14_C. The 14_C isotope is unstable, it decays slowly and it is naturally present in all living organisms. The activity of 14_C in living organisms is very stable since it is related to the concentration of 14_C in the environment, which is constant. The organism ceases absorbing the 14_C isotope from the environment once it has died. The isotope's natural decay causes the concentration of 14_C to fall from that point on. Its half-life is 5.700 years. In the time span of a human life, this is hardly noticeable, but after 50.000 years, the 14_C content falls to an unmeasurable level. This indicates that there is a negligible 14_C concentration in fossil materials. Certification schemes, and resulting product labels, based on the method of 14_C isotope exist, such as the "OK Biobased" label, founded by Vinçotte and currently managed by TÜV AUSTRIA, based on the American standard ASTM D6866 (Standard test methods for determining the biobased content of solid, liquid, and gaseous samples using radiocarbon analysis*)*, which uses a star system to indicate the bio-based content of a certified product: the more stars the label carries, the higher the bio-based content. The DIN-Geprüft Biobased label, which was introduced in 2010 by the certification body DIN CERTCO, is another label based on the 14_C method. It features three quality levels that indicate a product's bio-based content and it is determined using the certification schemes ASTM D6866, UNI EN 17228 (Bio-based polymers, plastics, and plastics products - Terminology, characteristics and communication), or ISO 16620 (Platics - biobased content - Part 2: determination of biobased carbon content) (Cescot Veneto and Università degli Studi di Padova, 2020; Horvat et al., 2012). All these labels are presented in Table 2.5.4.

Table 2.5.4: Labels for plastics based on renewable resources (Horvat et al., 2012).

Country	Organization	Biobased Content	Certification label
Germany	DIN CERTCO: Additional requirement: volatile solids > 50 % (mass)	20 – 50 %	BIOBASED 20 - 50 % DIN Geprüft
		50 – 85 %	BIOBASED 50 - 85 % DIN Geprüft
		> 85 %	BIOBASED > 85 % DIN Geprüft
Belgium	Vinçotte	20 – 40 %	★ OK biobased VINÇOTTE

		40 – 60 %	★★ OK biobased ✓ VINÇOTTE
		60 – 80 %	★★★ OK biobased ✓ VINÇOTTE
		> 80 %	★★★★ OK biobased ✓ VINÇOTTE

3. BIOPLASTIC MANAGEMENT AND ITS CRITICISMS

Misconceptions about the terms "biodegradable", "compostable" and "bioplastic" have led to great confusion in understanding which end-of-life management option is the best for different types of bioplastics. In this chapter, the meaning of these three terms, the main End of Life (EoL) options and the criticisms related to bioplastics management are presented.

3.1 Differences between Biodegradability, Compostability and Bioplastic terms

Biodegradability, compostability and bioplastics have three different meanings. As mentioned in the previous chapter, it is not certain that a bioplastic is also biodegradable, nor that a biodegradable plastic is also biobased, nor that a bioplastic is also biobased. It is also not said that all bioplastics are compostable; their compostability implies biodegradability (following the EN 13432:2000), but their biodegradability does not necessarily imply their compostability.

Biodegradability means that a material is biodegraded, through the action of microorganisms and bacteria, into simpler chemical elements, aerobically or anaerobically (Harrison et al., 2018). Compostability refers to the ability of an organic material to biodegrade and to turn into compost, which is defined, following the (EN ISO 17088:2021, 2021), as an “organic soil conditioner obtained by biodegradation of a mixture consisting principally of vegetable residues, occasionally with other organic material and having a limited mineral content”. With the word “Bioplastic” it is indicated a wide family of polymers which may be: biobased and biodegradable, biobased and not biodegradable, partially biobased and biodegradable, partially biobased and not biodegradable, fossil based and biodegradable (European Bioplastics, 2022).

3.1.1 Biodegradability

There is no one single definition of "biodegradable", and among the existing ones, none establishes criteria that must be met in order to label a substance as “biodegradable”. The term “biodegradable” does not contain any information on the environmental conditions (temperature, presence of microorganisms etc.), timescale and extent of the decomposition process. Therefore, all materials are inherently biodegradable, whether it takes few weeks or million years to break down into water, carbon dioxide and methane. It can therefore be concluded that this term only makes sense if it is specified in which environment (temperature, presence of microorganisms, etc.) and within what time frame its degradation takes place (Harrison et al., 2018).

According to the standards ASTM D5488-94d and EN 13432:2000, “biodegradable” is a compound that decomposes into carbon dioxide (under aerobic degradation conditions), or methane and carbon dioxide (under anaerobic conditions), inorganic compounds and new cell biomass.

There are also definitions of biodegradable plastic/polymers, but they are all qualitative. Following the ASTM D833 “biodegradable plastic is a degradable plastic in which the degradation results from the action of naturally occurring micro-organisms such as bacteria, fungi, and algae”. According to the same standard “degradable plastic is a plastic designed to undergo a significant change in its chemical structure under specific environmental conditions resulting in a loss of properties as measured by standard test methods appropriate to the plastic and the application in a period of time that determines its classification”. The ASTM sub-committee D20.96 defined degradable plastics as “plastic materials that undergo bond scission in the backbone of a polymer through chemical, biological and/or physical forces in the environment at a rate which leads to fragmentation or disintegration of the plastics”.

Following (Narayan, 2009), “biodegradability is an end-of-life option that allows one to harness the power of microorganisms present in the selected environment to completely remove plastic products designed for biodegradability from the environmental compartment via the microbial food time chain in a timely, safe and efficacious manner”. All the different definitions of the terms “biodegradable” and “biodegradable polymers” are very similar, but none define the parameter levels to be verified in order to concretely know in which cases a plastic is biodegradable or not, unlike the case of compostability (EN 13432:2000, 2000).

Plastics are considered as “biodegradable” on the market, not referring to a specific standard which define the biodegradability in general terms, but according to norms established for composting or for anaerobic digestion processes (like ISO 18606:2018 and EN 13432:2000). In contrast, the standardization of plastics' biodegradability in soil, freshwater, and the ocean left a significant gap.

3.1.2 Compostability

Unlike the definition of “biodegradable”, the definition of “compostable” contains requirements to be verify in order for a plastic item to be defined as “compostable”.

In Europe a bioplastic can be labeled as “compostable” if it meets the requirements listed in the norm EN 13432:2000. The counterparts of this standard are ASTM D6400 and ASTM D6868 (US), AS 4736 (Australia), ISO 17088 and ISO 18606 (worldwide) (Rujnić-Sokele et al., 2017). According to the European standards EN 13432 –Packaging: requirements for packaging recoverable through composting and biodegradation and EN 14995 – Plastics - Evaluation of compostability - Test scheme and specifications, the requirements to be verify in order for a plastic item to be defined as “compostable” are (as already explained in the subchapter §2.5.2.1): biodegradability, disintegrability, absence of negative effects on the composting process, low levels of heavy metals and no toxicity on the final compost. The first criterion includes a definition of biodegradation, which however is only applicable when referring to composting. So, every time a product is labeled as "biodegradable" it always refers to an industrial composting environment, and not to biodegradability in other mediums such as home compost, soil, aquatic environment etc.

3.1.3 Bioplastics

The term "bioplastic" does not imply any information on the biodegradability or compostability of a product. With this term a wide family of materials with different properties and different applications is indicated. A bioplastic can be: biobased and biodegradable, biobased and not-biodegradable, partially biobased and biodegradable, partially biobased and not biodegradable, fossil-based and biodegradable. In addition, the term biobased does not indicate that a polymer is automatically compostable or biodegradable, it indicates only the biobased carbon content of the bioplastic (Cescot Veneto and Università degli Studi di Padova, 2020; European Bioplastics, 2022). The methodology used to determine this quantity is the measure of the content of the isotope 14 of carbon (14_C), as already mentioned in the sub-chapter §2.5.2.2.

Therefore, it can be concluded that a biobased bioplastic or a biodegradable bioplastic are not automatically biodegradable or compostable.

3.2 Biodegradable bioplastic waste management options

The choice of the bioplastics End of Life (EoL) depends on both polymer characteristics and on economic aspects. BPs can be recycled as conventional plastics (in a separate stream), but only if the BPs stream is enough large to be economically convenient to be recycled. If the properties of the bioplastic are suitable, it can be biologically recycled through composting and anaerobic digestion (AD). BPs can also be incinerated, thus recovering energy, but this waste management option is not so appreciated in the waste hierarchy (Figure 3.2.1a). As the last preferred option, consulting the waste hierarchy, bioplastic can be landfilled, thus losing the intrinsic value of the material. Thus, the main end-of-life options for biodegradable bioplastics include (Rujnić-Sokele et al., 2017), (Figure 3.2.1b):

3.2.1. Recycling (and reprocessing);
3.2.2. Biological waste treatments: anaerobic digestion and composting. To date, composting is the main management option of BPs (Zhu et al., 2020).

3.2.3. Incineration (and other the energy recovery options);

3.2.4. Landfilling.

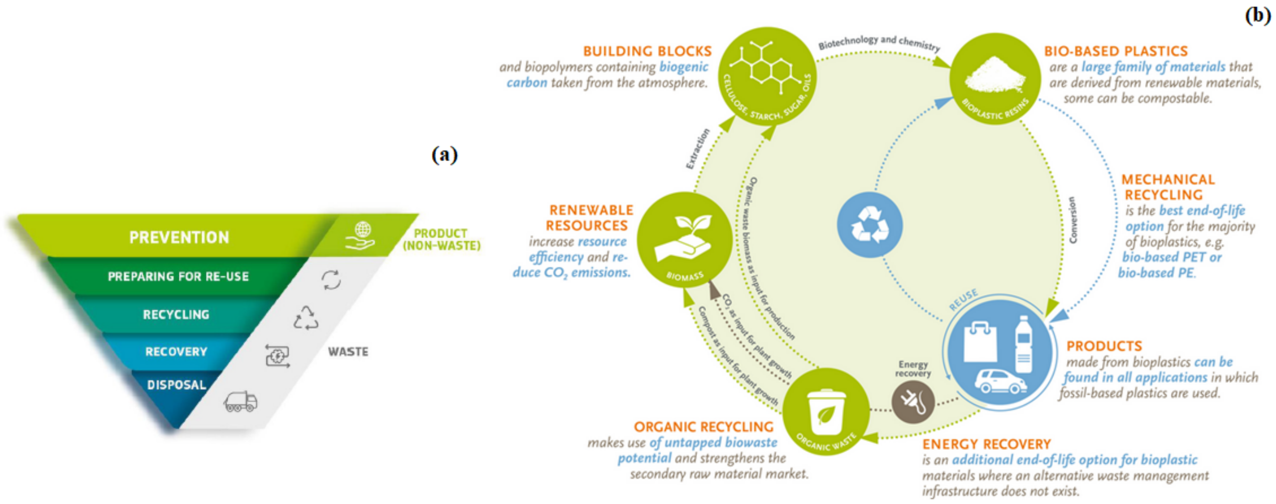


Figure 3.2.1: Graphic representation of (a) waste management hierarchy adopted in the EU (Fredi et al., 2021) and (b) waste management options for bioplastics (European Bioplastics, 2022).

3.2.1 Recycling

Since the biodegradable components of BPs could affect the reprocessed conventional plastic's qualities (such as strength, durability, etc.), the waste stream of biodegradable bioplastics must be separated from the waste stream of regular plastic. This problem is particularly relevant because biodegradable and conventional plastics have similar weights and densities, which prevent any easy mechanical separation and their distinguishing by the optical systems generally used for waste separation (La Mantia et al., 2013). Therefore, it may be preferable that biodegradable bioplastics are not collected with the conventional plastic stream but rather with the organic waste stream.

In Europe, separate collection of bioplastics with bio-waste, i.e. with the organic fraction of municipal solid wastes (OFMSW), was prescribed initially in 1994 and it is actually recommended (Cucina et al., 2021). However, there is a need for a stronger certification system and more precise handling guidelines for bioplastics. In fact, it was just recently brought up that bioplastic packaging had lower correct disposal rates than petroleum-derived packaging. This was most likely due to consumers' favourable opinions of the biodegradability of bioplastic packaging, which caused them to collect and dispose of these products less carefully (Cucina et al., 2021).

Another problem is that the recycling of bioplastic polymer waste is currently less financially advantageous than the recycling of conventional plastics due to the lack of a consistent and reliable source of bioplastic polymer waste in a significant quantity. Moreover, another problem is that it is feasible to mechanically recycle some bioplastic polymers, such as PLA, only few times before reaching a significant reduction in properties (Rujnić-Sokele et al., 2017).

Recent research indicates that whereas starch-based waste exhibits very poor recycling properties, Poly-L-lactic acid (PLLA) and aliphatic polyester can be recycled 5 to 10 times with a possible loss of thermal and mechanical properties (J. P. Lopez et al., 2012). Contrary to PLA, there aren't many investigations on the mechanical recycling of other biopolymers. PHA can be mechanically regenerated, however some of its molecular weight and mechanical properties will be lost (Sayantani et al. 2021).

(Piemonte et al., 2013) demonstrated that depolymerizing PLA by glucose fermentation, a chemical recycling of PLA, is energy efficient.

3.2.2 Biological waste treatments

There are two main options for the biological waste treatment of bioplastics: anaerobic digestion (AD) and composting.

3.2.2.1 Anaerobic digestion (AD)

Organic wastes are anaerobically broken down by AD into biogas and digestate through four successive phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Burning biogas in boilers or combined heat and power plants can turn it into an alternative energy source. Given its abundance in organic matter and plant macronutrients (N, P, and K), digestion waste is frequently used as a potential organic fertiliser (Cucina et al., 2021).

According to the data collected by (Cucina et al., 2021) during AD, it was recognized that PHAs blends degraded more quickly than other bioplastics. In fact, the estimated time for complete degradation of PHAs blends was found to be 31 ± 20 days under mesophilic conditions and 36 ± 28 days under thermophilic conditions. On the other hand, the degradation of PLA and starch-based blends was slower. In mesophilic and thermophilic conditions, PLA blends show to fully degrade in 423 ± 76 and 116 ± 48 days, respectively.

Temperature also plays a key role in starch-based blends degradation during AD. When AD was carried out under thermophilic conditions, these bioplastics demonstrated a considerable reduction in the amount of time needed to reach full breakdown (- 60%) (Cucina et al., 2021). Therefore, at the end, it was observed that PHAs blends degrade much faster that starch-based blends, which in turn degrade at an equal rate or faster than PLA. These differences can be related to the differences in chemical composition of the different bioplastics. PHA polymers and copolymers degrade more quickly since they are made directly from microbes. In contrast, there are few species that can break down the chemical structure of manufactured biopolyesters, such PLA. In order to maximise biogas production and waste volumes processed, AD of OFMSW is frequently carried out with short hydraulic retention periods (HRT), such as20–30 days). It is clear, from the data previously presented, that only PHA blends were acknowledged as being consistent with AD processes carried out with traditional HRT. In fact, for a biomass suited for AD, 80% of degradation (on a weight basis) is typically thought to be a target that should be obtained at the conclusion of the HRT, and PHAs blends are able to meet this requirement. Under mesophilic conditions, PLA and starch-based blends took roughly 10–15 times longer to totally degrade compared to a normal HRT of 30 days. The degradation kinetic improved as the thermophilic regime was adopted, however it appears that the goal of decomposing 80% (on a weight basis) of the bioplastics in the that HRT cannot be met. The high amount of bioplastics' residues in the digestate may also led to difficulties for its subsequent utilization (Cucina et al., 2021).

3.2.2.2 Composting

Composting is the accelerated breakdown of heterogeneous organic matter by a diverse microbial population under controlled circumstances in a wet, aerobic environment. The three main stages of the self-heating process of composting are (1) mesophilic (25–40 °C), (2) thermophilic (55–65 °C), and (3) maturation. Compost is a nutrient-rich organic amendment able to provide to soil N, P, K and organic matter. Prior to composting, suitable BP should be collected and separated through a separate collection scheme and transported to industrial composting facilities (Cucina et al., 2021).

According to the data reported by (Cucina et al., 2021), higher degradation kinetic constants were found under thermophilic composting conditions compared to those reported for AD. The estimated time for complete degradation of bioplastics was equal to 84 ± 47 days, 124 ± 83 days and 119 ± 43 days for PLA, PHAs and starch-based blends, respectively.

Several factors affect bioplastics degradation in composting environments, with temperature and bioplastics chemical composition being the most important. The high temperatures of the active phase of composting (>55 ◦C) allow the reaching of the glass transition temperature of the most common bioplastics, leading to the passage from the the crystalline status to the amorphous one, and therefore increasing polymer hydrophilicity, leading to higher hydrolyzation and enhancing the kinetic of bioplastics degradation during composting (Cucina et al., 2021). The standard composting of

OFMSW can successfully break down bioplastics; in fact, composting takes longer time than AD and typically lasts for around 90 days.

One issue of composting regards the fact that many composting facilities only handle garden waste and are not designed to process compostable packaging. To ensure an effective packaging compostable process, these facilities would need to undergo numerous technical modifications, especially at the level of pre-processing (Rujni-Sokele et al., 2017).

However, all the previous considerations are related to industrial composting only, because, during home composting temperature peaks do not usually exceed 35–40 °C and this could lead to issues such as bioplastics accumulation in the compost produced in this way (Guidoni et al., 2018).

3.2.3 Incineration

The majority of common plastics have Gross Calorific Values (GCVs) that are equal or higher than the one of coal. Thus, incinerating (with energy recovery) may be a wise choice. Incineration of petrochemical carbon, which has already been utilised for a high-value purpose, is a more environmentally friendly alternative than burning oil straight up (Song et al., 2009). In 2021 the 42% of the plastic waste collected in the EU27+3 were used for energy recovery (PlasticsEurope, 2021). Energy recovery by incineration is considered as a suitable option for all bioplastic polymers and renewable (bio)resources in bioplastic polymer products are considered to contribute with renewable energy production when incinerated. Natural cellulose fibre and starch have a lower GCV than coal, but are similar to wood and thus still have considerable value for incineration (Song et al., 2009). However, although theoretically feasible, energy recovery by incineration may negate many of the advantages of the material's potential for biodegradability (Mudgal et al., 2012).

3.2.4 Landfill

Landfilling of plastic waste is the least favoured option in the waste hierarchy. The landfilling of biodegradable materials, including biodegradable polymers, garden and kitchen waste, presents the problem consisting in the production , under anaerobic conditions, of methane, a greenhouse gas with 25 times the global warming effect of CO_2. Landfill gas is mostly captured (this is mandatory in Europe under the Landfill Directive) (Council Directive 1999/31/EC, 1999) and used as an energy source. However, thousands of illegal dumps and many landfills do not yet have any sort of gas collection system. The inclusion of biodegradable packaging could then result in the emission of more greenhouse gases for these facilities' operating outside of the Landfill Directive's standard (Mudgal et al., 2012; Song et al., 2009).

3.3 Bioplastics: Pros & Cons

Bioplastic has been presented as a valid alternative to conventional plastic, but is this true?
The main advantages of BPs are:

- Using of rrenewable resources for their production. This permits to conserve non-renewable fossil raw materials and to reduce the release of GHG during this stage (Anjoran, 2020).
- Using of agricultural and forestry wastes for biobased bioplastic production (Chen, 2013).
- Production of alternative products not deriving form petroleum oil. These latter, increases tremendously the concentration of GHG in the atmosphere and their stock will end in the near future (Chen, 2013).
- Using of less energy input for the BPs production than those used for the conventional plastics. The fossil fuel-based energy required to produce bioplastics, 44 MJ/kg of bio-resin, is much lower than the one used to manufacture petrochemical-based plastics, which is 78–88 MJ/kg of resin (Nanda et al., 2022).
- A potentially much lower carbon footprint. A plastic made from a biological source sequesters the CO_2 captured by the plant in the photosynthesis process. If the resulting bioplastic degrades back into CO_2 and water, this sequestration is reversed. But a durable bioplastic, made to be similar to polyethylene or other conventional plastics, stores the CO_2 and even if the plastic is recycled many times, the CO_2 initially taken from the atmosphere remains sequestered (Chen, 2013).
- Reduction in litter and improved compostability. The best advantage of biodegradable bioplastics lies in the reduction of permanent litter. Single Use Plastics (SUPs) shopping bags are the most obvious example of how plastics can pollute the environment, with a very long persistency. A large fraction of the litter in our oceans is made of disposable plastic bags. Cities and countries around the world are acting against the litter, sometimes by banning non-degradable plastic bags entirely (at the European level, through the SUP Directive, Directive EU 2019/904).
- Eco-safety. Bioplastic doesn’t contain any toxins (Anjoran, 2020).
- Energy crops as non-food incentives for farmers in industrialised countries with surplus food production.
- Positive effects on the market and the economy. The large-scale production of bioplastics can positively affect the global market and the bioeconomy along with addressing several problems related to environmental pollution (Arikan et al., 2015).

The main disadvantages of bioplastics are:

- The actual constituents of the bioplastics may themselves have adverse environmental impacts. For example, PLA is derived from corn starch which emits methane, a much more potent greenhouse gas than carbon dioxide, as it decomposes. Production of PHA requires the use of harsh chemicals like pyridine and diethyl ether which have potential occupational hazards (Lackner, 2015).
- Marine pollution. If dispersed in water environment, due to the low presence of microorganisms able to degrade bioplastics and due to the low temperature presents in the water bodies, bioplastics tend to be fragmented, due to physical forces, into micro or nano bioplastics, as happens to conventional plastics. Microplastics can be ingested by the fauna or can became a disease vector. In addition, BPs can be more easily decomposed into microplastics that conventional plastics and may be more easily used as carriers of microorganisms. As plastic debris migrates, microorganisms may spread into other ecosystems, affecting the local microbial community (Zhu et al., 2020).
- Biodegradability issues. BPs include both partially-biodegradable and fully biodegradable polymers. Partially-biobased bioplastics are generally a blend of bio-based and fossil-based, and the latter do not biodegrade. The fully biodegradable plastics are only theoretically completely degradable under specific conditions, and their degradation in the natural environment is still full of uncertainty (Lackner, 2015).
- End-of-life management options. BPs may not be fully transformed into carbon dioxide, water, and biomass. The most robust evidences are recent studies published by (Napper and Thompson, 2019) and (Nazareth et al., 2019). In particular, (Napper and Thompson, 2019) showed that BPs would not degrade rapidly in the ocean, soil, and open-air, and maintains its function in the environment for more than 3 years.
- Conventional plastic recycling stream contamination. The lack of bioplastic-specific recycling facilities increases the risk of degradation products getting collected through existing recovery systems for conventional plastics and contamination of the recycling stream itself. As a result, the mechanical properties of conventional recycled plastics will change significantly, and the quality of the recycled material will be lowered (Zhu et al., 2020).
- Lack of appropriate composting facilities. Sometimes the lack of composting facility suitable for receiving compostable BPs makes the 'green' bioplastics ending up in landfills, where they do not break down much easier than regular non-biodegradable plastics (Anjoran, 2020).
- Landfilling problems. The specific conditions required for the biodegradation of bioplastics such as temperature, pH and humidity are not necessarily achieved in landfills. Therefore,

direct landfilling of biodegradable polymers might not be feasible. Moreover, the installation of specific sites for the disposal of bioplastics requires land space, a controlled environment, regular monitoring, facilitated microbial proliferation, inoculation of specific polymer-degrading microorganisms along with their nutrient supply, routine mining of intermediates and by-products as well as capital cost (Arikan et al., 2015).

- Competition with food. The use of biobased feedstock leads to the use of fields, which are normally used to grow food, for the cultivation of vegetable raw materials to make bioplastics. According to the production of 0.18 ha of arable land per ton of BPs, if the packaging plastic that accounts for 40% of total European plastic demand is replaced by BPs, the area of arable land needed for its production is larger than the land area of Belgium (Napper and Thompson, 2019).
- The supply of raw materials for bioplastics depends on natural phenomena. Crop-based bioplastics require fertile land, water, fertilizers, and good weather conditions (Lackner, 2015).
- Intensified farming, extensive use of fertilizers, deforestation and grassland conversion causes drastic increases of greenhouse gas emissions (Lackner, 2015).
- Use of transgenic plants and genetically modified bacteria (Rujnić-Sokele et al., 2017).
- Competition with conventional plastics. Bioplastics must compete with the existing petrochemical-based conventional plastic manufactures in terms of low production cost, bulk manufacturing, compatibility with existing infrastructures, waste management, effective end-of-life remediation, consumer awareness, waste sorting at the consumer level and several other factors. Bioplastics are generally not cost-competitive compared to their oil-based counterparts. They are generally two or three times more expensive than the major conventional plastics such as PE or PET (Anjoran. R., 2020). Moreover, generally bioplastics have a lower mechanical strength respect to conventional plastics.
- Standardization problems. Plastics are considered as "biodegradable" on the market, not referring to a specific standard which define the biodegradability in general terms, but according to norms established for composting or for anaerobic digestion processes (like ISO 18606:2018 and EN 13432:2000). In contrast, the standardization of plastics' biodegradability in soil, freshwater, and the ocean is still missing (Zhu et al., 2020).
- Lack of legislation. An important increase in the production and consumption of bioplastics is expected but to date many countries have no legislations about their production, usage or waste management (Arikan et al., 2015).

- Bioplastics may encourage people to litter more. Improper use of green marketing is a deception for consumers and may lead to improper disposal of these materials (Zhu et al., 2020).
- BPs may become greenwashing in the process of marketing. The greenwashing on BPs will seriously affect the consumption of biodegradable products and can lead to the improper disposal of these materials in the environment.
- Misunderstanding of terms. The description of bioplastic as compostable can be confusing. All bioplastics are not compostable at home like organic food waste but usually require an industrial composting treatment (Arikan et al., 2015).

4. MATERIALS AND METHODS

The activity timeline that has been followed during the preparation of this thesis is presented in Figure 4.1.

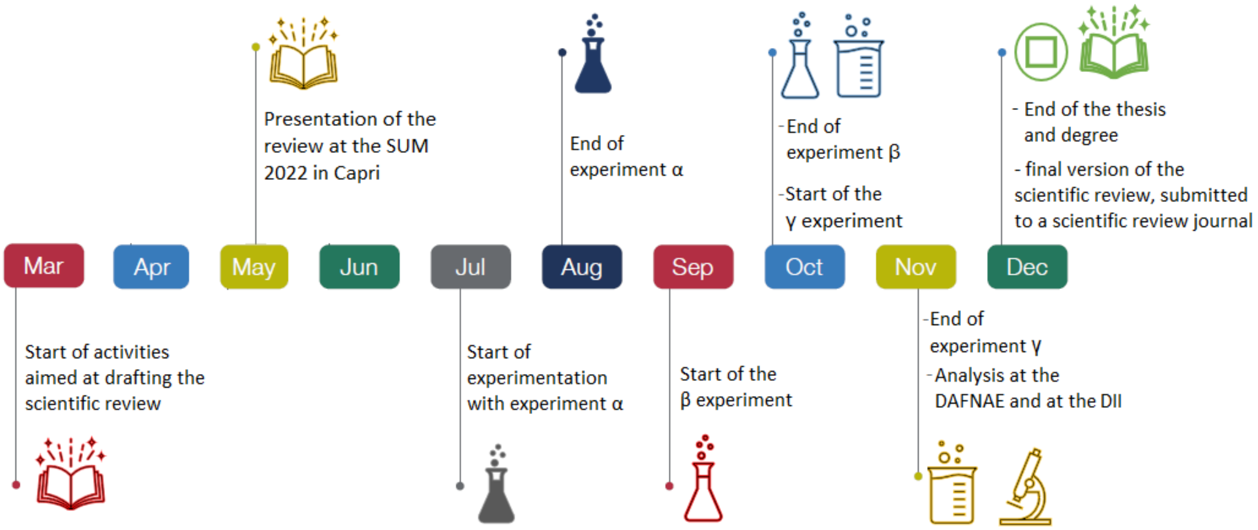


Figure 4.1: Timeline that includes all the activities carried out during the thesis.

The activities done during this year can be summarized into two main macro-topics: the development of a literature review on the biodegradability of bioplastics and the implementation of lab experiments in order to verify the biodegradability of PLA pellets. The materials and methods used for the development of these two activities are presented in the following chapters §4.1 and §4.2.

4.1 Scientific review

With the aim of collecting information on the types of biodegradability tests performed by different research laboratories and of extrapolating range of parameters that can be considered useful in order to define the biodegradability of the different groups of bioplastics in aquatic environment (fresh and salt water) unambiguously, the articles obtained from the available scientific literature on the topic of our interest have been analysed and discussed, also highlighting the knowledge gaps that should be remediated to better address the research.

The literature review has been done using Scopus and Web Of Science (WOS) as databases, using the following construction for the three keywords strings formulation:

$$BP+BIODEG+AE \qquad (4.1.1)$$

Where:

BP= “bioplastics” or its synonyms. Subsequently, for more specific research, the name of the most used biopolymers (PLA, PHA and PHB and PHBV, PBS, PES, PBSA, PGA, PCL, PBAT, PVA, TPS and cellulose) has been used.

BIODEG= “biodegradable” or its synonyms.

AE= “aquatic environment” which can be salt- or fresh-water. Synonyms have been also used such as “fresh water”, “river”, “lake”, “eutrophic reservoirs”, “sea”, “marine environment”, “salt water” and “ocean”.

Twenty-four strings have been used: twelve for fresh water and twelve for salt water. The research was conducted on title, abstract and keywords. All the scientific review papers found were automatically removed from the selected articles. In this scientific review only research papers were accepted. This search resulted in a total of 527 articles for fresh water and 993 articles for salt water. Subsequently these articles have been subjected to two consecutive screenings. The first screening was performed in order to exclude or accept an article in the review, according to its consistency to the research aim, through the interpretation only of the title and the abstract. The articles passing the first screening have been 87 for freshwater and 222 for saltwater. The reasons of exclusion, divided by fresh and salt water, used in the first screening are presented in Figure 4.1.1.

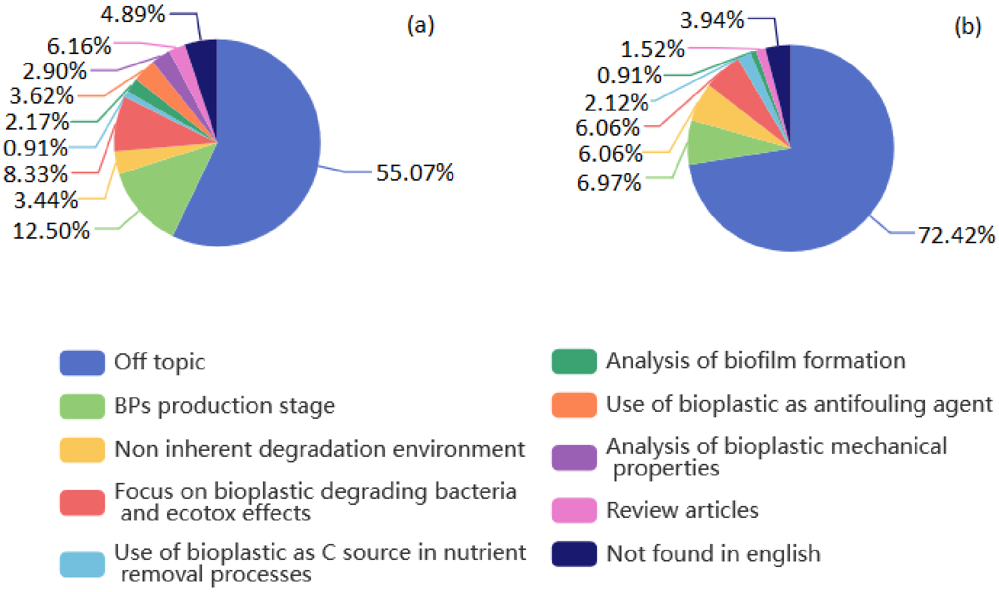


Figure 4.1.1: Reasons of exclusion used in the first screening for saltwater (a) and freshwater (b).

The reasons of exclusion are nearly the same in freshwater and saltwater, the only difference is the percentages of their occurrence. The two principal reasons of exclusion regard the presence of off topic articles and the presence of articles focusing on the production of bioplastics, instead of their degradation.

The article passing the first screening have been therefore analysed through the second screening, which consisted in the reading of the whole article texts and accepting an article in the review only if consistent with the aims of the search. At the end of this phase 25 articles were accepted for the freshwater and 67 articles for the saltwater.

The reasons for exclusion, divided by fresh and salt water, used in the second screening are presented in Figure 4.1.2.

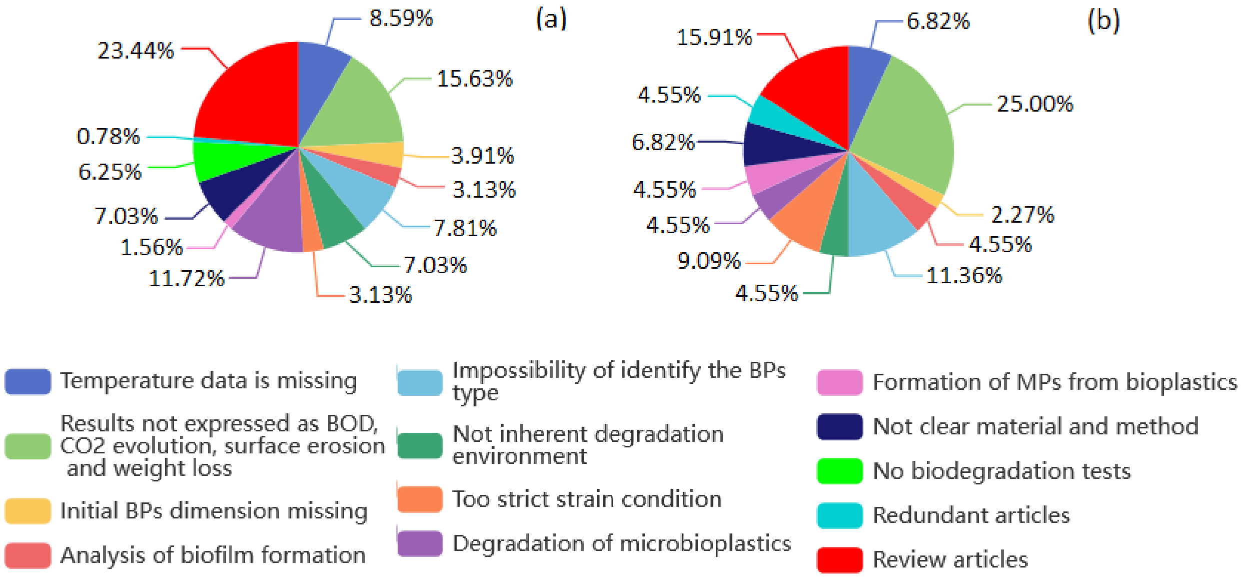


Figure 4.1.2: Reasons for exclusion used in the second screening for saltwater (a) and freshwater (b).

For both freshwater and saltwater, the reasons for exclusion are the same, but the percentages of their occurrence are different. The two overriding reasons for exclusion, both in fresh and salt water, are the use of unconventional units of measurement for detecting the biodegradability of bioplastics, and the presence of review articles, which have not been excluded in the first stage and that are not considered in this scientific review. The absence of a clear standardized procedure for carrying out biodegradability tests on plastics leaves decision leeway to scientists performing the tests, generating many results expressed with different units of measurement. All these reasons make it difficult to compare the results of the different biodegradability tests.

The data present in these articles have been analysed, re-processed and used to understand the state of the art on the biodegradability of bioplastics. This data re-processing lead to the production of the results and graphs presented in the “Results and discussion section”, in the chapter § 5.1.

Among the articles kept for this literature review, the most frequently used test medium has been saltwater (76.60%), (Figure 4.1.3). From this data, it can be deduced that scientists are more interested into the knowledge of the fate of bioplastics when released into the seas/oceans and in the understanding weather bioplastics tend to generate bioplastic islands like conventional plastics (oceanic plastic gyres). This great interest in the behavior of bioplastics in salt water is reflected in the fact that significantly more articles have been found for salt water rather than for fresh water in the literature databases (993 in SW versus 527 in FW).

Among the different groups of bioplastics, the most tested one are the biobased (55.60% in FW and 63.60% in SW) (Figure 4.1.3). Within this group, PHA family and the PLA family have been found to be the most popular (Figure 4.1.4). This result is in line with the fact that the most produced bioplastics belong in these two families (European Bioplastics, 2021). Furthermore, this literature review shows that in the market exists an extremely large number of different bioplastics and this number is expected to continue to grow in the future. Given this broad panorama, the definition of a biodegradability standard can be of great complexity.

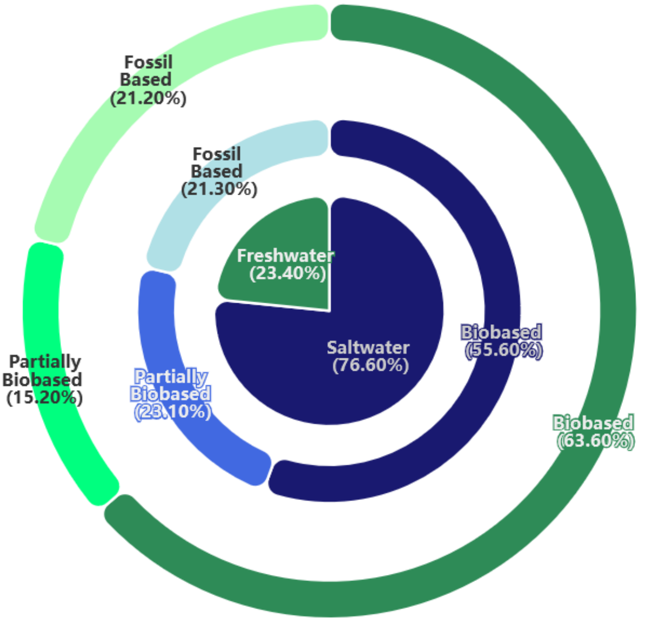
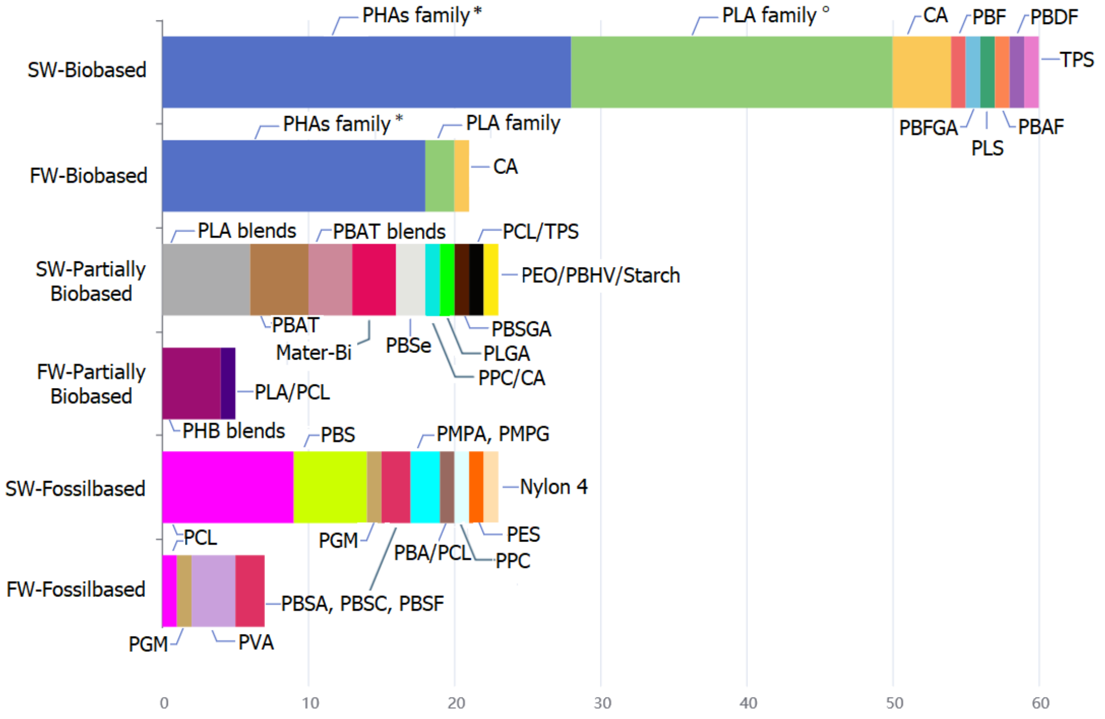


Figure 4.1.3: Percentage of biodegradation test (belonging to the literature review) carry out in salt and fresh water and subdivision of these tests according to the group of bioplastics tested, in each of the two mediums. Blue is used for saltwater, green in used for freshwater.



* includes: PHB, PHB/PHV, BIOPOL, P(3HB-co-4HB), P(3HB-co-3HV-co-3HB), PHBHH,

° includes: PLA, PLLA, PDLA, PLLA/PDLA

Figure 4.1.4: Number of times in which each type of polymer is tested within the literature review, divided according to the test medium and to the origin (biobased, partially biobased and fossil based). The acronyms provided by European Bioplastics have been used (European Bioplastics, 2021).

4.1.2 Standards regarding plastic biodegradation

The main standards concerning the biodegradation of plastics, in various environments (soil, compost, aquatic environment) in the presence or absence of oxygen, divided according to the standardization body that issued them (mentioned in Chapter §2.5), are listed in the following tables (Tables 4.1.1-4.1.4).

Table 4.1.1: ISO standards regarding the biodegradation of bioplastics.

ISO	Description
BS EN ISO 14851:2019	Determination of the ultimate **aerobic** biodegradability of plastic materials in an **aqueous medium**. Method by measuring the oxygen demand in a closed respirometer.
BS EN ISO 14852:2021	Determination of the ultimate **aerobic** biodegradability of plastic materials in an **aqueous medium**. Method by analysis of evolved carbon dioxide.
BS EN ISO 14853:2017	Plastics — Determination of the ultimate **anaerobic** biodegradation of plastic materials in an **aqueous system** — Method by measurement of biogas production.
BS EN ISO 10210:2017	Plastics - Methods for the preparation of samples for biodegradation testing of plastic materials.

BS EN ISO 18830:2017	Plastics - Determination of **aerobic** biodegradation of non-floating plastic materials in a **seawater/sandy sediment interface** -Method by measuring the oxygen demand in closed respirometer.
BS EN ISO 19679:2020	Plastics - Determination of **aerobic** biodegradation of non-floating plastic materials in a **seawater/sediment interface**. Method by analysis of evolved carbon dioxide.
BS EN ISO 22404:2021	Plastics. Determination of the **aerobic** biodegradation of non-floating materials exposed to **marine sediment**. Method by analysis of evolved carbon dioxide.
BS ISO 15314:2018	Plastics. Methods for **marine exposure**.
BS EN ISO 20200:2015	Plastics. Determination of the **degree of disintegration** of plastic materials under simulated **composting** conditions in a laboratory-scale test.
BS ISO 13975:2019	Plastics — Determination of the ultimate **anaerobic** Biodegradation of plastic materials in controlled **slurry digestion systems** — Method by measurement of biogas production.
BS EN ISO 17556:2012	Plastics — Determination of the ultimate **aerobic** biodegradability **in soil** by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved.
BS ISO 17088:2021	Plastics. Organic recycling. Specifications for **compostable** plastics.
BS EN ISO 14855-1:2012	Determination of the ultimate **aerobic** biodegradability and disintegration of plastic materials under controlled **composting** conditions—method by analysis of evolved carbon dioxide.
BS EN ISO 14855-2:2018	Determination of the final **aerobic** biodegradability of plastic materials under controlled **composting** conditions. Method by analysis of the developed carbon dioxide. Part 2: Gravimetric measurement of carbon dioxide developed in a laboratory test.
BS EN ISO 16929:2021	Plastics—determination of the degree of **disintegration** of plastic materials under defined **composting** conditions in a pilot-scale test.
BS EN ISO 15985:2017	Plastic materials - Determination of the final **anaerobic** biodegradability and disintegration in conditions of **anerobic digestion** of high-solids - Method by analysis of the released biogas.

Table 4.1.2: CEN standards regarding the biodegradation of bioplastics.

CEN	DESCRIPTION
BS EN 14047:2002	Packaging. Determination of the ultimate **aerobic** biodegradability of packaging materials in an **aqueous medium**. Method by analysis of evolved carbon dioxide.
BS EN 14048:2002	Packaging. Determination of the ultimate **aerobic** biodegradability of packaging materials in an **aqueous medium**. Method by measuring the oxygen demand in a closed respirometer.
BS EN 17033:2018	Plastics. **Biodegradable mulch films** for use in **agriculture** and **horticulture**. Requirements and test methods.
BS EN 13432:2000	Requirements for packaging recoverable through **composting** and **biodegradation**—test scheme and evaluation criteria for the final acceptance of packaging.
BS EN 14045:2003	Packaging. Evaluation of the **disintegration** of packaging materials in practical oriented tests under defined **composting** conditions.
BS EN 14046:2003	Packaging—evaluation of the ultimate **aerobic biodegradability** of packaging materials under **controlled composting conditions**—method by analysis of released carbon dioxide.
CEN/TR 15822:2009	Plastics: biodegradable plastics in or on **soil**—recovery, disposal and (under approval) related environmental issues.
BS EN 14806:2005	Packaging. Preliminary evaluation of the **disintegration** of packaging materials under simulated **composting** conditions in a laboratory scale test.

Table 4.1.3: ASTM standards regarding the biodegradation of bioplastics.

ASTM	Description
D5209 – 92	Standard Test Method for Determining the **Aerobic** Biodegradation of Plastic Materials in the Presence of **Municipal Sewage Sludge.**
D5271 – 02	Standard Test Method for Determining the **Aerobic Biodegradation** of Plastic Materials in an **Activated-Sludge-Wastewater-Treatment System**.
D6691-17	Standard Test Method for Determining **Aerobic** Biodegradation of Plastic Materials in the **Marine Environment** by a Defined Microbial Consortium or Natural Sea Water Inoculum.
D5437-93	Standard Practice for **Weathering** of Plastics Under **Marine Floating Exposure**.
D5247-92	Standard Test Method for Determining the **Aerobic** Biodegradability of Degradable Plastics By **Specific Microorganisms**.
D5511-18	Standard test method for determining **anaerobic** biodegradation of plastic materials under high-solids **anaerobic-digestion** conditions.
D5988–18	Standard test method for determining **aerobic** biodegradation of plastic materials in **soil**.
D7473–21	Standard Test Method for **Weight Attrition** of Plastic Materials in **the Marine Environment** by Open System Aquarium Incubations.
D7991–22	Standard test method for determining **aerobic** biodegradation of plastics buried in **sandy marine sediment** under controlled laboratory conditions.
D6400-21	Standard Specification for **labelling of plastics** designed to be **aerobically composted** in municipal or industrial facilities.
D5338-15 (2021)	Standard Test Method for Determining **Aerobic** Biodegradation of Plastic Materials Under **Controlled Composting Conditions**, Incorporating Thermophilic Temperatures.
D5526-18	Standard test method for determining **anaerobic** biodegradation of plastic materials under accelerated **landfill** conditions.
D6002-96	Standard guide for assessing the **compostability** of environmentally degradable plastics.
D7081-05	Standard specifications for **non-floating biodegradable plastics in the marine environment**.
D5210-92 (2007)	Standard Test Method for Determining the **Anaerobic** Biodegradation of Plastic Materials in the Presence of **Municipal Sewage Sludge**.
D7475-20	Standard test method for the determination of **aerobic** degradation and anerobic biodegradation of plastics in **landfill conditions** with accelerated bioreactor.
D5509– 96	Standard Practice for Exposing Plastics to a Simulated **Compost** Environment.
D5512 – 96	Standard Practice for Exposing Plastics to a Simulated **Compost** Environment Using an Externally Heated Reactor.
D6003-96	Standard Test Method for Determining Weight Loss from Plastic Materials Exposed to a Simulated Municipal Solid Waste (MSW) Aerobic **Compost** Environment.
D5525– 94	Standard Practice for Exposing Plastics To a Simulated **Landfill** Environment.

Table 4.1.4: Standards regarding the biodegradation of bioplastics issued by other certification bodies.

Others Certification Bodies	Description
OECD 310	An **aerobic** biodegradation test that predominantly measures ready biodegradability by the evaluation of CO_2 in sealed vessels.
OECD 306	Biodegradability in **Seawater**.

OECD 301-92: - OECD 301B - OECD 301C - OECD 301D - OECD 301F	Guidelines to establish that a test **substance** is regarded as **''readily biodegradable''**: - Modified Strum test (evolved CO_2) - Modified MITI test - Closed bottle test - Manometric respirometric test
OECD 302B OECD 302C OECD 302A	Modified Zahn-Wellens/EMPA test Modified MITI test Inherent biodegradability in soil
AS 4736–2006	Biodegradable Plastics Suitable for **Composting** and other Microbial Treatment.
AS 5810–2010	Biodegradable plastics suitable for **home composting.**
JSA- JIS K 6950-94	Plastics - Testing Method for **Aerobic** Biodegradability by **Activated Sludge**.
DIN V 54900	Determination of the **aerobic** biodegradability of polymeric materials in **aquatic** batch tests.
AFNOR NF U52–001	Biodegradable materials for use in **agriculture** and **horticulture** - Mulching products - Requirements and test methods.

Among all these standards not many evaluate the biodegradability of plastic in an aquatic environment. Most of them tend to evaluate its compostability. In fact, plastics are considered to be "biodegradable" on the market according to definitions contained into norms established for composting or for anaerobic digestion processes (like ISO 18606:2018 and EN 13432:2000), even though a specific standard which defines the biodegradability in general terms does not exist. Therefore, norms defining the biodegradability of bioplastics in engineered environment exist, but, on the contrary, there are no norms defining the biodegradability in the natural environment, such as in soil, in the freshwater, and in the ocean.

In this thesis development, the focus was posed on those standards relating to the biodegradation of plastics in aquatic environments under aerobic conditions, because the primary objective of this work is to define what happens to bioplastics when dispersed into the aquatic environment, to assess whether they undergo biodegradation processes. For what concern the water environment, many different internationally recognized standards have been set in order to assess the capability of a plastics to be biodegraded (Table 4.1.5), but these methodologies do not provide clear targets to be satisfied in order to label a bioplastic as biodegradable. Moreover, the different methodologies give great leeway regarding the ranges to be chosen for the environmental parameters (such as temperature, timeframe, inoculum concentration) and on the bioplastic shape and size. For these reasons the comparison of the results coming from different studies is challenging.

Table 4.1.5: Standards on the biodegradability of plastics in aqueous environment (the OECD standards are not included in the table below as they mainly relate to organic chemicals soluble in liquids).

Standard	Description	Publication Date
BS EN ISO 14851:2019	Determination of the ultimate **aerobic** biodegradability of plastic materials in an **aqueous medium**. Method by measuring the oxygen demand in a closed respirometer.	1999
BS EN ISO 14852:2021	Determination of the ultimate **aerobic** biodegradability of plastic materials in an **aqueous medium**. Method by analysis of evolved carbon dioxide.	1999
BS EN ISO 18830:2017	Plastics - Determination of **aerobic** biodegradation of non-floating plastic materials in a **seawater/sandy sediment interface** -Method by measuring the oxygen demand in closed respirometer.	2016
BS EN ISO 19679:2020	Plastics - Determination of **aerobic** biodegradation of non-floating plastic materials in a **seawater/sediment interface**. Method by analysis of evolved carbon dioxide.	2018
BS EN ISO 22404:2021	Plastics. Determination of the **aerobic** biodegradation of non-floating materials exposed to **marine sediment**. Method by analysis of evolved carbon dioxide.	2019
BS ISO 15314:2018	Plastics. Methods for **marine exposure**.	2005
BS EN 14047:2002	Packaging. Determination of the ultimate **aerobic** biodegradability of packaging materials in an **aqueous medium**. Method by analysis of evolved carbon dioxide.	2002
BS EN 14048:2002	Packaging. Determination of the ultimate **aerobic** biodegradability of packaging materials in an **aqueous medium**. Method by measuring the oxygen demand in a closed respirometer.	2002
ASTM D6691-17	Standard Test Method for Determining **Aerobic** Biodegradation of Plastic Materials in the **Marine Environment** by a Defined Microbial Consortium or Natural Sea Water Inoculum.	2010
ASTM D5209 – 92	Standard Test Method for Determining the **Aerobic** Biodegradation of Plastic Materials in the Presence of **Municipal Sewage Sludge.**	1992
ASTM D5271 – 02	Standard Test Method for Determining the **Aerobic Biodegradation** of Plastic Materials in an **Activated-Sludge-Wastewater-Treatment System**.	1993
ASTM D7991–22	Standard test method for determining **aerobic** biodegradation of plastics buried in **sandy marine sediment** under controlled laboratory conditions.	2015
ASTM D7081-05	Standard specifications for **non-floating biodegradable plastics in the marine environment**.	2005
JSA- JIS K 6950-94	Plastics - Testing Method for **Aerobic** Biodegradability by **Activated Sludge**.	1994
DIN V 54900	Determination of the **aerobic** biodegradability of polymeric materials in **aquatic** batch tests.	1998

Among the 54 standards presented in Tables 4.1.1-4.1.4, only 16 deal with the aerobic biodegradation of bioplastics in aquatic environment.

The standards EN ISO 14851 and EN ISO 14852 are the oldest. The first evaluates biodegradation by measuring the Biochemical Oxygen Demand (BOD), the second measures biodegradation by measuring the CO_2 produced. Both standards are used for freshwater. EN ISO 18830 is based on EN ISO 14851 by changing the type of test medium (passing form freshwater to saltwater). The same can be said for the standard EN ISO 19679, which is based on EN ISO 14852, but using salt water instead of fresh water. Both EN ISO 18830 and EN ISO 19679 are used to evaluate the biodegradation of plastic at the interface between seawater and sandy marine sediments. ASTM D5209 was procedurally similar to EN ISO 14852; it evaluates the biodegradability by measuring the carbon dioxide evolved, the residual polymer weight and the Soluble Organic Carbon (SOC) content. ASTM D5271 is the equivalent of EN ISO 14851; it measures the biodegradability through the measure of the BOD and the residual polymer weight. The test medium used, in both standards, is the freshwater. ASTM D6691 evaluates biodegradation by measuring the CO_2 evolution of a test material immersed in saltwater medium. The only standard that does not define a test method, but the requirements which must be achieved to label a product as “marine disposable”, in accordance with the guidelines issued by the Federal Trade Commission, is the ASTM D7081. Following this standard, a finite product can be labelled as “marine disposable” if it reaches these requirements:

1. **Disintegration During Marine Degradation**—A product or material disintegrates during marine water exposure such that any remaining residuals (plastic or substrate) are not readily distinguishable from other organic materials or particulates normally present in this environment. A monomaterial product (that means a product made only with one polymer) demonstrates satisfactory disintegration if after twelve weeks in controlled test situations (described in Test Method D6691), shows a dry remaining weight of less than 30 % of its original dry weight, after sieving on a 2.0 mm side mesh sieve.
2. **Inherent Biodegradation**—A level of inherent biodegradation for plastics has to be established, and this should be comparable to the one of some known marine biodegradable materials (for example, Kraft paper). A product demonstrates inherent biodegradability if the criteria in the following a) and b) points are met or exceeded.
    a. The plastics have to demonstrate that 30% or more of their organic carbon is converted to carbon dioxide within 180 days at 30°C (using Test Method D6691), when compared to the positive control. Also, they have to demonstrate that 90% undergoes biodegradation in an active environment like composting (in accordance with Test Method D5338). As alternative tests, which demonstrate the test material biodegradability by means of its microbial assimilation, the standards, ISO 14851:2019, ISO 14852:2021, and ISO 14855:2018 can also be used. Compliance to Specification D6400 is a necessary, but not sufficient prerequisite.
    b. In order to demonstrate that a material is inherently biodegradable, a satisfactory rate of biodegradation must be verified through a test method designed to optimize microbial activity. The test method can be Test Method D5338. To fulfill the requirements of this method, plastics have to achieve one of the following ratios of

conversion to carbon dioxide (I-III), within the time periods specified in IV or V. If the plastic is used as a coating or a binder, then the substrates shall be tested separately and shall meet the requirements of II, within the time periods specified in IV or V.

   I. For products consisting of a single polymer (homopolymers), 60% of their organic carbon must be converted to carbon dioxide by the end of the test, when compared to the positive control.
   II. For all the other polymers and substrates, 90% of their organic carbon must be converted to carbon dioxide by the end of the test period, when compared to the positive control.
   III. For products consisting of more than one polymer, each individual polymer component, present at more than 1 % concentration, must achieve the same specification for homopolymers, as described in I.
   IV. For materials that are not radiolabeled[1] the test period shall be shorter than 180 days.
   V. If radiolabeled materials are used, then the test period shall be no longer than 365 days.

3. **No Adverse Environmental Impacts**—The tested products or materials shall not adversely impact other marine organisms nor the ecosystem, once placed in the marine environment. Additionally, the polymeric products must not introduce unacceptable levels of regulated metals or other toxic substances into the environment. The plastic products shall undergo toxicity tests chosen between the following options: Polytox (microbial oxygen absorption) test, Microtox (microbial bioluminescence) test, fish Acute Toxicity (static conditions) OPPTS 850.1075 test, Daphnia test, Acute Toxicity (static conditions) OPPTS 850.1010, or Static Algal Toxicity Test OPPTS 850.5400. The regulated heavy metals contained in the plastic products should have concentration lower than 25% of those prescribed in the country where the product is sold.

A conformity label has also been developed for products described as biodegradable in seawater (Vinçotte OK Biodegradable MARINE), based on ASTM D7081. In addition, Vinçotte introduced the “OK Biodegradable WATER” conformity label, based on EN ISO 14851 and on EN ISO 14852 standards, to define a product as “degradable in natural freshwater environment”. If a product degrades at a rate of 90% during 56 days of incubation at a temperature of 20–25°C, it can be marked. These labels are not widely used, because there are not attractive neither for the producer nor for the consumer. In the case of these two labels, the requirements have been defined only in order to label the products as biodegradable, but not in order to define the biodegradability of the materials used.

In addition, these standards (EN ISO 14851 and EN ISO 14852) are difficult to apply for a product as it is (without making any reduction in size), as the label would require.

The main conditions defined by the most used standards (EN ISO 14851, EN ISO 14852, ASTM D5271, ASTM D5209, EN ISO 18830, EN ISO 19679 and ASTM D6691) are summarized and compared in Table 4.1.6 and Table 4.1.7. The first table include standards which were developed for freshwater, the second those which were developed for saltwater.

Table 4.1.6: Comparison of the contents of the most common standards used for freshwater.

	ISO 14851	**ASTM 5271**	**ISO 14852**	**ASTM 5209**
TEST MEDIUM	Produced	Produced	Produced	Produced
STIRRING	yes	yes	yes	yes
TEST MATERIAL CONCENTRATION	100 - 2000 mg/l of TOC or with a ThOD of at least 170 mg/l	60- 2000 mg/l of TOC	100- 2000 mg/l of TOC (also 20 mg/l of TOC can be tested)	Not specified
TEST MATERIAL SHAPE	Powder, films, pieces, fragments or shaped articles	Powder, films, pieces, fragments or shaped articles	Powder, films, pieces, fragments or shaped articles	Powder, films, pieces, fragments or shaped articles
REFERENCE MATERIAL	Not specified	Cellulose	Not specified	Not specified
NEGATIVE CONTROL	Not specified	Polyethylene	Not specified	Not required
INOCULUM	Activated sludge	Activated sludge, compost or soil	Activated sludge	Activated sludge
INOCULUM QUANTITY	Activated sludge:30-1000 mg/l of TSS or a percentage by volume of 1% to 5%	Activated sludge: 30-1000 mg/l of MLVSS. Compost or soil: concentration of 1 to 5% (V/V)	30-1000 mg/l of suspended solids	1% (V/V) inoculum for each flask
T(°C)	20-25°C	23°C	20-25°C	23°C
PH	7	7	7	7
CO_2 FREE AIR FLOW (ml/min)	/	/	50-100	50-100
DURATION	max 2 months	Min of 4 to 6 weeks to a max of 6 months	max 2 months (for specific cases also 6 months can be reached)	Not specified
LIGHT CONDITION	Dark or diffuse light	Dark or diffuse light	Dark or diffuse light	Not specified
CRITERION	BOD	BOD, SOC, weight variations	CO_2	CO_2, SOC and weight variations

Table 4.1.7: Comparison of the contents of the most common standards used for saltwater.

	ISO 18830	**ISO 19679**	**ASTM D6691**
TEST MEDIUM	Taken or produced	Taken or produced	Taken or produced
STIRRING	Yes (not disturbing the sediment-water interface)	Yes (not disturbing the sediment-water interface)	Yes, 175 rmp
TEST MATERIAL QUANTITY	The mass should correspond to a ThOD of 170 mg/l or to a TOC of 60 mg/l	Between 150 to 300 mg/l of seawater plus sediments	Usually 20 mg (verify that the carbon content is at least 20%)

TEST MATERIAL SHAPE	Films or sheet. Cut the test material sample in disks	Films or sheet. Cut the test material sample in disks	Powder (mean particle size < 25mm), films, pieces, fragments, formed articles.
TEST MATERIAL POSITION	Interface between seawater and seafloor	Interface between seawater and seafloor	Not specified
REFERENCE MATERIAL	Ashless cellulose filters	Ashless cellulose filters	Cellulose, chitin, and Kraft paper or all three
NEGATIVE CONTROL	Not specified	Not specified	Solitary inoculum
INOCULUM	Natural seawater	Natural seawater	Natural seawater/ an inoculum formed from a population of at least 10 ten aerobic marine microorganisms of know genera
INOCULUM QUANTITY	Not specified	Not specified	100 µL (in case of natural seawater no inoculum is needed)
T(°C)	15-25°C	15-25°C	30°C
PH	Not specified	Not specified	Not specified
DURATION	Max 24 months	Max 24 months	From 10 to 90 days
LIGHT CONDITION	Dark or diffuse light	Dark or diffuse light	Not specified
CRITERION	BOD	CO_2	CO_2

From the assessment of the information given above, various issues can be found. It can be noted that each standard defines a value or a range for the different process parameters. Furthermore, the different methodologies give great leeway regarding the ranges to be chosen for the environmental parameters (such as temperature, timeframe, inoculum concentration) and on the bioplastic initial shape and size. This prevents comparable results to be obtained, either using different standards or the same one. Moreover, the durations given by test methods can significantly underestimate the durations required for a polymer to biodegrade within natural ecosystems. In the available standards, only the maximum duration of the test is indicated, thus letting individual scientists to decide the appropriate duration. Test duration, as well as temperature, and initial shape of the tested bioplastic substantially influence the biodegradation result of the test. It should therefore be important that the test methods define uniquely all these parameters, in order to obtain comparable results. According to the various standards, to facilitate the biodegradation of the test materials, they must be reduced in size, or powdered, before being immersed in the test medium. However, no indications on the size to be achieve are given within the standards. Nevertheless, since a plastic object is entire when thrown into the natural or aquatic environment, the shredding process alters its original form and therefore the true fate of the product. Moreover, the marks “OK Biodegradable MARINE” and “OK Biodegradable WATER” established by Vinçotte, which are the only ones that certify the biodegradability of a product in water, refer to ISO and ASTM standards which report the aforementioned ambiguity relating to size.

Continuing to list the issues related to the standards, various standards set the test temperature around 20-25 °C, which, most of the time, is not a representative temperature range of a natural aquatic environment (sea/ocean mean water temperature is usually below 13 °C).

Furthermore, in most of the standards, the test medium is produced in lab and then inoculated with activated sludge inoculum; this microbial consortium may not be representative of the actual microbial population present in a real aquatic environment.

To conclude, in most of the available standards to evaluate the biodegradability of bioplastics in water environment, toxicity tests for polymer degradation products are not included.

4.2 Research Program

The objective of the research program is to evaluate the biodegradability of PLA in freshwater following the test method presented in the standard BS EN ISO 14851:2019. With this purpose three tests have been done, two of which were used as preparatory tests to verify the procedure and to highlight existing criticalities (α and β), while the third was used as official test (γ).

4.2.1 BS EN ISO 14851:2019

This standard has been prepared by the Technical Committee ISO/TC 61 "Plastics" in collaboration with Technical Committee CEN/TC 249 “Plastics” (The British Standards Institution, 2019). The document specifies a method, by measuring the oxygen demand in a closed respirometer, for the determination of the degree of aerobic biodegradability of bio-plastic materials, including those containing formulation additives. The test material is exposed in an aqueous medium under laboratory conditions to an inoculum from activated sludge.

The main conditions defined by the standard are summarized in Table 4.2.1.

Table 4.2.1: Main conditions defined by the standard BS EN ISO 14851:2019.

Test Medium	Produced
Stirring	Yes
Test Material Concentration	100 – 2000 mg/l of TOC or with a ThOD of at least 170 mg/l
Test Material Shape	Powder, films, pieces, fragments or shaped articles
Reference Material	Well-defined biodegradable polymer. If possible, the TOC, form and size should be comparable to that of the test material
Negative Control	Non-biodegradable polymer in the same form as the test material
Inoculum	Activated sludge from a wastewater treatment plant treating domestic sewage
Inoculum Quantity	Percentage by volume of 1% to 5% of the test medium used

T(°C)	20-25°C
Test Medium pH	7
Duration	Max 2 months
Light Condition	Dark or diffuse light
Criterion	BOD
N° Of Flasks Required	11

4.2.1.1 Test procedure

The aqueous medium is produced following the indications presented in the standard. BS EN ISO 14851:2019provides indications for the preparation of two types of test medium (standard and optimized), but if the user wants to simulate a natural environment the use of the standard test medium is recommended. This medium is composed by the combination of four different solutions (The British Standards Institution, 2019):

- Solution A, made by anhydrous potassium dihydrogen phosphate (KH_2PO_4), anhydrous dipotassium hydrogen phosphate (K_2HPO_4), disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) and ammonium chloride (NH_4Cl).
- Solution B, composed by magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$).
- Solution C, made by calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$).
- Solution D, made by iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$).

The incubation shall take place in the dark or in diffuse light in an enclosure which is free from vapors inhibitory to microorganisms, and which is maintained at a constant temperature, preferably between 20 °C and 25 °C under stirring conditions. The test material shall be of known mass and contain sufficient carbon to yield a BOD that can be adequately measured by the respirometer. The standard recommends to use a test material concentration of at least 100 mg/l, corresponding to a ThOD of about 170 mg/l or a TOC of about 60 mg/l. If the user wants to simulate a biodegradation process in a natural environment, the use of a test-material concentration of 100 mg/l is recommended. The test material should preferably be used in powder form, but it may also be introduced as films, pieces, fragments or shaped articles. As reference materials the standard requires a positive and a negative control. Aniline and/or a well-defined biodegradable polymer (for example microcrystalline cellulose powder, ashless cellulose filters or poly-β-hydroxybutyrate) can be used as a reference material; if possible, the TOC, form and size should be comparable to that of the test material. As a negative control, a non-biodegradable polymer (e.g. polyethylene) in the same form as the test material can be used. The inoculum must be an activated sludge collected from a well-functioning wastewater treatment plant or from a laboratory facility that treats predominantly domestic wastewater. The sample must be well mixed, kept aerobically and preferably used on the day of collection, or at least, within 72 h. After decanting the inoculum sample for at least 30 minutes, the supernatant liquid should be collected and used to inoculate the different flasks, to obtain a concentration in the test medium equal to 1-5% as volume fraction. The standard requires the use of 11 flasks, eight of which are compulsory, and three are facultative. The compulsory flasks are:

a) three test flasks for the test material (FTs);
b) three test flasks for the blank control (FBs);
c) two test flasks for checking the inoculum activity using a reference material (FCs).

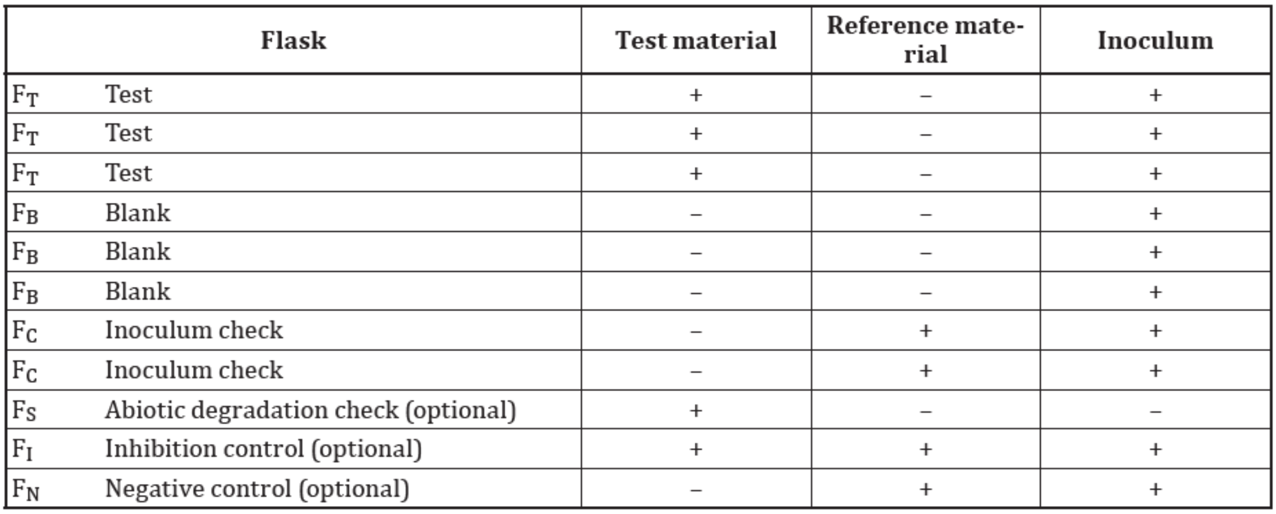
While the facultative ones are:

d) One flask for checking for possible abiotic degradation or non-biological change in the test material such as by hydrolysis (FS). The test solution in FS shall be sterilized. In our case the flask FS was sterilized through the use of hydrochloric acid (HCl).
e) One flask as a negative control (FN) using a non-biodegradable polymeric substance in the same form as the test material.
f) One flask for checking the possible inhibiting effect of the test material on microbial activity (FI).

Inside the flasks it is necessary to add the appropriate amounts of the test medium and the inoculum as indicated in Table 4.2.2. Then, the pH must be measured in the flasks and adjust to 7 if necessary. The carbon dioxide absorber must be added to the absorber compartments of the respirometer. The test material, the reference material and the material for the negative control must be added to the respective flasks as indicated in Table 4.2.2. As a final step, the flasks must be placed in a constant-temperature environment. At this point the experiment can begin. When a constant level of BOD is attained (plateau phase reached) and no further biodegradation is expected, the test is considered to be completed. The test period should not typically exceed 2 months.

At the end of the test, the pH must be measured and the concentrations of nitrate and nitrite must be determined in flasks FT (The British Standards Institution, 2019).

Table 4.2.2: Final distribution of test and reference materials, as indicated in the standard BS EN ISO 14851:2019 (The British Standards Institution, 2019).



Flask		Test material	Reference material	Inoculum
F_T	Test	+	−	+
F_T	Test	+	−	+
F_T	Test	+	−	+
F_B	Blank	−	−	+
F_B	Blank	−	−	+
F_B	Blank	−	−	+
F_C	Inoculum check	−	+	+
F_C	Inoculum check	−	+	+
F_S	Abiotic degradation check (optional)	+	−	−
F_I	Inhibition control (optional)	+	+	+
F_N	Negative control (optional)	−	+	+

4.2.1.2 Calculation and expression of results

The specific biochemical oxygen demand (BODs) of the test material must be calculated as the difference between oxygen consumption in the test flasks FTs and the blanks FBs, divided by the concentration of the test material, using Formula (4.2.1) (The British Standards Institution, 2019):

$$\mathrm{BOD_S} = \frac{\mathrm{BOD}_t - \mathrm{BOD}_{\mathrm{B}t}}{\rho_{\mathrm{TC}}} \tag{4.2.1}$$

Where:

BODs is the specific BOD, in milligrams per gram of test material;

BOD*t* is the BOD of the flasks FT containing test material at time *t*, in milligrams per litre;

BODB*t* is the BOD of the blank FB at time *t*, in milligrams per litre;

*ρ*TC is the concentration of the test material in the reaction mixture of flask FT, in grams per litre.

Then the percentage biodegradation Dt must be calculated as the ratio of the specific biochemical oxygen demand to the theoretical oxygen demand (ThOD, in milligrams per gram of test material), using Formula (4.2.2) (The British Standards Institution, 2019):

$$D_t = \frac{\mathrm{BOD_S}}{\mathrm{ThOD}} \times 100 \tag{4.2.2}$$

In the same way the BOD and percentage biodegradation of the reference material FC and, if included, the abiotic degradation check FS, the inhibition control FI and the negative control FN can be calculated (The British Standards Institution, 2019).

4.2.1.3 Validity of results

The test is considered valid if (The British Standards Institution, 2019):

a) the degree of biodegradation of the reference material (inoculum check FC) is > 60 % at the end of the test;

b) the BOD of the blank FB at the end of the test does not exceed an upper limiting value obtained by experience (this value depends on the amount of inoculum and is, for example, in the case of 30 mg/l dry matter, about 60 mg/l as interlaboratory tests have shown);

c) the BOD values of the three blanks FB and of the three test flasks FT are within 20 % of the mean at the plateau phase or at the end of the test.

d) If in flask FI (inhibition check, if included) the percentage biodegradation is <25 % and no significant degradation of the test material is observed, it can be assumed that the test material is inhibitory.

e) If in flask FS (abiotic degradation check, if included) a significant amount (>10 %) of BOD is observed, abiotic degradation processes may have taken place.

f) If flask FN (negative control) was included, no significant amount of BOD shall be observed.

If these criteria are not fulfilled, the standard recommends repeating the test using another inoculum (The British Standards Institution, 2019).

4.2.2 Preparatory tests (α and β)

The α experiment started on 20/07/2022 and it lasted 32 days. The β experiment started on 13/09/2022 and it lasted 35 days. They have been performed at the LISA laboratory of the ICEA department, in Voltabarozzo (PD). They have been made following the standard BS EN ISO 14851: 2019. The test material used is PLA, the chosen positive control is starch and the chosen negative control is HDPE (Figure 4.2.1). PLA and HDPE have been used in the form of granules of variable diameters. Starch has been used in powder form. The eleven flasks required by the standards have been filled with the test medium, the inoculum, the test material and the reference material (as indicated in Table 4.2.2), occupying a volume of 300 ml. The reactors have been inserted into the Sapromat respirometer and immersed in a bath maintained at 20 °C for the entire duration of the test, in the dark, without stirring. In the first experiment (α) the inoculum used has been taken from the activated sludge tank of the domestic wastewater treatment plant in Ca’ Nordio (Padova, Italy) on 18/07/2022. In the second experiment (β) the inoculum has been taken from the recirculation well of the secondary settle tank of the domestic wastewater treatment plant in Cà Nordio (Padova, Italy) on 12/09/2022, in order to have a more concentrated inoculum. It was decided to take the inoculum from two different points of the treatment plant in order to understand which was the most appropriate one to use in the final experiment (γ).

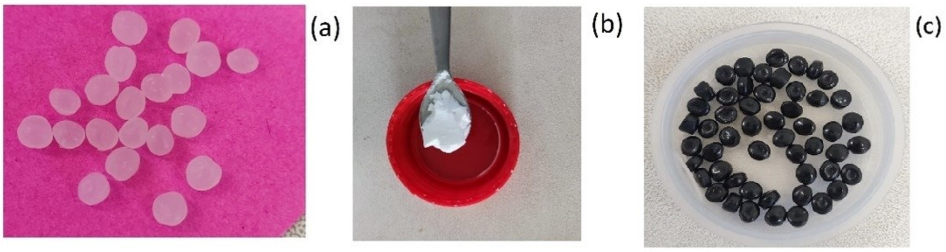


Figure 4.2.1: PLA granules (a), starch powder (b) and HDPE granules (c) used in the α and β experiments.

4.2.2.1 Apparatus used

The apparatus used is composed as follows:

I. Closed respirometer (Sapromat), composed of test vessels (glass flasks) located in a thermostatic apparatus (e.g. water-bath). The Sapromat is composed by three units: a computer where the data regarding the quantity of oxygen produced by the electrolytic cells are saved (a); the thermostatic apparatus where the flasks are immersed (b); and the data logger that records the units of volumes of produced oxygen (c), as shown in Figure 4.2.2.

Figure 4.2.2: The three units composing the Sapromat.

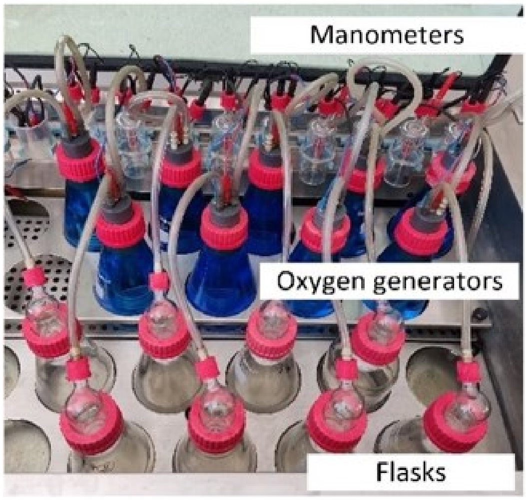


Figure 4.2.3: Components of the thermostating apparatus.

Inside the thermostatic apparatus 12 workspaces are presents (as shown in Figure 4.2.3), each composed by three elements: a flask containing the test material/reference materials, inoculum and test medium, an electrolytic oxygen generator and a manometer consisting in two electrodes responsible for the opening or closure of the circuit according on the pressure gaps. At the beginning of the test all the flasks are saturated with oxygen (in the form of O_2), then, when the oxygen is consumed (being converted into CO_2 and being trapped by the caustic soda which is located in the head space of the flasks), the manometer detects the depression, activating the electrolytic cell, which produces and provides oxygen to the flasks until the pressure is balanced.

The data logger used for the calculation of the Respirometric Index (RI) provided results in terms of units of volume of produced oxygen, which in turn can give the amount of oxygen provided to the system and thus the consumption of oxygen. In detail, one unit of volume corresponds to an absolute oxygen quantity of 0.25 mg. The calculation of the amount of oxygen consumption from the number of units of volume is done using Formula (4.2.3) and Formula (4.2.4):

$$IR\left(\frac{mgO_2}{g\ materials}\right) = \frac{Ncounts}{Pc * \frac{ST}{100}} * 0.25 \qquad (4.2.3)$$

Where:

Ncounts: number of units of volume relative to the number of days provided by the instrument;

P_C: weight, in g, or the material inserted in each flask;

ST: value, in percentage, of the total solid of the sample.

$$IR\left(\frac{mgO_2}{g\,C}\right) = \frac{Ncounts}{Pc * TOC} * 0.25 \qquad (4.2.4)$$

Where:

Ncounts: number of units of volume relative to the number of days provided by the instrument;

P_C: weight, in g, or the material inserted in each flask;

TOC: value, in ${}^{gC}/_{gmaterial}$ of the total organic carbon content of the sample.

II. pH analyzer. It has been used to measure the pH of the test medium mixed with the supernatant inoculum at the beginning and at the end of the test (Figure 4.2.4).

Figure 4.2.4: pH analyzer.

III. Filtration apparatus for the measure of Total Suspended Solids (TSS). This apparatus is composed by: a vacuum pump, a graduate funnel, a collection flask and a 0.45 µm filter (Figure 4.2.5).

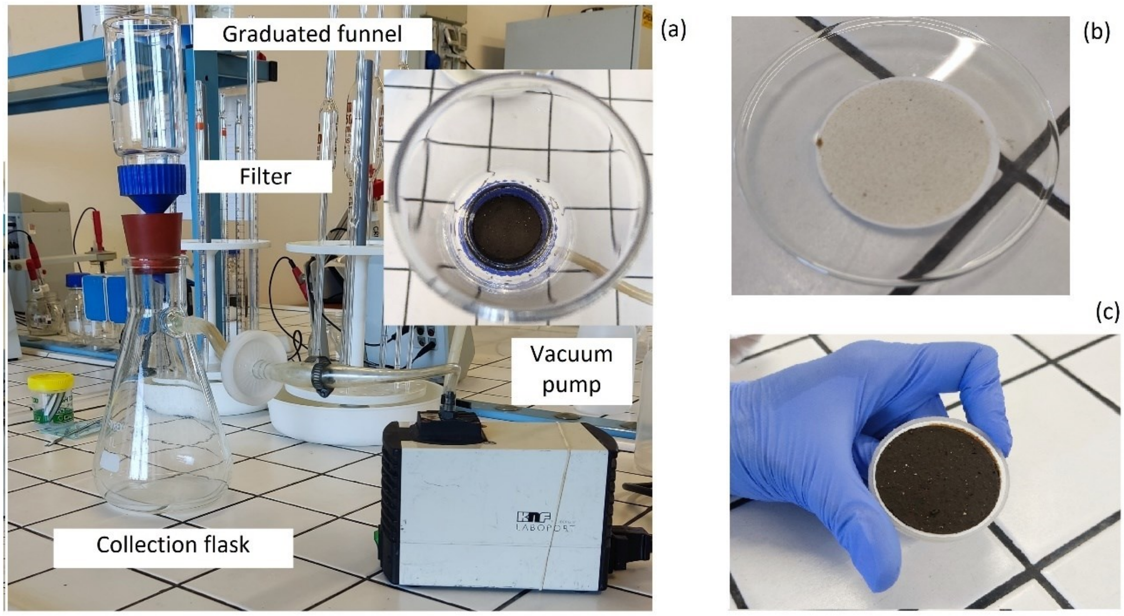


Figure 4.2.5: Filtration apparatus (a), filter after the filtration of the supernatant inoculum (b), filter after the filtration of the inoculum in an unaltered state (c).

The filtration procedure is composed of the following steps: the vacuum pump is switched on and the liquid sample is poured inside the graduated funnel. At the end what is obtained is: a filtered liquid, passing through the membrane, which is collected inside the collection flask, used in a second moment to measure the Dissolved Organic Carbon (DOC); a membrane rich in suspended solids, which will then be dried in an oven (at 105 °C) until its weight no longer varies, and weighted, in order to calculate the TSS.

IV. An oven and a muffle for drying the samples and for measuring the Total Suspended Solids (TSS), the Total Solids (TS) and the Volatile Solids (VS) (Figure 4.2.6). The samples are dried in the oven, and the weighted, in order to measure the TS and the TSS. The VS, instead, are measured after that the samples are dried first in the oven and then in the muffle, and the weighted. The oven works at 105 °C and is used to evaporate all the water contained in the samples, the muffle works at a temperature between 550-900°C and it is used to evaluate the content of the volatile solids present in the sample. Volatile solids pass into gas phase at this temperature, while the non-volatile ones remain in the crucibles.

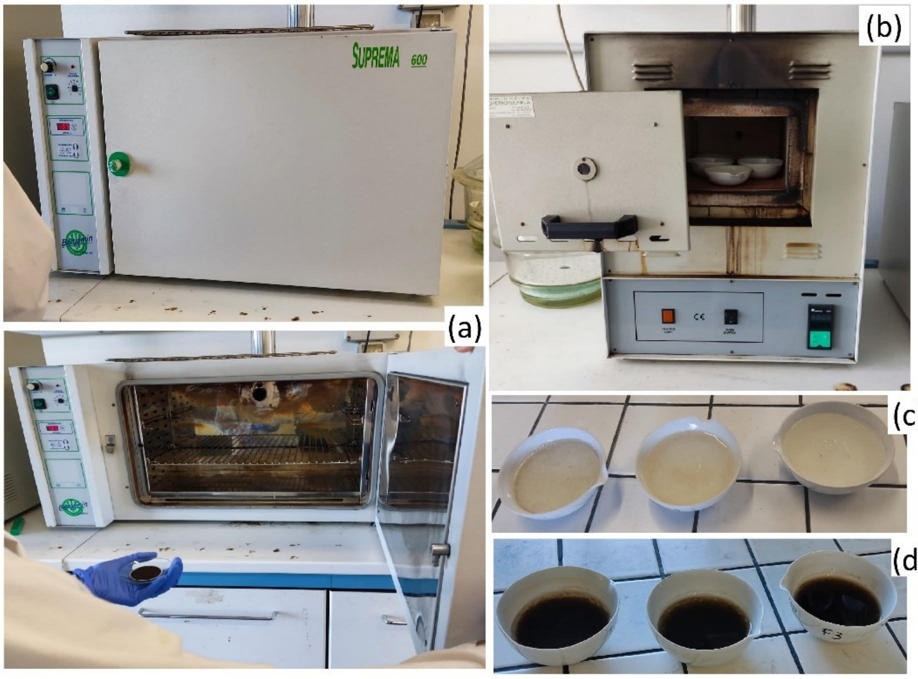


Figure 4.2.6: Oven open and closed (a), muffle (b), samples of supernatant inoculum (c) and inoculum in an unaltered state (d) inside the crucibles.

V. Total Organic Carbon (TOC) analyzer (Figure 4.2.7). This machine is used to measure the total organic carbon content of both liquid and solid samples. The solid sample is first dried in the oven (in order for the water to evaporate), then inserted into a crucible and finally put inside the TOC machine. This device calculates the Total Carbon (TC) and the Inorganic Carbon (IC), and the difference is the TOC. The process for measuring the TC is a combustion process: the sample is brought to a temperature of 900°C and air is blown. All the carbon contained in the sample, at a temperature of 900 ° C, is converted into CO_2, which is detected and measured by the machine through an infrared analyzer. Inorganic carbon can exist in the form of carbonates and bicarbonates. To measure the IC the machine working temperature is lower, about 300 °C, and an acid is added inside the sample. The acid converts all the inorganic carbon present in the sample into CO_2, which is detected and measured by the infrared analyzer. For liquid samples TOC is not calculated as the difference between TC and IC, but through an automatic machine function called Non Purgeable Organic Carbon (NPOC). Also the DOC is measured using the NPOC function.

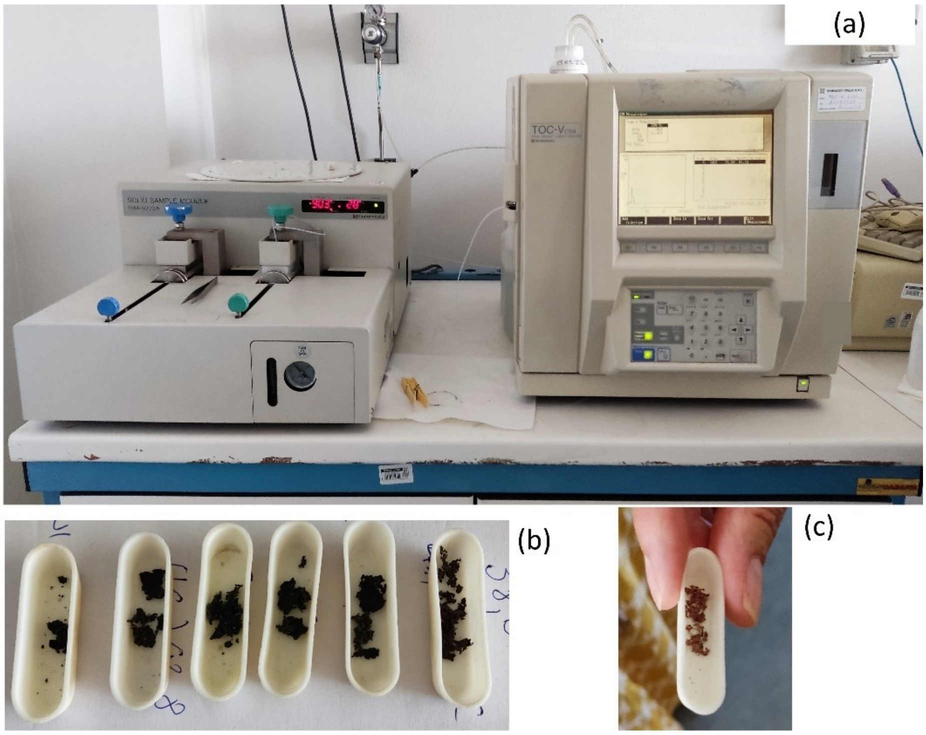


Figure 4.2.7: TOC analyzer machine (a), solid samples inside the crucibles before the TOC analysis (b) and solid sample inside the crucible after the TOC analysis (c).

VI. A digester and an automatic titrator for the measurement of the Chemical Oxygen Demand (COD) (Figure 4.2.8). This test is used to measure the total content (both biodegradable and not biodegradable) of organic compounds in a sample. The amount of organic matter is expressed as the total amount of oxygen required to chemically oxidize all the organics. The operating principle is based on the fact that all the organic matter can be oxidized to $\mathrm{CO_2}$ and $\mathrm{H_2O}$ by a strong oxidizing agent under acid conditions. To measure the COD value, the sample is mixed with strong acids then is placed into a digester and, when the digestion is complete (in general it takes two hours), the sample is titled with the Mohr’s salts. The oxidizing reagent used is potassium dichromate. The reaction that develops by putting the sample in contact with the acidified dichromate solution and keeping it at a high temperature (during digestion) causes the reduction of the dichromate from hexavalent to trivalent chromium. The analytical procedure involves working with an excess of oxidant, to be sure that all the organic substance is oxidized. The amount of remaining dichromate is measured by titration with ferrous-ammonium sulphate (Mohr's salt) which splits the dichromate ion into trivalent chromium ion and water. The concentration of COD is proportional to the amount of potassium dichromate consumed.

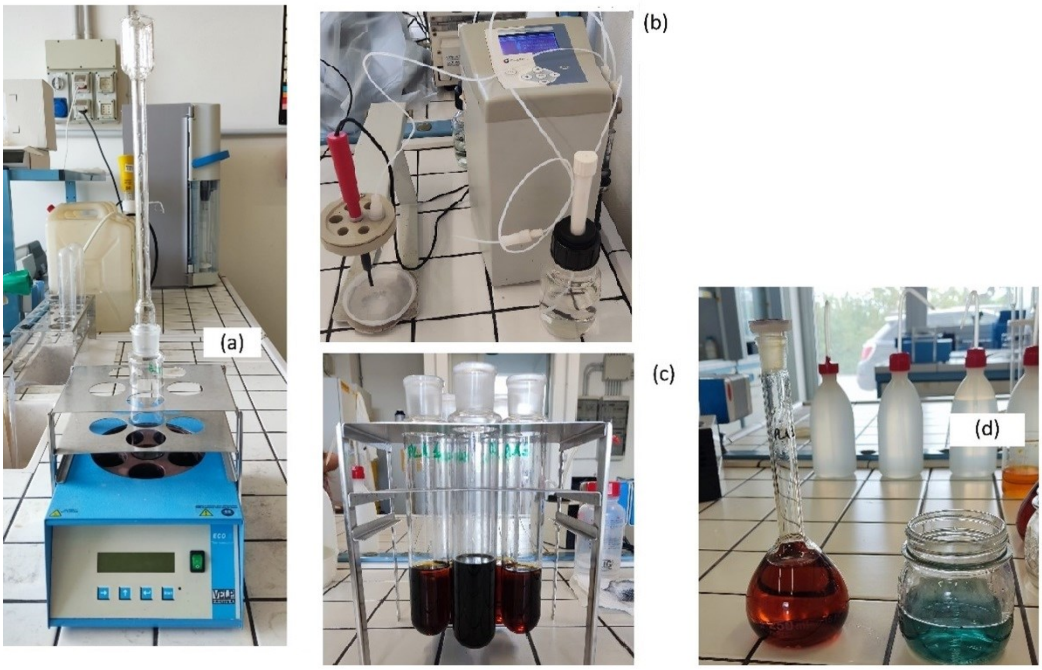


Figure 4.2.8: Digester (a), automatic titrator (b), samples exiting form the digestor (c), samples before (left) and after (right) the titration (d).

VII. NOx analyzer. A UV-Vis spectrophotometer (Figure 4.2.9) has been used for the measurement of nitrites and nitrates. Inside the liquid samples some chemicals are added. These chemicals react with NOx, coloring the solution in fuchsia, for nitrites (NO_2) and in yellow for nitrates (NO_3). Then the samples are placed into the UV-Vis spectrophotometer which reads the absorbance (ABS) and therefore the nitrites and nitrates concentration. This concentration is measured because some inorganic compounds, such as nitrogen, can be bio-oxidized by autotrophic microorganisms causing an additional oxygen consumption to that necessary for heterotrophic microorganisms to biodegrade the organic substance, causing an overestimation of the measured BOD values.

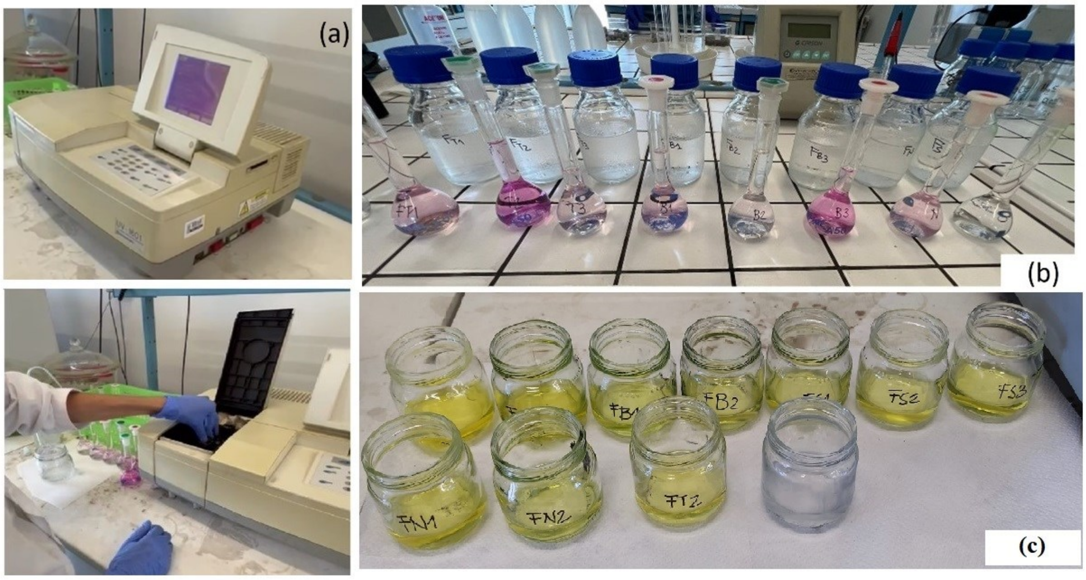


Figure 4.2.9: UV-Vis spectrophotometer (a), samples used to measure the $\mathrm{NO_2}$ content becoming fuchsia (b), samples used to measure the $\mathrm{NO_3}$ content becoming yellow (c).

4.2.2.2 Materials preparation

To identify the mass of materials to put inside each flask, the only guideline provided by the BS EN ISO 14851:2019 is to "use a test material concentration of at least 100 mg/l, corresponding to a ThOD of about 170 mg/l or a TOC of about 60 mg/l". This affirmation is valid only for the test material, the standard in fact does not specify the ThOD, or the TOC, nor the minimum concentration for both the positive and the negative controls. It only requires that they have a shape and a TOC value comparable to those of the test material. Nevertheless, it has been decided for positive and negative control materials (starch and HDPE) to impose the same ThOD value used for calculating the PLA mass.
So, the first thing to do consists in the calculation of the ThOD of PLA, HDPE and starch using the formula provided by the standard BS EN ISO 14851:2019 within the Annex A:

$$ThOD\left(\frac{mg\ ThOD}{g\ substance}\right)=\frac{16[2c+0.5(h-cl-3n)+3s+2.5p+0.5na-o]}{Mr} \quad (4.2.5)$$

Where:

the formula of the substance is the following " CcHhClclNnSsPpNanaOo ".
Mr is the molar mass in g/mol.

Using the molecular formula and the molar mass of the three substances the ThODs have been calculate (Table 4.2.3).

Table 4.2.3: Molecular formula, molar mass and ThOD of the three substances used in the experiments.

Substance	Molecular formula	Mr (g/mol)	ThOD (mg/g)
PLA	$(C_3H_4O_2)_n$	72.063	1332.168
HDPE	$(C_2H_4)_n$	28.054	3421.972
Starch	$(C_6H_{10}O_5)_n$	342.20*	561.0754

*this molar mass is taken from the technical datasheet of the starch used, it is not calculated using the stoichiometrical formula.

Now, imposing a ThOD of 170 mg/l and knowing the liquid volume in each flask (300 mL), the minimum quantity of PLA to be placed in the flasks was calculated using formulas 4.2.6 and 4.2.7. The same calculations were also done for starch and HDPE (Table 4.2.4). Considering that the minimum amount of HDPE calculated is very low, it has been decided to put an amount of HDPE comparable to the amount of PLA used.

$$ThOD_{material}\left(\frac{mg\ ThOD}{g_{material}}\right) * x\left(\frac{g_{material}}{l}\right) = 170\ \frac{mg\ ThOD}{l} \longrightarrow x\left(\frac{g_{material}}{l}\right) = \frac{170}{ThOD_{material}} \quad (4.2.6)$$

$$g_{material} = x\left(\frac{g_{material}}{l}\right) * liquid\ volume\ of\ the\ flasks(l) \quad (4.2.7)$$

Table 4.2.4: Minimum quantity of PLA, HDPE and starch needed to satisfy the ThOD minimum value imposed by the standard (using a flask with a liquid volume of 300 mL) for both the preparatory tests.

Substance	g of material
PLA	0.0382836
HDPE	0.0149037
Starch	0.090896874

The amount of material placed in each of the eight flasks used in the first experiment is presented in the Table 4.2.5 (only eight flasks have been used because the positive control was not available), while the amount placed in each of the ten flasks used in the second one is presented in Table 4.2.6 (only ten flasks have been used because one broke during the experiment). The position of the flasks inside the Sapromat, for the first and the second experiments, is shown in Figure 4.2.10 and in Figure 4.2.11, respectively.

Table 4.2.5: Grams of material placed into each flask in the α experiment (the values for the flasks of the blank – FB – has not been reported in the table because in those flasks no material was added).

Flask	**Material**	**g of material**
αFT1	PLA	0,0479
αFT2	PLA	0,0687
αFT3	PLA	0,0496
αFS	PLA	0,0507
αFN	HDPE	0,0609

Table 4.2.6: Grams of material placed into each flask in the β experiment (the values for the flasks of the blank – FB – has not been reported in the table because in those flasks no material was added).

Flask	**Material**	**g of material**
βFT2	PLA	0.0493
βFC2	Starch	0.0896
βFC3	Starch	0.0903
βFN1	HDPE	0.0688
βFN2	HDPE	0.0685
βFS1	PLA	0.0546
βFS2	PLA	0.0501
βFS3	PLA	0.0494

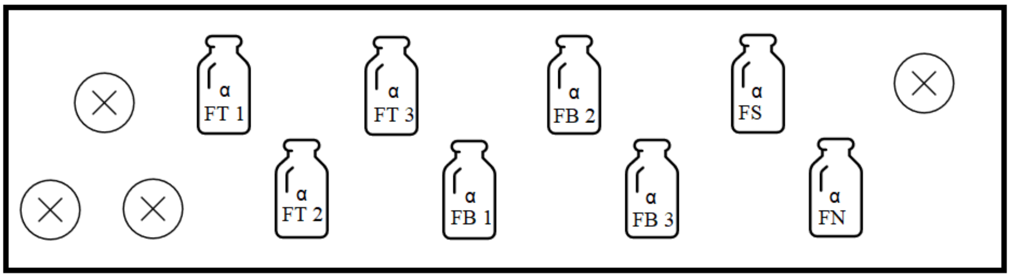


Figure 4.2.10: Position of the 8 flasks inside the respirometer in the experiment α (the circles containing the "x" indicate a position not in use).

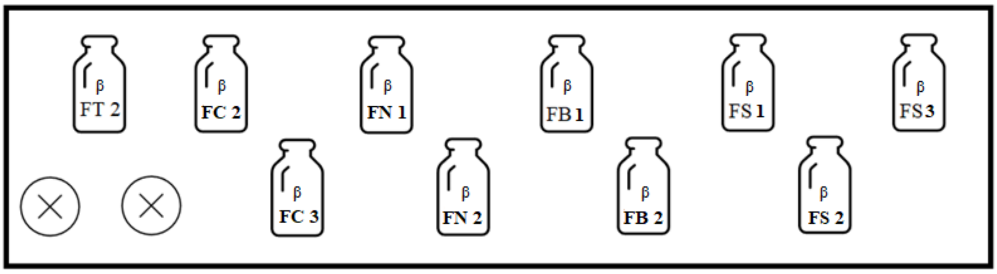


Figure 4.2.11: Position of the ten flasks inside the respirometer in the experiment β (the circles containing the "x" indicate a position not in use).

The test medium has been prepared as indicated in the standard, the incubation temperature used was 20°C, without stirring (due to a malfunction of the machinery). In each flask in which the inoculum should be present, an inoculum quantity equal to 3% of the total liquid volume was added in the first experiment, while equal to 5% in the second one. The percentage by volume of the added inoculum has been changed from the first to the second test, because the ISO standard gives the possibility to add a percentage between 3% and 5%, and we wanted to evaluate which one was the best percentage of inoculum to use, then, in the third experiment (γ).

4.2.2.3 Initial and final analyses

At the beginning and at the end of both the preparatory tests, the analysis presented in Table 4.2.7 and Table 4.2.8 have been performed. Devices and methods used for the analyses are summarized and listed in Table 4.2.9.

Table 4.2.7: Initial analysis performed for the experiments α and β. (TM: Test Medium; SI: Supernatant Inoculum; TM+SI: Test Medium mixed with Supernatant Inoculum; UI: Unaltered inoculum; PLA: polylactic acid).

α		**β**	
Analysis	***Analysed medium***	***Analysis***	***Analysed medium***
pH	TM+SI	pH	TM+SI
TSS	UI; SI		
TS and VS	UI; SI; PLA	TS and VS	UI; SI; TM+SI
TOC	UI; SI; TM+SI; PLA		
DOC	UI; SI	TOC	UI; SI; TM+SI; Starch
COD	PLA		

It was decided to measure the TSS only once, since the inoculum used in the two experiments was the same, not to measure the DOC in the second test, since the measured TOC value in the same test was very low (the TOC is always greater than the DOC) and to measure only once the COD of the PLA, since the polymer used in the two experiments is the same.

Table 4.2.8: Final analysis performed for the experiments α and β. (TM: Test Medium; SI: Supernatant Inoculum; TM+SI: Test Medium mixed with Supernatant Inoculum; UI: Unaltered inoculum; PLA: polylactic acid; HDPE: High-Density Polyethylene).

α		**β**	
Analysis	***Analysed medium***	***Analysis***	***Analysed medium***
pH	TM+SI	pH	TM+SI
Nitrate and Nitrite	TM+SI	Nitrate and Nitrite	TM+SI
TS and VS	TM+SI	TS and VS	TM+SI

TOC	TM+SI	TOC	TM+SI
Final weight	PLA; HDPE	Final weight	PLA; HDPE

Table 4.2.9: Devices and methods used for operation, monitoring and analytical activities in the preparatory tests.

Analysis	**Device**	**Method**
TOC (%)	TOC -V CSN Shimadzu Italia S.R.L	UNI EN 13137
DOC (%)	TOC -V CSN Shimadzu Italia S.R.L	UNI EN 13137
COD (%)	ECO 6 Thermoreactor – VELP SCIENTIFICA	IRSA - CNR Q. 29/03 vol. 2 n. 5130
U (%) and TS (%)	Suprema 600 Stoven Benjamin by Vittadini	IRSA - CNR Q. 64/84 vol. 2 n. 2
VS (%)	Gefran 1001 Muffle	IRSA - CNR Q. 64/84 vol. 2 n. 2
pH	Crison pH-meter GLP 22	IRSA - CNR Q. 64/85 vol. 3 n. 1
RI (mgO_2/l)	Sapromat E Voith Sulzer Papertechnology	Sapromat method
Nitric nitrogen (NO_3^-)	UV-1601, UV-Visible Spectrophotometer SHIMADZU	IRSA - CNR Q. 29/03 vol. 2 n. 4040A1
Nitrous nitrogen (NO_2^-)	UV-1601, UV-Visible Spectrophotometer SHIMADZU	IRSA - CNR Q. 29/03 vol. 2 n. 4050
TSS (%)	Suprema 600 Stoven Benjamin by Vittadini	IRSA - CNR Q. 29/03 vol. 1 n. 2090B

4.2.2.4 Meaning of the measured parameters

SOLIDS

The word “solids” refers to the dry matter that remains as residue after the evaporation inside an oven at 105°C until there are no more variations in the weight of the sample. The residues are called “Total Solids” (TS). They can be expressed as:

- for solid sample: $TS\ (\%) = \frac{\text{(gross weight after 105 ° C−crucible weight)}}{\text{raw weight}}$ (4.2.8)
- for a liquid sample: $TS\ (mg/l) = \frac{\text{(gross weight after 105 ° C−crucible weight)}}{sample\ volume}$ (4.2.9)

Total Solids can be further classified according to:

- their response to ignition, as Volatile Solids (VS) and Non-Volatile Solids (NVS).
- their response to sedimentation, as Settleable Solids and Non Settleable Solids.
- their response to filtration (size selection), as Total Suspended Solids (TSS) and Dissolved Solids (DS).

VS are those solids that pass into the gaseous phase in a temperature range between 550-900 °C., This occurs because of oxidation and volatilization of the organic substance. The materials that remain inside the crucible are Non-Volatile Solids (or ash). Volatile solids represent an estimate of the organic matter content of the sample. They can be expressed as:

- for solid sample: $VS(\%) = \frac{(\text{gross weight after 105°C}-\text{gross weight after 550°C})}{(\text{gross weight after 105°C}-\text{crucible weight})}$ (4.2.10)
- for a liquid sample: $VS\ (mg/l) = \frac{(\text{gross weight after 105 ° C}-\text{gross weight after 550°C})}{sample\ volume}$ (4.2.11)

Total Suspended Solids (TSS) are those, according to the Italian legislation, that are retained by a filter of porosity of 0.45μm. Therefore, they are found as residues on the membrane. The particles passing through the filter are called Dissolved Solids (DS), and they are dissolved into the liquid.

For the calculation of the TSS, first of all, a virgin membrane is weighed. After the filtration process and after the drying process in the oven at 105 °C, the membrane (presumably with some solid particles on it) is weighted again. TSS measure is obtained by the difference between these two weights, according to the formula (4.2.12).

$$TSS\ (\frac{mg\ TSS}{l}) = \frac{(gross\ weight\ after\ 105°C-membrane\ weight)}{sample\ volume} \qquad (4.2.12)$$

The value of the TSS must always be lower than the one of the TS because the TS also include the dissolved solids.

ORGANIC SUBSTANCE

The Theoretical Oxygen Demand (ThOD) expresses the stoichiometric oxygen demand necessary for the complete oxidation of an organic compound. Its evaluation is based on the knowledge of the molecular formula of an organic compound, assuming that all the carbon presents will be oxidized into CO_2 and that all the nitrogen presents will be oxidized into NO_3.

The Total Organic Carbon (TOC) measure is used to evaluate the total organic carbon content of a sample. It is the quantity of CO_2 that is formed by the combustion of the organic substance. CO_2 is detected and measured directly by an infrared analyser (TOC analyser). This analyser measures TOC as the difference between total carbon (TC) and inorganic carbon (IC). Some organic compounds, however, may not be completely oxidized, so the carbon value obtained with this analysis may be lower than that actually present in the sample.

The Dissolved Organic Carbon (DOC) is measured as the TOC but in this case, before the measure, the sample il filtrated with a 0.45 μm membrane, and the filtered liquid, rich in dissolved solids, is used for the measure of the DOC. TOC is always greater than DOC because it includes also the suspended organic carbon.

The Chemical Oxygen Demand (COD) test is used to measure the total content (both biodegradable and non-biodegradable) of organic compounds. It is based on the fact that all the organic matter can be oxidized by a strong oxidizing agent to CO_2 and H_2O under acid conditions. To measure it, the material to be analysed is mixed, in a sample tube, with mercury sulphate ($HgSO_4$), an oxidizing solution, a concentrated H_2SO_4 and silver sulphate (Ag_2SO_4). It is then connected to an air cooler, and to the anti-splash bell and it is placed in a digester. The content of the sample tube is then titrated with Mohr's salt. The titrator provides the volume of the used titrant, therefore, to calculate the COD value, firstly, the exact titer of Mohr's salt has to be determined (formula 4.2.13) and then the formula 4.2.14 has to be used in order to calculate the mg of O_2 per kg of PLA.

$$Ntit = \frac{Nbic * Vbic}{Vtit} \qquad (4.2.13)$$

Where:

Ntit: normality of Mohr's salt;
Nbic: normality of potassium dichromate;
Vbic: volume, in ml, of potassium dichromate;
Vtit: volume, in ml, of Mohr salt used for the blank.

$$COD = \frac{(Va - Vb) * Ntit * 8000}{Mc} \qquad (4.2.14)$$

Where:

Va: volume, in ml, of titrant used for the blank;
Vb: volume, in ml, of titrant used for the sample;
Ntit: normality of Mohr's salt;
8: equivalent weight of oxygen;
Mc: weight, in g, of the used sample.

NITRITE AND NITRATE

Nitrite and nitrate have also to be measured in order to assess whether the nitrification process occurred. Ammonium salts and nitrogen-containing test compounds can be bio-oxidized, by Nitrosomonas and Nitrobacter microorganisms, using oxygen as final electron acceptor, causing an overestimation of the oxygen consumed by heterotrophic microorganisms, for the biodegradation of the organic matter. The standard expressly requires the calculation of these concentrations (formula 4.2.15 and 4.2.16), and when they are high, a correction of the BOD values obtained has to be done, by removing the oxygen consumed for the nitrification process.

$$BOD_N = (\rho_{NO3} \times 4{,}57) + (\rho_{NO2} \times 3{,}43) \quad (4.2.15)$$

Where:

ρ_{NO3}: is the measured concentration of nitrate-nitrogen (N-NO3) in the flasks FT at the end of the test, in milligrams per litre;

ρ_{NO2}: is the measured concentration of nitrite-nitrogen (N-NO2) in the flasks FT at the end of the test, in milligrams per litre;

4,57: is the oxygen demand factor for the formation of nitrate;

3,43: is the oxygen demand factor for the formation of nitrite.

$$BOD_C = BOD_G - BOD_N - BOD_{Bt} \quad (4.2.16)$$

Where:

BOD_G: is the measured BOD of the flasks F_T at the end of the test, in milligrams per litre;

BOD_{Bt}: is the BOD of the blanks F_B at the end of the test, in milligrams per litre.

BOD_C: corresponds to BOD_t and it is used for calculating BOD_S and D_t, see Formulae (4.2.1) and (4.2.2) in §4.2.1.2.

4.2.3 Official Test (γ)

The third experiment started on 05/10/2022, and it lasted 37 days. Also in this case the followed standard was the BS EN ISO 14851:2019 and the materials used were the same as in the previous experiments, but a second positive control, cellulose in powder form, has been used, in addition to the firs one. The respirometric test has been performed in an external laboratory, outside the university, since unexpected results have been obtained in the first two experiments (carried out in the LISA laboratory of the University of Padova). The owner of the laboratory is the S.E.S.A. S.P.A. company, in Monselice (PD). The respirometer used was "Gas Endeavour" produced by BPC INSTRUMENTS. The liquid volume of the flasks was 750 ml. The reactors have been kept in a bath at 20°C, in the dark, and they have been stirred through an automatic apparatus working in continuous. The inoculum has been taken from the recirculation well of the secondary settle tank of the domestic wastewater treatment plant in Ca’ Nordio (Padova, Italy) on 03/10/2022. The choice of using the inoculum taken from the recirculation well derived from experimental consideration by comparing α and β test results. All the initial and final analysis were carried out at the LISA laboratory in Voltabarozzo (Padova, Italy).

At the end of the experiment, in order to understand if microorganisms had developed both in the liquid phase and in adherence to the plastic granules, some microbiological analyses have been performed: both the liquid phase has been observed through a fluorescent microscope, while both the liquid phase and the plastic granules have been used for the production of bacterial cultures through a procedure called "Serial Dilution". The microbiological analyses have been done in the Department of Agronomy Food Natural Resources Animals and Environment (DAFNAE) of the University of Padova.

In addition, collaborating with the Industrial Engineering department (DII) of the University of Padova, the Differential Scanning Calorimetry (DSC) andthe Fourier-transform infrared spectroscopy (FT-IR) analysis have been performed, to understand, if on a physical level, the bioplastic granules have been affected by biodegradation processes.

4.2.3.1 Apparatus used

The respirometer used in the ϒ test works differently from the one used in the α and β tests. It is composed of three units (Figure 4.2.12): a sample incubation unit with mechanical agitation, composed by 15 glass reactors which can be mixed at adjustable interval, speed, and rotation directions, (a); a gas absorption unit, which is composed by 15 gas trap bottles filled with 80 ml of NaOH, used to trap the CO_2 produced by microorganisms during the biodegradation process (b); the flow meter array and the DAQ unit, which is composed by 15 independent gas flow measurement units and built-in sensors, able to detect how much volume of oxygen has been fed into the flasks. They have a measuring resolution of 2 or 9 ml (in the experiment 2 ml has been used). After that the volume of oxygen has been detected, the little green triangle inside (Figure 4.2.13) rises, and the 2 ml of oxygen required by the flasks are recorded by the software. At the same time in which the 2 ml of oxygen pass from this unit to the flasks, the green triangle goes down, and so on (c).

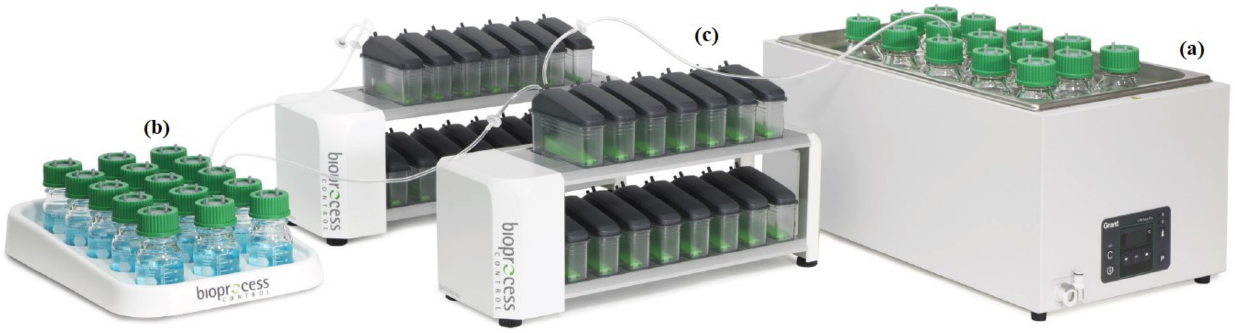


Figure 4.2.12: Gas Endeavour respirometer components.

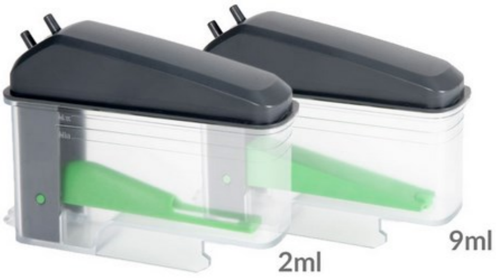


Figure 4.2.13: Zoom of one of the gas flow measurement units. The green part moves when the oxygen enter inside the transparent container. It can have a sensitivity equal to 2 or 9 ml of gas volume.

The flow meter array and the DAQ unit are connected to oxygen containing bags and to the 15 reactors present in the sample incubation unit, which are, in turn, connected to the gas absorption unit. When oxygen is consumed by the microorganisms present inside the flasks, a vacuum is generated which triggers the supply of oxygen: the oxygen exits from the bags, enters in the flow meter array and the DAQ unit, where of the volume of oxygen (2 ml) that enters in the flasks are detected by a software, and finally reaches the flasks, where it is used by microorganisms and converted into carbon dioxide, which escape form the reactors and is trapped in the gas absorption unit. The amount of oxygen used is provided in Nml (at 20°C and 1 atm), and then is converted into mg of oxygen consumed through the use of the molar weight of oxygen and the equation of state of ideal gases.

To investigate the microorganisms presence in each flask at the end of the experiment, some microbiological analyses have also been performed. The first microbiological analysis consisted in the observation of the microorganisms found in the liquid samples extracted from the flasks through the use of a manual fluorescence microscope. One milliliter of liquid was taken, centrifuged, and placed inside a vortex to concentrate the sample in order to obtain solid pellets, which have been observed at the microscope. This analysis was done on the flasks FT1, FC1, Fce1, FB3, FN1, FI and FS2. The microscope used was the Olympus BX60 (Figure 4.2.14), the lens used was the 100x.

Figure 4.2.14: Olympus BX60 microscope (https://webstore.diaginc.com/v/vspfiles/photos/WS-BX60-0170-2.jpg).

Furthermore, bacterial viability was tested using the Live/Dead BacLight bacterial viability kit (Molecular Probes Inc., Eugene, OR, USA). The kit contains two dyes, Syto 9 and propidium iodide, both staining DNA. Propidiun iodide enters only cells with damaged membrane (red staining), while Syto 9 labels cells with intact or damaged membrane (green staining). The two dyes were inserted into the liquid samples analysed under the fluorescence microscope, and in particular for the liquid contained in the flasks FT1, FC1, Fce1 and FS2. This coloring tests is useful in order to understand if the microorganisms are alive (green colored microorganisms) or dead (red colored microorganisms). TDC (total direct count) and viability were observed by fluorescence microscopy (Olympus fluorescent microscope BX60) equipped with a blue 420-nm exciter filter (Olympus BP 490) using an objective 100X.

In addition, bacterial cultures have been made, using the technique of the "Serial dilutions", with scalar dilutions up to the dilution level -5. Serial dilution is a systematic reduction of a known or unknown entity (a solute, an organism, etc.) by successive resuspension of an initial solution (solution0) in fixed volumes of a liquid diluent (blanks). These blanks are usually made from 0.45% saline (distilled water and sodium chloride). 1 ml of solution0 (that is, the liquid contained in each analysed flask) is taken and added to 9 ml of physiological solution, obtaining solution1. This solution is at dilution -1 because it contains 1/10 of the initial solution and 9/10 of the blank. Then, 1 mL of solution1 is added to another 9 mL of saline to produce the solution at dilution -2 (solution2). And so on until the -5 dilution is reached. These analyses have been made both by spreading the liquid and the plastic granules (coming from the ϒ test) on the Plate Count Agar (PCA). The bacterial cultures have been done for the same flasks analysed under the microscope. The culture medium used is the PCA above which 100 microliters of liquid and the granules taken from the various flasks are placed at the different dilution levels (from -1 to -5) and undiluted (therefore as it is). Finally, the plates are placed in an incubator at 30 ° C for 48 hours.

After the microbiological analysis, also some plastic characterization tests have been performed, in order to investigate the physical degradation of the plastic granules after the ϒ experiments. These tests consisted in the FT-IR analysis and in the DSC analysis, which are described hereafter. In the FT-IR analysis procedure, plastic samples are subjected to infrared radiation (IR). When a molecule is hit by an IR radiation at a certain frequency, the energy released by the radiation is converted into rotational and vibrational energy and the molecule can rotate or vibrate. Not all the times and not all the molecules vibrate if irradiated, but instead the vibration occurs only if the frequency of the radiation has a very precise value that depends on the chemical characteristics of the molecule under investigation. Plotting the absorbance and/or transmission of energy of the material’s chemical bonds (y-axis) against the wavenumber (x-axis), the FT-IR spectrum of the material under investigation is obtained. The intensity of the obtained peaks depends on how much the radiation has been absorbed/transmitted (Grossule at al., under publication). Peak wavenumbers in the FT-IR spectra reveal details on the material's chemical composition (Nandiyanto et al., 2019). The peak intensity variations represent qualitative data able to suggest that some chemical modifications have occurred to the polymeric structure during the degradation process (Grossule at al., under publication). FT-IR spectra were collected in attenuated total reflection mode (ATR) using a Thermo Scientific™ Nicolet™ iS™50 FT-IR Spectrometer with 2 cm^{-1} spectral resolution (Figure 4.2.15). Procedurally, first of all the background is acquired by recording the environmental spectrum with an empty sample port, which will be automatically subtracted from the spectrum of bioplastics by the machine itself. Then the plastic granules have been placed in the sample holder, and the analysis has begun. These analyses were performed for the virgin PLA and HDPE granules and for the granules extracted from the FT2, FS1, FN1 and FI flasks at the end of the experiment.

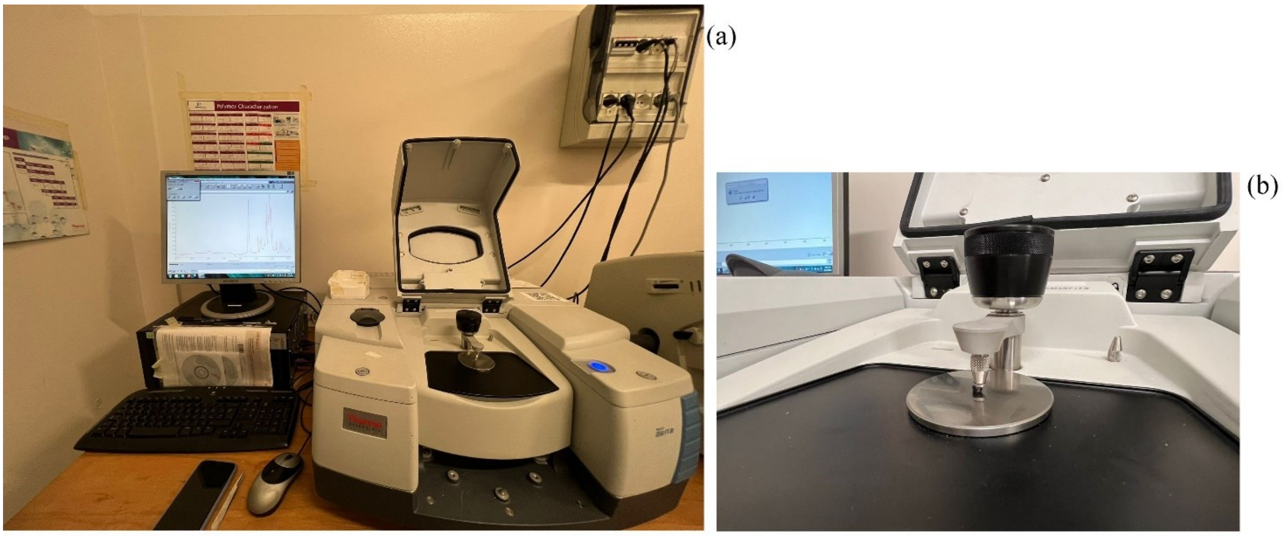


Figure 4.2.15: FT-IR machine (a) with a focus on the sample holder where a HDPE granule has been inserted (b).

The DSC analysis enables to examine the physical changes of the plastic material in response to a temperature variation, by plotting the heat flow absorbed or released by the material against temperature. The sample is subjected to a first heating cycle (to erase the thermal history to which the material is subjected during its processing), then to a cooling cycle and, in turn, to a second heating cycle. During the heating and cooling cycles, endothermic (heat absorbed) and exothermic (heat released) heat flows can be detected. When semicrystalline substances are analysed, as the PLA and the HDPE, the crystalline portion experiences endothermic reactions during the heating cycle, until it melts at its melting temperature (Tm) and exothermic reactions during the cooling cycle, until it crystallizes (at its crystallisation temperature, Tc). On the other hand, the amorphous fraction undergoes glass transition, going from a stiff glassy state below Tg to a rubbery solid state above Tg. By a comparison of the temperature melting peaks in the third heating cycle of a virgin granule and of a biodegraded one, the presence of bio-degradation phenomena can be supposed, as well as the presence of additives in the polymer formulation (Grossule at al., under publication).

Being both PLA and HDPE semicrystalline polymers, they have both the Tg and the Tm (amorphous polymers do not melt). Differential Scanning Calorimetry (DSC) was performed using a heat-cool-heat cycle, from 0°C to 250°C, being the Tg and the Tm of HDPE, respectively equal to -80/90°C and 130°C and those of PLA equal to 50/60°C and 210°C (the Tg of HDPE is not detected with the chosen temperature range). The heating rate and the cooling rate chosen are both of 10°C/min. The test is carried out in an inert atmosphere. Procedurally, a portion of the sample with a mass between 5 and 20 mg is placed inside a sample holder made of aluminium, which is placed in the machine, thus starting the analysis. This analysis has been performed for the virgin PLA and HDPE granules and for those extracted from the FT2, FS1, FN1 and FI flasks at the end of the experiment. The machine used is the DSC Q200 (Figure 4.2.16).

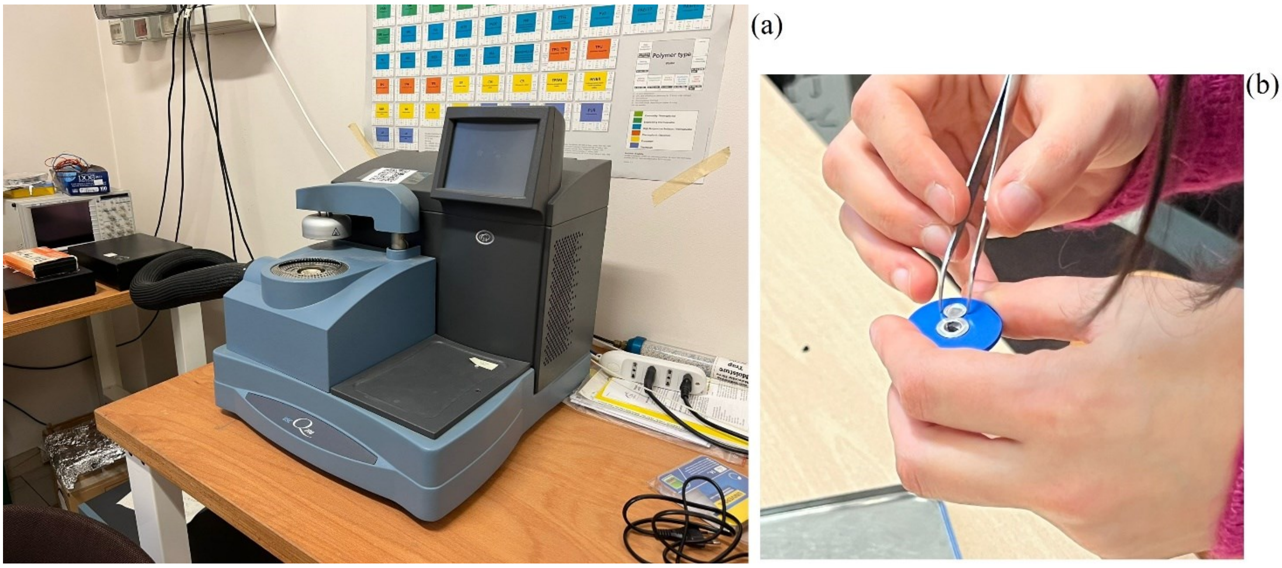


Figure 4.2.16: DSC machine (a) and the sample holder made of aluminium with inside a sample of HDPE (b).

The other instruments used are the same as described in the sub-paragraph § 4.2.2.1.

4.2.3.2 Materials preparation

To identify the mass of materials to put inside each flask, the same procedure and calculations described in subsection § 4.2.2.3 has been used. In this experiment, also cellulose is present as a positive control, in addition to starch. Its molecular formula and the ThOD are the same of those of starch (Table 4.2.10).

Table 4.2.10: Molecular formula, molar mass and ThOD of the substances used in the experiment.

Substance	**Molecular formula**	**Mr (g/mol)**	**ThOD (mg/g)**
PLA	$(C_3H_4O_2)_n$	72.063	1332.168
HDPE	$(C_2H_4)_n$	28.054	3421.972
Starch	$(C_6H_{10}O_5)_n$	342.20*	561.0754
Cellulose	$(C_6H_{10}O_5)_n$	342.20*	561.0754

*this molar mass is taken from the technical datasheet of the starch used, it is not calculated using the stoichiometrical formula.

Being the liquid volume of the flasks higher respect to those used in the in the first two experiments (750 ml), the minimum amount of PLA, HDPE, starch and cellulose needed to satisfy the ThOD imposed by the ISO (calculated following the equation 4.2.7) is greater (Table 4.2.11).

Table 4.2.11: Minimum quantity of PLA, HDPE, starch and cellulose needed to satisfy the ThOD minimum value imposed by the standard (using a flask with a liquid volume of 750 mL).

Substance	g of material
PLA	0.095709
HDPE	0.037259
Starch	0.227242
Cellulose	0.227242

The amount of material placed in each of the 15 flasks used in the first experiment is presented in Table 4.2.12. Also in this case, it was decided to use a quantity of polyethylene comparable to the one of PLA, even though the minimum quantity required is lower. The position of the flasks inside the Gas Endeavour respirometer is shown in Figure 4.2.17.

Table 4.2.12: Grams of material placed into each flask (the values for the flasks of the blank – FB – has not been reported in the table because in those flasks no material was added).

Flask	Material	g of material
γFT1	PLA	0.0971
γFT2	PLA	0.1171
γFT3	PLA	0.1116
γFC1	Starch	0.2964
γFC2	Starch	0.2806
γFN1	HDPE	0.1079
γFN2	HDPE	0.1016
γFS1	PLA	0.1094
γFS2	PLA	0.1106
γFI	PLA Starch HDPE	0.1104 0.2934 0.1110
γFCe1	Cellulose	0.2620
γFCe2	Cellulose	0.2649

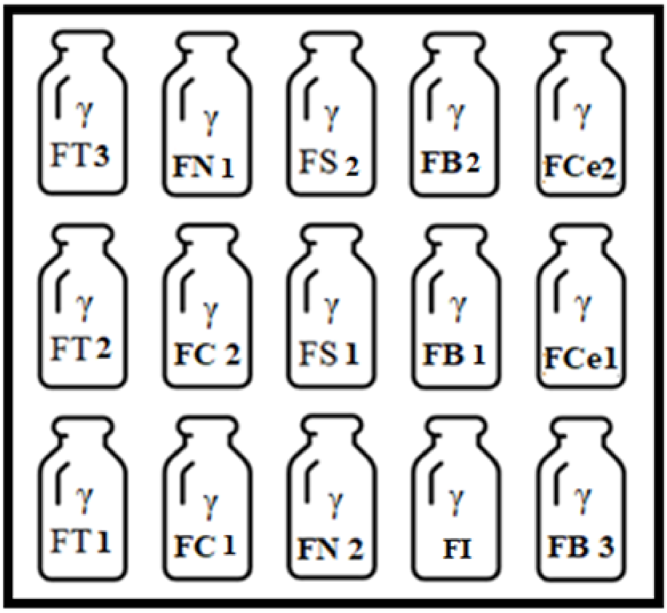


Figure 4.2.17: Position of the 15 flasks inside the respirometer.

The test medium has been prepared as indicated in the standard, the incubation temperature used is 20°C and mechanical stirring was provided. In each flask in which the inoculum should be present, an inoculum quantity equal to 5% of the liquid volume was added, as in the β experiment.

4.2.3.3 Initial and final analyses

At the beginning of the test the following analyses have been done:

- pH of the test medium mixed with the supernatant inoculum.
- Total Solids (TS) and Volatile Solids (VS) of the inoculum in an unaltered state, of the supernatant inoculum and of the mixing between the supernatant inoculum and the test medium.
- Total Organic Carbon (TOC) of the test medium mixed with the supernatant inoculum.
- TOC of cellulose and HDPE.

At the end of the test the following analyses have been done:

- TS and VS of the test medium mixed with the supernatant inoculum for each of the flasks tested.
- TOC of the test medium mixed with the supernatant inoculum.
- Nitrites and nitrates of the test medium mixed with the supernatant inoculum
- pH in each of the flasks tested.
- Final weight of the PLA and HDPE granules.

5. RESULTS AND DISCUSSION

In this chapter the results obtained through the scientific review, the three oxygen consumption experiments, the microbiological analyzes (ϒ experiment) and the plastic characterization analyzes (ϒ experiment) will be presented.

5.1 Scientific review

The data, already divided between fresh and salt water and between biobased, partially biobased and fossil-based polymers, have been further subdivided according to the test temperature, since it is considered one of the most important control parameters of the biodegradation process (Folino et al, 2020; Zhu et al., 2020).
Analyzing the number of tests done on site and those done off site, it was found that, in the case of freshwater, most of the tests have been done off site (68.00%), while in the case of saltwater, the on-site tests have been the most performed (55.60%). The preference for on-site tests, in the case of saltwater, may be due to the difficulty of reproducing the marine environment in the laboratory (temperature, water composition, microbial community) and due to the desire to understand what happens to bioplastics if released in a real natural environment. The temperature range and the average temperature for in situ and off-site tests carried out in salt and fresh water has been summarized in Table 5.1.1.

Table 5.1.1: Temperature range and average temperature for in site and off-site tests carried out in salt and fresh water (in the case of in situ tests, it is impossible to calculate the average temperature, due to the periodic variations of the operating temperature; N/A = non-available data).

	Freshwater		**Saltwater**	
	In Situ	*Off Site*	*In Situ*	*Off Site*
Temperature range (°C)	3.5-31	20-60	-1.7-32	10-60
Average Temperature (°C)	N/A	26	N/A	24.50

These results shown a wide variability of the used temperature in the various in site and off-site tests. Following the literature review, the average temperature of the tests carried out off site in both aquatic environments is around 25 °C, therefore this temperature has been chosen for the division and the representation of the various biodegradation rates: the results of the tests carried out at a temperature below 25 °C were divided from those of tests carried out at a temperature higher than (or equal to) 25 °C.
The results representation has been divided according to the type of indicator used to represent the level of biodegradability of the bioplastic. These indicators are:

- Weight Loss (WL) [% of the initial weight];
- Biochemical Oxygen Demand (BOD) [% of the ThOD];
- Biochemical Oxygen Demand (BOD) [mg/l];
- Carbon dioxide evolution (CO_2) [% of the $ThCO_2$];
- Surface Erosion (SE) [% of the initial surface].

Table 5.1.2 gives a quantitative indication of the number of test results expressed through each of the five biodegradation rate indicators listed above.

Table 5.1.2: Number of test results expressed through the five biodegradation rate indicators (the horizontal bars indicate that no tests have been done for that category using that type of indicator).

			WL	**BOD (%)**	**BOD (mg/l)**	**CO_2**	**SE**
BIOBASED	**FW**	T<25°C	15	-	4	-	-
		T≥25°C	18	8	-	-	-
	SW	T<25°C	69	-	-	1	12
		T≥25°C	45	15	2	2	-
PARTIALLY BIOBASED	**FW**	T<25°C	-	-	2	-	-
		T≥25°C	-	19	-	-	-
	SW	T<25°C	54	-	-	-	2
		T≥25°C	19	9	7	4	1
FOSSIL BASED	**FW**	T<25°C	1	-	-	-	-
		T≥25°C	2	5	-	4	-
	SW	T<25°C	35	-	-	-	-
		T≥25°C	8	8	2	-	-

As already discussed, the definition of biodegradability is set within the standards regarding compostability (like ISO 18606:2013 and EN 13432:2000). In particular, a material is defined as “biodegradable in aerobic conditions” if it is converted into CO_2, water and biomass, reaching a biodegradation level of 90% within six months. The European standard 13432 recommends to verify the conversion of the tested material into CO_2, following the ISO 14855:2004 or, in case of polymeric material, using the ISO 14851:2021 or the ISO 14852:2019, which respectively measure the biodegradation in terms of oxygen consumption (BOD) and of CO_2 evolution.

As can be seen form Table 5.1.2 most of the results have been provided in term of weight loss, which is a parameter that gives information regarding the material degradation (including both the biotic and abiotic parts), but that is never cited in the norms mentioned above.

In some tests the results are expressed both as Weight Loss (WL) and as Surface Erosion (SE), which is in a sign of disintegration of the material. This latter parameter is considered by the standard EN 13432:2000, as a parameter important to be evaluated in order to verify whether a material can be considered as compostable or not.

Due to the previous discussion and since the most frequently used indicators are WL and BOD (%), the elaboration of data is hereafter presented only using these two parameters. Two different graphical representation are also presented: boxplots and points when a number of results higher than 8 are found for that category (therefore in the case in which the number of the results is statistically significant number) and only points in the other cases (Figure 5.1.1).

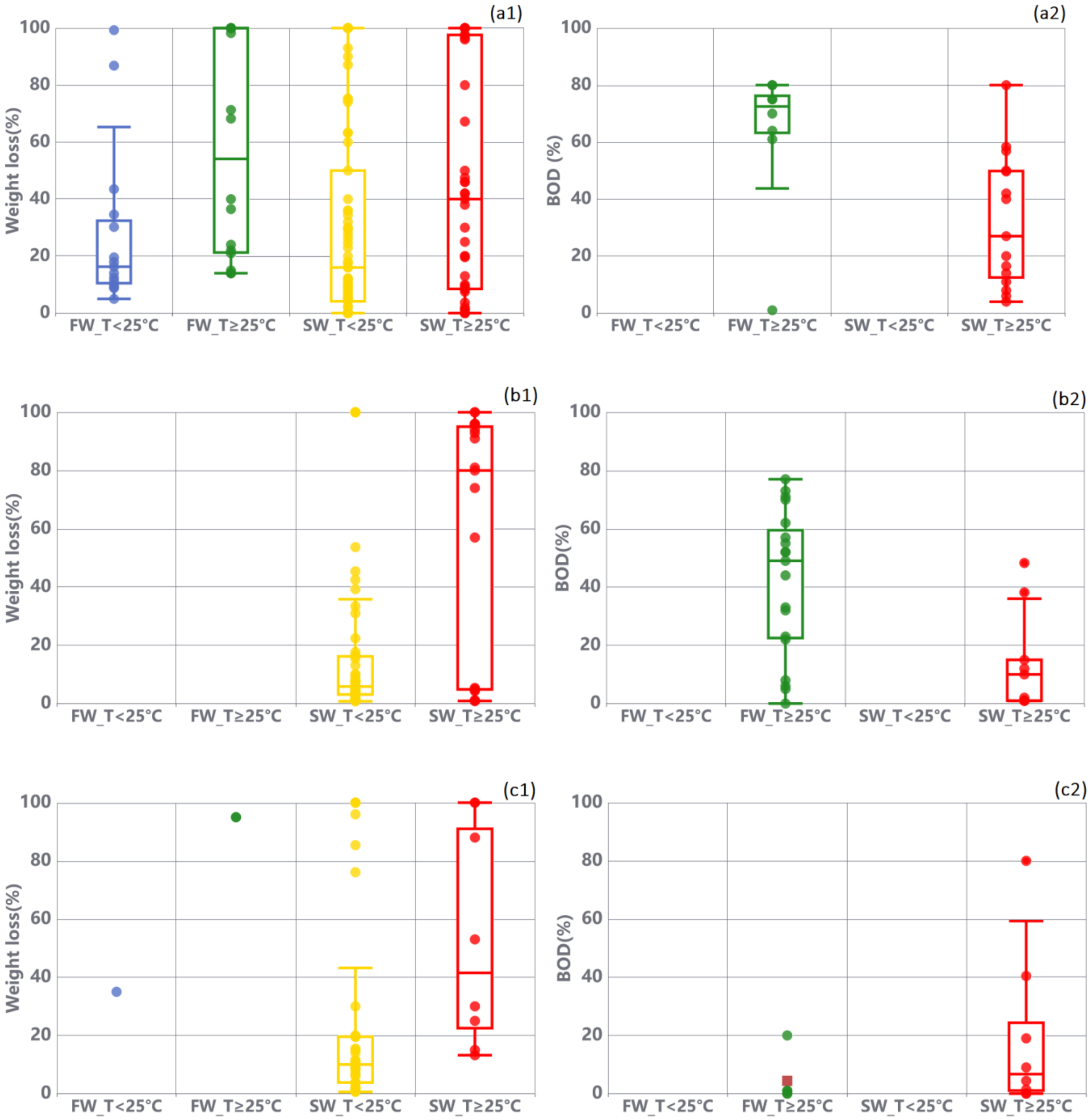


Figure 5.1.1: Results on the biodegradability of bioplastics (biobased (a), partially-biobased (b), fossil based (c)) expressed as weight loss (1) and BOD (%) (2). Each point represents a test result. Boxplots

was used only if the number of results was higher than 8. The red square (c2) or the horizontal lines represents the average value.

Speaking about the biodegradability rate of bioplastics great variability has been observed both in terms of weight loss and BOD (%). Most of the examinated tests analyze biobased biodegradable bioplastics in all the different conditions (freshwater and saltwater, temperature higher and lower to 25°C). Regarding the partially-biobased and the fossil based biodegradable bioplastics most of the tests have been carried out in saltwater, confirming the interest of scientists regarding the End of Life (EoL) of bioplastics in marine environments.

In Figure 5.1.1 the average value is evidence by the inner horizontal line in the box plots. In general, lower temperatures shows a lower biodegradation rate, in terms of both weight loss and BOD, analyzing the average values. This trend was expected, because with the increase in temperature, the degradative kinetics of microorganisms also increase as has been illustrated by (Miksch et al., 2022). Moreover, analyzing the graphs (a1), (a2) and (b2), bioplastics in freshwater seems to have a higher biodegradation rate both in terms of WL and BOD, by comparing the average values, in the same temperature range. This may occur because the marine environment could result more inhospitable for the microbial community, respect to the freshwater environment (Chen et al., 2019).

Table 5.1.3 shows the duration of the tests, in days, from which a high variability can be seen: the average duration of the tests varied between 21 and 365 days. This reflects the fact that in the standards regarding the biodegradation into the water environments the criteria for defining a polymer as biodegradable are not unequivocally defined. Only the maximum duration of the tests is given, and therefore some researchers referred to the maximum test duration expressed in those standards, while others to the definition of biodegradable material provided by the composting standards, i.e., the conversion of the 90% of the material into CO_2 in six months.

Table 5.1.3: Test duration in days, for each one of the five indicators (the horizontal bars indicate that no tests have been done for that category using that type of indicator).

			WL		**BOD (%)**		**BOD (mg/l)**		**CO_2**		**SE**	
			ave	**range**	**ave**	**range**	**ave**	**range**	**ave**	**range**	**ave**	**range**
BIOB	**FW**	T<25°C	180	22-480	-	-	25	14-28	-	-	-	-
		T≥25°C	74	15-365	28	21-30	-	-	-	-	-	-
	SW	T<25°C	217	36-600	-	-	-	-	195	195	59	22-120
		T≥25°C	193	30-365	31	25-82	30	30	280	200-360	-	-
PART. BIOB	**FW**	T<25°C	-	-	-	-	28	28	-	-	-	-
		T≥25°C	-	-	29	28-30	-	-	-	-	-	-
	SW	T<25°C	259	42-440	-	-	-	-	-	-	365	365
		T≥25°C	234	21-392	28	28	154	30-236	280	200-360	90	90
FOSSIL BASED	**FW**	T<25°C	294	294	-	-	-	-	-	-	-	-
		T≥25°C	42	27-57	30	30	-	-	21	21	-	-
	SW	T<25°C	104	35-440	-	-	-	-	-	-	-	-

T≥25°C	78	21-364	28	25-28	30	30	-	-	-	-

Comparing the biodegradability rate of each type of biopolymer with the average biodegradability rate of the group to which they belong (biobased, partially-biobased, fossil based) a qualitative assessment has been done (Figure 5.1.2). The polymers chosen for this representation are those with more results found in the literature.

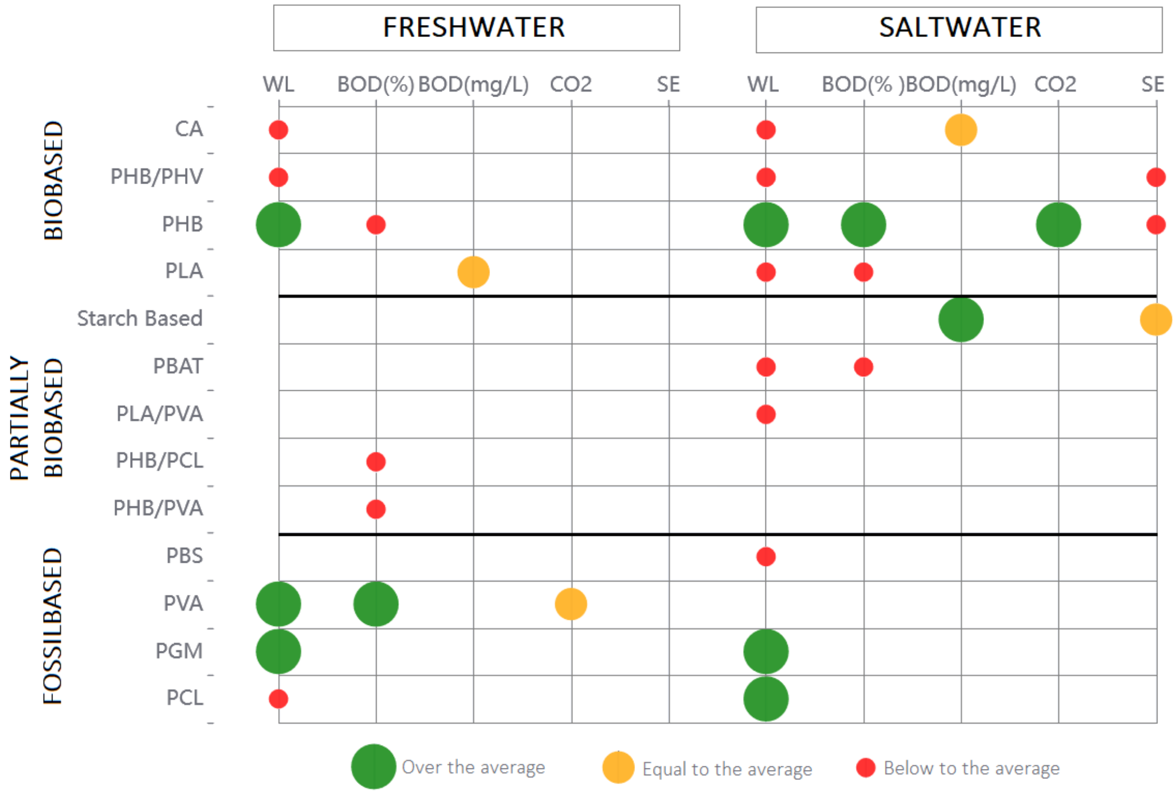


Figure 5.1.2: Qualitative representation of the biodegradability of specific bioplastic types compared to the average of each of the three group of bioplastics (biobased, partially-biobased, fossil based). Green dots means that the polymer biodegradation rate is higher than the average biodegradation rate of the group to which that polymer belongs; yellow dots means that the polymer biodegradation rate is equal to the average biodegradation rate of the group to which that polymer belongs; red dots means that the polymer biodegradation rate is lower than the average biodegradation rate of the group to which that polymer belongs.

The biodegradable bioplastics that have been design on purpose to be biodegradable (the partially biobased and the fossil-based ones) show a lower biodegradation level than the average biodegradation of their respective category and this is especially true for partially biobased bioplastics.

Among biobased bioplastics, PHB confirms to be the one more biodegradable of its category, both in FW and SW. Despite the PLA is the most used and produced bioplastic, as said before, it is the one that seems to have the most abundant criticality.

Between the partially biobased biodegradable bioplastics, no one apart from the starch-based bioplastics, which, however, present results only for salt in literature, seems to biodegrade in an appreciable way.

Among the fossil-based group, PVA shows a biodegradation level above the average (even though only tests carried out in fresh water have been found in the literature); PCL seems to degrade adequately into saltwater, but not much in freshwater; PBS has only been tested in saltwater, but it didn't give the expected results.

5.2 Research program

In this section the results concerning the parameters (presented in the subchapter § 4.2.2.3) used for the initial and the final characterization of the materials used in the oxygen consumption tests and the results concerning the Respirometer Index (RI) are presented for the three experiments (α, β and ϒ). In particular, the graphs showing the Respirometer Index (RI) curves are presented both in $mg_{O_2}/g_{material}$ and in $mg_{O_2}/g_{C_{org}}$ and with respect to the ThOD (in %, called Dt). For the ϒ experiment, the results of the microbiological analyses (through a fluorescence microscope and through the use of bacteria culture plates) and of the plastic characterization tests (FT-IR and DSC) are also shown.

5.2.1 Preparatory tests (α and β)

The values of the parameters (presented in the subchapter § 4.2.2.3) used for the initial characterization of the materials used in the first two experiments have been summarized in Table 5.2.1.

Table 5.2.1: Results of the initial analysis done to characterize the materials used in the αand βexperiment. The parameters marked with (*) have been made in duplicate or triplicate, so the value shown in the table corresponds to the average value.

Analysis	Target	Result		Unit of measurement
		α exp	**β exp**	
pH	Test medium+supernatant inoculum	7.50	7.24	-
TSS	Unaltered inoculum	4226	-	mg/l

TSS	Supernatant inoculum	4	-	mg/l
TS*	Unaltered inoculum	5036	9849	mg/l
VS*	Unaltered inoculum	2772 (56%ss)	5508 (56%ss)	mg/l
TS*	Supernatant inoculum	480	411	mg/l
VS*	Supernantant inoculum	121 (25%ss)	181 (44%ss)	mg/l
TS	Test medium+supernatant inoculum	598	830	mg/l
VS	Test medium+supernantant inoculum	148 (25%ss)	321 (39%ss)	mg/l
TS*	PLA	99.99	-	%
VS*	PLA	100	-	%ss
TC* (=TOC)	Unaltered inoculum	32.10	29.58	%ssC
IC	Unaltered inoculum	<1	<1	%ssC
TC	Supernatant inoculum	64.42	68.02	mg/l
NPOC	Supernatant inoculum	<10 (5.55)	<10 (6.21)	mg/l
TC	Test medium+supernantant inoculum	<5	<10 (6.50)	mg/l
NPOC	Test medium+supernantant inoculum	<5	<5	mg/l
TOC*	PLA	49.70	-	%ssC
DOC (TC)	Inoculum after a 0.45 µm filtration	60.36	-	mg/l
TC	Starch	-	40.94	%ssC
DOC (NPOC)	Inoculum after a 0.45 µm filtration	<10 (8.70)	-	mg/l
COD*	PLA	206’300	-	mg/kg

The average value of the TS measured on the inoculum in an unaltered state is higher in the β experiment, but the average value of the VS is equal to 56% of the TS, exactly as in the first experiment, indicating that half of the solids contained in the sample are organic. This means that the organic content of the two inocula are the same, it is only the inorganic content that changes.

The values of the TS and VS of the supernatant inoculum are higher in the β experiment. The VS are the 44.15% of the TS (in the α experiment the VS are the 25.12% of the TS), showing an increased organic content (being the VS an indicator of the organic content of a sample). The same comment can be made by analyzing the VS (38.58%ss instead of 24.67%ss) values measured in the test medium mixed with the supernatant inoculum.

In general, it can be observed that the content of total and volatile solids (and therefore of the organic matter) is greater in the inoculum taken from the outlet well of the secondary settle tank rather than the one taken from the activated sludge tank.

The VS of PLA are equal to 100% of its dry weight, indicating that the polymer is all composed by organic material.

The average value of the TC measured on the inoculum in an unaltered state in the β experiment (29.58 mgC/g sample) is similar to the one measured in the first experiment (32.10 mgC/g sample). They are relatively low, and visually, inside the crucibles extracted from the machine can be seen a residue, resistant to very high temperatures. This residue may be identified as very resistant oxides and/or sand. The measure of the IC is in both cases lower than1%, indicating that the TC of the inoculum in an unaltered state can be assumed as equivalent to the TOC.

The TOC (NPOC) of the supernatant inoculum, in both the experiments, is lower than the 10% of the TC, so the IC content is the prevalent part, meaning that the carbon content in the supernatant liquid is almost entirely composed by inorganic matter.

In both the tests, the TOC (NPOC) of the supernatant inoculum mixed with the test medium is less than the sensitivity of the instrument.

The TOC of starch (which correspond to the TC) is around 41% (mgC/g sample) and the one of PLA is around the 50% (mgC/g sample). Using the stoichiometrical formula of starch and PLA, the TOC can be calculated, and it results to be equal to 44.44% for starch, and to 50% for PLA. The measured values are very closed to the calculated one.

The IC content of the inoculum after a 0.45 µm filtration correspond to 51.66 mg/l (which is the 85.6% of the TC). This value is quite high; therefore, it can be concluded that this liquid has a high level of inorganic carbon, mainly due to the presence of inorganic dissolved compounds. This result is in line with those obtained before.

The average COD value of the PLA correspond to 206’300.35 $\mathrm{mg\ O_2}/\mathrm{kg_{PLA}}$. This value is relatively low, in fact, usually, the COD of an organic compound is around one million.

At the end of both experiments, PLA and HDPE granules were clearly visible inside the respective flasks. After air drying and weighing of the granules it was found that the final weight was unaltered compared to the initial weight. In some cases, it slightly increased, maybe due to the presence of water residues or some microorganisms attached around the granules (biofilm). In both the experiments, the pH, in each flask, has become slightly more basic. In the α test, both TS and VS have increased at the end of the experiment in each flask, with the greatest growth found in the FS flask, i.e., the one without inoculum. Instead, in the β test, both TS and VS have decreased, especially in the FN flask. The final TOC values, like the initial ones, are lower than the sensitivity of the machinery used for their measurement, therefore no variation with respect to the initial condition was found for this parameter. Moreover, at the end of the experiments the formation of nitrites, but especially nitrates, can be observed (Table 5.2.2 and 5.2.3), with higher values of nitrates in the β test.

From these results we can conclude that the negligible variation in the weight of the granules may indicate the fact that the polymer has not been affected by biodegradation processes or that its weight loss is not detectable by the analytical balance (i.e., the variation is lower than the sensitivity of the instrument). The slight increase in pH may be due to the formation of carbonates, which make the solution more basic. The increase of TS and VS, in the α test, indicates an increase of the solids content and of the organic substances in the various flasks, that can be attributed to the development of the microbial population. However, since neither a decreasing in the TOC content nor an increasing in the weight of the granules of the various polymers was observed, the developed microbial population can be autotrophic and not heterotrophic. This can be sustained also by the fact that nitrite has been formed (nitrification process is performed by autotrophic bacteria). This trend in the TS and VS values, however, is found only in the α experiment, while the production of nitrites is also observed in the other two experiments (β and ϒ).

The decrease of TS and VS in the β test, indicates a decrease of the solids content and of the organic substances in the various flasks, maybe due to the fact that heterotrophic bacteria, when they don't have an organic substrate with which to feed, tend to self-digest, reaching an endogenous phase and, consequently, to die. The fact that TOC value does not increase and that the weight of the various granules does not decrease also support this thesis.

Table 5.2.2: Final values of the parameters investigates in the α experiment. The values of pH, TS, VS, TOC, NO_2^- and NO_3^- are average values. (*) = the lower limit of the NO_2^- machine sensitivity is 0.02 N-NO_2/l, which converted in mg NO_2^-/l gives a value of 0.06569).

Flasks	**Parameters**						
	Weight (g)	**pH**	**TS (mg/l)**	**VS (mg/l)**	**TOC (mg/l)**	**NO_2^- (mgNO_2^-/l)**	**NO_3^- (mgNO_3^-/l)**
αFT1	0.0477	7.70	771	274 (36%ss)	<5	<0.06569*	2.641326
αFT2	0.0691						

αFT3	0.05						
αFB1	-	7.80	758	275.33 (36%)	<5	<0.06569*	2.729862
αFB2	-						
αFB3	-						
αFS	0.0508	7.70	824	308 (37%ss)	<5	<0.06569*	2.2134015
αFN	0.0611	7.80	800	288 (36%ss)	<5	<0.06569*	3.01022604

Table 5.2.3: Final values of the parameters investigates in the α experiment. The values of pH, TS, VS, TOC, NO_2^- and NO_3^- are average values. (*) = the lower limit of the NO_2^- machine sensitivity is 0.02 N-NO_2/l, which converted in mg NO_2^-/l gives a value of 0.06569).

Flasks	**Parameters**						
	Weight (g)	**pH**	**TS (mg/l)**	**VS (mg/l)**	**TOC (mg/l)**	**NO_2^- (mgNO_2^-/l)**	**NO_3^- (mgNO_3^-/l)**
βFT2	0.0495	7.70	648	76 (12%ss)	<5	< 0.06569*	6.551668
βFB1	-	7.60	631	56 (9%ss)	<5	< 0.06569*	7.503431
βFB2	-						
βFN1	0.0688	7.30	598	30 (5%ss)	<5	< 0.06569*	7.0164828
βFN2	0.0684						
βFS1	0.0547	7.30	635	69 (11%ss)	<5	< 0.06569*	6.492644
βFS2	0.0504						
βFS3	0.0498						
βFC2	-	7.40	610	51 (8%ss)	<5	< 0.06569*	14.874058
βFC3	-						

The oxygen consumption curves obtained during the two experiments are presented in Figure 5.2.1 as $mg_{O_2}/g_{material}$, in Figure 5.2.2 as $mg_{O_2}/g_{C_{org}}$ and in Figure 5.2.3 as %Dt**.** The presented values are mean values for those flasks that have been set in duplicate or triplicate.

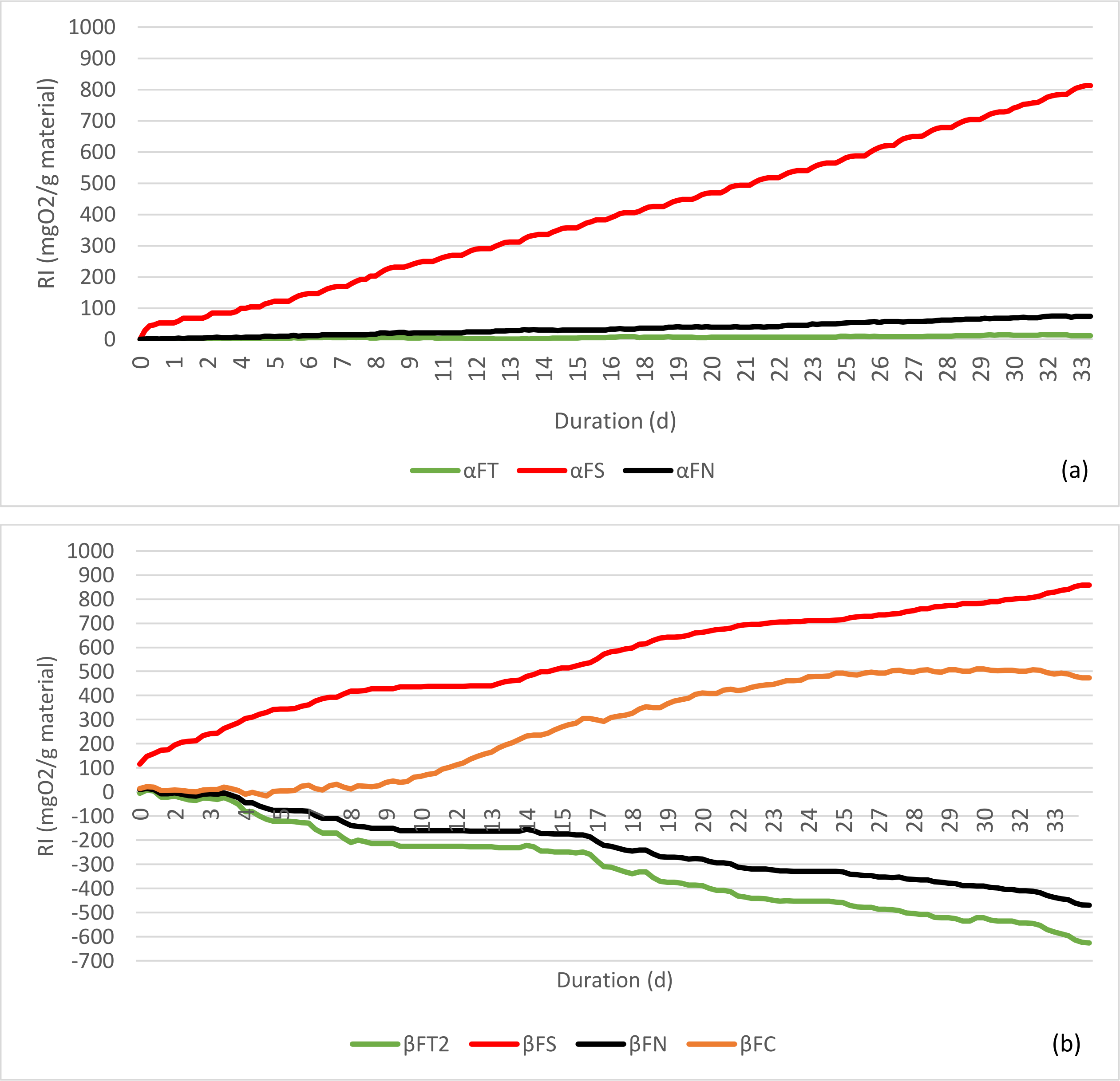


Figure 5.2.1: Respirometric Index (RI) curve in $mg_{O_2}/g_{material}$ obtained from the α (a) and β (b) experiments (in the α trial the positive control is missing because starch was not available at the beginning of the experiment). The values presented are net of blank values (i.e., endogenous respiration is not considered) and are mean values for those flasks that have been set in duplicate or triplicate.

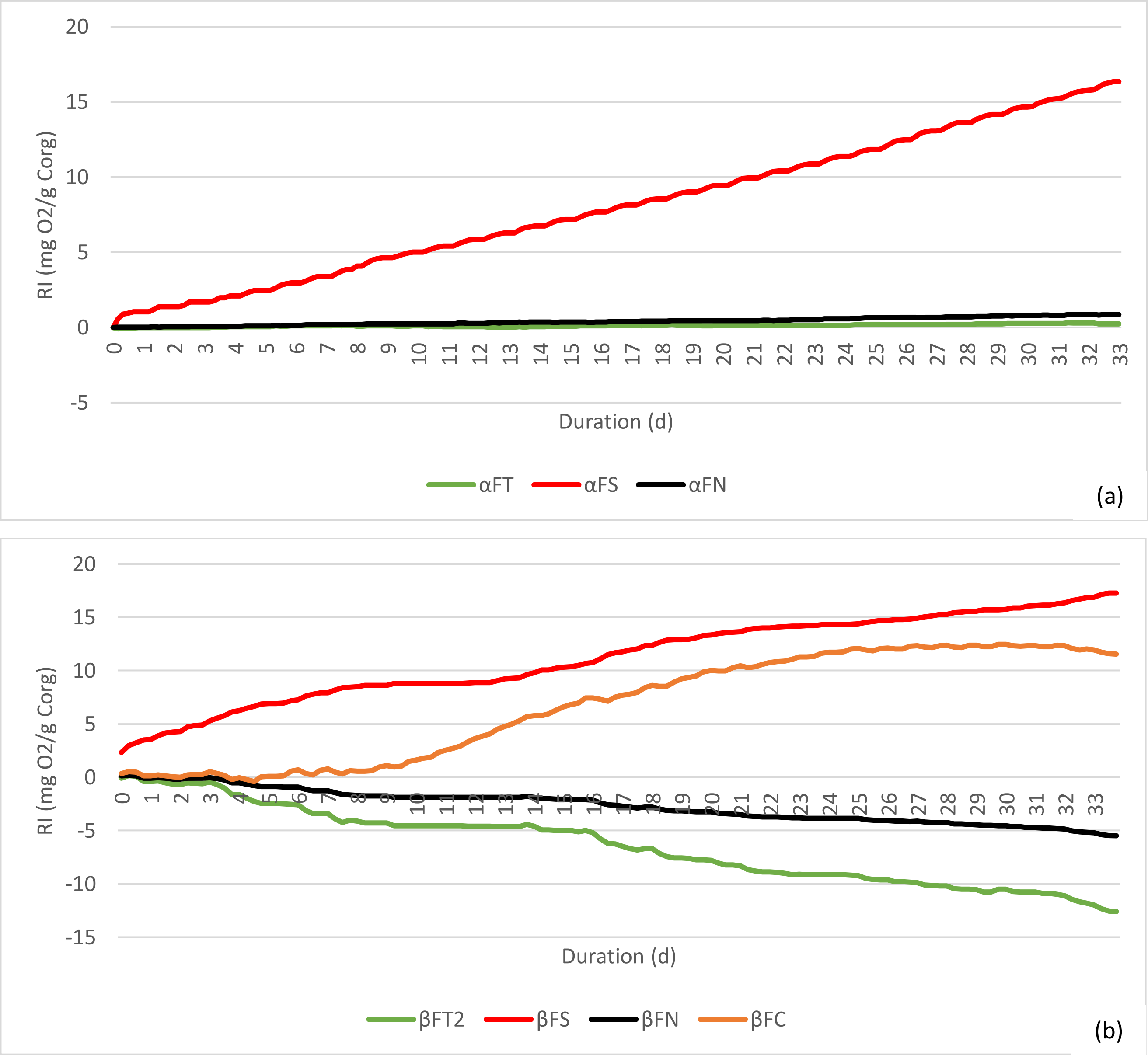
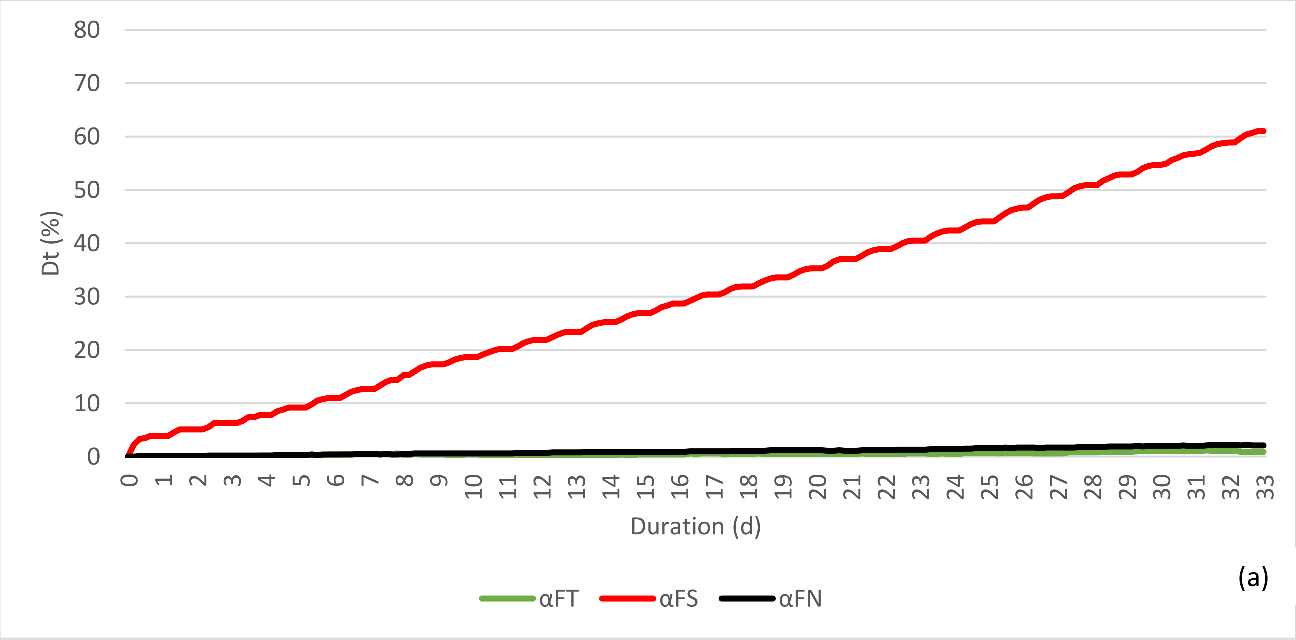


Figure 5.2.2: Respirometric Index (RI) curve in $mg_{O_2}/g_{C_{org}}$ obtained from the α (a) and β (b) experiments (in the α trial the positive control is missing because starch was not available at the beginning of the experiment). The values presented are net of blank values (i.e., endogenous respiration is not considered) and are mean values for those flasks that have been set in duplicate or triplicate.



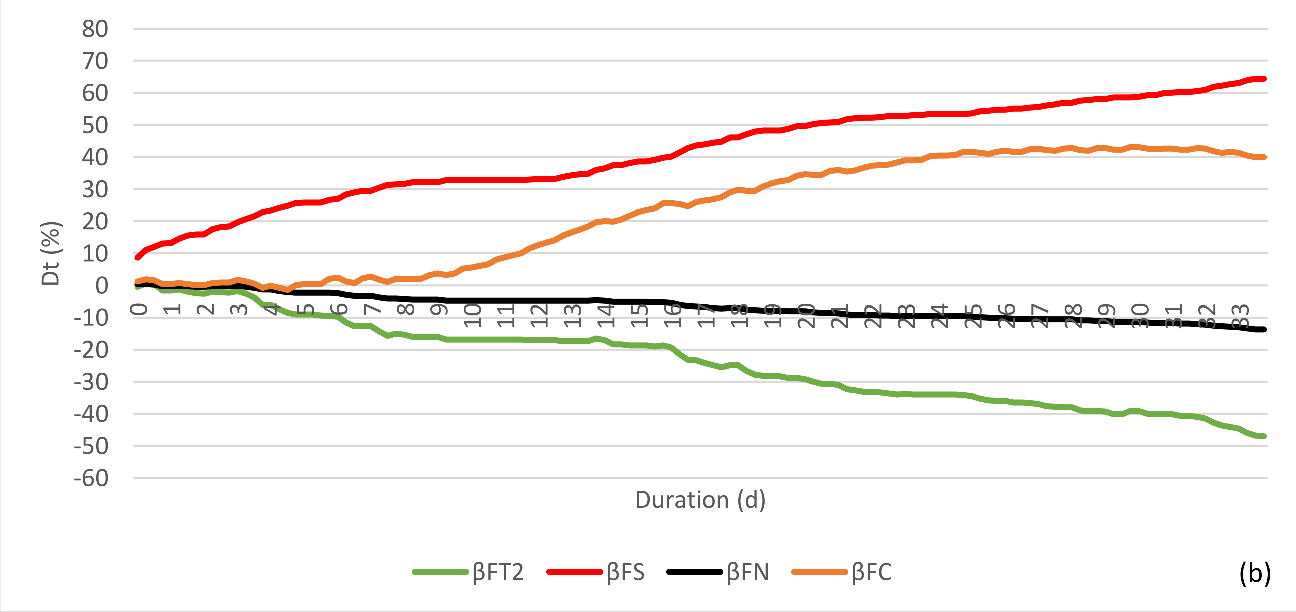


Figure 5.2.3: Respirometric Index (RI) curve in %Dt obtained from the α (a) and β (b) experiments (in the α trial the positive control is missing because starch was not available at the beginning of the experiment). The values presented are net of blank values (i.e., endogenous respiration is not considered) and are mean values for those flasks that have been set in duplicate or triplicate.

In all the graphs the curve with the highest respiration is the one corresponding to the flask FS, meaning that the flasks without the inoculum are the ones with the highest Respiration Index. This is probably because these flasks are not sterile, even though they were sterilized through the use of hydrochloric acid (HCl). In the α trial, the respiration produced by the flasks FT and FN is almost the same and negligible. In the β trial, their respiration is lower than zero (this is due to the fact that the values presented in the graphs are net of the respiration values found in the blanks). However, a value lower than zero doesn't have a physical value, and therefore those value can be considered equal to zero.

These first two tests have been used as preparatory tests, in order to verify the procedure given by the ISO standard. They have been useful in view of the last experiment (γ), because for example they gave us an information on the inoculum percentage to add to each flask and on the optimal duration of the test. The ϒ test has been performed using a more sophisticated and automated respirometer, supplied by the laboratory of the S.E.S.A. S.P.A. of Monselice (Padova, Italy).

5.2.2 Official test (γ)

The results of the oxygen consumption test (respirometric test) carried out in the S.E.S.A laboratory, the results of the microbiological tests (obtained through a fluorescence microscope and through the bacterial cultures) developed at the DAFNAE laboratory and the results of the plastic characterization tests (obtained through DSC and FT-IR) done at the in the laboratory of the Industrial Engineering department are shown and discussed in the following paragraphs.

5.2.2.1 Oxygen consumption test

The values of the parameters (presented in the subchapter § 4.2.3.3) used for the initial characterization of the materials have been summarized in Table 5.2.4. The chemical physical meaning of the parameter is the same presented in the subchapter § 4.2.2.4.

Table 5.2.4: Results of the initial analysis done to characterize the materials used in the ϒ experiment.

Analysis	**Target**	**Result ϒ exp**	**Unit of measurement**
pH	Test medium+supernatant inoculum	7.40	-
TS	Unaltered inoculum	8824	mg/l
VS	Unaltered inoculum	5408 (61%ss)	mg/l
TS	Supernatant inoculum	431	mg/l
VS	Supernantant inoculum	161 (37%ss)	mg/l
TS	Test medium+supernatant inoculum	605.55	mg/l
VS	Test medium+supernantant inoculum	152.05 (25.11%ss)	mg/l
TOC	Test medium+supernantant inoculum	4.9	mg/l
TOC	Cellulose	49.60	%ssC
TOC	HDPE	83.40	%ssC

Comparing the results with those obtained in the β experiment, having used the same percentage by volume of inoculum, it can be stated that the VS of the unaltered inoculum are a bit higher in the ϒ test and that the VS of the supernatant inoculum are a bit lower. Also, the value of the VS of the supernatant inoculum mixed with the test medium is lower and more similar to the value found in the α experiment. This means that, in the β experiment, the supernatant inoculum had a higher organic content. The TOC of the test medium mixed with the supernatant inoculum is very low and in line with the values found in the other two experiments, so that the organic carbon content of the liquid can be considered very poor.

At the end of the experiment, lasted for 37 days, the PLA and HDPE granules were clearly visible inside the respective flasks. After air drying and weighing, it was found that the final weight of the granules did not decrease. For all the flasks within this experiment, the pH decreases, meaning that a more acid environment formed. Both the TS and the VS have a decreasing trend (but smaller than the one found in the β experiment), in most of the flasks. The greatest decrease occurred in the flask FN. Only in the flask FC the average TS and VS have an increasing trend. The final TOC values, like the initial ones, are lower than the sensitivity of the machinery used for their measurement, therefore no variation with respect to the initial condition was found for this parameter. At the end of the experiment the formation of nitrites, but especially nitrates, can be observed (Table 5.2.5).

From the abovementioned results we can conclude that the negligible variation in the weight of the granules may indicate the fact that the polymer has not been affected by biodegradation processes, or that its weight loss is not detectable by the analytical balance (i.e., the variation is lower than the sensitivity of the instrument). The slight decrease in pH may be due to the dissolution of the carbon dioxide in the liquid, in fact, CO_2 reaches the trapping unit only when its equilibrium between the liquid and the gas phases has been reached. The decrease of TS and VS in the various flasks indicates a decrease of the solids content and of the organic substance, maybe due to the fact that heterotrophic bacteria, when do not have an organic substrate easily available for their feeding, tend to self-digest, reaching an endogenous phase and, that leads to their death. The fact that the TOC does not increase and that the granule weights do not decrease also support this theory. In this experiment, however, the decrease of TS and VS is quite slight, therefore this reduction could be attributed to a systematic error deriving from the weighing through the analytical balance. of the presence of nitrates and nitrites indicates that the nitrification process took place inside all the flasks, thus suggesting the presence, in the microbial mix, of autotrophic bacteria.

Table 5.2.5: Final values of the parameters investigated in the γ experiment. The values of pH, TS, VS, TOC, NO_2^- and NO_3^- are average values. (*) = the lower limit of the NO_2^- machine sensitivity is 0.02, but, actually the machine also reads below that value, so all these data are reported as <0.01 mg N-NO_2^-/l, which converted in mg NO_2^-/l gives a value of 0.03285 (°) = the lower limit of the NO_3^- machine sensitivity is 0.5 that converted in mg NO_3^-/l corresponds to 2.2134).

Flasks	**Parameters**						
	Weight (g)	**pH**	**TS (mg/l)**	**VS (mg/l)**	**TOC (mg/l)**	**NO_2^- (mgNO_2^-/l)**	**NO_3^- (mgNO_3^-/l)**
γFT1	0.0971	6.70	515	83 (16%)	<5	0.05584	10.8309
γFT2	0.1171						
γFT3	0.1120						
γFC1	-	6.70	614	185 (30%)	<5	<0.03285*	<2.2134 °
γFC2	-						
γFN1	0.1083	6.60	462	56 (12%)	<5	<0.03285*	<2.2134 °
γFN2	0.1016						
γFS1	0.1101	6.80	537	93 (17%)	<5	0.302177	6.2861
γFS2	0.1107						
γFI	HDPE: 0.1108 PLA: 0.0940	6.60	562	204 (36%)	<5	<0.03285*	<2.2134 °
γFB1	-	6.80	511	82 (16%)	<5	<0.03285*	10.5063
γFB2	-						
γFB3	-						
γFCe1	-	6.40	549	143 (26%)	<5	<0.03285*	<2.2134 °
γFCe2	-						

The oxygen consumption curve obtained during the 37 days of experiment duration are presented in Figure 5.2.4 as $mg_{O_2}/g_{material}$, in Figure 5.2.5 as $mg_{O_2}/g_{C_{org}}$, and in Figure 5.2.6 as %Dt**.** The presented values are mean values for those flasks that have been set in duplicate or triplicate.

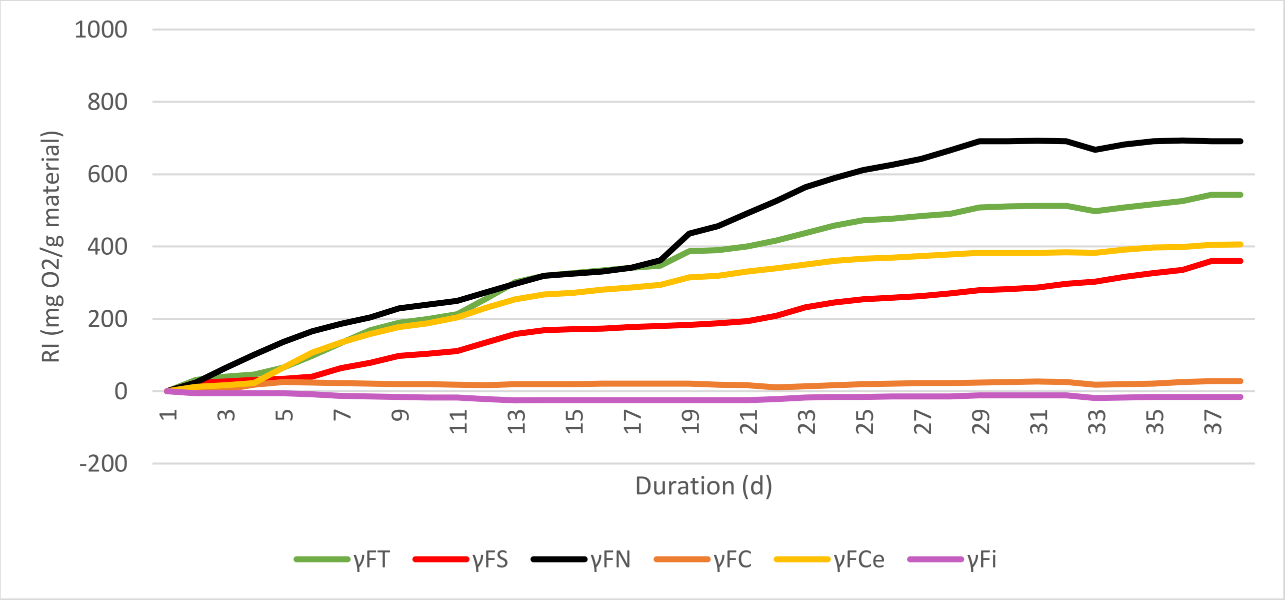


Figure 5.2.4: Respirometric Index (RI) curve in $mg_{O_2}/g_{material}$ obtained from the ϒ experiment. The values presented are net of blank values (i.e., endogenous respiration is not considered) and are mean values for those flasks that have been set in duplicate or triplicate

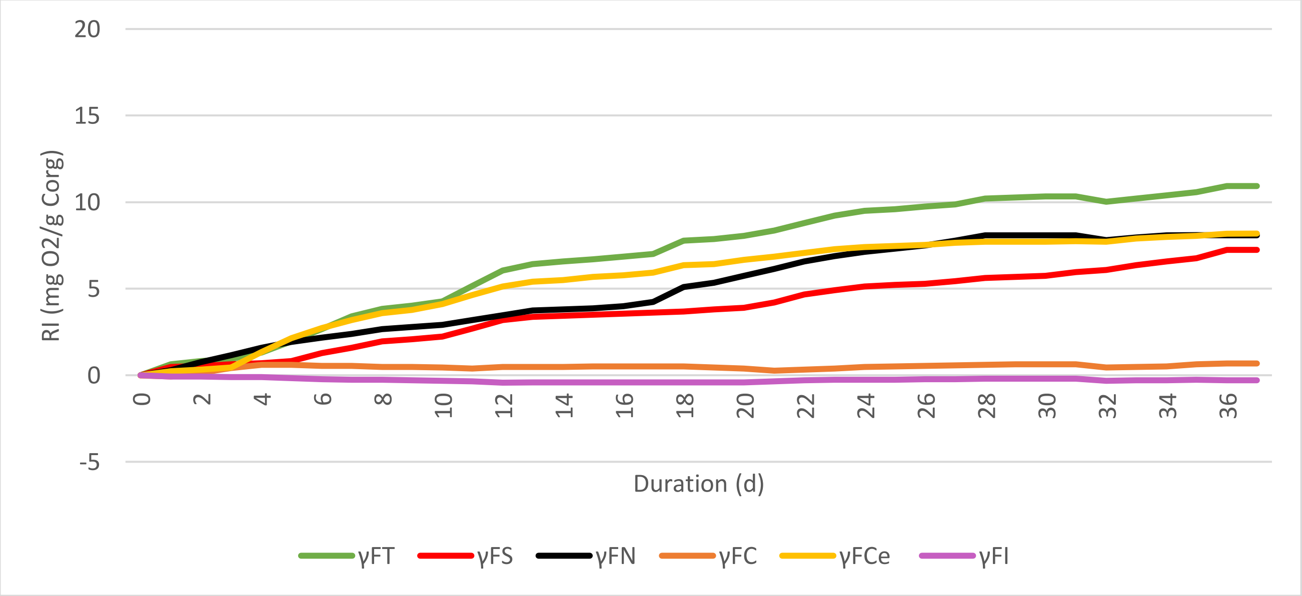


Figure 5.2.5: Respirometric Index (RI) curve in $mg_{O_2}/g_{C_{org}}$ obtained from the ϒ experiment. The values presented are net of blank values (i.e., endogenous respiration is not considered) and are mean values for those flasks that have been set in duplicate or triplicate.

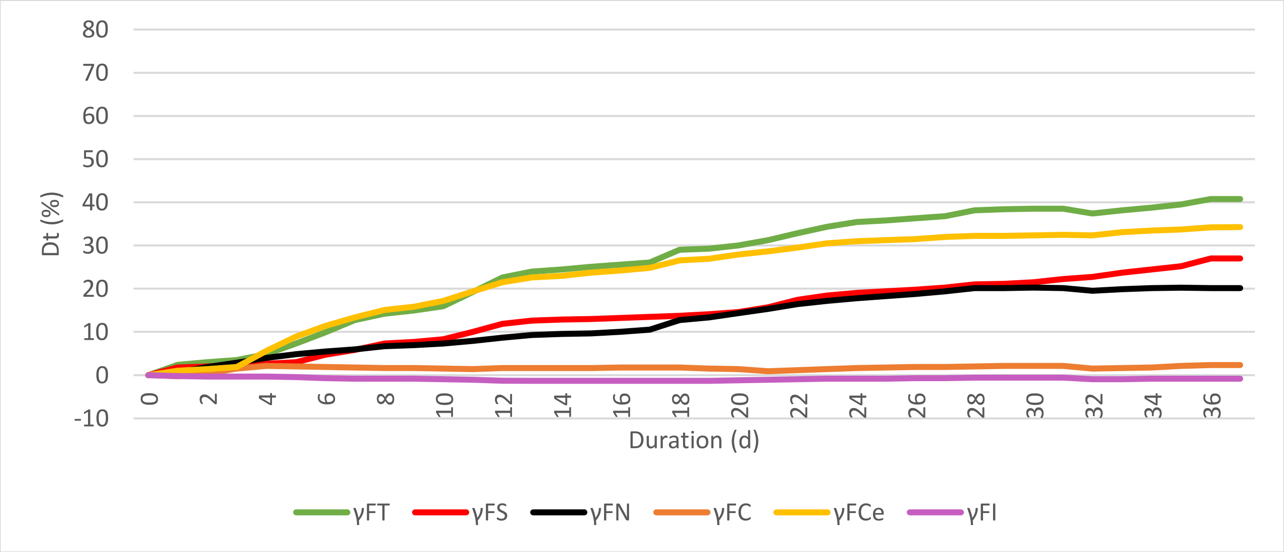


Figure 5.2.6: Respirometric Index (RI) curve in %Dt obtained from the ϒ experiment. The values presented are net of blank values (i.e., endogenous respiration is not considered) and are averaged in the case in which the same type of flask is made in duplicate or triplicate.

The relative position between the curves FT, FN, Fce and FS changes in each of the three different representations. The only constant concerns the position of the FC and FI curves, which reach slightly negative biodegradation levels (which is not physically significant and therefore these values have to be considered equal to zero) or tending to zero.

Considering only the Figure 5.2.6, some unexpected things are encountered. First, since the curves FC and FCe contains respectively starch and cellulose, which are polymers formed by chains of the same monomers, it was expected that the final biodegradation rate would be similar, instead cellulose reaches a final Dt of 35%, while starch does not even reach the 5%. This discrepancy is not supported by microbial cultures by liquid observation under the microscope, because for both flasks a good bacterial abundance was find (Figure 5.2.8, 5.2.9 and Table 5.2.6). Another anomaly is encountered for the flask FN: in fact, in this flask there is a polymer certified to be non-biodegradable (HDPE) and therefore we did not expect it to reach an appreciable final biodegradation level, instead at the end of the experiment, it reaches a Dt of 20%. This result could be due to the presence of antioxidants inside the PE granules, which during the experiment tend to oxidize by consuming oxygen. Moreover, also the respiration of the flask FS, which is the one without the inoculum, reaches a final Dt of about 30%. This can be justified by the presence of microorganisms, even though they were not expected to be present: this hypothesis is also supported by the results presented in the subsection §5.2.3.2, where the presence of microorganisms was observed in the liquid under the fluorescence microscope and bacterial colonies grew on the plate count agar (PCA) (Figure 5.2.8, 5.2.9 and Table 5.2.6). For what concern the flask FT, at the end of the 37 days, the curve reaches a Dt of around 40%. This value is the highest measured in this third experiment.

Considering now the validity criteria presented in the sub-chapter §4.2.1.3 imposed on the flasks FC, FI, FS and FN, it can be stated that:

- In the flask FS the Dt is higher than the 10%. This means, following the instructions given by the ISO, that the abiotic degradation takes place. This conclusion is not fully true because, as explained before, microorganisms have been found in this flask, so the high Dt value may be induced by their respiration.
- In the flask FN the Dt value is not negligible. This condition does not respect the validity criterion imposed by the followed ISO. This is probably due to the presence of antioxidants in the HDPE pellets.
- In the flask FC and FCe the Dt is lower than the 60%. This condition does not respect the validity criterion imposed by the followed ISO.
- In the flasks FI the Dt is lower than 25%. This means, following the instructions given by the ISO, that the tested material and the reference materials are inhibitors with respect to the microorganisms used.

5.2.2.2 Microbiological tests

In this section, the results of the microbiological tests (obtained through a fluorescence microscope and through the bacterial cultures) developed at the DAFNAE laboratory are presented.

By observing the liquid portion of the flasks FT1, FC1, Fce1, FB3, FN1, FI and FS2, with the use of a fluorescence microscope it was noted that (Figure 5.2.7):

- in the flask FT1 and FI various aggregates of microorganisms and also single microorganism cells were found. After the addition of the two dyes to the liquid, it was observed that the microorganism aggregates and cells colored in green and therefore they are alive.
- in the flask FC1 different microorganisms of different types are found (probably cocci, rods, protozoa). More single cells than aggregates are observed. In this sample the microorganisms are fast-moving, abundant and colored in green (therefore alive).
- in the flask FCe1 both microbial aggregates and single cells have been found. In FC1 and FCe1 a comparable quantity of living microorganisms can be seen.
- in the flasks FB3 and FN1 few living microbial aggregates have been found.
- in the flask FS2, which should be sterile and without inoculum, some microbial single cells are found, which are colored in green and therefore alive. This result is important because it

explains why in the Fs flasks the Respiration Index is greater than zero. Probably these microorganisms have been added to the FS flasks through the PLA granules.

Therefore, it can be concluded that living microorganisms have been found in each of the analysed samples. The fact that the microorganisms are alive is also supported by the oxygen consumption tests, in fact every curve (representing the different flasks) shows an increasing trend until the end of the experiment, and the plateau phase is not achieved.

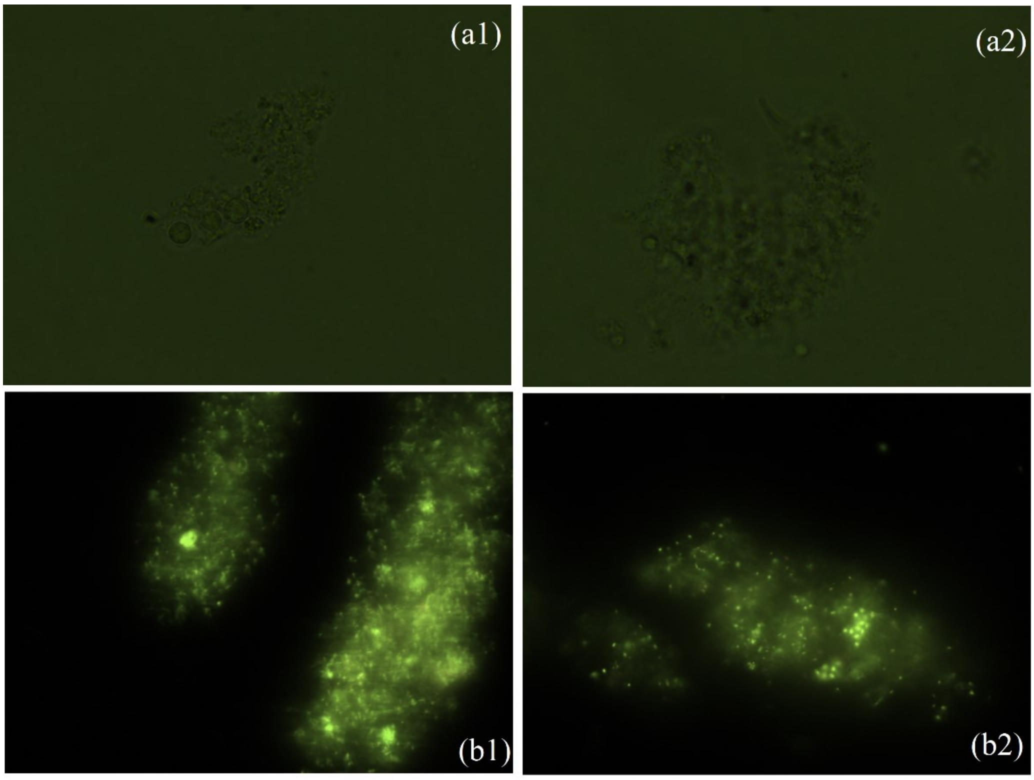


Figure 5.2.7: Images obtained through the fluorescence microscope with (b) and without (a) the fluorescence of the liquid extracted from the flask FT1 (1) and FCe1 (2). In the first two figures (a1 and a2) some aggregates are present, which, thanks to figures (b1) and (b2), can be determined as living cells (given the green color).

These results are also supported by the results of the bacterial cultures analyses, cultivated on the Plate Count Agar (PGA). The bacterial cultures have been made starting from the liquid phase of the different flasks (after the "serial dilution" procedure) but also from the plastic granules (which have been passed on the PGA and then removed). In general, it can be stated that microorganisms have been found in all the plates for all the different dilutions (up to -5 dilution level) (Figure 5.2.8). In the various plates different typology of microorganisms have been developed (Figure 5.2.9). Only in the plates on which the granules from the flask FN1 have been spread yellow colonies have developed. In the plates on which the granules coming from the flasks FI, FC1 and FCe1 have been spread pinkish colonies have developed. It can be therefore assumed that these colonies are able to biodegrade glucose (being starch and cellulose composed of monomers of this organic compound).

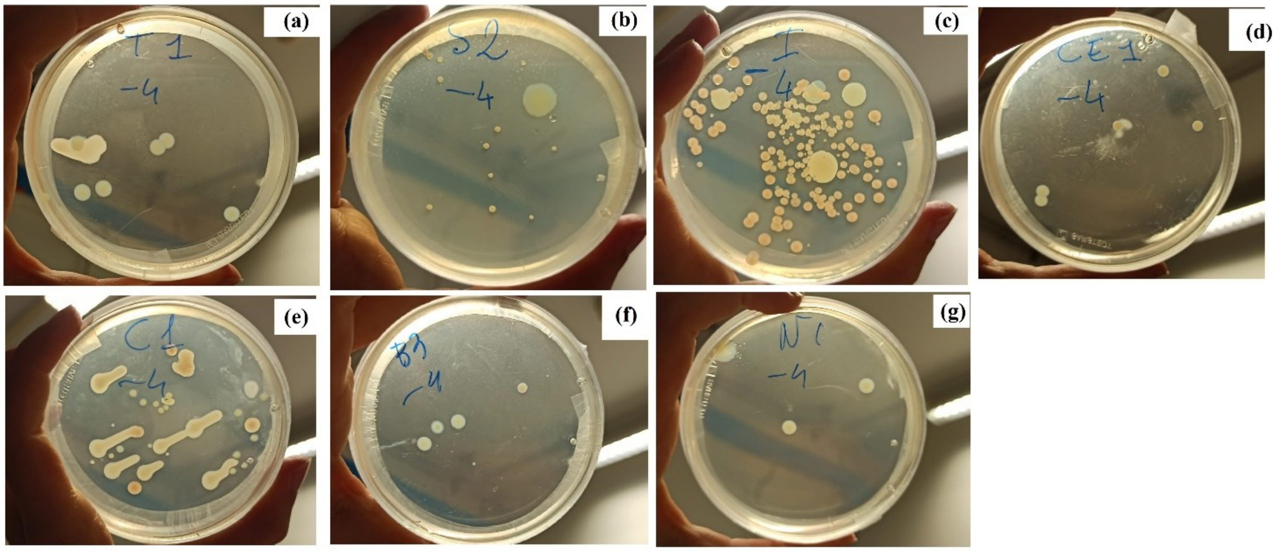


Figure 5.2.8: Bacterial cultures at the dilution -4 after 48h of incubation. Cultures of microorganisms developed from the liquid extracted from the flask FT1 are shown in figure (a), those developed from the liquid of the flask FS2 are shown in figure (b), those developed from the liquid of the flask FI in figure (c), those developed from the liquid of the flask FCe1 in figure (d), those developed from the liquid of the flask FC1 in figure (e), those developed from the liquid of the flask FB3 in figure (f), and those developed from the liquid of the flask FN1 are shown in figure (g).

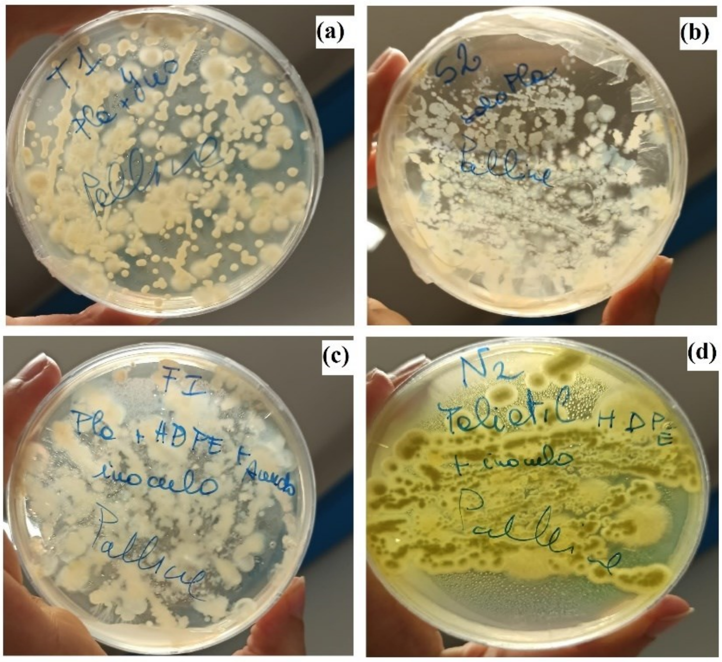


Figure 5.2.9: Bacterial cultures obtained by passing the plastic granules onto the Plate Count Agar after 48h of incubation. Cultures of microorganisms adhering to the PLA granules extracted from the

flask FT1 are shown in plate (a), those adhering to the PLA granules extracted from the flask FS2 in plate (b), those adhering to the HDPE and PLA granules extracted from the flask FI in plate (c) and those adhering to the HDPE granules extracted from the flask FN1 in plate (d).

The concentration of colonies developed on each Plate Count Agar, expressed as Colony Forming Unit per microliter, is presented in Table 5.2.6. In general, the values obtained are a the medium-low order of magnitude.

Table 5.2.6: Concentration of colonies (expressed as CFU/mL) counted on each Plate Count Agar.

FLASK	**CFU/mL**
FI	2,4 x 10^7
FB3	4,6 x 10^6
FC1	2 x 10^6
FS2	1,2 x 10^6
FT1	7 x 10^5
FCe1	5 x 10^5
FN1	3 x 10^5

The colonies concentration values (expressed in CFU/mL) are not coherent with the respiration levels found in the Figure 5.2.6. The Dt trend of the flask FI is circa zero, but the value of CFU/mL is the highest recorded in the table. This could be explained considering a possible inhibitory effect of the materials used on the microbial population. In the flask FS, although it should have been sterile, a good bacterial abundance was recorded, that could be due to the presence of bacteria originally adhered to the PLA granules. This hypothesis is also supported by the respiration level found in Figure 5.2.6 for the flask FS The bacterial abundance (in CFU/ml)found in the flask FC1 is an order of magnitude higher than the one found in the flask FCe1 but this evidence is not supported by the RI curves (Figure 5.2.6): in fact the final Dt (%) reached by the flask containing the cellulose is much higher than the one achieved by the flask containing the starch.

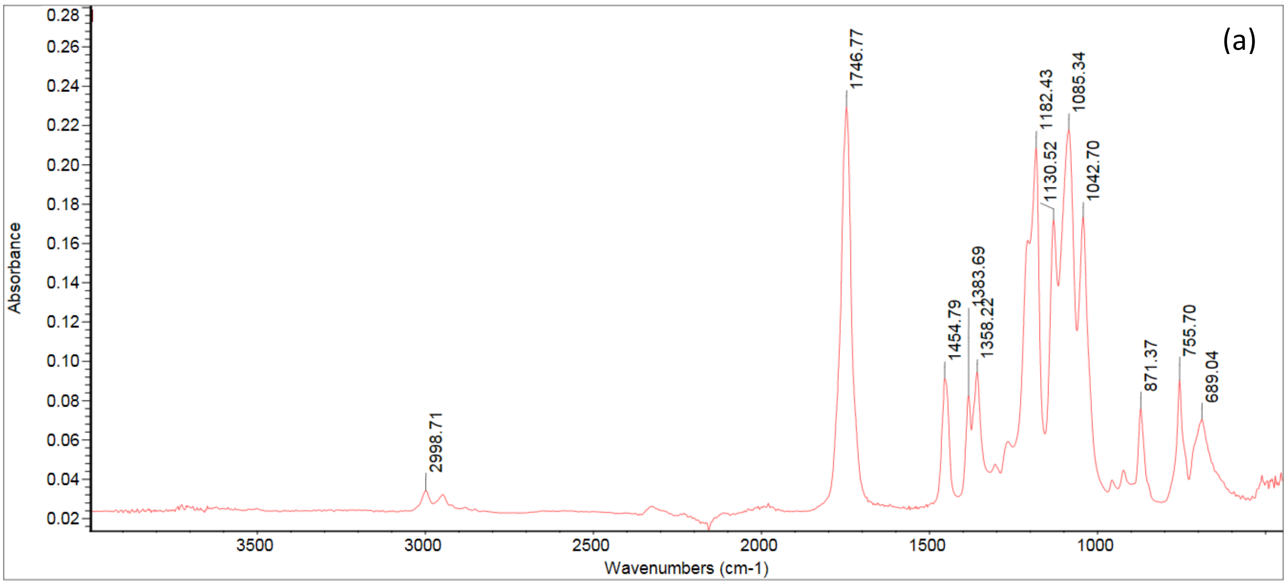
5.2.2.3 Plastic granules characterization tests

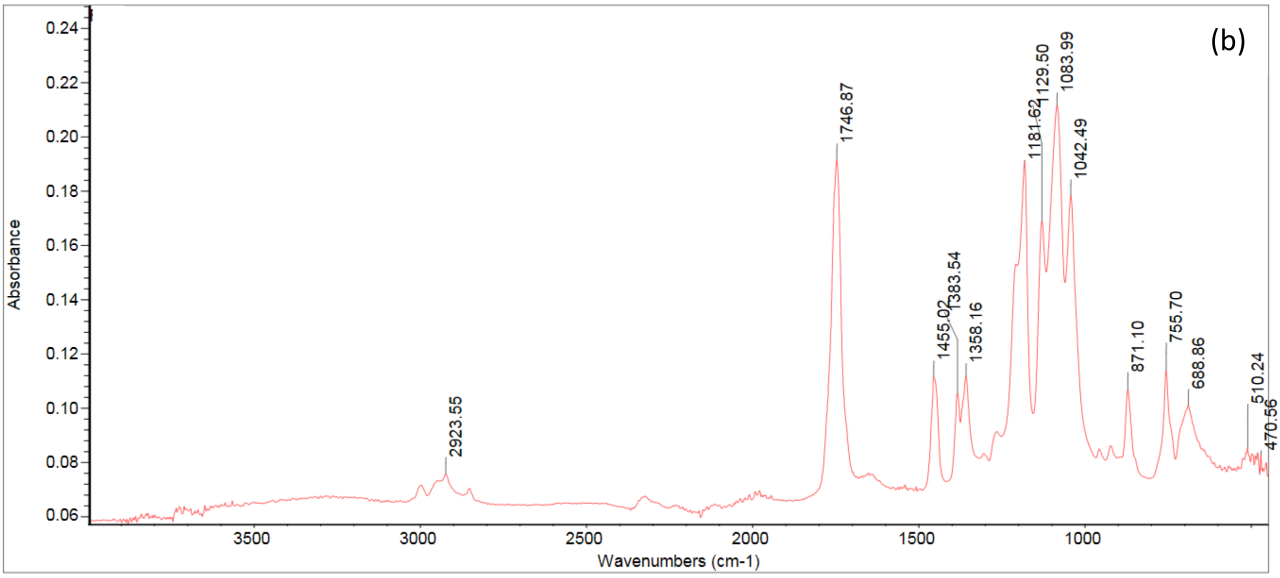
In this section the plastic characterization test results (obtained through DSC and FT-IR), done at the in the laboratory of the Industrial Engineering department, are shown and discussed.

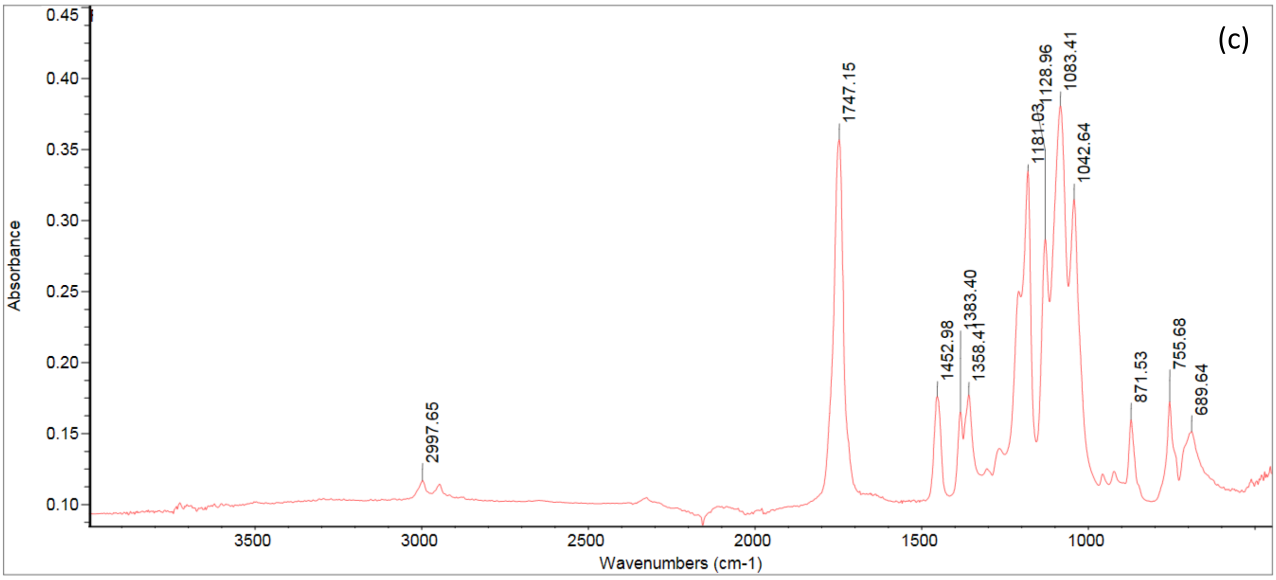
The output of the FT-IR analysis is a graph in which the wavelength (x-axis) is plotted versus the absorbance (y-axis). This analysis provides information regarding the chemical structure of polymers, so that any changes in the chemical structure of bioplastic granules due to the degradation process can be accessed. The output of the analysis is a spectrum, specific for every material, in which the peaks, corresponding to a certain wavelength, represent a specific type of bond present in the polymer (Grossule at al., under publication).

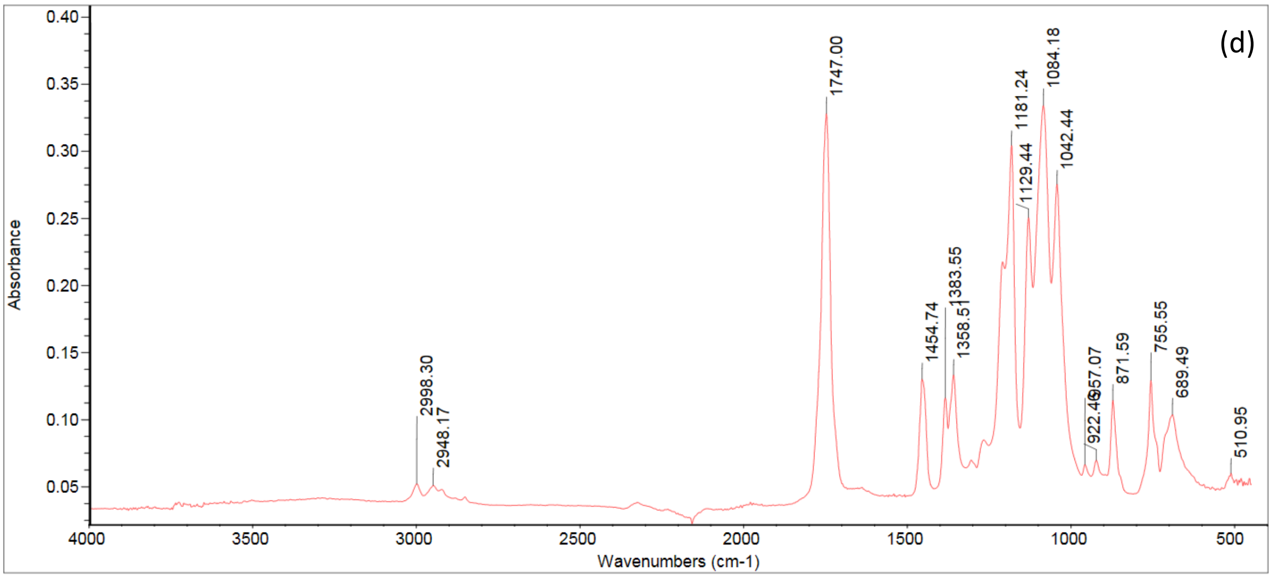
The final purpose of this analysis is to compare the spectra obtained from the analysis of the virgin PLA and of PLA extracted from the FT2, FS1 and FI flasks, in order to understand if the biodegradation process started during the oxygen consumption experiment and how the process affected the PLA granules. To do so it is necessary to compare the relationship between the different obtained spectra, by using as a reference one stable peaks of the virgin PLA. If a stable peak is changed (lowered or shifted to different wavelengths) in the polymer spectrum subject to the biodegradation process, compared to the peak in the virgin spectrum, it means that the corresponding bond is also altered, indicating a possible degradation of the polymer (Grossule at al., under publication). The same procedure has been applied also to HDPE granules, also in this case by comparing the spectra obtained from the analysis of the virgin HDPE and of the HDPE extracted from the flasks FN1 and FI, in order to assess if this polymer has been degraded during the experiment.

In Figure 5.2.10 the absorbance spectra of virgin PLA and of PLA contained in the flasks FT2, FS1 and FI are presented. In Figure 5.2.11 those of virgin HDPE and of HDPE contained in the flasks FN1 and FI are presented. All the spectra od PLA and HDPE are also represented in the same graph, in order to allow possible comparisons.




(b)
Absorbance
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
3500
3000
2500
2000
1500
1000
Wavenumbers (cm-1)
2923.55
1746.87
1455.02
1383.54
1358.16
1181.62
1129.50
1083.99
1042.49
871.10
755.70
688.86
510.24
470.56


(c)
Absorbance
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
3500
3000
2500
2000
1500
1000
Wavenumbers (cm-1)
2997.65
1747.15
1452.98
1383.40
1358.41
1181.03
1128.96
1083.41
1042.64
871.53
755.68
689.64


(d)
Absorbance
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)
2998.30
2948.17
1747.00
1454.74
1383.55
1358.51
1181.24
1129.44
1084.18
1042.44
957.07
922.49
871.59
755.55
689.49
510.95

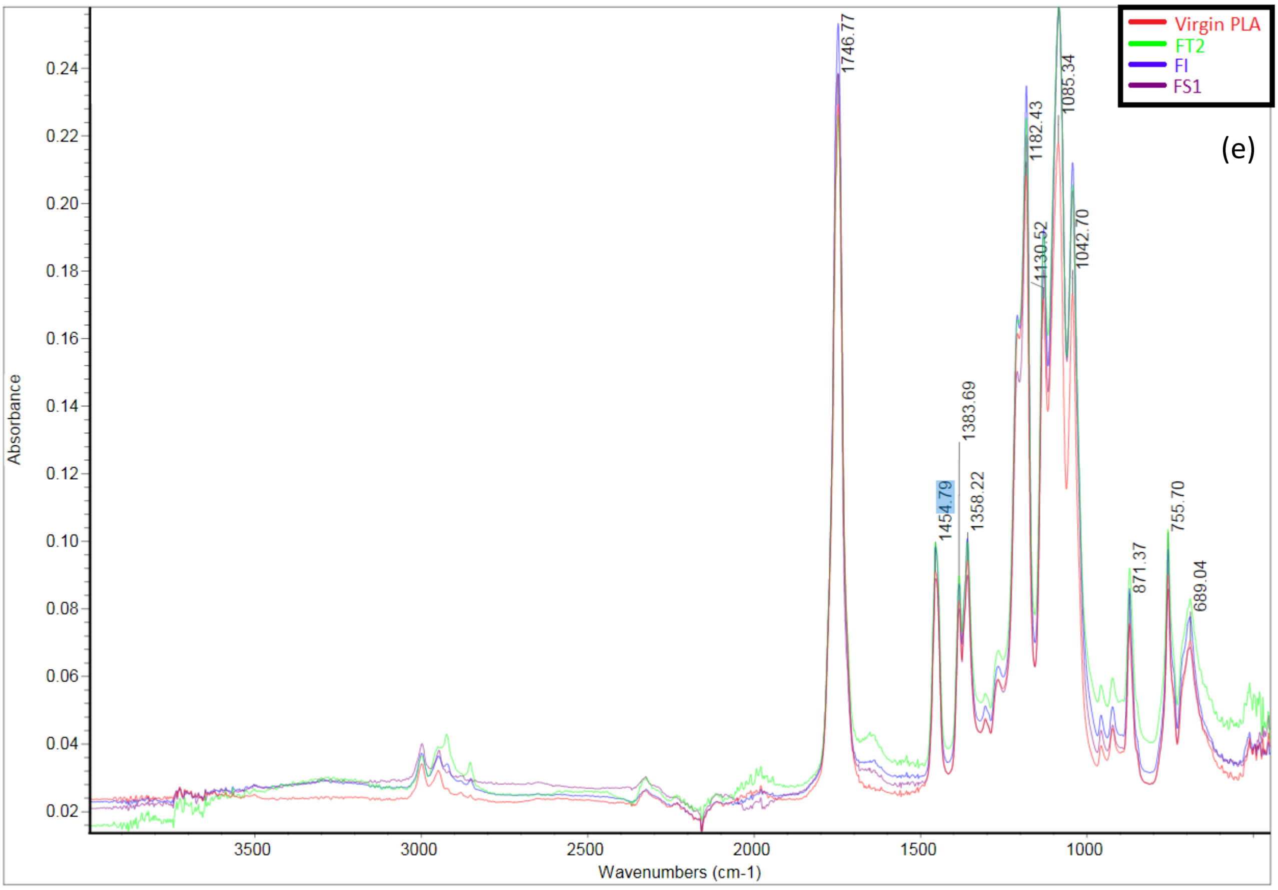
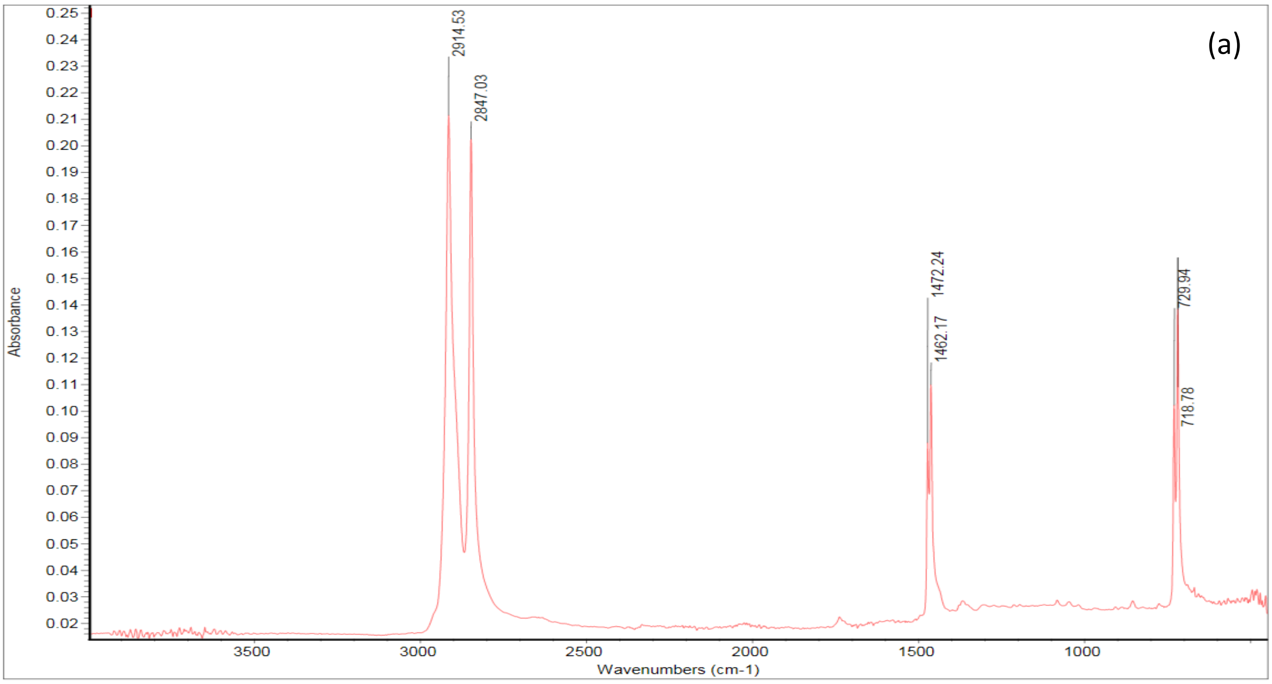
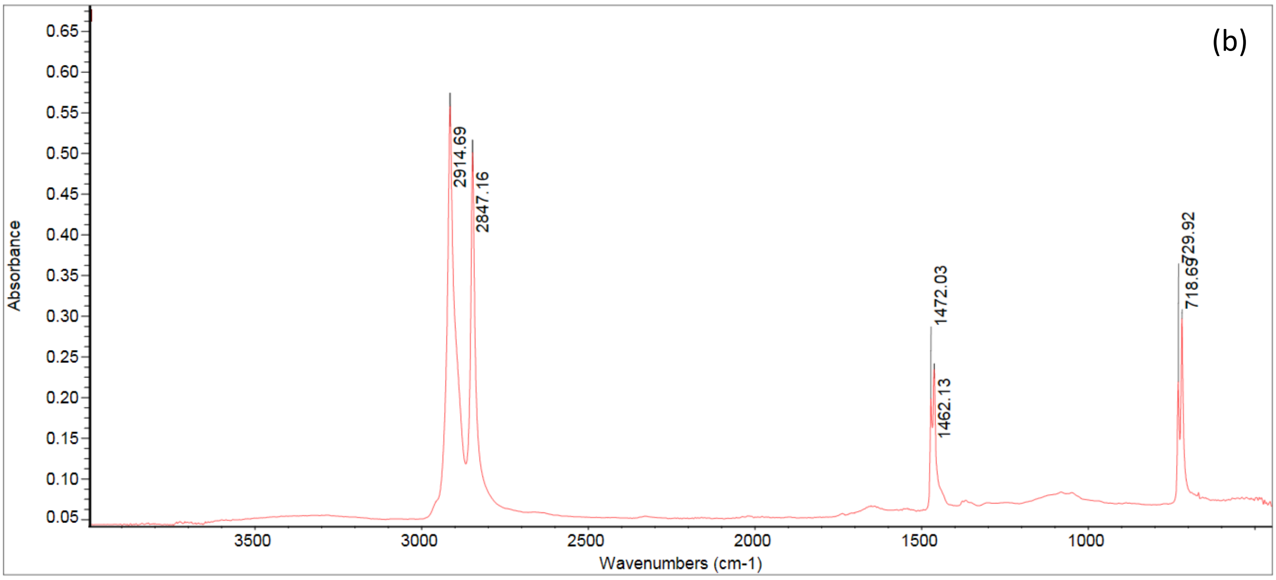
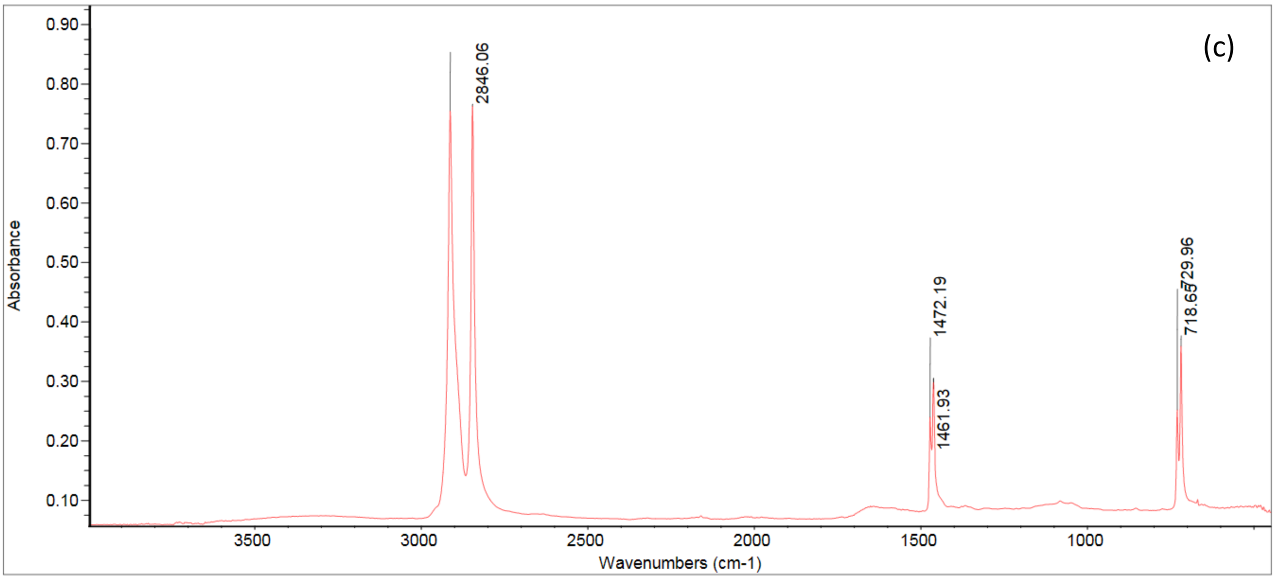


Figure 5.2.10: Spectra obtained through the FT-IR analysis for virgin PLA (a), PLA extracted from the flask FT2 (b), PLA extracted from the flask FS1 (c), PLA extracted from the flask FI (d). (e) shows all the different spectra together, in order to facilitate a comparison.




(b)
Absorbance
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
2914.69
2847.16
1472.03
1462.13
729.92
718.65
3500
3000
2500
2000
1500
1000
Wavenumbers (cm-1)


(c)
Absorbance
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
2846.06
1472.19
1461.93
729.96
718.65
3500
3000
2500
2000
1500
1000
Wavenumbers (cm-1)

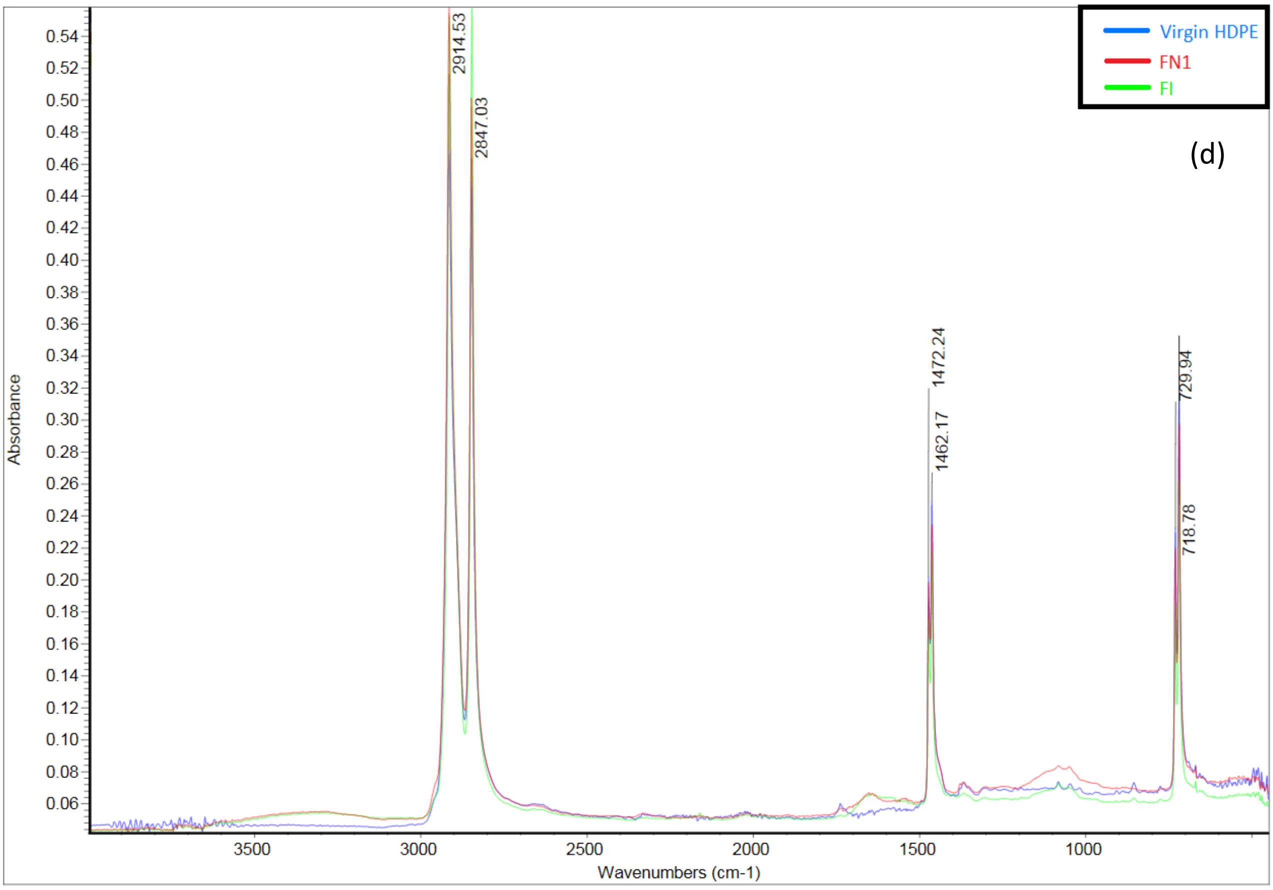
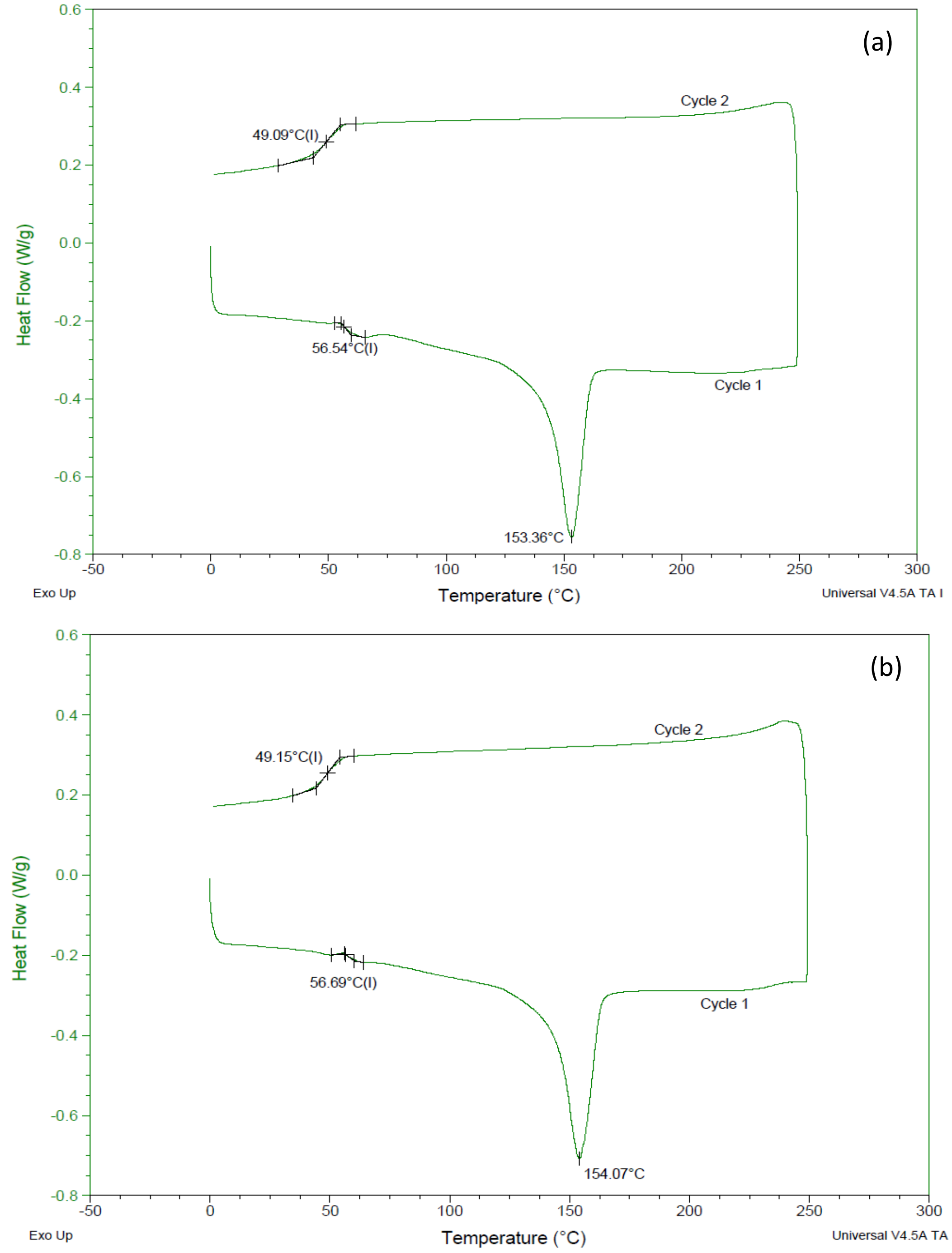
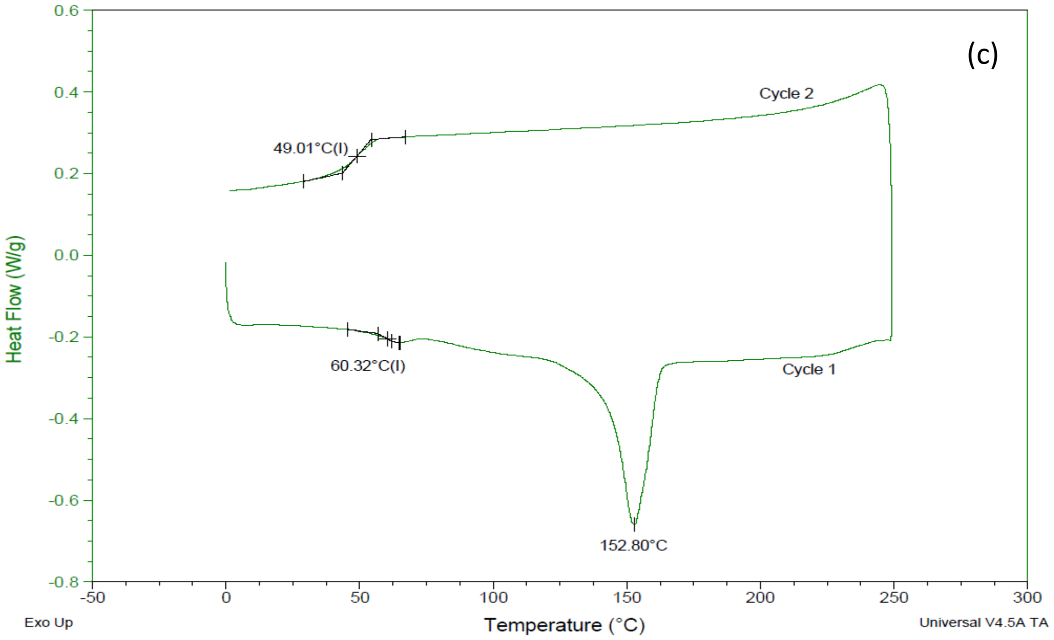


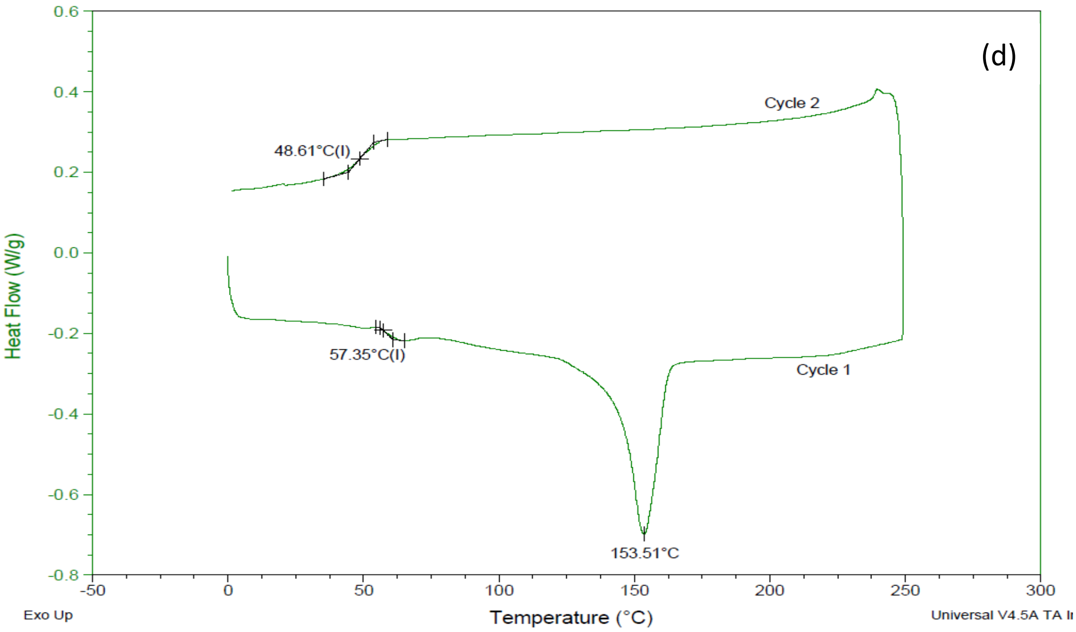
Figure 5.2.11: Spectra obtained through the FT-IR analysis for virgin HDPE (a), HDPE extracted from the flask FN1 (b) and HDPE extracted from the flask FI (c). (d) shows all the different spectra together, in order to facilitate a comparison.

As it can be seen from Figure 5.2.10 and 5.2.11, some very small differences in the spectra of virgin PLA and HDPE compared to the spectra of PLA and HDPE coming from the oxygen consumption test, can be detected (especially for PLA). However, making a comparison on the single peaks does not make any sense, since those difference can also derive from systematic errors in the use of the machine. Therefore, the comparison has to be done on the ratio between a stable peak (suitably identified) of the virgin PLA or HDPE and the peaks deriving from PLA and HDPE after the oxygen consumption test. Due to the time required to carry out these calculations, the results will not be available for this thesis.

The output of the DSC analysis is a graph in which the heat flow absorbed or released by the material (y-axis) is plotted against temperature (x-axis). The sample is subjected to a first heating cycle (to erase the thermal history of the material), then to a cooling cycle and, in turn, to a second heating cycle. During the heating and cooling cycles, endothermic (heat absorbed) and exothermic (heat released) heat flows can be detected. By a comparison of the temperature melting peaks in the third heating cycle of a virgin granule and of a biodegraded one, the presence of bio-degradation phenomena can be supposed (Grossule at al., under publication). Differential Scanning Calorimetry (DSC) has been performed using a heat-cool-heat cycle, from 0°C to 250°C with a heating and cooling equal to 10°C/min. This analysis has been performed for the virgin PLA and HDPE granules and for those extracted from the FT2, FS1, FN1 and FI flasks at the end of the experiment. In Figure 5.2.12 and 5.2.13 the results for PLA and HDPE are shown.




(c)
Heat Flow (W/g)
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
Cycle 2
49.01°C(I)
60.32°C(I)
Cycle 1
152.80°C
-50
0
50
100
150
200
250
300
Exo Up
Temperature (°C)
Universal V4.5A TA


(d)
Heat Flow (W/g)
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
Cycle 2
48.61°C(I)
57.35°C(I)
Cycle 1
153.51°C
-50
0
50
100
150
200
250
300
Exo Up
Temperature (°C)
Universal V4.5A TA I

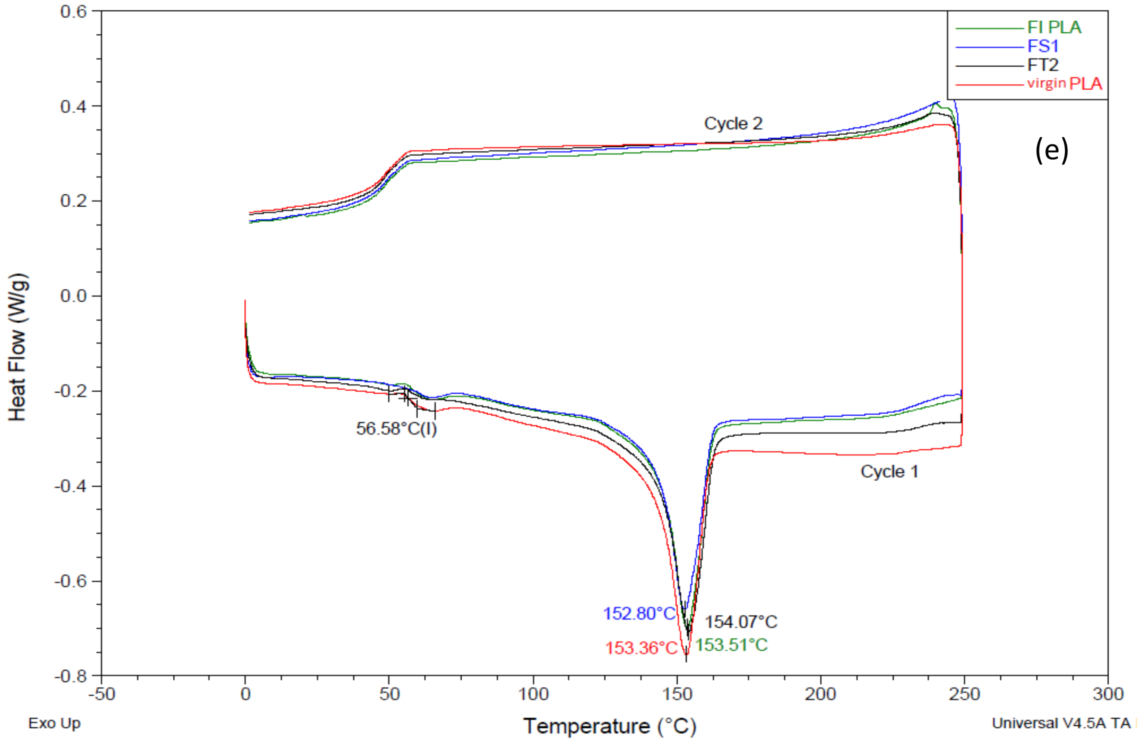
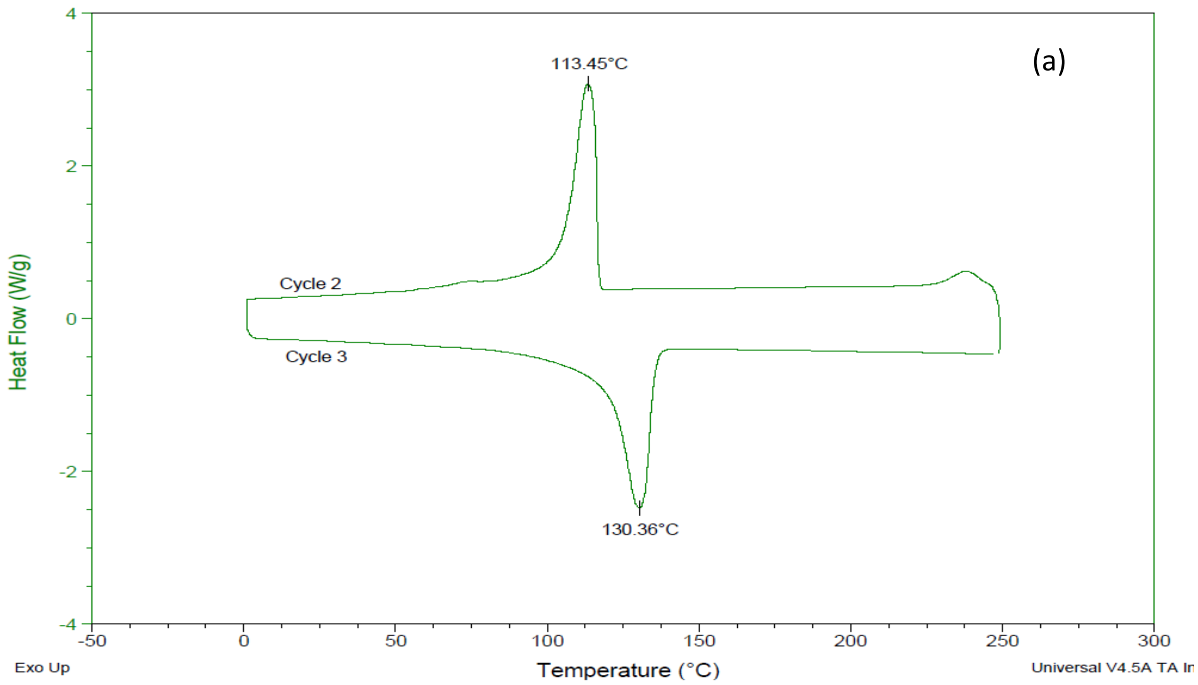
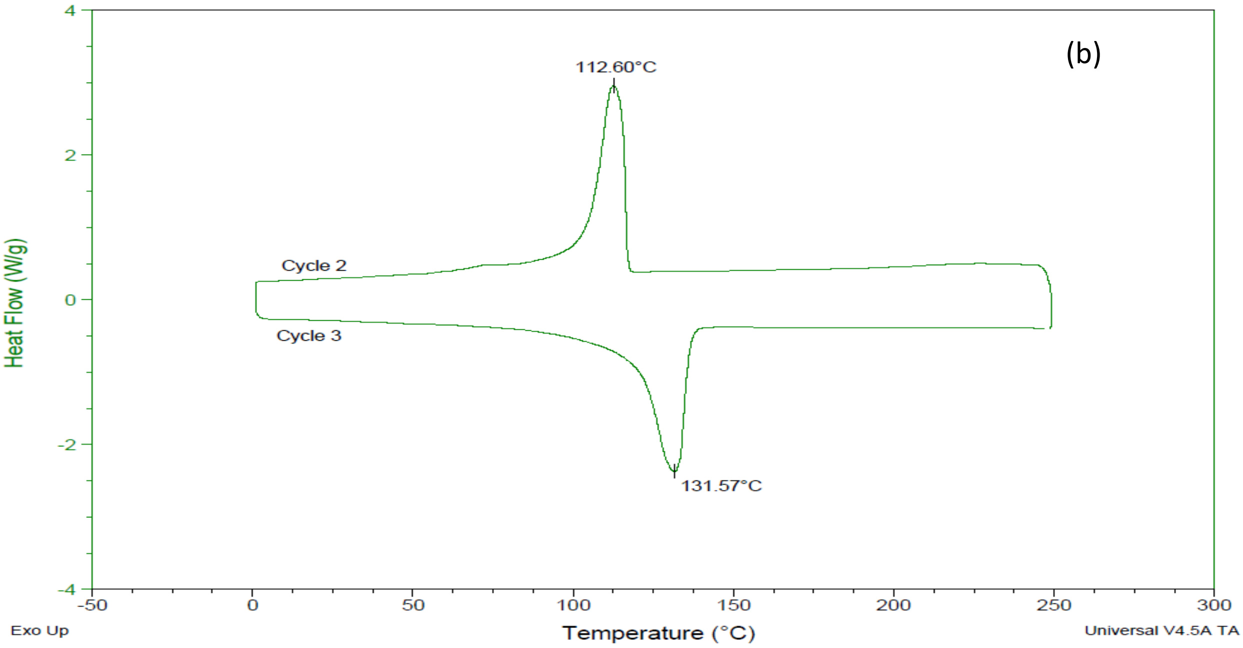
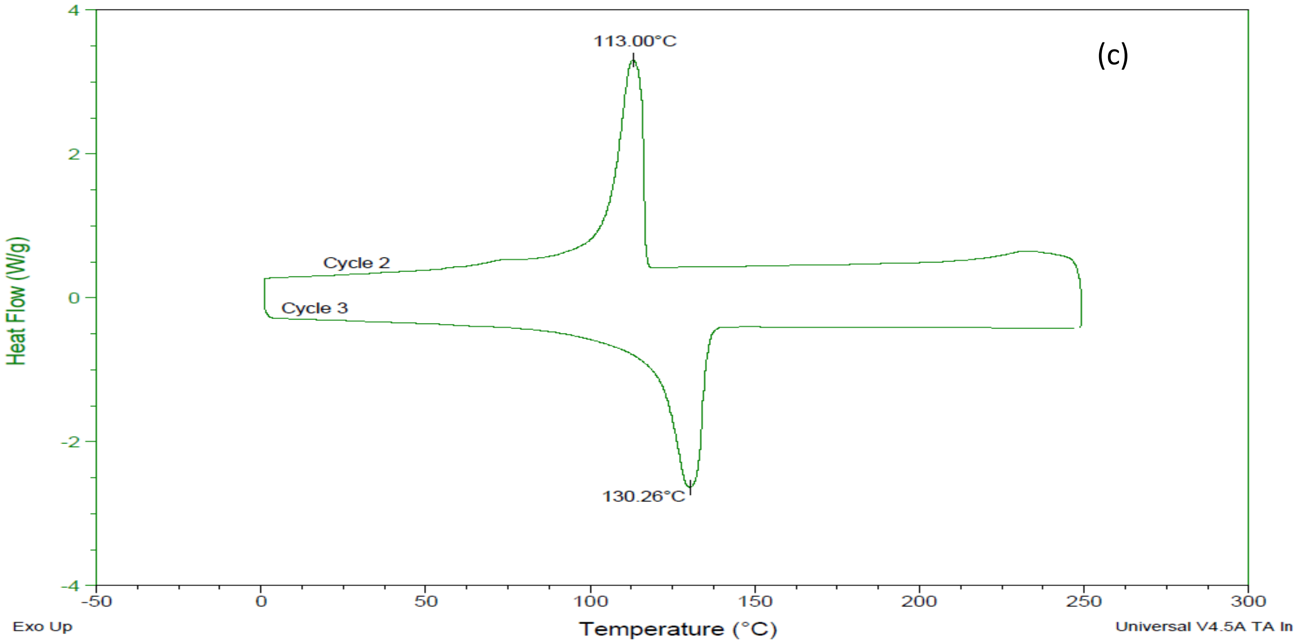


Figure 5.2.12: DSC curves of virgin PLA (a), PLA extracted from the flask FT2 (b), PLA extracted from the flask FS1 (c), PLA extracted from the flask FI (d). (e) shows all the different curves together, in order to facilitate a comparison.




(b)
112.60°C
131.57°C
Cycle 2
Cycle 3
Heat Flow (W/g)
4
2
0
-2
-4
-50
0
50
100
150
200
250
300
Temperature (°C)
Exo Up
Universal V4.5A TA


(c)
113.00°C
130.26°C
Cycle 2
Cycle 3
Heat Flow (W/g)
4
2
0
-2
-4
-50
0
50
100
150
200
250
300
Temperature (°C)
Exo Up
Universal V4.5A TA In

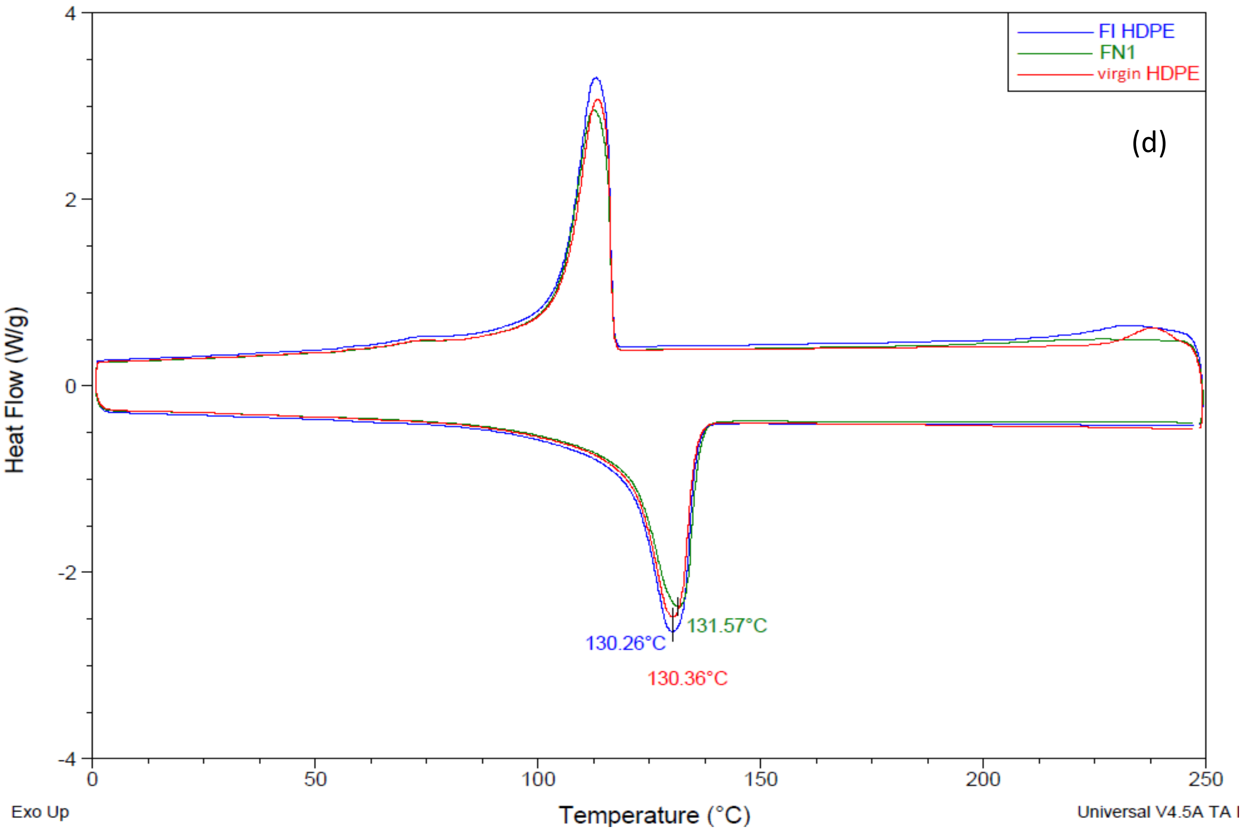


Figure 5.2.13: DSC curves of virgin HDPE (a), HDPE extracted from the flask FN1 (b) and HDPE extracted from the flask FI (c). (d) shows all the different curves together, in order to facilitate a comparison.

As it can be seen from Figure 5.2.12 and 5.2.13, some differences between the melting temperature of virgin PLA and HDPE compared to the melting temperature of PLA and HDPE coming from the oxygen consumption test can be detected, but these differences are small and always lower 1.5 °C between the virgin and the “degraded” material. This result is coherent with the one obtained through the FT-IR analysis and from the results coming from the oxygen consumption experiment.

6. CONCLUSIONS AND FUTURE PRESPECTIVES

The results coming from the literature review show a great heterogeneity of methodologies for verifying the biodegradability of bioplastics. The non-existence of a standard that set parameter levels to be met in order to label a polymer as "biodegradable" has led to the usage of various test methods with different test durations, temperatures, test medium composition, tested material forms and sizes, and different methodologies for the calculation of the degree of biodegradability. This makes comparisons of the biodegradability level of different bioplastics, and even the definition of the biodegradability level of the same type of bioplastic, difficult. Test methods set by existing standards (for both fresh and salt water) are sometimes not even follow by researchers, resulting in highly variable, and sometimes discordant results. The durations of the tests can significantly underestimate the durations required for polymer biodegradation within natural ecosystems. In the available standards, only the maximum duration of the test is indicated, thus letting individual scientists to decide the appropriate duration. Test duration, as well as temperature and initial shape of the tested bioplastic substantially influence the biodegradation result of the test. It is therefore important that test methods define uniquely all these parameters, in order to obtain comparable results.

According to the various standards, to facilitate the biodegradation of the test materials, polymers have to be reduced in size or powdered, before their immersion in the test However, the dimensions to be achieved are not indicated within the standards. Nevertheless, generally a plastic object is entire when it ends up in an aquatic environment, and therefore the shredding process alters the true fate of the product. Furthermore, the marks "OK Biodegradable MARINE" and "OK Biodegradable WATER" established by Vinçotte, which are the only ones that certify the biodegradability of a product in water, refer to ISO and ASTM standards which report the aforementioned ambiguity relating to size.

Various standards set test temperature around 20-25 °C, which, most of the time, is not a representative temperature range of a natural aquatic environment (sea/ocean mean water temperature is usually below 13 °C). Extremely variable results have been found in the literature review, most of them expressed as weight loss (without specifying the initial size), which does not allow comparisons in order to identify the effective bioplastics biodegradability.

Moreover, an appropriate standard regarding the biodegradability of bioplastics in an aquatic environment should include an analysis of the by-products, in order to evaluate their ecotoxicity.

To conclude, although the perception of bioplastic by citizens, is of a green biodegradable material, the majority of the existing biodegradable biopolymers show very different biodegradability levels according to the case study under consideration. The bioplastics that seem to really biodegrade in all the most varied conditions (temperatures, sizes, different degradation environments) are PHB, among biobased bioplastics, starch based, among the partially biobased group, PVA and PGM among fossil-based bioplastics. All these conclusions have been drawn from the analysis of the literature, which however highlighted the lack of homogeneity in testing these materials.

The results obtained from the three oxygen consumption experiments on PLA, made by following the standard BS EN ISO 14851:2019, confirm the issues highlighted by the review and bring out other criticalities.

Very often the technical data sheet of the bioplastics, that should contain the chemical formula and an indication on the presence of additives, are not provided by the companies, thus preventing a correct interpretation of the collected results. An example of this is found in the ϒ test, where a high HDPE respiration has been observed and it has not been possible to understand if it derives by the presence of antioxidants.

Observing the final Dt (%) graphs, we can affirm that PLA granules ere not degradable in a natural environment, such as the one we reproduced, at least under the condition given by the BS EN ISO 14851:2019. Moreover, it should be added that the results expressed as Respirometric Index (as it is required by the ISO) may not be an adequate way to describe the biodegradation rate of biodegradable bioplastics. In fact, there are many non-transparent elements, such as the inoculum which appeared to be not appropriate (it worked poorly), even though this inoculum derived from a domestic wastewater treatment plant, as explicitly required by the standard. Another factor supporting the inadequacy of the inoculum can be seen by comparing the Dt (%) graph, obtained in the last experiment, for the flask FI, and the results of the bacterial cultures and the related microorganism concentration (expressed in CFU/mL). From the bacterial culture results it can be seen that the bacterial count in the FI flask is the highest, while from the biodegradation test results it can be observed that the final Dt tends to zero. This means that the bacteria are present, but they do not oxidize the PLA granules.

The standard requires to sterilize the flask FS and to keep it without inoculum, but both the experiment and the bacterial cultures showed that the microorganisms inside this flask were present. Therefore, the sterilization procedure, as presented by the standard, is not sufficient. More investigations for understanding the processes the tested PLA underwent are necessary: with the help of the DAFNAE, microorganisms capable of degrading PLA could be isolated, and with the help of the Industrial Engineering Department, other analyses should be done in order to understand if the PLA and HDPE granules have been biologically attacked by the microbial activity.

An idea for future developments of this research could be to use the procedure contained in the BS EN ISO 14582: 2021 standard, in which the biodegradability of a bioplastic is investigated accordingly to the carbon dioxide production.

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