UNIVERSITAT POLITÈCNICA DE CATALUNYA
BARCELONATECH
Escola d'Enginyeria de Barcelona Est

FINAL MASTER THESIS

Master in Chemical Engineering – Smart Chemical Factories

BIOREMEDIATION OF PLASTICS USING MARINE BACTERIA

Report and Annex

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Convocatory: June 2022

Resumen

En este estudio teórico realizado para la biorremediación de plásticos mediante bacterias marinas, se realiza un amplio estudio de las estrategias actuales para la gestión de residuos de los plásticos y toda la situación presente. Entre las diferentes opciones que se presentan hoy en día como muy prometedoras, se encuentra la biorremediación de estos plásticos, que será el núcleo del proyecto. Se analizan diferentes opciones de estudios de laboratorio de la biodegradación de diferentes plásticos utilizando bacterias marinas y se ha desarrollado un enfoque industrial, con una propuesta de esquema de una pequeña planta para tratar 1000 kg/año de polietileno -tereftalato (PET) a partir de botellas de PET.

Este estudio también se centra en la comprensión de la situación real y cómo las bacterias marinas pueden ser un actor clave en los próximos años para el tratamiento de los materiales plásticos que se generan en nuestra sociedad. Se revisan y analizan los mecanismos y la información más importantes que se pueden obtener para estas bacterias marinas específicas que son capaces de biorremediar plásticos para dar una buena visión de los problemas reales y futuros a los que se enfrenta la sociedad humana y la salud pública debido a la producción de micro y nanoplásticos, proporcionando una posible solución factible para reducir el impacto que pueden producir estos problemas. Para esto, se ha seleccionado una bacteria como el candidato más prometedor y se ha utilizado para modelar el esquema propuesto que se presenta en este estudio.

Resum

En aquest estudi teòric realitzat per a la bioremediació de plàstics mitjançant bacteris marins, es realitza un ampli estudi de les estratègies actuals per a la gestió de residus dels plàstics i tota la situació actual. Entre les diferents opcions que es presenten avui dia com a molt prometedores, hi ha la bioremediació d'aquests plàstics, que serà el nucli del projecte. Es van analitzar diferents opcions d'estudis de laboratori de la biodegradació de diferents plàstics, es va seleccionar la més prometedora i es va desenvolupar un enfocament industrial, amb una proposta d'esquema d'una petita planta per tractar 1000 kg/any de polietilè-tereftalat (PET) a partir de ampolles de PET.

Aquest estudi també se centra en la comprensió de la situació real i com els bacteris marins poden ser un actor clau en els propers anys per al tractament dels materials plàstics que es generen a la nostra societat. Es van revisar i analitzar els mecanismes i la informació més importants que es poden obtenir per aquests bacteris marins específics que són capaços de bioremediar plàstics per tal de donar una bona visió dels problemes reals i futurs als quals s'enfronta la societat humana i la salut pública a causa dels micro i nanoplàstics, proporcionant una possible solució factible per reduir l'impacte que poden produir aquests problemes. Per això, es va seleccionar un bacteri com el candidat més prometedor i es va utilitzar per modelar l'esquema proposat que es presenta en aquest estudi.

Abstract

In this theorical study performed for the bioremediation of plastics using marine bacteria, a broad study of the actual strategies for the waste management of the plastics and whole situation is performed. Among the different options that are present nowadays as very promising, it's the bioremediation of this plastics, which will be the core of the project. Different options from laboratory studies of the biodegradation of different plastics were analyzed and the most promising one was selected and an industrial approach was developed, with a proposed scheme of a small plant to treat 1000 kg/year of polyethylene-terephthalate (PET) from PET bottles.

This study also is focused on the understanding of the actual situation and how the marine bacteria can be a key player in the next years for the treatment of the plastic materials that are generated in our society. The most important mechanisms and information that can be obtained for these specific marine bacteria that are able to bioremediate plastic was reviewed and analyzed in order to provide a good view of the actual and future problems that human society and public health is facing because of the micro and nanoplastics and provide a possible feasible solution to reduce the impact that these problems can produce. For this, one bacteria was selected as the most promising candidate and was used to model the proposed scheme that is presented in this study.

Appreciations

Thanks to my friends and family for the support and help during my academic career and thanks to my tutor, Jordi Bou, because of propose me and let me work in a topic that I consider so important and that will make the difference in the future.

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1. Introduction

Nowadays the consumption model generates a lot of waste of many kinds, that is a growing problem as the typical strategies are reaching a non-functional point. The plastic waste is one of the main concerns as affects in a huge way to not only the soil or the humans in a direct way but also the biosphere and the oceans and seas. Plastic waste can be bioaccumulated on the edible animals and plants and also on the water to feed them, so finally affects also in a non-direct way with a bioaccumulation on the trophic chain and it's understood that will generate so many problems to public health on the next years.

The plastic waste from the petroleum derived products is very common in the normal life and are very hard produced and consumed by the different applications in society. As there are many problems with them, novel forms of treatment and waste management are needed in order to approach to a more sustainable and effective way that avoid the generation of the bioaccumulated components.

1.1. Motivation

As the typical ways to manage the waste generated on our society are reaching a breakdown point, it's mandatory to explore new ways to obtain an environmentally friendly and viable economical way to manage at least a part of this waste and indeed start a new way of thinking and focus on sustainable ways to change not only the economic model, but also the way of consume and search for new ways to live.

In this context, the bioremediation of the plastic waste through microorganism is really promising as it's considered as an environmentally friendly and also a possible economically feasible way to manage this waste. The usage of the microorganism can be rally profitable in the sense of searching microorganisms that could manage this waste in an appropriate way and then free a part of the pressure putted on the waste management industry by the problems that our actual society suffer and will suffer in a harder way. This document will focus on the usage of marine bacteria as the main microorganism that will drive this bioremediation approach.

1.2. Objective

Taking into account the issue presented, this project will offer a feasible approach of bioremediation of the plastic waste with a certain strain of marine bacteria. The approach its focused on a small plant approach, providing also the information of the whole process needed to manage the plastic waste. It will have the following points:

- Definition of the problem and the scope of the approach
- Understanding of the metabolic and microbial processes that allows the bacteria to degrade the non-biodegradable plastic waste
- Selection and study of a certain strain of marine bacteria that could allow to manage the type of plastic waste decided and its growth and mechanism characteristic
- Construction of the whole process and equipment description
- Rigorous design of the bioreactor and specific definition of the conditions needed as growth media, dimensions, materials and operation data.
- Economic and environmental analysis of the approach to be able to evaluate the performance and actual viability of the provided process

1.3. Scope

The scope of the project presented in this document will be determined by the novel approach that it represent for the bioremediation field, as the marine bacteria usage is a very innovative approach for the management of the plastic waste. Taking into account the lack of information that is present in the field, some assumptions had to be made in order to give a more practical approach of an industrial process and evaluate the availability or suitability of this technology in an industrial world.

Once this is made, the desired process with the selected candidate will be performed to give an industrial and practical approach in terms of viability in a small plant scale. The supposed capacity of the plant will be of 1000 kg of plastic degraded per year, as will be commented and justified later.

2. State of the art

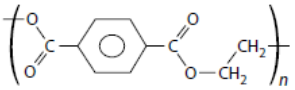
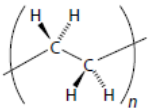
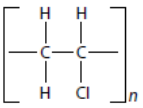
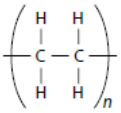
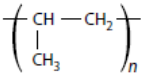
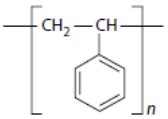
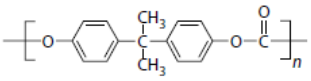
Plastic pollution is nowadays one of the main threats for the ecosystem and also the society. This threat appears as it affects also the biotic and the abiotic components of the biosphere and also the trophic chain with bioaccumulation problems. On 2020, the global production of plastic reached 367 million tons. From this data China is the highest contributor with a 32% of the total, Europe with a 15% follows and the conglomerate of Mexico, Canada and US under the name NAFTA (North American Free Trade Agreement) has a 19%. [1]

The key of the problem presented before appears not on the increasing amount of plastic produced, but on the increasing number of plastic debris that is thrown on the water bodies and the soils without a proper waste management and bioaccumulates on the biosphere with awful consequences. It's estimated that around 4.8 to 12.8 million tons of plastic debris are thrown on seas and oceans without the proper management strategies to reduce or eliminate the risk that surrounds it. A clear example of this problem nowadays could be seen with the example of the two most contaminated water bodies on the worlds, the river Yangtze in China with an input plastic waste of almost 310000 tons and the river Ganges on India with an input plastic waste of 115000 tons. [2]

It's estimated that more than the 80% of the world plastic use is related with the petrochemical plastics. Between the most used ones they are polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC). [2]

The most used ones are presented on the **Table 2.1** with the chemical structure, symbol associated and most typical applications:

***Table 2.1**: Most widely used plastics with additional information and typical applications. Source: [3]*

Plastic	Symbol	Structure	Applications
Polypropylene terephthalate	01 PET		Soft drink, water and dressing bottles, peanut butter, and jam bars
High-density polyethylene	2 HDPE		Milk, juice and water bottles, trash and retail bags
Polyvinyl chloride	3 PVC		Juice bottles, cling films, raincoats, visors, shoe soles, garden hoses, and electricity pipes
Low-density polyethylene	4 LDPE		Frozen food bags, squeezable bottles, flexible container lids
Polypropylene	5 PP		Bottle caps, drinking straws, medicine bottles, car batteries, disposable syringes
Polystyrene	6 PS		Packing materials, laboratory ware, disposable cups, plates, trays, and cutlery
Others (often polycarbonate)	07 O		Beverage bottles, baby milk bottles, electronic casing

As these materials are an essential part of the economic network and worldwide economy, it's not possible to just ignore the issues related with them. Indeed, most of the plastics that are most used are non-degradable and remains for a really extended period on the biosphere as was commented before. The presence of the plastics that remain on the soil or the water bodies, generate small particles that are the real concern of the plastic bad waste management and bioaccumulation problems. These small particles are known as [2]:

- Microplastics (MPs): If the size is between 0.1 µm and 5 mm.
- Nanoplastics (NPs): If the size is between 0.001 µm and 0.1 µm.

These two types of particles are the main drivers of the bioaccumulation, toxicity and pollution derived from plastic waste. The main problems that are related with the plastic debris and the presence of micro and nanoplastics are mainly the following [4]:

- Entanglement on the plastic debris with serious injury on the growth, restriction of the movement, prevention from properly feeding and in case of mammals.
- Accumulation on key organs as kidney, liver or lungs with severe consequences as cancer, malformations or diseases.

- Accumulation on digestive system leading to decreased stimuli for feeding, possible blockage on gastrointestinal zones, decreased hormone and enzyme production and finally reproduction problems associated.

The plastic particles have a typical high toxicity as can be found numerous toxic chemicals and organic pollutants on the plastic debris as polychlorinated biphenyls (PCBs), nonylphenol (NP), organic pesticides as dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and bisphenol A (BPA) mainly. These different compounds are really dangerous for the wildlife and can be bioaccumulated representing a potential risk to human health. These risks associated to human health are developmental impairment, cancer, endocrine disruption, neurological behavioral changes, diabetes, arthritis and DNA hypomethylation. [4]

All the effects commented before can be schematized as it's presented on the **Figure 2.1**:

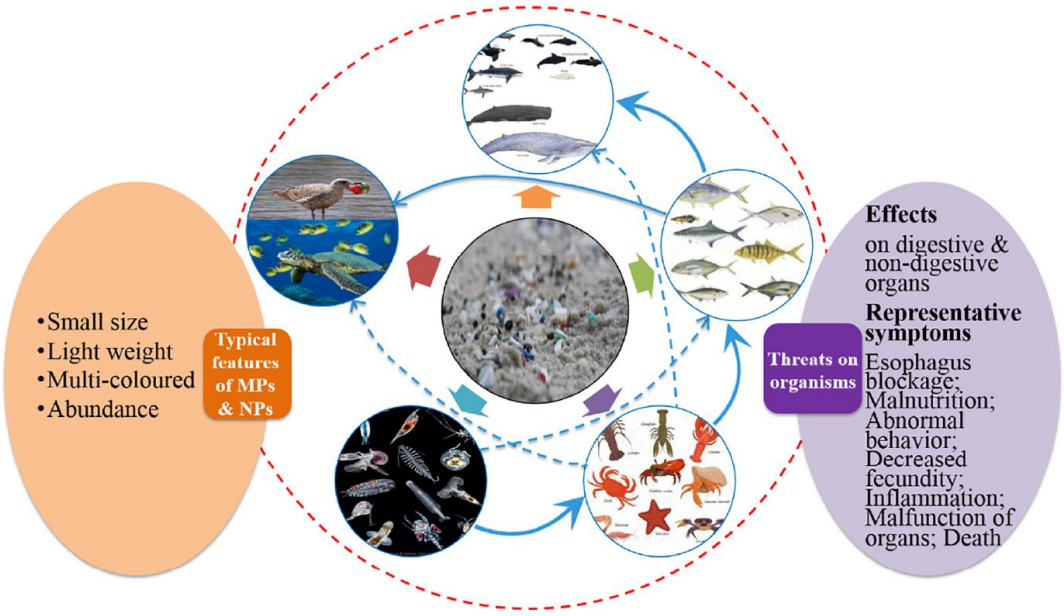


***Figure 2.1**: Micro and nanoplastics effects on marine organisms. Source: [5]*

Other derived problem, as commentary, is that the decrease on the health of the indigenous fauna generate opportunities to the invasive and destructive species to migrate to new territories and disrupt the environmental equilibrium. [4]

2.1. Production and situation of micro and nanoplastics in nature

First of all, to be able to design and obtain new ways to manage the plastic waste and avoid the actual problems with them it's needed to understand firstly how the plastic waste that is able to bioaccumulate and generate health and environmental problems (nanoplastics and microplastics) is typically generated.

The microplastics and nanoplastics can be divided into primary and secondary according to the source where they come:

- Primary MPs/NPs: Those that are released into the environment directly in form of micro-pellets, microbeads, microfibers or nanofibers and other different forms in an accidental or intentional way. They are produced as intended products, wastes from manufactures or erosion of large plastic wastes as wheels or boards for example. [5]
- Secondary MPs/NPs: Those that are derived directly from the breakdown of large or mesoplastic litters that are on the environment by direct action of physical, chemical and biological forces. Some of them are for example mechanical forces, heat, oxidation and ultraviolet light. The highest amount of micro and nanoplastics are directly generated as secondary micro/nanoplastics. [5]

This information can be easily understood, among the accumulation on the food chain and the possibility of scaling to health problems that were commented before in **Figure 2.2**:

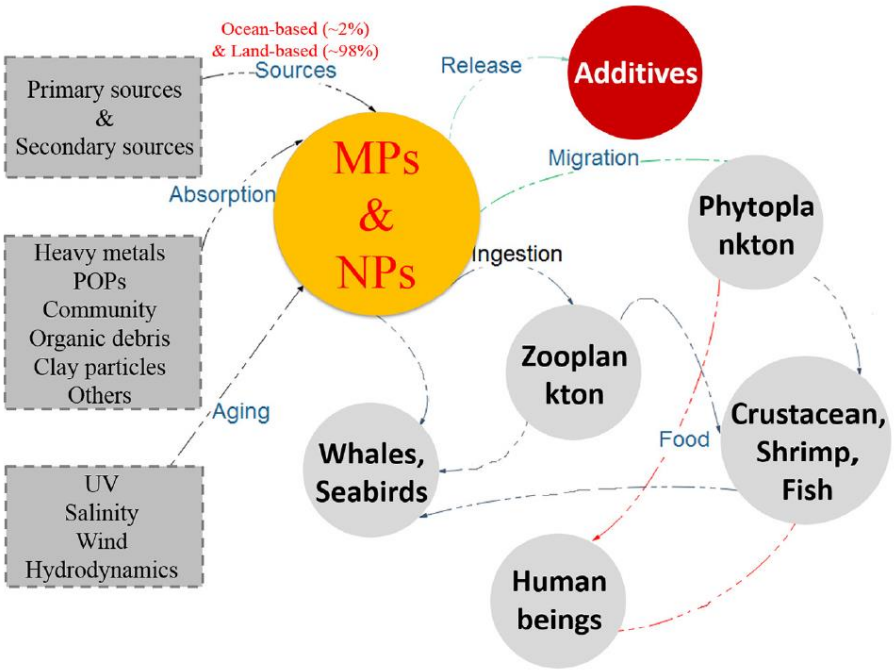


***Figure 2.2**: Micro and nanoplastics routes and bioaccumulation sources. Source: [5]*

As can be seen on **Figure 2.2**, the outstanding majority of the micro and nanoplastics sources are land based as the most human activities are land based also. The plastic litters are usually directly discharged to lands, promoting that fact. The presence of this components is directly related also to a bad waste management, but also to illegal dumping or even accidental discharges that can be related to activities as for example construction, manufacturing or farming. [5]

It's true that an immense majority of the micro and nanoplastics are land-based but the main problem come when they are accumulated as micro and nanoplastics on the water bodies. So, to understand that, it's important to analyze also how the land-based plastic waste in transferred into the water bodies as rivers and oceans.

The entrance of the micro and nanoplastics can be mainly drive by three pathways, that are wind or air pathway, soil pathway and the water or riverine pathway that is the major route. The water bodies as rivers take these plastic wastes from many different parts as sewer overflows, air precipitation, dust or other discharges and carry them into the ocean. As the density of the plastics is usually lower than the water one, a high fraction of this micro and nanoplastics carried by the rivers reach finally the ocean. [5]

The air/wind can also transfer plastics at high distances from the coast regions to sea surfaces, making a direct discharge. Nonetheless, the high distance transportation it's only expected in such special cases as hurricanes, storms or typhons. The agricultural activities generate also dangerous plastic wastes as pesticide and fertilizers packaging in soils, allowing the entering of these dangerous components accumulated on the soil into the water bodies during the rain runoffs or tidal washings. [5]

The transfer of the nano and microplastics into water bodies (specially oceans) is directly related with [5]:

- Physicochemical characteristics of the plastics: Such as chemical composition, surface charge, hydrophobicity/hydrophilicity, density, shape and size.
- Ocean dynamic conditions: Influence and changes on wind, waves, currents, water flows and tidal regimes.
- Ocean geometry: Mainly terrain and slope.
- Shoreline characteristics: As for example coastal vegetation, soil type or bioturbation.
- Biological interactions: As biofouling or the possible ingestion of them by organisms as was commented before.
- Human activities: Activities as tourism, fishery or urban development can facilitate the transportation.

As example of this transportation possibilities for the micro and nanoplastics, and the incredible possibility of high persistence and also high movement is given by the [5] as foamed polystyrene

particles (with a density of 1,05 g/cm^3) that are able to be transferred along all the Baltic Sea, that means around 250 km, in one day if significant windage is present. That give the idea of the real problem that is facing in that document, with not only bioaccumulation or diseases associated but also high mobility and persistence that reinforces the idea that this issue can't be avoided and new ways of management are needed. Some of the main micro and nanoplastics on the main oceans are presented on the **Table 2.2** :

***Table 2.2**: Micro and nanoplastics present on the main water bodies and oceans around the world. Source: [5]*

Location	Net size (μm)	Dominant MPs	MPs size (mm)	Abundance (p/m^3)	Reference
North Yellow Sea, China (Asia)	30	Film, fiber, granule, pellet	<0.5	545 ± 282	(Zhu et al., 2018)
Yangtze Estuary	333	Fibers, granules, films	0.5–5	4137.3 ± 2461.5 (Estuarine); 0.167 ± 0.138 (Sea)	(Zhao et al., 2014)
Tokyo Bay, Suruga Bay, Ise Bay, Seto Inland Sea (Asia)	350	Spherical particle, microbead	<2 (most)	0.03–0.075	(Isobe et al., 2015)
South-eastern coastline of South Africa	80	Fiber	0.08–5	257.9 ± 53.36–3308 ± 1449	(Nel and Froneman, 2015)
Guanabara Bay, Rio de Janeiro, Brazil	300	Fragment, film	0.3–1	1.4–21.3	(Olivatto et al., 2019)
North Eastern Mediterranean Sea			<5	42 ± 46.7	(Guven et al., 2016)
South Funen Archipelago, Baltic Sea in Europe	300	Fragment, fiber	0.3–0.63 (most)	0.07 ± 0.02	(Tamminga et al., 2018)
Cape cod to the Caribbean in Atlantic	947	Spherule	1	6.06×10^{-5}-8.32×10^{-3}	(Colton et al., 1974)
Louisiana coast in the northern Gulf of Mexico	335	Fibers	<0.1	5.0–18.4	(Mauro et al., 2017)
Northwestern Pacific	330	Fiber, fragment, film		0.13 ± 0.11	(Mu et al., 2019)
from Fremantle to Hobart, Australia	350	Fragment, fiber		0.031	(Isobe et al., 2016)
South and southwest of Svalbard, Norway		Fiber, fragment, film	1.93 ± 1.22	0.34 ± 0.31	(Lusher et al., 2015a)
Arctic Central Basin	250	Fiber, fragment	1–2	0.7	(Kanhai et al., 2018)
NorthWest Europe	333	Fibers and spheres	0.355–5	0–1.5	(Maes et al., 2017)

Notes: Unit of p/m^2 or p/m^3, g/m^3 indicates the piece of MPs per square meter or cubic meter, and gram of MPs per cubic meter of water, respectively. The omitted unit of abundance is "p/m^3" by default, while other units are presented in the table.

As can be seen on the **Table 2.2**, the accumulation of micro and nanoplastics it's critical in certain water bodies as for example on the North Yellow Sea in China or in certain zones of the South Africa coastline, making it a worldwide problem and not only a developed or non-developed countries problem.

2.2. Typical plastic waste management options

Right now, it's clear that the actual strategies for plastic waste management are not good enough or not efficient enough to face the problems that appeared on the last years and the awful forecasts in terms of public and environmental health. To understand the changes that are appearing in terms of investigation and inversion by the European Union, it's mandatory to first stablish the framework from which the actions taken are based on.

The trends nowadays on the whole waste management strategies is based on the legal framework provided by the directive 2008/98/CE. This directive stablishes the preferred options in terms of waste management. The preferred solution, from up to down, it's presented on :

***Figure 2.3**: Waste management framework representation. Source: [6] [7]*

As can be seen on **Figure 2.3**, the waste management framework picks as preferred option the prevention and less produced amount of waste. As this is not the case that concern this study, nor the re-use, the focus will be on the three main strategies provided by the Directive 2008/98/CE [6]:

- Recycling (Preferred one)
- Recovery (Energy recovery on this case)
- Disposal (Landfill)

So, it's possible to see that this directive stablishes some options that need to be focused on to develop a more environmentally friendly and also circular system and also set some requirements to the waste treatment such as insurance on that the waste will be managed without endangering the human health and the environment, without risk natural resources and adversely affect people and landmarks. [6]

This framework also set some different objectives for these last years as for example [7]:

- By 2020 increase the amount of reused and recycled waste materials (paper, metal, plastic and glass) to a minimum of 50% and usage of reused and recycled materials for non-hazardous construction and substitution of materials increased by a minimum of 70%.
- By 2025 increase of the reuse and recycling of MSW up to 55% (2025), 60% (2030) and 65% (2035) by weight as minimum.

With these objectives settled up, the traditional strategies seem to be not enough accounting the problems that are present nowadays. According to the framework stablished by [6], the traditional plastic waste management strategies that are going to be evaluated are the ones that implies some waste management as recycling, energy recovery and landfill. Among those ones, the most typical ones are presented.

First of all, mechanical recycling. This is one of the actual main parts on the reprocessing of the recycled plastic materials. First of all, the different plastic materials are recovered, sorted and separated due to shape, density, size, color or chemical composition. Once this is done, it's washed to remove of the possible contaminants that they can have. Then they are grinded, reducing its size from the initial product to form of flakes. Finally, some of them are reprocessed into granulates, to facilitate their usage on the converters. This alternative, that is usually coupled with some thermal treatment, has some drawbacks and one of the main is the possible degrading of the polymers under some conditions like oxidation, light, hydrolysis or mechanical stress. [8]

Other of the forms that has increased its importance is chemical recycling. Chemical recycling started to be an interesting and ascending option as can be suitable for the production of valuable chemicals and also fuels. The amount on oxygen of the plastic feedstock is really low comparing to biomass, which allows to obtain a higher carbon efficiency and then really interesting chemicals with a good margin. Some of the technologies that are used into this alternative are gasification, pyrolysis, fluid-catalyzed cracking and hydrocracking. [8]

An example of this chemical recycling routes is provided for PET on **Figure 2.4**:

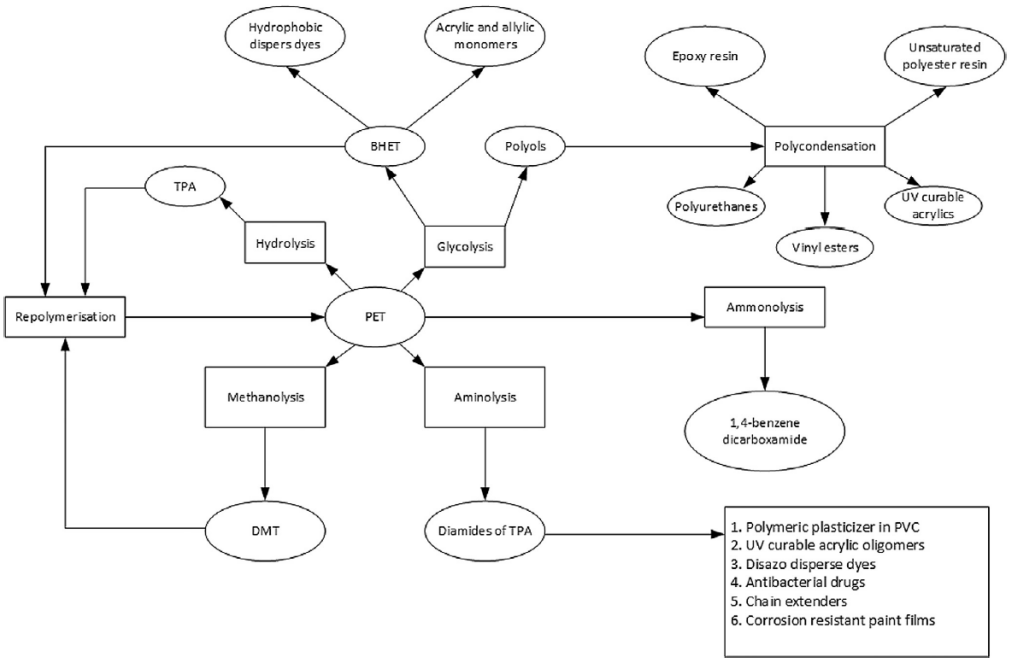


***Figure 2.4**: Chemical recycling route of PET. Source: [8]*

The next preferred option according to **Figure 2.3** is the energy recovery of the plastic waste. The energy recovery of these waste is often driven by incineration or thermal processes. For incineration, it's one of the simplest processes but has many drawbacks that makes it each year less attractive at not only governments but also society. The energy that is recovered through this

alternative is at the same order than the virgin polymer. Nonetheless, the amount is estimated to being depleted to even the half of the initial value due to the degraded quality. Also, the greenhouse gases emissions and also the contaminants generated make it a non-preferred option.[9]

One example of this contaminants generated is presented on the **Table 2.3** for the case of PVC incineration:

***Table 2.3**: Contaminant compounds and health effects of the incineration of PVC. Source: [10]*

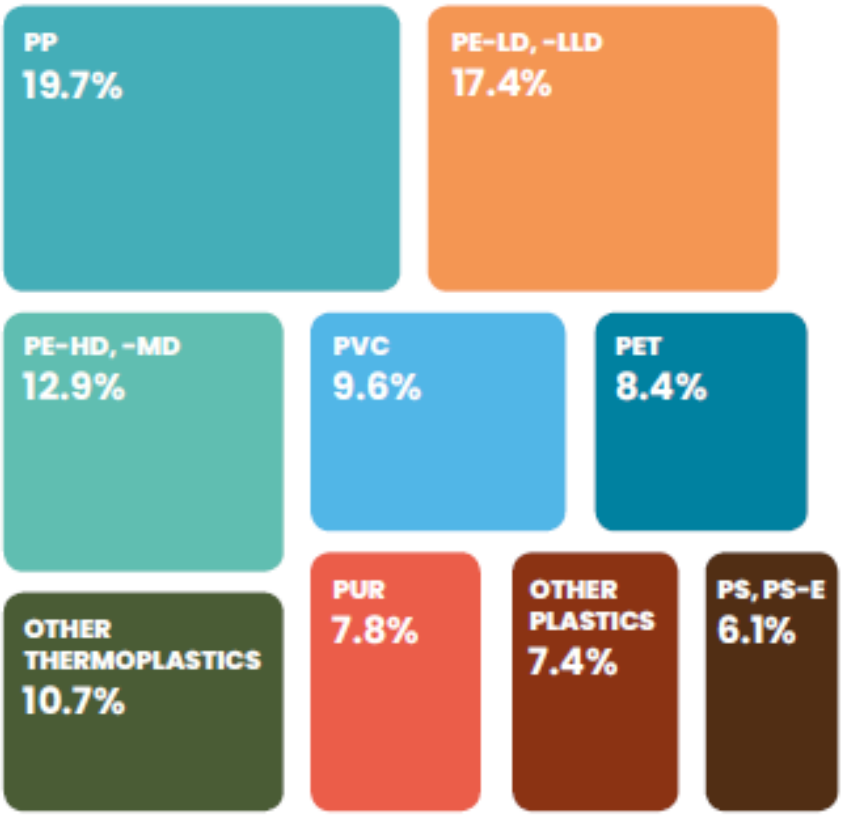
Compound	Health effect(s)
Acetaldehyde	It damages the nervous system, causing lesions.
Acetone	Irritates the eyes, the respiratory tract.
Benzaldehyde	Irritates the eyes, skin, respiratory system, limits brain function.
Benzole	Carcinogenic, adversely effects the bone marrow, the liver, the immune system.
Formaldehyde	Serious eye damage, carcinogenic, may cause pulmonary oedema.
Phosgene	Gas used in the WWI. Corrosive to the eyes, skin and respiratory organs.
Polychlorinated dibenzo-dioxin	Carcinogenic, irritates the skin, eyes and respiratory system. It damages the circulatory, digestive and nervous system, liver, bone marrow.
Polychlorinated dibenzofuran	Irritates the eyes and the respiratory system, causes asthma.
Hydrochloric acid	Corrosive to the eyes, the skin and the respiratory tract.
Salicyl-aldehyde	Irritates the eyes, the skin and the respiratory tract. It can also affect the central nervous system.
Toluene	Irritates the eyes and the respiratory tract, can cause depression.
Xylene	Irritates the eyes. It can also affect the central nervous system, reduces the level of consciousness and impairs learning ability.
Propylene	Damages the central nervous system by lowering of consciousness.
Vinyl chloride	Carcinogenic, irritating to eyes, skin and respiratory system. Effect on the central nervous system, liver, spleen, blood-forming organs.

Other of the energy recovery treatments that have some interesting situation as a way of recover this energy as fuel or chemicals is the pyrolysis. This process is based on the breakdown of long polymer chains in an environment of oxygen depletion and high temperature. Different fuels and chemicals can be obtained depending on the usage or not of catalyst and also on the catalyst selected and its selectivity towards some specific chemical route.[9]

Landfill is finally the least preferred option and constantly being depleted against other more environmentally friendly and more safe options. Landfill is the disposal of the plastic wastes untreated at a special place prepared to the storage during a certain time (20 years or more). The lack of space, the growing concern about public health and environment and also the potential leachate of toxic chemicals that can lead to soil and groundwater contamination for long terms, made this option an actual least preferred and all the legislation provided by the European Union make its possibilities as an option decrease each year. [10]

2.3. Actual situation of plastic waste management on EU-27

To understand the necessities of Europe and the effort putted on the development of new materials and new strategies to the management of waste and specially on plastic waste, it's mandatory to first take a look around the whole picture that is the plastic waste management and plastic environment on Europe. The distribution of demand on main plastic products for economic activities on the EU are presented on **Figure 2.5**:



PP: Polypropylene

PE-LD, -LLD: Polyethylene low density (LD) and linear low density (LLD)

PE-HD, -MD: Polyethylene high density (HD) and medium density (MD)

PVC: Polyvinyl chloride

PET: Polyethylene terephthalate

Other thermoplastics: Includes polyacetals, polyesters resins, etc.

PUR: Polyurethane

Other plastics: Adhesives, coatings and sealants

PS, PS-E: Polystyrene (PS) and expandable polystyrene (PS-E)

***Figure 2.5**: Distribution of polymer demand by type of polymer on EU-28. Source: [1]*

As can be seen on **Figure 2.5**, the demands are mainly dominated by polypropylene and polyethylene of different types that will depend on the final use that will be provided to them. The whole demand of the European market and this percentages are associated to the amount of 49.1 mega tons of total plastic demanded and used on the industry on the year 2020. [1]

For the post-consumer plastic waste, in 2020 was collected on the EU28 around 29.5 mega tons. That amount doesn't match the demand amount as the life span of the plastic products have various ages (ranging usually from 1 to 50), making it not the same. Behind this 29.5 mega tons the distribution of the plastic waste management is presented on **Figure 2.6**:

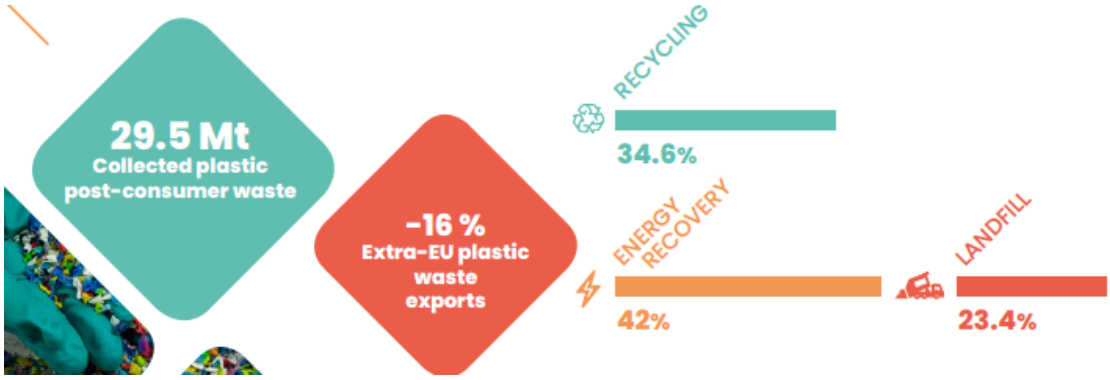


***Figure 2.6**: Distribution of plastic waste management on EU-28 during 2020. Source: [1]*

As can be seen on **Figure 2.6**, right now more than a third of the plastic waste is collected and recycled in the corresponding facilities with a good result overall. On the other hand, the energy recovery option is presented as the main option nowadays with the contamination (air contamination mainly). The landfill option represents almost a quarter of the total amount, which represents also a discard of this wastes and then a remanent problem, which despite the different protection of the landfills to avoid leaches maybe could generate the problems considered before.

It's important also to check how the plastic waste management was moving around these last years to check possible trends and see the ideas and projects behind them. **Figure 2.7** presents the plastic waste management trend between the years 2006-2020:

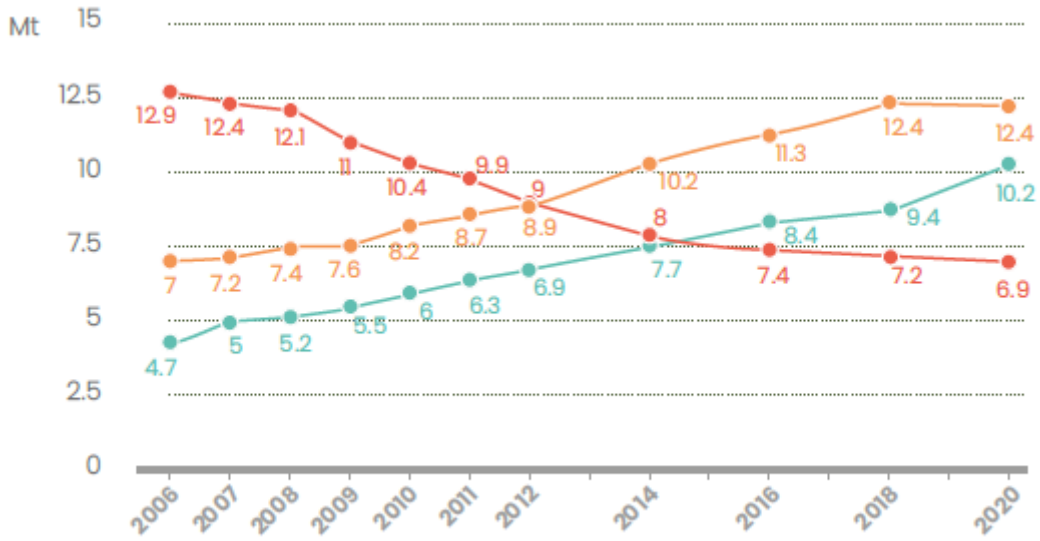


***Figure 2.7**: Evolution of plastic waste management on EU-28 during 2006-2020. Source: [1]*

As can be seen on **Figure 2.7**, some trends are possible to be analyzed. First of all, it's possible to see an increase on the total amount of plastic waste collected from 2006 (24.5 Mt) to 2020 (29.5 Mt).

Also, the different treatments experimented some increases or decreases on the time depending mainly on the efforts putted by the European Union and other governments to fix the problems derived from the traditional plastic waste management, which demonstrated to be not sufficient nowadays. The recycling (blue) and energy recovery (yellow) options experimented a huge increase along the years, being an increase of 117.7% for recycling and a 77.1% for energy recovery. On the other hand, the landfill (red) option had decreased on a really important amount, that is estimated as a decrease of 46.4%.

This trend can be matched with the overall strategy of waste management of the European Union of the last years and also the forecasts made for the next years.

3. Bioremediation strategies and bacteria available

As was commented before, the actual strategies for the plastic waste management are not good enough. These different strategies are not fulfilling the needs of the next steps for our society and also are a huge concern because of the contaminants, micro and nanoplastics and also soil and water bodies contamination through this. So, attending to this trend new approaches were searched for match the necessities of nowadays. One of the main strategies that is being developed and studied because of the environmentally friendly and commercial possibilities characteristics is the bioremediation.

The bioremediation is defined in [11] as: '' *A natural phenomenon but seldom induced to clean up the environmental contamination and is carried out by deteriorating microbes (mostly fungi and bacteria)''*

The bioremediation uses as key carriers the microorganisms to clean or restore the contaminated environment at low cost and with eco-friendly methods. The microorganisms that surround this contaminated environment, either soil either water bodies, they suffer very extreme conditions in terms of toxic concentrations on a certain recalcitrant or very toxic compound and/or elements. Anyway, they are able to produce some special mechanisms that allow them to survive in those harsh conditions. This has two important implications that are [11][12]:

- Usage of very specific organisms for specific toxic compounds/elements.
- Usage of autochthonous microorganisms (as far as possible) from the same polluted environment that is intended to be recovered.

On the other hand, different type of strategies can be used in remediation approaches. Mainly they are divided into in-situ and ex-situ depending on where the remediation action is done. The in-situ approach is based on remove directly the contaminants that are present on the soil or the water bodies directly without any physical movement of the media. Some of the main in-situ techniques are [12][13]:

- Biological treatments: Land farming, natural attenuation, bioremediation, bioventing or phytoremediation.
- Physico-chemical treatments: Solidification and stabilization, soil flushing, fracturing, electroreclamation or adsorption.
- Thermal treatments: Soil vapor extraction and vitrification.

The ex-situ approach is based on extract physically the media that is contaminated to be treated on a specific location (far away from the initial place). Some of the main ex-situ techniques are [12][13]:

- Biological treatments: Biological dehalogenation, bioremediation and constructed wetlands.

- Physico-chemical treatments: Excavation and disposal, pump and treat, oxidation, adsorption, ion exchange, physical separation, dehalogenation and solidification.
- Thermal treatments: Incineration, pyrolysis.

Despite the huge available number of techniques, the focus is on the bioremediation strategy for this work. As was commented before, the bioremediation is an environmentally friendly and low-cost remediation technique that is based on the special mechanisms of certain microorganisms that are able to treat certain pollutants. Usually, two different types of bioremediation techniques that are the solid phase bioremediation and slurry phase bioremediation.

The solid phase bioremediation is the name provided for the techniques based mainly on composting as biopiles or land farming. This option is typically used for the treatment of polluted soils that has petroleum products on them. First of all, the pile of contaminated soil is made and then is artificially aerated through venting or mechanical moving. Then some water is added and the temperature, pH and aeration have to be controlled. Some other substrates can be added to enhance the microbiological processes that allow to degrade the petroleum compounds. The hydrocarbon degrading microorganisms are the main drivers to transform the organic contaminants into stable innocuous products. [13]

The main parameters that have to be checked during the solid phase bioremediation are recovered on **Table 3.1**:

Table 3.1*:Range of conditions for solid phase bioremediation. Source: Data extracted from [13]*

Oxygen	**10-15**	**%**
Temperature	50-65	ºC
Moisture	50-55	%
C:N ratio	30:1	
pH	6-9	
Porosity	1-5	cm

For slurry phase bioreactor notice that this technique is for the treatment of polluted wastewater. Is considered as a slurry bioreactor, really close to the secondary treatment scheme of a wastewater treatment plant, and considered as one of the best bioremediation techniques. In this technique, to eliminate the pollutants the wastewater is treated into a bioreactor that provide the biologically

active environment to have the desired efficiency of a certain strain selected previously. The microorganisms that are available to this treatment are the bacteria, fungi or microalgae. [13]

In the case of this project, the aim is directly putted on the slurry phase bioreactor as is the one that matches the best with the marine bacteria. But as was commented, many microorganisms are available to evaluate the bioremediation techniques. As the microorganisms represent nowadays the vanguard for the prevention of the bioaccumulation of xenobiotic compounds on humans and also animals, it's important to know and to understand the role that they can play in our society and the many benefits that can provide. The ability of these microorganisms to degrade the synthetic plastic is directly related with their adaptation to be able to use them as chemicals for their growth and energy demands. The strategy that they have is the usage of different enzymatic systems that allow them to convert the initial plastic into intermediates that they can assimilate and metabolize. This will be explored in more detail further on. [14]

The main possibilities that are present as microorganisms are the following:

- Algae: They represent an interesting option in bioremediation and industrial applications even if they are photosynthetic or heterotrophic. They are microorganisms that have the ability to effectively remove inorganic and also organic pollutants using accumulation, absorption or metabolizing them into reasonable and safe levels. Two of the main drawbacks that they present as an option is the lack of investigation directly into the bioremediation of plastic wastes and that their metabolic pathways are not well oriented into the mineralization (final conversion into soluble inorganic components) of the plastics. This final case makes the plastics products and intermediates still able to bioaccumulate and infiltrate into the food chain. [14]

- Fungi: They are one of the main drivers in the biosphere of the biogeochemical cycles for the essential nutrients as nitrogen or sulfur on earth. Fungi are one of the better options to biodegrading and assimilate synthetic polymers as their primary carbon source for growth. One of the problems that the fungi present, despite to be a good degrader and enzyme producer for polymer degradation, it's the very dependance into the efficiency on the state of the substrate (plastic waste). This is related with the ability to colonize the surface of the substrate, that is not really good without a prior pretreatment and some level of surface degradation into the plastic waste. [14]

- Bacteria: They represent the other main driver in the biosphere for the transformation and cycles of the main nutrients. They are able to generate carbon and nutrients from complex polymers that can be natural or synthetic. They are also one of the main studied microorganisms for bioremediation strategies as their ability to degrade numerous materials depending on the strain selected as for example petroleum, plastic, metals or organic compounds. These microorganisms are also able to be used in couples or groups to synergize into the degradation of certain polymers as the thermoplastics. The bacteria availability to degrade some polymeric substrates is also very related to external factors.

Bacteria can also be used in aerobic or anaerobic conditions and control the degradation of the plastic waste through physical, chemical or enzymatic actions. [12][14]

As was commented briefly before, the bioremediation strategies represent the usage of microorganisms and their enzymes to degrade and transform the plastic wastes into different intermediates and products that finally are non-toxic for the environment. This method efficiency is directly related to the usage of certain enzymes to the degradation of certain polymers and also to a lot of different environmental parameters as pH, temperature or moisture. These parameters can hardly vary the efficiency and rate of degradation of the microorganisms. [15]

A general scheme of the methodology behind this enzymatic perspective is provided on **Figure 3.1**:

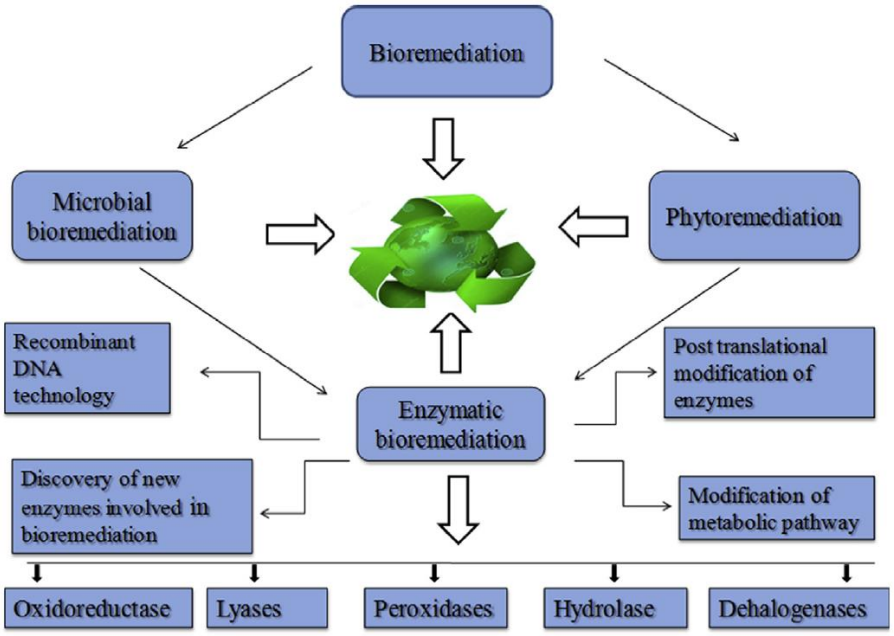


***Figure 3.1**: Overview of enzymatic methodology on bioremediation. Source: [15]*

Enzymes are complex macromolecular compounds that are able to catalyze some metabolic reactions and determine the availability of the microorganisms to do a certain work, as in this case the degradation of the plastic waste. Generally speaking, the degradation of the contaminants with microorganisms is understood as a slow process. Some approaches to speed up the whole process were the extraction of the enzymes and their direct usage or combine some strains. Nonetheless, some intracellular pathways are lost on this approach that is a main drawback but a needed one in the case of a slow growth of the microbe on the polluted media. In the case of the marine bacteria, this will not be a problem as will be commented after, as they are robust and very adaptable microorganisms. [15]

A general scheme of the different enzymes that are used on the bioremediation and their function is presented on **Table 3.2**:

***Table 3.2**: Enzymes used on bioremediation and their objective. Source: [15]*

Enzyme classification	Examples	Functions	Reference
Oxidoreductase	Oxygenases	Catalyze oxidation of aromatic compounds such as chlorinated biphenyls, aliphatic olefins by incorporating one or two molecules of oxygen and making them prone to further transformation and mineralization.	Chakraborty et al., 2014
	Laccases	Cleave ring present in aromatic compounds and reduce one molecule of oxygen in water and produces free radicals.	Shraddha et al., 2011
	Peroxidases	Catalyze reduction reaction in the presence of peroxides, such as hydrogen peroxide (H_2O_2) and generate reactive free radicals after oxidation of organic compounds.	Bansal and Kanwar, 2013
Hydrolases	Lipases	Break triglycerol into glycerol and fatty acid and widely used for wastewater treatment, polyaromatic hydrocarbon degradation etc.	Mehta et al., 2017
	Cellulases	Break down complex cellulosic materials into simple sugars and commonly used in the treatment of agricultural residues such as cotton waste, sawdust of *Khaya ivorensis* and rice straw.	Bhardwaj et al., 2017
	Carboxylesterases	Catalyzes the hydrolysis of carboxyl ester bond present in synthetic pesticides such as organophosphates with addition of water.	Singh, 2014
	Phosphotriesterases	Catalyze hydrolysis of phosphotriesters, the main components of organophosphorus compounds used worldwide in pesticides, causes severe poisoning and death.	Santillan et al., 2016
	Haloalkane dehalogenases	Used for biodegradation of halogenated aliphatic compounds such as 1,2,3-Trichloropropane.	Nagata et al., 2015

To improve the performance of the enzyme-based technology, as bioremediation through microorganisms is, some new approaches are made trying to obtain more flexible and suitable systems. Notice that this perspective of use enzymes as an extracellular product in most of the cases requires many things, but mainly that the desired enzymes are able to be extracted and stabilized and also that the microorganisms is able to produce enough quantity to be suitable for medium or large-scale applications. Some of the techniques that are focused on the last years are the genetic engineering enzyme engineering (change on basic amino-acid structure of enzymes) and some other as immobilized enzyme technology or nanozymes (use of nanoparticles as mimics of enzymes). [15]

Once this first approach is covered, the main point to understand the suitability and great potential of the microorganism, and more focused on marine bacteria, as driver to degrade synthetic polymers is to know and understand the mechanism that allows the bacteria to degrade the plastic and finally obtain some non-toxic inorganic components.

The microbial degradation of the plastic waste is driven by five main steps, that are colonization, biodeterioration, biofragmentation, assimilation and mineralization. [16]

A general scheme is presented in **Figure 3.2**:

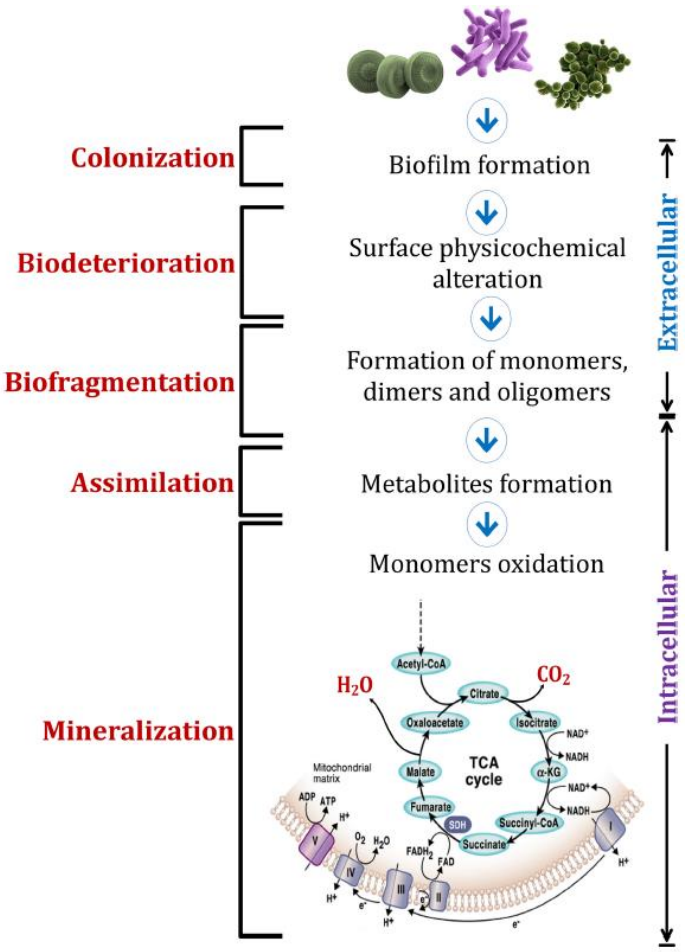


***Figure 3.2**: General scheme of microbial degradation of plastic waste. Source: [16]*

Using **Figure 3.2** as basis, it's important also to know in a proper way how all of the five main steps work. The first step is the colonization. The colonization is the deposit of the microbial species into the polymer surface. This deposit leads to a biofilm formation, which generates damage to the polymer surface. The microbes perform this step with the production of proteins and polysaccharides that modify the pore size, increasing the number of cracks and the size of the pores. [16]

As a direct consequence of this step, the biodeterioration occurs naturally because of the activity of the microbe adhesion on the polymer surface. The biodeterioration directly modifies the physico-chemical properties of the polymer surface and backbone. Different kinds of biodeterioration can occur, being the main ones for the bacteria the physical biodeterioration and enzymatic biodeterioration. Notice that the penetration rate of a certain strain can be enhanced through the secretion of some extracellular substances that modify the hydrophilic and hydrophobic phases of the backbone and increase the accumulation of pollutant, which enhances the rate of microbial growth and the biodeterioration rate. Physical biodeterioration can be performed by bacteria and also fungi, invading the material surface and increasing the pores and the cracks on the backbone,

augmenting the specific surface and increasing also the rate of the process. For enzymatic biodeterioration, a lot of different extracellular enzymes are produced by the microorganism to deteriorate the polymer surface as for example peroxidases. It's important to know that some of the main polymers, as PU and PVC, are certainly resistant to this type of degradation and other enzymes as lipases, esterases or proteases are needed to reduce and overcome the polymer crystallinity. [16]

The next step is the biofragmentation, that consists in the degradation of the polymers into monomers, dimers and/or oligomers. The cleave of the polymers occur in different manners as for examples secretion of certain enzymes as oxidoreductases and/or free radicals. The enzyme concentration varies depending on the tine and the activity, which is nor immediately started. Because of the crystalline and hydrophobic nature of the polymers, some of the cleave reactions can be very complicated. This can be understood as the need of more enzymes to be able to change the polymer structure. The presence of the free radicals, that will lead to a formation of a hydroxyl/carbonyl or carboxyl group, can help as the formation of these groups increase the polarity and hygroscopic properties of the polymer and then the microbial attack and the biofragmentation rate. [16]

Once the polymer backbone is converted into monomers, dimers and oligomers, the assimilation of the species occurs. This assimilation provides of energy and carbon the microbial cell, which allow it to grow up. Some of the monomers can be able to penetrate the membrane cells through some specific membrane carriers, but other can't be assimilated due to the cell membrane permeability. This fact doesn't represent a real problem as the microbial cell is able to use these non-assimilated substances using a biotransformation process through an enzyme-catalyzed conversion into energy carrying molecules as adenosine triophosphate (ATP). When the monomers are finally assimilated, non-toxic inorganic soluble components are produced and the bioremediation process can be considered as finished. [16]

An example of this process is presented in **Figure 3.3**:

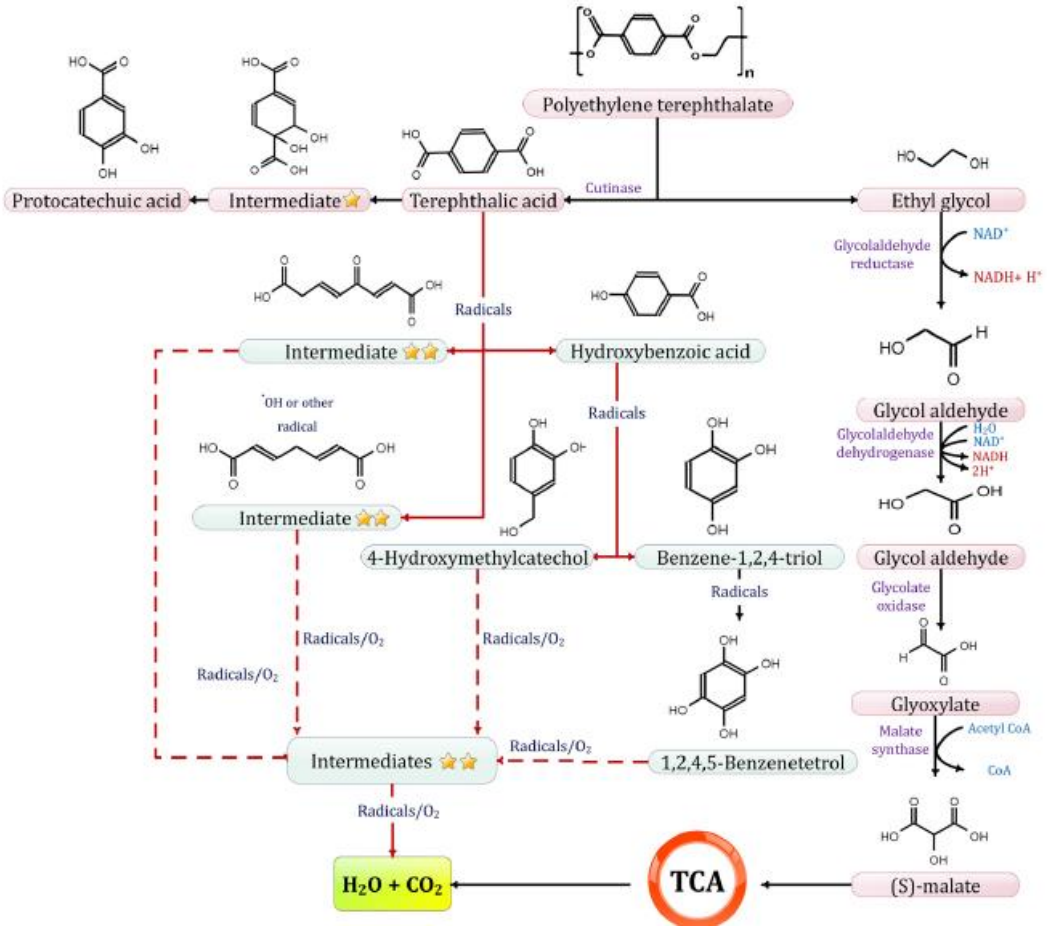


***Figure 3.3**: Mechanism that leads to PET biodegradation. Source: [16]*

In **Figure 3.3** is possible to see a case where the intermediates are assimilated through an enzyme-catalyzed conversion. The degraded monomers are oxidized with some microbial catabolic pathways that produce energy-carrying molecules as ATP. The ATP molecule is very important as in involved into three key pathways that are aerobic respiration, anaerobic respiration and fermentation. This also gives the idea that is suitable process for both aerobic and anaerobic conditions, as the energy carrier generated is key for both type of microorganisms. [16]

3.1. Advantages and drawbacks of marine bacteria

Despite the multiple options for bioremediation of plastic waste, one of them has grown into popularity and number of studies due to their properties and possibilities. This option is the marine bacteria.

Marine bacteria have some very representative characteristics that makes it an interesting option in terms of selection for the scope of this project. First of all, marine bacteria represent a huge amount of the microorganisms that are responsible for the primary production and also cycling of

nutrients. Most of the different bacteria that are present in the seawater is viable for applications but have some problems in terms of growth, which usually leads to genetic modification. [17]

Other important feature of the marine bacteria is that they are very robust and respond really quickly to any changing environmental pattern. This makes them also suitable as bioindicator. Some of the typical changes on the marine environment are changes on sea surface temperature, pH of the environment, changes in the sea level, etc. Marine bacteria need to be able to handle all these constant changes in order to survive. Some of these changes are fixed with a change in the bacteria location, searching for more favorable conditions, but in other cases some adaptation mechanisms as chemotaxis or adhesion of some receptors occur. From the different changes commented, the most critical seems to be the pH of the environment. Marine bacteria need sodium and potassium ions, among others, to maintain the osmotic balance of the cell and allow their growth. The change in the environment pH will modify some of the microbial loops and nitrogen cycle, modifying the growth of the bacteria and also having a direct impact into the availability of this bacteria in the marine environment. Anyway, marine bacteria are considered as more capable of handle pH changes than terrestrial bacteria or other marine microorganisms, but the adaptation in this field is nonetheless poor. [17] [18]

So, the main advantages and drawbacks for the selection of marine bacteria as main driver for the bioremediation of plastic waste are presented in **Table 3.3**:

Table 3.3*: Advantages and drawbacks of marine bacteria for bioremediation. Source: Extracted from [17][18]*

Main advantages	Main drawbacks
Very resistant in adverse and diverse conditions	Don't perform well in cases of mixed contaminants, hard to find coupling of bacteria that work well and don't compete
Very ample opportunity for bioremediation in-situ or ex-situ	Lethal products may form (In the case of polyaromatic hydrocarbons, organic pollutants or lethal substrates)
Cost-effective compared to chemical processes	High production of biomass, which can lead to biofouling if it's not well handled
Highly specific, which reduces the formation of possible harmful products	Recombined strains can lead to instability when new generations of the bacteria appear
Able to consistently reduce environmentally harmful compounds into inorganic soluble forms	

3.2. Possible candidates and options available

Once the framework is presented, now a study of the different possibilities that seem interesting for their application into the plastic waste bioremediation will be performed. The objective is to determine the better candidates for this theorical study and pilot plant approach. Some things have to be taken into account beforehand:

- The high dependency of the performance and availability of the technique depending on the selection of a certain strain will determine the operation conditions and components of the process.
- The selection of a certain strain will fix the substrate (type of plastic waste) that has to be used and also the growth media associated.
- The expected operation time to biofilm formation and demineralization will be in a scale of days or weeks, as this process is still slow.
- As the growth media has to be directly specified for the strain selected, will be considered to produce them directly in the process of the pilot plant, which means that no differentiation will be made in terms of growth media conditions between the bacterial strains.
- The optimal temperature of growth will be a key factor in order to achieve good operating conditions and satisfactory yields.

So, when this is established a presentation and evaluation of the most promising candidates will be performed.

In order to achieve a good understanding of the options that can be provided and the different developments that were made in the last years, some of the most promising options will be evaluated beforehand select anyone. It's reported that around 90 species of marine microorganisms are able to degrade plastic waste, which made the options limited. Nonetheless, the scope of this project is directly aimed to explore this option and try to evaluate good options to possibly scale up these promising species.

Table 3.4 presents the marine bacteria options that appear as most promising:

***Table 3.4**: Marine bacteria candidates for bioremediation. Source: Specified into table*

Bacteria strain or consortia	General characteristics	Reference
Pseudomonas sp.	Zone of isolation: Gulf of Mannar, India Plastic substrate: HDPE Plastic weight loss: 15% Experiment time: 30 days	[19]
Arthrobacter sp.	Zone of isolation: Gulf of Mannar, India Plastic substrate: HDPE Plastic weight loss: 12% Experiment time: 30 days	[19]
Brevibacillus borstelensis	Zone of isolation: Tamil Nadu, India Plastic substrate: HDPE Plastic weight loss: 11,4% Experiment time: 30 days	[20]
Lysinibacillus sp. + Salinibacterium sp.	Zone of isolation: Antwerp, Belgium Plastic substrate: Linear LDPE (LLDPE) and HDPE Plastic weight loss: 15% (LLDPE) and 5,2% (HDPE) Experiment time: 6 months	[21]
Bacillus sphericus ***Bacillus cereus*** ***Vibrio fumisii*** ***Brevundimonas vesicularis*** ***(No consortia)***	Zone of isolation: Chennai, India Plastic substrate: Nylon 6 Plastic weight loss: 2% (*B.vesicularis*), 2,1% (*B.cereus*), 0,4% (*V.fumisii*) and 0,5% (*B.vesicularis*) Experiment time: 3 months	[22]

Bacillus sphericus ***Bacillus cereus*** ***Vibrio furnisii*** ***Brevundimonas vesicularis*** ***(No consortia)***	Zone of isolation: Chennai, India Plastic substrate: Nylon 66 Plastic weight loss: 4,5% (*B.vesicularis*), 7,5% (*B.cereus*), 1,5% (*V.fumisii*) and 0,8% (*B.vesicularis*) Experiment time: 3 months	[22]
Bacillus sphericus ***Bacillus cereus*** ***(No consortia)***	Zone of isolation: Chennai, India Plastic substrate: LDPE Plastic weight loss: 20% (*B.sphericus*) and 25% (*B.cereus*) Experiment time: 12 months	[23]
Bacillus sphericus ***Bacillus cereus*** ***(No consortia)***	Zone of isolation: Chennai, India Plastic substrate: HDPE Plastic weight loss: 9,7% (*B.sphericus*) and 7% (*B.cereus*) Experiment time: 12 months	[23]
Lysinibacillus fusiformis ***Bacillus cereus*** ***(No consortia)***	Zone of isolation: West coast of India Plastic substrate: PE Plastic weight loss: 17,56% (*L.fusiformis*) and 6% (*B.cereus*) Experiment time: 8 months	[24]
Vibrio sp.	Zone of isolation: Bay of Bengal (West Bengal), India Plastic substrate: PET Plastic weight loss: 35% Experiment time: 6 weeks	[25]
Bacillus paralicheniformis	Zone of isolation: Arabian Sea Plastic substrate: PS Plastic weight loss: 34% Experiment time: 6 weeks	[26]

In **Table 3.4**, as was commented, are represented the most promising ones that was able to collect enough information about them. As was considered in first place, the bacterial strain that will be considered as the selected one will depend into different factors referenced to the overall performance in the plastic biodegradation and also the importance of the plastic waste that is available to biodegrade. It seems reasonable to prioritize the most abundant plastics that represent the main body of the problem described into the first parts of this study.

Two main competitors appear as the main options, that are *Vibrio sp.* (with PET degradation) and *Bacillus paralicheniformis* (with PS degradation). These two options not only generate the highest degradation of the options, either do it in the less time (2 months). Applying a calculation on a year basis, also these two are the main options as the other two possible competitors could be the *Arthrobacter sp.* and *Pseudomonas sp.* Taking into account that the experiment time is the half and assuming a ''constant'' degradation rate in the time, they would provide a 30 and 24% of degradation into HDPE. These values are still lower than the performance provided by the *Vibrio sp.* and *Bacillus paralicheniformis*.

Between these two options, the most reasonable one appears to be the *Vibrio sp.* as the PE and PET represent between a 70 and a 75% of the plastic produced in the world and also represent the hugest fraction of plastic waste (represented for PET for one usage water bottles for example).

So, finally the option of the *Vibrio sp.* for degrading PET will be selected. In the next section, the conditions of the experiment referenced in their studio will be evaluated in order to emulate the conditions as much as possible and have the basis to estimate some equal results.

3.3. Conditions of the selected marine bacteria strain

In order to stablish properly the characteristics of the proposed scheme and operation, the conditions and important parameters for the correct performance of the *Vibrio sp.* will be studied and evaluated. Some of the key parameters will be:

- Growth media
- Temperature
- Concentration of substrate used
- pH
- Usage of chemicals for pre/post treatment

Strain used is the *Vibrio sp.* at GenBank accession nº KY941137.1 strain PD6. [25]

The growth media used in that study is represented by a pseudo-typical composition for saline water microorganisms with some chemicals like potassium dihydrogen phosphate, sodium nitrate, potassium chloride, etc.

The composition of this growth media is presented in **Table 3.5**:

***Table 3.5**: Growth media for Vibrio sp. Source: [25]*

Component	Chemical formula	Concentration (g/L)
Potassium dihydrogen phosphate	KH_2PO_4	1
Sodium nitrate	$NaNO_3$	2
Magnesium sulphate heptahydrate	$MgSO_4 * 7\ H_2O$	0,5
Potassium chloride	KCl	0,5
Ferrous sulphate heptahydrate	$FeSO_4 * 7\ H_2O$	0,01
Ammonium chloride	NH_4Cl	1

In that growth media is possible to see the presence of the saline components (Sodium, magnesium and chloride) and also the presence of some minor components for the growth as functional groups (Sulphate, nitrate or ammonium) and other typical components like iron.

The study considers also an evaluation of the samples of PET through a scheme of 1,2,3,4,5 and 6 weeks. There is no specific concentration reported, but the used samples are squares of 1 cm size. These squares are washed with ethanol and vacuum dried after their presence in the bioreactor and evaluated for known the degradation rate with three different techniques. These techniques are scanning electron microscopy (SEM), Fourier-transformed infrared radiation (FTIR) and X-ray diffraction analysis (XRD).

Scanning electron microscopy is used to study the fixation of the microbial isolates into the polymer films. This technique allows to identify the morphological changes that occurred into the polymer structure. Using this technique was clear that the films before degradation show a smoother surface than the one present after biodegradation. After the degradation process, the surface of the polymer is compromised and cracks and mass loss are clear. [25]

In **Figure 3.4**, the SEM images obtained are presented:

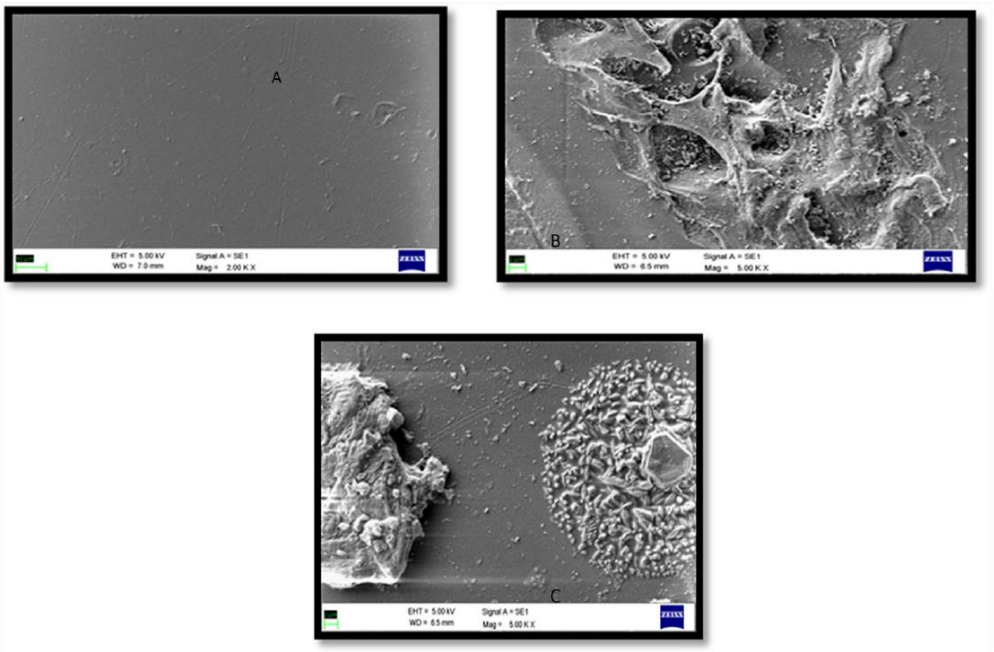


***Figure 3.4**: Scanning electron microscopy study of surface degradation. Source: [25]*

X-ray diffraction analysis is used for study the crystallinity of the sample before and after the degradation by the bacteria. This study was performed at room temperature and the crystallinity was studied through a direct interpretation of the diffraction schemes provided by the technique. [25]

The obtained pattern of the polymer film is presented into **Figure 3.5**:

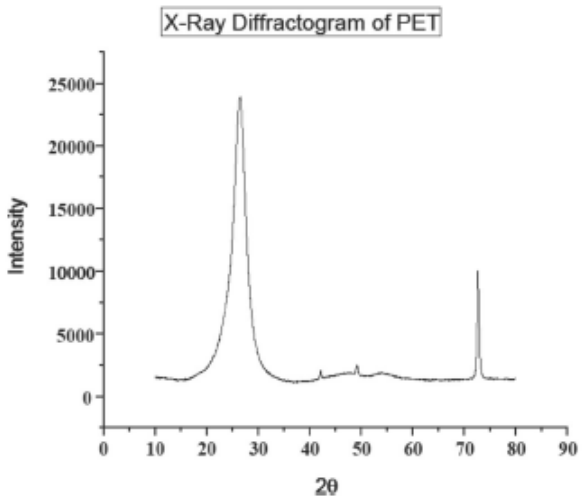


***Figure 3.5**: X-ray diffraction study of surface degradation. Source: [25]*

The pattern shows that the crystallinity of the polymer film decreases as the intensity of the film increases. As the film intensity increases, the diffraction patterns will show a decreasing trend that can be interpreted as a decrease into the crystallinity. [25]

FTIR is performed to evaluate the polymer film obtained as the final part of the degradation by fungi and bacteria, but only the bacteria results will be considered. This technique allows to analyze the polymer structure through identification of the present bonds in a sample. The difference between the both cases (after and before degradation) will allow to evaluate the real degradation of the structure. [25]

The results of the FTIR are presented into **Figure 3.6**, being the left side the reference PET and the right side as the obtained by the degradation:

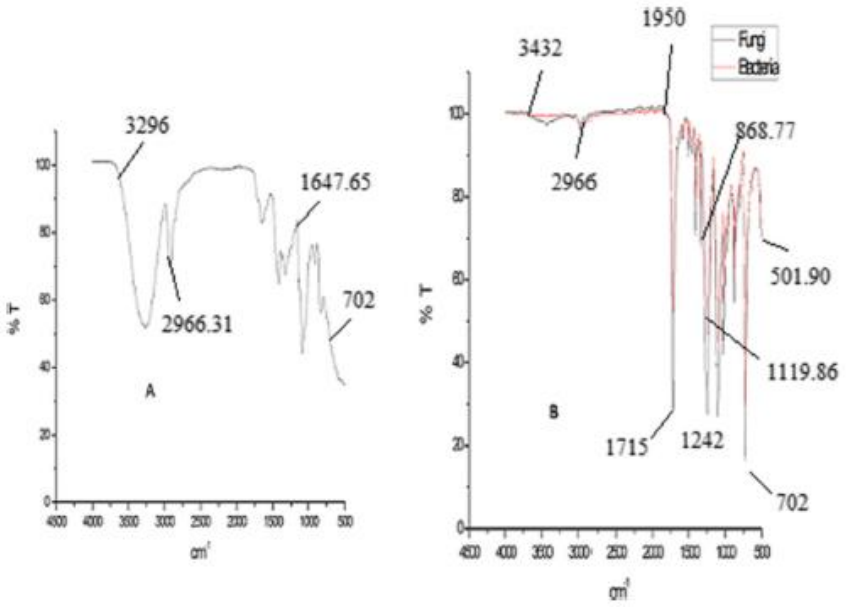


***Figure 3.6**: FTIR study of surface degradation. Source: [25]*

The bond break into the C-H bonds, as the trend decreases with wavenumber, shows that really the degradation has occurred into the polymer structure. The main absorption bonds that represent an important result for PET are the hydroxyl bond (3600-3200 cm^{-1}), carbon oxygen stretching C-O (1150-1050 cm^{-1}) and ester ethylene/carbon hydrogen stretching C-H (3200-2700 cm^{-1}). [25]

There is no reported change into the peaks that are referred to -OCH_3 group, which allows to maintain the functional structure into the secondary components commented into **Figure 3.3**. The C=O bonds seem to disappear (806-769 cm^{-1}) when the degradation is performed due to an asymmetric structure containing carbonyl group and an increase in the C-H bond in the degraded polymer because of the breaking bonds and degradation of the polymer structure. [25]

When the degradation of the polymer structure is confirmed with the analytical methods, a sensibility analysis into three different parameters is performed to be able to achieve an optimum behavior of the bacteria *Vibrio sp.* into the PET degradation. Temperature, pH and inoculum dose are evaluated.

For temperature analysis, temperatures between 25 and 45ºC with an interval of 10ºC are evaluated during 3 weeks and a constant pH of 7. An optimal temperature of 35ºC was obtained, as the degradation rate is higher at this temperature than the other two cases considered (25 and 45ºC) as can be seen in **Figure 3.7** [25]:

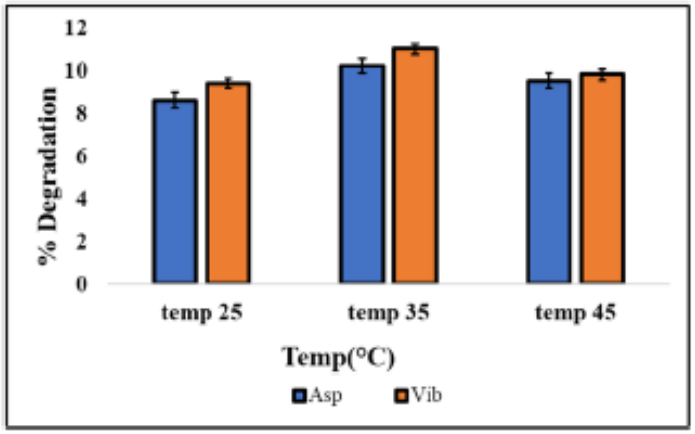


***Figure 3.7**: Optimal temperature study for microbial strains, Vibrio sp. at orange bars. Source: [25]*

The degradation, as marine bacteria is used, plays a key role into the degradation of the polymers. To obtain the better option, a wide range of pH were considered (from 1 to 11) during a period of 3 weeks. The other parameters were considered constant (temperature and inoculum) to achieve a control experiment and be able to properly consider the influence of this parameter into the degradation rate. [25]

The results are presented into **Figure 3.8**:

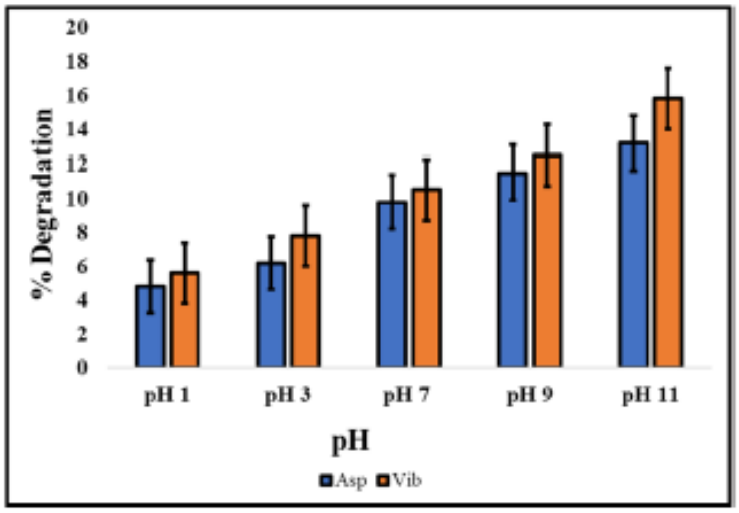


***Figure 3.8**: Optimal pH study for microbial strains, Vibrio sp. at orange bars. Source: [25]*

It's observed that the increase in the pH of the solution leads to higher degradation rates, which can be directly related to various facts, but mainly is expected that the low pH restrings the microbial growth of this marine bacteria. On the other hand, an alkaline pH will be closer to the typical pH of the environment for this marine bacteria growth, which generate higher microbial growth and then higher degradation rate. [25]

The last parameter to be evaluated is the inoculum dose that should be used for the polymer degradation. This parameter could not appear as key in the operation but indeed it is. A small inoculum dosage can be insufficient to degrade the polymer backbone and a large dosage can lead to poor degradation rates of the polymer. The microbial species were incubated during 3 weeks and different dose were studied from 1 mL/75 mL to 7 mL/75 mL fixing 35ºC and pH 7. [25]

The results are presented in the **Figure 3.9**:

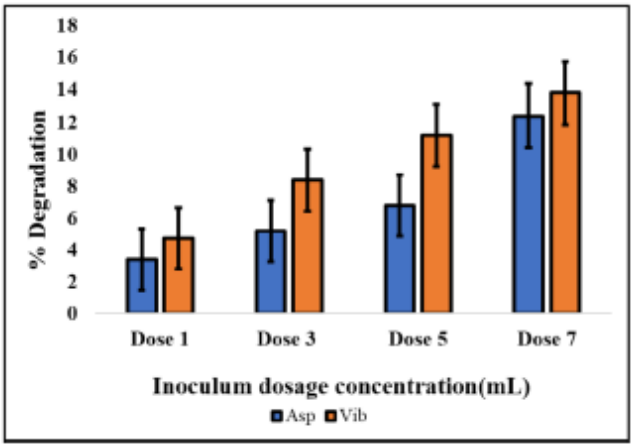


***Figure 3.9**: Optimal inoculum dosage (per 75 mL) for microbial strains, Vibrio sp. at orange bars. Source: [25]*

As can be seen, the increase into the dosage concentration will lead to higher degradation rates, which will enhance the ability of the microbial strain to degrade the PET substrate.

Once the optimal parameters are obtained (pH 11, 7 mL of inoculum per 75 mL of dissolution and 35ºC), a final study is performed to obtain the real capacity of the bacterial strain to degrade the PET substrate under the optimal conditions.

Figure 3.10 represents the obtained results for *Vibrio sp.*:

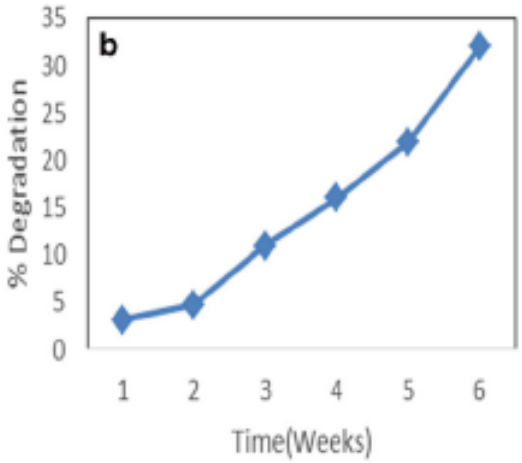


***Figure 3.10**: Degradation % for PET of Vibrio sp. at optimal conditions. Source: [25]*

These final results allow to consider the 35% of degradation at 6 weeks with the optimal conditions referenced before. [25]

Based into this study and the data extracted, a base process to process a certain part of PET plastic mass will be proposed using this bacterial strain and the conditions commented and presented as optimal ones.

4. Modelling of bacteria and bioreactor performance

As the approach of this study it's a theorical study of a selected strain and a novel process, it will be needed to develop a theorical model that allows to predict and study the effect of the selected variables and possible solutions into the performance of the system. Also, the bioreactor and its performance it's really important because will determine the steps needed before and after it. As was commented, some optimal conditions as pH or inoculum concentration with the growth media for the strain was commented and set up. Nonetheless, the values generated are useful as laboratory guidance but they are not really suitable for a higher scale (as it's the approach of this study). Then using some reference knowledge and approximation studies, a theorical and practical model for the main reactions that occur and also the initial and final conditions expected there will be developed.

As there is a lack of information because the experiment is developed at a laboratory scale, some assumptions were made in the development of a reasonable model for the prediction and analysis of the viability of this option. The different assumptions or correlations that have been made will be commented as soon as needed.

4.1. Bacteria empirical formula and initial biomass concentration

In order to be able to set the equations and the prediction of the system in terms of biomass and other products it's needed to stablish in the first place the bacteria chemical formula and also the initial biomass concentration to obtain the prediction of the final effluent obtained from the bioreactor and also the plant scheme suggested by the author.

Figure 4.1 was used as a basis for the typical composition of bacterial cells in biochemistry process and was used as basis:

Constituent or element	Percent of dry weight
Major cellular material	
Protein	55.0
Polysaccharide	5.0
Lipid	9.1
DNA	3.1
RNA	20.5
Other (sugars, amino acids)	6.3
Inorganic ions	1.0
Sum:	100%
As cell elements	
Carbon	50.0
Oxygen	22.0
Nitrogen	12.0
Hydrogen	9.0
Phosphorus	2.0
Sulfur	1.0
Potassium	1.0
Sodium	1.0
Calcium	0.5
Magnesium	0.5
Chlorine	0.5
Iron	0.2
Other trace elements	0.3
Sum:	100%

***Figure 4.1**: Typical bacterial cells composition basis. Source: [27]*

Taking into account the composition of the five main components (C, H, O, N and P) and assuming that the bacterial chemical equation will have the form $C_xH_yO_zN_aP_b$ it's possible to estimate the empirical formula of a bacteria as that form presented.

Assuming 100 kg of dry weight and performing an analysis in terms of molar mass, the formula can be obtained. The empirical obtained formula it's presented in **Table 4.1** and **Figure 4.2**:

***Table 4.1**: Empirical formula of bacteria calculation. Information obtained from [27]*

	% Mass	MW (g/mol)	Molar content	Base P	Base C
C	50	12	4,17	65	1
O	22	16	1,38	21	0,32
N	12	14	0,86	13	0,2
H	9	1	9,00	140	2,15
P	2	31	0,06	1	0,0153
			Molecular weight (g/mol)	1472,5	22,07

$$C_{65}H_{140}O_{21}N_{13}P$$

$$CH_{2,15}O_{0,32}N_{0,2}$$

***Figure 4.2**: Empirical formula of bacteria cells. Own elaboration.*

As usually the nitrate is used as limiting nutrient for bacterial growth and it's known that the molar relative proportion of the nitrogen and phosphorus is 13:1, the simplified formula will be used as basis for the next steps. To evaluate if this approximation was reasonable, some other typical approaches for empirical bacteria formula were revised and the obtained result fit with the literature founding as can be seen in **Table 4.2**:

***Table 4.2**: Empirical formula of bacteria cells from literature. Source: [28]*

Microorganisms	Chemical formula	Ref.
Bacteria	$CH_{1.666}O_{0.27}N_{0.20}$	Abbott and Clamen (1973)
Bacteria	$CH_{2.0}O_{0.50}N_{0.27}$	van Dijken and Harder (1975)
Candida utilis	$CH_{1.83}O_{0.54}N_{0.10}$	Herbert (1976)
C. utilis	$CH_{1.87}O_{0.56}N_{0.20}$	
C. utilis	$CH_{1.83}O_{0.46}N_{0.19}$	
C. utilis	$CH_{1.87}O_{0.56}N_{0.20}$	
Klebsiella aerogenes	$CH_{1.75}O_{0.43}N_{0.22}$	
K. aerogenes	$CH_{1.73}O_{0.43}N_{0.24}$	
K. aerogenes	$CH_{1.75}O_{0.47}N_{0.17}$	
K. aerogenes	$CH_{1.73}O_{0.43}N_{0.24}$	
Saccharomyces cerevisiae	$CH_{1.64}O_{0.52}N_{0.16}$	Harrison (1967)
S. cerevisiae	$CH_{1.83}O_{0.56}N_{0.17}$	Kok and Roels (1980)
S. cerevisiae	$CH_{1.81}O_{0.51}N_{0.17}$	Wang et al. (1976)
Paracoccus denitrificans	$CH_{1.81}O_{0.51}N_{0.20}$	Stouthamer (1977)

Then, the next step will be to evaluate the initial biomass concentration of the inoculum used by the experiment described in [25]. They define a concentration of 7 mL of inoculum of unknown concentration into 75 mL of dissolution. It's known that usually a commercial inoculum has at least 10^7 cells/mL. So, to set a non-overoptimistic scenario this limit concentration will be assumed as the one used by the authors of [25]. To be able to perform the calculations, it's needed to know the approximate weight of a simple cell. This information was extracted from [29] that assume literally that: '' *1.600.000.000 cells of Escherichia coli would weigh a gram*''. So then, the approximate mass of a cell can be estimated as 6.25 x 10^{-10} g/cell.

As this data is obtained, it's possible to evaluate the approximately initial concentration of biomass used by the reference experiment. The results are presented in **Table 4.3**:

***Table 4.3**: Initial biomass concentration. Information obtained from [29]*

Initial inoculum			**Final result**		
Cell concentration	1,00E+07	cells/mL	**Cell concentration**	9,33E+05	cells/mL
Volume	7	mL	**Volume**	75	mL
Nº cells	7,00E+07		**Nº cells**	7,00E+07	
Biomass concentration	0,00625	g/mL	**Biomass concentration**	0,000583	g/mL
	6,25	g/L		0,583	g/L

As the number of cells transferred it's the same as taken, then it's possible to obtain these results and estimate that the initial biomass concentration needed for the development of the process is of 0,583 g/L of the bacterial strain defined in last sections.

4.2. Validation of assumptions with growth media

To validate the assumption of using the nitrogen as the limiting nutrient and not taking into account the phosphorus in the general balances for the following steps, it's needed to evaluate the molar concentration of the main components of the growth media to check if the assumption can be sustained through the next calculations. Based on the growth media explained before and calculating the main nutrients for bacterial growth, it's obtained in **Table 4.4**:

***Table 4.4**: Growth media molar concentration. Own elaboration.*

Growth media			**Molecular weight**		**Molar concentration**	
KH_2PO_4	1	g/L	136,09	g/mol	7,35E-03	mol/L
$NaNO_3$	2	g/L	85	g/mol	2,35E-02	mol/L
$MgSO_4$*7 H_2O	0,5	g/L	246,48	g/mol	2,03E-03	mol/L
KCl	0,5	g/L	74,55	g/mol	6,71E-03	mol/L
$FeSO_4$*7 H_2O	0,01	g/L	278,05	g/mol	3,60E-05	mol/L
NH_4Cl	1	g/L	53,49	g/mol	1,87E-02	mol/L
NO_3^-	1,46	g/L	62	g/mol	2,35E-02	mol/L
PO_4^{3-}	0,698	g/L	94,97	g/mol	7,35E-03	mol/L

As can be seen, the nitrate molar concentration is behind the stoichiometric relation (N/P = 13) so it can be assumed that only nitrate will be relevant in the following calculations and the assumption made in the first steps it's validated.

4.3. Chemical reaction modelling of bioreactor

Once the initial basis for the chemical set of reactions is established, the next step will be to develop the whole chemical equation set for the action of the bacteria and also a general chemical equation that allow to predict the behavior of the bacterial strain through a black box model. This approach will be used as the full developed model it's very challenging and not well defined at this point. Nonetheless, a detailed view will be made in order to understand properly how it's the mechanism that leads to the biodegradation of the PET polymer and which components can be expected as final products or intermediate if it's needed.

The simple theorical modelling of the biodegradation of PET follows the structure that can be seen in **Figure 4.3**:

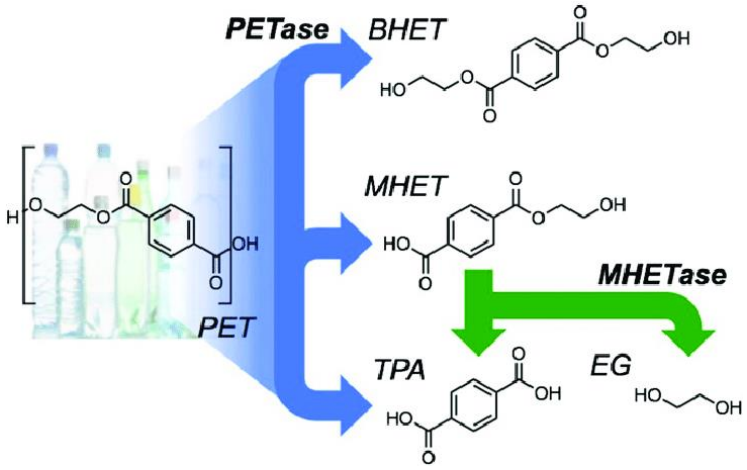


***Figure 4.3**: Simple model for enzyme degradation of PET. Source: [30]*

Figure 4.3 presents a simple approach that is useful to have the idea of the main feature that makes the biodegradation of this high crystallinity polymer by the action of the microorganisms. It's also reported in [30] and [31] that the amount of BHET present in the degradation it's at trace levels if it's not specially catalyzed, which makes it not really interesting to take into account. As was explained before, the bacteria it's not only able to transform the polymer into the polymerization reactants but also degrade it into a mineralization level and use some easy organic molecules to transform into biomass, energy and mineralization products. In the case of this marine bacteria, as was presented in **Figure 3.3**, the carbon source it's the molecule that will be involved into the growth and respiration cycles through the TCA cycle, that will be the ethylene glycol. Also, the terephthalic

acid will be decomposed into the mineralization products but through the action of the bacteria but not used for their growth as a main carbon source. This is reasonable if it's taken into account that the carbon sources usually are molecules that can be easily taken for the bacteria and helps to continue the growth and develop the colony, so the easiest to degrade and in structure will be the ethylene glycol and will be considered as the main carbon source from the degradation of the PET.

To be able to develop a most accurate model for the degradation of PET, will be needed to take into account the needs in terms of other chemical molecules, as oxygen for example, in the whole process of degradation into the final mineralization products (H_2O and CO_2). [31] offers a more detailed mechanism using the canonical esterase reaction mechanism with hydrolases. This mechanism is presented in **Figure 4.4**:

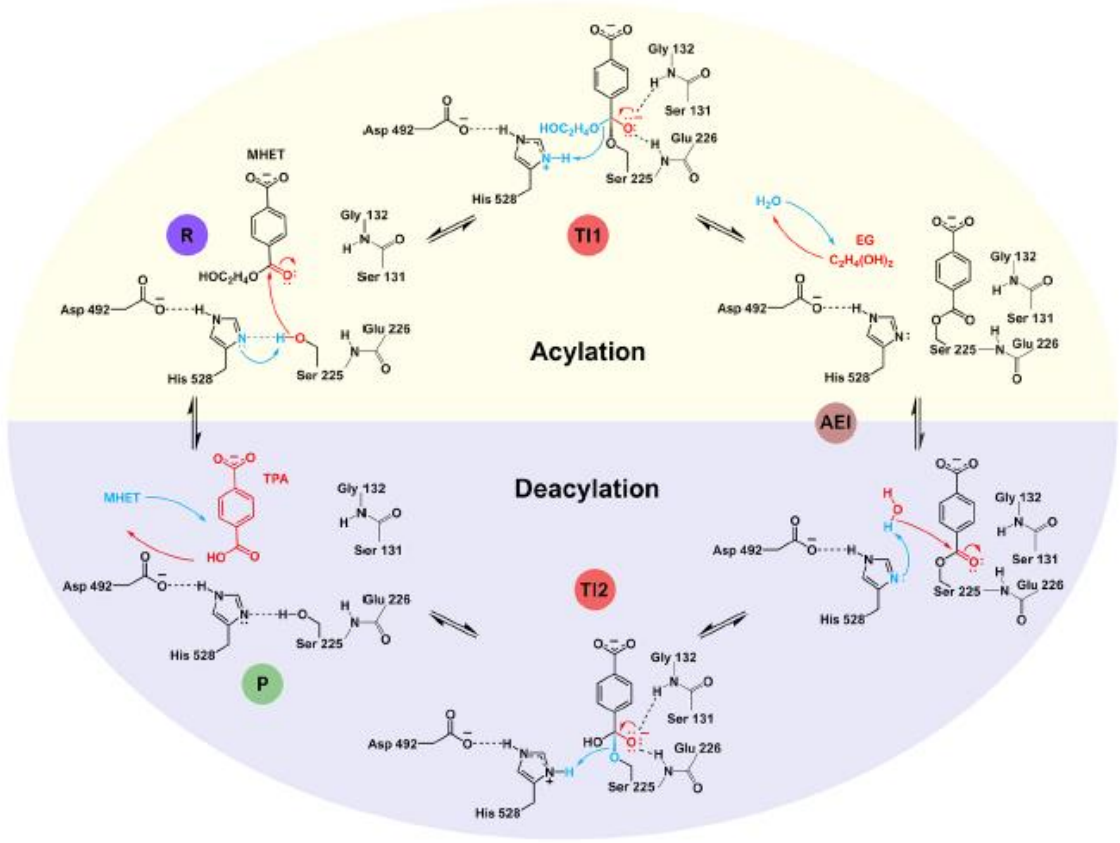


***Figure 4.4**: Proposed MHET degradation mechanism. Source: [31]*

As can be seen in **Figure 4.4**, it's needed some other molecules as oxygen and water to be able to model correctly the whole degradation process. Using the facts described before and the mechanism that is presented in the last figure, a suitable model for the biodegradation of PET was proposed to apply into the mass and energy balances of the proposed process for this project.

First of all, the structure of the PET polymer follows the form $(C_{10}H_8O_4)_n$ with an approximate number of monomers in the polymer of 125. So, for the general equation of the polymer, the chemical reaction for the action of the PETase, that is the first step of the degradation, will be:

$$(C_{10}H_8O_4)_n + H_2O \xrightarrow{PETase} (C_{10}H_8O_4)_{n-1} + C_{10}H_{10}O_5(MHET) \quad \textbf{(Eq. 1)}$$

As was commented before, the BHET concentration was stated as trace amount so it's not relevant in the development of the model. As the information of a certain number of monomers being cut from the whole polymer structure it's missing, then the model will be developed for a single monomer. This will fit also with the desired view as using a monomer from the structure will be equivalent in terms of molecular mass and then the model can be suitable even with that. Other point of view is also that approach can be swiped also into the polymer approach just converting the number of moles and using the right molecular weight.

So, for a single monomer the equation will be:

$$C_{10}H_8O_4 + H_2O \xrightarrow{PETase} C_{10}H_{10}O_5(MHET) \quad \textbf{(Eq. 2)}$$

Once the monomer it's cut from the whole polymer structure, the action of the MHETase takes place, which generates the polymerization reactants through another hydrolysis as the following one:

$$C_{10}H_{10}O_5 + H_2O \xrightarrow{MHETase} C_8H_6O_4 + C_2H_6O_2 \quad \textbf{(Eq. 3)}$$

Once the monomer is broken into the two polymerization reactants (TPA and EG), each one follows a different route into the final mineralization products. Simplifying the whole sets of reaction and intermediates that are needed, the final expected products for both are the following:

$$C_8H_6O_4 + \frac{15}{2}O_2 \rightarrow 8\,CO_2 + 3\,H_2O \quad \textbf{(Eq. 4)}$$

$$C_2H_6O_2 + \frac{5}{2}O_2 \xrightarrow{TCA\ cycle} 2\,CO_2 + 3\,H_2O \quad \textbf{(Eq. 5)}$$

As was commented before, the ethylene glycol can be assumed as the main carbon source for the bacterial growth of the colony of *Vibrio sp*. This means that the theorical decomposition it's not enough, as the nitrate and the biomass have to take place into the decomposition equation. So, taking this into account, the ethylene glycol decomposition is expected to have the form:

$$C_2H_6O_2 + a\,NO_3^- + b\,O_2 \xrightarrow{TCA\ cycle} c\,CH_{2,15}O_{0,32}N_{0,2} + d\,CO_2 + e\,H_2O \quad \textbf{(Eq. 6)}$$

It's needed to specify the stoichiometric coefficient for the biomass. As no real information was found in the reference study and more detailed experiments should be done to obtain that information, another reference study will be used. Using the same values from [32] for biomass coefficient (0,826) and balancing the equation, the result obtained it's the following:

$$C_2H_6O_2 + 0{,}165\, NO_3^- + 1{,}114\, O_2 \xrightarrow{TCA\ cycle} 0{,}826\, CH_{2,15}O_{0,32}N_{0,2} + 1{,}174\, CO_2 + 2{,}112\, H_2O \quad \textbf{(Eq. 7)}$$

In order to obtain the whole view of the set of equations developed and also refer it to the monomer consumption rate, that is the main information of the reference study, adding the equation (2), (3), (4) and (7) the final chemical equation it's:

$$C_{10}H_8O_4 + 8{,}614\, O_2 + 0{,}165\, NO_3^- \rightarrow 0{,}826\, CH_{2,15}O_{0,32}N_{0,2} + 9{,}174\, CO_2 + 3{,}112\, H_2O \quad \textbf{(Eq. 8)}$$

With the development of the equation (8), then it's possible to predict the performance of the bioreactor system and estimate how much PET can be degraded with the final concentrations of the main components of the bioreactor.

4.4. Predicted growth and operation of bioreactor

Once the theorical framework for the system is established it's possible to develop the expected performance for the bioreactor with the selected conditions and with the information of weight loss from [25]. As no real information, more than the weight loss percentage, it's given by [25] then some options and some assumptions will be considered. As basis for the analysis:

- Total consumption of the limiting nutrient: To be able to present the maximum potential of the model and as no more information is provided, it was decided to considerate the total consumption of the nitrate content of the growth media during the 6 weeks.
- Three cases were considered:
  - 1º case: Total consumption of nitrate
  - 2º case: Total consumption of nitrogen (nitrate + ammonia)
  - 3º case: Addition of nitrate up to stoichiometric relation with phosphate
- The initial concentration of PET considered was calibrated to give the maximum amount possible of PET degraded considering the complete depletion of nitrate/nitrogen sources and also taking into account that the maximum weight loss is of 35% at 6 weeks.
- The percentages of the degradation each week is directly estimated from **Figure 3.10**.
- The concentrations of phosphate will follow the stoichiometric relation of N 13:1 P.
- The base chemical reaction for calculation of the molar concentrations is the **Eq. 8.**

In the modelling of the bacteria performance, the specific growth can be calculated in order to give the idea of the evolution of the system in these three cases and during the whole operation time. The specific growth calculations were made using [33] model, that will be described.

Two models were considered, but both of them has the same basis that is the assumption of a homogeneous colony without mortality that will growth with an exponential model. Assuming this, then the change in the microbial biomass during the time will be represented by [33]:

$$\frac{dB_t}{dt} = \mu B_t \quad \textbf{(Eq. 9)}$$

Where B_t is the biomass concentration at time t (mass/volume) and μ it's the specific growth rate (time^{-1})

The standard solution of the equation (9) it's the following:

$$B_t = B_o e^{\mu t} \quad \textbf{(Eq. 10)}$$

Where B_o it's the biomass at time t=0

Changing the equation to allocate the specific growth rate, it's possible to formulate the following equation:

$$\mu = \ln\left(\frac{B_t}{B_o}\right)/\Delta t \quad \textbf{(Eq. 11)}$$

This model assumes that the growth rate is equivalent to a semi-logarithmic slope, that can be assumed as true when there is no mortality or better referred as the growth really overcomes the mortality and always is a net growth of the colony. Other model that can be used is the based-on productivity. The productivity it's the slope of the first derivate of biomass against time, following the next formula [33]:

$$P = \frac{dB_t}{dt} \quad \textbf{(Eq. 12)}$$

Where P is the productivity (mass/time units)

Assuming that this derivative will dominate the growth of the bacteria and not the semi-logarithmic slope, then it's possible to formulate the biomass at a time t as [33]:

$$B_t = P\ x\ T + B_o \quad \textbf{(Eq. 13)}$$

Taking into account then the equation (13) and the equation (11) it's possible to formulate the final model as:

$$\mu = ln\left[\left(\frac{P\ x\ T}{B_o}\right)/B_o\right]/T \quad \textbf{(Eq. 14)}$$

And making some simplifications, then the final model will be:

$$\mu = ln\left({}^{P}/_{B_o} + 1\right) \quad \text{(Eq. 15)}$$

This final model will be true if the time interval T it's considered as 1, that in this case will be. As the model it's stablished, then the results for the 3 cases considered are presented on **Table 4.5**, **Table 4.6** and **Table 4.7**:

***Table 4.5**: Performance of the bioreactor in the case 1. Own elaboration.*

Case 1: Only use of primary nitrogen source (Nitrogen present as nitrate)

	PET degradation	C PET (mol/L)	C biomass (mol/L)	C NO3 (mol/L)	C PO4 (mol/L)	C biomass (g/L)	Week to week	Week to week	Week to week
Week 0	0,00%	0,407	0,026	0,024	0,0073	0,583	**Eq. (11) ($week^{-1}$)**	**Eq. (15) ($week^{-1}$)**	**Eq. (12) (g/week)**
Week 1	3,00%	0,395	0,037	0,022	0,0072	0,806	0,324	0,324	0,223
Week 2	5,00%	0,387	0,043	0,020	0,0071	0,954	0,169	0,227	0,149
Week 3	12,00%	0,359	0,067	0,015	0,0067	1,474	0,435	0,638	0,520
Week 4	16,00%	0,342	0,080	0,013	0,0065	1,771	0,184	0,412	0,297
Week 5	23,00%	0,314	0,104	0,008	0,0062	2,291	0,257	0,638	0,520
Week 6	35,00%	0,265	0,144	0,000	0,0055	3,183	0,329	0,928	0,891

***Table 4.6**: Performance of the bioreactor in the case 2. Own elaboration.*

Case 2: Complete depletion of primary and secondary nitrogen

	PET degradation	C PET (mol/L)	C biomass (mol/L)	C NO_3 (mol/L)	C PO_4 (mol/L)	C biomass (g/L)	Week to week	Week to week	Week to week
Week 0	0,00%	0,731	0,026	0,042	0,0073	0,583	**Eq. (11) ($week^{-1}$)**	**Eq. (15) ($week^{-1}$)**	**Eq. (12) (g/week)**
Week 1	3,00%	0,709	0,045	0,039	0,0071	0,983	0,522	0,522	0,400
Week 2	5,00%	0,695	0,057	0,036	0,0069	1,249	0,240	0,377	0,267
Week 3	12,00%	0,643	0,099	0,028	0,0062	2,182	0,558	0,956	0,933
Week 4	16,00%	0,614	0,123	0,023	0,0059	2,716	0,219	0,649	0,533
Week 5	23,00%	0,563	0,165	0,014	0,0052	3,649	0,295	0,956	0,933
Week 6	35,00%	0,475	0,238	0,000	0,0041	5,248	0,364	1,320	1,599

***Table 4.7**: Performance of the bioreactor in the case 3. Own elaboration*

Case 3: Nitrogen up to stoichiometric relation with phosphate

	PET degradation	C PET (mol/L)	C biomass (mol/L)	C NO_3 (mol/L)	C PO_4 (mol/L)	C biomass (g/L)	Week to week	Week to week	Week to week
Week 0	0,00%	1,654	0,026	0,096	0,007	0,583	**Eq. (11) ($week^{-1}$)**	**Eq. (15) ($week^{-1}$)**	**Eq. (12) (g/week)**
Week 1	3,00%	1,605	0,067	0,087	0,007	1,488	0,937	0,937	0,905
Week 2	5,00%	1,571	0,095	0,082	0,006	2,091	0,340	0,710	0,603
Week 3	12,00%	1,456	0,190	0,063	0,005	4,202	0,698	1,531	2,111
Week 4	16,00%	1,390	0,245	0,052	0,004	5,408	0,252	1,121	1,206
Week 5	23,00%	1,274	0,341	0,033	0,003	7,519	0,330	1,531	2,111
Week 6	35,00%	1,075	0,505	0,000	0,000	11,137	0,393	1,975	3,619

As can be seen, the results give similar results for the three cases, but the results from **Eq. (15)** gives higher results that can be related to an overestimation of the growth rate. This overestimation is driven mainly by the oscillations in the productivity, that is related to the increasing amount of PET degraded from week to week. This slow start approach can be related with some lag time for the organism or the presence of some inhibition by some components that can be no further analyzed as the lack of information from [25]. From the three cases the selected case for the mass balance and final calculation will be the 2º case, as gives an idea of the better performance available without any change in the growth media composition and then it's closer to the information given by [25].

A representation of the evolution of the growth rate by **Eq. (11)** for the three cases will be provided to allow and easily observe the trends at **Figure 4.5**:

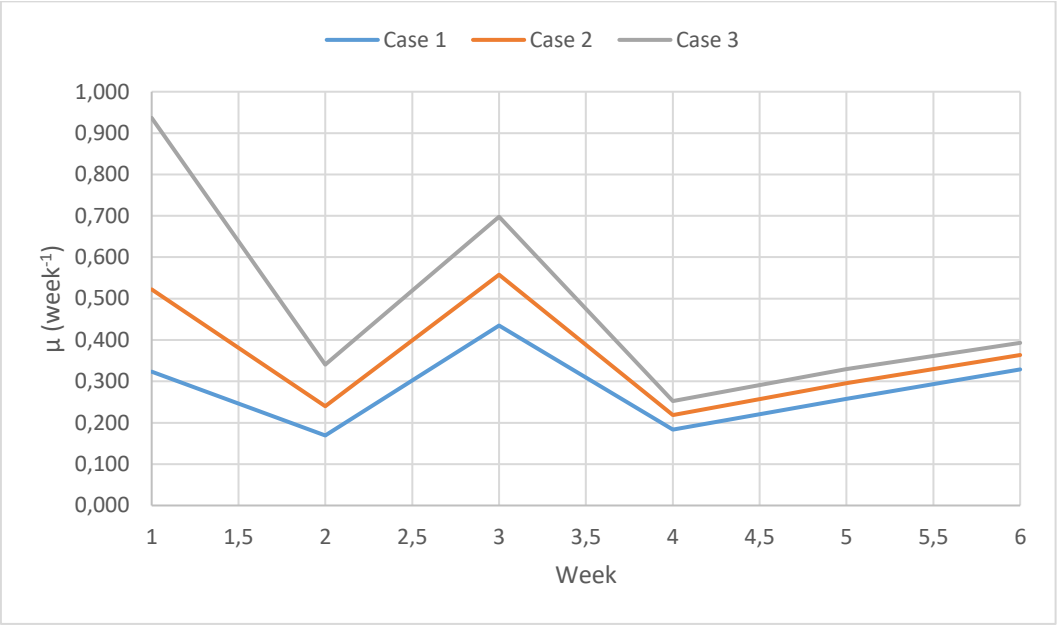


***Figure 4.5**: Growth specific rate evolution for the three cases considered. Own elaboration.*

It's needed to take into account that the logarithmic approach will give higher values for the last cases as the difference it's higher in the first weeks but mainly the peak of the performance it's obtained through week 3. The third case as was commented, it's not really very suitable as modifies the concentrations of the growth media chosen by the authors of [25], which makes it not really good to take into account as a possibility without further studies or information. Nonetheless, this case results are presented to give an idea of other cases model for the information obtained and given by the authors of [25].

4.5. Operation parameters, heat transfer and chemical dose

As a final review of the modelling and different needs of the operation of the bioreactor following the different chemicals used in the reference [25] it's needed to evaluate the operation parameters, the heat transfer needed and the chemical dose of the used chemicals outside of the ones that conform the growth media composition.

Table 4.8 present the different values commented:

***Table 4.8**: Operation parameters and chemicals. Own elaboration.*

Temperature	**35**	**ºC**
pH	11	
Inoculum dose	0,583	g/L
Auxiliar chemicals		
Glutaraldehyde	2,50%	m/m
Ethanol	0,1	mol/kg

For the auxiliar chemicals used, glutaraldehyde and ethanol, they are just fixing agents for the biomass at the start of the degradation facilitating the action of the biofilm formation and are not reported to be consumed at any point of the biodegradation. Because of this reason, they will be represented in the mass balance but not considering any change during the whole process. To well set the operation of the bioreactor and the whole process, the dose needed was calculated. As both chemicals are referred to PET amount, then the final values will be of 2,5 kg of glutaraldehyde per 100 kg of PET and 0,004607 kg ethanol per kg of PET.

Other point that is needed to calculate it's the dose of sodium hydroxide for reaching the desired pH of 11. From the different chemicals that are present in the growth media and also the system, the ones that will affect the pH the most and will be considered are potassium dihydrogen phosphate and ammonium chloride plus the action of the sodium hydroxide. To be able to calculate the addition of each specie to the pH, and assuming a total pH + pOH of 14 (water dissociation constant (pK_w) of 14) the model for acids and base dissociation and calculations it's the following:

$$K_a = \frac{[Acid\ specie^-][H^+]}{[HA]} \qquad \textbf{(Eq. 16)}$$

$$K_b = \frac{[Basic\ specie^+][OH^-]}{[BOH]} \qquad \textbf{(Eq. 17)}$$

Taking also into account that pK_a, pK_b and pH are the $-\log_{10}[x]$ and knowing the dissociation constants it's possible to calculate the needed dose of NaOH for reach the desired pH. It's important to notice that the NaOH as a strong base, will have a pK_b of -1, which means a huge dissociation into hydroxyl ions in the dissolution. Comment also that for the potassium dihydrogen phosphate, the

compound will suffer a 3-step dissociation following the chemical equilibrium presented in the equation (18):

$$KH_2PO_4 \overset{Ka_1}{\longleftrightarrow} K^+ + H_2PO_4^- \overset{Ka_2}{\longleftrightarrow} H^+ + HPO_4^{2-} \overset{Ka_3}{\longleftrightarrow} H^+ + PO_4^{3-} \quad \textbf{(Eq. 18)}$$

The logarithmic form of dissociation constants values are presented in **Table 4.9**:

***Table 4.9**: Data used for the NaOH dose calculation. Own elaboration.*

Specie	Molar concentration (M)	Dissociation constant	Reference
KH_2PO_4	0,00735	pKa_1= 2,15 pKa_2= 6,82 pKa_3= 12,38	[34]
NH_4Cl	0,0187	pKa= 9,24	[35]
NaOH	Unknown	pK_b= -1	-

Making the calculations with the data and the model presented, the dose of NaOH that is needed it's of 0,035 M, which means a mass concentration of 1,4 g/L of the final liquid volume in the bioreactor.

In the case of the final effluent, if no neutralizer is used, the expected pH of the water will be too high to discharge. In order to change this, hydrochloric acid is proposed as the possible neutralizer of the high pH. The problem is that when it has to be used, at sedimentation tank, no real information of the conformation of the mixture elements in terms of the remaining growth media components is known. This makes unavailable a proper approach for the estimated consumption in general terms. As no more information is known, a rough estimation will be made with the known composition calculated in **Table 4.9** to just have a general idea of the dose.

It's important to notice that really the molar concentrations in the sedimentation tank should be lower (as ammonium chloride is considered as total consumed and dihydrogen monobasic phosphate should be consumed in a certain quantity) and the amount of water increases without increase in the amount of chemicals as an effect of the products of the biochemistry reaction, so probably the real required dose will be lower than the one calculated here.

Taking as basis the commented before, the model developed and that hydrochloric acid is a strong acid (pK_a=-1), the estimated amount will be of 0,022 mol/L which means a mass concentration of 0,803 g/L respect to the bioreactor volume.

As a final comment, no consideration will be taken about the hydrochloric acid in the total consumptions as no real information of the need it's available but will be taken into account for the safety study of the proposal as it is for sure that it will be present in the operation of the process.

5. Process description, equipment and mass balance

For the case described before an initial structure for a pilot plant will be proposed. In order to properly stablish the alternative proposed the main parameters as type of equipment, mode of operation, capacity of the plant, general mass balance and a block flow diagram (BFD) will be presented. Before that, individual mass balances for the sections of the pilot plant will be performed and also a rigorous design proposal to the main equipment that is the bioreactor.

The proposed block flow diagram for the pilot plant is the presented in **Figure 5.1**:

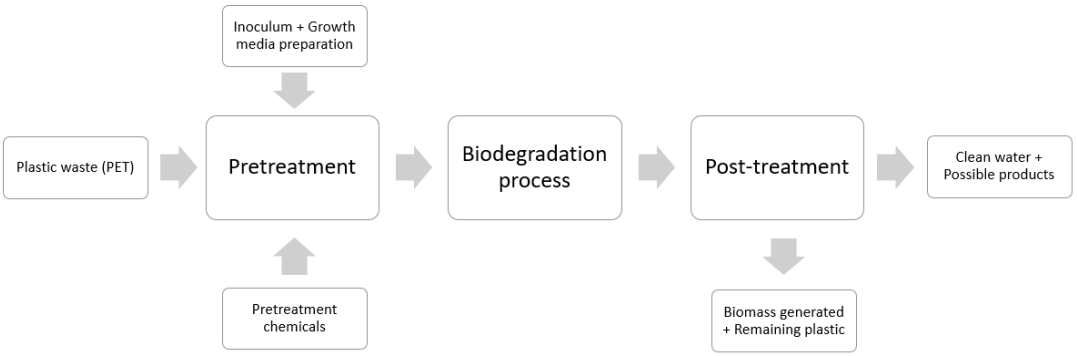


***Figure 5.1**: Block flow diagram (BFD) for the pilot plant proposed. Source: Own elaboration.*

The whole plant will be divided into three different sections by self-decision to define in a proper way the different operations that are involved into the whole operation of the bioremediation process.

The first section (Section 100) will be the pretreatment zone. In this zone two main operations of the plant will be performed. In first place the PET will be received, prepared into proper pieces and disinfected to avoid any cross contamination with the bacterial inoculum that is intended to use and stablish a non-competitive system. If the disinfection was avoided, competitive bacteria will appear into the biodegradation process, leading to an unsuccessful yield of degradation and also can be expected a lot of different operation problems as bacterial cell death, appearance of biofouling, etc. On the other hand, the initial inoculum will be prepared with the adequate growth media (following the procedure of [25]) and this prepared mixture will be used as the initial biomass concentration for the following section.

The second section (Section 200) will be the biodegradation process in a bioreactor. In this section, the bioreactor will operate in batch mode as the time needed for the biodegradation is of 2 months. The operating conditions of the bioreactor will be of 35ºC and a pH of 11 as is reported in [25]. The

initial concentration of biomass is no reported directly but will be estimated in the mass balances, as the chemical formula of the bacteria and other modelling parameters.

The last section (Section 300) will be the post-treatment of the treated plastic and the biomass generated. It's expected to obtain a clean water effluent with some products from the total mineralization of the PET and other effluent where the remaining plastic and the generated biomass are obtained and will be separated in order to allocate them into different possible applications. Also in the sedimentation tank, some neutralizer will be used to avoid high pH in the final water. The neutralizer selected will be the most usual one, that is hydrochloric acid.

The process will operate in batch mode as the needed time for the biodegradation it's of 6 weeks, which makes unfeasible the continuous mode for the operation of this plant. The mass balances and different calculations will be performed according to the cycles that will be made. It's considered that a year has 52 weeks, so 8 cycles during a year will be considered and 4 weeks for contingencies and any possible problem that appear during the normal operation. The objective of the plant will be of 1000 kg of PET degraded per year, which accounts for 125 kg of PET degraded per cycle. For the characteristic volume of each equipment, a fulfilling of 85% of the total volume for normal conditions will be considered and the densities and water volume needed.

5.1. Equipment list

In **Table 5.1**, **Table 5.2** and **Table 5.3** it's provided the list of the equipment necessary for the proposed scheme for the whole process, separating it by the different sections:

***Table 5.1**: Equipment list for Section 100. Source: Own elaboration.*

Section	Name tag	Equipment type
Pretreatment and conditioning (Section 100)	***V-101***	Growth media and inoculum preparation vessel
	V-102	PET auxiliary chemicals addition vessel
	S-101	PET shredder
	P-101	Centrifugal pump
	E-101	Heat exchanger

***Table 5.2**: Equipment list for Section 200. Source: Own elaboration.*

Section	Name tag	Equipment type
Biodegradation and reaction (Section 200)	***R-201***	Biodegradation vessel

***Table 5.3**: Equipment list for Section 300. Source: Own elaboration.*

Section	Name tag	Equipment type
Separation and conditioning (Section 300)	***F-301***	Small size screening equipment
	V-301	Sedimentation vessel

The different equipment used will have the following objectives:

- Vessel V-101: Vessel for the preparation of the liquid growth media and the inoculum preparation for the posterior conditioning and introduction to the reaction vessel.
- Vessel V-102: Vessel where the auxiliary chemicals to help the formation of the biofilm by the bacteria (glutaraldehyde and ethanol) are added to the existent PET in the desired form.
- Shredder S-101: Mechanical shredder to reduce the size of the PET inlet to the desired 1 cm diameter pellets following the equivalent size used at [25].
- Pump P-101: Centrifugal pump to impulse the growth media from the vessel V-101 to the reactor R-201.
- Heat exchanger E-101: Heat exchanger to elevate the temperature of the growth media entering the reactor R-201 up to 35ºC.
- Reactor R-201: Reaction vessel where the biodegradation of the PET and the growth of the bacteria occurs.
- Screening panel F-301: Small size screening panels that will generate two different streams that mainly will be composed of:
    - One stream with the remaining PET and some biomass and chemicals that are associated with them.
    - Other stream where the majority of the water and the remaining chemicals are present.
- Vessel V-301: Sedimentation vessel that will concentrate and separate the remaining biomass and chemicals, leaving a clean effluent of water.

5.2. General mass balance and proposed process flow diagram

For the next calculations that will be performed, some assumptions and information have to be taken into account:

- The calculations and data supposed will be adequate for 1 cycle of the operation of 6 weeks, taking into account that a total amount of biodegraded PET of 1000 kg/year during 8 cycles of 6 weeks in batch operation was considered.
- Only the PET biodegradation and bacterial mass generated, that was described and modeled in the last part, will be considered as chemical reaction of the process.
- The streams that have no change in the composition will not be taken into account in the definition of the mass balance i.e., 7 and 8.
- The oxygen needed for the biomass reaction will be considered as entering in the system with the inlet of water as dissolved oxygen.

Taking into account this information, then the operating conditions and important characteristics of the different equipment is in the following table:

***Table 5.4**: Operation characteristics for equipment of the process. Source: Own elaboration.*

Operation	Equipment	Conditions
Mix and preparation of the growth media	Vessel V-101	Total volume: 3 m^3 Liquid volume: 2,544 m^3 Temperature: 20ºC Full mixing condition
Reduction of volume and preparation of the PET	Shredder S-101	Shredding capacity: 500 kg/h Power consumption: 7,5 kW Minimum shred size: 8 mm Obtained from [36]
Addition of auxiliary chemicals for PET biodegradation	Vessel V-102	Total volume: 0,35 m^3 Occupied volume: 0,265 m^3 Temperature: 20ºC

Biodegradation of PET and bacterial biomass growth	Reactor R-201	Total volume: 3 m^3 Liquid volume: 2,544 m^3 Temperature: 35ºC pH 11 Batch operation during 6 weeks
Elimination of remaining PET	Screening F-301	Elimination of all PET with 10% of total biomass, 10% of total water and all auxiliary chemicals
Sedimentation of the biomass	Vessel V-301	Elimination of remaining biomass and some water, assuming a 40% of biomass and 60% of water in a mass basis

Taking into account the different conditions and assumptions commented before, the proposed process flow diagram (PFD) for the whole process is presented in **Figure 5.2**:

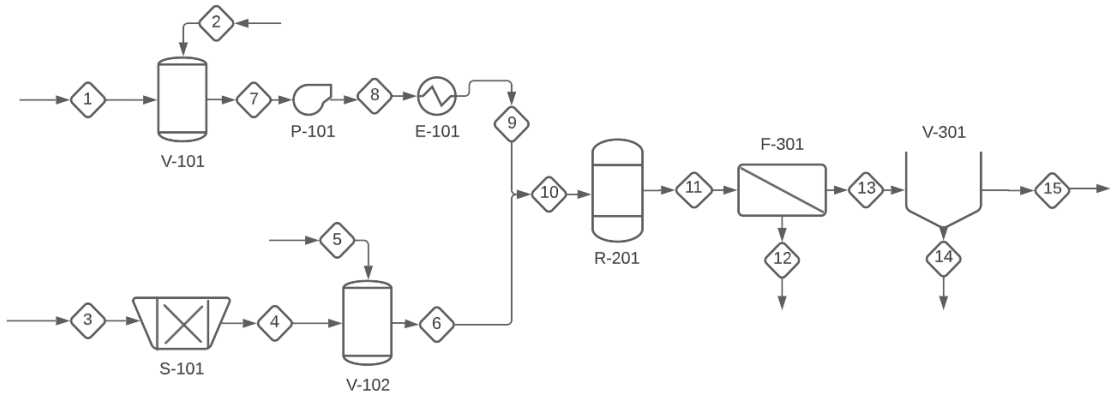


***Figure 5.2**: Process flow diagram of the process. Source: Own elaboration.*

Taking into account the proposed process flow diagram for the process, the general mass balance will be performed. The general mass balance takes into account the inlets and outlets of the process in order to close the mass balance and corroborate the quality of the calculations. Using the stream number used in **Figure 5.2**, the results are presented in the following tables:

***Table 5.5**: Inlets of the process. Source: Own elaboration.*

Component (kg/cycle)	Water and oxygen inlet 1	Growth media and inoculum 2	PET inlet 3	Auxiliary chemicals inlet 5
Water	2544,00	0,00	0,00	0,00
PET	0,00	0,00	357,13	0,00
Biomass	0,00	1,48	0,00	0,00
Nitrate	0,00	6,66	0,00	0,00
Glutaraldehyde	0,00	0,00	0,00	8,93
Ethanol	0,00	0,00	0,00	1,65
CO2	0,00	0,00	0,00	0,00
O2	179,45	0,00	0,00	0,00
Total amount (kg/cycle)	2723,45	8,14	357,13	10,57
Total inlet (kg/cycle)	3099,29			

For the outlets of the process, the results are:

***Table 5.6**: Outlets of the process. Source: Own elaboration.*

Component (kg/cycle)	PET and auxiliary outlet 12	Biomass outlet 14	Clean effluent 15
Water	258,05	28,49	2293,93
PET	232,13	0,00	0,00
Biomass	1,33	11,40	0,60
Nitrate	0,00	0,00	0,00
Glutaraldehyde	8,93	0,00	0,00
Ethanol	1,65	0,00	0,00
CO2	0,00	0,00	262,79
O2	0,00	0,00	0,00
Total amount (kg/cycle)	502,09	39,89	2557,31
Total outlet (kg/cycle)	3099,29		

As can be seen with the total outlet and inlet, the mass balance close perfectly with a difference between inlets and outlets of 0 kg per cycle. As this value is the desired, it's considered that the mass balance and the proposed process flow diagram are correct taking into account the framework that was established for the performance of this study.

5.3. Individual mass balance

Once the general mass balance is achieved without any difference between inlets and outlets, now the individual mass balance for each equipment piece will be performed to evaluate the operation of each single equipment present in the project. As a comment, the s elected conditions and separations are the ones presented in **Table 5.4**.

5.3.1. V-101 and growth media preparation

In first place, water will be taken and some inoculum and growth media components will be added in order to prepare the main driver of the biodegradation of the PET. In the mass balance, the main components will be considered and also the ones that it's known how it's their performance to make the approximation more valuable from the operation point of view. The scheme and mass balance are presented in **Figure 5.3** and **Table 5.7**:

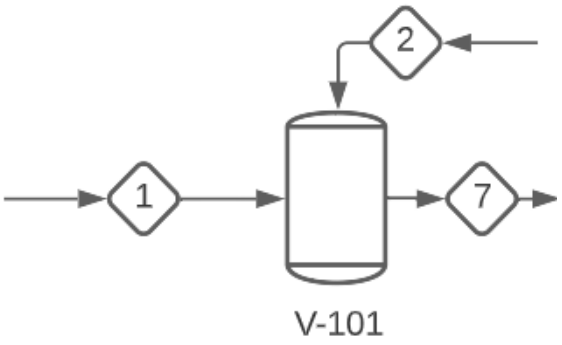


Figure 5.3: *Inlets and outlets of the vessel V-101. Source: Own elaboration.*

Table 5.7*: Mass balance for vessel V-101. Source: Own elaboration.*

Component (kg/cycle)	Water and oxygen inlet 1	Growth media and inoculum 2	Prepared bacteria for biodegradation 7
Water	2544,00	0,00	2544,00
PET	0,00	0,00	0,00
Biomass	0,00	1,48	1,48
Nitrate	0,00	6,66	6,66
Glutaraldehyde	0,00	0,00	0,00
Ethanol	0,00	0,00	0,00
CO2	0,00	0,00	0,00
O2	179,45	0,00	179,45
Total amount (kg/cycle)	2723,45	8,14	2731,59

5.3.2. S-101 and V-102

The idea of this two equipment is the preparation of the PET that enters the process with a reduction of their size and accommodation for the desired size. Once this is achieved, the vessel V-102 offers the support to introduce the desired chemicals that help the formation of the bacterial biofilm in the PET surface, making the biodegradation easier. The scheme and the mass balance for this part is presented in **Figure 5.4** and **Table 5.8**:

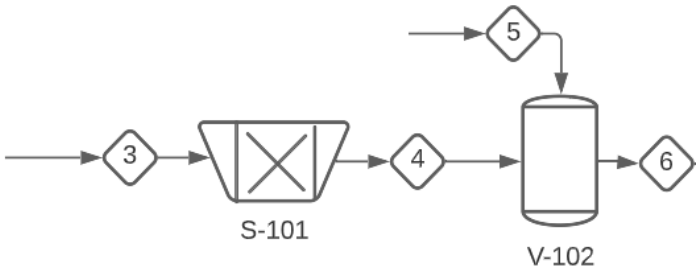


***Figure 5.4**: Inlets and outlets of the shredder S-101 and vessel V-102. Source: Own elaboration.*

***Table 5.8**: Mass balance for the shredder S-101 and vessel V-102. Source: Own elaboration.*

Component (kg/cycle)	PET inlet 3	Auxiliary chemicals inlet 5	Prepared PET 6
Water	0,00	0,00	0,00
PET	357,13	0,00	357,13
Biomass	0,00	0,00	0,00
Nitrate	0,00	0,00	0,00
Glutaraldehyde	0,00	8,93	8,93
Ethanol	0,00	1,65	1,65
CO2	0,00	0,00	0,00
O2	0,00	0,00	0,00
Total amount (kg/cycle)	357,13	10,57	367,70

5.3.3. R-201

This equipment will be the main core of the process, as it's the one where the biodegradation of the PET occurs and the bacterial biomass is generated. The scheme is presented in **Figure 5.5**:

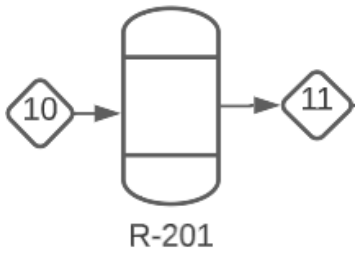


Figure 5.5: *Inlets and outlets of reactor R-201. Source: Own elaboration.*

For the proper analysis of the mass balance on this equipment, not only the mass amount is presented. First the general mass balance will be presented in **Table 5.9**:

Table 5.9*: Mass balance for the reactor R-201. Source: Own elaboration.*

Component (kg/cycle)	Reactor inlet 10	Reactor outlet 11
Water	2544,00	2580,47
PET	357,13	232,13
Biomass	1,48	13,33
Nitrate	6,66	0,00
Glutaraldehyde	8,93	8,93
Ethanol	1,65	1,65
CO2	0,00	262,79
O2	179,45	0,00
Total amount (kg/cycle)	3099,29	3099,29

For the chemical reaction considered in this process, the molar and mass balance for the involved components are presented in **Table 5.10**:

Table 5.10: *Molar and mass balance for the involved components in the reactor R-201. Source: Own elaboration.*

Stream	Reactor inlet 10	Reactor outlet 11	Reactor inlet 10	Reactor outlet 11
	Molar amount (kmol/cycle)		Mass amount (kg/cycle)	
Biomass	0,07	0,60	1,48	13,33
Nitrate	0,11	0,00	6,66	0,00
PET	1,86	1,21	357,13	232,13
CO2	0,00	5,97	0,00	262,79
Water	141,33	143,36	2544,00	2580,47
O2	5,61	0,00	179,45	0,00
Total amount	148,98	151,15	3088,72	3088,72

5.3.4. F-301 + V-301

This final section is intended to separate the different products and non-reacted elements that goes out of the reactor. In the screening of the F-301 the Pet is recovered with some biomass and chemicals. Following this, the sedimentator V-301 eliminates the remaining biomass and generates a clean effluent. The scheme and the results are presented in **Figure 5.6** and **Table 5.11**:

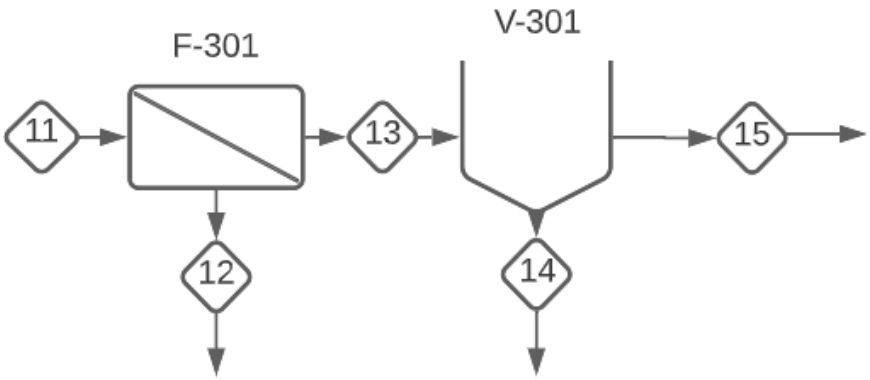


***Figure 5.6**: Inlets and outlets of screening F-301 and sedimentator V-301. Source: Own elaboration.*

***Table 5.11**: Mass balance for screening F-301 and sedimentator V-301. Source: Own elaboration.*

Component (kg/cycle)	Reactor outlet	PET and auxiliary outlet	Sedimentator inlet	Biomass outlet	Clean effluent
	11	12	13	14	15
Water	2580,47	258,05	2322,42	28,49	2293,93
PET	232,13	232,13	0,00	0,00	0,00
Biomass	13,33	1,33	12,00	11,40	0,60
Nitrate	0,00	0,00	0,00	0,00	0,00
Glutaraldehyde	8,93	8,93	0,00	0,00	0,00
Ethanol	1,65	1,65	0,00	0,00	0,00
CO_2	262,79	0,00	262,79	0,00	262,79
O_2	0,00	0,00	0,00	0,00	0,00
Total amount (kg/cycle)	3099,29	502,09	2597,20	39,89	2557,31

5.4. Energy consumptions

For the operating expenses, different approaches can be made in order to establish the range of money that will be needed. As this is an initial and brief approach of this process and some information is missing, in this case the operating expenses of the whole process will be mainly represented by the energetic consumptions of the main equipment and the chemical consumption.

In this approach, three energy consumptions will be considered as the most important ones. The first will be the energy consumption of the shredder machine, that will be extracted from [36], as a commercial equipment. The shredding capacity is of 500 kg/h and the power consumption is of 7,5 kW, so taking into account the amount needed for 1 cycle (357,13 kg) the total consumption can be obtained as the time of operation will be of 42,85 min.

Knowing this, other main energy consumption will be the maintenance of the temperature during the bioreactor operation. As no information is obtained from the reference document, as was done with the biomass stoichiometric coefficient, the heat of reaction from [25] will be taken as reference.

The value of the heat reaction then is of 45,1 kJ/mol, which indicates that is an endothermic reaction. With this value known and also as the molar amount of PET that reacted is known, then is possible to know the necessary heat per cycle in the bioreactor R-201. The total molar amount of PET that is biodegraded is of 0,65 kmol, which means an amount of 650 mol and the total energy can be obtained.

Finally, the last energy consumption will be to elevate the temperature of the growth media and inoculum up to 35ºC from the initial 20ºC in the heat exchanger E-101. Assuming that the specific heat of the mixture is the same as water (4,18 kJ/kg K), the calculation can be done directly. All the results are recovered in **Table 5.12**:

***Table 5.12**: Energy consumption of the main process equipment. Source: Own elaboration*

Equipment	Use or change	kJ/cycle	kWh/cycle	kWh/year	% of total
Shredder S-101	Shredding of 357,13 kg of PET	19285,02	5,36	42,86	8,77
Reactor R-201	Maintenance of temperature up to 35 ºC during biodegradation 6 weeks	29315,00	8,14	65,14	13,33
Heat exchanger E-101	Temperature rises from 20ºC up to 35 ºC of stream 8	171270,69	47,58	380,60	77,90
Total		219870,71	61,08	488,60	100,00

As can be seen the most critical point is the temperature rise of the stream 8, as the amount of the stream (2731,59 kg) is very huge and almost represent a 78% of the total energy consumption making it a really critical point in terms of energy consumption. So, as a general comment, some energy recuperation or integration could be done in that point for the improvement of the economics of the process and the environmental impact of the process.

5.5. Chemical consumption

The other main point for the operating expenses of this process will be the chemical consumption that is done along the different operations of the process. For this chemical consumption calculation, the mass balance amounts and also the known concentrations for the chemicals that conform the growth media are taken into account to achieve the most accurate approximation that can be done with the scope of this project.

Taking into account this, the obtained chemical consumption for each cycle and for a total year operation are presented in **Table 5.13**:

***Table 5.13**: Chemical consumption of the process. Source: Own elaboration.*

Chemical	Concentration		Amount per cycle (kg)	Amount per year (kg)
NaOH	1,4	g/L	3,56	28,49
Glutaraldehyde	2,5 % (m/m) respect to PET		8,93	71,44
Ethanol	0,1 M respect to PET		1,65	13,20
KH_2PO_4	1	g/L	2,54	20,35
$NaNO_3$	2	g/L	5,09	40,70
$MgSO_4$ x 7 H_2O	0,5	g/L	1,27	10,18
KCl	0,5	g/L	1,27	10,18
$FeSO_4$ x 7 H_2O	0,01	g/L	0,025	0,20
NH_4Cl	1	g/L	2,54	20,35
Total			26,89	215,10

With this total information, it's possible to make a proper estimation of the total operating expenses for the process operation during the normal operation of it.

6. Economic analysis

For the economic analysis of the process operations, to main approximations were made:

- Capital expenses (CAPEX) and operating expenses (OPEX) of the process following the information of consumptions provided by **Table 5.12** and **Table 5.13**.
- Budget estimation taking as equipment purchase the CAPEX estimation and allocating different percentages.

6.1. CAPEX and OPEX estimation

The first calculation will be the operating expenses of the process. The operating expenses of the process account for the chemicals and utilities consumption that is needed for the operation. In the case of this process the main operating expenses that was taken into account are the energy consumption and the chemical consumption.

For the energy consumption, the information provided by **Table 5.12**, was taken into account as basis. For the calculation of the cost associated to the energy consumption, the standard price for the non-household consumers per kilowatt per hour was consulted. The information that was taken into account for price is presented in the **Figure 6.1**:

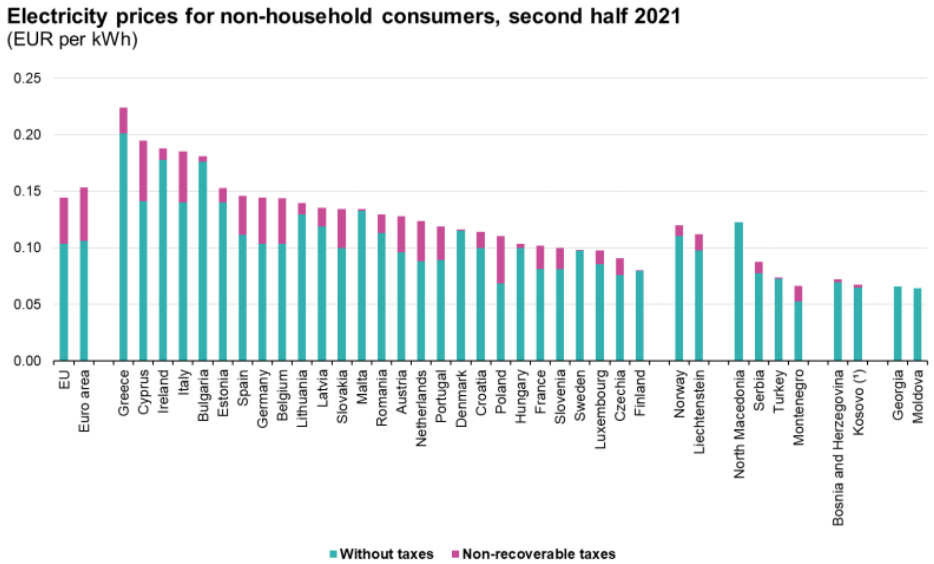


***Figure 6.1**: Electricity price in EU-27 for non-household consumers for the second half of 2021. Source: [37]*

With this information, it can be estimated that the cost per kWh for electricity in Spain is about 0,145 euros/kWh. Using the information of **Figure 6.1** and **Table 5.12**, the **Table 6.1** is provided:

***Table 6.1**: OPEX for energy consumption. Source: Own elaboration.*

Equipment	Use or change	Euros/cycle	Euros/year
Shredder S-101	Shredding of 357,13 kg of PET	0,78	6,21
Reactor R-201	Maintenance of temperature up to 35 ºC during biodegradation 6 weeks	1,18	9,45
Heat exchanger E-101	Temperature rise from 20ºC up to 35 ºC of stream 8	6,90	55,19
Total (Euros)		8,86	70,85

The next step will be the calculation of the OPEX related with the chemical consumptions, determined by **Table 5.13**. The prices were consulted in [34], taking into account the purest form possible that was specified as viable to cell cultivation or growth media formation. The specific links will be provided in the annex. The obtained values are presented in **Table 6.2**:

***Table 6.2**: OPEX for chemical consumption. Source: Own elaboration.*

Chemical	Price (Euros/kg)	Cost (Euros/cycle)	Cost (Euros/year)	Reference
NaOH	42,30	150,66	1205,25	[34]
Glutaraldehyde	82,50	736,73	5893,80	
Ethanol	119,93	197,88	1583,05	
KH_2PO_4	156,00	396,86	3174,91	
$NaNO_3$	153,00	778,46	6227,71	
$MgSO_4$ x 7 H_2O	132,00	167,90	1343,23	
KCl	172,00	218,78	1750,27	
$FeSO_4$ x 7 H_2O	205,00	5,22	41,72	
NH_4Cl	145,00	368,88	2951,04	
Total		3021,37	24170,98	

For the CAPEX calculations, commercial prices for real equipment were searched to give the most accurate approach that was possible. The material was selected as stainless steel in each of the equipment that has and expected high pH. The stainless steel has some problems with the high pH and the presence of sodium hydroxide but only at high temperatures. As no high temperatures are present, then the material it's adequate. The specific links of the equipment will be provided at annex. Taking into account the information of characteristics of equipment from **Table 5.4**, the is obtained:

Table 6.3: *CAPEX calculation for the main equipment. Source: Own elaboration.*

PFD tag	Type of equipment	Material	Capacity or characteristics	Cost (Euros)	Reference
V-101	Mixing tank	Stainless steel	3 m^3	2350	[38]
V-102	Mixing tank	Stainless steel	0,35 m^3	263,2	
S-101	Commercial shredder for PET bottles	Carbon steel	500 kg/h	6000,00	[36]
R-201	Reactor vessel with heat exchange	Stainless steel	3 m3	2350	[38]
F-301	Fine screening	Stainless steel	1 mm grid From 10 to 500 m^3/d	4230,00	
V-301	Sedimentation vessel	Carbon steel or stainless steel	3 m^3	2237,20	
Total				17430,4	

As can be seen, in the case of this process, the CAPEX and the OPEX of the plant will represent a 42% and 58% respectively. The main costs will be allocated to the chemical consumption as the biodegradation of PET requires of very specific conditions and some additional chemicals that will facilitate the work of the marine bacteria.

6.2. Budget

For the estimation of the budget, an allocation system following the methodology from [39] will be performed. In this case, it's important to have some fixed value to be able to estimate it properly, that will be the CAPEX of the plant as purchase of equipment. This methodology allocates specific percentages for each need of the budget according to a given range for multipurpose plants. As no real information it's known, the medium value of the range will be supposed. The supposed values are presented in **Table 6.4**:

***Table 6.4**: Allocated percentages for budget. Information obtained from [39], own elaboration.*

Cost percentages for multipurpose plants				
	Inferior limit	Superior limit	Assumed percentage	Normalized percentage
Direct cost				
Purchase of equipment	15%	40%	27,5%	21,8%
Equipment installation	6%	14%	10,0%	7,9%
Instrumentation and control	2%	8%	5,0%	4,0%
Piping	3%	20%	11,5%	9,1%
Electric installation	2%	10%	6,0%	4,8%
Building	3%	18%	10,5%	8,3%
Terrain improvement	2%	5%	3,5%	2,8%
Installation services	8%	20%	14,0%	11,1%
Terrain purchase	1%	2%	1,5%	1,2%
Indirect cost				
Engineering and supervision	4%	21%	12,5%	9,9%
Construction cost	4%	16%	10,0%	7,9%
Contractor fee	2%	6%	4,0%	3,2%
Contingencies	5%	15%	10,0%	7,9%
Total			126,0%	100,0%

The assumed percentages were normalized to still have a 100% basis. So, with the information obtained from **Table 6.4** and **Table 6.3**, knowing that 21,8% of the budget will be the CAPEX (17430,40 Euros) then the budget will be presented.

6.2.1. Direct costs

For the direct costs of the budget that account for the purchase, installation, construction of the structure and the needs for the process establishment the obtained data is presented in **Table 6.5**:

***Table 6.5**: Direct cost allocation. Source: Own elaboration.*

Allocation	Accounts for	Total cost	
Purchase of equipment	Searching, dealing and final purchase of equipment	17430,4	Euros
Equipment installation	Cementation, platforms and construction operations	6338,33	Euros
Instrumentation and control	Purchase, installation, calibration of systems and software	3169,16	Euros
Piping	Materials, valves, paint and coating	7289,08	Euros
Electric installation	Electric equipment, cables and lighting	3803,00	Euros
Building	Process building, maintenance and utility building	6655,24	Euros
Terrain improvement	Access, roads, cleaning and terrain operations	2218,41	Euros
Installation services	Fire prevention equipment and waste management	8873,66	Euros
Terrain purchase	Purchase of the desired terrain and needed documentation	950,75	Euros
Total direct cost (Euros)		56728,03	

6.2.2. Indirect costs

The indirect costs of the process account for the engineering work, supervision, contingencies and the fee to the contractor's work. The values are covered in **Table 6.6**:

***Table 6.6:** Indirect cost allocation. Source: Own elaboration.*

Allocation	Accounts for	Total cost	
Engineering and supervision	Engineering consulting and supervision of the work	7922,91	Euros
Construction cost	Temporary construction work, documentation and taxes	6338,33	Euros
Contractor fee	Payment for the contractor	2535,33	Euros
Contingencies	Estimation errors, changes of legislation or unknown problems	6338,33	Euros
Total indirect cost (Euros)		23134,89	

6.2.3. Cash-flow and total budget

The amount of direct and indirect costs for the process, accounting the information from **Table 6.5** and **Table 6.6**, it's of 79862,92 Euros (**Seventy-nine thousand eight hundred sixty-two euros and ninety-two cents**).

The cash-flow allocation accounts for the typical purchase and sell operations made in the process for the payment of chemicals, maintenance, salaries and so on. Taking into account the information from [39], that usually is estimated that the cash-flow is of 20% of the total budget, so 80% of the total budget will be of direct and indirect costs as fixed costs, the needed cash-flow will be of 19965,73 Euros (**Nineteen thousand nine hundred sixty-five euros and seventy-three cents**).

Making the direct addition of the two values, the total estimated budget for this process will be of 99828,65 Euros (**Ninety-nine thousand eight hundred twenty-eight euros and sixty-five cents**).

7. Environmental study

This environmental impact study has as objective to evaluate the possible effects and impacts on the environment of the proposed process for the PET biodegradation that can be directly related with the normal operation of the plant. These effects are directly related with the execution, operation and dismantling of the process and some corrective measures and important points will be evaluated.

A brief analysis of the main points will be done as the scope of this project is to evaluate in a theorical way the viability and possibility of this process in a small scale, looking forward to more improvements in the operation that can make it available and suitable for higher quantities of PET. The legal framework of this study will be the Spanish Law 21/201, of December 9th, on environmental assessment that establish the bases that define the assessment of this study to ensure a high level of environmental protection and sustainable development.

The carriage of this study will be done following as mandatory as is stated in the Annex I, Group 5, 2º group, iii) because of the presence of bases as sodium hydroxide. The project is classified then in the Chemical, petrochemical, textile and paper industries group [40]

7.1. Impact identification

7.1.1. Construction phase

The construction phase represents the construction of the plant, with the operations of assembly, structures, equipment and also machinery with the needed start tests.

During this phase the main impacts that can be identified are the derived directly from the site preparation, installation of the equipment, traffic created by the handling of material and equipment and soil movement.

The main impacts that can be allocated during this phase are related with the emissions of greenhouse gases by the machinery and the erosion of the soil due to land movement, levelling of the place and the noise raise due to construction activity.

The positive effect can be related with the economic impact of the work, that will generate jobs for construction personnel.

7.1.2. Exploitation phase

The exploitation phase represents the industrial activity of the biodegradation of PET. For the proposed process, the main impacts can be directly associated with chemical and utilities consumption and the generation of carbon dioxide by the biodegradation of the PET.

About the electric consumption, the main electric consumption will be driven by the heat up of the water mixture before the entrance in the reactor. There is no expected high consumption of water and energy. The main water consumption will be to prepare the growth media for the bioreactor. It's expected that for a whole year more or less 20 m^3 will be expected, which is not a big consumption.

The usage of some chemicals as glutaraldehyde and sodium hydroxide will represent also an impact as the pH will be really high in some equipment and the related hazards for the chemicals. Also has to be taken into account the production of CO_2 by the biological reaction.

The impacts that are derived from the use of basic mixtures will not be accounted as it's expected to use some neutralizer (as HCl) in the sedimentation tank to neutralize the effect of the sodium hydroxide.

7.1.3. Dismantling phase

The dismantling phase represent the closure of the plant due to the end of its useful life. The impacts that can be accounted are very similar to the ones accounted in the construction phase. The highest difference will be the presence of a huge amount of solid waste in this phase due to the dismantling of the equipment, that some of them can be maybe classified as hazardous materials. Washing water and wastewater will be increased due to the cleaning operations and the soil that was used will start to recover but will need a very high time.

7.2. Cause-effect matrix

From this initial analysis, the method to make the estimation on the different severity of the impacts with the cause-effect matrix. The cause-effect matrix will account for the intensity and the probability of the impact. The obtained matrixes are **Table 7.2**, **Table 7.3** and **Table 7.4**:

***Table 7.1**: Legend of cause-effect matrix. Source: Own elaboration.*

	Positive impact
	Low impact
	Medium impact
	High impact
	Critical impact

***Table 7.2**: Cause-effect matrix for construction phase. Source: Own elaboration.*

	Land preparation	**Machinery operation and traffic**	**Construction operations**	**Waste generation**
Climate				
Air Quality				
Flora and Fauna				
Soil				
Hydrology				
Noise and Vibrations				
Aesthetic-cultural factor				
Socioeconomic factor				

Table 7.3: *Cause-effect matrix for exploitation phase. Source: Own elaboration.*

	Process operation	Resources consumption (Water, electricity…)	Maintenance and repair	Waste generation
Climate				
Air Quality				
Flora and Fauna				
Soil				
Hydrology				
Noise and Vibrations				
Aesthetic- cultural factor				
Socioeconomic factor				

Table 7.4*: Cause-effect matrix for dismantling phase. Source: Own elaboration.*

	Plant demolition	Machinery operation and traffic	Waste generation
Climate			
Air Quality			
Flora and Fauna			
Soil			
Hydrology			
Noise and Vibrations			
Cultural factor			
Socioeconomic factor			

7.3. Related CO2 generation of process

One important measurement in terms of environmental impact is the kg of carbon dioxide that can be generated by the operation. In the case of this project, the main actions that can be directly related to carbon dioxide producing due to the process directly are:

- Energy consumption of equipment
- Carbon dioxide generation through bioreaction

So, taking into account that [41] accounts for a factor of 0,3 kg CO_2/kWh for electric consumption in non-household and the carbon dioxide generated through biochemistry is directly obtained by the mass balance the obtained results are in **Table 7.5**:

***Table 7.5**: Carbon dioxide generation by main operations of the process. Source: Own elaboration.*

Equipment	kWh/cycle	kWh/year	kg CO2 per cycle	kg CO2 per year
Shredder S-101	5,36	42,86	1,61	12,86
Reactor R-201 heating	8,14	65,14	2,44	19,54
Heat exchanger E-101	47,58	380,60	14,27	114,18
Reactor R-201 CO_2 generated	-	-	262,69	2101,52
Total	61,08	488,60	281,01	2248,10

So, this project is estimated that will generate 2,25 tons of carbon dioxide per year. This consumption can be expected as low comparing to chemical process but can be also related to the small size of the plant proposed. Taking into account the capacity of the plant, the direct generation of carbon dioxide per product processed is of **2,25 kg CO_2/kg PET degraded**.

7.4. Impact prevention and mitigation measures of the most important points

As the main environmental related impacts are accounted and evaluated, some mitigation measures and also preventions that should be taken into account to avoid the most critical points of the proposed plant and process.

The mitigation and prevention techniques per phase are the following:

- Construction phase: The mitigation and prevention techniques for this phase should be focused in the correct maintenance of the machinery that is present, limit the operations that can generate dust, manage waste generated in a proper way, minimize the operating time of machinery that can be considered as very noisy and finally use visual barriers to minimize the visual impact during the construction operations.

- Exploitation phase: The mitigation and prevention techniques for this phase should be focused in the proper analyze and determine the state of the waste streams that are generated, determine properly the hydrochloric acid dose needed to neutralize the water effluent and not allow the discharge without neutralize, correct management of waste generated, limitation of speed of vehicles inside the plant and direct compliance with regulation on the emission levels generated.

- Dismantling phase: The mitigation and prevention techniques for this phase should be focused in the same type than for construction phase. Correct waste management, moderation of operations that rise dust, minimize the operating time of high noise levels and also help the characteristics of the land that were prior to the construction of the plant.

8. Safety study

In this section, a brief safety analysis will be performed to have a general idea and some precautions about the use of some chemicals among a reactivity analysis made with the software ''*Chemical Reactivity Worksheet (CRW)*'' developed by AIChe. The data for the safety sheets will be provided at the annex.

First of all, the hazard level of the substances that are present in the process that was proposed will be evaluated using the recognized system known as REACH. REACH system accounts for *Registration, Evaluation, Authorisation and Restriction of Chemicals* following the legal framework stablished by the European Union in the Directive EC/1907/2006.

Other of the main parts for the specification of the different hazards is the Directive EC/1272/2008 that is known as the CLP framework, that accounts for *Classification, Labelling and Packaging* of chemical substances and establish a common framework with the global harmonized system (GHS) and the European system. The pictograms and the hazard phrases will follow this legal framework. All the information that could be needed will be directly referenced to the safety data sheet with REACH approval that was used for each chemical.

First of all, the pictograms that are present in the substances of this process will be presented in **Table 8.1**:

***Table 8.1**: Pictogram information. Source: European Chemical Agency (ECHA). Available at: https://echa.europa.eu/es/regulations/clp/clp-pictograms*

Pictogram	Category
	GHS02 - Flammable
	GHS03 - Oxidizing warning / Comburent
	GHS05 - Corrosive

	GHS07 - Irritant
	GHS08 - Systemic health hazard
	GHS09 – Environmental warning

Table 8.2 will present the hazard phrases that were present in the different chemicals that are involved in the process:

***Table 8.2**: Hazard phrases information. Information obtained from REACH safety sheets*

Name tag	Hazard associated
H225	Highly flammable liquid and vapor
H272	May intensify fire; oxidizer
H290	May be corrosive to metals
H302	Harmful if swallowed
H314	Causes severe skin burns and eye damage
H315	Causes skin irritation
H317	May cause allergic skin reaction
H318	Causes serious eye damage
H319	Causes serious eye irritation
H332	Harmful if inhaled
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled

H335	May cause respiratory irritation
H410	Very toxic to aquatic life with long lasting effects

Once this is established, the information obtained from each chemical safety data sheet is presented in **Table 8.3**:

***Table 8.3**: Hazard analysis of the process chemicals. Source: Own elaboration, information obtained from REACH safety sheets*

Substance	Pictogram	Hazard phrases
Sodium hydroxide		H290, H314, H318
Glutaraldehyde		H302+ H332, H314, H317, H334, H335, H410
Ethanol		H225, H319
Dihydrogen monobasic phosphate	N.C	N.C
Sodium nitrate		H272, H319
Magnesium sulphate heptahydrate	N.C	N.C
Potassium chloride	N.C	N.C
Iron (II) sulphate heptahydrate		H302, H315, H319

Ammonium chloride		H302, H319
Hydrochloric acid		H290, H315, H319, H335

N.C: Not classified

For the interaction between the different chemicals that are present a reactivity analysis was made using the chemicals that are present in the CRW. Using these chemicals, the following results are obtained:

Y : Compatible
N : Incompatible
C : Caution
SR: Self-Reactive
*: Changed by user

NFPA Health	NFPA Flammability	NFPA Instability	NFPA Special	CRW PET Compatibility Chart	AMMONIUM CHLORIDE	ETHANOL	FERROUS SULFATE	GLUTARALDEHYDE SOLUTION	HYDROCHLORIC ACID, SOLUTION	POTASSIUM CHLORIDE	SODIUM HYDROXIDE, SOLID	SODIUM NITRATE	WATER				
				AMMONIUM CHLORIDE													
2	3	0		ETHANOL	Y												
				FERROUS SULFATE	Y	N											
				GLUTARALDEHYDE SOLUTION	N	C	N	SR									
3	0	1		HYDROCHLORIC ACID, SOLUTION	C	C	N	N									
				POTASSIUM CHLORIDE	Y	Y	N	C	N								
3	0	1		SODIUM HYDROXIDE, SOLID	N	N	Y	N	N	Y							
				SODIUM NITRATE	N	N	N	N	N	C	Y						
				WATER	C	Y	N	C	C	Y	C	Y					

***Figure 8.1**: Chemical reactivity analysis for the process chemicals. Source: CRW.*

As can be seen in **Figure 8.1**, some critical interactions can be present in the process if the different chemicals are not handled in a proper way. On the other hand, no critical interactions are expected during the normal development of the operation because the concentrations of chemicals are very small and they are very diluted in the water media and the different mixtures that are present during the normal operation conditions. Nonetheless, some precautions should be taken into account for the handling of the chemicals and to avoid some non-desired problems:

- The mixture of glutaraldehyde and ethanol has to be taken with extreme caution as the interaction between these two chemicals can generate some undesired products if they are mixed without care and heat release is expected.
- For the water and growth media, the introduction of water and ferrous sulphate should be introduced with care as the interaction is undesired. Nonetheless, the concentration of this chemical (0,01 g/L) is very low, so no real problems should be expected.
- Some of the growth media components can release heat when interact with water, which should be taken into account.
- No direct interaction between the growth media pure components and auxiliary chemicals for the PET biodegradation should be allowed to avoid any undesired generation of heat or chemical products.
- The handling of the glutaraldehyde should be careful as it's the most dangerous chemical in the process and also the storage of this chemical can lead to self-reactive scenarios if it's not stored in optimal conditions. A further analysis, special storage and periodic check of the quality is recommended for this specific chemical.

Conclusions

By the finishing of this study and the development of the different methodologies to evaluate the results and information obtained, some conclusions can be extracted:

- In terms of the state of art, the bioremediation strategies appear as a very interesting and promising alternatives that are being pushed by the authorities for the further improvement in the waste management and the reduce of waste generation. Among the different options that are available for the plastics, some of them that are very crystalline or toxic can be easily handled by the bacteria to non-hazardous components and mineralization products.
- For the different options that are available to bioremediate the plastics in terms of marine bacteria, the overall characteristics of the marine bacteria are very interesting and beneficious for the further operation and scaling up. Nonetheless, it's very specific the usage that can be done of the different strains. Also, the mechanism that is driven it's not fully developed and understood, which makes it a non-suitable already alternative for bigger scales.
- For the selected strain (*Vibrio sp* Strain PD6*)*, there is a lot of missing information that had to be assumed and in general for these studies, the lack of information it's very difficult to model properly the operation. On the other hand, it's very promising that exists strains of marine bacteria that are able to reduce in a good quantity the amount of plastic with a non-strict operation. As could be seen by the modelling, the needs of the process proposed are very reasonable and the estimated operation it's very promising for further studies and developments on the genetic engineering for marine bacteria strains. The main drawback will be the elevated time of operation and that the plastic structure it's still present.
- For the proposed scheme and the analysis, also the economy, environmental and safety analysis provide a good view of what would be a real scale small process. The results are very interesting as it seems to be really scalable if the marine bacteria strain performance can be improved in a sufficient way, but as was commented more research and development is need as the amount of plastic that can be degraded nowadays is very poor to the needs of the actual society.

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Annex

This annex will cover the specific additional bibliography and information that can be needed for the safety sheets, equipment cost and chemical cost consultation.

Chemical prices:

- Sodium hydroxide. Reference: *S5881*. Available at: https://www.sigmaaldrich.com/ES/es/substance/sodiumhydroxide40001310732
- Glutaraldehyde. Reference: *340855*. Available at: https://www.sigmaaldrich.com/ES/es/product/sial/340855
- Ethanol. Reference: *493546*. Available at: https://www.sigmaaldrich.com/ES/es/search/ethanol?focus=products&page=1&perpage=30&sort=relevance&term=ethanol&type=product
- Dihydrogen monobasic phosphate. Reference: *P5655*. Available at: https://www.sigmaaldrich.com/ES/es/search/kh2po4?focus=products&page=1&perpage=30&sort=relevance&term=kh2po4&type=product
- Sodium nitrate. Reference: *S5022*. Available at: https://www.sigmaaldrich.com/ES/es/search/sodium-nitrate?focus=products&page=1&perpage=30&sort=relevance&term=sodium%20nitrate&type=product
- Magnesium sulphate heptahydrate. Reference: *230391*. Available at: https://www.sigmaaldrich.com/ES/es/search/magnesium-sulfate-heptahydrate?focus=products&page=1&perpage=30&sort=relevance&term=magnesium%20sulfate%20heptahydrate&type=product
- Potassium chloride. Reference: *P9333*. Available at: https://www.sigmaaldrich.com/ES/es/search/potasium-chloride?focus=products&page=1&perpage=30&sort=relevance&term=potasium%20chloride&type=product
- Iron sulphate heptahydrate. Reference: *F8633*. Available at: https://www.sigmaaldrich.com/ES/es/search/iron(ii)-sulfate-heptahydrate?focus=products&page=1&perpage=30&sort=relevance&term=iron%28ii%29%20sulfate%20heptahydrate&type=product
- Ammonium chloride. Reference: *A9434*. Available at: https://www.sigmaaldrich.com/ES/es/search/ammonium-chloride?focus=products&page=1&perpage=30&sort=relevance&term=ammonium%20chloride&type=product

Equipment cost:

- Vessel V-101 and reactor R-201. *Factory manufacturer stainless steel reactor tank/reaction kettle/jacketed chemical vessel*. Available at: https://www.alibaba.com/product-detail/Stainless-Steel-VesselFactoryManufacturerStainless_1600229783657.html?spm=a2700.7724857.normal_offer.d_title.6c071264rMjZbG&s=p

- Vessel V-102. *20L~500L customized stainless steel storage tank / vessel*. Available at: https://www.alibaba.com/product-detail/20L-500L-customized-stainless-steel-storage_62490343444.html?spm=a2700.7724857.normal_offer.d_title.651f1628tPyTKA
- Screener F-301. *Efficient rotary mechanical bar screen for municipal wastewater treatment plant*. Available at: https://www.alibaba.com/product-detail/efficient-Rotary-mechanical-bar-screen-for_60645450156.html?spm=a2700.galleryofferlist.normal_offer.d_title.54492517NdIt8q&s=p
- Sedimentation vessel V-301. *High Efficiency Sedimentation Tank Machine Tank Sedimentation*. Available at: https://www.alibaba.com/product-detail/Sedimentation-Tank-High-Efficiency-Sedimentation-Tank_1600462164931.html?spm=a2700.7724857.normal_offer.d_title.58cb5025Nzlm82&s=p

REACH safety sheets:

- Sodium hydroxide. Available at: https://www.fishersci.co.uk/store/msds?partNumber=10234590&productDescription=10KG+SODIUM+HYDROXIDE+98%2B%25+FOR+ANALYSIS&countryCode=GB&language=en
- Glutaraldehyde. Available at: https://www.sigmaaldrich.com/ES/en/sds/sial/g5882
- Ethanol. Available at: https://www.carlroth.com/medias/SDB-9065-AU-EN.pdf?context=bWFzdGVyfHNlY3VyaXR5RGF0YXNoZWV0c3wyNjA5NjF8YXBwbGljYXRpb24vcGRmfHNlY3VyaXR5RGF0YXNoZWV0cy9oNjYvaDI5LzkwMjc4MDM2NDM5MzQucGRmfDEyMTNkODhhY2U3MzIwZTc2MGYyZjc0YTg2N2NlM2IyOWIyNjdiMDk0YTY3MTExNGU2MzdlNmVlZDJiNDU5M2Y
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- Iron sulphate heptahydrate. Available at: https://www.carlroth.com/medias/SDB-P015-AU-EN.pdf?context=bWFzdGVyfHNlY3VyaXR5RGF0YXNoZWV0c3wyMzIyODZ8YXBwbGljYXRpb24vcGRmfHNlY3VyaXR5RGF0YXNoZWV0cy9oYTIvaDRlLzg5Njk3MzMzMDg0NDYucGRmfGZkN2RhZmI1NWRiNDY1ZTM4NDFkZmE4YmM0OWRlZmExOGJkZDIxY2M1ZGY0OWRmNTY5ODhlMDE3MDExNjg3YzM

- Ammonium chloride. Available at: https://www.carlroth.com/medias/SDB-5470-MT-EN.pdf?context=bWFzdGVyfHNlY3VyaXR5RGF0YXNoZWV0c3wyNDcwODZ8YXBwbGljYXRpb24vcGRmfHNlY3VyaXR5RGF0YXNoZWV0cy9oOGQvaDU5LzkwNTU1MTIwNjgxMjYucGRmfDcwMzc1NzgyOWU3N2ZjMzFlMTQ4YjQxNzk3MmEwZjU5MGFkNGNmNDdlNGExNDIwMWU2MDg0ZTY3NDhmM2Y2YTA