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FACULTY OF CHEMISTRY

DISSERTATION THESIS

Master: CHEMISTRY OF ADVANCED MATERIALS

Student: FTODIEV ANDREEA-IULIANA

Coordinator: Conf. Dr. MĂDĂLINA SĂNDULESCU

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UNIVERSITATEA DIN BUCUREȘTI

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FACULTY OF CHEMISTRY

DEPARTMENT OF ORGANIC CHEMISTRY, BIOCHEMISTRY AND CATALYSYS

Valorization of plastic polymers leading to value-added products

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Summary

Introduction

The production of plastic dates back to 1950' and since then they have become one of vital materials used in everyday life applications. The variety of plastics available and the cheap production of those are the main reasons of the grow of plastic industry [1]. Although the plastic industry consumed 6% of world oil production in 2014, from the 26% used in packaging applications, only 5% was recycled causing a huge economic loss. By 2050 the plastic industry is estimated to consume 20% of the world oil production [1].

The plastic wastes accumulation has been growing in the recent years and it became one of the most severe environmental and social issue [2]. It is estimated that between 2010 and 2025 100 MT of plastic waste will enter in the ocean [1]. It is predicted that by 2025 for each three tons of fish, there will be one tone of plastic in the ocean [3]. This will cause widespread contamination of marine ecosystems since the microplastics (smaller fragments of plastics which was degraded) can be ingested by zooplankton and phytoplankton which will have a negative impact on their health. Since approximatevely 70% of the world's oxygen is produced from the photosynthesizing of marine plants, the plastic will have a huge impact in climate change and global warming [1].

Since the plastics with polymer structures are especially designed to mentain optimal material properties, most of the plastics can not be attacked by microorganisms. The evolution could not develop enzymes to degrade these man made materials and therefore usually the plastics do not rot in the biological environment [4].

With the discovery of Tokiwa and Suzuki in 1977 of some lipases that are able to attack the ester bonds in some aliphatic polyesters and can depolymerize such materials, more attempts were made to design similar enzymes [4]. Also with the increasing problem of plastic waste, the pursuing of making biodegradable plastics started [4].

Theoretical part

1. Synthetic plastics – general consideration

The term ''plastics'' generally refers to synthetic polymers that are omnipresent in modern society. Plastics are that common in our everyday life, that it is estimated each person consumes 50 kg per year and European Union and 68 kg per year in the United States [5].

Plastics find applications in a different domains such as packaging, biomedical devices, clothing and sport equipment, electronic components [5].

Unfortunately, the main problems of them are that they are obtained from the nonrenewable sources of petroleum/natural gas and the deposition rate accelerated past the rate of production [5][6].

The global production and consumption of plastics increased at an alarming rate over the last few decades accumulating persistent in the landfills and the environment, only 9% of plastic waste being successfully recycled in 2015 in the United States [5].

2. Types of synthetic plastics

Synthetic plastics like polystyrenes (PS), polyethylene (PE), polyurethane (PUR), polypropylene (PP), polyvynyl chloride (PVC) and polyethylene teraphtahlate (PET) have a very important role to almost every aspect of our lives [2].

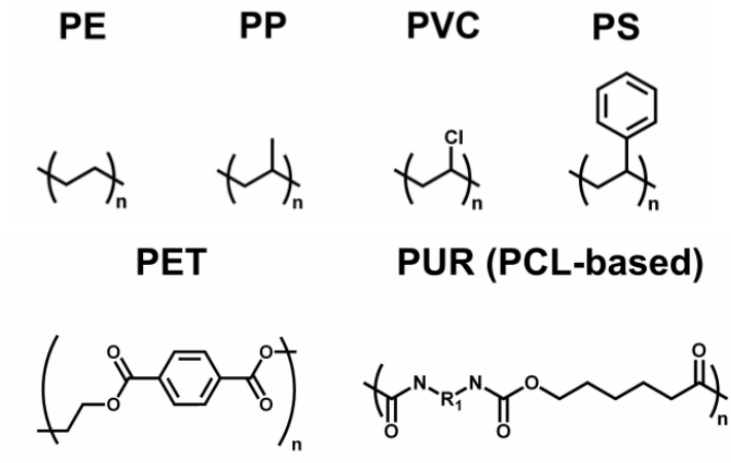


Figure 1. The main types of synthetic plastics [2]

2.1 Polystyrene (PS)

Polystyrene is the simplest aromatic hydrocarbon polymer based on the monomer styrene having a hard texture, high tensile strength and excellent transparency [7][8]. Since it can be monoextruded, coextruded with other types of plastics, injection molded or foamed, it can generate a large range of products [7]. Polystyrene is generally used in the food industry having applications like protective packaging (for eggs, meat, fish etc.) and disposable plastic silverware (lids, plates, bottles, cups etc.) [7][8]. It is one of the most widely used plastics, mostly because it is an inexpensive resin per unit weight [9].

PS is a clear, odorless, hard, tasteless, colorless material with outstanding properties including thermal stability, mechanical strength, relatively low density and low production cost. The particular reason for its highly stable structure and resistance to decomposition is its structure of phenyl groups and single C-C bonds [10].

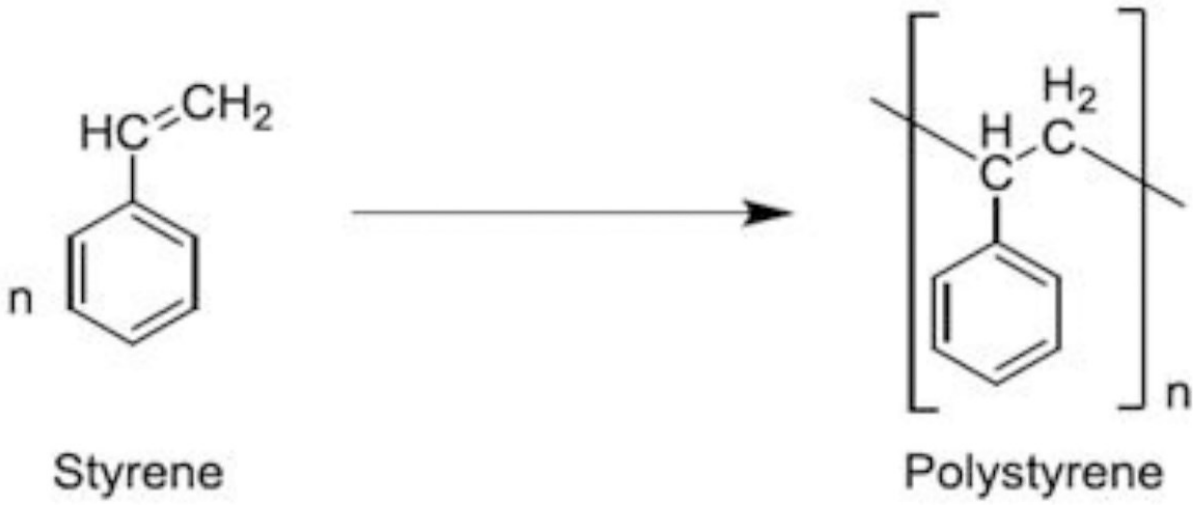


Figure 2. Polymerization of styrene to polystyrene [10]

Naturally polystyrene is transparent, but it can be coloured with different colourants. Being a thermoplastic polymer, polystyrene is in solid state at room temperature, but above 100°C it starts to flow [11].

Polystyrene is rather chemically inert being waterproof and resistant to many acids and bases, this being one of the reasons of its popularity in fabricating many objects of commerce. But it can be easily attacked by organic solvents like acetone, aromatic hydrocarbon solvents and chlorinated solvents [11].

Polystyrene can be used in either solid and expanded forms, both of which can be recycled. Solid PS such as coffee cups, trays etc. can be recycled and transformed into office equipements, videocassette cases etc [12].

PS is manufactured in three main commercial forms: expanded polystyrene (EPS), general purpose polystyrene (GPPS) and high-impact polystyrene (HIPS). There is a fourth type of fabricated PS with the name of syndiotatic polystyrene (SPS), but it has a relatively minor grade [10].

2.2 Polypropylene (PP)

Polypropylene is one of the most popular plastics because of its low density and excellent chemical resistance. It can be processed through many converting methods including injection molding and extrusion [13].

Polypropylene is an olefin polymer, thermoplastic with a low melting poing. PP fiber is the fourth largest volume artificial fiber, the 2014 worldwide production value being US$56.73 million. PP's major use is in industrial applications like geotextiles, ropes, carpets, surgical sutures and sanitary products [14].

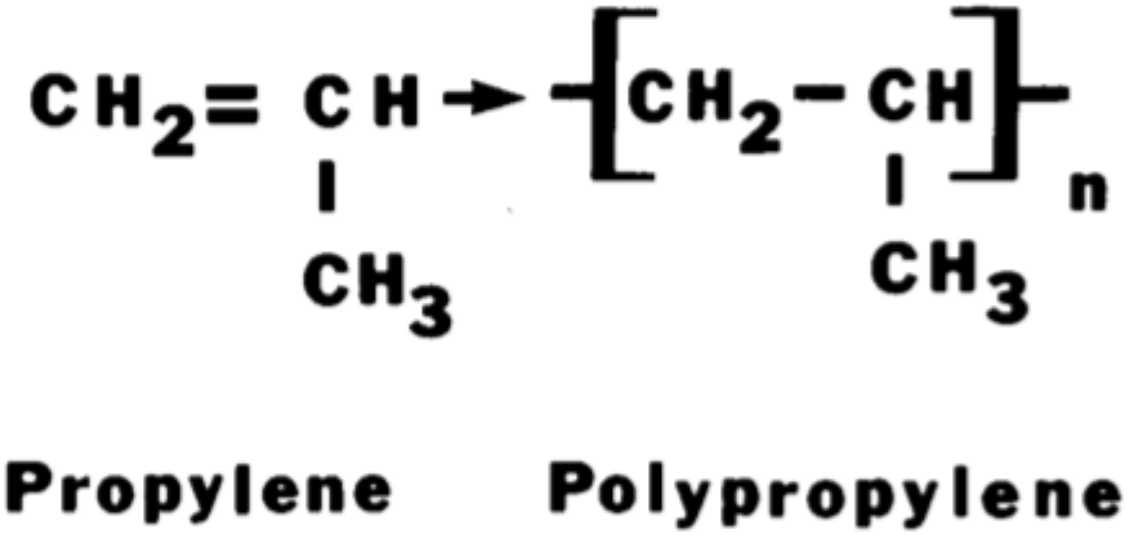


Figure 3. Polymerization of propylene to polypropylene [15]

Polypropylene is a transparent, free-color material and it is produced through a process of monomer connection called addition polymerization [13]. PS is formed from propylene, a liquefiable hydrocarbon gas [15].

Three stereoisometic forms of PP exist: isotactic, syndiotactic and atactic. Isotactic PP has methyl groups on one side of the polymer chain, syndiotactic PP has methyl groups that alternate along the chain and atactic PP has methyl groups arranged randomly. Between all three types of PP, only the isotactic one has commercial importance [15].

Its high temperature resistantace makes polypropylene suitable for items such as funnels, bottles, trays, pails and jars that have to be sterilized frequently [13]. The melting poing of PP is around 165°C, but this can vary with the degree of chemical and steric purity [15].

2.3 Polyethylene (PE)

Polyethylene is the most common plastic in use today having the simplest molecular structure of any polymer [16][17]. It is primarily used for packaging and it is the largest tonnage plastic material [16][17]. Polyehylene represents 34% of the total plastic produced annually with over 100 million tonnes [16].

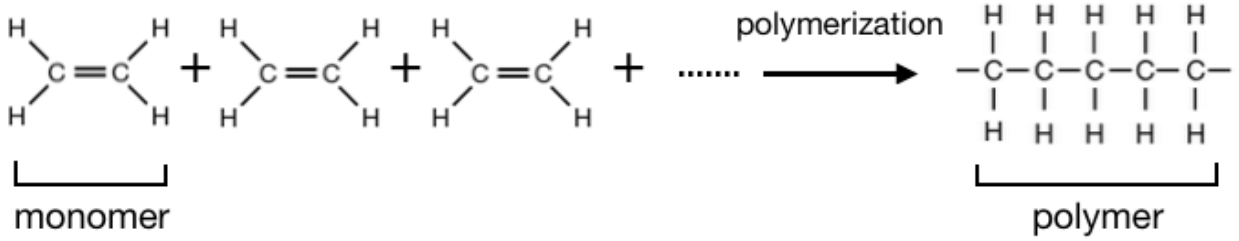


Figure 4. Polymerization of ethylene to polyethylene [17]

PE is an inert material and it is very difficult to degrade in the environment. In a study made with a polyethylene sheet, only after 12-32 years it showed partial degradation and negligible weight loss being kept in a moist soil. The particular reason for this circumstance is the water insolubility, the hydrophobicity due to high molecular weight, presence of linear backbone of carbon atoms and its degree of crystallinity [17].

PE is a mixture of similar polymers of ethylene. It can be low density or high density, depending on the pressure and temperature applied when manufacured: the low density PE (LDPE) is prepared at high pressure and high temperature and high density PE (HDPE) is prepared at low pressure and low temperature [16].

LDPE is inert at room temperature, but it can be attacked by strong oxiding agents and some solvents. LDPE is characterized by tear strength, tensile strength, opacity, rigidity and

chemical resistance due to its degree of crystallinity within the range of 50-60%. It is generally used coating on paper, textiles, mulching agricultural fields and constructing polyhouse [17].

HDPE is produced during a catalytic process and has little branching. It provides stronger intermolecular forces and greater tensile strength than LDPE. It is widely used industrial and day-to-day applications like milk jugs, carry bags, margarine tubs, detergent bottles, water pipes etc. due to its opacity, hardness and durability at higher temperatures [17].

2.4 Polyurethane (PUR)

Polyurethane is a very versatile polymer having the structure property relationship of diisocyanates and polyols providing ample customization to the manufacture [18].

Polyurethane is formed by the reaction between di/poly isocyanate and a diol or polyol, creating repeated urethane linkage in the presence of chain extender and other additives [18].

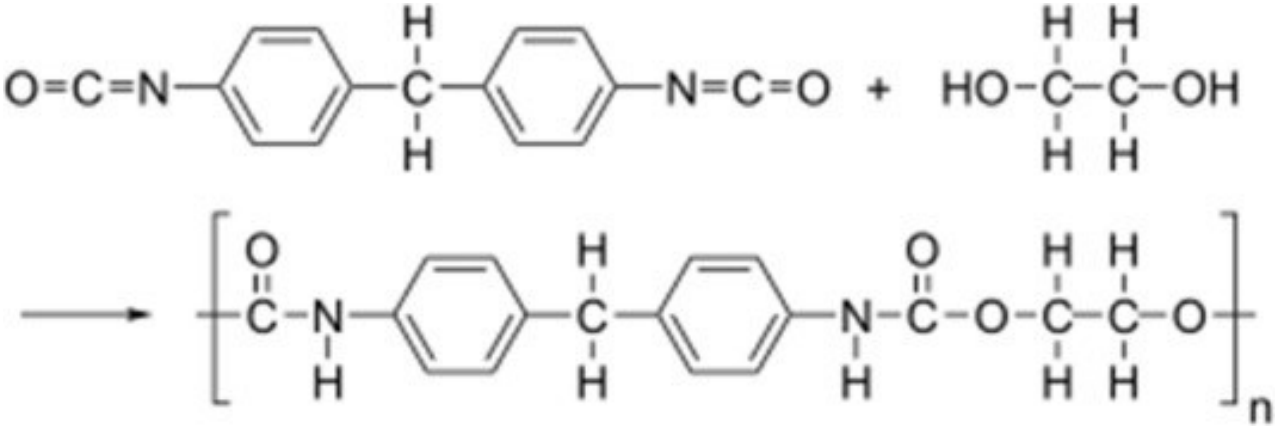


Figure 5. Polyurethane formation [18]

The properties of polyurethane are diverse, these can range from soft touch coatings to very hard rigid material used in construction. PUR has attracted not only the scientific community, but also the industries due to its properties and ease of tailoring [18].

PUR has many different applications in different domains. Due to the advances in different techniques of modern times, manufactures are able to produce this polymer in a wide range of polyurethane apparel. Some applications of PUR in this department are: manmade skin and leathers, sports clothes, and a variety of accessories [19].

PUR find other application in major appliances like rigid foams for refrugerator and freezer thermal insulation systems, in household materials such as flexible foam padding cushions, floors, in modern material science like composite woods [19].

2.5 Polyvinyl chloride (PVC)

Polyvinyl chloride is a widely used polymer and it is one of the most valuable products of the chemical industry. PVC is produced from its polymer, vinyl chloride, and is a hard plastic that it can be made softer with the help of plasticizers. Over 50% of PVC manufactured is used in construction being inexpensive, hard and easy to assemble [20].

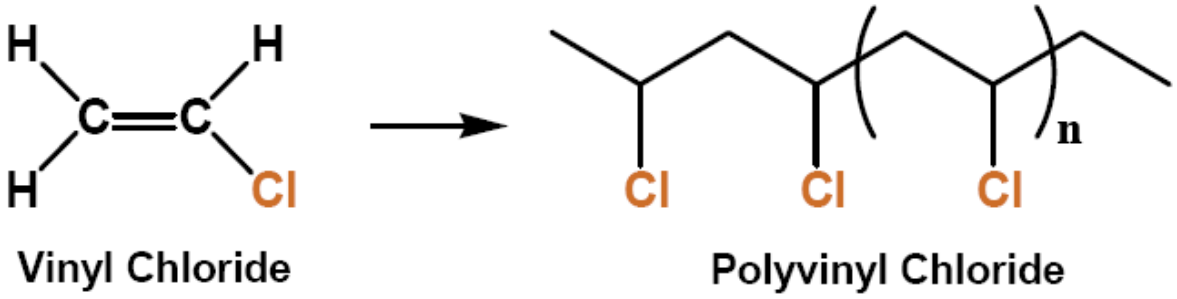


Figure 6. Polymerization of vinyl chloride to polyvinyl chloride [21]

PVC with 57% of mass by chlorine is an 'infrastructure thermoplastic' material. Being thermoplastic, PVC softens when heated and hardens when it cools. Because of this property, PVC can be subjected to different techniques: extrusion, calendering, injection and blow molding [22].

Due to its low density, PVC provides low material cost on a volume basis [22]. Although appearing to be an ideal bulding material, replacing in recent years the traditional building materials as wood and concrete, concerns have been raised about the environmental and human health costs of PVC [20].

2.6 Polyethylene teraphtahlate (PET)

Polyethylene teraphtahlate, commonly referred as polyester or PET, is a semiaromatic polymer synthesized from ehylene glycol and terephtalic acid [23].

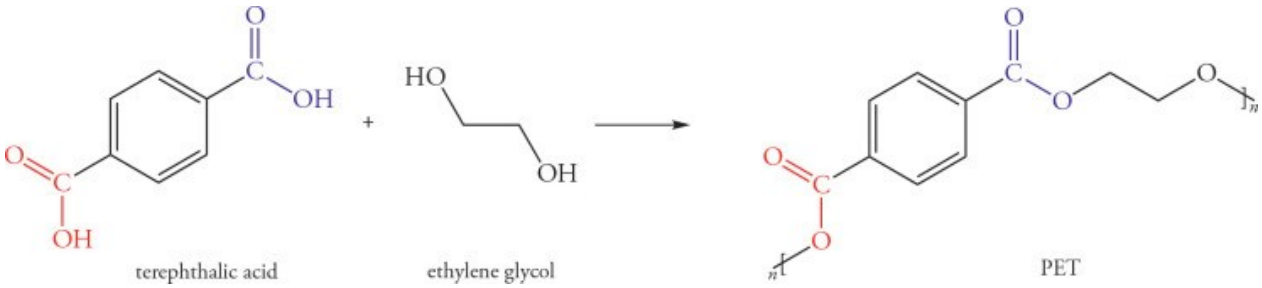


Figure 7. Polymerization of terephthalic acid and ethylene glycol to PET [24]

PET is used in industrial applications due to its excellent moisture and fair oxygen barrier characteristics having a glass transition temperature of around 67-81°C and a melting poing of 260°C [23].

The half life of the polymer at 37°C in a normal saline environment is of 700 years [25]. Due to its very important property to be colourless and transparent (if amourphous) or translucent (if semi-crystalline), the consumers can see the content from the bottles [26].

PET is lightweight (compared to a 750 ml wine bottle, a 1L PET bottle weights 335 less grams), thermoplastics, semi-rigid to rigid, robust and mechanically resistant to impact. It is extremelly inert compared with other plastics and it does not contain plasticizers (in the case of PVC the use of plasticizers is essential), but it can be blended with other polymers to improve certain properties [26].

Because all of these properties, PET is the third most commonly used plastic in the packaging industry with a continuous growing demand [26].

3. Plastic waste

3.1 Methods of disposing plastic waste

The current methods for disposing of plastic wastes mainly include landfilling, producing the same or similar product (primary recycling), mechanical recycling (secondary recycling), chemical recycling (tertiary recycling) and incineration (quaternary recycling) [1][2]. Landfilling is the major method due to its low cost and operability, especially in the developing countries [2].

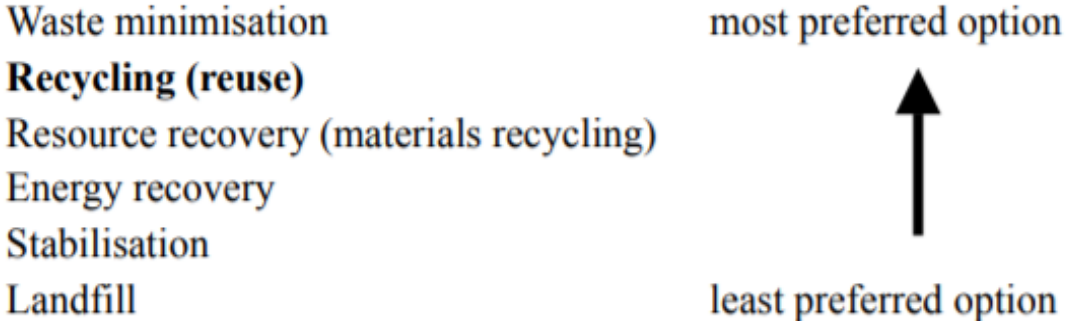


Figure 8. Generally accepted European Community Strategy for dealing with waste [27]

Primary recycling refers to either reusing the material or recycling the plastic to produce products with the original structure. It is a closed loop recycling method and can be only used on high quality plastic [1].

Secondary recycling indicates the conversion of waste plastic into a less demanding product via mechanical tranformations. Mechanical recycling has various advantages over chemical recycling (tertiary recycling): lower global warning potential, less acidification and eutrophication, more renewable energy use and last, but not least, a lower processing cost [1].

Tertiary recycling describes the chemical reaction used to depolymerise and degrade plastic waste into monomers or into other useful materials. There are many chemical recycling methos and these depends on the polymer type and on tehniques used, each of them having advantages and disadvantages. Although mechanical recycling presents some advantages over chemical recycling, the tertiary recycling has also some advantages over the secondary one: the potention of producting circular polymer since recovered monomers can be repolymerised and the opportunity to achieve new materials with added value [1].

Quaternary recycling indicates the energy that is recovered via incineration of low grade plastic waste. When plastics are burned, they result heat energy that is used to generate steam and electricity. The quaternary recycling should only be used as a last solution since the imbedded energy of the polymers molecular structure is lost and harmful chemicals and dioxins are released into the atmosphere [1].

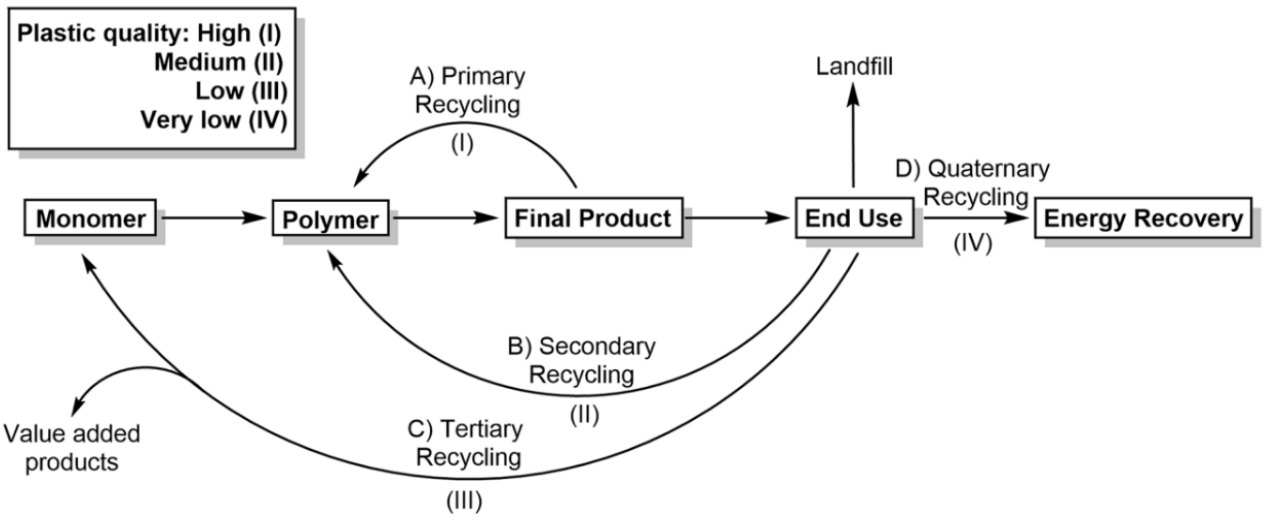


Figure 9. Diferent plastic waste treatment options and associated plastic quality [1]

3.2. Methods of disposing PET wastes

3.2.1 Mechanical recycling

Because of the large amount of PET circulating, its mechanical recycling is well established and the recycled PET has applications in a variety of domains. In the process of mechanical recycling, PET usually suffers the process of yellowing discoloration, a nontoxic procedure. Around 72% of recycled PET found its use in fibre applications. If the PET becomes so low grade that it can not be mechanically recycled anymore, then chemical recycling should be used to recover its monomers [1].

PET can be subjected to mechanical recycled via melt extrusion up to 40 cycles without a significant chance to be observed in its mechanical properties. After this, the melting temperature is dropping due to recuded crystallinity of the polymer. PET is highly stable to any type of solvolysis [1].

3.2.2 Hydrolysis

PET can be hydrolysed into its monomers terephthalic acid (TA) and ethylene glycol (EG) and the conditions are either acidic, alkaline or neutral. Hydrolysis has some disadvantages like high temperature and pressure requirements in addition to long reaction times [1].

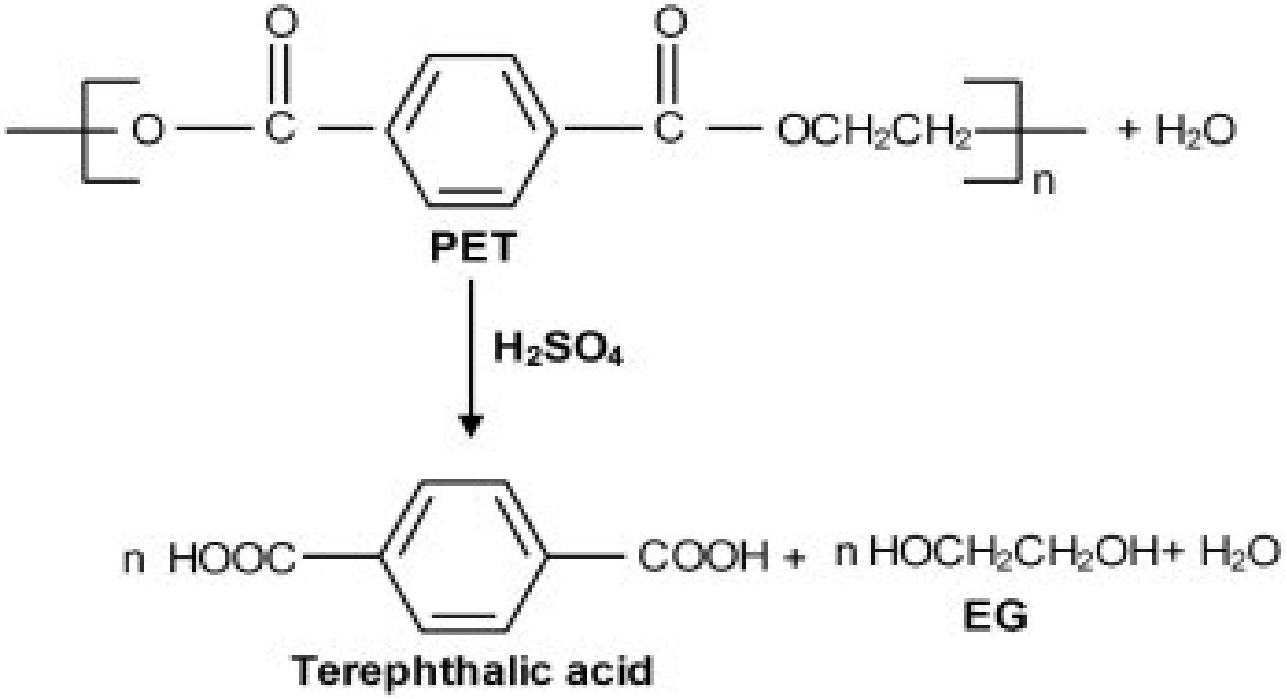


Figure 10. Hydrolysis of PET [28]

Acidic hydrolysis takes place using concentrated acids like phorphoric, nitric or sulfuric acid. The yields obtained from this methods are high, but the main disadvantage is that the

separation of EG from the highly acid solution is difficult. Additionally, the high amount of acid needed to industralize this method poses economic and environmental problems [29].

Alkaline hydrolysis is typically carried out in aqueous solutions of 4–20 wt% NaOH. This process has relatively good yields, but the longer reaction times and high temperatures are some drawbacks of the method [29].

Neutral hydrolysis uses also high temperature and elevated pressures. Without the need for stoichiometric acid or base, this type of hydrolysis would be the ideal one, but this process usually proces low purity monomers and have a slow rate of reaction [29].

3.2.3 Glycolysis

Glycolysis is the most used chemical recycling method for PET and it consists in an insertion of a glycol into the PET chains. The glycol breaks the ester linkages and it replaces with hydroxyl terminals for producing Bis 2-Hydroxyethyl Terephthalate (BHET), oligomers and dimers. For obtaining mostly BHET with a very little amount of oligomers the optimum parameters for glycolysis are: a pressures of 0.1–0.6 Mpa, a temperature range of 180–240 °C, a transesterification catalyst, between 0.5 and 8 h for completion and a high EG/PET ratio [1].

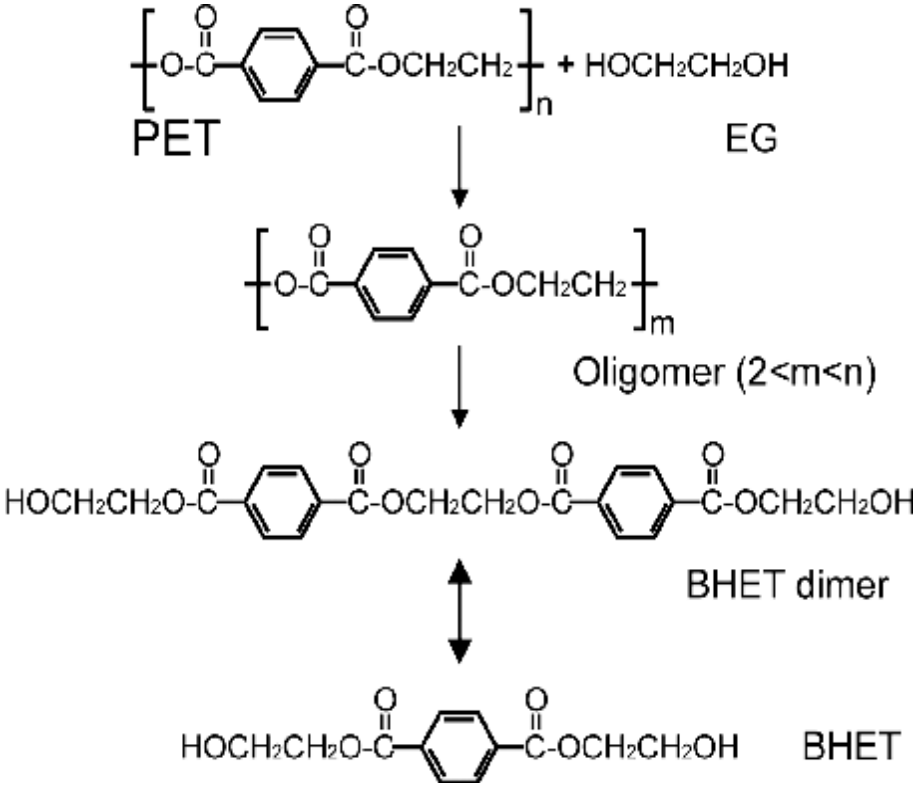


Figure 11. Glycolysis of PET [30]

Glycolysis takes place using a very large variety of glycols such as EG, diethylene glycol (DEG), propylene glycol (PG) and dipropylene glycol. Usually, the catalysts used are metal acetates. The zinc acetate is considered the best among them. Although, these catalysts are often used in industry, they have some disadvantages like: difficulty separating the catalyst from the products, side reactions and product impurities. For more recyclable and simpler purification, ionic liquid catalyst can replace the usual ones [1].

3.2.4 Pyrolysis

There are two major types of pyrolysis of PET: thermal pyrolysis and catalytic pyrolysis [29]. The thermal pyrolisis of PET takes place at high preassure and temperature leading to the formation of a solid char and a volatine fraction. Then, the fraction can also be separated into a condensable hydrocarbon oil and a noncondensable gas. The hydrocarbon oil is composed of a mixture of organic compounds like toluene, styrene, ethylbenzene etc [1].

Catalytic pyrolysis utilizes a catalyst for reducing the temperature and reaction time and thus improving the selectivity and economic viability [29]. Although catalytic pyrolysis has a narrower distribution of hydrocarbon products, it presents a higher market value. Catalysts like silica alumina, ZSM-5 and zeolites are usually used and it was proven that some of them achieved a higher conversion rate of valuable aromatic compounds in the oil compared with the thermal pyrolysis [1].

3.2.5 Alcoholysis

PET can be depolymerised via methanolysis resulting a stoichiometric mixture of its monomers N,N-Dimethyltryptamine (DMT) and ethylene glycol (EG). Methanolysis is carried out at high temperatures and pressures using divalent metal catalysts. The methanolysis also generates several byproducts like Bis(2-Hydroxyethyl) terephthalate (BHET), 2-hydroxyethyl methyl terephthalate (MHET), oligomers, and dimers of DMT and BHET [1].

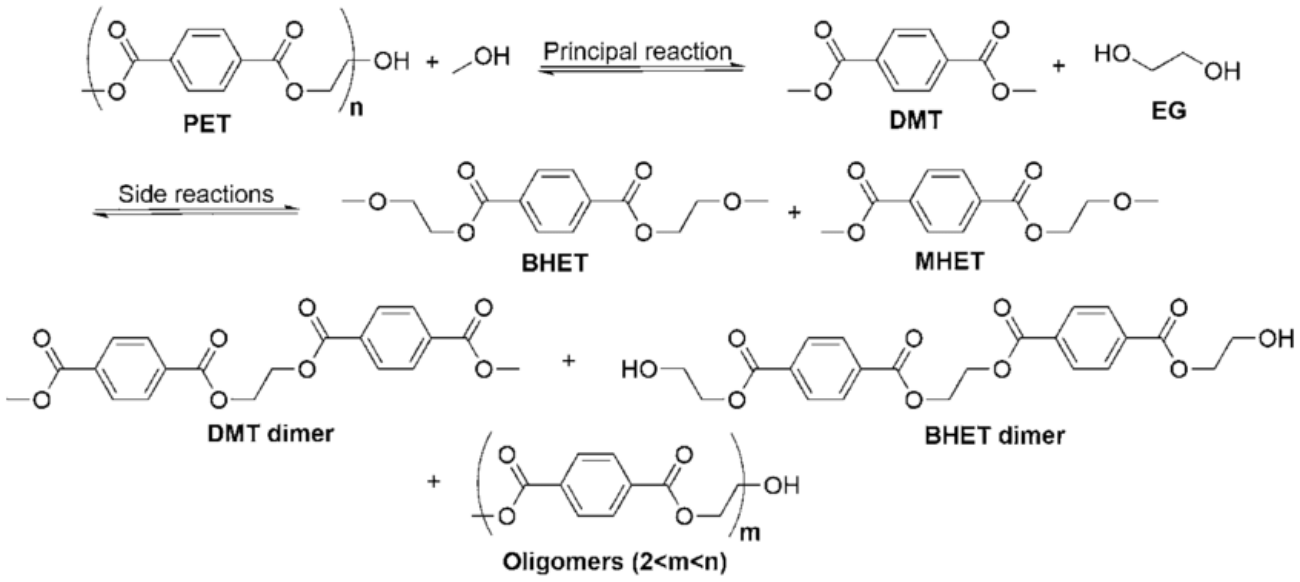


Figure 12. Overall reactions for PET alcoholysis using supercritical methanol [1]

Although in this process the products obtained have high value, it has some major problems. Firstly, it is a costly process because of the separation and refinement of the byproducts produced. Secondly, the major product of this process is DMT and nowadays the majority of PET is synthesis from terephthalic acid (TA). Therefore, an additional conversation of DMT to TA is needed in order to complete the cycle [1] .

4. Additional steps for the PET degradation/decomposition

4.1 Pre-treatment and post-treatment of plastic waste

In order to improve the process performance for PET recycling (degradation/ fragmentation/ decomposition), PET samples are often pre-treated in different ways. PET fabrics were cut into pieces of 0.5 - 1 g and only after that were incubated in glass vessels. [31, 32]. Another method involved to cut into pieces smaller than 20 x 20 mm. Then, the samples were subjected of a solution of Tween-80 at 2% v/v at 50°C for 1 hr. The last step of the pre-treatment consisted in washing of the samples with distilled water for 1 h and drying in an oven at 40°C for 24 h [33].

The PET can be designed as a film of size 1.0 / 0.5 cm which was washed with an aqueous Na_2CO_3 solution (2g l^{-1}) at 37°C for 0.5 h followed by washing twice with distilled water [34]. Also, the PET films were prepared by melting in a heated hydraulic press. Then, each film was washed with ethanol and placed in a 10 ml glass vial. The glass vials contained 5 ml of phosphate

buffer (Na_2HPO_4/KH_2PO_4, 100 x 10^{-3} M, pH 7) [4]. There was another alternative for which a circular film (Ø 64 mm) was punched out of the polyester films. It was cleaned with ethanol and placed in a 300 ml glass beaker. Then 10 ml of 25 mM $NaH_2PO_4 \cdot H_2O$ buffer was added [35].

After degradation process, the plastic samples were often treated before analysis. So that, they were simple washed with water and dried in air [32], or washed with 2g L^{-1} Na_2CO_3 at 60°C for 1 h. Finally, these were double-washed with deionized water for 1 h [31]. Sometimes, the samples were washed in a solution of 2g/L of Na_2CO_3 for 2 h in order to stop the enzymatic reaction. At the end, the polymers were washed firstly in 10g/L Tween-80 at 25°C for 1 h and then in distilled water [33].

Other alternatives could be washing with water and ethanol and dried overnight at 50°C [4] or only the ajustment of the solution pH to 5-6 with a small amount of 1 M HCl.

After the enzymatic treatment, until the HPLC analysys, the solution was stored at 4°C [35].

Another type of treatment is the UV pre-treatment before enzymatic degradation. In this case, amourphous PET films had a thickness of 250 µm. Then, UV irradiation of PET samples was carried out over 14 days using a 1-kW xenon arc lamp. A water filter was applied to filter the IR radiation and a water bath was used for further cooling during irradiation [36].

4.2 Analytical methods for monitorization of PET degradation (decomposition)

The samples after degradation process could be considered the pieces of PET and also the solution resulted after degradation process. The solid samples samples were analysis in order to identify any modifications of the surface morfology. In this case, the characterization techniqued for solid surface were useful (FTIR, AFM, XRD, SEM/TEM, TSC-TGA, XPS) [31]. The second direction of analysis was focused on the solution. In this case, HPLC and GC couled with MS for identification and DAD/RID for quantification were performed [34, 35].

5. Derivatization of PET using biocatalytic methods

5.1 Surface modification of poly(ethylene terephthalate) (PET) fibers by a cutinase from *Fusarium oxysporum*

Cutinases are serine esterases that have the role of hydrolysis of ester bonds in cutin and belong to the hydrolase fold family. They have the capacity to hydrolyze the ester bonds of synthetic polyesters and this makes them proper for the surface modification of PET [31].

The polyester fabrics were cut in pieces and incubated in glass vessels. It was applied the enzymatic treatment and then the fabrics were washed with Na_2CO_3 for an hour. At the end, it was double-washed with deionized water [31].

The whole enzymatic process was proved to be environmentally friendly and without affecting the thermal and mechanical properties of the PET fabric. The changes were confirmed by DSC-TGA analyses, tensile tests, FT-IR ATR analysis, XPS and SEM. The free hydroxyl and carboxyl groups were detected with the help of FT-IR ATR and XPS analyses [31].

It was concluded that the enzyma *F. oxysporum* cutinase is capable of derivatization of PET without compromising the polymer structure and properties [31].

5.2 Production of heterologous cutinases by *E. coli* and improved enzyme formulation for application on plastic degradation

The aim of this work was to optimize the process of degradation of polyethylene terephthalate using an enzyme from *E. Coli*. The hydrolytic action of the enzyme was applied to the degradation of the plastic [33].

The enzyme used was cutinase. Two types of cutinase were prepared for this experiment: one wild type form from Fusarium solani pisi and its C-terminal fusion to cellulose binding domain N1 from Cellulomonas fimi. The cultures used were *E. Coli* CUT for the first type and *E. Coli* CUT-N1 for the second type [33].

Both cutinases were treated first with ampicillin and isopropyl β-D-1-thiogalactopyranoside. The optimum pH of both cutinases was around 7.0 and they were stable between 30 and 50°C. By addition of glycerol, PEG-200 and $(NH_4)_2SO_4$ to the metabolic liquid, followed by ultra filtration, the mixture became stable during 60 days at 28°C. Treating the PET with the help of cutinase led to a weight loss of 0.90% [33].

In conclusion, recombinant microbial cutinases have advantages in the treatment of PET using enzymatic treatments [33].

5.3 Rapid Hydrolyse of Poly(ethylene terephthalate) using a hydrolase from *T. Fusca*

Due to the incresing problem of plastic waste at the end of 1980, there were attempts do design biodegradable plastics. But most of the biodegradable plastics are based on aliphatic polyesters which exhibit limited useful properties. Aromatic polyesters such as PET or poly(butylene terephthalate) (PBT) which provide excellent properties can not be attacked by hydrolytic enzymes and can not serve as biodegradable source of plastic [4].

But the reason of missing biodegradability of aromatic polyesters was found by Marten at al.,: the mobility of the polymer chains in the crystalline part controls the biodegradability. This can be correlated with the temperature difference between the melting point and the temperature at which degradation takes place [4].

This work present the ability of a hydrolase isolated from the actinomycete Thermobifida fusca to depolymerize the aromatic polyester PET at a higher rate than other hydrolases such as lipases [4].

The experiment started with the characterization of the samples of PET. For the degradation process, the materials were melted using a heated hydraulic press. The samples were washed with ethanol and placed in a glass vial containing phosphate buffer. Degradation was started by adding the enzyme solution. At the end, the vials were placed in a rotational shaker and thermostated [4].

The results of the experiment demonstrated that commercial PET can be hydrolized by an enzyme. Within 8 weeks, microbial action resulted in an approx. 15% weight loss of the PET fibers [4].

5.4 Degradation of Poly(ethylene terephthalate) Catalyzed by Metal-free 2 Choline-Based Ionic Liquids

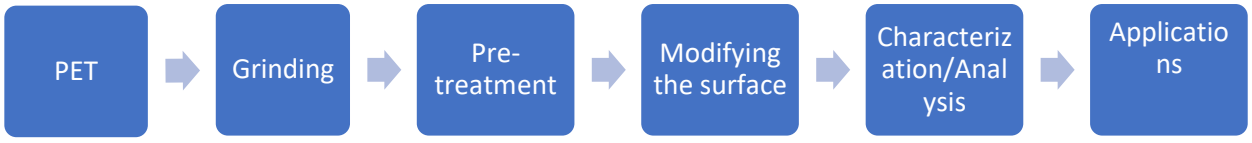
The glycolysis of PET is an expected way for degradation of PET to its monomer bis(hydroxyethyl) terephthalate (BHET) since BHET can be polymerized again to form new PET materials and provides possibilities for a permanent loop recycling. However, most of the used glycolysis catalysts are metal-based which have a high cost and present a negative environmental impact [37].

This study aims to develop a series of choline-based ionic liquids with a role in the glycolysis of PET without using any metals [37].

The catalyst used was Choline acetate [Ch][OAc], a cheaper , more biologically compatible and environmentally friendly substitute of the conventional imidazolium metal-based ionic liquids. It was found that under the optimum conditions(PET (5.0 g), ethylene glycol (EG) (20 g), [Ch][OAc] (5 wt %), 180 °C, 4 h, atmospheric pressure), the choline acetate can achieve even better performance than the initial metal based catalyst, the yield of BHET reaching up to 85.2 % [37].

6. The aim of the thesis

We proposed a detailed study for developing a technology for PET recycling. So that, our study will be directed to set up and optimise an enzyme biocatalysis for PET degradation/ fragmentation/ decomposition. Screening of enzymes will allow to decide and choose the best biocatalyst for process performance. Detailed optimization of the biocatalytic method will be considered. The system performance will be monitored directed the analysis to the characterization of the plastic surface and also looking for the composition of the process environment after incubation time. For the determination of any modifications of the surface morphology, the techniques such as FTIR, XPS, DSC-TGA, AFM, SEM/Tem will be used. HPLC-DAD/RID and/or GC-MS/FID will be performed for the evaluation of the reaction phase containt after incubation time.



Experimental part

1.1 Substances and reagents

Commercial Bis(2-hydroxyethyl) terephthalate (BHET) was purchased from Sigma-Aldrich and needed for optimizing the system for future use in PET degradation.

An attempt was made to build reaction systems based on different types of DES. Six types of DES were created starting from the solubility of BHET in different solvents: DES 1 (one part acetic acid, three parts ethylene glycol), DES 2 (one part acetic acid, three parts glycerol), DES 3 (one part oleic acid, three parts ethylene glycerol), DES 4 (one part oleic acid, three parts glycerol), DES 5 (one part octanoic acid, three parts ethylene glycol) and DES 6 (one part octanoic acid, three parts glycerol).

The system of BHET and DES was completed by adding free (lipase from *Aspergillus niger*) and immobilized enzymes (Lypozime RMIM, Lypozime TLIM, Novozyme 425 and Transenzyme) as catalysts.,. Lipozyme® TL IM –*Thermomyces lanuginosus* in silica gel, Novozym® 435 – lipase B from *Candida antarctica* in PMMA, Transenzyme – lipase in PMMA (no additional information found), Lipozyme® RM IM –*Rhizmucor miehei* in anionic exchange resin.

PET from four different sources and with different durity was used in the experimental processes and was noted with initials according to their origin: ST (PET from a bottle of juice), TA (PET from a packing tray), CU (PET from an ice cream box) and CF (PET from a bottle of Cif). The PET was cut into pieces of around 0.5 cm x 0.5 cm.

PET was subjected to the reaction with dimethyl carbonate (DMC) which was anhydrous, ≥99%, of HPLC purity and purchased from Sigma-Aldrich.

The catalyst used in the degradation of PET was the enzyme *Aspergillus niger*.

PET was subjected to the reaction with dimethyl carbonate, thiols and aniline.

Dimethyl carbonate (DMC) was anhydrous, ≥99%, of HPLC purity and purchased from Sigma-Aldrich. Aniline was also purchased from Sigma-Aldrich.

The reaction medium was the buffer Tris hydrochloride with the concentration of 10 mM and a pH of 8.3.

The catalyst used in the reaction with DMC and thiols was an immobilized enzyme Novozyme 425

1.2 Method for grinding/chopping PET

PET was subjected to grinding firstly using a Ultra centrifugal mill ZM 200 from Retsch. The mill has been set at 400 rot/min and the PET was introduced multiple times until tiny pieces resulted. For a better performance and for obtaining a powder texture, the PET was then introduced in a Ball mill PM 100 from Retsch and was let for half an hour at 400 rot/min.

1.3 Methods of sample pre-treatment

Both PET pieces and PET powder were pre-treated using five different methods.

Table 1. Pre-treatment of PET samples

<table>
<tr><td>Method 1</td><td>Method 2</td><td>Method 3</td><td>Method 4</td><td colspan="3">Method 5</td></tr>
<tr><td>Immersion in aqueous solution 2g/L Na_2CO_3 at 37℃ (over the weekend)</td><td>Immersion in 2% Tween 80 solution at 50°C, for 1 hour</td><td>Immersion in 2% Tween 80 solution at 50°C, for 1 hour</td><td>Immersion in 20% ethanol aqueous solution for 1 hour</td><td>Simple</td><td>In distilled water (AD)</td><td>In hydrogen peroxide (AO)</td></tr>
<tr><td>Washing with distilled water</td><td>Immersion in distilled water for 1 hour under stirring at room temperature</td><td>Repeated washing with distilled water</td><td>Washing with distilled water</td><td colspan="3">Exposure to UV lamp for several days</td></tr>
<tr><td>Dry in the oven at 40℃</td><td>Dry in the oven at 40℃</td><td>Immersion in aqueous solution 2g/L Na2CO3 at 37℃ for 1 hour</td><td>Dry in the oven at 40℃</td><td colspan="3">Dry in the oven at 40℃</td></tr>
<tr><td></td><td></td><td>Washing with distilled water</td><td></td><td colspan="3"></td></tr>
<tr><td></td><td></td><td>Dry in the oven at 40℃</td><td></td><td colspan="3"></td></tr>
</table>

Samples were washed with Na_2CO_3 and distilled water in order to clean and remove finishing agents.

1.4 Methods of sample preparation

1.4.1 Sample preparation for BHET hydrolysis

The experiment involved the hydrolysis of 0.001 g BHET with 1 mL of different types of DES and 0.001 g of each type of enzyme mentioned above. The reaction was left in a thermoshaker for 24 hours under agitation at 60°C.

1.4.2 Sample preparation for PET degradation

The PET was pretreated using five different methods. After the pre-treatment, the PET pieces were put in vials with 500µL DMC, 500 µL Tris hydrochloride and 2 mg of the Aspergillus niger enzyme. The vials were put in a thermoshaker at 60°C for three days.

1.5 Methods for modifying PET surface

1.5.1 Reaction of PET with DMC

PET was put in reaction with 500 µL DMC, 500 µL Tris hydrochloride and 1 mg of enzyme Novozyme 425. The vials were left in a thermoshaker for different periods of time at 60° C.

1.5.2 Reaction of PET/ BHET with thiols

PET and BHET were put in reactions with thiols. The thiols used were 2-mercaptoethanol, 2-aminothiophenol and 4-acetamidothiophenol.

Firstly, 0.001 g of BHET (5 mM) reacted with 25 mM of each thiol, 1000 µL Tris hydrochloride and 1 mg of enzyme Novozyme 425. The reaction was left in a thermoshaker at 60° C for one day.

For PET reaction, 0.01 g of PET (45 mM) with 90 mM of thiols, 1000 µL Tris hydrochloride and 1 mg of Novozyme 425 were put in vials. These were put in a thermoshaker at 60° C for 3 days.

1.5.3 Reaction of PET/ BHET with aniline

For the BHET reaction, all the peroxidases described at 2.1 Substances and reagents were used. For each peroxidase, 100 µL were put in reaction with 894 µL Tris hydrochloride, 5 µL H_2O_2, 1 µL aniline and 0.001 g BHET for 1 day at 40° C.

After the liquid phase from BHET reactions was analysed using HPLC and the results were examined, the peroxidases with the best results were chosen: Lacase M120, Peroxidase EP010 and Versatile peroxidase 2-1B Variant 12,97 ABTS. These were put again in the reaction in the same amount, but with 0.01 g PET (45 mM), 5 µL H_2O_2, 9 µL anilina and 886 µL Tris hydrochloride for 3 days at 40° C.

1.6 Method of pre-treatment before analysis

Sample was mixed with an equal volume of the mobile phase for removing the enzyme and the salt content (provided by the buffer solution), and also for adjusting the polarity of the sample comparing to the mobile phase. The resulted mixture was centrifugated and the supernatant was collected and acidified with 1 µL HCl for neutralizing the potential acidic products from the sample.

1.7 Method of sample PET characterization

1.7.1 FT-IR

FTIR spectra were recorded using a Spectrum Two FTIR spectromer (Perkin Elmer, Hamburg, Germany) equipped with a total attenuated reflectance cell in the range of 8300-350 cm^{-1}.

1.7.2 Dinamic light scattering

The particle size was determined by the DLS method using a Mastersizer 2000 device with Hydra 2000S accessory, equipped with two light sources: HeNe red laser (632 nm) and blue LED (455 nm). Water was used as a dispersion medium.

1.8 Method of sample analysis after reaction

Monitorization of the content of the reaction phase was performed based on HPLC-DAD analysis using a modular system (Agilent 1260) equipped with a C18 column (Poroshell 20) and DAD detector. The HPLC-DAD system was set up for injecting 10 µL sample and the analysis was performed at 25℃ with a flow rate of 1 µL/min mobile phase (20 % acetonitrile and 20 % H2SO4 (10 mM) dispersed in distilled water). The detection was performed at 241 nm, ie the

specific wavelength for TPA and its derivatives. Retention time of the substrate and the products are: 1 min for TA, 1. 14 for MHET and 1.31 min for BHET.

1.9 Method for the preparation of the enzyme entrapment

Carbohydrate biopolymers using Na-alginate and K-carrageenan were created for entrapment of enzyme. A 4% Na-alginate solution of 3 mL was dropped with disposable pippete in a 5% CaCl2 solution. For the K-carrageenan beads, a 1.4 wt% solution was made and dropped in a 0.5 M KCl solution [9].

In some beads, PET was added to test if it influences the specific activity of the enzyme.

2. Results and discussions

2.1 The PET degradation

The degradation of PET was carried out in two steps: the first one being the pretreatment of the PET surface and the second one the degradation reaction itself.

Five different methods of pre-treatment listed at 2.3 were used.

For each sample, the specific chromatograms have been recorded. It is not possible to identified all the peaks from the chromatograms since most of them are small polymeric fragments (oligomers) from PET structure. So that, the quantification of the system performance involed the total sum of the peak area from the chromatograms which were not present in the initial phase of the reaction. Relative area of these sum for each type of PET and each pre-treatment method were calculated. The results for all the methods were plotted as in the graphic below:

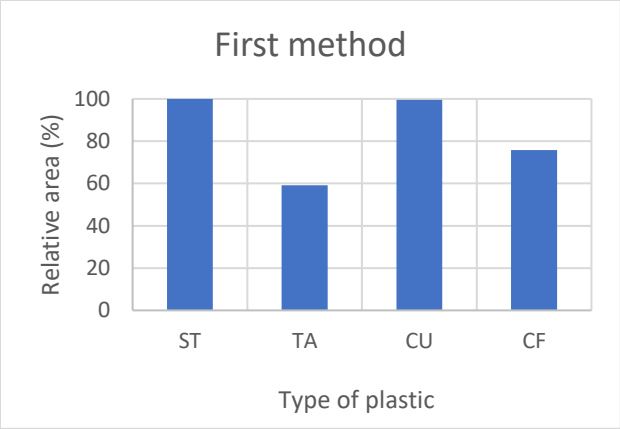


Figure 13. Relative area calculated for each type of plastic that was pretreated with the first method

Table 2. Sums of the relative areas

Sum of the relative areas	
First method	334
Second method	251
Third method	346
Fourth method	265
Fifth method	214
Fifth method (AD)	236
Fifth method (AO)	239

It can be observed that first and third method have the biggest sums of relative areas, so the degradation went better in these conditions. Although the second method had an error (the liquid phase of the CF reaction evaporated), it can be considered a valid method.

The PET samples were weighed before the reaction and after. The masses were listed in the tables below.

Table 3. The masses of PET samples before and after reactions

	Method 1				Method 2				Method 3				Method 4			
	ST	TA	CU	CF	ST	TA	CU	CF	ST	TA	CU	CF	ST	TA	CU	CF
Before reaction (mg)	0,0122	0,0159	0,0197	0,0420	0,0220	0,0216	0,0276	0,0326	0,0106	0,0163	0,0197	0,0321	0,0218	0,0197	0,0284	0,0369

After reaction (mg)	0,0130	0,0163	0,0194	0,0418	0,0240	0,0216	0,0272	0,0328	0,0112	0,0170	0,0194	0,0320	0,0220	0,0201	0,0283	0,0373
Difference (mg)	+0.8	+0.4	-0.3	-0.2	+2	0	-0.4	+0.2	+0.6	+0.7	-0.3	-0.1	+0.2	+0.4	-0.1	+0.4

Method 5										
Simple				Distilled water				Hydrogen peroxide		
ST	TA	CU	CF	ST	TA	CU	CF	ST	TA	CU
0,0186	0,0193	0,0256	0,0429	0,0286	0,0148	0,0262	0,0553	0,0259	0,0213	0,0260
0,0196	0,0193	0,0255	0,0430	0,0305	0,0159	0,0260	0,0555	0,0275	0,0216	0,0260
+1	0	-0.1	+0.1	+1.9	+1.1	-0.2	0	+1.6	+0.3	0

With red were listed the samples which had a mass increase after reaction, with green the samples that had a mass decrease. after reaction and with grey the ones that had no mass change. It can be observed that the samples which had a higher durability, CU and CF, were the only ones with a decrease of the mass.

Pretreatment method 1,2 and 3 allowed to achieve the most degraded PET surface. Positive difference between masses could be the effect of DMC attached on the PET surface (carboxy methylation).

2.2 BHET system

BHET is one of the most useful substrate which can mime very well the PET behavior. So that, BHET was mixed with free/immobilized lipase enzyme in DES environment. DES composition of two substances (one as H donnor and the other as H acceptor) was prepared and used as the reaction environment. The components were organic acids and alcohol (see table 4). Both of them can interact with the products of BHET hydrolysis. In this way, the equilibrium of hydrolysis could be shefted to the more products and finally the total conversion of the process could be improved.BHET hydrolysis takes place according to the following scheme.

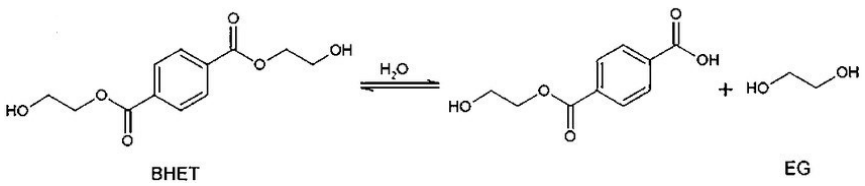


Figure 14. BHET hydrolysis

Table 4. The compositions of DES

DES COMPOSITION											
1		2		3		4		5		6	
25%	75%	25%	75%	25%	75%	25%	75%	25%	75%	25%	75%
acetic acid	ethylene glycol	acetic acid	glycerol	oleic acid	ethylene glycerol	oleic acid	glycerol	octanoic acid	ethylene glycol	octanoic acid	glycerol

After the HPLC analysis, the conversion for each type of DES and each type of enzyme was calculated. Graphics were made to see which type of DES is the best system for each enzyme.

In figure 10, experimental results for BHET system using free lipase from *Aspergillus niger* are presented. DES5 and 6 exhibited maximum conversion of 17.7% and 16.6%. Low conversion was noticed for DES3.

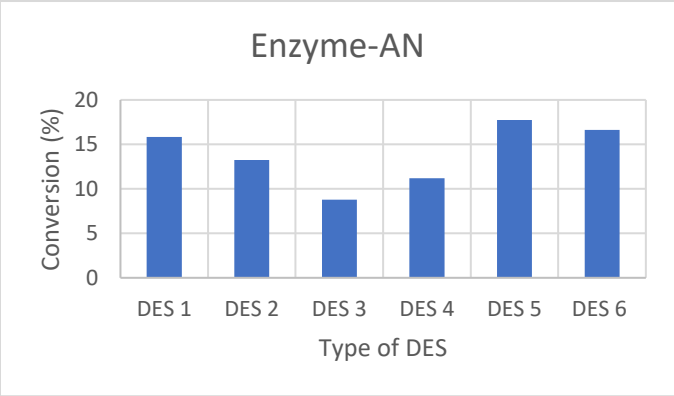


Figure 15.Conversion according with each type of DES for the enzyme Aspergillus niger

Experimental results for BHET system using Immobilized lipase Transenzyme are presented: DES 5 and 6 exhibited maximum conversion of 23.3% and 28.1%. Low conversion was noticed also for DES3.

Experimental results for BHET system using Immobilized lipase Lypozime TL1M were: DES 5 and 6 exhibited maximum conversion of 17.9% and 21.9%. Low conversion was noticed also for DES3

Experimental results for BHET system using Immobilized lipase Novozyme 425 are given: DES 5 and 4 exhibited maximum conversion of 26.9% and 18.5%. Low conversion was noticed for DES 1, 2 and 3.

Experimental results for BHET system using Immobilized lipase Lypozime RMIM were: DES 5 and 6 exhibited maximum conversion of 22.5% and 21.9%. Low conversion was noticed for DES 2 and 4.

As a general remarks analysing the graphics, it can be seen that the best systems are: DES 1, 3 and 5 with Transenzyme, DES 6 with Lypozime TLIM, DES 5 with Novozyme 425 and DES 5 and 6 with Lypozime RMIM. The reactions with these systems were repeated, but varying the quantity of the enzyme: 2 mg and 5 mg were used instead.

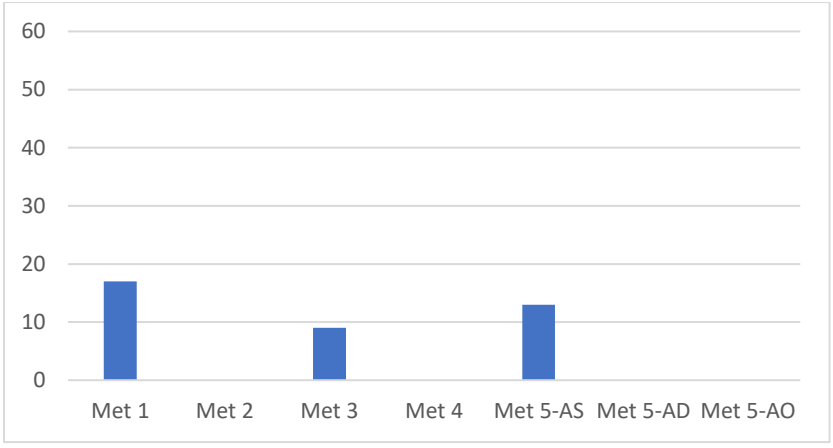
1.3. Choosing the pre-treatment approach

Both pieces of PET and PET powder treated with all 5 pre-treatment methods were put in reactions with DMC and had their liquid phase analysis by HPLC.

Using HPLC, the interest compounds solubilized in the liquid phase that results from the reaction such as terephtalic acid (TA), Bis(β-hydroxyethyl) terephthalate (MHET) and Bis(2-Hydroxyethyl) terephthalate (BHET) were identified.

In the graphs below all the areas of the interest compounds from HPLC have been noted.

(A)



(B)

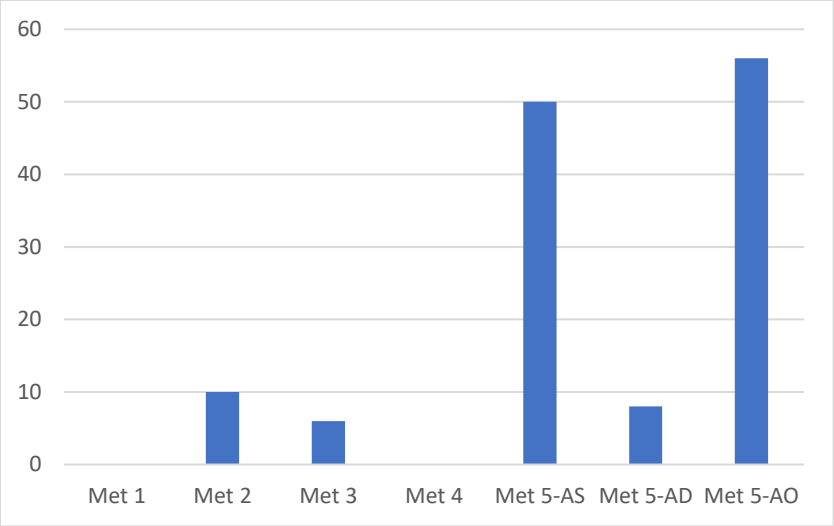


Figure 16. Influence of the pre-treatment approach on the PET derivatization. (A) PET film and (B) PET powder

From the figure 1(A and B), it is obvious that the best results were for PET powder which was pre-treated with method 5 (exposure to UV lamp for several days) in nothing (AS) and in H_2O_2 (AO). The research was continued using these two types of pre-treatment and only using grinding PET.

2.4 Reactions of BHET with thiols

BHET has a structure very similar with PET, so it can mime very well the PET behavior. Before the reaction with PET, a hydrolysis of BHET was tried.

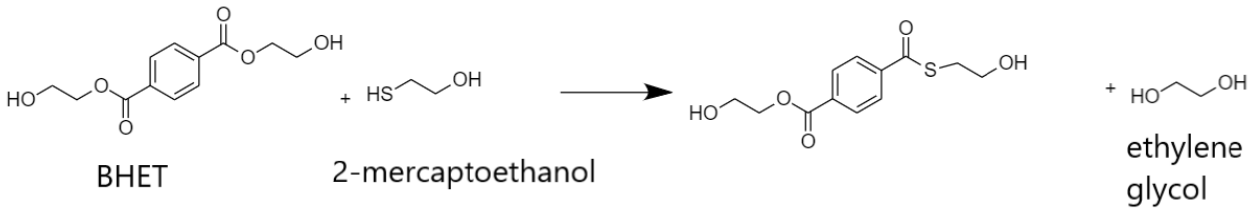


Figure 17. Reaction of BHET with 2-mercaptoethanol

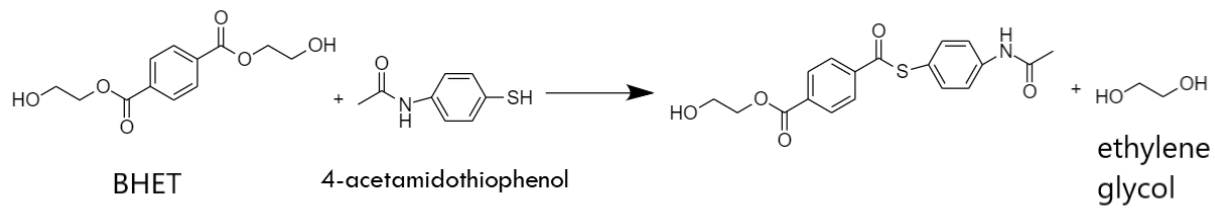


Figure 18. Reaction of BHET with 4-acetamidothiophenol

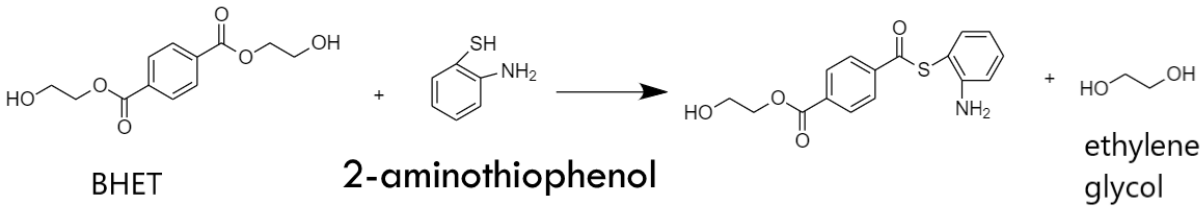
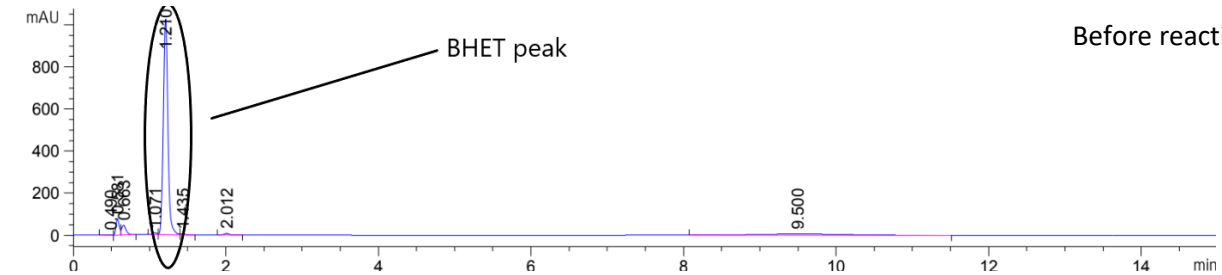


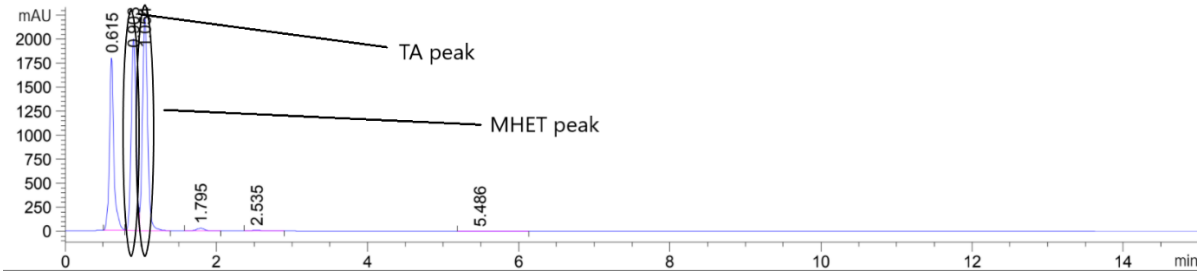
Figure 19. Reaction of BHET with 2-aminothiophenol

BHET was subjected in a reaction with thiols. The liquid phase from the reaction was analyzed using HPLC. For a better analysis of the results, control sample containing the same compounds were also analyzed using HPLC.

(A)

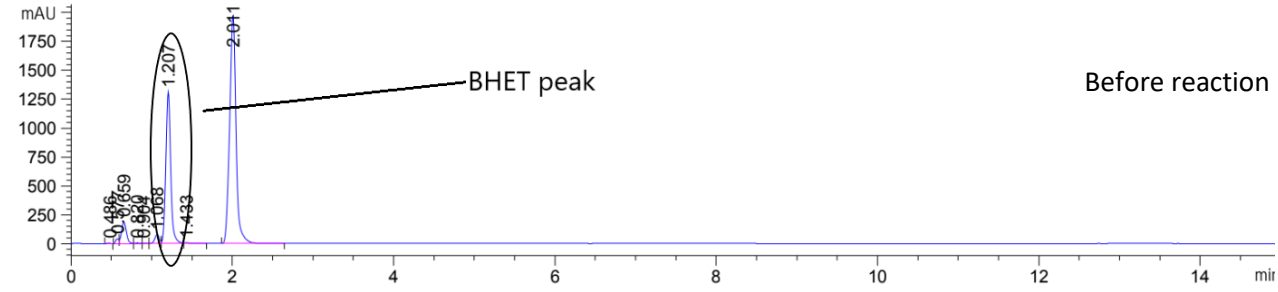


Before reaction



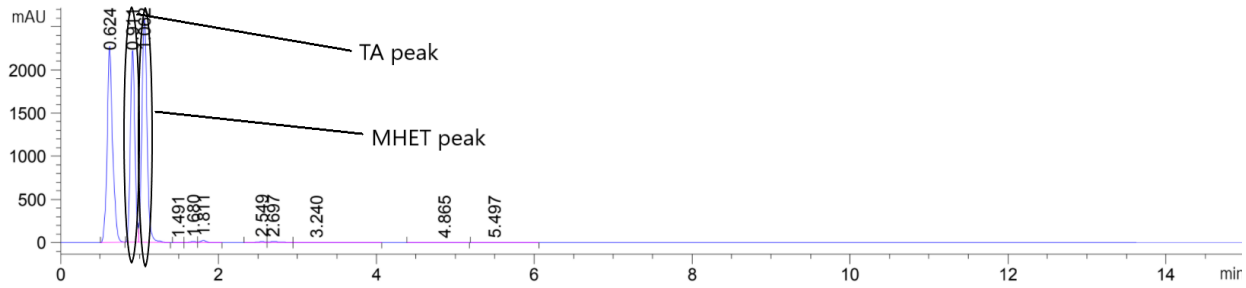
After reaction

(B)



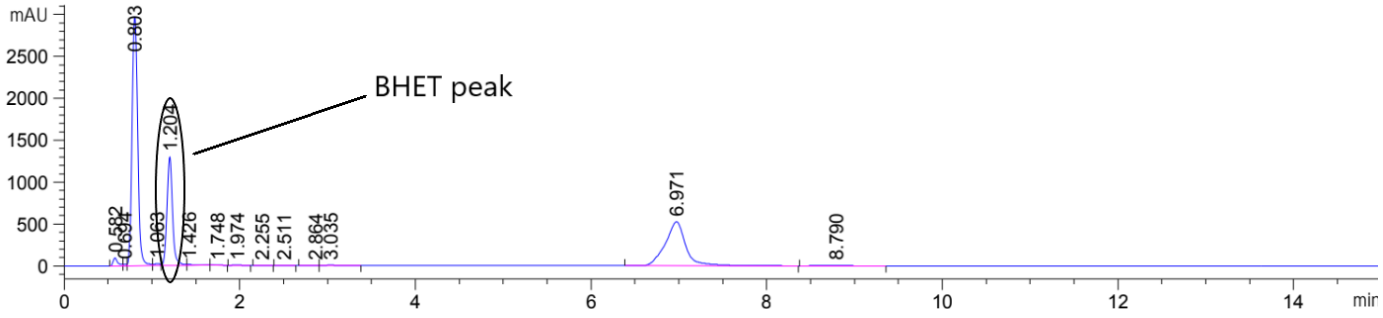
Before reaction

After reaction



(C)

Before reaction



After reaction

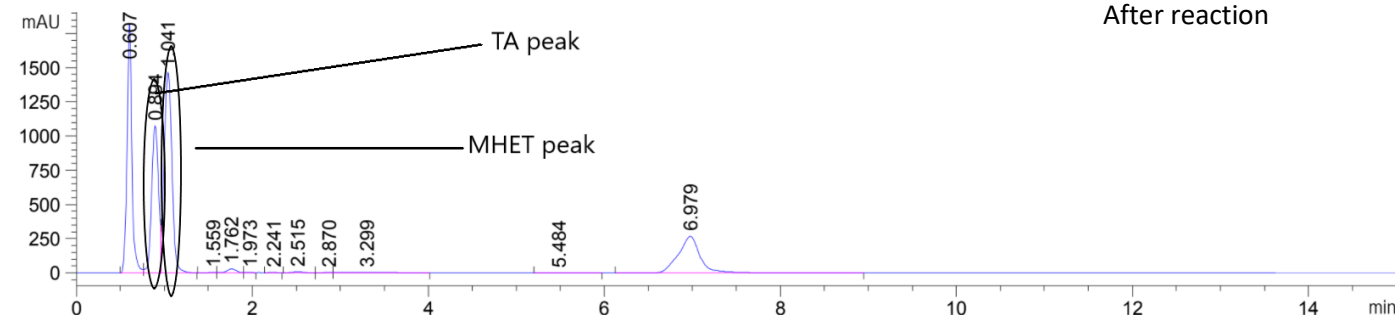


Figure 20. Reaction of BHET with (A) 2-mercaptoethanol, (B) 4-acetamidothiophenol and (C) 2-aminothiophenol

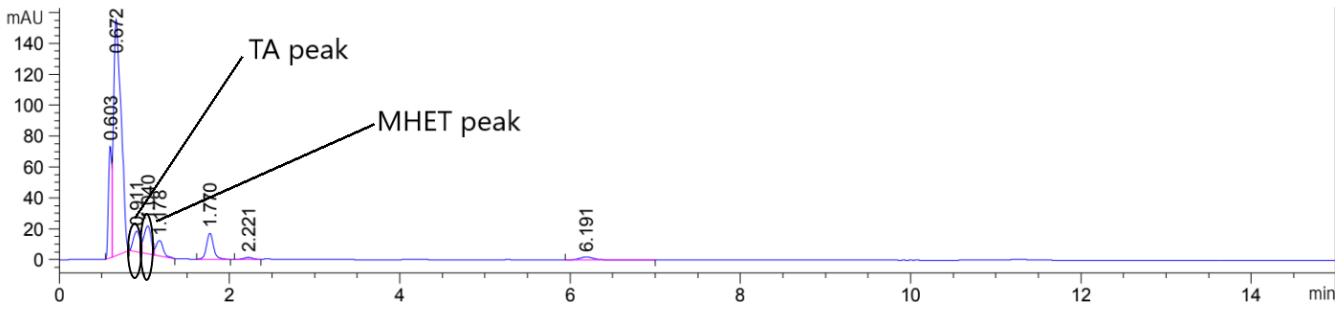
From the control sample chromatograms, it can be observed the BHET peak disappears in the chromatograms realized after the reactions. This has been replaced with the peaks for TA and MHET, its products after the hydrolysis.

After analysing the HPLC chromatograms, it is clear that the hydrolysis of BHET takes place in the presence of thiols so the research was continued with PET.

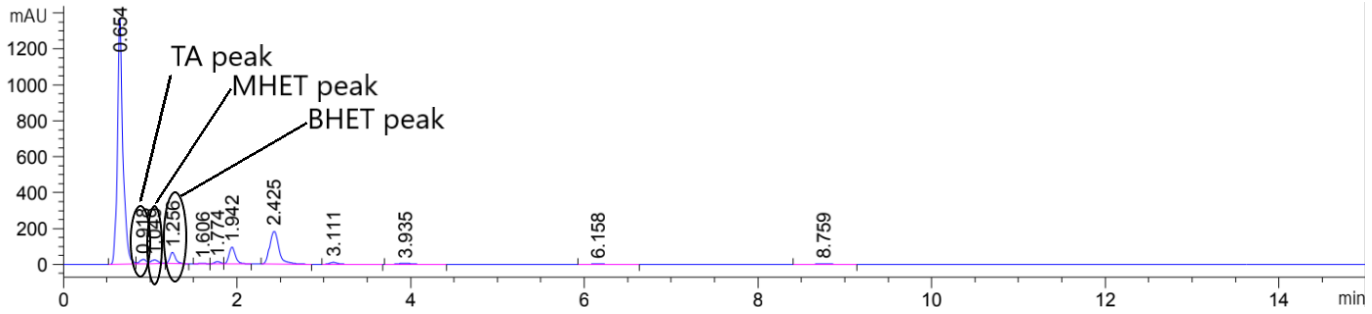
2.5 Reaction of PET with thiols

The liquid phase from the PET reaction with thiols was also analyzed using HPLC.

(A)



(B)



(C)

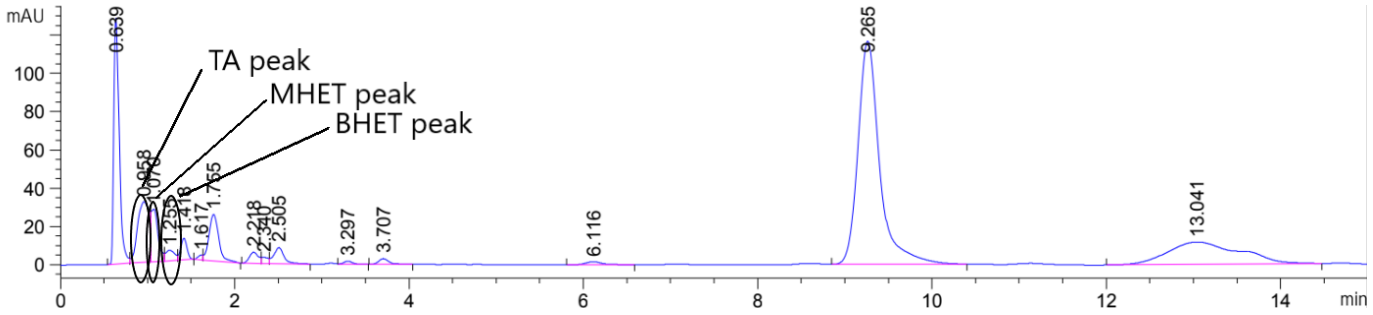
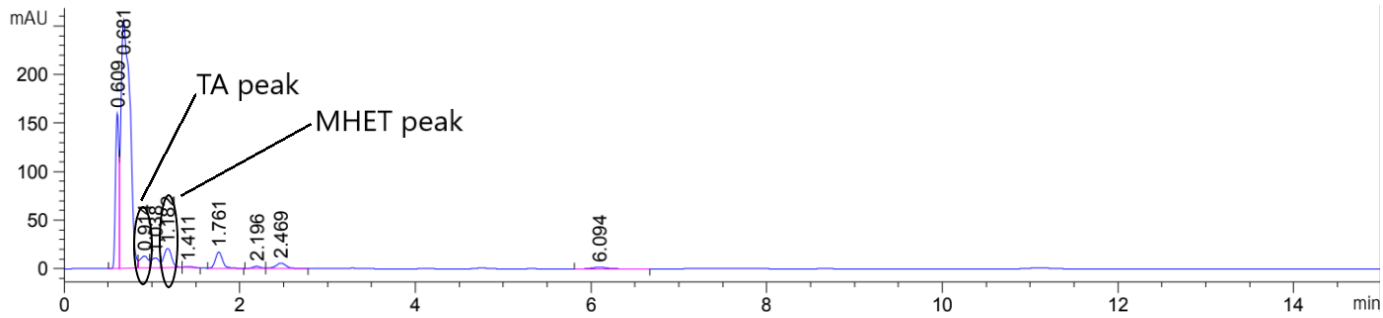
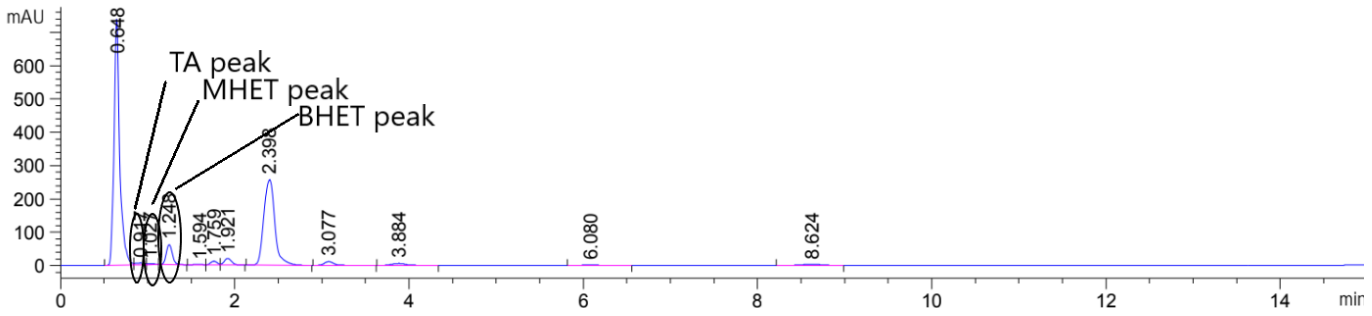


Figure 21. Reaction of PET with (A) 2-mercaptoethanol, (B) 4-acetamidothiophenol and (C) 2-aminothiophenol using method AO of pretreatment

(A)



(B)



(C)

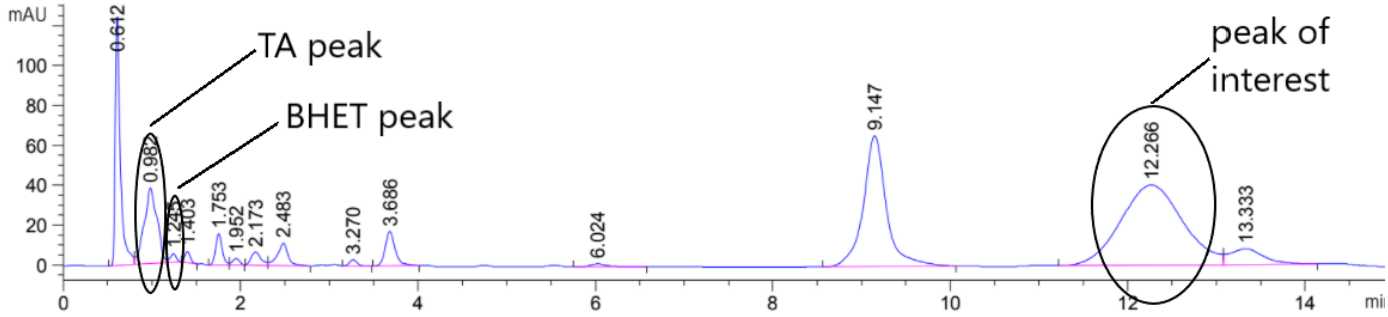


Figure 22. Reaction of PET with (A) 2-mercaptoethanol, (B) 4-acetamidothiophenol and (C) 2-aminothiophenol using method AS of pretreatment

Similar with the BHET reaction, peaks for TA and MHET were observed in the chromatogram meaning the hydrolysis of PET took place. BHET peaks also appeared.

All the chromatograms were examined and the ones with the best results were chosen for FTIR. The second one (the reaction with PET-AO and 4-acetamidothiophenol) due to the high area of the TA, MHET and BHET peaks and the last one (the reaction with PET-AS and 2-amicemidothiophenol) due to the interesting peak that appeared at the retention time of 12.2 min were the one characterized by FTIR.

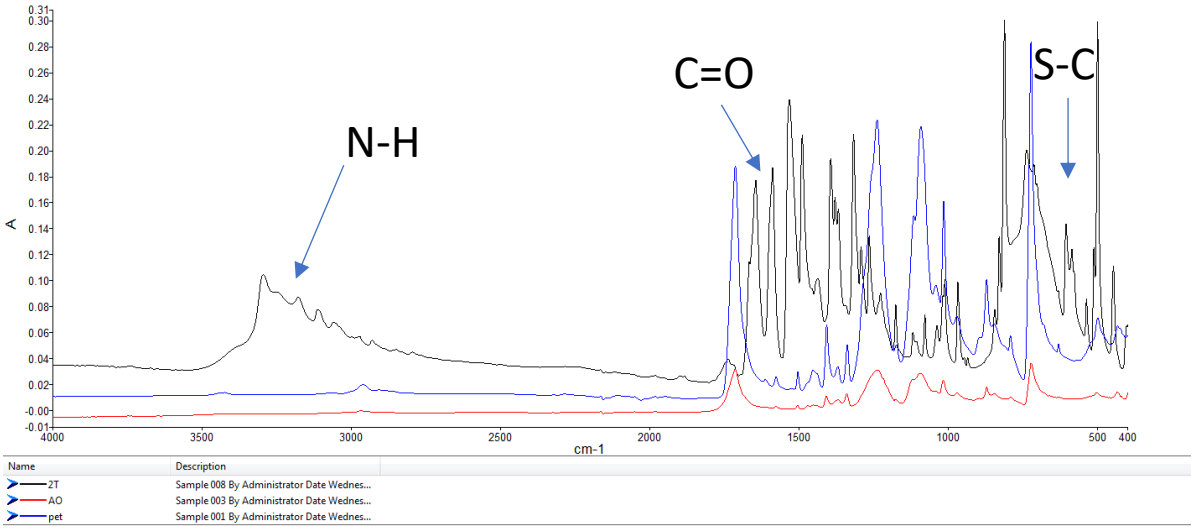


Figure 23. Overlayed spectrums of PET before pre-treatment (blue), PET after pre-treatment (red) and PET after reaction with 4-acetamidothiophenol (black)

New bands can be observed around the wave number 3000-3400 cm^{-1}. These can correspond to N-H stretching. The ones at the wave number 1500-1700 cm^{-1} can be a new bound C=O. The new bands appeared at 500-7000 cm^{-1} can be the bounds S-C that are formed after PET reactioned with thiols.

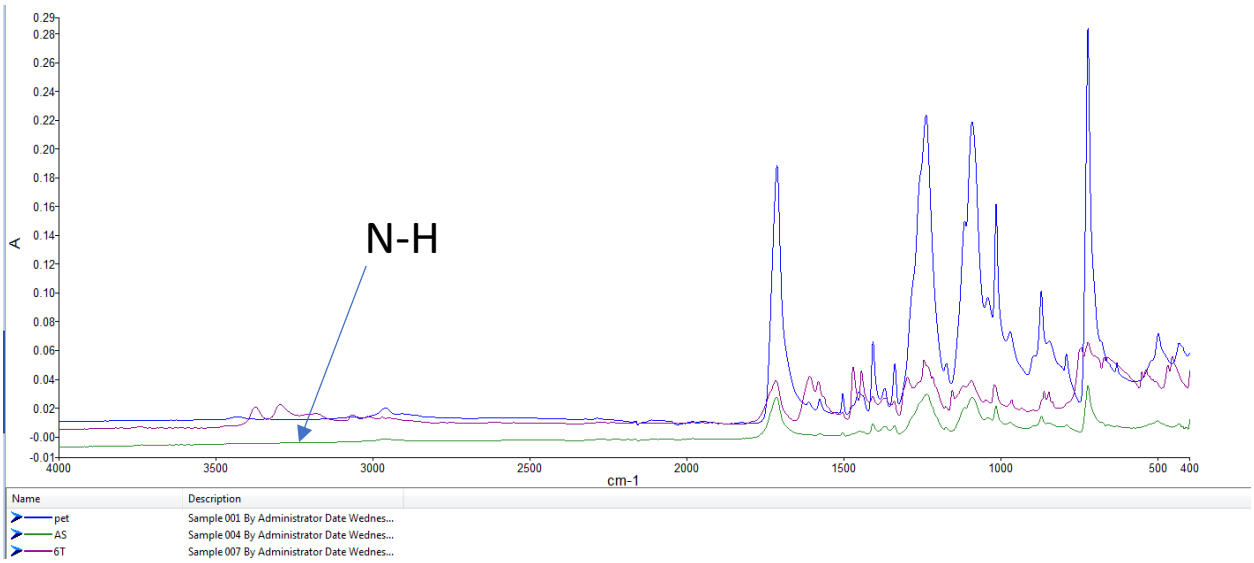


Figure 24. Overlayed spectrums of PET before pre-treatment (blue), PET after pre-treatment (green) and PET after reaction with 2-aminothiophenol (purple)

New bands can be observed around the wave number 3000-3400 cm^{-1}. These can correspond to N-H stretching.

2.6 Reaction of BHET with aniline

The same method with BHET as in 3.4 was used, but this time different peroxidases and aniline were put in the reaction. The liquid phase was analysed using HPLC.

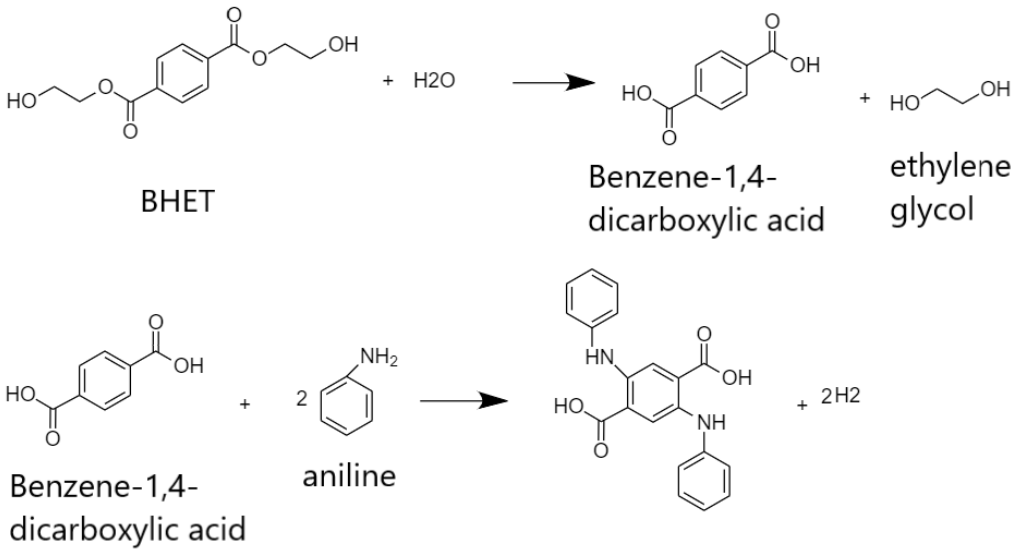


Figure 25. Reaction of BHET and aniline

From the chromatograms, using the area peaks of TA, MHET and BHET, the conversation rate of obtaining TA and MHET was calculated for each peroxidase.

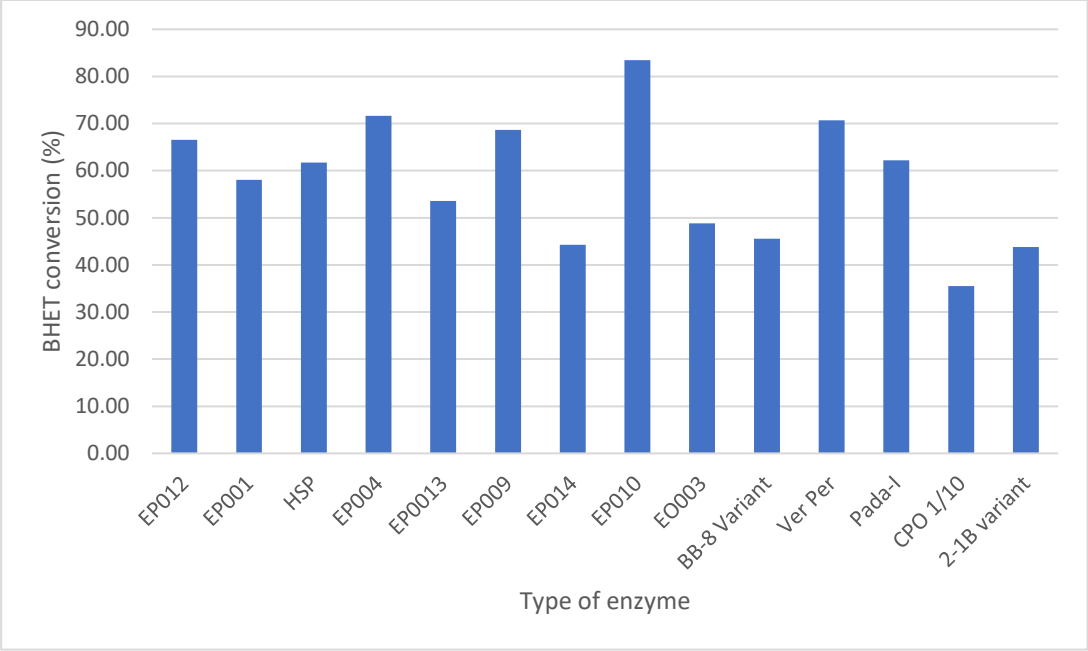


Figure 26. Enzyme effect on the biocatalytic reaction between BHET and aniline. Experimental conditions: 0.001 g BHET (5 mM), 5 µL H2O2, 1 µL anilina (10 mM), 100 µL enzyme and 894 µL Tris hydrochloride for 24 hours at 40° C and 1000 rotation/min

Peroxidase/laccase are catalysts for oxi-polymerization of aniline attached on the PET surface via o/o' benzene positions of TPA or TPA derivatives. The peroxidase with the best conversion rate (EP010, EP004 and Versatile Peroxidase) were chosen for reactions with PET.

2.7 Reaction of PET with aniline

As described previously aniline interacted with PET surface. the reaction was catalysed by three different types of peroxidases. Both liquid phase of the reaction and PET surface were evaluated. The liquid phase was again analyzed with HPLC.

Table 5. Abbreviation of aniline-peroxidase sample

Sample	Abbreviation
Reaction of PET pretreated with method AS with peroxidase EP010	9A-AS
Reaction of PET pretreated with method AO with peroxidase EP010	9A-AO
Reaction of PET pretreated with method AS with Versatile Peroxidase	14A-AS

Reaction of PET pretreated with method AO with Versatile Peroxidase	14A-AO
Reaction of PET pretreated with method AS with Lacase M120	4A-AS
Reaction of PET pretreated with method AO with Lacase M120	4A-AO

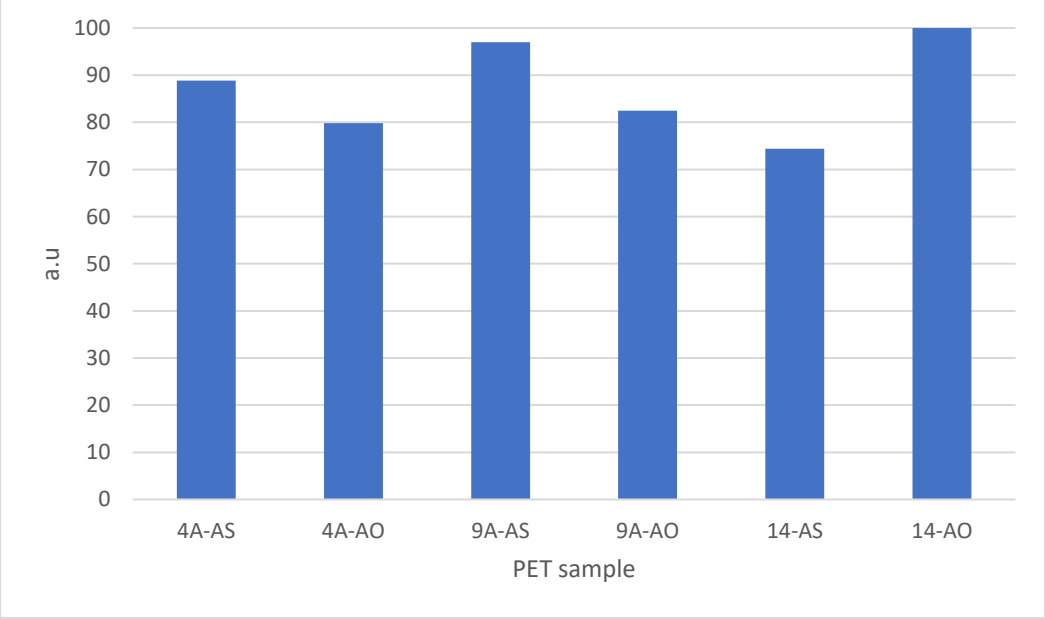


Figure 27. Evaluation of the liquid phase after the reaction between PET and aniline(peroxidase enzyme as biocatalyst). Experimental conditions: 0.01 g PET (45 mM), 5 µL H2O2, 9 µL anilina, 100 µL of peroxidase and 884 µL Tris hydrochloride for 72 hours at 40° C

All the areas from the chromatogram were calculated. Then, the relative area for each one of the reaction was put in the graph from above. It can be seen that the reaction with Versatile Peroxidase using the AO method of pre-tretment had the best results. The sample was characterized with FTIR after.

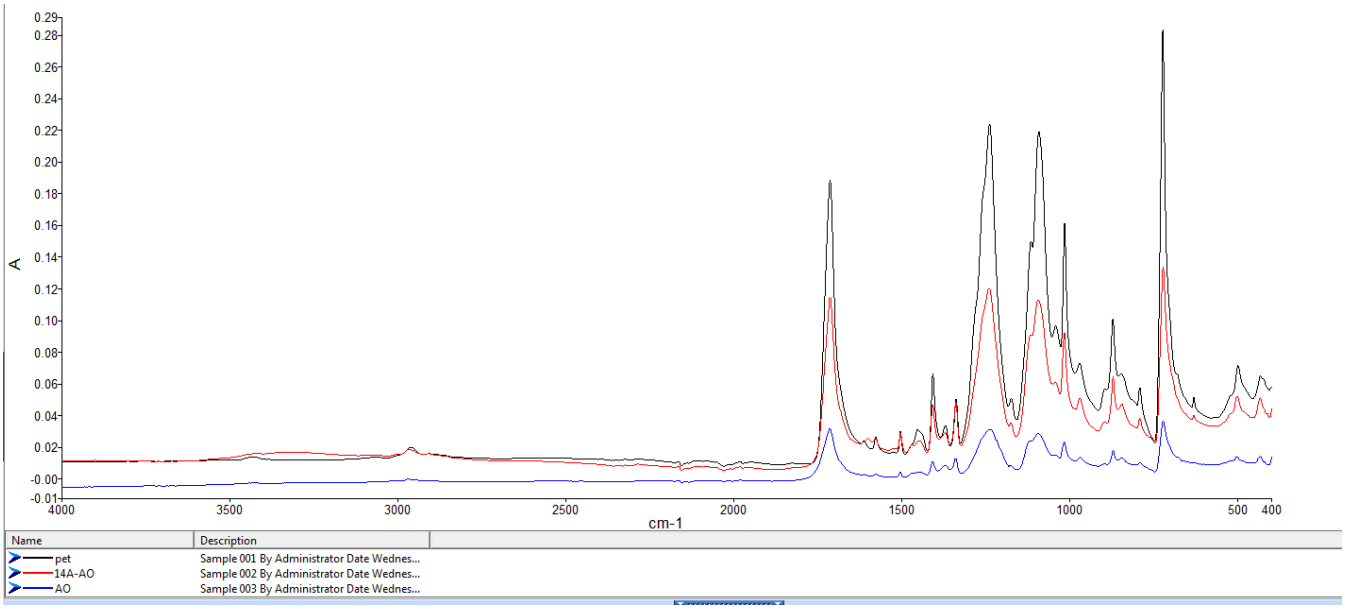


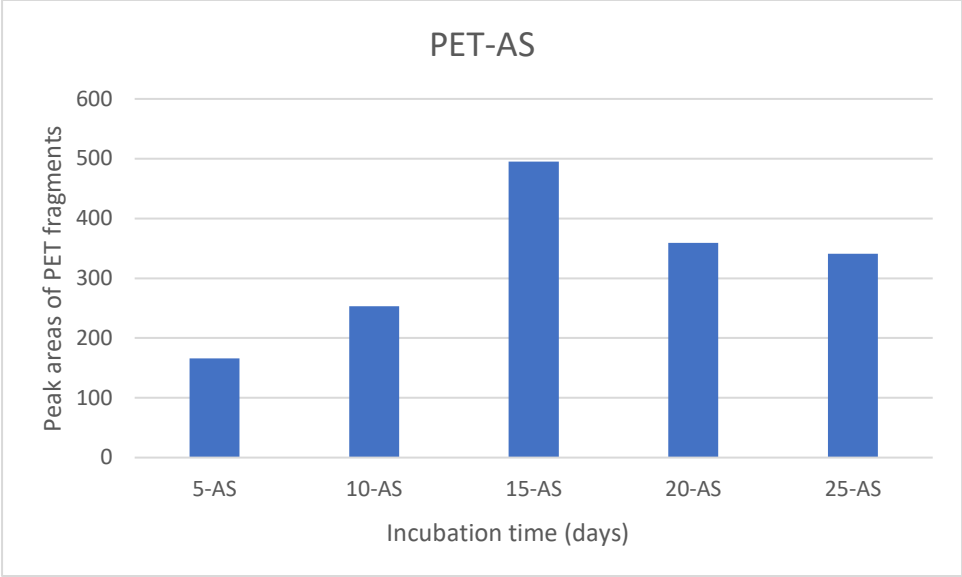
Figure 28. Overlayed spectrums of PET before pre-treatment (black), PET after pre-treatment (blue) and PET after reaction with aniline-peroxidase (red)

The spectrum of PET before pre-treatment and the PET after pre-treatment (AO) was overlayed with the one of the sample after reaction with aniline-peroxidase. Unfortunately, no change can be observed in the spectrum.

2.8 Reaction of PET with DMC

PET reacted with DMC during different periods of time for studying if the reaction time affect in a positive way the final products. The periods of time studied were: 5, 10, 15, 20 and 25 days.

At the end of the reactions, the samples were analyzed using HPLC.



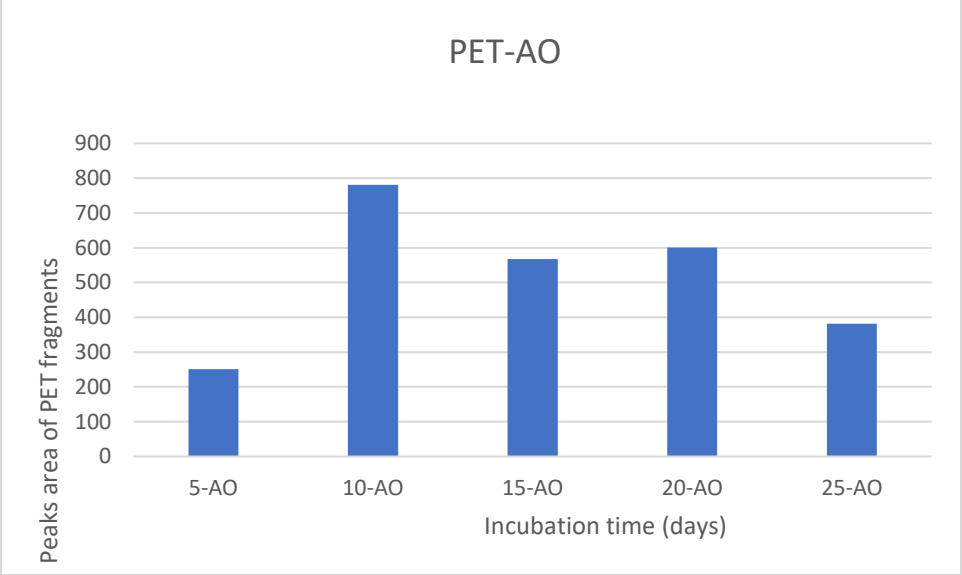


Figure 29. Area of peaks of interest for PET pre-treated using method AO and AS during different periods of time

It can be seen that for the method of pre-treatment AS, the best results were for the reaction left for 15 days and for the method of pre-treatment AO, the best results were for the reaction left for 10 days. FTIR characterization was succeeded by this analysis.

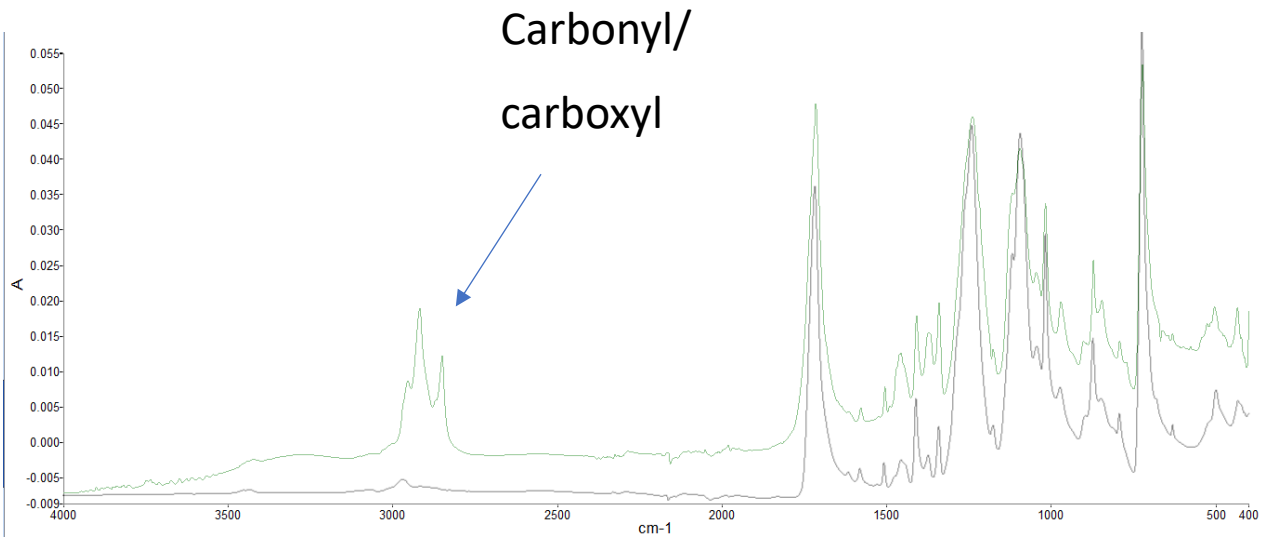


Figure 30. Overlayed spectrum of PET before pre-treatment (gray) and PET after a 15 days reaction with DMC (green)

New bands can be observed around the wave number 2700-3000 cm^{-1}. These can correspond to new aldehydes, carbonyl groups and carboxyl groups that were formed after the reaction with DMC.

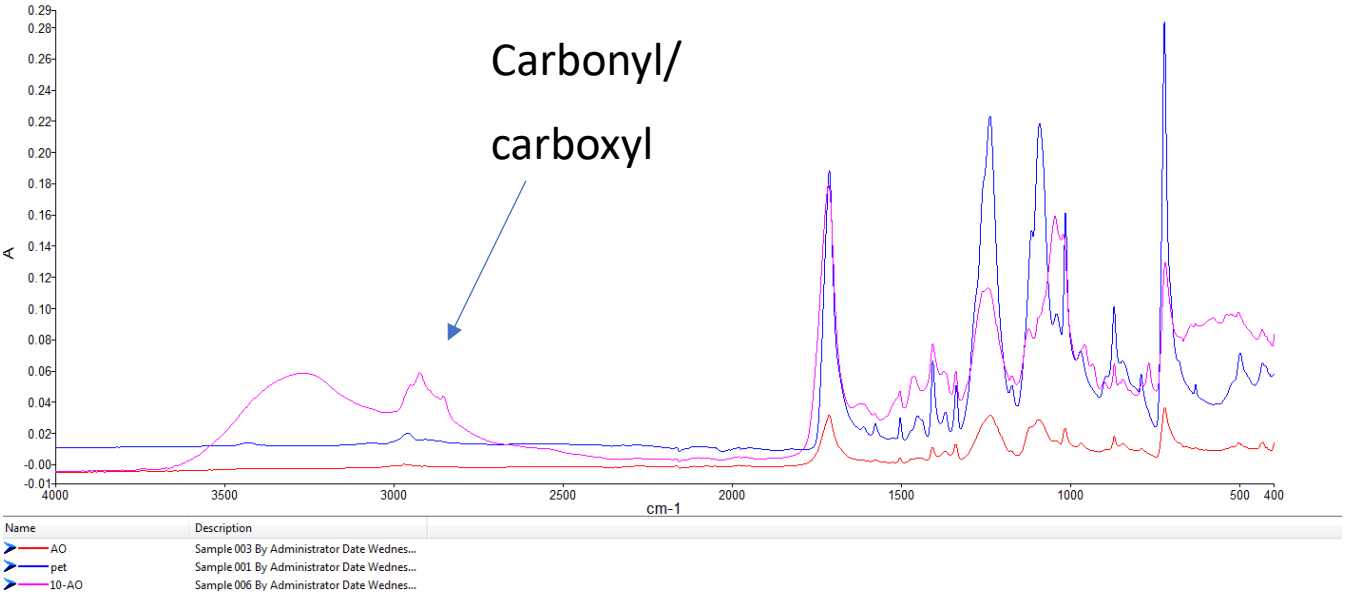


Figure 31. Overlayed spectrum of PET before pre-treatment (blue), PET after pre-treatment (red) and PET after a 10 days reaction with DMC (purple)

New bands can be observed around the wave number 2700-3000 cm^{-1} and a very large and wide one around the wave number 3000-3400 cm^{-1}.

The bands from 2700-3000 cm^{-1} can correspond to aldehydes, carbonyl groups and carboxyl groups that were formed after reaction similar to the previous FTIR spectrum.

2.9 DLS

The PET before pre-treatment was subjected to a DLS characterizations for measuring the dimension of the PET particles after grinding.

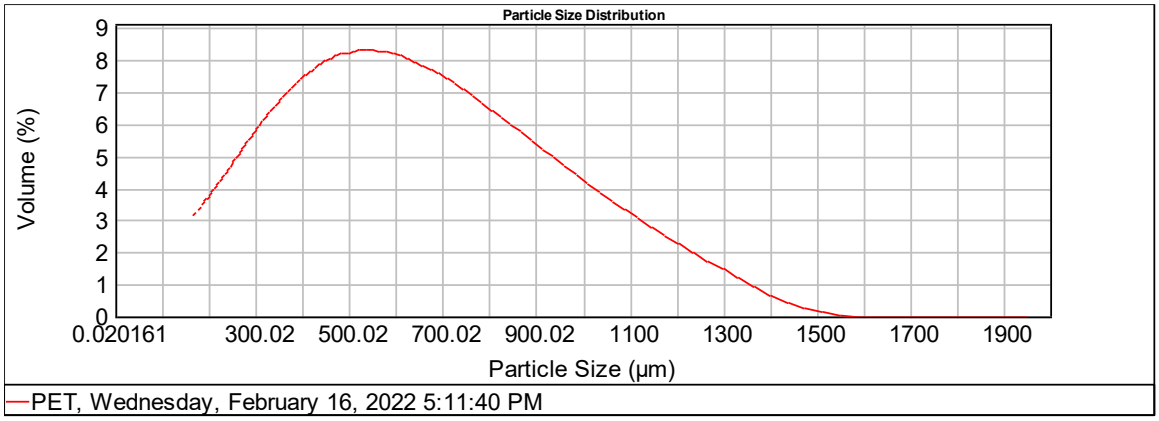


Figure 32. DLS spectrum

Table 6. Results from DSL characterization

Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.020	0.00		0.00	1.002	0.00	7.096	0.08	50.238	0.83	355.656	5.36
0.022	0.00	0.159	0.00	1.125	0.00	7.962	0.09	56.368	0.95	399.052	5.80
0.025	0.00	0.178	0.00	1.262	0.00	8.934	0.10	63.246	1.08	447.744	6.10
0.028	0.00	0.200	0.00	1.416	0.00	10.024	0.12	70.963	1.22	502.377	6.24
0.032	0.00	0.224	0.00	1.589	0.00	11.247	0.14	79.621	1.37	563.677	6.15
0.036	0.00	0.252	0.00	1.783	0.02	12.619	0.17	89.337	1.52	632.456	5.83
0.040	0.00	0.283	0.00	2.000	0.07	14.159	0.21	100.237	1.67	709.627	5.27
0.045	0.00	0.317	0.00	2.244	0.07	15.887	0.25	112.468	1.82	796.214	4.53
0.050	0.00	0.356	0.00	2.518	0.08	17.825	0.29	126.191	1.98	893.367	3.63
0.056	0.00	0.399	0.00	2.825	0.09	20.000	0.34	141.589	2.16	1002.374	2.71
0.063	0.00	0.448	0.00	3.170	0.09	22.440	0.39	158.866	2.38	1124.683	1.80
0.071	0.00	0.502	0.00	3.557	0.09	25.179	0.43	178.250	2.66	1261.915	0.89
0.080	0.00	0.564	0.00	3.991	0.09	28.251	0.48	200.000	3.00	1415.892	0.09
0.089	0.00	0.632	0.00	4.477	0.09	31.698	0.53	224.404	3.39	1588.656	0.00
0.100	0.00	0.710	0.00	5.024	0.08	35.566	0.58	251.785	3.85	1782.502	0.00
0.112	0.00	0.796	0.00	5.637	0.08	39.905	0.65	282.508	4.35	2000.000	
0.126	0.00	0.893	0.00	6.325	0.08	44.774	0.73	316.979	4.87		
0.142		1.002		7.096		50.238		355.656			

The average dimension of the particles was around 500 µm. The dimension range of all particles started with 2 µm and ended with 1400 µm.

2.10 Enzyme entrapment

Using the method from 2.8, different types of beads were prepared. PET-AO derivatized with 4-acetamidothiophenol, PET-AS derivatized with 2-amicemidothiophenol, PET-AS after 15 days in reaction with DMC and PET-AO after 10 days in reaction with DMC were the reactions with the best results. The PET from these reactions was put in the entrapment formed.

Specific enzymatic activity was calculated for all types of beads, either containing PET or not with the help of para nitrophenyl butyrate. The results from the enzymatic activity were listed in the table below.

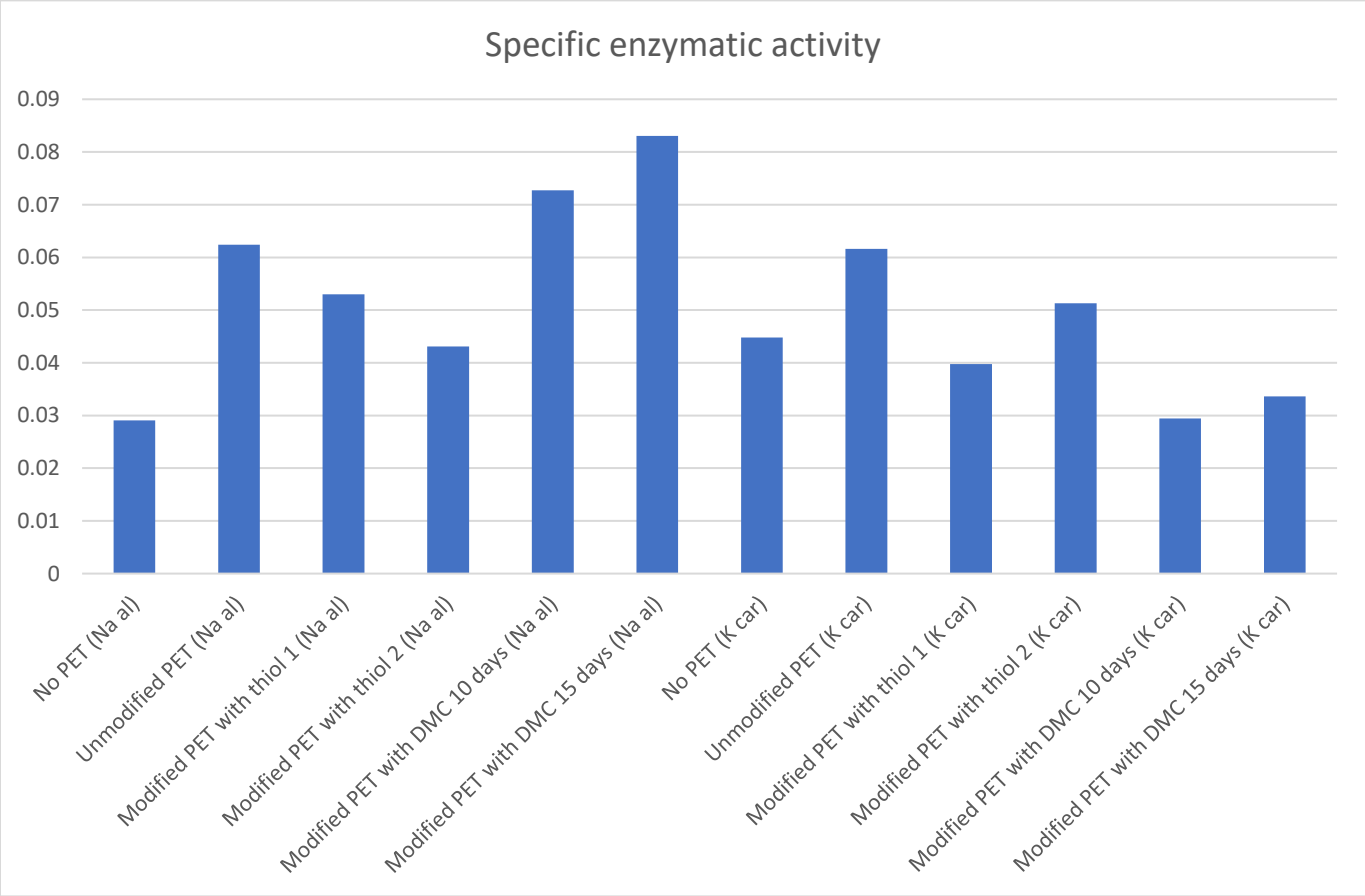


Figure 33. Specific enzymatic activity for different enzyme entrapment

It can be seen that the enzymatic activity raised in most of the cases were Pet was added in the enzyme entrapment. This it is possible due to the fact that the enzyme became more stable after the addition of PET.

For testing the enzymes, these were used in a reaction between sylibin and methyl palmitate with the enzyme cold active.

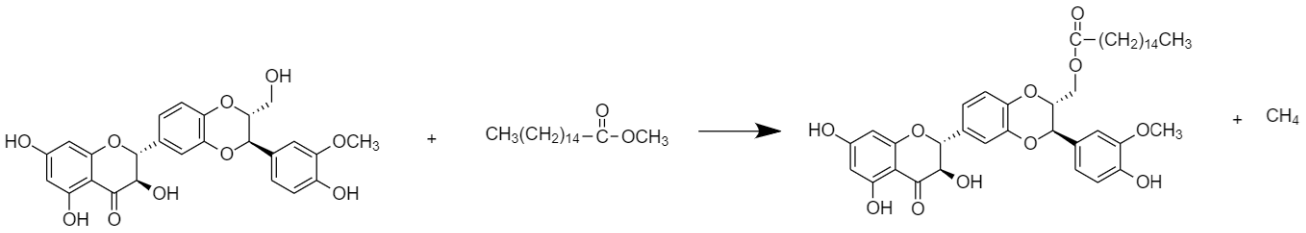


Figure 34. Reaction between sylibin and methyl palmitate

The reaction took place at 25° C for 24 hours in tetrahydrofuran. After the reaction, the liquid phase, after it was dried in an oven, was analyzed using HPLC. All the peaks were calculated and the conversions were listed in the graph below.

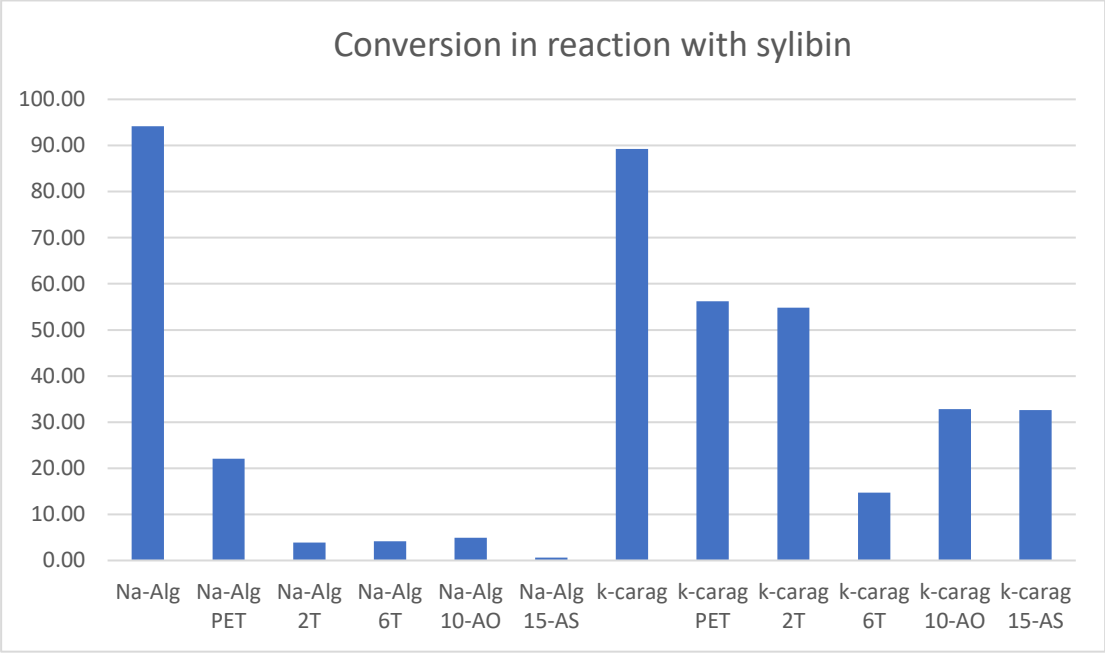


Figure 35. Conversion in reaction with sylibin

In the graph it can be seen that a better conversion rate was for the beads formed with K carrageenan. The study was continued further using only K carrageenan in making the beads.

Another study made followed the changing of the conversion rate after adding three beads in the reaction and not just one. The study also tracked the variation of the conversion rate modifying the temperature.

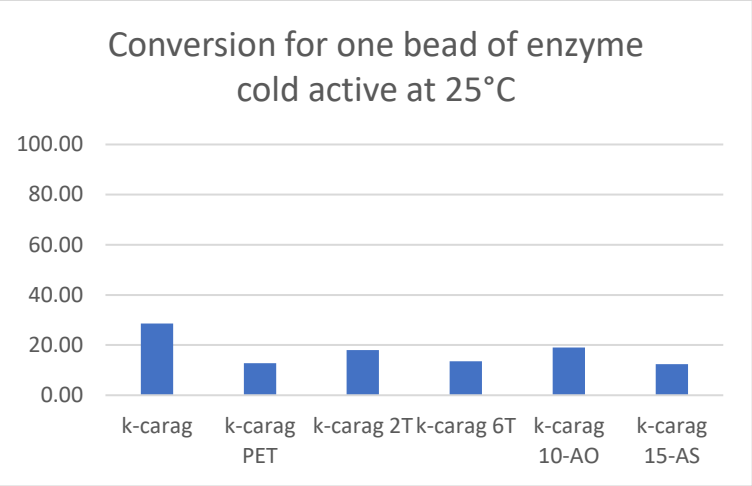


Figure 36. Conversion for one bead of enzyme

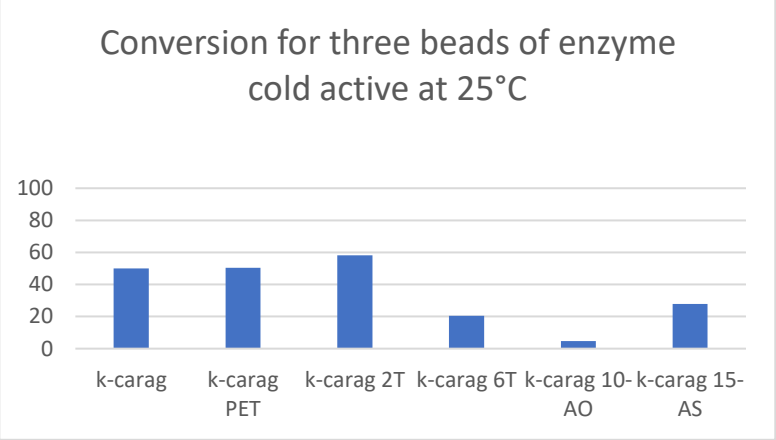


Figure 37. Conversion for three beads of enzyme

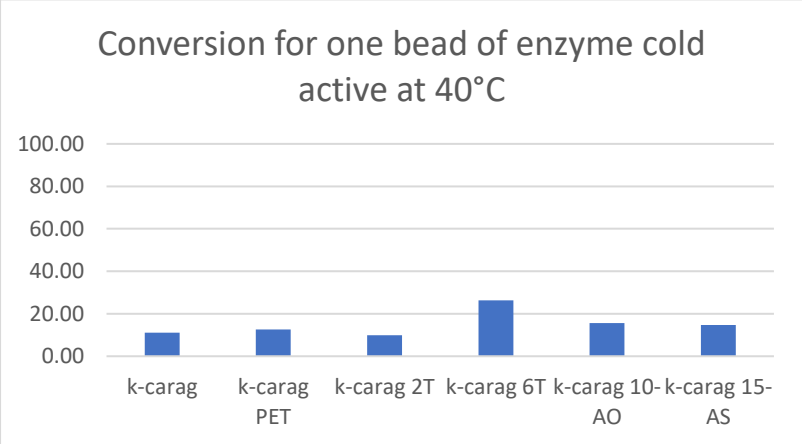


Figure 38. Conversion for one bead of enzyme

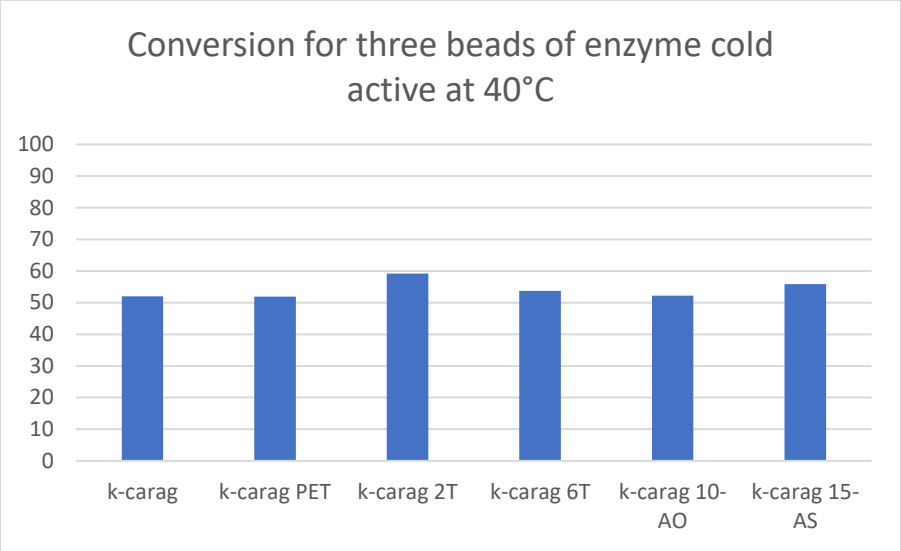


Figure 39. Conversion for three beads of enzyme

As it can be seen from the graphs from above, the conversion rate has increased with the increasing of number of beads in the reaction.

In the initial reaction, 8.1 mg of methyl palmitate, 988 µL tetrahydrofuran (THF), 1 mg of sylibin and 1 bead of enzyme were used. For getting a better view of the perfect conditions for the reaction, these reactants were changed as in the table from below. The beads with Pet-AO derivatized with 4-acetamidothiophenol(2T) and Pet-AS derivatized with DMC for 15 days were chosen to continue this study with.

Table 7. The name of the reacions when reactants were changed

Type of Pet	Name of the reaction	Reactants
2T	2TA	8.1 mg of methyl palmitate, 988 µL THF, 1 mg of sylibin and 4 beads of enzyme
2T	2TB	8.1 mg of methyl palmitate, 988 µL THF, 1 mg of sylibin and 6 beads of enzyme
2T	2TC	8.1 mg of methyl palmitate, 988 µL THF, 1 mg of sylibin and 8 beads of enzyme
2T	2TD	4 mg of methyl palmitate, 988 µL THF, 1 mg of sylibin and 4 beads of enzyme
2T	2TE	12 mg of methyl palmitate, 988 µL THF, 1 mg of sylibin and 4 beads of enzyme

2T	2TF	8.1 mg of methyl laurate, 988 μL THF, 1 mg of sylibin and 4 beads of enzyme
2T	2TG	8.1 mg of methyl oleate, 988 μL THF, 1 mg of sylibin and 4 beads of enzyme
2T	2TI	8.1 mg of methyl palmitate, 988 μL ethanol, 1 mg of sylibin and 4 beads of enzyme
15-AS	15-ASA	8.1 mg of methyl palmitate, 988 μL THF, 1 mg of sylibin and 4 beads of enzyme
15-AS	15-ASB	8.1 mg of methyl palmitate, 988 μL THF, 1 mg of sylibin and 6 beads of enzyme
15-AS	15-ASC	8.1 mg of methyl palmitate, 988 μL THF, 1 mg of sylibin and 8 beads of enzyme
15-AS	15-ASD	4 mg of methyl palmitate, 988 μL THF, 1 mg of sylibin and 4 beads of enzyme
15-AS	15-ASE	12 mg of methyl palmitate, 988 μL THF, 1 mg of sylibin and 4 beads of enzyme
15-AS	15-ASF	8.1 mg of methyl laurate, 988 μL THF, 1 mg of sylibin and 4 beads of enzyme
15-AS	15-ASG	8.1 mg of methyl oleate, 988 μL THF, 1 mg of sylibin and 4 beads of enzyme
15-AS	15-ASI	8.1 mg of methyl palmitate, 988 μL ethanol, 1 mg of sylibin and 4 beads of enzyme

All the liquid phases from the reactions was analyzed after using HPLC. The conversion rates were calculated and listed in the graphs from below.

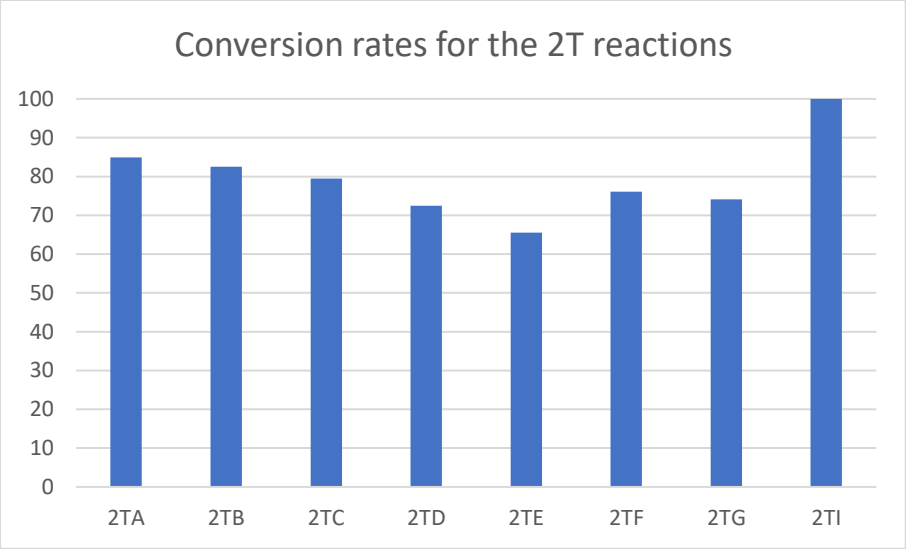


Figure 40. Conversion rated for the 2T reactions

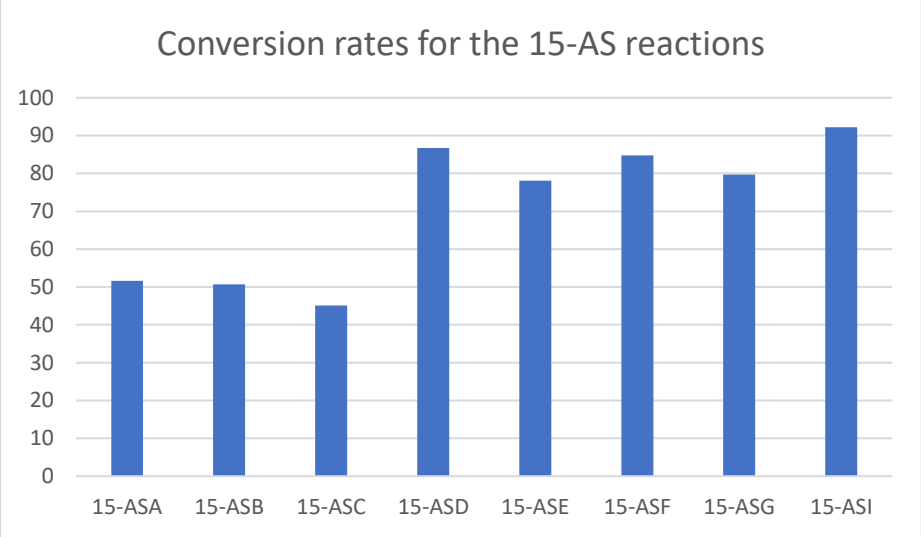


Figure 41. Conversion rated for the 15-AS reactions

From the graphs it can be concluded that after 4 beads, the conversion rate do not increase with the increase of number of beads. It can be seen that the conversion rates start to decrease a little with rising the number of beads. Also, it can be observe that methyl laurate has decent conversion rate results can be used as a substituent for methyl palmitate. Good conversion were also seen in the case when ethanol was used instead of tetrahydrofuran.

3. Conclusions

1. Reaction with thiols

4-acetamidothiophenol and 2-aminothiophenol offered best performance for PET(AO) and PET(AS) derivatization, respectively. FTIR analysis confirmed the thiol insertion on the PET surface.

2. Reaction with aniline

Enzyme screening was performed for BHET and aniline interaction. The best ones Laccase M10, EP010 and Versatile peroxidase were selected. Unfortunately, PET surface cannot be modified using peroxidase biocatalysis.

3. Reaction with DMC

PET derivatization with DMC has been performed. The best results were obtained for PET(AS) at 15 days and for PET(AO) at 10 days. FTIR spectra confirmed the acyl insertion on PET surface.

Modified PET was used as stabilizer for polysaccharide (alginate/carageenan) cavity prepared for enzyme immobilization. Cold active lipase was evaluated for silybin esterification. A solution was the entrapment of the lipase in polysaccharide cavity protecting the proteinic structure against solvent attack. The Pet used for the entrapment was chosen to be the one derivatized with 4-acetamidothiophenol, 2-aminothiophenol and DMC at 10 and 15 days.

Multiple studies were made for seeing if the which were the best parameters and reactans for the enzyme cold active trapped in beads with Pet. It was concluded that the bead with carageenan had the best stability. We also examined if the number of beads that affect the reaction and we saw that with the increasing of beads up to four, the reaction will have a better conversion rate. After four beads, the conversion rate starts to drop.

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