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VIRTUTE ET SAPIENTIA

FACULTY OF CHEMISTRY

EXPERIMENTAL REPORT

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

Master: CHEMISTRY OF ADVANCED MATERIALS

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1. Introduction

The production of plastic dates back to 1950' and since then they have become one of vital materials used in everyday life applications [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].

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].

Due to the fact that PET is almost impossible to degradate and has such a negative impact on the environment, new methods for PET recycling are constantly being search. In order to improve the degradation process and to prevent the release of microplastics in the natural environment, it is important to understand the degradation process [7].

Many plastics have a poor tendency to bend to other materials and to other substances due to their inherent inert chemical structure. In order to improve the process performance for PET recycling (degradation/ fragmentation/ decomposition), PET samples are often pre-treated in 5

different ways. An effective way of pretreatment might include cleaning and activation of the polymer surface [8].

It is more difficult to obtain good adhesion to polymers compared with metals. There are several reasons to this: plastics contain numerous components and these can vary considerably and some of the components, like lubricants and plasticizers, hinder adhesion severely, the temperature dependence of the mechanical properties of plastics is very high, all plastics have a low surface energy and they necessitates some kind of pretreatment to make plastics wettable by adhesives [8].

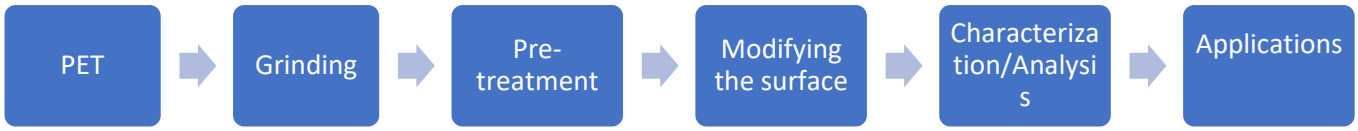
The research community has already started to search for new alternative of the plastics valorization and the general strategies seem to be the chemical/biochemical transformations. Usually, chemical modification of the plastic by degradation/decomposition leads to value-added chemicals which can be a convenient avenue to supplement current recycling processes.

We consider that biocatalysis could be an alternative for plastics (PET) recycling. Continued efforts need to be focused on enzyme biocatalysts (cutinase/lipase/carbonic alhydrase) to improve the overall efficiency with minimum energy consumption for the set up process.

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.

In this report we proposed to test and to optimize different methods of PET pretreatment and grinding/chopping of the plastic (PET). Then, different methods for modifying the PET surface were tested starting from a model with BHET that mime the structure of the PET. Different reagents were used for the derivatization such as DMC, thiols and aniline. Analysis of the liquid phase after PET derivatization was performed using HPLC. In this way, the process efficiency was evaluation monitoring the reaction products (based on the peaks area). Additionally, the characterization of the PET surface after derivatization have been done using FTIR technique.

Identification of new band in the PET spectrum after derivation was a clear evidance of the succes of the derivation approach. Based on the experimental data, optimum experimental conditions were set up for developing new materials as dericatized-PET useful for the preparation of stable entraped cavity of immobilised enzyme.



2. Experimental part

2.1 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.

Polyethylene terephthalate (PET) was procured from the bottles bought from the store.

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.

2.2. 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.

2.3 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

Method 1	Method 2	Method 3	Method 4	Method 5		
Immersion in aqueous solution 2g/L Na_2CO_3 at 37℃ (over the weekend)	Immersion in 2% Tween 80 solution at 50°C, for 1 hour	Immersion in 2% Tween 80 solution at 50°C, for 1 hour	Immersion in 20% ethanol aqueous solution for 1 hour	Simple	In distilled water (AD)	In hydrogen peroxide (AO)

Washing with distilled water	Immersion in distilled water for 1 hour under stirring at room temperature	Repeated washing with distilled water	Washing with distilled water	Exposure to UV lamp for several days
Dry in the oven at 40℃	Dry in the oven at 40℃	Immersion in aqueous solution 2g/L Na2CO3 at 37℃ for 1 hour	Dry in the oven at 40℃	Dry in the oven at 40℃
		Washing with distilled water		
		Dry in the oven at 40℃		

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

2.4 Methods for modifying PET surface

2.4.1 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.

2.4.2 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.

2.4.3 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.

2.5 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.

2.6 Method of sample PET characterization

2.6.1 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}.

2.6.2 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.

2.7 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.

2.8 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.

3. Results and discussions

3.1 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.

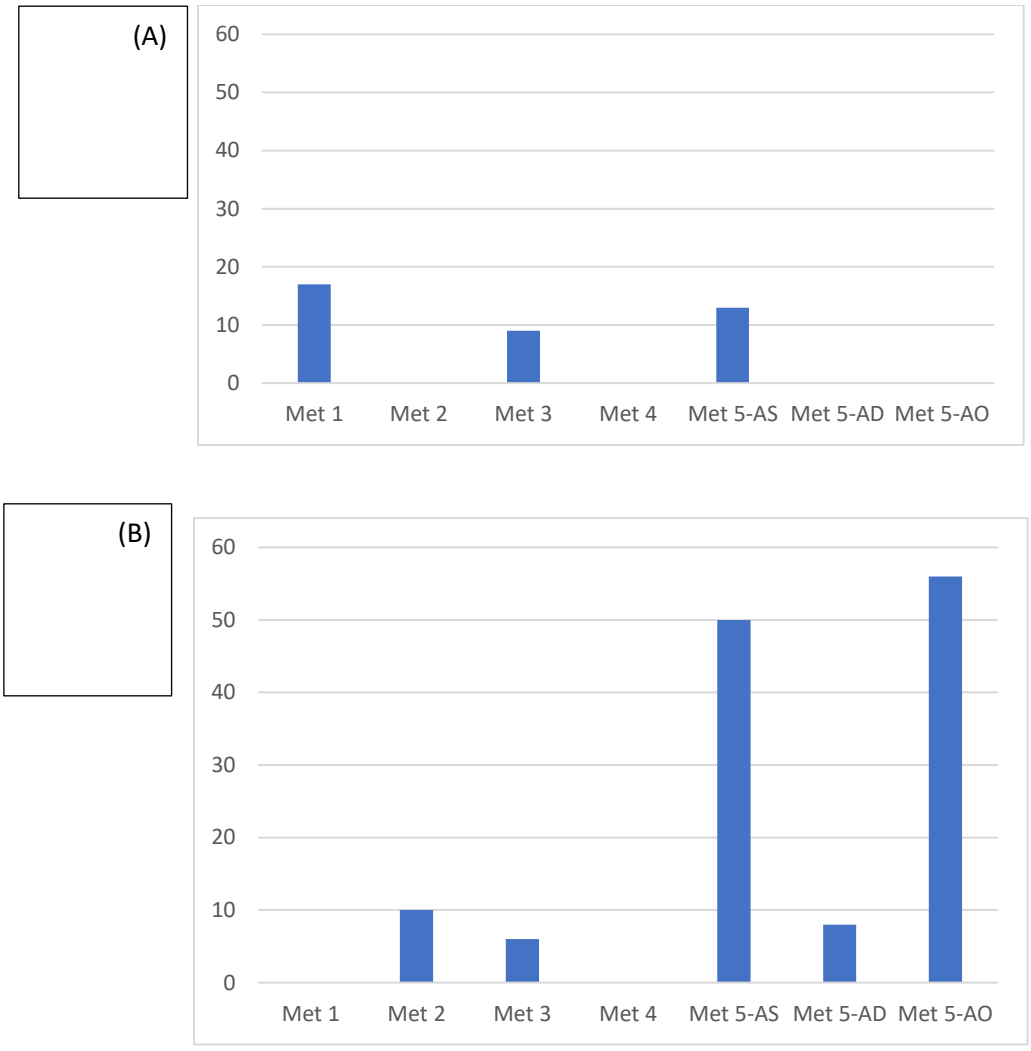


Figure 1. influence of the pre-treatment approach on the PET derivatization. (A) PET film and (B) PET powder

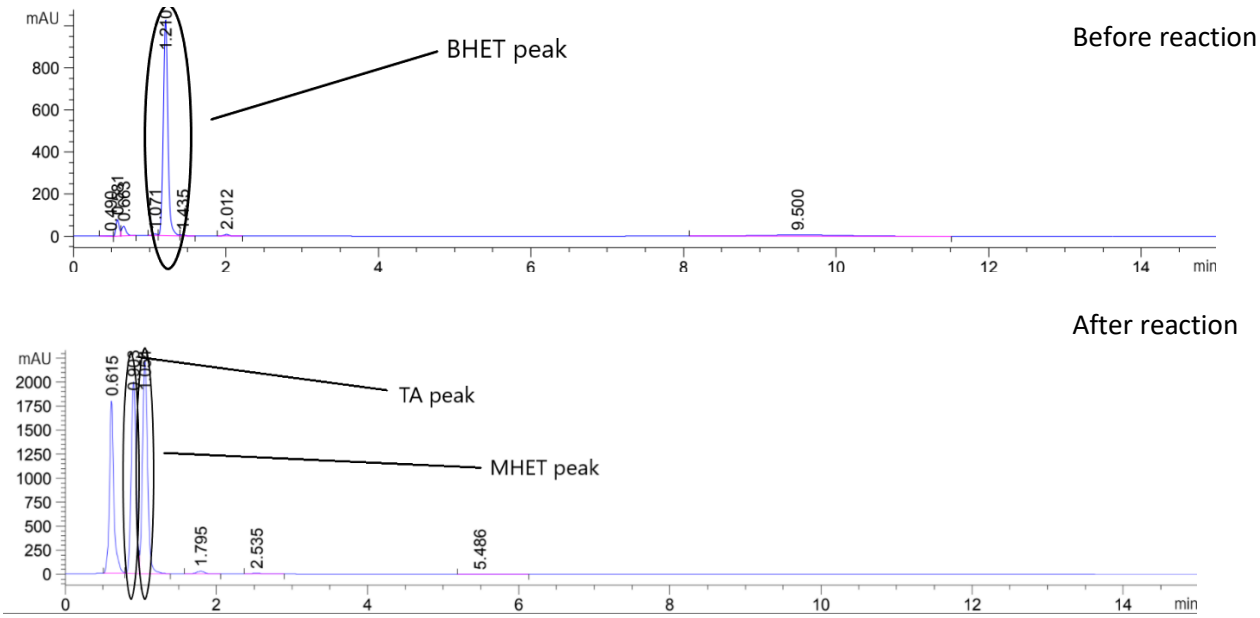
From the figure 1(A and B), it is obvious that the best results were for PET powder which was pre-treated with method 5 (exposure to UV lamp for several days) in nothing (AS) and in H_2O_2 (AO). The research was continued using these two types of pre-treatment and only using grinding PET.

3.2 Reaction of BHET with thiols

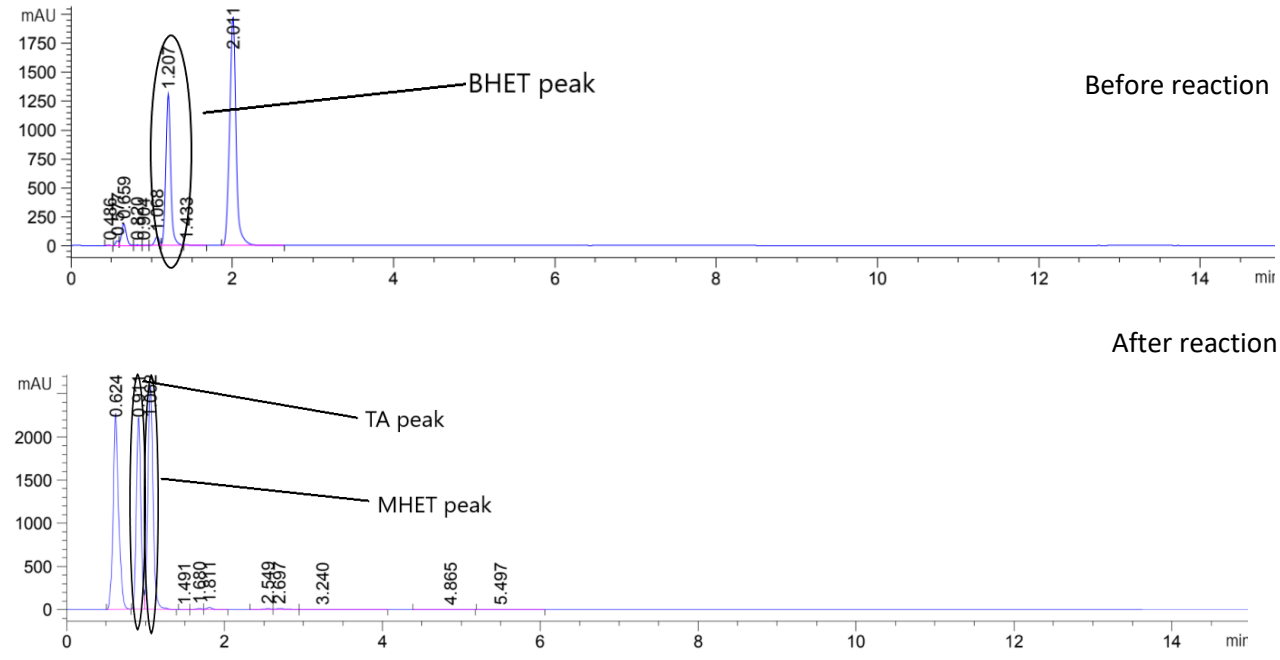
BHET has a structure very similar with PET, so it can mime very well the PET behavior. Before the reaction with PET, a hydrolysis of BHET was tried.

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)



(B)



(C)

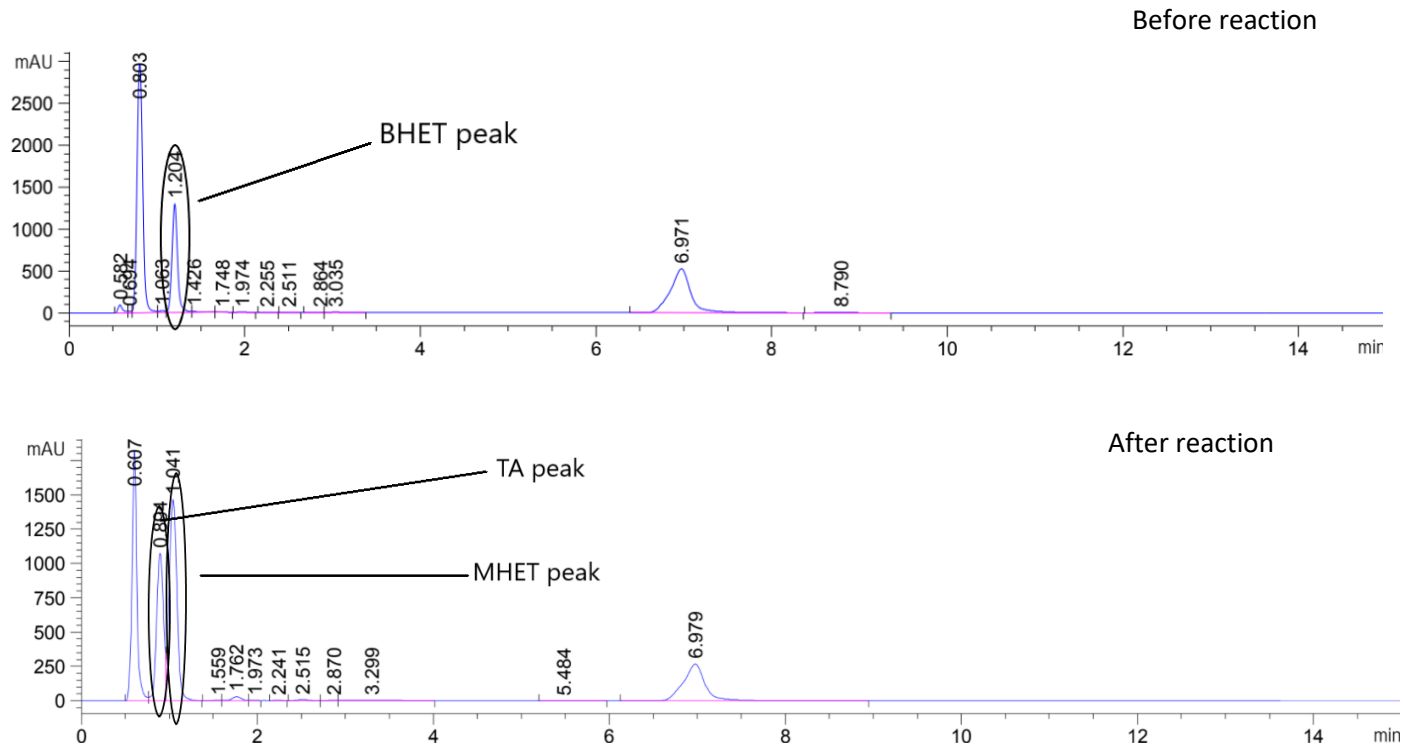


Figure 2. Reaction of BHET with (A) 2-mercaptoethanol, (B) 4-acetamidothiophenol and (C) 2-aminothiophenol

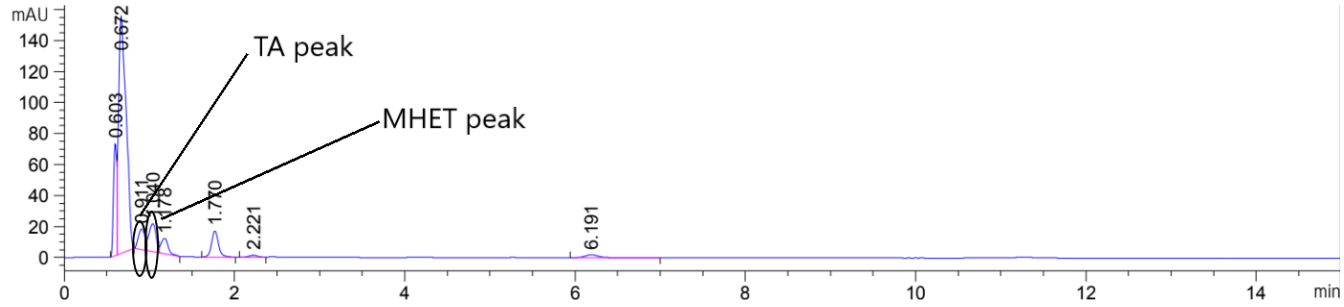
From the control sample chromatograms, it can be observed the BHET peak disappears in the chromatograms realized after the reactions. This has been replaced with the peaks for TA and MHET, its products after the hydrolysis.

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.

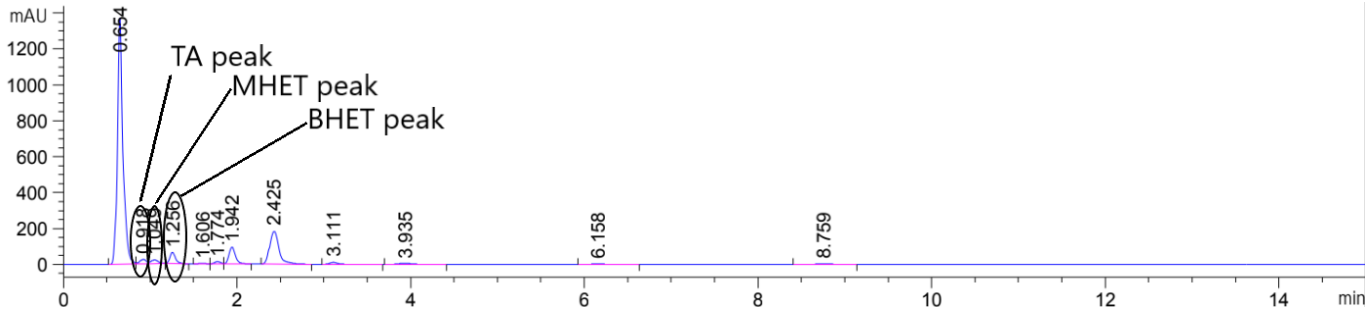
3.3 Reaction of PET with thiols

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

(A)



(B)



(C)

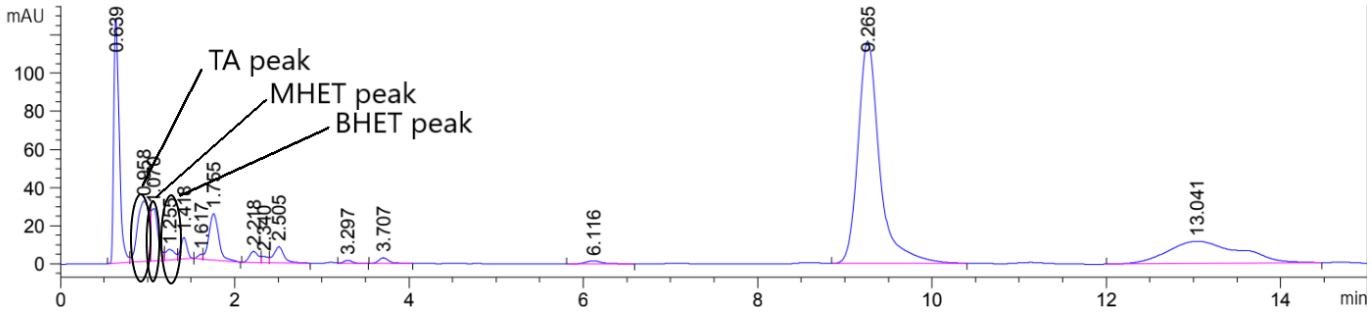
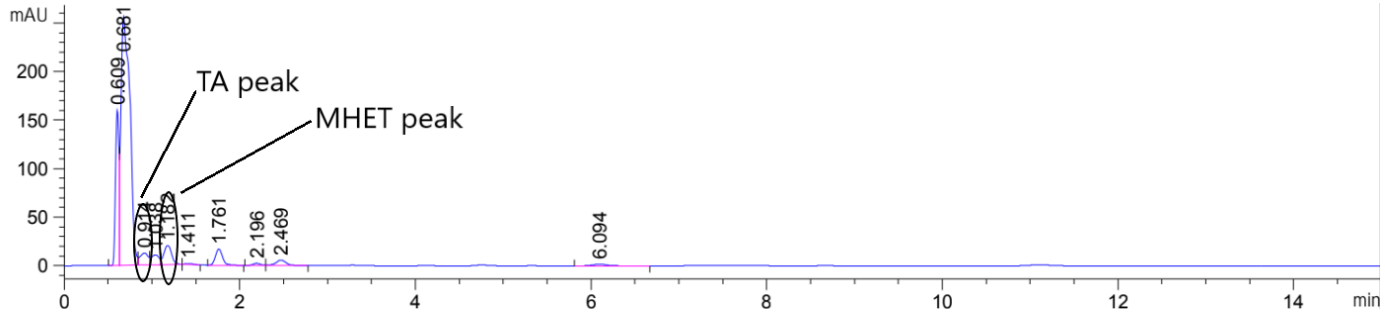
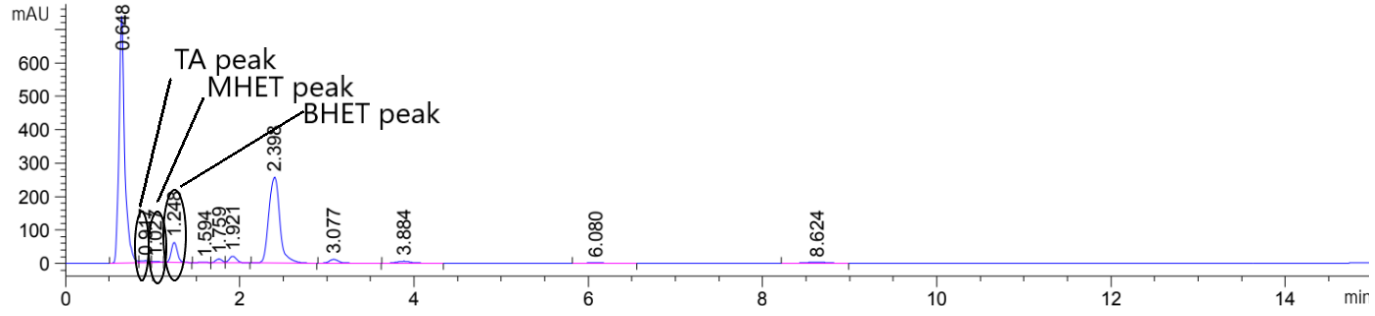


Figure 3. Reaction of PET with (A) 2-mercaptoethanol, (B) 4-acetamidothiophenol and (C) 2-aminothiophenol using method AO of pretreatment

(A)



(B)



(C)

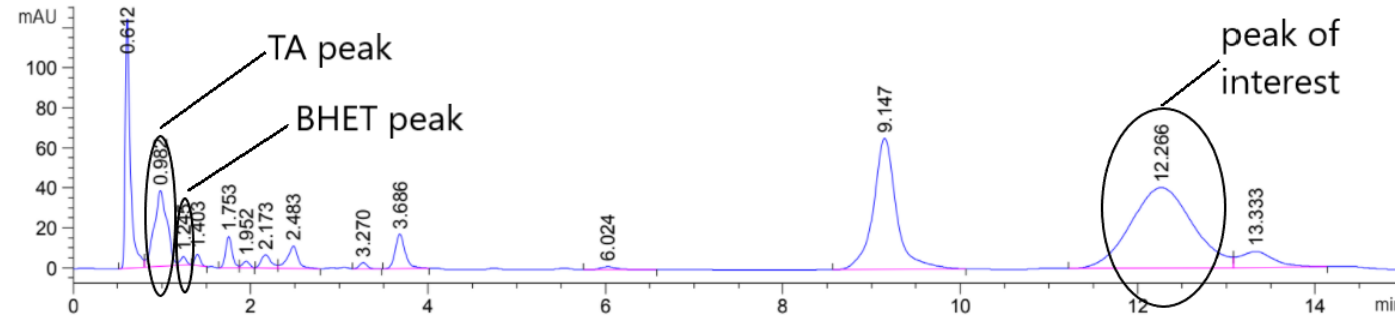


Figure 4. Reaction of PET with (A) 2-mercaptoethanol, (B) 4-acetamidothiophenol and (C) 2-aminothiophenol using method AS of pretreatment

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.

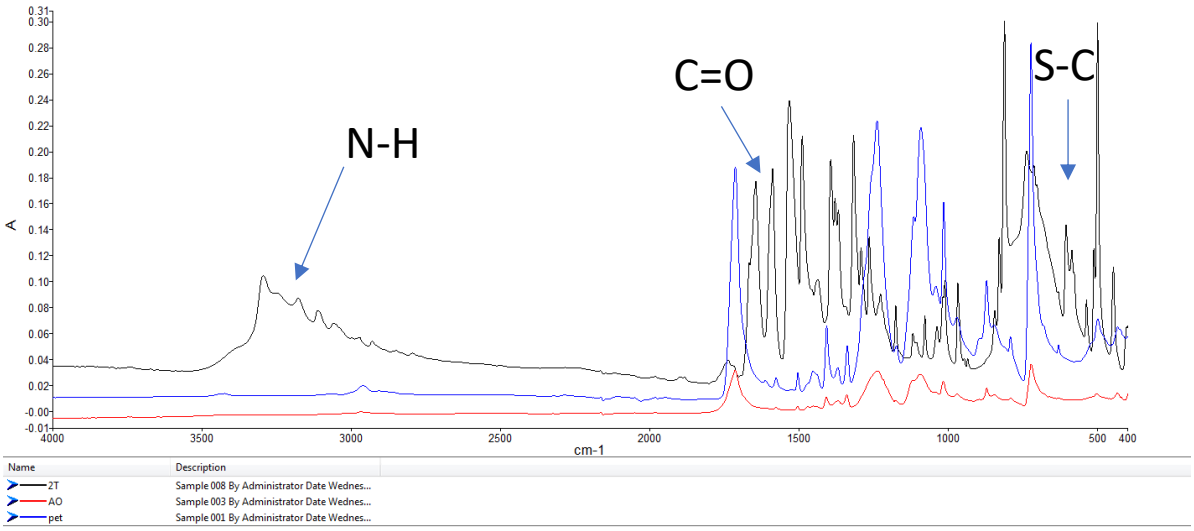


Figure 5. Overlayed spectrums of PET before pre-treatment (blue), PET after pre-treatment (red) and PET after reaction with 4-acetamidothiophenol (black)

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.

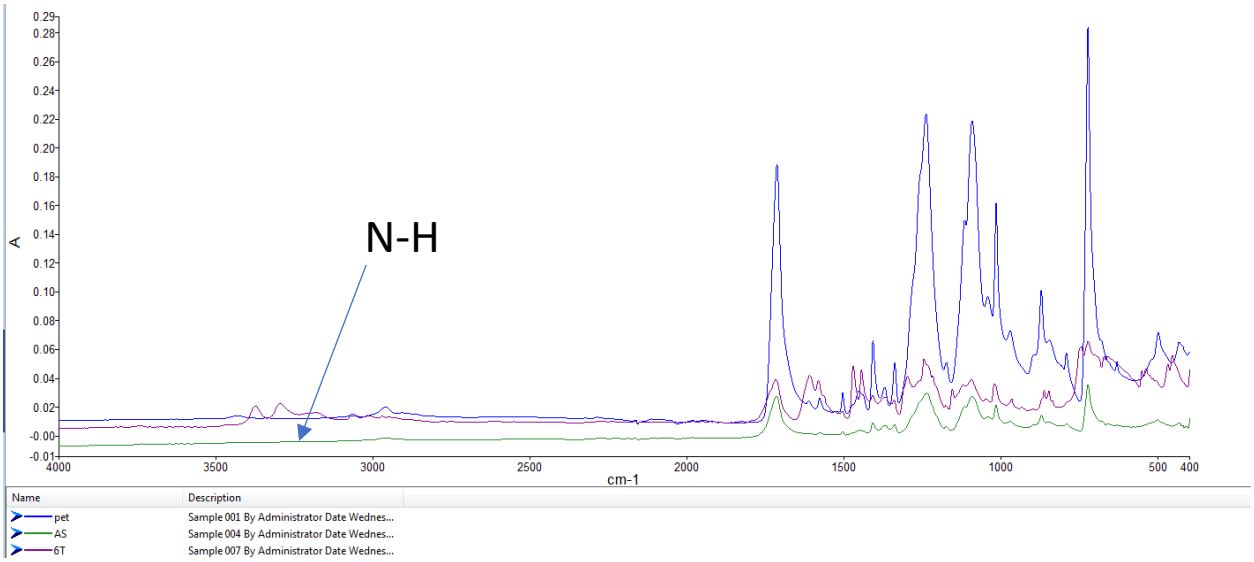


Figure 1. Overlayed spectrums of PET before pre-treatment (blue), PET after pre-treatment (green) and PET after reaction with 2-aminothiophenol (purple)

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

3.4 Reaction of BHET with aniline

The same method with BHET as in 3.2 was used, but this time different peroxidases and aniline were put in the reaction. The liquid phase was analysed using HPLC.

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.

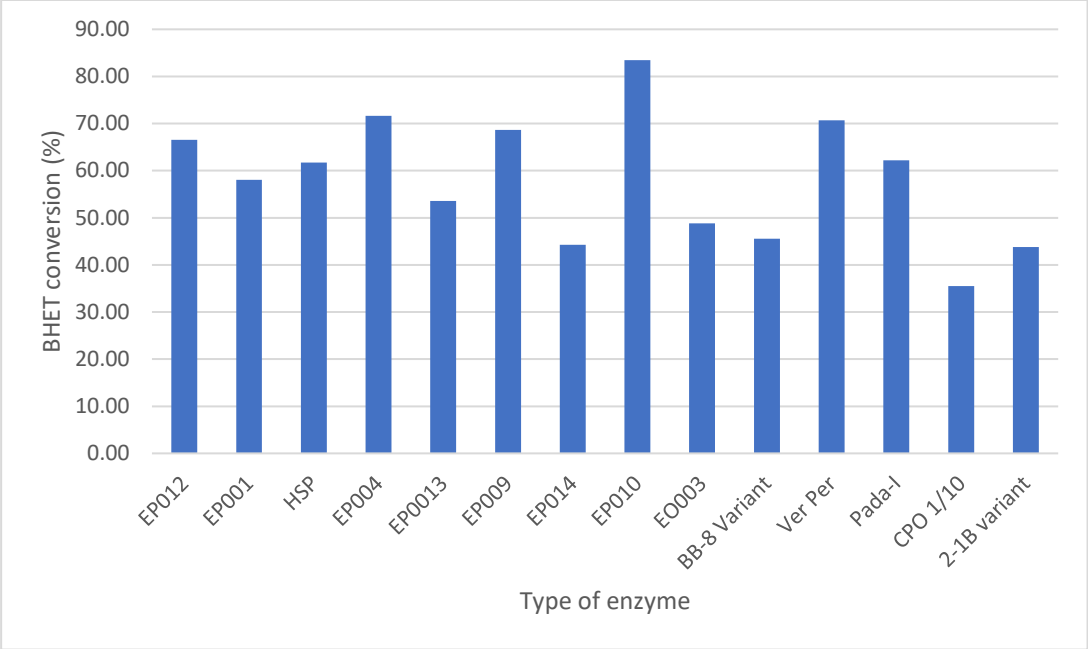


Figure 7. Enzyme effect on the biocatalytic reaction between BHET and aniline. Experimental conditions: 0.001 g BHET (5 mM), 5 µL H_2O_2, 1 µL anilina (10 mM), 100 µL enzyme and 894 µL Tris hydrochloride for 24 hours at 40° C and 1000 rotation/min

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.

3.5 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 2. 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

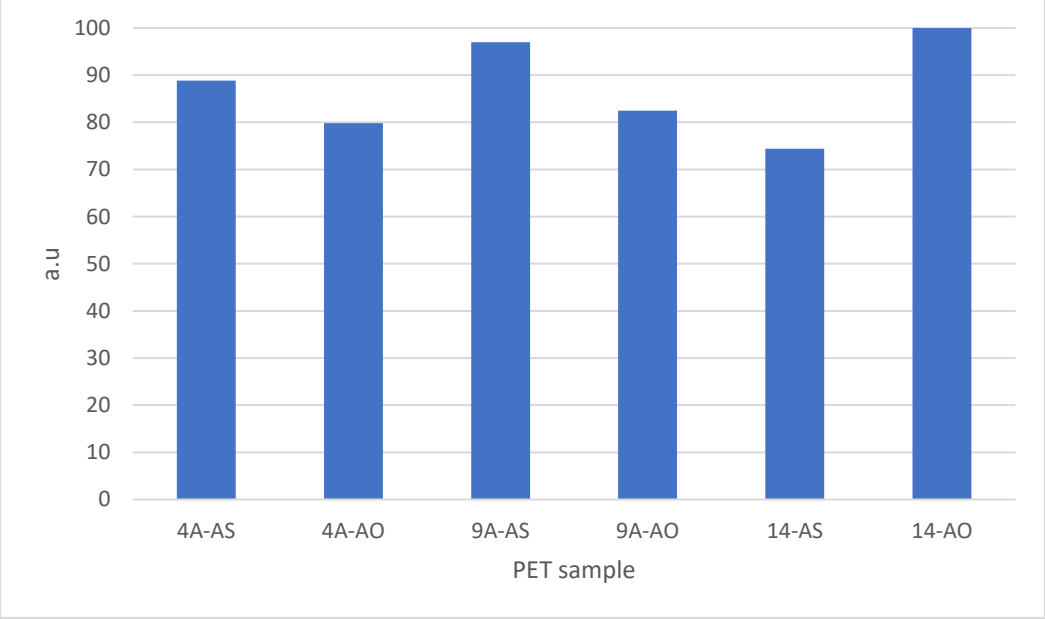


Figure 8. Evaluation of the liquid phase after the reaction between PET and aniline(peroxidase enzyme as biocatalyst). Experimental conditions: 0.01 g PET (45 mM), 5 µL H_2O_2, 9 µL anilina, 100 µL of peroxidase and 884 µL Tris hydrochloride for 72 hours at 40° C at 1000 rotation/min

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.

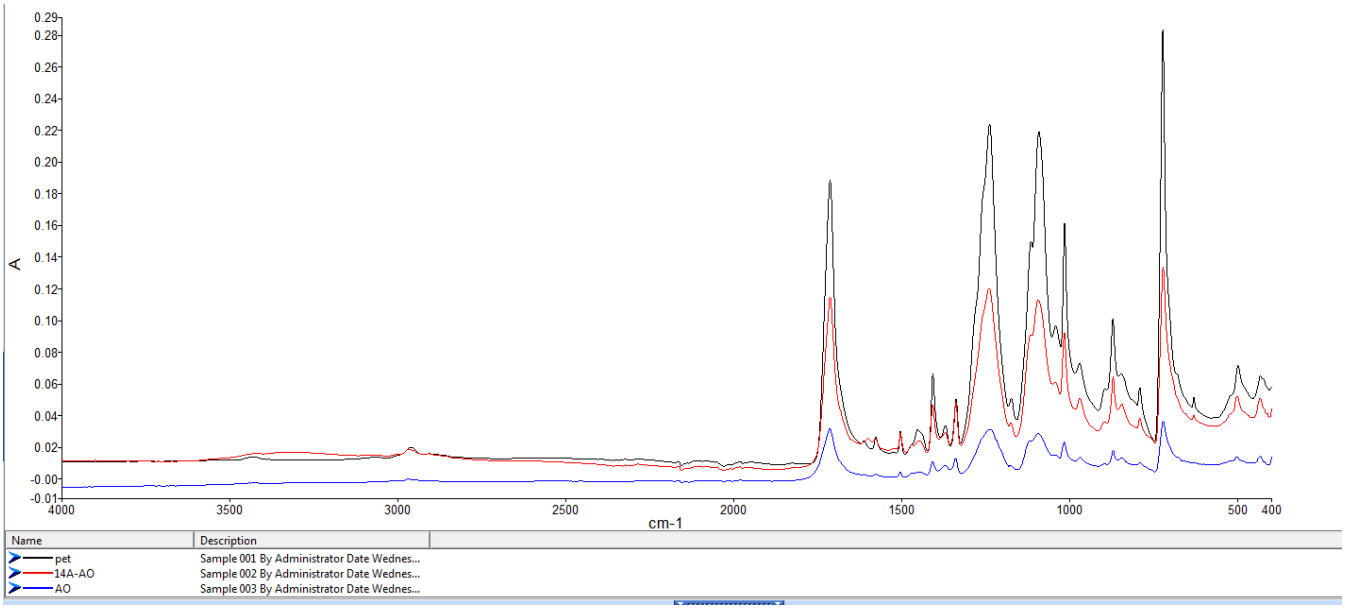


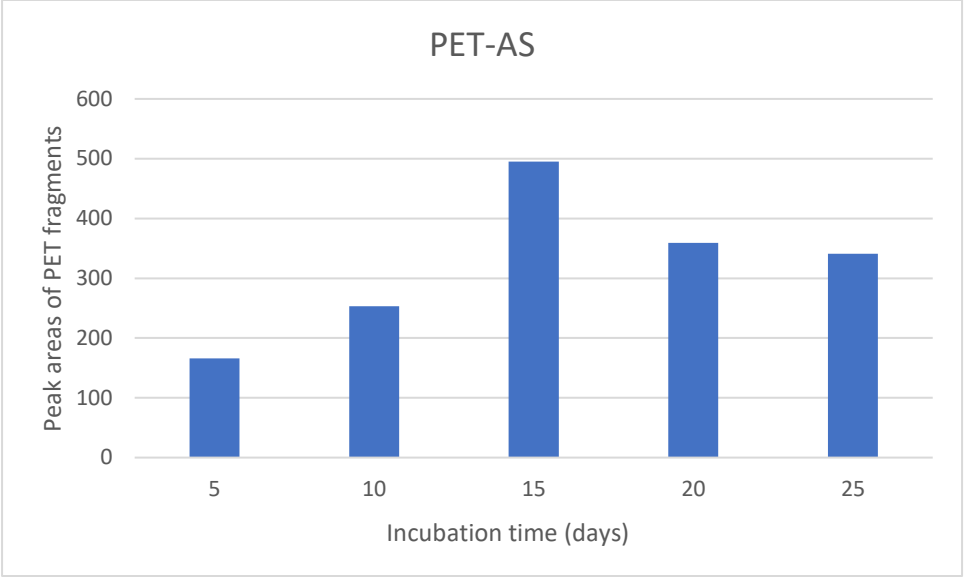
Figure 2. Overlayed spectrums of PET before pre-treatment (black), PET after pre-treatment (blue) and PET after reaction with aniline-peroxidase (red)

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.

3.6 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.



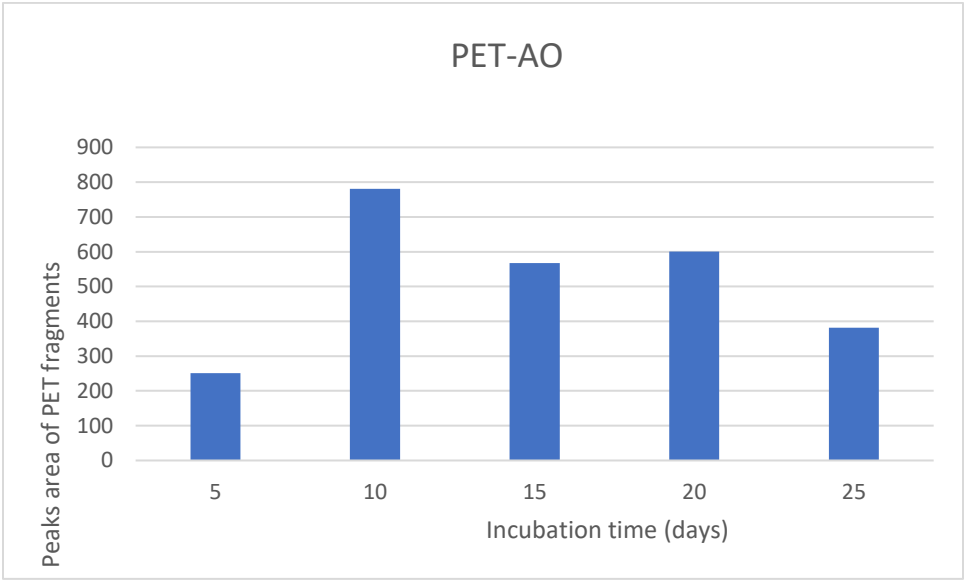


Figure 9. Area of peaks of interest for PET pre-treated using method AO and AS during different periods of time

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.

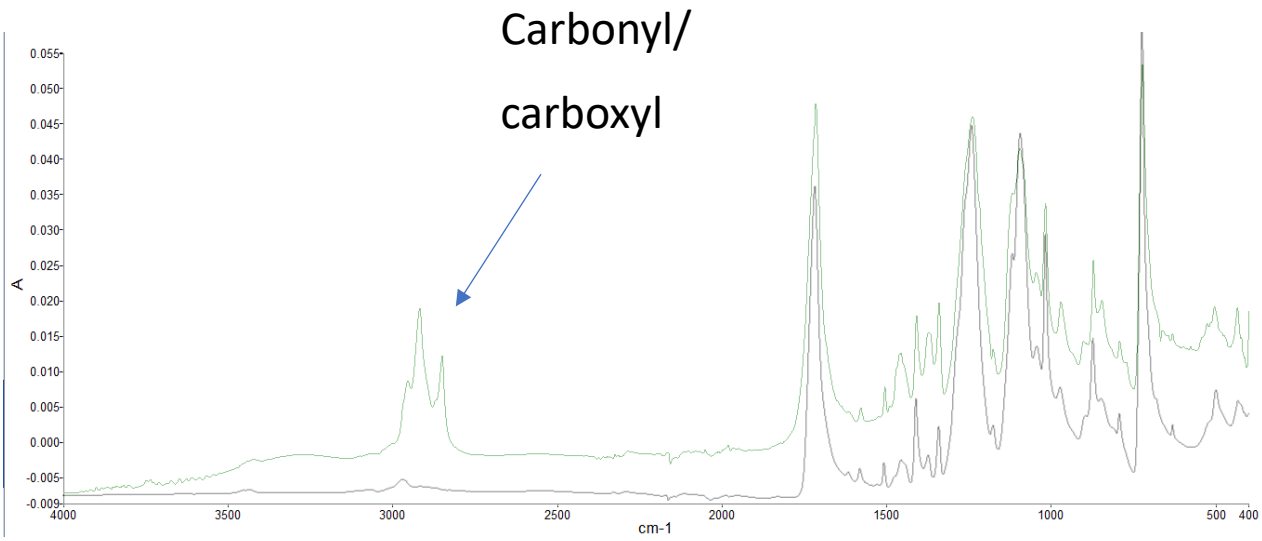


Figure 10. Overlayed spectrum of PET before pre-treatment (gray) and PET after a 15 days reaction with DMC (green)

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.

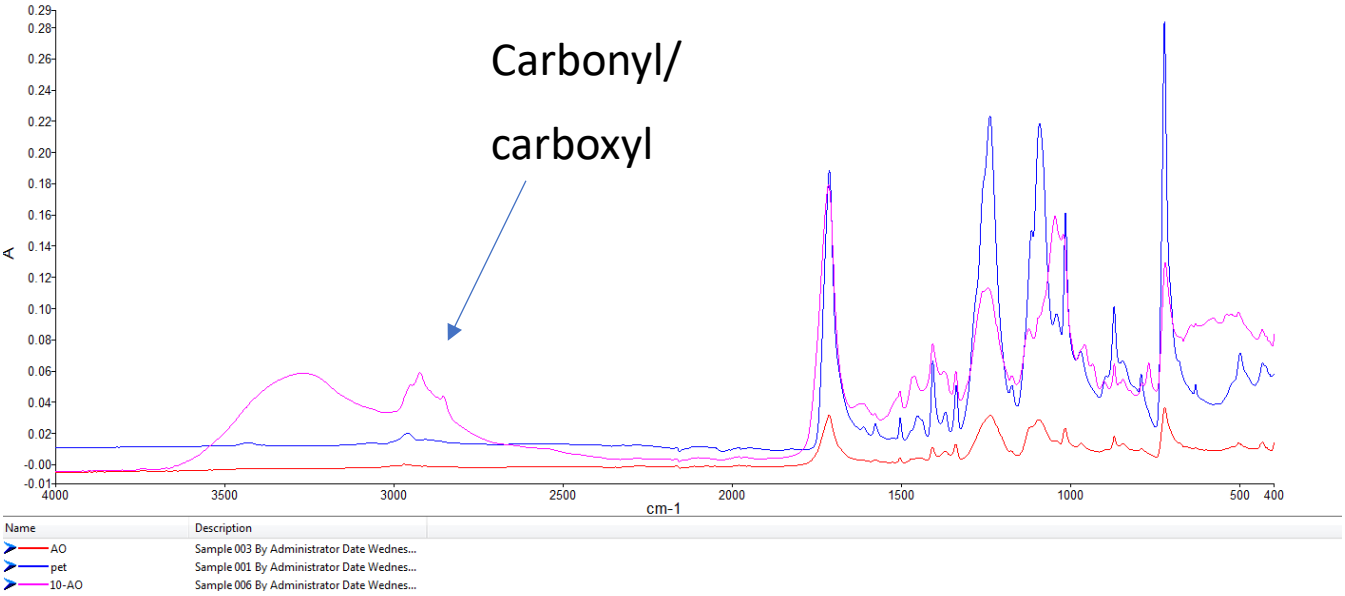


Figure 11. Overlayed spectrum of PET before pre-treatment (blue), PET after pre-treatment (red) and PET after a 10 days reaction with DMC (purple)

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.

3.7 DLS

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

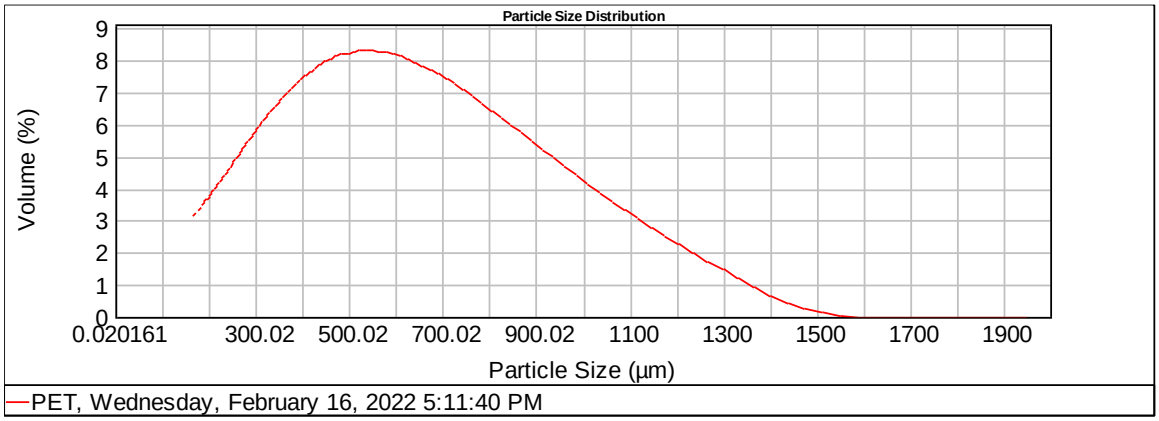


Figure 12. DLS spectrum

Table 3. 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	0.08	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.

4. 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 will be used as stabilizer for polysaccharide (alginate/carageenan) cavity prepared for enzyme immobilization. Cold active lipase was evaluated for silybin esterification. Strong solvent effect was detected on the enzyme activity. In this case, a solution can be the entrapment of the lipase in polysaccharide cavity protecting the proteinic structure against solvent attack. Usually, such kind of immobilization leads to unstable structures.

We proposed the used of modified PET as stabilizer of the enzyme immobilization cavity. Modification of the PET surface involved the insertion of -NH2, -SH or -CO- groups. All the insertion can be ionised and finally the PET surface can interact with the proteinic structure of the enzyme inside of the polysaccharide cavity. In this way, two aspects can be covered: (i) enzyme space will be conserved during the biocatalytic reaction and (ii) enzyme structure can be preserved due to the proximity of the PET surface (immobilized lipase more stable than the free lipase!).

5. References

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