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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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FACULTY OF CHEMISTRY

DEPARTMENT OF ORGANIC CHEMISTRY, BIOCHEMISTRY AND CATALYSYS

Enzymatic degradation of PET

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Summary

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

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 propose to test and to optimize various methods of PET pretreatment before decompozition in the enzymatic system. In this way, the PET surface will be destabilised and the enzyme attach through hydrolysis will be easier performed. PET decomposition should exhibit better conversion.. Characterize of the treated surfaces and also th identification of the products resulting from the PET degradation will be the aims for next experiments. For this time, a BHET model system has been also tested for DES conditions. 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. 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.

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

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

2.2. Methods of sample preparation

2.2.1 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.

2.2.2 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.

2.3 Methods of sample pre-treatment before analysis

2.3.1 Pre-treatment of BHET samples

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.

2.3.2 Pre-treatment of PET samples

After the reaction, the PET pieces were washed with distilled water and then weighed. Their final weight was compared with their initial weight.

The liquid phase was pretreated as decribed in section 2.3.1. Pre-treatment of BHET samples

2.4 Method for analysis

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˚C with a flow rate of 1 µL/min mobile phase (20 % acetonitrile and 20 % H_2SO_4 (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 AT, 1. 14 for MHET and 1.31 min for BHET, as it can be observed in the chromatograms.

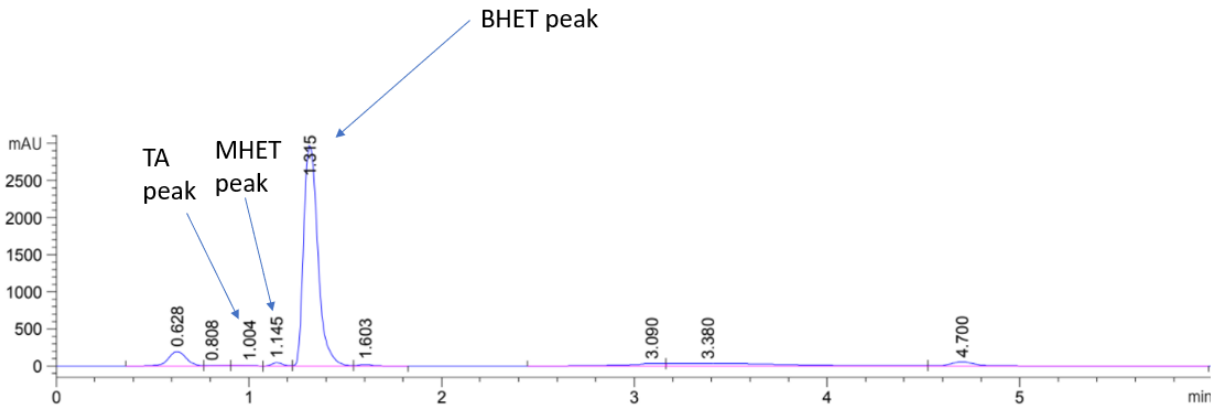

Figure 1. Chromatogram of the reaction mixture after BHET hydrolysis in DES 1 using lipase from Arpergillus niger

2.5 Results and discussions

2.5.1 The PET degradation

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

Five different methods of pre-treatment were used and listed in the table below.

Table 1. Different methods for the pre-treatment of PET

Method 1	Method 2	Method 3	Method 4	Method 5		
Immersion in aqueous solution 2g/L Na_2CO_3 at 37°C (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°C	Dry in the oven at 40°C	Immersion in aqueous solution 2g/L Na2CO3 at 37°C for 1 hour	Dry in the oven at 40°C	Dry in the oven at 40°C
		Washing with distilled water		
		Dry in the oven at 40°C		

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

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 were plotted in the graphics below.

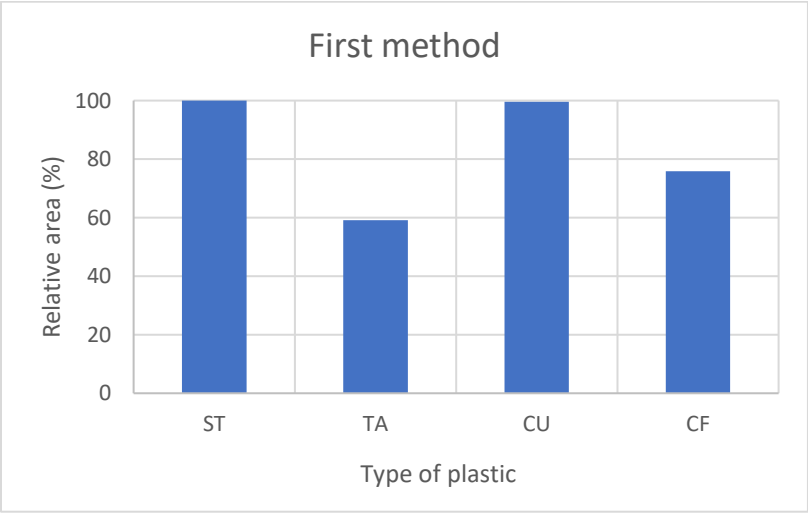

Figure 2. Relative area calculated for each type of plastic that was pretreated with the first method

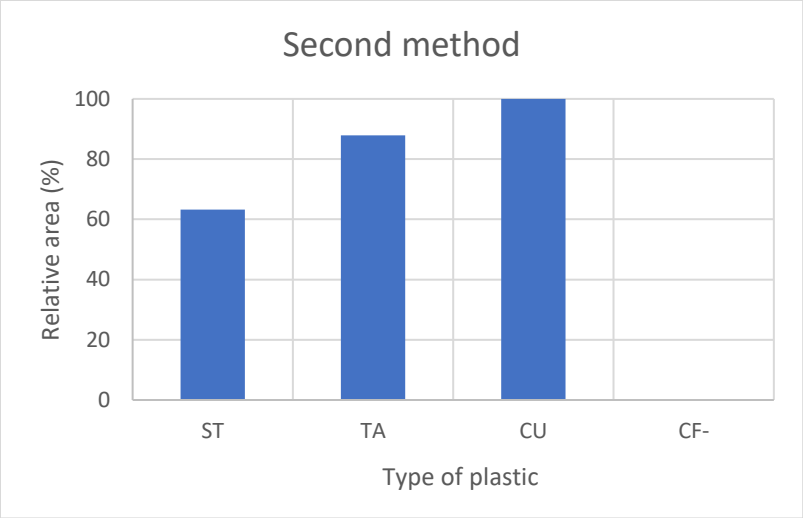

Figure 3. Relative area calculated for each type of plastic that was pretreated with the second method

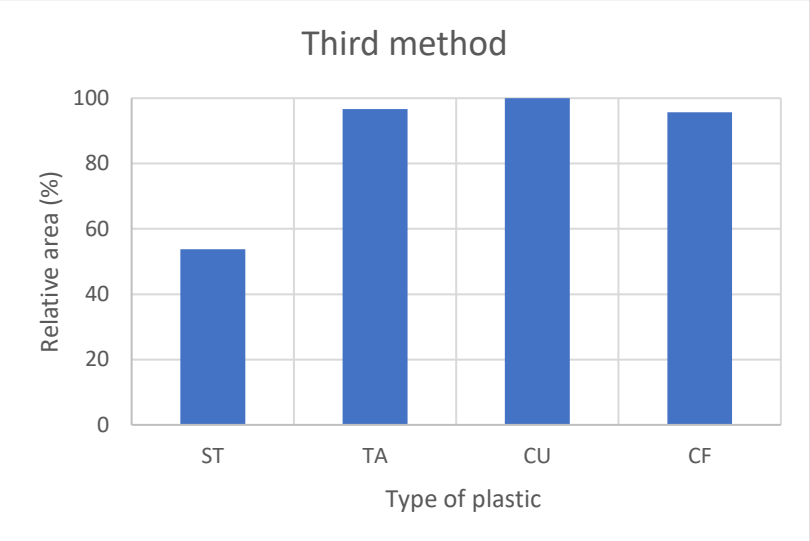

Figure 4. Relative area calculated for each type of plastic that was pretreated with the third method

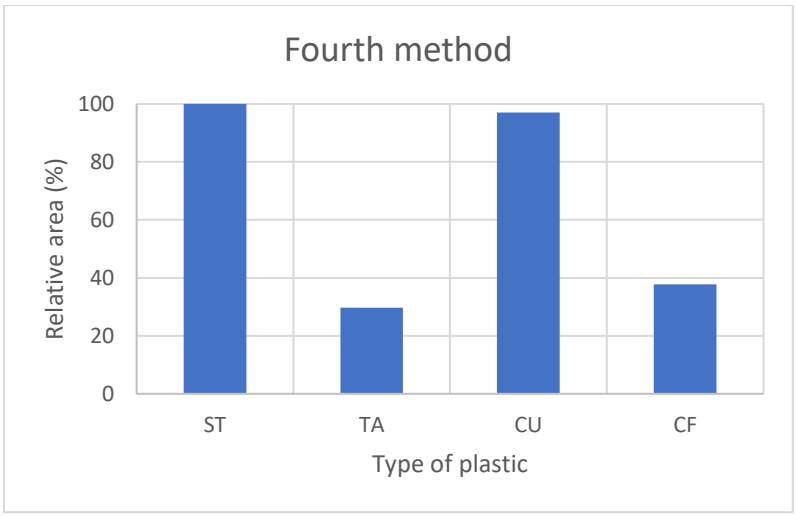

Figure 5. Relative area calculated for each type of plastic that was pretreated with the fourth method

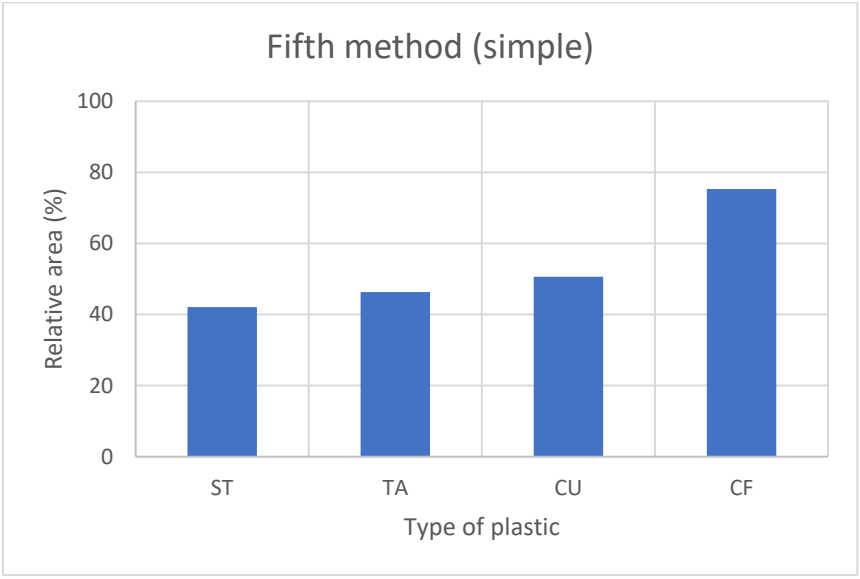

Figure 6. Relative area calculated for each type of plastic that was pretreated with the fifth method

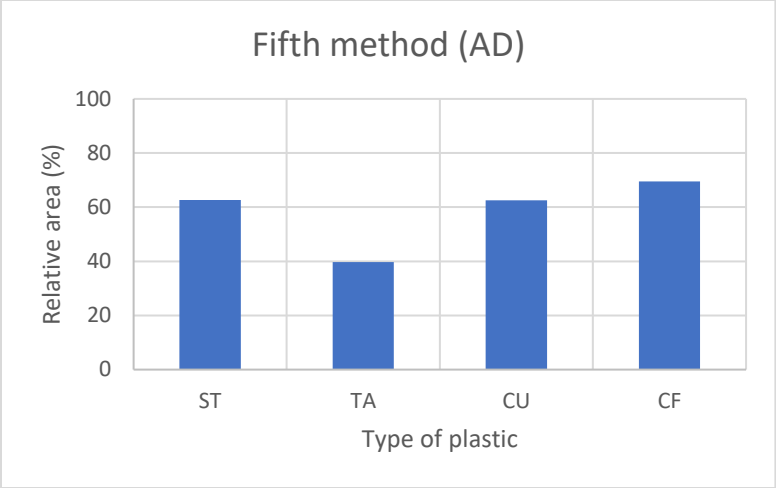

Figure 7. Relative area calculated for each type of plastic that was pretreated with the fifth method (in distilled water)

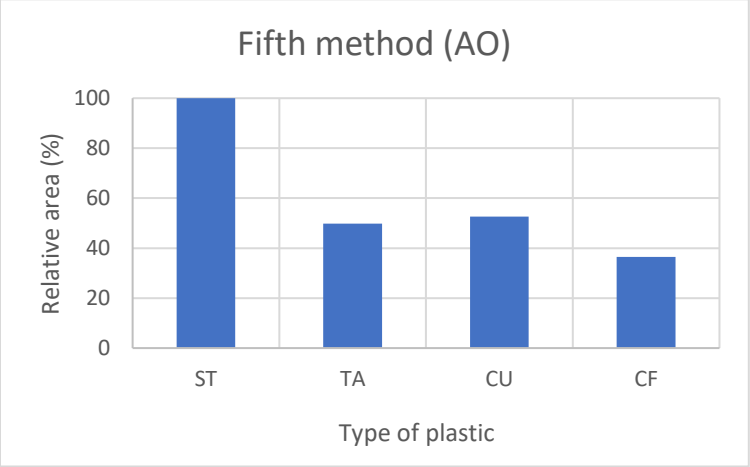

Figure 8. Relative area calculated for each type of plastic that was pretreated with the fifth method (in ***hydrogen peroxide)***

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 consider.

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).

2.5.2 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.

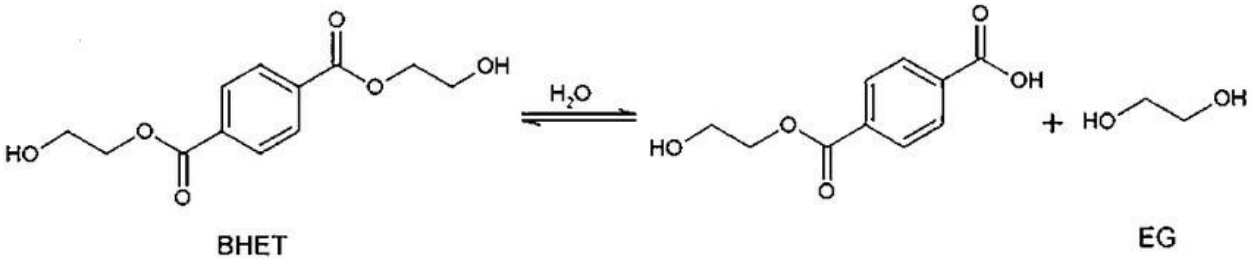

Figure 9. BHET hydrolysis [9]

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.

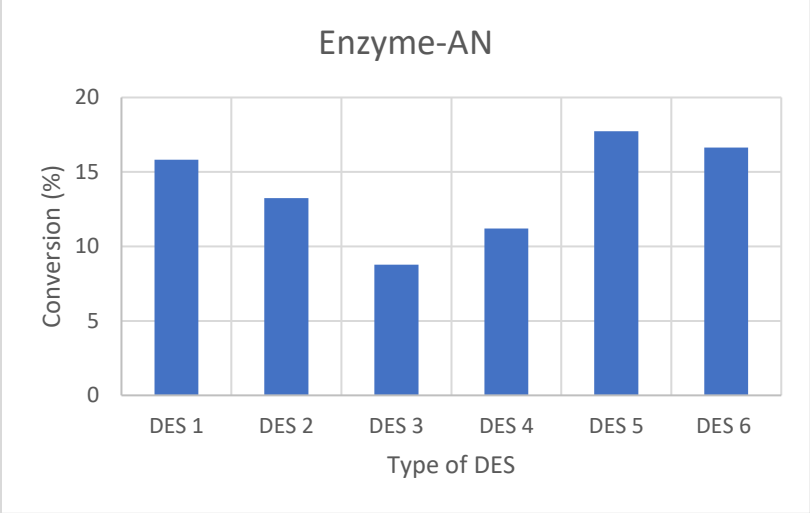

Figure 10. Conversion according with each type of DES for the enzyme Aspergillus niger

In figure 11, 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.

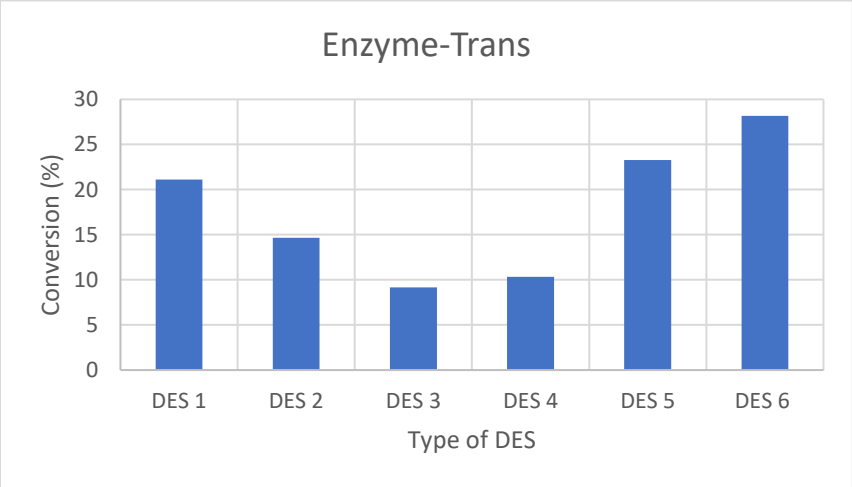

Figure 11. Conversion according with each typed of DES for the enzyme Transenzyme

In figure 12, experimental results for BHET system using Immobilized lipase Lypozime TL1M are presented. DES 5 and 6 exhibited maximum conversion of 17.9% and 21.9%. Low conversion was noticed also for DES3

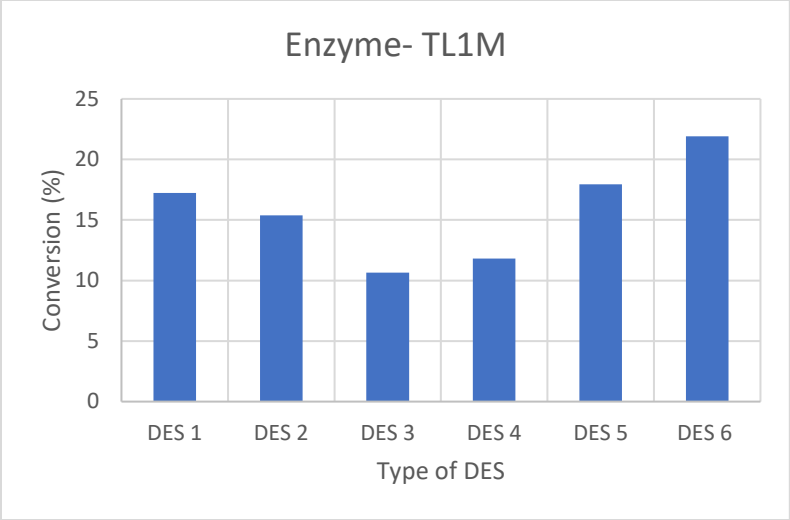

Figure 12. Conversion according with each typed of DES for the enzyme Lypozime TLIM

In figure 13, experimental results for BHET system using Immobilized lipase Novozyme 425 are presented. DES 5 and 4 exhibited maximum conversion of 26.9% and 18.5%. Low conversion was noticed for DES 1, 2 and 3.

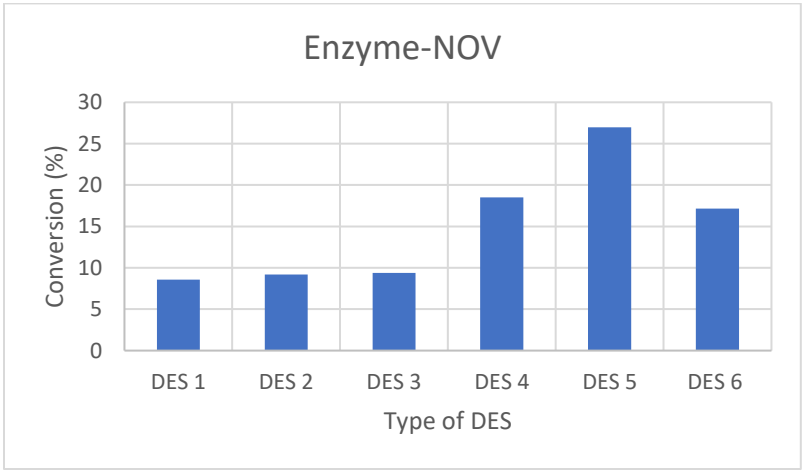

Figure 13. Conversion according with each typed of DES for the enzyme Novozyme 425

In figure 14, experimental results for BHET system using Immobilized lipase Lypozime RMIM are presented. DES 5 and 6 exhibited maximum conversion of 22.5% and 21.9%. Low conversion was noticed for DES 2 and 4.

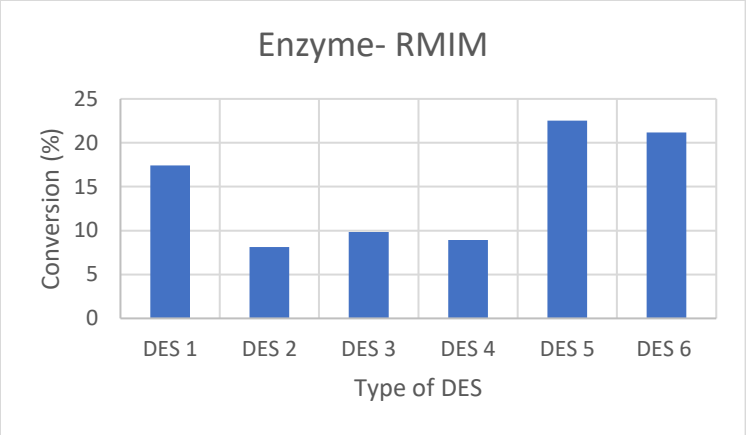

Figure 14. Conversion according with each typed of DES for the enzyme Lypozime RMIM

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.

3. Conclusions

In this experimental study different pretreatments of the PET surface have been performed. Then, the PET samples were degraded based on lipase catalysis in the presence of DMC. Best performance was noticed for methods 1,2 and 3.

On the other hand, BHET model system was evaluated in DES environment based on the lipase catalysis activity. Different types of DES were tested taking into account the solubility of BHET in DES phase. After that, BHET was hydrolyzed in DES with several types of enzymes. The best couples enzyme-DES chosen for next experiments 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 study will be continued on both direction: BHET system in DES will be optimized and adapted for PET degradation. Also, sample pretreatment of the PET will be coupled to the degradation system in order to improve the accessibility of the enzyme on the PET surface for a better PET degradation.

4. References

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