

A Persistence Search for the Most Appropriate Process of PET Recycling: A Review Paper

KEY WORDS: Plastic, PET Recycling

ABSTRACT

One of the most serious threats to the environment in today's world is plastic pollution. The reason for widespread of plastic is its poor disposal management, indiscriminate use of plastic and its related products. There was a tremendous increase in the production of plastic from the start of 21st century due to its high demand which tripled the waste in these two decades. This review paper aims at providing the understanding of various techniques used for PET plastic degrading process and currently used in large scale that is quite detrimental to the environment. Further, the recent discovery of the bacteria eating enzyme provided a shaft of light in waste green recycling process. Adding to this, there is an outline provided for bioengineering of the most preferred enzymes for hydrolysis process result is compared that which one is more efficient one. Comparing them and trying to exploring the potential of various mutated enzymes for hydrolyzing of plastic waste formulated by various researchers to identify the Nobel PET catalyst which can solve the massive environment crisis when used in large scale.

INTRODUCTION

Polyethylene terephthalate (PET or PETE) aromatic polyester of family polymer is a general-purpose thermoplastic POLYMER that consists of non-hydrolyzable covalent bond (Levchik, S.V 2004). When used for fabrics it is known as polyesters and in case of bottles, container, packaging applications it is called as PET or PET Resin (Müller, 2006). There are two ways to obtain the product from poly-condensation reaction of monomers.

- Trans-esterification occurring between the monomers named terephthalic acid and ethylene glycol.
- Esterification reaction of monomers, ethylene glycol and terephthalic acid (Lechner, 2014; Burnley, 2015).

PET is colorless, flexible, and depending upon its process, it can be semi-rigid or rigid. In natural form it is found in semi-crystalline state and is most widely used by

packaging industries because of its thermostability and durability. Prominently, most often it is used in food jars, plastic bottles of soft drinks and plastic film (Joo S et al. 2018).

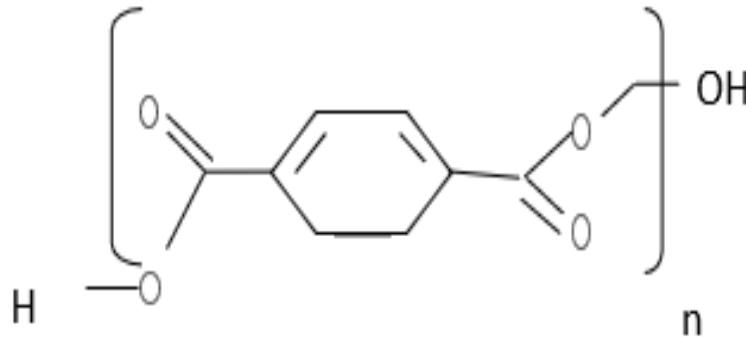


Figure 1: Polyethylene Terephthalate Molecular Structure [Chemical formula: $(C_{10}H_8O_4)_n$]

HISTORY

During the mid-1940s, the first PET was synthesized in the U.S. by Dupont chemists while searching for polymers for making new textile fibers. Later, these fibers came to be known as Dacron. In the late 1950s, to create PET films a way was found by researchers to stretch these thin sheets of PET today which is used as X-ray film, packaging material and photographic etc. With advancement in technology, PET was blow-stretched molded into strong, shatterproof, and lightweight bottles which gained market acceptance in 1973. In the end of 1970s, the very first PET bottle was recycled.

CONVENTIONAL METHODS FOR THE RECYCLING PET PLASTIC

Most conventional methods used for degradation process until now are mechanical, chemical, and biological. Land filling of plastic is the easiest one used worldwide but it has disadvantages to it. Due to anaerobic conditions the rate of degradation is quite low. There is no effect of solar radiation and UV on the degrading process of these are dumped in rivers, oceans, landfills including the terrestrial environment that is lethal to marine animals (Cleary, 2014; Abdel-Shafy, 2018; Palm, 2019).

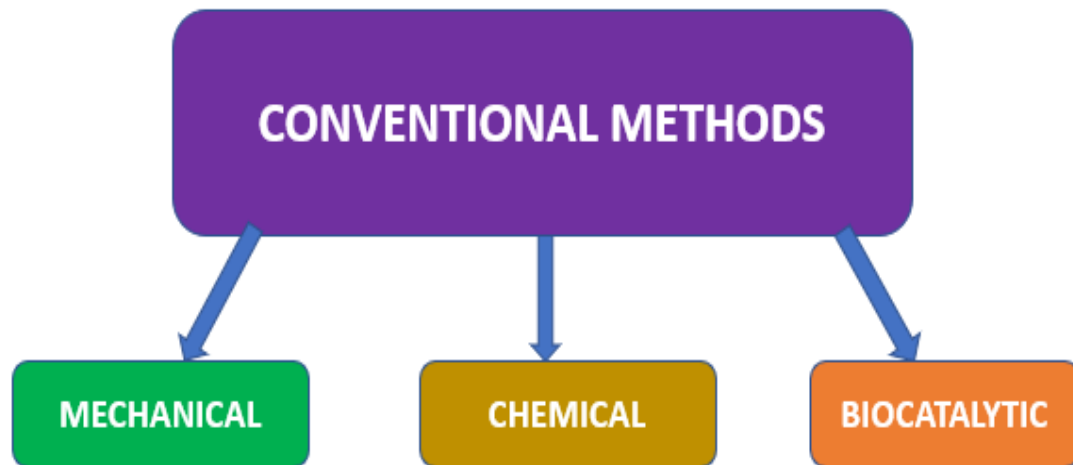


Figure 2: Conventional Methods for the Recycling PET Plastic

An alternate method to overcome this problem is converting this waste into reusable material by recycling process. Three main methods known today for recycling processing are:

1. Mechanical Treatment:

This method is one of the most widely used in recycling PET waste where the basic structure remains the same. This technique is performed into stages, firstly the sorting, shredding, melting of the plastic waste is being done that return plastic to its original form (Grigore, 2017).

2. Chemical Treatment: Through acidic, alkaline and neutral approaches chemical treatment is being done for de-polymerization of plastic into its constituent monomer by hydrolysis and glycolysis. (Garcia, 2017).

3. Biocatalytic Treatment: Involvement of microorganism for the degrading process is involved in this type of treatment. Through enzymatic activity cleavage of polymer bonds occur (Ribitsch 2011; Kale 2015).



Figure 3: PET Bottle Benefits (Amcor plc 2020)

Biological approach for this process that includes enzymatic hydrolysis due to the presence of the bond. Through hydroxylation by hydrolytic enzyme PET converted into its monomers TPA, MHE, EG and BHET (Ribitsch, 2011; Qasaimeh 2016). Although there were numerous enzymes reported for this Degradation process, the rate of process was low. Factors includes crystallinity, hydrophobicity, and structure most often limits enzyme function in the flowchart, there discussed various enzymes used in degrading process. Major source for cutinase is bacteria and fungi. It is a serine esterase consist of catalytic triad of residue Ser-his-asp residue (Cleary, J. 2014 Müller, R.J 2005). Cutinase is viewed as most promising enzyme because of versatility of its hydrolysing property, catalyze esterfication including transesterification reaction. It is also used as major substitute to harsh chemicals. Lipase is well known for surface modification. It is more effective when produced by bacteria in conversion of PET to its intermediate product MHET, increased by 50 folds higher while comparison done, with fungal one. When used singly, this enzyme have lower efficiency towards hydrolysis of PET(Cleary, 2014). Leaf-branch compost cutinases (LCC), a lipolytic esterolytic enzymes belongs to the family of lipases but the two enzyme families are showing different catalytic behavior toward PET degradation(Longhi and Cambillau 1999).

	Mechanical Treatment	Chemical Treatment	Catalytic Treatment
Advantage	Cost effective, most commonly used, plastic products reused without any alteration in its structure, degrading rate is quite fast.	Simple method for treatment of enzymes, degrading rate is fast.	Byproduct formed can be used for different applications. Highly time consuming, eco-friendly.
Disadvantage	Limited to monomer plastic, not environment friendly, cannot be handled because of Temperature sensitivity.	Catalyst required are of high cost and energy consuming, eco friendly, from economical point of view not so effective.	When compared With chemical as well as mechanical treatment, this is quiet time consuming.

Table 1: Comparison of Advantages and Disadvantages of Major Types of Treatment Methods for Recycling Process used for recycling purpose (Joo et al., 2018).

ADVENT OF PETase ENZYME

In 2016, Yoshida and his team isolated a novel bacterium named Ideonella Sakaiensis 201-F6 while screening natural community of bacteria that was exposed to PET in the environment. These bacteria used PET as energy and carbon source. Also produced two

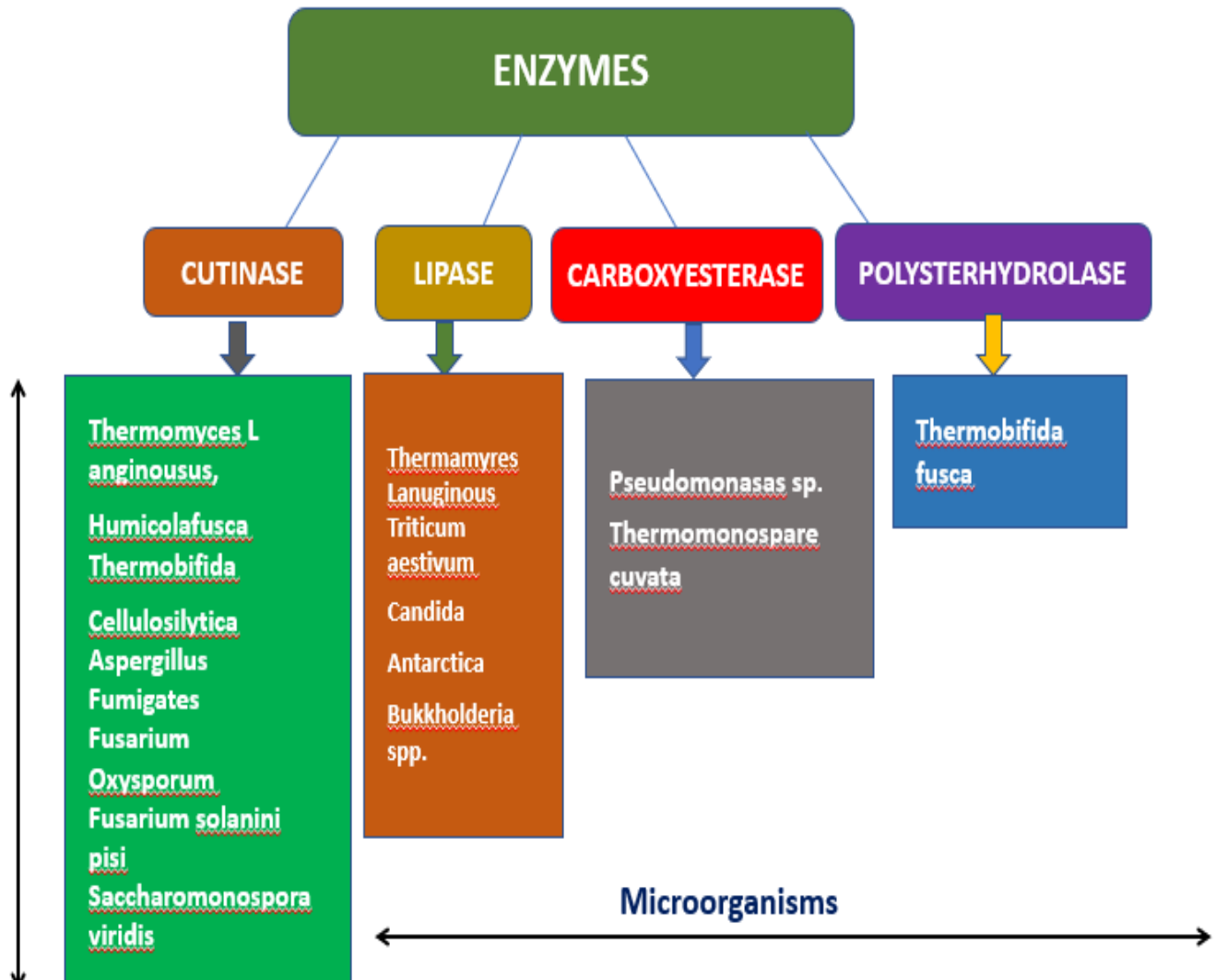


Figure 4: Various Microbial Enzymes which are Known to Hydrolyze PET

enzymes when grown on PET that were capable of hydrolyzing PET (Austin,2018).Two enzymes identified by researchers from the bacteria were:

1. PETase (Han, 2017)
2. Mono (2hydroxy ethyl) terephthalic acid hydrolase(MHETASE)(Longhi and Cambillau,1999).

Both of these enzymes were required to enzymatically PET into its constituent monomers, terephthalic acid (TPA) and ethylene glycol (EG). It was found that much of the work was done by PETase enzyme so further analysis was done on this enzyme and very less information is there about MHETase which act on Mono-(2-hydroxyethyl)terephthalic acid (Yang 2013).



Figure 5: Systematic Diagram Depicting the Bacteria Producing Enzyme and the Complete Mechanism of Degradation (Jang et al. 2017).

ENGINEERING PLASTIC EATING ENZYME

The University of Campinas in Brazil and Scientist at University of South Florida during structure study of PETase enzyme discovered that PETase is quite similar to cutinase but differ by having more active site. In order to get a better understanding of the enzymes Gregg Beckham at NREL and Professor John McGeehan of Portsmouth after the discovery of bacteria solved PET structure by using 3D information and inadvertently engineered an enzyme whose degrading rate was good when compared with the one that evolved in nature (Yang, 2016; Joo 2018). The Goal was to determine the structure and they just ended up moving a step further by engineering more efficient enzyme. This suggested the researcher that there is a room for further improvement of these enzymes. Further the process of bioengineering becomes more appealing due to the presence of computer aided engineering of enzymes. There was a way explored when solved the structure of LC cutinase, IsPETase, and Tfcutinase. Active site modification in protein engineering is considered to be the most effective one via site active mutagenesis (Yang, 2013; Tournier, 2020).

ENZYME	PRODUCT	MUTATION(s)	RESULT(Biological Effects)	REFERENCE
Leaf branch compostinase	TPA, EG	F243I/D238C/S283C/Y127 GF243I/D238C/S283C/N246M 6MF243W/D238C/S283C/Y127 GF243W/D238C/S283C/N246M	Achieved 92% of PET degradation into monomers, including productivity of 16.7 g of terephthalate/L/h or 10 h. improve melting temperatures by 9.8°C which is higher than wildtype LCC (18).	(Tournier et al. 2020)
IsPETase	MHET, EG, TPA	S160A D206A H237A Y87A W185A M161A I208A W159A N241A S238A R280A C203A/C239A	R280A increased PETase catalytic activity by 22.4% and 32.4% in 18 h and 36 h, respectively compared with IsPETase wildtype (17).	(Joo et al. 2018)
IsPETase	MHET, EG, TPA	W185A S238F/W159H	The absolute crystallinity loss is 4.13% more than IsPETase wildtype (16)	(Austin et al. 2018)
MHETase	TPA, EG	S419G, S419G_F424N, W397A	S419G, S419G_F424N: That enhance the enzyme affinity and activity toward BHET, W397A: Enhanced activity of enzyme toward MHET (19)	(Palm et al. 2019)
IsPETase	BHET,	S214H-I168R15-W159H-	Enhanced IsPETase	(Cui et

		S188Q-	thermostability for 3	al.,2019)
	MHET,TA	R280A-A180I-G165A- Q119Y-L117F-T140D (DuraPETase)	Enhanced activity	
IsPETase	MHET,EG, TPA	R61A,L88F, I179F	Enhanced catalytic activity of PETase by 1.4, 2.1, and 2.5 fold more than the wild type. I179F mutant showed highest degradation rate (22.5 mg. μmol/L)(21)	(Maetal.2018)

Table2: Different Types of Mutations Performed to Improve Productivity and Catalytic Performance of PET Hydrolysis.

CONCLUSION REMARKS

From time to time there has been a great advancement in the treatment of the waste accumulated through plastic. Among various methods available for PET recycling, the most environmentally friendly method considered is the enzymatic one. The variant of LCutinase through protein engineering strategy proved to be most effective one but the cost of enzyme production is high. Currently there are some researchers who still believe that IsPETase is the best one for hydrolysis purpose and some improvements need to be done on the basis of the viewpoint of material and enzymatic approach. Research for finding the most potential biological treatment strategy for recycling purpose is not moving in one direction. Recently, a study was performed where the functional expression in model green microalgae was demonstrated via HPLC analysis which was the first reported success for producing PETase in green microalgae that provides a standard for biodegradation purpose. Another study done last year, where these enzymes found in the bacteria named MHETase structure was studied and the was combined with PETase enzymes. The chimeric protein of PETase and MHETase improved degradation rate. SEM analysis of the digest ed amorphous films of PET confirmed degradation moving forward the design of multi enzyme system for mixed polymer waste is quite promising and also fruitfull area for continued investigation in a hope that PETase is the best suited one for hydrolysis of PET plastic.

A massive accumulation of plastic in landfill and water bodies is getting problematic. Traditional methods used recently seem bit detrimental leading to harm life forms in some way. Arrival of the bacteria that degrades plastic gave a ray of hope in the path of green recycling scheme. Besides this, the utilization of bioengineering strategy in industrial process will boost the productivity by improving thermo-stability; enhance performance of catalysts which will lead to reduction in PET crystallinity. Above all engineering that was being performed it was concluded that the engineered LCC enzyme was best candidate for hydrolysis of PET that's how, 90% depolymerization within 10hr with 16.7g TPA productivity/L/h. So further study in field of genetic improvement and large-scale production will fill need to resolve the problem in the most subtle way by bringing the recycling process closer to commercial as well as practical reality.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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