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

ABSTRACT

One of the most serious threats to the environment in today's world is plastic pollution. Thereason for widespread of plastic is its poor disposal management, indiscriminate use of plasticanditsrelatedproducts.Therewasatremendousincreaseintheproductionofplasticfromthe start of 21st century due to its high demand which tripled the waste in these two decades.This review papers aims at providingthe understanding ofvarious techniques usedfor PETplastic degrading process and currently used in large scale that is quite detrimental to theenvironment. Further, the recent discovery of the bacteria eating enzyme provided a shaft oflight in waste green recycling process.Adding to this, there is an outline provided forbioengineering of the most preferred enzymes for hydrolysis process result is compared thatwhich one is more efficient one. Comparing them and trying to exploring the potential ofvarious mutated enzymes for hydrolyzing of plastic waste formulated by various researchersto identify the Nobel PET catalyst which can solve the massive environment crisiswhenused in largescale.

KEY WORDS: Plastic, PET Recycling

INTRODUCTION

Polyethylene terephthalate (PET or PETE) aromatic polyester of family polymer is ageneral-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).Therearetwo ways toobtain theproduct from poly-condensation reactionofmonomers.

- Trans-esterification occurring between the monomers named terephthalicacidand ethyleneglycol.
- Esterificationreactionofmonomers,ethyleneglycolandterephthalicacid (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 packagingindustries because of itsthermostability and durability. Prominently, most oftenit is used infoodjars, plasticbottles ofsoftdrinks and plasticsfilm (JooS et al. 2018).

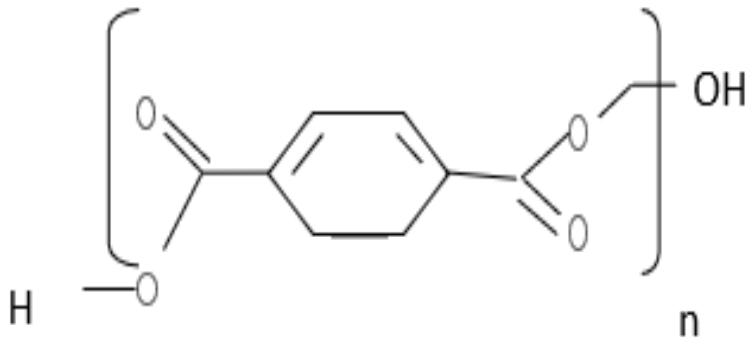

Figure 1: Polyethylene Terephthalate Molecular Structure [Chemical formula: $(C_{10}H_8O_4)n$]

HISTORY

During the mid-1940, first PET was synthesized in U.S by Dupont chemists while searching for polymers for making new textile fibers. Later, these fibers came to be known asDacron. In the late 1950s, to create PET films a way was found by researchers tostretch these thin sheets of PET today which is used as X-ray film, packaging material andphotographic etc. With advancement in technology, PET was blow-stretched molded intostrong, shatterproof, andlightweight bottles which gained market acceptance in 1973.In theendof1970s,thevery first PET bottlewas recycled.

CONVENTIONAL METHODSFOR THE RECYCLINGPETPLASTIC

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 disadvantage to it. Due to anaerobic conditions the rate of degradation is quite low. There is no effect of solar radiation and UV on degrading process of these are dumped in river, oceans, landfills including terrestrial environment that is lethal to marine animals (Cleary, 2014; Abdel-Shafy, 2018; Palm, 2019).

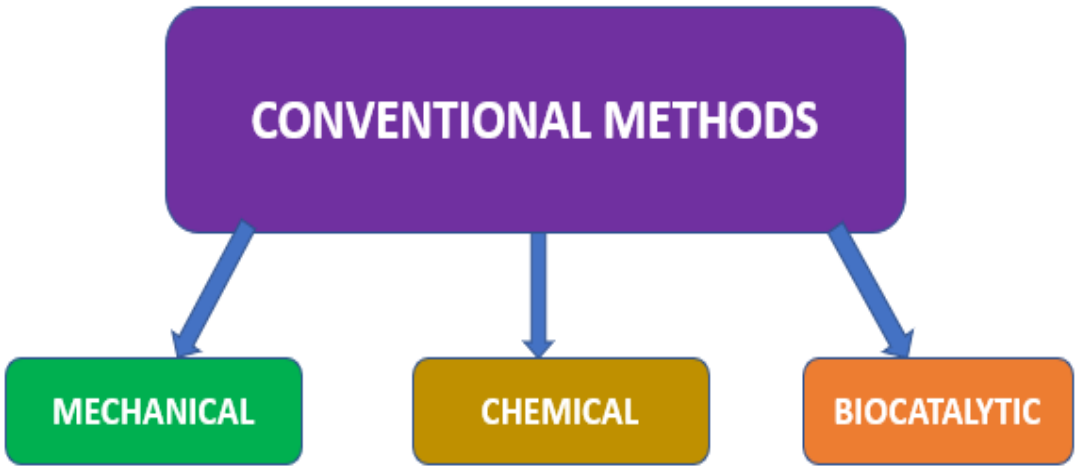

Figure 2: Conventional Methods for the Recycling PET Plastic

An alternate method to overcome thisproblem isconvertingthiswasintoreusablematerial byrecyclingprocess.Threemainmethodsknown today for recycling processing are:

1. **MechanicalTreatment**:
   Thismethodisoneofthemostwidelyusedoneinrecycling PETwastewherethebasicstructureremainssame.Thistechniqueis performed into stages,firstlythesorting,shredding,meltingoftheplasticwasteisbeingdone thatreturn plastic toits original form(Grigore, 2017).

2. **Chemical Treatment**: Throughacidic,alkalineandneutralapproacheschemical treatment is being done for de-polymerization of plastic into its constituentmonomerby hydrolysisand glycolysis. (Garcia, 2017).

3. **Biocatalytic Treatment**: Involvementofmicroorganismfordegradingprocess is involved in this type of treatment. Through enzymatic activity cleavage ofpolymerbond occur (Ribitsch2011; 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 IdeonellaSakaiensis 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

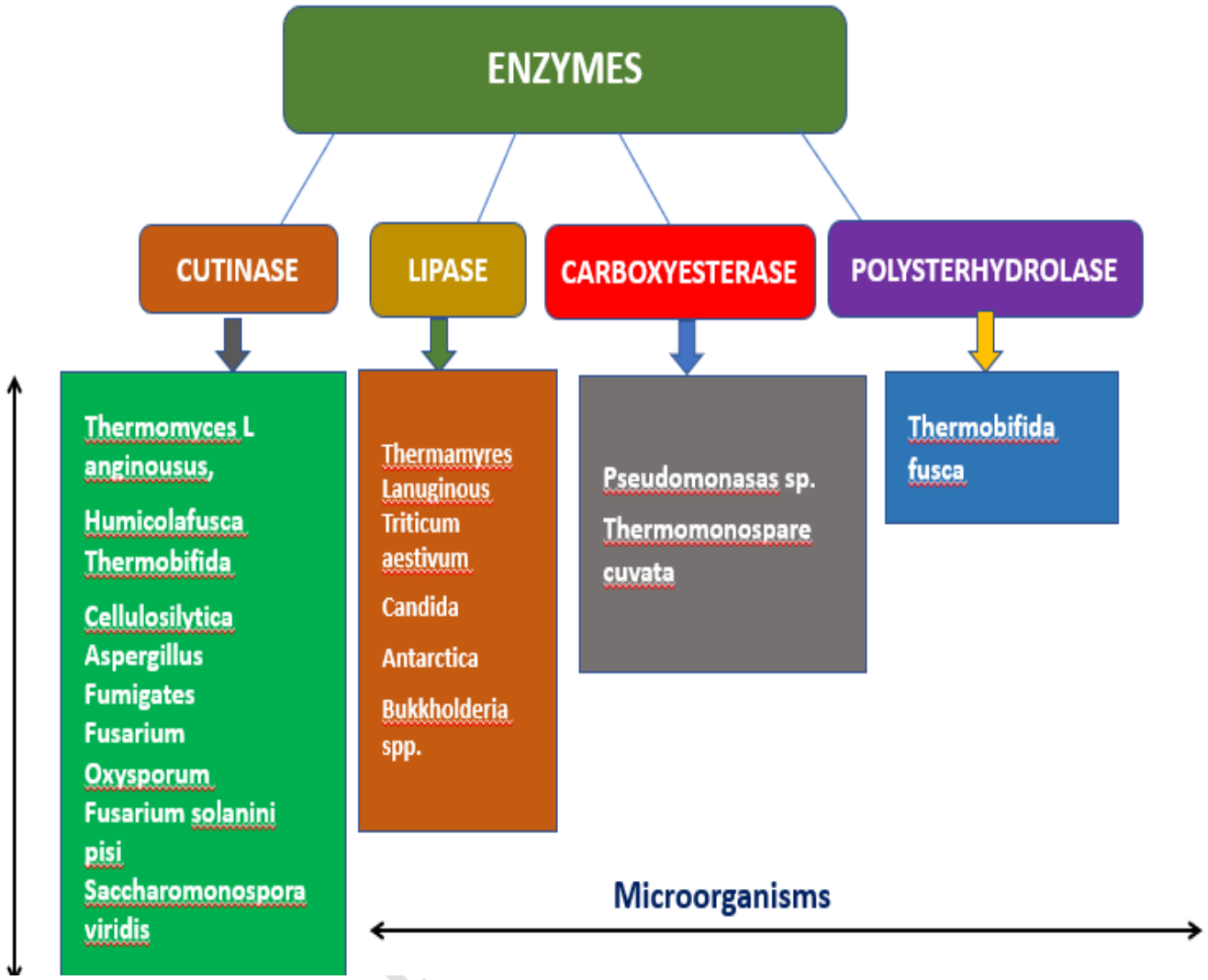

Figure 4: Various Microbial Enzymes which are Known to Hydrolyze PET

enzymes when grown on PET that were capable of hydrolyzing PET (Austin,2018).Two enzymes identified byresearchersfrom thebacteria were:

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

Bothoftheseen enzymeswererequiredtoenzymaticallyPET intoitsconstituentmonomers, terephthalic acid (TPA) and ethylene glycol (EG). It was found that much of thework was done by PETase enzyme so further analysis was done on this enzyme and very less information is there aboutMHETase which act on Mono-(2-hydroxyethyl)terephthalic acidone(Yang2013).

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

ENGINEERINGPLASTICEATINGENZYME

The University of Campinas in Brazil and Scientist at University of South Florida duringstructure study of PETase enzyme discovered that PETase is quitesimilar tocutinase butdiffer 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 discoveryof bacteria solved PET structurebyusing 3D information and inadvertently engineered an enzyme whose degrading rate wasgood 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 byengineering more efficient enzyme. This suggested the researcher that there is a room forfurther improvement of these enzymes. Further the process of bioengineering becomes moreappealing due to the presence of computer aided engineering of enzymes. Therewas a wayexplored when solved the structure of LC cutinase, IsPETase, and Tfcutinase. Active sitemodification in protein engineering is considered to be the most effective one via site activemutagenesis (Yang, 2013; Tournier,2020).

ENZYME	PRODUCT	MUTATION(s)	RESULT(Biological Effects)	REFERENCE
Leaf branchcompostutinase	TPA, EG	F243I/D238C/S283C/Y127GF243I/D238C/S283C/N246MF243W/D238C/S283C/Y127GF243W/D238C/S283C/N246M	Achieved92% of PET degradation intomonomers, includingproductivityof16.7gof terephthalate/L/hover10h. improve meltingtemperaturesby9.8◦Cwhichishigher thanwildtypeLCC(18).	(Tournieretal.2020)
IsPETase	MHET, EG, TPA	S160A D206A H237A Y87AW185A M161A I208AW159AN241AS238A R280AC203A/C239A	R280A increasedPETasecatalyticactivityby22.4%and32.4% in 18 h and 36 h,respectivelycomparedwithIsPETase wildtype (17).	(Jooet al.2018)
IsPETase	MHET,EG, TPA	W185AS238F/W159H	The absolutecrystallinitylossis4.13% more than IsPETasewildtype(16)	(Austinetal.2018)
MHETase	TPA,EG	S419G, S419G_F424N,W397A	S419G,S419G_F424N: ThatenhancetheenzymeAffinityandactivitytowardBHET,W397A:Enhancedactivityof enzyme towardMHET(19)	(Palm etal.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	Enhancedcatalyticactivityof PETaseby1.4,2.1,and2.5folds more than the wild type.I179Fmutantshowedhighestdegraderate(22.5mg.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, wherethese 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 edamorphous films of PET confirmed degradation moving forward the design of multi enzyme system for mixed polymer waste is quite promising and alsofruitfull area for continued investigation in a hope that PETaseis the best suited one for hydrolysis of PET plastic.

A massive accumulation of plastic in landfill sand 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 infield 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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