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Review on Efficacy of Microbial Degradation of Polyethylene Terephthalate and Bio-upcycling as a Part of Plastic Waste Management

Menaka Devi Salam*, Ajit Varma, Rishabh Prashar, Divya Choudhary

Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Noida, Uttar Pradesh, India
*Corresponding author: salammenaka@gmail.com

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Abstract Poly (ethylene terephthalate) (PET) is a very common and excessively used plastic polymer. The accumulation of PET waste in the environment has led to increasing global concerns because of the extremely low degradation properties of this polymer. Only a few ways have been identified to biologically degrade PET. Although none of these methods has been brought into use industrially, but the evolution of microbes leading to abilities to degrade certain polymers is quite promising and gives us the opportunity to identify and utilize these properties to solve the long time existing problem of plastic waste. Different types of PET hydrolases have been isolated from fungi as well as bacteria and some of them have shown remarkable degradation of crystalline PET with upto 50% weight loss and in about two to three weeks time. Some of the PET hydrolases have been characterized to be stable at high temperatures of 50 to 70°C which is an advantage for industrial application as efficient degraders of PET. This review article gives a brief overview on the various methods of PET degradation and focuses mainly on the microbes which have been identified to be capable of degrading PET. The enzyme involved in degradation for each microorganism is being explored in order to get a better understanding of the degradation mechanism. In addition, current status of bio-upcycling of PET is being discussed which is an alternative way of managing the ever increasing build of plastic waste.

Keywords: *PET hydrolases, MHET hydrolases, polymers, monomers, depolymerization*

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1. Introduction

PET, also known as poly(ethylene terephthalate) is a plastic polymer which is used to produce single use plastic. This is used for making objects like plastic wrappers, plastic bottles, packets etc. which usually end up in the environment rather than a dustbin. PET is a copolymer formed by a polymerization reaction of terephthalic acid and ethylene glycol.

After the World War II, the use of plastic increased many folds due to lack of revenue around the world as it was a cheaper and stable option. Since then, the ever-increasing demand has led us into a situation that we neither have the resources, nor the methods to deal with the waste produced as a by-product of this demand. The increasing plastic waste in the environment has largely affected the developing Asian and African countries due to their less efficient garbage collection system. Globally, about half a portion of the plastic wastes either enters landfill or is disposed off into the environment [1]. Also, every year about 8 million tons of plastic waste enters the ocean. This has lead to the formation of "the great pacific garbage patch" which spans from the west coast of North America to Japan. To give an idea of the magnitude of this patch – it ranges to an area of over 1.6 million square kilometres, which is 3 times the size of France. In the sea, environmental factors like sun, wind, and waves break the plastic waste into micro-plastic which is even more dangerous to the environment as its particles can travel with air. Traces of micro-plastic have actually been found on Mount Everest (the largest peak in the world) and even in the Marina trench (the deepest trench on earth).

At present, mechanical and chemical degradation processes of plastics for their recycling are commonly adopted. However, both the methods remain unattractive due to lower quality product in case of mechanical degradation and high cost and pollution effects in case of chemical degradation. Biological degradation serves to be eco-friendly and cost-effective alternative. The present review article is intended to go across all the important research works that have been done in order to find a way to biologically degrade PET. This will give us an idea of how microbes are evolving to their environments and developing ways to deal with it. It will also bring into light the future prospects of using micro-organisms to degrade plastic waste in the environment and how it is

going to be an extremely important area of research in the upcoming future.

Table 1. Some important properties of PET

Chemical formula	$(C_{10}H_8O_4)n$
Molar mass	Variable (10-50 kg/mol)
Density	Amorphous PET: 1.33 g/cm^3 (25°C) Crystalline PET: 1.50 g/cm^3 (25°C)
Melting temperature	250°C
Boiling Temperature	350°C
Solubility in water	Insoluble
Refractive index	1.57
Tensile strength (psi)	6600
Compression strength (psi)	14000
Monomers	Terephthalic acid, Ethylene glycol
Glass transition temperature (Tg)	Amorphous PET: 67 °C Crystalline PET: 81 °C

2. PET (Polyethylene Terephthalate)

PET is a thermoplastic polymer which belongs to the polyester family of polymers. This homopolymer is known for its excellent mechanical, thermal and chemical resistance. Other properties which make PET one of the most used polymers is its flexibility, colourlessness, resistance to moisture, alcohol and other solvents which is the reason it is used to make plastic bottles for mineral water and carbonated drinks. Depending on the processing, it can be semi-rigid to rigid in nature.

PET, with a recycling code of 01, is also known as the most recycled thermoplastic and is generally recycled to make fibres, fabrics, sheets of packaging etc. The general properties of PET are as given in Table 1.

Commercially, to improve the impact strength and surface finish of the product, glass fibre and carbon nanotubes are used as fillers which are mixed with PET. PET is a homopolymer but can be made to a copolymer i.e. PET-G (polyethylene terephthalate glycol). This modification is done by replacing ethylene glycol or terephthalic acid with cyclohexane dimethanol and isophthalic acid respectively [2].

2.1. Production of PET

The raw materials for PET production are highly purified ethylene glycol and dimethyl terephthalate (DMT) or terephthalic acid. The main steps of production are esterification or transesterification and poly-condensation. The initial process is through an esterification step where DMT and excess ethylene glycol are reacted at 150-200°C at atmospheric pressure with a heterogenous basic catalyst of antimony or titanium. Methanol is removed by distillation and excess of ethylene glycol is removed with vacuum driving the process during poly-condensation. A trans-esterification reaction is carried out at 270-280°C.

In a similar way, the esterification reaction of ethylene glycol and terephthalic acid is conducted at moderate pressure (2.7-5.5 bars) and high temperature (258 -263°C) with a base catalyst solution and water is eliminated in the reaction with continuous removal by distillation. The product then goes through a poly-condensation process which is operated in a reactor under vacuum and increased temperature above the melting point of the polymer. The polyester melt obtained is process as fibres or filaments and also bottle grade chips after undergoing through further condensation process. Recent advances have reduced the steps of PET production by adopting various advanced design and techniques of reactor systems and thus reducing the cost of production.

2.2. Degradation of Plastic

Degradation of plastic polymers involves change in its properties under the influence of environmental factors like heat, light, acid, alkalis etc. The process of degradation of plastic is extremely useful for carrying out the process of recycling, but plastic needs to be protected from degradation, immediately after its manufacturing. This is done with the help of stabilizers like Hindered amine light stabilizers (HALS) and UV-absorbers which protects against weathering and antioxidants which terminate the chain reactions initiated by UV radiations [3]. These stabilizers actually make the process of degradation even more challenging.

Talking about commodity polymers (the polymers that are used in high volume and wide range of applications), the ones which are commonly used are polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), PET, polystyrene (PS) or Styrofoam, polyurethane (PU), polycarbonate, and poly(methyl methacrylate) (PMMA). They constitute 98% of polymers produced worldwide [4]. Changes in polymer properties arise as a result of chemical, physical or biological reactions which break the bonds and lead to chemical transformations.

Photo-degradation: Polymers can degrade by the process of photolysis i.e. absorbing the energy of visible light or higher spectrum live UV radiation or gamma rays. It can be understood that sensitivity of any polymer to photolysis is determined by its ability to absorb harmful part of the radiations [4].

UV rays and gamma rays are responsible directly for photo-degradation, while visible part of sunlight accelerates the process.

Photolysis occurs because the polymer absorbs UV portion of the spectrum which excites electrons to higher energy state and results in reactions like oxidation, cleavage and thus results in degradation.

Thermal degradation: Occurs by overheating. Due to high temperatures, the polymer backbone breaks and reacts randomly, thus leading to changes in the properties of the polymer, and thereafter physical and chemical changes [5].

Thermal degradation involves changes to the molecular weight of the polymer and other changes that might occur are reduced ductility, chalking, colour changes etc. The materials generated after degradation depends on several factors like additives, colorants used, specific temperature, exposure time and other environmental factors [6].

Chemical degradation: This is a type of polymer degradation in which changes in the polymer properties occur due to a chemical reaction with the surroundings of the polymer. The reaction results in the breaking of double bonds within the polymer structure. The chemical reactions that results in polymer degradation are solvolysis, ozonolysis and oxidation [7].

Solvolysis: Solvolysis involves the breaking of C-O or C-N backbone in high volume polymers and involves water or alcohol as one of the reagents. Common solvolytic processes are glycolysis, methanolysis and hydrolysis. It is applicable to automobile shredder residues such as PU and post consumer plastics such as PET and PE [8].

Ozonolysis: An organic reaction in which ozone attacks the unsaturated polymers in the double bond and causes chain scission and oxidation ultimately leading to decrease in cross-link density. Ozone readily attacks unsaturated polymers but saturated polymers also get degraded at a slower rate. The process is often applicable to elastomers e.g. rubber [9]. Action of ozone on polyethylene and polystyrene have been studied at temperatures of 25° to 109°C and 55° to 154°C respectively. Aldehydes and ketones were obtained from polyethylene, whereas ozonide or peroxidic complex was produced from polystyrene ozonization [10].

2.3. Biological Degradation of PET

Micro-organisms such as bacteria and fungi are responsible to biodegradation of waste PET in the environment [11]. The degradability of plastic also depends on the type of environment it is present in, such as the type of soil because different micro-organisms are responsible for degrading a certain type of plastic polymers and these micro-organisms are present in different soil conditions according to their requirements of survival. Plastics act as substrates or carbon source for the heterotrophic microorganisms. Another important thing is to evaluate the distribution and population of polymer-degrading micro-organisms in various ecosystems. The major mechanism involved in the biodegradation is the adherence of micro-organisms to the surface followed by the colonization of the exposed surface [6].

2.4. Factors Affecting Biodegradability of Plastic

Both physical and chemical factors determine the biodegradation of a polymer and the rate of degradation. The surface condition of the plastic, first order structure and high order structure plays important role in the process of biodegradation [12].

Polyesters with side chains are less assimilated than the ones without side chains. Molecular weight of the polymer is another factor that plays its role in determining the degradability of the polymer. The polymers with higher molecular weight are less susceptible to degradation by micro-organisms than the ones with lower molecular weight. Moreover, the morphology of the polymer affects the process. The degree of crystallinity is also important as micro-organisms tend to attack the more amorphous polymer. Thus, more the crystalline nature of a polymer lesser the chances of it getting degraded by any microbe.

The major reason for the inefficiency in biodegradability of aromatic polymers was explained by Marten et al. (2005) [13]. They showed that the mobility of polymer chain in the crystalline part controls the biodegradability of the polymer. Mobility was dependent on the difference between the melting temperature of the polymer and the temperature at which biodegradation starts. In case of aromatic polymer, the melting temperature is very high so the mobility of polymer chain becomes low and thus the rate of biodegradation is also low [13].

2.4.1. The Process of Biodegradation

The process of degradation involves the conversion of the polymers into monomers and then mineralization of the monomers (e.g. PET= terephthalic acid + ethylene glycol). This is because of the large size of polymers which does not allow the polymer to pass through the cellular membranes, thus they are first depolymerised to monomers.

This initial breakdown can occur as an outcome of various physical and chemical forces like heating, cooling, wetting, wind etc. which cause mechanical damage to the polymer [14]. The colonization by fungus can also cause damage to the structure of polymer as fungus can penetrate the polymer solids [15].

Polymers can also be degraded my microbial enzymes, after which the monomers are absorbed into the microbial cell and used as a carbon source, for example, degradation by *Ideonella sakaiensis* [16]. It involves the adherence of microbes to the polymers and thereafter colonization. The enzymes released by microbes also attach to the polymer followed by hydrolytic division. The monomers, dimers, oligomers thus formed are later on converted into carbon dioxide and water by mineralization [12]. Under aerobic conditions, oxygen is used as an electron acceptor followed by the synthesis of carbon dioxide and water as the final products [17].

2.4.2. Standard Methods Used for Testing Biodegradation of PET

One of the most common preliminary checking of PET degradation is physical observation of visible changes in the structure of PET film or pellets. Different observation on the basis of vision can be described as roughening of surface, formation of holes or crack, change in colour, de-fragmentation etc. Another type of physical testing method used is observation of changes in mechanical properties especially in the cases where the degradation is carried out by abiotic factors [18]. However, in the case of degradation carried out by enzymes, the mechanical properties only change if there is a significant change in the molar mass of the specimen. Another method of observation is weight loss measurement and residual PET but the method does not give a direct proof of the degradation and variations may arise due to improper cleaning of the material. To prove that biodegradation has taken place, advanced techniques can be used to study the surface like Scanning electron microscopy (SEM), Atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC), Nuclear magnetic resonance spectroscopy (NMR), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD) [6].

Alternative and more reliable methods of testing biodegradation of PET are measurement of CO_2 evolution and O_2 consumption [19], radiolabelling, clear zone formation and enzyme testing [6]. It is observed that a combination of all these standard assessment techniques would be a reliable approach for final confirmation of the biodegradation ability.

2.5. Microbial Biodegradation of PET

Leaf branch compost: In a very recent study, leaf branch compost (LCC) as a PET hydrolase was recorded to achieve over 90% degradation in just 10 hours against amorphous PET. The study reported depolymerisation by LCC to be 33 times more effective than other enzymes isolated from microorganisms. It also showed highest thermostability (84.7°C) compared to the entire PET degrading enzymes tested [20].

No inhibition was observed by depolymerisation products terephthalic acid and ethylene glycol in the reaction. With enzyme engineering technology, mode of binding to the polymer and thermal stability were studied and improved variants were also developed having increased depolymerisation than wild type LCC [20].

Using LCC to recycle PET, it produced 0.6 kg of sodium sulphate for 1 Kg of PET recycled. This meant that for every 100,000 tons of PET recycled every year, 60,000 tons sodium sulphate was produced. This is about 0.28% of the world's market of sodium sulphate.

2.5.1. *Ideonella sakaiensis*

Ideonella sakaiensisis a Gram negative, aerobic bacterium that was first discovered in 2016 by a group of scientist from Kyoto Institute of Technology and Keio University, Japan, while trying to explore microorganisms from samples of soil and wastewater obtained from localities near plastic recycling centres in Sakai, Japan [21]. The scientists in their study obtained this bacterium from a consortium (consisting of bacteria, yeast and protozoa) which was able to degrade PET film at a rate of 0.13 mg cm-2day-1 at 30°C. After further investigation of the microbial components in the consortium, the novel strain named as *Ideonella sakaiensis* 201-F6 was discovered which had the property of degrading PET and was able to use it as the sole source of carbon. Also this strain had the ability to grow at a wide range of pH and temperature (pH 5.5 to 9 and 15 to 42°C) [22]. *Ideonella sakaiensis* adhered to the PET film with its bacterial appendages and secreted an enzyme known as PET hydrolase (PETase), which is responsible for generating the intermediate mono (2-hydroxyethyl) Terephthalic acid (MHET).This MHET is taken up by *I. sakaiensis* and then hydrolyzed by the second enzyme i.e. MHET hydrolases (MHETase), to produce terephthalic acid and ethylene glycol [23]. Crystal structure of MHETase and its binding to MHET analogue have been studied and show that MHETase is highly substrate specific and has induced-fit mode of substrate binding [24].

PETase from *Ideonella sakaiensis* 201-F6 was shown to exhibit 5.5- to 120-fold higher activity than similar enzymes like lipase and cutinase. The crystal structure of PETase and its active sites have been studied. It was found that DS1and DS2 (two intramolecular disulphide bridges near the catalytic centre) and W156 in the catalytic centre (Tryptophan residue which displays variable conformations) are important for PETase activity [25]. The swaying conformation of W156 has been reported to play a role in binding of substrate and thereafter conversion to product [26]. Improvement in the activity of PETase by creating mutation in active site residues has also been reported proving that protein engineering is yet another scope for providing better performance by biodegradation. Variants of *Ideonella sakaiensis* have been made by protein engineering which showed increased melting temperature by 8.81°C and PETase activity increased by 14 fold to that of the wild type strain at milder condition of 40°C [27]. Also PETase was reported to degrade polyethylene-2,5-furandicarboxylate (PEF) which is a bio derived alternative of PET [28].

2.5.2. *Bacilus subtilis*

Bacillus subtilis has also been reported to degrade PET. In one such study, *Bacillus subtilis* produced p-nitrobenzylesterase (BsEstB) which hydrolyzed bis(benzoyloxyethyl) terephthalate (3PET) to produce terephthalic acid (TA), benzoic acid (BA), 2-hydroxyethyl benzoate (HEB), and mono-(2-hydroxyethyl) terephthalate (MHET). BsEstB enzyme showed optimum temperature at 40°C and pH 7 [29]. *Bacillus subtilis* MZA-75 isolated from soil was able to degrade polyurethane films. This was shown by appearance of surface cracks under scanning electron microscopy (SEM), decrease of the functional group ester in fourier transform infra-red spectroscopy (FT-IR) and increase in polydispersity index indicating chain scission when observed through gel permeation chromatography (GPC) [30]. *Bacillus subtilis* has also been reported to produce surfactin (biosurfactants) which aides in the degradation of polyethylene films. The polyethylene films of 18 µ thickness, which were pre-treated with ultraviolet radiation, when incubated with *Bacillus subtilis* showed 9.26% weight loss in 30 days [31]. In one study, the gene coding PETase from *Ideonella sakaiensis* was isolated, synthesized and expressed in *B. subtilis* WB600. Thereafter, five signal peptides from *Bacillus subtilis* were analysed for the secretion of PETase from *Bacillus subtilis* and it was found that SPamy signal peptide induced secretion of PETase at the highest and also enhanced the degradation process [32].

2.5.3. *Thermobifida fusca*

Polyesterase from the actinomycete, *Thermobifida fusca*, was first reported by a German research team and was found to be highly thermostable with enzyme activity showing upto 70°C [33]. The enzyme was also referred to as cutinase and it has been extensively studied for the degradation of PET. It was also shown that this enzyme was able to depolymerize PET at a rate of 8 to 17 µm per week at 55°C [33]. The PET hydrolase from *T. fusca* was crystallized by Roth et al., 2014[34] and explained about its high thermostability. Later on, Then et al. (2015) reported that Ca^{2+} and Mg^{2+} increased the Tm values of PET hydrolase from *Thermobifida fusca* by 10 to 14°C [35]. Barth et al., (2016) studied a dual enzyme system consisting of an engineered PET hydrolase from *T. fusca* and an immobilized carboxylesterase from the same strain for efficient degradation of PET [36,37]. The study demonstrated that MHET obtained after the reaction was low as detected by HPLC indicating the degradation of the intermediate MHET by the carboxylesterase. Further studies to improve the PET hydrolase activity were done by various research groups through protein engineering such as site directed mutagenesis of a carbohydrate

binding module which was used to develop a fusion protein with the *T. fusca* cutinase [38]. Site directed mutagenesis of the active site of *T. fusca* cutinase was done through comparision studies with PETase of *Ideonella sakeiensis* by another research group. They also experimented on the effect of surfactants on the activity of the mutated form of *T. fusca* cutinase and found that the cationic surfactant, dodecyl trimethyl ammonium, greatly enhanced its activity upto 13-fold greater than that without the cationic surfactant. This showed that the cationic surfactant helped in attracting the enzyme to the low crystallinity PET film used in the experiment [39].

2.5.4. *Thermomyces insolens*

Thermomyces insolens (formerly *Humicola insolens*) which is a type of fungus was found to produce a thermostable polyester hydrolase which was a cutinase. It catalysed the hydrolysis of low crystalline PET film upto 97% at 70°C in 96 h [40]. The study was done along with two other PET degrading microrogansims i.e. *Pseudomonas mendocina* and *Fusarium solani* which also produced cutinases. The cutinase of *Thermomyces insolens* was found to be the most efficient, making it to be the fungal cutinase having the highest PET hydrolysing activity so far. Analysis of its hydrolysis products showed to consist exclusively of terephthalic acid and ethylene glycol.

2.5.5. *Penicilium* Species

Fungi are known to degrade a variety of environmental contaminants and polymeric materials [41]. *Penicillium* species has been found to have significant degrading potential of polyethylene and its related plastic materials [41,42,43]. *Penicillium oxalicum* NS4 and *Penicillium chrysogenum* NS10 isolated from plastic dumping grounds of Kolkata, India, have been reported to degrade plastic sheets (HDPE and LDPE) significantly. The percentage weight losses of the HDPE plastic sheets after 90 days incubation were 58.6% by the isolate NS10 and 55.34% by isolate NS4. In case of LDPE plastic sheets, the percentage of weight losses were 34.35% by isolate NS10 and 36.6% by isolate NS4 [44]. These show significant amount of degradation of polyethylene by *Penicillium* species, but recent investigations demonstrated that fungi degrading polyethylene are uncommon and they have more potential to degrade polyurethane [45]. Comparative studies of bacteria and fungi for polyethylene degradation have shown that bacteria are more efficient than fungi in their degradation [46]. This has been explained as due to fungal nature of growing as mats on the surface of plastic materials and hydrolyzing the upper layers only instead of penetrating the polymer chains whereas bacteria can penetrate and hydrolyze the inner polymer chains [47].The confirmation of these findings, however, needs more validation such as characterization of the enzyme and biochemical mechanisms underlying the hydrolysis.

2.5.6. *Streptomyces* sp.

Report on the ability of *Streptomyces* sp. to degrade plastics has been demonstrated way back in 1991 by Lee and his research group [48]. They studied on three strains of *Streptomyces* i.e. *Streptomyces viridosporus* T7A, *S. badius* 252, and *S. setonii* 75Vi2 which were known to be lignin degraders. The degradation abilities of these species of *Streptomyces* were studied on heat and UV light treated plastics by incubating in shaker flasks, and results showed significant reduction in percent elongation and molecular weight of polyethylene when compared to uninoculated controls [48]. In another report, *Streptomyces* sp. was studied for its degradation ability of commercial PET bottles which were cut into small pieces of different mesh sizes (420, 300 and 212 µm). To 50 mg of powdered PET, 0.5 ml of microbial suspension and 0.5 ml Mc Farland standard were added and incubated in a shaking incubator at 28°C, 120 rpm for 18 days. For extraction of residual PET, toluene was used as a solvent. Biodegradation rate (calculated as weight loss per unit time) after 3, 6, 12 and 18 days, the results showed that till 3rd day of incubation all the particle sizes had same degradation rate. After that, till 6th day of incubation, 212µm had the most degradation rate while 300, 420, 500µm had almost the same rate. After 12 days, 212µm and 300µm particle sizes had higher rate of degradation while that of 420 and 500µm had almost the same rate [49]. In an attempt to search for PET hydolase like enzymes in *Streptomyces* species, in silico based approached was adopted by one research group and they found that out of 52 genomes of *Streptomyces* analyzed, three PETase homologues were identified from marine sponge associated *Streptomyces* sp. The protein sequences were compared with PET-ase of *Ideonella sakiensis* and were found to have the same conserved regions of catalytic sites. PETase like gene from isolate M14 was expressed in *E. coli* with the native signal peptide of the isolate and qualitative analysis on petriplates with the substrate polycaprolactone confirmed polyesterase activity [50]. *Streptomyces albogriseolus* LBX-2 isolated from a soil sample in China was found to be able to use polyethylene as the sole carbon source. Degradation studies were done with polyethylene and it was found that significant degradation was observed after a period of 15 days. The complete genome was sequenced and it was found to contain 21 monooxygenase and 22 dioxygenase genes which are known to code for enzymes linked with polyethylene degradation [51].

3. Whole Cell or Purified Enzyme

The investigations on various microorganisms for their ability to degrade PET have been done either with whole cell or with purified enzyme. Whole cell biodegradation system has the advantage that they are less time consuming and cost-effective as it does not require enzyme purification steps [52]. Also whole cell biodegradation system can be used for simultaneous production of the enzyme and degradation of the plastic, ultimately giving rise to low-cost and easy operation system. But the whole cell system also requires certain characteristics which will help in the efficient degradation of the plastic. The microorganism should be able to adsorb well to the surface of PET material. It is preferable that the

microorganism is thermotolerant so that it can perform degradation of semi-crystalline PET which has a high Tg of around 70°C. So far, there are no reports on thermophilic microorganisms able to degrade plastic but engineering of thermophilic microbes by thermophilic hydrolases to confer the ability to degrade plastic at high temperatures has been done by some researchers, and this strategy has been found to be very promising. Very recently, LCC has been cloned and expressed using *Clostridium thermocellum* which is a thermotolerant bacterium and showed 62 % weight loss of plastic after 14 days incubation at 60°C [53]. The results of this study clearly showed the potential of using thermotolerant microorganism along with theromophilic hydrolase for application in plastic waste bioremediation. PET hydrolase producing microorganisms with special features of binding or adsorbing to surface materials can also possess high efficiency of PET degradation. These microorganisms generally have the ability to form biofilm and secrete exopolysaccharides through which they adhere to the solid surface such as plastic [54]. Hydrolysis of PET by PETase to MHET is the rate limiting step, since MHET is rapidly converted to its monomeric products by MHETase [21]. Therefore, in order to increase PETase activity, researchers have mainly focussed on the production of mutant form of PETase through protein engineering. In this process, during the selection of mutants, rapid screening and assay of PETase activity is important. Use of recombinant whole cell, like *E.coli*, for the production of the modified form of PETase has the disadvantage of inability to secrete the enzyme extracellularly. Various other host cells such as *Saccharomyces cerevisiae* have been experimented in this regard. But, in an attempt to find a more faster method of screening of the enzyme activity, cell free protein synthesis which is the production of protein from nucleic acid templates through in vitro transcription and translation, has found to be quite beneficial [55]. Through this method manipulation for functional protein synthesis can be easily done as it is an open system. The selection method can be enhanced by high throughput screening through fluorescence labelling and plate reader platform. Thus, it is shown that cell free protein synthesis method is beneficial in screening procedures of PETase mutants obtained by protein engineering.

4. Bio-upcycling for Plastic Waste Management

Biodegradation and bio-upcycling is considered one of the most optimal recycling routes in the present scenario for manangement of plastic waste [56]. PET made from renewable feedstocks i.e. from plants is of major interest in PET manufacturing industries to save the use of non-renewable fossil feedstocks. Many industries have started manufacturing bio-PET with the feedstocks derived from plant sources but the recycling of bio-PET has similar challenges as recycling of petroleum based PET bottles. Hence new technologies to improve the recycling process with overall minimum consumption of energy and which is cost effective and eco-friendly would contribute significantly in proper management of plastic waste. Therefore, focus has been on the search of microbial enzymes which can degrade the petroleum based plastics to give rise to its degradation products which can be recycled into useful polymers in a closed loop system of reprocess [37]. Tiso et al. (2020) established a novel bio-upcycling system of PET using purified LCC for the degradation of PET to its monomers which were later converted into important biopolymers using *Pseudomonas putida* strain [57]. This strain was able to metabolize ethylene glycol to produce polyhydroxyalkanoate (PHA) intracellularly. The strain was engineered to produce hydroxyalkanoyloxy-alkanoates (HAAs) extracellularly which can be polymerized into bio-based polyurethane (PU) by chemical process. In another recent study of production of value added compounds from plastic waste materials, polyoxymethylene (POM) polymer transformation into cyclic acetals, which is industrially important, has been elucidated using combined approach of reaction of POM with biomass derived diols and the study showed a new sustainable approach for upcycling of POM [58]. Standard recycling strategies, such as mechanical and incineration processes, give rise to plastics of low quality whereas upcycling of degraded products through catalytic transformations give rise to high quality value added products. Biocatalysis is one of the important strategies through which we can obtain high quality polymers. Therefore, various microbial communities can be explored and their ability to degrade plastics as well as upcycling can be studied to find a sustainable solution to the ever increasing plastic waste (Table 2).

Table 2. Upcycling processes studied for obtaining value added products

S. No.	Plastic monomers/polymer	Upcycling Process	Value added products	Reference
1.	Ethylene glycol and terephthalic acid	Enzymatic degradation of PET by LCC polyester hydrolase; Upcycling by engineered *Pseudomonas* sp.	Polyhydroxyalkanoate (PHA) and poly(amide urethane) (PU)	[57]
2.	1,4-Butanediol	Engineered *Pseudomonas putida*	Polyhydroxyalkanoate (PHA)	[59]
3.	Polystyrene	*Cupriavidusnecator*	Polyhydroxyalkanoate (PHA)	[60]
4.	Terephthalic acid (TA)	*Pseudomonas putida* strains and *Pseudomonas frederiksbergensis*	Polyhydroxyalkanoate (PHA)	[61]
5.	Polystyrene	*Pseudomonas putida*	Polyhydroxyalkanoate (PHA)	[62]
6.	Benzene, toluene, ethylbenzene, and xylenes (BTEX compounds)	*Pseudomonas putida*	Polyhydroxyalkanoate (PHA)	[63]
7.	Polystyrene	*Pseudomonas putida*	Polyhydroxyalkanoate (PHA)	[64]
8.	Polyaromatic hydrocarbons	*Pseudomonas* sp.	Polyhydroxyalkanoate (PHA)	[65]

5. Conclusion

This review covers the major concern of increasing PET waste in the environment, and how some microbes have evolved the ability to degrade PET, provided that the conditions are suitable. This is done by utilizing PET as a carbon source when the surroundings are devoid of more prominent source of carbon or easily available carbon.

Starting from the late 1900's, some huge strides have been taken towards developing ways of degrading PET biologically. A large number of microbes were identified to possess this property like *Thermobifida fusca*, *Ideonella sakaiensis*, *Bacilus subtilis* and more as discussed in the review. The latest enzyme to join this list i.e. LCC (leaf branch compost cutinase) has been found to be very effective with degradation capacity of 90% of PET in just 10 hours. Also, it is thermostable, and not inhibited by its end products.

It can be concluded that microbes which secrete enzymes having depolymerisation properties can degrade PET in suitable conditions i.e. optimum temperature and pH and PET should be the only carbon source. The ability of these micro-organisms to depolymerise a polymer can be enhanced by genetic manipulations and protein engineering.

The technique used for analysing PET degradation, in many cases, is percent weight reduction test. However, it has many limitations as weight loss may be contributed by other factors. Further confirmatory tests should be followed like SEM, XRD, and spectrophotometric methods like FTIR, NMR etc. Degradation products analysis, type of enzyme and mechanism of enzyme reaction should also be carried out to finally confirm the microbial reaction against PET.

It is preferable that the enzyme hydrolysing PET is thermostable as it can then be used along with physical treatment i.e. high temperature. Attempts have been made through genetic manipulations and enzyme engineering to obtain thermostable PET hydrolases. Also, gene cloning of thermostable PET hydrolase in host cell which can withstand high temperature have been studied with fruitful results.

While the management of plastic waste can be done upto some level by reducing and recycling, but a lot of it depends on the development of efficient upcycling processes. Therefore, the focus has been made in recent years on the bio-upcycling of plastic waste to obtain value added products. For this, we need to explore novel microorganisms and enzymes which can hydrolyse PET efficiently and also those enzymes which can synthesise valuable materials from the degraded products. This can be done by integrating metagenomics, protein engineering, and synthetic biology to find efficient ways of bio-upcycling plastic waste that can be industrially applicable throughout the globe.

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