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Microbes in Plastic Degradation

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ABSTRACT: Due to increasing production of plastic and its piling up is a critical concern hence ways to degrade the plastic needs to be sought out. Microplastics (MPs) of the size of micron or less have been found everywhere, even in human blood, highlighting the adversity of the situation. Natural and microbial degradation mechanisms of MPs, focusing on polyethylene (PE) and polyethylene terephthalate (PET) have been explored by the study. Natural degradation is hindered by stability and hydrophobic properties of the polymers. Despite recycling efforts, a significant portion of PET waste ends up in landfills and the environment, posing threats to ecosystems and organisms. MPs are ingested by aquatic organisms, serve as substrates for unwanted microbes, and act as vectors for toxic chemicals. Atmospheric and aquatic fluxes contribute to the transport of MPs from production sources to marine environments. Microorganisms like bacteria, can degrade plastic polymers. Bacterial cultures are found to be effective in degrading MPs through extracellular and intracellular enzyme systems. Bacterial degradation of PE and PET has been demonstrated in laboratory conditions, with varying removal efficiencies and degradation durations. Abiotic factors like oxygen and light help in the PE degradation, while hydrolase enzymes are involved in PET degradation. For effective plastic management, a cyclic bioeconomy based method is necessary, which involves reconsideration of the entire value chain of plastic. Future-proofing plastic waste management requires delinking plastic production from fossil-based raw materials and energy sources. Additionally, addressing agricultural and food waste losses can contribute to reducing the carbon footprint associated with plastic production. Overall, understanding the microbial degradation of plastics offers promising avenues for combating plastic pollution and achieving a more sustainable plastic waste management system.

KEY WORDS & ABBREVIATIONS: Polyethylene terephthalate (PET), Terephthalic Acid (TPA), ethylene glycol (EG), Microplastics (MPs), polyvinyl chloride (PVC), polypropylene (PP), polyethylene (PE), polystyrene (PS), mono-[2-hydroxyethyl] terephthalate (MHET), bis-[2-hydroxyethyl] terephthalate (BHET).

1. INTRODUCTION

Plastic consumption is an essential part of our civilization and yearly production has reached more than 350 million tons [1]. Among the plastics, PET, a synthetic polymer which is derived from petroleum, is highly used plastics [2]. In 2017, the production capacity of PET was more than 30 million tons per year [3]. Nearly, half of a trillion PET bottles had been produced in 2016, and a calculated estimation of about 0.6 trillion plastic bottles were produced in 2021 [4].

In the year 2021, China, North America & European Union being rich economy countries together produced more than sixty percent of plastic production worldwide. [Fig:2]. In the Indian scenario, we see however being a developing country, also sharing a significant amount of production. Telangana and Tamilnadu are top plastic producing states of India [Fig: 3 & 4].

PET polymer is made of repeating units of Terephthalic acid (TPA) and ethylene glycol (EG). PET is ideal because of low weight and durability, state of production and utility. One of the primary reasons for PET's popularity is due to its exceptional clarity, strength, and having lightweight nature. These characteristics make it an ideal material for making bottles and containers for beverages such as water, juices and other food items. The transparency of PET allows consumers to see the contents, while its strength and lightweight make it convenient for transportation and handling. PET's role in the packaging industry cannot be undermined. It offers excellent barrier properties, effectively preventing the permeation of oxygen, carbon dioxide, and moisture. This feature helps to maintain the freshness, flavour, quality of packaged goods and extend their shelf life. Additionally, PET's shows resistance to impact and breakage which ensures the protection of fragile products during transit.

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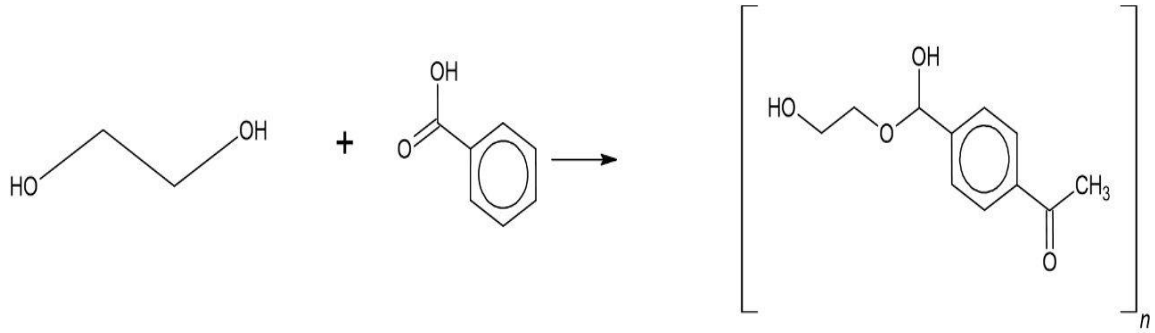

Fig : 1 Polymerisation reaction showing PET formation (Monomer inside bracket)

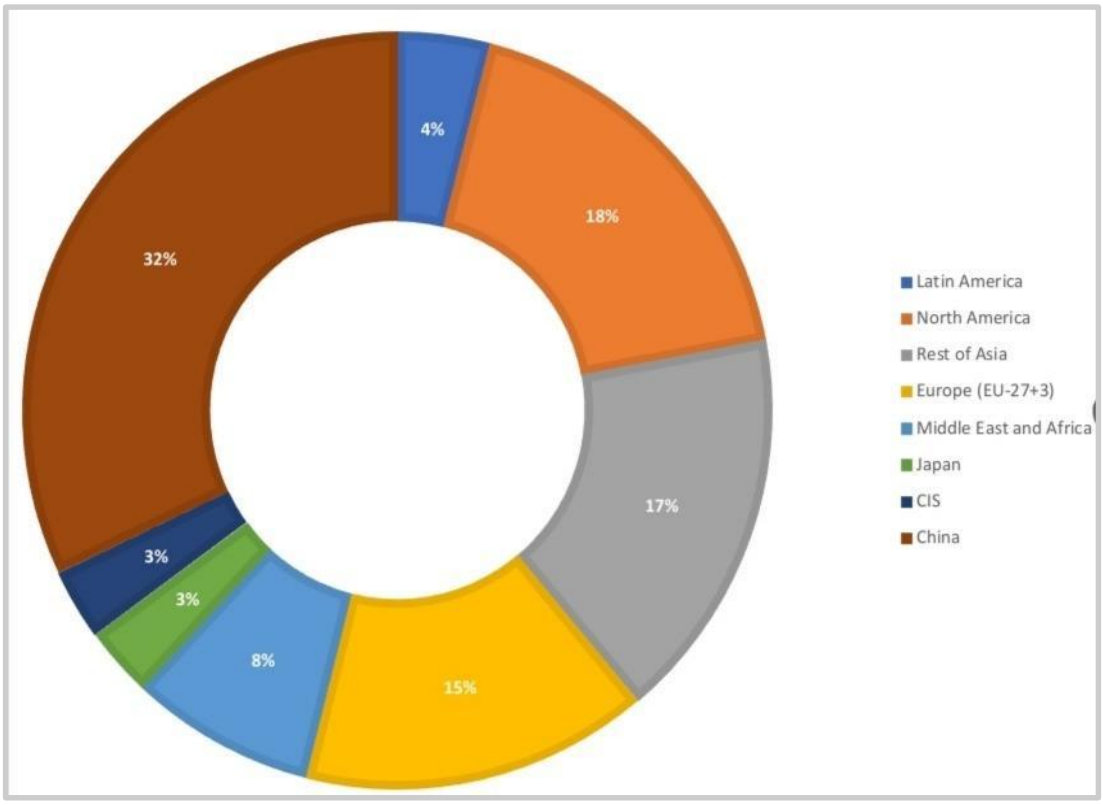

Fig: 2 Contribution of countries in global plastic production 2022 [72]
**** North America represent Canada USA Mexico**
***** Commonwealth of independent States**

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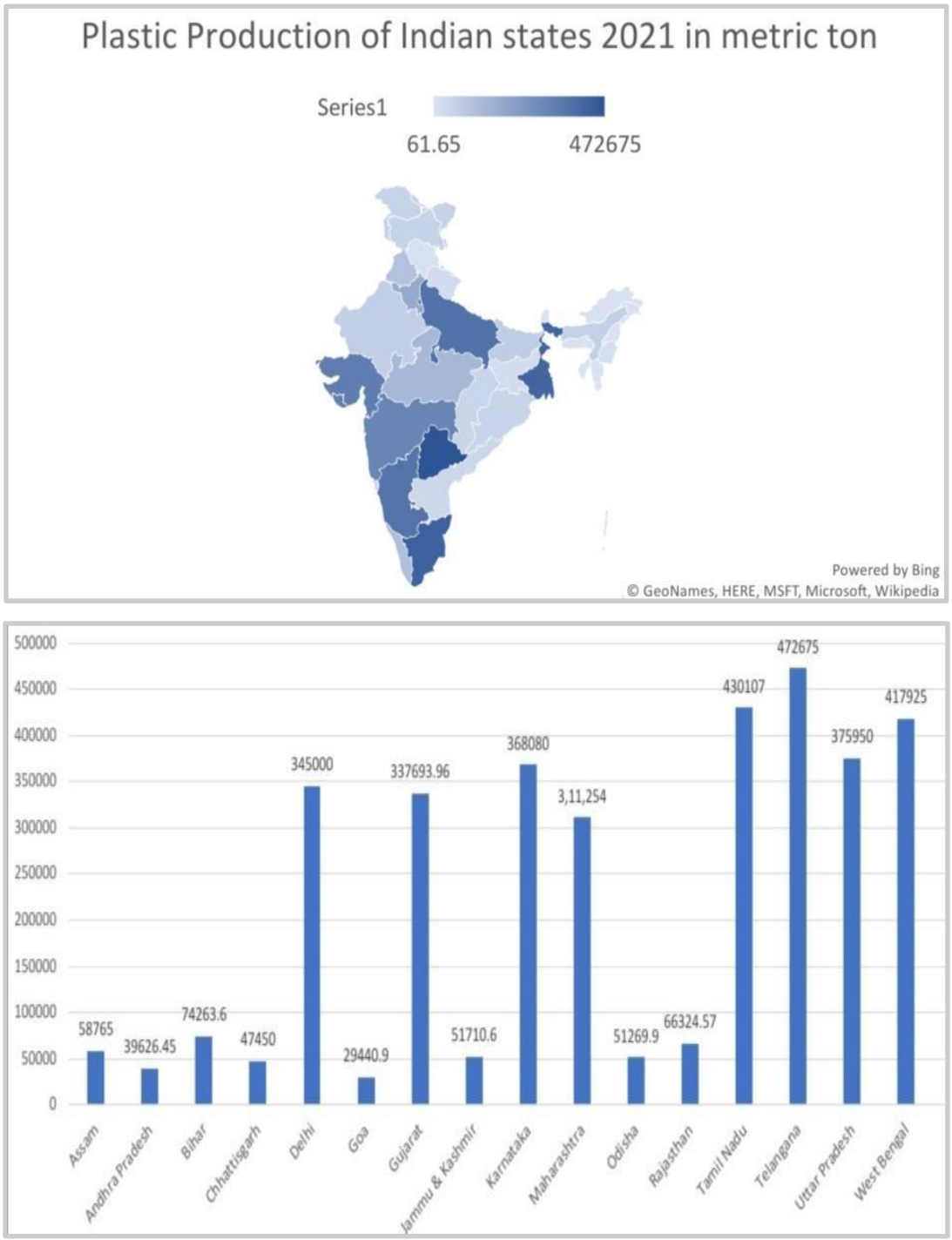

Fig : 3&4 Plastic production of Indian States in 2021 (CPCB -2020-21), made using excel mobile.

Many factors like crystalline nature, surface hydrophobicity and polymer backbone stability affect natural diffusion of these MPs [4]. Because the major use as plastic is packaging, PET is mostly used for short-term or one time applications. In Spite of the existence of a recycling system, more than half of the PET produced ends up landing in landfills. Only a certain amount of PET is recycled to its recyclable form, and most of it ends up as low-quality, non-recyclable plastic [5]. PET waste remains in the oceanic and terrestrial areas, which is potentially fatal to many life forms including human beings. Plastic pollution turns out to be a reason

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for the death of nearly one million seabirds, as well as around one lakh marine mammals and turtles each year [6]. It becomes more dangerous and creates the worst situation when MPs produce from them in the order of micron size or lesser. In recent studies MPs have been detected inside the blood of Humans also.

2. MICROPLASTICS AND IT'S DEGRADATION

Presence of MPs has been reported in human blood shows the alarming situation. MPs are ubiquitous worldwide due to the influence of wind and ocean currents [7]. There are two classes of plastics, primary and secondary plastic. [8,9]. Primary MPs belong to various microbes used in cosmetics and steel production whearas secondary microplastics are generated during breakdown of larger plastics. Disposable cosmetics contain many basic MP removers which remove plastic from skin pores. As removal of Microplastic is tough during the treatment plant, they should be carefully released.

MPs have small size, due to which they have low surface to volume ratio. They are also easy to disperse, and they make their environmental impact which is concern for pollutant absorption capacity [10]. The ubiquity of MPs can lead to risks for the ecosystem, but the debate continues about their environmental impact. MPs are found in all aquatic places whether it's water surface, groundwater column, polar regions or deep-sea surface [11-15]. Aquatic organism may ingest it,in nutrition process [16], also causes the unwanted microbial growth [17], and may lead the generation of vectors for toxins. [18]

The major routes of MP transports to water bodies starting from generation has been reported earlier [19]. Wind movement is responsible for MP transportation and higher dispersal found in places of high population. In watery areas, transport and deposition is determined by water flow.

Microplastic degradation refers to the process by which microplastics, plastic particles < 5 mm in size, are broken down and ultimately transformed or eliminated from the environment. While microplastics pose a significant environmental concern due to their widespread presence in various ecosystems, the degradation of these particles is a complex and challenging task. There are two ways in which these microplastics can be degraded, natural degradation and microbial degradation (especially by bacteria).

2.1 Natural Degradation Mechanism of Microplastics

The natural degradation of microplastics refers to the process by which these small plastic particles break down and undergo transformation in the environment without human intervention. While microplastics are known for their non biodegradability. Natural degradation processes can contribute to their breakdown over time.

Its mechanism of polymers is a modern phenomenon usually involving physical (due to abrasion, absorption swelling, light (photogenic), chemical (due to oxidation, hydrolysis), and biological factors (caused by microbes) [40, 15]. The result of the mechanisms depends on the properties of polymer and environmental conditions.

Oxidation by oxygen or ozone has nothing to do with anaerobic degradation. However, photodegradation can lead to radical formation and which in turn leads to the breaking of polymer chains into small molecules which are consumed by microbes.

It's important to recognize that natural degradation processes for microplastics are relatively slow, and the complete degradation of microplastics can take an extensive amount of time. Additionally, the microplastics breakdown can create smaller particles known as nanoplastics, which may also have environmental implications.

While natural degradation processes can contribute to the breakdown of microplastics, they are not enough to address the large scale of microplastic pollution on their own. However, their overall effectiveness in completely eliminating microplastics is limited. Effective management strategies should focus on reducing the sources of microplastics, improving waste management practices, and implementing policies to prevent further plastic pollution.

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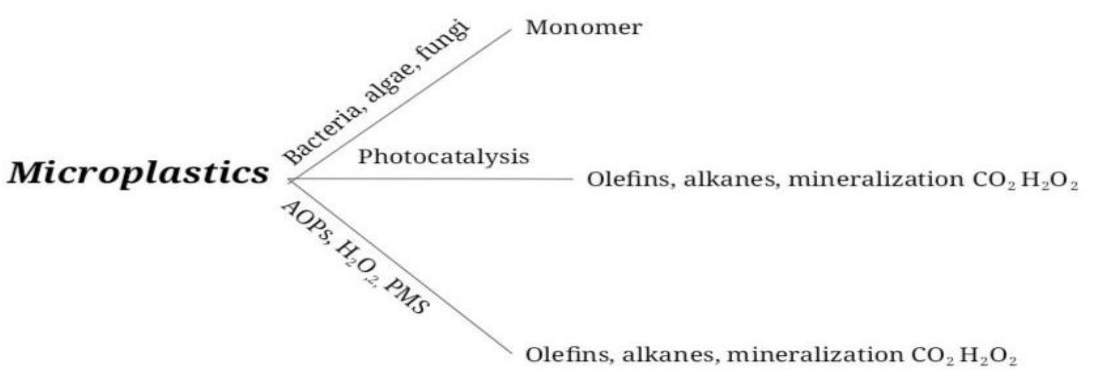


Fig: 5 Microplastics degradation through physical chemical and biological processes

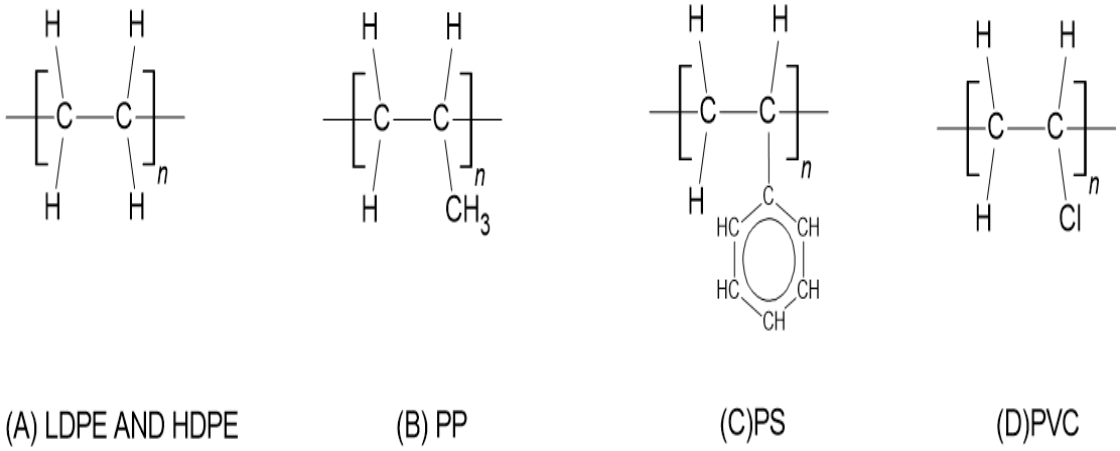
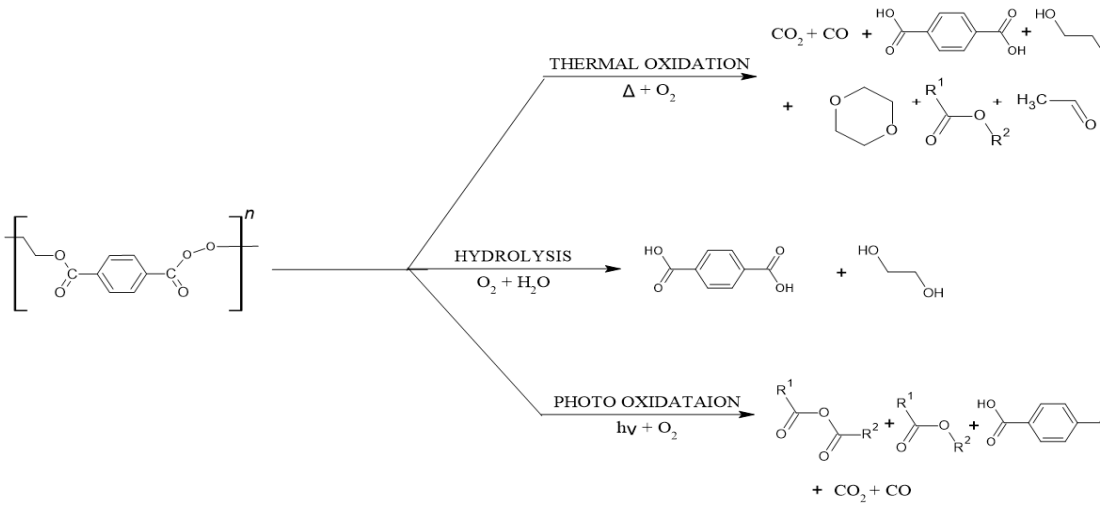


Fig: 6 Some examples of plastic polymers [20]



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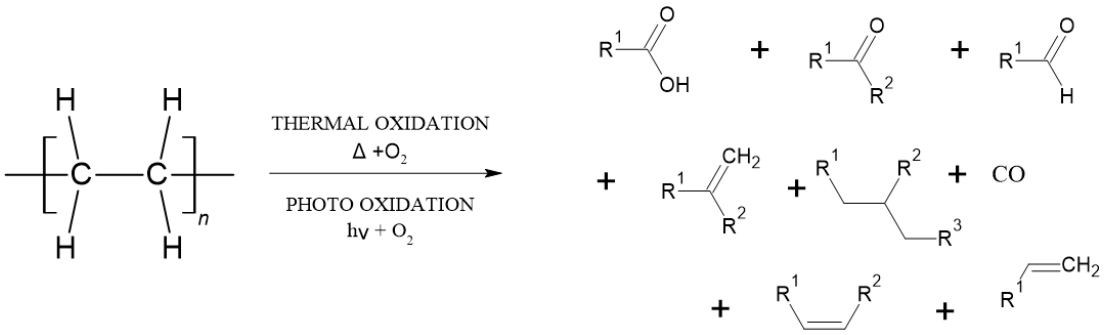

Fig: 7&8 natural plastic Degradation by thermal, hydrological, photological means

2.2 Bacterial Degradation Mechanisms

Bacteria are microorganisms mainly unicellular; they are found everywhere. They can metabolize the MPs and produce simple molecules [21]. Bacterial cultures have been used to degrade MPs in laboratory conditions. Their effectiveness were reported earlier by several studies. In general, MP degradation by bacteria involves various enzyme systems. Extracellular hydrolytic enzymes like lipase, xylanase etc. are secreted by bacteria and abiotic factors have a major role in degradation. The degradation mechanisms of different plastics are discussed in the following table below.

Table I : List of bacteria that can degrade plastic

Bacteria	Plastic-Type	Removal Efficiency	Degradation Duration	Limitation	References
Bacillus cereus	PE, PET & PS	1.6%, 6.6% &7.4%	40 days	Insignificant efficiency	22
Bacillus strain 27	PP	6.2%,3.0% &5.8%	40 days	Requires a Bushnell Hass medium	23
Rhodococcus ruber	PE	6%	7 days	Requires biofilm activation (long time)	24
Escherichia coli	PUR	1-2%	3 days	Insignificant efficiency	25
Enterobacter asburiae YT1	PE	6.1%	28 days	Vague enzymes present	26
Pseudomonas citronellolis	PVC	10-19%	45 to 90 days	Requires biofilm activation (long time)	68
Bacillus flexus	PVC	10-19%	45 to 90 days	Requires biofilm activation (long time)	68
Clostridium thermocellum	PET	60%	14 days	—	69

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Pseudomonas fluorescens,	*PEU*	5.3%	5 months	Less effective against aromatic PEU	70
Pseudomonas denitrificans,	*PEU*	2.5%	5 months	Less effective against aromatic PEU	70
Yarrowia lipolytica	*PEU*	6.7%	5 months	Less effective against aromatic PEU	70
Bacillus subtilis	*PEU*	6.8%	5 months	Less effective against aromatic PEU	70
P. stutzeri	PEG	NR	1 - 3 days	Degrad only smaller PEG	71

2.3 Abiotic Degradation of PE by Oxygen and Light

Here UV exposure is the initiating factor for PE degradation [27-30] followed by a complex set of reactions [29,31] leading to formation of lighter molecules such as ROH, $RCOR^1$ (R^1 and R being a wild card group) [27]. The process is oxygen dependent as oxygen acts as adduct to polymer [29]. Random chain cuts produce compounds of low molecular mass whereas Random cross linking results in compounds of increased molecular mass. During the process various alkanes and alkenes of 2 to 6 carbon are produced. [30,32]. Methyl group of terminal position can be attacked by Microorganisms during PE biodegradation its speed is fast if molar mass is less than a half of kg [27]. Small polymer fragments that are formed from abiotic reactions may be bio-degraded into short segments such as esters and acids [27,32]. Humus under enzymetic reaction make CO_2 and H_2O as final products [27,32]. The service life of PE is application dependent, so its estimation varies. Vasile in 2005 showed that in a temperate climate PE having cross links can serve up to 15-20 years [27]. Another study found that the carbon chain of the polymer is converted to CO_2 by biodegradation at the rate of about 0.1% per year. Under ideal laboratory conditions the degradation process will be slower because the polymer degradation conditions in the marine environment are not optimized [33].

2.4 Role of Hydrolases Enzyme used for Degradation of PET

It's thought previously that microbes can't degrade Synthetic polymers. Recently it was found that some microbes can degrade or modify PET by their hydrolases. In the due course of evolution Microbes have developed a way to utilize plastic and other natural polymers(like polyester) to ensure survival in polluted areas by producing many hydrolase. Enzymes that exhibit PET hydrolysis activity include carboxyl ester hydrolase enzymes such as cutinase, lipase, and esterase [34].These hydrolases have been isolated from sources like *Ideonella sakaiensis, Thermobifida fusca, Fusarium solani, Humicola insolar* and *Aspergillus oryzae* [35].

These are generally serine hydrolases and characterized by the trio of amino acids serine, histidine, and aspartic acid and α / β hydrolase bundle in their active site [62]. Most functionally tested PET hydrolases contain a disulfide bond at carboxy terminus that is responsible for making them more thermodynamically and kinetically stable [63].

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Nowadays genetic engineering and protein engineering, both tools are increasing the capacity of microorganisms to degrade plastics and enzymes respectively [40]. Rise in structural information of enzymes and its availability has immensely helped in targeted engineering approaches [41]. For example, by mutation , enzyme variants of LC-kinase, actinobacterial cutinase from *Thermobifida fusca* (TfCut2) , and Polyethylene-ase from *Ideonella sakaiensis* (IsPETase) were created and exhibited PET hydrolysis activity due to factors such as product inhibition and optimal catalytic activity [42].

Table II : List of enzymes used to degrade plastics

Enzyme	Microbial Source	PBD/uniprot code	Sequence Length (Kd)	Reaction Temp [c]	Substrate	Reported Degradation	References
HiC	*Humicola insolens*	4OYY	194	30-85	IcPET (7%)	97% +/- 3% IcPET ~~weight loss~~	36
IsPETase	*Ideonella sakaiensis 201-F6*	6ILW	290	20-45	IcPET (1.9%)	TPA, MHET, EG release	37, 68
LCC	Uncultured bacterium	4EBO	293	50-70	PET film	<5% weight loss	37,38
PET12	*Polyangium brachysporum*	A0A0G3BI90 (uniprot)	298	50	Nanoparticle agar	Zone of clearance	39
PET2	Uncultured Bacterium	7ECB	308	50	Nanoparticle agar	Zone of clearance	39
PE-H	*Pseudomonas aestusnigri*	6SBN	312	30	PET film	MHET release	40
Alkene monooxygenase	*Rhodococcus sp. TMP2*	B0I533	510	20	pristane	Temperature sensitive	64
Lip And MnP	*Phanerochaete chrysosporium (MTCC-787)*	*Q02567 P49012*	378 372	37	LDPE	70%weight loss Of PE	65
NylC	*Agromyces sp.*	Q1EPR5 3AXG	355	30	Nylon-6	—	66
PUR esterase	Commanus Acidovorans	Q9WX47	548	30	PUR	optimum pH 6.5 temperature 45°C.	67

2.5 Role of Biofilms in Degradation

Bacteria have the ability to attach on the surface polymeric plastic and degrade them but the speed of the process depends on the morphology of polymers [61]. Polymer are made of organic compounds with low polarity resulting hydrophobicity but cells surface are hydrophilic hence bacteria cannot attach easily to polymer but if hydrophilic functional groups are attached, the bacteria can

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attach to polymer and degrade polymer effectively by its secretions or the other way may be by making cell surface hydrophobic which can be done by biofilm development [43]. For example biofilm-adapted *Pseudomonas* sp. AKS2 cells have more hydrophobic cell surface and reduce more Low Density Polyethylene (LDPE) than planktonic cells. In addition, cells in biofilms have been found to release some chemicals like exopolysaccharides which help them to attach to plastic polymers [44].

2.6 Fate of PET and PE

PET can be hydrolysed in TPA and EG by enzymes.. Intermediates from the incomplete hydrolysis of PET include mono-[2-hydroxyethyl] terephthalate (MHET), which is in the truncated shell, and bis-[2-hydroxyethyl] terephthalate (BHET), which can then be hydrolysed to TPA and EG. Likewise, EG can be oxidized in the glyoxylate which is used in the Tri Carboxylic Acid cycle by the glyoxylate pathway in the (The steps shown below may and may not represent elementary reactions. Asterisks indicate addition of new functional groups) [45].

Small aliphatic hydrocarbons can move into cells of bacteria without further cleavage. Twisted arrows are shown in the diagram when more than one reaction occurs. [40].

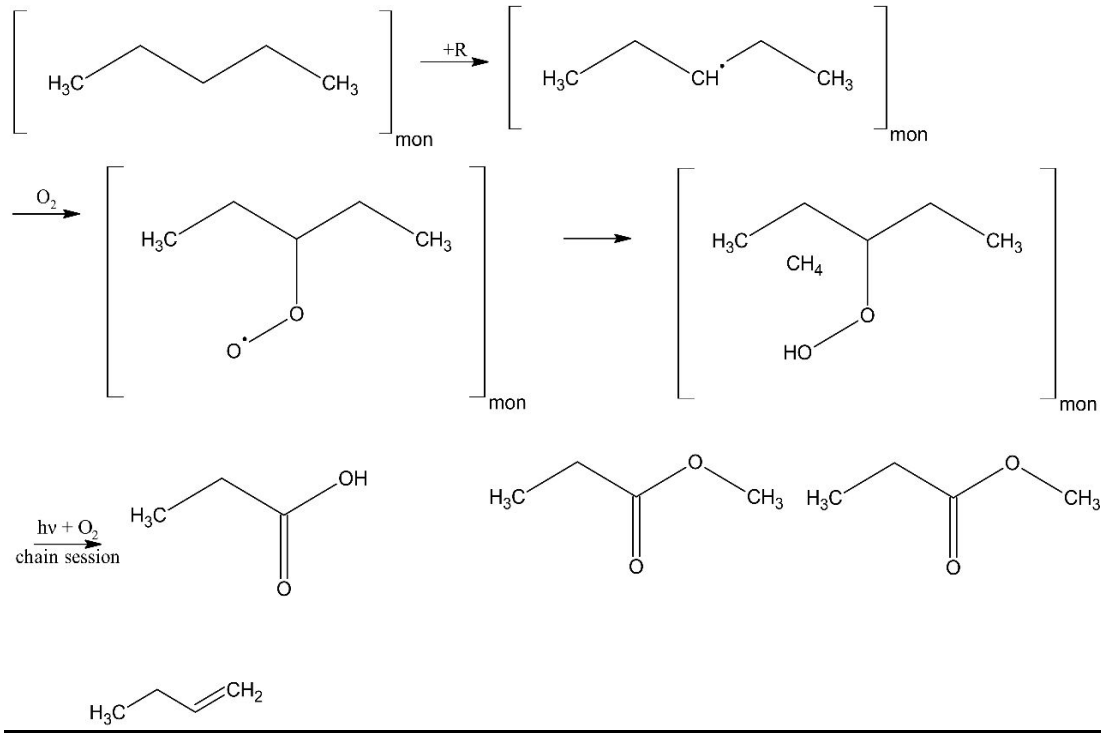

Fig: 9 Reaction involved in Abiotic degradation of PE by oxygen and light.

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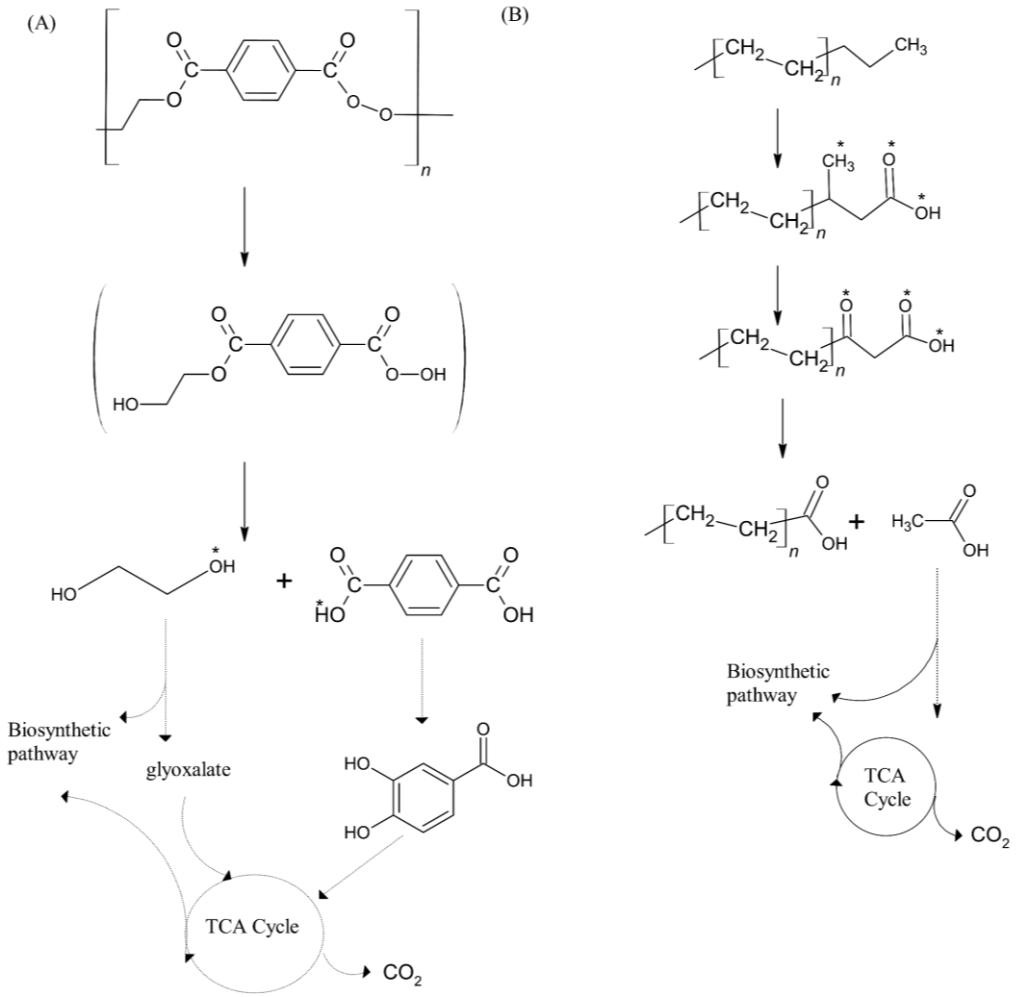

Fig: 10 The path includes several steps of oxidation, dehydration, and carbon-carbon bonding to produce acetic acid, which can enter the TCA cycle.

1. Future initiative for plastic waste management

A major move towards the future economy is rethinking the entire plastic value chain, delinking them from fuel (i.e. raw material and energy production) such an economy is known as circular bioeconomy [46]. More than 99% plastic is fossil based, mostly durable and non-biodegradable, which damages the environment and causes severe economic losses [47, 48].

Every year, 370 crore (3.7 billion) tons of agricultural waste and 130 crore (1.3 billion) tons of food waste are produced worldwide [49], which can be lost at any stage of the supply chain whether it is during manufacturing or distribution. which result directly in 3.3 billion tons of CO_2 emissions and loss of 750 USD per year (excluding fish and seafood) [50].

According to the concept of biorefinery various useful chemicals like flavonoids, can be extracted from such waste through bioprocessing which can be used to make high value products [51]. Many reviews have addressed the green assessment waste such as agro industrial waste, forest waste and food waste [52, 53, 54], highlighting their advantages and limitations.

Some international standards set requirements for end-of-life, deficiency (controlled conditions, inadequate replication, etc.) reveals when biodegradation level is checked, that limits identification and fate of material in the environment [55,56]. As most consumers don't know what is biodegradable and what is not even worse they also don't know what biodegradable means resulting in pollution and improper disposal [57,58] .

Solution is as follows, Using chemical markers enable auto identification of PEF or PLA bottles. Mechanical processing is environmentally friendly for driving plant based food products and chemical processing can be done for algal feedstocks [14, 60,

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61]. For social benefits, biobased polymers must be considered throughout their life cycle without interfering with traditional plastic recycling or waste management in multiple streams. Plastics reduction should be the top priority of policy makers [37].

3. CONCLUSION

MPs pollution is a whole biosphere and therefore a lot of research has been going to solve the problem. Various studies have been done, some of them are discussed above in relation to effectiveness of various degradation pathways like chemical, physical, biological. Along with their limitations. In general, biological and light based degradation are more suitable for complete degradation and mineralization but they take a longer time than other methods. High-efficiency chemical techniques usually require input of chemicals and high heat, and the intermediate and product production during these methods have been shown to be non-toxic to microorganisms.

Efforts to mitigate microplastic pollution involve not only tackling the sources of microplastics but also developing strategies for their effective removal and degradation. Research into innovative technologies, such as using specialized enzymes or engineered microorganisms, is ongoing to enhance the degradation process and promote the environmentally friendly disposal of microplastics.

In conclusion, microplastic degradation encompasses the physical, chemical, and biological processes involved in breaking down microplastic particles. While physical and chemical weathering can fragment microplastics, microbial degradation holds promise as a potential solution for their complete breakdown. Understanding and advancing our knowledge of microplastic degradation processes are crucial in addressing the environmental impacts of microplastic pollution and developing effective strategies for its remediation.

Till now the data of appendages removal during MP degradation is inconclusive and not enough. It is hoped that policymakers will become more aware of the MP issues as the time goes and more research is done.

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