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An Updated Review on the Bioremediation of Marine Plastic Pollution

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ABSTRACT
Almost 300 million tonnes of plastic waste are generated annually. Although this issue could be overcome by switching to biodegradable polymers, the existing detrimental effects of synthetic plastic wastes must be dealt with. Various modes of plastic degradation have been tried so far which include the Physical, Thermal, and Chemical means of degradation. Recently biodegradation of synthetic polymers has caught the eyes of researchers and a wide range of microorganisms have been found as potential degraders of these plastics. A concern to protect the environment and human safety led us to explore and research to fill this knowledge gap. Microorganisms have been found to have the capability to adapt themselves to the environment and alter their catabolic pathways in such a way that they either directly utilize these plastic wastes as a carbon source or produce by-products that target the polymer structures. This review paper deals with plastic pollution in the marine environment and how biodegradation could be a solution to it. It shows the various issues faced by marine wildlife and draws a focus on the microplastics that act as a pelagic habitat for the microorganisms. It also talks about the potential microbes from marine sources that can degrade plastics and potential enzymes produced by some of them. These findings pave the way to further enhance the development of environment-friendly degradation processes and products by protein engineering of these enzymes, strain engineering, understanding the genomics and proteomics of the enzymes, and generating an enzyme-based product for large-scale plastic waste management.

KEY WORDS: BIOREMEDIATION, ENZYMES, MARINE POLLUTION, MICROORGANISMS, PLASTIC DEGRADATION.

INTRODUCTION

Since the early 1950's synthetic plastics have gained huge importance for their astonishing physical and chemical properties. Now they have become a crucial part of our lives. Tonnes of plastics are produced every year and about 50% of them are designed for single use. Over the years their use has been exploited by mankind and now plastic wastes have become omnipresent. Almost 300 million tonnes of plastic waste are generated annually. According to marine researchers, the plastic debris could serve as a geological indicator of the Anthropocene epoch. Although this issue could be overcome by switching to biodegradable polymers, the existing detrimental effects of synthetic plastic wastes must be dealt with (Peng et al., 2020).

A concern to protect the environment and human safety led us to explore and research to fill this knowledge gap. Various modes of plastic degradation have been tried so far which include the Physical, Thermal, and Chemical means of degradation. Recently biodegradation of synthetic polymers has caught the eyes of researchers and a wide range of microorganisms have been found as potential degraders of these plastics. Microorganisms have been found to have the capability to adapt themselves to the environment and alter their catabolic pathways in such a way that they either directly utilize these plastic wastes as a carbon source or produce by-products that target the polymer structures. This review paper focuses on plastic pollution in the marine environment and how biodegradation could be a solution to it (Miraj et al., 2019, Peng et al., 2020).

Plastics and the Marine Environment: In the past 70 years, a major concern regarding the marine environment is the marine pollution that ranges from the surface till the deepest of waters. Chemicals such as persistent organic

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pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs) and plastic polymers bio-accumulate and harm the marine life According to Reddy et al., (2006), on an average, for every 1 kg of intertidal sediments about 81mg of small plastics fragments were collected. Upon examining under Fourier Transform Infra Red Spectroscopy (FT-IR) and scanning electron microscope (SEM) they were found to be polyurethane, nylon, polystyrene, polyester and glass wool (Reddy et al., 2006, Miraj et al., 2019, Catania et al. 2020). The accumulation of these small fragments is not yet completely understood. This problem is pervasive throughout the world and is evident in the terrestrial environments, the oceans, on the shores and even in freshwater ecosystems (Barnes et al., 2009).

Figure 1: FT-IR spectra of small plastic fragments in the sediments of Alang- Sosiya ship-breaking yard. (a) Thermocol (polyurethane), (b) styrofoam (polyurethane), (c) nylon, (d) transparent plastic (polystyrene), (e) colored plastic (polyester), (f) glass wool (Reddy et al. 2006)

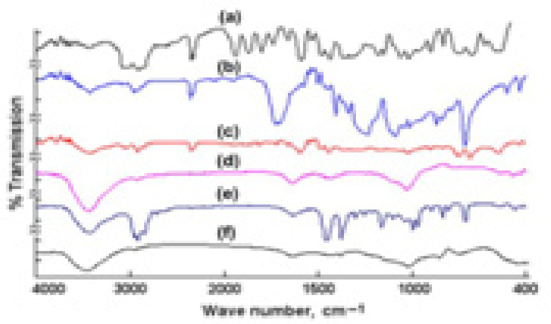
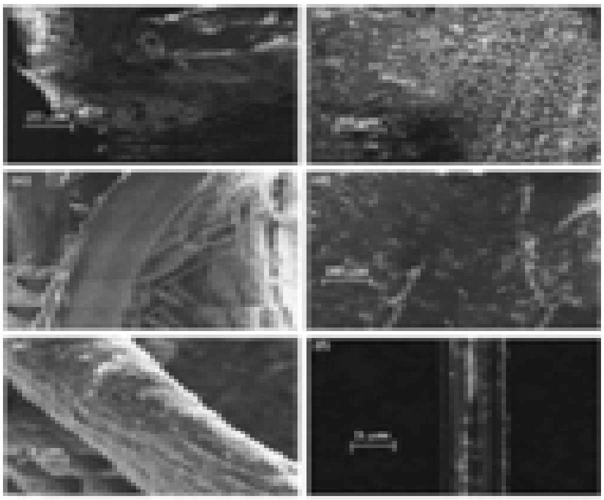
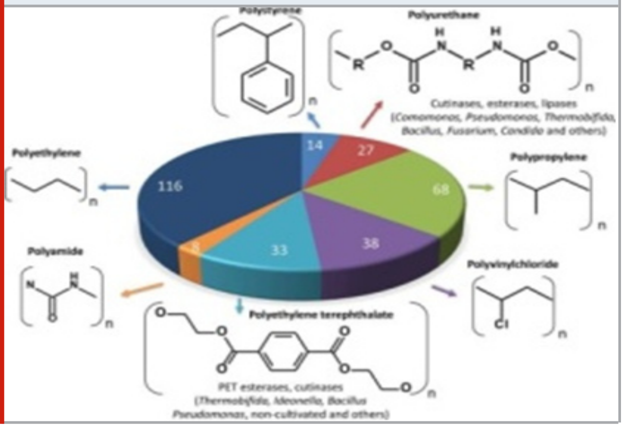


Figure 2: Scanning electronic microscopic (SEM) images of small plastic fragments. (a) Thermocol (polyurethane), (b) Styrofoam (polyurethane), (c) nylon, (d) poly- styrene, (e) polyester, (f) glass wool present in sediments of Alang-Sosiya ship-breaking yard. (Reddy et al. 2006)



Dangers to the Marine Wildlife: Ingestion, entanglement and chemical contamination effects of the plastic waste are the major problems faced by the marine wildlife due to the persistent forms of marine debris. The study was focussed on three major marine taxa, viz. seabirds, sea turtles and marine mammals. Used buoys, traps, pots, fishing nets, monofilaments, plastic bags, some plastic utensils, balloons, food packaging and other EPS packaging posed a huge risk of entanglement of the marine wildlife. The ingestible debris included the plastic utensils, plastic bags, butts, caps, balloons, monofilaments, food packaging and other EPS packaging. Chemical contamination, a secondary consequence of ingestion, was found to be mainly caused by hard plastic containers, plastic bags, butts, plastic utensils and other EPS packaging materials. Straws, stirrers, takeout containers, plastic lids, beverage bottles, cups, plates and cans were other sources of marine debris which posed minor threats to ingestion and chemical contamination. Around 8 million tons of plastic debris is dumped into the oceans each year. A majority of this is due to the intentional disposal of plastics into the sea/oceans. In this crucial phase, policy-based changes as well as consumer driven changes are essential in order to protect our marine wildlife. (Wilcox et al., 2016).

Figure 3: Main synthetic polymers globally produced in 2016. Numbers in the chart indicate the global annual production (millions of tons) of the specified synthetic polymer.Indicated are the names of bacterial genera producing verified enzymes with available protein sequences that are known to be involved in the breakdown of the high-molecular-weight polymers. (Danso.et al. 2019)



Issues with microplastics: The large sized plastics that once were a huge threat now seem negotiable in front of the microplastics. Unlike the mega- or macroplastics that remain floating in the waters, these microplastics can travel to considerable distances deep into the ocean (Barnes et al., 2009). The fragmentation of the large plastics is related to the chemical, thermal and photo and biological degradation. These involve the processes such as UV induced degradation, chemical leaching, ingestion by animals and birds (Barnes et al., 2009). According to Jayasiri et al., (2013), in comparison with the meso, macro and mega plastics, the microplastic litter were found in abundance along the coasts of recreational beaches in Mumbai. The Juhu beach showed the highest number of about 55.33 % of microplastics.

This poses a high risk to the marine beings as there is a huge possibility of ingestion. It is also reported that the beaches are more contaminated by smaller fragments of plastic than by virgin plastic pellets. Upon investigation it was revealed that land-based sources are responsible for the plastic pollution in these beaches (Jayasiri et al., 2013 Miraj et al., 2019).

Table 1. Microorganisms isolated from Marine sources that are capable of degrading different types of plastic wastes:

Microorganism	Source of the Microorganism	Type of Plastic	Reference
Bacillus cereus, Bacillus sphericus	Shallow Marine water from Indian Ocean	Low and High Density Polyethylene (LDPE and HDPE)	Sudhakaret al. 2008
Bacillus sp.	Coastal Marine Water	Polyvinylchloride (PVC), LDPE, and HDPE	Kumariet al. 2019
Pseudomonas, Alcanivorax, Tenacibaculum	Deep sea water	Aliphatic polyesters poly(ε-caprolactone) [PCL], poly(β-hydroxybutyrate /valerate) [PHB/V], and poly (butyrene succinate) [PBS]	Sekiguchiet al. 2011
Brevibacillus	Marine water, soil *borstelensis* spilled marine water	HDPE sediment and oil	Mohanrasu et al., 2018
Lysini bacillus, *Salini bacterium*	Marine water from Coastal sites in Northern Crete;	Linear Low Density Polyethylene (LLDPE)	Syranidou et al.,2017
Alcanivorax	Agios Onoufrios Marine sediments and	LDPE	Delacuvellerie et
borkumensis *Kocuria palustris, Bacillus pumilus,*	water-sediment interface Pelagic Waters, Arabian Sea	LDPE	al.2019 Harshvardhan and Jha 2013
Bacillus subtilis *Pseudomonas spp, Streptococcus spp, Staphylococcus spp, Micrococcus* spp and *Moraxella spp, Bacillus subtilis,*	Choked Sewer Line	LDPE and Starch Blend	Prabhat et al., 2013
Bacillus amylolyticus, Arthobacter defluvii Vibrio alginolyticus,	Benthic zone sediments of	LDPE, Blends	Raghul et al., 2014
V.parahaemolyticus Bacillus sp.,	various marine environments Mangrove sediments	of PVA- LLDPE Microplastics of	Auta et al., 2018
Rhodococcus sp. Muricauda sp.,	Marine Water	polypropylene (PP) Polyethylene	Debroas et al. 2017
and *Thalassospira sp.* *Alphaproteobacteria,* *Gammaproteobacteria, Bacteroidetes, Planctomycetes, Erythrobacter,*	Seawater in the area of Cape Tonkiy	terephthalate (PET) Polystyrene (PS)	Tourova et al., 2020
Maribacter, and *Mycobacterium* *Alpha proteobacteria, Gamma proteobacteria, Bacteroidetes, Pseudomonas,*	Industrial Water	PS	Tourova et al. 2020

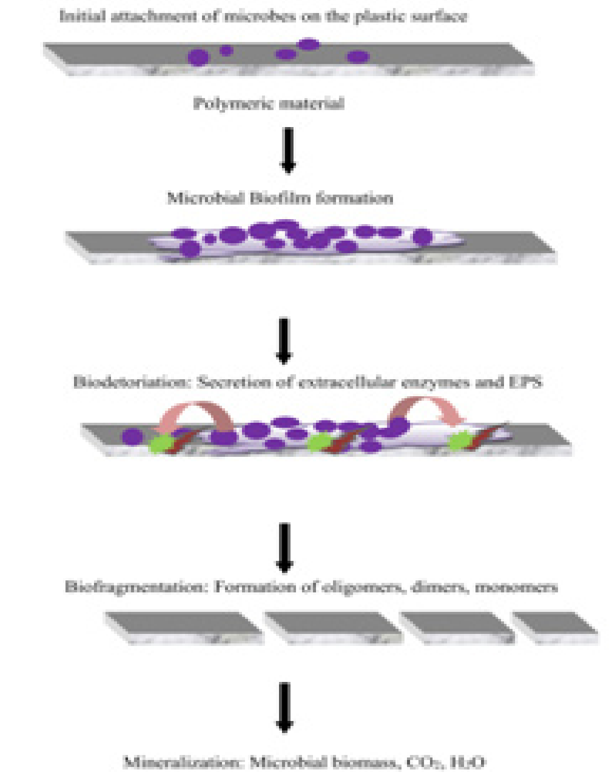
Arenimonas, Acidovorax, and *Mycobacterium*			
Thalassospira	Marine Waters	Polyvinyl-alcohol (PVA)	Nogi et al. 2014
povalilytica sp. nov. **II. Fungi**			
Aspergillus niger, Aspergillus glaucus	Choked Sewer Line	LDPE and Starch Blend	Prabhat et al. 2013
Aspergillu stubingensis,	Marine Coastal	HDPE	Devi et al. 2015
Aspergillus flavus **III. Algae**	Dumpyard		
Alariaesculenta, Palmariapalmata *Diatoms: Amphora, Achananthes, Cocconeis, Cymbella, Grammatophora, Haslea, Licmophora, Mastogloia, Nitzschia,*	Benthic Marine Water Marine Surface Waters	Nylon, PP, Polyethylene (PE) Microplastics of PS, PE, PP	Welden and Cowie 2017 Reisser et al. 2014
Microtabella, Minidiscus, Thalassionema, Thalassiosira Coccolithophores: Calcidiscus, Emiliania, Gephyrocapsa, Umbellosphaera, Umbilicosphaera,	Marine Surface Waters	Microplastics of PS, PE, PP	Reisser et al. 2014
Coccolithus,			
Calciosolenia **IV. Barnacles** Lepas	Marine Surface Waters	PS, PP, PE	Reisser et al. 2014

Microplastics are plastics that are less than 5mm in size. These are most abundant in the surface sea waters and are known to be supporting the lives of many microbes and small invertebrates. Upon observing under the scanning electron microscope, around 14 genera of diatoms, 7 genera of Coccolithophores, Bryozoans, Barnacles, a Dinoflagellate, an Isopod, a marine worm, marine insect eggs, as well as bacteria, Cyanobacteria, and fungi were found to be present on the surface of these microplastics. The surface also had a textured appearance which indicated that these microbes enhanced their degradation. In big picture, since these microplastics are found in floating water, they are believed to be game changers in the ecological niche, organism scattering and ocean productivity. (Reisser et al., 2014). According to Peng et al.,. (2020), microplastics cause malnutrition, inflammation, chemical poisoning, growth thwarting, decrease of fecundity and death in marine life due to destruction in the internal organs/tissues. Also, research shows that nanoplastics have the potential to cross biological barriers which results in their bioaccumulation in the important organs of the marine animals, (Peng et al., 2020).

International Policies to mitigate plastic use and wastage: Globally, the governments have made policies for reducing the use of plastics by banning plastic bags, making them taxable for those who sell them, etc., While some countries, such as North America, Australia and the United Kingdom, have imposed partial bans few other countries in Europe have imposed fees per bag. Several other countries in Africa and Asia have imposed complete ban on the usage of plastic bags. Microbeads are another type of single use plastics. Various governments have also imposed policies against the use of these microbeads, but no strict ban had been imposed as of in 2017 (Xanthos and Walker, 2017). India banned the plastic bags sized less than 20μm, in 2002, in order to arrest the clogging of municipal drainage systems and put a stop of mortality of cows due to ingestion of plastics. But this was enforced only in 2005, with a ban of bags sized less than 50 μm. In 2016, Karnataka imposed a complete ban on the use of plastic bags. Also, India is committed to ban all single use plastics by 2022. But although a lot of policies have been imposed against the use of plastics, many countries fall short of execution approaches. The short- and long-term impacts of these measures must be researched and various campaigns for the public could bring awareness among the public (Xanthos and Walker, 2017 Miraj et al., 2019).

How far are degradable plastics really degradable?: These days the focus of research has shifted to research in bio-based polymers as they have similar properties and are environmentally friendly. This is believed to be a sustainable solution in managing the growing plastic use and wastage. (Catania et al., 2020). According to O'Brine et al., (2010), compostable plastic bags tend to degrade faster when compared to oxo-biodegradable plastic bags and conventional plastic bags. This was observed by comparing the decrease in tensile strength. The compostable plastic bags were completely degraded between 16 and 24 weeks whereas about 98% of the other plastic bags remained even after 40 weeks. This reveals that the so called degradable or biodegradable plastic bags usually last longer (approx. 18 months) than they are thought of and hence they must be reused and recycled rather than being used for a single application. Hence even though the degradable plastics seem convincing, there are certain limitations to its degradability which might affect their applications. (O' Brine et al., 2010).

Figure 4: Schematic illustration of plastic biodegradation by microorganisms. (Kumar Aet et al., 2020)



Degradation of the Plastic Wastes: The plastic wastes in the marine environment undergo weathering and degradation due to their exposure to the sunlight, oxidants and physical stress. Such abiotic degradation is usually followed by biological degradation mechanisms. Hence the pathways of degradation and their products must be analysed from an environmental chemist point of view in order to evaluate their properties and potential risks to the environment. Plastics such as polypropylene (PP), polyethylene (PE), polystyrene (PS) and polyvinyl chloride (PVC) have a carbon backbone whereas polyethylene terephthalate (PET) and polyurethane (PU) have carbon and hetero atoms in their backbone (Gewert et al., 2015). The plastics having a carbon backbone at first undergo the photo-initiated oxidative degradation. This breaks the polymers into smaller fragments that can easily pass through the microbial cell membrane and undergo biodegradation. Biodegradation causes the polymers to break into monomers and the monomers undergo mineralization. The degree and rate of degradation depends on the amounts of additives present in the plastic as additives tend to inhibit degradation. PET and PU on the other hand have an increased thermal stability and undergo hydrolytic cleavage at their ester or amide groups. This is followed by biodegradation (Gewert et al., 2015 Miraj et al., 2019).

Biodegradation: A solution to the issue: Biodegradation seems to be a promising solution as it is eco-friendly and affordable. The plastic wastes span the marine sources right from the surface till the ocean bed. The microbes present in each of these niches are capable of easily adapting to the plastic wastes and are likely to form biofilms on the surface of the plastic debris. Various factors play key roles in the biodegradation mechanisms, of which, the polymer characteristics and environmental conditions are the most important ones (Kumar et al., 2020).

The marine debris is broken down by microbes in one of the two ways

- The microbes utilize these chemicals as their carbon source with the help of certain key catabolic enzymes.
- The microbes produce by- products that attack the polymer structure

Immobilized enzymes offer a greater potential for treating wastewaters polluted with recalcitrant materials (Catania et al., 2020).

The degradation mechanism by the microbial enzymes involves the following steps:

- Formation of microbial Biofilm: Initial attachment and formation of plastisphere.
- Biodeterioration: Action of microbial exoenzymes on the mechanical, chemical and physical properties of the plastics.
- Biofragmentation: Enzymatic depolymerization into oligomers, dimers or monomers
- Assimilation: Plastic is converted into Carbon-dioxide, water, methane and biomass (Lucas et al., 2008; Kumar et al., 2020).

Products formed after biodegradation of plastics: Plastics upon biodegradation initially form smaller subunits which further get degraded into small inorganic molecules such as carbon dioxide and water (Andrady, 1998). According to Lucas et al., (2008) and

Restrepo-Flórez et al., (2014), "Once the molecular size of the synthetic polymers has been reduced to a range of 10–50 carbon atoms, the degradation products can be taken up into the cell for further metabolization" (Wei and Zimmermann, 2017).

Table 2. Potential Microbial enzymes those are capable of degrading different types of plastic wastes:

Microorganisms	Enzymes	Plastic	References
Aspergillus clavatus			Ishii et al., 2007
Alcaligenes faecalis	PHB depolymerise	PHB and PHB valerate (PHBV)	Kita et al., 1995 Mabrouk and Sabry, 2001
Streptomyces sp. SNG9			
Candida antarctica	Lipase B	Polyurethane (PUR)	Shibasaki et al. 2009
Ideonella sakaiensis *Paraglaciecola agarilytica,*	PETase	PET	Palm et al., 2019
Marinobacterium litorale *Penicillium* sp.,	Styrene monooxygenases Oxidase, Hydrolase	Styrene	Pu et al., 2018
Geotrichum fermentans	and Dehydrogenase	PVA	Kawai and Hu, 2009
Pestalotiopsismicrospora	Serine hydrolase	Polyester	Jonathan et al., 2011
Pseudomonas chlororaphis	Polyurethanases	PUR	Howard et al., 2007
Pseudomonas protegens	Lipase	PUR	Hung et al., 2016
Sphingomonas terrae	PEG-Dehydrogenase	Polyethylene glycol (PEG)	Sugimoto et al., 2001
Thermobifida fusca	Hydrolase	PET	Muller et al., 2005
B. cereus, B.sphericus	Peroxidase	HDPE and LDPE	Sudhakar et al., 2008
Nocardia	Esterase	PET	Sharon et al., 2012

Table citation: (Kumar A et al. 2020)

CONCLUSION

This review paper analyses marine plastic pollution, the various factors that cause it, and how it can be treated using biodegradation by microorganisms. It shows the problems faced by marine wildlife and draws a focus on the microplastics that act as a pelagic habitat for the microorganisms. It also talks about the potential microbes from marine sources that can degrade plastics and potential enzymes produced by some of them. These findings pave the way to enhance the development of environment-friendly degradation processes and products by protein engineering, strain engineering, understanding the genomics and proteomics, and generating an enzyme-based product for large-scale plastic waste management.

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