Bioremediation of Synthetic Polymers: Present and Future Prospects of Plastic Biodegradation

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A B S T R A C T

An alarming challenge of environmental pollution in today's world has been increasing ever since man has come to know about the ways of exploiting the mother earth leading to irreparable damage. A considerable mismatch between the ever-increasing use of plastics and their proper waste management has raised questions about the sustainability of environment and its safety. Polymer degradation is given importance to reduce the ponderance of plastic in the environment through physical, chemical and microbial methods over various disposal methods because of their superior efficiency. With respect to the potentiality of microbial communities to biologically convert certain plastic polymers into simpler and safer products, current understanding for characterizing new microbial strains and their mechanisms to degrade fossil- based polymers play deciding factor to ensure environmental safety. This review summarizes current knowledge on different types of plastics from which microbial community can derive their nutrition through bioremediation process by using enzyme or non-enzyme based high molecular weight plastic degradation. It has also covered the major concerns about the natural and synthetic polymers, their types, uses, problems associated with their accumulation and factors affecting their biodegradability through bioremediation. It has looked at the disposal methods and the standard methods used in assessing the extent of polymer degradation. Biodegradability tests of synthetic polymers are important parameters to

Introduction

Plastics are artificially synthesized long chain polymeric molecules (Scott, 2000) consisting of a wide range of synthetic or semi-synthetic organic and inorganic compounds (Saminathan *et al*., 2014). The word plastic originated from the Greek word "plastikos", meaning 'able to be molded into different shapes (Joel, 1995). More than half a century ago synthetic polymers started to gain popularity which has led to present day indispensability of plastic in our daily life. Stability and durability of plastics have been

improving ever since, and hence this group of materials is now considered as materials being resistant to many environmental influences. Plastics have replaced paper and other cellulose-based products owing to their better physical and chemical properties (Rivard *et al*., 1995).

Commercial production of plastics has reached the present global annual production of 330 million metric tonnes (Mt) (Plastics Europe, 2017). If the present rate of growth continues then plastics production is estimated to be doubled within the next 20 years. Polymeric materials such as polyethylene (PE), polycaprolactone (PCL), polyurethane (PUR), polyhydroxybutyrate (PHB), polyhydroxyalkanoate (PHA), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polybutylene succinate (PBS), polylactic acid or polylactide (PLA), polypropylene (PP), and polystyrene (PS) are in common practice of day to day use (Muhamad *et al*., 2015; Yoshida *et al*. 2016). Majority of these fossil- based and bio-based plastics are non-biodegradable, e.g., PE, PET, PP, PS, and PVC. Thus, these non-biodegradable plastics impose a serious threat to environment by accumulating in large quantities due to improper waste management and uncontrolled disposal. As plastics are not readily degraded due to their stability in the ambient environment, their disposal has currently created a considerable pollution problem and thus, becoming a serious threat to our planet (USEPA, 2005; Sharma and Dhingra, 2016; Krueger *et al.,* 2015). There are different ways through which polymers can be degraded (Table 1). The success rate of bio-based biodegradable plastics in substituting the traditional plastics is still at a basal level due to their complex structure and lack of knowledge about optimized conditions for fast degradation (Rujnic-Sokele and Pilipovic, 2017).

Different plastic disposal methods and their limitations

Even though burying in landfill, incineration and recycling are some of the plastic waste disposal methods (Zhang *et al*., 2004) but each of them has their own limitations. Persistence of plastic components in the landfill as waste for years (Tansel and Yildiz, 2011) is due to the anaerobic condition and limited availability of oxygen in landfills (Massardier-Nageotte *et al*., 2006; Tollner *et al*., 2011). In addition to it increase in production of hydrogen sulphide by sulphate-reducing bacteria in soil are potentially lethal (Tsuchida *et al*., 2011). Heavy metals, oxygen-based free radicals and greenhouse gases are released when plastics are incinerated (Astrup *et al*. 2009; Khoo and Tan, 2010; Shen *et al*., 2010; Simoneit *et al*., 2005). Significant environmental drawbacks of plastic disposal *via* both landfill and incineration were the driving force behind the development of plastic recycling processes. While recycling processes is a relatively expensive and inefficient process (Zhang *et al*., 2004; Yamada-Onodera *et al*., 2001) due to the presence of additives and impurities during the recycling procedure which decrease both the yield and quality of the recovered product (Zhang *et al*., 2004; Awaja and Pavel, 2005) with potential health hazards (Villain *et al*., 1995; Demertzis *et al*., 1997). These inefficient and inappropriate plastic polymer disposal methods have left us with nothing but the bioremediation method as a viable option of microbial mediated plastic degradation.

Bioremediation

Many studies have revealed the potentiality of certain microorganisms in fast degradation of polymers under stress conditions by producing exoenzymes such as proteases, lipases, and cutinases and other related

products (Tokiwa *et.al*., 2009; Muhamad *et al*., 2015; Mohanty *et al*., 2000; Sharma *et al*., 2003; Ghosh *et al*., 2013). Mechanisms underlying microbial mediated polymer degradation include either direct use of plastic fragments as microbial nutritional source or indirect action of various microbial enzymes.

Deterioration in the functionality of polymers due to chemical, physical or biological reactions resulting in bond excision followed by chemical transformations known as polymer degradation (Pospisil and Nespurek, 1997).Various terms such as environmental degradation, photo degradation, thermal degradation, and biodegradation are used interchangeably in the context of biodegradation of plastic-based materials (Potts, 1978). Biodegradation refers to the degradation and assimilation of various polymeric plastic materials by living microorganisms (such as bacteria, fungi, and algae) to produce degradation products such as CO_2, H_2O, CH_4, and biomass (McCarthy, 2003). Enzymatic degradation of plastic materials is also considered biodegradation, and the term "enzymatic biodegradation" is widely used (Li and Vert, 1995). Microbial and enzymatic biodegradation of plastics are achieved under relatively milder environmental conditions of pH, temperature, and pressure. Bio mineralization, a similar process to bioremediation in which organic matter is converted into products such as CO_2, H_2O, CH_4 (Frazer, 1994; Hamilton *et al*., 1995). Aerobic and anaerobic biodegradation occur in wild nature and sediments or landfills respectively where as in composts and soil the degradation process shares both aerobic and anaerobic mechanisms (Gu *et al*., 2000). Fragmentation of polymers to constituent monomers followed by excretion and using the monomers as nutrients through mineralization requires several different microorganisms (Microbial metabolism, 2007).

Microbial biodegradation of plastics

Abiotic hydrolysis serves as a priming reaction in initiating the environmental degradation of synthetic polymers (Gopferich, 1997) by enhancing the surface area of the polymer and reducing its molecular weight (Singh and Sharma, 2007). The initial breakdown of a polymer can result from a variety of environmental (physical and chemical) forces (Swift, 1997) which cause mechanical damage to the polymeric materials (Kamal and Huang, 1992). The growth of microorganisms can cause minute swelling and bursting, as the cells penetrate the polymers (Griffin, 1980). High molecular weight polymers are poor in solubility thus making them unfavorable for microbial attack. Moreover polymers have to have accessibility to bacterial cellular membrane in order to get assimilated and then degradation by cellular enzymes. Microbial extracellular and intracellular depolymerase enzyme mediated depolymerization (Doi, 1990; Gu *et al*., 2000) followed by biomineralization result in degradation of higher molecular weight synthetic polymers, after which the smaller monomers are absorbed into microbial cells through their outer semi permeable membrane and biodegraded by utilizing them as carbon and energy sources (Goldberg, 1995). Microbial biodegradation (bioremediation) process involves both aerobic and anaerobic mechanisms (Shah *et al*., 2008). Aerobic microorganisms yield CO_2 and H_2O using oxygen as electron acceptor (Seymour, 1989) whereas anaerobic consortia of microorganisms yield CO_2, H_2O and CH_4 as the final products of polymer deterioration (Barlaz *et al*., 1989; Fig. 1).

Anaerobic biodegradation is an important component of the natural attenuation of contaminants at many hazardous waste sites. Degradable plastics is a plastic designed to undergo a significant change in its chemical

structure under specific environmental conditions resulting in a loss of some properties that may vary as measured by standard test methods appropriate to the plastic and the application in a period of time that determines its classification (Albertsson *et al*., 1987). Microbial degradation of both natural and synthetic polymers is equally important in order to understand the mechanism of bioremediation that involves the interactions among materials, microorganisms (bacteria, fungi, and actinomycetes) and the biochemical changes involved in it (Albertsson *et al*., 1987; Andersson and Karlsson, 1990).

Incorporation of starch and prooxidants in the synthetic plastics facilitates the microbial biodegradation process by decreasing the inertness and resistance to microbial attack thus results in an efficient fragmentation of high molecular weight polymers (Vijaya and Reddy, 2008). Lack of efficiency in the biodegradability of several biodegradable plastics (bioplastics) in the last 10 years has restricted the market popularity of bioplastics and imposed an urgency to develop efficient microorganisms and their products to solve this global issue.

Classification of plastics based on biodegradability

Based on biodegradability there are two groups of plastics, i.e., non-biodegradable plastics and biodegradable plastics. Non-biodegradable plastics are further categorized into fossil-based (conventional synthetic plastics) and bio-based (biodegradable plastics or bioplastics) polymers. Non-biodegradable plastics include routinely used plastics *viz.* polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyurethane (PU) (Pavia *et al*., 1988). Depending on the degree of biodegradability

and microbial assimilation both bio-based and fossil-based polymers can be included in biodegradable plastics or bioplastics which undergo enzymatic and non- enzymatic hydrolysis (Wackett and Hershberger, 2001). Bio-based biodegradable plastics (cellulose, starch, and starch-based polymers) are derived from renewable resources and thus preferred from the environmental point of view due to their ability to be completely degraded biologically owing to their ability of being hydrolyzed by microbial enzymes (Kale *et al*., 2007). The majority of the fossil-based plastics are non-biodegradable hence pose a serious threat to the environment in terms of their inefficient disposal and handling (Hoshino *et al*., 2003; Vert *et al*., 2002). Even though the bioremediation processes need to be optimized for various environmental conditions for effective and speedy biodegradation of plastics but the current research is focused on finding microorganisms capable of degrading the fossil-based plastics in the atmosphere through enzyme mediated bioremediation (Vijaya and Reddy, 2008).

Mechanism of plastic degradation by microorganisms

Major steps involved in the biodegradation of plastics are given as follows. Bio-deterioration is a natural process in which mild microbial degradation results in chemical, physical and mechanical modifications of plastic facilitated by abiotic forces to weaken the polymeric structure (Helbling *et al*., 2006; Ipekoglu *et al*., 2007; Jakubowicz *et al.,* 2006). The formation of biofilm over the surface of plastic triggers this process depending upon the chemical composition and physical structure of the plastic. Formation of biofilm depending upon the chemical composition, physical structure of the plastic and the microenvironment leads to the growth of microbial consortia thus

making the degradation process fast forward (Zettler *et al*., 2013) through deterioration of structural integrity of plastics (Bonhomme *et al*., 2003). Bio-fragmentation refers to the process of cleaving the polymeric plastics into oligomers, dimers or monomers. Polymers have to have accessibility to bacterial cellular membrane in order to get degraded by cellular enzymes and then assimilation into microbial cells. Microbial extracellular and intracellular depolymerase enzyme mediated depolymerization (Doi, 1990; Gu *et al*., 2000) followed by biomineralization result in degradation of higher molecular weight synthetic polymers, after which the smaller monomers are absorbed into microbial cells through their outer semi permeable membrane and biodegraded by utilizing them as carbon and energy sources (Tsuchida *et al*., 2011). Bacteria and fungi degrade plastics through their extracellular enzyme mediated cleavage of long chain polymers. Bacterial enzymes such as lipase, various serine hydrolase class enzymes (Tokiwa *et al*., 2009; Muhamad *et al*., 2015; Abou-Zeid *et al*., 2001) and fungal enzymes such as glycosidase, catalase, cutinase, manganese peroxide, various serine hydrolase class enzymes (Tokiwa *et al*., 2009; Muhamad *et al*., 2015; Howard , 2002; Russell *et al*., 2011) participate in plastic biodegradation. Bacteria and fungi are the pivotal players in biodegradation of polymeric hydrocarbons in the environment whereas the role of algae and protozoa in aquatic and terrestrial ecosystems is still a mystery.

These above mechanisms are followed unanimously by all the microbes for plastic degradation. Biodiversity and prevalence of synthetic polymer degrading microbes depends upon the surrounding environment. It is important to determine their distribution and population in different ecosystems to avail their beneficial property of degrading the synthetic polymers. The main mechanism behind the microbial degradation of synthetic

polymers is the adherence of microbes over the surface of plastic followed by their colonization.

Synthetic plastics such as polyethylene degraded by *Brevibacillus borstelensis*, *Penicillium simplicissimum* (Yamada-Onodera *et al*., 2001; Hadad *et al*., 2005), polyurethane degraded by *Comamonas acidovorans*, *Curvularia senegalensis, Fusarium solani, Aureobasidium pullulans, Pseudomonas chlororaphis* (Howard, 2002; Akutsu *et al*., 1998; Zheng *et al*., 2005), polyvinyl chloride degraded by *Pseudomonas putida, Pseudomonas fluorescens, Aspergillus niger* (Anthony *et al*., 2004; Mogilnitskii *et al*., 1987). Natural plastics such as Poly(3-hydroxybutyrate-co-3-mercaptopropionate) degraded by *Schlegelella thermodepolymerans* (Elbanna *et al*., 2004), Poly(3-hydroxybutyrate) degraded by *Pseudomonas lemoignei* (Jendrossek *et al.,* 1995), Poly (3-hydroxybutyrate-co-3-mer-captopropionate) degraded by *Pseudomonas indica* (Elbanna *et al*., 2004), Poly(3-hydroxybutyrate) poly(3-hydroxybutyrate-co-3-hydroxyvalerate) degraded by *Streptomyces sp*. (Mabrouk and Sabry, 2001), Poly(3- hydroxybutyrate-co-3 hydroxy-propionate) degraded by *Acidovorax sp., Ralstonia pikettii* (Wang *et al*., 2002), Poly(3-hydroxybutyrate) poly(3-hydroxypropionate) poly(4-hydroxybutyrate) poly(ethylene succinate)poly(ethylene adipate) degraded by *Alcaligenes faecalis, Pseudomonas stutzeri, Comamonas acidovorans* (Kasuya *et al*., 1999), Polycaprolactone degraded by *Clostridium botulinum, Clostridium acetobutylicum, Fusarium solani* (Abou-Zeid *et al*., 2001; Benedict *et al*., 1983), Polylactic acid degraded by *Fusarium moniliforme, Penicillium Roquefort, Amycolatopsis sp., Bacillus brevis, Rhizopus delemer* (Torres *et al*., 1996; Pranamuda *et al*., 1997; Pranamuda and Tokiwa, 1999; Tomita *et al*., 1999; Fukuzaki *et al*., 1989), polymer blends such

as starch/polyethylene/polyster degraded by *Aspergillus niger, Penicillium funiculosm, Streptomyces sp., Phanerochaete chyrsosporium* (Lee *et al*., 1991).

Polyethylene (PE) is a long chain polymer of ethylene produced as either high-density (HD-PE) or low-density polyethylene (LD-PE). Bacteria belongs to Gram-negative (*Pseudomonas, Ralstonia* and *Stenotrophomonas*) and Gram-positive (*Rhodococcus, Staphylococcus, Streptomyces, Bacillus*) genra are found to have associated with PE-degradation (Kumar and Raut, 2015; Restrepo-Florez *et al*., 2014). In addition fungal genera (*Aspergillus, Cladosporium, Penicillium*) affiliated with assumed PE-degradation were also reported (Yamada-Onodera *et al*., 2001; Bonhomme *et al*., 2003; Kumar and Raut , 2015; Restrepo-Florez *et al*., 2014; Pathak and Navneet, 2017; Ojha *et al*., 2017; Veethahavya *et al*., 2016; Vimala and Mathew, 2016). Gut-associated microbiome of invertebrates is also noted to degrade PE (Yang *et al*., 2015; Yang *et al*., 2014).

Polyethylene terephthalate (PET) is a polar linear polymer resulted from the repeating units of the aromatic terephthalic acid and ethylene glycol (Gubbels *et al.,* 2018). PET hydrolyzing enzymes (PET hydrolases) have relatively low turnover rates and appears to be limited to a few bacterial phyla. Out of which most members belong to Gram-positive phylum Actinobacteria (Acero *et al*., 2011) and genera *Thermobifida* or *Thermomonospora* (Kleeberg *et al*., 1998; Hu *et al*., 2010; Wei *et al*., 2014; Wei *et al*., 2014; Chen *et al*., 2008; Zimmermann and Billig, 2011; Ribitsch *et al*., 2012; Kawai *et al*., 2014). Degradative enzymes for PET (e.g. PET hydrolase and tannase, MHETase) are typically serine hydrolases e.g. cutinases (EC 3.1.1.74), lipases (EC 3.1.1.3) and carboxylesterases (EC 3.1.1.1). The α/ß-

hydrolase fold and the catalytic triad are the mechanisms underlying PET hydrolysis (Wei et *al*., 2014; Ollis *et al*., 1992). Besides the actinobacterial PET hydrolases, fungal cutinases of the phyla Fusarium and Humicola showed substrate specificity for PET (Carniel *et al*., 2017).

Polyvinylchloride (PVC) and Polypropylene (PP) are the third most frequently produced polymers. PVC is composed of repeating chloroethyl units while PP is a polymer of repeating units of propane-1,2-diyl units (Fischer *et al*., 2014; Karger-Kocsis and Barany, 2019). Only very few reports have been published that describe regarding degradation of any of these polymers.

Polystyrene (PS) (poly(1-phenylethene) polymer consists of styrene monomers. Inspite of the fact that there is no such ideal enzyme known to date which can degrade high molecular weight polymers, reports showed half reduction in the molecular weight of PS by employing brown-rot fungi followed by depolymerization with (Krueger et al., 2015). Similarly co- incubation of white rot fungi and brown rot fungi together shown to have good biodegradability of PS (Milstein *et al*., 1992). It is found that PS degradation is effective in presence of a large number of bacterial genera compared to a single bacterium (Ho *et al*., 2018; Mooney *et al*., 2006; Dobson *et al*., 2002; Tischler, 2015; Oelschlagel *et al.,* 2018).

Biodegradability tests

The extent and stage of biodegradation are important to know. The analytical tools used to monitor the biodegradation process include several processes. Effects such as the roughening of the surface, formation of holes or cracks, de-fragmentation and changes in colour or formation of biofilms on the surface are some of the visual cues through which the

progress of biodegradation can be accessed. These changes ensures the microbial attack has occurred but do not prove the advancement of biodegradation in terms of metabolism (Ikada, 1999). Change in the physical properties of a polymer such as density, viscosity, loss in molecular weight, melting temperature, loss in tensile strength, changes in the crystalline structure (Witt *et al*., 2001; Sowmya *et al*., 2014; Erlandsson *et al*., 1997). Changes in the chemical properties of the polymer in synthetic media through FTIR, including the formation or disappearance of functional groups as determined by techniques such as TLC, GCMS, NMR and FTIR can be measured (Arutchelvi *et al*., 2008).

Bio-conversion of the carbon backbone of the polymer to metabolic end product during bioremediation process can be estimated by measuring CO_2 evolution and O_2 consumption (Hoffmann *et al*., 1997). Evolution of CO_2 and methane during microbial anaerobic degradation of polymers as the end products can be tested by using gas chromatography or manually by titration (Gartiser *et al*., 1998; Reischwitz *et al*., 1997). A zone clearance test is performed on suitable agar medium by dispersing fine particles of polymers that has to be checked for its degradability by certain organisms.

A clear halo around the colony of inoculated microorganisms indicates the ability of that particular organism to depolymerize the polymer, which is the first step of biodegradation.

This method is usually employed to screen organism's ability to degrade a certain polymer in question (Abou-Zeid *et al*., 2004; Nishida and Tokiwa, 1993) which is further analyzed for its metabolic activity measurement by ATP assays, protein analysis and FAD analysis (Arutchelvi *et al*., 2008).

Factors affecting biodegradation of plastics

Various factors have been attributed to influence the biodegradation of plastics (Fig. 2). Although such materials contain energy sources for microorganisms but the timing of those materials to be identified as their energy source play a crucial role in degradation (Voet *et al*., 2006; Ramos *et al.,* 1994; Nisbet and Sleep, 2001; Szathmary and Smith, 1995). The lack of available geometrical compatibility between substrate (synthetic plastic) and microbial enzyme due to high molecular weight (Shah *et al*., 2008; Kelen, 1983) and branched cross-linked rigid structures (Omichi, 1992) makes the biodegradation process even harder (Voet *et al*., 2006; Bailey and Ollis, 1986; Koshland, 1994). Hydrophobicity of plastic-based materials has also been attributed to make them nonbiodegradable (Nakajima-Kambe *et al*., 1999). In case of fungus mediated biodegradation of plastics, the formation of bio-film play a sensitive influential role. The presence of associated contaminants like carbohydrates (such as glucose) make the biodegradation process of synthetic plastic slower as the earlier is more preferred carbon source than plastic (Jang *et al*., 2002). Presence and absence of oxygen especially low partial pressure of oxygen can significantly slow down the rate of degradation (Jakubowicz, 2002).

In conclusion, indispensible uses of plastic polymers in huge amounts in every part of the world are increasing incessantly. The poor disposal methods of these polymers often end up in causing significant environmental issues. Even though bio- and fossil-based biodegradable plastics in certain applications like packaging, agriculture, and health industry polymers are reported to be environmentally safe but the nescience of their structure and optimal degradation conditions have made the exploration of non-biodegradable petrochemical products to a

greater extent causing a great threat to the environment especially in the absence of waste management facilities and littering control. An alternative approach of bioremediation for such plastic based polymers has solved multifaceted role in terms of safe disposal and resource recovery through the utilization of degradation byproducts. The problems underlying the low degradability of synthetic polymers can be alleviated either by chemically modifying them or by searching new alternatives for their degradation by any of the following mechanisms; environmental erosion, photo degradation, thermal degradation and microbial biodegradation (bioremediation). The main bottleneck of initial breakdown of the high molecular weight polymer and its crystalline structure through microbial enzyme mediated degradation can be brought forward by researching more on diversity of known enzymes and microbes acting on synthetic polymers. A sound knowledge about the different steps of bioremediation with the associated factors affecting this process could provide better exploration of a cost effective, high efficient and eco-friendly technology capable of reducing and eliminating synthetic plastics.

Table.1 Various polymer degradation routes

Factors (requirement/ activity)	Photo-degradation	Thermooxidative degradation	Biodegradation
Active agent	UV-light or high-energy radiation	Heat and oxygen	**Microbial agents**
Requirement of heat	Not required	Higher than ambient temperature required	**Not required**
Rate of degradation	Initiation is slow. But propagation is fast	Fast	**Moderate**
Other consideration	Environment friendly if high-energy radiation is not used	Environmentally not acceptable	**Environment friendly**
Overall acceptance	**Acceptable but costly**	**Not acceptable**	**Cheap and very much acceptable**

(http://www.envis-icpe.com)

Fig.1 Mechanism of biodegradation of polymers (Alshehrei, 2017)
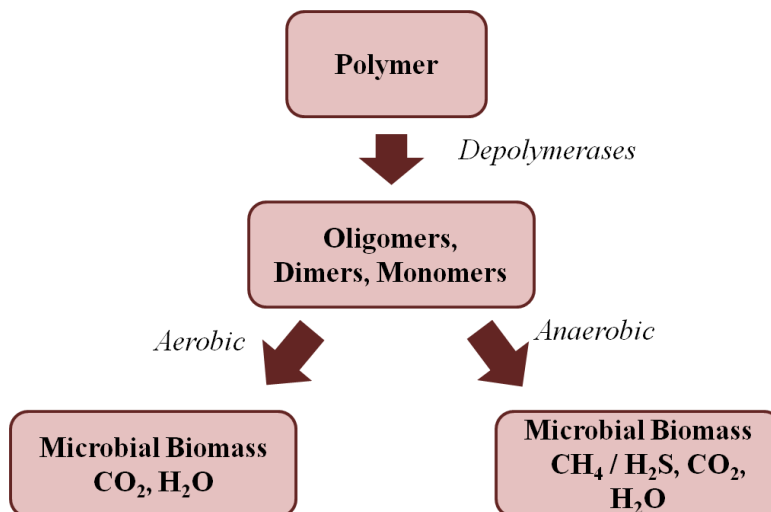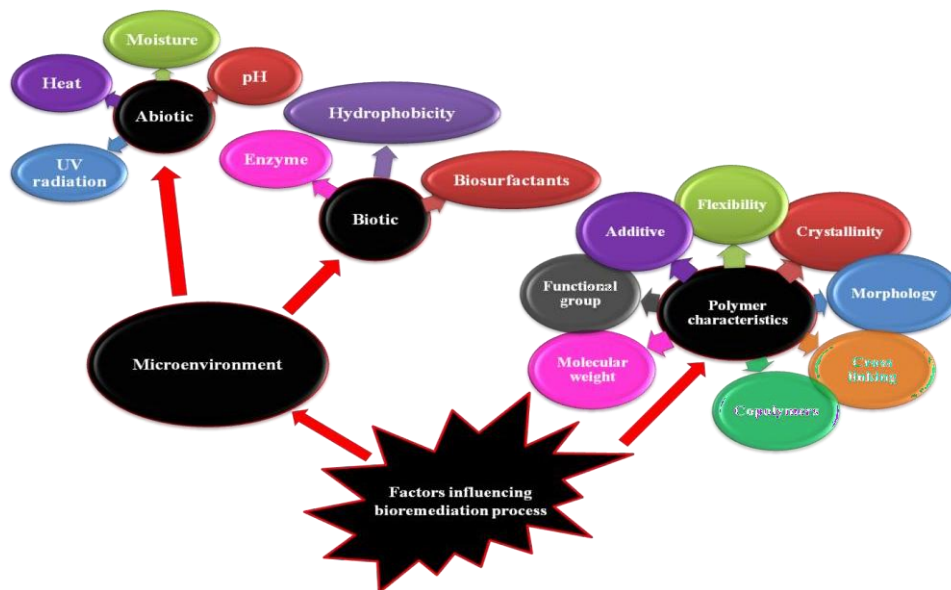

Fig.2 Various factors affecting the biodegradation of plastics

There is an urgent need to standardize all details related to screening of organisms which degrade polymers or produce enzymes that degrade polymers. Since current understanding about the identification of highly active enzymes for synthetic polymers has not up to the mark, the analytical study of global metagenomes might offer a promising source for the identification of such biocatalysts. The study of molecular mechanisms involved in the process of biodegradation like bio-fragmentation, bio-assimilation, and bio-mineralization is still in their nascent stage which can be evolved as a major thrust area of research in future. Therefore, there is a huge hue and cry for conducting research and large-scale bioremediation studies in this field to sort out environmental and resource depletion problems of the world.

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