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Approaches for Enhancing Microbial Degradation of Synthetic Plastics: A Review

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A B S T R A C T

The term plastic is given to various polymers which can be moulded in different shapes as per the requirement in different sectors. Some plastics are biodegradable while others are not. The biodegradability of plastics depends upon many factors such as polymer characteristics and growth conditions. Because of complex polymeric structure and high molecular weight microorganisms cannot degrade plastics efficiently. So, scientists discover new ways to improve biodegradation. In this review, factors that can enhance plastic degradation by microorganisms including pre-treatment and blending of plastics, use of potent strains, modification in growth medium and biofilm formation are discussed.

Introduction

Plastics are polymeric substances made up of various synthetic or semi-synthetic compounds (Saminathan *et al.*, 2014). They are synthesized from petrochemicals which are obtained from natural gas, coal and oil. The most common plastics used in different industries and agriculture are polyethylene (PE), polycaprolactone (PCL), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PUR), polyhydroxyalkanoate (PHA), polyhydroxybutyrate (PHB), polybutylene succinate (PBS), polyethylene terephthalate (PET), polylactide (PLA), and polystyrene (PS) (Muhamad *et al.*, 2015; Yoshida *et al.*, 2016). Most of these plastics including PE, PP, PS, and PVC are non-biodegradable and get accumulated in the environment in large amount because of improper management of waste. It resulted in a big threat to all forms of life on the planet (Sharma and Dhingra, 2016). It not only caused serious health issues but also affected soil fertility severely. The rate of generation of plastic waste is about 57 million tons annually, as per estimation on global level (Vijaya and Reddy,2008).

Moreover, deposition of plastic waste in oceans is much larger than land which is a serious threat to life of aquatic animals and fishes (Comăniță *et al.,* 2016). So, it is a big concern to scientists to deal with the problem of increasing accumulation of non-biodegradable plastic waste in the environment day by day (Tokiwa and Calabia, 2008). One way to overcome this problem is the use of biodegradable plastics in different sectors. But because of low stability their use in industries is limited (Rujnić-Sokele and Pilipović, 2017). Plastics are complex molecules made up of long chains of repeating units of carbon atoms. The monomers are connected to each other with strong carbon-carbon bonds. Because of their polymeric structure and hydrophobicity they are very resistant to degradation (Ghosh *et al.,* 2013). They are deliberately made in that way so that they can persist without damage for long times. Because of this it is very difficult for microorganisms to attack them (Vijaya and Reddy, 2008). However, slow breakdown of plastics over long period of time is observed in nature. Also, many studies have been conducted which showed the partial degradation of non-biodegradable plastics by various microorganisms including bacteria and fungi. Microorganisms employ different methods for degradation of such plastics including use of plastic fragments as a source of carbon and energy or indirectly by using exoenzymes (Mohanty *et al.,* 2000; Sharma *et al.,* 2003; Ghosh *et al.,* 2013). Many bacterial enzymes play important role in biodegradation (Tokiwa *et al.,* 2009; Muhamad *et al.,* 2015). Moreover, enzymes lipases and esterases, secreted by *Rhizopus delemar*, *R. arrhizus, Achromobacter sp*. and *Candida cylindracea*, have been reported to work on complex polymers like poly (ethylene adipate) and PCL (Jin *et al.,* 2000; Lam *et al.,* 2009). Since degradation of plastics in nature by microorganisms is very slow and extent of degradation is also very less. So, this review is focused on different approaches which enhance microbial degradation of synthetic polymers. As described above, due to their resistance microorganisms cannot decompose intact plastics so, before microbial degradation plastics are pretreated with heat, UV rays, light or different chemicals (Shah *et al.,* 2008; Arkatkar *et al.,* 2010; Wilkes and Aristilde, 2017). Also, blending of non-biodegradable plastics with biodegradable polymers like starch fastens the process of breakdown (Kim, 2003). Moreover, enhanced degradation of plastics by the use of efficient strains obtained from sited where plastic is being dumped for very long periods including both land and marine environments has been studied by various researchers (Singh *et al.,* 2014;Sen and Raut, 2015; Iakovlev *et al.,* 2017). In addition, use of microbial consortium, strain improvement by mutation and biofilm formation by microbes are also covered in this review.

Factors influencing plastic biodegradation

The process of biodegradation is affected by a number of factors including properties of polymers, conditions in which degradation occurs and characteristics of enzymes involved in degradation. Microbes require water for their growth as well as activity, so moisture rich conditions fastens the process of degradation (Ho *et al.,* 1999). Various microbes require different but specific pH range for growth. Degradation process of polymers produces some products which change the acidity of medium which in turn change the activity of microbes and further degradation (Auras *et al.,* 2004; Henton *et al.,* 2005). Similarly, temperature also affect the process of degradation as polymers with high melting point are difficult to degrade and enzyme activity also diminishes at higher temperatures (Tokiwa and Calabia, 2004; Tokiwa *et al.,* 2009). Different enzymes obtained from bacterial and fungal species are involved in the degradation of various polymers because of presence of different active sites (Kale *et al*., 2007). Various polymers differs in characteristics properties including molecular weight, shape, size and additives. Polymers with high molecular weight are more resistant to microbial degradation (Tokiwa *et al.,* 2009). Similarly, large surface area of polymers increases the rate of degradation (Stevens, 2003; Kijchavengkul and Auras, 2008). In addition, presence of additives in polymers also affects degradability of polymers significantly (Yang *et al.*, 2005).

Approaches for improving biodegradation of synthetic plastics

Many bacterial and fungal genera have been identified which possess the capability of degrading different types of plastics (Shimao, 2001). But the rate and extent of degradation process is very low because of high molecular weight and hydrophobicity of polymers (Shah *et al.*, 2008b; Ali *et al.*, 2014). As plastic pollution is increasing day by day in globe, there is dire need to find better ways to combat this problem (Walker *et al.*, 1997; Barnes, 2002; Moore, 2008). Microbial degradation is a cheap and eco-friendly method of plastic degradation (Grover *et al.*, 2015). So, scientists find some ways of enhancing degradation by potential microbes.

Biodegradable polymer blends

Biodegradation of resistant and inert polymers can be stimulated by addition of additives such as starch, which are biodegradable in nature and serve as nutrient source for microbes and can be easily digested. It was reported that biodegradation ability of *Pseudomonas sp*. increased by the addition of alternative substrates (Singh and Sharma, 2008). Similarly, *P. aeruginosa* ATCC13388 showed higher degradation of hydroxypropylated starch blended PE film than nonblended film (Kim, 2003). However, the mechanical strength and durability of plastic get reduced by the addition of such sugar additives (Kim, 2003). Therefore, the nutrient stimulant should not be blended in the polymer structure but added externally.

Promising microorganisms

Many microorganisms including bacterial and fungal species are involved in the process of biodegradation of different plastics. The degradation abilities of different organisms are different depending upon the type of polymer, growth conditions and enzymes secreted. A number of bacteria and fungi isolated from soil in plastic dumping sites and plastic surfaces have been discovered which effectively degrade synthetic plastics like PE (Veethahavya *et al.*, 2016; Vimala and Mathew, 2016; Ojha *et al.*, 2017), PP, PVC (Iakovlev *et al.*, 2017), PS (Milstein *et al.*, 1992), etc. Various thermophilic microbes are found to be highly potent in degrading different synthetic plastics because of their ability to grow in extreme conditions and secreting many plastic degrading enzymes (Skariyachan *et al.*, 2016; Ganesh *et al.*, 2017). Moreover, many marine microbes possess good biodegradation abilities. As we know, plastic waste is being dumped in oceans from a long period of time. Due to high amount of plastic in water many benthic organisms have developed the abilities to degrade them. This may be because they utilize this waste as new carbon source (Pauli *et al.*, 2017). It was speculated that development of ability of microbes to utilize new carbon sources generated unique features or enzymes in them which are very helpful in dealing with the problem of plastic pollution (Rampelotto, 2014). Among the potential microbial species being employed in the process of biodegradation *Pseudomonas, Flavobacterium, Micrococcus, Subtercola, Polaromonas, Leifsonia, Agreia* and

Cryobacterium were isolated from the cryoconite of three glaciers located in northwest Spitsbergen (Singh *et al.,* 2014). Moreover, it was studied that invertebrates possess the capability of degrading different plastics (Bombelli *et al.,* 2017; Brandonet *et al.,* 2018). This may be because of microbe present in the gut of the worms in addition to mechanically grinding and shredding of plastics by these organisms. Lastly, microbes can be manipulated genetically by mutation with mutagens such as ultraviolet rays and ethyl methane sulphonate to overproduce the desired enzymes for effective plastic degradation (Muralidhar *et al.,* 2014).

Microbial consortium

Biodegradation of plastics is a complex and multistep process. It requires breakdown of polymeric compounds into monomeric components and subsequent degradation and assimilation by microbes. It needed a number of enzymes which are highly efficient. All bacteria do not possess all enzymes responsible for effective degradation. So, symbiotic bacterial consortium can degrade resistant plastics more effectively. It was reported that when *P. aeruginosa* MZA-85and *Bacillus subtilis* MZA-75 were used in consortium for PU degradation resulted in the highest weight loss and esterase activity as compared to the individual strains (Shah *et al.,* 2016). One more reason for enhanced biodegradation capabilities in a consortium is microbial interactions with each other. *P. putida* VM15A and *Pseudomonas sp*. VM15Cwere capable of utilizing PVA as a sole carbon source only when grown together symbiotically but not individually (Shimao, 2001). Similarly, co-culturing of a *Flavobacterium* sp. with a *Pseudomonas* sp, resulted in the degradation of PEG due to removal of toxic by-products of PEG degradation produced by *Flavobacterium* sp. (Gu, 2003).

Pre-treatment of plastics

As mentioned above, complex synthetic plastics are very difficult to degrade by microbes because of their high molecular weight and inertness. Microbes cannot attack directly on polymeric molecules. So, before microbial degradation polymers needs to be converted into smaller molecules so that microbes can act on them and degrade further (Gilan *et al.,* 2004; Arkatkar *et al.,* 2010; Devi *et al.,* 2016). For this purpose, plastics are treated with different physical and chemical agents prior to biodegradation.

Thermal degradation

The breakdown of complex molecules into simpler onesby the use of heat is known as thermal degradation however, in the presence of oxygen degradation is known as thermal oxidation. In this process peroxide radicals are produced when products of long chain polymers react with oxygen (Ghosh *et al.,* 2013). Thermal degradation takes place in the presence of visible light while thermal oxidation requires infrared radiations. Many properties of the polymers change due to change in polymeric structure such as optical property, reduction in malleability and molecular weight and many others (Shah *et al.,* 2008).

Photodegradation

It is the use of high intensity photon particles for the breakdown of long chain polymers into monomeric units. Polymers are able to entrap the solar radiations because of their unique physical and chemical properties. In the presence of oxygen free radicals are created and (Krueger *et al.,* 2015) chain cleavage of polymeric molecules occurs. In this way, smaller molecules are generated which are easily degraded and consumed by different microbial species (Wilkes and Aristilde, 2017).

Chemical treatment

A number of chemicals such as nitric acid, sulphuric acid, hydrogen peroxide and hydrochloric acid are used for this purpose. These chemicals create hydroxyl group radicals which in turn oxidize the surface of polymers (Arkatkar *et al.,* 2010; Sen and Raut, 2015). Moreover, it was found that prooxidant additives increase the hydrophobicity of polymeric polyethylene by producing lower molecular weight products and creating carbonyl functional groups (Chiellini *et al.,* 2006; Singh and Sharma, 2008).

Growth medium

The biodegradation of different plastics is greatly affected by the nature of the growth medium. Microbes need various nutrients for growth and multiplication. Optimum conditions and nutritional requirements of different microbes are different. It was reported by many scientists that some nutrient sources enhance the degradation process while others diminish it. As reported by Hung *et al.* (2016), if citrate or succinate were added in the medium degradation of PU by *P. protegens* Pf-5 greatly enhanced. A reduction in the cell surface hydrophobicity and attachment of *Pseudomonas sp*. A to the polymer surface was observed when exposed to carbon- rich and nitrogen-starved conditions (Sanin *et al.,* 2003). This happened due to secretion of extracellular polysaccharides because of overflow of excess carbon (Sanin *et al.,* 2003). So, the increase in concentration of extracellular polysaccharide resulted in improved hydrophobicity of the cell surface (Sanin *et al.,* 2003). Accordingly, cell surface hydrophobicity was increased in *Pseudomonas sp*. AKS2 on addition of ammonium sulphate to the growth media (Tribedi and Sil, 2013b). The activity of metabolic enzymes is also influenced by trace elements which in turn affect biodegradation of plastics. Moreover, iron and manganese can act as a pro-oxidants of polymers (Singh and Sharma, 2008). However, PVA degradation by *Pseudomonas sp.* O-3 decreased by the addition of nickel and cobalt in the medium as they serve as inhibitors of enzymes involved in the degradation (Suzuki, 1976). These studies showed the importance of manipulating medium composition for increasing hydrophobicity and attachment of microbial cells to the plastic polymers which in turn enhance biodegradation.

Biofilm formation

The structure of polymer surface plays very important role in deciding the ability of bacterial cell to attach to the polymer surface (Donlan, 2002). Bacteria tend to attach to hydrophilic surfaces more easily so creation of hydrophilic functional groups on the surface of polymers enhances the attachment of bacteria to plastics. In addition, higher roughness of surfaces not only enhance the attachment but also increases the accessibility of extracellular bacterial enzymes to plastics (Sanin *et al.,* 2003; Tribedi and Sil, 2013c; Nauendorf *et al.,* 2016). The plastic polymers such as PE which has high molecular weight and hydrophobicity require changes in the surface by oxidation reactions or addition of certain chemicals to make it feasible for bacterial colonies to adhere to such polymers (Shah *et al.,* 2008; Sivan, 2011). The bacterial species which possess the ability of biofilm formation on unmodified plastic surfaces have comparatively higher hydrophobicity of their surfaces (Gilan *et al.,* 2004; Tribedi and Sil, 2013b; Devi *et al.,* 2016). As studied by Tribedi *et al.,* (2015), *Pseudomonas sp.* AKS2 which showed higher potential for degradation of LDPE possesses greater hydrophobicity of their cell surfaces. In addition, it was found that exopolysaccharides are secreted by the cells in the biofilm which helps in the attachment (Tribedi and Sil, 2013c). However, it was determined that *Pseudomonas sp*. AKS2 was not good at degrading other plastics because of differences in their surface structures. Furthermore, biofilm formation is also affected by the nutritional status of the medium (Sivan, 2011). Bacterial cell surface hydrophobicity was found to increase with decreasing glucose and increasing ammonium sulphatecontents (Tribedi and Sil, 2013b). Similarly, there was minimal breakdown of PE in marine sediments which are rich in organic carbon because of inability of bacterial biofilm formation (Nauendorf *et al.*, 2016). Therefore, biofilm formation directly enhances the process of plastic degradation by bacterial species.

Future prospects

A limited number of microbes and enzymes are known which can degrade synthetic plastics. Moreover, their degradation capabilities are also very less. Synthetic plastics can be degraded only to some extent. In addition, microbes require pretreatment of these stable plastic polymers with different chemical and physical agents. Therefore, novel microbes are required which are highly potent in degrading synthetic plastics without pretreatment and to a larger extent. Furthermore, currently known microbes can be genetically manipulated by mutation or genetic engineering methods so that the production of plastic degrading enzymes can be increased. Likewise, highly potent enzymes which can degrade most dominant polymers can be identified from non-cultivated microorganisms (i.e., global metagenomes). Moreover, structures similar tocellulosome can be developed in microbes which can attack inert and resistant plastics. The biodegradable additives can be added in the plastics which enhance the biodegradability without affecting their useful properties. Lastly, such enzymes which can not only degrade plastic polymers into smaller units but also convert these products into new biopolymers or other beneficial products can be obtained. This may cause significant reduction in the global plastic problem.

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