BIODEGRADATION OF PLASTICS USING BACTERIA - A REVIEW

Saima Mirza¹, Brishti Sasmal¹, Meghna Goswami² and Priyanka Patel³*

¹Research Trainee, Rapture Biotech, Ahmedabad, Gujarat, INDIA
²Research Assistant, Rapture Biotech, Ahmedabad, Gujarat, INDIA
³Director, Rapture Biotech, Ahmedabad, Gujarat, INDIA

Email: codonbiosolutions@gmail.com

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Saima Mirza1, Brishti Sasmal1, Meghna Goswami2 and Priyanka Patel3*

1Research Trainee, Rapture Biotech, Ahmedabad, Gujarat, INDIA
2Research Assistant, Rapture Biotech, Ahmedabad, Gujarat, INDIA
3Director, Rapture Biotech, Ahmedabad, Gujarat, INDIA

Email: codonbiosolutions@gmail.com

ABSTRACT

Plastic has caused adverse effects in ecosystem and that particularly concerns our health as well as the environment. Plastic decomposition takes thousands of years in the landfills. Also, large amounts of plastics are not recycled ending up in landfills and water bodies. Some portions are burned up in incinerators resulting in release of many harmful gases in to the environment. Plastics entrapped in different ways causes distress. Microbes play an important role in degradation and decomposition of wastes. Some of the microbes have ability to degrade environmental plastic. Some of these microbial species utilize carbon as its sole source of energy and produce certain enzymes which accounts for plastic degradation. Microorganisms which have been reported to be able to degrade different kinds of polymers are Pseudomonas sp., Proteobacteria sp., Bacillus sp., Stenotrophomonas sp., Staphylococcus sp., Rhodococcus sp., Arthrobacter sp. Ideonella sp., Comamonas sp., Streptomyces sp. and others. This review focuses on bacterial degradation of different forms of synthetic plastics such as Polyurethanes (PUR), Polythene (PE), Polyethylene terephthalate (PET), Polyamides, Styrene.

Keywords: Bacterial plastic degradation, Biodegradation of plastic, Environment and Enzyme.

INTRODUCTION

Plastics are the synthetic and semi-synthetic organic man-made polymers, mainly composed of various elements such as carbon, hydrogen, oxygen, nitrogen, chlorine, and sulphur. Plastics usage and demand has been growing exponentially. Now-a-days plastics have become an inseparable need of our life. Widespread use of plastic is due to its influential properties such as water resistance, versatility, durability, thermal and electrical insulation. For the same, it has easily replaced materials such as woods, metals, and glasses, which were being used. Hence, production of plastics is rapidly increasing with increased amounts of left-over plastics into the environment becoming a big threat to our ecosystem. These plastics in form of micro plastics are also entering the human food chain causing many health hazards. Disposal of plastics such as Polyethylene, Polypropylene, Polystyrene, Polyvinyl chloride, Polyethylene terephthalate etc. is a matter of concern due to its non-biodegradable nature [1-3]. Presently, plastic wastes are subjected to thermal treatment, combustion or pyrolysis, land-filling, incineration and mechanical or chemical recycling. However, any of these methods are not environment friendly and do release contaminants in the environment. There is an urgent need to overcome this challenge of plastic waste degradation. This can be fulfilled in an eco-friendly manner with plastic degrading microorganisms. These microbes are also capable of producing enzymes which can degrade plastic. Microbes proclaimed to be efficient in plastic degradation are Pseudomonas sp., Proteobacteria sp., Bacillus sp., Stenotrophomonas sp., Staphylococcus sp., Rhodococcus sp., Arthrobacter sp. Ideonella sp., Comamonas sp., Streptomyces sp. and others. These microbes have been noted to degrade and decompose all types of plastic as polyethylene, polypropylene, polystyrene, polyurethanes and polyvinyl chloride [4].
Table: 1- A representation of different types of plastic usage and their degradation by bacteria

<table>
<thead>
<tr>
<th>Plastic</th>
<th>Uses</th>
<th>Bacteria involved in plastic degradation</th>
<th>Enzymes used for microbial degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyurethanes (PUR)</td>
<td>Used in elastomeric wheels, tyres, rigid foams, furniture, adhesives, coatings, textiles, and other industrial areas</td>
<td>Comamonasacidovorans TB-35</td>
<td>Esterase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pseudomonas chlororaphis</td>
<td>PueB lipase/ PueA lipase</td>
</tr>
<tr>
<td>Polyethylene (PE)</td>
<td>Used in mostly all known things of routine life ranging from plastic bags, water packing, milk bottles, food packaging, toys</td>
<td>Pseudomonas putida GPo1</td>
<td>Alkane hydroxylases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pseudomonas aeruginosa E7</td>
<td>AlkB enzyme</td>
</tr>
<tr>
<td>Polyethylene Terephthalate (PET)</td>
<td>Widely used in food packaging of beverages, milk and soft-drink bottles. Its use as fibres in materials as rayon, wool and cotton.</td>
<td>Ideonellasakaiensis 201-F6</td>
<td>PETases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ideonellasakaiensis</td>
<td>MHETases</td>
</tr>
<tr>
<td>Polyamides</td>
<td>Used to produce synthetic fibres, textile, automobile, rope, industries, carpet, kitchen utensil, sport wears etc.</td>
<td>Pseudomonas strain ND1</td>
<td>Various extracellular enzymes</td>
</tr>
<tr>
<td>Styrene</td>
<td>Used in the production of plastics such as polystyrene (PS), acrylonitrile butadiene styrene (ABS), polyethylene (PE) and styrene acrylonitrile (SAN).</td>
<td>Pseudomonas putida SN1</td>
<td>monooxygenase enzyme</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pseudomonas putida ST201</td>
<td>Epoxystyrene isomerise, Phenylacetaldehyde dehydrogenase</td>
</tr>
</tbody>
</table>

Polyurethanes (PUR)

Polyurethanes (PUR) is a thermosetting plastic material. Otto Bayer, a German professor, in 1937 produced the first PUR by a polymerization reaction of polyisocyanates and polyester diol. Polyurethanes are monomers of urethane groups. PUR obtained from different sources have variation in their di-isocyanates polyols side chain, as different poly-ol type provides variation in properties and result into different type of polyurethane such as rigid polyurethane foam, flexible polyurethane foam, thermoplastic polyurethane, and polyurethane ionomers etc. that are suitable for different application [5]. It is synthesized easily because of its varying nature and degree of cross-linking. For example, polyurethanes or polyether which contains hydroxyl groups can be synthesized using polyester or polyether resins [6]. Polyurethane has wide range of uses in variety of industries due to its heat insulation capacity, desirable strength to weight ratio, versatility, and durability. It is also consumed in different industrial sectors to produce elastomers, adhesives, foams, coatings, textiles, and other industrial areas. Due to its vast applications, PUR is produced in high quantities. The production of synthetic polymers has resulted in plastic wastes which has greatly polluted the environment as well as effects human health and aquatic life adversely. However, different microorganisms have showed the ability of degrading polyester PUR [7].

Bacterial degradation of PUR

Comamonasacidovorans is a gram-negative, aerobic, non-spore forming, rod-shaped bacterium belonging to the genus Comamonas and family Comamonadaceae, mostly found in soil and water [8]. PUR esterase is a PUR degrading enzyme, derived from Comamonasacidovorans TB-35, which utilizes polyester PUR as its carbon source. It produces two kinds of esterases; one is released in the media and the other is surface bound with-in the cell. However, it was observed that surface bound esterase only had the ability to degrade PUR. It was noted that enzymes degrading PUR work in two steps as described further. Firstly, hydrophobic adsorption onto the PUR surface followed by hydrolysis of the ester bonds of PUR. The cell-bound surface is hydrophobic in nature and
this hydrophobic surface binding domain has also been observed in other solid-polyester-degrading enzymes degrading polyhydroxyalkanoates (PHA). However, there is no homology between the amino acid sequence of PUR esterase and PHA degrading enzymes [9,10].

Betaproteobacteria from the genus Pseudomonas, a gram-negative bacterium has been frequently linked with PUR activities. PueB lipase from Pseudomonas chlororaphis was the first enzyme identified to act on PUR [11]. Pseudomonas chlororaphis is a heterotrophic soil bacterium, this organism codes for an additional enzyme active on PUR, known as PueA lipase. The secreted hydrolase degrades PUR and degradation is regulated tightly by mechanism of control carbon catabolite and both the lipase genes. It has also been reported that Pseudomonas putida degrades PUR in high rates by consuming the added colloidal PUR [12,13].

PUR biodegradation has also been reported by other microbial species as Corynebacterium species, Enterobacter agglomerans, Pseudomonas chlororaphis, Pseudomonas putida and Bacillus subtilis. PUR can also be efficiently degraded by a few enzymes produced by certain moderate thermophilic actinomycetes including, Thermobifida alba AHK119, Thermobifida fusca, Thermomonospora curvata DSM43183 and Saccharomonospora viridis AHK 190 [6,12-16].

**Polyethylene (PE)**

Polyethylene is thermoplastic and become thermoset plastic when modified by addition of cross-linkers. Ethylene monomers polymerize with Ziegler and metallocene catalysts which results in the production of PE. It is classified into various types based on density of polymer and their degree of branching, such as High-density polyethylene (HDPE), Ultra-high-molecular-weight polyethylene (UHMWPE), linear low-density polyethylene (LLDPE) and Low-density polyethylene (LDPE). This synthetic plastic is used in mostly all known things of routine life ranging from plastic bags, water packing, milk bottles, food packaging, toys etc. attributed to its good processability, water resistance and low oxygen barrier properties. Polyethylene contributes to 36% of total non-fibre plastics production. The highly recalcitrant hydrophobic backbone and inert nature of PE makes it hard to decompose and nearly non-biodegradable [17,18].

**Bacterial degradation of Polyethylene**

Many of the species known to degrade PE are capable of hydrolyzing and metabolizing linear n-alkanes. Alkane hydroxylases (AHs) are the key enzymes involved in aerobic degradation of alkanes by bacteria. The first step involves hydroxylation of C-C bonds to release primary or secondary alcohols. The Alkane hydroxylase system has been well studied in P. putida Gp01. AlkB enzyme of P. aeruginosa strain E7 played a central role resulting in the biodegradation of LMWPE into CO₂ and other organic compounds. The alkB gene was cloned in Pseudomonas sp. E4, and the AlkB enzyme expressed from the recombinant strain participated in the early stage of LMWPE biodegradation, in the absence of the other specific enzymes like rubredoxin and rubredoxin reductase Pseudomonas sp. has been studied extensively for its demonstrated abilities to degrade various synthetic plastic polymers. Pseudomonas degradation of polyethylene is caused majorly by oxidation and/or hydrolysis. Pseudomonas sp. produces enzymes that can cleave the chain of high molecular weight polymer into low molecular weight monomers. The degree of biodegradation is dependent on structural arrangement of polymer and strain type. Pseudomonas putida S3A uses polyethylene as carbon and nitrogen source for its metabolism. Research reports have mentioned that an engineer strain of Pseudomonas is able to oxidize aromatic, aliphatic, terpenic and polyaromatic hydrocarbons. [19,20] However, without any chemical pre-treatments, Pseudomonas sp. AKS2 was able to degrade LDPE films, 5% of the total mass of 300 mg was degraded within 45 days [21]. Also, an uncharacterized Pseudomonas sp. was observed to degrade 28.6% of 5% dry weight of low MWPE (MW=1700Da) without pre-treatment in 40 days [22].

Majority of these microbes are found to be capable to deteriorate surface of PE and form a biofilm on PE. S. maltophilia adheres to plastic surfaces and are known to form bacterial films (biofilms) [12,23]. For complete degradation of plastic, the polymers are required to breakdown into smaller monomers first, which than can pass through cell membrane of microorganism followed by subsequent intracellular metabolism. Stenotrophomonas sp., has been observed to grows on a polyethylene component (novel poly-β-
Microorganisms as *Pseudomonas sp.*, *Ralstonia sp.*, *Stenotrophomonas sp.*, *Klebsiella sp.*, *Acinetobacter sp.* from Gram negative category and *Rhodococcus, Staphylococcus, Streptococcus, Streptomyces, Bacillus* from Gram positive have been reported to degrade PE. Analysis of 16S rDNA of bacteria belonging to genera *Brevibacillus sp.*, *Cellulosimicrobium sp.*, *Lysinibacillus sp.* has also shown possibility to degrade PE [24].

**Polyethylene Terephthalate (PET)**

Polyethylene terephthalate (PET) widely known as polyester is a thermoplastic polymer made from polymerization of ethylene glycol and terephthalic acid in presence of certain catalysts. It is generally stiff and has high strength attributed to aromatic rings present in its monomer units. First synthesis of PET was carried out by DuPont (North American Chemist) in mid 1940s, whose method was further modified in 1950s resulting in the formation of thin extruded sheets. [25, 26]. The stiffness and wrinkle-free property of PET makes it appropriate for use as fibres in association with materials as rayon, wool and cotton. Due to its light weight nature, it has been extensively accepted in liquid packaging, and its heavy mechanical properties make it an appropriate substrate for thin films in solar cells, taping films etc. It has also been used universally for waterproofing of underwater cables. [27-30] It is 18% total plastic production of the world. PET can be recycled but major portions are not recycled and remain in the environment causing damage to different life forms in different ways, for example, large number of plastics ends up in the ocean, where they are fragmented into microplastics being a major threat to zooplanktons and other aquatic species by entering the ecosystems [29,31].

**Bacterial degradation of PET**

A *Betaproteobacteria*, genus *Ideonella*, named *Ideonellasakaiensis* 201-F6 was isolated in 2016 been reported to be capable of utilizing PET monomers as energy and carbon sources. *Ideonellasakaiensis* 201-F6 secretes enzymes that are involved in PET degradation. This microbe secretes PETase, an enzyme with hydrolysing properties for PET mono- and oligomers. These microbes generally form a film on surface of PET and release the enzymes onto it, causing PET degradation. *Ideonellasakaiensis* also produces an enzyme known as MHETase (tannase enzyme) which is capable of degrading an intermediate product released during the reaction of PETase on mono- and oligomers. This product is hydrolysed by MHETase, providing metabolized compounds which work as energy and carbon source for these bacteria. Some researchers have also mentioned MHETase to hydrolyse ethylene glycol and terephthalic acid [29,32]. Plastic bottles made of PET have also been studied to be degraded by a bacterium belonging to phylum *Actinobacteria*and *Thermomonospora* by producing hydrolytic enzyme which can hydrolyse ester bonded monomers of PET [27-39].

**Polyamides**

Polyamides are polymers which contain amide linkage. It is made of monomer units which might be aliphatic, semi-aliphatic or aromatic linked by amide bond i.e, (R―CO―NH―R’). Polyamides includes protein polymers. It is an important synthetic polymer mostly manufactured as nylon. Polyamides have wide range of applications from being ideal choice in many engineering fields to its usage in textile, automobile, rope, carpet, kitchen equipments, sport wears etc, mainly because of polyamide’s high durability, strength, high temperature bearing and electrical resistance properties. Polyamides are generally formed from petrochemicals and are non-biodegradable in nature. It causes environmental hazards as increased greenhouse gas emissions, environmental pollution and affect human health adversely [24,33].

**Bacterial degradation of Polyamide**

As other plastic polymers, microbial degradation of polyamides has also been documented. *APseudomonas sp.*, (strain ND11) was grown successfully on a minimal medium containing Polyamide (PA4) as sole carbon and nitrogen source indicative that it was capable of breaking polyamides and utilizing it. The amide-bonds of polymer were hydrolysed by its extracellular enzymes and GABA was produced as degradation intermediate [34]. Also, a wide range of bacteria have been observed to be able to grow on various oligomers of Nylon. Nylon-6 uses e-Caprolactam as monomer, which is a man-made toxic xenobiotic compound and nylon manufacturing industries realise this toxin in water.
bodies along with 6-aminohexanoic acid cyclic dimer. These 6-aminohexanoic acid oligomers accumulate in water bodies and cause water pollution. This compound serves as carbon and nitrogen source to Arthrobactercitreus, Rhodococcusrhodochrous and Bacillus sphaericus and it is utilized in short periods removing it from waste streams. The degradation of e-Caprolactam in wastewater was found to be optimal over a wide range of pH from 5.0 to 9.0, at a temperature of 30°C [35, 36]. Recently, Pseudomonas aeruginosa MCM-407 strain was observed to be able to efficiently degrade 6-aminohexanoate linear dimers enzymatically and remove e-Caprolactam with reduction in chemical oxygen demand [37, 38]. Arthrobacter sp. strain K172 has genes responsible for 6-aminohexanoate metabolism, a by-product of Nylon production. It has nylD1 and nylE1 genes which get translated into enzymes diamers: cyclic-dimer hydrolase (NylA), dimer hydrolase (NylB), and endo-type-oligomer hydrolase (NylC), which is obligatory for hydrolysis of nylon oligomers. [39] Also, as earlier many marine originated bacteria such as Bacillus cereus, Bacillus sphaericus, Vibrio furnissi, and Brevundimonasvesicularis have potential and can play major role in degrading polyamide like nylons in a time span of few months (35°C, pH 7.5) [40].

**Styrene**

Styrene is a homopolymer made of phenylethane. It is also known as vinylbenzene or phenyletheene. Styrene is a colorless, aromatic monomer with a sweetish odor. Styrene is an important mono-aromatic compound which is produced industrially in a very large scale. In 1851, M. Berthelot, a French chemist introduced the production of styrene by catalytic dehydrogenation of benzene with ethylene. Styrene occurs naturally in liquid form and therefore is an essential component used in making variety of strong, light-weight and flexible products. They are mostly found in gasoline and other fuel constituents. They are used in the production of plastics such as polystyrene (PS), Acrylonitrile butadiene styrene (ABS), Butadiene (SB) and Styrene acrylonitrile (SAN) [41-45].

**Bacterial degradation of Styrene**

Styrene can be degraded by bacteria using aerobic metabolism. The initial process of styrene using aerobic breakdown proceeds through an attack on its aromatic ring. The attack on ring could occur through either a mono-oxygenase or di-oxygenase attack followed by ring cleavage. Pseudomonas putida SN1, a gram-negative bacteria strain was identified to have high-styrene degrading property. Pseudomonas putida SN1 converts mono-oxygenase enzyme to styrene oxide which acts as a chiral building block in organic synthesis. These bacteria could only grow on styrene and styrene oxide but not on toluene and benzene. The optimal property was shown at 30°C, pH 7.0 and estimated as 170 unit/gm [46-48].

Pseudomonas putida ST201 bacterial strain, isolated from soil, is also capable of degrading styrene. Due to its high tolerance to styrene, it could degrade styrene completely within 48 hours at concentration up to 600 mg/l. It was also able to degrade a mixture of benzene, ethylbenzene, toluene, and p-xylene. Nucleic acid of this bacterium has codes for the oxidation of styrene to phenylacetic acid. Four open reading frames have been mentioned by the researchers as styA, styB, styC and styD. First two encodes for styrene monoxygenase which is responsible for transforming styrene to epoxy styrene, styC codes for epoxy styrene isomerase (used in the enzymatic pathway that converts epoxy styrene to phenylacetaldehyde) and styD helps production of phenylacetaldehyde dehydrogenase (causes oxidation of phenylacetaldehyde to phenylacetic acid) [49,50].

Another strain of Pseudomonas putida, CA-3 contains genes responsible for styrene degradation pathway. The pathway has been divided into two parts, upper pathway (Genes styS and styR - converts styrene to phenylacetic acid) and a lower pathway (degrades phenylacetic acid) Though the best performing isolate of Pseudomonas putida is reported as NBUS12 strain. Genetic studies of this strain have mentioned it as different from existing phenotypically similar bacterial strains [51,52].

**CONCLUSION**

Plastic polymers have been proven as root cause of many environmental and health hazards. Over exploitation of plastics and improper waste management systems leads to the accumulation of plastic in environment causing deterioration of our ecosystem. The accumulated plastics may take up to hundreds of years to decompose. These ruthlessly discarded plastic accumulates are found to be responsible of leaching of many hazardous pollutants into the surrounding soils and water bodies. Hence, an
urgent requirement for development of different disposal methods for plastics is an essential need of the hour. Different mechanical, chemical and biotic methods are being developed for plastic degradation and disposal. Biotic methods are newer eco-friendly alternatives for plastic degradation. Biotic methods decompose plastics using microorganisms and do not produce any major pollutants in the process. There are many bacterial and fungal species reported by the scientists which help in degradation of plastics. These microbes produce enzymes to metabolize plastic polymers and use it as their energy sources. There are published research reports mentioning genetic modification of microbes to convert plastic into biodegradable compounds. The possibility of using microbes as plastic degradation machineries is advantageous in every aspect with respect to energy, time and resources. Most of the microorganisms studied till date utilize polymers either as a carbon source or release enzymes that can degrade them for consumption in their metabolic pathways adequately. Furthermore, research studies are required for screening and isolation of microbial species which can effectively degrade and decompose various plastic polymers. The implementation of microbial methods for plastic degradation can open newer avenues and help reduce the global problem of plastic waste management.

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