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Review Article

Effect of different bacteria on the biodegradation of polyurethane

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ABSTRACT

Polyurethane has been used and over-exploited worldwide in the manufacture of different goods, but it is hard to break down and represents an important contaminant due to its accumulation when discarded. Recent research findings have shown that several bacteria and their enzymes can biodegrade various plastics, such as polyurethane. In this review, we sought to group, analyze, and relate the techniques used by different bacterial species to biodegrade polyurethane that have been identified in different studies by searching databases, such as PubMed, Web of Science, and Scopus. Different species of proteobacteria, actinobacteria, and endobacteria biodegrade polyurethane by oxidation and hydrolysis to obtain carbon and nitrogen sources. Changes as weight loss, tensile strength, and chemical and surface changes were observed in polymer properties, showing that biological technologies have a direct impact on polyurethane by modifying the molecule in different ways.

Key words: bacteria; biodegradation; poly(ether urethane); biotechnology.

RESUMEN

El poliuretano se usa y sobreexplota alrededor del mundo en la fabricación de diversos productos, sin embargo, es un material de difícil degradación y, por su acumulación, un contaminante importante al ser desechado. Los resultados de investigaciones recientes demuestran el potencial de diferentes bacterias y sus enzimas para biodegradar diferentes plásticos, como el poliuretano. En esta revisión se buscó agrupar, analizar y relacionar las técnicas utilizadas por diferentes especies bacterianas para biodegradar poliuretano, identificadas en diferentes estudios, buscando en bases de datos como PubMed, Web of Science y Scopus. Se encontraron diferentes especies de proteobacterias, actinobacterias y endobacterias que biodegradan el poliuretano por oxidación e hidrólisis para obtener fuentes de carbono y nitrógeno. Se observaron cambios como pérdida de peso, fuerza de tensión y cambios químicos y de superficie en las propiedades del polímero, mostrando que las tecnologías biológicas tienen un impacto directo sobre el poliuretano al modificar la molécula de diferentes maneras.

Palabras clave: bacterias; biodegradación; poli(éter uretano); biotecnología.

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INTRODUCTION

Polymer biodegradation is widely applied as a solution to plastic pollution. A higher biodegradation efficiency has been observed in studies where individual strains are isolated and consortia of microorganisms with degradation capacity are used, as they eliminate potentially toxic intermediates in the environment. Therefore, they are a promising tool for the degradation and bioremediation of plastic waste.[1] About 140 million tonnes of plastics, synthetic polymers, are consumed annually as packaging materials, and the number increases continuously day by day. Polyurethanes (PUs) are synthesized polymers containing polyethers or polyesters (polyols), diisocyanates, diols or diaminescan and a carbamate group as a linker. They constitute an important group among synthetic polymers in terms of their widespread application and unique properties. They have been used in the materials sector as constituents of mattresses, shoe soles, chairs, and even elements for refrigerators and panels, where they are found as coatings, supports, and foams. All those applications make PU the polymer with the biggest production.[2,3] Plastics are highly chemically and biologically resistant and have a high resistance to stretching. For that reason, they are used in medicine and different industrial products, such as foams, adhesives, construction materials, fibers, and coatings.[4] PUs have become a fundamental material in human life because their versatility allows them to be used as a substitute material to create several products. For that reason, the consumption of this plastic has increased worldwide and, therefore, its accumulation at the end of its useful life.[5] In 2014, an accumulation of 4.4 million tonnes of PU was registered in Europe alone. This is an alarming amount since it is known that PU degradation takes hundreds of years. The real problem is that there is no correct method to carry out its disposal.

The current options are chemical and mechanical recycling as well as incineration; however, none of these methods are effective or safe for the environment.[6,7] It is known that bacteria, both natural and genetically modified, degrade contaminants into simpler and less toxic forms, transforming them into other compounds. In natural and contaminated sites, the interaction of microbial communities with a metabolism regulated to obtain carbon and energy has garnered interest in environmental biotechnology and research. The latter focuses on the biodegradative capacity and adaptability of their metabolic processes to make use of chemicals found in the environment.[8] PU can undergo different alterations, starting with changes at molecular level, reflected in chemical and physical changes.

The aim of this review is to find and group the processes by which different types of bacteria carry out their effective biodegradation. With this information, these biological technologies can be considered a potential alternative solution for PU contamination.

METHODS

Databases PubMed, Web of Science, and Scopus were used for the search of scientific information. Boolean operators and the keywords biodegradation OR biological degradation AND microorganisms OR bacteria AND plastic OR polyurethane NOT bioplastic OR biodegradable plastic were used. Using the filters, we obtained theses and articles from 2015 to date, in Spanish o English, published in systematic review or journals.

Inclusion criteria:

- Bacteria with faster growth rate
- Bacteria with a high rate of PU degradation in short periods
- Articles published in 2015 and on
- Direct biodegrading activity on PU
- Basic Research Articles
- Articles in English and Spanish

Exclusion criteria:

- Non-pathogenic bacteria
- Articles published before 2015
- Yeast or fungal analysis
- Review articles
- Meta-analyses

RESULTS

Microbial degradation of polyurethane

The degradation of PU has been investigated, including changes in its properties through biological reactions caused by microorganisms that cause its loss of function and lead to the degradation of the material.[8] An example of the above are bacteria, as presented by Oceguera-Cervantes, et al.[3] They discovered *Corynebacterium spp.*, *Pseudomonas fluorescens*, *Pseudomonas chlororaphis*, and *Bacillus subtilis* use PU as a carbon and nitrogen source when strains are grown in PU media supplemented with yeast extract or glucose. On the other hand, in their study on microbial degradation in plastic, Peng Y, *et al.*[9] leave aside bacteria as an entity and focus on identifying bacterial enzymes for process optimization. The latter are responsible for degrading PU and products of this biodegradation. The authors also mention experimentation with aliphatic polyester and polyester PU under the activity of microorganisms through the hydrolytic breaking of the ester or urethane bonds in PU structures. Bacterial degradation, also used in other biotechnological processes, has been demonstrated in food production; the lipase and hydrolases secreted by *Pseudomonas chlororaphis* act over the PU.[4]

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Biodegradation mechanism

The environment in which the PU is found will determine the degradation mechanism since the presence of microorganisms and even acidic, alkaline, or oxidative conditions are potential degraders of this polymer. Microbial communities can adapt to the presence of different components and use them as sources of energy and growth, since they can degrade polymers into intermediate products by enzymatic systems.[6,10] These degradative enzymes produced by microorganisms have been classified into intracellular and extracellular enzymes, where we find both oxidative and hydrolytic action, in which in the PU hydrolysis mechanism a series of steps have been observed in the presence of hydrolase type enzymes, where depolymerization of the long carbon chains of the plastic polymers is carried out causing a decrease in molecular weight and viscosity and the rupture of all the chains. Other microbial enzymes with a similar mechanism have been discovered, such as laccases, peroxidases, lipases, esterases, and cutinases.[9,10]

Polyurethane biodegradation by different bacteria

Bacteria have the function of making the transformation and flow of nutrients to the soil and, therefore, the environment. It has been shown that the decomposition of different complex polymers by bacteria allows the release of carbon and nutrients. These degradative bacteria are both gram positive and gram negative, including *Pseudomonas*, *Burkholderia gladioli*, and *Bacillus subtilis* species (Table 1) and their activity was measure in contaminated media.[4,9,10]

PU degradation is directed by carbon catabolic controls, using two types of lipases (one encoded by the *pueE* gene and the other encoded by the *pueB* gene), to obtain dispersal growth.[11] *Pseudomonas putida* is reported to work in this way, obtaining a high degradation of PU in a couple of days.[9] A significant activity in the degradation of PU is also observed by enzymes from *Pseudomonas spp*. and *Bacillus spp* with esterase activity. In comparison with these two types of enzymatic reactions, lipase activity has a greater significance when obtaining the data analyzed by Nuclear Magnetic Resonance (NMR) and Infrared (FT-IR) Spectroscopy.[12] Stepien AE, et al.[4] performed biodegradation tests with *Pseudomonas denitrificans* with commercial PUs.

The changes in the chemical structure of the polymer were evaluated using methods as mass loss analysis to assess the degradation process during the incubation of the polymer in the bacterial environment. The activity is related to the oxidative action of enzymes released by the microorganisms into the polymer. According to Glaser JA[13], *Pseudomonas otitidis* biodegrades PU by an enzymatic reaction that causes depolymerization. It has urease enzymes that degrade urea linkages as well as proteases and esterases that hydrolyze ester bonds to depolymerize PU.[9] Although the individual biodegradation of each bacterium is effective, an efficient process can be obtained by using the activity of two or more bacteria together (consortium). For example, the urethane bonds in the polyester immersed in the PU are broken by fusing the polyamidase from *Nocardia farcinica* with a receptor of the polymer found in a polyhydroxyalkanoate depolymerase from *Alcaligenes faecalis*.[14] The biodegrading activity of

Table 1. Bacteria proven to biodegrade polyurethane. Contaminated sites-samples from contaminated sites (e.g., landfill/dump sites, activated sludge, contaminated soils, etc.). PU (polyurethane) biodegradation.

Polymer	Sample origin	Phylum	Bacteria	References
PU	Contaminated	Firmicutes	*Bacillus sp. AF8* *Bacillus subtilis*	(4)
PU	Contaminated	Proteobacteria	*Pseudomonas chlororaphis, P. denitrificans ATCC 19244*	(4)
PU	Contaminated	Proteobacteria	*Burkholderia gladioli, Pseudomonas otitidis, P. putida*	(9)
PU	Contaminated	Firmicutes	*Bacillus subtilis*	(17)
PU	Contaminated	Actinomycetes	*Thermomonospora curvata*	(15)
PU	Contaminated	Actinomycetes	*Saccharomonospora viridis*	(16)
PU	Contaminated	Actinobacteria	*Nocardia farcinica*	(14)
PU	Contaminated	Proteobacteria	*Alcaligenes faecalis*	(14)

actinomycetes *Thermomonospora curvata* DSM4318312 and *Saccharomonospora viridis* AHK 190 is produced by hydrolyzing enzymes. They break the polyester bonds by adding a water molecule that reacts chemically with the macromolecule. The activity of these enzymes occurs at moderate temperatures.[15,16] In a recent publication, Schmidt J, et al.[17] used polyester hydrolases—previously described—such as LC-cutinase, TfCut2, Tcur1278, and Tcur0390, to demonstrate the biodegradation of PU by this type of enzymatic activity. The tests were carried out over long periods of time and at elevated temperatures, resulting in significant weight loss in the tested media.[17] *Thermobifida*-derived cutinases performed the highest biodegradation of PU due to their nonspecific nature, producing up to 78 different substrates by lipolysis.[18]

Experimental conditions modify the degradation activity

The microbial metabolism requires certain conditions for its degradation activity, so it is necessary to know the growth media, temperature, and activity time of the bacteria.[19] In some of the experiments, a pre-treatment is applied to increase the bioavailability of the polymers. The materials are washed with distilled water or ethanol, which align the polymer structure and change the polarity of the material to non-polar, causing certain structures to break and be retained by other molecules forming clathrate. Alternatively, materials are exposed to high temperatures or UV radiation. This last pre-treatment causes abiotic degradation, weakening the polymer structure and thus promoting the biodegradation process.[20-23]

Growth conditions

The growth and survival of bacteria with degradation activity are mainly affected by temperature. High levels of temperature during experiments show that an increase in the metabolism of microorganisms also requires an increase in environmental temperature and, therefore, in plastic degradation.[24] Other growth factors of the bacteria reported by the investigations are pH and the culture medium, where often the bacteria were grown *in vivo* and the most used was the mineral salt medium, as well as Bushnell-Haas or the liquid basal medium free of carbon and some with added glucose, where the bacteria are forced to use the carbon of the plastics and thus have a greater biodegradation of the plastics.[24,25]

Common changes in the polymers during degradation

Molecular Weight loss

The alteration in the physical properties of the polymer during its biodegradation is reflected as molecular weight reduction in which it is mainly demonstrated as percentage weight reductions calculated from the difference between the initial polymer weight and the weight after the exposure to bacteria that achieved biodegradation. The PU molecules change their bones and release compounds leading to a weight loss of the molecules. These observations were typically combined with another method, such as surface changes and/or FT-IR (Fourier Transform Infrared) spectroscopy.[26]

Tensile strength

The changes in tension originate from the breaks between PU molecules, resulting in a polymeric matrix with a lower order in the crystalline domains and fewer cross-links compared to the original material. Subsequently, Young's modulus decreases and the material becomes more brittle and less stiff.[27] Changes in plastic tensile strength are typically calculated using a tensile machine (e.g. INSTRON 5566) and determined in megapascals (MPa), as the percentage loss in tensile strength, elongation at break point (percent) or ultimate tensile strength (i.e. the stress the material experiences when extended to break point).[28]

Surface changes

In 60% of the studies, surface alterations (cracks, pores, and holes) in plastics were assessed by noticeable changes in the *before* and *after* SEM (Scanning Electron Microscopy) images. SEM image analysis determined the degradation of PU by bacteria and evidenced the formation of biofilms occurred within 15 days; the HDPE (High Density Polyethylene) films were cracked and developed holes when incubated with *Klebsiella pneumoniae.*[20] Atomic force microscopy is used to characterize the surface of relatively flat, solid and semi-solid samples. The technique provides morphological information in 3D from topographic images at a nanometric scale. It also provides surface parameters as roughness and distribution (homogeneity) of particles on various materials, such as plastic sheets.[29]

Chemical changes

Polymers undergo chemical changes at the time of biodegradation. FT-IR is the most used method (60.9% of the studies) to evaluate this type of chemical changes. The spectra prove chemical changes because they demonstrate the intensity variations of the carbonyl bands due to changes in the double or triple bonds or the methyl groups that show as flat-spectra with different ratio peaks. The analysis of this technique relies on the fact that most molecules absorb in the infrared region. This absorption corresponds specifically to the vibration modes of the different bonds present in the analytes

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and provide detailed information on the chemical structure of the polymer. Because of its accuracy and rapidity, FT-IR is especially common for PU degradation analysis. It is a non-destructive method: The sample is recovered without damage after the analysis. To assess superficial biological degradation, surface analysis can be easily performed.[30] As time progresses, chemical changes continue according to biodegradation that is taking place: decrease of carbonyl groups and an increase in the number of unsaturated hydrocarbons resulting from the conversion of the carbonyl groups. Another method to evaluate these changes is through the thermal profiles using Thermal Gravimetric Analysis (TGA), obtaining the degradation curves product of the digestion of the hydrocarbon skeleton, where there is a modification in the molecular weight results in a low molecular weight. Analyzing the culture medium is another way to detect changes with HPLC (High Pressure Liquid Chromatography) to seek for intermediate molecules of degradation,[30] and due to the accessibility of this.

DISCUSSION AND FUTURE PERSPECTIVES

The review of the information shows the need for improving the research by comparing tests between degrading bacteria and their plastic-enzyme activity in the near future. This will allow to focus on their effectiveness as a biodegradation technology, considering time and yield. Similarly, further studies should be developed without PU pre-treatment and under natural environmental conditions. These observations will lead to the use of specific bacteria in different ecosystems. It is necessary to identify and characterize depolymerases, because they lead the biodegradation procedure and over expression of these enzymes might have a greater benefit.

CONCLUSION

Polyurethanes are versatile polymers with highly variable structures, chemical compositions, formulations, morphologies, and shapes. Due to accumulation, they have become a severe environmental and social issue, so innovative approaches have been developed to reduce these persistent contaminants. Some of the most relevant innovations are the reports on their biodegradation by microorganisms or their enzymes isolated from polyurethane-degrading environment. This biological treatment was transformed into a technology to reduce plastic waste. These biological technologies have direct impact on the biodegradation mechanisms and kinetics by modifying physical characteristics of PU.

Several authors have reported different kinds of bacteria that degrade polyurethane or its plastic-degrading enzymes. Most of them are relevant to the research of biological technologies aimed at reducing polyurethane contamination. The bacteria that biodegrade polyurethane are from different phylum, like protobacteria, actinobacteria, and firmicutes; each one has different biodegradation properties that confer different processes, time, speed, growth conditions, among others to make the molecule decomposition. Degradation occurs when the main molecule undergoes changes in its bonds, making it weaker or releasing molecules; therefore, the structure and chemical properties are affected and it is possible to measure them by looking for the changes in the molecule before and after the process; in the same way, the activity of the bacteria is measured to see its efficiency in the degradation process and its characteristics. The research showed that authors measured this activity according to tensile machine measuring, FT-IR spectroscopy, surface changes method, TGA, or HPLC. Structural and chemical changes evidenced the deterioration of the polymer under experimental conditions since natural environmental conditions were not reported. However, the experiments were performed with degradation pretreatment of the polyurethane molecules to promote enzymatic degradation in the structures.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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AUTHOR CONTRIBUTIONS

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