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SYSTEMATIC REVIEW

REVISED Efficiency of Microorganisms and Effectiveness of Biodegradation Techniques on LDPE Plastics: A Systematic Review [version 2; peer review: 2 approved]

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Abstract

Introduction

The aim of the research was to demonstrate the efficiency of microorganisms and the effectiveness of biodegradation techniques on Low-density polyethylene (LDPE) plastics. The research question was: What is the efficiency of *LDPE*-degrading microorganisms and the effectiveness of biodegradation techniques?

Methods

The systematic review was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Articles were obtained from Scopus, Web of Science (WOS), Embase, and Google Scholar. The DeCS/Mesh search terms were: Low-density polyethylene, efficiency, biodegradation, microbial consortia, fungi, bacteria. Inclusion criteria were: scientific articles that included bacteria, fungi, and microbial consortia reported as *LDPE* degraders that report the percentage of weight loss; articles published from January 2010 to October 2022, and publications in Spanish and English with open access. Exclusion criteria were: studies that do not report

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gravimetry, the biodegradation time of *LDPE*, and the genus or species of the polyethylene-degrading microorganism.

Results

Out of 483 studies found, 50 were included in this Systematic Review (SR). The most frequent study techniques were scanning electron microscopy (SEM), gravimetry, and fourier transform infrared spectroscopy (FTIR), and in the case of microorganisms, the most studied belonged to the genus Pseudomonas, Bacillus, and Aspergillus. Regarding the isolation place, the most frequent mentioned in the reviewed articles were landfill soil and sanitary landfill soil. The efficiency of *LDPE*-degrading microorganisms was higher in bacteria such as *Enterobacter spp.*, *Pantoea spp.*, *Pseudomonas spp.*, *Escherichia coli*, and *Bacillus spp.*, which obtained a range of DE of 9.00-70.00%, 24.00-64%, 1.15 – 61.00%, 45.00%, and 1.5-40% with DT of 4-150, 120, 4-150, 30, and 30-120 days, respectively; in the case of fungi, the main microorganisms are *Neopestalotiopsis phangngaensis*, *Colletotrichum fructicola*, and *Thyrostroma jaczewskii* with efficiencies of 54.34, 48.78, and 46.34%, in 90 days, respectively; and the most efficient microbial consortia were from *Enterobacter spp.* and *Pantoea sp.* with 38.00 – 81.00%, in 120 days; and, *Pseudomonas protegens*, *Stenotrophomonas sp.*, *B. vallismortis* and *Paenibacillus sp.* with 55. 00 – 75.00% in 120 days.

Conclusions

The most efficient microorganisms in *LDPE* degradation are *Enterobacter spp.*, *Pantoea spp.*, *Pseudomonas spp.*, *Escherichia coli*, and *Bacillus spp.*; in fungi *Neopestalotiopsis phangngaensis*, *Colletotrichum fructicola*, and *Thyrostroma jaczewskii*; and in microbial consortia, those formed by *Enterobacter spp.* and *Pantoea sp.*, and that of *P. protegens*, *Stenotrophomonas sp.*, *B. vallismortis* and *Paenibacillus sp.*; and the most effective techniques used in *LDPE* biodegradation are SEM, gravimetry, and FTIR.

Keywords
Low-density polyethylene; efficiency; biodegradation; microbial consortia; fungi; bacteria.

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REVISED **Amendments from Version 1**

Based on the reviewers' comments, the relevant changes were made; hence, the changes made relate to: Paragraph 7 of the article was modified to indicate more precisely and specifically the different types that are used in the degradation process of LDPE plastics, including photo-oxidation, thermal degradation, ozonation, mechano-chemical degradation, catalytic degradation, and biodegradation. Paragraph 8 was separated from paragraph 7 to contextualize the importance of biodegradation and the action of microorganisms on polymers through different enzymes.

In the method section, the title of Table 1 was modified because the column containing the titles of the publications was excluded; on the other hand, a column equivalent to the sample used in the biodegradation was added; furthermore, the methods, microorganisms identified, and the analysis techniques were broken down into columns.

Any further responses from the reviewers can be found at the end of the article

Introduction

Plastics are synthetic polymeric molecules characterized by their versatility, lightness, low cost, and high durability.[1] Among the most common are polypropylene, polyethylene, nylon, and polycarbonate, which are considered highly persistent with a capacity for bioaccumulation; they also contaminate the soil, mainly cultivable areas, thus reducing the water filtration capacity and fertilization of plants.[2,3] Currently, they have become one of the most significant pollutants in marine ecosystems where most of these float and disintegrate into small fragments when exposed to the sun, taking the name of microplastics.[4]

Plastics are classified into[1]: easily degradable, which includes biologically phase plastics such as compostable and biodegradable ones, and[2] difficultly degradable, among which thermoset and thermoplastic plastics such as polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polytetrafluoroethylene (PTFE), and low-density polyethylene (LDPE) can be mentioned.[5] The latter, globally, are the most marketed, with a production of 390.7 million tons in 2021, of which 50% was produced in Asia and 22% in America.[6] Polyethylene is the most commonly used plastic in everyday life, accounting for 96% of all plastics on the market.[7]

LDPE accounts for 64%, and is primarily used in the form of bags, wrappings, and containers, which are discarded after use.[8] The mismanagement of plastic waste increases daily, mainly in Asian countries such as China, Indonesia, the Philippines, Vietnam, Malaysia, Thailand, and in Western countries like the United States. Of the plastics produced, considered to be 18 billion metric tons, 6% are incinerated, 23% are reused, 62% are disposed of in landfills, and 9% are considered recycled.[1]

It has been demonstrated in-vitro that the ingestion of plastics by living beings produces a high impact on fauna. It is mentioned that they cause neurotoxic and degenerative damages in rodents, marine invertebrates, fish, and mammals, who are exposed to the presence of high levels of microplastics.[9] Some studies in fish indicate that microplastic particles can cause oxidative damage to lipids in the gills and muscles, as well as neurotoxicity through the inhibition of acetylcholinesterase and alterations in neurotransmitter levels.[10]

Humans, as an important component of the ecosystem, are also affected by plastic waste. It has been estimated that a weekly intake of microplastics (MPs) with values ranging between 0.1 and 5 g can be found bound to food and drinking water, thereby generating adverse health effects.[11] In the city of Beijing, China, an analysis of feces conducted on young people between 18 to 25 years old who consumed water and food revealed the presence of microplastics such as polypropylene with a size of 20 – 800 nm.[12] Another study in Mexico found up to 30 microplastic particles in a series of foods such as energy drinks, tea, sodas, and beers[13]; another work conducted in Iran, in the analysis of bottled mineral water, found values of 8.5 ± 10.2 particles/L of PET, PS, PP.[14]

MPs are a globally recognized problem due to their prevalence in natural environments and the food chain, as well as their high impact on human health. Plastics directly affect living beings, either through ingestion or toxicity. It is noted that they could act as vehicles for invasive species and by adsorption on their surface of other synthetic chemical pollutants such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), or organochlorines, currently used by the chemical industry, thus potentiating or synergizing their toxic power due to components they contain such as plasticizers, heavy metal additives, etc.[15] Studies on microbiota have allowed the assessment of the effect of MPs, especially PETs, on microbiota, demonstrating that they would act at the colon level, decreasing the values of *Staphylococcus spp.*, *Bifidobacterium spp.*, and *Clostridium spp.*[16]

An important aspect to consider is the degradation process of plastics, such as with LDPE, which can take up to 400 years to decompose.[17] Different types of degradation are used como son: (a) Mechanisms of photooxidation, this process uses light absorption, acting by photooxidation and photodegradation; (b) Thermal degradation, is carried out by depolymerization or accidental reaction, using initially high temperature and ultraviolet light; (c) Ozonation, the ozone present in the atmosphere causes the degradation of polymers, transforming them into so-called reactive oxygen species (ROS), which are a group of free radicals capable of producing oxidative damage; (d) Mechanochemical degradation, the process breaks the polymer chains by exposing them to mechanical stress and ultrasonic irradiation; (e) Catalytic degradation, residual polymers are catalytically transformed into hydrocarbons producing oils and gases; y, (f) Biodegradation, the process involves various microorganisms, mainly bacteria (aerobic or anaerobic) and fungi.[5]

The capability of hydroxylases, lipases, and laccases enzymes, secreted by LDPE-degrading microorganisms, which are responsible for breaking the polymer chain into low molecular weight fragments, must be mentioned.[18] Extracellular enzymes play a very important role in biodegradation through the depolymerization of LDPE to form intermediate products that can be used as a carbon source by microorganisms,[19] as they oxidize, reduce, hydrolyze, esterify, and cut the internal molecular structure of the polymer.[20]

Microorganisms accelerate and increase the degradation process, making them an alternative to reduce the accumulation of petroplastics in the environment.[21] There are reports of bacteria (*Pseudomonas spp. and Bacillus spp.*) and fungi (*Aspergillus spp. and Fusarium spp.*) that can degrade this plastic under laboratory conditions.[22,23] The use of more efficient microorganisms in the degradation of LDPE will allow the proper selection of bacteria or fungi with greater action and degradative efficiency of plastic.[24] At the industrial level, it will involve the handling of various effective methods of detection and quantification, such as gravimetry, scanning electron microscopy (SEM),[25–30] Fourier transform infrared spectroscopy (FTIR), and gas chromatography coupled with mass spectrometry (GC-MS), which complement the study of the polymer's natural degradation.[31]

The purpose of this systematic review is to demonstrate the efficiency of LDPE-degrading microorganisms and the efficacy of the main biodegradation techniques on this type of plastics.

Methods

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) methodology was used, which is established for systematic reviews and meta-analysis statements.[32] The information was extracted from articles obtained from various databases such as: Scopus, Web of Science (WOS), Embase, and Google Scholar. The identification, screening, and eligibility of scientific articles were organized through the Zotero Bibliographic Manager.[33] The protocol of the systematic review was registered in PROSPERO (International prospective register of systematic reviews) under the number CRD42024506168.

The search strategy in all databases consisted of managing Boolean operators (AND, OR, and NOT), keywords (biodegradation, low-density polyethylene), years of publication (2010 – 2022), type of document (original article), language (Spanish, English), and open access publications. The DeCS/Mesh search terms were: Low-Density Polyethylene, LDPE, efficiency, biodegradation, microbial consortia, fungi, and bacteria.

The auxiliary search strategy included:

✓ **Scopus**

((TITLE-ABS-KEY (BIODEGRADATION) AND TITLE-ABS-KEY (LOW DENSITY POLYETHYLENE)) AND (LIMIT-TO (LANGUAGE,"English") OR LIMIT-TO (LANGUAGE,"Spanish")) AND (LIMIT-TO (PUBYEAR,2010) OR LIMIT-TO (PUBYEAR,2011) OR LIMIT-TO (PUBYEAR,2012) OR LIMIT-TO (PUBYEAR,2013) OR LIMIT-TO (PUBYEAR,2014) OR LIMIT-TO (PUBYEAR,2015) OR LIMIT-TO (PUBYEAR,2016) OR LIMIT-TO (PUBYEAR,2017) OR LIMIT-TO (PUBYEAR,2018) OR LIMIT-TO (PUBYEAR,2019) OR LIMIT-TO (PUBYEAR,2020) OR LIMIT-TO (PUBYEAR,2021) ORLIMIT-TO (PUBYEAR,2022)) AND (LIMIT-TO (EXACTKEYWORD,"Article")) AND (LIMIT-TO (DOCTYPE,"ar")) AND (LIMIT-TO (SRCTYPE,"j")))

✓ **Web of Science (WOS)**

(ALL=(biodegradation)) AND ALL=(low density polyethylene) and 2022 or 2021 or 2020 or 2019 or 2018 or 2017 or 2016 or 2015 or 2014 or 2013 or 2012 or 2011 (Publication Years) and 28 Article (Document Types) and Article (Document Types) and All Open Access (Open Access) and English (Languages)

✓ **Embase**

"biodegradation AND polyethylene AND low AND density AND [2010-2022]/py AND [article]/lim AND ([english]/lim OR [spanish]/lim"

✓ **Google Scholar**

The advanced search in this database included the exact phrase "LDPE biodegradation"; at least one of the following terms: Fungi, bacteria, or microbial consortia; terms mentioned in all scientific articles. It was also possible to delimit the years and languages of publication for each study.

Selection criteria

Inclusion criteria

- Scientific articles that included bacteria, fungi, and microbial consortia reported as LDPE degraders.
- Scientific articles that report the percentage of weight loss after the process.
- Articles published from January 2010 to October 2022.
- Publications in Spanish and English with open access.

Exclusion criteria

- Studies that do not report gravimetry.
- LDPE biodegradation time.
- Genus or species of the polyethylene-degrading microorganism.

The coordination and development of the review activities were carried out through the Zoom video chat software. To include the studies, their relationship with the research question was verified, based fundamentally on the terms: Biodegradation, LDPE, bacteria, and fungi. Then, for the quality assessment of the Systematic Reviews, Meta-analysis and a scientific article evaluation scale were used to ensure strict compliance with the inclusion and exclusion criteria mentioned in previous paragraphs. The web application used throughout the process of identification, selection, eligibility, and inclusion was Zotero. To collect relevant data from each report, PRISMA 2020 was used. Empirical articles were evaluated using an analytical rubric designed according to the parameters founded on the SSAHS scale by López-López E, Tobón S, JuárezHernández LG) for the consideration of scientific articles. Systematic reviews were evaluated using the Quality Assessment of Systematic Reviews and Meta-Analyses, utilizing an observation guide (checklist style as indicated by the National Heart, Lung, and Blood Institute).[34] Data systematization tables were used considering year, author(s), sample, study type, methods, identified microorganism, and the study technique (TE) were included. The variables for which relevant information was sought were: degradation efficiency (DE) of LDPE degrading microorganisms, biodegradation of plastics, degradation time (DT), and percentage of weight loss.

Results

Figure 1 shows the PRISMA methodology, whose search protocol identified 483 primary articles, of which 133 duplicates, 279 by title, 5 by abstract, 11 by access, and 20 for not meeting the inclusion terms were excluded. A total of 35 full-text articles were obtained, and 15 were included from previous review.

Table 1 indicates the number of articles found, totaling 50, with the highest quantity produced between the years 2018 to 2022. Also, it is observed that 100% of the articles correspond to experimental research works. Likewise, it points out the study techniques, with the most frequent being: SEM, gravimetry, and FTIR. Regarding the microorganisms identified in each of the studies, the most frequent in the phylum bacteria belonged to the genera *Pseudomonas* and *Bacillus*; as for the phylum of fungi, *Aspergillus spp.* predominated. As for the isolation site, the most used and mentioned in the reviewed articles were landfill soil and sanitary landfill.[32]

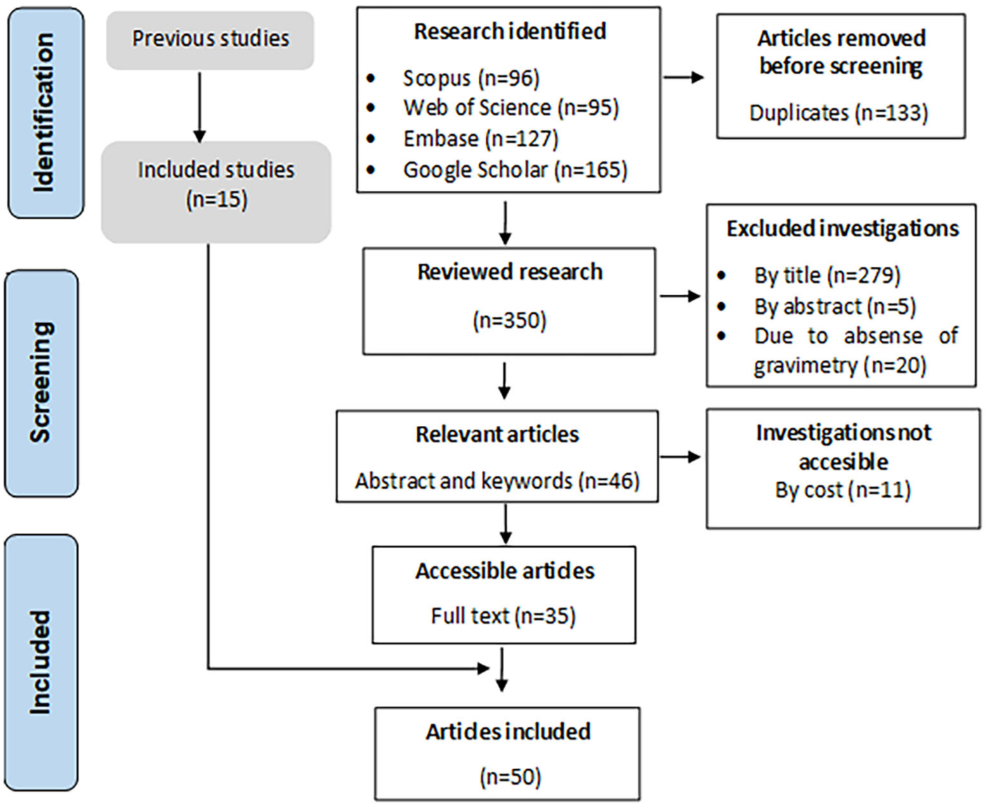

Figure 1. Flowchart of identification, screening, eligibility, and included articles.

Table 2 allows for the descriptive observation of microorganisms classified into bacteria, fungi, and microbial consortia, in quantities of 23, 17, and 9 respectively. Also, it specifies the species and the efficiency of LDPE-degrading microorganisms expressed in weight loss from highest to lowest and the days used for complete degradation, highlighting among bacteria *Enterobacter spp.*, *Pantoea spp.*, *P. spp.*, *Escherichia coli*, and *B. spp.* which obtained an ED range of 9.00-70.00%, 24.00-64.00%, 1.15 – 61.00%, 45.00%, and 1.50-40.00% with TD of 4-150, 120, 4-150, 30, and 30-120 days, respectively; in the case of fungi, the main microorganisms are *Neopestalotiopsis phangngaensis*, *Colletotrichum fructicola*, and *Thyrostroma jaczewskii* with ED of 54.34, 48.78, and 46.34%, respectively, and TD of 90 days; and, the most efficient microbial consortia were from *E. spp.* and *Pantoea sp.* with ED of 38.00 – 81.00%, and TD of 120 days; and, *P. protegens*, *Stenotrophomonas sp.*, *B. vallismortis*, *Paenibacillus sp.* with ED of 55.00 – 75.00% and TD of 120 days.

LDPE: Low-density polyethylene. IL: Isolation Location. MI: Identified Microorganism. TE: Study Technique. FTIR: Fourier Transform Infrared Spectroscopy. FTIR-ATR: Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance. SEM: Scanning Electron Microscopy. GC-MS: Gas Chromatography–Mass Spectrometry. EDS: Energy Dispersive X-ray Spectroscopy. FE-SEM: Field Emission Scanning Electron Microscopy. HT-GPC: High Temperature Gel Permeation Chromatography. TGA: Thermogravimetric Analysis. AFM: Atomic Force Microscopy. XRD: X-ray Diffraction. BATH: Bacterial Adhesion to a Hydrocarbon. GC-FID: Gas Chromatography-Flame Ionization Detector. DSC: Differential Scanning Calorimetry. NMR: Nuclear Magnetic Resonance.

Discussion

According to the data analyzed (Table 1), in recent years there has been an increase in studies on LDPE-degrading microorganisms with the aim of minimizing environmental impacts through bioremediation.[78] The requirement for special technologies allows understanding the degree of polymer disintegration and the nature of its resulting products. In the SR, up to 20 study techniques used in the biodegradation of LDPE have been detected. From our point of view, we consider highlighting in this article those methods that, due to frequency and especially efficacy, stand out among others. These methods include the following: *SEM*,[67,69–71] *gravimetry*,[25,26,27,28,29,30,35,36,39,40] and *FTIR*.[20,28,60,66]

Table 1. Articles Classified by Year, Author, Study Type, Sample, Methods, Identified microorganisms and Study Technique.

N°	Year	Authors	Study type	Sample	Methods	Identified microorganisms	Study Technique
1	2010	Uribe D, Giraldo D, Gutiérrez S, Merino F.[35]	Experimental	LDPE from landfill	Biological	*Pseudomonas sp.* MP3a., MP3b., *Penicillium sp., Rhodotorula sp., Hyalodendrun sp.*	Gravimetry, FTIR
2	2012	Kyaw B, Champakalakshmi R, Sakharkar M, Lim C, Sakharkar K.[28]	Experimental	Strain identified	Biological	*P. aeruginosa* PAO1 (ATCC15729), *P. aeruginosa* (ATCC15692), *P. putida* (KT2440 ATCC47054), *P. syringae* (DC3000 ATCC10862)	Gravimetry, FTIR-ATR, SEM, GC-MS, Tensile strength
3	2013	Tribedi P, Sil A.[30]	Experimental	Solid waste landfill soil	Biological	*Pseudomonas sp.* AKS2	Gravimetry, SEM, AFM, Tensile strength
4	2014	Bhatia M, Girdhar A, Tiwari A, Nayarisseri A.[25]	Experimental	Sanitary landfill soil	Biological	*P. citronellolis* EMBS027	Gravimetry, FTIR, SEM, TGA
5	2014	Muthumani S, Anbuselvi S.[36]	Experimental	Polyethylene trash	Biological	*Streptococcus sp., E. coli, Klebsiella sp., Bacillus sp., Pseudomonas sp., Saccharomyces sp., A. niger, A. flavus, Streptomyces sp.*	Gravimetry, Optical microscopy
6	2014	Das M, Kumar S.[26]	Experimental	Solid waste soil	Biological	*Aspergillus sp.* (FSM-3, 5, 6, 8), *Fusarium sp.* (FSM-10)	Gravimetry, FTIR, SEM, Optical microscopy
7	2015	Quinchía A, Maya S.[29]	Experimental	Strain identified	Biological	*Pycnoporus sanguineus* UTCH03	Gravimetry, FTIR, SEM, DSC
8	2015	Duddu M, Tripura K, Guntuku G, Divya D.[37]	Experimental	Not mentioned	Biological	*Streptomyces coelicoflavus* nbrc 15399	Gravimetry, FTIR, Optical microscopy, BATH
9	2015	Das M, Kumar S.[38]	Experimental	Strain identified	Biological	*B. amyloliquefaciens* (BSM-1 and BSM-2)	Gravimetry, FTIR, SEM, Sturm
10	2015	Deepika S, Jaya Madhuri R.[39]	Experimental	Garbage soil	Biological	*Streptomyces sp., Pseudomonas sp., A. niger, A. flavus*	Gravimetry
11	2016	Gajendiran A, Krishnamoorthy S, Abraham J.[27]	Experimental	Landfill soil	Biological	*A. clavatus* JASK1	Gravimetry, FTIR, SEM, Sturm, AFM
12	2016	Abraham J, Gosh E, Mukherjee P, Gajendiran A.[40]	Experimental	Sludge plus garden soil	Biological	*Streptomyces sp., A. nonius*	Gravimetry, FTIR, GC-MS, Sturm, AFM
13	2016	Skariyachan S, Manjunatha V, Sultana S, Jois C.[41]	Experimental	Plastic waste from rural and urban areas	Biological	*Proteus spp., Enterobacter spp., Enterobacter spp., Pseudomonas spp., Pantoea spp.*	Gravimetry, GC-FID, SEM, Tensile strength, FTIR

Table 1. *Continued*

N°	Year	Authors	Study type	Sample	Methods	Identified microorganisms	Study Technique
14	2017	Skariyachan S, Setlur A, Naik S, Naik A, Usharani M, Vasist K.[42]	Experimental	Landfill soil	Biological	*P. protegens* bt-dsce02, *Stenotrophomonas* sp. bt-dsce03, *B. vallismortis* bt-dsce01, *Paenibacillus* sp. bt-dsce04.	Gravimetry, FTIR, SEM, EDS, NM
15	2017	Gajendiran A, Subramani S, Abraham J.[43]	Experimental	Landfill soil	Biological	*A. versicolor*	Gravimetry, FTIR, SEM, Sturm, AFM
16	2017	Ojha N, Pradhan N, Singh S, Barla A., Shrivastava A, Khatua P, Rai V, Bose S.[44]	Experimental	Landfill soil	Biological	*P. oxalicum* NS4 (KU559906), *P. chrysogenum* NS10 (KU559907)	Gravimetry, FTIR, FE-SEM, AFM
17	2017	Awasthi S, Srivastava N, Singh T, Tiwary D, Kumar P.[45]	Experimental	Not mentioned	Biological	*Rhizopus oryzae*	Gravimetry, SEM, AFM, Tensile strength
18	2018	Denisse Yans Z. Dela Torre, Lee A. Delos Santos, Mari Louise C. Reyes and Ronan Q. Baculi.[46]	Experimental	Water from rock crevices	Biological	*B. krulwichiae*, *B. pseudofirmus*, *Prolinoborus fasciculus*, *Bacillus sp*.	Gravimetry, SEM, FTIR
19	2018	Munir E, Sipayung F, Priyani N, Suryanto D.[47]	Experimental	Sanitary landfill soil	Biological	*Streptococcus sp*. Sp2, *Streptobacillus sp*. Sp4	Gravimetry, FTIR, SEM
20	2018	Munir E, Harefa R, Priyani N, Suryanto D.[48]	Experimental	Landfill soil	Biological	*Trichoderma viride*, *A. nonius*	Gravimetry, Tensile strength
21	2018	Hikmah M, Setyaningsih R, Pangastuti A.[49]	Experimental	Strain identified	Biological	*Trichoderma spp*. (TL1), *Trichoderma spp*. (TL2), *Trichoderma spp*. (TL3)	Gravimetry, SEM, DSC, BATH
22	2018	Thamizhmarai T, Kannahi M.[50]	Experimental	Vedharaniyam waste soil	Biological	*Pseudomonas sp., A. niger, A. flavus, A. oryzae*	Gravimetry, FTIR, SEM
23	2018	P. Priyadarshini, Summera Rafiq, SK. Jasmine Shahina, K. Vijaya Ramesh.[51]	Experimental	Solid waste landfill	Biological	*Nocardiopsis alba*	Gravimetry, FTIR, SEM
24	2018	Jayaprakash V, Palempalli U.[52]	Experimental	PE bags in soil for 6 months	Biological	*A. oryzae*	Gravimetry, FTIR, SEM
25	2019	Sáenz M, Borodulina T, Diaz L, Banchon C.[53]	Experimental	Mangrove (Santay Island Ecuador)	Biological	*A. terreus, A. niger*	Gravimetry, SEM

Table 1. *Continued*

N°	Year	Authors	Study type	Sample	Methods	Identified microorganisms	Study Technique
26	2019	Bardají D, Furlan, J, Stehling E.[54]	Experimental	Solid waste landfill and incinerator	Biological	*Paenibacillus sp.*	Gravimetry, FTIR, SEM
27	2019	Islami A, Tazkiaturrizki T, Rinanti A.[55]	Experimental	Strain identified	Biological	*Thiobacillus sp.* K29:AA29p, *Clostridium sp.*	Gravimetry, SEM
28	2019	De Silva J, Jayasekera G, Nanayakkara C.[56]	Experimental	Landfill soil	Biological	*Fusarium sp.*_PS3, *Penicillium sp.* Ps2, *A. niger*	Gravimetry, FTIR, SEM, Optical microscopy
29	2019	Kartikey Kumar, Deepa Devi[57]	Experimental	Soil adhered to plastic	Biological	*Bacillus sp*. ISJ51, ISJ55, ISJ57	Gravimetry, FTIR, SEM, BATH
30	2020	Montazer Z, Najafi M, Levin D.[58]	Experimental	Larvae	Biological	*Cupriavidus necator* H16, *P. putida* LS46, *P. putida* IRN22, *Lysinibacillus fusiformis*, *B. aryabhattai*	Gravimetry, GC-FID
31	2020	Butrón S.[59]	Experimental	LDPE from dumpsite	Biological	*P. aeruginosa*	Gravimetry, optical, fluorescence microscopy
32	2020	Gupta K, Devi D.[20]	Experimental	Strain identified	Biological	*P. aeruginosa* ISJ14	Gravimetry, FTIR, FE-SEM, BATH
33	2020	Dey A, Bose H, Mohapatra B, Sar P.[60]	Experimental	Plastic landfill waste	Biological	*Stenotrophomonas sp*. P2, *Achromobacter sp.* DF22	Gravimetry, FTIR, SEM, AFM, BATH
34	2020	Sarker R, Chakraborty P, Paul P, Chatterjee A, Tribedi P.[61]	Experimental	Agricultural soil	Biological	*Enterobacter cloacae* AKS7	Gravimetry, SEM, BATH, Tensile strength, Fluorescence microscopy
35	2020	Samanta S, Datta D, Halder G.[62]	Experimental	Landfill	Biological	*B. tropicus* MK318648	Gravimetry, FTIR, SEM, AFM, Tensile strength
36	2021	Glen Cletus DSouza, Ryna Shireen Sheriff, Varun Ullanat, Aniruddh Shrikrishna, Anupama V. Joshi, Lingayya Hiremath, Keshamma Entoori[63]	Experimental	CI	Biological	*A. niger, A. flavus, A. oryzae*	Gravimetry, FT-IR and SEM
37	2021	Maroof L, Khan I, Yoo H, Kim S, Park H, Ahmad B, Azam S.[19]	Experimental	Landfill soil	Biological	*B. siamensis, B. cereus, B. wiedmannii, B. subtilis, P. aeruginosa, Acinetobacter iwoffii*	Gravimetry, FTIR, FE-SEM, XRD, Carbon analysis

Table 1. *Continued*

N°	Year	Authors	Study type	Sample	Methods	Identified microorganisms	Study Technique
38	2021	Soleimani Z, Gharavi S, Soudi M, Moosavi Z.[64]	Experimental	Plastic landfill soil	Biological	*Streptomyces sp.* IR-SGS- T10(MK719894.1), *Streptomyces sp.* IR-SGS-Y1 (MK719896.1, *Streptomyces sp.* IR-SGS-T5 (MK611552.1) *S. alborgiseolus* IR-SGS- T10(MK719894.1), *Streptomyces sp.* IR-SGS-K3 (MK608706.1), *S. gancidicus* IR-SGS-K2 (MH819728.1), *Streptomyces sp.* IR-SGS-K1 (MK608363.1), *Rhodococcus ruber* IR-SGS-T6 (MK611559.1), *R. ruber* IR-SGS-T7 (MK611560.1), *Nocardia sp.* IR-SGS-T9 (MK719893.1), *N. farcinica* IR-SGS-T8 (MK719892.1)	Gravimetry, FTIR, SEM, Tensile strength
39	2021	Waqas M, Haris M, Asim N, Islam H, Abdullah A, Khan A, Khattak H, Waqas M, Ali S.[65]	Experimental	Strain identified	Biological	*B. safensis, B. amyloliquefaciens*	Gravimetry, SEM
40	2021	Khruengsai S, Sripahco T, Pripdeevech P.[66]	Experimental	Institute of Excellence in Fungal Research	Biological	*D. italiana, T. jaczewskii, C. fructicola, S. citrulli, A. niger*	Gravimetry, FTIR, SEM, GC-MS
41	2021	Khandare S, Chaudhary D, Jha B.[67]	Experimental	Strain identified	Biological	*Cobetia sp., Halomonas sp., Exiguobacterium sp., Alcanivorax sp.*	Gravimetry, FTIR-ATR, FE-SEM, AFM, TGA
42	2021	Nadeem H, Alia K, Muneer F, Rasul I, Siddique M, Azeem F, Zubair M.[68]	Experimental	Solid waste landfills	Biological	*Serratia sp., Stenotrophomonas sp., Pseudomonas sp.*	Gravimetry, FTIR
43	2021	Chaudhary A, Chaitanya K, Dalmia R, Vijayakumar R.[69]	Experimental	Strain identified	Biological	*Thermomyces lanuginosus*	Gravimetry, FTIR, SEM, TGA
44	2021	Skariyachan S, Taskeen N, Kishore A, Krishna B, Naidu G.[70]	Experimental	Landfill soil	Biological	*Enterobacter sp.* nov. bt DSCE01, *E. cloacae* nov. bt DSCE02, *P. aeruginosa* nov. bt. DSCE-CD03	Gravimetry, FTIR, SEM, AFM, XRD, EDS
45	2021	Zahari N, Abdullah S, Tuah P, and Cleophas F.[71]	Experimental	Strain identified	Biological	*B. subtilis, C. tropicalis*	Gravimetry, FTIR, SEM
46	2021	Perera P, Deraniyagala A, Mahawaththage M, Herath H, Rajapakse C, Wijesinghe P, Attanayake R.[72]	Experimental	Dry reserve forest	Biological	*Phlebiopsis flavidoalba, Schizophyllum commune, Phanerodontia chrysosporium*	Gravimetry, FTIR, SEM, Tensile strength

Table 1. *Continued*

N°	Year	Authors	Study type	Sample	Methods	Identified microorganisms	Study Technique
47	2022	Saira A, Maroof L, Iqbal M, Farman S, Lubna, Faisa S.[73]	Experimental	Peshawar district landfills	Biological	*A. Niger, A. flavus, Penicillium*	Gravimetry, FTIR
48	2022	Maleki M, Moghimi H, Azin E.[74]	Experimental	Compost	Biological	*Achromobacter denitrificans* Ebl13	Gravimetry, Sturm, FTIR, SEM, TGA
49	2022	Khruengsai S, Sripahco T, Pripdeevech P.[75]	Experimental	Institute of Excellence in Fungal Research	Biological	*Neopestalotiopsis phangngaensis*	Gravimetry, SEM, Sturm, Tensile strength
50	2022	Liu X, Zhang Y, Sun Q, Liu Z, Zhao Y, Fan A, Su H.[76]	Experimental	Household waste landfill	Biological	*B. velezensis* C5	Gravimetry, FTIR-ATR, SEM, EDS, FE-SEM, HTGPC, GC-MS

Table 2. Degradation efficiency (ED) of LDPE by microorganisms expressed as weight loss (%) and degradation time (TD) in days, 2010 – 2022.

Microorganisms		Degradation time (Days)	Degradation efficiency weight loss (%)
Bacteria			
	Enterobacter spp.[41,61,70]	45-120	9.00-70.00
	Pantoea spp.[41]	120	24.00-64.00
	Pseudomonas spp.[19,20,25,28,30,36,39,41,50,59,68]	4-150	*1.15*-61.00
	Escherichia coli[36]	30	45.00
	Bacillus spp.[19,36,38,47,57,58,62,65,71,76]	30-120	1.50-40.00
	Proteus spp.[41]	120	16.00-59.00
	Streptomyces spp.[36,37,39,40,64]	28-90	2.31-46.70
	Serratia sp.[68]	150	40.00
	Nocardiopsis alba[51]	150	32.25
	Stenotrophomonas spp.[60,68]	100-150	7.54-32.00
	Paenibacillus sp.[54]	90-120	11.60-30.80
	Klebsiella sp.[36]	30	21.00
	Achromobacter spp.[60,74]	60-100	7.45-12.30
	Lysinibacillus fusiformis[58]	18	8.20
	Rhodococcus spp.[64]	60	3.01-6.23
	Nocardia spp.[64]	60	3.60-5.98
	Prolinoborus fasciculus[47]	90	5.10
	Halomonas sp. H-255[68]	90	1.72
	Cobetia sp. H237[68]	90	1.40
	Exiguobacterium sp. H256[68]	90	1.26
	Alcanivorax sp. H265[68]	90	0.97
	Acinetobacter iwoffii[19]	90	0.76
	Streptococcus spp.[36,47]	30	0.16
Fungi			
	Neopestalotiopsis phangngaensis[75]	90	54.34
	Colletotrichum fructicola[66]	90	48.78
	Thyrostroma jaczewskii[66]	90	46.34
	Stagonosporopsis citrulli[66]	90	45.12
	Diaporthe italiana[66]	90	43.90
	Saccharomyces[36]	30	43.00
	Aspergillus spp.[26,27,36,39,40,43,48,50,52,53,56,66,73]	30-270	4.90-40.60
	Penicillium chrysogenum NS10*(KU559907)*[73]	90	0.35-36.60
	Schizophyllum commune[72]	60	9.65
	Thermomyces lanuginosus[69]	30	9.21
	Fusarium spp.[26,56]	60-90	0.59-9.00
	Rhizopus oryzae[45]	30	8.40
	Trichoderma spp.[48,49]	35-45	4.87-7.51
	Candida tropicalis[71]	7	3.20
	Phlebiopsis flavidoalba[72]	60	2.60
	Phanerodontia chrysosporium[72]	60	2.50
	Pycnoporus sanguineus UTCH03[29]	180	0.66

Table 2. *Continued*

Microorganisms		Degradation time (Days)	Degradation efficiency weight loss (%)
Microbial Consortia			
	Enterobacter spp., Pantoea sp.[41]	120	38.00-81.00
	P. protegens, Stenotrophomonas sp., B. vallismortis, Paenibacillus sp.[42]	120	55.00-75.00
	*Enterobacter sp.*nov. bt DSCE01, *E. cloacae nov.* bt DSCE02, *P. aeruginpsa* nov. bt. DSCE-CD03[70]	160	64.25
	Lysinibacillus xylanilyticus, *A. niger*[77]	126	15.80-29.50
	A. niger, A. flavus, A. oryzae[63]	55	26.15
	Cupriavidus necator H16,[58] *P. putida* (LS46,IRN22)[58]	18	13.50
	Thiobacillus sp. K29, Clostridium sp.[55]	30	5.30-6.40
	Pseudomonas spp. (MP3a, MP3b)[35]	60	5.40
	Penicillium sp., Rhodotorula sp., Hyalodendron sp.[35]	60	4.80

The SEM study technique,[19,42–44,46,49–54,56,62,63,70,72] is used to detect the biodegradation of LDPE and is employed to monitor changes on the surface of the LDPE film.[72,74–76] The adhesion of microorganisms to the surface is essential for biodegradation.[65] After incubating LDPE with selected degrading microorganisms on the surface, some characteristics such as erosion, holes, and cavities are observed, which are attributed jointly to the formation of bacterial film and the penetration of fungal hyphae.[60,68] Erosion is considered the primary cause of the mass reduction of the surface due to the secretion of enzymes and microbial extracellular metabolites.[20]

Another frequently used technique in the reviewed articles is gravimetry,[20,42–46,48–55,68–71] which is a simple and highly precise test to determine the polymer weight reduction, originating as a consequence of being used as a source of carbon and energy by microorganisms.[19,62–65,72–74,76] This weight loss is considered proportional to the surface area, as biodegradation starts on the polymer's surface.[38] With Gram-positive bacteria, the degradation efficiency (DE) in terms of LDPE weight loss has been reported: *Streptomyces sp.* DE 5.2% and degradation time (DT) of 90 days[40] and *Bacillus amyloliquefaciens* DE 11% and DT 60 days.[38]

It was found in the review that various articles mentioned the FTIR technique,[19,25,26,27,28,29,30,35,37,40,44,46,50,51,64,67–70,74–76] as the third most frequent of those applied to determine biodegradation; in addition, the cited studies consider it analytical and efficient, useful for identifying the chemical configuration of organic, polymeric, and inorganic material, and the morphological changes, which are supported by the chemical structural changes at the level of the carbon chains, observing new functional groups (alkoxy, acyl, carboxyls, and nitro) or absence of them, and modifications in the chains such as breaks, stretches, and formation of double bonds; moreover, this technique determines the carbonyl index (CI), which measures the degree of degradation of the LDPE and in which its value depends on the degraded carbonyl bonds.[28] In reality, it involves measuring the concentration of carbonyl groups (CG) corresponding to acids, aldehydes, and ketones.[35] In the process of LDPE biodegradation, the initial weight corresponds to the oxidation of the chain that leads to the formation of CG, and subsequently, these form carboxylic groups that are degraded by β-oxidation and then through the citric acid cycle to CO_2 and H_2O.[77]

In Table 2, as observed, the microorganisms frequently reported in the articles analyzed in Table 1 include bacteria from the genera *Bacillus*, *Brevibacillus*, *Cellulosimicrobium*, *Comamonas*, *Delftia*, *Enterobacter*, *Escherichia*, *Idonella*, *Kocuria*, *Lysinibacillus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhodococcus*, *Rhodotorula*, *Stenotrophomonas*, and *Streptomyces*,[25,28,30,42,46,58,59] with the genera *Pseudomonas*, *Bacillus*, and *Streptomyces* predominating. Other microorganisms are fungi, with *Aspergillus sp.* most frequently cited.[39,48,50,52,63] Among the less frequently cited species include *Rhizopus oryzae*, *Paenibacillus sp.*, *Streptomyces coelicoflavus*, *Thiobacillus*, *Clostridium*, *Achromobacter denitrificans*, *Penicillium oxalicum*, *P. chrysogenum*, *Pycnoporus sanguineus*, *Enterobacter cloacae.*[29,37,44,45,54,55,61,74]

In the same table, it is analyzed that among the most efficient bacteria in the degradation of LDPE according to the weight loss of the polymer include several species such as *Enterobacter spp.* with an ED of 9.00 – 70.00%, and TD of 4 -150, *Pantoea spp.* with an ED of 24.00 – 64.00% and TD of 120, *Pseudomonas spp.* with an ED of 1.15 – 61.00% and TD of

4-150, *Escherichia coli* with an ED of 45.00% and TD of 30, and finally, *Bacillus spp.* with an ED of 1.50 – 40.00% and TD of 30-120. *Bacillus sp.* is also considered as another important species in the biodegradation process, having a consumption rate of 0.0019 g of the polymer per day[45]; or, participating in consortia such as the one constituted by *Bacillus vallismortis*, *Pseudomonas protegens*, *Stenotrophomonas sp.*, and *Paenibacillus sp.*[42]

It has been determined that *P. aeruginosa* cultured on LDPE as the only carbon source has an ED of 0.0015 g of LDPE per day and a TD of 462 days to reduce a polyethylene film from 1g to 0.5g[20]; *Enterobacter cloacae* AKS7 and *Escherichia coli* possess another type of degradative action, and it is due to the secretion of extracellular polymeric substances and the high hydrophobicity of the microorganism's cell wall, which allows a greater formation and adherence of the bacterial biofilm.[36,61] In the case of *Pantoea sp.*, its efficiency can be measured either individually or in consortium with *Enterobacter.*[41]

Fungi (Table 2), like bacteria, are considered LDPE-degrading microorganisms. The most efficient are: *Neopestalotiopsis phangngaensis*, *Colletotrichum fructicola*, and *Thyrostroma jaczewskii* with EDs of 54.34, 48.78, and 46.34%, respectively, and a TD of 90 days. The mycotic activity is considered to be due to their great capacity for adherence. In the polymer biodegraded for 40 days, the biofilm formed by the strongly adhered fungi is observed; at 80 days, surface deformation is evident and microcracks are differentiated.[28] Other efficient species are also reported, such as *A. clavatus* with an ED of 35.00% and a TD of 90 days[27] and *A. versicolor* with 40.60% and a TD of 90 days.[43]

The degradative efficiency of microbial consortia has also been reported in different studies,[35,41,63,70] showing the most efficient to be the one formed by *Enterobacter spp.* and *Pantoea sp.*, and the one of *Pseudomonas protegens*, *Stenotrophomonas sp.*, *B. vallismortis*, and *Paenibacillus sp.* The cooperation of different microorganisms allows the use of different and complementary metabolic capacities for their growth, forming pure or mixed biofilms (fungi and bacteria), more resistant and metabolically more active.[79]

LDPE-degrading microorganisms form a biofilm on the polymer and use it as a carbon source for consumption, an event that is reflected in weight loss. The biodegradation by microorganisms is a process of high metabolic activity, in which the count of viable cells, the concentration of surface proteins, and the efficiency in degradation expressed as polymer weight loss must be taken into account.[20] The most frequent place of isolation of microorganisms with LDPE-degrading capacity came from landfill soil and sanitary landfills with the presence of plastics. Various studies have indicated that bacteria and fungi adapt under different environmental conditions, a process mediated by complex cellular changes at the enzymatic level,[19,56] maintain physiology and metabolism, thus ensuring the survival of microorganisms. It has been demonstrated that bacteria such as *Pseudomonas putida* is a resistant and efficient xenobiotic decomposer because it presents an effective efflux pump; similarly, *Streptomyces atacamensis* shows xerotolerant latency and spore response to desiccation, and upregulation of proteins that are functional during xeric stress,[80] which probably explains why certain microorganisms are more efficient at degrading LDPE compared to others.

Considering that degradation is a slow process (this activity occurs before 60 days of incubation), and that degradation methods are heterogeneous, some authors conclude that there is no standard methodology in relation to analytical methods.[15] However, in this article, we present the various biodegradation techniques, so a more precise vision could be had to assess which of them is the most consistent and effective according to their ED and TD.

Finally, based on the detection and quantification tests of polymer degradation, the exposed microorganisms constitute a sustainable alternative, useful for bioremediation and minimization of environmental impacts, with the aim of reducing environmental pollution by LDPE.

Conclusions

- The microorganisms with the highest degradation efficiency on LPDE-type plastics in bacteria are *Enterobacter spp.*, *Pantoea spp.*, *Pseudomonas spp.*, *Escherichia coli*, and *Bacillus spp.*; in fungi *Neopestalotiopsis phangngaensis*, *Colletotrichum fructicola*, and *Thyrostroma jaczewskii*; and in microbial consortia, those formed by *Enterobacter spp.* and *Pantoea sp.*, and the one by *P. protegens*, *Stenotrophomonas sp.*, *B. vallismortis*, and *Paenibacillus sp.*

- The most effective techniques used in LDPE biodegradation are SEM, gravimetry, and FTIR.

Limitations

The results obtained allow for the identification of a lack of studies on microorganisms efficient in the biodegradation of LDPE, which limits the possibility of expanding their number and understanding their efficiency. Moreover, there are few studies on alternative methods that are effective in biodegradation. These limitations should be taken into account for the guidance and development of new research.

Ethics and consent

Ethical approval and consent were not required.

Data availability

No data are associated with this article.

Reporting guidelines

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Open Peer Review

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Reviewer Report 30 July 2024

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✔ **Magaly de la Cruz Noriega**
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This research work is very interesting and relevant, as it gives us updated information on the role of microorganisms in degrading low-density plastic. But for its indexing some details are required.

In the introduction, it is necessary to include a more detailed paragraph on the plastic degradation techniques such as (thermal, radiation, mechanical, chemical and biological), then put emphasis on the biological ones, indicating the advantages and limitations in the biodegradation of low-density plastic, to have a clearer picture of this review, then eliminate the research question rather place the implication or impact that this work has.

Regarding the results, consider in Table 1 to consider the classification as follows: (Year, Authors, Type of study, type of sample or sample, Technique (physical, mechanical or biological), identified microorganisms, Analysis in the detection of Degradation.

The discussion needs to go deeper into Table 1.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are the conclusions drawn adequately supported by the results presented in the review?

Partly

If this is a Living Systematic Review, is the 'living' method appropriate and is the search schedule clearly defined and justified? ('Living Systematic Review' or a variation of this term should be included in the title.)
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: biotechnology, bioremediation, electromicrobiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 06 Aug 2024

Jorge Guillermo Morales Ramos

The observations made in terms of classifying degradation types and highlighting biodegradation among them were raised. The second point on table 1, the table was constructed on the basis of the suggestions, introducing the sample and techniques.

Competing Interests: None

Reviewer Report 25 July 2024

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The article titled "Efficiency of Microorganisms and Effectiveness of Biodegradation Techniques on LDPE Plastics: A Systematic Review" was published in F1000Research. This systematic review, based on the PRISMA method, examines studies published between January 2010 and October 2022, analyzing the degradation of LDPE by bacteria, fungi, and microbial consortia, as well as the techniques used to evaluate this degradation.

The study is particularly relevant due to the growing concern about plastic accumulation in the environment and the negative impacts associated with these materials, such as soil and water contamination and the effects on human health and wildlife. The results of this study provide a comprehensive view of the biodegradative capabilities of different microorganisms and effective methodologies for evaluating plastic degradation, which can guide future research and industrial applications in plastic waste management.

Main Comments

Conceptualization of Biodegradation Techniques:
Comment: It would be beneficial for the introduction of the study to include a detailed conceptualization of different biodegradation techniques, covering aerobic and anaerobic processes, necessary environmental conditions, types of microorganisms involved, and specific applications for LDPE plastics.
This would help readers understand how these techniques are applied and evaluated in the context of plastic biodegradation, providing a clear and complete theoretical framework from the outset.

Secondary Comments

Diversity of Evaluation Techniques:
Comment: The study highlights several evaluation techniques such as SEM, gravimetry, and FTIR but would benefit from a more in-depth discussion of the specific advantages and limitations of each technique.
Rationale: A detailed comparison would help readers choose the most suitable techniques for their own research and practical applications.

Future Research in Microbial Consortia:
Comment: The review mentions the effectiveness of microbial consortia in LDPE degradation but could be expanded to suggest additional studies exploring the synergistic interaction between different microorganisms.
Understanding how microbial consortia interact and enhance the degradation of plastics can open new avenues for research and development of more efficient biotechnologies.

Environmental and Human Health Impact:
Comment: Although the impact of plastics on the environment and human health is addressed, a section specifically dedicated to discussing these effects with recent data and concrete examples would strengthen the study's argument.
Providing detailed information on the environmental and public health consequences of plastic accumulation underscores the importance of developing and applying effective biodegradation techniques.

The article offers a comprehensive and detailed overview of the biodegradative capabilities of various microorganisms and the effective methodologies for the degradation of LDPE. However, it could benefit from further conceptualization of biodegradation techniques and a more detailed explanation of the inclusion and exclusion criteria. Additionally, expanding the discussion on the advantages and limitations of evaluation techniques, synergy in microbial consortia, and the environmental and human health impacts would provide a more complete and applicable perspective.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are the conclusions drawn adequately supported by the results presented in the review?
Yes

If this is a Living Systematic Review, is the 'living' method appropriate and is the search schedule clearly defined and justified? ('Living Systematic Review' or a variation of this term should be included in the title.)
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioremediation and Biodegradation of organic contaminants

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 06 Aug 2024

Jorge Guillermo Morales Ramos

We are grateful for this review of our article.

Competing Interests: None