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Systematic Review and Meta-analysis on the Application of Bacteria for the Biodegradation of Plastics

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Abstract*– The accumulation of plastic waste is a problem that affects terrestrial and marine ecosystems, in addition to altering the quality of life of humans. The purpose of this research was to evaluate, through a systematic review and meta-analysis, the biodegradation of plastics using different species of bacteria. The study had a quantitative approach, applied type, non-experimental design of documentary review and descriptive-explanatory level. A total of 502 studies were collected from Scopus and Web of Science databases, in the period from January 2012 to September 2021. The results showed that Pseudomona sp. bacteria biodegraded low-density polyethylene (LDPE) by 1.8%, while Pseudomona AK31 reduced the mass of polystyrene (PS) by 19.9%. Regarding colonial growth, a bacterial consortium had a growth of 1.9E+06 CFU/mL and the bacterium Bacillus paralicheniformis had a growth of 6E+5 CFU/mL, both on polyurethane films for 30 days. Finally, it is concluded that bacterial strains can degrade plastics and for the evaluation it is necessary to know their bacterial population and the mass reduction of the polymer.

***Keywords*-- plastic, biodegradation, bacteria, enzyme, systematic review, meta-analysis.**

I. INTRODUCTION

The accumulation of plastic waste generates negative impacts on the environment because polymeric compounds are resistant to degradation and very persistent, reaching the point of saturation and generating contamination of the planet's soil, oceans and air [1]-[5]. This impact is increased because humanity has produced more than 6 billion metric tons of plastics, of which only 9% is recycled [6]. Another aspect is that plastic has diverse applications substituting glass, wood, metal and other products, being an important part of the world economy [7]. So, if this indicator is maintained, in 2050 there will be about 96 billion tons of plastic waste [8], [9].

In view of this problem, several studies show the use of bacteria as a possible solution for the biodegradation of plastics. Among the microorganisms studied, bacteria such as Arthrobacter sp, Pseudomonas sp, Thermobifida fusca, Ideonella sakaiensis were used in the biodegradation of high-density PET plastics [6] and [10]. The strains of Pseudoxanthomonas sp. NyZ600 that biodegrade bisphenol-A(PC) polycarbonate [11], prokaryotes such as Alcanivorax and Marinobacter that biodegrade polyethylene (PE) and polypropylene (PP) [12]-[14], Paenibacillus and Corynebacterium that biodegrade polystyrene foam (EPS) [15], the bacterial strain AKS31 that biodegrades polyurethane (PUR) [16] and the bacterium Alcaligenes faecalis LNDR-1 that biodegrades polyethylene bags [17]. These microorganisms are an innovative field for research and can be applied in the control of these synthetic solid wastes.

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Bacteria are prokaryotic organisms present in all types of marines, terrestrial and aerial habitats. The aerobic and anaerobic condition of these microorganisms such as methanotrophs (methane producers) or the Pseudomonas group, are exploited in the biological degradation of synthetic plastics [18]. The main component of bacteria are enzymes that degrade a specific type of polymer, even these enzymes can be synthesized in laboratories for a larger scale application for the solution of this problem [19].

The research developed contributes to have an alternative for the treatment of plastics that alter the living conditions of living beings. The application of bacteria in the process of biodegradation of plastics is considered an effective and accepted method since it is a process that does not generate negative impacts on the environment. On the other hand, the use of bacteria represents variable costs according to the development of the technology, requiring laboratory practices to carry out diverse tests and to be able to identify the types of potential bacteria, as well as the synthesis of effective enzymes in the biodegradation of plastics. In addition, the use of microorganisms could be an alternative in the development of an eco-efficient management system in the reduction of solid waste, thus reducing the impact on the environment.

Therefore, the present research through systematic review evaluated the effectiveness of the application of bacteria in the biodegradation of various types of plastics between the years 2012 to 2021, identifying the groups of bacteria that have higher efficiency in the biodegradation of plastics, determining the operational conditions in the process of biodegradation of plastics by bacteria, and determining the process of reduction of plastics in biodegradation.

II. METHODOLOGY

A. Approach, type, design and level of research

The study had a quantitative approach, applied type, non-experimental design of documentary review and descriptive-explanatory level.

B. *Sources of information and search strategy*

The systematic review of the present study was developed based on the statistical approach of meta-analysis. The scientific articles were extracted from reliable database sources, such as Scopus, Web of Science and ScienceDirect. Regarding the time of publication, it was limited from January 2012 to September 2021. The search for information in the database was carried out systematically using keywords referring to plastic. The search string established was ((bacteria OR bacterium OR bacterial) AND ("plastic biodegradation" OR "biodegradation of plastic" OR "plastic bio-degradation" OR "bio-degradation of plastic" OR "plastic degradation" OR "degradation of plastic" OR "polymer bio-degradation" OR "bio-degradation of polymer" OR "polymer degradation" OR "degradation of polymer")). All the information search was performed in the English language. In addition, the references of the reviews were taken into account to analyze the most relevant studies.

C. *Inclusion and exclusion criteria*

For the research, duplicate papers were eliminated based on title and keywords. The abstracts of the selected articles were then reviewed and articles unrelated to the research topic were excluded. Subsequently, the articles were downloaded for full-text review.

The different inclusion criteria are defined as follows:

1. All research that showed the degradation of various types of plastics by different types of bacteria were included. The selection of this criterion was based on the process of biodegradation of plastics by a specific type of bacteria or a potentially active consortium.

2. All the articles selected were those that identified the operational conditions of the process of biodegradation of plastics by bacteria. The parameters studied are temperature (T°), pH, bacterial nature, types of enzymes, degradation time, among others.

3. All researches with insufficient data on operational conditions were excluded.

4. All investigations that considered the mass reduction of plastic by bacteria were included; also, bacterial growth in the plastic degradation process was considered.

5. All investigations that considered the degradation of plastics by bacteria without presenting data such as the level of polymer mass reduction and colonial growth were excluded.

6. All investigations that considered degradation of plastics by other organisms (fungi, insects, algae, etc.) were excluded.

D. *Article selection and information extraction*

Each potentially eligible article was reviewed for data collection (using validated data collection forms). The information of each article was extracted and summarized considering the following factors: (a) author(s) and year of publication; (b) types of bacteria with plastic degrading capacity; (c) operational conditions in the biodegradation process (T°, pH, contact time); (d) reduction of plastic mass (mg); (e) initial and final population of bacterial colonies (CFU/mL); (f) types of enzymes; (g) bacterial nature (CFU/mL).

E. *Evaluation of methodological quality*

The Newcastle-Ottawa scale was used to evaluate the methodological quality of the included investigations. This checklist was adapted according to the interest of the present investigation, analyzing 2 dimensions: selection and outcome. The selection indicates whether the different types of bacteria presented the ability to degrade the different types of synthetic plastics under different conditions. While, the result section was established by the growth of the bacterial population in the degradation process of the plastics, and the mass reduction of the plastic by bacterial activity. Finally, each section (selection and result) was evaluated with the criteria of "Yes" and "No" to determine the quality standards of information whether it is good, acceptable and bad [20]-[23].

F. *Data meta-analysis*

RevMan 5.4.1. software, widely used in data review and analysis, was used. This statistical program is applied in systematic reviews and meta-analysis. For the heterogeneity of the investigations, the forest diagram was used, which is elaborated with the Chi2 and I2 statistical tests.

Dichotomous data were used, which were presented and compared with the Odds Ratio (OR) meta-analysis criterion. Effect estimates were developed with 95% confidence intervals. Heterogeneity of the investigations was assessed through visual analysis of the forest plot in order to determine the overlap of confidence intervals. In addition, the Chi2 statistic indicates the differentiation of the results, as well as the compatibility of them by performing a random selection of data. The p-value determines the heterogeneity of the investigations.

The results of each investigation were directed to an environmental approach, and the use of bacteria were subject to bacterial growth in the plastic biodegradation process (either as a single species or the application of a bacterial consortium) and the mass reduction of plastic by bacterial activity.

III. Results and Discussion

A. *Inclusion and exclusion of studies*

The meta-analysis follows a specific sequence and therefore Figure 1 shows the flow diagram of the selection of the research included in the meta-analysis. A total of 502 articles were identified through the search of the databases described above. The different inclusion criteria defined were evaluated as explained below. Of the 502 selectable articles, 131 studies were excluded because of the type of document (reviews, conference paper, data paper, among others), 128 studies were excluded because they were duplicated in the databases (Scopus and Web of Science) and the rest because they were not related to the subject of the study. A total of 183 studies were obtained for the full-text review, and 168 studies were excluded for not having arguments related to the subject matter, such as the application of other microorganisms, insufficient data on operational conditions, among others. In total, 15 studies were selected and registered for the final evaluation. For the meta-analysis, only 10 studies were analyzed, which complied with the same working conditions, considered a minimum of two tests to evaluate the reduction in mass of plastic by bacterial activity and used additives or bacterial consortia.

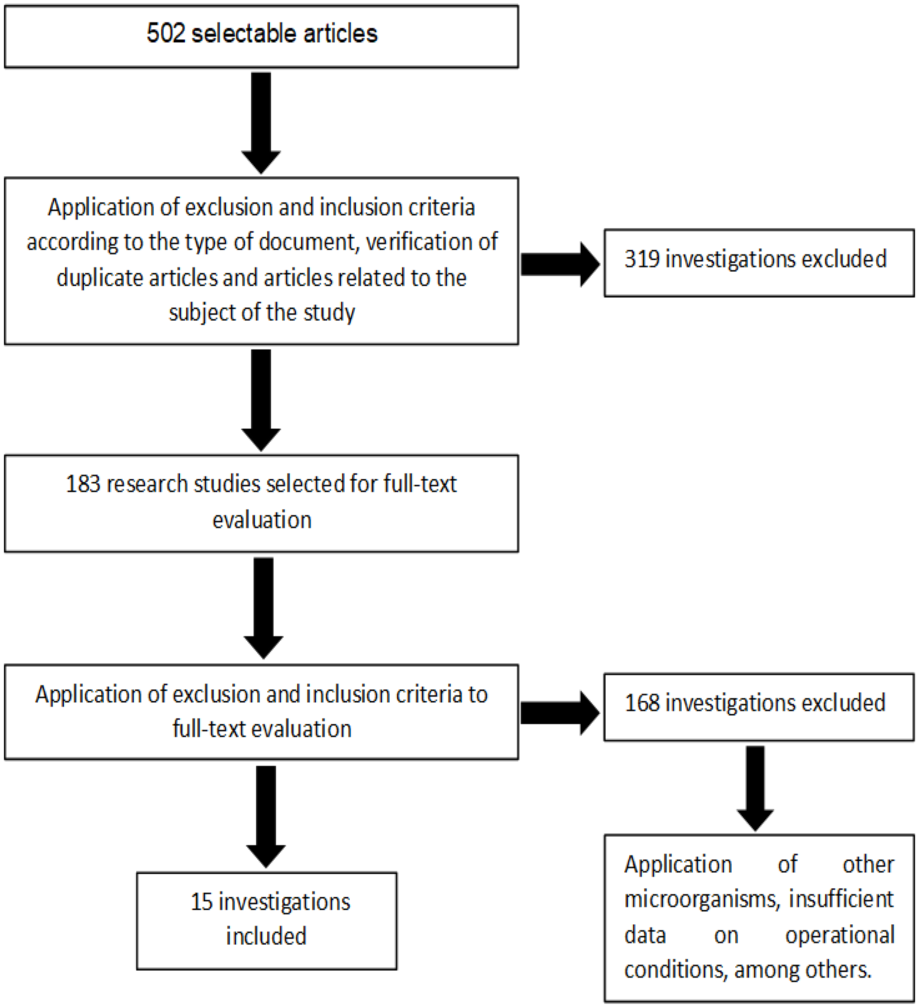

Fig. 1 Flowchart for selection of included and excluded research

B. Description of the included research

For the description of the included research, Table 1 shows that the majority of selected research was located in the Web of Science database with 9 articles versus 6 articles in the Scopus database. Each study evaluated the effectiveness of bacteria, either a species or a consortium with biodegrading function on plastics. A variety of bacteria were identified in the selected studies, with the genus Pseudomona and the genus Bacillus being the bacterial strains with the greatest application in the degradation process. The countries where studies of bacteria for the biodegradation of plastics were carried out were India, China and the Republic of Korea. The operational conditions taken into account were T° and pH as fundamental factors in the process of biodegradation of plastics by bacteria. Suitable conditions for an efficient biodegradation process were at a temperature of 30 to 37 °C and pH of 7 to 9.

TABLE I
CHARACTERISTICS OF STUDIES FOR THE SYSTEMATIC REVIEW AND META-ANALYSIS

Nro.	Journal	Database	Bacterium	Geographical scope	Author (s)
1	MDPI	Web of Science	*Pseudomona aeruginosa*	Seul, Korea	[1]
2	Marine Pollution Bulletin	Scopus	*Alltermonas australica* *Cobetia sp.*	Gujarat, India	[24]
3	Journal of Hazardous Materials	Web of Science	*Rhodobacterales* *Oceanospirillales* *Burkholderiales*	Chania, Greece	[25]
4	International Biodeterioration y Biodegration	Web of Science	*Brevibacillus sps* *Aneurinibacillus sp.*	Belagavi, India	[26]
5	International Biodeterioration y Biodegration	Web of Science	*Methylocella sp.* *Methylocystis sp*	Bangkok, Thailand	[18]
6	Journal of pure and applied microbiology JPAM	Web of Science	*Enterobacter cloacae* *AKS7*	Bengal, India	[27]
7	Environmental science and pollution research	Web of Science	*Alcaligenes faecalis* *LNDR-1*	Kolkata, India	[17]
8	Environ Sci Pollut Res	Scopus	*Pseudomonas putida*	Taipei China	[28]
9	Chemosphere	Scopus	*Bacillus sp.* *Paenibacillus s.*	Incheon, República de Korea	[29]
10	Heliyon	Scopus	*Pseudomona* *aeruginosa strain ISJ14*	Uttarakhand, India	[30]
11	Polymer Degradation and Stability	Web of Science	*Pseudomonas sp.* *Bacillus sp.*	Peshawar, Pakistan	[31]
12	Science of the Total Enviroment	ScienceDirect / Scopus	*Bacillus* *paralicheniformis G1*	Chennai, India	[32]
13	Science of the Total Enviroment	ScienceDirect / Scopus	*Pseudomona sp.* *YJB6*	Hong Kong, China	[32]
14	Journal of Environmental Management	Web of Science	*Enterobacter sp.* *Pseudomonas sp.*	Karnataka, India	[34]
15	Biotech	Web of Science	*Pseudomona* *AK31*	Kolkata, India	[16]

In relation to the Scopus database (Figure 2) it was observed that publications on microplastic contamination developed from 2018 and had a significant increase from 2019 to 2020. While, in the Web of Science database (Figure 3), the scientific production was increasing from the year 2011 to 2020, being the last two years with the highest amount of research.

It is evident that in the last years (2019 - 2020) there was a growing interest of the scientific community on microplastic pollution in aquatic environments due to the fact that ensuring the quality of water resources is of utmost importance to conserve aquatic species and for human health.

C. Bacterial growth and plastic mass reduction

The growth of the bacterial population is related to the degradation of plastics. Figure 2 indicates the relationship of bacterial growth, and Figure 3 mentions the reduction of plastic mass by bacterial action. Microorganisms require a favorable medium for obtaining compounds and energy to develop; therefore, polymers serve as a nutritional and energy source for bacteria. [18] performed the inoculation of 2E+11 CFU/mL of Rhodobacteriales strains, incorporating oxygen and methane gases in doses of 18 and 10%; obtaining values of 4.5E+11 and 2.7E+6 CFU/mL and a polymer reduction of 20%. On the other hand, [27] increased the bacterial population by 2.2E+6 CFU/mL and 2.4E+6 CFU/mL, using doses of mineral oil (d1=0.01 and d2=0.05%) obtained a polymer reduction of 26.7 and 25.5 mg respectively. These studies showed that colonial growth and plastic reduction are related, especially if an additive is added to improve the effectiveness of polymer mass reduction.

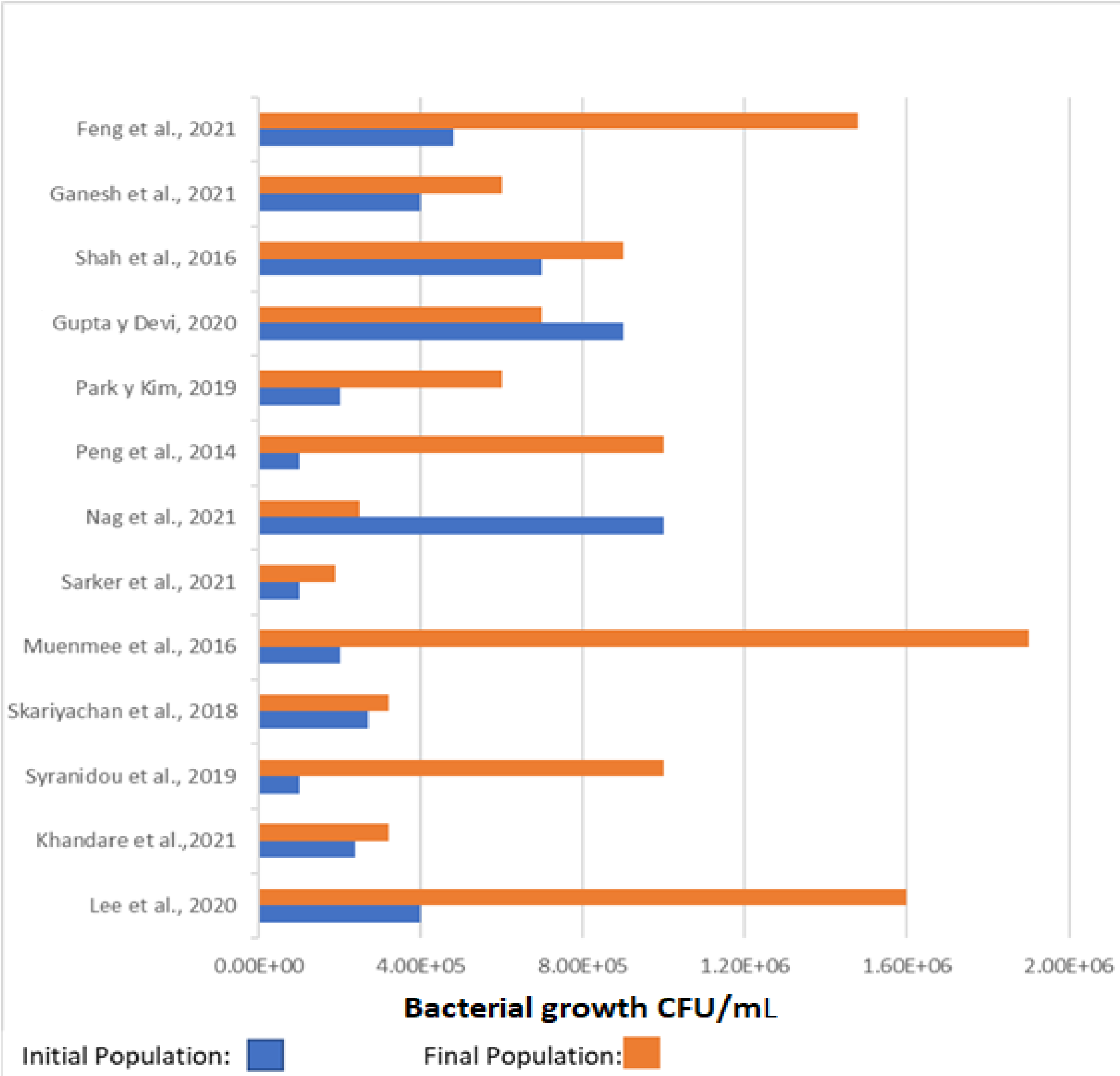

Fig. 2 Level of bacterial growth on plastic films or dust

D. Meta-analysis

For the meta-analysis of the use of bacteria in the mass reduction of different plastics, the Table 2 and the forest plot (Figure 4) show the 10 articles included. These were worked up considering the mass reduction aspect of the plastic by the action of bacterial degradation either by applying an additive or using a bacterial consortium. This is represented by the polygon at the bottom of the graph, and the edges represent the limit of the confidence interval. In addition, in the same graph (Figure 4), the Odds Ratio presented a value of 0.79, indicating that the reductions in plastic mass increased by 21% from trial 1 to trial 2. Another aspect to consider is that the investigations included obtained a statistical heterogeneity of P=0.01 and I2=58 %, indicating that the results of trials 1 and trial 2 present a moderate variability. Regarding the weight values (Weight), the studies of [16], [28] and [18] presented values of 19.9%, 17.5% and 15.1%, respectively, which indicated that these studies were able to degrade the polymers to a greater extent by using additives or bacterial consortia.

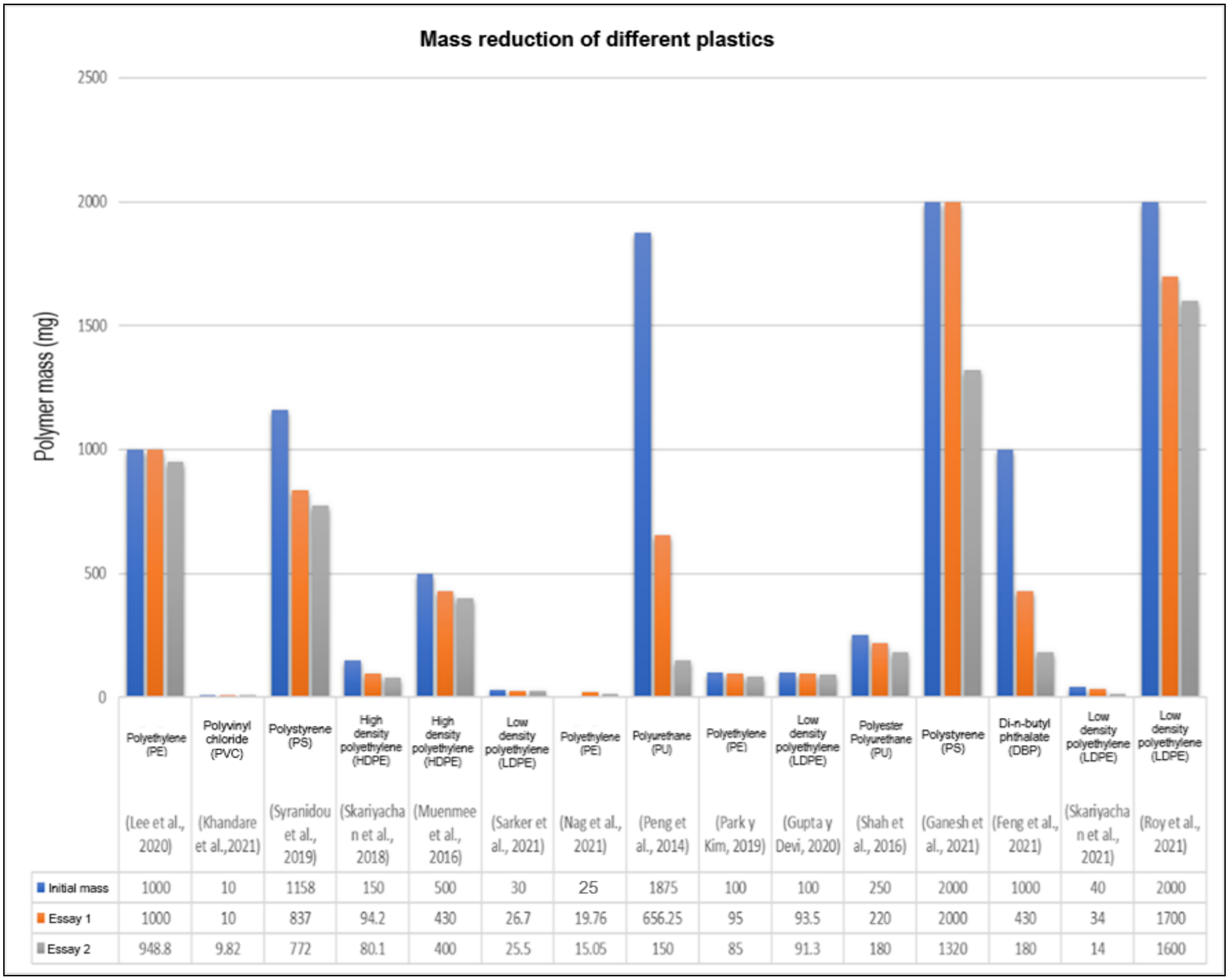

Fig. 3 Mass reduction of different types of plastic by bacterial action

TABLE II
USE OF DIFFERENT ADDITIVES OR BACTERIAL CONSORTIA TO IMPROVE THE BIODEGRADATION OF PLASTICS BY BACTERIA

Use of additives (mL)		**Polymer mass reduction (mg)**			**Application of additives or bacterial consortia**	**Authors**
Dose 1	**Dose 2**	**Initial Mass**	**Essay 1**	**Essay 2**		
1	2	150	94.2	80.1	E1: polymer portion E2: polymer strips	[26]
1	2	500	430	405	MOR CH4 E1: L1: 15;70 and 140 cm E2: L2:140 cm	[18]
1	5	30	26.7	25.5	Mineral oil	[27]
0.2	1.5	1875	712.5	656.3	pH control according to additives	[28]
2	5	100	95	85	E1: control	[29]

					E2: bac. inoculation	
6.5	8.7	100	93.5	91.3	E1: MSM E2: BHM	[30]
10	30	250	220	180	E1: 1 strain E2: bacterial consortium	[31]
1	5	250	220	180	E1: PVA+YJB6 E2: SA + PVA + YJB6	[33]
15	25	40	34	14	Cons. Bac. + Dung Bac/bacterial consortium	[34]
100	10	2000	00	1700	E1: laboratory E2: microcosm	[16]

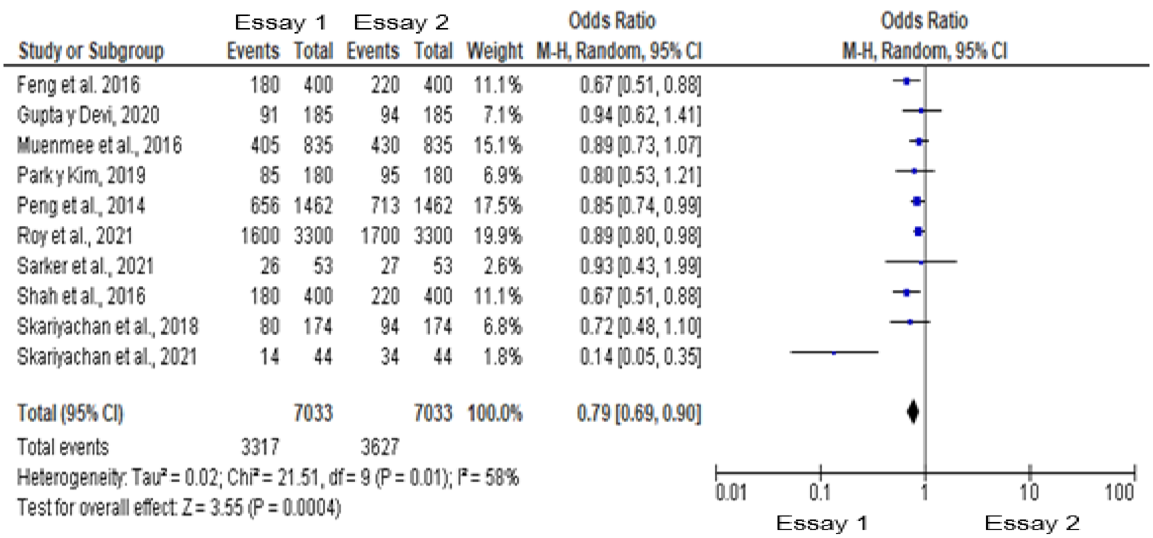

Fig. 4 Meta-analysis of mass reduction by bacterial activity using additive

IV. Conclusions

The systematic review and meta-analysis showed that the use of bacteria in the biodegradation of plastics is favorable, obtaining biodegradation percentages of 19.9%. Among the main results were:

1. Bacteria of the genus Pseudomona and Bacillus were recognized as the most used microorganisms in the biodegradation of plastics due to the fact that these strains are effective in the biodegradation of polymers.

2. It was determined that the operational conditions that most influence the biodegradation process of plastics is the application of additives to improve bacterial activity, obtaining maximum mass reduction percentages of 19.9%. In addition, most research indicated that the average temperature of bacterial activity is 25 to 50°C and they work at pH values of 7.5 to 8.

3. The relationship between bacterial growth and the reduction in mass of the films or powder of a polymer was determined, indicating that the higher the bacterial concentration, the greater the reduction in the mass of the plastic. As an example, inoculation of the bacterium Methylocella sp with an initial population of 4. E+07 CFU/mL in a 2000 mg polystyrene (PS) sample increased the bacterial population to 3E+8 CFU/mL and the mass of the PS was reduced by 1320 mg.

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