CHEMICAL ENGINEERING TRANSACTIONS

VOL. 99, 2023

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Sauro Pierucci, Flavio Manenti

ISBN 978-88-95608-98-3; **ISSN** 2283-9216

DOI: 10.3303/CET2399067

Evaluation of the Biodegradation Process of Low-density Polyethylene by *Aspergillus brasiliensis* in Soil

Lina F. López, Diana Morales-Fonseca*

Department of Chemical and Environmental Engineering. Fundación Universidad de América, Ak 1#20-53, Bogotá, Colombia.
diana.morales@profesores.uamerica.edu.co

Due to the impact that plastics generate in ecosystems, the management of this type of plastic waste has become a growing problem, so it is extremely important to focus efforts on research and knowledge generation around this topic. This research was focused on the evaluation of the biodegradation process of low-density polyethylene sheets using inoculum treatments of *Aspergillus brasiliensis*, in a compost-type soil matrix in order to determine the percentage of biodegradability and the state of the surface of the material by Scanning Electron Microscope (SEM) analysis. The percentages of LDPE biodegradation 3.60 % were evidenced in 10 weeks under conditions such as 6.3 pH, 55% humidity and 20 °C. The studies carried out with this strain are still limited within the scientific bibliography, therefore, this project is considered relevant in the characterization of the biodegradation processes of complex polymers.

1. Introduction

The advantage of plastics over other materials is due to the versatility offered, depending on the type of polymer, it is possible to heat, sterilize and manipulate this type of material maintaining its structural properties (Lear et al., 2021). Although polymers are currently essential in human life in recent years the management of plastic waste has become a growing problem, so it is of uttermost importance to focus efforts on research and the generation of knowledge around this topic.

Globally it is estimated that only 10% of plastics are recycled and 14% are incinerated; the remaining 76% go to landfills or enter the natural environment (Horton, 2021). Plastics impact ecosystems because this type of waste is easily incorporated into the food chain, causing huge problems for fauna (Srikanth et al., 2022). Contrary to what was previously believed, plastics are not inert, plasticizing chemicals commonly leach, forming chemical and biological associations (Lear et al., 2021). Another major problem related to this waste is its longevity in the environment given the resistance provided by the material.

Plastic production has increased widely exponentially since the 1950s, with an approximate production of 8.3 billion metric tons of plastic. This production is expected to continue to increase with an annual production rate of 1,100 tons by 2050 (Lear et al., 2021).

This environmental issue increases the necessity to be aware of the anthropogenic impact on our environment one example of this is how plastic waste is affecting aquatic ecosystems and even though nowadays there are alternatives for conventional plastics where the replacement would base the plastic industry on renewable resources and furthermore lead to a reduction of fossil CO_2 emissions (Koch and Mihalyi, 2018).

Low-density polyethylene LDPE is one of the best-known polymers for its large number of applications and its low production cost. Among its properties are tensile strength, rigidity, chemical resistance, and even flexibility at low temperatures (Kumar and Raut, 2015). Low-density polyethylene is widely used for the manufacture of single-use bags, globally between 500 billion and 1 trillion polyethylene bags are used (Srikanth, M et al., 2022). The degradation rate of low-density polyethylene in landfills is relatively slow, being degraded in 500 years (Grover et al., 2015). It is important to mention that the average degradation rates for LDPE are slightly higher in aquatic environments compared to degradation on land (Chamas et al., 2020).

Paper Received: 18 November 2022; Revised: 24 March 2023; Accepted: 2 May 2023
Please cite this article as: Lopez L.F., Morales-Fonseca D., 2023, Evaluation of the Biodegradation Process of Low-density Polyethylene by Aspergillus brasiliensis in Soil, Chemical Engineering Transactions, 99, 397-402 DOI:10.3303/CET2399067

Microplastics accumulate in the soil, changing the physical properties and communities of microorganisms (Rong et al., 2021). In fact, low-density polyethylene microplastics can stimulate soil enzymatic activity by changing the composition of bacterial communities in it. This represents threats to the biogeochemical cycles of the earth (Huang et al., 2019).
Biodegradation can be defined as the decomposition of materials mainly by microorganisms such as fungi or bacteria, acting on substrates such as leaves, grass, and even food waste (DSouza et al., 2021). That makes biodegradation a natural process. This mechanism involves the action of different enzymes on the surface of the material (Srikanth et al., 2022). It is also important to recognize that microbiomes frequently work in conjunction with abiotic factors such as temperature and light to impact the structural integrity of polymers and the accessibility to enzymatic attack (Lear et al., 2021).
Currently, microorganisms capable of degrading LDPE are limited to 19 genera of bacteria and 12 genera of fungi, where fungi stand out for their ability to survive in environments with low nutrient availability, low pH, and low humidity (Kumar and Raut, 2015). Fungi play a fundamental role in low-density polyethylene biodegradation due to their ability to secrete degrading enzymes such as lipases, proteases, and laccases. In fungi, the genus *Aspergillus sp.* is known for its relevance when it comes to degrading LDPE, there are investigations with approximately 15 strains of the genus for the evaluation of the biodegradation process of LDPE (Acuña, 2017). Low-density polyethylene biodegradation can occur in soil matrix such as composting and liquid matrix in submerged fermentation, in soil matrix the use of biological processes for the mineralization of polymers occurs. Therefore, the present investigation was focused on evaluating the biodegradation process of low-density polyethylene sheets in composting assemblies by *Aspergillus brasiliensis* strain.

2. Materials and methods

A single-factor design experiment was used, and each treatment was worked under duplicate. The treatments were named (1) LDPE + *A. brasiliensis*, (2) LDPE control (without inoculum), and (3) PLA polylactic acid + *A. brasiliensis.*

2.1 Microorganism

The *Aspergillus brasiliensis* strain was donated by the National University of Colombia, it was preserved in a 25 % glycerol solution in Sabouraud broth at 4 °C. The strain was activated on Sabouraud agar, depositing an agar disc with mycelium in the central part of the culture medium and incubated for 7 days at 30 °C.

2.2 Inoculum production

Biomass propagation in liquid medium was carried out in 100 mL Sabouraud broth previously sterilized at 121 °C and 15 psi. The *A. brasiliensis* strain was inoculated with 6 discs in broth and incubated for 8 days at 30 °C and 120 rpm.After 8 days, cell suspension in form of pellets was taken to a homogenizer. The concentration of biomass inoculated by each experimental unit corresponds to 0.0106 g/mL in dry weight.

2.3 Construction of composting assemblies

In plastic pots with a volume of 0.897 L, the assemblies were made by adding 180 g of compost, one sheet of low-density polyethylene with dimensions of 7 x 7.5 cm, inoculated with the microorganism followed by an additional 100 g of compost. Control treatments used sheets of polylactic acid with dimensions of 7 x 7.5 cm. These assemblies were subjected to weekly monitoring of temperature, pH, and humidity for 68 days.
For the weekly monitoring of temperature, a conventional thermometer was used, it was introduced into the pots and later the data recorded on the thermometer was read. For the weekly monitoring of the pH, a sample was taken from each pot, mixed in a 1:1 ratio with distilled water and the pH value was taken with a potentiometer. In order to monitor the humidity, a sample was also taken from each pot, approximately 1 g, it was placed in a muffle at 100 °C for 3 hours to eliminate the water content and then the sample was weighed again to determine the percentage of humidity.

2.4 Percentage weight loss (% biodegradability)

A widely used method to determine biodegradation is the weight loss of materials (Kumar and Raut, 2015). Therefore, the foils were weighed on an analytical balance before being subjected to the biodegradation process and after each treatment, carefully removing them from the mounts, cleaning with distilled water and being placed in an oven at 100 °C for 2 hours. Equation 1 was used to specify the biodegradability percentage of each assembly.

$$\%\ biodegradability = \frac{Initial\ weight - Final\ weight}{Initial\ weight} * 100 \qquad Eq\ 1$$

2.5 Scanning Electron Microscope Analysis

Prior to carrying out the SEM analysis, the samples were gold-plated due to they are nature non-conductive. The analysis was made at VEGA3 TESCAN microscope. Backscattered electron images were taken to observe the morphology of the polyethylene surfaces. The LDPE surface was captured at a magnification of 50x and 1000x.

3. Results

The following variables such as pH, humidity, and temperature were important to ensure microbial activity.

3.1 Variable monitoring

pH is a key variable in the activity of microorganisms and their survival, according to Esmaeili, et al., 2013 it is estimated that pH for biodegradation of fungi in soil matrix must be between 6.0 and 8.0 appropriate pH ranges for the action of the enzymes in the biodegradation process (Kumar and Raut, 2015). The pH values for *A. brasiliensis* treatments with their respective control and the LDPE control can be seen in Figure 1 where the record of the data obtained during 10 weeks is shown, as can be seen, the pH values varied over time, with a standard deviation of 0.2571 for LDPE + A. *brasiliensis* treatment, although the pH remained within the range necessary for the survival of the microorganism.

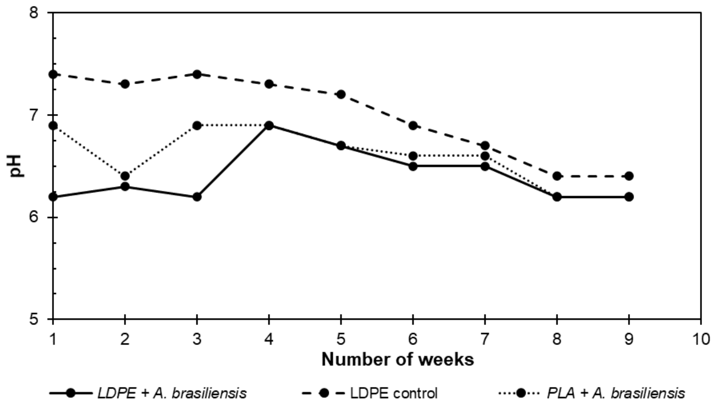

Figure 1: pH monitoring during 10 weeks

The activity of water is a key factor for the functioning of the cells, so the humidity in the matrix has a direct effect on the growth and the metabolic activities of the microorganisms, especially with the enzyme activity related to the biodegradation of this kind of polymers (Dsouza, et al., 2021). The moisture content in the compost should be between 40 – 60 % (Garcia, 2022). Therefore, the monitoring of the humidity variable was also necessary to ensure the microbial activity within the different treatments. Figure 2 shows the humidity record for A. *brasiliensis* treatments with their respective control and the LDPE control for 10 weeks.

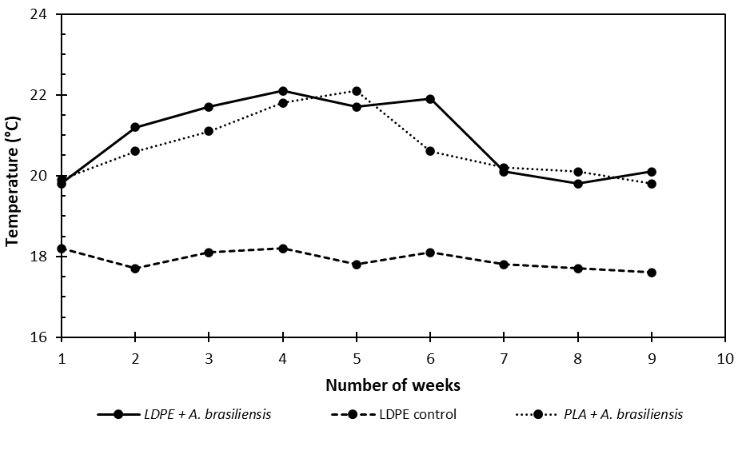

Figure 2: Humidity monitoring during 10 weeks

Same as in pH, temperature also affects the enzymatic activity and the colonization of the polymer. This variable should be found between the range of 20 - 30 °C (Gonzales, 2019). It is possible to conclude that the temperature for the treatments was found in the range of 19.8 to 22.6 °C, temperature was within the established range to favorable enzymatic activity.

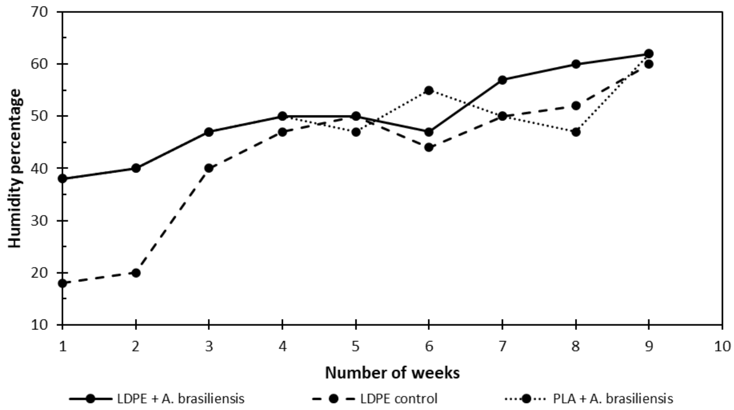

Figure 3: Temperature monitoring during 10 weeks

3.2 Biodegradability

In LDPE biodegradation, the change in one of the physical properties attributed is weight loss (DSouza, et al., 2021). This allows this percentage of weight loss to be interpreted as a percentage of biodegradation. As previously mentioned, filamentous fungi play a key role when it comes to biodegrading polymeric materials due to their ability to secrete enzymes that affect the structure of the material, such as proteases, talking in general terms of the *Aspergillus* genre (Srikanth et al., 2022). Initially, the polymer is broken down into smaller subunits which are subsequently enzymatically degraded into intermediate products that can be assimilated by microbial cells and used as carbon sources leading to the production of energy, water, and carbon dioxide (Ndahebwa, 2018).

In this investigation, a factor to consider is that in composting, microorganisms can be found naturally, which are in charge of naturally decomposing organic matter. Compost is formed when organic compounds are naturally degraded into nutrient-rich products (Ahmad et al., 2020). However, knowing in detail the micro-organisms that participate exactly in the composting process is complex since the communities can vary depending on variables such as temperature, nutrients, humidity, pH and nutrients present in the compost (Camacho, et al., 2014).

In the case of this research, the best results of the *A. brasiliensis* treatments can be seen in Table 1, with a percentage of biodegradation of 3.601% in a period of 68 days. Although the information about the *Aspergillus brasiliensis* strain degrading LDPE is not widely documented, as is the case with other species of the genus *Aspergillus*, in submerged liquid fermentation for the biodegradation of low-density polyethylene by *A. brasiliensis* it can be reached biodegradation percentages of 1.90% in periods of 30 days. (Garcia, 2022). While in humus-type soil matrix where microbial interactions by *A. brasiliensis* and humus-specific microbiota occur, LDPE biodegradation percentages can be obtained for sheets with and without pretreatment of 1.527 and 0.621 respectively, in periods of 3 months (Calcetero and Morales, 2021).

Table 1: Loss of weight due to biodegradation.

Treatment	Initial weight	Final weight	Percentage of Loss weight
LDPE + A. brasiliensis	0.1083	0.1044	3.6011 %
LDPE control	0.1077	0.1075	1.80 %
PLA + A. brasiliensis	0.3291	0.1724	47.62 %

The control treatment with sheets of polylactic acid (PLA) allowed evidencing the degrading activity (47% biodegradation) by *A.brasiliensis* under the experimental conditions implemented.

PLA is a compostable biopolymer and it is commonly used for packaging mulch films and compost bags but the use of microorganisms could influence this composting process due to the nature of its structure (Aziz et al., 2020).

In fact, these values obtained can be comparable to those reported by biodegradation of polylactic acid films in activated sludge bioenhanced with *A. brasiliensis* inoculums, where the great metabolic versatility of the microbial communities typical of activated sludge is used. and of the enzymes generated by the microorganism reporting biodegradation percentages of 20.41% (Mogollón H., et al., 2020).

3.3 Scanning Electron Microscope analysis

The LDPE samples were subjected to 50x and 1000x magnification in a Scanning Electron Microscope to observe the surface morphology after biodegradation. The resulting SEM image of control and biodegraded sample based on the highest percentage loss of weight are shown in figure 4. The results showed surface erosion, pitting in the form of cracks, holes, scions, and small cavities. Observation that is related and consistent with studies of low-density polyethylene degradation by *Aspergillus brasiliensis* (Garcia, 2022).

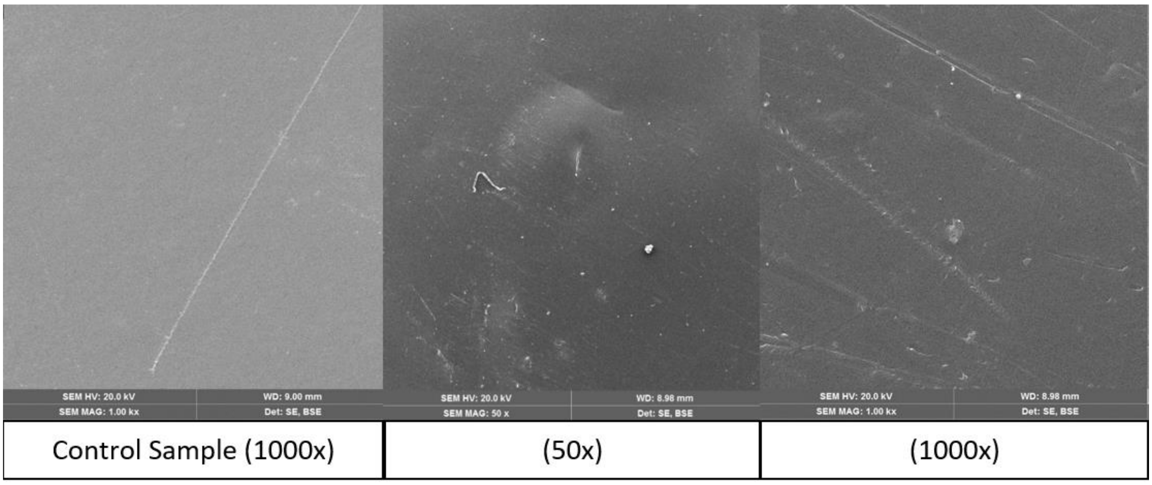

Figure 4: Surface morphologies of polyethylene samples within highest weight loss percentage and control sample

4. Conclusions

The biodegradation of the LDPE sheets by the *A. brasiliensis* strain in soil matrix was evidenced with a percentage of weight loss of 3.60%, observing the attack on the morphology of the sheets by means of SEM images, verifying the activity of the microorganism when degrading low-density polyethylene. Checking by monitoring variables such as pH, humidity and temperature that the parameters for microbial/enzyme activity are given in a composting-type matrix.

References

Acuña. N. (2017). Literature review on low-density polyethylene biodegrading microorganisms and their effects on the material (undergraduate thesis). Francisco José de Caldas District University, Bogotá, Colombia.

Ahmad Sanadi N.F.B., Lee C.T., Ibrahim N., Klemes J.J., Li C., Gao Y., 2020, Effect of Compost Maker on Composting and Compost Quality, Chemical Engineering Transactions, 78, 181-186.

Aziz Z., Keshavarz T., Kyazze G., 2020, Recycling and the Environment: a Comparative Review Between Mineral-based Plastics and Bioplastics, Chemical Engineering Transactions, 79, 355-360.

Calcetero. L. and Morales. D. (2021). Biodegradation of low-density polyethylene LDPE sheets pretreated by UV radiation, in earthworm humus and Aspergillus brasiliensis. In DEVELOPMENT AND INNOVATION IN ENGINEERING. Edition 6 (pp. 425–433). Bogotá, Colombia: Editorial Antioqueño Research Institute. (In Spanish)

Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J. H., Abu-Omar, M., Scott, S. L., & Suh, S. (2020). Degradation rates of plastics in the environment. ACS Sustainable Chemistry & Engineering, 8

DSouza, G. C., Sheriff, R. S., Ullanat, V., Shrikrishna, A., Joshi, A. V., Hiremath, L., & Entoori, K. (2021). Fungal biodegradation of low-density polyethylene using consortium of Aspergillus species under controlled conditions. Heliyon, 7.

Esmaeili A, Pourbabaee AA, Alikhani HA, Shabani F, Esmaeili E. (2013). Biodegradation of Low-Density Polyethylene (LDPE)by Mixed Culture of Lysinibacillus xylanilyticus and Aspergillus niger in Soil. PloS ONE 8(9) : e71720. doi:10.1371/journal.pone.0071720.

Garcia. D. (2022). Development of a low-density polyethylene (LDPE) biodegradation model by Aspergillus braziliensis in a bioreactor (undergraduate thesis). University of America Foundation, Bogota

Gonzales. V. (2019). Biodegradative capacity of filamentous fungi against polyethylene (undergraduate thesis). Graduate School, National University of the Altiplano, Puno, Peru.

Grover. A., Gupta. A., Chandra. S., Kumari. A., Khurana. P. (2015). Polythene and environment. INTERNATIONAL JOURNAL OF ENVIRONMENTAL SCIENCES, 5.

Horton, A. A. (2022). Plastic pollution: When do we know enough? Journal of Haz-ardous Materials, 422.

Huang, Y., Zhao, Y., Wang, J., Zhang, M., Jia, W., & Qin, X. (2019). LDPE micro-plastic films alter microbial community composition and enzymatic activities in soil. Environmental Pollution (Barking, Essex: 1987)

Kumar Sen, S., & Raut, S. (2015). Microbial degradation of low-density polyethylene (LDPE): A review. Journal of Environmental Chemical Engineering, 3, 462–473.

Koch D., Mihalyi B., 2018, Assessing the Change in Environmental Impact Categories when Replacing Conventional Plastic with Bioplastic in Chosen Application Fields, Chemical Engineering Transactions, 70, 853-858.

Lear, G., Kingsbury, J.M., Franchini, S. (2021) Plastics and the microbiome: impacts and solutions. Environmental Microbiome ,16.

Mogollon, H., Sarmiento. L., Algecira. N. and Morales. D. (2020). Use of microbial inoculums for the biodegradation of polylactic acid films. In Formative Research in Engineering (pp. 616–623). Bogotá, Colombia: Antioqueño Research Institute Editorial. (In Spanish)

Ndahebwa Muhonja, C., Magoma, G., Imbuga, M., & Makonde, H. M. (2018). Molecular characterization of low-density polyethene (LDPE) degrading bacteria and fungi from Dandora dumpsite, Nairobi, Kenya. International Journal of Microbiology, 2018

Rong, L., Zhao, L., Zhao, L., Cheng, Z., Yao, Y., Yuan, C., Wang, L., & Sun, H. (2021). LDPE microplastics affect soil microbial communities and nitrogen cycling. The Science of the Total Environment, 773

Srikanth, M., Sandeep, T. S. R. S., Sucharitha, K., & Godi, S. (2022). Biodegradation of plastic polymers by fungi: a brief review. Bioresources and Bioprocessing, 9.