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Preliminary study on degradation efficiency of expanded polystyrene by Pseudomona Flourescens

Carolina Hernández Contreras[1], Carlos Alberto Amaya Corredor[2,*] Natalia Alexandra Bohórquez Toledo[3], and Carlos Alberto Rodríguez Pérez[4]

[1,2,3,4]Environmental Engineering Programa, Faculty of Engineering, Unidades Tecnológicas de Santander, Colombia

Abstract

The following work is a preliminary investigation that aimed to calculate the efficiency of Pseudomonas fluorescens in expanded polystyrene degradation, developing a biological treatment system; in order to reduce the time degradation and the environmental impacts by this residue. P. fluorescens bacteria initially the two means of liquid culture, meat broth (nutrient) and distilled water was inoculated; where three types of assemblies, aerobic, anaerobic and anaerobic to above ambient temperature worked. The selected material were 6 grams of a sheet and a plate of expanded polystyrene for food. As a result it was found that 67% of the samples the biodegradation process developed by the action of the bacterium P. fluorescens, the process being effective in assemblies whose temperature was maintained at an average of 28 ° C, developing a means of alkaline pH neutrally.

Keywords: Biodegradation, Polystyrene, Pseudomonas fluorescens

Introduction

In Colombia and the rest of the world, the segregation of waste, commonly referred to as garbage, has generated great concern due to the deficient planning, organization and execution in the management, characterization, treatment and final disposal of waste and, subsequently, due to the negative impact it is having on the environment (fauna, flora, resources, ecosystems, etc.). (MinAmbiente, 2016).

The problem of solid waste management is associated with the growing generation of waste caused by the absence of responsibilities of the productive sector in the generation, management and disposal of post-consumer waste, the loss of the potential for utilization and use of waste, the social responsibility of the population, the lack of adherence and compliance with established environmental regulations in the different sectors (industrial, commercial, residential, institutional, special, etc.), the lack of markets for the commercialization of these materials, since there is no culture or motivation to carry out activities for the use of solid waste (Bucaramanga, 2015).

One of the most consumed products in the world are plastics, being of outstanding production and consumption the expanded polystyrene, commonly known in Colombia as "icopor".

EVAL (Regional Evaluation of Municipal Solid Waste Management in Latin America and the Caribbean) 2010 (Martínez, Daza, Tello, Soulier, & Terraza, 2010) estimated that, the per capita generation of MSW (household solid waste) in Latin America and the Caribbean reaches 0.63 kg/inhab/day, while that of MSW (urban solid waste) amounts to 0.93 kg/inhab/day. The per capita indicators obtained for the region imply a daily urban generation of approximately 295,000 tons of MSW and 436,000 tons of MSW (PAHO, 2010), 20% of which is plastic waste..

In the environmental and sustainable development aspects, the United Nations Conference Paris 2015, which established the 2030 agenda and the Sustainable Development Goals SDG (ONU, 2015) recommends minimizing the generation of solid waste, recycling and reusing it as much as possible, treating and disposing of it properly, and increasing collection coverage and other elements of the service.

The national government of Colombia has been working on the regulation of various guidelines (laws, decrees, resolutions, etc.) for the different productive sectors of the national territory, from the purchase of raw materials for the production of a product to the waste generated throughout the production and consumption chain, in order to preserve and conserve the environment. (López Bolaños, 2020). This has been developed in conjunction with the Ministry of Environment and Sustainable Development and each of the support entities in terms of environmental control of each department and municipality, evidencing the initiative of the standardization of waste management since 2000, with Decree 2676/2000, which is currently abolished by Decree 351 of 2014. (MinAmbiente, 2016)

Despite the large repertoire of environmental regulations covering the Colombian territory, there are still evident problems in terms of adequate treatment and final disposal systems for industrial, commercial and residential waste (Sáez & Urdaneta, 2014); this has led to the need to find a method to degrade waste, without affecting the environment and allowing the sustainable development of a territory. Especially for waste such as expanded polystyrene, whose production and consumption is generated in large quantities and that currently there is no treatment in Colombia for its elimination without affecting the environment. (ARANDES, 2004)

Microbial biotechnology is an option that is increasingly impacting the field of cleaner production (Lukashe, Mupambwa, Green, & Mnkeni, 2019). Therefore, in the exploration of an alternative to traditional methods, it has been found that some microorganisms such as Pseudomonas are able to process, integrate and react to a wide variety of changing conditions in the environment and show a high capacity to react to physico-chemical and biological signals (ARUTCHELVI J., Biodegradation of polyethylene and polypropylene, 2007) (BAIRRD, 2001) (BELTRAN, 2012). Strains have been described that are able to acquire resistance to heavy metals, solvents and detergents, which allows them to exploit a range of carbon sources as nutrients, as well as to colonize environments and niches that are difficult for other microorganisms to colonize. (Martin Amor, 2011) (ALANIS GARCIA, 2004) (BENAVIDES LÓPEZ DE MESA, 2006).

This project is a preliminary study conducted by students of the Technological Units of Santander, Colombia, in which they want to determine if the bacterium Pseudomona fluorescens could be an effective biological treatment in the degradation of expanded polystyrene, with the purpose of reducing the degradation time and the environmental impacts generated by the waste.

Materials and Methods

The field work was carried out in two stages. In the first phase, the purchase of the Pseudomona fluorescens bacteria strain was made through the Punto Clínico laboratory in the city of Medellín. After the purchase of the strain, the P. fluorescens bacteria were sowed. (Lukashe, Mupambwa, Green, & Mnkeni, 2019) in 6 samples for the development of the procedure, managing the sowing in Agar-Blood and Agar-MacConkey in BHI nutrient broth medium. (Moya-Salazar, Pio-Dávila, Terán-Vásquez, & Olivo-López, 2016). They were organized by number, letter, number #X# indicating: number: treatment; letter: sample; number: process.

The second phase was developed in the laboratory facilities of the Santander Technological Units, where the different quantitative and qualitative analyses required for the development of the P. fluorescens bacteria in the degradation of two types of expanded polystyrene products were carried out. (Arthuz-López & Pérez-Mora, 2019). Table 1 shows the treatments applied to each sample and the process followed.

Table 1. Treatments used in the experiment

Aerobic Treatment At Ambient Temperature	Anaerobic Treatment At Ambient Temperature	Hermetic Box At Increased Temperature	Description
1A[1]	2C[1]	3E[1]	P fluorescens + Liquid Culture Medium with kitchen seasoning + Expanded Polystyrene Sheeting
1A[2]	2C[2]	3E[2]	P fluorescens + Liquid Culture Medium with cooking spice + Expanded Polystyrene Meal Dish
1B[1]	2D[1]	3F[1]	P fluorescens + Liquid Culture Medium + Expanded Polystyrene Foil
1B[2]	2D[2]	3F[2]	P fluorescens + Liquid Culture Medium + Expanded Polystyrene Food Dish

The area destined to make the culture medium was cleaned with soap and water and disinfected with sodium hypochlorite at 5000ppm. The 4 lids of the glass flasks required for the aerobic assembly were drilled, taking into account that the hole in the lid should fit the diameter of the hose with the help of an aquarium oxygen pump.

Dehydration of the degraded material, to determine weight loss, the samples were weighed with the liquid culture medium on the balance, the hoses, tapes, adhesives and/or labels were removed from each sample, and the samples were put into the oven by assembly; first the assembly 1 (Aerobic at ambient T°), then assembly 2 (Anaerobic at ambient T°) and finally assembly 3 (Anaerobic at increased T°), the oven was programmed at +/- 100°C to 110°C for a period of 24 hours, and repeated for each assembly, Once the cycle was finished, the oven door was opened and the samples were allowed to cool, the dehydrated samples were weighed and finally the final weight of the degraded material was calculated (weight of the dehydrated sample - weight of the glass bottle). The study of the differences was carried out by means of a one-factor analysis of variance.

Results and Discussion

Two types of materials of different density and shape of expanded polystyrene were selected, a sheet and a plate for food. The amount of expanded polystyrene sample to be degraded was established, taking 6 g as a pilot sample. The types of assemblies to be carried out were determined, taking into account the absence and presence of oxygen and the temperature variable. A dehydration test was performed at the end of the mounting time, to determine the amount of weight loss of each sample and the percentage of efficiency of P. fluorescens as a degrading agent of expanded polystyrene.

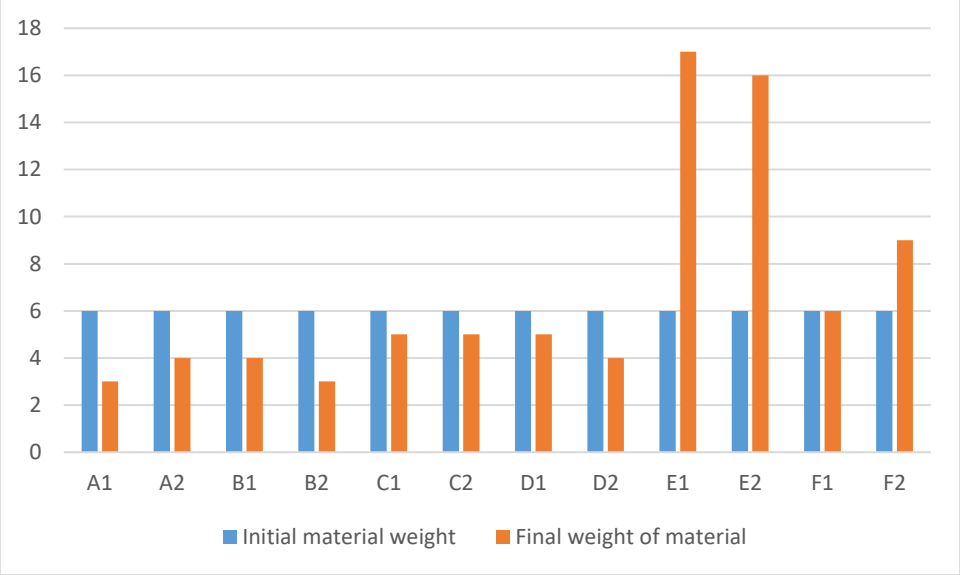
Two types of mounting were performed, one at room temperature (25°C to 30°C) and the other at a higher temperature than the environment (40°C to 55°C), considering one of the characteristics of this bacterium in adapting to different temperatures and environments. Each set-up was carried out in aerobic and anaerobic medium. It was verified by taking turbidity, if the bacteria are developing in each of the samples. By means of the dehydration practice, the weight loss of expanded polystyrene of each of the samples was determined, to analyze at what temperature the P. fluorescens bacteria obtained more development and the biodegradation process was more effective. (Lukashe, Mupambwa, Green, & Mnkeni, 2019)

A qualitative analysis was performed (Hernandez, Fernández, & Baptista, 2014), each sample was weighed and three types of data were recorded: the weight of the samples with the remaining liquid culture medium, the weight of the samples without the liquid culture medium and the weight of the samples with the dehydrated material. The dehydration practiced to the expanded polystyrene once the liquid culture medium was removed, was by gravity drying in a closed glass box with the glass jars open and a heat

medium (bulb) was conditioned to accelerate the drying, for a period of 7 days under a recorded temperature of 51°C. (Martin Amor, 2011)

After 7 days, the analysis of weight loss of the expanded polystyrene to be degraded (6 g) was carried out with the weight of the dehydrated material, where it was analyzed that all the samples of aerobic set-up A and B and anaerobic set-up C and D handled at room temperature (25°C to 30°C) presented degradation of the material, registering weights lower than 6 g; on the other hand, the samples of anaerobic set-up E and F that were handled at a temperature higher than the environment did not present any record of degradation of the material. That is to say, of the 100% of the samples worked, only 67% of the samples (8) presented the process of biological degradation by P. Fluorescens and the remaining 33.3% did not develop the degradation process. (Lukashe, Mupambwa, Green, & Mnkeni, 2019) (Martin Amor, 2011).

Figure 1. Differences between the final weight and the initial weight of polystyrene.



Source: Authors

Is important take into account that the technique used for the dehydration of the expanded polystyrene was handmade and therefore it can be reflected in the values of the final weight of the degraded material; using a sophisticated dehydration technique, weights lower than those recorded could have been obtained. For this reason it can be seen evidenced in samples E1, E2 and F2, which registered values higher than 6 grams (initial dry weight of the sample to be degraded) because the samples still have moisture content. (BENAVIDES LÓPEZ DE MESA, 2006)

The technique used for dehydration of the degraded material was chosen due to the availability of equipment (autoclave or oven) and personnel for its execution (laboratorian). Additionally, an analysis of variance was performed to observe the significant differences between treatments, resulting in a probability of P = 7.1031E-10 confirming that there are no significant differences between treatments, see Table 2.

Analysis of Variance

Origin of Variations	Sum of squares	Degrees of freedom	Mean squares	F	Probability	Critical value F
Between Groups	375,1666667	5	75,033333	122,7818182	7,10317x10-10	3,105875239
Within groups	7,33333	12	0,611111			

Total	382,5	17

Discussion

After 8 weeks of mounting, it was observed that the P. fluorescens bacteria in aerobic and anaerobic medium carried out the degradation process of the two types of expanded polystyrene worked (sheet and food plate) under stable pH and temperature conditions, calculating its efficiency as a degrading agent at 67%, which corresponds to the 8 samples worked at room temperature (Mounting A, B, C and D). It should be noted that the expected efficiency of the bacteria could have been higher if the temperature conditions had been more stable for assemblies E and F (assembly with T° > 45°C).

Initially, the P. fluorescens had as a nutrient source the meat broth (Ricostilla) that was added to each of the mounts as a primary food source for the development and growth of the bacteria. After the second week, it was observed that the plastic hoses of the aerobic mounts had a variety of perforations which were located in the part of the hose that was submerged in the culture medium. In the third week the plastic part of the hose was already degraded by the bacteria and an accumulation of very fine particles began to be seen at the bottom of the flasks of assemblies A, B, C and D, and some were still in suspension. As the weeks went by, more particles were evidenced at the bottom of the flasks and at week 8 (last week), by measuring the weight of each flask by the dehydration technique, it was evident that the assemblies A, B, C and D reduced the weight of the polystyrene; establishing that the level of acceptance of the expanded polystyrene as food for P. fluorescens had been positive. (BENAVIDES LÓPEZ DE MESA, 2006) (Martin Amor, 2011)

Finally, two types of expanded polystyrene materials with different characteristics were chosen in order to analyze the degradation process of a porous and compact material. For this purpose, the laminate (porous material) and the food plate (compact material) were chosen; during the course of the project it was observed that the green pigmentation characteristic of P. fluorescens appeared first in the porous material (laminate) and then in the compact material (food plate), this being a warning sign of the presence of the bacteria in the sample, taking into account that color pigmentation is not always visible. As the weeks went by, it was observed that the compact material (dinner plate) presented more sedimented and suspended particles in the bottles than the bottles of the porous material (lamella). Even taking into account the density and type of materials handled, the biodegradation process was present in both, arriving at this discussion based on the difference in weights obtained from the polystyrene at the end of the assembly.

The assemblies worked on were divided into two; one assembly was handled at room temperature (25°C to 30°C) and the other was handled at increased temperature (> to 45°C), in order to determine if the biodegradation process of expanded polystyrene can also occur at high temperatures, taking into account that the P. fluorescens bacteria has the eurythermic capacity that allows it to adapt to different environments. As a result, it was obtained that the biodegradation process only occurred in the assembly handled at room temperature, establishing that the optimum temperature in the research project for the degradation of expanded polystyrene by means of the P. fluorescens bacteria is from 25°C to 30°C, corresponding to 100% of the temperature range presented by the samples that developed the biodegradation process. (Lukashe, Mupambwa, Green, & Mnkeni, 2019) (BENAVIDES LÓPEZ DE MESA, 2006)

Conclusion

When evaluating the biological degradation method by the action of the bacterium P. fluorescens, it can be concluded that this bacterium is an efficient biological agent for the degradation of expanded polystyrene

both in aerobic and anaerobic conditions; as observed in the assemblies A, B, C and D; managing stable physical-chemical conditions such as pH and temperature.

The characteristic as eurythermic organism allowed P. fluorescens to adapt in such a way that restricting any type of carbon reception and having no other source of food and energy, it was forced to take expanded polystyrene as its direct nutritional medium in the case of the anaerobic mounts. In the aerobic setups, it was evident that the bacteria first attacked the plastic hose as a food source and then the expanded polystyrene; this was due to the fact that the chemical structure of polyethylene is easier to degrade because of its linear chain than that of polystyrene, which has benzene branches; therefore, expanded polystyrene is identified as a source of nutrition and energy accepted by P. fluorescens, with results of degradation in setups A, B, C and D.

The density of a material is a property to be taken into account in the degradation process, since the less dense the material is, the faster its degradation will be. In the case of the icopor sheet (expanded polystyrene), being less dense than the icopor plate (compact expanded polystyrene), it was the first to show changes both in acquiring green pigmentation and in having the greatest weight loss; but even so, in the final results of weight loss of the material, it was the icopor plate that showed the greatest weight loss (sample D2). Therefore, it is not possible to point out which of the materials had more influence on the P. fluorescens bacteria in the biodegradation process.

The best conditions for biodegradation of expanded polystyrene by P. fluorescens were at a pH range of 6.4 to 8.6 (neutral-basic) and a temperature (psychrotrophic) of 27°C to 30°C, which corresponded to 100% of the samples that presented the biodegradation process.

The biodegradation process was presented in the aerobic A and B and anaerobic C and D assemblies, handled at room temperature with a range of 25°C to 30°C (psychrotrophic bacteria), concluding that only 67% of the expanded polystyrene samples achieved the biodegradation process.

The light green pigmentation observed in samples A2, B2, C2 and D2, was generated by the fluorescein fluorescent pigment characteristic of P. fluorescens; which is activated or reacts in alkaline media or solutions. Where, the pH history recorded by samples A2, B2, C2 and D2 was higher than 6, taking into account that these samples were handled in distilled water whose pH is alkaline.

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