ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 17, Issue, 11, pp.35365-35368, November, 2025
DOI: https://doi.org/10.24941/ijcr.49813.11.2025

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

THE BIODEGRADATION OF POLYSTYRENE BY AN ACETIC ACID BACTERIUM

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ARTICLE INFO

Article History:
Received 27th August, 2025
Received in revised form
18th September, 2025
Accepted 24th October, 2025
Published online 30th November, 2025

Keywords:
Biodegradation, Polystyrene, Bacteria, Acetic-acid, *Gluconacetobacter diazotrophicus.*

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ABSTRACT

The biodegradation of materials that pollute the environment is a topic of research worldwide. Various methods for degrading commonly used polymeric materials such as polystyrene have been documented; however, these methods are often costly or have an unfavorable environmental impact. Polystyrene and its derivatives are used in the manufacture of utensils and food and beverage packaging. Studies have been reported showing the toxic properties of these substances. Biodegradation using microorganisms such as bacteria and fungi has yielded significant results. This paper shows some data related to the ability of the nitrogen-fixing bacterium *G. diazotrophicus* to biodegrade polystyrene.

Citation: Flores-Encarnación, M., Jiménez-Flores Y.A., Cabrera-Maldonado C. and Xicohtencatl-Cortes J. 2025. *"*The biodegradation of polystyrene by an Acetic acid bacterium .*"*. ***International Journal of Current Research, 17, (11), 35365-35368.***

INTRODUCTION

As is well known, plastic pollution is a global problem affecting soil and water worldwide. These materials take a very long time to decompose. Pollution refers to the introduction of contaminants into the natural environment that cause adverse changes (Ho*et al*., 2018). These contaminants can be in various forms such as chemicals, particles, or energy, and they can originate from natural sources or human activities. Pollution can affect air, water, and soil, as well as ecosystems, wildlife, and human health (Chanda *et al.*, 2024; Obebe *et al*., 2020). Plastic pollution has emerged as one of the most pressing environmental challenges of the 21st century. With the widespread use of plastics in various industries and consumer products, the accumulation of plastic waste in the environment has reached alarming levels. From oceans and waterways to terrestrial ecosystems, plastic pollution poses significant risks to biodiversity, ecosystem integrity, and human well-being (Bank and Hansson, 2019; Chanda *et al.*, 2024). One of those pollutants in the environment is polystyrene, widely used for the manufacture of food or liquid beverage containers (thermoplastic polymer). Polystyrene is an aromatic polymer formed as a result of polymerization of styrene (vinylbenzene) monomers (Kik *et al*., 2020) (Fig. 1). Polystyrene is a durable thermoplastic that is generally believed to be nonbiodegradable. Biodegradation of polystyrene does occur but at a very slow rate in natural environments and therefore polystyrene persists for long periods of time as solid waste.

It has been reported that a sheet of polystyrene buried in soil for 32 years had no sign of degradation (Ho*et al*., 2018; Otake *et al.*, 1995). Therefore, strategies are being sought to facilitate the biodegradation of polluting plastics such as polystyrene. This work shows some data regarding the biodegradation of polystyrene using a novel acetic acid bacterium.

MATERIAL AND METHODS

Source of material: In this study, the polystyrene was obtained from fragments of a commercial thermal container made of that material. It was obtained from a grocery company in Puebla, México.

Biological material: The strain of *Gluconacetobacter diazotrophicus* PAL5 was used. Bacterial strain was stored in cryovials at -40°C until analysis.

Culture: *G. diazotrophicus* strain was grown in modified LG liquid medium containing (g/L): K_2HPO_4, 0.2 g; KH_2PO_4, 0.6 g; $MgSO_4.7H_2O$, 0.2 g; $CaCl_2.2H_2O$, 0.02 g; $Na_2MoO_4.2H_2O$, 0.002 g; $FeCl_3.6H_2O$, 0.01 g, $(NH_4)_2SO_4$ 0.1321 g; sucrose, 10 g. The pH was ajusted to 6.8 with hydrochloric acid (Reis *et al.*, 1994). Culture was grown at 30°C in a 250 mL Erlenmeyer flask containing 50 mL of LG liquid medium stirred at 150 r.p.m. for 48 - 72 hours. Growth was determined by measuring the absorbance at 560 nm.

Polystyrene biodegradation test: *G. diazotrophicus* strain was grown in LG liquid medium lacking sucrose, containing (g/L): K_2HPO_4, 0.2 g; KH_2PO_4, 0.6 g; $MgSO_4.7H_2O$, 0.2 g; $CaCl_2.2H_2O$, 0.02 g; $Na_2MoO_4.2H_2O$, 0.002 g; $FeCl_3.6H_2O$, 0.01 g, $(NH_4)_2SO_4$ 0.1321 g. The pH was ajusted to 6.0 with hydrochloric acid. For that, 3 mL of an active culture of *G. diazotrophicus* (72 hrs of culture, Ab_{560nm}=1.12) were used to inoculate each 250 mL Erlenmeyer flask containing 100 mL of LG liquid medium. Before inoculating the bacteria, the LG medium was supplemented with 1 g of polystyrene obtained from fragments of a commercial thermal container. This material was used as a carbon source and was autoclaved before use. Culture was grown at 30°C under steady-state conditions for 8 weeks. Each day the cultures were manually agitated for 1 min. As a control, LG medium without sucrose or polystyrene was inoculated with *G. diazotrophicus*.

Biofilm detection: To demonstrate the formation of biofilm by *G. diazotrophicus* on fragments of polystyrene commercial thermal container, the calcofluor white staining was used, according to the modified methodology described by Flores-Encarnación *et al.*, (2016). For this, some polystyrene fragments were extracted from the Erlenmeyer flask where *G. diazotrophicus* was cultured (in the presence of polystyrene fragments) for at least 14 days. The polystyrene fragments were placed with the help of metal forceps into a sterile 1.5 mL centrifuge tube containing 1 mL of calcofluor white 0.02% dye and it was incubated for 1 hour at room temperature in the dark. Then, the polystyrene fragments were placed in a glass Petri dish and were exposed to UV light. Fluorescence emitted showed the exopolysaccharides produced by the bacteria attached to the polystyrene fragments confirming the formation of biofilm.

RESULTS

In this study, *G. diazotrophicus* (an acetic acid bacterium) was used to promote the biodegradation of polystyrene. Fragments of commercial polystyrene were used. *G. diazotrophicus* was grown in modified LG as described in Materials and Methods. In order to induce in *G. diazotrophicus* use of polystyrene as a carbon source, sucrose was eliminated in the preparation of the LG culture medium. *G. diazotrophicus* was grown at 30°C under steady-state conditions for 8 weeks.

As can be seen in Fig. 1B, the LG medium is transparent. Inoculation of the culture medium with *G. diazotrophicus* and the addition of polystyrene fragments does not change the color of the culture medium (Fig. 1C). As can be seen in Fig. 1D, *G. diazotrophicus* was able to grow in the LG culture medium lacking the carbon source that is frequently used for the preparation of this culture medium (sucrose). Instead, 1 gram of polystyrene was added to 100 mL of LG culture medium. After two weeks of steady-state culture, degradation of the polystyrene was observed. This was indicated by the deposition of small white clumps at the bottom of the Erlenmeyer flask (Fig. 1D).

After 2 months of incubation, deposits of white clumps accumulated at the bottom of the Erlenmeyer flask, while others remained in suspension, as shown in Fig. 1E. On the other hand, to demonstrate the contact of the bacteria with the polystyrene, the formation of biofilm by *G. diazotrophicus* was determinated using calcofluor white staining. The results are shown in Fig. 1F. As can be seen in Fig. 1F, the polystyrene fragment emitted fluorescence on its surface when exposed to UV light, which denoted the presence of exopolysaccharide-producing bacteria. *G. diazotrophicus* grew attached to the surface of polystyrene and also in suspension in the LG culture medium. The growth of *G. diazotrophicus* was dependent on the polystyrene used as a carbon source in the LG culture medium. As a control, only polystyrene was dyed with calcofluor white. As seen in Fig.1G, the polystyrene did not emit the white fluorescence observed in Fig. 1F. The Petri dish used for the calcofluor white assay was also irradiated with UV light. The Petri dish did not exhibit autofluorescence (Fig. 1H).

DISCUSSION

The world has witnessed significant changes through past centuries due to incredible inventions such as plastics (Pilapitiya and Ratnayake, 2024). Plastic consumption has increased about 180 times from 1950 to 2018. The worldwide plastic production is calculated to be 400.3 million tons in 2022. Plastic production is thus expected to increase exponentially hereafter (Pilapitiya and Ratnayake, 2024; Rafey and Siddiqui, 2021). Plastics often break down into smaller fragments through degradation or fragmentation, weakening the bonds and strength of particles and causing them to become brittle. Ultimately, they break down into a powdered form as microparticles, referred to as secondary microplastics. Secondary microplastics include polyethylene, polypropylene, and polystyrene particles (Hwang *et al*., 2020; Schymanski *et al*., 2018; Siddiqui *et al*., 2023; Stolte *et al*., 2015). Polystyrene microplastic pollution is a major environmental issue due to its extensive use, persistence, toxicity and resistance to degradation (P *et al*., 2025). Direct contact between humans and microplastics is a potentially critical concern. This contact can occur through various sources, such as food packaging materials, containers, personal care products, biomedical products, and disposable water bottles (Siddiqui *et al*., 2023).

Widespread use of polystyrene microplastics in the food chain has raised global concerns regarding marine and human health. Polystyrene is extensively used at the industrial level because of its low cost and chemical stability mainly in scrubs, handwashing soaps, cleansers, toothpaste, or biomedical products (Dauvergne, 2018; Fendall and Sewell, 2009; Gregory, 1996; Napper *et al*., 2015; Sharma and Chatterjee, 2017; Siddiqui *et al.*, 2023; Weber *et al*., 2020). Prolonged exposure to polystyrene microplastics has adverse effects on male reproductive health. The human body is exposed to plastic, including plastic bottles, liquid carry bags, and plastic food containers, leading to the ingestion of microplastics. For example, the increased utilisation of such straws necessitates an evaluation of their impact on the male testicular system, as microplastics have been found to inf luence sperm production and erectile processes. It has been reported that microplastics have a significant impact on male reproductive functions, affecting both sperm production and erectile processes. This results were obtained using microplastic-polluted water on mice and focusing on testosterone levels and associated abnormalities (Dilip, 2024). As the usage of everyday plastic items continues to rise, the absorption and accumulation of microplastics pose a serious threat to human and animal health. However, it remains uncertain whether direct microplastic contamination from plastic packaging poses a risk to human health (Deng *et al*., 2022; Dilip, 2024). Therefore, different strategies are being investigated to allow the removal or degradation of polystyrene in the environment.

In the present work, some data were obtained regarding the ability of a novel acetic-acid bacterium (*G. diazotrophicus*) to degrade polystyrene. *G. diazotrophicus* is a Gram-negative, acid tolerant, obligate aerobe and the cells are rod shaped with rounded ends (0.7-0.9 μm by 1-2 μm) having lateral or peritrichous flagella (Cavalcante and Döbereiner, 1988; Chawla *et al.*, 2014; Gillis *et al.*, 1989; Muthukumarasamy *et al*., 2002). *G. diazotrophicus* belongs to the selected group of bacterial species endowed with the capacity for nitrogen fixation. It has been reported that *G. diazotrophicus* in culture performed nitrogen fixation under aerobic conditions and that this peculiar life-style required an efficient mechanism for protection of nitrogenase activity from deleterious oxygen (Flores-Encarnación *et al.*, 1999; Kennedy and Tchan, 1992; Kim and Rees, 1994; Stephan *et al.*, 1991). In this work, fragments of commercial polystyrene were used as a carbon source and *G. diazotrophicus* was grown at 30°C under steady-state conditions for 8 weeks in modified LG. As mentioned earlier, sucrose was eliminated in the preparation of LG culture medium. Instead, 1 gram of polystyrene was added to LG culture medium. The results indicated that *G. diazotrophicus* was able to grow in the LG medium (lacking the carbon source that is frequently used for the preparation). It was proposed that the removal of the carbon source (sucrose) compromised the metabolic activity of

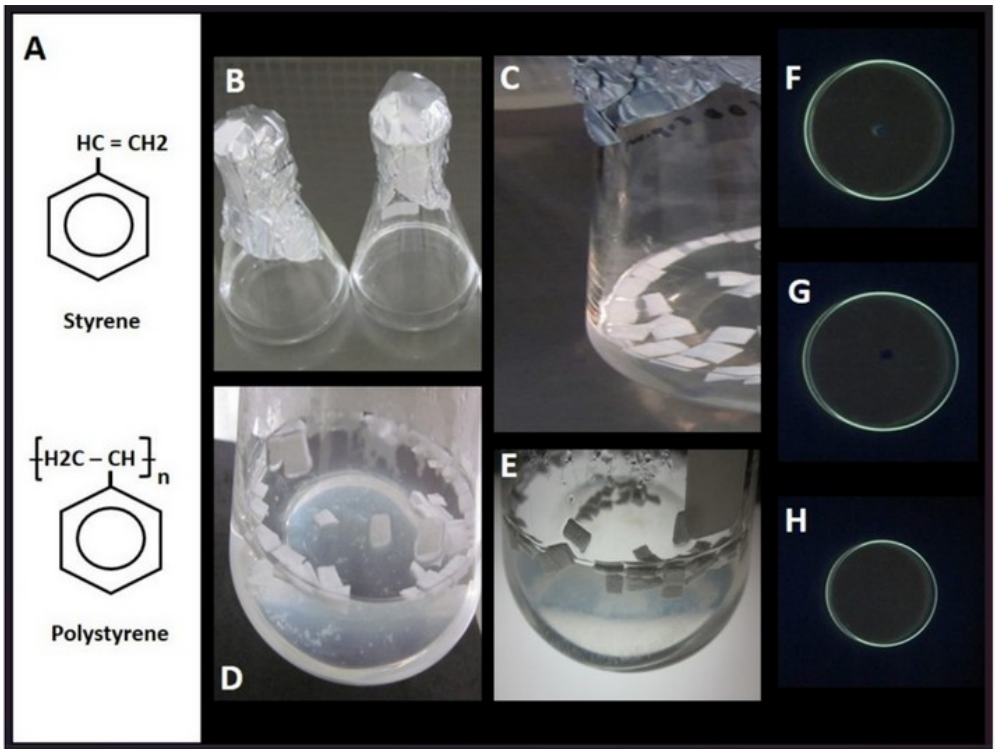

Fig. 1 Degradation of polystyrene by G. diazotrophicus.A. Structure of styrene and polystyrene. B. Uninoculated LG culture medium. C. LG culture medium with and polystyrene fragments. D. Growth of G. diazotrophicus and degradation of polystyrene. E. Degradation of polystyrene after 2 months of incubation with G. diazotrophicus. F. Formation of bacterial biofilm on the surface of polystyrene. G

G. diazotrophicus. However, *G. diazotrophicus* was able to replicate, probably by synthesizing some enzymes that could act on the polystyrene to obtain energy and continue its growth. So, after two weeks of steady-state culture, degradation of the polystyrene was observed with the appearance of small white clumps. It has been reported the biodegradation of modified polystyrene using *Pseudomonas aeruginosa*; strains of *Rhodococcus ruber* were also used to degrade pure standard polystyrene flakes, polystyrene powder, and ELISA 96-well microtiter plates manufactured from pure polystyrene (Mor and Sivan, 2008; Shimpi *et al*., 2012). However, biodegradability of polystyrene materials is complex and not fully understood (Ho *et al*., 2018).

It has been observed that mostof the bacterial cells adhered to polystyrene surface within few hours, forming a biofilm and a small reduction inthe polystyrene weight (0.8% of gravimetric weight loss) was found after 8 weeks incubation (Mor and Sivan, 2008). Ward *et al.* (2006) used styrene oil as the sole source of carbon and energy in a strain of *Pseudomonas* putida. Those authors observed that one gram of styrene oil was converted to 62.5 mg of polyhydroxyalkanoate and 250 mg of bacterial biomass in shake flasks. Motta *et al.* (2009) used species of fungus *Curvularia* to investigate degradation of polystyrene. The results showed hyphae adhering to and penetrating the polymeric surface and forming spores in all the treated samples after 9 weeks. These data showed that biodegradation of polystyrene material. The results presented in this study are consistent with data reported by other authors, showing biofilm formation on the surface of the tested polystyrene material.

Furthermore, because polystyrene is a lightweight material, it remained floating on the surface of the LG culture medium, where *G. diazotrophicus* remained attached to the polystyrene given its aerobic requirements. After 2 months of incubation, deposits of white clumps accumulated at the bottom of the Erlenmeyer flask, while others remained in suspensión. As in other studies previously reported by other authors in other microorganisms, *G. diazotrophicus* adapted its metabolism to be able to grow using polystyrene as the only source of carbon and energy.

This is the first report on the role of *G. diazotrophicus* as a bacterium with properties for the biodegradation of polystyrene. It has been reported that conventional removal methods, including filtration, flotation, coagulation, adsorption, and bioremediation, often struggle with inefficiencies, high costs, and secondary pollution. Therefore, the degradation of polystyrene using microorganisms should be a viable alternative to combat environmental pollution caused by this substance, however techniques such as biodegradation, photocatalysis, and thermal or chemical treatments face challenges related to effectiveness, prolonged treatment durations, and sustainability (P *et al.*, 2025). Therefore, further studies are needed to improve techniques, especially those related to the biodegradation of polystyrene.

CONCLUSION

The use of plastics revolutionized the contemporary world. However, the excessive increase in plastic materials and their improper disposal have had serious consequences for humanity. Today, we find them as pollutants in the environment, present in aquatic environments, the air, soil, and even as part of the tissues of aquatic organisms. Therefore, the search for strategies that allow the degradation of microplastics such as polystyrene is crucial. This work presented some data regarding the biodegradation of polystyrene using the nitrogen-fixing bacterium *G. diazotrophicus*.

ACKNOWLEDGEMENTS

Thank to Facultad de Medicina-BUAP and Grupo de Académicos de Puebla SC for the facilities provided for the development of this work.

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