

Biodegradation of cotton-elastane textile material by antarctic *Bacillus* spp.: Effect of biostimulation

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ABSTRACT

The accumulation of textile waste, especially that composed of blends of natural and synthetic fibers, poses an environmental challenge due to its limited degradation under natural conditions. This study evaluated the bioremediation potential of bacteria of the genus *Bacillus*, isolated from Antarctic ecosystems, for the degradation of denim-type textile material (98% cotton and 2% elastane). Two strains (*Bacillus albus* and *Bacillus wiedmannii*) were used in a biostimulated system with molasses as a carbon source, incubated at room temperature for 240 days. Degradation was assessed through mass loss, visual analysis, and temporal monitoring. The results showed a progressive reduction in the textile material in the inoculated treatments, reaching values exceeding 80% mass loss, in contrast to the abiotic control. The process exhibited three distinct phases: adaptation, active degradation, and stabilization, associated with the system's dynamics and substrate availability. It was observed that the cellulose fraction was preferentially degraded, while the presence of synthetic components limited total degradation. The addition of molasses may have promoted microbial activity in the system. Overall, the results demonstrate the potential of Antarctic bacteria of the genus *Bacillus* in bioaugmentation and biostimulation processes for the partial degradation of textile waste, contributing to the development of sustainable strategies within the framework of the circular economy.

Keywords: bioaugmentation, bioremediation, biostimulation, cellulose.

INTRODUCTION

Cellulose is the most abundant biopolymer in the biosphere and constitutes the main structural component of lignocellulosic biomass found in agricultural, forestry, and industrial waste (Datta, 2024; Klemm et al., 2005; Ma et al., 2024). This polysaccharide consists of long chains of β -1,4-D-glucose organized into highly crystalline microfibrils, which give it remarkable resistance to natural degradation. Due to this complex structure, its decomposition depends largely on the activity of microorganisms capable of transforming complex organic compounds into simpler, usable forms (Madhushree et al., 2024).

In addition to agricultural waste, the textile industry represents a significant source of environmental pollution due to the generation of solid waste that is difficult to degrade (Sandin and Peters, 2018). Among the most commonly used materials are cotton, a natural fiber composed primarily of cellulose, as well as synthetic fibers such as elastane (Spandex), which imparts elasticity and strength to the textile material (Haslinger et al., 2019). Denim-type textiles, widely used globally, combine natural fibers with synthetic components, which hinders their complete degradation under natural conditions. The presence of cellulosic fractions in textiles opens up the possibility of applying biological strategies for their partial transformation, particularly through the

use of microorganisms with the ability to utilize these compounds as a carbon source (Ejaz et al., 2021). However, the incorporation of synthetic fibers limits the efficiency of these processes, posing a challenge for the development of textile waste treatment technologies.

In this context, the study of microorganisms from extreme environments has gained prominence in environmental biotechnology. Psychrophilic microorganisms, adapted to low-temperature conditions, possess physiological characteristics that allow them to maintain metabolic activity under limiting conditions (D'Amico et al., 2006; Rozman et al., 2021). These adaptations include structural modifications in their enzymes, which enhance their activity in conditions where other microorganisms are less efficient.

Antarctica is one of the most extreme ecosystems on the planet and an important reservoir of microbial diversity (Bowman, 2018). Despite adverse conditions – such as low temperatures, high ultraviolet radiation, and limited nutrient availability – various microorganisms have developed mechanisms that allow them to survive and perform key ecological functions (Cary et al., 2010). Recent studies have demonstrated the presence of diverse bacterial communities with potential for biotechnological applications (Ortiz et al., 2021). Among these microorganisms, species of the genus *Bacillus* have been isolated from Antarctic environments and have demonstrated the ability to adapt to extreme conditions (Pudasaini et al., 2017). These bacteria have been studied for their potential to transform complex organic compounds, making them candidates for applications in bioremediation processes.

Despite advances in characterizing microbial diversity in polar environments, there are still gaps in our understanding of the applied potential of isolated bacteria in the degradation of complex materials (Ramasamy et al., 2023a). In particular, there are few studies that experimentally evaluate the ability of Antarctic bacteria of the genus *Bacillus* to contribute to the degradation of textile materials containing a cellulose fraction. In this context, the objective of this study was to evaluate the bioremediation potential of bacteria of the genus *Bacillus* from Antarctica in the degradation of denim-type textile material composed of 98% cotton and 2% spandex, by assessing mass loss and observing the degradation process under controlled experimental conditions.

MATERIALS AND METHODS

Microorganisms

Two bacterial strains belonging to the genus *Bacillus* (*Bacillus albus* and *Bacillus wiedmannii*) were used; these had been previously isolated from soil samples collected in terrestrial ecosystems in Antarctica and were stored in the institutional microbiological strain collection. The selection of the strains was based on their ability to grow under variable environmental conditions and on preliminary evidence of degradative activity observed during their microbiological characterization.

The strains were stored at $-80\text{ }^{\circ}\text{C}$ in nutrient broth supplemented with 20% (v/v) sterile glycerol. For reactivation, the cultures were inoculated onto nutrient agar (NA) and incubated at room temperature ($25 \pm 2\text{ }^{\circ}\text{C}$) for 24–48 h. Subsequently, a single colony from each strain was transferred to 250-mL Erlenmeyer flasks containing 100 mL of nutrient broth (NB) and incubated under constant orbital agitation (120 rpm) until the exponential growth phase was reached.

The use of bacteria of the genus *Bacillus* from Antarctic environments is based on their ability to adapt to extreme conditions, such as low temperatures, nutrient limitation, and environmental variations, which enhances their potential in the transformation of complex organic compounds. Recent studies have shown that Antarctic soils harbor diverse microbial communities with functional and biotechnological potential, including microorganisms associated with organic matter degradation processes (Qu et al., 2024; Ramasamy et al., 2023).

Preparation of the inoculum

The bacterial inoculum was prepared in a liquid medium consisting of sterile distilled water supplemented with molasses as an easily assimilable carbon source. Molasses was added at a concentration of 2% (v/v) to promote bacterial growth prior to inoculation (Correa da Silva et al., 2024).

The *Bacillus* strains, which had been previously reactivated, were inoculated into this medium and incubated at room temperature ($25 \pm 2\text{ }^{\circ}\text{C}$) under moderate orbital agitation for 24 h, until an active culture was obtained. Subsequently, the cell concentration was adjusted spectrophotometrically to an optical density of 0.5 ± 0.05 at 600 nm (OD₆₀₀), equivalent to approximately 1×10^8 CFU/mL.

The use of molasses as a supplement is based on its content of simple sugars and organic compounds that promote microbial growth. In this study, its incorporation allowed for the production of an active inoculum suitable for use in the experimental textile degradation process.

Preparation of the textile substrate

Denim fabric fragments composed of 98% cotton and 2% elastane (spandex) were used. The samples were cut into 3 × 3 cm pieces, washed with sterile distilled water to remove surface dirt and any adhering contaminants, and then dried at 30 °C for 24 h. Once dry, the samples underwent surface disinfection using UV-C radiation for 15 min before being incorporated into the experimental units. The initial mass of each fragment was determined using an analytical balance with an accuracy of ± 0.0001 g.

Soil preparation

Soil was used as the base matrix for the experimental systems. The soil was first air-dried and sieved to remove coarse particles and large organic debris. It was then subjected to a sterilization process to reduce interference from the native microbiota in the experiment. For sterilization, the soil was treated in an autoclave at 121 °C for 15 minutes in two consecutive cycles, ensuring the inactivation of any present microorganisms.

Once sterilized, 300 g of soil were placed in individual plastic containers, which were used as experimental units for each treatment and evaluation time point. The soil was kept under ambient conditions until it was used in the experiment.

Experimental design

The experiment was conducted using a completely randomized design with three treatments: two biological treatments inoculated with *Bacillus albus* (T1) and *Bacillus wiedmannii* (T2), and an abiotic control without bacterial inoculation (T3). A destructive sampling scheme was employed over time, establishing eight evaluation time points distributed over 240 days, with monthly intervals. Each experimental unit corresponded to an independent sampling time point; therefore, biological replicates were not considered within each evaluation time point due to the destructive sampling design.

Each experimental unit consisted of a system contained in individual plastic containers with 300 g of previously sterilized soil and a piece of denim-type fabric. In the biological treatments, the previously standardized bacterial inoculum (~10⁸ CFU/mL) was added, while the control received only the base solution without microorganisms. The units were incubated at room temperature (25 ± 2 °C) for 240 days. No external water was added; moisture was maintained based on the system's initial conditions and the internal dynamics generated during the process (Table 1).

Each experimental unit consisted of a system contained in individual plastic containers, into which the textile material was incorporated along with the previously prepared soil matrix.

For the biological treatments (T1 and T2), the previously standardized bacterial inoculum was added, while the control treatment (T3) received only the base solution without microbial inoculation.

The experimental units were incubated at room temperature (25 ± 2 °C) for a period of 240 days. During this time, no external water was added; the system's moisture content was maintained

Table 1. Experimental design of the treatments evaluated for the degradation of denim-type fabric

Treatment	Treatment description	Substrate	Inoculum	Experimental design	Experimental units	Incubation time
T1	Biostimulated system (water + molasses) with <i>Bacillus albus</i>	Denim-type fabric (98% cotton, 2% spandex)	Strain 1 (~10 ⁸ CFU/mL)	Plastic containers with 300 g of sterile soil	8 (one for each sampling time)	240 days
T2	Biostimulated system (water + molasses) with <i>Bacillus wiedmannii</i>	Denim-type fabric (98% cotton, 2% spandex)	Strain 2 (~10 ⁸ CFU/mL)	Plastic containers with 300 g of sterile soil	8 (one for each sampling time)	240 days
T3 (Control)	Abiotic system (no bacterial inoculation)	Denim-type fabric (98% cotton, 2% spandex)	No inoculum	Plastic containers with 300 g of sterile soil	8 (one for each sampling time)	240 days

based on the initial conditions of the substrate and the internal dynamics of the experimental system.

This design allowed us to evaluate the individual effect of each bacterial strain on the degradation of the textile material, as well as to compare the results with an abiotic control, which made it possible to rule out physical or environmental effects not associated with microbial activity (Kudus et al., 2024).

Incubation conditions

The systems were incubated at room temperature (24–28 °C) for a period of 240 days, simulating natural environmental conditions. Humidity was maintained at a stable level due to the closed nature of the system, which allowed for the preservation of a humid environment without the need for external water addition.

Degradation assessment

The degradation of the textile material was evaluated every 30 days by:

- Mass loss (%)
- Loss of area (cm²)
- Visual and photographic analysis

The samples were removed, washed with distilled water, dried at 60 °C for 24 h, and weighed again.

Data analysis

The data obtained were analyzed descriptively, considering the temporal evolution of the textile material's mass loss under each treatment. Degradation trends over time were evaluated to identify patterns in the behavior of the experimental system. Comparisons between treatments were performed using graphical and descriptive analysis, without applying inferential statistical tests, in accordance with the experimental design based on independent units by sampling time.

RESULTS AND DISCUSSION

Degradation of the textile material

Over the course of the 240-day incubation period, a progressive reduction in the mass of the textile material was observed in the treatments

inoculated with *Bacillus*. Treatment T1 exhibited a mass loss of 83.3%, while T2 reached 84.6%, indicating similar behavior between the two strains.

In contrast, the control treatment showed a considerably lower mass loss (9.4%), suggesting that the degradation observed in the inoculated treatments is primarily associated with the biological activity of the system.

The degradation process consisted of three phases: an initial adaptation phase (0–60 days), an active phase (60–150 days) characterized by a sustained increase in mass loss, and a stabilization phase (150–240 days), during which the degradation rate tended to decrease (Chukwuma et al., 2021a; Ejaz et al., 2021; Miao et al., 2021).

This behavior could be associated with the microorganisms' gradual adaptation to the substrate and the subsequent utilization of the most accessible fractions of the material (Danso et al., 2019; Korsá et al., 2023; Wei and Zimmermann, 2017). Although no direct measurements of enzymatic activity were performed, previous studies have reported that bacteria of the genus *Bacillus* may participate in the degradation of lignocellulosic materials through the production of extracellular enzymes (Zhang et al., 2023) (Figure 1).

Overall, the results obtained demonstrate the potential of bacteria of the genus *Bacillus* to contribute to the partial degradation of textile materials under environmental conditions. This approach has been proposed as a sustainable alternative for the recovery of lignocellulosic waste and the reduction of textile waste accumulation (Ghosh et al., 2025; Suen et al., 2023).

Degradation behavior over time

The temporal analysis showed that the degradation of the textile material followed a progressive trend in both treatments evaluated. During the first 60 days (Figure 2), the mass loss was moderate (≈36–42%), suggesting an initial phase of adaptation of the system to the substrate.

Subsequently, between 60 and 150 days, a sustained increase in mass loss was observed, indicating a higher intensity of the degradation process. This behavior has been reported in biodegradation studies, where an initial phase is followed by an increase in microbial activity associated with the transformation of organic compounds (Di Pippo et al., 2023; Skilbeck et al., 2025).

Starting on day 90, mass loss exceeded 50%, coinciding with the period of greatest degradation

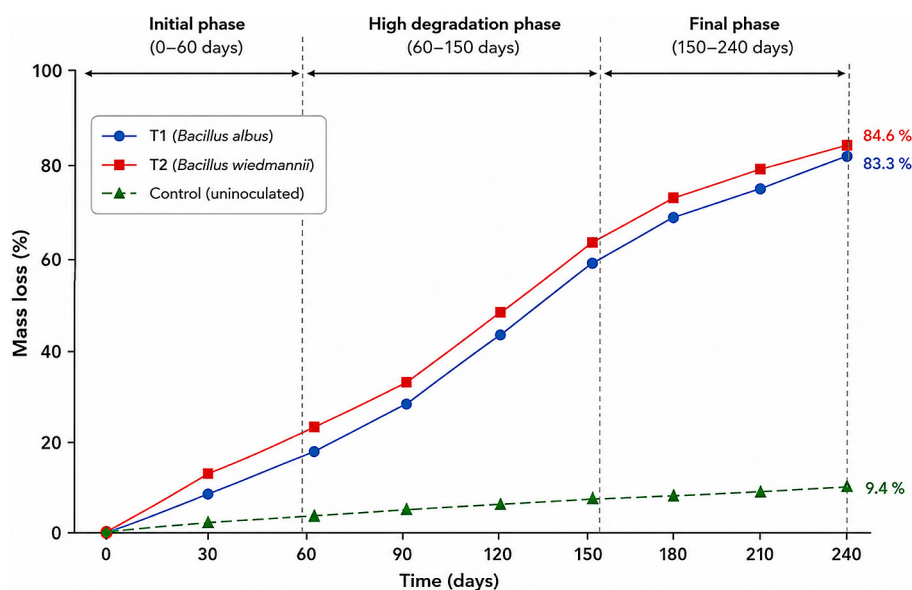


Figure 1. Mass loss (%) of the textile material as a function of time for the different treatments

observed in the system. This pattern is consistent with previous studies describing a progressive utilization of the cellulose fraction in lignocellulosic materials (Hsin et al., 2025; Hu et al., 2024; Ma et al., 2024).

Finally, between days 150 and 240, mass loss continued to increase, albeit at a slower rate, suggesting a deceleration of the degradation process. This behavior has been described when the most easily degradable compounds have been consumed, leaving fractions that are more resistant to microbial action. In blended textiles, this limitation may be related to the presence of synthetic components and the reduced availability of substrates accessible to microorganisms (Janković et al., 2025; Skilbeck et al., 2025).

pH variation during the process

The pH of the system remained relatively stable throughout the experimental period. In T1, values ranged from 6.20 to 6.45, while in T2, values ranged from 6.00 to 6.45 (Figure 3). These ranges indicate conditions ranging from slightly acidic to near-neutral, commonly reported as suitable for the development of microbial processes associated with the degradation of organic compounds.

Several studies have indicated that extracellular enzymes produced by bacteria of the genus *Bacillus* are stable within pH ranges close to neutrality (Fouda et al., 2024; Ugras et al., 2024). Likewise, recent research indicates that these microorganisms can maintain their activity within slightly acidic to

neutral ranges, which favors the transformation of lignocellulosic materials under variable environmental conditions (Sharma et al., 2025).

In treatment T2, a slight initial decrease in pH was observed, followed by a gradual stabilization throughout the experiment. This behavior could be associated with the temporary formation of compounds derived from the degradation of organic matter during the early stages of the process. Subsequently, the stabilization of the pH could reflect a balance between the production and transformation of these compounds within the system.

Overall, the results indicate that the pH remained within a stable range during the experimental process, suggesting that it did not represent an obvious limitation to the observed degradation of the textile material.

Temperature behavior

The temperature of the experimental system ranged from 24.0 °C to 28.5 °C in both treatments, with no marked differences between T1 and T2. A slight increase was observed during the first 60 days, followed by a gradual decrease toward values close to room temperature (Figure 4).

This behavior has been described in biological systems for the transformation of organic matter, where temperature variations associated with system activity may occur during the early stages of the process (Cai et al., 2024; A. Wang et al., 2023).

The gradual decrease in temperature observed after the first 60 days could be related to

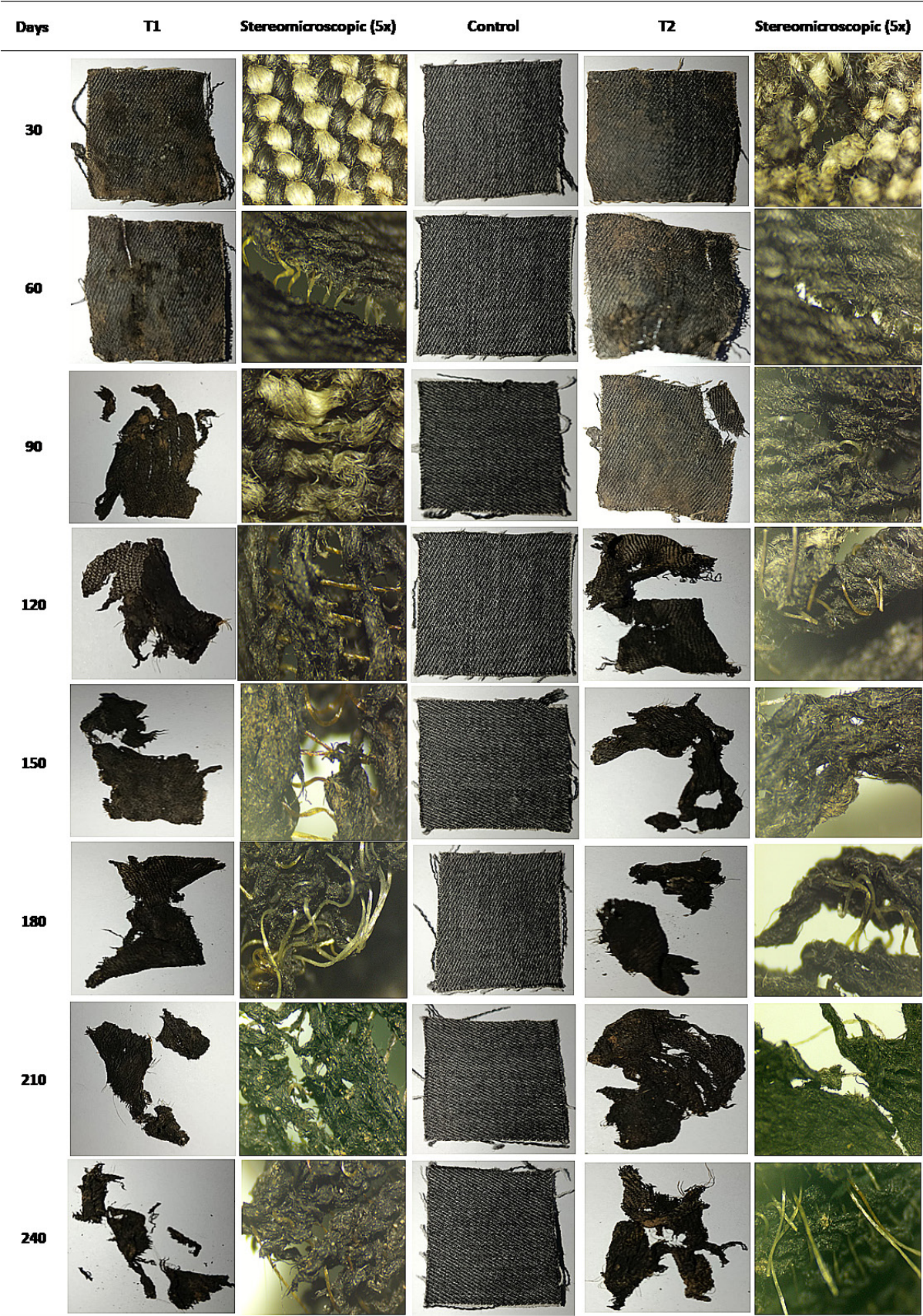


Figure 2. Progressive degradation of a denim-type textile material (98% cotton and 2% elastane) in the presence of *Bacillus albus* and *Bacillus wiedmannii*. Macroscopic and stereomicroscopic (5×) images of treatments T1 and T2 are presented, compared to the abiotic control, over a period of 240 days

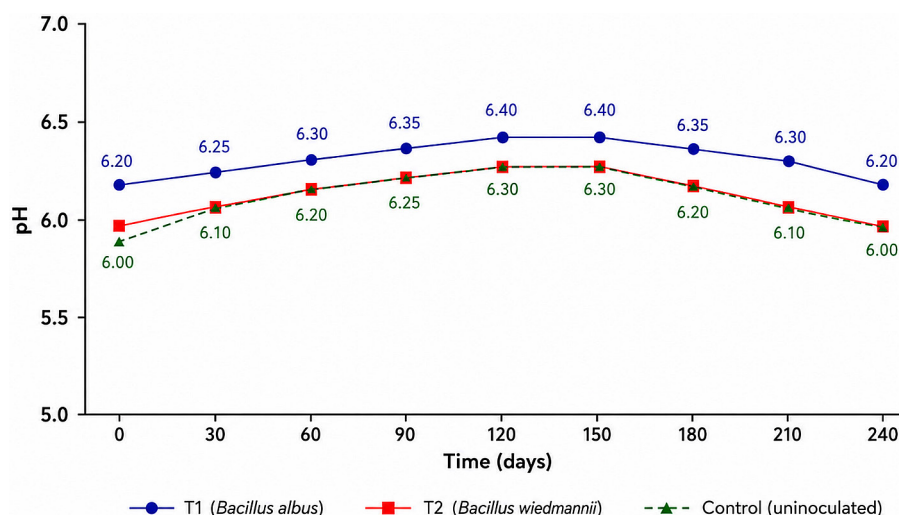


Figure 3. pH evaluation during the experiment (240 days)

changes in the availability of readily degradable compounds over time. Previous studies have indicated that degradation efficiency depends not only on the capacity of microorganisms but also on the structural complexity of the substrate and the accessibility of its fractions (Hsin et al., 2025; Ma et al., 2024).

Overall, the results indicate that the thermal conditions of the system remained within a relatively stable range during the experiment, suggesting that they did not represent an obvious limitation to the observed degradation process.

Comparison of treatments

The differences observed between the inoculated treatments and the abiotic control show a

greater loss of mass in the textile material in the systems containing bacteria (Figure 5). The lower degradation recorded in the control suggests that the mass loss observed in T1 and T2 cannot be attributed solely to physical or chemical factors in the system but is associated with the biological activity present in the inoculated treatments.

The greater degradation observed in the inoculated treatments is consistent with previous studies that have reported the ability of different species of the genus *Bacillus* to participate in the transformation of lignocellulosic materials (Wang et al., 2024). Furthermore, it has been reported that different strains of this genus may exhibit variations in their degradative performance, which could explain the differences observed between T1 and T2 (Cai et al., 2024).

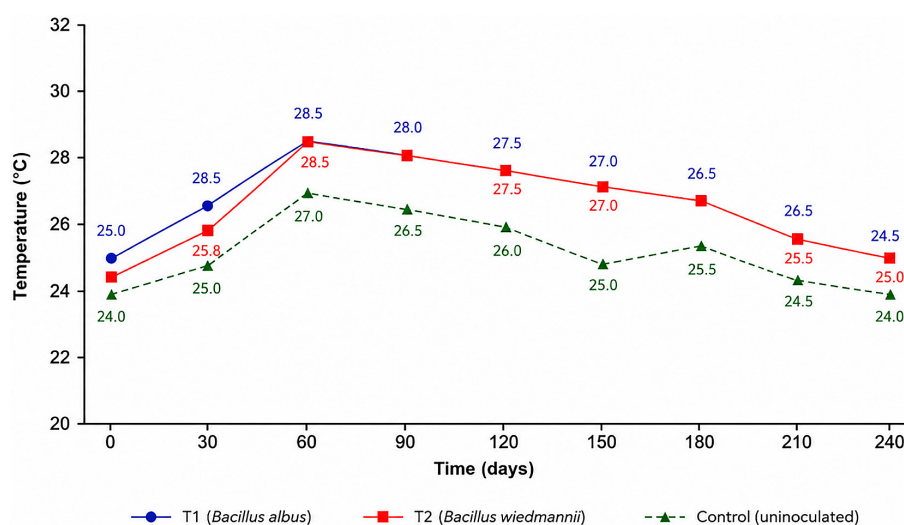


Figure 4. Temperature (°C) over the course of the experiment (240 days)

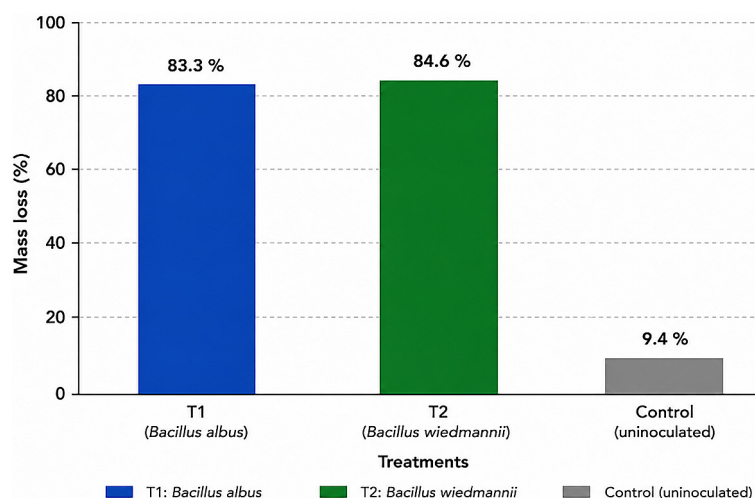


Figure 5. Comparison of mass loss (%) of the textile material at the end of the experiment (240 days)

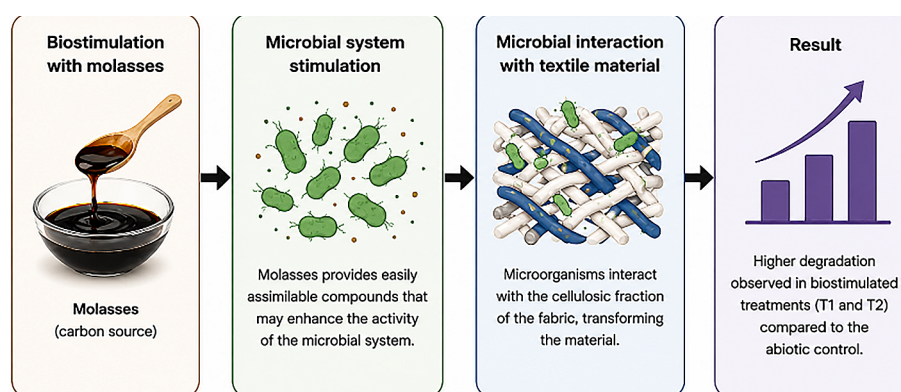


Figure 6. Mechanism of biostimulation with molasses and its effect on enzymatic activation and degradation of textile material by *Bacillus* sp.

The differences between the two treatments suggest functional variability among the strains evaluated. This behavior has been linked in the literature to differences in substrate utilization capacity and in the physiological characteristics of each isolate (Li et al., 2020; Chukwuma et al., 2021). In this context, even microorganisms with similar degradative capacities may exhibit different performance under comparable experimental conditions.

Effect of the biostimulated system

The use of molasses as a carbon source may have contributed to the degradation process observed in the inoculated treatments (Figure 6). The addition of readily assimilable compounds has been reported as a strategy that promotes microbial activity in organic matter degradation systems (Correa da Silva et al., 2024; Islam and Roy, 2018).

In this context, the greater degradation observed in the inoculated treatments suggests a combined

effect of bacterial inoculation and the availability of an additional carbon source. This behavior has been documented in studies where biostimulation improves the degradation efficiency of lignocellulosic materials (Korsa et al., 2023; Zhang et al., 2023).

However, in this study, no direct measurements of enzymatic activity or microbial growth were not performed in this study; therefore, the specific mechanisms involved cannot be confirmed. Overall, the results demonstrate the potential of biostimulated systems to contribute to the partial degradation of textile waste under environmental conditions.

CONCLUSIONS

The results show that bacteria of the genus *Bacillus* have the potential to partially degrade textile materials composed of cotton–elastane (spandex) blends under environmental conditions. The progressive decrease in mass and the

loss of structural integrity of the material suggest the involvement of biological processes in the degradation, particularly regarding the cellulose fraction of the substrate.

The temporal analysis identified a characteristic behavior of the biodegradation process, with an initial adaptation phase, followed by an active degradation phase and a final stabilization phase. This pattern suggests that the process dynamics are influenced by substrate availability. In this regard, differences were observed among the treatments, suggesting that degradative performance may be influenced by the specific functional characteristics of each strain. This result highlights the importance of microbial selection in the development of bioremediation applications.

The use of molasses as a carbon source may have contributed to the degradation process observed in the inoculated treatments. The addition of readily assimilable compounds has been reported as a strategy that promotes microbial activity in organic matter transformation systems. In this context, the results obtained suggest that the combination of bioaugmentation and biostimulation could represent a promising alternative for the partial degradation of textile waste under environmental conditions.

Overall, this study provides experimental evidence regarding the potential of these strategies in processes for the valorization of lignocellulosic materials. Finally, it is recommended that future studies focus on the enzymatic characterization of the strains used, as well as on the optimization of operating conditions and the scaling up of the process for its potential industrial application.

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