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Original Research Article

Evaluation of polyester polyurethane-degrading capability of bacteria isolated from landfill sites

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Abstract

Background: Polyurethane (PU) is one of the most widely used categories of plastics in modern life. The enormous demand for PU has contributed to the global plastic crisis. In recent years, microbial strains capable of degrading plastics have garnered significant scientific interest. In the study, bacterial strains were isolated from natural environment, such as soil and waste plastics, and screened for PU-degrading activity using Impranil as a model substrate.

Materials and Methods: The PU-degrading activity was evaluated on both liquid (Impranil) and solid (PU foam) substrates. Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM) analyses were used to detect chemical and physical changes.

Results: FTIR spectra reveal that the urethane, and ester components, along with carbon chains in Impranil and PU foam, were degraded by the microorganisms. On the liquid substrate (Impranil), degradation was observed in urethane, and ester bonds. On the solid substrate (PU foam), it is likely that the urethane component was attacked by the bacterial strain. SEM images disclosed that fiber density of PU foam in inoculated nutrient broth (NB) ppeared to be lower compared to the control. The PU-degrading strains were identified as *Bacillus velezensis*.

Conclusion: These findings demonstrate that microorganisms from natural environment could play a significant role in addressing the global plastic pollution.

Keywords: Polyurethane, biodegradation, Impranil, PU foam, *Bacillus* velezensis.

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1. Introduction

Since their invention in 1907 by Leo Baekeland, plastics have become an essential part of modern life due to their outstanding characteristics compared to other materials, such as durability, light weight and low cost.[1] Today, plastics are indispensable across various sectors, including automotive, agricultural, healthcare, construction, packaging, and textiles. However, the continual rise in plastic production has led to their emergence as major contaminant in both terrestrial and aquatic ecosystems. Plastic waste can currently be recycled, disposed of in landfills, incinerated or biodegraded. Globally, only 9% of plastic waste is recycled, 19% is incinerated, 50% is deposited in landfill, and 22% escapes waste management systems, and ending up in uncontrolled dumpsites.[2,3] Improperly discarded, plastic waste pollutes and harms the environment, acting as a major driver of biodiversity loss and ecosystem degradation. Plastic production and disposal are estimated to contribute around 3.3% of global greenhouse gas emissions.[4] Plastic pollution poses significant threats to human health, impacts food and water safety, disrupts economic activities, and exacerbates climate change. Therefore, the development of innovative approaches is urgently needed to solve the problem of “white pollution”. Plastic degradation is typically achieved through photodegradation, thermooxidative degradation, hydrolytic degradation, and biodegradation. Among these, microbial biodegradation using bacteria and fungi has gained significant attention as an eco-environmental approach for effectively managing plastic waste. Numerous studies have

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focused on isolating microorganisms and evaluating their biodegradation potential.[5-12] However, the effectiveness of microbial degradation is not yet sufficient to address plastic waste on a large scale. As a result, research into microbial strains capable of degrading plastic waste remains ongoing.

Polyurethane (PU) accounts for approximately 8% of the total mass of plastics produced, making it the sixth most widely used polymer globally.[13] This versatile polymer is commonly utilized in coatings, adhesives, foams, shoe soles, synthetic leather, and bumpers. PU is synthesized through the condensation of three components: isocyanates (R-N=C=O), polyol (R'-OH), and chain expanders, which are classified as polyester (PS) or polyether (PE) types depending on the polyol used. The most important and resistant unit in polyurethane strucuture is urethane bond (-NH-COO-) (**Figure 1**).[14] Due to its highly complex polymer structure, PU is typically discarded in landfills or incinerated for heat recovery. Among these methods, landfilling remains the most widely used approach for managing PU waste.[12,13]

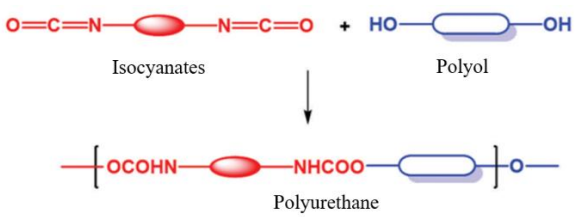

Figure 1: General reaction for the synthesis of polyurethane

The decomposition of PU is slow and releases harmful pollutants. Many recent studies have reported that fungi are the predominant microorganisms involved in the biodegradation of polyester PU.[5,15-18] Additionally, bacteria isolated from natural environment also plays a significant role in this process.[8,11,19-23] These microorganisms degrade plastics by producing plastic-degrading enzymes (PDE) like lipase, protease, urease and esterase.[12,15,19,24] Microbial enzymes can degrade PU without causing negative environmental impacts. However, the biodegradation of PU waste remains highly limited due to its inherent resistance. Therefore, exploring more effective microbial strains for PU decomposition, particularly on a large scale, is highly desirable.

In this context, the present study primarily focuses on selecting bacterial strains isolated from natural environment (soil and plastic waste) based on their PU-degrading ability. The results were analyzed using Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM).

2. Materials and Methods

2.1. Polyurethane forms used in the study

Two different forms of polyurethane were used in the study, including liquid and solid substrates. The liquid substrate, known as Impranil DLN-SD (referred to as Impranil) was supplied by the headquarters of Covestro company in Vietnam. Impranil is a water-based anionic aliphatic polyester polyurethane colloid dispersion commonly used as a substrate in polyurethane degradation studies. Its popularity stems from its commercial availability, application as a coating material in the aviation and leather industries, and several advantageous properties. These include a relatively temperature tolerance of up to 80°C, stability to hydrolysis with a pH range of 4-8, and a faster dissolution rate compared to other polyurethanes. Furthermore, this substrate is easily degraded and assimilated by microorganisms, appearing as a white, milky suspension containing 40% polymer. The addition of Impranil to a liquid culture medium has been shown to upregulate the expression of numerous genes encoding enzymes responsible for PU degradation, such as lipases, proteases, and oxidoreductases.[6,12,15,19,24] PU foam, commonly used to manufacture household scrub sponges such as those of the Scotch - Brite brand, was employed as the solid substrate of PU in the study. This flexible material is valued for its lightweight, strong, supportive, and comfortable properties. These characteristics contribute to its widespread use, accounting for 31% of global PU production[3].

2.2. Isolation of bacteria from natural environment and preliminary screening for Impranil-degrading ability

Soil, and plastic waste (e.g., bottle, bag) from plastic-polluted sites was collected and stored in sterilized zip-lock plastic bags to isolate Impranil-degrading bacteria. For each sample, 10 grams of soil or plastic waste were mixed with 90 ml of physiological saline solution, shaken at room temperature for 30 minutes, and then serially diluted. From the serially diluted samples, 100 µl was evenly spread onto the surface of Nutrient Agar (NA), and incubated at 37°C for 24 hours to obtain single, isolated bacterial colonies. These colonies were subsequently transferred onto NA plates (HiMedia, India) supplemented with 3% Impranil (v/v). When dispersed in the agar medium, Impranil forms a whitish, opaque texture. Impranil was added to the culture medium after autoclaving to prevent denaturation. Bacterial isolates capable of degrading Impranil form clear hydrolysis zones around their colonies after 3 days of incubation at 37°C, serving as an indicator of Impranil degradation. The two bacterial isolates demonstrating the strongest Impranil-degrading capacity were selected for identification and experiments on both liquid and solid PU substrates. The isolates were preserved in BHI broth (HiMedia, India) supplemented with 30% glycerol and stored at -20°C until use.

2.3. Identification of the bacterial strains

The bacterial strains selected for the study were sent to P&Y laboratory (Vietnam) for identification through PCR and 16S rRNA gene sequencing. Genomic DNA was extracted from isolated colonies using GenJET Viral DNA and RNA purification kit (Thermo Fisher Scientific, USA). Amplification was carried out using 63F and 1387R primer

sets[25] in a thermocycler. The program consisted of denaturation at 95°C for 5 minutes, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute 30 seconds. The PCR products were then sequenced and compared against the National Center for Biotechnology Information (NCBI) database using BLAST to identify the bacterial strain.

2.4. Evaluation of the PU-degrading ability of the bacterial strains isolated using a liquid substrate (Impranil)

The Impranil-degrading ability of the isolated bacterial strains was evaluated in Nutrient Broth (NB) supplemented with 1, 3, and 5% (v/v) Impranil. The bacterial strains were thawed at room temperature and transferred to BHI broth, followed by incubation at 37°C for 24 hours. Subsequently, 50 µl of the bacterial suspension was inoculated into 50 ml of NB containing 1, 3, and 5% Impranil. The samples were incubated at 37°C and shaken daily for 30 minutes at 200 rpm using a shaker incubator (HB-201SF - Korea). The disappearance of Impranil-related opaque cloudiness in the liquid medium after 19 days of incubation indicated the PU-degrading ability of the tested bacterial strains. The samples containing 1 and 3% Impranil were subsequently processed for FTIR analysis as follows: After centrifugation at 6,000 rpm for 5 minutes, the supernatant was collected and concentrated at room temperature using a vacuum concentrator (Hei-VAP Core ML/G3 XL P/N: 572-01305-00, Germany) with a pressure of 25 mbar. The dried mass was incubated at 40°C for 24 hours prior to FTIR spectral measurement. The control sample, prepared without bacterial inoculation under identical conditions, was used for comparison.[6-8,10,12]

2.5. Evaluation of the PU-degrading ability of the bacterial strain isolated using a solid substrate (PU foam)

Firstly, PU foam was cut into small cubes (1×1×1 cm) and sterilized at 121°C for 15 minutes before use. The samples were prepared as follow: 50 µl of the bacterial suspension, cultured overnight at 37°C, was transferred into 50 ml of sterilised NB. Twenty sterilized PU cubes were added to each sample. Control samples were prepared under the same conditions, consisting of 50 ml of sterilized NB and twenty PU cubes. All the samples were incubated at 37°C and shaken daily for 30 minutes at 200 rpm using a shaker incubator (HB-201SF - Korea) at 200 rpm. Every three weeks, the PU cubes in all the samples were retrieved and transferred to new bottles containing 50 ml of sterilized NB and 50 µl of freshly cultured bacterial suspension. This process was conducted under aseptic conditions. After three months, the PU cubes were recovered, thoroughly washed with sterile distilled water, and air dried. The dried cubes were then subjected to FTIR spectroscopy and SEM analysis to evaluate the PU degradation.[8,10-12]

2.6. Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was performed using Bruker Lumos FTIR microscope to detect changes in the chemical bonds of Impranil and PU foam at the end of the experiment. Spectra were recorded over a frequency range of 4000 to 400 cm^{-1}, averaging 32 scans at a resolution of 4 cm^{-1}. The data were plotted and analyzed using OriginPro software (2024b version). Functional groups were identified and compared with those of the original Impranil and PU foam to assess chemical modifications resulting from bacterial degradation.

2.7. Scanning electron microscopy (SEM) analysis

Scanning Electron Microscopy (SEM) analysis was conducted to directly observe morphological changes, such as the formation of holes and erosion, on the PU foam surface. These changes were examined at magnifications of ×50, ×250, and ×500 using a Hitachi S-4800 SEM. The samples were sent to the Institute of Chemical Technology at the Vietnam Academy of Science and Technology for SEM analysis. Before observation, the PU foam samples were coated with platinum to enhance conductivity. For comparison, the PU foam immersed in pure NB was employed as the control.[11]

3. Results

3.1. Isolation of bacteria from natural environment and preliminary screening for impranil-degrading ability

Seventy-five samples (30 from soil, and 45 from plastic waste) were collected to isolate Impranil-degrading bacteria. A total of 150 bacterial strains were rapidly screened on NA supplemented with 3% (v/v) Impranil. After 3 days of incubation at 37°C, clear zones were observed around colonies of 23 strains, indicating their ability to degrade Impranil (**Figure 2**). Based on the diameter of the clear zones, two bacterial strains demonstrating the strongest Impranil-degrading ability (named as D 3.2.1 and M 1.2.1) were selected for identification and further experiments involving on liquid (Impranil) and solid (foam) PU substrates.

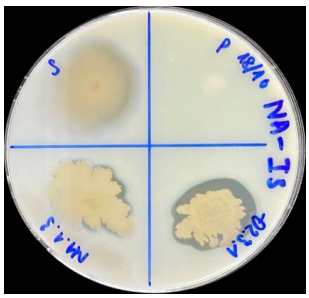

Figure 2: Growth of bacterial isolates from soil, plastic waste on NA plate supplemented with 3% Impranil after 3 days of incubation at 37°C. Zones of clearing around the bacterial colonies indicated Impranil-degrading activity

3.2. Identification of the bacterial strain

The bacterial strains were identified by PCR amplification combined with 16S rRNA gene sequencing. The resulting sequence was compared against nucleotide sequences in the NCBI database using the BLAST alignment tool. The results

reveal that the strain D 3.2.1 and M 1.2.1 share 100%, and 99, 83% similarity with *Bacillus velezensis*, respectively. Therefore, we designated these strains as *Bacillus velezensis* D 3.2.1 and *Bacillus velezensis* M 1.2.1. The 16S rRNA gene sequences are available on the NCBI database under the accession number PQ821362.1 (https://www.ncbi.nlm.nih.gov/search/all/?term=PQ821362.1) for *Bacillus velezensis* D 3.2.1, and PQ821361.1 (https://www.ncbi.nlm.nih.gov/search/all/?term=PQ821361.1) for *Bacillus velezensis* M 1.2.1 and also presented in supplementary data.

3.3. Evaluation of the PU-degrading ability of the bacterial strains isolated using a liquid substrate (Impranil)

The PU-degrading ability of two bacterial strains (*Bacillus velezensis* D 3.2.1 and M 1.2.1) was evaluated in NB supplemented with 1, 3, and 5% (v/v) Impranil. The results are presented in **Figure 3**. After 19 days of incubation at 37°C, the milky white color of the culture medium, resulting from the presence of 1% Impranil, completely disappeared for both bacterial strains. At 3% Impranil, the medium color faded by approximately 50% compared to the control, indicating partial degradation of the substrate. The strain D 3.2.1 exhibited slightly better Impranil-degrading ability compared to the strain M 1.2.1. However, at 5% Impranil, no significant change in the medium color was observed in two samples after 19 days of incubation at 37°C. Based on these results, the samples from the strain D 3.2.1 grown in 1, and 3% Impranil were selected for FTIR analysis to gain further insight into the degradation mechanism. The FTIR results are presented in **Figure 4**.

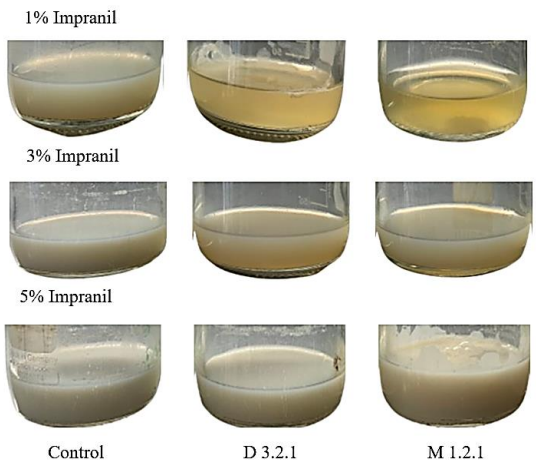

Figure 3: Degradation of Impranil (1, 3, and 5%) added to NB by the strains D 3.2.1 and M 1.2.1 after 19 days of incubation at 37°C

Impranil is often used as a PU substrate in biodegradation experiments because it is easily degraded and assimilated by microorganisms.[7,9,19,26] However, this material contains more ester bonds than urethane bonds, and the biodegradability of ester bonds is much higher.[27] Although a variety of microorganisms have been reported to successfully degrade Impranil, they exhibit weak capabilities in degrading solid polyester polyurethane substrates such as polyurethane film, foam, and elastomers.[19] In the next step, PU foam will be used as a real substrate to evaluate PU degradation by the strain D 3.2.1.

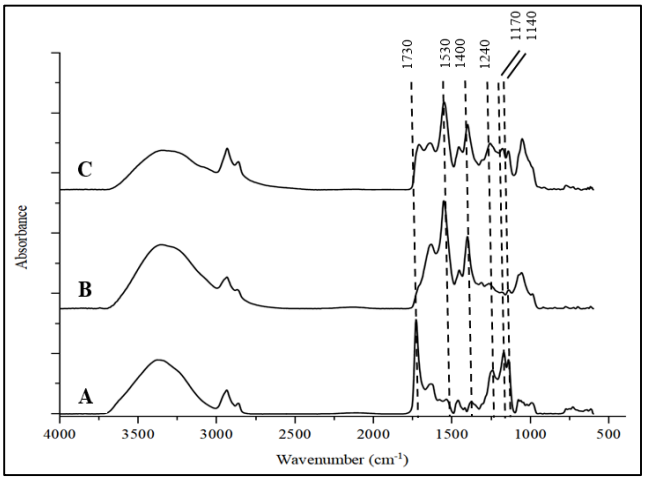

Figure 4: FTIR spectra of the original Impranil (A) and inoculated NB supplemented with 1% Impranil (B), and 3% Impranil (C), incubated at 37°C for 19 days

3.4. Evaluation of the PU-degrading ability of the bacterial strain isolated using a solid substrate (PU foam)

In evaluating polyester polyurethane degradation on a real and commonly used substrate, a scrub sponge made of polyester polyurethane foam was chosen. The PU foam was cut into small cubes (1×1×1 cm) in order to facilitate the attachment and penetration of the bacterium, and improve the biodegradation rate. When the PU cubes were added to NB and NB inoculated with the strain D 3.2.1, all of them were observed to float on the surface. After 3 days of incubation at 37°C and shaken 30 minutes per day, the PU cubes in the inoculated NB began to sink to the bottom of the flagon due to the attachment and penetration of the bacterium. After three months of incubation, all the PU cubes in this group were observed to float again on the surface of the culture medium. The cubes were then removed, washed and sent to a commercial lab for FTIR measurement and SEM analysis to determine chemical and physical changes. The PU cubes immersed in NB floated continuously from the beginning until the end of the experiment.

FTIR spectra of the PU foam immersed in NB and inoculated NB after three months of incubation at 37°C, with shaking for 30 minutes per day are showed in **Figure 5**. The positions and assignements of the main spectral peaks in the PU foam are summarized in **Table 1**. In general, all the spectra show the usual peaks of polyurethane foam. Based on FTIR analysis, only a subtle decrease in the intensity of the peak at 1085 cm^{-1} (associated with C-O stretching in C-O-C=O of urethane) is observed in the FTIR spectrum of the PU foam in inoculated NB compared to those in NB after three months of incubation at 37°C, with shaking 30 minutes per day.

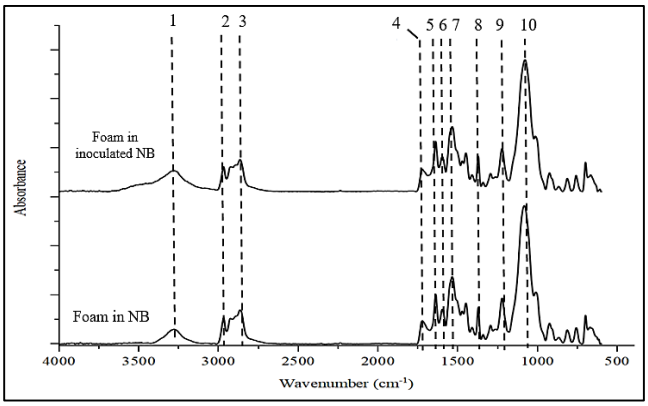

Figure 5: FTIR spectra of the PU foam immersed in NB and NB inoculated with the strain D 3.2.1 after three months of incubation at 37°C, with shaking for 30 minutes per day

Table 1: Positions and assignments of the main spectral peaks found in the PU foam

Order	Wavenumber (cm^{-1})	Peak assignment	References
1	3276	N-H stretching vibration of urethane	28-30
2,3	2976, and 2864	CH_2 stretching vibrations	29-33
4	1727	C=O stretching vibration of free urethane	28, 29,34,35
5	1640	C=O stretching vibration of amides	30
6	1600	C=O hydrogen in urethane	36
7	1537	N-H bending vibration of urethane	29, 30, 34,37,38
8	1375	CH_2 and CH_3 in carbon backbone	35
9	1222	C-N stretching vibration of urethane	11, 28
10	1085	C-O stretching in C-O-C=O of urethane	29,30,33

3.5. Scanning electron microscopy (SEM) analysis

Scanning Electron Microscopy (SEM) was used to assess the morphological modifications on the surface of the PU cubes. This technique enables a qualitative evaluation of surface degradation after biological treatment by observing cracks or holes on the degraded polymers. SEM images of the PU foam in NB, and inoculated NB are presented in **Figure 6**.

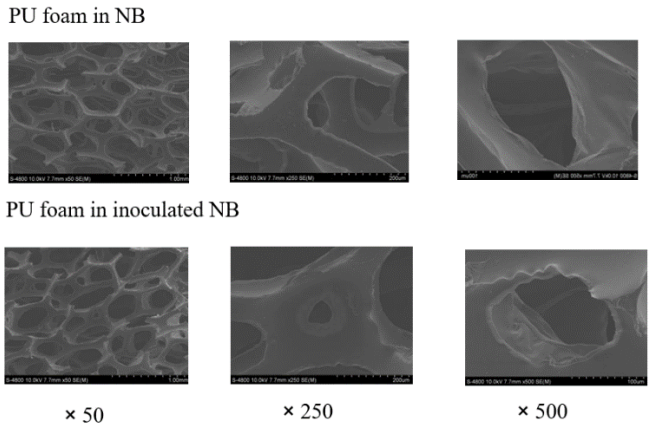

Figure 6: Scanning electron microscopy view (×50, 250, and 500 magnification) of the PU foam immersed in NB, and inoculated NB after three months of incubation at 37°C, with shaking for 30 minutes/day

4. Discussion

Bacillus velezensis is an aerobic, Gram-positive, endospore-forming, and free-living soil bacterium first described by Ruiz-Garcia et al., 2005.[39] In recent years, the bacterium has gained scientific interest as a potential biocontrol agent against phytopathogens, and as a microbial inhibitor. It also shows potential for food preservation due to its ability to produce antimicrobials, volatile organic compounds, bioactive enzymes, and plant growth-promoting substances.[40-42] Moreover, *Bacillus velezensis* exhibits promising probiotic properties, including high bile salt tolerance, absence of antibiotic resistance and virulence factors, and a high success rate of colonization in the intestinal mucosa. Additionally, it demonstrates the ability to degrade mycotoxins such as zearalenone.[43] Additionally, the ability to form endospores is a significant advantage for *Bacillus velezensis*, allowing it to survive in unfavorable environments, such as high temperatures, desication, and exposure to gastric juices. A study conducted by Gui Z et al.,[6] demonstrated that *Bacillus velezensis* GUIA, isolated from a deep-sea environments, is capable of degrading waterborne polyurethane (Impranil), with the oxidoreductase Oxr-1 identified as the key enzyme responsible for degradation. Zeng et al.,[44] reported that *Bacillus velezensis* MB01B, isolated from landfill soil, can degrade commercial PUR materials, including Impranil, TPU film and PUR desk mats. *Bacillus velezensis* D 3.2.1 and M 1.2.1 have the ability of degrading Impranil stronger than other bacteria in some previous studies.[45,46]

Changes of chemical groups during plastic biodegradation can provide insight into which part of the polymer molecule is being degraded. Polyester polyurethane contains both urethane and ester bonds within its molecular structure, so degradation occurs primarily through the cleavage of these bonds. Several studies have reported a decrease in the abundance of carbonyl groups detected by FTIR, indicating that the degradation predominantly affects

the soft segments of the polymer.[16,26,38,47] Consistent with this observation, the functional groups within the frequency range of 1730 cm^{-1} to 1000 cm^{-1} shows significant changes in this work. The FTIR spectrum of the orginal Impranil has a large absorption peak at 1730 cm^{-1} related to C=O stretch in the ester fraction.[38,46,48] A complete loss of this peak is observed in the FTIR spectra of inoculated NB containing 1% or 3% Impranil. This result indicates the hydrolysis of the ester bond in the urethane linkage. This finding aligns with the results reported in other studies.[8,38,49,52] A sharp increase in the peak at 1530 cm^{-1}, which is typically attributed to the nitrogen of the urethane moieties, is clearly observed in both 1% and 3% Impranil samples. According to Oprea,[53] an increase in this peak is associated with urethane bond hydrolysis. However, other studies have suggested that a decrease in this peak results from urethane bond degradation.[10,33,49,54,55] A sharp increase in the peak at 1400 cm^{-1}, associated with the CH_2 bond, is clearly observed in FTIR spectra.[56,57] This observation aligns with the study conducted by Nakkabi et al.,[46] on the biodegradation of polyester polyurethanes by *Bacillus subtilis*. In addressing the degradation of PU, the cleavage of the urethane bond appears to be a key factor. A decrease in the band at 1240 cm^{-1}, corresponding to the C-O-C elongation vibration of the urethane group, is observed in the sample supplemented with 1% Impranil.[16,49,50] A reduced intensity of the peaks around 1140 cm^{-1} and 1170 cm^{-1}, corresponding to C-O stretching, indicates a change in the ester component.[8,20,54] The results show that both the ester and urethane components in Impranil structure are degraded by the strain D 3.2.1 isolated from soil in a plastic landfill.

Evaluating PU foam degradation is much more challenging than Impranil, as the substrate is a highly complex system based on crosslinked architectures with various components and additives. Thanks to its alveolar structure, microorganisms can easily attach to, colonize the surface of PU foam, initiating microbial biodegradation. In most plastic degradation studies, solid substrates are generally pre-treated with UV irradiation, thermal and chemical treatments to modify the polymers and facilitate the plastic degradation by microorganisms.[2,58] However, the PU foam used in this work was not subjected to any pretreatments, such as physical and chemical agents, to alter its structural and morphological characteristics and facilitate microbial degradation. Therefore, the time during which the PU substrate was immersed in the culture medium inoculated with the bacterium needs to be longer to obtain chemical and physical changes as indicators of the biodegradation process. The strain D 3.2.1 was isolated from landfill soil. Microbial biodegradation of PU plastics is primarily mediated by the enzymatic action of hydrolases, including esterases, ureases, proteases, and amidases. Esterases hydrolyze the ester bonds in the soft segment of polyester-based PU plastics, resulting in the release of carboxylic acid and alcohol end-groups. Ureases are capable of degrading urethane bonds in selected polyurea-urethane polymer, releasing two amines and carbon dioxide. Proteases and amidases are two additional enzymes involved in PU degradation, these enzymes hydrolyze peptide or amide bonds and have also been reported to attack urethane bonds.[7,20,27,59-61] One of the major challenges in the enzymatic degradation of PU is that only the ester bonds in the soft segments of polyester-based PU are typically hydrolyzed, with few reports on the biodegradation of urethane bonds.[26] In the study, the FTIR results from PU foam indicate that the urethane components (the peak at 1085 cm^{-1}) were degraded by the strain D 3.2.1 isolated from soil after three months of incubation in NB. The finding is consistent with the study led by Orts et al.[11]

The fibrous structure of the PU foam facilitates the attachment and colonization of the bacterium on the substrate, thereby enhancing the biodegradation rate. However, this structure makes it relatively difficult to observe morphological changes in the SEM images. The fiber density of the PU foam in inoculated NB appears to be lower than that of the foam immersed in NB. Based on the FTIR analysis, the biodegradation process in the experimental lot has only just begun after three months of incubation at 37°C. Consequently, significant morphological changes in the PU foam may not be clearly visible yet.

5. Conclusion

The bacterial strain (*Bacillus velezensis* D 3.2.1), isolated from landfill soil, was evaluated for its polyester polyurethane-degrading activity. FTIR analysis confirms that this strain is capable of degrading ester and urethane bonds in a liquid substrate (Impranil). On a solid substrate (PU foam), only urethane component was attacked by the bacterial strain. These findings highlight the potential use of microorganisms isolated from natural environments for polyester polyurethane degradation, offering a promising alternative to mitigate the global plastic crisis. However, further research is needed to identify the key enzymes involved in polyester polyurethane degradation and to optimize enzymatic conditions for industrial-scale applications.

6. Source of Funding

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7. Conflict of Interest

The authors declare no conflict of interest with the present study.

8. Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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