

Biodegradation of polyurethane by the microbial consortia enriched from landfill

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Abstract

Polyurethanes (PU) are one of the most used categories of plastics and have become a significant source of environmental pollutants. Degrading the refractory PU wastes using environmentally friendly strategies is in high demand. In this study, three microbial consortia from the landfill leachate were enriched using PU powder as the sole carbon source. The consortia efficiently degraded polyester PU film and accumulated high biomass within 1 week. Scanning electron microscopy, Fourier transform infrared spectroscopy, and contact angle analyses showed significant physical and chemical changes to the PU film after incubating with the consortia for 48 h. In addition, the degradation products adipic acid and butanediol were detected by high-performance liquid chromatography in the supernatant of the consortia. Microbial composition and extracellular enzyme analyses revealed that the consortia can secrete esterase and urease, which were potentially involved in the degradation of PU. The dominant microbes in the consortia changed when continuously passaged for 50 generations of growth on the PU films. This work demonstrates the potential use of microbial consortia in the biodegradation of PU wastes.

Key points

- Microbial consortia enriched from landfill leachate degraded polyurethane film.
- Consortia reached high biomass within 1 week using polyurethane film as the sole carbon source.
- The consortia secreted potential polyurethane-degrading enzymes.

Keywords Plastic biodegradation · Polyurethanes · Microbial consortia

Introduction

Plastic wastes have become a significant threat to the environment ever since their invention (Liu et al. 2021a; Ru et al. 2020). In particular, with the spread of the new coronavirus, global production and consumption of synthetic polymers continue to increase (Kannan et al. 2023). Polyurethane (PU) is the 6th most used class of heteropolymer, which is widely used as coatings, adhesives, foams, shoe soles, synthetic leather, and bumpers (Akindoyo et al. 2016; Vargas-Suarez et al. 2021). PU is synthesized by the condensation

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Qingsheng Qi qiqingsheng@sdu.edu.cn between three components: isocyanates, polyols, and chain expanders, which can be classified as polyester (PS) or polyether (PE) types depending on the type of polyol (Silva and Bordado 2004). The polyester polyols are mostly made from dicarboxylic acids and diols such as adipic acid (AA), butanediol (BDO), and ethylene glycol, which are polymerized through the ester bonds. Due to their high resistance to degradation, large quantities of PU waste permanently persist in the environment and become potentially hazardous compounds, threatening the human health and the integrity of various ecosystems (Geyer et al. 2017). Although the various physical or chemical methods such as landfilling and incineration are available to treat the PU waste, it still generates negative impact on the environment (Cregut et al. 2013; Gaytan et al. 2019). Instead, biodegradation has been gaining high attention as the promising green alternative (Jin et al. 2022; Li et al. 2022).

Biodegradation of the PU waste is still challenging due to its complex molecular composition (Jin et al. 2022; Magnin

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et al. 2020). PU has a crystalline "hard chain segment" (polyisocyanate) which contributes to hardness and tensile strength and an amorphous "soft chain segment" (chain extender with polyol) which contributes to elasticity (Tang et al. 2001). The urethane bonds and the polyol segments are relatively sensitive to the microbial attack (Magnin et al. 2019). It has already been reported that microbial communities or pure microbes can attack the refractory PU waste (Crabbe et al. 1994; Shah et al. 2013a). The degradation capabilities of several bacteria and fungi over different types of PU or its model substrates such as the waterborne PU dispersion Impranil DLN, toluene-2,4-carbamic acid diethyl ester (TDCB), and 1-methoxypropan-2-yl (4-nitrophenyl) carbamate have also been assessed (Akutsu-Shigeno et al. 2006; Gamerith et al. 2016; Peng et al. 2014). Among these, microbial consortia consisting of two or more species can greatly promote the degradation of polymers, especially the PU waste with complex compositions and multiple different chemical bonds (Vargas-Suarez et al. 2019, 2021).

Microbial consortia are the collections of microorganisms that live together in a particular environment. These microorganisms interact in various ways such as mutualism, predation, or competition (Faust and Raes 2012; Jiang et al. 2022). In particular, the mutualism relationship plays an impressive synergistic role in the degradation of different organic pollutants and xenobiotics including synthetic polymers (Gao and Sun 2021; Sha et al. 2022; Yin et al. 2020). It was observed that microbial consortia from PU-rich environments such as landfill leachate and wastewater treatment plants were effective in degrading PU and better than the pure culture, as measured by weight loss, O2 consumption, CO2 release, and Fourier transform infrared (FTIR) spectroscopy (Cosgrove et al. 2007; Shah et al. 2008, 2016; Zafar et al. 2013). The recycling of certain plastics such as polyethylene terephthalate (PET) using the full biotechnological route has been recently reported (Liu et al. 2021b; Wang et al. 2022). However, the current biodegradation of PU is still very limited and cannot meet the practical application of recycling (Liu et al. 2021a; Magnin et al. 2020). Therefore, it is highly desirable to explore more effective microbial consortia for PU degradation.

In this context, the present study is primarily focused on screening microbial consortia that can efficiently degrade PU films. The microbial composition of the consortia and the activities of associated depolymerases were analyzed to further understand the potential mechanisms of PU biodegradation.

Materials and methods

Materials and growth mediums

The polyester polyurethane (PS-PU) powder used in this study was purchased from BASF (Germany). Waterborne

PU dispersion Impranil DLN was purchased from Covestro (Germany). Other biochemical reagents were purchased from Sinopharm Reagent (China). PU films were prepared by dissolving 500 mg PU powder in 25 mL tetrahydrofuran and then pouring into a glass Petri dish. After tetrahydrofuran volatilization, PU formed a film. The PU film was removed from the glass and cut to size, then soaked in 75% alcohol for 30 min, and left to dry under a UV sterilizing lamp (Khan et al. 2017). The minimum essential medium (2.26 g/L KH₂PO₄, 5.37 g/L K₂HPO₄-3H₂O, 2.53 g/L NaH₂PO₄-2H₂O, 3.34 g/ LNa₂HPO₄, 0.85 g/LNaNO₃, 0.66 g/L(NH₄)₂SO₄, 0.4 g/ LMgSO₄-7H₂O, and trace elements) was supplemented with either the PU substrate or Impranil DLN as required (Peng et al. 2014). The × 1000 trace element solution consisted of 5 g/L MnCl₂-4H₂O, 1.5 g/L CuSO₄-5H₂O, 50 g/L Na₂EDTA, 1.0 g/L (NH₄)₆Mo₇O₂₄-4H₂O, and 5.5 g/L CaCl₂. LBD agar plates were prepared by adding 1% Impranil DLN and 1.5% agar into Luria broth (LB; 10 g/L NaCl, 10 g/L tryptone, and 5 g/L yeast extract). The Tween 20 plates used for the qualitative enzyme assay of esterase consisted of 10 g/L peptone, 5 g/L NaCl, 0.1 g/L CaCl₂, 10 mL/L Tween 20, and 12 g/L agar powder. The urea phenol red plates used for the qualitative enzyme assay of urease consisted of 20 g/L urea, 5 g/L NaCl, 2 g/L KH₂PO₄, 1 g/L peptone, 1 g/L glucose, 0.012 g/L phenol red, and 15 g/L agar powder.

Screening method for the PU-degrading bacteria

Twenty soil and leachate samples obtained from the Xiaojianxi landfill were taken in sterile water, shaken, and left to stand; the supernatant was diluted appropriately to make a bacterial suspension. The suspension (50 μ L) was incubated in minimum essential medium containing 2 g/L yeast extract and 2 g/L PU powder at 30 °C and 150 rpm. After cell growth to stationary phase, 1 mL of culture was taken in a 1.5 mL Eppendorf tube and centrifuged at 12,000 g for 1 min to remove the supernatant. After washing three times with 0.85% saline, the cells were re-inoculated into minimum essential medium supplemented with 2 g/L PU powder as the sole carbon source at 30 °C and 150 rpm for screening. Cell growth was determined by measuring the OD₆₀₀ with a spectrophotometer (Shimadzu UV2500, Japan).

Continuous passage culture of the consortia

The continuous passage culture was established in minimum essential medium supplemented with 5 g/L PU powder as the sole carbon source, and each generation was incubated at 30 °C and 150 rpm for 60 h. After 60 h of incubation, the cells were collected and washed three times, and then re-inoculated into fresh medium with an initial OD_{600} of 0.1 for the next generation of culture.

Amplicon sequencing of 16S rRNA

The genomic DNA of the consortium was extracted according to the instructions of the genome extraction kit (TIANGEN BIO-TECH, China). The primers 338F (ACTCCTACGGGAGGC AGCAG) and 806R (GGACTACHVGGGTWTCTAAT) were used to amplify the V3-V4 region of the 16S rDNA with the Phanta Max Super-Fidelity DNA Polymerase (Vazyme, China) in a 50-µL final volume (Su et al. 2021). The PCR conditions were as follows: initial denaturation at 95 °C for 3 min; 30 cycles of denaturation at 95 °C for 10 s, annealing at 56 °C for 15 s, and extension at 72 °C for 1.5 min; then final extension at 72 °C for 10 min. The expected length of the PCR product was 1.6 kb. A total of 100 ng purified DNA product was prepared for amplicon sequencing using the NovaSeq platform (Allwegene, China). At least 40,000 reads were acquired for each sample. The sequence data has been deposited in the GenBank database under the Bio-Project accession number PRJNA888221.

Physical characterization of the degradation to the PU films

Scanning electron microscopy (SEM)

For SEM analysis, LB medium containing 3.3 g/L PU film was used for incubation of the microbial consortia. After 48 h of incubation, the PU film was removed and soaked in a 1:1 mixture of ethanol and tert-butanol for 15 min. The mixture was poured off, and the ethanol was replaced twice with pure tert-butanol for 15 min. The PU film was pre-frozen at -80 °C for 2 h and then freeze dried. The dried film was sprayed with gold, and the surface morphology was observed at $\times 2000, \times 10,000$, and $\times 20,000$ magnification with scanning electron microscopy (SEM; Quanta 250 FEG; FEI, USA). For comparison, the untreated PU film was employed as a control for the SEM analysis.

Fourier transform infrared (FTIR) spectroscopy

After 48 h incubation with the microbial consortium in LB medium, the PU film was removed and sonicated in a 1% SDS in distilled water for 15 min, then soaked in a 75% ethanol solution for 15 min. The naturally dried PU films were subjected to FTIR spectroscopy analysis with the Nicolet iS50 spectrometer (Thermo Fisher, USA) in attenuated total reflection mode from 500 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹. The functional groups in the FTIR spectra were identified and compared with the original PU film according to McCarthy et al. (1997).

Contact angle

The sample handling for contact angle determination was the same as for FTIR. The measurement was carried out by placing a drop of 10 μ L of distilled water onto the surface of the PU film (3 cm×6 cm), obtaining an image of the drop shape using a microscope head with the camera, and then using a digital image processing algorithm to calculate the contact angle (Rhim et al. 2006). Three biological replicates were analyzed for each microbial consortium.

Analysis of degradation products by high performance liquid chromatography (HPLC)

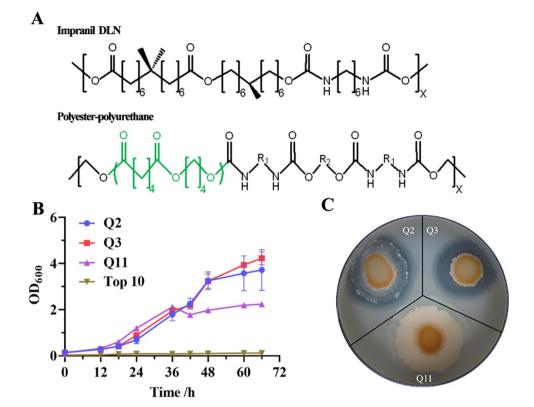
The concentrations of the degradation products adipic acid (AA) and butanediol (BDO) from the PU film were determined by HPLC as previously reported (NakajimaKambe et al. 1997; Shigeno and Nakahara 1991). A sample of the culture (1 mL) was taken at different time points during the incubation of PU film with the consortium. The supernatant was collected by centrifugation for 1 min at 4200 g. The HPLC system (Shimadzu, Japan) equipped with a differential refraction detector and an AminexR HPX-87H ion exchange column (300 mm \times 7.8 mm) was used for the sample analysis. The sample volume was 10 µL, and the mobile phase was 5 mM sulfuric acid at a flow rate of 0.6 mL/min. The detection time was 25 min, and the column temperature was 65 °C. Three biological replicates were analyzed for each experimental condition.

Enzymatic assays of the esterase and urease

Esterase

The esterase activity was qualitatively identified by incubating on Tween 20 plates. After 24 h of incubation in the LB-PU medium (LB medium supplemented with 5 g/L PU film), 20 µL culture was spread onto the Tween 20 plates and incubated at 37 °C with E. coli DH5a as the control. The Tween 20 substrate was catalyzed by esterase and formed a chelate with Ca²⁺ as a fatty acid calcium salt (Sierra 1957), which appeared as a white halo around the consortium. To quantify the esterase activity, 45 mL of culture grown in LB or LB-PU medium for 24 h was centrifuged at 8000 g for 20 min. The supernatant was concentrated to 1.5 mL using an ultrafiltration tube to obtain the crude enzyme. The esterase activity was quantified by absorbance at 410 nm using *p*-nitrophenyl palmitate (p-NPP) as the substrate, as previously reported with slight modifications (Peng et al. 2014): the 80 µL reaction mixture contained 8 µL crude enzyme, 3 g/L p-NPP, and 100 mM phosphate buffer, pH 7. The reaction was carried out at 37 °C for 15 min and then terminated by adding $80~\mu L$ of 95% ethanol. The absorbance at 410~nm was measured using a microplate reader (BioTek, USA). The amount of p-nitrophenol (µmol) produced per minute was defined as one esterase unit (U).

Fig. 1 The microbial consortia O2, O3, and O11 can degrade PU. A The proposed chemical structure of Impranil DLN and the polyester-polyurethane powder used in this study. The green color in the chemical structure of polyester-polyurethane represents the polyester polyol. **B** Growth curves of Q2, Q3, and O11 consortia in minimum essential medium with 5 g/L PS-PU powder as sole carbon source. Error bar indicates the standard deviation with three repetitions. C The degradation of Impranil DLN by Q2, Q3, and Q11 consortia. The clear areas in the Impranil DLN agar plates can demonstrate the degradation of Impranil DLN by the consortia



Urease

The urease activity was qualitatively identified using urea phenol red plates. After 24 h of incubation in LB-PU medium, 20 µL of the culture was spread onto plates and incubated at 37 °C with E. coli DH5a as the control. The urea substitute is catalyzed by urease and produces alkaline NH_4^+ , causing the pH to increase and the phenol red indicator to turn red, producing a pink color around the consortium. The urease activity was quantified by absorbance at 639 nm using urea as the substrate, as previously reported with slight modifications (Oceguera-Cervantes et al. 2007): the 60 µL reaction mixture contained 4 µL crude enzyme, 20 µL 5 M urea, and 36 µL 50 mM phosphate buffer, pH 7. The reaction was terminated by adding 20 µL of the urease developer I (70 g/L phenol, 3.4 g/L sodium nitrosocyanide) and 40 µL of urease developer II (14.8 g/L NaOH, 10 g/L NaClO) for 3 min at 37 °C. The absorbance at 639 nm was measured using a microplate reader (BioTek, USA). The amount of NH_4^+ (µmol) produced per minute was defined as one urease unit (U).

Results

Screening of PU-degrading microbial consortium

The diversity and function of the microbial consortia are frequently dependent on their habitats. Therefore, to screen

the microbes capable of degrading PU, we collected 20 soil and leachate samples from the Xiaojianxi landfill in Qingdao, and then shook them with sterile water. The supernatant was added to minimum essential medium containing both yeast extract and PS-PU powder for enrichment. PS-PU is composed of polyester polyol bonded to an isocyanic acid and then cross-linked by the chain extender. PU has a wide range of compositions, and the definitive components are not informed by the supplier. However, the proposed chemical structures of the PS-PU used in this study are shown in Fig. 1A. After 4 days of incubation, all the samples appeared significantly turbid, indicating microbial growth. The cultures were further enriched using minimum essential medium with PS-PU powder as the sole carbon source. After 66 h of incubation, a significant increase in biomass was found in three samples, labeled Q2, Q3, and Q11. To exclude the interference of residual cellular nutrients, these three samples were continuously passaged three more times, and the samples from each passaging could grow in minimum essential medium with 5 g/L PS-PU powder.

In the third round of passages, the OD_{600} of Q2, Q3, and Q11 gradually increased, whereas the *E. coli* TOP10 strain, used as the negative control, did not grow. After 60 h of incubation, the growth of the three samples reached stationary phase, with Q2 and Q3 growing better and the final OD_{600} of Q3 reaching approximately 4.0 (Fig. 1B). Microscopic analyses showed that all three microbial samples were composed of a variety of microorganisms.

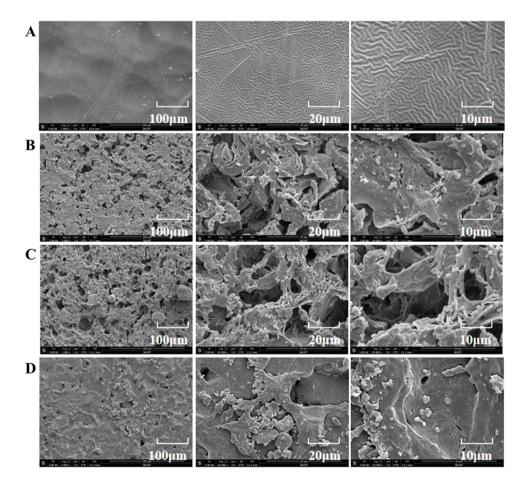
To further verify the degradation of PU by the three microbial consortia, the PU waterborne dispersion Impranil DLN was added to the LB medium to make an LBD agar plate. Impranil DLN is a colloidal PS-PU dispersion with estimated sizes in the sub-micrometer range. The proposed structure of Impranil DLN has been reported (Liu et al. 2021a) and is shown in Fig. 1A. When Impranil DLN is used as a substrate, the degradation by microbes is easily observed by the formation of clear areas on the agar plates (Liu et al. 2021a). When the microbial consortia were incubated on the LBD plates, clear areas were apparent around the Q2 and Q3 consortia, indicating degradation of Impranil DLN (Fig. 1C). Surprisingly, the Q11 consortium did not form clear areas on the LBD plate, even though Q11 was able to grow in liquid culture with PS-PU powder as the sole carbon source.

Characterization of PU film degradation by the microbial consortia

Commercial PU usually appears in the form of foams and films (Liu et al. 2021a). Therefore, PS-PU films were prepared to characterize the PU-degradation capability of the three consortia. After 48 h incubation, the PU films gradually fragmented and were degraded by all three consortia. SEM images showed that the surface of the PU film became rough and even formed holes (Fig. 2), indicating surface degradation (Alvarez-Barragan et al. 2016; Nakkabi et al. 2015). The initial untreated PU film was smooth and intact with only a few vein lines (Fig. 2). Q3 consortium caused the most damage to the PU film, suggesting that Q3 may have the highest PU-degradation activity, consistent with the results obtained from the growth in PS-PU powder.

The structural changes of the functional groups such as the representative ester, urethane, and amide in the PU can be observed through FTIR spectroscopy (Akutsu et al. 1998; Oprea and Doroftei 2011). FTIR analysis was used to characterize the structural changes in the PU films after degradation by the consortia. Compared with the non-inoculated control, several functional groups in the PU films had different intensities after incubation with the three consortia (Fig. 3). The peak at 1724 cm⁻¹, associated with the C=O stretch, decreased in the Q3 and Q11 consortia, indicating the hydrolysis of the ester functional group. The peak at 2918 cm⁻¹, associated with the aliphatic CH₂ stretch, decreased in all three consortia, suggesting a break in the carbon backbone. The peak at 1536 cm⁻¹, associated with the N–H stretch, decreased in intensity in all three consortia, implying the hydrolysis of urethane (Fig. 3). However, the alteration with FTIR spectroscopy is less

Fig. 2 Surface changes of the PU films observed by SEM after 48 h incubation with the consortia at $\times 2000$, $\times 10,000$, and × 20,000 magnification. A The initial untreated PU film. B PU film incubated with the O2 consortium. C PU film incubated with the Q3 consortium. D PU film incubated with the Q11 consortium. The initial untreated PU film was smooth and intact, whereas the surface of the PU film incubated with the consortia became rough and even formed holes



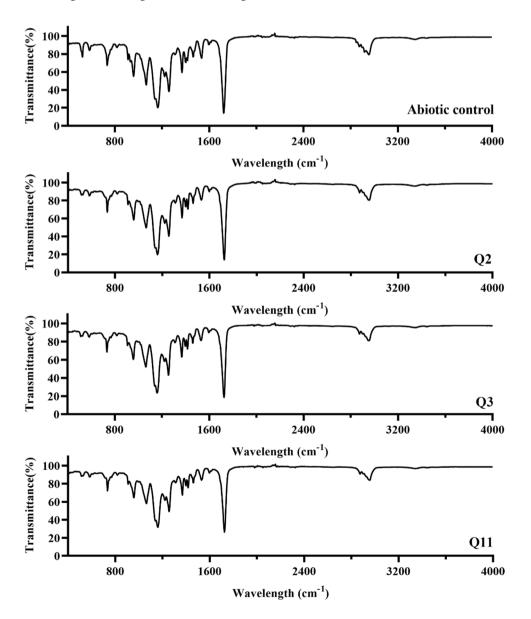
obvious and can only be used for qualitative analysis of functional group changes.

The contact angle can indicate the hydrophilic/hydrophobic properties of a material, and a larger contact angle indicates stronger hydrophobicity (Han and Krochta 1999; Uscategui et al. 2016). Compared with the control, the contact angles of the PU films decreased by different amounts after incubation with the three consortia (Table S1), indicating that the PU films were degraded and became more hydrophilic. When the films were incubated with the Q3 consortium, the contact angle had the highest reduction from $91.8^{\circ} \pm 0.7^{\circ}$ to $49.9^{\circ} \pm 0.9^{\circ}$, suggesting that Q3 may have the highest degradation performance.

More importantly, although there was a lag period of approximately 30 h, the Q2, Q3, and Q11 consortia were able to grow with 3 g/L PU films as the sole carbon source, whereas there was no significant change in the blank control (Fig. 4A). Among the three consortia, Q11 had the shortest lag period and the fastest initial growth rate, with an OD_{600} 3.5 after 114 h of incubation. While the initial growth rates of Q2 and Q3 were slower, the final biomass of Q2 was comparable to that of Q11, and Q3 had the highest biomass with a final OD_{600} of 4.0 (Fig. 4B).

The degradation of PU could generate AA and BDO (NakajimaKambe et al. 1997; Shah et al. 2013a, b). To analyze the small molecule monomers generated by biodegradation of the PU films, the supernatants of the consortia were analyzed by HPLC. After 24 h incubation, AA begin to accumulate in the Q2 and Q3 consortia (Fig. 5A). The concentration of AA continuously increased with incubation time and reached 315.2 and 436.5 mg/L in the Q2 and Q3 consortia, respectively, at 72 h, indicating that the PS-PU films were gradually degraded and released AA (Fig. 5A). The monomer BDO accumulated after 24 h incubation in the Q3 consortium and reached a maximum of 384.9 mg/L after 48 h. The concentration of BDO then started

Fig. 3 Comparison of the FTIR spectra obtained from the PU film incubated with the consortia and the abiotic control. The PU samples were analyzed after 48 h incubation with the microbial consortia at 37 °C and 150 rpm. The noninoculated medium was used as the abiotic control. The peaks at 1536, 1724, and 2918 cm⁻¹, representing the N-H stretch, the C = O stretch, and the aliphatic CH₂ stretch, were slightly different, indicating that the functional groups of the PU films changed after incubation with the consortia



to decrease to a final concentration of 212.3 mg/L after 72 h (Fig. 5B). The Q2 consortium had a similar BDO concentration curve as the Q3 consortium, but the Q2 consortium began to accumulate BDO after 36 h, slightly later than Q3 (Fig. 5B).

Interestingly, Q11 had a similar degradation of PU films as Q2 and Q3, as shown by the morphological changes and the consortia growth on PU films (Figs. 2 and 4B). However, the Q11 consortium did not have any accumulation of AA and BDO after incubation with the PU films, and the difference in the composition of the microbial consortia may have resulted in Q11 breaking different chemical bonds in the PU films, such as urethane, and producing different monomers.

Analysis of the microbial composition of the consortia

To enrich and analyze the composition of the microbial consortia involved in degradation of PU, the consortia were cultured in successive passages using minimum essential medium supplemented with 5 g/L PU powder as the sole carbon source. Taking 60 h of incubation as one generation, the three consortia were continuously passaged

for 50 generations, and PU-degrading consortia with stable microbial composition were obtained.

As shown in Fig. 6, the biomass of the consortia significantly fluctuated during the passage culture. For example, the OD600 of Q3 began to drop sharply after the fourth passage, and growth was restored by the 9th passage. After the 14th passage, the biomass decreased again until the 28th passage when growth was recovered; the biomass increased significantly from the 31th passage, and growth became stable after the 40th passage (Fig. 6). The fluctuation in biomass may be related to multiple factors such as the proportion of different microbesin the initial inoculation or domestication by the PU substrate. Overall, the biomass of the three consortia did not significantly change after the 40th passage, suggesting that the composition and proportions of the microbes tended to be stable.

To analyze the microbial compositions, amplicon sequencing of the 16S rRNA was performed for the three consortia before and after passage. At least 40,000 reads were obtained for each sample, sufficient to cover all microbes in the consortia. These sequences were classified into 80 operational taxonomic units (OTUs) by cluster analysis. Each consortium contained at least 38 OTUs, and 33

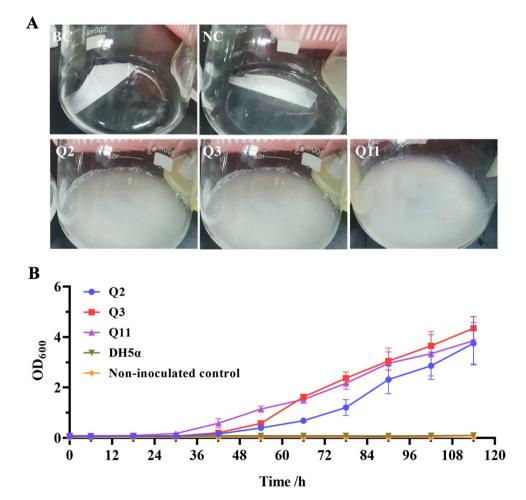


Fig. 4 The consortia can degrade and growth with the PU films. A After 114 h incubation, the three consortia could grow with the PU films as the sole carbon source, and the PU films were completely fragmented. In contrast, there was no significant microbial growth in the non-inoculated control (BC) and the E. coli DH5α control (NC) with the PU films remaining intact. B The growth curves of Q2, Q3, and Q11 consortia in the minimum essential medium with PU films as the sole carbon source. Error bar indicates the standard deviation with three repetitions

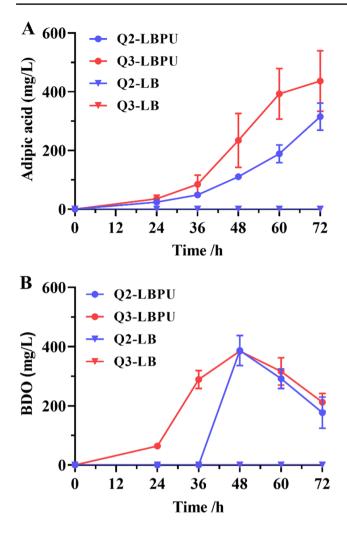


Fig. 5 Accumulation of the degradation monomers AA (**A**) and BDO (**B**) after incubating PU film with the consortia. PU film 3 g/L was added to the minimum essential medium and incubated with the consortia. Error bar indicates the standard deviation with three repetitions

OTUs were present in all three consortia (Fig. S1), indicating that the microbial composition of the three consortia was highly similar. However, there were also clear differences in the dominant microbes of each consortium (Fig. 7). Before passaging, the dominant microbe in Q2 and Q11 was Pseudomonas, accounting for over 60% of the total sequences. The most abundant microbe in the initial Q3 consortium was Sphingomonas, with a percentage of 49%. After passaging, the dominant microbe in each of the three consortia significantly changed. Specifically, the dominant microbes in Q2 changed from Pseudomonas to Cupriavidus, Pseudomonas, and Pigmentiphaga. In addition to the original Sphingomonas, Acinetobacter became a dominant microbe in the Q3 consortium after continuous passage for 50 generations. In the Q11 consortium, Pseudomonas decreased from 67 to 11%, and Cupriavidus increased from 10 to 59%.

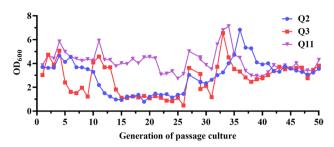
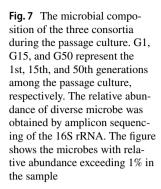


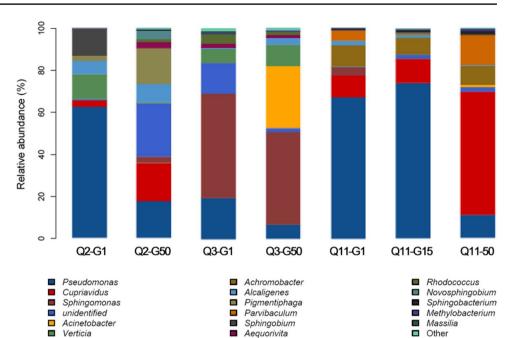
Fig. 6 Continuous passage culture of the consortia in minimum essential medium with 5 g/LPU powder as the sole carbon source. Each generation was incubated at 30 °C and 150 rpm for 60 h. After 60 h of incubation, the cells were collected and washed three times, and then re-inoculated into fresh medium with an initial OD_{600} of 0.1 for the next generation of culture. OD_{600} indicates the final cell density of the consortia after 60 h of incubation

The consortia secrete extracellular enzymes related to PU degradation

Biodegradation of PU relies on microbially produced enzymes, especially free enzymes secreted outside the cells (Magnin et al. 2020). Therefore, the supernatant of the consortia after incubation with PU film was collected, and extracellular proteins were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Compared with the control, the protein bands significantly increased in all three consortia after incubation with PU films, indicating that PU can induce the expression and secretion of certain proteins (Fig. S2). The molecular weight of proteins was largely distributed between 20 and 70 kDa, and these proteins are likely involved in the degradation of PU.

Esterase and urease may be involved in breaking the crucial chemical bonds in PU (Biffinger et al. 2015; El-Morsy et al. 2017; Phua et al. 1987; Ruiz et al. 1999). Therefore, we separately evaluated esterase and urease activities in the consortia using enzyme assay agar plates (Fig. 8). Compared with the negative control strain E. coli DH5 α , the Q2 and Q3 consortia had obvious white halos when grown on Tween 20 plates, indicating the presence of esterase, whereas Q11 did not show this pattern. In contrast, only the Q11 consortium turned red on the urea phenol red plate, indicating that urea was degraded and generated alkaline NH₄⁺, whereas Q2 and Q3 consortia did not show obvious urease activity. These may explain that O2 and O3 consortia can degrade the soft segment polyols of PU with esterase and produce AA and BDO, whereas Q11 only had urease activity that degraded the hard segment of PU and could not degrade polyols to AA and BDO because it lacked esterase. Furthermore, since the definitive degradation products were not detected, we cannot exclude the possibility that Q11 degraded the additives added to the PU.





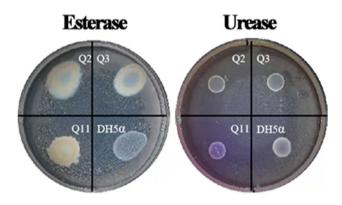


Fig. 8 Qualitative identification of esterase and urease activities in the consortia using the enzyme assay agar plates. After 24 h of incubation in the LB-PU medium (LB medium supplemented with 5 g/L PU film), 20 μ L culture was spread onto the plates and incubated at 37 °C with *E. coli* DH5 α as the control. The esterase activity was determined using the Tween 20 plate. The Tween 20 substrate can be catalyzed by the esterase and forms the chelate with Ca2⁺, which appeared as a white halo around the consortium. The urease activity was determined using the urea phenol red plate. The urea substitute can be catalyzed by urease and produces alkaline NH₄⁺, causing the pH to increase and the phenol red indicator to turn red, producing a pink color around the consortium

To further analyze PU degradation-related enzymes, the activity of esterase and urease in the three consortia was quantitatively measured (Fig. 9). Both Q2 and Q3 secreted esterase regardless of the presence of PU, whereas Q11 had almost no esterase activity (Fig. 9A).

For the urease activity, there are some inconsistencies between the urease activity of the crude enzyme supernatant and the plate activity assay. Specifically, the supernatants of all three consortia showed varying degrees of urease activity, and the activity of Q2 and Q11 decreased with the addition of PU film, whereas Q3 showed an increase in urease activity with the addition of PU film (Fig. 9B). However, in the plate assay, only Q11 consortium turned red around, indicating a significant urease activity. Q2 and Q3 consortia did not appear to show obvious urease activity (Fig. 8). We speculate that the difference may be due to the sensitivity of various analysis methods, the amount of enzyme secreted under liquid or solid culture conditions, etc. Nevertheless, the identification of potential urease among the consortia that may degrade PU is required in the following work.

Discussion

In this study, we screened three microbial consortia from landfill leachate that efficiently degraded PS-PU film within 1 week. Microbial consortia are usually more effective than pure cultures in degrading natural polymers, especially PU waste with complex compositions and multiple chemical bonds, which may be attributed to the collaboration between multiple organisms (Vargas-Suarez et al. 2019, 2021). Obvious physical and chemical changes in the PU film were observed after incubation with the consortia. First, the microbes in the consortia caused visible breaks and holes in the PU films. Second, FTIR spectroscopy results indicated possible hydrolysis of the ester and urethane functional groups, as well as breakage of the carbon backbone in the PU film after incubation. Similar changes in PU after microbial incubation have been previously reported (Fuentes-Jaime

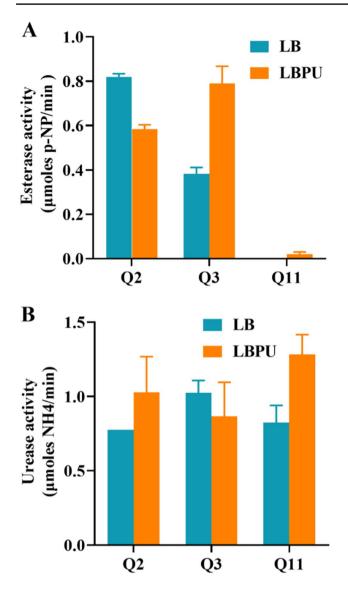


Fig. 9 Quantitative assay of the esterase (**A**) and urease (**B**) activities for the three PU degrading consortia. The crude enzyme used for the activity assay was prepared by concentrating 50 mL of consortium supernatant to 1.5 mL using the Millipore ultrafiltration tube. Esterase activity was assayed with 3 g/L p-nitrophenyl palmitate (p-NPP) as substrate and measured at 410 nm. Urease activity was determined with 1.6 M urethane as substrate and measured at 639 nm. Three replicate experiments were performed for each consortium, and average values with standard deviation were presented

et al. 2022; Shah et al. 2013a), further implying that the consortia can attack specific groups in PU and cause degradation. More importantly, the consortia secreted potential PU-degrading enzymes including esterase and urease. Esterase is the most prevalent category of enzyme involved in PU degradation and results in the release of carboxylic acids and alcohols (Magnin et al. 2020). Some purified esterases such as PU esterases A (PueA) and B (PueB) from *Pseudomonas chlororaphis* and PU esterase PudA from *Comamonas* *acidovorans* (Howard et al. 2001; Nomura et al. 1998) have been shown to degrade PU, suggesting that the presence of specific esterase in the consortia played an essential role in PU degradation. Because enzyme activity was determined using the crude enzyme in the supernatant, and the results could be interfered with by many factors such as protein secretion, culture medium components, and the other secreted proteins; therefore, these results can only be used for a rough analysis. Identifying the key enzymes related to PU degradation and performing more detailed enzymatic characterization should be considered for future work.

Compared with pure bacteria, consortia can not only effectively degrade PU but also use the degradation products to grow rapidly. The consortia could grow using PU powder or films as their sole carbon source, and the cell density indicated by OD_{600} of the Q3 consortium reached above 4.0 after 114 h of incubation (Fig. 4B). However, we also observed that the growth state of the consortia was not stable with PU as the sole carbon source, especially for Q11 that had significant fluctuation in biomass during successive passages (Fig. 6). The biodegradation and assimilation of PU may require the collaboration of multiple microorganisms, and the microbial composition significantly changed during the multiple passages of the culture using PU films as the sole carbon source. The change in the microbial abundance may imply an adaptation of the consortium to the PU substrate, which establishes a functional balance between depolymerase generation and PU-degrading monomer assimilation of the consortium ecosystem. To clarify this point, subsequent work should explore the cooperative relationship between different microorganisms and the key role of pure cultures in the consortium.

PU has a complex and variable composition and structure; therefore, clarifying the composition of degradation products from PU is important for the study of the biodegradation process. We detected the polyester polyol monomers AA and BDO in the PU cultures by HPLC, similar to what was previously reported for PU biodegradation (NakajimaKambe et al. 1997; Shah et al. 2013a). However, there may be other small molecules produced from the biodegradation of PU that were not detected. Searching for PU substrates with more defined components and identifying other small molecules derived from PU degradation using gas chromatography–mass spectrometry or HPLCmass spectrometry will rule out the possibility that additives present in PU are being degraded and will identify the key enzymes involved in PU biodegradation.

Finally, the extent of degradation of the PU film by these three consortia was quite significant compared with previous reports based on SEM and cell growth (Cregut et al. 2013; Jin et al. 2022). This indicates that there is great potential for biodegradation of PU and biotransformation into high market-demand bioproducts and bioplastics. Further research on pure microbes from the consortia and the key enzymes associated with PU degradation is required to gain insight into the biochemical mechanisms of PU biodegradation and to further enhance biodegradation efficiency.

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

Declarations

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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