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Rapid biodegradation of renewable polyurethane foams with identification of associated microorganisms and decomposition products

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ABSTRACT

The goal of this research was to determine if we could develop commercially-relevant polyurethane products that can biodegrade in the natural environment. We developed polyester polyols from algae oils and formulated those into polyurethane foams that meet the standards for footwear, while maintaining a chemical structure that would allow them to biodegrade. These foams were incubated in compost and soil, and lost 30% and 71% mass, and 41% and 71.5% compression force, respectively, after 12 weeks. Several bacteria and fungi grew abundantly on the polyurethane and we were able to isolate microorganisms from compost and soil capable of growth with polyurethane as the sole carbon source. Scanning Electron Microscopy and Imaging Mass Spectrometry were used to visualize biodegradation activity. Enzymatic hydrolysis confirmed that breakdown products were reproductions of the original monomers. These results demonstrate that it is possible to create polyurethane products that have an end-of-life biodegradation option.

1. Introduction

Over the last 50 years, humans have generated over 6 billion metric tons of plastic waste. Of this, only about 9% was recycled, 12% was incinerated, and 79% was left to accumulate in landfills or the natural environment. If current production and waste management trends continue, roughly 96 billion tons of plastic waste will be in landfills or in the natural environment by 2050 (Geyer et al., 2017). In addition to the large carbon footprint and water consumption of petroleum and plastic production, the overwhelming majority of plastics endure in the environment for hundreds of years, where they can degrade into microplastics that are first consumed by primary consumers, but eventually move up the food chain, interfering with metabolism, reproduction, and increased mortality rates (Anbumani and Kakkar, 2018; Betts, 2008; Masnadi et al., 2018; Moore, 2008; Sun et al., 2018). The adverse effects of petroleum-based plastic production on health and the environment are well-documented. Pollution emitted during petroleum drilling, refining, and waste management practices leads to cardiovascular disease and respiratory conditions, whereas toxic emissions or leachables from degrading plastics act as endocrine disruptors and can cause cancer (Azoulay et al., 2019). Given these deleterious environmental effects, there is a need for plastics that can be sourced from renewable feedstocks and can undergo biodegradation at the end of their useful life.

A potential source for plastic precursors is algae, which have fast growth rates under photosynthetic conditions and can be grown with non-potable water and non-arable land (Doron et al., 2016). Additionally, algae possess unique metabolic pathways for producing hydrocarbons that can be converted into valuable chemicals, including plastic precursors (Roesle et al., 2014; Phung Hai et al., 2020).

Among the wide variety of materials used to make plastics, polyurethane (PU), especially polyester PU, have the potential to be renewably sourced and subsequently biodegraded (Howard, 2002; Nguyen et al., 2010; Sonnenschein, 2014). Unlike many other polymers, they can be synthesized from a diverse set of monomers. A typical PU contains repeating urethane bonds produced by linking a polyol, a hydrocarbon with at least two hydroxyl groups, and a diisocyanate, but the identity of the polyol or the molecule carrying the isocyanates can vary widely in composition and size, making PUs an ideal canvas for innovation in renewable and biodegradable plastics.

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Previous studies have demonstrated that polyester PUs, in which the polyol contains repeating ester linkages, can degrade under various chemical and biological conditions through cleavage of the ester bonds (Osman et al., 2018). Several chemical methods, including hydrolysis and glycolysis with alcohols, are proven to cleave polyesters (Sheel and Pant, 2018; Wu et al., 2003). In previous literature, various bacteria and fungi have been found to degrade polyester PUs through the use of esterase, urease, amidase, and protease enzymes (Magnin et al., 2019). PU biodegradation is largely controlled by the activity of secreted or surface-bound enzymes and their ability to access the soft segment ester groups (Santerre and Labow, 1997). In particular, esterase enzymes, such as lipases and LC cutinases, have been shown to hydrolyze polyester PU bonds (Howard, 2002). However, the possibility of using enzymes to fully depolymerize a product remains understudied. Developing a PU that consists of ester and urethane bonds that can be naturally hydrolyzed establishes a practical way to depolymerize PU products, resulting in biodegradation in natural environments. Studying the microbial community responsible for PU degradation and monitoring the potential breakdown products allows us to examine how these biodegradation processes occur, enabling future innovation in sustainable plastic production.

In this study, we used a multi-disciplinary approach to analyze microorganismal degradation of a renewable, commercially-relevant PU product. We began with algae-based polyester polyols that were formulated into PU foams to meet specifications for real-world commercial products, specifically flip-flop footbeds and shoe midsoles (Fig. 1). This PU foam had a 52% biological content by mass, as the isocyanate used was derived from petroleum sources. Degradation of the PU was examined under two conditions: through exposure to compost and soil environments, and through in vitro enzymatic hydrolysis. Utilizing new technology, such as metagenomic sequencing, mass spectrometry, and various imaging tools, gave us an in-depth analysis of the chemical and biological processes taking place during the PU biodegradation. In this paper we demonstrate rapid biodegradation of high performance PU foams in several environments, identify consortia of organisms responsible for consuming the PU, and determine the primary molecular breakdown products of our foams, including intact PU monomers that reveal the potential to use polyester PU to create commercial products that are sustainably-sourced, biodegradable, and potentially recyclable.

2. Materials and methods

2.1. Environmental Incubation of polyurethane cubes

PU foam composed of algae-based, linear polyester polyols, a diacid, and 4,4′-methylene diphenyl diisocyanate (MDI), was polymerized in a 2 cm-thick mold and cut into 2 cm cubes. These were subject to incubation in two separate environments: compost and soil. Cubes were weighed and compression-tested prior to being incubated in containers filled with either compost or soil material, and placed at 30 °C under continuous high humidity. A set of control cubes were tested in empty containers under the same incubation conditions in order to confirm that physical degradation occurred as a result of exposure to the compost or soil and not simply to the high humidity condition. Compost was collected from UCSD Roger's Community Gardens and soil was collected from UCSD Solis Hall.

2.2. Physical analysis of degrading PU foam samples

Physical tests were conducted on the compost and soil-incubated cubes in triplicates at 0, 4, 8 and 12 weeks. Cubes were washed with MilliQ water three times and dried at room temperature overnight before measuring mass and compression force deflection (CFD). Samples were compression-tested according to ASTM D3574 C- 50% Compression Force Deflection using an AFG 2500N compression tester (MecMesin Inst) equipped with a MultiTest-dV sample stage at a rate of 100 mm/min for 10 cycles (ASTM International, 2017). Data was reported for the 10th cycle for all cubes. Initial, pre-degradation mass and CFD measurements were taken prior to incubation and marked accordingly to monitor specific cubes and account for minor variations between cubes. The mass loss and maximum force were normalized as a percentage of initial, pre-degradation values.

2.3. Metagenomic sequencing to identify organisms associated with biodegradation

PU cubes were removed from the incubation environments at 4, 8 and 12 weeks and cut into approximately 2 × 2 × 1 mm sections with a sterile razor. Sections from the exterior and interior surfaces of the cube were used for separate sequencing analyses. Compost and soil material, marked in Fig. 3 as environment, were collected at the same time points and analyzed in the same manner as background analyses. The DNA library was prepared following the Earth Microbiome Project protocols

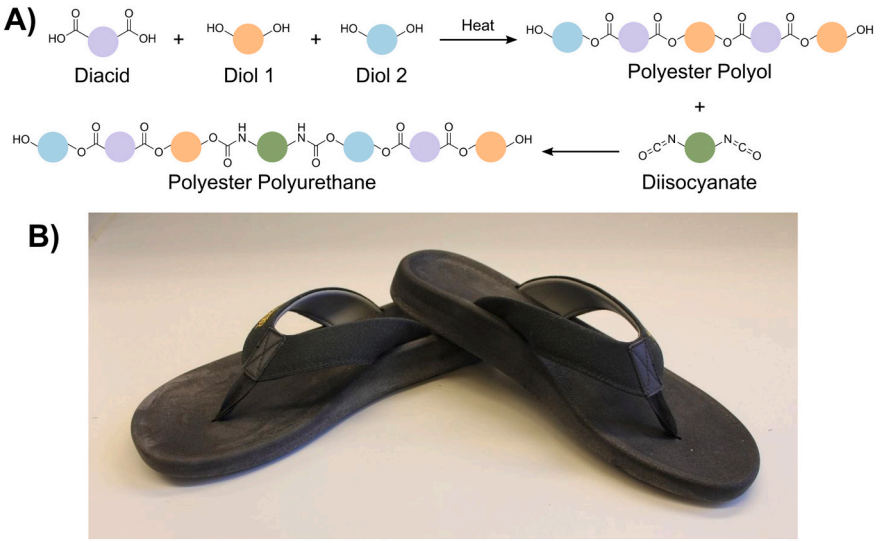

Fig. 1. A) General polyester polyol and polyurethane (PU) syntheses and structure. **B)** Algenesis algae-based PU flip-flop prototype.

for 16S (bacteria) and ITS (fungi) sequencing (Thompson et al., 2017). DNA was extracted with Qiagen MagAttract KingFisher PowerSoil DNA Kit (cat #: 27000-4-KF) with 0.15 g to 0.20 g starting material. The final pool was cleaned using Promega Wizard SV Gel and PCR Clean-Up System (cat#: A9281). Next-Generation Sequencing was performed on the Illumina MiSeq system with 2 × 250 bp reads. The sequencing was conducted at the IGM Genomics Center, University of California, San Diego, La Jolla, CA. Sequences were analyzed using the QIIME 2 pipeline and reads were trimmed based on quality dropoff–220 bp for 16S reads and 230 bp for ITS reads (Bolyen et al., 2019). Reads were then classified based on reference libraries from SILVA for 16S and UNITE for ITS (Quast et al., 2013; Nilsson et al., 2018).

2.4. Isolation of microorganisms capable of utilizing PU as sole carbon source

To identify organisms from compost and soil capable of utilizing the PU to grow, organisms were inoculated into M9 minimal media, designated in the study as minimal media, with PU as the sole carbon source. PU foam was frozen in dry ice and pulverized with a high-speed blender to create fine particulates and then autoclaved to ensure sterility. In 125 mL Erlenmeyer flasks, 25 mL of minimal media and 0.5 g PU particulate were prepared. 1 g of material from either compost or soil, designated as the inoculum, was added to separate flasks, along with environmental control flasks with inoculum but no PU and a control flask with PU minimal media but no inoculum. Flasks were shaken at 100 rpm at room temperature. 1 mL of the liquid in the flask was then used to inoculate a fresh minimal media flask for the subsequent passage. Fresh flasks were inoculated weekly for 8 weeks and then bi-weekly at weeks 10 and 12 for a total of 10 passages. At the end of each passage, 1:1000 and 1:10,000 dilutions were prepared and 50 µL was plated onto lysogeny broth (LB) and potato dextrose agar (PDA) media and grown at room temperature for 48 h. Individual colonies with unique morphology were picked from each plate from the 10th passage. ThermoFisher Phire Plant Direct PCR Master Mix (cat# F160S) with appropriate primers for 16S (515F, 806R) and ITS1 (ITS1-F, ITS2) were used to PCR amplify each selected colony (Parada et al., 2016; Apprill et al., 2015; Gardes and Bruns, 1993; White et al., 1990). The samples were sent to Eton Biosciences for Sanger sequencing.

2.5. Scanning Electron Microscopy of biodegrading PU

PU cubes were cut with a sterile razor into 1–2 mm thick slices, approximately 4 × 4 mm in size, and autoclaved. For each inoculant, one slice from the open-celled interior and one slice from the closed-cell exterior surface (skin) were prepared. Each set of slices was placed in 15 mL culture tubes with 5 mL of minimal media and inoculated with 500 µL of liquid culture, as described in the Supplementary Methods. The samples were incubated shaking at room temperature for 72 h. To fix samples after incubation and prior to imaging, PU slices were placed in a gradient of 55%, 70%, 85% and 100% ethanol for 10 min in each concentration of ethanol based on suggestions for SEM bacteria imaging in the literature (Kalab et al., 2008). After attaching the foam slices to aluminum stubs using carbon tape, an Emitech K575X Sputter Coater was used to deposit an iridium layer on the foam slices, excess of which was dusted off with compressed air prior to imaging. All samples were imaged at high vacuum using an FEI Quanta FEG 250 scanning electron microscope, at magnifications ranging from 600× to 20,000×. Each micrograph, including controls, was visually inspected for the presence of fungal and bacterial colonies, as well as changes in topography of the foam.

2.6. Imaging Mass Spectrometry of biodegrading PU films

Minimal media agar plates covered with a thin film of PU, designated here as PUM9 film-agar plates, were prepared as described in the Supplementary Methods. To allow microbes access to the minimal media nutrients under the PU film, a small rectangular section was excised from the film using a sterile razor. These exposed agar sections were inoculated with 200 µL samples from the 8th passage soil and compost shaker flasks from Section 2.4 and grown in a humidified container at room temperature for a week, then transferred to a MALDI target plate and analyzed via MALDI-TOF IMS according to standard protocols (Yang et al., 2012). See Supplementary Methods for further details.

2.7. PU foam sample preparation for enzymatic degradation

PU foam was frozen using liquid nitrogen and crushed with a Qiagen TissueLyser (cat# 85300). Foam particulate was washed with MilliQ water to remove any soluble contaminants and then dried overnight in a desiccator. Initially, four commercial enzymes were screened for PU biodegradation activity: lipase from *Aspergillus niger* (cat# 62301), lipase from *Candida rugosa* (cat# L1754), esterase from *Bacillus subtilis* (cat# 96667) and cholesterol esterase from *Pseudomonas* species (cat# C9281). Bovine serum albumin (BSA) (cat# 9048-46-8) was used as a negative control. Foam was added to 1.5 mL Eppendorf tubes to a mass of 2.4 ± 0.2 mg. 1.2 mL of 400 µg/mL enzyme solution in phosphate-buffered saline (PBS) was added. All samples were prepared in triplicate. In addition, tubes containing enzymes without foam and foam without enzymes were prepared as controls. Samples were shaken for 24 h at 37 °C, then frozen immediately to prevent further enzyme activity.

2.8. Identification of PU degradation products using Gas Chromatography–Mass Spectrometry and Liquid Chromatography–Mass Spectrometry

For Gas Chromatography–Mass Spectrometry (GCMS), samples were acidified and the products were extracted with ethyl acetate (EtOAc), derivatized with MSTFA, and run on a GCMS similar to the method described in (Gautam et al., 2007). Chromatograms were created by subtracting the no-substrate control from an average of the triplicate sample chromatograms. 0–100 ppm standards containing diol 1, diol 2, and diacid 1 diluted in PBS were treated to the same extraction and GCMS method. Each peak was integrated using the instrument's integration tool. The mass percent of each compound in the proprietary PU foam was known. From these values, the expected concentration of each compound at 100% PU foam degradation in parts per million ($C_{100\%\ degradation}$) and the percent degradation of the PU foam from each product ($m/m\%_{product}$) was calculated according to formulas 1 and 2, where $m/m\%_{compound}$ is the mass percent of compound in the foam formulation, m_{foam} is the mass of foam added to the enzyme sample, m_{sample} is the total enzyme sample mass, and $C_{product}$ is the product concentration in the enzyme sample. Values are reported to a 95% confidence interval.

$$C_{100\%degradation} = (m/m\%_{compound}) \times (m_{foam}/m_{sample}) \times 10^6 \quad (1)$$

$$m/m\%_{product} = (C_{product}/C_{100\%degradation}) \times 100\% \quad (2)$$

For Liquid Chromatography–Mass Spectrometry (LCMS), a 50 ppm standard containing all four expected breakdown products was prepared by gentle heating in MilliQ water for several hours until complete dissolution. The standard and triplicates of the *Pseudomonas* enzyme degraded PU samples and BSA controls were filtered with a 0.22 µm syringe filter. 10 µL was injected into a Waters Acquity SQD LCMS system with a 2.1 × 150 mm 3 µm ACE C18-PFP column. The running buffers were 0.1% formic acid at pH 3.35 (A) and 100% ACN (B). The run was held at 99% A at 0.2 mL/min for 7 min, then ramped to 100% B over the course of 5 min and held for 8 min before returning to the original eluent conditions over 1 min and held for 5 min to re-equilibrate. The MS had an ESI probe set at 3.5 kV and 350 °C with positive

mode scan ranges from 70 to 500 m/z. Triplicate mass traces were averaged and the average traces for enzymes incubated with no foam were used for background subtraction.

2.9. Calculation

For cube degradation mass and CFD measurements, all data were performed in triplicate. We determined p-values using a two-sided Welch's t-test with the null hypothesis that the control and sample cubes had the same mean and variance (Ruxton, 2006). For metagenomic sequencing of compost- and soil-incubated PU cubes, the ratio, I:B, was used to calculate the fold-change of the relative abundance of each genus in the PU interior compared to the background environment at 12 weeks, according to Eq. (3).

$$I{:}\ B = (\%Abundance_{PU\ interior})/(\%Abundance_{background\ environment}) \quad (3)$$

Log-scaled scatter plots were used to compare the abundance of the top 10 species over time in the PU interior and abundance in the background environment. For identification of the enzyme degradation products from the in vitro samples, experiments were performed in triplicate and the uncertainty calculated to a 95% confidence interval. One sample was further hydrolyzed to identify the total percentage of the PU solubilized by the enzyme catalysis. Error values for this percentage were reported to a 95% confidence interval and were calculated from the error in the calibration curves for each monomer according to Eq. (4), where S_{Ca} is the uncertainty in the concentration, S_r is the standard deviation about the regression, b is the slope, m is the number of samples, n is the number of standards, S_{samp} and $\bar{S}_{std}$ is the sample signal and mean standard signal, C_{stdi} is the concentration of the standards, and $\bar{C}_{std}$ is the mean standard concentration.

$$S_{C_a} = S_r/b\sqrt{1/m + 1/n + \frac{(S_{samp} - \bar{S}_{std})^2}{b^2 \sum_i (C_{std_i} - \bar{C}_{std})^2}} \quad (4)$$

3. Results and Discussion

3.1. Environmental Incubation of PU cubes

PU foam cubes showed marked degradation after incubation in compost and soil, compared to controls (Fig. 2A). The top and bottom of all pre-incubation cubes had a smooth and closed-cell exterior skin, while the four sides of the cubes were cut to expose the porous interior. Over the course of the 12 weeks all six sides became noticeably porous, to the point where there was little to differentiate the sides from the top and bottom. The color also varied between the controls and incubated samples. It is important to note that while yellowing is a typical sign of PU degradation, some of the color difference may have come from the organisms or substances in the environments themselves (Rosu et al., 2009).

3.2. Physical analysis of degrading PU foam samples

In addition to visible observations, mass loss and reduction in CFD occurred over time from the degraded PU cubes in Section 3.1. Triplicate measurements normalized against pre-degradation values for each cube are pictured in Fig. 2B and C. After 12 weeks, samples in compost lost 30 ± 3% mass and 41 ± 3% CFD. Samples in soil showed greater degradation with 71 ± 9% mass loss and similar 71.5 ± 0.8% CFD decrease. The decrease in maximum force over time can be partially explained by the loss in mass through a change in surface area where the force is applied and a change in surface density, resulting in more stress per molecule and therefore a lower force. Decreased chain length, physical orientation of the remaining polymer chains, and degraded PU cell structure within the cubes may also be a factor in the CFD decrease over time.

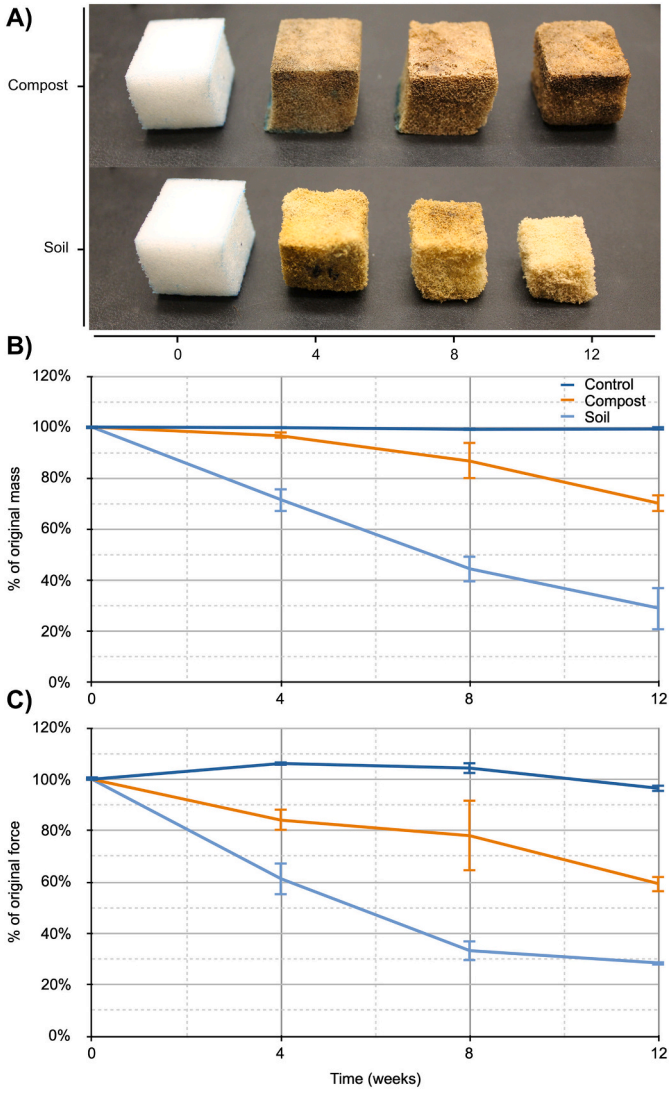

Fig. 2. Biodegradation of PU cubes over 12 weeks. Degradation was analyzed through **A)** change in appearance, **B)** cube mass, and **C)** maximum force at 50% compression force deflection (CFD). Error bars indicate sample standard deviations of the triplicate measurements. For compost and soil mass loss, $p < 0.01$, and for compost and soil CFD, $p < 0.01$ (Table S2).

3.3. Metagenomic sequencing to identify organisms associated with biodegradation

To identify the consortia of bacteria and fungi present on the PU cubes as mentioned in Section 3.1, 16S and ITS metagenomic sequencing was employed. After 12 weeks in compost and soil, several microorganisms were present in greater abundance on the PU interior and PU exterior than in the background microbial community, as highlighted in Fig. 3. An uncharacterized fungus, which was heavily present in both compost and soil, was most similar to a soil fungus with GenBank accession number JX489840.1 (Zhou and Wu, 2012). Table 1 highlights which bacteria and fungi are enriched on the PU interior at 12 weeks by analyzing the fold-change of the relative abundance of each genus in the PU interior from their relative abundance in the background environment. Notably, bacteria *Pigmentiphaga*, *Roseomonas*, and *Phenylobacterium*, and the fungus, *Arthrographis* were greatly enriched in the PU from both compost and soil environments. Previous literature supports this finding. The species *Pigmentiphaga daeguensis* has been shown to biodegrade aniline, which is a precursor to PUs and other materials (Huang et al., 2018). *Roseomonas* sp. has been used to

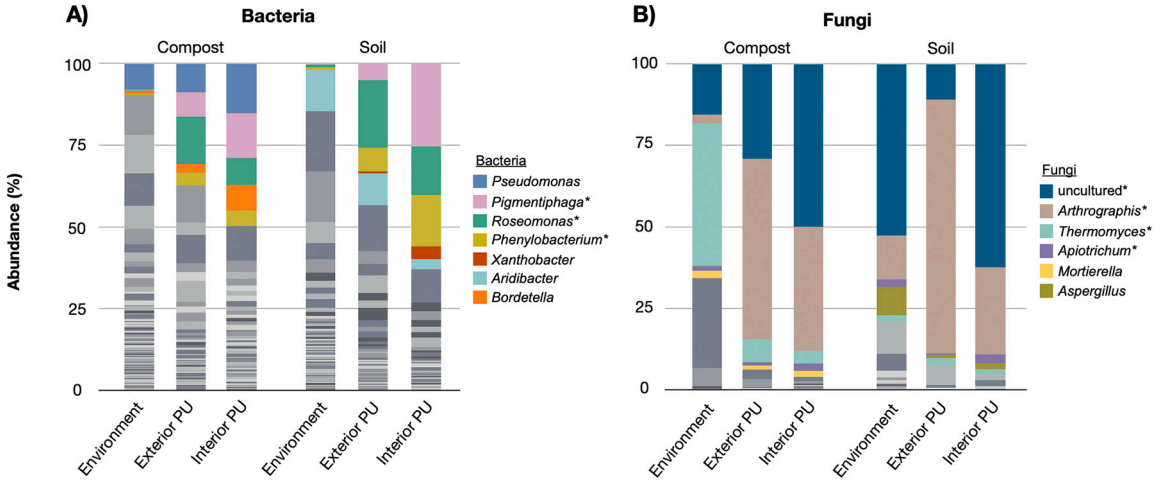

Fig. 3. Biodiversity associated with biodegradation of PU in compost and soil by **A)** bacteria and **B)** fungi at 12 weeks. Genus-level analysis of the environment, the exterior surface of PU and the interior section of PU were compared. The 5 most abundant genera found on the PU interior of each sample are colored and labeled. *Found in both compost and soil samples.

Table 1
List of top 5 bacterial (16S) and fungal (ITS) genera in compost and soil and the fold-change of their relative abundance in the PU interior from the background environmental media. I:B is the ratio of relative abundance in PU interior to relative abundance in background environment. n/a: *Xanthobacter* showed no abundance in the media and 3.84% abundance in interior PU at 12 weeks. ❖ I:B ≥ 1.5, at least 50% greater relative abundance in PU interior compared to background environment.

	Top 5 bacteria		Top 5 fungi	
Environment	Genus	Fold change (I:B)	Genus	Fold change (I:B)
Compost	*Pseudomonas*	1.95❖	Uncultured	3.24❖
	Pigmentiphaga	211.08❖	*Arthrographis*	13.06❖
	Roseomonas	19.88❖	*Thermomyces*	0.09
	Bordetella	9.36❖	*Apiotrichum*	1.49
	Phenylobacterium	21.68❖	*Mortierella*	0.72
Soil	*Pigmentiphaga*	504.60❖	Uncultured	1.19
	Roseomonas	18.11❖	*Arthrographis*	2.00❖
	Phenylobacterium	29.11❖	*Apiotrichum*	1.39
	Xanthobacter	n/a❖	*Aspergillus*	0.22
	Aridibacter	0.25	*Thermomyces*	1.00

Table 2
List of bacteria enriched from compost and soil, which were able to survive using PU as a sole carbon source after 10 serial passages.

Environment	Family	Genus
Compost	*Burkholderiaceae*	*Achromobacter*
	Brucellaceae	*Brucella*
	Pseudomonadaceae	*Pseudomonas*
	Rhizobiaceae	*Rhizobium*
	Xanthomonadaceae	*Stenotrophomonas*
Soil	*Flavobacteriaceae*	*Chryseobacterium*
	Oxalobacteraceae	*Herbaspirillum*
	Brucellaceae	*Ochrobactrum*
	Nocardiaceae	*Rhodococcus*
	Xanthomonadaceae	*Stenotrophomonas*

remediate crude-oil contaminated soil in a consortium of bacteria, with 52.1% crude-oil removal (Zhao et al., 2011). Similarly, *Phenylobacterium* has been used as an effective petroleum degrader in contaminated soil (Huang et al., 2019). Additionally, *Arthrographis kalrae* has been found to be a dominant species on composted polyester PUs (Zafar et al., 2014). It is likely that organisms able to degrade hydrocarbon structures in other polymers possess enzymes that can cleave ester and urethane bonds in polyester PUs. The loss of structural integrity of the PU over the 12 weeks was most likely a result of the growth of organisms that were enriched on the PU.

3.4. Isolation of microorganisms capable of utilizing PU as sole carbon source

To enrich and isolate microorganisms responsible for degradation of the foams, we serially-passaged microbial samples derived from soil or compost through 10 passages in minimal media, where PU was the sole carbon source. Plates of the cultures showed that consortia of organisms from compost and soil were surviving, while control flasks lacking PU had few surviving organisms, indicating that the PU was being used as a nutrient source. Notably, the surviving organisms (Table 2) are different from the top organisms found in Section 3.3, with the exception of *Pseudomonas* from compost. It is expected that organisms able to adapt to use the PU for energy may not be the same as those able to grow in a natural environment. By the 10th passage, no fungi were found to survive in the consortium with PU as the sole carbon source. Since we did not observe significant enrichment of fungi over time in Section 3.3, it is possible the same occurred here; however, fungal communities have been shown in previous literature to biodegrade PUs in soil environments as well as in liquid cultures (Filip, 1979; Cosgrove et al., 2007). Rather, a potential explanation for this finding is that bacteria outcompeted fungi in the consortia.

Several of these bacteria genera have been shown in literature to degrade plastic products and precursors. Recently, a strain of *Pseudomonas* has been shown to grow on several PU precursors and intermediates, and the enzymatic pathways were studied by genetic analysis (Espinosa et al., 2020). *Rhodococcus* species have been found to degrade crude-oil contaminated soils (Hamamura et al., 2006). A *Chryseobacterium* has been isolated that was capable of degrading polyethylene (Jeon and Kim, 2014). *Rhizobium* has been found to degrade phthalate esters (Tang et al., 2016).

3.5. Scanning electron microscopy of biodegrading PU

SEM imaging provided a qualitative look at the organisms' interactions on the surface of the PU foam. Inoculants from both soil and compost passaging experiments were imaged on the PU surface (Fig. 4). Observations of the soil-isolated bacteria on the closed-cell skin surface of the PU showed an abundance of open-celled areas with bacteria, suggesting the ability for bacteria to degrade and penetrate the skin of the PU (Fig. 4B). We also observed the presence of biofilms, which

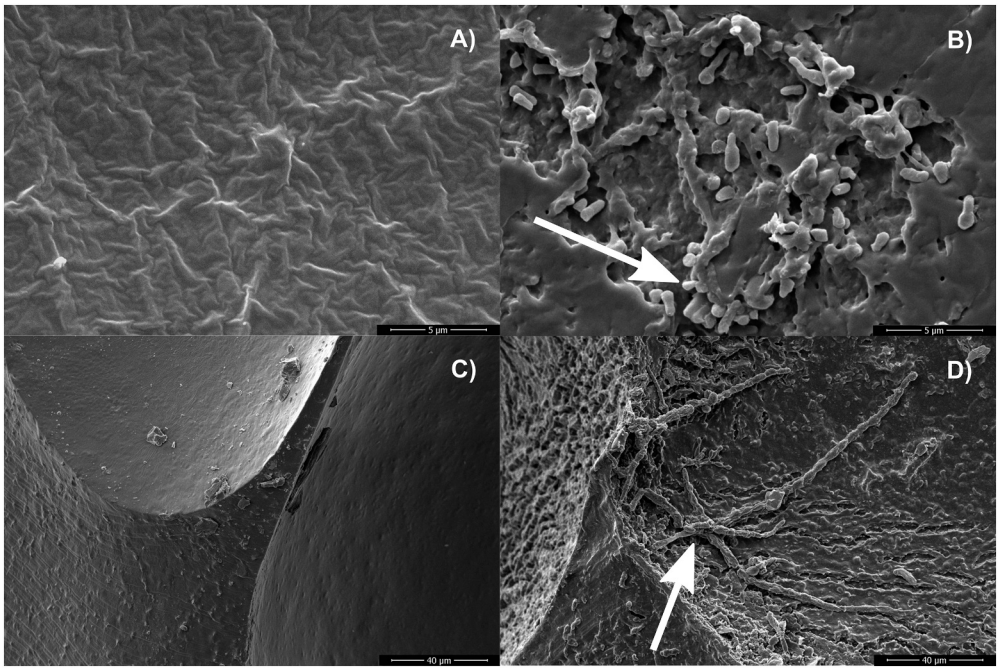

Fig. 4. SEM micrographs of PU foam. Arrows indicate growth of organisms of interest. **A)** Control sample of the PU closed-cell exterior, taken at 8000× magnification. **B)** Exterior surface of the PU with a consortium of soil bacteria able to utilize PU as sole carbon source, taken at 8000× magnification. **C)** Control sample of the PU open-celled interior, taken at 1000× magnification. **D)** Interior of the PU with *Cladosporium* sp., taken at 1000× magnification.

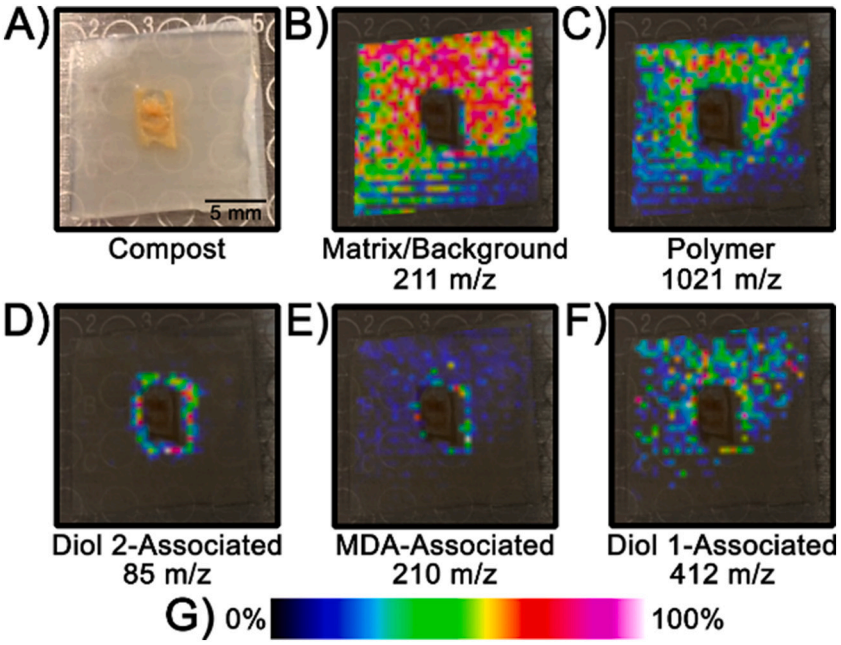

Fig. 5. IMS of compost-derived organisms growing on PUM9 film-agar plates. **A)** Photograph of the culture growth one week after incubation, with scale bar for all images. **B-F)** Mass distributions indicating location and relative intensity of the given m/z value and its molecular association. **G)** Relative intensity scale for B-F. See Supplementary Data for evidence of molecular assignments.

could be a mechanism of bacterial attachment to the PU surface. Several studies have concluded that bacterial growth on plastic tends to be related to the formation of biofilms (Sanin et al., 2003; Sivan, 2011). Previous literature indicates that several fungi are capable of degrading polyester PUs (Russel et al., 2011). During the experiment from Section 3.4, a fungus, *Cladosporium* sp., contaminated several flasks of minimal media with PU, indicating that the fungus was capable of living on our PU, despite not surviving in the consortia of organisms in Section 3.4. To confirm biodegradation activity, *Cladosporium* sp. was then tested individually in the minimal media with PU as a sole carbon source. The culture was able to successfully grow over the course of a week, corroborating findings in previous studies where *Cladosporium* from soil grew on PU-agar plates (Crabbe et al., 1994). To understand interactions of this fungus with the PU, PU slices were inoculated with an isolate of *Cladosporium* sp. and visualized. Comparison between the PU control (Fig. 4C) and PU inoculated with *Cladosporium* sp. (Fig. 4D) shows extensive topographical changes. Significant surface degradation and extensive binding of *Cladosporium* sp. with the foam indicates degradation of the PU for utilization by the fungus for growth.

3.6. Imaging Mass Spectrometry of biodegrading PU films

2D Matrix-Assisted Laser Desorption/Ionization-Imaging Mass Spectrometry (MALDI-IMS), which provides spatial resolution of chemicals across a solid sample, was applied to provide further evidence of biodegradation of the PU. The consortia of passaged compost and soil microbes, as well as the isolated *Cladosporium* sp., were separately grown on minimal media agar plates covered with a thin film of PU (PUM9 plates) and analyzed by IMS after visible accumulation of microbial biomass over a one week incubation. Because the polymer

precursors hypothesized to result from biodegradation were unlikely to be directly observed due to their size, and charge constraints of the instrumentation, we also analyzed an uninoculated PUM9 plate spotted with 1 μL drops of concentrated chemical standards of the two diols, one diacid, and 4,4′-methylenedianiline (MDA, the expected enzymatic degradation product of MDI), to allow recognition of matrix-derivatized ions related to the polymer precursors. Following matrix application, we noted that exposed M9 agar rejected the universal MALDI matrix, resulting in regions devoid of signal (Fig. 5). Based on spatial distributions of mass signals on the uninoculated standards sample and known polymer spectral patterns, we identified molecular signatures associated with the matrix, the polymer, or the polymer precursors. On the biodegrading samples (Fig. 5), matrix or PU polymer associated signatures showed broad and relatively uniform distributions (Fig. 5B,C) where PU was present on the sample, while ion signatures associated with the PU polymer precursors were localized around the sites where the organisms contacted the PU substrate (Fig. 5D–F). These distributions provide further evidence that the organisms are degrading the PU films to produce, but not necessarily consume, the expected PU degradation products. In addition, we observed a number of ion distributions, such as 206 *m/z*, that also localize around the organisms but were not present on the control sample except as low intensity or non-uniform noise. We hypothesize that these are either products of the organisms' metabolism or are unknown by-products of the PU film degradation.

3.7. Identification of PU degradation products using Gas Chromatography–Mass Spectrometry and Liquid Chromatography–Mass Spectrometry

To test the ability of known polyester degrading enzymes, specifically esterases, to break down our PU foam into monomers, four commercially-available esterases were assayed for their ability to depolymerize our PU foams: lipase from *Aspergillus niger*, lipase from *Candida rugosa*, esterase from *Bacillus subtilis* and cholesterol esterase from *Pseudomonas* species. The microorganisms from which the enzymes were derived have been cited to have biodegradation activity (Osman et al., 2018; Gautam et al., 2007; Rowe and Howard, 2002; Das and Mukherjee, 2007). Cholesterol esterase from *Pseudomonas* species performed the best with the highest production of diols over the course of 24 h. The *Pseudomonas* genus is a species shown by metagenomic sequencing to be abundant in our compost sample in Section 3.3. As this enzyme hydrolyzes ester bonds found in sterol esters, it was hypothesized that the ester and urethane bonds found in our PU foams would hydrolyze to produce the starting polyol monomers—diol 1, diol 2, and diacid 1—and MDA, the breakdown product from cleavage of the urethane bond formed by the isocyanate MDI used to make our PU foams (Uwajima and Terada, 1976; Santerre and Labow, 1997). This in vitro method served to model the degradation products expected in the environmental samples.

After 24 h of incubation, the triplicate samples were analyzed for the presence of diols and diacids, the expected depolymerization products of the polyester polyols, by LCMS and GCMS. Standards in the phosphate-buffered saline (PBS) matrix and foam incubated with BSA were also run to compare retention times and to account for other peaks present solely in the matrix. Each peak identity was also confirmed by its mass spectra in both the GCMS and LCMS of standards and samples. The LCMS chromatograms in Fig. 6A–D show all four breakdown product peaks, which were not present in the BSA negative controls (Fig. 6E–H). In the GCMS chromatogram (Fig. 6J), diols 1 and 2 and diacid 1 were identified, as well as four prominent peaks at 9.8, 10.1, 11.27, and 11.47 min, which were identified by their unique mass spectra as partial degradation products: dimers and trimers of diols and diacids. To further validate the identity of the four peaks, one of the degraded samples was treated with a strong base to hydrolyze the remaining water-soluble polymer fragments. Notable in the base-hydrolyzed GCMS chromatogram was the complete disappearance of the partial PU fragment peaks and an increase of all three final product peaks, corresponding to 39 ± 3%, 43 ± 4%, and 32 ± 2% PU degradation into diol 1, diol 2, and diacid 1, respectively. These data indicate that the cholesterol esterase degraded 38 ± 6% of the PU foam into a combination of polyol monomers and water-soluble dimers and trimers in 24 h.

4. Conclusions

Here, we show that algae-based PU foams degrade with significant loss of structural integrity after only 12 weeks of incubation in compost and soil. We identified microorganisms that grew abundantly in close association with the degrading PUs. We downselected for bacteria that were able to utilize the PU as a sole carbon source. *In vitro*, we confirmed that a cholesterol esterase from Pseudomonas species depolymerized 38% of the PU back into monomers and oligomers over 24 h of enzymatic treatment. This study demonstrates the possibility of creating commercially-viable biodegradable PU products, and shows the potential of using recombinant enzymes to depolymerize and recycle PU products.

Credit author statement

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: M.D.B., R.S.P. and S.P.M. are founders of, and hold an equity position in Algenesis Materials, and M.T. and N.K.N. are employees and shareholders in Algenesis Materials, a company that could benefit from this research.

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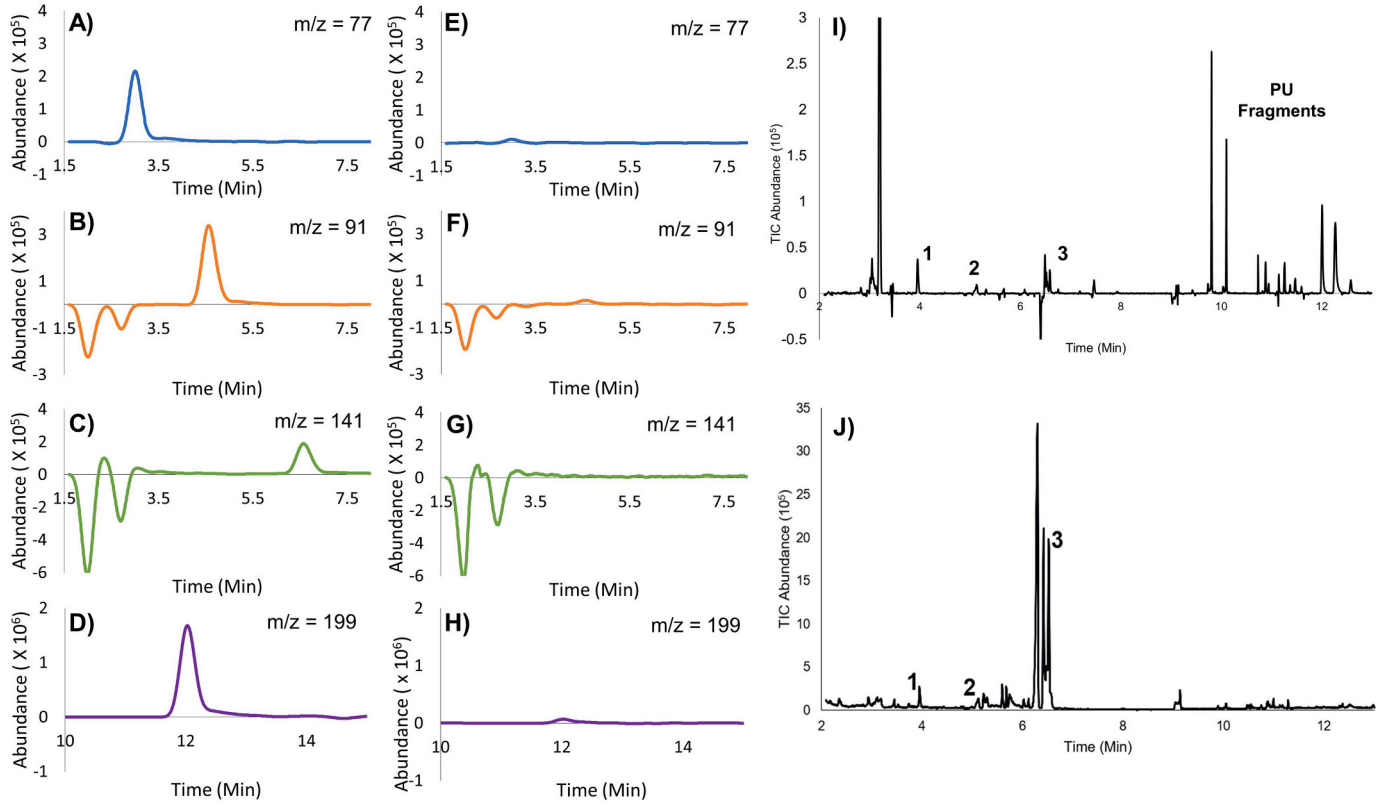

Fig. 6. LCMS (left) and GCMS (right) chromatograms of enzyme-degraded PU foam. From top to bottom LCMS mass traces correspond to diol 1, diol 2, diacid 1, and MDA. **A-D)** Foam degraded with *Pseudomonas* species cholesterol esterase for 24 h. **E-F)** A negative control of foam reacted with BSA for 24 h, showing no degradation products. **I)** GCMS of *Pseudomonas* species cholesterol esterase degraded foam. 1, 2, and 3 correspond to diol 1, diol 2, and diacid 1, respectively, and PU fragments are present from 9.5 to 13 min. **J)** The same sample chemically degraded to identify the PU fragments. Note the disappearance of the peaks from 9.5 to 13 min and the increase in abundance of peaks 1, 2, and 3 to ~10× that of the original sample. Unlabelled GCMS peaks are PBS media traces.

experiment to isolate and identify organisms capable of degrading PU, and Christina C. Saak and Jennifer M. Michaud for their contribution on how to approach metagenomic sequencing data analysis.

Data availability

Supplementary Information and Source Data is available for this paper. Sequence read data is available in the BioProject database via accession numbers PRJNA625105 and PRJNA625106. Other supporting data from this study are available from the corresponding authors upon reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2020.100513.

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