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bioMATADOR: biocompatible Material Agnostic Testbed for Autonomous Degradation of Recalcitrants

by Patrick B. Kruk, Jose A. Wippold, Sunny Karnani, and Joseph M. Pennington IV

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14. ABSTRACT
Plastic polymers are ubiquitous in both the modern world and U.S. Army fielded material due to being highly recalcitrant and nonbiodegradable, giving rise to their pervasive presence in military packaging, infrastructure, supply chain logistics, and sustained operational readiness. The ability to break down, degrade, or reduce and reuse these plastics with biological enzymes shows promise to ease this logistical burden and improve battlefield sustainability while reducing the demands of deployment. Yet large-scale material studies remain limited to short-duration manual benchtop procedures not conducive to meaningful polymer degradation. Here we describe a novel microfluidic device, the bioMATADOR (biocompatible Material Agnostic Testbed for Autonomous Degradation of Recalcitrants). The bioMATADOR accommodates a variety of material packing during fabrication along with solvent turnover and volume fill rates and flow patterns, while maintaining high optical clarity and light transmittance to allow for easy viewing and microscopy. The bioMATADOR presents a cost- and time-effective platform to both accelerate discovery of polymer-degrading enzymes and streamline material degradation analysis by enabling custom, long-term, autonomous experiments.

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1. Introduction

Polymer plastics have become ubiquitous in the modern world, including in U.S. Army fielded material (Nayanathara Thathsarani Pilapitiya and Ratnayake 2024). These plastics provide the strength necessary to withstand battlefield and deployment wear and tear (i.e., they are highly recalcitrant, inert, and nonbiodegradable). However, this strength comes at the cost of waste generation, as polymer plastics take hundreds of years to degrade (Geyer et al. 2017). The degradation of plastic materials is a significant concern for the Army, as plastics are ubiquitous in military equipment, packaging, and infrastructure, and their breakdown can impact operational readiness, environmental sustainability, and logistical demands. Plastic materials are used in everything from ammunition casings and vehicle components to food packaging and shelter materials, making them a pervasive presence in military operations.

The Army's widespread use of plastics translates into large-scale amounts of plastic waste being generated, which can persist in the environment for centuries if not properly managed, highlighting the need for effective plastic degradation and recycling strategies. After initial use, they present no benefit to the warfighter, serving only as a burden on logistics. Current waste reduction and management techniques such as “burn pits” have proven detrimental to the health of both deployed military personnel and the surrounding environment (Smith et al. 2009; Szema et al. 2011; Woodall et al. 2012). While these consequences have since been limited with new DoD guidance regulating the burning of plastics, this has done nothing to alleviate the logistical challenges. As such, any potential for polymer plastic degradation promises to streamline Army logistics and improve operational readiness. A proposed solution is to investigate polymer degradation induced by bacterial enzymatic mechanisms. To facilitate this research, there is a need for high-throughput systems to investigate polymer degradation. This need has not been met yet due to the challenging nature of degradation experiments—in particular, these studies have been limited by the continuous and exceedingly long time spans necessary to achieve polymer degradation through biological means.

Here we propose bioMATADOR to meet these challenges. The biocompatible Material Agnostic Testbed for Autonomous Degradation of Recalcitrants is a novel testbed invented, designed, and tested at the U.S. Army Combat Capabilities Development Command (DEVCOM) Army Research Laboratory (ARL) to be a valuable tool for high-throughput and autonomous enzyme degradation studies. Microfluidics has proven to be an enabling tool capable of providing tangible benefits to many realms of life sciences (Whitesides 2006; Li et al. 2018; Convery and Gadegaard 2019; Di Carlo 2019; Sollier et al. 2011). This testbed can be used

to screen large libraries of enzymes for their ability to degrade specific polymers, allowing for high-throughput identification of novel enzymes with potential biotechnological applications. Additionally, the testbed can be automated, enabling autonomous studies of enzyme activity and substrate specificity over the long timescales necessary to evaluate recalcitrant polymers and their optimal reaction conditions (Figure 1). This testbed can accelerate the discovery of new enzymes and the optimization of existing ones, which can be used to develop more efficient biodegradation processes. bioMATADOR aims to be a powerful tool for advancing, accelerating, and better facilitating our understanding of enzyme-directed polymer degradation function as well as developing innovative solutions for plastics management in the future.

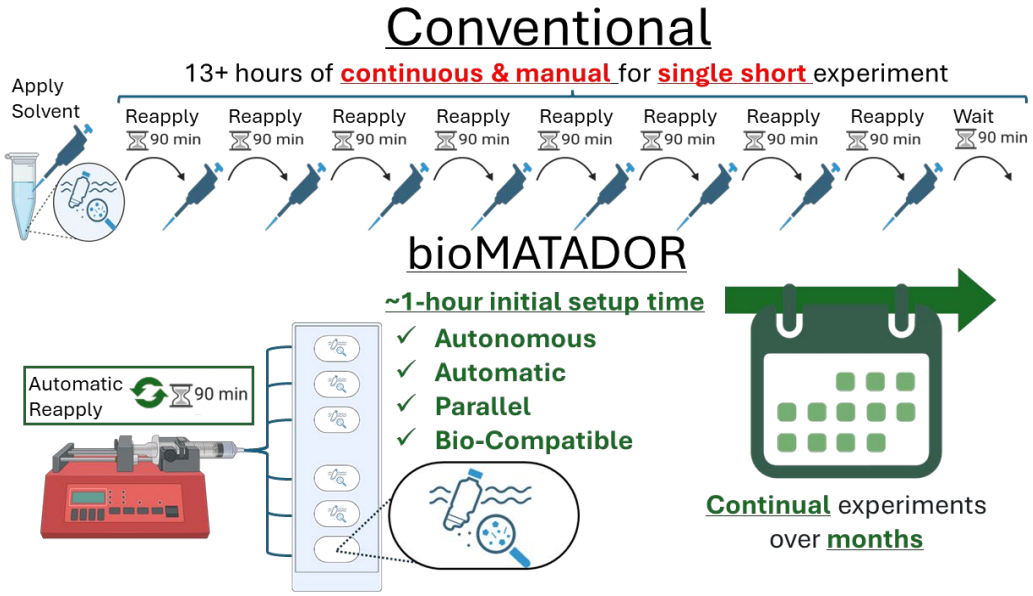

Figure 1. Comparison of conventional means of long-term degradation experiments, with the bioMATADOR device and system as well as its main corresponding advantages. Conventional method adapted from Sanluis-Verdes et al. (2022).

The bioMATADOR has the enhancing characteristic features of being optically clear, compatible with traditional and fluorescent microscopy, and allows for a continual and autonomous degradation scheme. This platform enables autonomous biodegradation of materials on the scale of weeks to months—a great improvement from current benchtop laboratory capabilities. The design of bioMATADOR is readily compatible with standard laboratory benchtop research capabilities such as fluorescence and brightfield microscopy, flow cytometry, optical profilometry, and downstream analytical techniques (gas chromatography–mass spectrometry, sequencing, etc.) via collection of degradation products in the effluent. This ease of use is also present in the multichamber design with the double-sided glass slides. The six chambers allow multiple samples to be observed and collected at once, as

well as making it easier to run control, duplicate, and triplicate experimental regimes simultaneously. All components of the bioMATADOR from the resin of the main body to the microfluidic components of the tubing, and especially the epoxy and glass of the chamber windows, are readily available in most biological laboratory environments. These constituent components are also cost effective to implement into the fabrication protocol of the bioMATADOR. This allows it to be fabricated at larger scales required for rigorous degradation testing. The bioMATADOR presents a cost- and time-effective device to facilitate rapid advancements in polymer degradation research to meet U.S. Army and national defense needs.

2. Experimental and Numerical Approach

2.1 bioMATADOR Design Iterations and Improvements

The original inception of the bioMATADOR started with a two-chamber design meant to fit a laboratory standard 25-mm × 25-mm × 1-mm glass microscopy slide on one side to act as a window. Following extensive testing, a second six-chamber design was created to easily run more experiments in parallel and triplicate. This chamber was designed to fit a 25-mm × 75-mm × 1-mm laboratory standard glass microscopy slide. We used Fisherbrand plain microscope slides (Waltham, Massachusetts); however, the bioMATADOR is designed to fit any slides that adhere to the laboratory standard specifications above. Through more experience and testing with device fabrication, filleting the lead edge and including an inset to the tubing inlet point facilitated better construction of the device, which decreased the frequency of leakages. This greatly decreased early fabrication problems with excess epoxy clogging channels, as well as inserted tubing bending or kinking to block flow. After additional rounds of testing, the bottoms of the chambers were removed and replaced by a second inset glass slide that was epoxied into place under the same conditions as the initial one. This allowed for complete viewing of the samples in the experimental chambers from above and below, while also drastically increasing clarity. This improved design around v10 was referred to as the "plug-and-play" variant. Following improvements to clarity and experimental function, there were improvements to ease of use. The most prominent improvement was the addition of a "ledge" to allow for easy holding and labeling of the device in v11. The most recent design iteration (v12) of the device can be seen in Figure 2.

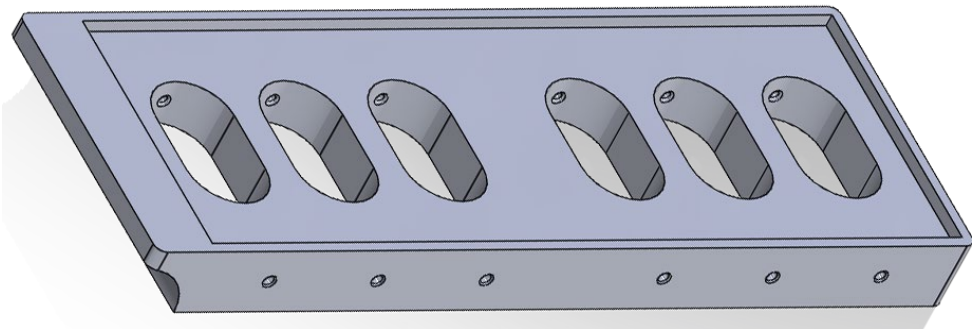

Figure 2. Most recent design version (v12) of the "plug-and-play" 3D-printable bioMATADOR main body.

2.2 Flow Testing

Preliminary flow testing was done on a bioMATADOR with chambers filled with bulk cellulose, nylon, and polyester. The filled chambers were saturated with water and then placed under a Canon Powershot SX70 HS camera (Tokyo, Japan). A 50-mL syringe of water with 10 drops of red food coloring was then flowed into each chamber using hand pressure. All tests were recorded to determine flow and fill patterns. Testing was carried out on earlier prototypes of the bioMATADOR device (v1–9) without the double glass slide design element. However, the internal volume, geometry of the chambers, and inlet and outlet channels remained constant across all tests.

The flow was then tested at specified flow rates with the help of a Chemyx F200X syringe pump (Stafford, Texas) and the same syringe and tubing layout as the preliminary testing regime. The same testing was conducted with later iterations of the bioMATADOR device (v10+) that incorporated the double glass slide design. As before, all experiments were recorded to better determine flow and fill patterns within the chambers. The specified flow rates tested were 60, 600, and 6000 μL/min (or 1, 10, and 100 μL/s, respectively). Snapshots were taken of the beginning, middle, and end of the chamber filling cycle. The beginning (t = 0 s) was defined as when the injected/inflowing (colored) water had filled the inlet channel yet had not entered the main chamber. The middle was judged to occur when the chamber volume appeared to be half filled with the new colored inflowing water. The end point was defined as when both the entire chamber volume and outlet channel were filled with the new colored water. The time for all points to be reached was recorded and compared to COMSOL's (Burlington, Massachusetts) system modeling.

2.3 Leak Testing

Structural integrity of the bioMATADOR device is paramount to its usefulness as any unintended inflow or outflow would compromise the reliability of any obtainable results from experimentation. The bioMATADOR's promise of long-term and continuous experimentation rests on its ability to withstand consistent internal pressure without leakage. To test this ability, a fully assembled device (of the most recent design iteration v12) was connected to an Auto Joe air compressor (Glendale, Arizona) at the designated inlet tubing. The corresponding outlet tubing was then clipped shut to create a sealed system, and the entire device was placed under water. Any resultant loss of integrity or escape of pressure on the main body of the device would then produce visible bubbles as the air escapes. All experiments were again recorded with the Canon camera. Air flow was applied for a minimum of 10 s to ensure pressure buildup. Testing began at 10 psi and was held for 10 s, then raised to 20 psi and held an additional 10 s. Pressure was then continuously increased by intervals of 20 psi and held for 10 s until the air compressor maxed out at 120 psi.

2.4 Finite Element Flow Simulations

Essential to the design of a high-throughput testing platform is the careful management of enzymes under evaluation by limiting their waste. To that end, 3D computational fluid dynamics simulations were undertaken using COMSOL Multiphysics (COMSOL 2024). Two types of simulations were run: (1) tracking a dilute species introduced at the inlet to track the time and fluid volume needed to replace the previous volume of fluid as a function of flow rate, and (2) a two-phase flow simulation using a level-set method with a wetted wall to track the liquid–air interface in the case of an empty volume either being filled for the first time, or as part of an intermediate step where the previous fluid is first drained before a new enzyme solution is introduced. The level-set modeling approach was applied in the case where the bioMATADOR volume was operated vertically to leverage gravity in both filling and clearing the chamber. In both simulations, the Navier–Stokes (Equation 1) and continuity (Equation 2) equations are solved, and a species transport equation (Equation 3) was included in the dilute species tracking simulations. In the level-set approach, an interface tracking the partial differential equation is also solved (Equation 4).

Navier–Stokes: $$\rho\left(\frac{\partial u}{\partial t} + u \cdot \nabla u\right) = -\nabla p + \nabla \cdot \tau + f \quad (1)$$

Continuity: $$\rho \nabla \cdot u = 0 \quad (2)$$

Species transport: $$\frac{\partial c_i}{\partial t} + u \cdot \nabla c_i = \nabla \cdot (D_i \nabla c_i) \quad (3)$$

Species transport: $$\frac{\partial \phi}{\partial t} + u \cdot \nabla \phi = \gamma \nabla \cdot (\epsilon_{ls} \nabla \phi - \phi(1-\phi)\frac{\nabla \phi}{|\nabla \phi|}) \quad (4)$$

The flow is assumed laminar and incompressible due to the small length scales, low flow rates, and the fluid properties (water-like). Inlet boundary conditions track experimental conditions. The outlet has a constant pressure boundary condition, and a no-slip condition is applied to the wall.

The simulations were designed to simulate a hypothetical workflow where the chamber starts filled from a previous round of testing and a "fresh" solution is introduced to the bioMATADOR volume. In this case, the concentration of species being tracked is set to zero in the domain, a 1 mol/m^3 concentration of a dilute species with a diffusivity resembling food dye (294 μm^2/s) is introduced at the inlet, and the volume contains no source terms. Integrating the product of flow rate and species concentration over the inlet and outlet boundaries for different run times and the average concentration in the bioMATADOR chamber provides insight into the average concentration in the volume and the amount of new fluid "carried over" to the exit, both as a function of time and flow rate.

3. Results and Discussion

3.1 Device Design and Fabrication

3.1.1 3D Printing Main Body

All 3D printing was done on stereolithography 3D printers from Formlabs (Somerville, Massachusetts) to design specifications. Both the Form4 and Form3 printers were used throughout design and testing with no noticeable difference found in the final print surface quality and fidelity to designed specifications (Table 1). The Clear (v4) resin used on Form3 printers was found to "yellow" more during the recommended curing time. However, as the curing time of later bioMATADOR devices was reduced, this effect was minimized. Clear resin was selected to ensure the visibility and optical clarity of the internal testing chamber of the device. However, the addition of the second bottom layer glass slide in v10 of the device removes the strict necessity of this clear resin, opening the possibility of using other resins to construct the main body. Other resins such as Rigid 10k or Black V5 would offer various advantages, such as the ability to be autoclaved or decreased autofluorescence. This warrants further investigation and raises the potential for using different resins to allow for various continual optical detection methods depending on user need.

Table 1. bioMATADOR 3D printing and curing process settings.

Settings	Parameters	Value
Job settings	Printer version	From 4
	Material	Clear (V5)
	Layer thickness	0.025 mm
	Print setting	Default
Support settings	Raft type	Mini rafts
	Touchpoint density	1.0
	Touchpoint size	0.45 mm
	Internal supports	☑
	Bundled supports	☒
Cure settings	Cure time	5 min
	Cure temperature	60 °C

3.1.2 Glass and Tubing Fabrication

Once fully printed and cured, the bioMATADOR was dusted using compressed air to remove any stray particulates. Gorilla Glue (Cincinnati, Ohio) fast-acting epoxy was then applied liberally to the inset surface of the device (Figure 3, highlighted in red). Any area of the inset surface not initially covered in epoxy posed the risk of bubble formation when the glass slide was pressed down into the empty space. Any bubbles proved almost impossible to remove and were found to be the leading cause of leakages as liquid would flow out the chamber and between the slide and main device body. At higher pressures, this occurred frequently to any visible surface imperfections or bubbles in the epoxy layer. Bubbling is easy to prevent by ensuring that the surface is completely covered with epoxy before adhering the glass slide. The slide was adhered by inserting it into the precise sized inset and pressing down with hand pressure for at least 20 s. This pressure allowed the epoxy to adhere the slide to the main body of the device, while also forcing excess epoxy to be pressed out between the slide and main body on either the top surrounding the glass or on the edges of the internal chambers. The epoxy was then left to dry for the recommended 24 h. After sufficient drying, the bioMATADOR was flipped over and the epoxy and slide adhesion steps were repeated on the remaining inset surface. Finally, 0.01-inch inner-diameter/0.03-inch outer-diameter TYGON Plastic Tubing (Kalamazoo, Michigan) was inserted into the inlets and outlets. The tubing was inserted until encountering resistance due to the channel constriction incorporated through earlier experimentation. Epoxy was then applied around the circumference of the inserted tubing to prevent leakage. Following the recommended 24-h drying time, the device was ready for experimentation.

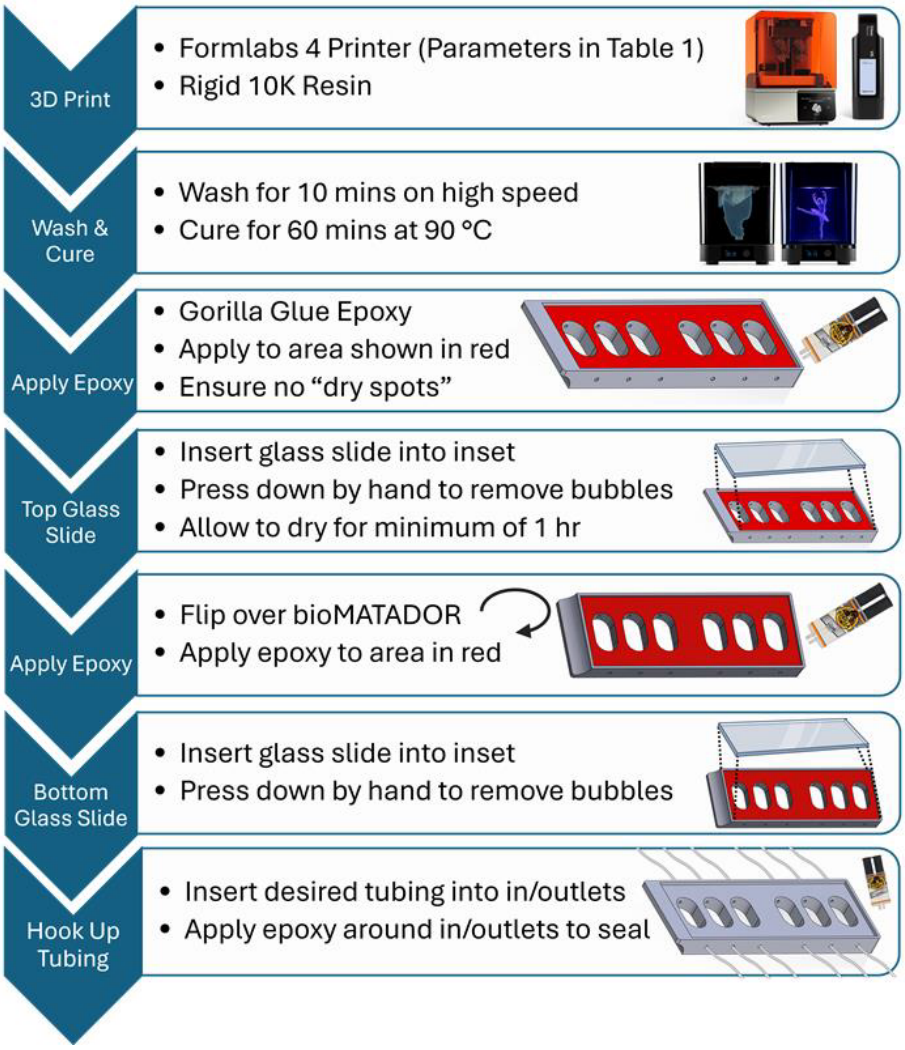

Figure 3. Flowchart of fabrication of bioMATADOR device from initial 3D printing to application of glass microscopy slides and inlet and outlet tubing systems.

3.2 Flow Testing and COMSOL Modeling

Preliminary flow tests were conducted to assess the impact of flow rate and geometry on solvent turnover and dead volume formation (Figure 4). The various flow rates of the bioMATADOR demonstrated different flow patterns of injected liquid and thereby differing fill patterns. Low flow rates (60 µL/min) demonstrated a more uniform fill pattern from the inflow point (left) to the outflow point (right) with limited turbulent or chaotic flow. However, more mixing of the original liquid inside the chamber with the new inflow could be seen in the lighter (i.e., more diluted) color of the inflowing and filled chambers. This contrasts with the increasing flow rates of 600 µL/min and 6000 µL/min, where a greater flow instability is noted but less dilution (and thereby mixing) of the inflow liquid with the original fill was observed. This instability is most noticeable at 6000 µL/min

when the chamber is half-filled (1.18 s). The chamber can be seen to backfill as the injected colored flow is shown to rush past the original filled volume and directly out before cycling through the entire volume of the chamber and eventually back-filling through a combination of mixing and a vortical flow induced by an interaction with the chamber wall.

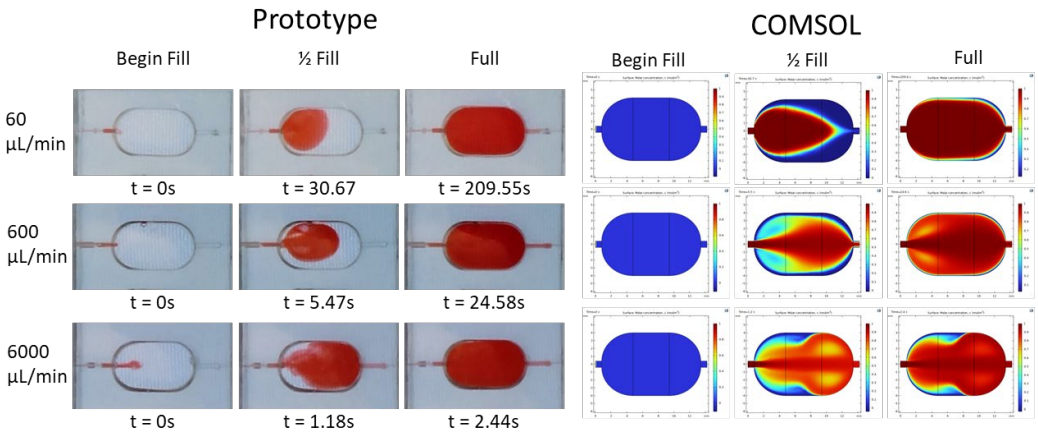

Figure 4. Flow testing and COMSOL modeling of bioMATADOR prototype.

To understand this behavior numerically, a series of flow simulations were designed to track the concentration of a dilute species at three different flow rates. Simulation run time and the results presented (Figure 5) are scaled based on flow rate and chamber volume to evaluate the average concentration in the bioMATADOR chamber as a function of the number of equivalent fluid volumes introduced. At the lowest flow rates evaluated (1 µL/s), approximately three chamber volumes of "fresh" enzyme are required to achieve an average concentration greater than 96%. At 10 µL/s, a 90% average concentration is reached with a tenfold reduction in fill time. Increasing the flow rate to 100 µL/s enables a further tenfold reduction in fill time but results in a lower average concentration of 83%. Regardless of flow rate, roughly three chamber volumes are needed to establish average concentrations that broadly reflect the composition of the introduced flow (>80%) (Figures 5 and 6). These trends hold even as the volume of the chamber is reduced. These results suggest a potential loss of up to two-thirds of the introduced enzyme before the chamber reaches near steady-state concentrations.

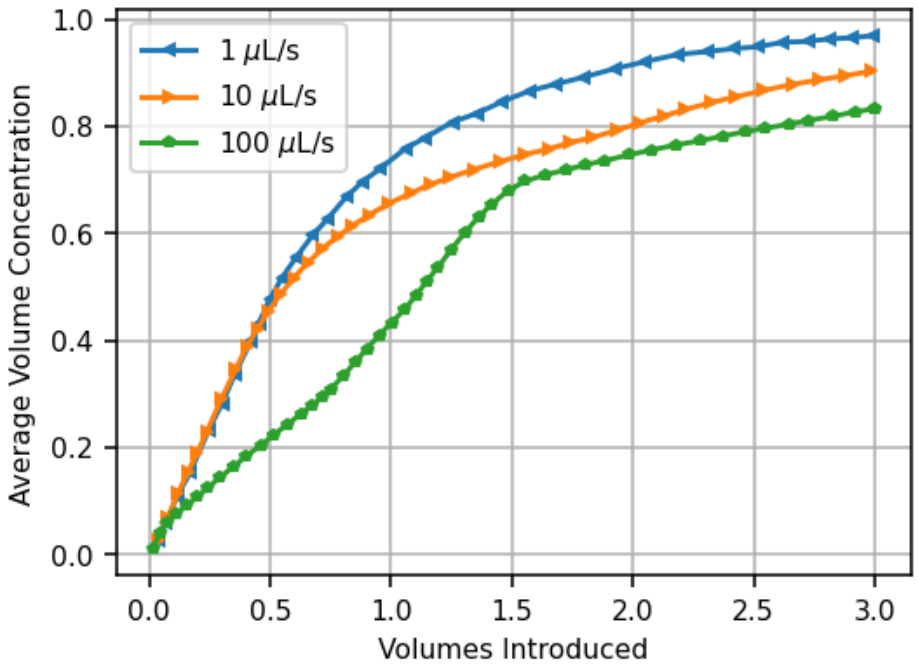

Figure 5. Average chamber concentration as a function of volumes of fluid introduced (3D simulations).

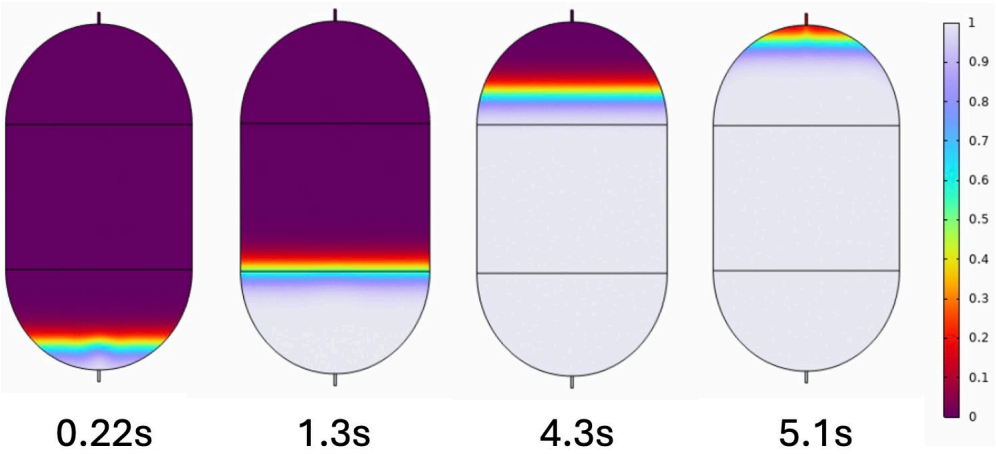

Figure 6. Images tracking the time evolution of the interface between the "fresh" enzyme solution and the unfilled region of the bioMATADOR chamber.

A potential solution to address the excessive carry-over losses observed in the horizontal chamber configuration is to orient the chamber vertically to leverage gravity to uniformly fill the chamber and minimize waste. In this approach, it was assumed that the chamber would be fully drained of the previous fluid via the entrance port prior to refilling with the "fresh" enzyme solution. Under these conditions, carry-over losses were effectively eliminated, and the required fill time could be accurately estimated by dividing the chamber volume by the flow rate.

3.3 Leakage Testing

The bioMATADOR was able to stay air-tight and intact while undergoing a maximum of 120 psi of internal pressure (Table 2). This pressure was held for a minimum of 10 s and was applied to the same device that had undergone all previous testing (six rounds of testing with increases of 20 psi). Pressure was held for a minimum of 10 s at each interval, with the bioMATADOR showing no signs of leakage, which is defined as the development of bubbles during submersion of device. The highest pressure tested was 120 psi due to limitation of the pump; higher pressures would require more testing if needed for specific experimental setups. Initial pressure testing supports the integrity of the device under intended operational conditions. The bioMATADOR could be used to facilitate experimental degradation conditions under moderate pressures.

Table 2. Pressure testing of bioMATADOR device (v12).

Pressure applied (psi)	Pass/fail
10	Pass
20	Pass
40	Pass
60	Pass
80	Pass
100	Pass
120	Pass

3.4 Applied Testing

The bioMATADOR was designed to allow for easy viewing and optical measuring of the internal chamber throughout continuous degradation experiments. To determine the effectiveness of the design for this purpose, the bioMATADOR underwent both brightfield and fluorescence microscopy imaging. These were designed to mimic standard experimental procedures as closely as possible to determine the performance of the bioMATADOR under experimental conditions.

The bioMATADOR achieved great clarity across a range of microscopy conditions (Figure 7). The background signals present in the chamber were minimal under both brightfield and fluorescence imaging as shown by the phosphate-buffered saline (PBS)-filled control chamber in Figure 4. This was important to determine as both the glass slides and main 3D-printed resin body of the bioMATADOR were designed to have minimal optical effects on (or distortions to) signals in the chambers. Multicolored beads suspended in PBS were shown to fluoresce brightly under microscopy across a range of colors. Beads were visible under blue, green, and red filters, demonstrating the high transmittance capability of the double glass bioMATADOR. The beads could also be seen clearly under brightfield imaging

conditions. The clear v4 resin used to manufacture the device was shown to fluoresce brightly; however, as can be demonstrated in the stitched images of the entire bioMATADOR chamber, this effect was limited to the edges of the chamber and caused minimal distortion to the chamber's center.

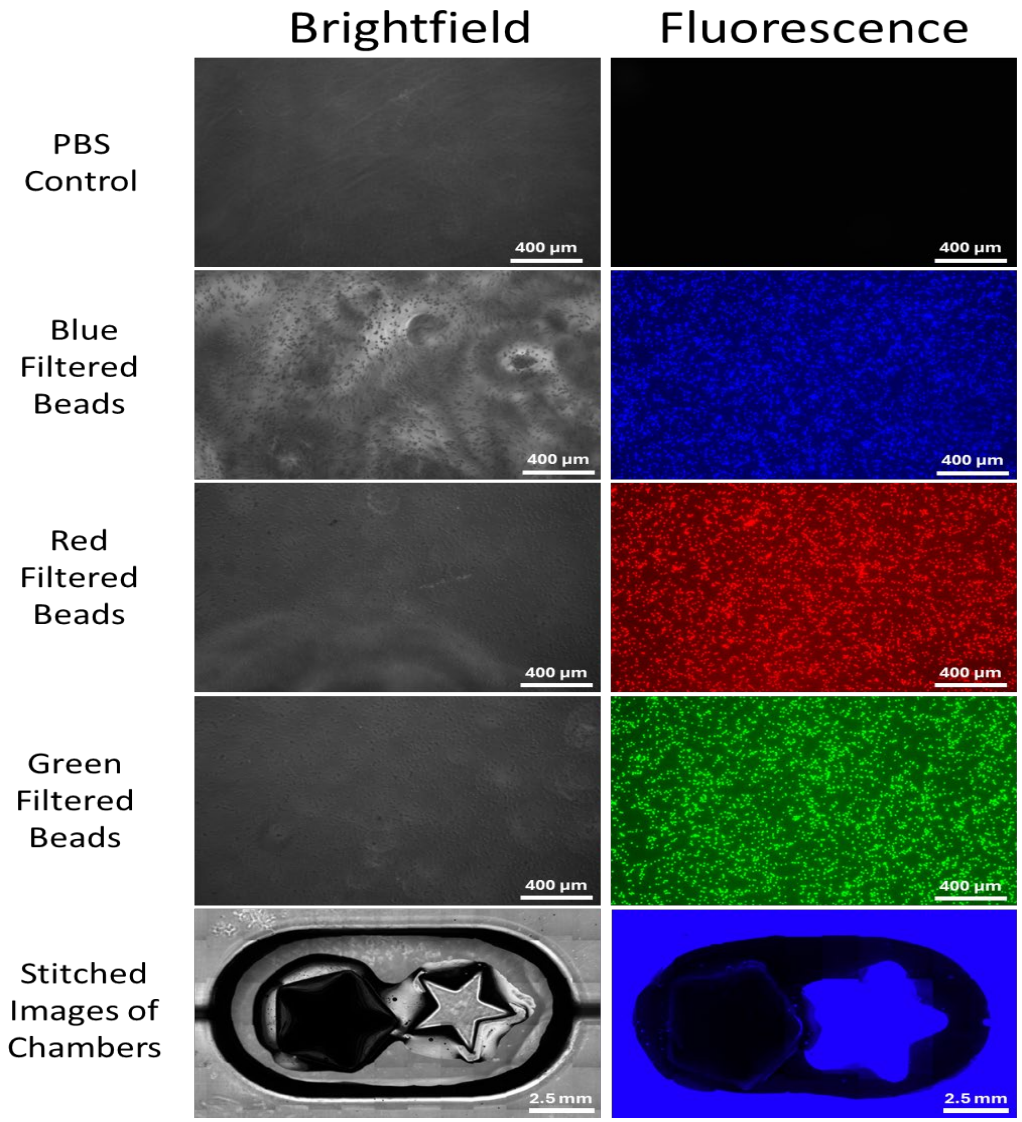

Figure 7. Brightfield and fluorescence imaging of the internal chambers of the bioMATADOR device through the double glass design.

The epoxy used was also found to be a source of distortion depending on its placement. While the entire surface surrounding the chamber was coated in epoxy, only the portions of epoxy that were exposed to air within the chamber due to overflow caused image distortion. The areas of epoxy sandwiched between glass and the main body showed minimal distortion under brightfield imaging. Further investigation is warranted to determine the optimal procedure for adhering samples to the glass slides within the chamber to maintain optical integrity and image clarity.

3.5 Material Packing

A key component of the bioMATADOR is the ability to remain material agnostic, allowing researchers to choose the material (object, device, sample, etc.) to insert into the chambers and tailor it to their experimental requirements.

The bioMATADOR was demonstrated to be fully compatible with both bulk and loose material, as well as designed architectures (Figure 8). Bulk material packing was demonstrated with cellulose, nylon, and polyester, while loose material packing was demonstrated with 3D-printed stars adhered to the "lower" glass slide with the same epoxy used in device fabrication. The addition of these materials into the chambers was not found to impede flow in a way that invalidates internal degradation experiments or compromise the integrity of the device. The process to insert these materials was relatively simple, with low costs in time, energy, and laboratory resources. These initial material packing results show the versatility of the bioMATADOR to facilitate real-time observation and collection of degradation products for further analysis autonomously.

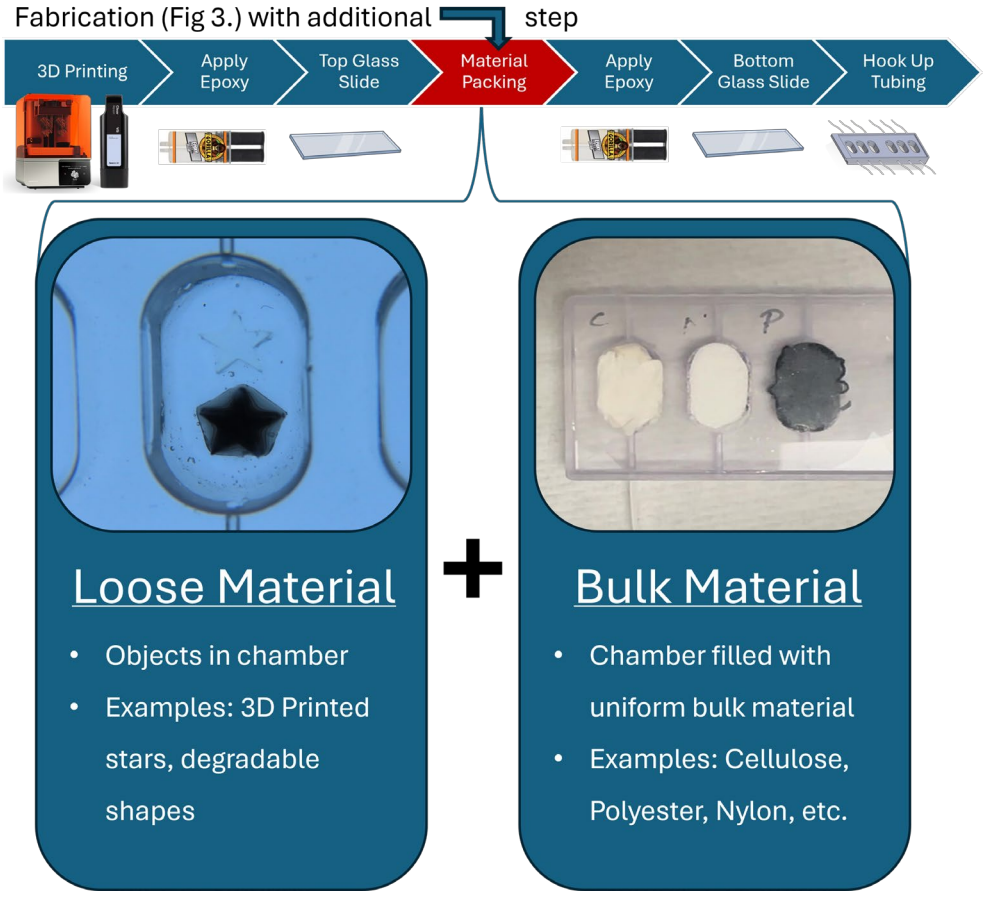

Figure 8. Material packing into the bioMATADOR can be tailored to the requirements of individual experiments. Both loose and bulk material packing are added prior to full assembly.

4. Conclusions

The bioMATADOR has proven effective at facilitating longer-term biodegradation experimental regimes throughout its prototyping and initial testing phases. It offers a wide array of unique and distinct advantages over conventional degradation experimental regimes. These conventional experimental regimes require great investments in time and resources that drastically limit their scope, reach, and scientific applicability. A representative degradation experimental regime from a study of wax worm enzymes took 13 h of continuous manual laboratory work (Sanluis-Verdes et al. 2022). The length of any such research is directly limited by the time commitments of its researchers and their capability for continuous manual labor. The bioMATADOR device streamlines these requirements, allowing for autonomous degradation research continually over much greater time frames that are more applicable to biological degradation timescales. Compared with the hours-long time frames of traditional research, bioMATADOR is designed to allow for experiments over the course of weeks to months to potentially years. It does this while allowing a researcher to precisely control the rate of addition of new solvent with a laboratory standard, easily accessible syringe pump. The design of the bioMATADOR also allows for easy sample collection, observation, storage, and experimentation for both the solvent and material under investigation. The six-chamber design allows for multisample observation and collection, making it easier to run control, duplicate, and triplicate experimental regimes simultaneously. This drastically increases the scalability of the bioMATADOR to help test large libraries of degradation enzymes. bioMATADOR seeks to meet the increased demand for a broad range of material testing necessary to evaluate new and improved enzymes in support of the Army's goals of a reduction in supply chain logistics and new material design in circular manufacturing.

5. Future Work

The bioMATADOR has already proven effective at providing a novel testbed for degradation experiments; however, room remains for design improvements and additional biological testing.

5.1 Potential Device Iterations

Various improvements to the bioMATADOR have been proposed that warrant further investigation. These can be broadly divided into two categories: changes to the architecture of the internal experiment chamber and external additions to the device to improve functionality.

The most drastic proposed change to the architecture of the experiment chamber would be to alter its shape from the current "football" into a volume more resembling a torpedo. This change would involve engineering a more gradual opening and narrowing of the inlet and outlet points. The advantages could be better flow and filling patterns through the chamber volume, thus benefiting the turnover or replacement rate of the target enzyme, saving both time and resources as well as ensuring more consistent experimental results. Similarly, the inlet and outlet points could be shifted from their placement at the midpoint of the height of the chamber. The inlet would be moved to intersect the floor of the chamber, while the outlet would be moved to intersect the chamber ceiling. This could help achieve a "bottom-up" fill pattern that should minimize mixing and work to optimize the turnover rate.

Another improvement aimed at optimizing the turnover rate could be to implement a disruption point at the inlet that would break up the inlet flow. This could optimize the turnover rate but must be weighed against the possible increase in mixing due to the more turbulent flow at the inlet. A comparatively simple change that would also help optimize the turnover rate and minimize mixing would be to lower the height of the device to create a more shallow chamber. This would result in a more even fill and less turbulent flow but must be weighed against the volume requirements of the experiments the bioMATADOR will facilitate later. In all cases, the fill material to be tested will greatly impact the flow and mixing; future design choices could be tailored to specific needs as they arise.

The most immediate potential external addition to the device to improve its functionality would be the development of a more defined collection system or chambers. The outflow from running bioMATADOR degradation experiments contains all the critical degradation products and other relevant data. Future iterations of the bioMATADOR could therefore incorporate a collection chamber to store the outflowing solvent for future testing. This system would need to allow flow in only one direction (outward from the device) to remain free from contamination. This could be achieved by incorporating duckbill valves; however, the exact use of these valves in the bioMATADOR process warrants further study.

Another potential external change to the bioMATADOR would be to change the resin used to 3D print the main body of the device. The clear v4 resin of the main body showed high fluorescence during microscopy, which could interfere with signals from the experimental chamber. Changing to a black, gray, white, etc., resin could mitigate these issues but must be weighed against decreased clarity and the ability to see all angles of the chamber. Testing should also be done to determine the optimal alternative resin.

The last potential addition to the device would be the ability to control and maintain different temperatures, which could be accomplished with a Peltier chip incorporated into the device or broader system.

5.2 Device Degradation Testing

The long-term viability of the bioMATADOR also warrants further examination. Once a more finalized design with the incorporation of the collection system is reached, it will be important to test the device's biocompatibility over the much longer time scales present in degradation experiments (ideally weeks to months). Full degradation testing regimes must be completed with the device remaining clear and avoiding biofouling and degradation of the main device. Preliminary results are promising, as the device was acid- and base-resistant up to 1 molar during a 24-h-long exposure period. Temperature stability remains to be tested with goals to test bioMATADOR stability between 4 and 95 °C.

6. References

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List of Symbols, Abbreviations, and Acronyms

3D	three-dimensional
ARL	Army Research Laboratory
DEVCOM	U.S. Army Combat Capabilities Development Command
bioMATADOR	biocompatible Material Agnostic Testbed for Autonomous Degradation of Recalcitrants
DoD	Department of Defense
PBS	phosphate-buffered saline

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