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Microfiber Acoustic Recycling with Enzyme-Assisted Valorization and Elimination (MARVEL): A Novel Approach for Microplastic Isolation from WWT Sludge

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Author Note

This research was conducted as part of the Technology Student Association (TSA) Biotechnology Challenge 2024 following the annual theme: Microplastics. We, Arsh and Ridhima, were the primary authors and contributors to this work. This solution was awarded 2nd place at the North Carolina TSA State Competition. With this in mind, the style and structure of the following paper slightly deviate from standard formal conference research papers. This presents a proof of concept and a potential design rather than a scientific process towards a proposed solution. There are no conflicts of interest to declare. Correspondence concerning this article should be addressed to Arsh Jha, arshj5093@gmail.com.

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Definition & Explanation of the Problem

Posing a pervasive environmental issue, synthetic microfiber plastics (MFPs)—a class of microplastics (MPs)—are fibrous plastic particles measuring under five millimeters. Engineered deliberately for durability from petroleum-based materials like polyester, polyamide, polymethyl methacrylate, and polyurethane, MFPs have an extremely slow natural biodegradation rate of 1000 years. To add, MFPs flood Earth at about 400,000 tons annually and constitute the largest share of plastic pollution; IUCN found that 35% of MPs in the environment originated from just the "laundry of synthetic textiles" as fibers shed in washing machines and synthetic textile factories each day.

Despite wastewater treatment (WWT) plants and septic tanks promising between 60-99% filtration of MFPs, a significant oversight persists: microplastic filtration from deposited sludge. Essentially, the reason treatment systems are so effective is due to the particle-retaining capability of sludge, given its cohesive properties. In this way, most particles settle down when sludge settles down during the primary and secondary clarifier steps (see Fig 1.1 & 1.2). However, sludge is often left untreated because of its costly nature. Henceforth, untreated sludge continuously releases MFPs when repurposed as fertilizer or incinerated in landfills. To add, the sheer volume of water processed means millions of MFPs are still released through "treated" effluent. A cycle of reentry begins, and such shortcomings make WWT the second leading source of primary microplastic loss (Boucher and Friot, 2017). Once part of the water cycle, these lightweight particles travel widely, seeping into soil, groundwater, the deep sea, and water bodies via runoff, and accumulating in food and water sources. In the rare case they are extracted, the fibers are recycled, which only adds to the problem: recycled polyester can shed twice the amount of fibers as new polyester (Fibershed 2022).

The multifaceted impact of MFPs is profound, particularly due to the capacity of MFPs to carry pollutants, toxins, pathogens, antibiotic-resistant bacteria, and even carcinogens (especially when covered in sludge) that have been deemed deadly and growth-hindering. (Ocean Conservancy, Liu J, et al. 2022). Posing a significant health concern, these contaminants now located in producers (plants) and consumers (like microorganisms & fish) alike cascade through the food web via bioaccumulation and biomagnification, ultimately affecting humans. MFPs may also clump together and act as large pieces of plastic, contributing to the death of over 100,000 marine animals yearly. Compounding the problem, MFPs in soil and groundwater end up in agriculture produce, jeopardizing food and water safety, as well as the revenue income of such environmental industries. Studies from the WHO and CDC accentuate the severity of these ramifications—upon repeated consumption, MFPs result in oxidative stress, DNA damage, organ dysfunction, metabolic disorders, weak immune systems, and several neurological, reproductive, and developmental issues (Danopoulos et al. 2022). In the long-term, MFPs contribute to an overarching issue of lower oxygen levels, biodiversity, and a greater carbon footprint, disrupting Earth’s established natural processes.

The urgent challenge pertaining to MFPs today arises from the ineffectiveness and absence of adequate microplastic removal mechanisms within worldwide WWT systems. Kupec highlights a concerning statistic: "95% of wastewater goes untreated" in developing countries, emphasizing the urgency of upgrading facilities. While traditional plant installations can cost $500,000-$2 million, decentralized options i.e. septic tanks range from $3000-$20000. Hence, recognizing global disparities in wastewater infrastructure, integrating cost-effective solutions into these systems is crucial, necessitating a comprehensive approach to extract and degrade microfiber plastics before they settle in sludge and eventually reach the environment.

Explanation of the Solution

Utilizing the force of sound waves and acoustophoresis, acoustic separation emerges as a pioneering remedy to segregate and eliminate microfiber plastics from wastewater treatment systems. While ultrasonic waves have long been instrumental in medical imaging and manipulating cells within the human body for therapeutic purposes due to their unparalleled precision (Figarol, Agathe et al.), their adaptability in targeting specific particles has prompted the development of a system tailored for microfiber extraction. This innovative technique is complemented by encapsulated enzymes, a breakthrough in bioremediation approaches, which effectively break down the harmful plastic particles. The fusion of these methodologies creates a comprehensive solution, decisively curbing the re-entry of microfiber plastics (MFPs) into the environmental cycle.

SCIENTIFIC REASONING & RESULTS

The technology behind acoustic separation involves the strategic arrangement of transducers and reflectors within a steel resonating chamber, seamlessly integrating into water pipes connected to wastewater treatment systems. This system generates a standing wave that plays a pivotal role in manipulating the dynamics of water particles. Within this setup, specific areas, termed nodes and antinodes, emerge, essential for directing the movement of MFPs (see Fig 2.2, 2.3 & 3.2). The resonating chamber's size, acoustic wavelength, and frequency influence vibrations that prompt the segregation of MFPs either at pressure nodes or antinodes. This occurs because standing waves create diverse pressure zones, prompting synthetic microfibers to gather around the nodal plane—a point of equilibrium (American 3:10-3:58). Research conducted at the New Mexico Institute of Mining and Technology highlighted that when using a frequency of

1.58 MHz and a chamber size of 486 µm, microfibers ranging from 6 to 180 µm gravitate towards the central pressure node, while larger ones spanning 180 to 300 µm concentrate towards the sides. Water that is not caught in these plastic-contaminated channels may continue through the system as normal, causing no disruptions in the pre established treatment process or the surrounding environment.

In its early phases, acoustic separation showcases impressive efficacy, clearing over 70% of smaller microfibers and 82% of larger ones—an unparalleled versatility ("Pulsing"). This method emerges as the ideal solution for plastic micro*fibers* especially, considering their propensity to morph and disintegrate further into nanoplastic fibers. Employing both curvature and water flow mechanisms, each contaminated water channel is guided into dedicated pipes linked directly to a degradation tank for specialized treatment. This controlled and streamlined process enables precise manipulation and removal of MFPs. By addressing the contaminants before the sludge deposition stage, the resultant treated wastewater and sludge is expected to contain fewer toxins, making it more conducive for repurposing as fertilizer, contributing significantly to a circular economy.

Plastics, structured with a carbon backbone, endure a slow natural degradation process spanning thousands or even millions of years. A potential solution, bioremediation, often demands specific environmental conditions and thus risks organismal death or mutation due to toxin accumulation. However, enzymes have showcased remarkable efficiency, operating hundreds of times faster and enduring multiple applications. For instance, a particular polyester, polybutylene succinate (PBS), underwent complete degradation in just 72 hours using enzymes (Thirunavukarasu et al., 2016). Derived from bacteria, fungi, and plants, hydrolytic enzymes within the hydrolase family provide a diverse toolkit for breaking down intricate fiber polymers in water (Seo et al., 2009). Shielding these sensitive enzymes from environmental fluctuations within treatment tanks, such as pH and temperature, is achievable through encapsulation within protective casings made from biodegradable polymers like polylactic acid (PLA), polyhydroxyalkanoates (PHA), or polyethylene glycol (PEG), along with silica, alginate nanospheres, or microgels. Upon exposure to plastic surfaces, enzymes like esterases, lipases, depolymerases, and PETases in an enzyme solution are attracted to the hydrophobic surface of plastic particles. Targeting the long carbon chains within the substrate, these enzymes efficiently cleave chemical bonds through hydrolysis, achieving an impressive breakdown efficiency of approximately 90% (Jyoti et al., 2021). Enzymes exhibit particular promise in degrading synthetic fibers, especially in the case of MFPs made of PET, as their ester bonds are comparatively easier to break than other plastics with pure carbon chains.

The breakdown process results in monomers and dimers, natural compounds that pose minimal risk to the environment. Therefore, after this compound-containing water is sent back through the regular WWT procedure and released into the environment and water supply, organisms can efficiently mineralize the compounds into water and gas, ensuring complete degradation of the synthetic particles. Notably, this degradation process might also generate biogases, such as methane, which can be captured and repurposed as a valuable fuel source. This underscores the positive prospective nature of enzyme degradation products, particularly in the context of MFPs where the byproducts could serve as an additional energy resource.

REJECTED SOLUTIONS

Several conventional methods are present for the purpose of extracting and breaking down microfibers, namely coagulation-flocculation, sludge filtration, and advanced oxidation processes (AOPs) and bioremediation, but each has its limitations. These shortcomings highlight acoustic separation paired with encapsulated enzymes as *the* ideal solution to the microfiber plastic problem.

Coagulation and flocculation methods utilize clumping agents to aggregate microfiber plastics into large clumps that can be removed, dried, and recycled fairly quickly. However, this process heavily relies on chemicals like Al and Fe salts, demanding substantial energy consumption from mechanisms like hydrocyclones to disperse the chemicals into water (Wei et al., 2022). The resulting ecologically harmful residue from the chemicals adds to the sludge volume, while intense stirring of the chemicals can lead to the fragmentation of microplastics into nanoplastics. Furthermore, real world application in wastewater treatment (WWT) plants has shown a modest removal rate of 40.5–54.5% for microplastics (Wang et al.). In contrast, acoustic separation doesn't rely on chemicals, operating as a contact-free method with no degradation or toxic byproducts. Its minimal energy requirement stems from adaptable transducers, compatible with various electricity sources.

To avoid such water related issues, filtering the sludge itself through may seem like a viable option. Methods such as Magnetic Seed Filtration (MSF), though capable of extracting microfibers by binding them with magnetite particles dispersed in sludge, demand intricate conditions and involve chemical interventions, like Fenton reactions and lime and bacterial treatments, leading to undesired byproducts. The challenge escalates when MFPs form deep bonds with other materials like biofilm (very common in sludge, a biomass), obstructing their extraction altogether. Even achieving a 75% removal efficiency in the case of PET microfibers demands repeated filtration (Frank et al.).The intricate entrenchment of microfiber plastics in sludge which causes changes in physical properties, along with the challenges of maintaining specific conditions and the production of toxic byproducts, underscores how extraction before sludge deposition through acoustic separation remains a much simpler, two-step process.

In contrast to enzymes, advanced oxidation processes (AOPs) rely on highly reactive molecules to break down microplastics through oxidation processes, often requiring additional chemical catalysts or UV radiation. These processes, while showcasing high efficiency in some instances such as Miao et al.'s electro-Fenton technology, frequently fail to truly address microplastics by only reaching surface-level degradation. This releases potentially toxic byproducts in the form of nanoplastics and their virulent byproducts. The need for complex reaction devices, high energy consumption, and chemical catalysts such as TiO2/C or UV rays further limits the global applicability of AOPs (Wei et al.). Conversely, enzymes, with their extensive research history, offer a more promising solution, facilitating complete degradation of microplastics into harmless compounds like CO2, H2O, CH4, and N2 gas, without generating harmful products.

ADVANTAGES

The proposed solution faces cost limitations like electricity reliance and efficient enzyme functionality, yet these are manageable. Transducers and reflectors have modest energy needs, due to their small size and cost-effective chamber construction (made of cheaper metal like steel), supporting economic viability. Further, although the MFP solution is reliant on the presence of treatment systems, this is in accordance with the UN's Sustainable Development Goal (SDG) 6 & 14 for 2030, through which decentralized treatment systems are only going to increase in number. The solution is adaptable to any pipes, resulting in a significantly lower cost version when employed in septic tanks (when compared with full scale WWT plants), which foster a circular economy in underprivileged nations. The rapid growth of genetic engineering—a field predicted to be growing at a rate of 15% by the Bureau of Labor Statistics (Carabetta 2022)—especially through CRISPR, indicates potential for swift enzyme development. In fact, a PETase enzyme was discovered and enhanced in just a few years (“Plastic” 00:00-7:51). Encapsulation specifically shields enzymes from otherwise concerning environmental factors, reducing monitoring needs and providing cost-effective compared to specific oxidation processes. Ethically, enzyme use involves much fewer concerns than bio organisms or just letting contaminated water enter the biosphere, given they only need to be successfully extracted once. Advancements and research promise to address these hurdles effectively.

Most importantly, the solution's adaptability is evident in its versatility. Acoustic separation customizes sound wave frequencies to diverse water pipe sizes worldwide. Acoustic separation has also been tested in both pure and extremely contaminated water (which has altered densities) and removal efficiency remained consistent, even with no change in the pipe structure (“Pulsing”). Within the degradation tank, a mix of enzymes, often termed an "enzyme soup," can be tailored to target specific microfiber plastics. This adaptability underscores the solution's effectiveness for widespread application, especially considering the different treatment system standards globally.

Ultimately, both acoustic separation and encapsulated enzyme technologies emerge as the pinnacle of exactness and reliability, excelling in the extraction and subsequent complete degradation of microfiber plastics. Acoustic separation distinguishes itself with its minimal environmental impact, preventing further hazardous breakdown of MFPs while precisely targeting microfibers of all sizes. In parallel, encapsulated enzymes, which are already being adjusted and improved day by day, offer a comprehensive, cost-effective solution that showcases remarkable efficiency and efficacy in the full breakdown of MFPs, ensuring pristine degradation devoid of toxic byproducts. Together, they mark a pioneering shift toward precision, environmental consciousness, and sustainable methodologies to remove microfiber plastic through wastewater—and even encouraging the installation of any form of wastewater treatment where not already present due to its promising efficiency—thus combatting the persistent challenge of microfiber plastic reentry and contamination in all parts of the world.

Real Life Applications

In the quest for innovative solutions to combat plastic pollution, acoustic separation and enzymatic degradation biotechnologies have emerged with promise. Despite being recently developed solutions, these technologies have already demonstrated their potential through groundbreaking developments and favorable outcomes in real-world scenarios.

The Hong Kong Research Institute of Textiles and Apparel (HKRITA) garnered recognition for developing Acousweep, earning them the prestigious Global Change Award. The machine utilizes transducers to generate ultrasound, effectively 'sweeping' particles in water to one side of the pipe for extraction into a collection tank (see Fig 1.4). Impressively, the lab-scale version demonstrates the capability to treat 5-10 tons of water per hour, with plans for commercialization in 2024. Employing an external approach to acoustic separation, Acousweep offers a 'plug-and-play' solution, easily adaptable to various wastewater treatment systems. Its contact-free and self-sustaining nature eliminates the need for constant monitoring or filter replacements ("Acousweep").

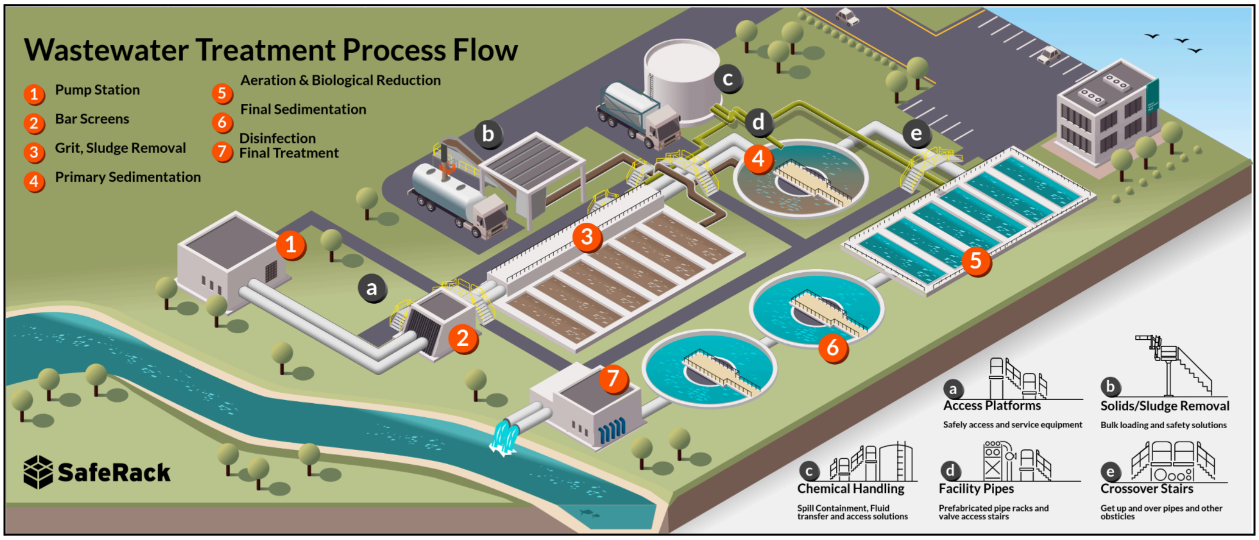
Similarly, researchers at the New Mexico Institute of Mining and Technology have developed a two-stage pipe system for microplastic extraction, with potential for scalability pending pipe widening. Initial experiments on real pond water with MFPs showed the prototype device filtering one liter of water in approximately one & a half hours, achieving removal efficiencies of 82% across varying contamination levels ("Pulsing"). Additionally, Shinshu University researchers devised a bulk acoustic wave system, leveraging piezoelectric elements to generate sound waves for microfiber plastic extraction. Demonstrating high efficiency in laboratory settings, specifically by extracting 95% of PET and 99% of Nylon 6 MFPs, this system is poised for commercial production, intended for integration directly into washing machine pipes (Coxworth 2019). Similar to the Acousweep, both systems mentioned were relatively simple and did not require much exterior manipulation.

In the realm of enzymatic degradation—a solution which only became possible in 2012 but has already skyrocketed in success—a breakthrough was achieved in 2020 by scientists from Carbios and Université de Toulouse. They engineered a PETase enzyme derived from the bacterium *Ideonella sakaiensis* strain 201-F6, capable of breaking down 90% of PET plastic from various sources within ten hours (Lao). Notably, modifications allow the enzyme to function at temperatures below 50 degrees Celsius, overcoming previous limitations in enzyme transportation and utilization (UT News). This enzymatic solution demonstrates efficacy even on complex and soiled plastics, presenting a significant advancement over traditional thermomechanical processes ("Carbios"). The initiation of industrial testing for the enzyme in 2021 highlighted the swift advancements within the bioenzyme industry. Carbios' ultimate strategy entails either allowing monomers to biodegrade into the environment, akin to the proposed solution, or recycling small plastics through repolymerization, both of which foster economic circularity within the textile industry where waste is minimized, and resources are utilized more efficiently. Furthermore, while the enzyme industry is currently in its nascent stages, with the increasing demand for enzymatic solutions and their growing recognition of environmental benefits, the enzyme industry and Carbios are praised for being at the front of rapid expansion, spurring economic growth within the bioenzyme sector in the near future.

As research, development, and commercialization efforts continue to accelerate, these technologies hold the promise of revolutionizing our approach to synthetic microfiber plastic treatment in wastewater. By fostering a more sustainable future, they pave the way for generations to come to inherit a cleaner, healthier environment.

Supplementary Information

Wastewater Treatment Plant System



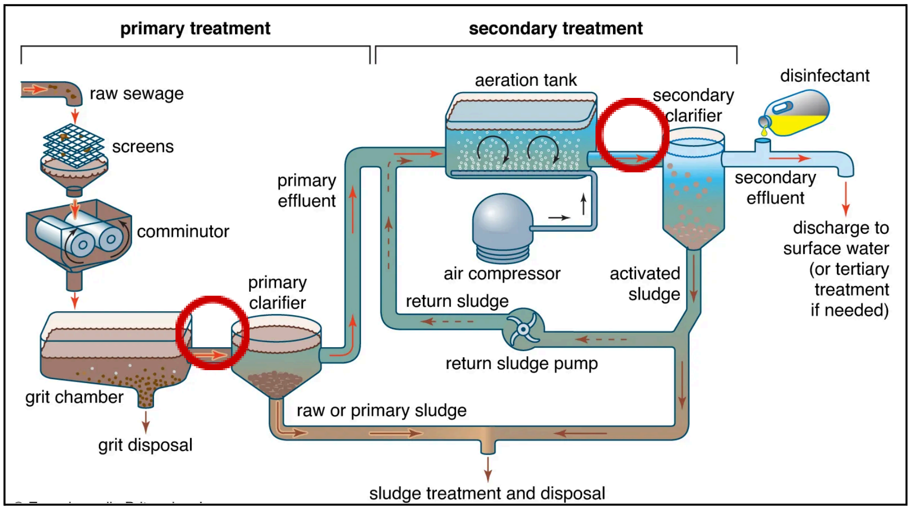
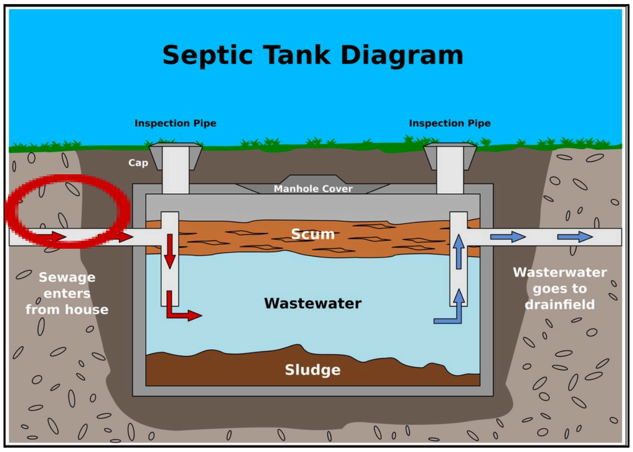


Fig 1.1 & 1.2: Water treatment through WWT plants is extremely efficient due to its several steps, but microfibers become stuck in sludge once deposition occurs. Acoustic separation is intended to be employed in pipes that lead out of the grit disposal step but before clarification steps, thus removing MFPs at the right time (red circles indicate where the acoustic chamber will be employed).

Septic Tank System

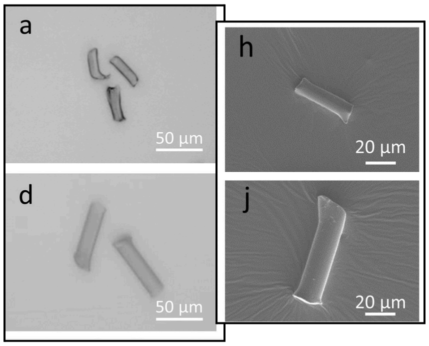
Fig 1.3: Although a much simpler process, this decentralized treatment still effectively filters water through sludge deposition and scum (oil and grease) flotation. Similar to WWT plants, the acoustic mechanisms can easily be employed in the pipe leading into the septic chamber, and the enzyme degradation tank may be added as an additional step. The pipes are made of PVC, vitrified clay, or ductile iron, all of which are

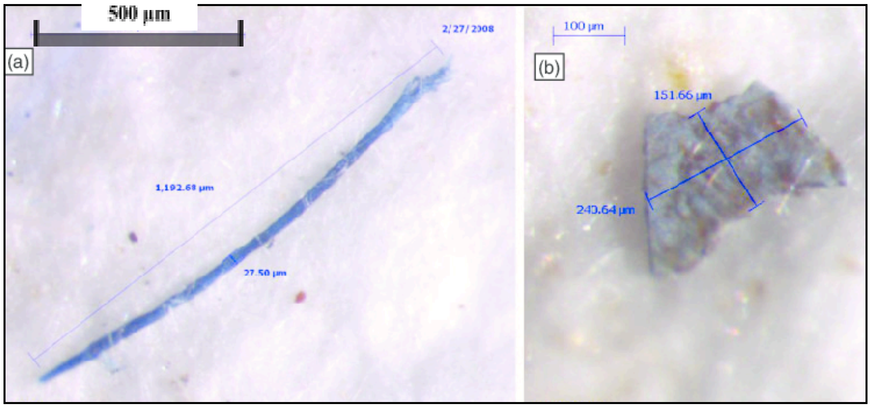


fairly cheap and durable materials, making it easy to build or rebuild the infrastructure to incorporate the resonating chamber for our solution.

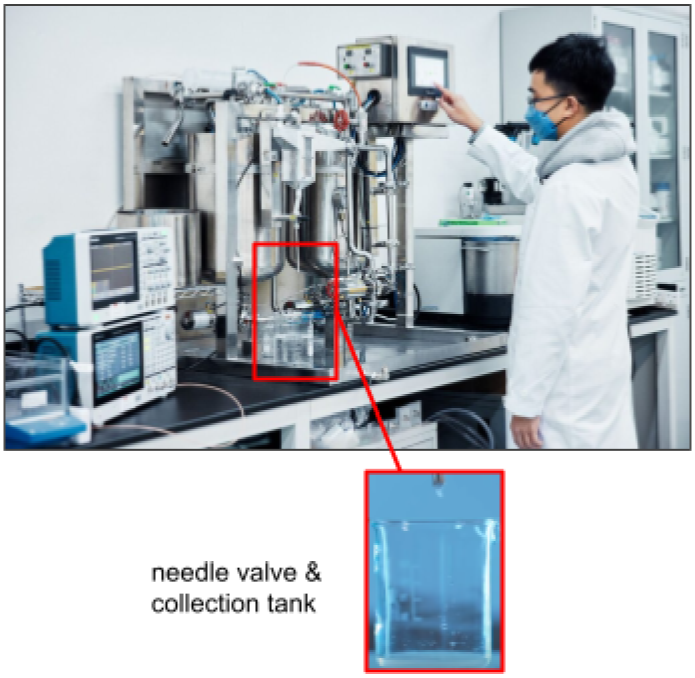
MFP Size & Shape,

Fig 1.5 & 1.6





Acousweep Mechanism



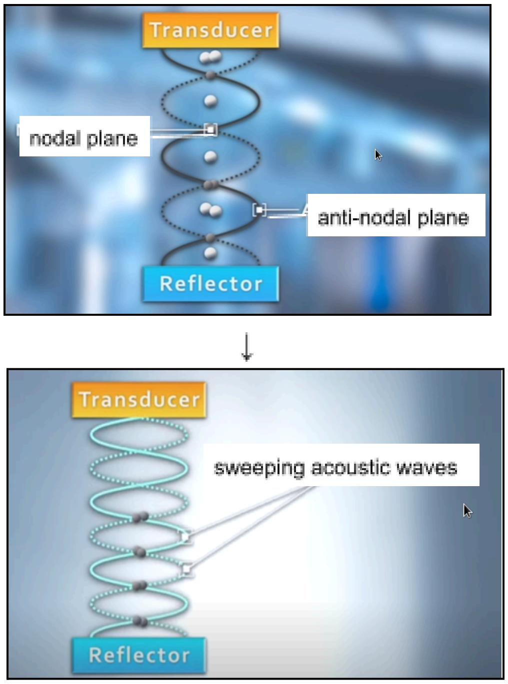
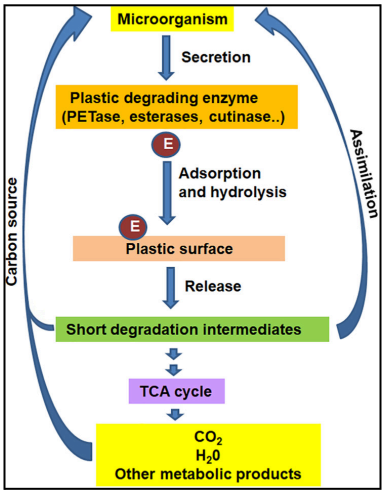


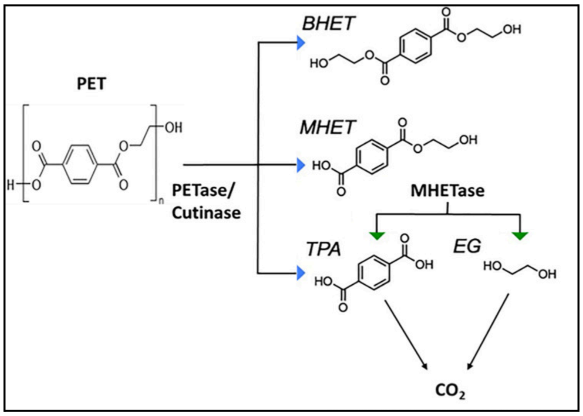
Fig 2.1, 2.2, & 2.3:

HKRITA uses transducers and reflectors to create sweeping waves instead of standing waves, a mechanism very similar to our solution. There is a needle valve at the apex of the reflector, which, when detects a significant concentration of MFPs, opens and releases the contaminated water into a separate tank for further treatment, such as enzymatic degradation or agglomeration (HKRITA). Fig 2.2 & 2.3 depict particles concentrated at the nodal planes of a sound wave.

Enzyme Cycle & Process

Fig 2.4 & 2.5: The general life cycle of an enzyme and its products is continuous; Fig 2.3 depicts how the products readily incorporate with the biotic and abiotic ecosystem. Fig 2.4 depicts products of the PETase and MHETase enzyme: nontoxic BHET; MHET; terephthalic acid (TPA), which can be turned into proto-catechuic acid, something with many uses in medicine and nutrition (Kincannon et al. 2022) and ethylene glycol, something used a lot commercially, as antifreeze ("Plastic" 3:40-4:05).





Enzyme Structure & Efficiency

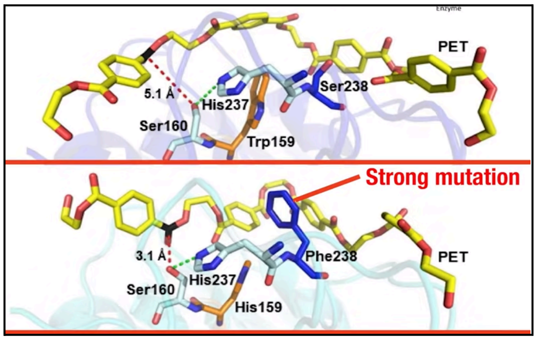


Fig 2.6: Cutin is nature's polyester, and several bacteria have evolved over thousands of years to break down this wax through cutinase. PETase is an enzyme that evolved from several cutinase enzymes in just fifty years, allowing it to break down synthetic polyesters. Since identifying its structure, scientists have been able to make small changes to the amino acid structure, which in turn, resulted in a 100 fold faster break-down rate. This depicts the original PETase enzyme in comparison to an only slightly modified one, which had a huge increase in the digestion rate of plastic, due to an increase in the crystalline structure.

Key Drawbacks of Other Common Solutions, 3.1 - Table 1:

Process description	Major mechanism	Lowest size of microplastic particle removed/finest mesh	Efficiency (%)	Challenges
Wastewater treatment plant processes	Primary, secondary, and tertiary	100 µm	99.9	Not possible to remove MPs of size <100 µm
	Secondary treatment	20 µm	95.6	Complete retention is not possible especially for smaller, malleable particles like fibers
	Tertiary treatment		97.2	
	Membrane bioreactor		99.4	
	Membrane bioreactor (MBR)	250 µm	99.3	Not possible to remove MPs of size <250 µm
Al and Fe salt	Coagulation	<0.5 mm	45.34±3.93	Low efficiency, chemical pollution

Electrocoagulation	Charge neutralization, flocculation	–	90 (pH 3–10) 99.24 (pH 7.5)	Operation time needs to lowered down, high energy consumption
Filtration with granular activated carbon (combined with coagulation and sedimentation)	Physical properties (size and shape)	1–5 µm	56.8–60.9	Process is slow and results in obstruction of the pores with time; costly; regeneration is tough
Pulse clarification with filtration	Entrapment in sludge blanket formed due to coagulation floats	<100 µm	85	Complete retention is not possible, requires repetition to be efficient
Advanced oxidation processes (AOPs)	Oxidation of particles through highly reactive molecules and chemical or physical catalyst	1 µm	85.3%	Efficiency relies on costly catalysts, complete degradation is not common, releasing toxins
Derived from Surya Singh, Madhanraj Kalyanasundaram, Vishal Diwan; Removal of microplastics from wastewater: available techniques and way forward. *Water Sci Technol* 15 December 2021; 84 (12): 3689–3704. doi: https://doi.org/10.2166/wst.2021.472				

Case Study & Our Acoustic Separation Solution

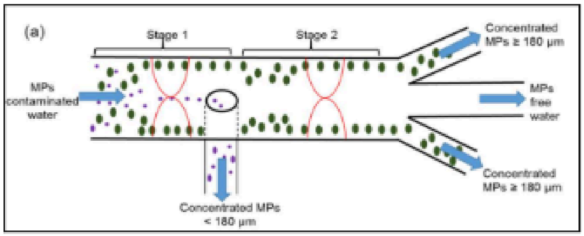
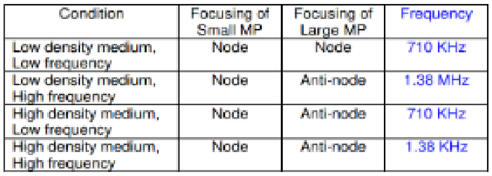


Fig 3.2 & 3.3: Researchers from the Department of Chemistry, New Mexico Institute of Mining and Technology have made this acoustic focusing device that effectively removes smaller MFPs the first time ultrasound is applied, and larger ones the second time. Our solution uses the same mechanism, leading the shown extraction pipes to an enzyme degradation tank.



Condition	Focusing of Small MP	Focusing of Large MP	Frequency
Low density medium, Low frequency	Node	Node	710 KHz
Low density medium, High frequency	Node	Anti-node	1.38 MHz
High density medium, Low frequency	Node	Anti-node	710 KHz
High density medium, High frequency	Node	Anti-node	1.38 KHz

Fig 3.4: The researchers found that the difference in where small and large MFPs focus on the nodal planes is dependent on the density of the wastewater, as well as the frequency of sound waves. These findings demonstrate that frequency can be adjusted based on pipe size and the typical density of each specific treatment system the solution is used in.

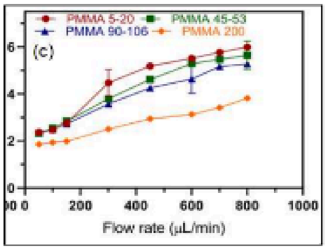


Fig 3.5 shows the voltage required (y-axis) to effectively focus one of the plastics used in their trials, PMMA. The energy requirement is low, and although it increases with high water flow rates, the rates can be managed using flow restrictors.

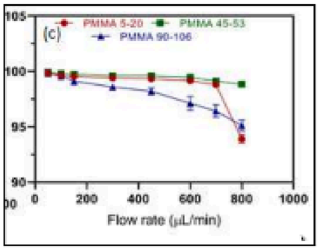
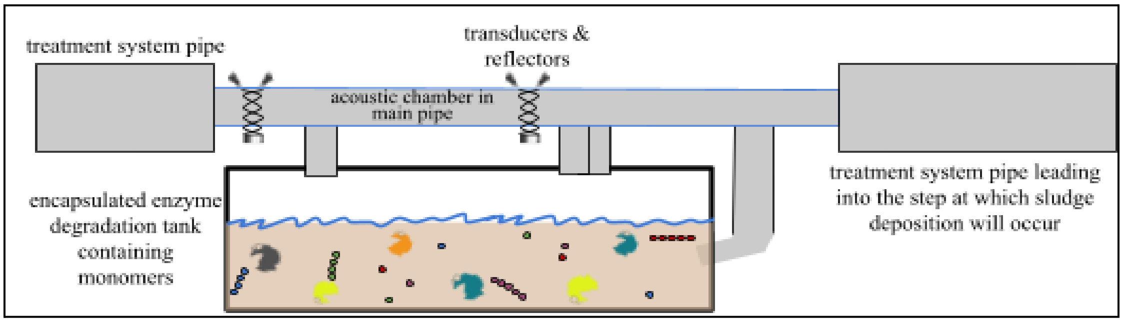


Fig 3.6 shows the focusing efficiency (y-axis) of soundwaves on different sized PMMA particles. Again, high flow rates can be regulated, keeping efficiency above 90%

Fig 3.7 depicts the structure through which the two parts of our solution may be connected.



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