Perspectives Article

The usage of bacteria to degrade microplastics in the Chesapeake Bay*

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The Chesapeake Bay's ecosystem is heavily impacted by microplastics, with 98% of water samples containing them. The bioaccumulation of microplastics has adverse effects on both the reproductive function and health of tertiary species, and the death of these animals can lead to ecosystem collapse. Bacteria that have been shown to degrade plastics in a lab could be a cheap and adaptive solution to remove these harmful pollutants. However, there must be consideration of competency for the bacteria species and the challenge of making them adhere to plastics. Additionally, establishing viable bacterial colonies within the bay is complicated because of the variability in salinity and temperature. The reproductive capability of the bacteria means that no further input is required after introduction into the ecosystem, and the ability to adapt to changing environments makes it a long-term solution. While the removal of plastic by bacteria has promise, there should be consideration for the byproducts of the degradation process and the potential effects on the native ecosystem. The plastic crisis causes both damage to the environment and to the humans that live within the watershed. The engineering of bacteria for variable ecosystems is essential in the fight against plastic pollution and should be pursued due to the severity of this crisis.

Keywords: Plastic degradation, environmental conservation, Chesapeake Bay, plastic pollution

The classical definition of plastic is "any of numerous organic synthetic or processed materials that are mostly thermoplastic or thermosetting polymers of high molecular weight and that can be made into objects, films, or filaments" (Merriam Webster, n.d.). To further define microplastics, it is important to understand their specifications. Microplastics are plastic particles ranging from 5 mm to 1 nm, whereas nano plastics are plastic particles smaller than 1 µm. The prevalence of these microplastics has been observed in every ecosystem on the planet, including uninhabited regions such as the antarctic tundra. As stated by the U.S. Environmental Protection Agency, "Plastics have become ubiquitous in natural and built environments, which has caused concern regarding potential harms to human and aquatic life" (EPA, 2022). Some of the plastic infiltrating the bay is a result of runoff from cities that occupy the watershed. This has led to legislation in Maryland being introduced specifically targeted at banning polystyrene (Nature-Pack, 2018). The Maryland Legislative Assembly passed the Expanded Polystyrene (EPS) Food Service Products Ban during the

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2019 legislative session, which banned the use of food service products made up of EPS, more commonly referred to as Styrofoam. The law went into effect in July of 2020 (Maryland Department of the Environment, n.d.). Although there has been legislation and other steps taken to prevent further increase of plastic pollution in the bay, the state of the bay is still abysmal. According to the 2022 State of the Bay report released by the Chesapeake Bay Foundation, the overall score of the bay remained at a 32 (D+), unchanged since 2020. This report takes into account changes in population from the main species inhabiting the bay such as blue crabs and oysters as well as toxic levels to which microplastics contribute. Toxic levels in the 2022 report were at 28 (D) showing no change from 2020 (Chesapeake Bay Foundation, 2022). This information further exemplifies the necessity of implementing some sort of microbial change to the ecosystem to further the decomposition of these harmful plastics, and importantly, one that is viable in the varying degrees of salinity found to be present in the Bay.

Current bacterial solutions

As the negative effects of plastic pollution have become increasingly prevalent in our everyday lives, the pressure for solutions has greatly escalated. There have been several studies conducted and steps made to understand how bacteria could be the solution to our plastic problem. One potential method is microbial degradation, a process in which biodeterioration, thermal decomposition, and integration take place and transform into gasses and a microbial community (Kumar et al., 2023). Biofilm formation is another natural method of plastic digestion in which bacterial cells are "self-immobilized in an extracellular polymeric matrix" (*Biofilm Formation,* 2019). Some bacterial biofilms can biodegrade polymers, including plastics. The effectiveness of these processes depends on the type of bacterial organism, as well as outside factors such as the climate. In ideal conditions, the waste plastic can be converted into methane, CO2, biomass, water, and inorganic chemicals (Mohan & Arunasri, 2019). These bacterial systems show immense potential for being utilized to degrade microplastics.

A more specific study conducted by the University of Cambridge with results published in *Nature Communications* found that, in 29 lakes studied in Europe, lake bacteria digested the plastic pollution in the water before other natural matter. Breaking down the plastics fed the bacteria and enabled them to metabolize other natural carbon compounds. When plastic pollution caused the carbon levels to rise by 4%, the rate of bacterial growth more than doubled. Though plastic pollution has far more negative effects than positive ones, the increased bacterial growth means more food for other organisms in the lake ecosystem, like fish. Another promising aspect of the study is that the lakes all differed in factors such as depth, surface temperature, and area, showing that the process could occur in many scenarios (University of Cambridge, 2022). While unfortunate that microplastics are so common they have become food, it could be the key to solving our plastic pollution crisis.

In a study conducted in 2022 and 2023 by North Carolina State University, researchers genetically engineered bacteria *Ideonella sakaiensis* and *Vibrio natriegens* to be able to break down PET microplastics. They took DNA from *I. sakaiensis,* a bacterium that produces enzymes able to metabolize PET, and inserted the genetic sequence into a plasmid. They then put that plasmid into *V. natriegens,* a bacterium that flourishes in salt water and speedily reproduces. Though only able to do so in salt water at room temperature, this is the first genetically modified bacteria able to break down PET. Even with its limitations, this still shows great promise for becoming a viable solution in the future (National Science Foundation, 2023). These studies are vital to improving our understanding of solutions for plastic pollution, but more in-depth research and time must be dedicated to the topic for progress to be made.

Possible solutions: Biological degradation

The degradation of plastics requires two things: a way to attach to the plastic and a way to degrade the plastic into a harmless or useful byproduct. Plastic biodegradation requires an adaptable solution because each plastic requires its own degradation pathway (Mohanan et al., 2020). The biodegradation of most plastics begins with an enzyme that needs to be able to attach to the plastic surface. Adsorption of the enzyme onto the plastic is hindered by the hydrophobicity of the plastics listed previously. The current enzymes that are able to both degrade and adhere to plastic are PETase, cutinase, esterases, etc. The factors of degradation include temperature, pH, moisture, UV, enzymes, and the properties of the plastics. The types of bonds present in the plastic mean that change what type of biodegradation is needed. The types are as follows: "(i) Polymers with carbon back-bones; (ii) Polymers with ester-bond back-bones and side-chains; and (iii) Polymers with hetero/carbamate(urethane) bonds" (Mohanan et al., 2020).

Polymers with only carbon backbones include PE, PP, PS, and PVC. The degradation pathways for this type of plastic are difficult, like in the case of PVC, which has a high hydrophobicity and stability. It is unlikely that a plasmid could be made from the existing genetic material of bacteria because the extreme variability in salinity and temperature within the Chesapeake Bay would hinder the fidelity of the enzyme (Kijchavengkul & Auras, 2008). A biological solution could be made by the utilization of either an already existent bacteria within the bay or through accelerated natural selection with bacteria already found within the bay. Currently, there are no known bacteria in the Chesapeake Bay that can degrade bacteria. However, due to the prevalence of microplastics within the watershed it is likely that there is some microorganism that can degrade plastic.

Accelerated natural selection has been used to evolve indigenous bacteria that can degrade pollutants in the environment (Ilmjärv et al., 2017). This works through the process of natural selection of favorable traits in response to environmental pressures. A visual explanation is shown above in Figure 1.

Accelerated natural selection could either be with plasmids or through the selection of survival conditions. The plasmid method

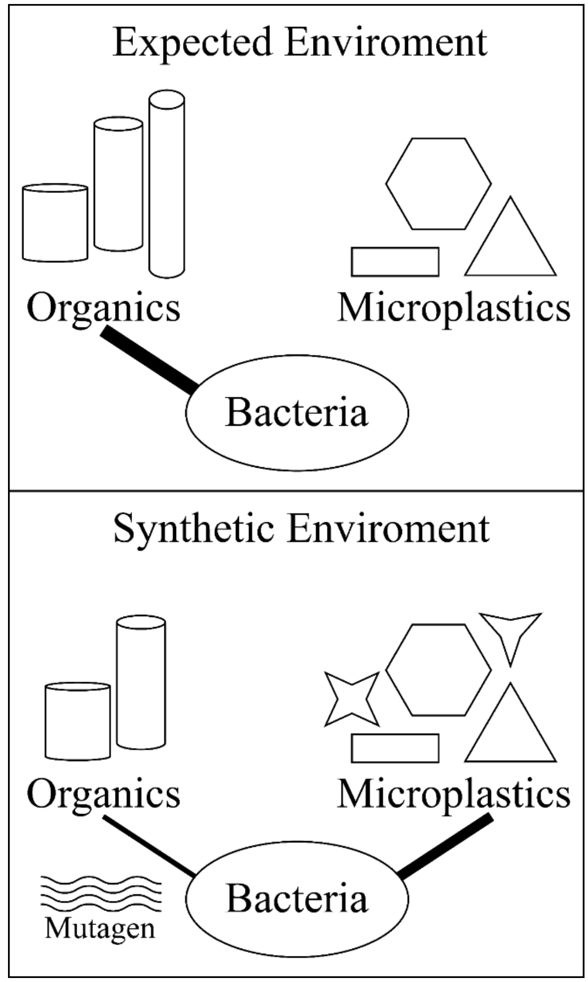

Figure 1. Illustration of a hypothetical artificial accelerated natural selection process.

would be as follows: locate enzymes that can degrade plastics, reverse transcriptase them into a plasmid, find a competent microorganism in the Chesapeake Bay, transform the plasmid into the microorganism, and test whether it can still survive in the harsh environment and make sure there are no harmful byproducts. The Chesapeake Bay has many variable and extreme conditions, so this method is unfavorable. Currently, the biological mechanisms available require very specific conditions.

There are two other possible solutions both utilizing accelerated natural selection. The first would be to place a microorganism that can already degrade plastic into a simulation of the harsh environments of the Chesapeake Bay with the main carbon source being plastic. This would probably require a slow introduction to the harsh conditions and many generations. The environment would also contain some mutagen, like a UV light, to accelerate the mutation rate and perhaps the adaptation rate.

The second method would be to place a microorganism native to the bay into a simulation of the bay, but with the main carbon source as plastic. This method is most compatible with the already occurring evolution of microorganisms, which can degrade plastics. The environment would differ in that it would have some mutagen and less nutrients. Scientists are finding microorganisms that can degrade plastics, and there may even be some already present within the Chesapeake Bay. As such, it is reasonable that the best a lab can do is exploit the mechanism of natural selection. A solution such as this would naturally fade away once the problem of plastic pollution is solved.

Setbacks

Currently, for the authors, it is only hypothetical. We are unable to achieve this due to our lack of resources and other necessary components. The enzymes needed for degradation (PETase, cutinase, and esterases) are not readily available in the environment, and even if they were, there are still some potential setbacks (Mohanan et al., 2020). The Chesapeake Bay is very unique in that it has polar variability in its salinity and temperature. Additionally, for degradation to happen, UV or oxygen is necessary to catalyze the enzymes. Unfortunately, those two factors are not always present in this bay (Mohanan et al., 2020). Finally, there has not been enough research done on the possibility that the degradation process could produce harmful byproducts that might have an unwanted and damaging effect on the native environment (NCBI, 2023). We believe that with proper government backing and advancement in the research on this topic, this solution to the plastic problem could present a significant turning point in the fight to save our earth.

Next steps

Hypothetically, it would be possible to develop a biological mechanism that could degrade plastic within the environment of the Chesapeake Bay through artificially accelerated natural selection. Just like with the case of CRISPR, research has found that instead of artificially inducing a plastic degrading mechanism, we would be more likely to succeed if we developed a microorganism plastic degrading mechanism. While this method would be time heavier than the alternative, it is much simpler than the enzymatic pathway. As it is derived from natural selection the process is more adapted to the environment, while a lab-based bateria would not be as safe in the bay and could not survive. This method would also reduce the risk that there would be byproducts harmful to the environment.

Author contributions

E.B. contributed the background research on the current conditions in the bay and the background on microplastics and the effects they have worldwide. Z.P. contributed an in-depth overview of the current bacterial solutions to this issue. A.O. discussed our proposed potential solutions to this widespread issue, and L.C. discussed the further steps and the setbacks we encountered.

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