Fall 2021

Utilizing plastic-degrading microbes for plastic degradation

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Global Sustainable Development

11/3/21

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"On my honor, I have not given, nor received, nor witnessed any unauthorized assistance on this work."

Introduction:

Pollution is an overwhelming problem that seems to accumulate more and more each year, faster than it can be processed for elimination. Much of this pollution gets dumped into the ocean and it suffers many effects because of it. One may think that the ocean is too vast for a petite amount of pollution to have any drastic effects on it. However, the ocean does not have any boundaries, and due to this, it can be very susceptible to several effects such as acidification, marine diseases, etc., that can derive from the presence of pollutants in the ocean. Many of these pollutants include, but are not limited to, persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs) from oil spills and combustion of fossil fuels (Abdel-Shafy & Mansour, 2016), as well as plastics. These pollutants linger in our ocean for an excruciatingly long time due to their resistance to degradation from the elements and long half-lives (Catania et al., 2020). While these persistent pollutants do damage to our oceans, studies have also shown that plastics are the main contributing factor responsible for the formation of biofilms. These biofilms are highly diverse bacterial communities that have congregated around these plastics due to their innate ability to break them down into carbon dioxide and biomass (Catania et al., 2020). Some of these microbes are able to break down plastics in under a short period of time, whereas others take a bit longer. These microorganisms are capable of breaking down these plastic debris with the help of specially evolved enzymes. Scientists have taken an interest in the key organisms with enzymes capable of degrading the polymers and isolated them to study and learn more about them. This ultimately leads to the idea of how plastics in our ocean affect the diversity of biofilm communities and how we can utilize these microbial organisms as a form of bioremediation in the ocean.

Plastics Debris:

Humans use a lot of plastic because it plays an important role in our daily lives, but this has also led to a dramatic increase in the production of plastic. We dump about 300 million metric tons of plastic in oceans every year (Naik et al., 2019). In the 1940s, 0.5 million tons of plastic was being produced annually, and that number has significantly increased to 550 million tons in 2018(Naik et al., 2019). Many of these plastic polymers include, polyethylene (PE), polypropylene (PP), polystyrene (PS), etc. This can pose a serious problem as plastic is not the most biodegradable material and able to persist in the environment just like POPs (Rodriguez et al., 2021). Plastic is not often recycled and in 2013, only about 14% of plastic packaging materials were recycled whereas a shocking 72% were simply thrown away in landfills or simply dumped straight into the ocean (Naik et al, 2019). Through fragmentation and natural weathering, both physical and chemical, the plastics eventually breakdown into what was mentioned as microplastics. These microplastics, due to their incredibly small size, are able to impact a wide variety of marine biota. These microplastics that are too small for the naked eye to see, usually ranging from about 100 nm to 0.5 cm (Naik et al., 2019), and these can be absorbed by organisms and impede their ability to develop and reproduce. In addition, there are hydrophobic POPs in the ocean that readily adsorb to the plastics and accumulate toxic chemicals that ultimately leech into the environment, affecting the marine organisms present in the ecosystem (Catania et al., 2020). When these POPs accumulate and spread across large areas, it is very difficult to contain, as they are able to easily get into the tissue of marine animals. Just how humans breathe in air filled with oncogenic pollutants, fish and other marine animals cycle water for oxygen, thus exposing them to the pollutants (Baines et al., 2021). Over time, the POPs accumulate in the tissues of organisms, and it can damage the DNA of their cells, leading to the development of cancer or variations of it (Baines et al., 2021). Ultimately, these plastics clearly have to be dealt with and marine microbes might hold the key to solving this plastic problem.

Effect on microbes:

While these pollutants travel through our oceans, a large number of microbes readily colonize the pollutants and form biofilm communities. They have derived special enzymes capable of altering the chemical composition of these pollutants and breaking them down in a way in which they can get energy out of it. However, while these microbes are breaking down these pollutants together, they are also competing against one another. The diversity of the biofilms is vast, consisting of heterotrophs, autotrophs, symbionts, etc. (Zettler et al., 2013). Some of these microbes are key players in breaking down these pollutants and others are there to use the by products as substrate for their own metabolic pathways. These key players are competing to reproduce and grow faster than the other degrading microbes, so while there is “cooperation” they are still competing against one another for the sake of survival (Oberbeckmann et al., 2016). In a study done by Oberbeckmann and her research team, they had wondered if the type of pollutant plays a role in the type of microbes present in the biofilms that form at them. After much tedious research in the North Sea, using polyethylene terephthalate (PET) and glass, they were able to conclude that the type of pollutant is not the determining factor of the diversity of biofilm communities, rather they are just diverse because those microbes found in that particular biofilm are present because they were able to establish themselves quick enough to the available pollutants in the water. The bacteria that were able to establish themselves on the PET were bacteria of the families Bacteriodetes, such as Cryomorpheaceae, Flavobacteria, and Saprospiraceae, as well as diatoms such as

Coscinodiscophytina and Bacillariophytina. They compared the diversity of communities on PET and glass and found a 57% divergence between the two. Furthermore, they were able to establish that while the pollutant was not a huge determining factor, the environmental conditions were. Depending on the physio-chemical conditions of the environment in which the pollutants are in, it helps better shape the dynamics of the species found in the biofilm communities, by narrowing the possible microbes capable of living under those conditions. Environmental conditions include temperature, pH. Oxygen availability, salinity levels, etc. Thus, environmental factors and geographical locations plays a much larger role in biofilm diversity rather than the substrate itself. This in turn, helps scientists predict what types of microbes to expect in these different biofilms, and that allows them to more quickly identify what microbes are key players in degrading pollutants. However, seasonal variables may alter the diversification of biofilm communities. Oberbeckman and co-workers, discovered that during the winter the microbial communities had the least amount of diversity and localized primarily on PET with few on glass. During the spring there was a 43% overlap between communities found on PET and glass. The summer had the most diversity between the two substrates.

In a similar study done by Yang et al., analyzing microplastics in the freshwater Pearl River Delta, China, they came to the conclusion that the biofilm communities were pretty similar to one another, in that, microplastics were not a huge factor in determining the diversity of the biofilm communities. They only differed greatly during the different seasons. Furthermore, they were able to determine that the key microorganisms present across most of the biofilms and seasons, were microbes belonging to Streptococcaceae, Deinococcaceae, Flavobacteriaceae, Aeromonadaceae, Comamonadaceae, Moraxellaceae, Pseudomonadaceae, and Enterobacteriaceae. These are all families containing microbes that could have the potential to degrade plastics. Do keep in mind, that these samples were taken from freshwater sources. Overall, with the exceptions of key plastic-degrading microbes, the diversity of other microbes that are present in the biofilm communities vary greatly. The relationship between microbes and pollutant is quite complicated and there is still much to learn and understand about it. However, as we do, we begin to discover more about it and more specifically the microorganisms that play a key role in their unintentional bioremediation, and the enzymes that allow it to happen.

Degrading organic pollutants:

As production of goods increase, so do the waste, especially if there is not an efficient way of disposing of it. Therefore, we have a large accumulation of waste present in our oceans due to a lack of waste management. The microorganisms that form biofilms on or around the waste, are able to degrade these pollutants to varying degrees, which we find potential in using those same enzymes for bioremediation. Thus, the idea of using the microbial organisms as a form of bioremediation has received a lot of attention as a viable solution to the pollution present in our oceans. In order to implement this form of bioremediation, scientists have decided to use the isolated enzymes from plastic-degrading microbes, and genetically engineer them to have a higher ability or efficiency in removing plastics, and then reintroducing them to the bacteria (Liu et al., 2019). However, while it does seem promising, like any system, it has its pros and cons. The benefits of genetically engineering bacteria are as what was mentioned already- efficient degradation of pollutants. Given that biofilms are a highly competitive ecosystem, and many microbes are reliant on the products of other microorganisms, it makes sense to include two or more bacteria that work together, rather than a single bacterium (Liu et al., 2019). Even though the genetically engineered microorganisms have high adaptability, they have better chances of surviving and degrading pollutants collectively (Liu et al., 2019). This form of remediation could also help solve the issue of finding ways to manage or degrade newly emerging pollutants. This makes sense as bacteria are able to rapidly adapt to changes in their environment, therefore they would be able to produce enzymes capable of breaking down the new pollutants in the environment. Now for the cons of this idea. If these genetically engineered microbes were to be introduced, we would hope that they would still be able to adhere to the right substrate and they would also degrade the pollutants as they were designed to do rather than get involved in other interactions (Liu et al., 2019), however that can be difficult in a biofilm where it is a complex setting. There is also the factor of genomic stress. By this I mean, having too many plasmids in a bacterial cell can induce cellular stress and cause a decrease in its growth and reproduction, and thus its efficiency to bioremediate (Catania et al., 2020). Furthermore, the recombinant DNA in the engineered bacteria could end up getting lost in reproduction. While loss of the plasmid is rare, it can happen, because as a bacterial colony grows and divides, some daughter cells will not receive the plasmid, and eventually the number of bacterial cells without the plasmid will outnumber those that do (Liu et al., 2019). Even when we do genetically engineer bacteria, we have to make sure that they do not produce any hazardous by products as they break down the pollutant (Catania et al., 2020). This would go against the whole concept of bioremediation. Lastly, if the microbes are successful in breaking down the pollutant in a given area, we would need to find a way to control their growth and prevent them from growing out of control and upsetting the balance of the local ecosystem.

There is a second method that has been considered as a possible approach to degrading plastics. Rather than using the bacteria themselves, scientists have thought about using only the enzymes themselves. Essentially, once the enzymes are identified, analyzed, and isolated. They will undergo a modification necessary for their efficient performance and then they would then be freeze-dried, turned into a powder, and finally introduced to a liquid. (Ellis et al., 2021). This liquid would contain the modified enzymes and they would then be introduced to the polymers and reduce them to monomers. These monomers can then be reused for other purposes.

Conclusion:

This world struggles with getting rid of waste in an efficient manner. Due to this, we have a large accumulation of it, in particular plastic waste. The presence of plastic in our ocean has some concerning effects on the marine organisms present in it, and effects on them come back to affect us, as everything is interconnected. Microbes that congregate on these plastics tend to have the ability to degrade them to carbon dioxide and biomass. They are able to do so because they possess enzymes capable of breaking polymers down. These microbes are being identified and isolated, so scientists can study the enzymes they possess and potentially use them for large-scale bioremediation. It seems that these plastic-degrading microbes have set a foundation for scientists to continue from and hopefully we can find a way to use them and their enzymes for this plaguing plastic problem.

Literature Cited:

Abdel-Shafy, H. I., & Mansour, M. S. (2016). A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egyptian Journal of Petroleum, **25** (1), 107–123.

Baines, C., Lerebours, A., Thomas, F., Fort, J., Kreitsberg, R., Gentes, S., Meitern, R., Saks, L., Ujvari, B., Giraudeau, M., & Sepp, T. (2021). Linking pollution and cancer in aquatic environments: A review. Environment International, **149**. https://doi.org/10.1016/j.envint.2021.106391

Catania, V., Diliberto, C.C., Cigna, V., Quatrini, P. (2020). Microbes and Persistent Organic Pollutants in the Marine Environment. Water Air Soil Pollution **231**, 1-10.

Ellis, L.D., Rorrer, N.A., Sullivan, K.P., Otto, M., McGeehan, J.E., Roman-Leshkov, Y., Wierckx, N., Beckham, G.T. (2021). Chemical and biological catalysis for plastics recycling and upcycling. Nature Catalysis **4**, 539-556.

Liu, L., Bilal, M., Duan, X., & Iqbal, H. M. (2019). Mitigation of environmental pollution by genetically engineered bacteria— current challenges and future perspectives. Science of The Total Environment, **667**, 444-454.

Naik, R.K., Naik, M.M., D'Costa, P.M., and Shaikh, F. (2019). Microplastics in ballast water as an emerging source and vector for harmful chemicals, antibiotics, metals, bacterial pathogens and HAB species: A potential risk to the marine environment and human health. Mar. Pollut. Bull. **149**, 110525.

Oberbeckmann, S., Osborn, A. M., & Duhaime, M. B. (2016). Microbes on a bottle: substrate, season and geography influence community composition of microbes colonizing marine plastic debris. PLoS One, 11, 1–24.

Oberbeckmann, S., Loeder, M.G.J., Gerdts, G., Osborn, A.M. (2014). Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in Northern European waters. FEMS Microbiology Ecology, 90, 478-492.

Rodriguez, J., Gallampois, M.J.C., Haglund, P., Timonen, S., Rowe, O. (2021). Bacterial communities as indicators of environmental pollution by POPs in marine sediments. Environmental Pollution, 268 115690

Yang, G., Gong, M., Mai, L., Zhuang, L., Zeng, E.Y. (2021). Diversity and structure of microbial biofilms on microplastics in riverine waters of the Pearl River Delta, China. Chemosphere, 272, 129870.

Zettler, E. R., Mincer, T. J., & Amaral-Zettler, L. A. (2013). Life in the "plastisphere": microbial communities on plastic marine debris. Environmental Science & Technology, 47(13), 7137– 7146.

Gibson, Tom. "Could Plastic-Eating Bacteria Save the Planet?" *Youtube*, uploaded by Bloomberg Quicktake, 14 December 2020, Could Plastic-Eating Bacteria Save The Planet? - YouTube