Biodegradation of Plastics by Microbes in the Marine Environment

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Research Context

In this paper, I provide the background, methodology, and findings of multiple studies of the multiple angles that plastics and their respective microbial communities have been researched. These perspectives on plastics in relation to microbes include determining how plastics impact the dynamic system known as the open ocean is not well understood, especially with regards to microbial communities, to determine if insight can be gained by testing community composition of samples. Amaral-Zettler et al. (2013), check to see if the plastics littering global waterways reflect the regions that the plastics are claimed to have originate in and that management of the issue will depend on regional cooperation, Amaral-Zettler et al. (2015). Additionally, a comparison of bacterial communities from several different substrates "…floating plastics, sediment-associated plastics and sediments from the Mediterranean Sea." , Delacuvellerie et al. (2019), successful indicators of biodegradation, Jacquin et al. (2019), and lastly, the plastic lifecycle, Amaral-Zettler et al. (2020).

Introduction

The general term "plastic" refers to an industrial material consisting of organic polymers that has served many purposes in human society as a whole. Plastics serve as a cost efficient and reliable material when it comes to mass production of many kinds of product. It requires much less effort and time to "forge" it into its desired shape and function. Additionally, plastic is surprisingly light in mass and can be used as substitute for many metals. For example, many components of cars are made of some kind of plastic, resulting in the final automobile being lighter than it normally would. This increases fuel efficiency of the car, without much loss (if any) to the safety of the driver and passengers. From mundane office appliances, all the way to state of the art medical equipment, plastics play a role in the economic stability and efficiency of society as we know it.

Plastics are currently the world's most abundant type of debris in the marine environment, depending on the size of plastic substrate in question, it could be that of a "microplastic". Typically, less than 5 millimeters (mm), make up a large portion of plastic marine debris (PMD). Based on the Amaral-Zettler et al. (2015) paper, plastics, specific to regions, find themselves in foreign water ways. Weather events, such as storms, high winds, tsunamis, etc. can be cause for a surge of un-recorded plastics to enter global water ways. There are observational methods to get an idea of how quickly plastic marine debris (PMD) can travel global waterways. These observed migrations are due to plastic's long half-life (time to naturally degrade). Some microbes identified are known to cause pathogens in marine animals and humans. Some microbes identified are known to cause pathogens in marine animals, including humans.

Due to publications in the past two decades regarding plastic marine debris and news outlets reporting on them, public awareness of the issue has drastically (and appropriately) increased. An advisory body known as Group of Experts on the Scientific Aspects of Marine There are observational methods to get an idea of how quickly plastic marine debris (PMD) can travel global waterways. These observed migrations are due to plastic's long half-life (time to naturally degrade). Environmental Protection (GESAMP) recommended the following "assessing the importance of plastics and microplastics as a vector for the transfer of organisms", Amaral-Zettler et al. (2015). The advice can be broken down into several aspects, to determine the species distribution of those relevant to colonizing plastic substrates, based on time and location, which have allowed researchers to determine how biogeography of these microbial communities differ on the local, regional, and global level?

More recent studies demonstrate that each piece of plastic (substrate) can serve as their own microgeographic barriers, affecting the potential for gene flow. In other words, the plastics themselves serve as a selective pressure to sustain microbes who have the ability to use it as an energy source. If the situation were more straightforward, observations of a consistent community with respect to a specific type of polymer would exist. Demonstrating the potential for invasive pathogens & organisms However, the ocean is a dynamic environment, and this tends to complicate things when studying microbial communities of plastic substrates.

Landfills are another way for plastics to enter the marine environment, with an estimate 32% of all landfill plastics finding themselves in global waterways. Plastic production since the Amaral-Zettler et al. (2013) paper, has only been increasing and now a 72% of plastics produced each year enters the terrestrial or marine environment. LDPE, PET, and PS are the ubiquitous and utilized in a variety of products, each having a different chemical composition, indicative of how long it may take to biodegrade the material.

Methodology

Sample Collection

Due to the vast bodies that compose global waterways, sampling was especially important to consider. The following is how samples were collected. Plastic marine debris (PMD) is collected at multiple location within the boundaries of the North Atlantic, Amaral-Zettler et al. (2013). Collected from sea via boat using a 333 μm mesh towed on the surface of the water pieces of plastic substrate are filtered with four liters of "clean" seawater & periodically freshwater flushed.

For the Amaral-Zettler et al. (2015) paper, samples are collected from the North Pacific and Atlantic subtropical gyres. Additionally, another set of samples collected along the longitudinal gradient at the Atlantic cruise intersection. From the Virgin Islands (12.0°N), up to Cape Cod (41.5°N), can be categorized into three types (based on kind of substrate), surface water, deep water, and plastics.

Lastly, there are samples from the Mediterranean Sea Delacuvellerie et al. (2019) consisting a total of three populations, made up of three different substrates, polystyrene (PS), polyethylene terephthalate (PET), and low-density polyethylene (LDPE).

Sample Analysis

North Atlantic microbial samples are analyzed phenotypically, via scanning electron microscopy (SEM) and genotypically, via next generation sequencing (Amplicon Pyrotag

Sequencing). Additionally, for identifying and differentiating types of plastic, Raman Spectroscopy is utilized and compared to a known database.

North Pacific and Atlantic subtropical gyre samples were analyzed using next generation sequencing, specifically the non-recombinant sequence of a small subunit of ribosomal rRNA (16s-rRNA), allowing for the direct comparison to a global database. Nonmetric multidimensional scaling (NMDS) and Analysis of Similarity (ANOSM) are performed on coastal and open water samples.

Mediterranean Sea samples were analyzed using next generation sequencing (similar to that of North Pacific and Atlantic subtropical gyre samples), for the same reasons. Additionally, alpha diversity was analyzed with support NMD analysis. And finally, enrichment experiments are performed to provide further insight on bacterial communities specific to plastic substrates, rather than being present by chance.

Results

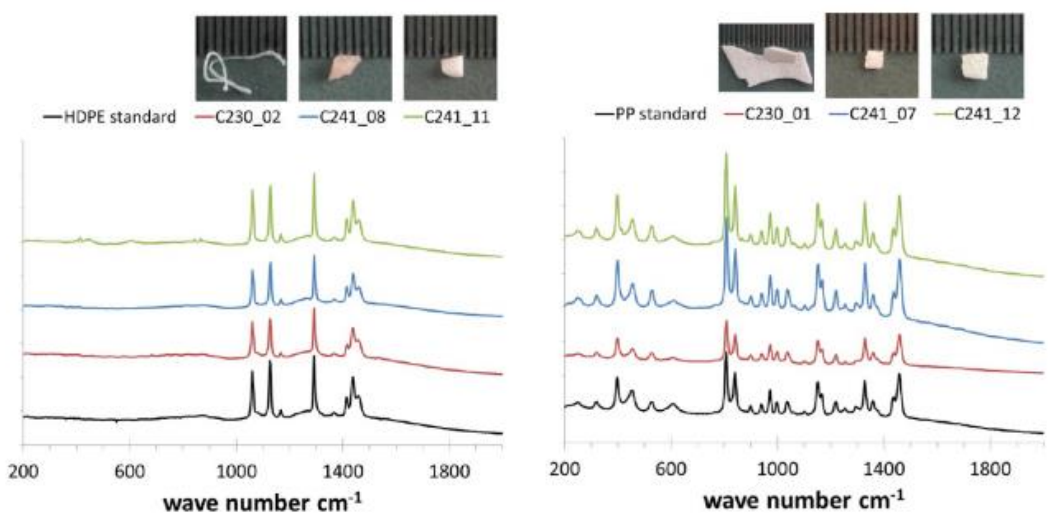


Figure 1 Amaral-Zettlet et al. (2013)***:*** Spectra produced by Raman spectroscopy, utilized to identify polymer (plastic) types collected.

It is reported that the known plastic substrates present are **polyethylene (PE)** & **polypropylene (PP)**. These types of plastic are from distinct areas surrounding the North Atlantic

Ocean, implying that the accumulation of plastic is due to many factors, and it is an issue the world as a whole must face and overcome.

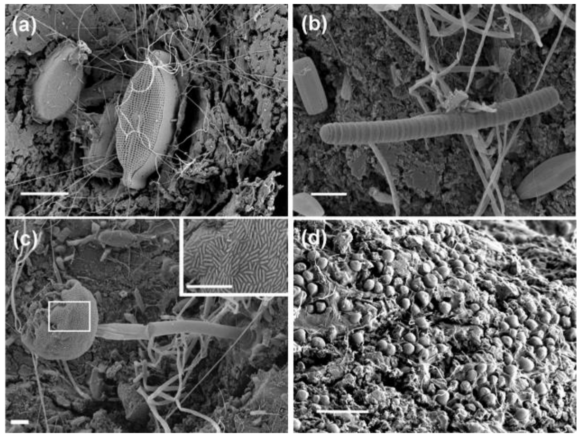


Figure 2 Amaral-Zettlet et al. (2013)***:*** High resolution images from SEM for phylogenetic inference.

Reveals a diverse bacterial & eukaryotic community existing on BOTH identified substrates (PE & PP). Over 50 morphotypes, which were found to cover about 0 – 8% of the plastic's surface.

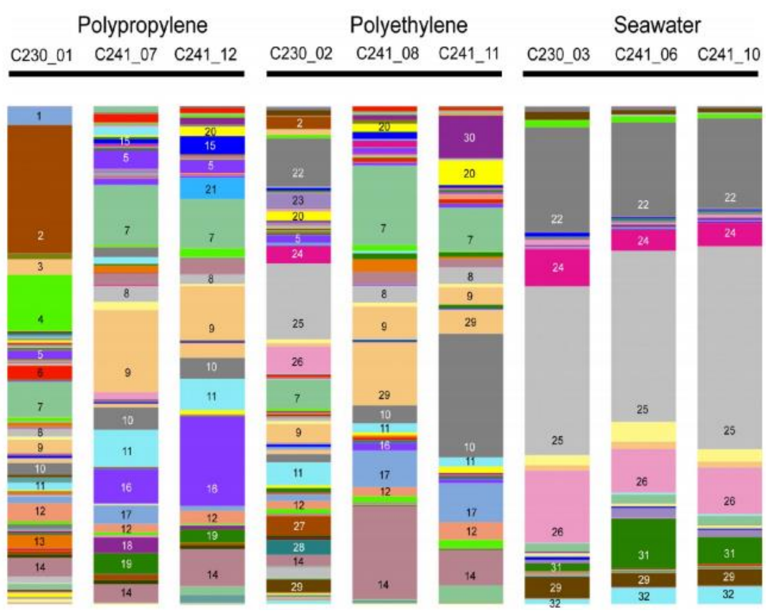


Figure 3 Amaral-Zettlet et al. (2013)***:*** Created using Global Alignment Sequence Taxonomy (GAST), showing community composition (based on OTUs) for each substrate type.

A unicellular bacterial known as *Prochlorococcus* was a dominant species for the microbial phototroph community. Other phototrophs identified include *Navicula*, *Nitzschia*, *Sellaphora*, *Stauroneis*, and *Chaetoceros*. Additionally, identified diatoms with photosynthetic abilities include the following, prasinophytes, rhodophytes, cryptophytes, haptophytes, dinoflagellates, chlorarachniophytes, chrysophytes, pelagophytes, and phaeophytes.

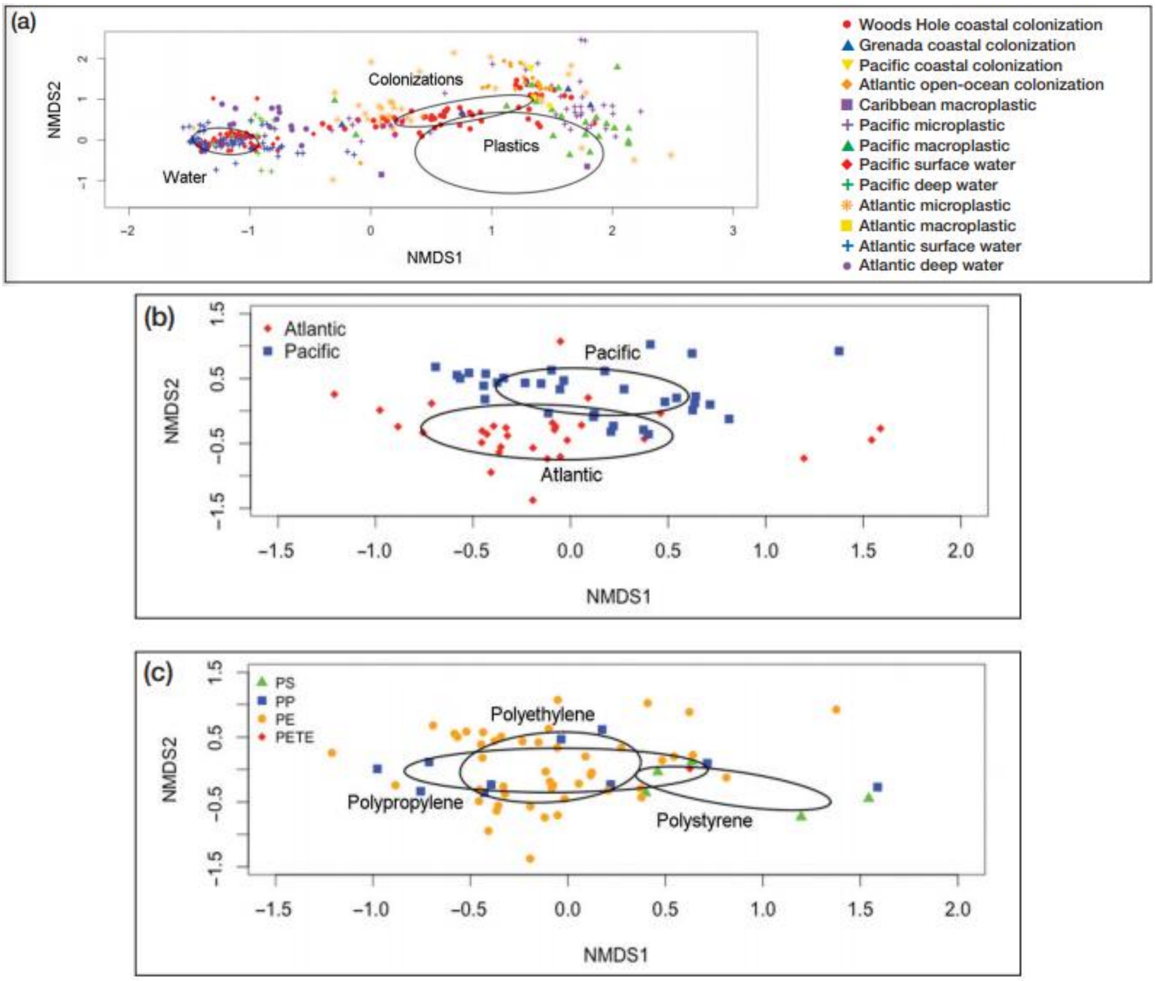


Figure 2 Amaral-Zettlet et al. (2015)***:*** NMDS for determining microbial clusters with respect to sample type. (a) is representative of plastics exposed to costal and open water, (b) is representative of the differences between Atlantic and Pacific samples, (c) is representative of microbial communities and their respective plastic substrates.

Table 1. Results from post-hoc analyses of ANOSIM tests

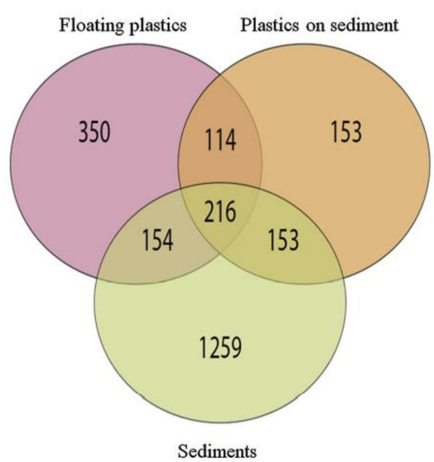
Groups	R statistic	Significance level	Possible permutations	Actual permutations	Number ≥ observed
water, plastics	**0.826**	**0.001**	**Very large**	**999**	**0**
water, colonizations	**0.858**	**0.001**	**Very large**	**999**	**0**
plastics, colonizations	**0.475**	**0.001**	**Very large**	**999**	**0**
PE, PP	0.085	0.19	Very large	999	195
PE, PS	**0.318**	**0.02**	**2598960**	**999**	**19**
PE, PETE	0.099	0.271	48	48	13
PP, PS	**0.254**	**0.043**	**3003**	**999**	**42**
PP, PETE	0.093	0.273	11	11	3
PS, PETE	−0.24	0.833	6	6	5
PE, PP, PS, PETE	**0.167**	**0.037**	**NA**	**999**	**36**
Atlantic, Pacific	**0.284**	**0.001**	**NA**	**999**	**0**
water, plastics, colonizations	**0.777**	**0.001**	**NA**	**999**	**0**

Notes: Groupings with significant *R* statistics are highlighted in bold. PE = polyethylene; PP = polypropylene; PS = polystyrene; PETE = polyethylene terephthalate. NA = global tests do not generate a value for possible permutations.

Table 1 Amaral-Zettlet et al. (2015)

With a global significance(α) value of 0.001, we can say that there were only differences in composition between PE vs PP OR PP vs PE. Remaining data implies that community clusters appear more dependent on geography, rather than plastic type (e.g. LDPE, PET, etc.).

A)



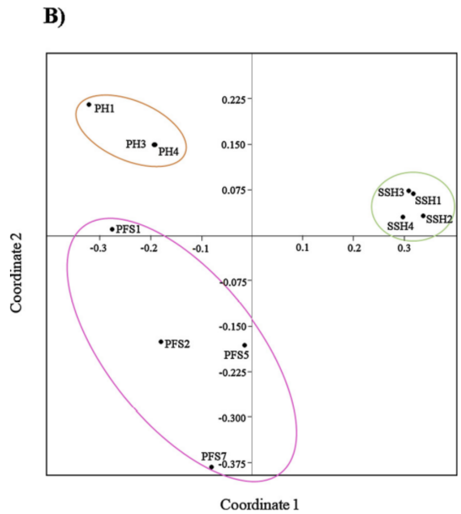


Figure 1 Delacuvellerie et al. 2019): (a) is a Venn diagram of bacterial OTUs present in the sample based on the type of substrate the bacteria were found on. (b) is Non-metric multidimensional scaling (nMDS) showing that each substrate does indeed have OTUs specific to them.

There were no significant differences in community composition when comparing floating and sediment plastic samples. Multivariate analysis, showing that the sediment community is mostly represented by *Proteobacteria*. Specifically, *Gamma*-, *Alpha*- and *Deltaproteobacteria*. Plastic bacterial communities were mainly composed of *Bacteriodetes* & *Proteobacteria (Gamma- & Alpha-)*

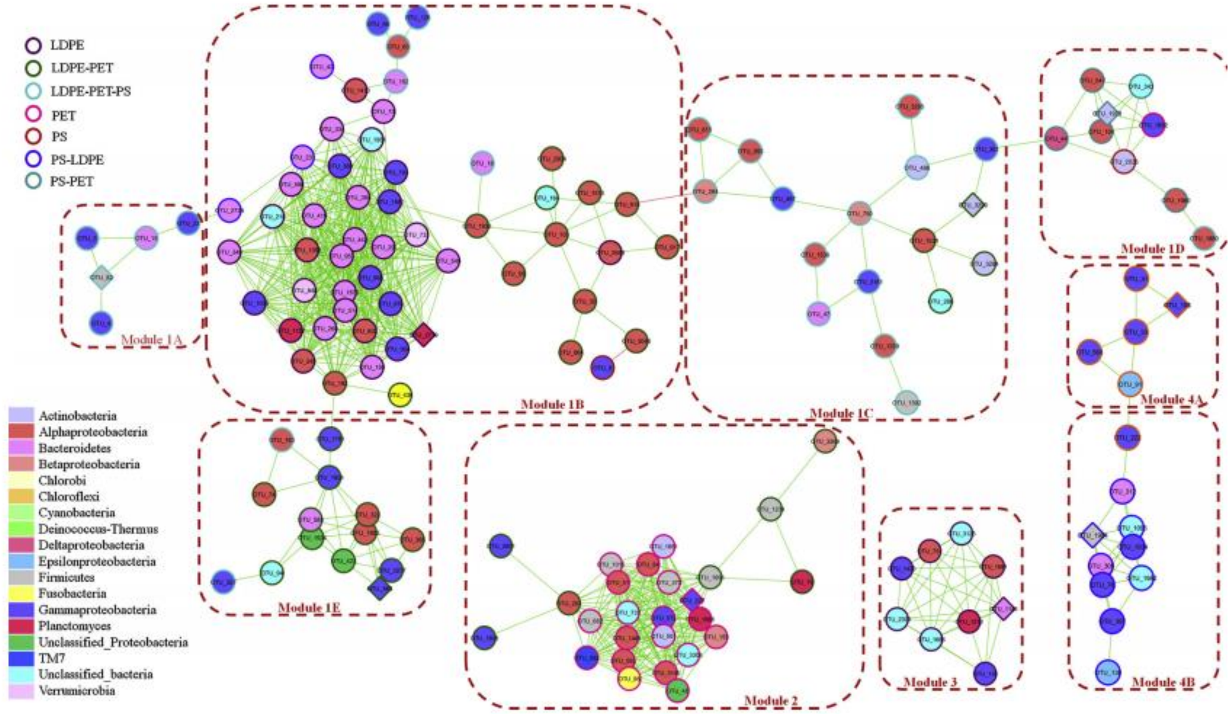


Figure 4 Delacuvellerie et al. (2019): Network diagram of OTUs post enrichment (in the lab setting)

Module 1B demonstrates that it consists of OTUs that are only observed on the plastic substrate LDPE. Other microbes associated with plastic degradation but found on other substrates were *Rhodobacteraceae, Flavobacteriales, Gramella, Oleibacter, Rhizobiales, Halomonas, and Alacanivorax.* Generally speaking, *Rhodobacteraceae* & *Flavobacteriales* were dominant microbes observed in plastisphere samples. Module 3 also containing microbes who only colonize LDPE. Containing *Rhodobacteraceae* & *Alacanivorax* Module 1C are OTUs of bacteria found on plastic substrates but were not associated with plastic degradation. Module 2 are OTUs of bacteria only found on PET. Containing *Clostridiales, Cellulosimicrobium, Stappia, and Rhizobium.* Which are known for aromatic hydrocarbon degradation Module 4A are OTUs of pathogenic bacteria that were present of the genus *Vibrio* & *Arcobacter*

Discussion

Pits in the plastic substrate were in the shape of bacteria, suggesting that the plastic is being used as a carbon source, via hydrolysis. It was discovered that a diverse microbial community exists, consisting of saprotrophs, symbionts, predators, heterotrophs, and phototrophs on the plastic substrate. This community existing on the plastic is referred to as the "Plastisphere". Past studies, such as Oberbeckmann et al. (2014) have concluded that microbial communities will change depending on the two variables, spatial & temporal (i.e. location & time). Plastisphere microbial communities demonstrated significant differences in composition when comparing them to the composition of communities using open water as a substrate, rather than plastic. Research findings led to the conclusion that plastisphere communities between the Atlantic and Pacific waterways were significantly different in comparison to one another. Plastisphere communities are a lot more dynamic, vs the open water communities, who tend to be more stable with regards to community diversity. Sample populations demonstrated that *Gammaproteobacteria* & *Bacteroides* were dominant.

Delacuvellerie et al. (2019) verifies that the unique environment known as the plastisphere does indeed host microbial communities. Additional plastics were identified in samples, such as polyvinyl Chloride (PVC) and polypropylene (PP). Lastly, *Alcanivorax borkumensis* was identified to do specifically degrade LDPE

Lastly, a paper from Jacquin et al. (2019), discusses possible indicators of successful biodegradation. Typically, micro plastics (less than 5mm long) are sampled in a finite space, that simulates the open ocean environment with microbes discussed in the results section.

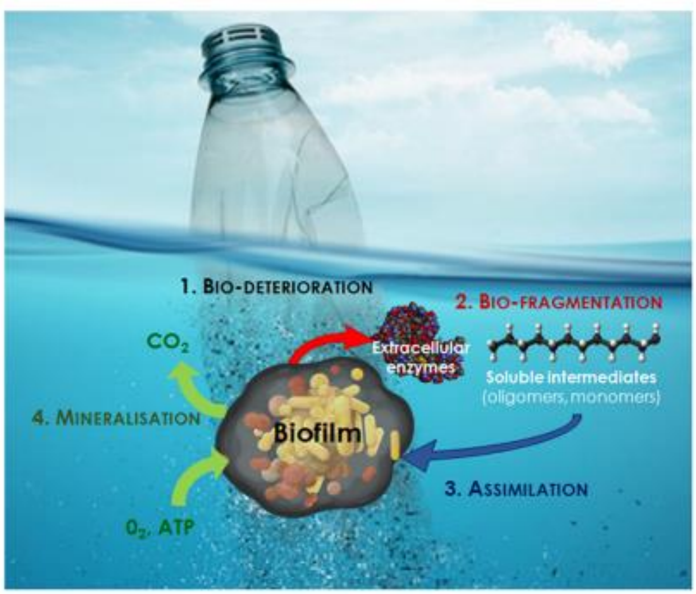


Figure 2 from Jacquin et al. (2020) paper

It was concluded that there are three total indicators of successful biodegradation, proportional to the size of the plastic in question. First, about 60-70% of ambient CO2 concentrations are due to microbes performing metabolic processes (i.e. using the plastic as an energy source). Second, biological oxygen demand should not exceed the amount of oxygen present in the ocean (within a given amount of space). Third, there is observational loss in mass or evidence of degradation, by the formation of microbial pits on the surface of the plastic. The field of microbial ecology has seen recent advances in methodology and understanding of plastic degradation by microbes in the Marine environment. Many limitations such as knowledge of carrying capacity of these microbial communities have yet to be discovered, and many perspectives (not colonization) have yet to be explored. It is expected to see more findings and nuances with regards to plastic degradation as time goes on.

Literature Cited

1. Amaral-Zettler, L.A. et al. (2020) Ecology of the plastisphere. *Nat Rev Microbiol* 18, 139–151.

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