Microbial degradation of plastics:

A tool for cleanup of the oceans?

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1. Abstract

In need for a functional tool to clean the oceans from the increasing plastic pollution it is an interesting approach to look into plastic degrading microorganisms. Plastics are being divided into different types which are considered more or less degradable. Nevertheless microorganisms that can even degrade supposedly non-degradable plastics like polyethylene are being discovered in an increasing rate. However mechanisms are not yet fully understood and the natural conditions of the oceans pose difficulties for microbial growth and enzyme activity. This essay provides an overview of the different types of plastics, their biotic and abiotic degradability as well as of plastic degrading microorganisms and enzymes. The applicability of plastic degrading microbes in the oceans is being estimated considering the current state-of-the-arts. Furthermore mechanical tools for ocean cleanup are being introduced.

2. Introduction

Plastic pollution is one of the main environment problems we are facing nowadays. An estimated 311 t of plastic are produced annually worldwide but only ~14 % of those are collected for recycling.[1] However, many particles will escape the recycling system and will get swept into the oceans. But plastic waste in the oceans is not only coming from landfill. Outworn nautical equipment and microbeads from cosmetics are just as big of a problem. In 2014 an estimated 5.25 trillion items, weighing more than 260,000 t were floating around in the oceans of our planet.[2] The natural currents in the oceans pull the light plastic particles along, causing them to accumulate in huge gyres. Those natural gyres now form enormous plastic islands. Over time, plastic particles experience weathering. Larger particles get degraded and turn into microplastics. The marine fauna ingest the plastic particles. Through consumption of seafood the plastic particles are even being ingested by us. But plastics cannot be digested by animals. It will always re-enter the ecosystem.

An approach to clean our oceans from plastic is thus urgently needed. While some projects to mechanically collect the particles are already up and running, making use of plastic-degrading enzymes expressed by microorganisms might be a valuable bioremediation tool. Studies have shown that some microorganisms are able to degrade plastics. The wide variety of plastic types and the persistence of these as well as the natural conditions in the oceans make this a difficult task.

3. Plastics: An overview

Plastics can be divided into two general groups: thermoplastics and thermoset plastics.[3] By heating and cooling these plastics can be shaped repeatedly. The molecules consist of long sole carbon chains that are joined end-to-end.[4] This mere carbon atom backbone results in a resistance to degradation of this type of plastic. They are thus considered non-biodegradable. Thermoset plastics cannot be repeatedly softened and hardened, as they solidify ultimately after melting.[3] The molecular structure of thermoset plastics is highly cross-linked and their main-chain is made out of heteroatoms. They are thus susceptible to degradation through hydrolytic cleavage of ester or amide bonds.[5]

Thermoplastics are the main type of plastic used in packaging. They make up 92 % of plastic in use worldwide. The main types of thermoplastics are polyethylene (PE), polyvinylchloride (PVC), polypropylene (PP) and polystyrene (PS). Thermoset plastics include amongst others polyester such as polyethylene terephthalate (PET) and polyurethane (PUR).[6]

Due to their non-biodegradability but tremendous abundance in plastic waste, thermoplastics pose a huge problem in plastic pollution in the oceans.

Plastic particles floating in the oceans have recently been divided into four size classes: small microplastics of 0.33 – 1.00 mm, large microplastics of 1.01 – 4.75 mm, mesoplastics of 4.76 – 200 mm and macroplastics larger than 200 mm.[2]

4. Degradation of plastics

4.1 Abiotic degradation

Plastic particles in the marine environment are exposed to various external conditions. During time and weather sunlight, oxidants and physical stress alter the state of the material and lead to its abiotic degradation.[7]

Depending on the type of plastic, the degradation process follows a different pathway.[7] About 80 % of plastic products used in Europe is made out polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyethylene terephthalate (PET) and polyurethane (PU).[8] These main types of polymers can be divided into two subgroups: polymers with a carbon-carbon backbone (PE, PP, PVC and PS) and polymers with a heteroatom located in the backbone (PET and PU).

In the polymers PE, PS and PP a photoinitiated-oxidative degradation causes a decrease in the molecular weight and formation of carboxylic end-groups. This abiotic degradation is initiated thermally, hydrolytically or by UV light and takes place before biodegradation occurs.[9] Since temperatures in the oceans are rather moderate, thermal activation is not relevant during plastic degradation in the marine environment. Thus sunlight and oxygen are the most relevant factors.[10] In PVC, dechlorination through UV-light precedes the photo-degradation.[11,12]

PET and PU are plastic polymers with a heteroatom in the backbone. In these polymers abiotic degradation takes place through hydrolysis and photo-oxidation. This leads to the formation of smaller fragments and carboxylic end-groups.[13,14]

Polymers are hardly ever used in their pure form[7] but additives are added to promote specific properties of the material. Additives like bisphenol A, phthalates and others are used to enhance stability, flexibility and resistance.[10,15,16] Additives are usually not covalently bound to the polymer, thus they can leach out into the water during plastic degradation.[17] These additives are a crucial factor in the degradation rate[18] and they at the same time form a group of potentially dangerous substances that enter the marine environment. As many factors influence the degradation of plastic polymers, some pathways are autocatalytic and different pathways can take place at the same time, it is almost impossible to predict which degradation products, additives or degradation products of additives specifically enter the marine environment.

Abiotic degradation is often a precursor for biodegradation.[9] Small fragments of polymers can pass through cell membranes of microorganisms and are digested by intracellular enzymes. Some microorganisms also excrete enzymes and allow for extracellular digestion.[19] During biodegradation the polymer fragments is converted to monomers which are then mineralized. Ideally the final products of biodegradation are CO_2, H_2O and humus. This is however dependent on the type of plastic and on the additives.[20,21]

4.2 Biotic degradation

Biotic plastic degrading activity has been observed by different microorganisms and on different types of plastic.

Degradation by microorganisms requires the formation of a biofilm on the surface of the plastic particles. Biofilms allow colonization of the microorganisms on the surface of the non-soluble plastic substrate. Polyethylene (PE), which is a highly abundant plastic, however has a hydrophobic surface, which poses a problem for biofilm formation.

Gilan et al. isolated a strain of the actinomycete species *Rhodococcus ruber,* which has higher cell-surface hydrophobicity than other strains of this species and is thus an interesting candidate for biofilm formation on polyethene.[22] For isolation of this strain C208 a two-step enrichment protocol was employed.

Rhodococcus ruber C208 was isolated from soil taken from a plastic waste burial site. The first enrichment took place in soil with PE and the second enrichment in liquid synthetic medium with PE as sole carbon and energy source.

To evaluate the biodegradation *Rhodococcus ruber* C208 was incubated with PE as sole carbon and energy source for 30 days at 30 °C. To simulate natural conditions the PE particles were pre-treated with an alternating exposure to humidity and UV-radiation. Indeed *R. ruber* C208 formed a biofilm on the surface of the PE and after 30 days 8 % of the dry weight of PE had been degraded. It was also shown that weathering of the plastic material enhanced the bacterial degradation. This treatment causes the formation of carbonyl groups and thus enhances the surface hydrophilicity of the material.[23–25]

Degradation of 8 % of the material within 30 days is a rather rapid process compared to previous observations which ranged from 3.5 % to 8.4 % in 10 years.[26–28] The rather rapid biodegradation with measurable degradation after 2 weeks is likely due to the effective colonization of PE by *R. ruber* C208 due to biofilm formation.[22]

Rhodococcus ruber C208 has also shown to be able to degrade the plastic polystyrene (PS), which is commonly considered non-biodegradable.[29] As with PE, the PS samples have undergone artificial weathering and thus been exposed to alterating UV-light and humidity. *R. ruber* C208 was incubated with the weathered PS flakes as a sole source of carbon and energy in a synthetic medium at 35 °C. Adhesion of the cells to the PS flakes and biofilm formation could be observed. The effective adhesion of the cells to the plastic is caused by the previously observed slight hydrophobicity of strain C208.[22] After 4 weeks of incubation a weight reduction of 0.5 % could be observed and of 0.8 % after 8 weeks.[29]

Biodegradation of PE has also been observed by the fungus *Penicillium simplicissimus* and the thermophilic bacterium *Brevibacillus borstelensis.*[30,31]

A PE degrading strain *P. simplicissimus YK* was isolated from soil and leaves.[30] Degradation of PE was tested with spores and hyphae of the fungus. The PE samples had been irradiated with UV light for 500 h to simulated weathering conditions. Incubation of *P. simplicissimus* with PE showed little or no reduction of weight after 6 months. Incubation of fungal hyphae with PE resulted in a significant weight reduction of low-molecular weight PE (MW = 1,000 – 10,000) after 1 month and reduction of high-molecular weight PE (MW = 4,000 – 28,000) after 3 months. These results suggest that extracellular enzymes of the hyphae are responsible for PE degradation.

An enhanced growth of *P. simplicissimus* on agar plates containing UV-irradiated PE again suggests that carbonyl groups on the plastic after irradiation are crucial for degradation. Effectiveness of degradation showed to be dependent on the growth phase of the fungus.

The thermophilic strain 707 of *Brevibacillus borstelensis* was isolated from soil and has shown to be more effective in the degradation of PE than *Rhodococcus ruber*.[31] Incubation of *B. borstelensis* with UV-irradiated PE as sole carbon and energy source resulted in an 11 % degradation of weight in 30 days at 50 °C. Only low biofilm formation and a low cell-hydrophobicity were observed for this organism. However the degradation rate increased with increasing irradiation time. This suggests that UV-irradiation increases the hydrophilicity of the plastic surface by introduction of carbonyl groups and allows colonization of microorganisms. Furthermore it was observed that *B. borstelensis* was able to degrade the CH_2 backbone of non-irradiated PE.

The plastic polyurethane (PUR) is generally susceptible to microbial degradation. Many microorganisms, especially fungi express PUR degrading enzymes like PUR esterase from *Comamonas acidovorans TB-35* or an esterase from *Pseudomonas chloroaphis.*[32,33]

In general biotic degradation possibilities are enhances following abiotic degradation. Different microorganisms are able to settle on hydrophilic plastic surface or have hydrophobic properties themselves that allow colonization. However, biodegradation of plastic is a very slow process and optimal temperatures have often shown to lie around 30-35 °C or even higher.

Another difficulty posed by the marine environment in the open oceans is the low abundance of microorganisms. Initiation of biofilm formation requires a certain cell density. Through quorum sensing signals attachment of cells to surfaces is then possible. Microcolonies are formed that develop into biofilms.[34] In order to isolate other plastic degrading organisms it would be interesting to look at marine microorganisms that already have developed methods to form biofilms on floating plastic debris. Previously it could be observed that specific microorganisms settle on plastic particles due to increased nutrient abundances on those compared to the open oceans.[35] It is thus likely that these organisms have developed methods to outcompete each other for example by using the plastic particle itself as a nutrient. Furthermore those marine microorganisms are more likely to have optimal temperatures that correspond to the temperatures in the oceans. This is crucial for utilization of microorganisms for plastic clean-up in a marine environment.

4.3 Plastic degrading enzymes

Poly(ethylene terephthalate) (PET) is a thermoset plastic and thus considered degradable. However its biodegradation has turned out to be difficult and only few organisms are known to be able to degrade PET. As about 56 million tons of PET have been produced worldwide in 2013 alone, this very frequently used type of plastic makes up for a large part of the plastic pollution.[1]

Until recently LC-cutinase was considered the most effective PET-degrading enzyme[36]. LC-cutinase was isolated from an unknown organism from a soil-leaf mixture. It showed PET-degrading activity of 12 mg h^{-1} mg^{-1} of enzyme at 50 °C, which is higher than other PET-degrading enzymes reported previously. It is able to degrade PET to terephthaltic acid and ethylence glycol. Very recently Yodisha *et al.* isolated the strain *Ideonella sakaiensis 201 – F6* from a PET bottle recycling site.[37] This strain was able to degrade the surface of a PET film at a rate of 0.13 mg cm^{-2} day^{-1} at 30 °C. At 28 °C 75 % of the degraded PET film carbon was further catabolized into CO_2. After only 6 weeks at 30 °C, an almost complete degradation of a small PET film was observed. Two enzymes were found to be responsible for this PET hydrolysis. Initiating the degradation the bacterial cell adheres to the PET surface. It then secretes a PETase which hydrolyses mono(2-hydroyxethyl) terephthaltic acid (MHET). MHET is then taken up by the cell and further hydrolyzed by the intracellular MHET hydrolase belonging to the family of tannases. This process can be seen in figure 1. Hence, *I. sakaiensis* uses the two forming monomers ethylene glycol and terephthalic acid for its growth. The genes for PETase and MHETase are strongly upregulated in the presence of PET and thus show to be unique for the degradation of PET. Furthermore PETase has shown to be more active at lower temperatures compared to other known PET-hydrolytic enzymes such as TfH, LCC and FsC. This makes this enzyme and interesting candidate for PET degradation under natural conditions. These rather recent results pose questions about the mechanism of adherence of the *I. sakaiensis* cells to the smooth PET surface. Investigation of this mechanism could deliver valuable information for using microorganisms as a bioremediation tool for plastic.

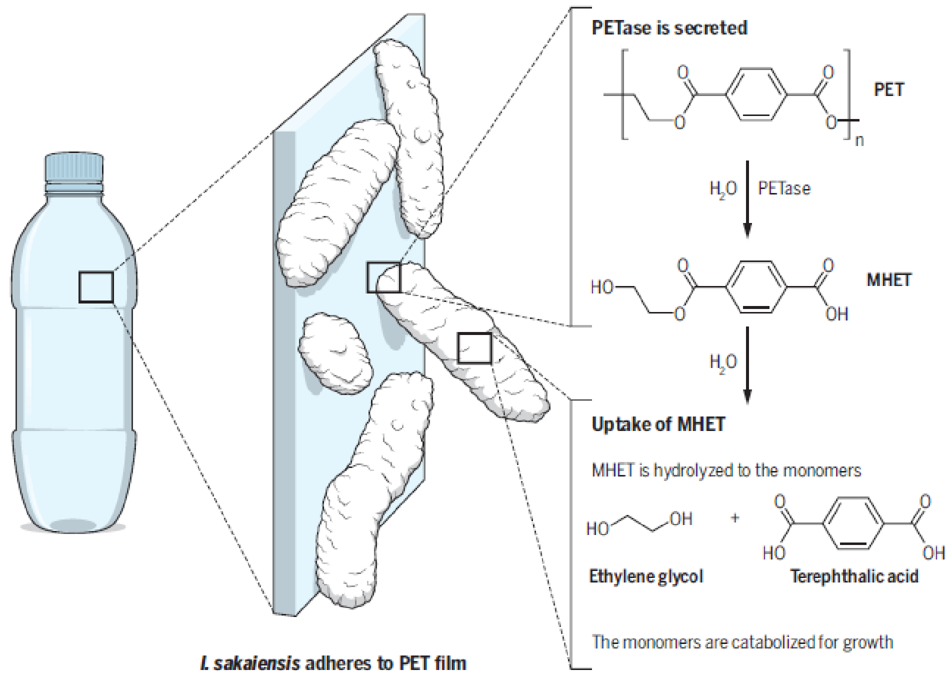

Figure 1: Degradation pathway of PET[38]

5. Mechanical removal of plastics in the oceans

5.1 The Seabin Project

Two surfers from Australia have developed a different approach to tackle waste in the oceans [39]. The Seabin, as seen in figure 2, resembles a floating trash can. It is a cylindrical bin fitted into a floating pontoon. A filter is fitted inside of it and a shore-based pump sucks water through the filter. A vortex forms around the opening of the bin, which is located just below the water surface. Due to the vortex water is sucked into the filter, flushing floating trash into it. The filtered water is then released back into the sea.

The filter has to be emptied and cleaned manually every day [40]. The floating pontoon allows accessibility at all tides. It is tested to be safe for marine animals which do not tend to swim close enough to the water surface to be in danger to be sucked into the vortex [41]. The filter even allows small particles like fish eggs to pass through [39]. Though, this aspect poses a problem when it comes to microplastics. These particles are also able to pass through the filter. The first design of the Seabin was aimed to be stationary in marinas and run on electricity provided by the marina [42]. Newer designs are modified to be attached to yachts and to run on their electricity [40]. Another design makes use of a solar-powered pump which would allow offshore use or cleaning of remote bays [39].

The filter can catch large waste debris and even more sophisticated filters can be used [39]. These allow cleaning the water from hydrocarbons like oil and fuel and from detergents.

While marine animals do not pose a problem for the Seabin, aquatic plants do [39]. Seagrass tends to grow in marinas in large amount and is prone to clog the Seabin. This problem has yet to be solved.

The company is aiming on shipping the Seabins in early 2017. The project is financed through crowdfunding [39].

The Seabin project is an important innovation for cleaning of marinas, which are a main source of pollution of the oceans. Newer design allow offshore usage and refrain from carbon-emitting fuel source, using solar energy instead [39].

Figure 2: A Seabin [Source: The Seabin Project]

5.2 The Ocean Cleanup

The Ocean Cleanup is a Dutch company founded in 2013. They have developed a system to mechanically remove plastic debris from the oceans.

By creating an artificial coastline they are able to collect dispersed floating plastic debris.

A V-shaped array is placed into the oceans current (figure 3). Solid screens catch the floating plastic particles, allowing sea animals to pass underneath (figure 4). Using these screen instead of nets prevent animals getting caught in them. The orientation of the arrays allows the debris to gather in an even more condensed way in the center of the screens (figure 5).

The debris is collected, the plastic extracted, shipped to land and sold to companies.

The system is solely powered by the ocean's natural current, thus no external energy source is needed.

A minimal amount of moving particles in the system allows a high degree of autonomy and minimizes the need for expensive offshore operations

So far the company is financed through corporate partnerships and philanthropic funding. Selling the extracted plastic to companies is to be

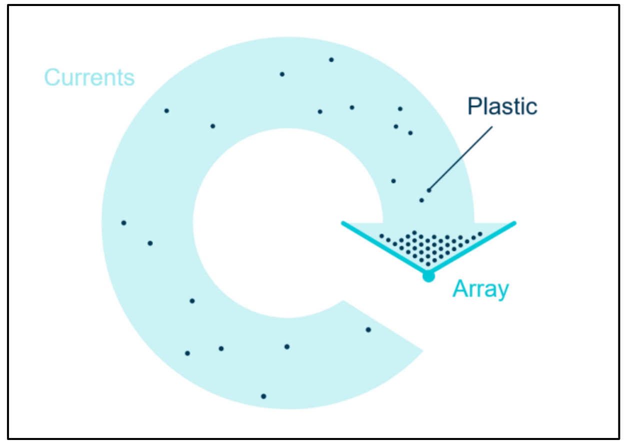

Figure 3: Currents [Source: The Ocean Cleanup]

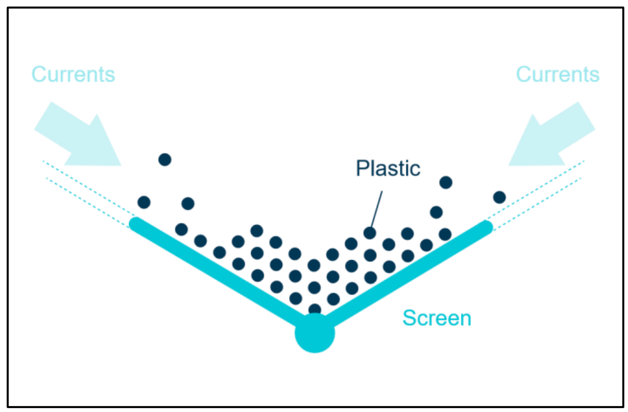

Figure 4: Catch [Source: The Ocean Cleanup]

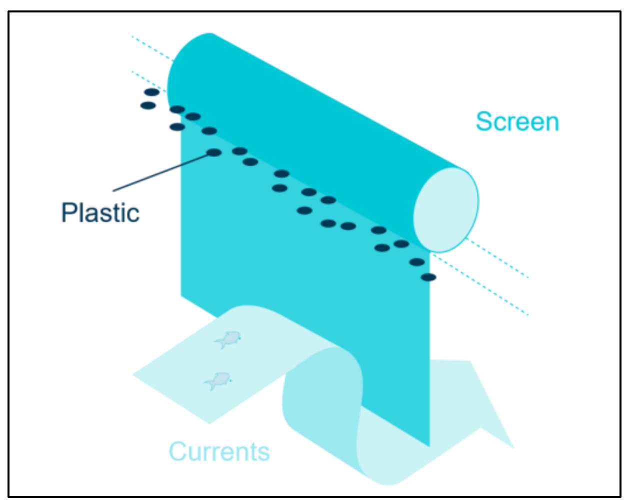

Figure 5: V-shaped array [Source: The Ocean Cleanup]

extended to make the system fully self-sustainable.

In 2016 The Ocean Cleanup installed a full-scale prototype in the North Sea. It is a 100 meter-long segment located 23 km off the coast of the Netherlands. It is set to remain for one year. The chosen location is highly storm ridden. Even minor storms in this area are more severe than rare strong storms in the final location in the pacific. From this prototype engineers are drawing data to develop a system that can endure the conditions in the Great Pacific Garbage Patch.

In late 2017 a working pilot system is to be installed in the Pacific Ocean. This is an operational testing platform set up to finalize the concept of the project.

The aim is to introduce the final system into the Great Pacific Garbage Patch and start cleaning it in 2020 (figure 6). The Ocean Cleanup claims that they will be able to remove half of the Great Pacific Garbage Patch within 10 years.

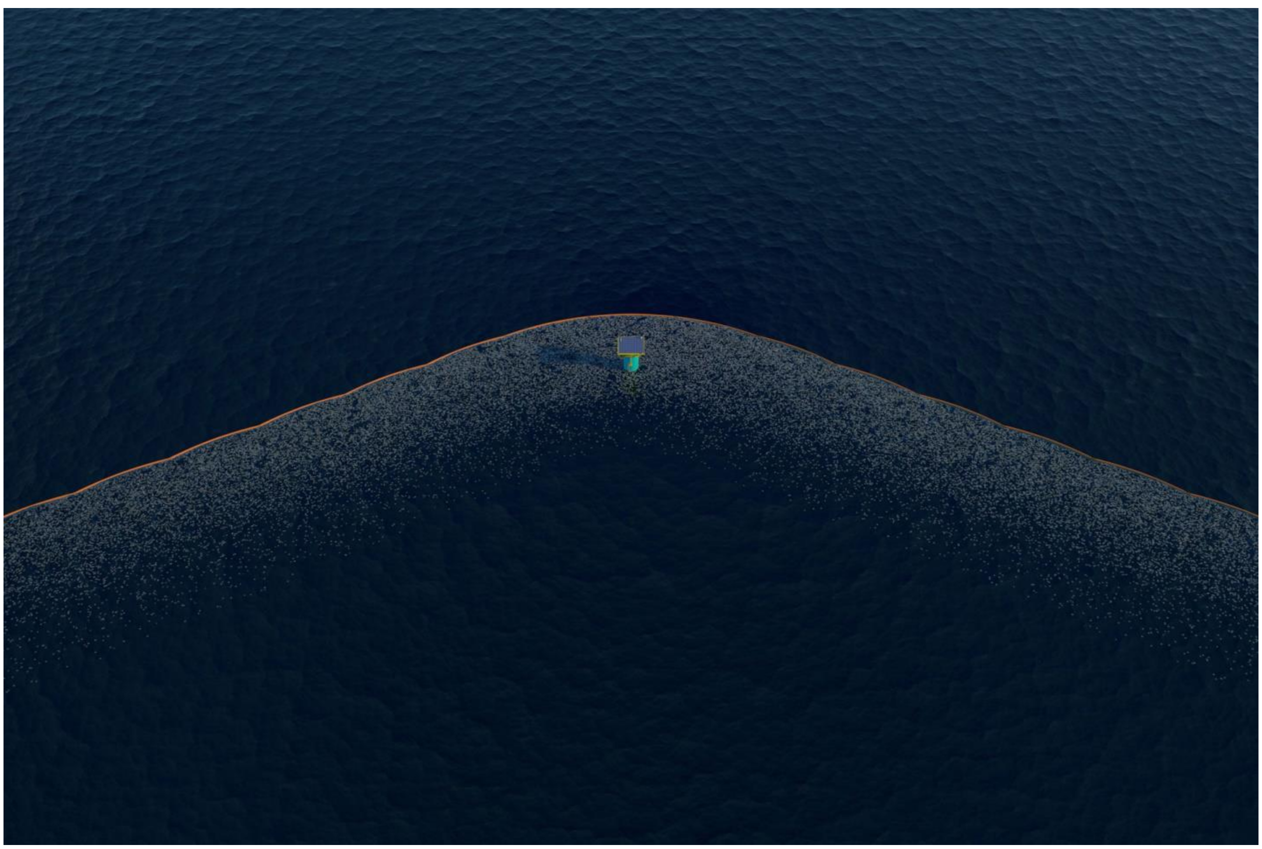

Figure 6: Artists Impressions Pacific Cleanup [Source: Erwin Zwart/ The Ocean Cleanup]

6. Discussion and Outlook

Many microorganisms show the ability to at least partially degrade different kinds of plastic. However, none of these pathways can yet be utilized for bioremediation of plastics in the oceans. Biodegradation of the plastics is often a process that is not yet complete elucidated and urgently needs further research. So far, most attempts show biodegrading activity at temperatures of at least 30 °C. For application in the oceans activity at far lower temperatures are needed. Furthermore efficient biofilm formation is needed to colonize the particles prior to degradation. Engineering of an organism that shows good biofilm formation efficiency and expression of plastic degrading enzymes that have their activity maximum at lower temperatures could be an interesting approach. However, in this case the common problem of synthetic biology is faced again as application of genetically engineered microorganisms in nature is not yet being approved of. Another approach could be isolation of microorganism from the marine environment, ideally organisms that already colonize plastic particles in the plastic debris patches. Studies showed that organisms like *I. sakaiensis* were able to develop certain activities in a rather short time span.[37] Therefore chances are that an effective plastic degrading organism has already developed in the plastic gyres. Culturing and directed application could then make it a successful bioremediation tool. Another problem is posed by the unknown long term effects that the degrading microorganisms might have on the marine environment or on ships and nautical equipment. The production of potentially harmful degradation intermediated is to be resolved. Plastic degrading organisms could also have a strong growth advantage in the ocean's plastic patched. This might lead to unwanted monoculturing of specific strains and would be unfavorable for the marine biodiversity. These microorganisms will also find their way into the food chain. Hence, the effects of this certainly need to be elucidated.

The application of microorganisms for bioremediation of plastics in the ocean still demands extensive research and possible paradigm shifts to allow application of genetically engineered organisms for the greater good of pollution-free oceans.

Until then the mechanical plastic removal methods such as the OceanCleanUp and The Seabin remain the most effective way to clean our oceans from plastic pollution.

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