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Microbial degradation of polyethylene terphthalate (PET): An outlook study

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Abstract
The bacteria, known as *Ideonella sakaiensis*, is capable of producing two enzymes known as PETase and MHETase. These enzymes can degrade PET (Polyethylene terephthalate) into ethylene glycol and terephthalic acid. The genes that code for these two enzymes can be transferred to a much suitable host that has a high enough copy number that can produce the enzymes in economically viable amounts. The mass-produced enzymes can then be made to react with PET materials which will get converted to the by-products. Following this the products, ethylene glycol and terephthalic acid can then be separated. Each of the products can further be treated to convert them into energy sources that can be utilized by other microorganisms. The ethylene glycol is converted into formaldehyde which can be utilized by various methylotrophic bacteria. The terephthalic acid that is produced can be used by *Arthrobacter sp*. bacteria. Thus, helping us to achieve the complete biodegradation of PET plastic waste materials.

Keywords: MHETase, polyethylene terephthalate, *Ideonella sakaiensis*, PETase

1. Introduction
Plastic pollution is the accumulation of plastic objects and substances, in the various components of the ecosystem. It was the major threat to wildlife, habitats, and also human life associated with these ecosystems (Moore, 2020) [10]. Plastic pollutants have been classified based on their size as, micro-debris, meso-debris, and macro-debris (Hammer *et al*., 2012) [4]. The low expense of manufacturing, high durability, and chemical structure, which makes them resistant to normal degradation procedures, have led to the rise of plastic products in our society or environment. Macro and Microplastics have shown maximum accumulation in the Northern hemisphere, mainly in waterfronts and urban centers. Plastic debris that starts as macro-debris can turn into meso or micro-debris as a result of degradation or collision (Hammer *et al*., 2012) [4]. Plastic pollution on land poses more of a threat than what is observed in the ocean. It affects plant life and animal life, including that of the humans who are settled on the land (Anderson *et al*., 2018) [1] The plastic wastes that are not properly managed varies from 60% in East Asia and Pacific to 1% in North America (Hannah *et al*., 2018) [6].
The number of plastic pollutants that are entering the ocean is increasing yearly. As of a recent survey that was carried out, it is expected that there are more amounts of plastic materials on the seabed as when compared to that of the ocean surface. The great Pacific Garbage Patch is an example of the harmful effects of plastic pollution in the ocean. According to Forbes, The Great Garbage Patch is about 1.6 million square kilometers in size as of 2020. Not just that the plastic debris that falls into the ocean destroys the aesthetic beauty of the ocean but it also causes major harm to marine life. Plastic can cause the release of toxic carcinogens such as diethylhexyl phthalate. Plastic can also pollute water bodies by the release of heavy metals such as cadmium, lead, and mercury.
Plastic pollutants not only harm the abiotic components of the ecosystems but also causes major harm to the flora and fauna of a region (aquatic or terrestrial). Plastic pollutants if accidentally consumed by organisms can likely lead to the death of the organisms as this can cause blockages within the digestive tract of the organism and ultimately lead to starvation and death of the organism. Likewise, the toxic substances that could be released as a result of plastic pollution can poison animals in a variety of ways which could in turn poison the food supply of other organisms including humans.

The plastic debris that is not visible to the naked eye could be scarier than what was anticipated earlier. Microplastics are a growing concern in our modern world. Microplastics can be of such small scales that they can be even found in the womb of pregnant women. (Ragusa *et al*., 2021) [14].

Plastic can also alter the chemistry of the soil drastically. The films formed as a result of plastic pollution could lead to increased evaporation of the soil of a region leading to the faster drying out of the soil of that specific area. The microplastics present in the soil could also lead to nutrient immobilization, leading to lower availability of nutrients to plants (Salt, 2019) [15].

1.1 About the problem

There are many issues related to the different ways in which plastic pollutants are dealt with. Conventional methods of plastic waste disposal are methods such as landfills and incineration. As mentioned, numerous drawbacks are associated with these methods, and they can end up causing more harm than good.

The landfill is one of the oldest methods of waste disposal. When rainfall occurs in areas of landfills, the water from the rain percolates through the garbage that is present and accumulates various toxic substances as it passes down. This forms what is known as "Leachate". If left unchecked, this leachate could cause major harm by polluting the groundwater sources. Depending on the constituents of plastic products the time it takes to degrade a plastic waste in a landfill can vary anywhere from ten years to more than centuries. Very little of the plastic waste that is being discarded daily is being recycled. Most of the plastic wastes either end up in large landfills or get discarded carelessly into open spaces. Another issue associated with discarding plastic wastes in the open is that there is a high chance of accidental consumption of the said plastic wastes by animals, which could prove to be fatal for the animal. The plastic waste that is consumed by the organism gets retained in its digestive tract and leads to the starvation of the organism and causes the death of the organism. Landfills could cause eutrophication of water bodies. It could also negatively impact the flora and fauna of a region by releasing toxic substances into the environment. Landfill leachate can affect both groundwater sources and surface water sources. The chemical composition of the leachate and the harm caused by it is mainly dependent upon various factors such as the composition of the waste, climatic conditions, age, and degradation of solid waste. The existing methods employed to treat the landfill leachate are either too expensive or can lead to the production of harmful by-products (Stefanakis *et al*., 2014) [16].

The leachate produced can also hinder the flourishing of soil microflora, thus reducing the quality of the soil, and adversely affecting the topography of an area. The plastic wastes that accumulate in an area can also cause the water to not percolate into the soil, which will deplete the groundwater reserve.

Another conventional way of dealing with plastic wastes is to incinerate them. There are numerous disadvantages to the burning of plastic wastes. It is deadly for both aquatic and terrestrial life. Various incineration techniques such as gasification, plasma arc, etc are employed to burn plastic wastes. Plastic at its core is petroleum-based and its incineration can cause the release of various toxic fumes which can cause various respiratory issues. The incineration of plastic can also cause the release of various greenhouse gases which can inadvertently lead to global warming. Not only that the fumes produced as a result of plastic burning can lead to respiratory issues, but they can also lead to various skin allergies or even cancer. As a result of plastic burning, air toxicity can also rise. In many areas, this can also contribute to the phenomenon known as smog. Prolonged exposure to plastic fumes as a result of plastic burning can also have varied neurological effects such as nausea, dizziness, etc due to the release of chemical components such as styrene, Butadiene, and acetone. The melting of plastics is also not a good alternative as it can release various dioxins into the environment. Home burning of plastics, which is a common practice in many rural areas, can cause the release of various harmful gases directly into the atmosphere without any treatment or any ways to contain it. Recycling is a good way to deal with plastic wastes that are being generated. But it is to be noted that almost 90% of the plastic that is being produced is not being recycled. Also, many of the garbage patches formed in the oceans are way beyond what can be easily recycled and dealt with. Another issue that of recycling plastic wastes is that it does not essentially reduce the amount of plastic that is present in the world. It is just being transformed from one form to another. As mentioned previously the complete degradation of any plastic wastes causing its complete breakdown is by burning or incinerating it and we saw why it is not at all safe to burn plastic wastes.

1.2 Objectives of the study

The study aims to develop a protocol for the complete biodegradation of plastic wastes using microorganisms and their enzymes. The objectives are,

1. Successful cultivation of bacteria Ideonella sakaiensis on a suitable growth medium.
2. Identification and Isolation of genes that code for the enzymes PETase and MHETase from the bacteria.
3. Cloning and expression of the genes in suitable vectors.
4. Separation of enzymatic degradation products of PET degradation as a result of bacterial action.
5. Conversion of ethylene glycol to formaldehyde using periodic acid.
6. Culturing of microorganisms on formaldehyde for its complete assimilation.
7. Culturing of microorganisms on terephthalic acid for its complete assimilation.

2. Review of literature

Ideonella sakaiensis strain 201-F6 is a bacteria that belong to the genus Ideonella. It has displayed the ability to break down polyethylene terephthalate (PET) and use it as a source of carbon (Yoshida *et al*., 2016 March) [20, 21]. *I. sakaiensis* was first discovered in Japan, in 2016. It was discovered by Kohei Oda and Kenji Miyamoto.

I. sakaiensis is a gram-negative bacterium. It is aerobic. Its morphology is rod-shaped. Cells show motility which is due to the presence of a single flagellum. *Ideonella sakaiensis* responds positively to both catalase and oxidase tests. *Ideonella sakaiensis* grows in a temperature range of 15-42 degrees celsius with an optimum of 37 degrees celsius. It grows at a pH range of 5.5-9 with an optimum of 7-7.7. Colony characteristics of *Ideonella sakaiensis* are as follows: colony colour- colourless; colony texture- smooth; colony shape- circular; colony size- 0.6-0.8 micrometre width and 1.2-1.5 micrometer length. It is observed that the bacteria can grow on PET surfaces by adhering to PET surfaces with the aid of thread-like appendages. Apart from the fact that these appendages serve the function of aiding in attachment, it also

might be helping with the release of PET degrading enzymes (Yoshida *et al*., 2016 March) [20, 21]. The cultivation medium used for the cultivation of the bacterium in laboratory conditions is NBRC no.802 agar medium. The medium components are as follows: Hipolypepton, Yeast extract, Magnesium sulphate, Dipotassium hydrogen phosphate, distilled water, and agar. Any temperature above 45 degrees celsius inhibits the growth of the organism. Also, salt (NaCl) concentration of 3% (w/v) prevented the growth of the organism. It is observed that the organism could not grow under anaerobic conditions with or without the aid of lighting. Furthermore, it is also observed that under aerobic conditions, the lighting to which the organism is subjected does not affect the growth of the organism. The DNA G+C content was found to be 70.4mol%. It is also observed that Strain 201-F6 has ubiquinone with 8 isoprene units. Some of the other members of the genus Ideonella include *I. dechloratans* and *I. azotifigens*. Based on various features such as physiological, biochemical, and various other data such as phylogenetic data, Strain 201-F6 was found to be representative of a new species that was never observed before. Hence it was given the name *Ideonella sakaiensis* (Kohei *et al*., 2016).

The partial sequence for the gene that codes for the 16S rRNA of *I. sakaiensis*, as obtained from NCBI is as follows:

ORIGIN

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2641 atatcgagac gggcctgcgg atccaatccg aaggcaacgg tgccgagcgc gtcgggattg
2701 tcgttcactg cgttgtcgtg acctccatcc gttgcgatgg tggcgaaatt gcgagacagc
2761 gcgcttgcga tctgtccccc accaatgctg ccggtagccg cggacagcga gccgttagtt
2821 ccgctgccgc cttccatgaa gaaacgaccg ttccattcgg cgggcatccg gaggcggaat
2881 ttgatttcgt agggatagcc gtcgatcccg gtgcgcttcg cgatggcgcc ggacacctcg
2941 cagtgttcgg gcagcgcagc cgcagatgcg gtggcgggcg cggcgtcacg ccaggcagcg
3001 acttccacga ccgtcgcggc atttggccac accatgtcgc catttccgtc cttcagagcc
3061 tcgcatgcgg cgcgcgaagc caagggcacc gggggcggcg gaggctcctg ctgcggcggc
3121 tgctgctgcg gcagcggcag cggcgtggat ccgccgccag cgcacgcggc tagggccacc
3181 gacgccagca gcatcgtggt gactgttgtc tgcatcttat gtctccttcg actggttcgc
3241 ggcattgatt tgcagccgcg ggcgcgatgg tgtgaccgaa ggcttgatgt agcgtcagga
3301 tatttgcgca tcgcgaaaga aacttggtcg ttccagggtt tcccctacga cgctttgcat
3361 cgaagtaccg agatgcccgc cgcagcggct ccactgcgcc cggcccgggt gcgcacaatc
3421 cgccggcaag cgggtcgcca gcgcatcgcg cgcacgacgc agcgccggca gcatggtcga
3481 acggaatccg ccatcgtcat acggtcggcc tggaccgcga tgctcatggc gccgacggta
3541 tcgcccgcac ggtcgaacac cgggatggcc atcgaccgca cgccgagttc cagttcttca
3601 tcgctctccg accagccctg ctcgcggcag cgggccacct cggccacgac gtcgttgcta
3661 cgccagaggg tgcgcggtgt cagtgcagcg cgctgcatct ggtcgacacg tctgcgtgct
3721 tccaggggct cgaacgcggc gagcaacacc ccgccgagcg ccgagcaata gctcggaagc
3781 cgcgatccga tggccagccc cgtcgaaagg ctgcgccggg caattgcgcg agcgacgatc
3841 accacatcgt cgcccagaag catgcccagg ctggccgact cacgggtgcg ctcagagaga
3901 gcgaccagca gcggctgcgc aagctgcggc atcgggcggg aactgatagg cgtgcgcgat
3961 cagcagcgcc ttgggcagca tccagaagcg cttgccgtcg ctgtcgaggt agccgagcgc
4021 ctgcagcgtg agcagcgagc gccgcgccga ggccggcgtg ctgccggtca ggcgcgccgc
4081 ctccgagacg gtgagccgcg cgtgctgccg gccgaagcag cccagcacct gcaggccctt
4141 gtcgagcgag ctcacgaagt tcttgtctgc catcgcatct tgatcctgcg catgccgggg
4201 cgtccatttt tgcgcatgcc gcacatccgg cttgtggcga aaggcatgcg gcccggacac
4261 tggatcaccc ccgacgaacg tcagacacgg agacacaaca ggatgacaac cgccctcgcg
4321 cggcgcctcg gcgccgcctg cctgaccgcg gcctgcctgc tcggcagcgc tgcggcacag
4381 actccgcaag ccctcaagat catcgtgccc tacccggccg gcggcacggc cgacatcctg
4441 ccgcgcgtgg tggccgagaa gctgcgcgcg cagttccccg ccggcgtgct gatcgacaac
4501 cgcaccggcg ccggcggcaa catcggcgcc gaggcggtgt tccgcgccga acccgacggc
4561 aacaccctgc tggcctcgcc gcccgggccg atcgccatca accaccacct gtaccggaag
4621 atggccttcg acccgtcgaa gtgggagccg gtcacggtgc tggcgacggt gcccaacgtg
4681 ctggtggtca atccgcggct gccggtgaag aacgtgcagg agttcatcgc ctacgccaag
4741 gccaacccgg gcaaggtgac ctacggctcg cagggcaacg gcaccacctc gcacctgacg
4801 gccagcctgt tcatgcagct cacgggcacg gagatggtcc acgtcccctа caagggcacg
4861 gcgccggcgc tggtggacct ggtgggcggg cagatcgacg tgttcttcga caacatcagc
4921 tcgtcgctgc ccttccacca ggccggcaag ctgcgcatcc tgggcgtggc cgacgagcag
4981 cgctccgccg cgctgcccga ggtgcccacc ttcgccgagc aggggctgcc gtcgatgaac
5041 gccgtgacct ggttcgcggt ggtggcgccg ccgggcaccc ccgcggccaa ggtggccgcg
5101 ctccagaaga gctttgccgg cgcactgacc cagccgagg tgcagcagaa gttcgccgag
5161 cagggcgccg agccgcgcgg ctgggacccg gcccgcaccg gccagttcat ccgcgccgag
5221 tccgccaagt gggaccgggt catccgcagc gccaacgtcc gcctcgactg agcaacccca
5281 tgcaagacac gtccaccctt caacccgtcc gctggtccgg ccccgggctc acgcgcatcc
5341 cctacggcgt ctaccgcgac gcgcagctcg ccgccgagga gcaggcgcgc atcttccagg
5401 gcgagacctg gaactacctg tgcctggagg ccgagctgcc cgaggccggc agctaccgca
5461 ccaccttcgt cggcgagacg ccggtggtgg tggtgcgca cgcggacggc gaggtctatg
5521 ccttcgagaa ccgctgcgcc caccgcggcg cgctgatcgc gctggagaag tcgggccgcg
5581 ccgagaactt ccagtgcgtc taccacgcct ggagctacaa ccggcagggc gacctgaccg
5641 gcgtggcctt cgagaaaggc gtcaagggcc ggggcggcat gccgcccggc ttctgcaagg
5701 aggcgcacgg cccgcgcaag ctgcgcgtgg cgaacttctg cggcctgctc ttcggcagct
5761 tcggcgacga ggtgcccggc atcgaggagt acctgggcga ggagatctgc gaacgcatcg
5821 agcgcgtgct gcacaagccg gtggaggtga tcgggcgctt cacgcaggcg ctgcccaaca
5881 actggaagct gtacgtggag aacgtgcgcg acagctacca cgccagcctg ctgcacctgt
5941 tcttcaccac cttcgagctc aaccgcctgt cgcagaaggg cggcgtgatc gtcgacgaaa
6001 gcggcggcaa ccacgtgagc tattccatgg tcgacgcgca ggccgagaag gacgcctcgt
6061 accgcgacca gggcctgcgc tcggacgatg cgaactaccg cctcaaggac ccgagcctgc
6121 tggccggctt ccaggaatac ggggacggcg tgacgctgca gatcctctcg gtcttccccg
6181 gcttcgtgct gcagcagatc cagaactgcc tggcggtgcg ccaggtgctg cccaggggcg
6241 tggagcgcac cgaactgaac tggacctacc tgggctatgc cgacgacacg cccgagcagc
6301 gccaggtgcg gctcaagcag tccaacctga tcgggccggc cggcttcatc tcgatggagg
6361 acggcgccgt cggcggcttc gtgcagcgcg gcatcgccgg cgcgggcgcg ctggaggcgg
6421 tggtggagat gggcggcgac ggcaccgcct cgagcgaggg ccgcgccacc gaggcctcgg
6481 tgcgcggctt ctggaaggcc taccggcagc acatgggcgt ctgagcacga cggccccaaa
6541 gagagagtga aagagcccca caccatgatc gacctgctgg cgctgtgcgc cttcaacgcg
6601 gcctacgccg agaccatcga cagcgacgcg ctggagcggt ggcccgactt cttcaccgac
6661 gactgccact accgcatcac ccacgccgag aacgagcgcg aggggctggc cgccggcatc
6721 gtctacgccg actcgcgcgc gatgctggag gaccgcatcg ccgcgctgcg cgaggccaac
6781 atctacgagc gccagcgcta ccggcacctg ctgggcatcc cgctgctcgg cgcgcaggac
6841 gacacgggcg ccgaggcgcg cacacctttc atggtggcgc gcatcatggc caccggccag
6901 accgagctgt tcgccagcgg catctaccgg gaccgcgtcg tgcgccagga cggcgggctg
6961 cggctgcgct cgcgcgtggc cgtgtgcgac agcacggtga cggacacgct gctggccctg
7021 ccgctgtgaa cgccacccct gcggccgtcg cgccgccccg gctggcgctg gcggtcggcg
7081 atcccaacgg catcgggccg gagatcgcgc tcaaggccct ggcggcgctg ccggccgcgg
7141 cgcgcgggcg catcacgctg tacggccccg cggccgtgct ggagcgcacg gcatcgcagc
7201 tcgggcagga ggcgctgctg cgcgagcagc ccgtggtgga cgccggcgcg ctgccggcgc
7261 aggcggcgcg gcccggccgc atcgacccgg cggcgggcgc ctcggccgtg gcctcggcca
7321 gcgccgccat cgaggcctgc cggcgcggcg aggccgacgc cgtggtcgcc tgcccgcacc
7381 acgagacggc gatccaccag gccggcatcg ccttcagcgg ctacccctcg ctggtcgcgc
7441 gcgtgtgcgg ccagccggag gacagcgtgt tcctgatgct ggtcggcggc ggcctgcgca
7501 tcgtgcacgc cacgctgcac gagagcgtgg cgcacgcgct gggccggctg cagcccgcgc
7561 tgatcgccgc ggcggcgcgc gccggcgcgc gggcctgcgc gcggctgggc gtcgccgatc
7621 cgcgcatcgg cgtcttcggc atcaacccgc acgcctcgga gggcgggctg ttcggccccg
7681 aggacgcgca gcacgtcgag ccggccgtgc aggcgctgcg tgccgaaggc ctgcgcgtgg
7741 acggcccgct gggcgccgac ctgctgctgg cgcagcggcg gcacgacctg tacgtcgcga
7801 tgctgcacga ccagggccac atcccggtca agctgctggc gccgaatgcg gccagcgcgc
7861 tgtccatcgg cgcgcaggtg ctgctgtcca gcgtcggcca cggcagcgcg atggacatcg
7921 ccggccgcgg catcgcggac cccggtgccg tgctgcgcac catcgcgctg ctgtcgggcg
7981 ccggcgtgga ggaagggacg gcatgaacca tcgcatcacc atcgaaggca gcgacgccgc
8041 cttcgactgc ggccccggcgc agagcgtgct cgacgccgcg ctgcgcgccg gcatcgagct
8101 gccctactcc tgccgcaaag gcgtgtgcgg caactgcgcc ggcgcggtgg ccgagggcga
8161 ggtggccggc ctcggcggcc cgatccgcaa cgagagctgc gcgcccgatc aggttctctt
8221 ctgcatgtgc gcgccgcgcg gcgacctgcg catccgcccc gccgcctggc accgcgtcga
8281 cccgtccgcc cgcaagcgct tcacggcgaa ggtgttccgc aaccagctcg ccgcgcccga
8341 cgtctcggtg ctgcagctgc gcctgccggc cgggcagcgg gcccgcttcc aggccggcca
8401 gtacctgcag ctcgcgctgc ccgacggcag cacccgctgc tactcgatgg cgaacccgcc
8461 gcacgagaac gacacgctga ccctgcacgt gcgccacgtg cccggcggcg ccttcagcgc
8521 gcgcgtgccc ggcctggcgc cgggcgacgc gatcgaggtc gagctgccgt tcggcgccgt
8581 cacgctggag gcggacgcgc gccggccgat cgtcttcgtg gtgggcggca ccggcttcgc
8641 gccggccagg tcgatcctgg acgacatggc gcgctggcgc gtggagcgcc cgatcacgct
8701 gatctggggc gcacgccgcg ccgagggcat ctacctgcgc ccggccattg ccaagtggca
8761 gcgccagtgg ccgcagggct tccgcttcgt cgaggcgctc agcggggagg cgcgcgaggg
8821 cgccttcgcc ggccgggtcg acgcggcgct gcgcgcgcac tgccccgacc tgtcgggcca
8881 cgagctgtat tgctgcggct cgccgggcat ggtgcagtcg gtgcgcgagg cggcggtgca
8941 ggcgctgggc ctgccggccg cgcgcttcca cgccgacgtg ttcgtcaacg ggccggcggc
9001 gggcgccgtg ccggcctgaa gacggccggc cccatagaga gatgccgcag gagcgtttca
9061 tggtgtcttc agtctcattc ggccacgagg acaaatggca tacagcgaat tccttcaaga
9121 aggcttgagc tctttttgca atatgtgaat cgactcctgg cctactagtc cattcgactc
9181 ggggcatcaa tgattgagct aagacacctg cggtacttca ttgcggtcgc ggaggaactg
9241 aatttccgcc gtgctgccga acgtgtgcac gtcgaccaga cgcctctgtc gcgcaccgtg
9301 cgcgatctgg aggagcaact gggtgtcacg ctcctcgtcc gcgcgccacg caggctgaaa
9361 ctgacccctg ccggatccaa gctgctcgag catgcccgca tgctcttcac gcgtctggag
9421 cgcatcaagc gggtcgtccg cgaaaccgat gcgcgctacc gcgcaccgct tcgcgtcggc
9481 gtggcggacg gcatggctca gctcaggctc tccgaatgct tcgccggctg gcgcgcactg
9541 gcgcccgaca tccgctgga gattgcggag atgcctgcgg ccgaactgtc gggcgcgttg
9601 cggcgcgagg aagtcgacat cggcttttct ttcggccttc cggacgatga cgccattgcc
9661 caggagatgg cctgggacta tccgctggtg gccctgctgc cgccggacca cgaactggcc
9721 acccgcgagg ttgtgcagac ctccgaactg ctgtcgtttc ccatgatcgc ctgccgcctc
9781 gaccgtctgc cgggcctgcg ccggcagatg gacgccgtgc gccagaacta cgctgcgcgg
9841 cccgtcatcg ccgccgaagc acggacgctg gtgggctacg tcatccgcgt cgccgcgggg
9901 ttgggtgtcg gcgtggcgga tgccggacac atggcgaccc tgcgccgcac cgatgtcgtc
9961 gtggtaccgc tggccgagga catacgcatc aagacctacg tgctgcacaa gcaccggcgc
10021 gacggcctgc cgcagatgct ccagcgattc ctcgcgtacg cccgaacctt gcatcagccg
10081 taacagcaca tcgccgtggg gcgatgtcgg ctcgcgccag aagccttgga cgcgtcgcgc
10141 gtcggctggc atgctaaccg ccgcctgact tggctgcgcc ctgcgagcag tctccaccac
10201 acctctggaa tcaacgaagc acccgctagc gtacgccgcg ccacgccctg atgggtgtgc
10261 ctcgcgggcg cttgatttca gacttgcacc tgcgtgtcgt ggtggcacgc gcattggaaa
10321 tcacgtgcct cgggaggtgg cctcatgccg acatcagcga ccggcatcct gttcggacag
10381 atcatcgtcg tgctatgcgt gccgctgggc ggcacgtggg ctgccacgca gtggacggcg
10441 cgggccttgg gctaccaggc cggactcggg ccggcctggt tccatgtgct cggcatgccg
10501 gcctacgagc cgtggaagct gttcgagtgg tggtatttct atggcgccta ttcgccccgc
10561 gtcttcgaac gcggagggat catggccgct tcgagcgggc tggccgccac cgccgcggcc
10621 atcgccatgg cggtgtggcg ggcccggctt gccgggcggg tcaccaccta cggctctgcc
10681 tgctgggccg aagccgaaga ggtggaaaag gccggcctgg ccggcgatgc cggggtcttc
10741 ctgggctgca tcgaaggcgt gggccgaaag ctgcgccgcc ggctgcgcac gtacctgcgt
10801 catgacggcc ccgagcacgt gctggccttc gcgcccacgc gttcgggcaa gggcgtgggg
10861 ctggtggtgc ccacgctgct cacctggccc ggctcggccg tgatccacga catcaagggc
10921 gaaaactgga atctcacggc cggctggcgc agtcggttca gccactgcct gctgttcaac
10981 ccgaccgatc cgcgctcggc cgcttacaac ccgctgctgg aagtgcgccg gggcgtgcac
11041 gaggtgcgca taggaatttc aaacggtc
//

[Accession number NCBI is:BBYR01000104, version number: BBYR01000104.1.]

There are a total of 11,068base pairs in the above-shown sequence.

It was observed that the organism would attach itself to the

PET surface and make use of an enzyme, PET hydrolase or PETase.

The PETase degrades PET and converts it into mono (2-hydroxyethyl) terephthalic acid (MHET). This MHET is a heterodimer that is made up of two major constituents, Ethylene glycol and terephthalic acid (TPA). The PET hydrolase or PETase found in I. sakaiensis functions by hydrolyzing the ester bonds that are found in PET. Upon further analysis, it was observed that Ideonella sakaiensis was able to produce a second enzyme called the MHETase enzyme or MHET hydrolase. This enzyme was found on the outer cell membrane. The MHETase is capable of degrading MHET, which is produced as a result of hydrolysis of PET by the PETase enzyme that was produced in the early stages of degradation by the organism, into Ethylene glycol and terephthalic acid (O. Kohei *et al*., 2016). PETase and MHETase share a similar structure and can be combined to form a super enzyme which can increase the activity of the enzymes. This was first discovered at the University of Portsmouth, United Kingdom. The super enzyme was formed as a result of the combination of the two separate enzymes, PETase and MHETase (Carrington, 2020) [2]. Polyethylene terephthalate hydrolase or PETase is coded by the gene ISF6_4831 and MHETase is coded by the enzyme ISF6_0224 (UniProtKB - A0A0K8P6T7 (PETH_IDESA) and UniProtKB - A0A0K8P8E7 (MHETH_IDESA)). According to the above-mentioned Shotgun sequences, Gene ISF6_4831 is observed in the 74th contig and the gene ISF6_0224 is observed in the 104th contig. The whole-genome shotgun sequence consists of 227 contigs. The whole organism consists of 6,142,063 bp. It has a total number of 5,527 proteins in it (NCBI accession number: BBYR01000000).

2.1 Polyethylene terephthalate Hydrolase (PETase)

PETase belongs to the class of enzymes designated as an esterase. Esterases are enzymes that hydrolases catalyze the splitting of esters into acid and alcohol with the aid of water. PETase is responsible for the catalysis of PET into its monomeric form mono-2-hydroxyethyl terephthalate (MHET) (Yoshida *et al*., 2016) [20, 21]. The PETase was first observed in Ideonella sakaiensis strain 201-F6. It was discovered in 2016.

The structure of PETase consists of three polypeptide chains, and these polypeptide chains were observed in an asymmetric unit. These three chains are denoted as A, B, and C chains. PETase adopts an alpha/beta-hydrolase fold. Within the fold is the highly conserved catalytic triad S131-H208-D177, found on the protein surface. The S131 part is what acts as the nucleophile. This nucleophile is located within a short distance that it can be polarized by the base H208. This is stabilized by acid D177. Many unique features are associated with the catalytic center of the enzyme. For instance, PETase forms two intramolecular disulphide bridges. These disulfide bridges are named as DS1 and DS2. This is only seen in PETase as all the other homologous enzymes only have one Disulfide bridge. The DS1 is what is specific to the PETase. DS2 connects the C-terminal helix and the last loop, while the DS1, which is only found in PETase, links the two loops that are associated with the catalytic acid and the base (Han. X *et al*.2017) [5].

PETase is coded by the gene ISF6_4831. The gene is observed in the 74th contig out of the 227 contigs. The sequence that codes for the enzyme is as follows:

1321 ctcgtgacac ctacaccaac aggagacaac atgaactttc cccgcgcttc ccgcctgatg
1381 caggccgccg ttctcggcgg gctgatggcc gtgtcggccg ccgccaccgc ccagaccaac
1441 ccctacgccc gcggcccgaa cccgacagcc gcctcactcg aagccagcgc cggcccgttc
1501 accgtgcgct cgttcaccgt gagccgcccg agcggctacg gcgccggcac cgtgtactac
1561 cccaccaacg ccggcggcac cgtgggcgcc atcgccatcg tgccgggcta caccgcgcgc
1621 cagtcgagca tcaaatggtg gggcccgcgc ctggcctcgc acggcttcgt ggtcatcacc
1681 atcgacacca actccacgct cgaccagccg tccagccgct cgtcgcagca gatggccgcg
1741 ctgcgccagg tggcctcgct caacggcacc agcagcagcc cgatctacgg caaggtcgac
1801 accgcccgca tgggcgtgat gggctggtcg atgggcggtg gcggctcgct gatctcggcg
1861 gccaacaacc cgtcgctgaa agccgcggcg ccgcaggccc cgtgggacag ctcgaccaac
1921 ttctcgtcgg tcaccgtgcc cacgctgatc ttcgcctgcg agaacgacag catcgccccg
1981 gtcaactcgt ccgccctgcc gatctacgac agcatgtcgc gcaatgcgaa gcagttcctc
2041 gagatcaacg gtggctcgca ctcctgcgcc aacagcggca acagcaacca ggcgctgatc
2101 ggcaagaagg gcgtggcctg gatgaagcgc ttcatggaca acgacacgcg ctactccacc
2161 ttcgcctgcg agaacccgaa cagcacccgc gtgtcggact tccgcaccgc gaactgcagc
2221 tgagtctcac cgtttccaat caggcgtgat gcctttggtg gccgcggctc gcaagagccg

The gene sequence is made up of 872 base pairs. The translation of the above-mentioned nucleotide yields a result as seen below:

MNFPRASRLMQAAVLGGLMAVSAAATAQTNPYARGPNPTAASLE
ASAGPFTVRSFTVSRPSGYGAGTVYYPTNAGGTVGAIAIVPGYTARQS
SIKWWGPRLA
SHGFVVITIDTNSTLDQPSSRSSQQMAALRQVASLNGTSSSPIYGKVDT
ARMGVMGWS
MGGGGSLISAANNPSLKAAAPQAPWDSSTNFSSVTVPTLIFACENDSI
APVNSSALPI
YDSMSRNAKQFLEINGGSHSCANSGNSNQALIGKKGVAWMKRFMD
NDTRYSTFACENP

NSTRVSDFRTANCS

There are a total of 290 amino acids in the sequence. The above-mentioned nucleotide and amino acid sequence can be obtained from NCBI using the accession ID: BBYR01000074.

The structure has been obtained from Uniprot, Accession ID: A0A0K8P6T7.

The enzyme, as mentioned previously is an esterase, more specifically it is a lipase.

2.2 Mono (2-hydroxyethyl) terephthalic acid hydrolase (MHETase)

MHETase belongs to the class of enzymes designated as hydrolases. It was first observed in 2016. As the name suggests the enzyme causes the hydrolysis of Mono(2-hydroxyethyl) terephthalic acid, which was formed as a result of the action of PETase enzyme on PET, into terephthalic acid and ethylene glycol(Yoshida *et al*., 2016) [20, 21]. The enzyme was first observed in Ideonella sakaiensis strain 201-F6. The structure of MHETase shares a close resemblance to tannase and feruloyl esterase. Like PETase, MHETase also displays an alpha/beta-hydrolase fold. Within the fold, the catalytic triad was formed by S225, H528, and D492. The catalytic triad is flanked by the presence of 5 disulphide bonds. The disulphide bonds also flank an oxyanion which comprises backbone amide nitrogen atoms of G132 and E226. Unlike PETase, the catalytic site of MHETase binds to the substrate very tightly. Structural analysis results of MHETase identified that the lid domain of the enzyme was the major difference to the closely related tannases and feruloyl esterases. MHETase might originate indeed from a loop modification in the lid domain. The major changes in the functioning of MHETase from that of the other enzymes originate due to loop insertion, deletions, and recombination (Weber. G *et al*.,2019)

MHETase is coded by the gene ISF6_0224. The gene was observed in the 104rth contig out of the 227 contigs. The sequence that codes for the enzyme is as follows:

1381 ggcgccacgc tacgtggccc ctatcaggga ggcgccgcgc aggcgaagtt cgcttcggtg
1441 ttgatgtctc cactaccctt gtagcgggcg atttggggat acgggcaaag tggccgcgtg
1501 cgtgccgcca ccccgaagta ccctggcgtg cccgaccagg cggagatctg atccggcgcc
1561 tcgccgcgct cgacccaggc gaccaagggc gtcagcatgt cgaaccggtc ggtcccggga
1621 ccgccgctgc aatgattcat acccggcacc agaaaaaggc gcgcgaatcc agcagcacct
1681 ggcatcgctg cgccaagtct ctcgtaatag tcggccgtgt ccagggctga gaacgcggcg
1741 tcggacatgc cgtggtacag gatcatcttg ccgccgcgat cgcggaaggc agcaaggtca
1801 gtagaagttg caccgtgcca gtccatgctg gattgggtga actgtccgct ggtcgcccaa
1861 atcttcaacg gatcgatgtc gaaatcgaat ttcatcatcc gcgcggcaac ctgcgtcatg

1921 ggcatcggtt cgggcggtgt ggcgaaatcg accagccagg agcgcgccga gaagccgctc
1981 acgcgctggg cgttgttggc actgctgttg aagctaccga gccaccacga gcgccacccc
2041 tggttatagg tcgtgccact cagtccgctc atgccagcgt cccaagccca tctgttgtac
2101 aacggcgttc ccgccgaatt cacgggcccc gccatcgcgc gcttgatcgc agtgacctgg
2161 acggggctca ggcaatccgc agtcttggcg ccaacgcact ggagcgcctg gccgtttgca
2221 ggattggctg ccgtggcggg gtcaaaggct gcctggcagg cccggtaatt gtcaacgatg
2281 ccatccgcaa ggccgtcgag ggcgtcacat gtgcccagga ttgcctgtga aagaaggtgc
2341 aggtcggcgt cagagaaaga cttattgata agcggcacgc cttgtgcatc caaaccgaca
2401 gcggcgggcg caagggactg tgtggtccac gcaccgctga tcccggcctt cggcaactgg
2461 tagcctggtg caccggcaac gatgccgtcg tagtgggaag ggaagcgctg cgagagcatc
2521 atgccctcgc ggccgccctc cgaacagccg atgaaatagg acttgtcggc agcgcgtccg
2581 tagaacctgg ccaccgcggc cttgcctgcc tgcgtgacct gatcgtacga gttgtagccc
2641 atatcgagac gggcctgcgg atccaatccg aaggcaacgg tgccgagcgc gtcgggattg
2701 tcgttcactg cgttgtcgtg acctccatcc gttgcgatgg tggcgaaatt gcgagacagc
2761 gcgcttgcga tctgtccccc accaatgctg ccggtagccg cggacagcga gccgttagtt
2821 ccgctgccgc cttccatgaa gaaacgaccg ttccattcgg cgggcatccg gaggcggaat
2881 ttgatttcgt agggatagcc gtcgatcccg gtgcgcttcg cgatggcgcc ggacacctcg
2941 cagtgttcgg gcagcgcagc cgcagatgcg gtggcgggcg cggcgtcacg ccaggcagcg
3001 acttccacga ccgtcgcggc atttggccac accatgtcgc catttccgtc cttcagagcc
3061 tcgcatgcgg cgcgcgaagc caagggcacc gggggcggcg gaggctcctg ctgcggcggc
3121 tgctgctgcg gcagcggcag cggcgtggat ccgccgccag cgcacgcggc tagggccacc
3181 gacgccagca gcatcgtggt gactgttgtc tgcatcttat gtctccttcg actggttcgc

The gene sequence is made up of 1811base pairs. The translation of the above-mentioned nucleotide yields a result as seen below:

MQTTVTTMLLASVALAACAGGGSTPLPLPQQQPPQQEPPPPPVP
LASRAACEALKDGNGDMVWPNAATVVEVAAWRDAAPATASAAAL
PEHCEVSGAIAKRT
GIDGYPYEIKFRLRMPAEWNGRFFMEGGSGTNGSLSAATGSIGGGQIA
SALSRNFATI
ATDGGHDNAVNDNPDALGTVAFGLDPQARLDMGYNSYDQVTQAG
KAAVARFYGRAADK
SYFIGCSEGGREGMMLSQRFPSHYDGIVAGAPGYQLPKAGISGAWTT
QSLAPAAVGLD
AQGVPLINKSFSDADLHLLSQAILGTCDALDGLADGIVDNYRACQAA
FDPATAANPAN
GQALQCVGAKTADCLSPVQVTAIKRAMAGPVNSAGTPLYNRWAWD
AGMSGLSGTTYNQ
GWRSWWLGSFNSSANNAQRVSGFSARSWLVDFATPPEPMPMTQVA
ARMMKFDFDIDPL
KIWATSGQFTQSSMDWHGATSTDLAAFRDRGGKMILYHGMSDAAFS
ALDTADYYERLG
AAMPGAAGFARLFLVPGMNHCSGGPGTDRFDMLTPLVAWVERGEA
PDQISAWSGTPGY
FGVAARTRPLCPYPQIARYKGSGDINTEANFACAAPP

There are a total of 600 amino acids in the sequence. The above-mentioned nucleotide and amino acid sequence can be obtained from NCBI using the accession ID: BBYR01000104.

The structure has been obtained from Uniprot, Accession ID: A0A0K8P8E7

The enzyme, as mentioned previously is a Hydrolase, more specifically chlorogenate esterase.

2.3 Cloning of PETase and MHETase)

The functional expression of PETase can be carried out in green microalgae (Chlamydomonas reinhardtii). This alga is a unicellular photosynthetic microorganism. The ability to incorporate the production of the enzyme PETase is achieved through the transformation with the aid of a recombinant vector pBR9_PETase_Cre (Ji Won Kim *et al*., 2020) [8].

The functional expression of the MHETase enzyme can be expressed in E.coli BL21 with the aid of the vector pUCIDT plasmid, which also constitutes a native signal peptide derived from *Ideonella sakaiensis* and a constitutive promoter J23106(Janatunaim, Rifqi Z. *et al*. 2020) [7].

The two enzymes can then be combined to form a super enzyme complex. This work was carried out at the University of Portsmouth, U.K, under the guidance of Prof. John.E.Mc Geehan. The combining of the two enzymes generated a super enzyme complex that was capable of degrading the PET six times faster (Carrington, 2020) [2].

2.4 Separation of Ethylene Glycol and Terephthalic acid

The action of the enzymes produced by the bacteria *Ideonella sakaiensis*, acts on and degrades Polyethylene terephthalate into MHET and then into two by-products, namely, Ethylene glycol and Terephthalic acid. Now, these two substances must be separated as they might be produced as a mixture.

An economical and rather environment-friendly method of separation of these two compounds was achieved by Gerald C. Tustin and his colleagues. The technique they developed involved the separation of terephthalic acid and ethylene glycol from a complex resin through a six-step method. Of this technique, only five steps is what we require. Cooling the mixture of these two compounds will leave the terephthalic acid in the solid phase and the ethylene glycol in the liquid phase. For obtaining the factory-grade forms of these compounds they have to be further treated. Ethylene glycol can be separated from the liquid portion using distillation techniques. The terephthalic acid can be recovered from the solid portion by heating it in the presence of water vapours at high enough temperatures. This acid-water vapour mixture can be cooled below the dew point of terephthalic acid. From this the polymer grade terephthalic acid can be isolated. (Tustin *et al*., 1995) [17].

2.5 Dealing with Ethylene Glycol

The ethylene glycol, which can be separated from the terephthalic acid, cannot be uptaken by many microbial species, owing to its high toxicity. For this reason, ethylene glycol must be converted into a less toxic form. To do this, it is treated with periodic acid which converts it into formaldehyde, which is arguably a less toxic form of ethylene glycol.

The reaction mechanism is named Glycol cleavage. It is a specific type of reaction in organic chemistry. This is a type of oxidation reaction.

As stated above, the ethylene glycol upon reacting with periodic acid can yield Formaldehyde as the reaction product. The formaldehyde so formed can be utilized by a variety of methylotrophic bacteria. This bacteria does not essentially utilize formaldehyde as a source of carbon. Instead, they utilize formaldehyde as a cofactor, for the degradation and assimilation of single carbon atom molecules, such as methane, methylamine, and dichloromethane, as a source of energy (Vorholt, 2002) [18].

2.6 Dealing with Terephthalic acid

Terephthalic acid is an important industrial chemical. But if left untreated or exposed to water, it can act as a major pollutant. Recent studies have shown that terephthalic acid can be degraded using physical and chemical methods. But these techniques are rather expensive and complex. Because of these reasons microbial degradation of terephthalic acid is being considered as a viable alternative for the degradation of terephthalic acid. This is also an environmentally friendly method. This was achieved by utilizing a bacterial species known as *Arthrobacter sp*. Rather than utilizing the enzyme produced by the organism, the resting cells are being used for the purpose. This is because the extraction of the enzyme is a rather expensive method and also within the cells the enzymes will be protected from the external environment (Yi-Mei Zhang *et al*., 2013) [19].

3. Methodology

The study with an extensive literature search using Google Scholar and Google search engine using the search string "Microbial degradation Polyethylene terephthalate".

3.1 Cloning and expression of PETase enzyme in Chlamydomonas reinhardtii

1. The amino acid sequence of PETase (ISF6_4831) obtained from UniProt.
2. The amino acid sequence of PETase can be reverse-translated, codon-optimized for C. reinhardtii, and synthesized by using the resulting pIDT_PETase_Opt. The codon-optimized PETase-encoding gene can be digested and cloned into pBR9_mCherry_Cre.
3. The digestion and cloning can be done by using XhoI and BamHI restriction endonucleases, generating pBR9_PETase_Cre.
4. With the transformation of PETase gene, expression confirmation, and plastic degradation, microalgal strains can be used.
5. For gene cloning, the ligated plasmid (PETase gene from pIDT_PETase_Opt + pBR9_mCherry_Cre) can be transformed into E. coli DH5α by using commercial heat shock competent cells.
6. The heat-shocked cells can then be spread on LB-agar plates (BD) containing 100 mg/L Ampicillin and the correct clones can then be confirmed by polymerase chain reaction (PCR) and DNA sequencing. From the final E. coli clone, pBR9_PETase_Cre plasmid can be purified from the 300 mL culture solution by using Nucleobond Xtra Midi Plus for the transformation of microalgae.
7. Competent cells of *C. reinhardtii* CC-124 and CC-503 can then be prepared by using MAX™ Efficiency Transformation Reagent for Algae. Each 400 µL of competent cells containing 10 µg of pBR9_PETase_Cre (linearized by PsiI) is to be subjected to electroporation.
8. The electroporation can be performed with Gene Pulser Xcell Electroporation systems under the conditions of 500 V, 50 µF, and 800 Ω.
9. After the pulse, 10 mL of TAP medium supplemented with 40 mM of sucrose is added to the cells immediately, and the cells are recovered for 16 h at 25 °C with shaking (80 rpm) under low light.
10. The cells can be plated on TAP-agar plates containing 10 mg/L Zeocin. Colonies from the TAP-agar plates can be inoculated into 96-well plates with 150 µL TAP medium containing 10 mg/L Zeocin. After cultivation, cells in green-colored wells can be transferred to 24-well and 12-well plates sequentially using the aforementioned conditions. For the selection of stable transformants, the cells from 12-well plates can be cultured with a 10 mL TAP medium in a T-flask. The well-grown cells can be chosen as the final clones. To verify the PETase gene integration into the nuclear genome of *C. reinhardtii*, the genomic DNA of the final clones can be extracted via boiling with specific solution (1 M KCl, 10 mM EDTA, 100 mM of Tris–HCl, pH 9.5) for subsequent PCR analysis.
11. The protein expression can be analyzed by Western blot analysis and SDS-PAGE analysis.
12. To analyze the catalytic activity of PETase produced from *C. reinhardtii*, a commercial PET beverage bottle (PepsiCo., NY, USA) can be used as the substrate. The PET powder for HPLC analysis can be prepared by grinding the bottle with sandpaper.
13. The ground PET was filtered using a sieve to remove large fragments. The PET films for electron microscopy were prepared by cutting a PET bottle into 2 cm × 1 cm fragments.
14. Wild-type *C. reinhardtii* were cultivated and harvested to obtain cell lysates. The harvested cells were resuspended in 1× PBS and Protease Inhibitor Cocktail.
15. The solution was disrupted by sonication on ice for 20 min with 3 s pulses at 7 s intervals.
16. After the preparation of all materials, PET powder and films were incubated with 1 mL and 5 mL of the cell lysates, respectively.
17. All of the reaction mixtures were incubated at 30 °C for 4 weeks, changing the cell lysate to a fresh one weekly to prevent contamination and PETase degradation (Ji Won Kim *et al*., 2020) [8].

3.2 Cloning and expression of MHETase in E. coli BL21

Methods: Construction of MHETase gene in pUCIDT plasmid with native signal peptide from I. sakaiensis 201-F6 and constitutive promoter J23106 can be expressed in *Escherichia coli* BL21 (DE3) by heat shock. Expression analysis using SDS-PAGE and activity of enzymes is analyzed by spectrophotometry method and SEM.

Results: MHETase gene protein can be successfully constructed in pUCIDT +Amp plasmid with native signal peptide from *Ideonella sakaiensis* 201-F6, T7 terminator, and constitutive promoter J23106. PCR analysis showed that the gene was successfully contained in the cells by band size (1813 bp) in electrophoresis gel. Analysis using SnapGene, pairwise alignment using MEGA X, and NCBI can be demonstrated that in the MHETase sequence the gene can be in-frame in pUCIDT plasmid.

Conclusion: MHETase gene can be successfully constructed in plasmids by the in-silico method. Synthetic plasmids transformed in E. coli BL21 contain MHETase gene sequences which were in the frame. Hence, the E. coli BL21 cells have the potential to produce MHETase proteins for the plastic degradation testing process (Janatunaim, Rifqi Z., *et al*. 2020) [7].

3.3 Separation of the products of enzymatic degradation by bacterial enzymes

(1) Cooling the mixture with water at about 70° C. to 100° C., filtering the solids, washing the solids, and then drying the solids at a temperature of from about 25 C. to 100° C. to provide a solid portion comprised of the terephthalic acid and a liquid portion composed of ethylene glycol.

(2) Recovering the ethylene glycol from the liquid portion of the mixture by a two-step distillation, wherein in a first step of the two-step distillation the water and low boiling components are removed at about 0.1 to 6 atmospheres pressure and temperatures of about 100 °C. to 170 °C., and wherein in a second step of the two-step distillation high boiling species are removed at about 1 mmHg to 10 atmospheres pressure and at a temperature range of about 50 °C. to 300 °C.

(3) Recovering the solid terephthalic acid by heating the solid portion above its dew point with a continuous stream of water vapor at a temperature of about 310 C. to 370° C. and a pressure of about 0.1 atmospheres to 1.2 atmospheres to produce a vapor comprised of the water and terephthalic acid.

(4) Cooling the vapor containing the terephthalic acid and the water to a temperature below the dew point of 2 the terephthalic acid and.

(5) Collecting the solid polymer grade terephthalic acid (Tustin *et al*., 1995) [17].

4. Expected results and discussion

4.1 Cloning and expression of PETase enzyme in *Chlamydomonas reinhardtii*

Firstly, expect to find the stable integration of the PETase encoding gene in the plasmid, pBR9_mCherry_Cre, and subsequently leading to the production of the stable recombinant plasmid pBR9_PETase_Cre. After this, it is expected that this plasmid can be successfully transferred to an E. coli DH5α host. Following this, the *E.coli* should be able to infect the *Chlamydomonas reinhardtii* and transfer the gene of interest into the nuclear genome of the *Chlamydomonas reinhardtii*. The Chlamydomonas should then be able to produce the enzyme PETase in an economically viable amount.

4.2 Cloning and expression of MHETase enzyme in E. coli B12

Here in this stage, expect to see the stable integration of the MHETase coding gene into the plasmid pUCIDT +Amp. From there expected that this recombinant synthetic plasmid can then be stably transferred into an E.coli B12 expression vector. Suppose to see that E.coli can produce the enzyme MHETase in an economically viable amount.

4.3 Separation products of enzyme degradation

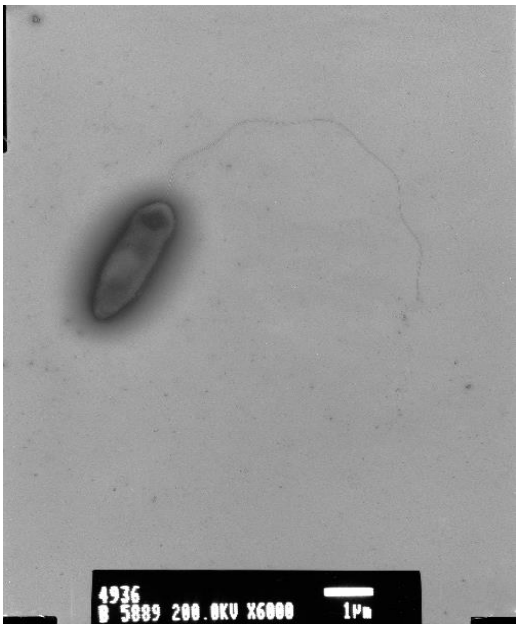
Following the enzymatic degradation of PET, left with Terephthalic acid and Ethylene glycol in the mixture. Following a variety of procedures, expected to separate the two components from the mixture with the maximum purity as much as it is possible.

5. Conclusions

Plastic pollution is a menace that is eating away at our planet with each passing day. With this review literature, we aim to bring to light a possible method to achieve the complete degradation of PET plastics, without involving any of the physical damage that any of the conventional methods would bring forward. Microbial degradation is the only solution to this problem. With the aid of the microbe *Ideonella sakaiensis* and the enzymes that it is capable of producing, the complex substance PET can be converted into much simpler forms, which can then be utilized by other organisms as a source of energy or potentially use it as a catalyst.

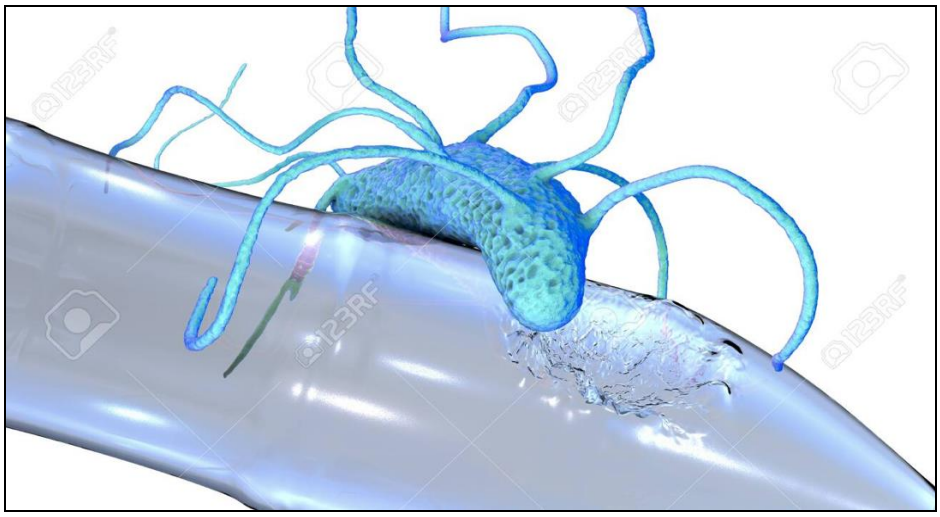
Some of the few limitations that can be faced while utilizing this method there can be some limitations. They are as follows:

1. *Ideonella sakaiensis* is native to Japan and it is rather costly to obtain, needing very specific growth medium components. This could pose a challenge.
2. The exact economic feasibility of the entire work is not determined.
3. The duration it takes to attain the complete biodegradation of PET is not exactly known. Depending on the size of the plastic pollutant the duration of the process might vary.



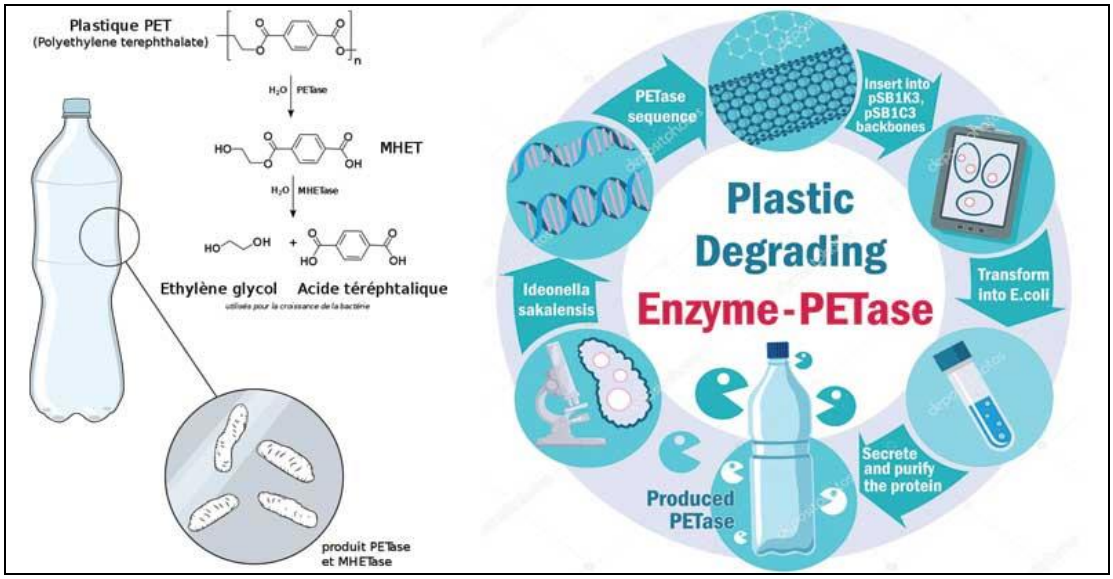
Source: https://www.microbiologyresearch.org/content/journal/ijsem/10.1099/ijsem.0.001058#tab2

Fig 1: Transmission electron micrograph of a cell of strain 201-F6T grew in NBRC no. 802 broth at 30 °C for 24 h. Bar, 1 µm.



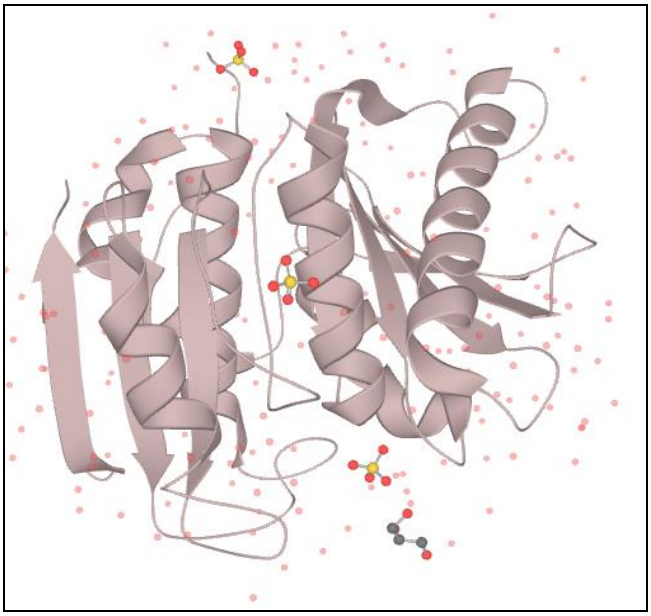
Source: https://www.123rf.com/photo_127986519_stock-illustration-plastic-degrading-bacteria-ideonella-sakaiensis-3d-illustration-recently-discovered-bacteria-that-ha.html

Fig 2: *Ideonella sakaiensis*, 3D illustration



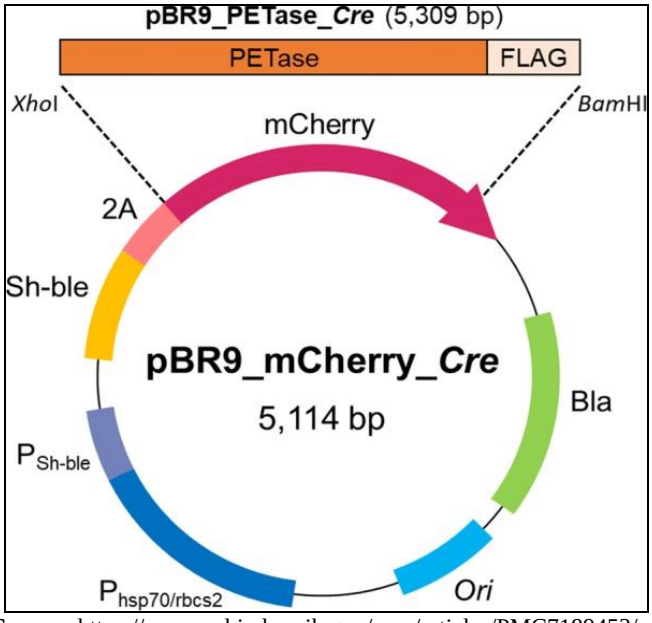
Source: https://thebiologynotes.com/ideonella-sakaiensis-plastic-eating-bacteria/

Fig 3: Action of *Ideonella sakaiensis* on plastics



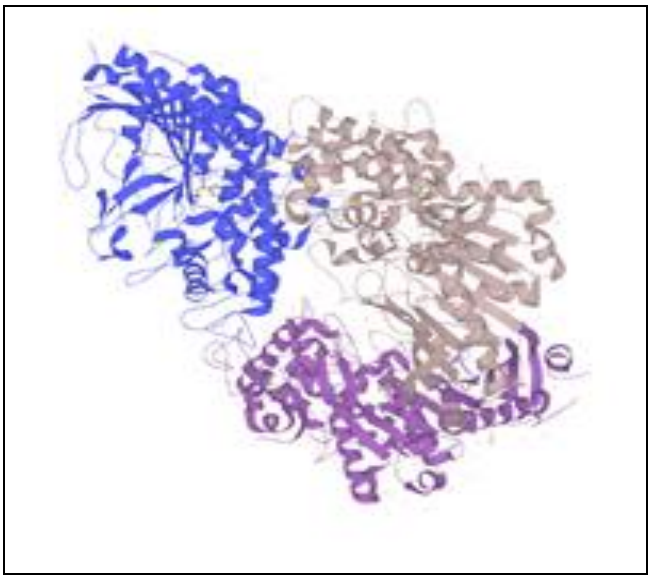
Source: https://www.uniprot.org/uniport/A0A0K8P6T7

Fig 4: Tertiary structure of the enzyme PETase.



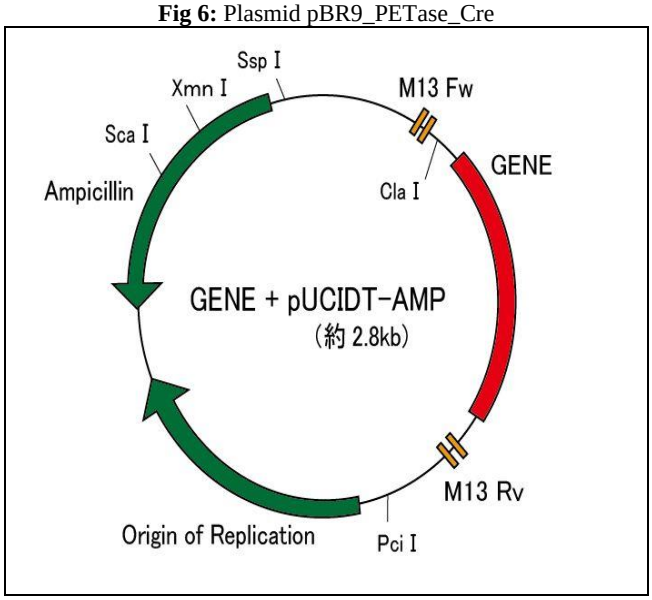
Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7189453/

Fig 6: Plasmid pBR9_PETase_Cre



Source: https://www.uniprot.org/uniport/A0A0K8P8E7

Fig 5: Tertiary structure of the enzyme MHETase.



Source: https://sg.idtdna.com/jp/site/custom_gene.html

Fig 7: Recombinant pUCIDT plasmid containing gene of interest.

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