Use of PETase for PET Degradation in a Bioreactor to Improve Effectivity of Recycling

Literature Review

Jessie Taubert

Mass Academy of Math and Science

Worcester, Massachusetts

Abstract

Plastic pollution is becoming increasingly detrimental to the environment as industries continue to mass produce products that rely on plastic as the basis. Because it is more financially beneficial for these corporations to use new plastic rather than recycled plastic, properly recycled plastic is not reused. Often times recycled plastics and other waste commercial and household wastes do not end up in the intended locations, but instead pollute land and waterways due to illegal dumping. With no effective way to collect plastic wastes and actively reuse them, the industry's habits have quickly become unsustainable.

Recently, a bacterial organism has evolved to contain an enzyme that allows the use of plastic for nutrients by breaking down the polymer. While the enzyme this organism contains has been deemed "not effective enough" for industrial implications based on the temperatures at which it can function and time it takes for the whole process to occur, it may still hold the key to creating a more effective recycling system. Many studies are already improving its effectivity by altering the enzyme, yet no real progress has been made to implement this technology at both the level it is functioning at or once it is developed for industrial use. This review will detail the current progress towards reversing plastic pollution through the use of the enzyme from this organism.

Keywords: enzyme, PETase, plastic pollution, recycling, bioreactor, bacteria, degradation

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Plastic pollution is becoming a major concern for the environment and is threatening the planet. This is largely due to ineffective recycling techniques in conjunction with the mass production of plastic. Because of this, waste plastics - along with other materials – are discarded and end up in rivers and across the land. While some materials are properly disposed of, throughout the recycling process a majority of these materials end up in landfills or disposed of with methods that release toxic pollutants into the air. Due to this, a better method for recycling is needed. One recent discovery by Yoshida et al. (2016) notes the findings of a bacteria with and enzyme that allows it to use plastic as a source of nutrients, ultimately breaking down the plastic. This technology, if developed further, could be used to combat the issue of plastic pollution.

Plastic Pollution

About 9 billion tons of plastic waste pollutes the earth. A majority of this was produced within the past two decades, evidence of the increased production of plastic in recent years to meet the higher demand due to its versatility. However, with this versatility comes a drawback, as these plastics are do not easily decay in nature (Demircan & Keskin, 2020). By 2050, it is expected that plastic production will grow by 70% to nearly 600 million tons per year (Cornwall, 2021). This is raising concern due to the alarming rate of increase in pollution of these disposable plastics. Countries are not properly disposing of this waste, and nearly 8 million tons of plastic waste end up in the ocean from coastal regions alone every year. Major rivers carry this discarded plastic, along with other trash, to the ocean and in some cases around the world. After World War II, the innovation of new products revolving around plastic increased - only 2.3 million tons of plastic were being produced in 1950, whereas 448 million tons were being produced in 2015 - and this created many new technologies that are inherent to our lives today. However, many of these products are not meant to last more than a moment, resulting in a “throw-away” culture and polluting the world. In fact, plastics that are only used once make up 40% of the annually produced plastic (Parker, 2019). Some researchers are looking at and developing biodegradable materials as plastic alternatives to combat this.

As plastics pollute the ocean and land, they begin to break down into smaller plastics into what are known as microplastics. Microplastics are any plastic under 5 millimeters in length, and they are extremely harmful to wildlife and humans, as they pollute drinking water and spread throughout the air (Parker, 2019). Aquatic species, meanwhile, face an additional struggle from plastic pollution, as they end up trapped and suffocated by waste plastic. No effective way has been discovered to remove plastics from these bodies of water. Rather, a more effective method would be to block discarded plastic from entering waterways initially and develop better methods of recycling to manage wastes (Parker, 2019).

Recycling

Recycling is one process that aims to reutilize wastes that would otherwise add to environmental pollution. While recycling is a good way to address plastic pollution, most manufacturers choose not to reuse plastic as producing new plastics costs less than producing plastic from recycled products. Other ways to remove plastic waste is to burn it, but this releases pollutant gases such as sulfur dioxide, hydrogen cyanide, and hydrogen fluoride, which have been shown to be toxic and poisonous (Bellini, 2019). Burying it is another option, but this pollutes the land and water, and mechanically breaking it down is expensive (Demircan & Keskin, 2020). Alternatives to common uses of plastic, such as aluminum or glass cans, release more carbon into the environment than plastic, making them less beneficial alternatives (Stanton et al., 2020). Even when the material changes, humans will continue to litter, making it essential to find a way to actively recycle and properly dispose of wastes (Stanton et al., 2020). Only 9% of all recyclable plastics end up being recycled in the US, since at every step of the process more and more of the recyclable material is disregarded, or it ends up in landfills and ultimately incorrectly disposed of (Sheth et al., 2019).

Developing countries that are moving towards becoming consumer-oriented and establishing a flourishing economy are encountering issues with waste control. These countries struggle to develop waste management systems since their economies are not able to support that system in the same way that more industrialized countries can. This lack of waste management impacts their purchasing habits, as they buy fewer products and try to reduce the amount of packaging used (Lemmons, 2021). Logically, these purchasing habits result in a reduced production of waste. However, these poorer nations do not have an organized waste management system, such as trash collection, to properly dispose of this waste. In some developing countries, attempts at implementing waste management systems have been made, however, these regulations are not enforced well and are ultimately disregarded by the people (Lemmons, 2021).

Because of the lack of a waste management system, waste accumulates in streams and on land, overall acting as a major concern to the health and environment of these countries. More urbanized areas that are developing in these nations have been found to struggle with the issue of waste management as well (Lemmons, 2021).

For example, the capital of the Philippines, Manila, creates 7,982 metric tons of trash every day, yet there is no collection of waste or recycling done to manage these wastes. This results in wastes piling up in landfills, and poorer citizens rummage through the trash to try and find things to help them make a living. One of these dumps collapsed in 2000 and killed 219 people. The government's response to this was to simply open a new dump (Lemmons, 2021).

China is also experiencing a rapid increase in waste production without a system to manage it, which has created health and environmental concerns, as toxic pollutants from the waste pollutes the air, water, and land. This has led to nearly half a million Chinese people dying from these hazards alone (Lemmons, 2021). While the government has attempted to address this, the people have not taken to their solutions well since their solutions reduce the consumerist leisure and the people are not daunted by the fines or consequences. It is speculated that if China continues on this path, their progress economically may not be able to grow at its maximum (Lemmons, 2021).

With all of this waste left in the environment due to ineffective waste manage systems, concern is arising in regard to how sustainable this behavior is. Synthetic polymers are particularly resistant to natural degradation due to their carbon-carbon bonds or carbon-oxygen bonds which are difficult to break, ultimately harming ecosystems (Austin et al., 2018). As previously mentioned, existing ways of recycling these polymers are harmful to the environment and the plastics do not always end up in a recycling center. Additionally, these existing methods of recycling deform the plastic and do not allow the industry to easily reutilize the plastic in a cost-effective manner (Demircan & Keskin, 2020).

PETase

Polyethylene terephthalate (PET) is one type of plastic polymer that is widely used in products such as water bottles and clothing. PET is made with different crystallinities to serve different functions (Wallace et al., 2020). While higher crystallinity PET holds its shape better due to the aligned concentration of atoms, lower crystallinity PET lacks this alignment and thus is more malleable, and these crystallinities are utilized separately or together based on the need of the product. Because of its structure, highly crystalline PET is less susceptible to degradation from enzymes (Wallace et al., 2020).

Some organisms have evolved due to exposure to plastic and have developed enzymes to break down plastic for energy (Hiraga et al., 2019). Microorganisms found in areas containing trash and vegetation, namely plastic landfills, have adapted to contain enzymes that can decompose plastic polymers, including the polymers that are used in bottles and clothing

(Cornwall, 2021). One organism, *Ideonella sakaiensis 201-F6*, was found in a landfill and was taken to lab and found to have properties to degrade PET. These properties were enzymes, namely PETase, which can dissolve PET by using it as a source of energy and carbon and ultimately breaking PET down into its monomers (Hiraga et al., 2019). The bacteria latch on to the PET and form a biofilm - a colony of bacteria that rely on the material for nutrients (O'Toole et al., 2000). However, some enzymes have been deemed to not work fast enough and others are only able to break down a specific plastic polymer, and these qualities are undesirable to the general public as it is harder to implement this technology when it takes a long time for the process to be completed (Cornwall, 2021). These limitations have prompted studies to improve the enzyme. While many experiments have looked at ways to improve the efficiency of PETase, little progress has been made to utilize this technology to address the growing issue of plastic pollution and ineffective recycling.

I. sakaiensis gets carbon and energy from PET in order to live and, because of this, has developed certain enzymes to aid in the process. One study used two enzymes, PETase and MHETase, as PET degrades to still have another polymer, MHET. This use of two enzymes helped develop the process of reducing the PET polymer into its monomers. Ethhylene glycol (EG) and terephthalic acid (TPA) were found to be products from this reaction (See Figure 1).

These products are what make up PET, and thus could be used to reproduce plastic.

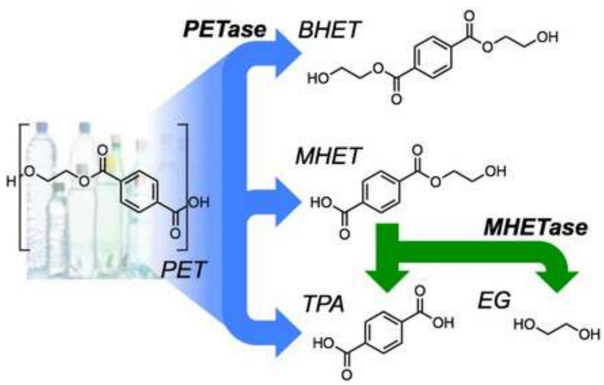

Figure 1. Molecular breakdown of PET into MHET, BHET, terephlatic acid (TPA) and ethylene glycol (EG). BHET can be broken down by PETase, but MHETase is required to break down MHET to TPA and EG (Austin et al., 2018).

When looking at the structure of PETase and MHETase, similarities are seen between these enzymes and hydrolase enzymes, as they share cutinase and lipase features. When altering the PETase active site cleft to more closely resemble a cutinase active site cleft, the degradation of crystalline PET was improved (Austin et al., 2018). Ester bonds link PET monomers which are hydrolyzed – or broken down with water - by hydrolase enzymes in nature, allowing the plastic to be biodegraded by breaking the carbon-carbon bonds (Hiraga et al., 2019). These carbon-carbon bonds are what make the polymer more difficult to degrade when compared to other plastics such as polyesters that have carbon-oxygen bonds (Cornwall, 2021). Other PET-degrading organisms with these hydrolase enzymes may be found in highly saline environments such as the ocean (Hiraga et al., 2019).

Bioreactors

Bioreactors have been used as tools to culture cells for research and other purposes. However, they have not been developed for economics or to be deployed in an industry setting due to some less-than-ideal statistics, including the high cost of stainless steel, which is what the typical bioreactor is made of, and low growth and productivity of the cells cultured in the bioreactor (Ducos et al., 2008). This technology may be able to be utilized in tandem with PETase to degrade plastic into its monomers, and then reuse these monomers to remake plastic, resulting in a cycle of recycling and producing plastic.

Bioreactors have different designs based on the microorganisms they are used with, but some features hold true for all circumstances. Mixing the contents of the bioreactor is a common component. Additionally, many different materials have been used in bioreactors. In one experiment, glass was used to create an airtight environment and a bottle was attached to the device to collect produced gases (Singh et al., 2011). In another study, PVC was used; however, they noted that it seemed like PVC would not be durable enough to support the weight if the volume increased. Because of this, PVC may not be the best material for large-scale bioreactors (Ducos et al., 2008). However, in this study the authors had two different designs. The first design was long and made of PVC with a raised side with jacks that aimed to utilize movement of the medium to mix the culture and aerate it, while their second design was tall and aimed to use bubbles from a valve with compressed air rather than waves to meet the same needs. The authors compared these designs with an Erlenmeyer flask and a stirred-tank bioreactor, two more common bioreactors, and looked at the reproduction of the species in a liquid medium. They found that the growth in the first and second designs was similar to that of the typical bioreactors, making them better options since they are efficient, more cost effective and disposable. They are also simpler and lighter than other bioreactors (Ducos et al., 2008).

Many studies have used similar endpoints and implemented similar gas pumps for aeration. Lin et al. (2020) set up a bioreactor with different sources of carbon and hydrogen to look at the growth of bacteria in aerobic and anaerobic conditions. This design utilized Petri dishes to allow for other nutrients to be added to the bacteria. They found that the optical density, as a measure of bacterial growth, of the cells in the liquid medium was low for succinate and pyruvate in anaerobic conditions, but xylose and glycose had relatively normal growth curves in anaerobic conditions (See Figure 2). With every trial, the substrate in the aerobic environment had a higher optical density than in anaerobic conditions (Lin et al., 2020). Because of this, growth curves may be a logical endpoint to find the best conditions to grow bacteria.

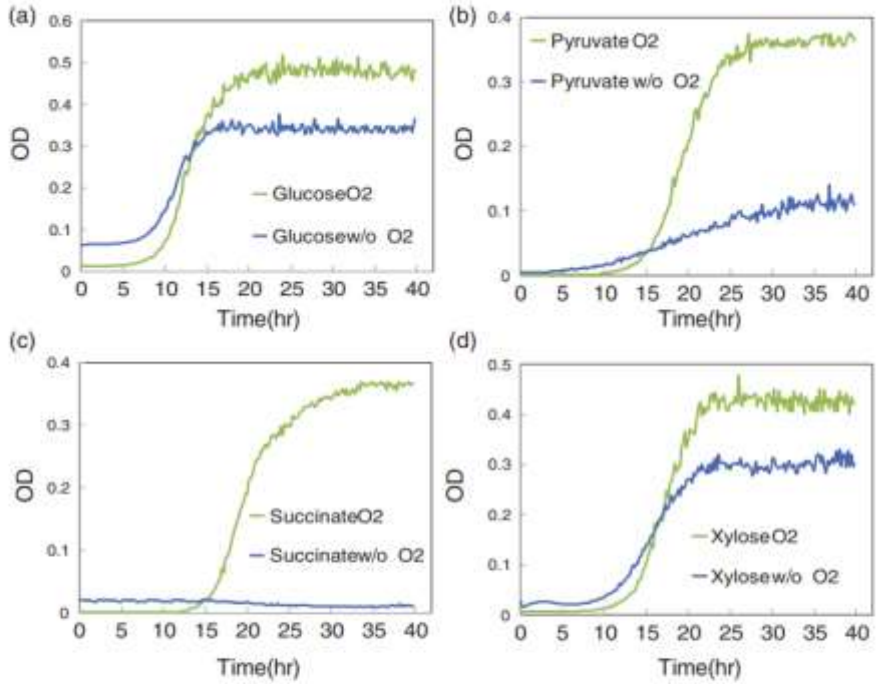

Figure 2. Graphical analysis of the optical density of bacteria cells in the different substrates. Each substrate was analyzed in both aerobic and anaerobic conditions over a period of 40 hours (Lin et al., 2020).

Conclusion

With progress being made to improve PETase, developing possible methods for application is increasingly important to actively reduce the harm that plastic pollution is causing to the planet. Implementing PETase in recycling methods could be the key for effective recycling as current methods are not sustainable. By breaking down the polymer and leaving the monomers to be remade into plastic, this enzyme allows for the creation of a cyclical pattern of degrading and remaking plastic rather than relying on new plastic and leaving wastes scattered, but more research may need to be done on how to isolate these monomers from the solution after the process has occurred. Developing a method to utilize PETase in industrial recycling processes could prove to be the next step to combatting plastic pollution.

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