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An Investigation into the Feasibility of using MHETase and PETase for Water Treatment Due to the Increase of Microplastic Ingestion through Tap Water

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ABSTRACT

Polyethylene terephthalate (PET) is the most abundantly produced plastic due to packaging and textiles, contributing to the widespread presence in the environment and the generation microplastics. Given microplastics high absorption rates and hydrophobicity they can accumulate toxic chemicals making them hazardous. When large quantities of microplastics infiltrate drinking water treatment facilities the public is exposed to these toxic chemicals, potentially resulting in various cancers and reproductive disorders. In response to the accumulating microplastics in the environment biochemical engineers have begun to analyze microbial solutions, such as enzymes MHETase and PETase, which can enzymically degrade PET polymers and metabolically assimilate them as a carbon nutrient source. However, it is unknown whether these enzymes can be adapted to drinking water treatment processes. To address this, an experimentation of inquiry was conducted to analyze the digestion rate of PET plastic when exposed to environments resembling those of drinking water treatment facilities. By introducing water samples collected from the Ann Arbor Drinking Water Treatment Facility and analyzing the change in phenol indicator color and mass of PET plastic samples. The research design concluded that despite the introduction of water samples there was no significant change in the digestion rate as compared to control samples, suggesting the feasibility of utilizing MHETase and PETase as a water treatment solution for PET microplastics within drinking water treatment facilities.

Introduction

The most abundantly produced synthetic polymer that is accumulating from packaging and textiles, according to The National Academy of Sciences, is known as Polyethylene terephthalate (PET).[1] In recent years plastic and plastic production, such as this, has gained popularity from the public due to concerns of environmental pollution and public health, and ultimately lacking sustainability. One of these growing concerns is microplastics. Microplastics are universally accepted as small plastics 5 mm in diameter.[2] After PET is discarded it can produce microplastics and infiltrate filtered water. According to The American Chemical Society, Americans who meet the recommended water intake consume on average 4,000 microplastics annually through tap water.[3] Although the long-term effects have not been foreseen, microplastics when ingested can be cancerous, and therefore a threat to public health.[4] Due to these rising concerns scientists began engineering enzymic solutions to rid the environment of microplastics. Two are known as PETase and MHETase, which are enzymes that can digest (PET) microplastics.[5] While these enzymes have been extensively studied, a gap can be identified through the unknown feasibility of adapting these enzymes to drinking water treatments specifically those used in the

US water treatment facilities. Therefore, the question remains: to what extent can PETase and MHETase be used as a water treatment in drinking water treatment facilities in the United States to rid it of microplastics?

Microplastics Necessity and Trend

The turning point for plastic was the onset of World War II; during this period the demand for cheap, strong, and long-lived material for military campaigns and other domestic applications arose.[2] As a direct result, the production of plastic increased exponentially. According to David Newton, an EdD graduate of Science Education at Harvard University, in 1950 the total worldwide plastic output was just 2 million tons, while in 2015 over 380 million tons were produced.[2] This overwhelming production is due to the excessive use of plastic and consumption behaviors. This is acknowledged by The Department of Political Science at the University of Lund in Sweden, plastics are undeniably fundamental to current materials and objects, and this can be demonstrated through a "broad range of applications from smartphones to food packaging and 3D printing."[6] Thus, fueled by constant public demand and necessity for future technology applications, it is estimated that the worldwide production of plastic in 2050 will be four times what is produced today.[2] The consistent increase in plastic production is correlational to microplastic production and exposure, pressing the issue for public health concerns.

Plastic Sources

Microplastics are classified by the mechanisms in which they were formed these categories are primary and secondary.[2] Newton describes primary microplastics as "wash off" or "leave on" products.[2] According to the National Library of Medicine, the majority of primary microplastics are "industrially manufactured as plastic fibers or particles in the micron size range and are used to manufacture products such as facial cleansers, cosmetics, or airborne media."[7] Surprisingly 85 percent of microplastics released in the environment originate from personal care and cosmetic products which include washing laundry.[2] This demonstrates that annual behaviors contribute to microplastic exposure and are likely to continue. Conversely, secondary microplastics are "formed from the breakdown of larger pieces of plastic."[2] These materials are commonly referred to when discussing microplastic pollution, they are a result of sunlight exposure and erosive actions on bigger plastic items such as bottles and reusable bags. Newton and the National Library of Medicines' conclusions introduce the many origins of primary and secondary microplastics and suggest that both are human behavior oriented whether it be intentional or nonintentional, and thus cannot be prevented.[2,7]

Microplastic Route

Regardless of where microplastics originate from, they always resonate or reach freshwater bodies. This can be extremely hazardous since US water treatment plants obtain water primarily from freshwater sources that include lakes, groundwater, and rivers. According to The Department of Biological Science at Wayne State University, even primary plastics that are designed for terrestrial systems such as recreational or industry, have the potential to reach freshwater sources.[8] Due to the purpose of primary microplastics, as mentioned, most are discharged into the environment from wastewater stations.[7] Dr. Sherri Mason, who holds a doctorate in Chemistry and is a leading researcher in freshwater plastic pollution, and her team conducted a study to test if microplastics are removed in wastewater treatment plants in the US. They collected 90 samples from 17 different facilities across the US. Wastewater facilities are specialized to remove fecal matter, nutrients, microbes, and other known matters that negatively impact the environment, they are not designed to remove microplastics.[9] So, it is not surprising that the results concluded, on average "each wastewater treatment facility was releasing more than four million pieces of microplastic into U.S. waterways every day: 60 percent fibers, 34 percent beads, and 6 percent

films and foams."[9] With the abundant amount of wastewater treatment facilities within the US, one can conclude that billions of microplastics are released into freshwater sources annually that are heavily relied upon, reinforcing the concern of microplastic ingestion through tap water. On the other hand, secondary plastics, once discarded, can erode in the soil or through UV exposure and travel through groundwater that will eventually lead to rivers, lakes, and any freshwater bodies.[2] If released in the atmosphere "they are eventually returned to earth's surface by rain, snow, sleet, or some other forms of precipitation."[2] As demonstrated by Newton and Mason it is blatantly clear that microplastics pose as a serious threat to water treatment facilities due to immense amounts of microplastics that enter water sources.[1,9] Therefore, considering the correlation and trend between plastic and microplastic production it can be implied that increased levels of microplastics will infiltrate drinking water treatment facilities.

Microplastic Properties

Once microplastics enter US waterways, small doses can advance to tap water that is ingested by American citizens. Microplastic infiltration is due to its properties. According to, Shampa Gosh, a chemical engineer, these particles are so small they route through water filtration techniques.[10] Regrettably, microplastics itself is not the only concern, according to the Department of Earth Sciences at the University of Naples Federico in Naples, Italy: because of their hydrophobicity and large surface area these materials have a high absorption potential.[11] High absorption rates increase the toxicity of microplastics by becoming a vector for hazardous chemicals and compounds. Continuously, Abdulkarim Hasan Rashed, a specialist in environment and sustainability development, chemicals are accumulated and absorbed on the surface of microplastics as they travel through wastewater sludge or organic fertilizers.[12] Some of these chemicals include "polyaromatic hydrocarbons, metals, and dioxins."[13]As detailed both primary and secondary microplastics are susceptible to chemical accumulation, increasing the toxicity and hazard of digesting them.

Literature Review

Current Treatment

Unfortunately, there is no current treatment for microplastics, and California is the only state that has mandated testing for microplastics in drinking water.[14] According to Juan Conesa, a chemical engineer, at present "treatment plants process water effectively, and the number of synthetic microparticles in effluents is usually very low. Still, discharge volumes from water-treatment plants are often elevated (reaching around 10^8 L/day), leading to the daily discharge of a substantial number of MPs and microfibers."[13] Water treatment differs by community, but general guidelines have been described by the Centers for Disease Control and Prevention (CDC). The first technique is coagulation, where chemicals such as salt, aluminum, or iron are added, and the positive charges of the chemicals neutralize the negative charges of dirt and other dissolved particles resulting in a bigger compound.[15] Following coagulation, flocculation, and sedimentation is the gentle mixing of water to form larger, heavier particles called flocs that will settle on the bottom of the water.[15] The first stage in the process, just described, on average removes 75% of microplastic.[13] After sedimentation, where the solids are separated, filtration begins, clear water passes through filters made of sand, gravel, and charcoal that remove "dissolved particles and germs, such as dust, chemicals, parasites, bacteria, and viruses."[13] The final technique used is disinfection, chemicals such as chlorine, chloramine, or chlorine dioxide might be added to kill remaining parasites, bacteria, or viruses.[15] When the final stages are complete removal efficiency increase to 91.9%.[13] Considering the high toxicity of microplastics 91.9% removal efficiencies on average are not safe or capable.

Health Risks

Although there is a moderate removal efficiency of microplastics it is still proven that microplastics are present in tap water. According to Jodi Sulpizio, a natural resource educator at Pennsylvania State University, on average 5.5 plastic particles are found per liter in tap water.[16] This poses as a serious threat to human health, they may "act as endocrine disruptors, altering hormonal signaling pathways that are essential for the proper functioning of reproductive systems."[10] This is further examined by Yonsei University of Medicine in South Korea, previously explored, due to microplastics' ability to absorb chemicals and its irregular shapes, it can physically stimulate the body, for when synthesizing plastic polymers, endocrine disruptors are used.[4] Endocrine disruptors are hormonally active agents, that can result in various cancers and reproductive disorders.[4] It is important to recognize that there is limited research on the adverse effects of microplastics, specifically long-term effects, but conversely, it cannot be ignored that microplastic ingestion is toxic and must be addressed.

MHETase and PETase

According to The American Chemical Society, PET accounts for the "sixth most produced polymer and the most commonly manufactured thermoplastic, principally for packaging purposes."[17] In 2016, a bacterium, *Ideonella sakaiensis*, was revealed to have the ability to grow in PET environments and use it as a carbon energy source.[17] This behavior was enabled by two enzymes PET hydrolase (PETase) and monoterephthalate hydrolase (MHETase). In summary, PETase can break PET polymers into monomers (MHET) then MHETase would break the molecule down into ethylene glycol and terephthalate, both of which can be metabolically assimilated by the bacteria.[17] PETase is currently the best wild type protein that can work in mild conditions, 40 degrees Celsius.[17] Thus, the goal of this research is to analyze the formation of these enzymes in PET environments that also mimic the conditions present in drinking water treatment facilities. The formation of the enzymes reacting with the environment will indicate the feasibility of utilizing them as a water treatment. However, a prediction can made that the formation of the enzymes will not be affected by the environment.

Method

This research study relies on scientific data analysis; thus, an experiment was conducted of inquiry. Data gathered was done by the student researcher, rendering it primary due to there being a lack of experimentation on PETase and MHETase in drinking water treatment facility climates. Most data are qualitative, relying on the phenol red indicator color and gas presence in the Durham fermentation tubes. Quantitative data was also collected through weight data analysis. Both quantitative and qualitative evidence is produced and analyzed furthering the reasoning for a scientific data analysis study. For the purpose of the experiment, all necessary materials were bought, refer to the appendix for an itemized list.

Material Specification

Materials such as Durham fermentation tubes, Compound Microscope, Bunsen Burner, Hot Plate, phenol red lactose broth, Autoclave, Agar slants, 37 degrees Celsius Incubator, Analytical Scale, and Inoculation loops were already made available to study without the need to order them.

For water sample collection, two Autoclave jars were used. The effectiveness of the jars is crucial to the experiment. The cap prevented any particles from entering or exiting the enclosed environment, protecting it from affecting the data due to contamination.

Water Selection

Two 500 millimeters (ml) of water were collected directly from the City of Ann Arbor Drinking Water Treatment Facility into Autoclave jars, this was taken before the water treatment process began. The City of Ann Arbor Drinking Water Treatment Facility's water source is the Huron River. So, this water sample will be referred to as River Huron Water. The other 500 ml water sample was collected before the filtration process. This water sample will be referred to as Pre-Filtration water. This was done to closely mimic the experiment to what is current in drinking water treatment facilities.

Plasmid Selection

Escherichia coli (E. coli) was purchased with a plasmid, pCJ189, from *AddGene*. pCJ189 has MHETase (Genbank GAP38911.1) linked to PETase (Genbank GAP38373.1) from *Ideonella sakaiensis*.

Procedure

To create bacterial growth, 300 ml of distilled water and two grams of phenol red lactose broth was mixed into a solution via a Hot Plate.

According to The University of Wyoming, during fermentation microorganisms produce gas, which may indicate bacterial growth, so Durham fermentation tubes trap the gas byproducts and appear as a bubble on the top of the tube.[18] Thus, 20 fermentation tubes were prepared, all consisting of phenol red lactose broth. To purify the subsamples an Autoclave was used. The purpose of this is to eliminate any competing bacteria to maintain a controlled system and ensure it is running under a single variable. The Autoclave was set at 125 degrees Celsius. After autoclaving the subsamples for 15 minutes they were transferred to an incubator for 24 hours at 37 degrees Celsius.

Of the 20, ten were inoculated with *E. coli* incubated with pCJ189. This *E. coli* will be known as Modified *E. coli*. The other ten were incubated with *E. coli* that didn't contain pCJ189. This will be referred to as Unmodified *E. coli*. Using inoculation loops and incubation techniques, the modified and Unmodified *E. coli* was transferred to their designated fermentation tubes. The purpose of testing Unmodified *E. coli* was to have one control to show that *E. coli* alone cannot digest the PET samples. After the transfer of *E. coli* 2 ml of Huron River water was added to three Modified *E. coli* and Unmodified *E. coli* fermentation tubes and another three of Modified *E. coli* and Unmodified *E. coli* consisted of 2 ml of water collected before the filtration process. The addition of these types of water is to analyze the behavior of the enzymes in water that are present in drinking water treatment facilities.

After incubation, six by one centimeters of pure PET plastic were cut and organized by numbers, and that is what the fermentation tubes will be referred to as. The samples were then introduced to eighteen fermentation tubes, and twenty-eight PET plastic samples were cut in total. The mass of each plastic was taken before it was introduced to other variables using an Analytical Scale, the purpose of this was to have a starting point in the data and have proper documentation. The plastics were then viewed under a compound microscope and pictures were documented for qualitative data analysis. Of the remaining fermentation tubes 2 were left as blanks that only had Modified or Unmodified *E. coli* present. This is to ensure consistency in the experiment.

It is recommended that the system be incubated at 37 degrees Celsius for top digestion results. After 96 hours the fermentation tubes were taken out of the incubator and the plastic was removed and rinsed with distilled water. This is to exterminate any remaining E. coli, so the mass is not affected. To analyze the changes in the plastic such as texture, size, and mass they were viewed under a Compound Microscope and weighed using an Analytical Scale. Pictures were then taken as qualitative evidence.

Results/Discussion

Table 1.1 Modified *E. coli* with 2 ml of Huron River Water

Fermentation Tube by Plastic number	Grams of PET before 96 hours	Grams of PET after 96 hours	Color before 96 hours	Color after 96 hours	Texture	Gas Production
5	0.0628	0.0624	Light Red	Dark Red	No Change	None
6	0.0804	0.0803	Light Red	Dark Red	No Change	None
7	0.0660	0.0659	Light Red	Dark Red	No Change	None

Table 1.2 Unmodified *E. coli* with 2 ml with Huron River water

Fermentation Tube by plastic number	Grams of PET before 96 hours	Grams of PET after 96 hours	Color before 96 hours	Color after 96 hours	Texture	Gas Production
8	0.0752	0.0752	Yellow	Yellow	No Change	Yes
9	0.0757	0.0757	Yellow	Yellow	No Change	Yes
14	0.0610	0.0610	Yellow	Yellow	No Change	Yes

The significance of this study is determined through not only a comparison of each of the same samples but also a comparison of which *E. coli* or type of water sample was utilized. Row 1 of the Tables organizes the fermentation tubes by plastic number. Due to larger pieces of plastic introduced to the *E. coli*, the experiment was held for a total of 96 hours. So, row 2 details the weight in grams of PET plastic samples before it was introduced to the experiment, and row 3 details the weight in grams of PET plastic after the experiment was completed (96 hours). Column 5 demonstrates the phenol red indicator color before 96 hours and column 6 then demonstrates the phenol red indicator color after 96 hours. Phenol red indicator color change will indicate bacterial growth due to the fermentation of sugars resulting in a more acidic solution. Column 7 characterizes if there was texture change, and finally, column 8 illustrates if there was gas production. Gas production also may indicate bacterial growth.

Table 1.1 characterizes the change of mass of three PET samples incubated with Modified *E. coli* and 2 ml of Huron River water, while Table 1.2 characterizes the change in mass of three PET samples incubated with Unmodified *E. coli* and 2 ml of Huron River water. PET sample 5 had a higher decrease in mass compared to samples 6 and 7, but not drastically. From the data shown in Table 1.1, the average decrease in mass was 0.002 grams. From the data shown in Table 1.2, there is no change in mass. There was also a change in phenol red indicator from light red to dark red in fermentation tubes with Modified *E. coli* and River Huron water as compared to Unmodified *E. coli* and River Huron water. While the Unmodified *E. coli* fermentation tubes and River Huron water had gas production whereas fermentation tubes with Modified *E. coli* and River Huron had no signs of production. There was also no change in texture for all fermentation tubes in Tables 1.1 and 1.2. This

proves that MHETase and PETase were both active during the 96 hours, even with the addition of Huron River water.

Table 2.1 Modified *E. coli* with 2 ml of Pre-Filtration Water

Fermentation Tube by PET sample number	Grams of PET before 96 hours	Grams of PET after 96 hours	Color before 96 hours	Color after 96 hours	Texture	Gas Production
1	0.0595	0.0593	Light Red	Dark Red	No Change	None
2	0.0709	0.0708	Light Red	Dark Red	No Change	None

Table 2.2 Unmodified *E. coli* with 2 ml of Pre-Filtration Water

Fermentation Tube by PET sample number	Grams of PET before 96 hours	Grams of PET after 96 hours	Color before 96 hours	Color after 96 hours	Texture	Gas Production
3	0.0742	0.0742	Yellow	Yellow	No Change	Yes
4	0.0613	0.0613	Yellow	Yellow	No Change	Yes
16	0.0575	0.0575	Yellow	Yellow	No Change	Yes

Table 2.1 shows the change in mass of three PET samples incubated with Modified *E. coli* with the addition of Pre-Filtration water, while Table 2.2 shows the change in mass of three PET samples incubated with Unmodified *E. coli* with the addition of Pre-Filtration water. PET sample 1 had a higher decrease in mass when compared to sample 2 by 0.001 grams. In line with the data from Table 2.1, the average decrease in mass was 0.0015. There is no change in mass as detailed in Table 2.2. As consistent with Tables 1.1 and 1.2, PET samples introduced to Modified *E. coli* and Pre-Filtration water experienced phenol red indicator color change from light red to dark red and no gas production, while fermentation tubes with Unmodified *E. coli* experienced no phenol red indicator color change but had substantial gas production. There was also no change in texture in all PET samples. This concludes that MHETase and PETase were both active during the 96 hours despite the addition of Pre-Filtration water.

Table 3.1 Modified *E. coli*

Fermentation Tube by PET sample number	Grams of PET before 96 hours	Grams of PET after 96 hours	Color before 96 hours	Color after 96 hours	Texture	Gas Production
18	0.0636	0.0635	Yellow	Yellow	No Change	None
19	0.0657	0.0656	Yellow	Yellow	No Change	None

Table 3.2 Unmodified *E. coli*

Fermentation Tube by PET sample number	Grams of PET before 96 hours	Grams of PET after 96 hours	Color before 96 hours	Color after 96 hours	Texture	Gas Production
10	0.0655	0.0655	Yellow	Yellow	No Change	Yes
11	0.0671	0.0671	Yellow	Yellow	No Change	Yes
15	0.0750	0.0750	Yellow	Yellow	No Change	Yes

Table 3.1 details two PET samples incubated with Modified *E. coli*, while Table 3.2 details three PET samples incubated with Unmodified *E. coli*. PET samples 18 and 19 both experienced a decrease in mass of 0.001 grams also resulting in the average to be 0.001 grams. PET samples 10, 11, and 15 all stayed consistent with absolutely no change in mass. However, there was no phenol red indicator color change for all fermentation tubes as shown in Tables 3.1 and 3.2. However, there was a production of gas in fermentation tubes with Unmodified *E. coli* as compared to Modified *E. coli*. Regardless of the absence of phenol red indicator color change in fermentation tubes with Modified *E. coli*, there persists an average decrease in mass that demonstrates that MHETase and PETase were both active during the 96 hours.

Overall, the results of this study strongly support the hypothesis: the environment present in drinking water treatment facilities will not affect the formation of MHETase and PETase. This can be established through consistent PET sample digestion. It was expected that the mass of PET samples in Tables 1.1, 2.1, and 3.1 will decrease while the mass of PET in table 1.2, 2.2, and 3.2 will stay consistent. This statement holds true in all fermentation tubes tested. Tables 1.1 and 2.1 establish evidence that the addition of Huron River and Pre-Filtration water has no negative effects on digestion rate but truthfully has a higher digestion average. The change in phenol red indicator color in Modified *E. coli* trials as compared to the consistent yellow color in Unmodified *E. coli* trials demonstrates that the Modified *E. coli* underwent different biochemical pathways to digest the plastic while the Unmodified *E. coli* experienced normal fermentation and growth. The texture for all trials despite fermentation tubes with Modified *E. coli* suggests no deformities or erosion of PET samples, and therefore the texture stayed unchanged. But even with a lack of texture change the decrease in mass did not change and therefore texture has no correlation and is not an indicator of PET digestion. Due to the consistency of results in all trials, the method of inquiry is replicable.

Conclusion

Based on the data collected and due to microplastics being in the micron size an answer can be formed for the research question, “to what extent can PETase and MHETase be used as a water treatment in drinking water treatment facilities in the United States to rid it of microplastics?”. Ascribed from the unvarying mass results despite the presence of Huron River or Pre-Filtration water as depicted in Tables 1.1 and 2.1, it is unlikely that the formation of the enzymes and digestion rate will be affected from coagulation to filtration. Hence, to a high extent, both MHETase and PETase can be habituated as a treatment for microplastics.

The findings in this study have the potential to aid chemical engineers and other scientists in identifying effective treatments for removing microplastics in drinking water. This study offers insight into enzymes that have high efficiency rates and provides valuable research that contributes to the body of literature surrounding

MHETase and PETase formation in varying environments. Using evidence in this study realistic plans can be introduced. For example, accordant with current water treatment, the Modified *E. coli*, theoretically, can be introduced at the beginning of coagulation along with other added chemicals such as chlorine. Coagulation is the first step in drinking water treatment, where larger compounds are formed using polar charges, but plastic does not have a charge. Due to plastic's absence of charge, this will allow optimal time for the Modified *E. coli* to metabolically assimilate PET microplastics. Then, during the slow mixture in flocculation and sedimentation, the Modified *E. coli* can continue to assimilate the PET microplastics, as heavier particles such as dirt separate and settle at the bottom. The process will be complete with filtration and disinfection, which will rid the water of bacteria, including the Modified *E. coli* that will be used. Consequently, MHETase and PETase can be used as a treatment leading to improved quality and sustainability of drinking water, thus avoiding health complications of the digestion of toxic microplastics.

Limitations

A drawback to the findings of this investigation is the lack of variables the study was conducted under. Drinking water treatment facilities use many chemicals to purify the water of bacteria already present, it is unknown how the modified *E. coli* will react with other bacteria and chemicals. Similarly, water samples were collected from the City of Ann Arbor Drinking Water Treatment Facility, and as aforementioned every community differs in water treatment. This may limit the confidence of the results because only one treatment facility was taken into consideration. However, the treatment process outlined by the CDC is like that of the City of Ann Arbor's. Furthermore, the experiment was held in a stationary phase, while majority of the treatment process consists of movement. It is unknown whether the flow of water will affect the rate of PET digestion or assimilation. However, the motion of water will unlikely affect the rate of fermentation because the bacteria itself has motility.

Future Directions

Regardless of governmental intervention, American citizens are still consuming microplastics at high rates pressing the concern for public health. Thus, new treatments such as those using PETase and MHETase must be investigated further. To begin, this study opens a new direction for future research regarding microbe treatment for microplastics in drinking water treatment facilities. In the future, altering the method of inquiry by adding new variables as stated in limitations can be introduced to the Modified *E. coli*. Introducing chemicals and other bacteria that are present in drinking water treatment facilities to the Modified *E. coli* with enzyme formation can add to the evidence present in this study. To add on, it would also be useful to experiment and research other enzymes besides MHETase and PETase. This could lead to comparing mechanisms, kinetic sustainability, and digestion rates that could narrow down the optimal enzymic solution that could be best suited to drinking water treatment facilities. Continuously, more samples should be taken from other drinking water treatment facilities this would extend the results and research. Furthermore, data that is collected from this experiment can be used to write a recommendation to drinking water treatment facilities in the US, to encourage further experimentation of these enzymes and possibly other microbes.

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Appendix

Table 4.0 Itemized List

Item Number	Product Name	Company	Quantity	Cost
162666	Plasmid pCJ189 expressed in E. coli	*AddGene*	1	$89