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ARTICLE

Biology

Nature's recycler, degrading plastic with the help of mycelium

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The first commercial plastic was invented in 1907 by Leo Baekeland and has become widely used since the 1960s (Plaine Products, 2019). Plastic's worldwide use comes from its durability, which results from the long hydrocarbon chains and chemical additives like thermal stabilizers that make up plastic (Hahladakis et al., 2018). This durability, along with the relatively recent discovery of plastic, has caused a lack of safe and cheap disposal methods. Of the over 380 tons of plastic produced annually, only 9% is recycled (OECD, 2022). All this plastic ends up in landfills and in the ocean where they form into huge plastic islands sometimes.

As a consequence of plastic not being disposed of correctly, over 1 million animals spanning 700 species are estimated to die yearly because of plastic pollution (Guilford County, 2019; Parker, 2019). When ingested, plastics can cause various health issues in animals and humans, such as malnutrition, internal injuries, cancer, and developmental problems (Lai, 2022). Similarly, the chemicals present in plastic can kill or stun the growth rate of microorganisms such as fungi (Tetu et al., 2019). This has added selective pressure to fungi because of plastic-filled environments, causing some mycelium species to develop plastic-degrading characteristics. In a study done by Alam and 12 other researchers on PETase, a plastic degrading enzyme, researchers found higher abundances of PETase in areas known to have a large load of plastic in their own environment (Alam et al., 2020). They also found that the enzyme was more effective in places where organic substrates were diluted, supporting the idea that plastic-filled environments put selective pressure on organisms like mycelium (Alam et al., 2020). Due to this, we can use mycelium's evolving plastic-degrading trait to our advantage for plastic disposal.

Mycelium, fungi, and bacteria use exoenzymes to catabolize organic substrates, and plastic while also catabolizing it using surrounding molecules, to turn it into smaller, digestible pieces. The exoenzymes of the mycelium collide and bind to the molecules that make up the main chain of the plastic and form an enzyme-substrate complex. This breaks the chain into smaller chains that can be absorbed (Infinita Biotech, 2021).

This paper investigates the ability of mycelium to degrade plastic using its enzymes. I investigated the degradation effects of mycelium on low-density polyethylene (LDPE) after UV exposure, measured by how much weight the plastic lost over time. At the end of my pretests, I decided to degrade the UV-treated-LDPE with *Pleurotus ostreatus* mycelium, grown in coffee beans, over nine weeks with the plastic being taken out and weighted on days 25, 35, 45, 55, and 65.

First, low-density polyethylene goes through photocatalysis, as shown in Figure 1. This is a process where UV rays pass through and excites electrons. Free radicals are created, which react with oxygen to form oxygen hydroperoxide and attach to LDPE as shown. The oxygen hydroperoxide and the excited electrons make LDPE more unstable, which weakens the plastic and makes it easier for the mycelium to

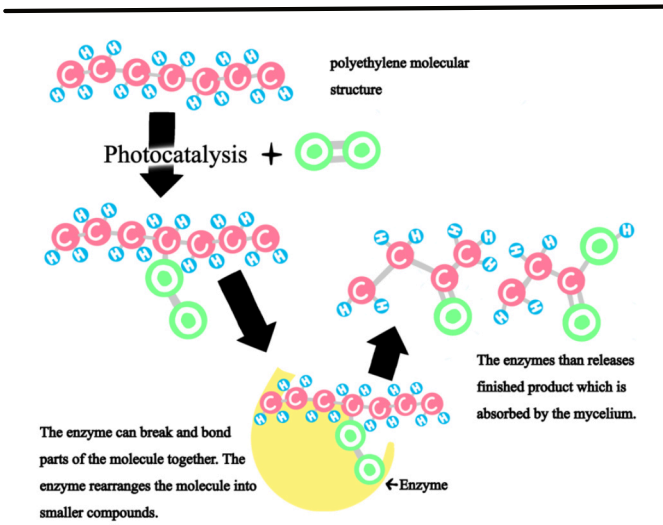

Figure 1: Lock and Key diagram showing how enzymes break molecules into smaller products the mycelium can absorb. Information compiled from Ghatge, 2020

break down (Cole-Parmer, n.d). Enzymes, shown in Figure 1, then break down the LDPE into smaller molecules that the mycelium can absorb and digest. The molecules produced depend on the enzymes, but after the mycelium absorbs them, these final molecules are broken-down molecules that get turned into ketones by esterase and lipase enzymes (Cole-Parmer, n.d). These molecules, which may differ depending on the substrate and enzymes, are then absorbed by the mycelium. Final molecules may differ but the process will be similar. Currently, it may be the only way for mycelium to break down plastic.

PRE-TESTS

I have done several experiments since the summer of 2021 to find the best method of degrading plastic and growing mycelium quickly and easily. I first learned how to grow mycelium from commercial *Pleurotus eryngii* mushrooms on cardboard. I sliced the stems into roughly 2 cm cubes and layered them on top of each other with wet sterilized cardboard. The mycelia grew quickly and in large amounts but also died off quickly, so I realized it might need a more nutritious substrate.

While trying to find a more nutritious substrate, I also wanted to see if mycelium from different mushrooms would grow better. I found nine different kinds around Fish Creek Forest in BC and Fort St. John, which I identified based on their appearances: *Panaeolina foenisecii, Lactarius vietus, Ramariopsis kunzei, Boletus sensibilis, Hygrocybe flavescens, Lycoperdon pyriforme, Coprinellus micaceus, Pleurotus ostreatus*, and a large unidentified mushroom with brown gills, a ring, and a round tan cap.

I put the sliced wild mushroom stems in nine trays, each containing four different substrates: coffee grounds, woodchips, soil, and a mix of coffee grounds and wood chips. The substrates were separated by cardboard. I also placed the sliced King Oyster mushrooms in two other trays, one with coffee grounds and the other with wood. The trays were opened daily for air and were sprayed with water every 3-5 days. I also tried growing the mycelium on agar, placing thinly sliced *Pleurotus ostreatus* stems on homemade agar, but it did not work well.

Through testing the substrate, I learned that wood chips, shown on the right side of Figure 3, and used coffee grounds were the most consistent in growing mycelium. Between them, I chose to use the coffee grounds as they were the easiest to keep moist, and they grew mycelium quickly with little effort. Using coffee grounds is also environmentally friendly because lots of people drink coffee which leads to large loads of coffee grounds being thrown out into the dump and producing significant methane emissions.

Among the different mushrooms, I chose to use *Pleurotus ostreatus* as it was readily available, low maintenance, and grew quickly. I used LDPE, identified as plastic number 4, due to its low crystallinity and lower density, making it relatively weak and making its bonds easier to break. LDPE is also commonly used as grocery bags and packaging and is often not recycled.

Through the data I gathered in my pretests, I decided that the best method was *Pleurotus ostreatus* (Pearl oyster) mycelium, grown from its mushroom stem, to degrade Low-Density Polyethylene (LDPE) in

Figure 2: Pleurotus ostreatus mushrooms (with mycelium) in wood chips (left). The tray has mycelium grown on wood chips after two weeks (right). Note: The mycelium's photo could not be captured well because the off-white mycelium blended into the tan wood chips on camera.

Figure 3: Jars with Pleurotus ostreatus mycelium in coffee grounds with low-density polyethylene Note: The jar on the left did not grow mold for a long time, but the jar on the right grew mold faster

coffee grounds. I also determined that a glass jar with holes poked in the lid was the best way to house the mycelium and provide air circulation as they did not grow mold for a long time, as shown in Figure 3.

MATERIALS AND METHODS

Research Question:
How much UV-treated LDPE, measured through weight (g) and length the plasic could strech (mm) , I will *Pleurotus ostreatus* mycelium degrade over nine weeks?

Materials used:
Pleurotus ostreatus stems, partially dry coffee grounds, and UV-treated LDPE was put in eleven 250 ml jars, one 500 ml, and two 1000 ml jars disinfected with 75% isopropyl alcohol. This was to see how much plastic the mycelium degraded. A scale was used to measure how much plastic went into each jar. To ensure the mycelium got ventilated air and humidity, three holes were poked into the lids with a nail and hammer. The jars were then put in a greenhouse made with a shelf and a plastic trap. A hygrometer and thermometer were placed in the greenhouse to measure humidity and temperature, respectively. A spray bottle filled with water was used during the experiment to keep humidity in the greenhouse. When the experiment ended, Ziploc bags were used to carry the plastic and mycelium separately. Then a ruler and marker were used to mark and measure how much to pull for the post-test.

Preparation:
Used coffee grounds from a local Tim Hortons were spread on a clean cloth and left to dry for two days to prevent water from the coffee grounds from pooling at the bottom and killing the mycelium due to excess moisture. The LDPE was hung outside in direct light for two weeks as the UV treatment.

Experiment
First, 15 jars were sterilized with 75% isopropyl alcohol (eleven 250 ml jars, two 100 ml jars, and one 500 ml jar). I layered 75 g of dried used coffee grounds and 1 gram of UV-treated LDPE with a sliced 7 cm *Pleurotus ostreatus* stem and put them in the middle of each of the eleven 250 ml sterilized jars. In the two 100 ml jars, jars 12 and 13, and the 500 ml jar, jar 15, the plastics to coffee ground ratio was increased because the jars were larger. Later, results from jars 12 and 13 were divided by 2 and 1.5 for jar 15; to see the percentage degraded as 2 and 1.5 grams of plastic were put in the bigger jars. Three holes were poked on the top of all lids with a nail to keep air and moisture flowing in. After, jars were put in a sterile dark mini greenhouse in a basement to decrease light exposure. The temperature was maintained between 14-18°C to prevent the mycelium from dying or growing mold.

Plastic from 3 jars was taken out every ten days after a 25-day initial growth period to let the mycelium grow and check if mycelium made any progress in degrading LDPE. The extracted plastic was washed with 75 % isopropyl alcohol to sterilize and remove coffee grounds and mycelium from the plastic. They were put in labeled Ziploc bags to weigh later in the college with a sensitive electronic scale.

After the experiment, I stretched the non-UV-treated plastic, UV-treated plastic, and UV-treated plastic in the mycelium to see if the plastic had weakened. A 3x1 cm piece of plastic from each category was cut out. A 1x1 cm square was marked in the middle of each one. The extra 2 cm of plastic was so I could hold onto the plastic properly. After ensuring that my fingertips lined up with the two lines marking the square, I stretched the plastic until it started ripping from one side. I measured the distance between the two marked lines to see how much it stretched before it began to break. This is to see if any part of the process, like UV treatment, weakened the plastic.

HYPOTHESIS

I believe the mycelium will further break down the already weakened bonds of the UV-treated plastic. LDPE has a branch-like structure which makes its intermolecular forces weaker. This will make it easier for the enzymes to catalyze the reaction of breaking their bonds. It also has a lower crystallinity than most plastics making it more reactive to enzymatic hydrolysis. The lower crystallinity will let enzymes have the surface area of the molecule needed to start the reaction (Mohanan, 2020).

However, I believe the mycelium will only degrade around 0.05 grams of plastic after nine weeks. I estimated this much because, in other experiments and prototypes such as the Fungi Mutarium, it took significantly longer to degrade a much smaller volume of plastic (Greene, 2022). There is a factor of uncertainty because I am doing this with quite a large amount of plastic, and there's a chance it may degrade below the estimated 0.05 grams because I'm using a much bigger piece of plastic.

RESULTS AND OBSERVATIONS

Figure 4 shows that plastic from jars 1-11 and 15 had minor variations. This happened because a less sensitive electronic scale was used at the start of the experiment. Some of the jars were different sizes so the amount of plastic was changed. Jars 12 and 13 were divided by 2 and 1.5 for jar 15; to see the percentage degraded as 2 and 1.5 grams of plastic were put in the bigger jars.

As shown in Figure 5, Mushroom urea, the waste product of the fungal metabolism of mycelium, was visible on the surface of the coffee grounds and mycelium in jars 12, 13, and 15. This could be a sign that the mycelium was degrading LDPE. Results from jar 14 were not used as jar 14 did not grow any mycelium.

As represented in Figure 4, plastic from the biggest jars, 12 and 13, had decreased in weight. Figure 6 shows that plastic taken out towards the end of the experiment was more fragile and could handle less stretching than UV-treated and plastic not treated with UV. Comparably, the non-UV-treated plastic could be stretched more, as shown in Figure 6.

DISCUSSION

According to the results, mycelium may have properties that can weaken and degrade LDPE. Mycelium in the largest jars, 12 and 13, degraded about 0.1 grams of the plastic. However, only the largest jars degraded some plastic.

I believe that the mycelium had more room to grow in larger jars because of the mycelium's increased surface area and mass (Ito et al., 2013). Because it is a

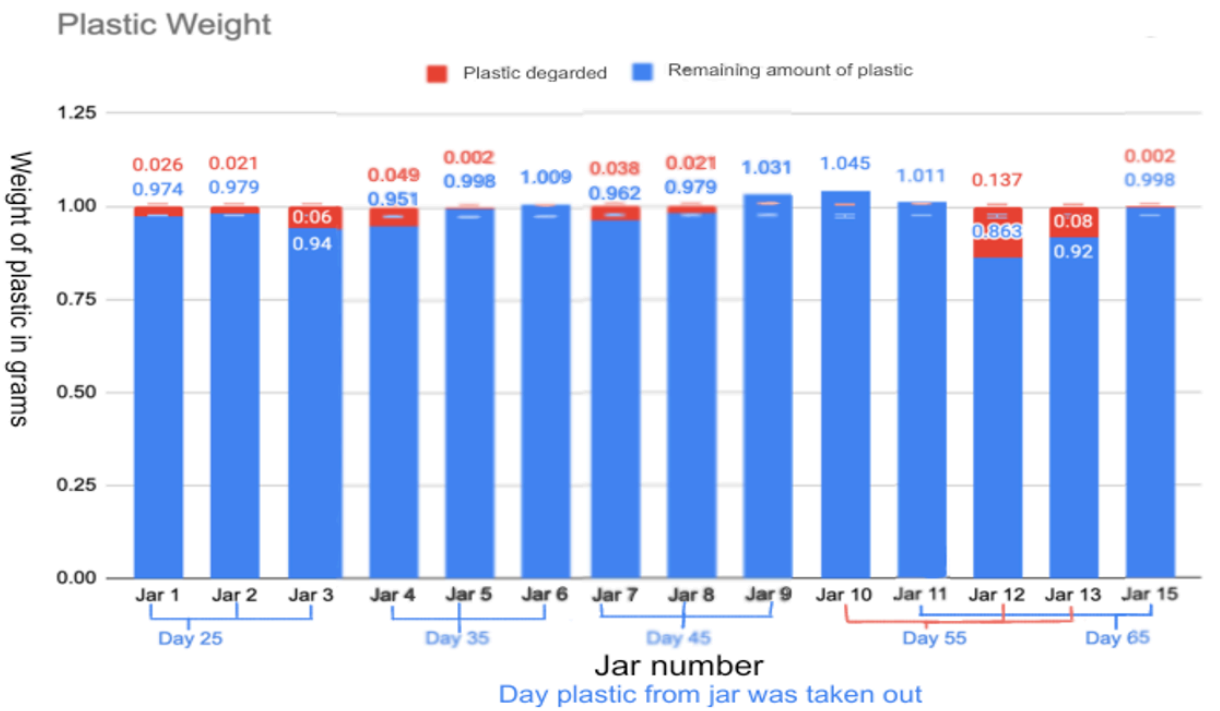

Figure 4: Stacked bar graph showing the weight of plastic taken out of jars of mycelium on different days. Note: Except for jars 12 and 13, plastic from other jars has most likely not degraded.

Figure 5: Mushroom urea sitting on the surface of coffee grounds in jar 15

larger organism, it needs more enzymes to break down more substrate. It will get more nutrients from this which is needed for it to support its daily function. Since there are a lot more enzymes in the bigger jars, there is a higher chance for the enzymes to collide with the plastic and break down its polymers, allowing the plastic to be absorbed and digested by the mycelium.

SOURCES OF ERRORS AND IMPROVEMENTS

There were three main sources of error in this experiment. The first error was a variation in the sensitivity of the scale. The scale I initially used to weigh the plastic and coffee grounds only measured up to 0.1 g and, therefore, could not measure minor variations in the weight of the plastic. This reduced the validity of the results because I couldn't detect minor changes, making it difficult to tell whether the mycelium had reliably degraded any plastic. To improve the validity of the results, I switched to a more sensitive scale that measured up to 0.001 g when measuring the weight of the plastic after taking them out of the mycelium.

The second error was not being able to measure the thickness of the plastic. Different parts of plastic may have variations in thickness which could influence the plastic's strength. Stretching plastic with the same thickness would yield more accurate and reliable results. Unfortunately, I could not find a vernier caliper precise enough to measure small differences in the thickness of the plastic. In future experiments, it is important to try and use plastic with even thickness throughout to increase reliability and accuracy.

The third error that may have affected the experiment is using my hands to stretch the plastic. I tried to pull each plastic with equal strength, but the results may not be as accurate or reliable as a machine doing it due to human error.

There are three other improvements I would also make in future trials. The first improvement is to do small and slightly bigger batches in larger jars over a

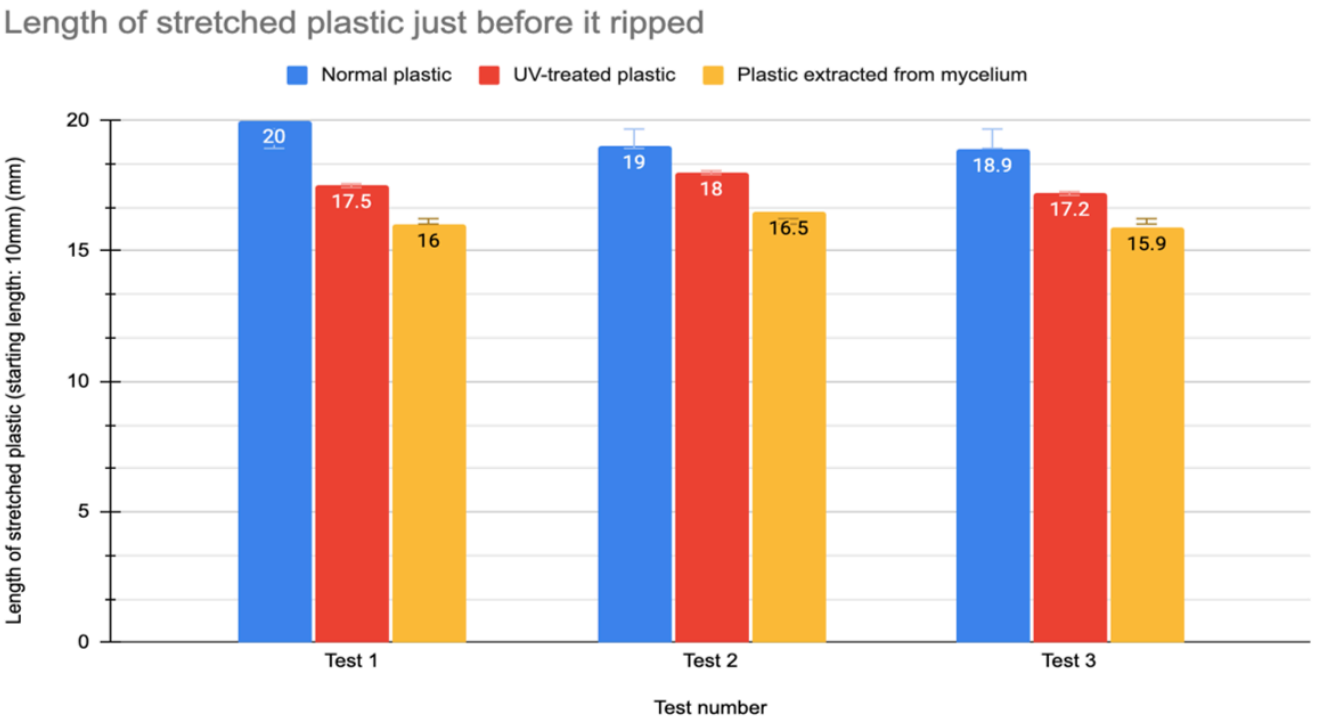

Figure 6: Bar graph showing the length of plastic stretched just before it started to rip. Starting length of the plastic was 10 mm.

long time. This will show if there is a difference in the weight of plastic degraded by mycelium depending on the size of the mycelia. This is done because in my experiment I found the larger jars degraded more plastic than the smaller jar. This improvement would be able to properly test the accuracy and reliability of this experiment's results. It may degrade more plastic and show better results. This would be done by putting more mycelium, plastic and coffee ground in the same ratio into a bigger jar. This may allow the mycelium to focus energy and time on degrading the plastic instead of survival.

The next is placing non-UV-treated LDPE in the mycelium as a control to confirm whether the UV treatment speeds up the degradation of LDPE. This will test if the UV takes part in degrading some of the plastic or weakening it to help the mycelium degrade it more easily. It will also help show if it is necessary to do so and can be used as a comparison point for the rest of the data and improve the validity of the results.

Finally, a third improvement is to use more mushroom stems with more mycelium to help the mycelium grow more quickly and speed up the experiment. This may show improved and sped-up results which is good because mycelium usually takes a long time to degrade plastic.

FURTHER RESEARCH

While conducting the experiment, I recognized three areas where further research is required. The first is to experiment with mycelium in places with lots of plastic, like landfills, or areas with significant plastic pollution. The mycelium there would have likely already evolved to be better adapted to a plastic-filled environment, which may help degrade plastics more efficiently. This is so we can test whether using mycelium from a plastic-filled environment would change degradation rates. This could be easily done by going to a landfill with lots of plastic or a place known to have a lot of plastic and collecting mycelium. This mycelium would then be compared to other mycelium and its effectiveness in degrading plastic.

Secondly, I want to test if breeding mycelium successfully degrades plastic or shows signs of successfully degrading plastic. This is to see whether breeding mycelium or making it mutate will increase the degradation rate of the enzymes it excretes. I would use mycelium mating, which is getting haploid parent cells of different sex and letting them fuse. If the mycelium species do not reproduce sexually, I will try growing it and then taking spores from the fruiting body to grow more mycelium. The mycelium may have a chance to mutate, causing the enzymes it produces to degrade plastic and be more efficient.

Finally, I would like to find the optimal size for the mycelium to grow so it is easy to take care of when degrading plastic at a large scale. With that, I would like to see if the amount and size of mycelium and plastic would affect how much plastic is being degraded. If mycelium were ever really effective at degrading plastic, then it would be good to find an optimal size for the mycelia to be to degrading plastic as quickly and efficiently as possible. This will help people degrade plastic quicker and may be able to reduce the cost of taking care of it or replacing dying mycelium. It would be done with trial and error, starting with large and small batches of mycelium and

seeing the degradation rate of plastic in different quantities.

CONCLUSIONS

I concluded that my hypothesis was partially correct in that it would degrade some plastic because, in my hypothesis, I thought that the mycelium would degrade about 0.05 grams of plastic, and since my results say that the bigger jars did degrade about 0.1 grams of plastic. However, even this number is inconclusive due to errors in measuring the plastic. Therefore, the actual weight of plastic degraded could be lower. From this, I concluded that mycelium has properties that may potentially degrade plastic and may be one of the best solutions for degrading plastic in the future. Although the experiment was mainly inconclusive, it could have potential in the future because of the minor weight errors that weren't possible to weigh without a sensitive scale. According to the results, the difference in weight of plastic in larger jars (12, 13) decreased on average by about 1 gram. If the mushroom urea had been tested for types of enzymes, I might have been able to find enzymes that may have degraded plastic and formed a more conclusive result. As mycelium continues to evolve and adapt to its environment, it may produce the enzymes needed to degrade plastic efficiently.

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My name is Swas Ghosh, and I am pursuing grade 10. I have been competing in science fairs since I was in grade 4 and this was my second time competing in the Canada-Wide Science Fair. I got interested in science fair when my grade 4 teacher first introduced me to it. I was also in the leadership club during my elementary and middle school before covid.

Some subjects I enjoy are Science, Mathematics and Art. I like soccer, swimming, reading, and photography. I want to continue to learn and explore the beautiful world of science.